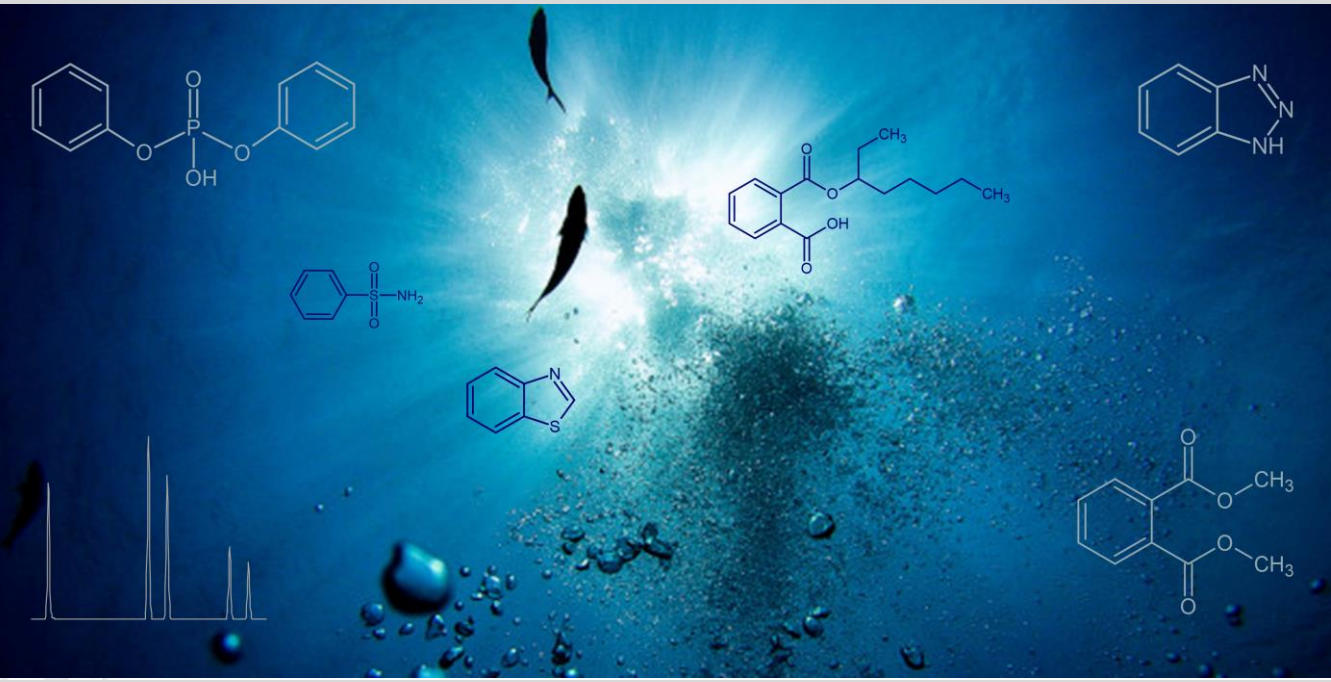
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS

Míriam Hidalgo Serrano

UNIVERSITAT ROVIRA i VIRGILI

Determinació i presència de compostos orgànics d'alt volum de producció, els seus productes de transformació i metabòlits en ambients aquàtics

MÍRIAM HIDALGO SERRANO



TESI DOCTORAL
2021

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

Determinació i presència de compostos orgànics d'alt volum de producció, els seus productes de transformació i metabòlits en ambients aquàtics

Míriam Hidalgo Serrano

TESI DOCTORAL

Dirigida per

Dra. Rosa Maria Marcé Recasens i Dra. Eva Pocurull Aixalà

Departament de Química Analítica i Química Orgànica

UNIVERSITAT ROVIRA i VIRGILI

Tarragona

2021

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

UNIVERSITAT ROVIRA i VIRGILI
Dept. de Química Analítica
i Química Orgànica

La Dra. ROSA MARIA MARCÉ RECASENS, Catedràtica del Departament de Química Analítica i Química Orgànica de la Facultat de Química de la Universitat Rovira i Virgili, i

La Dra. EVA POCURULL AIXALA, Catedràtica del Departament de Química Analítica i Química Orgànica de la Facultat de Química de la Universitat Rovira i Virgili,

FEM CONSTAR:

Que aquest treball, titulat "DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS", que presenta MÍRIAM HIDALGO SERRANO per a l'obtenció del títol de Doctor, ha estat realitzat sota la nostra direcció, a l'Àrea de Química Analítica del Departament de Química Analítica i Química Orgànica d'aquesta universitat.

Tarragona, 23 de novembre de 2021

Dra. Rosa Maria Marcé Recasens

Dra. Eva Pocurull Aixalà

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

Sembla increïble però això s'acaba. Jo, que mai havia pensat que faria un doctorat, que tot allò era massa per mi, he arribat fins aquí en gran part gràcies a la fantàstica experiència que va ser el treball de final de màster que em va obrir les portes al món de la recerca. És per això que no puc tancar aquesta etapa sense agrair l'ajuda i suport de tots els que han estat a prop meu aquests últims anys.

En primer lloc vull donar les gràcies a les meves directores de Tesi, la Dra. Rosa Maria Marcé i la Dra. Eva Pocurull, per la seva dedicació, ajuda i paciència i, sobretot, per compartir amb mi els seus coneixements. Em sembla increïble tot el que he après d'elles des que vaig començar aquesta Tesi; fos quin fos el problema amb què ens topàvem sempre tenien una resposta o un consell per solucionar-lo. També vull donar les gràcies al Dr. Francesc Borrull per brindar-me l'oportunitat de formar part de CROMA (que crec que és molt més que un grup d'investigació) i per tenir sempre un somriure, una paraula o una gràcia per mi quan ens creuàvem pel passadís o pel laboratori. Vull donar les gràcies també a la Dra. Núria Fontanals per la seva ajuda quan l'Orbi o el cromatògraf es resistien a funcionar (a vegades de manera simultània) i a les Dres. Marta Calull i Carme Aguilar per l'ajuda i els bons moments que hem compartit al grup. No voldria oblidar-me del personal del departament, la Tere, el Jaume, l'Avelina, la Dúnia i la Beatriz pels cops de mà que m'heu donat.

Agraeixo especialment la Tesi a tots els companys de laboratori que han estat un suport imprescindible aquests anys. A la Laura V, per l'ajuda, els consells, els dinars de pandèmia i els riures. A l'Albert, per la companyia des del màster, per les converses transcendentals i les recomanacions de llibres i de música. Al Joan Carles, per ser la única persona que es va deixar convèncer per venir a l'Orfeó a cantar, per les teves anècdotes... queden pendents un parell de partides de trivial. Al Sergi, per compartir amb mi l'iglú del 324, per ajudar-me a resoldre els dubtes tontos i per fer el friki amb mi parlant de Harry Potter (i per regalar-me el millor *felpudo*). A l'Óscar pels drames, els cotis i per ser el meu company de penes amb els ftalats i els peixos. Se t'ha trobat a faltar aquests últims mesos. També a l'Alba, l'Eugènia, la Tati, la Laura T, el Sameer, la Sílvia i l'Alberto.

Gràcies també a l'Ares, l'Imma, l'Olga i la resta de la gent de COVIDES per obrir-me les portes al vostre laboratori i al vostre celler aquests últims estius. He rigut molt amb vosaltres, he gaudit les veremes i a més m'heu ajudat a posar en perspectiva la Tesi. Ens tornarem a veure!

No menys important és la gent de fora del laboratori ja que sense el seu suport no hauria arribat fins on soc ara. Per una banda les amigues de tota la vida: l'Eva, que farda

de mi amb tothom encara que no m'ho mereixi i amb la que hem compartit converses congelades de matinada i els millors dinars de "gent gran"; i la Carme, que va compartir amb mi trens, llibres música i gats per ajudar-me a ser aquí avui, encara que ara estigui llunyet. Per altra banda als que no són de tota la vida però com si ho fossin. *A Pedro, porque aunque hablemos muy de vez en cuando echo de menos que seas mi compi de lab y lo malos que éramos a veces. Y a ti, Eleana, porque me da la vida que aunque quedemos solo para un café, ambas sabemos que se alargará a comida, merienda, cena y lo que surja.*

Finalment voldria donar les gràcies a la meva família. Als meus pares, pel suport infinit que m'han donat sempre, per animar-me a esforçar-me al màxim i per ser-hi sempre, entenguessin o no el que estava fent. Al meu germà per fer-me riure, per donar-me la tabarra i per apuntar-se a qualsevol complot amb mi. També a les iaies, que sempre han estat les que més ganes tenien que estudiés tot el que volgués ja que elles no ho havien pogut fer. I als avis, que tot i que fa temps que no hi són sempre he pensat que estarien orgullosos de mi. Per últim, *gracias a ti, Joshua. Gracias por tu paciencia infinita, por aguantar el estrés de estos últimos meses, por creer ciegamente en mí, por reírte de mis chistes de mierda, por apuntarte hasta a un bombardeo si es conmigo, por haber dejado tus bonitas islas para venirte a vivir conmigo... Sin ti esto no habría sido posible*. Per tot això i molt més, gràcies de tot cor. Aquesta Tesi és per vosaltres.

The saddest aspect of life right now is
that science gathers knowledge faster
than society gathers wisdom.

Isaac Asimov

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

ÍNDEX

RESUM

Els compostos orgànics d'alt volum de producció són substàncies orgàniques que es fabriquen o s'importen en volums de més de 1.000 tones en algun país l'Organització per la Cooperació i el Desenvolupament Econòmic. Són substàncies que s'emeten de manera continuada al medi ambient i que, a causa de les enormes quantitats en les quals s'empren a la indústria i de la seva toxicitat, poden comportar riscos ecotoxicològics per al medi ambient i per la salut pública. Els medis aquàtics són particularment sensibles als efectes negatius d'aquestes substàncies que trenquen el delicat equilibri que mantenen els ecosistemes marins i d'aigua dolça.

Així doncs l'objectiu principal d'aquesta tesi doctoral és el de desenvolupar nous mètodes analítics basats en la cromatografia de líquids acoblada a l'espectrometria de masses d'alta resolució per estudiar la presència de productes químics d'alt volum de producció, així com la dels seus productes de transformació i metabòlits, en mostres d'ambients aquàtics.

En la primera part d'aquesta tesi s'han analitzat mostres d'aigua del riu Ebre i mostres d'aigua de mar recollides a la costa de la ciutat de Tarragona per la determinació de derivats de la benzotriazola, la benzotiazola i la benzosulfonamida en aigües superficials. Per poder analitzar aquestes mostres s'ha adaptat i optimitzat un mètode basat en l'extracció en fase sòlida. En la segona part d'aquesta tesi s'han desenvolupat tres mètodes analítics diferents per la determinació d'èsters de l'àcid ftàlic i de l'àcid fosfòric en les espècies de peix i marisc més consumides per la població de Catalunya. Aquestes mostres representen un repte a l'hora de la seva extracció i neteja a causa de la seva complexitat, ja que contenen un gran nombre de substàncies que poden interferir amb la determinació dels compostos d'interès. En aquest apartat s'han utilitzat i comparat dues tècniques d'extracció: l'extracció amb líquids pressuritzats i l'extracció amb QuEChERS. També s'han avaluat diferents tècniques de neteja de mostra per obtenir extractes adients per la seva anàlisi. I a la tercera part d'aquesta tesi presentem els resultats preliminars d'un estudi de monitoratge al llarg d'un

any de la presència de dièsters de l'àcid fosfòric en les espècies de peix i marisc més consumides a Catalunya.

CAPÍTOL 1. INTRODUCCIÓ

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

1.1. Compostos orgànics d’alt volum de producció

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

La societat en què vivim avui en dia seria impensable sense l'ús de substàncies químiques d'origen antropogènic pensades per millorar la nostra qualitat de vida ja sigui amb finalitats industrials, mèdiques, agrícoles o cosmètiques. Com ja es reflectia a l'Agenda 21 promoguda l'any 1992 en una conferència de les Nacions Unides: *"Per arribar als objectius socials i econòmics de la comunitat mundial és indispensable utilitzar una gran quantitat de productes químics, i les millors pràctiques modernes demostren que aquests productes es poden utilitzar àmpliament, amb eficàcia econòmica i amb un alt grau de seguretat"* [1]. El que no es té en compte en aquesta afirmació (i que només es deixa intuir més endavant en el mateix escrit) és que l'ús de grans quantitats de substàncies químiques augmenta les probabilitats de contaminació química del medi ambient. Són diverses les rutes d'entrada de contaminants al medi ambient i a la **Figura 1** se'n mostren les principals.

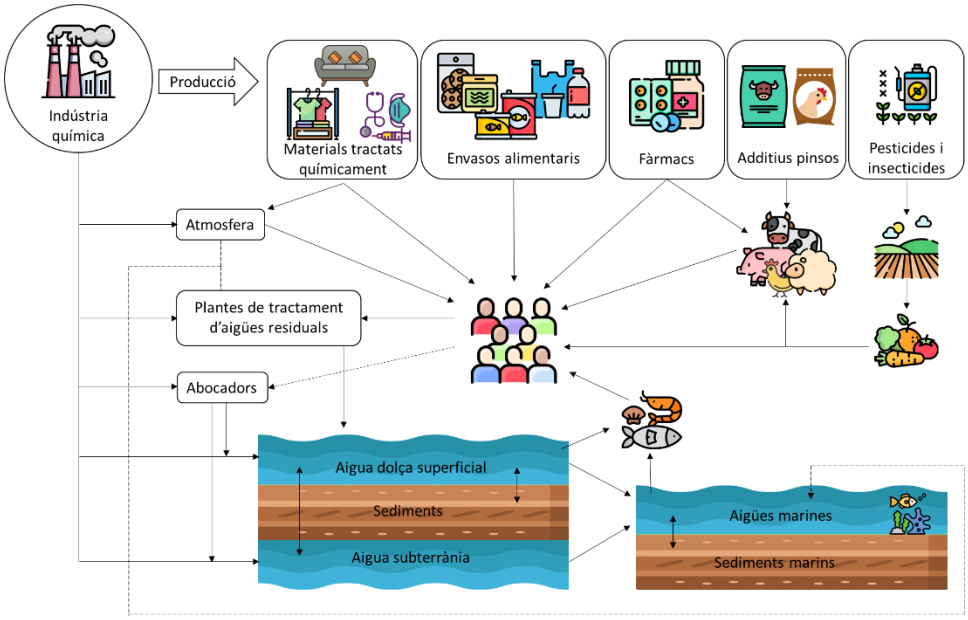


Figura 1. Distribució dels contaminants al medi ambient.

Moltes substàncies generades com a productes secundaris de processos químics són alliberades directament a l'atmosfera, l'aigua o el sòl i després passen a altres compartiments del medi ambient. Altres substàncies s'empren directament en el

tractament d'animals i plantes o terres de cultiu, però tard o d'hora passen a formar part d'aigües de rebuig i aigües superficials i subterrànies. Una altra via de contaminació és la degradació de productes que fem servir dia a dia, especialment plàstics. Molts dels additius afegits a aquests productes no s'hi troben lligats químicament i s'alliberen amb facilitat durant la vida útil dels objectes. Tot i que aquests contaminants afecten a tot el medi ambient, els medis aquàtics (formats per ecosistemes marins i ecosistemes d'aigua dolça) són particularment sensibles als seus efectes.

Els ecosistemes marins ocupen un 71% de la superfície del planeta i representen un 99.5% de la biosfera total. Contenen una gran biodiversitat, amb cadenes tròfiques fins a dos nivells més llargues que les d'altres ecosistemes, però també són dels més afectats pels estressos derivats d'activitats humanes, especialment en zones costaneres [2]. Un dels factors d'estrès és la destrucció física dels ecosistemes. Fa quinze anys s'estimava que s'havien perdut un 20% dels esculls de corall, un 35% dels manglars i fins a un 20% de les maresmes degut principalment al desenvolupament costaner i a pràctiques de pesca destructives i aquestes dades només incrementen amb el temps [3]. Un altre factor d'estrès és la contaminació deguda a abocaments de substàncies químiques i a l'acumulació d'escombraries als mars i oceans, especialment de microplàstics. Les diferents mides i densitats dels microplàstics fan que es trobin distribuïts àmpliament entre la superfície, les columnes d'aigua i els sediments de mars i oceans. Aquest fet, combinat amb la degradació dels microplàstics derivada de l'acció dels corrents marins, la llum solar i la calor fa que s'alliberin substàncies químiques de manera constant als ecosistemes marins. Aquesta contaminació està generant problemes no només a escala socioeconòmica i de biodiversitat, sinó que afecta la nostra pròpia salut a través de la ingesta de peix i marisc [4]. Mentre que l'aigua salada representa més d'un 97% de l'aigua de la Terra, l'aigua dolça es troba majoritàriament en forma de gel als pols (un 2%) i en aigües subterrànies (un 0.3%). Els rius, en canvi, només representen un 0.00009% de l'aigua total de la biosfera. Tot i això, són els ecosistemes que han patit més els estralls a causa de l'ús de la seva aigua per l'agricultura, la indústria i l'ús domèstic. En la majoria

de casos aquesta aigua es reintrodueix als rius contaminada per substàncies químiques i per matèria particulada, ja que les plantes de tractament d'aigües no són capaces d'eliminar alguns dels contaminants completament [2,5]. A més, durant els processos de tractament de les aigües residuals, especialment en processos tèrmics o a pHs alts, alguns d'aquests compostos es transformen en els seus productes de transformació i metabòlits que també poden tenir efectes ecotoxicològics negatius [6,7].

En aquestes últimes dècades, una de les principals preocupacions de la comunitat científica i dels governs mundials ha estat la contaminació dels productes químics d'alt volum de producció (AVP). L'Organització per la cooperació i el desenvolupament econòmic (OECD) i l'agència per la protecció del medi ambient dels Estats Units (EPA) defineixen aquestes substàncies com aquelles que tenen un volum de producció o d'importació anual de més de 1.000 tones [8,9].

El principal motiu pel qual aquestes substàncies causen alarma en aquestes organitzacions és perquè fan l'aproximació que una alta producció i importació significa una alta exposició i això comporta riscos tant per a la salut com per al medi ambient. Així doncs, tot i que algunes d'aquestes substàncies són innòcues, d'altres poden representar un perill a mitjà i a llarg termini no només per la flora i la fauna, sinó també per als éssers humans.

És per tot això que aquesta tesi doctoral se centra en la determinació de diverses famílies de productes AVP en medis aquàtics, seleccionades pel seu ús i efectes toxicològics: els derivats de la benzotriazola, la benzotiazola i la benzosulfonamida, els dièsters i monoèsters de l'àcid ftàlic i els dièsters de l'àcid fosfòric. Els següents apartats descriuen en detall les principals característiques d'aquests compostos així com els efectes que poden tenir en els éssers vius i les concentracions en què es troben en medis aquàtics.

1.1.1. Derivats de la benzotriazola, benzotiazola i benzosulfonamida

Els derivats de la benzotriazola, o benzotriazoles, són substàncies heterocícliques aromàtiques amb estructures basades en la fusió d'un anell de benzè amb un anell 1,2,3-tiazola. A causa de les característiques de la seva estructura, aquests compostos poden existir en dues formes tautomèriques (**Figura 2**) tot i que la primera és la predominant en un 99.9% a temperatura ambient [10]. A més, els seus derivats es poden formar tant per substitució a l'anell de benzè, com per substitució a la posició 1 de l'anell 1,2,3-tiazola.

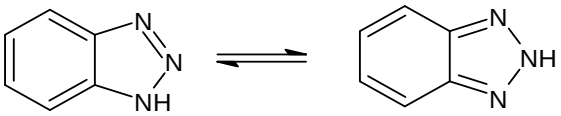
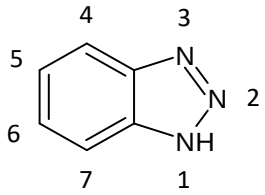


Figura 2. Estructures tautomèriques de la 1H-benzotriazola.

La versatilitat de la seva estructura fa que tant la benzotriazola com els seus derivats tinguin una gran varietat d'aplicacions. Per aprofitar les seves propietats inhibidores de la corrosió del coure i els seus aliatges les benzotriazoles s'utilitzen com a anticorrosius en líquids anticongelants en aviació, líquids de frens i detergents en pols [11]. Per altra banda, s'usen abundantment com a estabilitzadors UV en teixits i plàstics [12], com a agents antimicrobians i biocides [13]. La seva estructura també els fa extremadament útils pel desenvolupament de nous compostos farmacològicament actius [14].

Als registres de l'agència europea de productes químics (ECHA) es poden trobar els volums en els quals es fabriquen o s'importen algunes de les benzotriazoles a l'Àrea Econòmica Europea. Per exemple, la benzotriazola es registra en volums d'entre 1.000 i 10.000 tones anuals [15] i la 1-hidroxibenzotriazola entre 10 i 100 tones anuals [16]. La 4-metil-1*H*-benzotriazole, la 5-metil-1*H*-benzotriazola i la 5,6-dimetil-1*H*-benzotriazola es registren en volums d'entre 1 i 10 tones anuals [17–19]. Les benzotriazoles estudiades en aquesta tesi doctoral i l'estructura de la 1*H*-benzotriazola es poden trobar a la **Taula 1**.

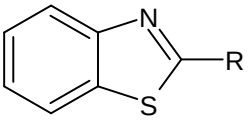
Taula 1. Benzotriazoles estudiades en la present tesi doctoral.



Abreviació	Nom del compost	CAS
BTR	1*H*-benzotriazola	95-14-7
1OHBTR	1-hidroxibenzotriazola	2592-95-2
4OHBTR	4-hidroxibenzotriazola	26725-51-9
4TTR	4-metil-1*H*-benzotriazole	29878-31-7
5TTR	5-metil-1*H*-benzotriazola	136-85-6
XTR	5,6-dimetil-1*H*-benzotriazola	4184-79-6
ClBTR	5-cloro-1*H*-benzotriazola	94-97-3

Els derivats de la benzotiazola, anomenades benzotiazoles, també són substàncies heterocícliques aromàtiques i la seva estructura es basa en la fusió d'un anell de benzè amb un anell 1,3-tiazola. Tots els seus derivats es formen habitualment per substitució a la posició 2, entre els àtoms de nitrogen i sofre, del grup químic d'interès. Les benzotiazoles, igual que les benzotriazoles, s'utilitzen habitualment com a inhibidors de la corrosió [20]. A més, es fan servir com a acceleradors per la vulcanització del cautxú, fungicides en la fabricació de paper i cuir, herbicides i fotosensibilitzadors [21,22]. Segons els registres de la ECHA, la benzotiazola es fabrica o s'importa a l'Àrea Econòmica Europea amb un volum aproximat de 10–100 tones anuals [23], la 2-mercaptobenzotiazola ho fa amb un volum de 1.000–10.000 tones anuals [24]. La 2-aminobenzothiazola i la 2-(metiltio)-benzotiazola es registren amb un volum de 1–10 tones anuals [25,26]. A la **Taula 2** es poden trobar les benzotiazoles estudiades en aquesta tesi doctoral i la seva estructura base.

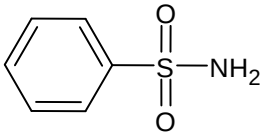
Taula 2. Benzotiazoles estudiades en la present tesi doctoral.



Abreviació	Nom del compost	CAS
BT	Benzotiazola	95-16-9
NH_2BT	2-aminobenzothiazola	136-95-8
OHBT	2-hidroxibenzotiazola	934-34-9
MeSBT	2-(metiltio)-benzotiazola	615-22-5
MCBT	2-mercaptobenzotiazola	149-30-4
MeBT	2-metilbenzotiazola	120-75-2

Els derivats de la benzosulfonamida, també anomenats benzosulfonamides, són substàncies aromàtiques amb un anell de benzè substituït amb un grup sulfonamida com a estructura bàsica. Els seus derivats es poden obtenir per substitució al grup amino de la sulfonamida i afegint un grup metil a l'anell de benzè per obtenir l'estructura de la toluensulfonamida. Les benzosulfonamides s'utilitzen a la indústria principalment com a plastificants i com a productes intermedis en la fabricació de productes com tints, tòners, desinfectants i productes fotoquímics [20,27,28]. Segons els registres de la ECHA tant la *orto*-toluensulfonamida com la *para*-toluensulfonamida es fabriquen o s'importen a l'Àrea Econòmica Europea en volums des de 10 fins a 100 tones anuals [27,28]. A més, la *N*-metil-*para*-toluensulfonamida i la *N*-metil-*para*-toluensulfonamida es registren amb volums de 1–10 tones anuals [29,30]. Les benzosulfonamides estudiades en aquesta tesi doctoral, així com l'estructura de la benzosulfonamida, es poden trobar a la **Taula 3**.

Taula 3. Benzosulfonamides estudiades en la present tesi doctoral.



Abreviació	Nom del compost	CAS
BSA	Benzosulfonamida	98-10-2
o-TSA	*orto*-toluensulfonamida	88-19-7
p-TSA	*para*-toluensulfonamida	70-55-3
Me-*p*-TSA	*N*-metil-*para*-toluensulfonamida	640-61-9
Et-*p*-TSA	*N*-metil-*para*-toluensulfonamida	80-39-7

Com ja hem comentat anteriorment, algunes d'aquestes substàncies tenen efectes toxicològics i, tot i que generalment el risc associat a aquests efectes és baix, poden arribar a representar riscos importants a causa de les grans quantitats en les quals s'utilitzen. Diversos estudis apunten que algunes benzotriazoles com la benzotriazola, la 5-metil-1*H*-benzotriazola i la 5-cloro-1*H*-benzotriazola poden causar toxicitat aguda i crònica [12,31] i a més tenen efectes disruptors endocrins en organismes aquàtics [32,33]. També s'ha demostrat que afecten negativament l'estrès oxidatiu de certes cèl·lules i poden provocar hepatotoxicitat en organismes aquàtics i els seus embrions [33–35]. Recentment s'ha observat certa cardiotoxicitat provocada per la 4-metil-1*H*-benzotriazola associada amb disfuncions mitocondrials [36].

Pel que fa a les benzotiazoles, aquestes poden tenir efectes tant citotòxics com genotòxics en organismes aquàtics [37,38] i en estudis *in vitro* s'ha observat que afecten algunes hormones tiroidals [39]. A més, algunes benzotiazoles podrien tenir efectes negatius en éssers humans. Sorahan *et al.* [40] van dur a terme un estudi per provar de relacionar diferents tipus de càncer en treballadors d'una planta de producció de 2-mercaptobenzotiazola al nord de Gales, amb l'exposició dels treballadors a aquest compost al llarg dels anys. Per una banda, els autors van observar un excés significatiu de la mortalitat en càncers a l'intestí gruixut i a la bufeta. Per altra banda, van observar un

excés significatiu de la morbiditat de càncer de bufeta i en diversos mielomes. Tenint en compte aquests resultats es pot considerar la 2-mercaptobenzotiazola com un potencial carcinogen humà, tot i que cal una avaluació més exhaustiva del risc per confirmar-ho. També s'han dut a terme diversos experiments que relacionen l'exposició de dones embarassades a benzotriazoles i benzotiazoles amb efectes negatius durant el període gestacional. Zhou *et al.* [41] van trobar una correlació entre concentracions altes en orina de 2-hidroxibenzotiazola a l'inici dels embarassos i riscos elevats de patir diabetis mellitus gestacional. A més, Chen *et al.* [42] van provar que l'exposició prenatal a diverses benzotriazoles i benzotiazoles es podia associar a alteracions en l'ADN mitocondrial trobat a la sang del cordó umbilical. La informació disponible respecte a la toxicitat de les benzosulfonamides és molt limitada comparada amb la de les benzotriazoles i les benzotiazoles. Només hi ha estudis per la *para*-toluensulfonamida, que ha resultat ser moderadament tòxica per algunes algues, però es considera no-tòxica per altres organismes aquàtics [43].

Tot i les evidències dels efectes perjudicials de moltes de les substàncies estudiades, per a la majoria d'elles no existeix cap tipus de regulació mediambiental. Tot i això, als registres de la ECHA es pot trobar la 1*H*-benzotriazola sota avaluació com a substància disruptora endocrina [15] i la 2-mercaptobenzotiazola com a sensibilitzadora de la pell [24]. A més, moltes de les benzotriazoles, benzotiazoles i benzosulfonamides es troben indexades en l'Annex III de la ECHA. En aquesta llista es compilen les substàncies que, segons resultats obtinguts amb models predictius, podrien tenir característiques carcinogèniques, mutagèniques o ser tòxiques per la reproducció, encara que la seva presència a la llista no significa que hi hagi evidències d'aquests efectes ni implica cap mena de regulació [44].

En general, les benzotriazoles, benzotiazoles i benzosulfonamides són substàncies molt polars i tenen coeficients de partició octanol-aigua (log K_{ow}) inferiors a 3 en la majoria de casos [22,45,46]. A més, algunes són resistents a la biodegradació [47] i s'ha demostrat que els processos que s'utilitzen en les plantes de tractament d'aigües residuals no són

gaire efectius en la seva eliminació [48]. Així doncs, la presència d'aquests compostos s'ha pogut confirmar en una gran varietat matrius biològiques i mediambientals. A la **Taula 4** s'inclou un recull de les concentracions de les benzotriazoles, benzotiazoles i benzosulfonamides incloses en aquesta tesi en matrius de medis aquàtics.

Taula 4. Benzotriazoles, benzotiazoles i benzosulfonamides determinades en medis aquàtics.

Substància	Aigua de riu (ng/L)	Aigües residuals (ng/L)	Aigua de mar (ng/L)	Peix i marisc (ng/g)	País	Referències
Benzotriazoles						
BTR	230–117.550	–	–	–	Turquia	[49]
	n.d.–1279	284-2132	–	n.d.–62,5	Xina	[50–55]
	39–526	-	–	–	Índia	[56]
	n.d.–710	62–2994	–	n.d.–2,43	Espanya	[45,57–60]
	15-23	1632–1802	–	–	Alemanya	[61]
	–	2.700–7800	–	–	Eslovènia	[46]
	–	–	–	42,5–71,3	Taiwan	[62]
	–	–	2,9–9,2	–	Itàlia	[63]
4TTR	10–73.700	–	–	–	Turquia	[49]
	4–150	352–566	–	n.d.–161	Xina	[51,54]
	n.d.–460	38–2149	–	–	Espanya	[45,57,59,60]
	38–42	2774–3026	–	–	Alemanya	[61]
	–	3.000–3400	–	–	Eslovènia	[46]
5TTR	30–33.030	-	–	–	Turquia	[49]
	n.d.–678	16–756	–	n.d.–67,9	Xina	[51–55]
	n.d.–350	22–2042	–	–	Espanya	[45,57,59,60]
	17–20	2129–2413	–	–	Alemanya	[61]
	–	3.400-4300	–	–	Eslovènia	[46]
	–	-	3,1–18,5	–	Itàlia	[63]
XTR	40–118.290	-	–	–	Turquia	[49]
	n.d.–15,6	n.d.–39	–	3,17–103	Xina	[52–54]
	–	1700–11.600	–	–	Eslovènia	[46]
	–	n.d.–162	–	–	Espanya	[57,59]
ClBTR	12-136	-	–	n.d.–146	Xina	[51,54]
	2–15	n.d.–58	–	–	Espanya	[57,59]
	9–13	384–500	–	–	Alemanya	[61]
	–	–	–	3,7–4,2	Taiwan	[62]

Taula 4. (Cont.).

Substància	Aigua de riu (ng/L)	Aigües residuals (ng/L)	Aigua de mar (ng/L)	Peix i marisc (ng/g)	País	Referències
Benzotiazoles						
BT	9.540–334.500	–	–	–	Turquia	[49]
	0,16–1082	1503–2023	–	132–13.400	Xina	[50,51,54]
	n.d.–286	n.d.–120	–	–	Espanya	[57,59]
	14–22	108–125	–	–	Alemanya	[61]
NH_2BT	4–43	n.d.–23	–	–	Espanya	[57,59]
	–	-	–	n.d.–15,2	Xina	[54]
OHBT	10–426	254–416	–	n.d.–512	Xina	[50,51,54,62]
	6–101	5–260	–	–	Espanya	[57,59]
	–	104-138	–	–	Alemanya	[61]
MeSBT	0,77–314	501–656	–	5,18–49	Xina	[50,51,54]
	–	n.d.–321	–	–	Espanya	[59]
	67–85	704–772	–	–	Alemanya	[61]
MCBT	360–23.540	–	–	–	Turquia	[49]
	1,37–1110	–	–	–	Xina	[50]
MeBT	n.d.–135	–	–	n.d.–114	Xina	[50,54]
Benzosulfonamides						
BSA	n.d.–71.870	n.d.–55	–	–	Turquia	[49]
	6–11	–	–	–	Espanya	[59]
o-TSA	8–20	n.d.–88	–	–	Espanya	[59]
p-TSA	27–69	n.d.–428	–	–	Espanya	[59]
Me-*p*-TSA	–	n.d.–5	–	–	Espanya	[59]
Et-*p*-TSA	2–25	2–74	–	–	Espanya	[59]

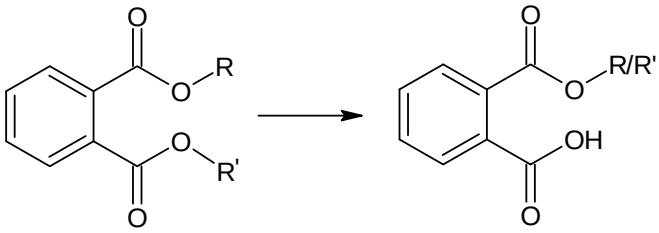
n.d. = no detectat

1.1.2. Dièsters i monoèsters de l'àcid ftàlic

Els dièsters de l'àcid ftàlic, anomenats ftalats de manera comuna, són substàncies aromàtiques sintètiques que es van introduir en adhesius a la indústria durant les dècades dels anys 20 i 30 del segle passat [64,65]. Els ftalats es van començar a sintetitzar mitjançant l'esterificació de l'anhídrid ftàlic amb diferents alcohols [66] i la seva estructura es basa en un anell de benzè amb dos grups èster en posició *orto*. A la **Taula 5**

es poden trobar els sis ftalats estudiats en aquesta tesi doctoral, així com la seva estructura química base.

Taula 5. Dièsters i monoèsters de l'àcid ftàlic estudiats en la present tesi doctoral.



Abreviació	Nom del compost	CAS
Dièsters de l'àcid ftàlic		
DMP	Dimetil ftalat	113-11-3
DEP	Dietil ftalat	84-66-2
DnBP	Di-n-butil ftalat	84-74-2
BzBP	Benzilbutil ftalat	85-68-7
DEHP	Bis(2-etilhexil) ftalat	117-81-7
DnOP	Di-n-octil ftalat	117-84-0
Monoèsters de l'àcid ftàlic		
MMP	Monometil ftalat	4376-18-5
MEP	Monoetil ftalat	2306-33-4
MnBP	Mono-n-butil ftalat	131-70-4
MBzP	Monobenzil ftalat	2528-16-7
MEHP	Mono(2-etilhexil) ftalat	4376-20-9
MnOP	Mono-n-octil ftalat	5393-19-1

Els ftalats es poden dividir en grups depenent de la longitud de les cadenes dels èsters. Aquells que només tenen un o dos àtoms de carboni s'utilitzen principalment en la producció de perfums i fragàncies, productes cosmètics i d'higiene i cura personal, productes de neteja, tintes i tòners, ceres, massilles, argila de modelar, i també en la síntesi de polímers i fàrmacs [67–69]. Els ftalats de baix pes molecular (de tres a sis àtoms de carboni), en canvi, es fan servir en adhesius, productes segelladors i de recobriment, i

sobretot es fan servir com a plastificants en productes de clorur de polivinil (PVC) [70,71]. Els ftalats d'alt pes molecular (de set a tretze àtoms de carboni) s'utilitzen principalment com a plastificants del PVC per incrementar-ne la flexibilitat, la mal·leabilitat i la durabilitat [72]. El paper dels ftalats com a plastificants és important, ja que representen aproximadament un 65% dels plastificants que utilitzats a escala mundial [73]. A més, el fet que aquestes substàncies siguin tan útils a la indústria fa que estiguin presents en una gran quantitat de productes quotidians com mobles, joguines, materials de construcció, roba i sabates, paper i cartró, plàstics alimentaris i material mèdic [67–71].

A causa d'aquest extens ús, la producció de ftalats no ha fet més que incrementar-se any rere any: als anys 70 la producció mundial estava per sota dels 2 milions de tones anuals; a mitjans dels 2000 ja superava els 6 milions de tones anuals; i l'any 2015 la producció es trobava per sobre dels 8 milions de tones anuals [65,74]. De tota la producció mundial de ftalats, Europa consumeix més d'un milió de tones [74], essent el bis(2-etilhexil) ftalat (DEHP) el més abundant de tots. Segons els registres de la ECHA, el DEHP es fabrica o s'importa a l'Àrea Econòmica Europea en volums d'entre 10.000 i 100.000 tones anuals [71]. Pel que fa a la resta de ftalats, la majoria es fabriquen o s'importen a l'Àrea Econòmica Europea en volums iguals o superiors a 1000 tones anuals [67–69], tot i que tant el di-n-octil ftalat (DnOP) com el benzilbutil ftalat (BzBP) ho fan amb volums entre 1 i 10 tones anuals [70,75].

Com hem mencionat anteriorment, una possible via de contaminació mediambiental, a part dels abocaments directes de substàncies químiques, és la degradació de productes d'ús quotidià. Aquesta via és especialment rellevant pels ftalats, ja que són substàncies que s'addicionen als materials plàstics únicament de manera mecànica. En no estar lligats químicament als plàstics, quan els productes es fan malbé o es deterioren els ftalats s'alliberen de la matriu polimèrica en la qual es troben i passen a diferents compartiments ambientals com l'atmosfera, aigües superficials, sòls i sediments o biota. És en aquests compartiments que els ftalats es poden degradar per vies aeròbiques o anaeròbiques mitjançant l'acció de bactèries, d'organismes complexos o per processos de fotòlisi i

hidròlisi [73,74]. Independentment de si la degradació es duu a terme en condicions aeròbiques o anaeròbiques, les vies de degradació principals són les mateixes i donen com a productes principals els monoèsters de l'àcid ftàlic. A la **Taula 5** es poden trobar els sis monoèsters estudiats en aquesta tesi doctoral, així com la seva estructura química base.

La immensa producció i ús de ftalats a nivell global és altament preocupant a causa dels efectes toxicològics de molts d'ells. Els efectes més rellevants s'atribueixen principalment al dietil ftalat (DEP), al di-n-butil ftalat (DnBP), al BzBP i al DEHP que es consideren disruptors endocrins a causa dels efectes estrogènics que tenen tant en organismes aquàtics com en amfibis, mamífers i cèl·lules humanes [76]. És a causa dels efectes disruptors que també es consideren extremadament tòxics per a organismes aquàtics, ja que afecten la seva reproducció. Diversos estudis han demostrat que l'exposició d'organismes aquàtics com el peix zebra (*Danio rerio*), anèl·lids o crustacis a aquestes substàncies porta a l'aparició d'anomalies i aberracions cromosòmiques en el seu desenvolupament [77,78]. Tot i això, aquests efectes no estan limitats a organismes aquàtics. L'exposició *in utero* tant al DEHP com al DEP pot afectar el sistema reproductor de les rates [79]. L'exposició de dones a diversos ftalats pot afectar les funcions ovàriques i incrementar el risc de patir una insuficiència ovàrica prematura [80]. També s'han descrit efectes negatius en nadons i infants, derivats d'una alta exposició de les mares a ftalats durant el període de gestació. Alguns d'aquests efectes són la disminució de la circumferència del cap de nadons nounats [81], afectacions en el desenvolupament reproductiu tant de nens [82,83] com de nenes [84], així com efectes negatius en el comportament i el desenvolupament cognitiu i psicomotor d'infants [85]. És interessant destacar que, en general, s'ha observat que els efectes negatius dels ftalats en els sistemes reproductius són més greus en individus masculins i que a més els seus efectes poden ser acumulatius en alguns casos. És a dir, que l'exposició a la combinació de diversos ftalats pot donar lloc a efectes molt pitjors que l'exposició a només un d'ells [79,84,86].

És a causa dels efectes tòxics dels ftalats que existeixen diverses regulacions arreu del món sobre el seu ús en productes quotidians. Pel que fa a la Unió Europea, el DnBP, el BzBP i el DEHP estan considerats juntament amb el diisobutil ftalat (DiBP) com a "substàncies extremadament preocupants" i requereixen d'autorització per poder ser utilitzades a la indústria [69–71]. A més, el seu ús està restringit per l'annex XVII de la normativa de registre, avaluació i autorització de substàncies químiques (REACH). L'entrada número 51 de l'annex estableix que l'ús d'aquests quatre ftalats no es pot trobar a concentracions majors del 0,1% en pes del material plàstic. Fins a mitjans de 2020 aquestes restriccions només s'aplicaven a materials plàstics destinats a productes i joguines per infants. El 7 de juliol de 2020, però, es van modificar les condicions de la restricció i des de llavors aquest límit s'aplica a qualsevol material plàstic destinat a la comercialització del producte (amb algunes excepcions descrites en altres Directives i Comissions Reguladores) [87]. En el mateix annex, a l'entrada 52, es poden trobar les restriccions establertes per al DnOP, el diisononil ftalat (DiNP) i el diisodecil ftalat (DiDP). Tot i que aquestes substàncies no es consideren perilloses per la salut, representen una gran part dels ftalats que s'utilitzen a Europa (un 80% l'any 2013) [88]. Com a mesura de prevenció es va decidir limitar la concentració de DnOP, DiNP i DiDP al 0.1% en pes del material plàstic pel seu ús en productes i joguines que els infants que es puguin dur a la boca [89]. L'agència europea de seguretat alimentària (EFSA) també ha estimat valors d'ingesta diària tolerable (TDI) pels ftalats més utilitzats per fabricar materials destinats al contacte amb aliments (DnBP, BzBP, DEHP, DiNP i DiDP), així com un TDI de grup per a la suma de DnBP, BzBP, DEHP i DiNP. Els valors de TDI van des de 0.01 fins a 0.5 mg/Kg de pes corporal al dia pels ftalats individuals i el TDI grupal està establert en 50 µg/Kg de pes corporal al dia [90]. Governs, organitzacions ambientals i agències de substàncies químiques d'altres països com Canadà, els Estats Units, Austràlia o Japó han establert els seus propis límits i restriccions [74,91–93].

La informació sobre la toxicitat dels monoèsters, en canvi, és més limitada i no existeixen regulacions per aquestes substàncies. Tot i això, hi ha diversos estudis que

relacionen l'exposició de peixos zebra a mono-n-butil ftalat (MnBP) i a mono(2-etilhexil) ftalat (MEHP) amb estrès oxidatiu al fetge, que podria estar relacionat amb l'acumulació de lípids en cèl·lules hepàtiques [94,95]. També s'han observat alteracions en la transcripció de gens involucrats en la síntesi d'hormones tiroidals en larves de peixos zebra [96]. Altres estudis *in vitro* amb cèl·lules de rates adverteixen que el MEHP pot afectar l'expressió genètica dels ovòcits de les femelles [97], així com interferir amb les funcions mitocondrials i la homeòstasi de cèl·lules del múscul esquelètic [98]. Pel que fa als seus efectes en humans, s'han observat efectes disruptors negatius causats pel MnBP i el MEHP en cèl·lules beta pancreàtiques que podrien estar lligats amb resistència a la insulina i diabetis de tipus dos [99].

Val la pena destacar, però, que diversos autors afirmen que els efectes negatius que s'associen als ftalats podrien ser provocats també pels seus respectius metabòlits. Per exemple, Kwack *et al.* [100] van dur a terme una comparació dels efectes que tenien diversos ftalats i metabòlits en rates mascle. Van avaluar els canvis en el pes dels animals, així com en el pes d'òrgans com el fetge o els testicles, canvis en els nivells de glucosa en sang, afectacions a enzims hepàtics i a la concentració de triglicèrids en sang. La conclusió principal que extreuen dels resultats és que els efectes tòxics provocats pels monoèsters són similars als dels respectius ftalats. De la mateixa manera, Ye *et al.* [86] afirmen que els efectes disruptors endocrins del DEHP en organismes aquàtics estan causats tant pel compost principal com pels seus metabòlits, així com Sun *et al.* [101] confirmen que tant el DEHP com el MEHP afecten de manera combinada el funcionament de l'esperma humà.

Degut a la gran diversitat d'estructures químiques tant dels ftalats com dels seus metabòlits, tenen propietats físico-químiques molt diverses. Alguns d'ells són altament solubles en aigua i és habitual determinar-los en mostres d'aigües superficials i residuals. D'altres són de naturalesa lipofílica i tenen log K_{ow} alts de manera que es poden absorbir en partícules en suspensió a l'aigua i acumular-se en els sediments. Alguns ftalats també són volàtils i es troben habitualment a l'atmosfera, des d'on es poden dipositar en altres

compartiments ambientals. Així doncs, no és d'estranyar que la presència tant de ftalats com de monoèsters s'hagi pogut confirmar en un gran número de mostres ambientals.

La informació sobre les concentracions de ftalats i els seus metabòlits al medi aquàtic està disponible a l'article de revisió enviat per la seva publicació a la revista *Trends in Analytical Chemistry* que està inclòs a l'apartat 1.2.3. d'aquesta tesi doctoral.

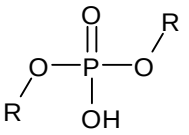
1.1.3. Dièsters de l'àcid fosfòric

Els dièsters de l'àcid fosfòric són els principals metabòlits i productes de transformació dels trièsters de l'àcid fosfòric (anomenats organofosfats de manera habitual). Aquests últims s'utilitzen de manera generalitzada a la indústria des que es va prohibir l'ús de retardants de flama bromats per la seva toxicitat, persistència en el medi ambient i potencial per bioacumular-se en éssers vius [102]. Tot i això, els organofosfats no només es fan servir com a retardants de flama sinó que també s'empren com a estabilitzadors, com a agents humectants i escumejants, i com a plastificants en mobles, materials de construcció, teixits i components electrònics, entre d'altres [103,104]. Els organofosfats, igual que els ftalats, no es troben lligats químicament als materials on s'addicionen i poden passar al medi ambient durant la vida útil dels productes. Així doncs, aquestes substàncies es detecten habitualment en matrius tan variades com sediments [105], aigües superficials i residuals [7,106,107], llots de depuradora [106,108–110], pols [111–113], biota [114–117] i fins i tot en aliments destinats al consum humà [104,118,119] de manera que aquestes substàncies arriben molt fàcilment al nostre cos. El fet que els organofosfats es trobin tan distribuïts al medi ambient resulta preocupant, ja que alguns d'ells es consideren disruptors endocrins i tòxics per la reproducció i el desenvolupament així com també poden ser tòxics per òrgans vitals com el cervell, el fetge i els intestins [120,121].

Un cop entren al nostre cos els organofosfats són ràpidament hidrolitzats donant com a resultats els respectius dièsters, que es poden utilitzar com a biomarcadors per avaluar

l'exposició humana als organofosfats [122]. El mateix procés de degradació també pot tenir lloc en animals i altres organismes o a causa de processos d'hidròlisi i fotòlisi al medi ambient [121,123]. Tot i això, la degradació dels organofosfats no és l'única via d'entrada dels dièsters al medi ambient, sinó que també s'utilitzen a la indústria com a catalitzadors, plastificants, retardants de flama i per la fabricació de pesticides i fàrmacs [103]. Els dièsters de l'àcid fosfòric estudiats en aquesta tesi doctoral es poden trobar a la **Taula 6**.

Taula 6. Dièsters de l'àcid fosfòric estudiats en aquesta tesi doctoral.



Abreviació	Nom del compost	CAS
BCEP	Bis(2-cloroetil) fosfat	3040-56-0
DPHP	Difenil fosfat	838-85-7
DNBP	Di-n-butil fosfat	107-66-4
BDCIPP	Bis(1,3-dicloro-2-propil) fosfat	72236-72-7
BBOEP	Bis(2-butoxietil) fosfat	14260-97-0
BEHP	Bis(2-etilhexil) fosfat	298-07-7

Segons els registres de la ECHA, tant el di-n-butil fosfat (DNBP) com el bis(2-etilhexil) fosfat (BEHP) es fabriquen o s'importen a l'Àrea Econòmica Europea en volums d'entre 100 i 1.000 tones anuals [124,125] i el bis(2-cloroetil) fosfat (BCEP), el difenil fosfat (DPHP) i el bis(2-butoxietil) fosfat (BBOEP) ho fan en volums de 1–10 tones anuals [126–128]. A més, s'estima que la producció global de dièsters és de més de 17.050 tones anuals [103].

Tot i que els estudis sobre la toxicitat dels dièsters de l'àcid ftàlic són molt limitats, la seva presència al medi ambient és igual de preocupant que la dels seus precursors ja que també poden tenir efectes negatius en éssers vius. La majoria estudis de toxicitat, però, adverteixen dels efectes disruptors endocrins i la toxicitat reproductiva i de

desenvolupament que provoquen tant del DPHP com del BDCIPP en els embrions d'aus i d'organismes aquàtics [129–132].

De manera similar al que passa amb els estudis de toxicitat, hi ha poca bibliografia que determini la presència dels dièsters de l'àcid fosfòric en medis aquàtics. A la **Taula 7** s'inclou un recull de les concentracions dels dièsters de l'àcid fosfòric inclosos en aquesta tesi en matrius de medis aquàtics.

Taula 7. Concentracions de dièsters de l'àcid fosfòric en medis aquàtics

Substància	Aigua de riu i llac (ng/L)	Aigües residuals (ng/L)	Peix i marisc (ng/g)	País	Referències
BCEP	–	2,1–80	–	Xina	[7]
	–	–	<0,001–0.26	Estats Units	[133]
DPHP	n.d.–35.4	4,9–1005	3,16–38,1	Xina	[7,134–137]
	–	50,8–1290	<0,17–1,62	Estats Units	[106,133]
DNBP	n.d.–390	1,6–4480	7,50–284	Xina	[7,134–137]
	–	–	<0,06–2,35	Estats Units	[133]
BDCIPP	n.d.–8,49	3,4–35	–	Xina	[7,135]
	–	610–4550	–	Estats Units	[106]
BBOEP	–	–	7,36–95,4	Xina	[136]
BEHP	–	1,4–3377	9,04–161	Xina	[7,136]

n.d. = no detectat

1.2. Determinació de compostos orgànics d'alt volum de producció en medis aquàtics

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

Com ja hem mencionat, els compostos orgànics AVP estan presents de manera habitual a diversos compartiments del medi ambient com a resultat del seu ús a la indústria. A causa de la gran varietat d'estructures que poden tenir, les seves propietats fisicoquímiques també poden variar molt, inclús entre substàncies de la mateixa família. Com que la majoria de compostos AVP són solubles en aigua, és usual trobar-los en matrius ambientals aquoses com poden ser aigües de rius, llacs i oceans. A més, alguns compostos AVP tenen propietats lipofíliques i tenen tendència a adsorbir-se en partícules en suspensió a l'aigua, sediments o sòls. És principalment a través d'aquestes vies que els compostos són ingerits per organismes aquàtics i introduïts a les cadenes tròfiques a través de les quals, després de passar per moltes baules, poden arribar a nosaltres. Tot i això, les concentracions dels compostos AVP a qualsevol dels compartiments ambientals esmentats solen ser de l'ordre parts per bilió o parts per trilió.

Es fa palesa, doncs, la necessitat de disposar de tècniques potents per extreure, preconcentrar i determinar de manera selectiva i amb la sensibilitat adequada els compostos de les mostres ambientals.

L'extracció en fase sòlida (SPE) és la tècnica d'extracció emprada de manera habitual per a matrius aquoses, ja que permet la preconcentració de grans volums de mostra. Aquesta característica resulta especialment rellevant per mostres recollides en cossos d'aigua de gran extensió com mars i oceans, ja que en aquestes zones els compostos se solen trobar a concentracions molt més baixes. Per matrius sòlides com poden ser sediments o biota, l'extracció per ultrasons (USAE) i els QuEChERS (abreviatura de *Quick, Easy, Cheap, Effective, Rugged and Safe)* són les tècniques d'extracció més emprades, tot i que també se'n poden utilitzar d'altres com l'extracció amb líquids pressuritzats (PLE) i l'extracció amb microones (MAE). Tanmateix, les mostres ambientals poden arribar a ser molt complexes i, com que no totes les tècniques que hem enumerat per la seva extracció són selectives, el seu ús comporta l'extracció simultània d'interferents que dificulten la determinació dels compostos d'interès. Així doncs, les tècniques d'extracció s'han d'acompanyar sovint de processos de neteja de l'extracte que es poden dur a terme mitjançant SPE, extracció en fase sòlida dispersiva (dSPE), o tècniques miniaturitzades com la microextracció en fase sòlida (SPME), per exemple.

Després dels processos d'extracció i neteja, l'ús de tècniques de separació i detecció adequades també és important per determinar correctament els compostos AVP a concentracions baixes. Una bona separació pot ajudar a separar els interferents de la matriu dels compostos d'interès. La cromatografia de gasos (GC) i la cromatografia de líquids (LC) són les dues tècniques més utilitzades. L'elecció entre una tècnica o l'altra

depèn, però, de les propietats fisicoquímiques dels compostos com són la volatilitat, l'estabilitat tèrmica i la polaritat, entre d'altres. A més d'una bona tècnica de separació és necessari disposar de tècniques de detecció selectives i sensibles. En aquest sentit, l'espectrometria de masses (MS) és la tècnica de detecció preferida per la determinació de compostos AVP tant en l'àmbit de la recerca com per determinacions rutinàries.

En els següents apartats es presenta una visió general de les diferents tècniques d'extracció i anàlisi emprades actualment per la determinació de les famílies de compostos incloses en aquesta tesi.

1.2.1. Tècniques d'extracció

Com ja hem mencionat, les tècniques d'extracció utilitzades són diferents depenent de si són matrius sòlides o líquides. Així doncs, els següents apartats se centren en les tècniques d'extracció emprades per matrius aquoses i matrius sòlides, ja que són les que s'estudien en aquesta tesi.

1.2.1.1. Tècniques d'extracció per matrius aquoses

En general, les tècniques d'extracció utilitzades per matrius aquoses es basen en l'equilibri de partició entre la matriu de la mostra (fase aquosa) i una altra fase. Quan l'altra fase és un dissolvent immiscible, parlem d'extracció líquid-líquid (LLE).

Tot i que la LLE ha estat una tècnica d'extracció molt utilitzada per l'extracció de compostos orgànics de mostres aquoses, durant les últimes dècades ha anat perdent popularitat, ja que presenta diversos inconvenients. Per una banda la LLE no és una tècnica adequada per l'extracció de la majoria de substàncies polars i, tot i que és una tècnica senzilla, a la vegada també és laboriosa, ja que sovint les dues fases no se separen adequadament a causa de la formació d'emulsions. En aquests casos cal repetir l'extracció diverses vegades, diluint així els anàlits en comptes de preconcentrar-los [138]. A més, en aquest tipus d'extraccions s'utilitzen grans volums de dissolvents orgànics tòxics i nocius pel medi ambient. En un món que intenta cada vegada més encaminar-se cap a la química verda, tècniques com la LLE han estat substituïdes per altres tècniques d'extracció amb una eficiència més alta i miniaturitzades per reduir tant els temps d'extracció com el volum de dissolvent usat [139]. Les principals tècniques de microextracció en fase líquida (LPME) són la microextracció en gota (SDME), la microextracció en fibra buida (HF-LPME) i la microextracció líquid-líquid dispersiva (DLLME) [140].

Pena *et al.* [141] van ser els primers a utilitzar la DLLME per la determinació de derivats de la benzotriazola i la benzotiazola en aigua riu, d'aixeta, aigües industrials i residuals. Van fer servir tri-n-butil fosfat per l'extracció i van obtenir recuperacions relatives superiors al 90% per totes les mostres excepte per les aigües residuals (60–88%). Uns anys més tard, Casado *et al.* [45] van proposar un altre mètode, només per la determinació de derivats de la benzotriazola, derivatitzant els anàlits d'interès abans de l'extracció per poder analitzar les mostres per GC-MS i fent servir toluè com a extractant. Amb aquest procés van obtenir recuperacions relatives similars a les del mètode anterior per a mostres d'aigua de riu i de l'aixeta, però van millorar notablement les recuperacions obtingudes per a mostres d'aigües residuals. La DLLME també s'ha utilitzat per l'extracció de ftalats de mostres d'aigua de mar, de riu i d'aixeta, obtenint bones recuperacions relatives (68–98%) per tots els tipus de mostra [142,143].

Una variant de la DLLME, la microextracció líquid-líquid dispersiva assistida per aire (AA-DLLME), també s'ha aplicat a la determinació de derivats de la benzotriazola, obtenint resultats similars als de la tècnica convencional [144]. El procés d'extracció és similar al de la DLLME convencional amb la diferència que en l'AA-DLLME tant la fase aquosa com la fase extractant s'emulsionen ràpidament amb una xeringa mitjançant cicles de succió-injecció. Després, la mescla se centrifuga o s'hi injecta aire per separar les fases. A la **Figura 3** es pot veure una descripció gràfica del procés.

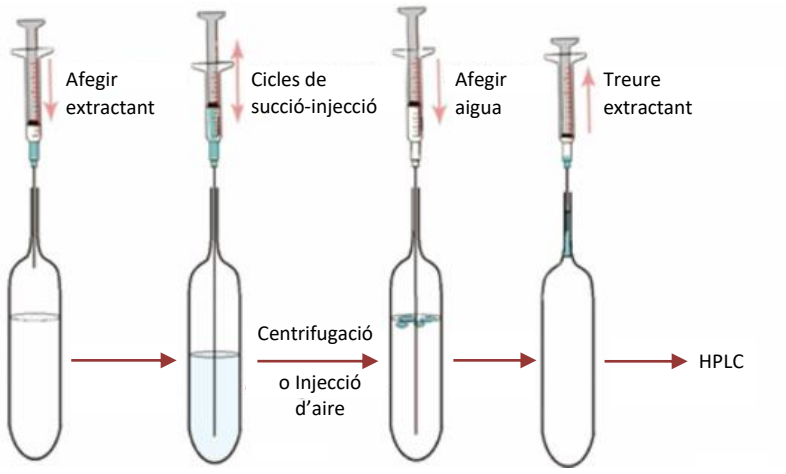


Figura 3. Esquema del funcionament de la tècnica AA-DLLME (Adaptat de [144]).

Recentment, Kraševec *et al.* [46] van desenvolupar un mètode basat en la HF-LPME per la determinació de derivats de la benzotriazola en aigua de riu, aigua potable i aigües

residuals. Per comprovar la seva eficàcia i aplicabilitat, van comparar el mètode desenvolupat amb la DLLME i la SPE convencional i van obtenir límits de detecció i valors de repetibilitat similars per tots tres mètodes. A l'hora de comparar la precisió dels mètodes, els valors obtinguts amb HF-LPME van resultar ser millors que els de la DLLME. També van dur a terme una avaluació de l'impacte ambiental dels mètodes proposats, sent la HF-LPME la que va donar millors resultats, seguida de la DLLME.

Tot i això, abans del desenvolupament i l'augment de les tècniques de LPME, la SPE ja havia desplaçat l'ús de la LLE. L'extracció en fase sòlida és una tècnica d'extracció exhaustiva que es va introduir a mitjans de la dècada de 1970 i des de llavors ha estat segurament la tècnica més utilitzada, no només per l'extracció de mostres aquoses, sinó també per la preconcentració i la neteja d'extractes degut a la seva simplicitat, versatilitat, rapidesa i als elevats factors d'enriquiment [145]. Per la SPE habitualment es fan servir sorbents empaquetats en cartutxos que són compatibles amb equips de buit, tot i que també es poden empaquetar en forma de discos o en plaques multipou [146,147]. El funcionament de la SPE es basa en la interacció dels anàlits presents en la mostra aquosa amb un sorbent sòlid, de manera que cal escollir un sorbent en el qual els anàlits quedin retinguts i els interferents no. Després, els anàlits s'elueixen del sorbent utilitzant dissolvents orgànics o dissolucions que més endavant es poden evaporar per incrementar el factor d'enriquiment i la selectivitat. Així doncs, els procediments habituals de SPE consten de quatre passos: condicionament del sorbent, càrrega de mostra, rentat del sorbent per eliminar interferències i elució dels anàlits. Tot i això, la SPE també es pot aplicar com a tècnica de neteja escollint un sorbent en el qual quedin retinguts els interferents i no els anàlits.

Pel que fa a l'elecció del sorbent, s'ha de triar tenint en compte les propietats fisicoquímiques dels anàlits, que seran les que definiran el tipus d'interaccions sorbent-anàlit i mostra-anàlit de les quals dependrà l'èxit de l'extracció o la neteja, tot i que altres paràmetres com el pH de la mostra i de l'eluent, el dissolvent triat per l'elució o el volum de mostra carregat també poden afectar l'eficiència de l'extracció [48]. Els primers sorbents que es van desenvolupar van ser sorbents amb base sílice enllaçada a cadenes alquíliques (C_{18} i C_8), a grups fenil i amino, entre d'altres. Tot i que són indicats per retenir compostos no polars, es tracta de sorbents inestables a pH extrems. Més endavant van aparèixer els sorbents basats en el carboni que tenen una gran capacitat de retenció per alguns anàlits, cosa que a vegades dificulta o inclús impedeix la seva elució [148]. Després, van aparèixer els sorbents polimèrics, principalment de poliestirè-divinilbenzè i després els sorbents polimèrics altament entrecreuats que presentaven una millor retenció dels compostos. Posteriorment, van aparèixer els sorbents polimèrics amb grups polars, com

els copolímers de N-vinilpirrolidona-divinilbenzè (comercialitzats principalment com a Oasis HLB) que presenten una millor retenció de grups polars i poden treballar en grans intervals de pH. Aquests últims presenten interaccions hidrofíliques i hidrofòbiques amb els anàlits que faciliten l'extracció de compostos àcids, bàsics i neutres de mostres ambientals [145]. A partir d'aquests sorbents polimèrics s'han desenvolupat sorbents d'intercanvi iònic que, a part de les interaccions no-selectives també presenten interaccions d'intercanvi iònic. Depenent dels grups funcionals, aquests sorbents es poden dividir en quatre grups: intercanvi catiònic fort (SCX), intercanvi aniònic fort (SAX), intercanvi catiònic feble (WCX) i intercanvi aniònic feble (WAX) [148,149].

El sorbent més habitual per l'extracció de les famílies de compostos estudiades en aquesta tesi és l'Oasis HLB comercialitzat per Waters i, per exemple, és l'únic sorbent que es fa servir per a l'extracció simultània de triesters i dièsters de l'àcid fosfòric d'aigua de riu, de llac, de l'aixeta i d'aigües residuals [7,106,134,135]. També s'ha utilitzat per a l'extracció de dièsters i monoèsters de l'àcid ftàlic de mostres tant d'aigua dolça com d'aigua de mar [150–154]. Pel que fa als derivats de les benzotiazoles, les benzotriazoles i les benzosulfonamides, tot i que l'Oasis HLB és el sorbent més emprat [59,61,63,155,156], també se'n fan servir d'altres com el Strata X [157] o sorbents d'intercanvi iònic de mode mixt com l'Oasis MAX i l'Oasis MCX [57,158].

Altres tècniques d'extracció derivades de la SPE, com la dSPE, la SPME i l'extracció mitjançant barres magnètiques agitadores (SBSE), han sorgit durant les últimes dècades per minimitzar els inconvenients que presenta la SPE.

Tot i que la dSPE es pot dur a terme amb sorbents convencionals com a etapa de neteja després d'una extracció, també es pot utilitzar com a tècnica extractiva. Com que el sorbent es dispersa directament en la mostra en comptes de trobar-se empaquetat, amb aquesta tècnica s'eviten problemes habituals de la SPE com són els blocatges del sorbent empaquetat o la necessitat de controlar constantment el flux de la mostra. En alguns casos també es pot reduir el temps d'extracció. Speltini *et al.* [21] han aplicat la dSPE a l'extracció de derivats de la benzotriazola, la benzotiazola i la benzosulfonamida de mostres d'aigua de l'aixeta, de riu i de canals. Fan servir nanotubs de carboni multicapa (MWCNTs) com a sorbents, que són un dels materials basats en el carboni més estudiats per aplicacions ambientals. Degut a la seva estructura única, els MWCNTs poden tenir interaccions fortes amb molècules orgàniques mitjançant forces no covalents (interaccions hidrofòbiques, enllaços d'hidrogen, forces de Van der Waals, etc.). A més, tenen una relació superfície-volum alta que afavoreix la retenció dels anàlits i existeix la possibilitat de funcionalitzar-ne la superfície per augmentar la seva selectivitat [159]. En

aquest cas, els MWCNTs tenen bona afinitat pels derivats de la benzotriazola, la benzotiazola i la benzosulfonamida i es poden obtenir resultats similars als de sorbents comercials en termes d'eficiència d'extracció, factor d'enriquiment i cost. Hi ha una variant d'aquesta tècnica en la qual s'utilitzen partícules magnètiques per l'extracció (m-dSPE). El principal avantatge de la m-dSPE és que les partícules magnètiques simplifiquen molt el procés de manipulació dels sorbents, ja que no cal filtrar la mostra sinó que el sorbent se separa apropant-hi un imant [145]. Jiménez-Skrzypek *et al.* [160] proposen un mètode que fa servir nanopartícules de magnetita (Fe_3O_4) recobertes de polidopamina per extreure ftalats de mostres d'aigua de mar. Les recuperacions relatives obtingudes amb aquesta tècnica són bones (79–110%) i els límits de quantificació són de l'ordre dels ng/L.

La SPME i la SBSE també s'han emprat per a l'extracció tant de benzotriazoles, benzotiazoles i benzosulfonamides [60,161], com de ftalats [162–164]. Tot i això, cal tenir en compte que tant les recuperacions com els factors de preconcentració i els valors de reproductibilitat obtinguts amb aquestes tècniques són inferiors als obtinguts amb SPE, ja que aquests mètodes es basen en processos d'equilibri [165].

1.2.1.2. Tècniques d'extracció per matrius sòlides

Les tècniques d'extracció per matrius sòlides es basen en l'extracció dels anàlits de la matriu de la mostra mitjançant un dissolvent (normalment orgànic). Les mostres sòlides mediambientals inclouen sediments, llots de depuradora, sòls i biota i, com ja hem comentat abans, solen ser matrius complexes que contenen molts compostos que poden interferir en l'extracció dels anàlits d'interès.

Les mostres necessiten sovint pretractaments com l'assecat, el congelat, la liofilització i l'homogeneïtzació abans de poder dur a terme l'extracció. A més, les concentracions resultants de les anàlisis es poden expressar segons el pes sec (d.w.), pes humit (w.w.), o pes en lípids (l.w.) de manera que la humitat i el contingut lipídic de la mostra també s'avaluen abans de l'extracció.

Avui dia, el mètode més utilitzat per l'extracció de compostos AVP és l'USAE. La seva simplicitat i versatilitat permeten l'ús de dissolvents de diferents polaritats per extreure els compostos d'interès, encara que normalment es requereixen dos o tres cicles d'extracció per obtenir bones recuperacions. Les benzotriazoles i les benzotiazoles se solen extreure amb la combinació de dos dissolvents orgànics com poden ser el diclorometà i l'acetonitril [50] o el diclorometà i l'hexà [54]. Els ftalats i els seus metabòlits

se solen extreure amb acetona [166–168] o amb acetonitril [160,169], i els metabòlits dels organofosfats amb metanol [106,108] o acetonitril [109,133,170]. La neteja dels extractes obtinguts per USAE normalment es duu a terme per SPE utilitzant cartutxos Oasis HLB, tot i que altres sorbents com el Florisil [108], el Strata X [157] i sorbents WAX [133] també s'han utilitzat. Altres processos de neteja com la digestió lipídica [171], la LLE [167], la m-dSPE [160] i la SPME [168,172] han donat bons resultats per la neteja d'extractes de biota i sediments en la determinació de ftalats i els seus metabòlits.

L'extracció per QuEChERS és la segona tècnica més utilitzada, sobretot per l'extracció de ftalats i dels seus metabòlits de mostres de biota. Es tracta d'una tècnica que consisteix en l'extracció de mostres amb continguts alts d'humitat mitjançant un dissolvent orgànic i sals per afavorir la separació de les dues fases. Quan les mostres han estat liofilitzades abans de l'extracció, s'afegeix una petita quantitat d'aigua a la mescla. Es tracta d'una tècnica molt versàtil, ja que es poden modificar varis dels seus components per incrementar l'eficiència de l'extracció. Per exemple, tot i que l'acetonitril és el dissolvent orgànic més emprat, també s'han utilitzat mescles de diclorometà amb acetat d'etil o hexà com a fases extractants [115,173]. A més, hi ha una gran varietat de mescles de sals disponibles comercialment. Les més destacades són les del mètode original (1 g de NaCl, 4 g $MgSO_4$) [174], les del mètode estàndard europeu EN 15662:2019 (1 g NaCl, 4 g $MgSO_4$, 1 g citrat de sodi, 0,5 g hidrogen citrat de sodi sesquihidratat) [175], i les del mètode oficial de l'associació internacional AOAC official (1,5 g acetat de sodi, 6 g $MgSO_4$) [176]. Després de l'extracció per QuEChERS, s'utilitza la dSPE per netejar la fase orgànica de l'extracció. Per extractes de mostres de biota se sol triar l'amina primària i secundària (PSA) o C_{18} com a sorbents per eliminar els lípids presents en la mostra [58,177,178]. La SPE també es pot incloure per netejar extractes de QuEChERS amb cartutxos també de PSA o C_{18} [179] o fins i tot Oasis HLB [180]. Així doncs, es podria dir que els principals avantatges d'aquesta tècnica són que no es necessita equipament especial, que es consumeixen pocs dissolvents i que el procés és ràpid [181]. A més, generalment els mètodes de QuEChERS donen bones recuperacions per la majoria d'anàlits. Álvarez-Muñoz *et al.* [58], per exemple, van utilitzar les sals del mètode EN per l'extracció de benzotriazoles de diverses espècies de musclos i després van aplicar una dSPE amb PSA i C_{18} com a neteja, obtenint com a resultat recuperacions relatives superiors al 90% i límits de detecció i quantificació de l'ordre dels ng/g (d.w.). Tsochatzis *et al.* [178], també van obtenir recuperacions relatives superiors al 90% i límits de l'ordre de ng/g (d.w.) fent servir les sals del mètode original i una dSPE com a neteja per la determinació de ftalats en musclos. En canvi, els QuEChERS no s'han utilitzat per a la determinació de dièsters de l'àcid fosfòric en mostres ambientals.

Hi ha dues tècniques que també s'han aplicat a l'anàlisi de mostres sòlides ambientals, però en menor mesura: la PLE i la MAE. La PLE es basa en l'extracció de mostres amb dissolvents a altes pressions i temperatures. La combinació d'aquests dos paràmetres, junt amb el temps d'extracció, milloren la solubilitat, difusió i desorció dels anàlits de la matriu, obtenint així mètodes ràpids i exhaustius. A més, la PLE utilitza volums baixos de dissolvent i és possible semi-automatitzar el procés. També existeix la possibilitat de dur a terme diversos cicles d'extracció o extraccions successives d'una mateixa mostra amb diferents dissolvents. Blair *et al.* [150], per exemple, van proposar un mètode de PLE per la determinació de metabòlits dels ftalats en sediments i biota. Els autors van extreure les mostres utilitzant aigua a 70 ^{o}C durant 5 minuts i 3 cicles. Després van netejar els extractes mitjançant una SPE (Oasis MAX) i van obtenir recuperacions superiors al 70% per la majoria de compostos en ambdós tipus de mostra. Hou *et al.* [136], van desenvolupar un mètode per l'extracció de metabòlits dels organofosfats de mostres de peix utilitzant acetona a 100 ^{o}C durant 3 cicles. Els extractes els van netejar amb cartutxos de SPE Bond Elut NH_2, que són intercanviadors aniònics febles, i van obtenir recuperacions superiors al 70% per tots els compostos i límits de quantificació per sota dels ng/g (d.w.). Tot i que no s'ha utilitzat la PLE per la determinació de benzotriazoles, benzotiazoles o benzosulfonamides en sediments o peixos, sí que s'ha fet servir per la determinació d'aquests compostos en llots de depuradora [182] i en matèria particulada [183].

La MAE utilitza l'energia de microones per escalfar el dissolvent d'extracció i accelerar així la cinètica del procés d'extracció, de manera que es poden dur a terme extraccions eficients amb menys volum de solvent i temps d'extracció més curts que amb l'USAE. Per exemple, Speltini *et al.* [184] van desenvolupar un mètode per determinar benzotriazoles, benzotiazoles i benzosulfonamides en mostres de sòl. En aquest estudi es van utilitzar 6 mL de metanol com a dissolvent d'extracció a 120 ^{o}C durant 10 min. Després es va centrifugar la mescla i la solució sobrenedant es va concentrar sota corrent de nitrogen i es va reconstituir per la seva posterior anàlisi. Zhang *et al.* [185] van fer servir 15 mL d'una mescla de metanol i acetat d'etil com a dissolvent per l'extracció de ftalats de mostres de sediment. Tot i això, els autors no indiquen en quines condicions de temperatura ni durant quant temps es va dur a terme l'extracció. Una de les grans limitacions de la MAE és que només es poden utilitzar dissolvents polars per les extraccions, ja que els dissolvents utilitzats en aquests mètodes han de poder absorbir energia de microones.

1.2.2. Tècniques de separació acoblades a l'espectrometria de masses

Com ja hem mencionat anteriorment, les tècniques més habituals per la separació de compostos AVP són la GC i la LC. En aquest apartat repassarem els usos més rellevants

d'aquestes tècniques de separació, així com de l'espectrometria de masses, per la determinació de les famílies de compostos incloses en aquesta tesi doctoral. Les tècniques cromatogràfiques acoblades a l'espectrometria de masses per la determinació de ftalats i dels seus metabòlits es troben descrites a l'article de revisió inclòs a l'apartat 1.2.3. d'aquesta tesi doctoral i no es comentaran en aquest apartat.

La LC és la tècnica cromatogràfica preferida per la determinació de benzotriazoles, benzotiazoles i benzosulfonamides degut a la seva baixa volatilitat. Les columnes més utilitzades per la determinació d'aquests compostos són les de fase estacionaria C_{18} [21,46,59,62,144,157,186,187], tot i que també se'n poden fer servir de fenil [54,60,158].

Pel que fa a la fase mòbil orgànica, habitualment el metanol dona bones separacions [21,62,158,187]. Tot i això, en alguns estudis s'ha comprovat que l'acetonitril produeix respostes més altes i millora les formes dels pics d'alguns compostos, de manera que també s'ha emprat en diverses ocasions [54,60,144,157]. Cal tenir en compte també l'existència de diverses espècies isomèriques com la 4TTR i la 5TTR o la *o*-TSA i la *p*-TSA. Amb l'ús a la fase mòbil de només metanol no es produeix la separació de cap de les parelles d'isòmers. En canvi, si s'afegeix tan sols un 2% d'acetonitril a la fase mòbil aquosa s'aconsegueix una bona separació de la 4TTR i la 5TTR i una lleugera separació de la *o*-TSA i la *p*-TSA [59,158].

La detecció de benzotriazoles, benzotiazoles i benzosulfonamides normalment es duu a terme per espectrometria de masses en tàndem (MS/MS) amb analitzadors triple quadrupol (QqQ) [157,158,184,187]. Tot i això, l'espectrometria de masses d'alta resolució (HRMS) també s'ha emprat en diverses ocasions amb analitzadors quadupol-temps de vol (qToF) [60,62] i Orbitrap [57]. Com que aquests compostos són relativament polars, la ionització per electrospray (ESI) és la interfase més comunament utilitzada. Generalment, la ionització es duu a terme en mode positiu per tots els compostos excepte per la OHBT, que s'ionitza bé tant en mode positiu com en mode negatiu [20], i per la BSA, la *p*-TSA i la *o*-TSA que només s'ionitzen en mode negatiu [21,59]. També és habitual afegir additius a les fases mòbils per millorar l'eficiència de la ionització, en concret concentracions baixes d'àcids com l'àcid fòrmic [54,60,62,157] i l'àcid acètic [59,184]. A més, alguns autors proposen l'ús d'acetat amònic per millorar la ionització de la OHBT i la MCBT, que no s'ionitzen bé en presència de l'àcid fòrmic [158].

La GC també s'ha utilitzat per determinar aquests compostos, tot i que en menor mesura. Les columnes emprades de manera habitual són columnes capil·lars amb fase estacionària de 5% difenil–95% dimetilpolisiloxà [45,50,61,161,183], tot i que també s'han

utilitzat columnes amb fase estacionària de 50% difenil–50% dimetilpolisiloxà [188]. Després de la separació, les benzotriazoles, benzotiazoles i benzosulfonamides s'ionitzen mitjançant la ionització electrònica i se solen detectar per MS o MS/MS. En concret, el quadrupol simple [45,61,183,188] i el triple quadrupol [50,161] són els analitzadors preferits per la detecció d'aquests compostos.

L'altra família de compostos estudiada en aquesta Tesi són els dièsters de l'àcid fosfòric. Cal destacar, però, que la informació sobre els mètodes de separació i detecció utilitzats per aquests compostos és molt limitada en comparació amb la de les altres famílies de compostos. La LC és la tècnica de separació preferida pels dièsters de l'àcid fosfòric, ja que en cas de voler-los separar per GC s'han de derivatitzar [189]. Les columnes més utilitzades són aquelles amb fase estacionaria C_{18} [7,108,113,133,136,170,189], altres fases estacionaries la de bifenil [190] o la cromatografia d'interacció hidrofílica (HILIC) [191] també han donat bons resultats. Generalment, tant el metanol [113,133,136,170] com l'acetonitril [7,108,189] donen bones separacions quan són utilitzats com a fase mòbil. A més, la separació d'aquests compostos es duu a terme habitualment o amb la fase mòbil aquosa a pHs àcids [108,192] o afegint-hi acetat amònic per aconseguir un pH més o menys neutre [113,117,190].

Després de la separació, els dièsters de l'àcid fosfòric s'ionitzen mitjançant la ionització per ESI en mode negatiu i es detecten per MS/MS amb analitzadors de triple quadrupol [108,113,133,136,170,190,191].

1.2.3. Phthalate esters in marine ecosystems: analytical methodologies, occurrence and distribution

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

PHTHALATE ESTERS IN MARINE ECOSYSTEMS: ANALYTICAL METHODS, OCCURRENCE AND DISTRIBUTION

Míriam Hidalgo-Serrano, Francesc Borrull, Rosa M. Marcé, Eva Pocurull

Universitat Rovira i Virgili, Department of Analytical Chemistry and Organic Chemistry, Marcel·lí Domingo s/n, Tarragona 43007, Spain

---

Abstract

Phthalate esters, which are widely used as additives in the plastics industry, are included in the list of High Production Volume Chemicals. Since they are not chemically bound to the plastics, they can leach into the environment through product degradation, wastewater and industrial discharge. They can also be transformed into corresponding phthalate monoesters via degradation in the environment or metabolization in complex living organisms. As numerous studies have reported the negative effects of phthalate esters and their transformation products on marine biota, these contaminants are an emerging concern.

Due to the impact these pollutants have on the marine environment, sensitive analytical methods are needed that can identify and quantify phthalate esters in marine environmental samples and evaluate their presence in the ecosystem. In this article we review the most important analytical methods for determining phthalate esters in seawater, marine sediments and biota. The “quick, easy, cheap, effective, rugged and safe” method and ultrasound-assisted extraction are the most common techniques for extracting the compounds of interest from solid samples, while solid-phase extraction is the most common technique for seawater samples.

We also describe the occurrence and distribution of the most frequently detected phthalate esters. Bis(2-ethylhexyl) phthalate, di-n-butyl phthalate and diisobutyl phthalate, as well as their metabolites and transformation products, are the main compounds detected in seawater, sediment and biota samples.

Keywords: *Analytical methods, Extraction techniques, Phthalate esters, Seawater, Marine sediments, Biota*

---

1. Introduction

Marine environments are delicate ecosystems that are highly sensitive to sources of chemical contamination, such as industry, cities and agriculture. One major worldwide environmental concern today is plastic pollution. According to the United Nations Environment Programme, over 300 million tonnes of plastic waste are produced each year [1], of which 1.7 to 4.6% are estimated to end up in the oceans [2]. Plastics can be subject to mechanical weathering and photodegradation, which fragment the products into particles called microplastics (MPs). MPs can be transported by ocean currents over vast distances and are known to have an immense impact on marine ecosystems [3]. In view of the above, it is important to determine how organic pollutants released from plastic materials affect marine environments. Phthalate esters (PAEs), which are not chemically bound to plastic products, can leach easily into the environment and become contaminants of concern.

PAEs are High Production Volume Chemicals commonly used as plasticisers to increase the flexibility and durability of plastics such as polyvinyl chloride. They can be divided into two groups depending on the length of their carbon chain: low-molecular-weight PAEs (3–6 carbon atoms) are mainly used in medical devices, adhesives, inks and paints, while high-molecular-weight PAEs (7–13 carbon atoms) are used, for example, in flooring, wires, coated fabrics and automotive applications. PAEs with only one or two carbon atoms in their chains are used as solvents and fixatives in personal care products but not as plasticisers [4]. However, emissions from commercial products are not the only route to PAE contamination as these compounds can also reach marine ecosystems through direct discharge, atmospheric deposition and surface runoff [5]. Moreover, their highly lipophilic nature and high octanol-water partition coefficient (log K_{ow}) mean they can be absorbed by suspended particles that can reach the bottom of aquatic environments and accumulate in the sediments [6]. Marine sediments can also act as an uptake source by organisms in these ecosystems [7].

Several studies have shown that when PAEs enter complex living organisms, they can be metabolised to the corresponding monoesters by hydrolytic, oxidative or conjugation processes in order to be excreted [8–10]. However, some of these derivates can also occur through the microbial degradation, photolysis and hydrolysis of PAEs in the environment [11].

PAEs are considered endocrine disruptors because of their estrogenic effects in aquatic organisms. They are also shown to cause acute developmental toxicity in zebrafish

embryos, which in turn leads to developmental abnormalities such as spinal curvatures, abnormal movements and pericardial oedema, and affects bone development and heart rate [12]. Di-n-butyl phthalate (DnBP) and benzylbutyl phthalate (BzBP) can also cause mortality in embryos even at low concentrations [12]. Some PAEs present chronic toxicity and are suspected of affecting thyroid hormone levels [13]. The toxic effects of PAEs are not limited to aquatic species, however, since they have demonstrated estrogenic effects also in human cells, mammals and amphibians [14]. PAEs can also impair ovarian function in women [15]. Moreover, they can pass through the placental barrier and affect the developing foetus *in utero* [16]. More specifically, exposure to bis(2-ethylhexyl) phthalate (DEHP), DnBP, BzBP and diethyl phthalate (DEP) during pregnancy is associated with worse behaviour, lower cognitive scores and psychomotor development effects in children [17].

Although information on the toxicity of phthalate metabolites, or phthalate monoesters (MPEs), is much more limited, several studies associate their presence with alterations of thyroid hormones [18]. MPEs can also affect human sperm function [19], be harmful to mitochondria functions [20], increase oxidative stress, and induce liver damage [21,22]. Some studies have suggested that the toxicity associated with PAEs may be caused solely by or in combination with their free metabolites [23,24].

In view of the endocrine-disrupting potential of PAEs and their widespread presence in the environment, several governmental and international organisations have regulated or issued statements on the use of some of these chemicals and established limits of intake [25–29].

Examining the presence of PAEs and MPEs and determining their occurrence and distribution in delicate ecosystems such as marine environments is essential for assessing their impact on the environment. One aim of this review is therefore to describe and compare the analytical methods used to determine the presence of the compounds of interest in marine compartments such as seawater, sediments and biota. Another is to describe their occurrence and distribution in marine environments while also highlighting the impact human activities responsible for industrial, plastic and microplastic pollution have on the environment.

2. Analytical methods

Like most emerging organic contaminants, PAEs and MPEs are found at very low concentrations in the environment (ng/g or ng/L), so suitable analytical methods are

needed in order to determine them in complex samples. These methods, which we discuss at length in this review, must include efficient extraction procedures and sensitive instrumental methods. Tables 1–2 summarise the most important analytical methods used to determine PAEs and MPEs in marine environmental samples.

2.1. Instrumental analysis

Gas chromatography (GC) and liquid chromatography (LC) are the most common instrumental techniques for determining these compounds (Tables 1–2).

Due to the low solubility and polarity on account of their long side chains, GC is the usual technique for determining PAEs. The usual columns for separating these compounds are fused silica columns coated with (5%-phenyl)-methylpolysiloxane [30–37], though 100% dimethylpolysiloxane columns [38,39] (35%-phenyl)-methylpolysiloxane [40] and low-polarity polymeric columns [41,42] have also been successfully used.

To determine PAEs in marine matrices, GC is most often coupled with mass spectrometry (MS) using single quadrupole analysers [6,30,31,33–35,37,39,41] or tandem mass spectrometry (MS/MS) with triple quadrupole (QqQ) analysers [32,36,40]. Electron ionisation (EI) is the preferred ionisation source for these compounds. Flame ionisation detectors (FID) [38,42] can also be used, though their selectivity and sensitivity may not be sufficient to determine the compounds of interest at low concentrations.

Due to their high polarity and low volatility, MPEs, on the other hand, need to be derivatised to be determined by GC [43,44]. Although Baini et al. [30] proposed a GC-MS method for simultaneously determining four PAEs and three MPEs without derivatising the MPEs, they did not provide an extended discussion of the development of the GC-MS method.

MPEs are therefore almost always determined by LC on their own or in combination with PAEs. Several reversed-phase columns have been used to separate PAEs and MPEs, the most common of which are C_{18} [44–52] stationary phase columns. C_8 [32] and C_{12} [43] columns have also provided good separation results.

Methanol (MeOH) and acetonitrile (ACN) are commonly used as organic modifiers for the mobile phase in LC methods. For PAEs, optimisation of the composition of the mobile phase is not described extensively in the articles. For MPEs, studies comparing separation

efficiency when using MeOH/water or ACN/water as the mobile phase have reported that results are better with the latter for several types of columns [32,53,54].

As we can see in Tables 1–2, LC is always coupled with mass spectrometers to determine PAEs and MPEs. The most common analysers are ion trap for MS [45], QqQ for MS/MS [32,43,46–49,51] and Orbitrap for high-resolution mass spectrometry (HRMS) [44,52,55].

Electrospray ionisation (ESI), which is the most common interface for these compounds, provides good ionisation efficiencies for PAEs in positive mode and for MPEs in negative mode [52]. However, atmospheric pressure chemical ionisation (APCI) has also been occasionally used for this purpose [56]. When a previous study by our group [52] also compared ESI and APCI for determining PAEs and MPEs, instrumental LODs and LOQs were similar with both interfaces for all compounds. Mobile phase additives are usually added to enhance the ionisation efficiency obtained. In particular, concentrations of 0.01–1% of acetic acid [32,43,45,47,48,52] or formic acid [46,49,51] are usually added to the mobile phases.

2.2. Extraction techniques and clean-up strategies

The most suitable strategies for sample extraction and clean-up depend on the characteristics of the matrix and the properties of the analytes of interest.

In the next section we review the most common and most interesting methods for extracting PAEs and MPEs simultaneously or separately.

2.2.1. Seawater samples

Several techniques have been used to extract PAEs and MPEs from seawater. The most common of these is solid phase extraction (SPE) (see Table 1), though microextraction techniques have also been used.

SPE, GC and LC are currently preferred due to their low consumption of organic solvents and the simplicity of the procedures. SPE can also provide high sample preconcentration factors because they can extract large volumes of sample. Since low concentrations of some PAEs and MPEs are expected in seawater samples, it is essential to increase the enrichment factor of the extraction methods used.

Table 1. Analytical methods for determining PAEs and MPEs in seawater.

Compounds	Extraction technique	Instrumental technique	LOD (ng/L)	LOQ (ng/L)	Ref.
PAEs: DMP, DEP, DnPrP, DiBP, DnBP, BzBP, DEHP, DnOP	SPE (Oasis HLB)	GC-MS	0.11–2.73	-	[34]
MPEs: MMP, MEP, MnBP, MiHP, MBzP, MiHpP, MEHP, MnOP, MiNP, MiDP	SPE (Oasis HLB)	LC-MS/MS	0.19–3.9	-	[43]
PAEs: DMP, DEP, DnBP, DnPP, BzBP, DCHP, DnHP, DBzP, DEHP, DiNP, DiDP **MPEs:** MMP, MEP, MnBP, MnPP, MCHP, MnHP, MBzP, MEHP, MiNP	SPE (Oasis HLB)	LC-HRMS	PAEs: 5–50 MPEs: 5–25	PAEs: 10–75 MPEs: 10–50	[44]
PAEs: DMP, DEP, DnBP, BzBP, DEHP, DnOP	SPE (MWCNTs : GO)	GC-FID	36-1410	120–4700	[42]
PAEs: DnPrP, DnBP, DnPP, DiPP, BzBP, DCHP, DEHP, DiNP, DiDP	m-dSPE (Fe_3O_4@pDA)	GC-MS	-	1.8–319	[33]
PAEs: DMP, DEP, DnBP, DiBP, DnPP, BzBP, DPhP, DCHP, DnHP, DEHP, DnOP, DiNP, DMEP, DEEP, DMPP, DBEP	SPME (PDMS)	TD-GC-MS	0.05–0.32	-	[39]
PAEs: DMP, DEP, DnBP, DEHP	SBSE (PDMS)	TD-GC-MS	5–21	-	[41]
PAEs: DMP, DEP, DnBP, DiBP, DnPP, BzBP, DPhP, DCHP, DnHP, DEHP, DnOP, DMEP, DEEP, DMPP, DBEP	SBSE (PDMS)	GC-MS	0.08–489	0.27–1630	[35]
PAEs: DMP, DEP, DAP, DnBP, DiBP, DiHpP, DEHP	DLLME	GC-MS	40–4520	-	[38]

Since PAEs and MPEs have a wide range of polarities, polymeric sorbents with a hydrophilic-lipophilic balance, such as Oasis HLB and Strata X, are also used to extract them. Paluselli et al. [34], for example, used Oasis HLB to extract eight PAEs from large volumes of seawater. These authors tested various elution solvents, i.e. acetone, dichloromethane (DCM), ethyl acetate (EtOAc), methanol and n-hexane, to increase extraction recoveries. N-hexane was useful only for eluting less polar PAEs such as DEHP and DnOP. In contrast, more polar solvents such as acetone and MeOH yielded low extraction recoveries for these compounds. DCM and EtOAc, with extraction recoveries above 81% and 85%, respectively, provided the best results. EtOAc was chosen as the best elution solvent. Sample volumes of 100, 200, 500 and 1000 mL and sorbent masses of 200 and 400 mg were also tested. Since the extraction recoveries were satisfactory for all combinations, the authors selected 1 L of sample to maximise the enrichment factor of the extraction step, and 200 mg of Oasis HLB to reduce the cost and time needed for the procedure. Even under optimised conditions, the extraction recoveries obtained for DEHP and DOP were 10%. The authors attributed the loss of DEHP and DOP to the absorption of the compounds onto glassware and suggested correcting it by using deuterated DEHP as a surrogate standard. With this method, relative recoveries ranged from 95% (DnOP) to 115% (DPrP), while LODs were 0.11–2.73 ng/L.

Similarly, Blair et al. [43] used 500 mg Oasis HLB cartridges to determine six single MPE isomers and four MPE isomeric mixtures in 500 mL of seawater. Ultrapure water and a 35% MeOH/water solution were used to wash the cartridges, and the compounds were eluted with EtOAc and ACN. The authors obtained recoveries between 58% (MiDP) and 122% (MBzP) and LODs of 0.19–3.9 ng/L. Huysman et al. [44] also used Oasis HLB to simultaneously extract 11 PAEs, nine MPEs and seven phenols from seawater.

Commercial sorbents are not the only option for extracting PAEs, however. Habibi et al. [42] proposed a simple SPE method combining multi-walled carbon nanotubes (MWCNTs) and graphene oxide nanosheets (GOs) as adsorbents to extract six PAEs. These authors tested several ratios of this combination and various amounts of sorbent to obtain the maximum extraction recoveries possible. They also tested several elution solvents and sample parameters. Briefly, tetrahydrofuran was more effective at eluting the compounds than other common solvents such as acetone, MeOH and n-hexane due to the strong, unique interactions of the compounds with the sorbents. Moreover, the volume and flow rate of the sample and the elution solvent were significant parameters for the extraction recoveries. Interestingly, adding a low percentage of organic modifier (MeOH) to the samples before loading greatly increased the extraction recoveries of the less polar compounds due to the greater solubility of the long-chain PAEs in water, which

hindered their adsorption onto glassware. However, the extraction recoveries obtained with this method ranged from 83 to 98%, which are similar to those obtained by methods using Oasis HLB. Also, the LODs were 36–1410 ng/L, which are between two and three orders of magnitude higher than those obtained by GC-MS (see Table 1) due to the low sample volume (100 mL) and the use of GC-FID.

Despite the good results, SPE has an important handicap when used to extract PAEs from any kind of sample. Most commercial sorbents are available only in the form of plastic cartridges, which can leach PAEs into the extract and alter the results of the analyses [57]. If glass SPE cartridges are unavailable, procedural blanks must be regularly performed to correct contamination from the plastic cartridges.

Dispersive solid-phase extraction (dSPE), a variant of SPE, has also been used to extract PAEs from seawater samples (Table 1). The main advantage of dSPE is that it is less time-consuming than SPE. It can also prevent some of the most common difficulties of sample-loading in SPE, such as cartridge blocking or the need for a controlled constant sample flow rate [58]. The fact that no plastic cartridges are needed for this procedure also minimises plastic-derived interferences. Jiménez-Skrzypek et al. [33] developed a magnetic-dispersive solid-phase extraction (m-dSPE) method using polymer-coated magnetic nanoparticles as the sorbent to extract 10 PAEs from 50 mL of seawater. Recoveries ranged from 79 to 116%. LOQs (using GC-MS) were 1.8–319 ng/L, which are slightly higher than those obtained with conventional SPE, probably because of a low enrichment factor.

Microextraction techniques such as solid-phase microextraction (SPME) followed by thermal desorption (TD) can also be used to extract PAEs from seawater samples. The main advantages of this technique are that it is both fast and simple because the sampling, extraction and preconcentration are conducted in a single step [59,60]. Also, desorbing the analytes from the probe does not require organic solvents, which makes it a promising extraction technique for green sample treatment methods [61]. It should also be borne in mind, however, that, due to their low volatility, this technique is not suitable for subsequently determining MPEs.

Zhang et al. [39] used a SPME fibre (coated with polydimethylsiloxane (PDMS)) to extract 16 PAEs from 10 mL of sample by direct immersion. These authors optimised extraction time and temperature, which affect the efficiency of the extraction, and evaluated the effects of salinity on extraction efficiency. Their results suggested that since salinity had little effect on recoveries, the method can be applied to samples with

different salinities without affecting the results. Good relative recoveries ranging from 77 to 114% were obtained for all compounds except DMP and DEEP. The low recoveries (55%) for DMP can be explained by its log K_{ow}, which is much smaller than that of the others. The LODs were 0.05–0.32 ng/L, which are similar to those obtained by GC-MS with conventional SPE methods (Table 1).

Another useful microextraction technique is stir-bar sorptive extraction (SBSE). Like SPME, SBSE can be followed by TD to extract the analytes from the sorbent. PDMS-coated magnetic stir bars have been used to extract DMP, DEP, DnBP and DEHP, with recoveries of 14%, 47%, 136% and 102%, respectively [41]. These large differences in recoveries are again caused by the log K_{ow} of the analytes. SBSE methods usually have more sensitivity than SPME methods due to the greater coating and, therefore, the greater surface contact area [35,59]. However, in this case the LODs ranged from 5 to 21 ng/L, which is slightly higher than the values obtained by SPME and GC-MS. However, the authors provided no information about the instrumental limits or the optimisation of the SBSE parameters, which makes it difficult to explain the results. SBSE can also be combined with liquid desorption, which makes the extraction technique also compatible with LC. Si et al. [35] developed a method to extract 14 PAEs from seawater by optimising both SBSE and desorption efficiency. Though they provided no information about extraction recoveries (comparing only peak areas for the optimisation), the LODs ranged from 0.08 to 489.13 ng/L. The authors pointed out that the LODs for high-molecular-weight PAEs were higher than those for the most hydrophilic compounds (such as DMP) since compounds such as DOP and DEHP interact strongly with PDMS and cannot be desorbed efficiently from the adsorbent. Although SBSE can present the same advantages as SPME in comparison with SPE, it should be borne in mind that SBSE often requires longer extraction times.

Dispersive liquid-liquid microextraction (DLLME) has also been used successfully to determine PAEs in seawater samples. The main advantages of this technique are its speed and simplicity. The dispersion of the extraction solvent in the liquid sample also leads to large enrichment factors, high recoveries, and low extraction times [62]. Although the solvent volumes are very small, the solvents themselves are non-environmentally friendly, which makes this technique incompatible with green-analytical-chemistry approaches [63]. When 20 µL of carbon tetrachloride dispersed in 0.5 mL of MeOH was used to successfully extract seven PAEs from seawater [38], the LODs were 40–4520 ng/L, which are similar to those obtained by Habibi et al. [42] (Table 1).

Although several microextraction techniques appear promising for extracting PAEs from seawater samples, SPE is the most common technique thanks to its high recoveries

and high enrichment factors (see Table 1). It should also be borne in mind that SPE carried out with a suitable sorbent/solvent combination enables the PAEs and their metabolites to be extracted simultaneously, thus making this procedure extremely useful for monitoring these contaminants in the environment. However, since SPE plastic cartridges are also a source of PAE and MPE contamination, procedural blanks should be taken regularly for adjustment purposes.

2.2.2. Solid samples

Sediments and biota are the most analysed solid samples from aquatic environments. Such samples are usually freeze-dried, homogenised and sieved before extraction, though there are also reports of extraction from wet samples. Moreover, the complexity of these samples means that a clean-up step is usually later required to remove any co-extracted matrix components that may interfere with analyses of the compounds of interest. The same clean-up procedures can be used for both sediment and biota samples. However, activated copper is sometimes added to sediment samples during extraction to remove sulphur interferents [6,31]. Note also, however, that as most authors do not report any clean-up optimisation or matrix effect values, it is difficult to evaluate the effectiveness of these procedures.

Several extraction techniques have been used to extract solid samples. These include ultrasound-assisted extraction (USAE), quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction, pressurised liquid extraction (PLE), and microwave-assisted extraction (MAE) (Table 2). The most common of these techniques is USAE [6,30,33,39,45,48–50,64]. USAE is simple and versatile and allows the use of lower quantities of solvent and shorter working times than other extraction techniques [65]. Also, it can be performed with organic solvents of different polarities to extract the compounds of interest. Studies in the literature on the extraction of PAEs and MPEs, for example, used acetone [45,48,49], ACN [33,50], MeOH [64], DCM [39] and solvent mixtures such as acetone/hexane (1:1) [6] and DCM/hexane (1:1) [30]. Major drawbacks with this technique, however, are the low power of ultrasound baths and the need for repeated extractions [61,66].

USAE has occasionally been used to extract a wide range of PAEs from sediments and biota without any clean-up step [6,45], yielding relative recoveries ranging from 67 to 111% and LODs ranging, respectively, from 7 to 27 ng/g (GC-MS) and from 1 to 5 ng/g (LC-MS). Similar recoveries and LOQs were obtained by Savoca et al. [50] with LC-HRMS by diluting the extract before injection. Lipid digestion with sulfuric acid [30] and liquid-liquid

Table 2. Analytical methods for determining PAEs and MPEs in solid samples.

Compounds	Matrices	Extraction technique and clean-up	Instrumental technique	LOD (ng/g)	LOQ (ng/g)	Ref.
PAEs: DMP, DEP, DnBP, DiBP, DnPP, BzBP, DCHP, DnHP, DEHP, DnOP, DnNP, DMEP, DEEP, DMPP, DBEP	Sediments	USAE (Acetone/Hexane 1:1)	GC-MS	7 – 27	23 – 74	[6]
PAEs: DEHP **MPEs:** MEHP	Krill, basking shark (muscle), stranded fin whale (blubber and muscle)	USAE (Acetone)	LC-MS	1 – 5	2 – 10	[45]
PAEs: DMP, DEP, DnBP, BzBP, DEHP, DnOP	Marine turtles (muscle, liver, gonad and fat tissues)	USAE (ACN)	LC-HRMS	-	0.1 – 10	[50]
PAEs: BzBP, DnHP, DEHP, DiOP, DnDP **MPEs:** MnBP, MBzP, MEHP	Neuston-plankton, cetaceans (Blubber)	USAE (DCM/Hexane) and lipid digestion with sulfuric acid	GC-MS	1 – 10	-	[30]
PAEs: DMP, DEP, DnBP, BzBP, DEHP **MPEs:** MEHP	Neuston-plankton, scleractinian coral	USAE (acetone) and LLE (water and hexane)	LC-MS/MS	0.7 – 21	-	[49]
PAEs: DMP, DEP, DnBP, DiBP, DnPP, BzBP, DPhP, DCHP, DnHP, DEHP, DnOP, DiNP, DMEP, DEEP, DMPP, DBEP	Sediments	USAE (DCM) and SPE (CNW-BOND Si)	GC-MS	0.12 – 1.60	-	[39]
PAEs: DnPrP, DnBP, DnPP, DiPP, BzBP, DCHP, DEHP, DiNP, DiDP	Sea sand	USAE (ACN) and m-dSPE (Fe_3O_4@pDA)	GC-MS	-	0.020 – 4.0	[33]

Table 2. (Cont.).

Compounds	Matrices	Extraction technique and clean-up	Instrumental technique	LOD (ng/g)	LOQ (ng/g)	Ref.
PAEs: DMP, DEP, DnBP, BzBP, DEHP, DnOP	Sediments	USAE (MeOH) and HS-SPME	GC-MS	1 – 79	1 – 142	[64]
PAEs: DMP, DEP, DnBP, BzBP, DEHP **MPEs:** MEHP	Scleractinian coral, sponge, clam, colonial hydrozoan	USAE (Acetone) and BioSPME (C_{18})	LC-MS/MS	0.2 – 2.1	1 – 5	[48]
PAEs: DMP, DEP, DAP, DnPrP, DiPrP, DnBP, DiBP, DnPP, BzBP, DPhP, DCHP, DnHP, DEHP, DnOP, DnNP, DMEP, DEEP, DMPP, DBEP	Fish	QuEChERS and dSPE (PSA, $MgSO_4$)	GC-MS/MS	0.01 – 10	0.05 – 20	[36]
PAEs: DMP, DnBP, BzBP, DEHP, DnOP **MPEs:** MnBP	Mussels	QuEChERS and dSPE (PSA, $MgSO_4$)	LC-MS/MS	3 – 15	9 – 45	[51]
PAEs: DMP, DEP, DnBP, BzBP, DEHP, DnOP **MPEs:** MMP, MEP, MnBP, MBzP, MEHP, MnOP	Seafood	QuEChERS and dSPE (PAEs: Lipifiltr®; MPEs: C_{18})	LC-HRMS	1 – 100	5 – 250	[55]
PAEs: DMP, DEP, DnBP, DEHP, DnOP **MPEs:** MMP, MEP, MnBP, MEHP, MnOP	Seafood	QuEChERS and SPE (PSA)	LC-MS/MS (MPEs) GC-MS/MS	PAEs: 0.034 – 2.2 MPEs:	-	[32]
PAEs: DMP, DEP, DnBP, DiBP, DnPP, BzBP, DPhP, DCHP, DnHP, DEHP, DnOP, DiNP, DMEP, DEEP, DMPP, DBEP	Seafood	QuEChERS and SPE (Cleanert® PAE)	GC-MS/MS	0.15 – 0.78	-	[40]

Table 2. (Cont.).

Compounds	Matrices	Extraction technique and clean-up	Instrumental technique	LOD (ng/g)	LOQ (ng/g)	Ref.
PAEs: DMP, DEP, DnBP, DiBP, BzBP, DEHP, DnOP	Posidonia oceanica (a), seafood (b), sediments (c)	QuEChERS	GC-MS	-	(a, b): 0.002 – 0.020 (c): 0.0003 – 0.0017	[31]
MPEs: MMP, MEP, MnBP, MiHP, MBzP, MiHpP, MEHP, MnOP, MiNP, MiDP	Sediments (a), seafood (b)	PLE (Water) and SPE (Oasis MAX)	LC-MS/MS	(a): 0.01 – 0.20 (b): 0.05 – 0.99	-	[43]
PAEs: DMP, DEP, DnBP, BzBP, DEHP	Seafood	PLE (MeOH), SPE (Bond Elut Plexa)	LC-HRMS	1 – 100	2.5 – 250	[52]
MPEs: MMP, MEP, MnBP, MBzP, MEHP, MnOP						
PAEs: DMP, DEP, DnPrP, DnBP, DiBP, DnPP, BzBP, DCHP, DnHP, DEHP, DnOP, DMEP, DEEP, DMPP, DBEP	Sediments	MAE (MeOH/EtOAc 1:1)	GC-MS	24 – 392[a]	-	[37]
MPEs: MMP, MEP, MnBP, MiBP, MBzP, MEHP, MnOP, MEOHP, MEHHP	Fish	Deconjugation and SPE (Oasis HLB)	LC-MS/MS	0.10 – 25.8	0.21 – 65.5	[46]
MPEs: MMP, MEP, MnBP, MiBP, MnPP, MiPP, MCHP, MnHP, MBzP, MnHpP, MEHP, MnOP, MnNP, MnDP, MEOHP, MEHHP	Harbour porpoises	Deconjugation and SPE (ABS Elut-NEXUS cartridges)	LC-MS/MS	0.17 – 0.84	0.50 – 2.50	[47]

[a]LOD expressed as ng/L instead of ng/g

extraction (LLE) with water and hexane [49] have been used as simple clean-up procedures to remove interferents from coral and neustonic/planktonic samples and from cetacean blubber. SPE and variants of the technique can also be used to remove matrix interferents from extracts and increase the selectivity of the methods. When, for example, Zhang et al. [39], conducted the clean-up step with a silica-based SPE cartridge, they obtained comparable recoveries and better LODs for the determination of PAEs in sediments than those obtained with no clean-up step by GC-MS. Similarly, Jiménez-Skrzypek et al. [33] successfully used a m-dSPE method developed for extracting PAEs from seawater as a clean-up step for sea sand extracts. SPME with PDMS-DVB [64] and biocompatible [48] fibre coatings have also been used with satisfactory results to clean up sediment and biota extracts.

QuEChERS has also widely been used to extract PAEs and MPEs from solid samples (Table 2). This technique involves initially extracting a wet sample with an organic solvent followed by a salting-out step to separate the two liquid phases (aqueous and organic). ACN is the usual organic solvent for most QuEChERS methods, though solvent mixtures such as hexane/DCM (1:1) [40] and DCM/EtOAc (1:1) [31] have also been reported. This first extraction step can also be complemented with USAE after adding the salts to the mixture [32,36,40]. NaCl and $MgSO_4$ are widely used in most QuEChERS methods for the salting-out step [67]. In a previous paper [55], we compared the extraction efficiency of PAEs and MPEs from fish with salt mixtures from three official QuEChERS methods: the original QuEChERS method (1 g NaCl, 4 g $MgSO_4$), the European standard method EN 15662:2019 (1 g NaCl, 4 g $MgSO_4$, 1 g sodium citrate tribasic dihydrate, 0.5 g sodium citrate dibasic sesquihydrate), and the AOAC official method (1.5 g sodium acetate, 6 g $MgSO_4$). We found no significant differences between the original method and the AOAC method. The EN method, however, yielded the best results, obtaining extraction recoveries ranging from 79 to 120% for most compounds.

After extraction by QuEChERS, a clean-up step with dSPE is usually added to remove interferents. For PAEs, 50–150 mg of PSA is reported to yield the best extraction efficiencies in biota samples [36,51], though other sorbents such as C_{18}, Florisil, alumina and combinations with graphitised carbon black (GCB) have also yielded good results. However, neither of the above studies evaluated the improvement or otherwise of the matrix effect after dSPE, which makes it difficult to evaluate the usefulness of the clean-up steps. However, in a previous study [55] we compared the matrix effect in seafood extracts after dSPE using three sorbents: PSA, Florisil and a novel sorbent named Lipifiltr®, which is indicated for removing lipids from samples following QuEChERS extraction. The lowest values of the matrix effect were obtained with Lipifiltr®. Little information is

available about dSPE as a clean-up step for MPEs following QuEChERS extraction. Only C_{18} and GCB are reported to yield good extraction efficiencies for a wide range of MPEs, with the best matrix effects being achieved with 200 mg of C_{18} [55]. SPE has also been used to clean up QuEChERS extracts from biota samples, with PSA cartridges [32] and specific SPE cartridges being used to extract PAEs [40]. These methods yielded similar LODs and LOQs to those obtained with USAE followed by LC-MS/MS and GC-MS, which shows that QuEChERS is a good alternative.

Castro-Jiménez et al. [31] proposed a different approach based on QuEChERS to extract PAEs and other compounds from sediments and biota: the extraction of lyophilised samples by sonication followed by two clean-up steps. First, they evaluated the extraction solvent mixture by comparing the background noise and blank levels of five organic solvent mixtures. They then compared a traditional QuEChERS first step (i.e. $MgSO_4$ and sodium acetate) with dSPE without extraction salts (i.e. $MgSO_4$, PSA and C_{18}) followed by sonication. The latter technique yielded slightly better results for most compounds and acted as an extra clean-up step. The authors proposed a second dSPE step with Florisil and alumina. As mentioned earlier, classic QuEChERS procedures involve extraction with an organic solvent of a sample with water content. Salts must then be added to promote the separation of phases and the partitioning of the analytes into the organic phase. Although the authors view this method as an innovative QuEChERS sequence, it could also be defined as a USAE followed by two dSPE clean-up steps since the water content of the sample and the salting-out step are completely disregarded in the extraction procedure. However, as Table 2 shows, the LOQs with this method are much lower than those achieved with traditional QuEChERS procedures and USAE methods (using GC-MS in all cases).

Blair et al. [43] developed a PLE method to analyse MPEs in sediments and seafood tissues. The extraction was performed with an accelerated solvent extractor, using water as the extraction solvent, at 70 ^{o}C for five minutes through three cycles. SPE with Oasis MAX cartridges was used to clean up the extracts. This method yielded high recoveries for most MPEs. The LODs ranged from 0.01 to 0.99 ng/g, which are similar to those obtained by USAE and QuEChERS followed by LC-MS/MS [32,48,51] (Table 2). In contrast, in a previous study [52] we proposed a PLE method to simultaneously analyse PAEs and MPEs in seafood in which, for the extraction, we used MeOH at 80 ^{o}C for 10 minutes. We then purified the extracts with a freezing lipid filtration to promote the precipitation of lipids and interfering compounds followed by SPE with Bond Elut Plexa cartridges. Since this method enables the simultaneous determination of PAEs and MPEs, it is a useful tool for monitorisation.

MAE [37] has also been used to extract PAEs from sediments. The main advantages of MAE are lower extraction times and less solvent consumption thanks to the use of microwaves to heat the solvent [68]. The extraction was performed with a microwave digestion system using a mixture of MeOH/EtOAc 1:1 (v/v) as the extraction solvent at 150 ºC for 20 minutes and a microwave power of 750 W. This method yielded recoveries ranging from 84 to 105% for all compounds.

As we mentioned earlier, when PAEs enter complex living organisms, they are rapidly transformed into MPEs and conjugated in order to be excreted. Most studies therefore only analyse what are called free-MPEs. To analyse all MPEs present, a deconjugation step is needed, which is performed by adding ammonium acetate (1 M) and β-glucuronidase to the sample before digestion at 37 ºC [46,47]. SPE can then be used to further extract and clean up the samples.

Although similar results are obtained with all the extraction techniques discussed (see Table 2), QuEChERS is the only one that can be performed without the need for special equipment such as an ultrasonic bath, a PLE or a microwave digestion system. This probably makes QuEChERS the fastest and simplest technique for analysing PAEs and MPEs in solid marine samples. However, since it also requires more manipulation than the other techniques, it can more easily lead to environmental contamination.

3. Occurrence and distribution

Although the occurrence of PAEs in environmental marine samples has been a focus of study in recent years, much less attention has been devoted to the occurrence of MPEs. In the next section we detail the occurrence of these compounds in marine ecosystems, discuss potential sources of PAE contamination, and analyse their spatial and temporal variations (see Figure 1).

3.1. Seawater

Numerous PAEs and several MPEs have been determined in seawater samples collected from water masses around the world [33–35,38,39,41–44]. Detailed information about the occurrence of these compounds in seawater is shown in Table 3.

According to the studies in our review, 23 PAEs have been detected, mainly on the coast of China (the Yellow Sea, the Bohai Sea and the East China Sea). DEHP, DnBP and

DiBP, with the highest concentrations and detection frequencies in most studies, are the main PAEs detected in the aquatic environment.

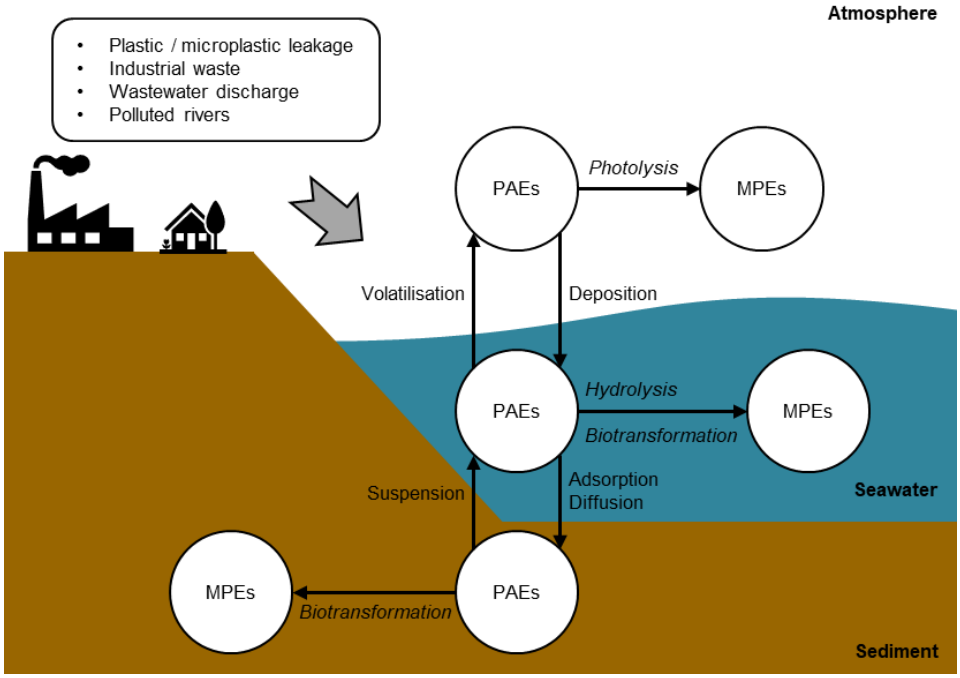


Figure 1. Sources, spatial distribution and degradation of phthalate esters in the environment.

As Table 3 shows, the highest DEHP concentration (168 µg/L) was recorded in Tunisia along the coastline of the Mahdia Governorate. In that study [69], the detection frequency of DEHP was 93%. Surface water samples were collected at sampling stations close to pollution sources such as factories, towns and tourist areas [69]. The second highest concentration of DEHP (9738 ng/L) was found at Hangzhou Bay in China (East China Sea). In this case the detection frequency was 100% with samples collected near Shanghai, the largest and most populated city in China [70]. Lower but still significant concentrations of DEHP were reported in the Gulf of Thailand (1160 ng/L) [71] and in the North Sea along the Belgian coastline (766 ng/L) [44]. In both cases, the samples were taken from coastal zones near populated areas. The lowest maximum concentrations (9.2 ng/L), on the other hand, were detected in the seamount area of the Tropical Western Pacific Ocean, far from any human activity [72]. Similar associations can be made with regard to DnBP and DiBP. The highest concentrations (30.5 µg/L for DnBP and 106 µg/L for DiBP) were also found in Tunisia, with detection frequencies of 89 and 93%,

respectively [69]. The second highest concentrations (17.952 µg/L for DnBP and 17.256 µg/L for DiBP) were also reported at Hangzhou Bay in China [70].

The sources of these compounds are diverse and include plastic leakage, industry and household waste. The high log K_{ow} and, therefore, the high hydrophobicity of DEHP, DnBP and DiBP also make their degradation by photolysis, hydrolysis or biological processes more difficult, which can facilitate their accumulation [73].

Table 3. Occurrence of PAEs and MPEs in seawater (ng/L) and sediment samples (ng/g)

Compounds	Seawater	Sediment	Sampling zone	Ref.
Phthalate acid esters				
DMP	0.08–830	4–4180	China	[5,37,70,73-78]
	1.4–6.4	<0.002–0.2	Mediterranean Sea	[31,34]
	–	70–6670	Persian Gulf	[6]
	20–100	–	South Korea	[41]
	n.d.–7	–	Tropical western Pacific Ocean	[72]
DEP	0.25–2404	2.5–1778	China	[5,37,70,73-78]
	6.9–50	<0.001–2.1	Mediterranean Sea	[31,34]
	<25–753	–	North Sea (Belgium)	[44]
	–	290–6360	Persian Gulf	[6]
	20–150	–	South Korea	[41]
	n.d.–2.1	–	Tropical western Pacific Ocean	[72]
	<10–17000	64–142	Tunisia	[69]
DAP	0.67–52	0.78–28	China	[70]
DPrP	–	1.12–492	China	[37, 70]
	1.30–1.31	–	Tropical western Pacific Ocean	[72]
	–	10.3–20.2	Tunisia	[69]
DiPrP	–	0.11–7.39	China	[70]
	n.d.–0.89	–	Tropical western Pacific Ocean	[72]
DnBP	11–17952	0.17–15103	China	[5,37,70,73-78]
	63.4–466	0.3–3.1	Mediterranean Sea	[31,34]
	<5–2645	–	North Sea (Belgium)	[44]
	–	720–12650	Persian Gulf	[6]
	40–360	–	South Korea	[41]
	230–770	n.d.–800	Thailand	[71]
	2.2–13	–	Tropical western Pacific Ocean	[72]
	<29–30500	42–82	Tunisia	[69]
DiBP	5–17256	1.78–7980	China	[5,37,70,73-78]
	1.9–14	–	Tropical western Pacific Ocean	[72]
	–	110–4238	Persian Gulf	[6]
	56.5–383.4	1.9–5.7	Mediterranean Sea	[31,34]
	<5–106000	152–394	Tunisia	[69]

Table 3. (Cont.).

Compounds	Seawater	Sediment	Sampling zone	Ref.
DnPP	0.04–146	0.13–2170	China	[5,37,70,75,77]
	–	80–1100	Persian Gulf	[6]
	n.d.–1.6	–	Tropical western Pacific Ocean	[72]
BzBP	0.09–344	0.29–1283	China	[5,37,70,73-75,77]
	3.2–4.8	0.8–1.9	Mediterranean Sea	[31,34]
	<10–343	–	North Sea (Belgium)	[44]
	–	450–10110	Persian Gulf	[6]
	n.d.–5.5	–	Tropical western Pacific Ocean	[72]
	–	<0.37–43	Tunisia	[69]
DPhP	0.27–1996	0.14–710	China	[5,70,75]
	0.84–5.3	–	Tropical western Pacific Ocean	[72]
DCHP	0.05–705	0.2–37	China	[5,70,75,77]
	–	100–2340	Persian Gulf	[6]
	1.1–7	–	Tropical western Pacific Ocean	[72]
DnHP	0.05–443	8.4–395	China	[5,37,75,77]
	<10–37	–	North Sea (Belgium)	[44]
	–	80–1530	Persian Gulf	[6]
DiHP	n.d.–2.3	–	Tropical western Pacific Ocean	[72]
DEHP	9–9738	0.31–22160	China	[5,37,70,73-75,77,78]
	102.9–296.5	4.2–319.5	Mediterranean Sea	[31,34]
	66–766	–	North Sea (Belgium)	[44]
	–	1080–35000	Persian Gulf	[6]
	30–300	–	South Korea	[44]
	310–1160	n.d.–1650	Thailand	[71]
	2.0–9.2	–	Tropical western Pacific Ocean	[72]
	<26–168000	4150–5240	Tunisia	[69]
DnOP	0.09–1386	0.12–970	China	[5,37,70,75,77]
	–	<0.003–0.1	Mediterranean Sea	[31]
	–	680–8589	Persian Gulf	[6]
	n.d.–1.7	–	Tropical western Pacific Ocean	[72]
DnNP	0.22–3.32	1–30	China	[70]
	–	120–3350	Persian Gulf	[6]
DiNP	0.13–126	10–70	China	[5,75,77]
DiDP	<25–108	–	North Sea (Belgium)	[44]
	n.d.–4.4	–	Tropical western Pacific Ocean	[72]
DMEP	0.2–811	0.3–585	China	[5,37,70,75-77]
	–	30–1620	Persian Gulf	[6]
DEEP	1.07–95	2–1670	China	[5,37,75]
	–	60–620	Persian Gulf	[6]
DMPP	1.57–579	8–280	China	[5,75,77]
	–	40–240	Persian Gulf	[6]
DBEP	0.64–234	0.40–879	China	[5,37,70,75,77]
	–	90–1020	Persian Gulf	[6]

Table 3. (Cont.).

Compounds	Seawater	Sediment	Sampling zone	Ref.
HEHP	–	40–510	Persian Gulf	[6]
Phthalate acid ester metabolites				
MMP	0.42–20	1.28–4.16	Canada	[43]
	<20–2542	–	North Sea (Belgium)	[44]
MEP	4.41–39	0.45–3.63	Canada	[43]
MnBP	51–108	5.3–20	Canada	[43]
	<5–292	–	North Sea (Belgium)	[44]
MnPP	<20–138	–	North Sea (Belgium)	[44]
MBzP	<0.22–6.05	0.19–3.02	Canada	[43]
	<5–58	–	North Sea (Belgium)	[44]
MiHP	<1.3–0.52	<0.07–0.53	Canada	[43]
MiHpP	2.71–6.61	0.05–0.07	Canada	[43]
MEHP	45–57	0.33–0.84	Canada	[43]
	<25–740	–	North Sea (Belgium)	[44]
MnOP	<0.5–1.06	0.04–0.2	Canada	[43]
MiNP	<0.48–29	0.01–1.83	Canada	[43]
MiDP	2.23–8.25	<0.13–0.14	Canada	[43]

n.d. = not detected

Since PAEs are not homogenous in seawater, their spatial (horizontal and vertical) distribution has been studied on various occasions. With regard to their horizontal distribution, most studies agree that the total concentration of PAEs is significantly lower in offshore areas than in inshore regions, which are strongly influenced by large rivers, ports and cities, all of which are affected by human activities that cause heavy pollution [5,41,70,72,73]. Coastal current transportation also seems to increase PAE concentrations in certain areas, probably due to the large amounts of plastic waste that are accumulated due to surrounding currents [74,75]. A significant positive correlation has been found between MP abundance and PAE concentration in surface water, since MPs tend to adsorb waterborne contaminants while simultaneously leaching their own plasticisers. The degradation and decomposition of the MPs and plastic debris then release a multitude of chemicals (such as PAEs) into the seawater [5,73]. By taking into account this correlation, the migration of organic contaminants such as PAEs can be used as an indirect method for tracing MPs in the environment. In addition to MPs, atmospheric deposition is also a potential source of PAEs [72,74].

With regard to the vertical distribution of PAEs, the concentration range seems to be more homogenous at offshore sites than at coastal sites due to the lack of heavily polluted inputs [74]. Most studies report high concentrations at the surface, followed by a slight

decrease at greater depths before a final increase near the bottom. The decrease in concentration with depth is probably due to microbial degradation, while the increase near the bottom appears to be caused by the remobilisation of sediments. Due to their log K_{ow}, PAEs tend to be adsorbed onto particulate matter which then settles on the seabed. Combined with the degradation of MPs and plastic debris accumulated on the seafloor, this means that the resuspension of sediments can easily cause PAEs to migrate from the sediments into the seawater [34,75,76].

Significant differences in the concentrations of PAEs have also been observed depending on the season and the weather. The total concentration of PAEs is generally higher in dry seasons than in wet seasons because seawater is somewhat diluted in wet seasons by increased river flows [41,70,76]. Moreover, as microbial bioreactivity and biomass levels drop in winter, there is less PAEs degradation, which could explain the higher PAE concentrations in areas where the dry season corresponds to winter [76]. Another factor that could influence PAE concentration is tide. In seasons when tides are stronger, they can enhance the resuspension of sediment, thus increasing the concentration of contaminants [70]. Heo et al. [41] also studied the influence of rainfall on PAE concentration by sampling the same areas before and after rainfall events. These authors found that the total concentration of PAEs and the individual concentrations of DnBP and DEHP fell by over 80% after rainfall whereas the concentrations of DMP and DEP remained stable. This can be explained by the fact that these compounds, which are typically present in personal care products (PCPs) [74], are more associated with proximity to large cities and wastewater discharges than with atmospheric deposition, sediment resuspension, plastic degradation or river influence. Since PAE concentrations seem to be sensitive to weather changes, in order to observe trends over time passive sampling should perhaps be included when monitoring these compounds.

Little information is available about the occurrence and distribution of MPEs in seawater. From the articles reviewed, we found that 11 MPEs have been determined in water masses from Canada and Belgium. MEHP and MnBP, which are the metabolites of the predominant PAEs in seawater, are the main MPEs detected in the samples. Table 3 shows that the highest MPE concentrations were reported in the North Sea along the Belgian coast. Specifically, the highest concentrations were 740 ng/L for MEHP and 292 ng/L for MnBP. This study also reported high concentrations of MMP, which may be linked to the use of PCPs [74].

More studies are needed, however, to better determine the occurrence of MPEs in seawater, ascertain whether these concentrations are correlated, and examine the sources of MPE pollution in seawaters.

3.2. Sediments

In addition to seawater samples, several PAEs and MPEs have been determined in marine sediment samples all over the world. For example, 21 PAEs have been determined in various sampling zones, with DEHP, DnBP and DiBP the main species in all studies. Other compounds, including DMP and DEP, were mainly determined in coastal areas on account of their relationship with PCPs [74].

Table 3 shows that the highest DEHP concentration (35 µg/g) was recorded in Asalouyeh, a town in the Persian Gulf where sediment samples were collected from sampling stations close to an industrial region. The detection frequency of DEHP in that study was 100% [6]. The second highest concentration (22 µg/g) was observed near Hangzhou Bay in the East China Sea. In this case, the detection frequency was also 100%, while DEHP concentrations were 59% of total PAE concentrations [5]. Lower concentrations of DEHP were also reported in Tunisia (5240 ng/g) [69] and the Gulf of Thailand (1650 ng/g) [71]. The highest concentrations for both DnBP and DiBP (15.1 µg/g and 7980 ng/g, respectively) were reported in the same sampling zones in the East China Sea [5]. The second-highest concentrations of DnBP and DiBP (12.7 µg/g and 4238 ng/g, respectively) were found in the Persian Gulf [6].

In general, most studies report that concentrations in the sediments are significantly higher than those in the surrounding seawater [5,69,70,75–77]. This can be explained by the lipophilic properties of PAEs, especially those with high molecular weights, which enable them to adsorb into carbon-rich particles and surfaces [6,69,75]. Wang et al. [70] concluded that the partitioning of PAEs between sediments and seawater depends on the properties of the compounds. More specifically, they found that while PAEs with low molecular weights tend to migrate from sediments to seawater and those with high molecular weights tend to diffuse from seawater to sediments, PAEs with medium molecular weights fall into a dynamic equilibrium distribution between both phases. This partitioning mechanism can explain why in some studies DEHP has the highest concentrations observed in sediments while the main species detected in the overlying water is DnBP [76].

The spatial distribution of PAEs has also been studied in sediments. All these studies agree that PAE concentrations are higher in coastal or inshore areas than in offshore areas owing to the large input of wastewater, sediments and contaminants from land carried mainly by rivers and water discharges [5,31,37,70,77,78]. For this reason, Arfaeinia et al. [6] evaluated how land use affects PAE concentration in marine sediment samples. These authors found that total PAE concentration was significantly higher in areas affected by industries or located near agricultural fields than in urban and natural areas. They also associated the concentrations in industrial regions with industrial wastewater discharges and those close to agricultural fields with the consumption of plastic films in agriculture (e.g. for greenhouses, plastic mulching, fertilisers and packaging) and the fact that the water used for irrigation usually comes from heavily polluted rivers located nearby.

Only Blair et al. [43] studied the occurrence of MPEs at significantly lower concentrations than those of PAEs. Unlike what is observed with seawater, MEHP was detected at very low concentrations in all sediment samples (Table 3), whereas low-molecular-weight MPEs such as MMP, MEP and MnBP were the predominant species in those samples. The fact that low-molecular-weight PAEs (such as DMP, DEP and DnBP) have higher degradation rates than high-molecular-weight PAEs (such as DEHP) may explain this change in distribution [79–81]. However, no definitive conclusions can be drawn from only one sampling zone. More studies are needed to confirm whether this trend can be extended to sediment samples from other areas.

3.3. Biota

A wide range of marine-inhabiting species, from simple living forms such as seagrass and invertebrates to extremely complex organisms like cetaceans and sharks, have been subject to the determination of PAEs and MPEs.

Table 4 shows that, like in seawater and sediment samples, the most frequently determined PAEs in biota are DEHP, DnBP and DiBP, followed closely by DEP. The highest concentrations were determined in muscle tissue samples from edible fish, crustaceans and molluscs. The highest concentration of PAEs, for example, was reported for DiBP in species of crab captured in Hangzhou Bay in China (5313 ng/g) [40], while the highest concentration of DEP was found in shrimp samples bought in Spanish markets (3393 ng/g) [52]. The highest DnBP concentration (2990 ng/g) was reported in gilt-head bream caught near the Tunisian coastline. This last study also reported that the highest concentrations of DiBP (1480 ng/g) and DEP (2700 ng/g) were in fish [69]. On the other hand, the highest

Table 4. Occurrence of PAEs and MPEs in biota samples (ng/g)

Compounds	Fish and cetacean	Crustacean	Mollusc	Invertebrates	Neuston/ plankton	Seagrass/ Posidonia	Sampling zone	Ref.
Phthalate acid esters								
DMP	–	–	19.6–27.3	–	–	–	Aegean Sea	[51]
	0.03–220	0.18–282	0.14–6.1	–	–	–	China	[8,40]
	<0.01–0.3	–	<0.01–0.3	–	–	<0.01–1.2	Mediterranean Sea	[31]
	<2.5–10	–	–	–	–	–	Spain	[55]
DEP	0.11–28	0.16–114	0.02–27	–	–	–	China	[8,40]
	–	–	2.3	0.4–2.2	1.4–18.3	–	Maldives	[48,49]
	1.6–7.0	29–230	2.4–3.1	–	–	2.7–8.9	Mediterranean Sea	[31,83]
	<50–492	<50–3393	53–401	–	–	–	Spain	[52,55]
	561–2700	–	–	–	–	32.3–80.9	Tunisia	[69]
DnPrP	<0.274–322	–	–	–	–	<0.133–38.6	Tunisia	[69]
DnBP	–	–	103–109	–	–	–	Aegean Sea	[51]
	0.22–491	0.47–437	1.51–138	–	–	–	China	[8,40]
	–	–	8.4	0.5–7.1	4.2–22	–	Maldives	[48,49]
	2.6–36.9	9–46	<0.002–17.6	–	–	12.1–166.7	Mediterranean Sea	[31,83]
	<50-373	–	<10–135	–	–	–	Spain	[52,55]
	<0.318–2990	–	–	–	–	307–491	Tunisia	[69]

Table 4. (Cont.).

Compounds	Fish and cetacean	Crustacean	Mollusc	Invertebrates	Neuston/ plankton	Seagrass/ Posidonia	Sampling zone	Ref.
DiBP	22–777	33–5313	46–78	–	–	–	China	[40]
	3.4–22.9	27–240	4.8–22.8	–	–	15.4–15.8	Mediterranean Sea	[31,83]
	434–1480	–	–	–	–	<0.094–120	Tunisia	[69]
BzBP	–	–	13.4–15.5	–	–	–	Aegean Sea	[51]
	–	–	1.9	0.5–1.6	3.2–25.1	–	Maldives	[48,49]
	<0.01–3.6	–	<0.01–17.5	–	54–475	<0.01–5.6	Mediterranean Sea	[30,31]
	<7.5–161	–	–	–	–	–	Spain	[52,55]
	<0.763–739	–	–	–	–	<0.510–171	Tunisia	[69]
DCHP	<0.19–127	<0.19–83	–	–	–	–	China	[40]
DnHP	–	–	–	–	17.47–889	–	Mediterranean Sea	[30]
DEHP	–	–	261–280	–	–	–	Aegean Sea	[51]
	3.9–1941	1.58–717	47.4–828	–	–	–	China	[8,40]
	–	–	28.4	2.1–20.2	18–228	–	Maldives	[48,49]
	11.4–12.3	15–300	53.5–638	–	34.95–2699	149.5–699.6	Mediterranean Sea	[30,31,83]
	<100–1562	<100–482	<100–656	–	–	–	Spain	[52,55]
	<12–398	–	–	–	–	–	Svalbard	[82]
	772–1460	–	–	–	–	465–845	Tunisia	[69]

Table 4. (Cont.).

Compounds	Fish and cetacean	Crustacean	Mollusc	Invertebrates	Neuston/ plankton	Seagrass/ Posidonia	Sampling zone	Ref.
DnOP	–	–	42.8–73.0	–	–	–	Aegean Sea	[51]
	1.54–28.1	0.51–0.91	1.65–4.96	–	–	–	China	[8]
	–	–	15.3–18.8	–	–	<0.02–23.6	Mediterranean Sea	[31]
DiOP	–	–	–	–	13.21–513	–	Mediterranean Sea	[30]
DnNP	<0.67–208	<0.67–109	–	–	–	–	China	[40]
DBEP	<0.3–291	<0.3–23	–	–	–	–	China	[40]
Phthalate acid ester metabolites								
MMP	–	–	4.43–21.82	–	–	–	Canada	[43]
	0.06–3.12	0.07–5.07	0.02–1.71	–	–	–	China	[8]
	5.67–5.73	–	–	–	–	–	Mediterranean Sea	[46]
	0.34–8.72	–	–	–	–	–	Norwegian Sea	[47]
MEP	–	0.29–2.61	5.63–25.54	–	–	–	Canada	[43]
	0.06–4.98	0.30–7.13	0.30–3.31	–	–	–	China	[8]
	–	–	4.5	1.1–3.2	1.9–27.1	–	Maldives	[48,49]
	32–34	–	–	–	–	–	Mediterranean Sea	[46]
	2.62–17.4	–	–	–	–	–	Norwegian Sea	[47]
	<5–178	<5-260	12-39	–	–	–	Spain	[52,55]

Table 4. (Cont.).

Compounds	Fish and cetacean	Crustacean	Mollusc	Invertebrates	Neuston/ plankton	Seagrass/ Posidonia	Sampling zone	Ref.
MnBP	6.63–60.9	8.66–38.2	75.0–585	–	–	–	Canada	[43]
	0.81–49.0	2.41–59.1	2.81–34.5	–	–	–	China	[8]
	141–207	–	–	–	8.5–590	–	Mediterranean Sea	[30,46]
	1.73–299	–	–	–	–	–	Norwegian Sea	[47]
	<5–10	<5	<5–72	–	–	–	Spain	[52,55]
MiBP	159–253	–	–	–	–	–	Mediterranean Sea	[46]
	3.78–419	–	–	–	–	–	Norwegian Sea	[47]
MnHP	0.17–4.64	–	–	–	–	–	Norwegian Sea	[47]
MiHP	–	–	0.61–1.55	–	–	–	Canada	[43]
MBzP	–	–	<0.06–1.74	–	–	–	Canada	[43]
	1.99-2.01	–	–	–	6.0–93.7	–	Mediterranean Sea	[30,46]
	0.69–7.29	–	–	–	–	–	Norwegian Sea	[47]
MnHpP	0.21–1.87	–	–	–	–	–	Norwegian Sea	[47]
MiHpP	–	0.017–0.31	1.83–2.97	–	–	–	Canada	[43]

Table 4. (Cont.).

Compounds	Fish and cetacean	Crustacean	Mollusc	Invertebrates	Neuston/ plankton	Seagrass/ Posidonia	Sampling zone	Ref.
MEHP	0.24–1.1	0.39–1.13	3.30–6.72	–	–	–	Canada	[43]
	1.34–56.3	2.9–72.9	6.72–11.6	–	–	–	China	[8]
	–	–	1.8	1.5	3.5–12.1	–	Maldives	[48,49]
	1.48–377.82	–	–	–	98.41–2709	–	Mediterranean Sea	[30,45,46,84]
	<25–162	7–40	35–117	–	–	–	Spain	[52,55]
MnOP	–	–	0.34–0.43	–	–	–	Canada	[43]
	0.05–8.56	0.04–1.74	0.20–2.15	–	–	–	China	[8]
	75–89	–	–	–	–	–	Mediterranean Sea	[46]
	0.19–5.53	–	–	–	–	–	Norwegian Sea	[47]
MnNP	24.0–98.9	–	–	–	–	–	Norwegian Sea	[47]
MnDP	0.25–17.6	–	–	–	–	–	Norwegian Sea	[47]
MEOHP	33–35	–	–	–	–	–	Mediterranean Sea	[46]
	0.20–2.12	–	–	–	–	–	Norwegian Sea	[47]
MEHHP	85–103	–	–	–	–	–	Mediterranean Sea	[46]
	0.17–5.93	–	–	–	–	–	Norwegian Sea	[47]

concentration of DEHP was found in neuston/plankton samples collected from the Mediterranean Sea near the coastline of Genoa [30].

Much more information is available about the occurrence of MPEs in marine biota than about their occurrence in seawater or sediment samples. MEHP, MnBP and MiBP, followed by MEP (Table 4), are the predominant MPEs in most species. Unlike with PAEs, the highest MPE concentrations were detected in neuston/plankton samples. Baini et al. [30] reported 2709 ng/g of MEHP and 590 ng/g of MnBP in neuston/plankton samples collected near the coastline of Genoa. Lower but significant concentrations of some MPEs were also found in samples of edible crustaceans and molluscs. Blair et al. [43] reported 585 ng/g of MnBP in blue mussels from Vancouver, while Hidalgo-Serrano et al. [52] found 260 ng/g of MEP in shrimp samples bought from Spanish markets.

Although all species have similar PAE and MPE detection frequencies, differences in PAE concentration levels have been observed. While some authors report a decrease in PAE concentrations as trophic levels increase, others report the opposite effect. Hu et al. [8], for instance, observed a slight trophic dilution in the food web between fish, molluscs and crustaceans that may be caused by a more efficient metabolic transformation at higher trophic levels. Routti et al. [82] also observed lower PAE detection rates in large mammals, which they attributed either to a lower presence of these contaminants in their food web or to a more rapid metabolisation and excretion of the pollutants. Lo Brutto et al. [83], on the other hand, reported higher concentrations of PAE in fish than in crustaceans.

These changes in the distribution of contaminants across trophic levels result from the interaction of many factors. On one hand, they may be associated with the feeding and living habits of each species. Several studies report greater contamination in molluscs than in fish or crustaceans, which suggests that the exposure of molluscs to PAEs is higher due to the pollution of marine sediments [8,43]. Higher PAE accumulation has also been observed in fish species that consume benthic organisms or in demersal fish species, which have higher bioconcentration factors [8]. Moreover, Rian et al. [47] found that PAE and MPE concentrations were lower in porpoises that inhabit less populated coastal areas than in those that inhabit more populated areas. However, these variations may also be associated with the bioavailability of PAEs and MPEs in the environment. Fourgous et al. [46] observed a temporal variation for DEHP metabolites in *Anguilla anguilla* between March, June and October which they attributed to a seasonal change in the exposure of eels to PAEs. These authors observed the highest concentrations of pollutants during the months when recreational activity in the area is most intense, when the release of

plasticisers into the environment is potentially boosted. Interestingly, regardless of these variations, no significant differences due to age, sex or body dimension have been observed in individuals of the same species [46,47].

MPs also seem to be a source of PAE contamination in marine biota. Saliu et al. [49], for example, found higher PAE concentrations in corals located in areas most affected by MP contamination. Baini et al. [30] also reported a positive correlation between PAE and MPE concentrations in neuston/plankton samples and MPs. However, more studies are needed to evaluate whether PAEs and MPEs can be used as markers of marine biota exposure to MPs.

4. Conclusions

PAEs and MPEs are present in marine matrices at detectable concentration levels in samples collected worldwide. The most common separation and detection techniques are GC for determining PAEs and LC for determining MPEs, both of which are usually coupled to MS, MS/MS or HRMS.

The optimal performance of the extraction techniques depends on the characteristics of the matrix and the properties of the analytes. SPE is the most common technique for seawater samples thanks to its simplicity, high recoveries and enrichment factors. Extraction from solid matrices is more challenging due to the high matrix effects usually present in such samples, which require clean-up steps to remove interfering matrix components. For these samples, USAE is the most common extraction technique, though QuEChERS has increasingly been used in recent years.

Numerous studies have determined the occurrence of PAEs in seawater, marine sediments and biota but less information is available about the occurrence of MPEs in those samples. Generally speaking, DEHP, DnBP and DiBP, as well as their main metabolites (MEHP, MnBP and MiBP), are the most frequently detected compounds in all samples regardless of their origin.

Polluted rivers and wastewater discharges from large cities and industrial zones appear to be the main sources of pollution in most areas, though coastal currents and atmospheric depositions are also potential sources of PAEs. Several studies have found a significant positive correlation between the abundance of MPs and plastic debris and PAE contamination in seawater, sediments and biota. This highlights the grave impact human activities and plastic pollution have on marine environments.

More information is needed on MPE concentrations in the environment since these are also contaminants with concerning effects on biota. Further investigation is also needed to evaluate whether PAEs and MPEs can be used as markers of marine plastic contamination worldwide.

References

[1] C. Giacovelli, Single-use plastic: A roadmap for sustainability, United Nation Environment Programme, 2018.

[2] J.R. Jambeck, R. Geyer, C. Wilcox, T.R. Siegler, M. Perryman, A. Andrady, R. Narayan, K.L. Law, Plastic waste inputs from land into the ocean, Science. 347 (2015) 768–771.

[3] G. Vered, A. Kaplan, D. Avisar, N. Shenkar, Using solitary ascidians to assess microplastic and phthalate plasticizers pollution among marine biota: A case study of the Eastern Mediterranean and Red Sea, Mar. Pollut. Bull. 138 (2019) 618–625.

[4] I. Katsikantami, S. Sifakis, M.N. Tzatzarakis, E. Vakonaki, O.I. Kalantzi, A.M. Tsatsakis, A.K. Rizos, A global assessment of phthalates burden and related links to health effects, Environ. Int. 97 (2016) 212–236.

[5] Z.M. Zhang, G.P. Yang, H.H. Zhang, X.Z. Shi, Y.W. Zou, J. Zhang, Phthalic acid esters in the sea-surface microlayer, seawater and sediments of the East China Sea: Spatiotemporal variation and ecological risk assessment, Environ. Pollut. 259 (2020) 113802.

[6] H. Arfaeinia, M. Fazlzadeh, F. Taghizadeh, R. Saeedi, J. Spitz, S. Dobaradaran, Phthalate acid esters (PAEs) accumulation in coastal sediments from regions with different land use configuration along the Persian Gulf, Ecotoxicol. Environ. Saf. 169 (2019) 496–506.

[7] S. Mohammadian, K. Ghanemi, Y. Nikpour, Competitive adsorption of phthalate esters on marine surface sediments: kinetic, thermodynamic, and environmental considerations, Environ. Sci. Poll. Res. 23 (2016) 24991–25002.

[8] X. Hu, Y. Gu, W. Huang, D. Yin, Phthalate monoesters as markers of phthalate contamination in wild marine organisms, Environ. Pollut. 218 (2016) 410–418.

[9] A.S. Valton, C. Serre-Dargnat, M. Blanchard, F. Alliot, M. Chevreuil, M.J. Teil, Determination of phthalates and their by-products in tissues of roach (Rutilus rutilus) from the Orge river (France), Environ. Sci. Pollut. Res. 21 (2014) 12723–12730. https://doi.org/10.1007/s11356-014-3213-0.

[10] Y. Wang, H. Zhu, K. Kannan, A Review of Biomonitoring of Phthalate Exposures, Toxics. 7 (2019) 21.

[11] R.J. Wright, R. Bosch, M.I. Gibson, J.A. Christie-Oleza, Plasticizer Degradation by Marine Bacterial Isolates: A Proteogenomic and Metabolomic Characterization, Environ. Sci. Technol. 54 (2020) 2244–2256.

[12] S.Y. Pu, N. Hamid, Y.W. Ren, D.S. Pei, Effects of phthalate acid esters on zebrafish larvae: Development and skeletal morphogenesis, Chemosphere. 246 (2020) 125808.

[13] J.W. Jung, J.S. Kang, J. Choi, J.W. Park, Chronic toxicity of endocrine disrupting chemicals used in plastic products in Korean resident species: Implications for aquatic ecological risk assessment, Ecotoxicol. Environ. Saf. 192 (2020) 110309.

[14] J. Mathieu-Denoncourt, S.J. Wallace, S.R. de Solla, V.S. Langlois, Plasticizer endocrine disruption: Highlighting developmental and reproductive effects in mammals and non-mammalian aquatic species, Gen. Com. Endocrinol. 219 (2015) 74–88.

[15] M. Cao, W. Pan, X. Shen, C. Li, J. Zhou, J. Liu, Urinary levels of phthalate metabolites in women associated with risk of premature ovarian failure and reproductive hormones, Chemosphere. 242 (2020) 125206.

[16] S. Dutta, D.K. Haggerty, D.A. Rappolee, D.M. Ruden, Phthalate Exposure and Long-Term Epigenomic Consequences: A Review, Front. Genet. 11 (2020) 1–27.

[17] Q. Zhang, X.Z. Chen, X. Huang, M. Wang, J. Wu, The association between prenatal exposure to phthalates and cognition and neurobehavior of children-evidence from birth cohorts, Neurotoxicology. 73 (2019) 199–212.

[18] W. Zhai, Z. Huang, L. Chen, C. Feng, B. Li, T. Li, Thyroid endocrine disruption in zebrafish larvae after exposure to mono-(2-ethylhexyl) phthalate (MEHP), PLoS One. 9 (2014) 1–6.

[19] X. Sun, W. Chen, S. Weng, T. Pan, X. Hu, F. Wang, T. Xia, H. Chen, T. Luo, Effects of the environmental endocrine disruptors di-2-ethylhexyl phthalate and mono-2-ethylhexyl phthalate on human sperm function in vitro, Reprod. Fertil. Dev. 32 (2020) 629–636.

[20] Y.H. Chen, Y.J. Wu, W.C. Chen, T.S. Lee, T.C. Tsou, H.C. Chang, S.W. Lo, S.L. Chen, MEHP interferes with mitochondrial functions and homeostasis in skeletal muscle cells, Biosci. Rep. 40 (2020) 1–14.

[21] C.G. Park, B. Sung, C.S. Ryu, Y.J. Kim, Mono-(2-ethylhexyl) phthalate induces oxidative stress and lipid accumulation in zebrafish liver cells, Comp. Biochem. Physiol. Part - C: Toxicol. Pharmacol. 230 (2020) 108704.

[22] Y. Jiao, Y. Tao, Y. Yang, T. Diogene, H. Yu, Z. He, W. Han, Z. Chen, P. Wu, Y. Zhang, Monobutyl phthalate (MBP) can dysregulate the antioxidant system and induce apoptosis of zebrafish liver, Environ. Pollut. 257 (2020) 113517.

[23] J. Mathieu-Denoncourt, S.J. Wallace, S.R. de Solla, V.S. Langlois, Influence of lipophilicity on the toxicity of bisphenol A and phthalates to aquatic organisms, Bull. Environ. Contam. Toxicol. 97 (2016) 4–10.

[24] T. Ye, M. Kang, Q. Huang, C. Fang, Y. Chen, H. Shen, S. Dong, Exposure to DEHP and MEHP from hatching to adulthood causes reproductive dysfunction and endocrine disruption in marine medaka (Oryzias melastigma), Aquat. Toxicol. 146 (2014) 115–126.

[25] Government of Canada, Phthalates, (2020). https://www.canada.ca/en/health-canada/services/chemicals-product-safety/phthalates.html (accessed September 23, 2021).

[26] U.S. Environmental Protection Agency, Phthalates action plan, US Environmental Protection Agency. (2012) 1–16.

[27] European Parliament, Directive 2005/84/EC of the European Parliament and the Council, Off. J. Eur. Union. 344 (2005) 40–43.

[28] EFSA CEP Panel, M.B. Baviera, C. Bolognesi, A. Chesson, V. Silano, P.S. Cocconcelli, R. Crebelli, D.M. Gott, K. Grob, E. Lampi, I. Steffensen, C. Tlustos, H. van Loveren, A. Mortensen, G. Rivi, L. Vernis, H. Zorn, J. Cravedi, C. Fortes, D. Arcella, C. Cascio,

A.F. Castoldi, K. Volk, L. Castle, Scientific opinion on the update of the risk assessment of di-butylphthalate (DBP), butyl-benzyl-phthalate (BBP), bis(2-ethylhexyl)phthalate (DEHP), di-isononylphthalate (DINP) and di-isodecylphthalate (DIDP) for use in food contact materials, EFSA J. 17 (2019) 1–85.

[29] National Industrial Chemicals Notification and Assessment Scheme, Phthalate esters: Environment tier II assessment, (2019). https://www.nicnas.gov.au/chemical-information/imap-assessments/imap-assessments/tier-ii-environment-assessments/phthalates (accessed September 23, 2020).

[30] M. Baini, T. Martellini, A. Cincinelli, T. Campani, R. Minutoli, C. Panti, M.G. Finoia, M.C. Fossi, First detection of seven phthalate esters (PAEs) as plastic tracers in superficial neustonic/planktonic samples and cetacean blubber, Anal. Methods. 9 (2017) 1512–1520.

[31] J. Castro-Jiménez, N. Ratola, An innovative approach for the simultaneous quantitative screening of organic plastic additives in complex matrices in marine coastal areas, Environ. Sci. Pollut. Res. 27 (2020) 11450–11457. https://doi.org/10.1007/s11356-020-08069-9.

[32] Y. Gu, X. Yu, J. Peng, S. Chen, Y. Zhong, D. Yin, X. Hu, Simultaneous solid phase extraction coupled with liquid chromatography tandem mass spectrometry and gas chromatography tandem mass spectrometry for the highly sensitive determination of 15 endocrine disrupting chemicals in seafood, J. Chromatogr. B. 965 (2014) 164–172.

[33] G. Jiménez-Skrzypek, J. González-Sálamo, D.A. Varela-Martínez, M.Á. González-Curbelo, J. Hernández-Borges, Analysis of phthalic acid esters in sea water and sea sand using polymer-coated magnetic nanoparticles as extraction sorbent, J. Chomatogr. A. 1611 (2020) 460620.

[34] A. Paluselli, Y. Aminot, F. Galgani, S. Net, R. Sempéré, Occurrence of phthalate acid esters (PAEs) in the northwestern Mediterranean Sea and the Rhone River, Progr. Oceanogr. 163 (2018) 221–231.

[35] Q. Si, F. Li, C. Gao, C. Wang, Z. Wang, J. Zhao, Detection of phthalate esters in seawater by stir bar sorptive extraction and gas chromatography-mass spectrometry, Mar. Pollut. Bull. 108 (2016) 163–170.

[36] Y. Xu, R. Weng, Y. Lu, X. Wang, D. Zhang, Y. Li, J. Qiu, Y. Qian, Evaluation of phthalic acid esters in fish samples using gas chromatography tandem mass spectrometry with simplified QuEChERS technique, Food Anal. Methods. 11 (2018) 3293–3303.

[37] B.T. Zhang, Y. Gao, C. Lin, W. Yang, T. Liu, X. Liu, Y. Wang, Spatial distribution of phthalate acid esters in sediments of the Laizhou Bay and its relationship with anthropogenic activities and geochemical variables, Sci. Total Environ. 722 (2020) 137912.

[38] B. Ajdari, M. Nassiri, M.M. Zahedi, M. Ziyaadini, Determination of phthalate esters in seawater of Chabahar Bay using dispersive liquid-liquid microextraction coupled with GC-FID, Water Sci. Technol. 77 (2018) 1782–1790.

[39] Z.M. Zhang, H.H. Zhang, J.L. Li, G.P. Yang, Determination of Phthalic Acid Esters in Seawater and Sediment by Solid-phase Microextraction and Gas Chromatography-Mass Spectrometry, Chinese J. Anal. Chem. 45 (2017) 348–356.

[40] H. Hu, L. Mao, S. Fang, J. Xie, M. Zhao, H. Jin, Occurrence of phthalic acid esters in marine organisms from Hangzhou Bay, China: Implications for human exposure, Sci. Total Environ. 721 (2020) 137605.

[41] H. Heo, M.-J. Choi, J. Park, T. Nam, J. Cho, Anthropogenic Occurrence of Phthalate Esters in Beach Seawater in the Southeast Coast Region, South Korea, Water. 12 (2020) 122.

[42] E. Habibi, K. Ghanemi, A. Larki, Efficient extraction of phthalate esters with different polarities from seawater samples using multi-walled carbon nanotubes/graphene oxide nanosheets, Anal. Methods. 9 (2017) 4425–4433.

[43] J.D. Blair, M.G. Ikonomou, B.C. Kelly, B. Surridge, F.A.P.C. Gobas, Ultra-trace determination of phthalate ester metabolites in seawater, sediments, and biota from an urbanized marine inlet by LC/ESI-MS/MS, Environ. Sci. Technol. 43 (2009) 6262–6268.

[44] S. Huysman, L. Van Meulebroek, O. Janssens, F. Vanryckeghem, H. Van Langenhove, K. Demeestere, L. Vanhaecke, Targeted quantification and untargeted screening of alkylphenols, bisphenol A and phthalates in aquatic matrices using ultra-high-performance liquid chromatography coupled to hybrid Q-Orbitrap mass spectrometry, Anal. Chim. Acta. 1049 (2019) 141–151.

[45] M.C. Fossi, D. Coppola, M. Baini, M. Giannetti, C. Guerranti, L. Marsili, C. Panti, E. de Sabata, S. Clò, Large filter feeding marine organisms as indicators of microplastic in the pelagic environment: The case studies of the Mediterranean basking shark (Cetorhinus maximus) and fin whale (Balaenoptera physalus), Mar. Environ. Res. 100 (2014) 17–24.

[46] C. Fourgous, M. Chevreuil, F. Alliot, E. Amilhat, E. Faliex, S. Paris-Palacios, M.J. Teil, A. Goutte, Phthalate metabolites in the European eel (Anguilla anguilla) from Mediterranean coastal lagoons, Sci. Total Environ. 569–570 (2016) 1053–1059.

[47] M.B. Rian, K. Vike-Jonas, S.V. Gonzalez, T.M. Ciesielski, V. Venkatraman, U. Lindstrøm, B.M. Jenssen, A.G. Asimakopoulos, Phthalate metabolites in harbor porpoises (Phocoena phocoena) from Norwegian coastal waters, Environ. Int. 137 (2020) 105525.

[48] F. Saliu, S. Montano, M. Lasagni, P. Galli, Biocompatible solid-phase microextraction coupled to liquid chromatography triple quadrupole mass spectrometry analysis for the determination of phthalates in marine invertebrate, J. Chomatogr. A. 1618 (2020).

[49] F. Saliu, S. Montano, B. Leoni, M. Lasagni, P. Galli, Microplastics as a threat to coral reef environments: Detection of phthalate esters in neuston and scleractinian corals from the Faafu Atoll, Maldives, Mar. Pollut. Bull. 142 (2019) 234–241.

[50] D. Savoca, M. Arculeo, S. Barreca, S. Buscemi, S. Caracappa, A. Gentile, M.F. Persichetti, A. Pace, Chasing phthalates in tissues of marine turtles from the Mediterranean Sea, Mar. Pollut. Bull. 127 (2018) 165–169.

[51] E. Tsochatzis, P. Karayannakidis, S. Kalogiannis, Determination of selected dichloroanilines and phthalates in lyophilised mussels samples with ultra-high performance liquid chromatography-tandem mass spectrometry after QuEChERS clean-up, Food Addit. Contam. Part A. 36 (2019) 1253–1260.

[52] M. Hidalgo-Serrano, F. Borrull, E. Pocurull, R.M. Marcé, Pressurised Liquid Extraction and Liquid Chromatography–High Resolution Mass Spectrometry for the Simultaneous Determination of Phthalate Diesters and Their Metabolites in Seafood Species, Food Anal. Methods. 13 (2020) 1442–1453.

[53] C. Deng, C. Li, J. Zhou, Q. Wang, H. Shao, J. Wang, Y. Wu, H. Zhang, M. Gao, X. Xu, F. Jin, Simultaneous Determination of Eight Monoalkyl Phthalate Esters in Porcine Tissue by Solid-Phase Extraction and Liquid Chromatography-Tandem Mass Spectrometry, J. Agric. Food Chem. 67 (2019) 7167–7173.

[54] Y.L. Feng, X. Liao, G. Grenier, N. Nguyen, P. Chan, Determination of 18 phthalate metabolites in human urine using a liquid chromatography-tandem mass spectrometer equipped with a core-shell column for rapid separation, Anal. Methods. 7 (2015) 8048–8059.

[55] M. Hidalgo-Serrano, F. Borrull, R.M. Marcé, E. Pocurull, Simple method for determining phthalate diesters and their metabolites in seafood species using QuEChERS extraction and liquid chromatography-high resolution mass spectrometry, Food Chem. 336 (2021) 127722.

[56] A.K. Sakhi, I.T.L. Lillegaard, S. Voorspoels, M.H. Carlsen, E.B. Løken, A.L. Brantsæter, M. Haugen, H.M. Meltzer, C. Thomsen, Concentrations of phthalates and bisphenol A in Norwegian foods and beverages and estimated dietary exposure in adults, Environ. Int. 73 (2014) 259–269.

[57] C. Luís, M. Algarra, J.S. Câmara, R. Perestrelo, Comprehensive insight from phthalates occurrence: from health outcomes to emerging analytical approaches, Toxics. 9 (2021) 157.

[58] B. Socas-Rodríguez, A. V. Herrera-Herrera, M. Asensio-Ramos, J. Hernández-Borges, Dispersive Solid-Phase Extraction, Anal. Sep. Sci. (2015) 1525–1570.

[59] H. Kataoka, Sample preparation for liquid chromatography, second ed., Elsevier, 2017.

[60] X.L. Cao, Phthalate Esters in Foods: Sources, Occurrence, and Analytical Methods, Compr. Rev. Food Sci. Food Saf. 9 (2010) 21–43.

[61] S. Armenta, S. Garrigues, F.A. Esteve-Turrillas, M. de la Guardia, Green extraction techniques in green analytical chemistry, Trends Anal. Chem. 116 (2019) 248–253.

[62] C. Almeida, J.O. Fernandes, S.C. Cunha, A novel dispersive liquid-liquid microextraction (DLLME) gas chromatography-mass spectrometry (GC-MS) method for the determination of eighteen biogenic amines in beer, Food Control. 25 (2012) 380–388.

[63] J.M. Kokosa, Dispersive liquid-liquid microextraction, Elsevier, 2019.

[64] V. Fernández-González, C. Moscoso-Pérez, S. Muniategui-Lorenzo, P. López-Mahía, D. Prada-Rodríguez, Reliable, rapid and simple method for the analysis of phthalates in sediments by ultrasonic solvent extraction followed by head space-solid phase microextraction gas chromatography mass spectrometry determination, Talanta. 162 (2017) 648–653.

[65] K. Duarte, C.I.L. Justino, A.M. Gomes, T. Rocha-Santos, A.C. Duarte, Green analytical methodologies for preparation of extracts and analysis of bioactive compounds, Elsevier, 2014.

[66] F. Chemat, N. Rombaut, A.G. Sicaire, A. Meullemiestre, A.S. Fabiano-Tixier, M. Abert-Vian, Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review, Ultrason. Sonochem. 34 (2017) 540–560.

[67] Á. Santana-Mayor, B. Socas-Rodríguez, A. V. Herrera-Herrera, M.Á. Rodríguez-Delgado, Current trends in QuEChERS method. A versatile procedure for food, environmental and biological analysis, Trends Anal. Chem. 116 (2019) 214–235.

[68] D. Pingret, A.S. Fabiano-Tixier, F. Chemat, Accelerated methods for sample preparation in food, Elsevier, 2012.

[69] A. Jebara, A. Albergamo, R. Rando, A.G. Potortì, V. lo Turco, H. ben Mansour, G. di Bella, Phthalates and non-phthalate plasticizers in Tunisian marine samples: Occurrence, spatial distribution and seasonal variation, Mar. Pollut. Bull. 163 (2021) 111967.

[70] L.Y. Wang, Y.Y. Gu, Z.M. Zhang, A.L. Sun, X.Z. Shi, J. Chen, Y. Lu, Contaminant occurrence, mobility and ecological risk assessment of phthalate esters in the sediment-water system of the Hangzhou Bay, Sci. Total Environ. 770 (2021) 144705.

[71] F. Malem, P. Soonthondecha, P. Khawmodjod, V. Chunhakorn, H.J. Whitlow, O. Chienthavorn, Occurrence of phthalate esters in the eastern coast of Thailand, Environ. Monit. Assess. 191 (2019) 4–6.

[72] Q. Zhang, J. Song, X. Li, Q. Peng, H. Yuan, N. Li, L. Duan, J. Ma, Concentrations and distribution of phthalate esters in the seamount area of the Tropical Western Pacific Ocean, Mar. Pollut. Bull. 140 (2019) 107–115.

[73] Y. Liu, Z. Li, I. Jalón-Rojas, X.H. Wang, E. Fredj, D. Zhang, L. Feng, X. Li, Assessing the potential risk and relationship between microplastics and phthalates in surface seawater of a heavily human-impacted metropolitan bay in northern China, Ecotoxicol. Environ. Saf. 204 (2020).

[74] A. Paluselli, S. Kim, Horizontal and vertical distribution of phthalates acid ester (PAEs) in seawater and sediment of East China Sea and Korean South Sea: Traces of plastic debris?, Mar. Pollut. Bull. 151 (2020) 110831.

[75] Z.M. Zhang, H.H. Zhang, J. Zhang, Q.W. Wang, G.P. Yang, Occurrence, distribution, and ecological risks of phthalate esters in the seawater and sediment of Changjiang River Estuary and its adjacent area, Sci. Total Environ. 619–620 (2018) 93–102.

[76] Z.M. Zhang, J. Zhang, H.H. Zhang, X.Z. Shi, Y.W. Zou, G.P. Yang, Pollution characteristics, spatial variation, and potential risks of phthalate esters in the water–sediment system of the Yangtze River estuary and its adjacent East China Sea, Environ. Pollut. 265 (2020) 114913.

[77] Z.M. Zhang, H.H. Zhang, Y.W. Zou, G.P. Yang, Distribution and ecotoxicological state of phthalate esters in the sea-surface microlayer, seawater and sediment of the Bohai Sea and the Yellow Sea, Environ. Pollut. 240 (2018) 235–247.

[78] X. Zhao, H. Jin, Z. Ji, D. Li, H.Y. Kaw, J. Chen, Z. Xie, T. Zhang, PAES and PAHs in the surface sediments of the East China Sea: Occurrence, distribution and influence factors, Sci. Total Environ. 703 (2020) 134763.

[79] B. V. Chang, C.S. Liao, S.Y. Yuan, Anaerobic degradation of diethyl phthalate, di-n-butyl phthalate, and di-(2-ethylhexyl) phthalate from river sediment in Taiwan, Chemosphere. 58 (2005) 1601–1607.

[80] W.J. Tang, L.S. Zhang, Y. Fang, Y. Zhou, B.C. Ye, Biodegradation of phthalate esters by newly isolated Rhizobium sp. LMB-1 and its biochemical pathway of di-n-butyl phthalate, J. Appl. Microbiol. 121 (2016) 177–186.

[81] S.Y. Yuan, C. Liu, C.S. Liao, B. V. Chang, Occurrence and microbial degradation of phthalate esters in Taiwan river sediments, Chemosphere. 49 (2002) 1295–1299.

[82] H. Routti, M. Harju, K. Lühmann, J. Aars, A. Ask, A. Goksøyr, K.M. Kovacs, C. Lydersen, Concentrations and endocrine disruptive potential of phthalates in marine mammals from the Norwegian Arctic, Environ. Int. 152 (2021) 106458.

[83] S. Lo Brutto, D. Iaciofano, V. Lo Turco, A.G. Potortì, R. Rando, V. Arizza, V. Di Stefano, First assessment of plasticizers in marine coastal litter-feeder fauna in the mediterranean sea, Toxics. 9 (2021) 1–9.

[84] M.C. Fossi, C. Panti, C. Guerranti, D. Coppola, M. Giannetti, L. Marsili, R. Minutoli, Are baleen whales exposed to the threat of microplastics? A case study of the Mediterranean fin whale (Balaenoptera physalus), Mar. Pollut. Bull. 64 (2012) 2374–2379.

Supplementary data

Table S1. Main characteristic phthalates

Name	Abbr.	Formula	CAS number	Log K_{ow}[a]	Water solubility (C_{sw}, mg/L)[a]
Phthalate acid esters					
Dimethyl phthalate	DMP	$C_{10}H_{10}O_4$	131-11-3	1.6	4000
Diethyl phthalate	DEP	$C_{12}H_{14}O_4$	84-66-2	2.4	1080
Diallyl phthalate	DAP	$C_{14}H_{14}O_4$	131-17-9	3.2	182
Di-n-propyl phthalate	DnPrP	$C_{14}H_{18}O_4$	131-16-8	3.2	180
Diisopropyl phthalate	DiPrP	$C_{14}H_{18}O_4$	605-45-8	2.8	-
Di-n-butyl phthalate	DnBP	$C_{16}H_{22}O_4$	84-74-2	4.5	11.2
Diisobutyl phthalate	DiBP	$C_{16}H_{22}O_4$	84-69-5	4.1	6.2
Di-n-pentyl phthalate	DnPP	$C_{18}H_{26}O_4$	131-18-0	5.6	0.8
Benzylbutyl phthalate	BzBP	$C_{19}H_{20}O_4$	85-68-7	4.7	2.69
Diphenyl phthalate	DPhP	$C_{20}H_{14}O_4$	84-62-8	4.5	-
Dicyclohexyl phthalate	DCHP	$C_{20}H_{26}O_4$	84-61-7	6.2	4
Di-n-hexyl phthalate	DnHP	$C_{20}H_{30}O_4$	84-75-3	6.8	0.05
Diisohexyl phthalate	DiHP	$C_{20}H_{30}O_4$	71850-09-4	6.3	-
Dibenzyl phthalate	DBzP	$C_{22}H_{18}O_4$	523-31-9	-	2.1
Bis(2-ethylhexyl) phthalate	DEHP	$C_{24}H_{38}O_4$	117-81-7	7.7	0.27
Di-n-octyl phthalate	DnOP	$C_{24}H_{38}O_4$	117-84-0	8.2	0.022
Diisooctyl phthalate	DiOP	$C_{24}H_{38}O_4$	27554-26-3	8.4	0.09
Di-n-nonyl phthalate	DnNP	$C_{26}H_{42}O_4$	84-76-4	8.6	$3x10^{-4}$
Diisononylphthtalate	DiNP	$C_{26}H_{42}O_4$	68515-48-0	8.6	-
Diisodecyl phthtalate	DiDP	$C_{28}H_{46}O_4$	26761-40-0	9.1	-
Bis(2-methoxyethyl) phthalate	DMEP	$C_{14}H_{18}O_6$	117-82-8	1.1	8500
Bis(2-ethoxyethyl) phthalate	DEEP	$C_{16}H_{22}O_6$	605-54-9	8.4	4
Bis(4-methyl-2-pentyl) phthalate	DMPP	$C_{20}H_{30}O_4$	84-63-9	5.5	-
Bis(2-n-butoxyethyl) phthalate	DBEP	$C_{20}H_{30}O_6$	117-83-9	4.1	300
Hexyl 2-ethylhexyl phthalate	HEHP	$C_{22}H_{34}O_4$	75673-16-4	-	-
Phthalate acid ester metabolites					
Monomethyl phthalate	MMP	$C_9H_8O_4$	4376-18-5	1.4	3738
Monoethyl phthalate	MEP	$C_{10}H_{10}O_4$	2306-33-4	1.9	1212
Mono-n-butyl phthalate	MnBP	$C_{12}H_{14}O_4$	131-70-4	2.8	126

Table S1. (Cont.).

Name	Abbr.	Formula	CAS number	Log K_{ow}[a]	Water solubility (C_{sw}, mg/L)[a]
Monoisobutyl phthalate	MiBP	$C_{12}H_{14}O_4$	30833-53-5	-	-
Mono-n-pentyl phthalate	MnPP	$C_{13}H_{16}O_4$	24539-56-8	-	350
Monocyclohexyl phthalate	MCHP	$C_{14}H_{16}O_4$	7517-36-4	-	300
Mono-n-hexyl phthalate	MnHP	$C_{14}H_{18}O_4$	24539-57-9	-	200
Monoisohexyl phthalate	MiHP	$C_{14}H_{18}O_4$	848131-14-6	3.9	12.8
Monobenzyl phthalate	MBzP	$C_{15}H_{12}O_4$	2528-16-7	3.1	52
Mono-n-heptyl phthalate	MnHpP	$C_{15}H_{20}O_4$	24539-58-0	-	-
Monoisoheptyl phthalate	MiHpP	$C_{15}H_{20}O_4$	92135-04-1	4.8	3.01
Mono(2-ethylhexyl) phthalate	MEHP	$C_{16}H_{22}O_4$	4376-20-9	4.7	1.49
Mono-n-octyl phthalate	MnOP	$C_{16}H_{22}O_4$	5393-19-1	5.3	0.97
Mono-n-nonyl phthalate	MnNP	$C_{17}H_{24}O_4$	24539-59-1	-	-
Monoisononyl phthalate	MiNP	$C_{17}H_{24}O_4$	68515-53-7	5.3	0.408
Mono-n-decyl phthalate	MnDP	$C_{18}H_{26}O_4$	24539-60-4	-	-
Monoisodecyl phthalate	MiDP	$C_{18}H_{26}O_4$	31047-64-0	5.8	0.129
Mono(2-ethyl-5-oxohexyl) phthalate	MEOHP	$C_{16}H_{20}O_5$	40321-98-0	-	-
Mono(2-ethyl-5-hydroxyhexyl) phthalate	MEHHP	$C_{16}H_{22}O_5$	40321-99-1	-	-

[a]Data obtained from the *Hazardous Subtances Data Bank* and from the following references:

- J.D. Blair, M.G. Ikonomou, B.C. Kelly, et al. Environ. Sci. Technol. 43 (2009) 6262–6268. https://doi.org/10.1021/es9013135.
- J. Mathieu-Denoncourt, S.J. Wallace, S.R. de Solla, et al. Bull. Environ. Contam. Toxicol. 97 (2016) 4–10. https://doi.org/10.1007/s00128-016-1812-9.
- S. Huysman, L. van Meulebroek, O. Janssens, et al. Anal. Chim. Acta 1049 (2019) 141–151. https://doi.org/10.1016/j.aca.2018.10.045.
- A. Jebara, A. Albergamo, R. Rando, et al. Mar. Pollut. Bull. 163 (2021) 111967. https://doi.org/10.1016/j.marpolbul.2021.111967.
- Q. Zhang, J. Song, X. Li, et al. Mar. Pollut. Bull. 140 (2019) 107–115. https://doi.org/10.1016/j.marpolbul.2019.01.015

1.4. Referències

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

[1] United Nations, Cumbre para la tierra. Programa 21. Programa de acción de las Naciones Unidas de Río, Programa 21. (1992). https://www.un.org/esa/dsd/agenda21_spanish/res_agenda21_19.shtml (Accés 4 de setembre de 2021).

[2] R.C. Nias, Endangered Ecosystems, Encyclopedia of Biodiversity, 2nd Ed., Academic Press, Cambridge, Massachusetts, 2013.

[3] Millennium Ecosystem Assessment, Ecosystems and human well-being: Synthesis, Island Press, Washington DC, 2005.

[4] G.G.N. Thushari, J.D.M. Senevirathna, Plastic pollution in the marine environment, Heliyon. 6 (2020) e04709.

[5] R.G. Wetzel, Freshwater Ecosystems, Encyclopedia of Biodiversity, 2nd Ed., Academic Press, Cambridge, Massachusetts, 2013.

[6] C. He, J. Li, G. Jiang, S. Chen, C. Niel, Z. Yuan, J.F. Mueller, P. Thai, Transformation of phthalates and their metabolites in wastewater under different sewer conditions, Water Res. 190 (2021) 116754.

[7] L. Xu, Q. Hu, J. Liu, S. Liu, C. Liu, Q. Deng, X. Zeng, Z. Yu, Occurrence of organophosphate esters and their diesters degradation products in industrial wastewater treatment plants in China: Implication for the usage and potential degradation during production processing, Environ. Pollut. 250 (2019) 559–566.

[8] Organisation for Economic Co-operation and Development (OECD), The 2004 OECD List of High Production Volume Chemicals, 2004. http://www.oecd.org/chemicalsafety/risk-assessment/33883530.pdf (Accés 4 de setembre de 2021).

[9] United States Environmental Protection Agency (EPA), High Production Volume List, 2014. https://comptox.epa.gov/dashboard/chemical_lists/EPAHPV (Accés 4 de setembre de 2021).

[10] V. Ji Ram, A. Sethi, M. Nath, R. Pratap, Five-Membered Heterocycles, 2019.

[11] Z.Q. Shi, Y.S. Liu, Q. Xiong, W.W. Cai, G.G. Ying, Occurrence, toxicity and transformation of six typical benzotriazoles in the environment: A review, Sci. Total Environ. 661 (2019) 407–421.

[12] A. Seeland, M. Oetken, A. Kiss, E. Fries, J. Oehlmann, Acute and chronic toxicity of benzotriazoles to aquatic organisms, Environ. Sci. Pollut. Res. 19 (2012) 1781–1790.

[13] L. Canova, M. Sturini, F. Maraschi, S. Sangiorgi, E.N. Ferri, A Comparative Test on the Sensitivity of Freshwater and Marine Microalgae to Benzo-Sulfonamides, -Thiazoles and -Triazoles, Appl. Sci. 11 (2021) 7800.

[14] I. Briguglio, S. Piras, P. Corona, E. Gavini, M. Nieddu, G. Boatto, A. Carta, Benzotriazole: An overview on its versatile biological behavior, Eur. J. Med. Chem. 97 (2015) 612–648.

[15] ECHA, 1H-benzotriazole Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.002.177 (Accés 8 de setembre de 2021).

[16] ECHA, 1-hydroxybenzotriazole Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.018.173 (Accés 8 de setembre de 2021).

[17] ECHA, 4-methyl-1H-benzotriazole Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.045.368 (Accés 8 de setembre de 2021).

[18] ECHA, 5-methyl-1H-benzotriazole Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.004.787 (Accés 8 de setembre de 2021).

[19] ECHA, 5,6-dimethyl-1H-benzotriazole Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.021.872 (Accés 8 de setembre de 2021).

[20] P. Herrero, F. Borrull, E. Pocurull, R.M. Marcé, An overview of analytical methods and occurrence of benzotriazoles, benzothiazoles and benzenesulfonamides in the environment, Trends Anal. Chem. 62 (2014) 46–55.

[21] A. Speltini, F. Maraschi, M. Sturini, M. Contini, A. Profumo, Dispersive multi-walled carbon nanotubes extraction of benzenesulfonamides, benzotriazoles, and benzothiazoles from environmental waters followed by microwave desorption and HPLC-HESI-MS/MS, Anal. Bioanal. Chem. 409 (2017) 6709–6718.

[22] C. Liao, U.J. Kim, K. Kannan, A Review of Environmental Occurrence, Fate, Exposure, and Toxicity of Benzothiazoles, Environ. Sci. Technol. 52 (2018) 5007–5026.

[23] ECHA, Benzothiazole Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.002.179 (Accés 8 de setembre de 2021).

[24] ECHA, 2-mercaptobenzothiazole Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.005.216 (Accés 8 de setembre de 2021).

[25] ECHA, 2-(methylthio)benzothiazole Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.009.471 (Accés 8 de setembre de 2021).

[26] ECHA, Benzothiazol-2-ylamine Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.004.790 (Accés 8 de setembre de 2021).

[27] ECHA, orto-toluensulphonamide Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.001.644 (Accés 8 de setembre de 2021).

[28] ECHA, para-toluenesulphonamide Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.000.674 (Accés 8 de setembre de 2021).

[29] ECHA, N-methyltoluene-4-sulphonamide Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.010.334 (Accés 8 de setembre de 2021).

[30] ECHA, N-ethyltoluene-4-sulphonamide Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.001.160 (Accés 8 de setembre de 2021).

[31] M.K. Durjava, B. Kolar, L. Arnus, E. Papa, S. Kovarich, U. Sahlin, W. Peijnenburg, Experimental assessment of the environmental fate and effects of triazoles and benzotriazole., Altern. to Lab. Anim. 41 (2013) 65–75.

[32] M. Giraudo, M. Douville, G. Cottin, M. Houde, Transcriptomic, cellular and life-history responses of Daphnia magna chronically exposed to benzotriazoles: Endocrine-disrupting potential and molting effects, PLoS One. 12 (2017) e0171763.

[33] T. He, T. Zhang, S. Liu, J. Shi, Y. sheng Huang, H. ping Zheng, W. hua Liu, Toxicological effects benzotriazole to the marine scallop Chlamys nobilis: a 2-month exposure study, Environ. Sci. Pollut. Res. 26 (2019) 10306–10318.

[34] H. Kim, B. Kim, Y. Shin, J. Kim, H. Kim, K. Kim, P. Kim, K. Park, Effect of benzotriazole on oxidative stress response and transcriptional gene expression in Oryzias latipes and Danio rerio embryo, Comp. Biochem. Physiol. Part - C: Toxicol. Pharmaco. 252 (2021) 109222.

[35] Z. Duan, Y. Xing, Z. Feng, H. Zhang, C. Li, Z. Gong, L. Wang, H. Sun, Hepatotoxicity of benzotriazole and its effect on the cadmium induced toxicity in zebrafish Danio rerio, Environ. Pollut. 224 (2017) 706–713.

[36] C. Yang, S. He, S. Lu, X. Liao, Y. Song, Z.-F. Chen, G. Zhang, R. Li, C. Dong, Z. Qi, Z. Cai, Pollution characteristics, exposure assessment and potential cardiotoxicities of PM2.5-bound benzotriazole and its derivatives in typical Chinese cities, Sci. Total Environ. (2021) 151132.

[37] F. Zeng, J.P. Sherry, N.C. Bols, Evaluating the toxic potential of benzothiazoles with the rainbow trout cell lines, RTgill-W1 and RTL-W1, Chemosphere. 155 (2016) 308–318.

[38] Y. Ye, J. Weiwei, L. Na, M. Mei, R. Kaifeng, W. Zijian, Application of the SOS/umu test and high-content in vitro micronucleus test to determine genotoxicity and cytotoxicity of nine benzothiazoles, J. Appl. Toxicol. 34 (2014) 1400–1408.

[39] M.W. Hornung, P.A. Kosian, J.T. Haselman, J.J. Korte, K. Challis, C. Macherla, E. Nevalainen, S.J. Degitz, In vitro, ex vivo, and in vivo determination of thyroid hormone modulating activity of benzothiazoles, Toxicol. Sci. 146 (2015) 254–264.

[40] T. Sorahan, Cancer risks in chemical production workers exposed to 2-mercaptobenzothiazole, Occup. Environ. Med. 66 (2009) 269–273.

[41] Y. Zhou, J. Qu, W. Liu, J. Liao, Y. Li, H. Zhao, J. Li, H. Jin, H. Liu, J. Fang, X. Sun, Y. Jiang, S. Xu, Y. Li, Y. Hong, W. Xia, Z. Cai, Early pregnancy exposure to benzotriazoles and benzothiazoles in relation to gestational diabetes mellitus: A prospective cohort study, Environ. Int. 135 (2020) 105360.

[42] X. Chen, Y. Zhou, C. Hu, W. Xia, S. Xu, Z. Cai, Y. Li, Prenatal exposure to benzotriazoles and benzothiazoles and cord blood mitochondrial DNA copy number: A prospective investigation, Environ. Int. 143 (2020) 105920.

[43] R. Meffe, C. Kohfahl, E. Hamann, J. Greskowiak, G. Massmann, U. Dünnbier, A. Pekdeger, Fate of para-toluenesulfonamide (p-TSA) in groundwater under anoxic conditions: Modelling results from a field site in Berlin (Germany), Environ. Sci. Pollut. Res. 21 (2014) 568–583.

[44] ECHA, Annex III list register. https://echa.europa.eu/es/information-on-chemicals/annex-iii-inventory/-/dislist/details/ (Accés 9 de setembre de 2021).

[45] J. Casado, R. Nescatelli, I. Rodríguez, M. Ramil, F. Marini, R. Cela, Determination of benzotriazoles in water samples by concurrent derivatization-dispersive liquid-liquid microextraction followed by gas chromatography-mass spectrometry, J. Chromatogr. A. 1336 (2014) 1–9.

[46] I. Kraševec, H. Prosen, Determination of polar benzotriazoles in aqueous environmental samples by hollow-fibre microextraction method with LC-MS/MS and its comparison to a conventional solid-phase extraction method, Microchem. J. 166 (2021) 106191.

[47] S.D. Richardson, S.Y. Kimura, Water Analysis: Emerging Contaminants and Current Issues, Anal. Chem. 88 (2016) 546–582.

[48] I. Kraševec, H. Prosen, Solid-phase extraction of polar benzotriazoles as environmental pollutants: A review, Molecules. 23 (2018) 1–14.

[49] S.M. Emadian, F.O. Sefiloglu, I. Akmehmet Balcioglu, U. Tezel, Identification of core micropollutants of Ergene River and their categorization based on spatiotemporal distribution, Sci. Total Environ. 758 (2021).

[50] L.X. Hu, Y.X. Cheng, D. Wu, L. Fan, J.H. Zhao, Q. Xiong, Q. le Chen, Y.S. Liu, G.G. Ying, Continuous input of organic ultraviolet filters and benzothiazoles threatens the surface water and sediment of two major rivers in the Pearl River Basin, Sci. Total Environ. 798 (2021) 149299.

[51] X. Han, Z. Xie, Y. Tian, W. Yan, L. Miao, L. Zhang, X. Zhu, W. Xu, Spatial and seasonal variations of organic corrosion inhibitors in the Pearl River, South China: Contributions of sewage discharge and urban rainfall runoff, Environ. Pollut. 262 (2020) 114321.

[52] J. Lu, H. Li, Z. Luo, H. Lin, Z. Yang, Occurrence, distribution, and environmental risk of four categories of personal care products in the Xiangjiang River, China, Environ. Sci. Pollut. Res. 25 (2018) 27524–27534.

[53] H. Mao, H. Li, Y. Li, L. Li, L. Yin, Z. Yang, Four typical personal care products in a municipal wastewater treatment plant in China: Occurrence, removal efficiency, mass loading and emission, Ecotoxicol. Environ. Saf. 188 (2020) 109818.

[54] J. Jia, Q. Zhu, N. Liu, C. Liao, G. Jiang, Occurrence of and human exposure to benzothiazoles and benzotriazoles in mollusks in the Bohai Sea, China, Environ. Int. 130 (2019) 104925.

[55] L. Yao, J.L. Zhao, Y.S. Liu, Q.Q. Zhang, Y.X. Jiang, S. Liu, W.R. Liu, Y.Y. Yang, G.G. Ying, Personal care products in wild fish in two main Chinese rivers: Bioaccumulation potential and human health risks, Sci. Total Environ. 621 (2018) 1093–1102.

[56] M. Williams, R.S. Kookana, A. Mehta, S.K. Yadav, B.L. Tailor, B. Maheshwari, Emerging contaminants in a river receiving untreated wastewater from an Indian urban centre, Sci. Total Environ. 647 (2019) 1256–1265.

[57] D. Salas, F. Borrull, R.M. Marcé, N. Fontanals, Study of the retention of benzotriazoles, benzothiazoles and benzenesulfonamides in mixed-mode solid-phase extraction in environmental samples, J. Chromatogr. A. 1444 (2016) 21–31.

[58] D. Álvarez-Muñoz, M. Rambla-Alegre, N. Carrasco, M. Lopez de Alda, D. Barceló, Fast analysis of relevant contaminants mixture in commercial shellfish, Talanta. 205 (2019) 119884.

[59] P. Herrero, F. Borrull, E. Pocurull, R.M. Marcé, Efficient tandem solid-phase extraction and liquid chromatography-triple quadrupole mass spectrometry method to determine polar benzotriazole, benzothiazole and benzenesulfonamide contaminants in environmental water samples, J. Chromatogr. A. 1309 (2013) 22–32.

[60] J. Casado, I. Rodríguez, M. Ramil, R. Cela, Polyethersulfone solid-phase microextraction followed by liquid chromatography quadrupole time-of-flight mass spectrometry for benzotriazoles determination in water samples, J. Chromatogr. A. 1299 (2013) 40–47.

[61] W. Xu, W. Yan, T. Licha, Simultaneous determination of trace benzotriazoles and benzothiazoles in water by large-volume injection/gas chromatography-mass spectrometry, J. Chromatogr. A. 1422 (2015) 270–276.

[62] C.H. Chen, W.H. Chung, W.H. Ding, Determination of benzotriazole and benzothiazole derivatives in marketed fish by double-vortex-ultrasonic assisted matrix solid-phase dispersion and ultrahigh-performance liquid chromatography-high resolution mass spectrometry, Food Chem. 333 (2020) 127516.

[63] R. Loos, S. Tavazzi, B. Paracchini, E. Canuti, C. Weissteiner, Analysis of polar organic contaminants in surface water of the northern Adriatic Sea by solid-phase extraction followed by ultrahigh-pressure liquid chromatography-QTRAP® MS using a hybrid triple-quadrupole linear ion trap instrument, Anal. Bioanal. Chem. 405 (2013) 5875–5885.

[64] N.Z.I.H. Harunarashid, L.H. Lim, M.H. Harunsani, Phthalate sample preparation methods and analysis in food and food packaging: a review, Food Anal. Methods. 10 (2017) 3790–3814.

[65] Y. Wang, H. Zhu, K. Kannan, A Review of Biomonitoring of Phthalate Exposures, Toxics. 7 (2019) 21.

[66] D. Salazar-Beltrán, L. Hinojosa-Reyes, E. Ruiz-Ruiz, A. Hernández-Ramírez, J.L. Guzmán-Mar, Phthalates in beverages and plastic bottles: Sample preparation and determination, Food Anal. Methods. (2017) 1–14.

[67] European Chemicals Agency (ECHA), Dimethyl phthalate Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.004.557 (Accés 12 de setembre de 2021).

[68] European Chemicals Agency (ECHA), Diethyl phthalate Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.001.409 (Accés 12 de setembre de 2021).

[69] European Chemicals Agency (ECHA), Dibutyl phthalate Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.001.416 (Accés 12 de setembre de 2021).

[70] European Chemicals Agency (ECHA), Benzyl butyl phthalate Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.001.475 (Accés 12 de setembre de 2021).

[71] European Chemicals Agency (ECHA), Bis(2-ethylhexyl) phthalate Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.003.829 (Accés 12 de setembre de 2021).

[72] I. Katsikantami, S. Sifakis, M.N. Tzatzarakis, E. Vakonaki, O.I. Kalantzi, A.M. Tsatsakis, A.K. Rizos, A global assessment of phthalates burden and related links to health effects, Environ. Int. 97 (2016) 212–236.

[73] R.J. Wright, R. Bosch, M.I. Gibson, J.A. Christie-Oleza, Plasticizer Degradation by Marine Bacterial Isolates: A Proteogenomic and Metabolomic Characterization, Environ. Sci. Technol. 54 (2020) 2244–2256. https://doi.org/10.1021/acs.est.9b05228.

[74] D.W. Gao, Z.D. Wen, Phthalate esters in the environment: A critical review of their occurrence, biodegradation, and removal during wastewater treatment processes, Sci. Total Environ. 541 (2016) 986–1001.

[75] European Chemicals Agency (ECHA), Dioctyl phthalate Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.003.832 (Accés 12 de setembre de 2021).

[76] J. Mathieu-Denoncourt, S.J. Wallace, S.R. de Solla, V.S. Langlois, Plasticizer endocrine disruption: Highlighting developmental and reproductive effects in mammals and non-mammalian aquatic species, Gen. Comp. Endocrinol. 219 (2015) 74–88.

[77] S. Net, R. Sempéré, A. Delmont, A. Paluselli, B. Ouddane, Occurrence, fate, behavior and ecotoxicological state of phthalates in different environmental matrices, Environ. Sci. Technol. 49 (2015) 4019–4035.

[78] S.Y. Pu, N. Hamid, Y.W. Ren, D.S. Pei, Effects of phthalate acid esters on zebrafish larvae: Development and skeletal morphogenesis, Chemosphere. 246 (2020) 125808.

[78] G. Hu, J. Li, Y. Shan, X. Li, Q. Zhu, H. Li, Y. Wang, X. Chen, Q. Lian, R.S. Ge, In utero combined di-(2-ethylhexyl) phthalate and diethyl phthalate exposure cumulatively impairs rat fetal Leydig cell development, Toxicology. 395 (2018) 23–33.

[80] M. Cao, W. Pan, X. Shen, C. Li, J. Zhou, J. Liu, Urinary levels of phthalate metabolites in women associated with risk of premature ovarian failure and reproductive hormones, Chemosphere. 242 (2020) 125206.

[81] M.S. Bloom, E.L. Valachovic, T.F. Begum, J.R. Kucklick, J.W. Brock, A.G. Wenzel, R.J. Wineland, L. Cruze, E.R. Unal, R.B. Newman, Association between gestational phthalate exposure and newborn head circumference; impacts by race and sex, Environ. Res. 195 (2021) 110763.

[82] A.P. Muerköster, H. Frederiksen, A. Juul, A.M. Andersson, R.C. Jensen, D. Glintborg, H.B. Kyhl, M.S. Andersen, C.A.G. Timmermann, T.K. Jensen, Maternal phthalate exposure associated with decreased testosterone/LH ratio in male offspring during mini-puberty. Odense Child Cohort, Environ. Int. 144 (2020) 106025.

[83] Y. Suzuki, J. Yoshinaga, Y. Mizumoto, S. Serizawa, H. Shiraishi, Foetal exposure to phthalate esters and anogenital distance in male newborns, Int. J. Androl. 35 (2012) 236–244.

[84] Y.E. Berman, D.A. Doherty, K.M. Main, H. Frederiksen, M. Hickey, J.A. Keelan, J.P. Newnham, R.J. Hart, Associations between prenatal exposure to phthalates and timing of menarche and growth and adiposity into adulthood: A twenty-years birth cohort study, Int. J. Environ. Res. Public Health. 18 (2021).

[85] Q. Zhang, X.Z. Chen, X. Huang, M. Wang, J. Wu, The association between prenatal exposure to phthalates and cognition and neurobehavior of children-evidence from birth cohorts, Neurotoxicology. 73 (2019) 199–212.

[86] T. Ye, M. Kang, Q. Huang, C. Fang, Y. Chen, H. Shen, S. Dong, Exposure to DEHP and MEHP from hatching to adulthood causes reproductive dysfunction and endocrine disruption in marine medaka (Oryzias melastigma), Aquat. Toxicol. 146 (2014) 115–126.

[87] European Chemicals Agency (ECHA), ANNEX XVII TO REACH. Entry number 51 - Conditions of restriction, 2020.

[88] P. Ventrice, D. Ventrice, E. Russo, G. de Sarro, Phthalates: European regulation, chemistry, pharmacokinetic and related toxicity, Environ. Toxicol. Pharmacol. 36 (2013) 88–96.

[89] European Chemicals Agency (ECHA), ANNEX XVII TO REACH. Entry number 52 - Conditions of restriction, 2018.

[90] European Food Safety Agency (EFSA), Update of the risk assessment of di-butylphthalate (DBP), butyl-benzyl-phthalate (BBP), bis(2-ethylhexyl)phthalate (DEHP), di-isononylphthalate (DINP) and di-isodecylphthalate (DIDP) for use in food contact materials, EFSA J. 17 (2019).

[91] Government of Canada, Phthalates, (2020). https://www.canada.ca/en/health-canada/services/chemicals-product-safety/phthalates.html (Accés 23 de setembre de 2021).

[92] U.S. Environmental Protection Agency, Phthalates action plan, US Environmental Protection Agency. (2012) 1–16.

[93] National Industrial Chemicals Notification and Assessment Scheme, Phthalate esters: Environment tier II assessment. https://www.industrialchemicals.gov.au/sites/default/files/Phthalateesters_ Environment tier II assessment.pdf (Accés 23 de juny de 2021).

[94] Y. Jiao, Y. Tao, Y. Yang, T. Diogene, H. Yu, Z. He, W. Han, Z. Chen, P. Wu, Y. Zhang, Monobutyl phthalate (MBP) can dysregulate the antioxidant system and induce apoptosis of zebrafish liver, Environ. Pollut. 257 (2020) 113517.

[95] C.G. Park, B. Sung, C.S. Ryu, Y.J. Kim, Mono-(2-ethylhexyl) phthalate induces oxidative stress and lipid accumulation in zebrafish liver cells, Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol. 230 (2020) 108704.

[96] W. Zhai, Z. Huang, L. Chen, C. Feng, B. Li, T. Li, Thyroid endocrine disruption in zebrafish larvae after exposure to mono-(2-ethylhexyl) phthalate (MEHP), PLoS One. 9 (2014) 1–6.

[97] E. Bonilla, J. del Mazo, Deregulation of the Sod1 and Nd1 genes in mouse fetal oocytes exposed to mono-(2-ethylhexyl) phthalate (MEHP), Reprod. Toxicol. 30 (2010) 387–392.

[98] Y.H. Chen, Y.J. Wu, W.C. Chen, T.S. Lee, T.C. Tsou, H.C. Chang, S.W. Lo, S.L. Chen, MEHP interferes with mitochondrial functions and homeostasis in skeletal muscle cells, Biosci. Rep. 40 (2020) 1–14.

[99] G. Karabulut, N. Barlas, The possible effects of mono butyl phthalate (MBP) and mono (2-ethylhexyl) phthalate (MEHP) on INS-1 pancreatic beta cells, Toxicol. Res. 10 (2021) 601–612.

[100] S.J. Kwack, E.Y. Han, J.S. Park, J.Y. Bae, I.Y. Ahn, S.K. Lim, D.H. Kim, D.E. Jang, L. Choi, H.J. Lim, T.H. Kim, N. Patra, K.L. Park, H.S. Kim, B.M. Lee, Comparison of the short term toxicity of phthalate diesters and monoesters in sprague-dawley male rats, Toxicol. Res. 26 (2010) 75–82.

[101] X. Sun, W. Chen, S. Weng, T. Pan, X. Hu, F. Wang, T. Xia, H. Chen, T. Luo, Effects of the environmental endocrine disruptors di-2-ethylhexyl phthalate and mono-2-ethylhexyl phthalate on human sperm function in vitro, Reprod. Fertil. Dev. 32 (2020) 629–636.

[102] X. Wang, W. Zhong, B. Xiao, Q. Liu, L. Yang, A. Covaci, L. Zhu, Bioavailability and biomagnification of organophosphate esters in the food web of Taihu Lake, China: Impacts of chemical properties and metabolism, Environ. Int. 125 (2019) 25–32.

[103] Y. Liu, S. Gong, L. Ye, J. Li, C. Liu, D. Chen, M. Fang, R.J. Letcher, G. Su, Organophosphate (OP) diesters and a review of sources, chemical properties, environmental occurrence, adverse effects, and future directions, Environ. Int. 155 (2021) 106691.

[104] L. Han, Y. Sapozhnikova, A. Nuñez, Analysis and Occurrence of Organophosphate Esters in Meats and Fish Consumed in the United States, J. Agric. Food Chem. 67 (2019) 12652–12662.

[105] D. Cao, J. Guo, Y. Wang, Z. Li, K. Liang, M.B. Corcoran, S. Hosseini, S.M.C. Bonina, K.J. Rockne, N.C. Sturchio, J.P. Giesy, J. Liu, A. Li, G. Jiang, Organophosphate esters in sediment of the great lakes, Environ. Sci. Technol. 51 (2017) 1441–1449.

[106] U.J. Kim, J.K. Oh, K. Kannan, Occurrence, Removal, and Environmental Emission of Organophosphate Flame Retardants/Plasticizers in a Wastewater Treatment Plant in New York State, Environ. Sci. Technol. 51 (2017) 7872–7880.

[107] Z. Zhang, H. Shao, M. Wu, J. Zhang, D. Li, J. Li, H. Wang, W. Shi, G. Xu, Occurrence, Distribution, and Potential Sources of Organophosphate Esters in Urban and Rural Surface Water in Shanghai, China, Arch. Environ. Contam. Toxicol. 77 (2019) 115–126.

[108] L. Fu, B. Du, F. Wang, J.C.W. Lam, L. Zeng, E.Y. Zeng, Organophosphate Triesters and Diester Degradation Products in Municipal Sludge from Wastewater Treatment Plants in China: Spatial Patterns and Ecological Implications, Environ. Sci. Technol. 51 (2017) 13614–13623.

[109] Y. Wang, P. Kannan, R.U. Halden, K. Kannan, A nationwide survey of 31 organophosphate esters in sewage sludge from the United States, Sci. Total Environ. 655 (2019) 446–453.

[110] F. Xu, Á. García-Bermejo, G. Malarvannan, B. Gómara, H. Neels, A. Covaci, Multi-contaminant analysis of organophosphate and halogenated flame retardants in food matrices using ultrasonication and vacuum assisted extraction, multi-stage cleanup and gas chromatography-mass spectrometry, J. Chromatogr. A. 1401 (2015) 33–41.

[111] S.H. Brandsma, J. de Boer, M.J.M. van Velzen, P.E.G. Leonards, Organophosphorus flame retardants (PFRs) and plasticizers in house and car dust and the influence of electronic equipment, Chemosphere. 116 (2014) 3–9.

[112] C. He, X. Wang, P. Thai, J.F. Mueller, C. Gallen, Y. Li, C. Baduel, Development and validation of a multi-residue method for the analysis of brominated and organophosphate flame retardants in indoor dust, Talanta. 164 (2017) 503–510.

[113] H. Tan, L. Yang, Y. Yu, Q. Guan, X. Liu, L. Li, D. Chen, Co-Existence of Organophosphate Di- and Tri-Esters in House Dust from South China and Midwestern United States: Implications for Human Exposure, Environ. Sci. Technol. 53 (2019) 4784–4793.

[114] S.H. Brandsma, P.E.G. Leonards, H.A. Leslie, J. de Boer, Tracing organophosphorus and brominated flame retardants and plasticizers in an estuarine food web, Sci. Total Environ. 505 (2015) 22–31.

[115] J. Castro-Jiménez, N. Ratola, An innovative approach for the simultaneous quantitative screening of organic plastic additives in complex matrices in marine coastal areas, Environ. Sci. Pollut. Res. 27 (2020) 11450–11457.

[116] S. Chu, R.J. Letcher, Determination of organophosphate flame retardants and plasticizers in lipid-rich matrices using dispersive solid-phase extraction as a sample cleanup step and ultra-high performance liquid chromatography with atmospheric pressure chemical ionization mass, Anal. Chim. Acta 885 (2015) 183–190.

[117] A. Strobel, W.G. Willmore, C. Sonne, R. Dietz, R.J. Letcher, Organophosphate esters in East Greenland polar bears and ringed seals: Adipose tissue concentrations and in vitro depletion and metabolite formation, Chemosphere. 196 (2018) 240–250.

[118] L. Pang, H. Yang, P. Yang, H. Zhang, J. Zhao, Trace determination of organophosphate esters in white wine, red wine, and beer samples using dispersive liquid-liquid microextraction combined with ultra-high-performance liquid chromatography–tandem mass spectrometry, Food Chem. 229 (2017) 445–461.

[119] Y. Wang, K. Kannan, Concentrations and Dietary Exposure to Organophosphate Esters in Foodstuffs from Albany, New York, United States, J. Agric. Food Chem. 66 (2018) 13525–13532.

[120] A.K. Greaves, R.J. Letcher, A Review of Organophosphate Esters in the Environment from Biological Effects to Distribution and Fate, Bull. Environ. Contam. Toxicol. 98 (2017) 2–7.

[121] C. Yao, H. Yang, Y. Li, A review on organophosphate flame retardants in the environment: Occurrence, accumulation, metabolism and toxicity, Sci. Total Environ. 795 (2021) 148837.

[122] E. Cequier, A.K. Sakhi, R.M. Marcé, G. Becher, C. Thomsen, Human exposure pathways to organophosphate triesters - A biomonitoring study of mother-child pairs, Environ. Int. 75 (2015) 159–165.

[123] R. Hou, C. Huang, K. Rao, Y. Xu, Z. Wang, Characterized in Vitro Metabolism Kinetics of Alkyl Organophosphate Esters in Fish Liver and Intestinal Microsomes, Environ. Sci. Technol. 52 (2018) 3202–3210.

[124] European Chemicals Agency (ECHA), Dibutyl hydrogen phosphate Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.003.191 (Accés 14 de setembre de 2021).

[125] European Chemicals Agency (ECHA), Bis(2-ethylhexyl) hydrogen phosphate Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.005.507 (Accés 14 de setembre de 2021).

[126] European Chemicals Agency (ECHA), Bis(2-chloroethyl) hydrogen phosphate Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.019.315 (Accés 14 de setembre de 2021).

[127] European Chemicals Agency (ECHA), Diphenyl hydrogen phosphate Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.011.507 (Accés 14 de setembre de 2021).

[128] European Chemicals Agency (ECHA), Bis(2-butoxyethyl) hydrogen phosphate Substance Information. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.034.657 (Accés 14 de setembre de 2021).

[129] Q. Zhang, C. Yu, L. Fu, S. Gu, C. Wang, New Insights in the Endocrine Disrupting Effects of Three Primary Metabolites of Organophosphate Flame Retardants, Environ. Sci. Technol. 54 (2020) 4465–4474.

[130] G. Su, D. Crump, R.J. Letcher, S.W. Kennedy, Rapid in Vitro metabolism of the flame retardant Triphenyl Phosphate and effects on cytotoxicity and mRNA expression in chicken embryonic hepatocytes, Environ. Sci. Technol. 48 (2014) 13511–13519.

[131] C.A. Mitchell, A. Reddam, S. Dasgupta, S. Zhang, H.M. Stapleton, D.C. Volz, Diphenyl Phosphate-Induced Toxicity during Embryonic Development, Environ. Sci. Technol. 53 (2019) 3908–3916.

[132] S.P. Funk, L. Duffin, Y. He, C. McMullen, C. Sun, N. Utting, J.W. Martin, G.G. Goss, D.S. Alessi, Assessment of impacts of diphenyl phosphate on groundwater and near-surface environments: Sorption and toxicity, J. Contam. Hydrol. 221 (2019) 50–57.

[133] G. Zheng, P. Miller, F.A. von Hippel, C.L. Buck, D.O. Carpenter, A. Salamova, Legacy and emerging semi-volatile organic compounds in sentinel fish from an arctic formerly used defense site in Alaska, Environ. Pollut. 259 (2020) 113872.

[134] Y. Li, C. Yao, Q. Zheng, W. Yang, X. Niu, Y. Zhang, G. Lu, Occurrence and ecological implications of organophosphate triesters and diester degradation products in wastewater, river water, and tap water, Environ. Pollut. 259 (2020) 113810.

[135] L. Xu, B. Zhang, Q. Hu, Y. Liu, T. Shang, X. Zeng, Z. Yu, Occurrence and spatio-seasonal distribution of organophosphate tri- and di-esters in surface water from Dongting Lake and their potential biological risk, Environ. Pollut. 282 (2021) 117031.

[136] R. Hou, C. Liu, X. Gao, Y. Xu, J. Zha, Z. Wang, Accumulation and distribution of organophosphate flame retardants (PFRs) and their di-alkyl phosphates (DAPs) metabolites in different freshwater fish from locations around Beijing, China, Environ. Pollut. 229 (2017) 548–556.

[137] L. Fu, L. Bin, J. Cui, D. Nyobe, P. Li, S. Huang, F. Fu, B. Tang, Tracing the occurrence of organophosphate ester along the river flow path and textile wastewater treatment processes by using dissolved organic matters as an indicator, Sci. Total Environ. 722 (2020) 137895.

[138] J. Stone, Sample preparation techniques for mass spectrometry in the clinical laboratory, Mass Spectrometry for the Clinical Laboratory, 1st Ed., Elsevier, 2017.

[139] S. Armenta, S. Garrigues, F.A. Esteve-Turrillas, M. de la Guardia, Green extraction techniques in green analytical chemistry, Trends Anal. Chem. 116 (2019) 248–253.

[140] A. Przyjazny, Extraction | Liquid-phase microextraction, Encyclopedia of Analytical Science, 3rd Ed., Elsevier, 2019.

[141] M.T. Pena, X. Vecino-Bello, M.C. Casais, M.C. Mejuto, R. Cela, Optimization of a dispersive liquid-liquid microextraction method for the analysis of benzotriazoles and benzothiazoles in water samples, Anal. Bioanal. Chem. 402 (2012) 1679–1695.

[142] B. Ajdari, M. Nassiri, M.M. Zahedi, M. Ziyaadini, Determination of phthalate esters in seawater of Chabahar Bay using dispersive liquid-liquid microextraction coupled with GC-FID, Water Sci. Technol. 77 (2018) 1782–1790.

[143] H. Farahani, P. Norouzi, R. Dinarvand, M.R. Ganjali, Development of dispersive liquid-liquid microextraction combined with gas chromatography-mass spectrometry as a simple, rapid and highly sensitive method for the determination of phthalate esters in water samples, J. Chromatogr. A. 1172 (2007) 105–112.

[144] J. LU, M.M. WANG, Q. WANG, H.P. LI, Z.G. YANG, Determination of Benzotriazole and Its Derivatives in Aqueous Sample with Air-assisted Liquid-Liquid Microextraction Followed by High-performance Liquid Chromatography, Chinese J. Anal. Chem. 46 (2018) e1817–e1825.

[145] B. Socas-Rodríguez, A. v. Herrera-Herrera, M. Asensio-Ramos, J. Hernández-Borges, Dispersive Solid-Phase Extraction, Anal. Sep. Sci. (2015) 1525–1570.

[146] B. Barnes, N. Snow, Recent advances in sample preparation for pesticide analysis, Comprehensive Sampling and Sample Preparation, Elsevier, 2012.

[147] H. Kataoka, Sample preparation for liquid chromatography, Second Ed., Elsevier, 2017.

[148] N. Fontanals, R.M. Marcé, F. Borrull, Porous polymer sorbents, Solid-Phase Extraction, 1st Ed., Elsevier, 2020.

[149] F. Augusto, L.W. Hantao, N.G.S. Mogollón, S.C.G.N. Braga, New materials and trends in sorbents for solid-phase extraction, Trends Anal. Chem. 43 (2013) 14–23.

[150] J.D. Blair, M.G. Ikonomou, B.C. Kelly, B. Surridge, F.A.P.C. Gobas, Ultra-trace determination of phthalate ester metabolites in seawater, sediments, and biota from an urbanized marine inlet by LC/ESI-MS/MS, Environ. Sci. Technol. 43 (2009) 6262–6268.

[151] S. Huysman, L. van Meulebroek, O. Janssens, F. Vanryckeghem, H. van Langenhove, K. Demeestere, L. Vanhaecke, Targeted quantification and untargeted screening of alkylphenols, bisphenol A and phthalates in aquatic matrices using ultra-high-performance liquid chromatography coupled to hybrid Q-Orbitrap mass spectrometry, Anal. Chim. Acta 1049 (2019) 141–151.

[152] A. Paluselli, Y. Aminot, F. Galgani, S. Net, R. Sempéré, Occurrence of phthalate acid esters (PAEs) in the northwestern Mediterranean Sea and the Rhone River, Prog. Oceanogr. 163 (2018) 221–231.

[153] P.W. Bartsch, T.M. Edwards, J.W. Brock, Prevalence of Eight Phthalate Monoesters in Water from the Okavango Delta, Northern Botswana, Bull. Environ. Contam. Toxicol. 103 (2019) 274–279.

[154] J. Jiang, D. Mu, M. Ding, S. Zhang, H. Zhang, J. Hu, Simultaneous determination of primary and secondary phthalate monoesters in the Taihu Lake: Exploration of sources, Chemosphere. 202 (2018) 17–24.

[155] M.D. Alotaibi, B.M. Patterson, A.J. McKinley, A.Y. Reeder, A.J. Furness, Benzotriazole and 5-methylbenzotriazole in recycled water, surface water and dishwashing detergents from Perth, Western Australia: analytical method development and application, Environ. Sci. Process. Impacts 17 (2015) 448–457.

[156] C.H. Loi, F. Busetti, K.L. Linge, C.A. Joll, Development of a solid-phase extraction liquid chromatography tandem mass spectrometry method for benzotriazoles and benzothiazoles in wastewater and recycled water, J. Chromatogr. A. 1299 (2013) 48–57.

[157] A.G. Asimakopoulos, A. Ajibola, K. Kannan, N.S. Thomaidis, Occurrence and removal efficiencies of benzotriazoles and benzothiazoles in a wastewater treatment plant in Greece, Sci. Total Environ. 452–453 (2013) 163–171.

[158] I. Carpinteiro, B. Abuin, M. Ramil, I. Rodríguez, R. Cela, Simultaneous determination of benzotriazole and benzothiazole derivatives in aqueous matrices by mixed-mode solid-phase extraction followed by liquid chromatography-tandem mass spectrometry, Anal. Bioanal. Chem. 402 (2012) 2471–2478.

[159] M.O. Barbosa, R.S. Ribeiro, A.R.L. Ribeiro, M.F.R. Pereira, A.M.T. Silva, Solid-phase extraction cartridges with multi-walled carbon nanotubes and effect of the oxygen functionalities on the recovery efficiency of organic micropollutants, Sci. Rep. 10 (2020) 1–12.

[160] G. Jiménez-Skrzypek, J. González-Sálamo, D.A. Varela-Martínez, M.Á. González-Curbelo, J. Hernández-Borges, Analysis of phthalic acid esters in sea water and sea sand using polymer-coated magnetic nanoparticles as extraction sorbent, J. Chromatogr. A. 1611 (2020) 460620.

[161] A. Naccarato, E. Gionfriddo, G. Sindona, A. Tagarelli, Simultaneous determination of benzothiazoles, benzotriazoles and benzosulfonamides by solid phase microextraction-gas chromatography-triple quadrupole mass spectrometry in environmental aqueous matrices and human urine, J. Chromatogr. A. 1338 (2014) 164–173.

[162] Z.M. Zhang, H.H. Zhang, J.L. Li, G.P. Yang, Determination of Phthalic Acid Esters in Seawater and Sediment by Solid-phase Microextraction and Gas Chromatography-Mass Spectrometry, Chinese J. Anal. Chem. 45 (2017) 348–356.

[163] H. Heo, M.-J. Choi, J. Park, T. Nam, J. Cho, Anthropogenic Occurrence of Phthalate Esters in Beach Seawater in the Southeast Coast Region, South Korea, Water. 12 (2020) 122.

[164] Q. Si, F. Li, C. Gao, C. Wang, Z. Wang, J. Zhao, Detection of phthalate esters in seawater by stir bar sorptive extraction and gas chromatography-mass spectrometry, Mar. Pollut. Bull. 108 (2016) 163–170.

[165] R. Urkude, V. Dhurvey, S. Kochhar, Pesticide Residues in Beverages, Elsevier, 2019.

[166] M.C. Fossi, D. Coppola, M. Baini, M. Giannetti, C. Guerranti, L. Marsili, C. Panti, E. de Sabata, S. Clò, Large filter feeding marine organisms as indicators of microplastic in the pelagic environment: The case studies of the Mediterranean basking shark (Cetorhinus maximus) and fin whale (Balaenoptera physalus), Mar. Environ. Res. 100 (2014) 17–24.

[167] F. Saliu, S. Montano, M. Lasagni, P. Galli, Biocompatible solid-phase microextraction coupled to liquid chromatography triple quadrupole mass spectrometry analysis for the determination of phthalates in marine invertebrate, J. Chromatogr. A. 1618 (2020).

[168] F. Saliu, S. Montano, B. Leoni, M. Lasagni, P. Galli, Microplastics as a threat to coral reef environments: Detection of phthalate esters in neuston and scleractinian corals from the Faafu Atoll, Maldives, Mar. Pollut. Bull. 142 (2019) 234–241.

[169] D. Savoca, M. Arculeo, S. Barreca, S. Buscemi, S. Caracappa, A. Gentile, M.F. Persichetti, A. Pace, Chasing phthalates in tissues of marine turtles from the Mediterranean sea, Mar. Pollut. Bull. 127 (2018) 165–169.

[170] G. Wang, H. Shi, Z. Du, H. Chen, J. Peng, S. Gao, Bioaccumulation mechanism of organophosphate esters in adult zebrafish (Danio rerio), Environ. Pollut. 229 (2017) 177–187.

[171] M. Baini, T. Martellini, A. Cincinelli, T. Campani, R. Minutoli, C. Panti, M.G. Finoia, M.C. Fossi, First detection of seven phthalate esters (PAEs) as plastic tracers in superficial neustonic/planktonic samples and cetacean blubber, Anal. Methods. 9 (2017) 1512–1520.

[172] V. Fernández-González, C. Moscoso-Pérez, S. Muniategui-Lorenzo, P. López-Mahía, D. Prada-Rodríguez, Reliable, rapid and simple method for the analysis of phthalates in sediments by ultrasonic solvent extraction followed by head space-solid phase microextraction gas chromatography mass spectrometry determination, Talanta. 162 (2017) 648–653.

[173] H. Hu, L. Mao, S. Fang, J. Xie, M. Zhao, H. Jin, Occurrence of phthalic acid esters in marine organisms from Hangzhou Bay, China: Implications for human exposure, Sci. Total Environ. 721 (2020) 137605.

[174] M. Anastassiades, S.J. Lehotay, D. Štajnbaher, F.J. Schenck, Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce, J. AOAC Int. 86 (2003) 412–431.

[175] EN 15662:2019, Foods of plant origin. Multimethod for the determination of pesticide residues using GC- and LC-based analysis following acetonitrile extraction/partitioning and clean-up by dispersive SPE. Modular QuEChERS-method, (2019).

[176] S.J. Lehotay, Official Method 2007.01: Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate, J. AOAC Int. 90 (2007) 485–520.

[177] Y. Xu, R. Weng, Y. Lu, X. Wang, D. Zhang, Y. Li, J. Qiu, Y. Qian, Evaluation of phthalic acid esters in fish samples using gas chromatography tandem mass spectrometry with simplified QuEChERS technique, Food Anal. Methods. 11 (2018) 3293–3303.

[178] E. Tsochatzis, P. Karayannakidis, S. Kalogiannis, Determination of selected dichloroanilines and phthalates in lyophilised mussels samples with ultra-high performance liquid chromatography-tandem mass spectrometry after QuEChERS clean-up, Food Addit. Contam. Part A. 36 (2019) 1253–1260.

[179] Y. Gu, X. Yu, J. Peng, S. Chen, Y. Zhong, D. Yin, X. Hu, Simultaneous solid phase extraction coupled with liquid chromatography tandem mass spectrometry and gas chromatography tandem mass spectrometry for the highly sensitive determination of 15 endocrine disrupting chemicals in seafood, J. Chromatogr. B. 965 (2014) 164–172.

[180] P. Herrero, F. Borrull, E. Pocurull, R.M. Marcé, A quick, easy, cheap, effective, rugged and safe extraction method followed by liquid chromatography-(Orbitrap) high resolution mass spectrometry to determine benzotriazole, benzothiazole and benzenesulfonamide derivates in sewage sludge, J. Chromatogr. A. 1339 (2014) 34–41.

[181] R. Perestrelo, P. Silva, P. Porto-Figueira, J.A.M. Pereira, C. Silva, S. Medina, J.S. Câmara, QuEChERS - Fundamentals, relevant improvements, applications and future trends, Anal. Chim. Acta 1070 (2019) 1–28.

[182] P. Herrero, F. Borrull, R.M. Marcé, E. Pocurull, A pressurised hot water extraction and liquid chromatography-high resolution mass spectrometry method to determine polar benzotriazole, benzothiazole and benzenesulfonamide derivates in sewage sludge, J. Chromatogr. A. 1355 (2014) 53–60.

[183] A. Maceira, R.M. Marcé, F. Borrull, Occurrence of benzothiazole, benzotriazole and benzenesulfonamide derivates in outdoor air particulate matter samples and human exposure assessment, Chemosphere. 193 (2018) 557–566.

[184] A. Speltini, M. Sturini, F. Maraschi, A. Porta, A. Profumo, Fast low-pressurized microwave-assisted extraction of benzotriazole, benzothiazole and benezenesulfonamide compounds from soil samples, Talanta. 147 (2016) 322–327.

[185] B.T. Zhang, Y. Gao, C. Lin, W. Yang, T. Liu, X. Liu, Y. Wang, Spatial distribution of phthalate acid esters in sediments of the Laizhou Bay and its relationship with anthropogenic activities and geochemical variables, Sci. Total Environ. 722 (2020) 137912.

[186] S.M. Ahmad, B.B.C. Calado, M.N. Oliveira, N.R. Neng, J.M.F. Nogueira, Bar adsorptive microextraction coated with carbonbased phase mixtures for performance-enhancement to monitor selected benzotriazoles, benzothiazoles, and benzenesulfonamides in environmental water matrices, Molecules. 25 (2020) 1–13.

[187] W. Li, J. Li, M. Deng, Y. Pan, L. Zeng, Benzotriazoles and benzothiazoles prevail in indoor dust from an E-waste dismantling area in South China: Elevated concentrations and implication for human exposure, Sci. Total Environ. 723 (2020) 137979.

[188] A. Nuñez, L. Vallecillos, R.M. Marcé, F. Borrull, Occurrence and risk assessment of benzothiazole, benzotriazole and benzenesulfonamide derivatives in airborne particulate matter from an industrial area in Spain, Sci. Total Environ. 708 (2020).

[189] E. Cequier, R.M. Marcé, G. Becher, C. Thomsen, A high-throughput method for determination of metabolites of organophosphate flame retardants in urine by ultra performance liquid chromatography-high resolution mass spectrometry, Anal. Chim. Acta 845 (2014) 98–104.

[190] Y. Wang, W. Li, M.P. Martínez-Moral, H. Sun, K. Kannan, Metabolites of organophosphate esters in urine from the United States: Concentrations, temporal variability, and exposure assessment, Environ. Int. 122 (2019) 213–221.

[191] A. Alves, A. Covaci, S. Voorspoels, Method development for assessing the human exposure to organophosphate flame retardants in hair and nails, Chemosphere. 168 (2017) 692–698.

[192] Y. Choi, J. Jeon, Y. Choi, S.D. Kim, Characterizing biotransformation products and pathways of the flame retardant triphenyl phosphate in Daphnia magna using non-target screening, Sci. Total Environ. 708 (2020) 135106.

CAPÍTOL 2. OBJECTIUS

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

L'objectiu principal d'aquesta tesi doctoral és determinar la presència de diverses famílies de contaminants orgànics d'alt volum de producció en ambients aquàtics. Les famílies de compostos estudiades han estat triades tenint en compte el seu ús a la indústria i en productes quotidians i inclouen els derivats de la benzotriazola, la benzotiazola i la benzosulfonamida, els dièsters de l'àcid ftàlic i els seus metabòlits, i els dièsters de l'àcid fosfòric que són metabòlits dels organofosfats.

Per assolir aquest objectiu es desenvoluparan mètodes analítics basats en la cromatografia líquida acoblada a l'espectrometria de masses d'alta resolució i s'utilitzaran tècniques d'extracció com l'extracció en fase sòlida, l'extracció amb líquids pressuritzats i l'extracció amb QuEChERS. Degut a la complexitat de les mostres, també s'avaluaran les millors estratègies per la neteja dels extractes obtinguts. Un cop desenvolupats, els mètodes s'aplicaran a mostres d'aigües superficials i a les espècies de peix i marisc de major consum de la zona de Tarragona per determinar la presència dels contaminants d'interès en ambients aquàtics.

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

CAPÍTOL 3. PART EXPERIMENTAL, RESULTATS I DISCUSSIÓ

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

Com s'ha comentat a la introducció d'aquesta tesi doctoral, molts compostos AVP no s'eliminen totalment a les plantes de tractament d'aigües on, a més, poden tenir lloc processos de degradació i transformació. Així doncs, els compostos poden arribar fàcilment a rius i mars degut als abocaments d'aigües residuals efluents. A més, degut a les seves propietats lipofíliques, diversos compostos AVP es poden bioacumular en organismes aquàtics.

La definició de compostos AVP engloba un gran nombre de substàncies químiques. Algunes d'elles s'estudien des de fa anys i tant els seus efectes tòxics com la seva presència al medi ambient estan ben documentats. Altres substàncies, però, s'han estudiat en menor mesura i la informació respecte a la seva toxicitat, capacitat bioacumulativa i presència en matrius mediambientals és molt més limitada.

La recerca d'aquesta tesi doctoral se centra en la determinació de diverses famílies de compostos orgànics AVP en matrius mediambientals com aigües superficials i organismes marins. Els grups de compostos estudiats són els derivats de la benzotriazola, la benzotiazola i la benzosulfonamida, els dièsters i monoèsters de l'àcid ftàlic i els dièsters de l'àcid fosfòric. Aquesta tesi doctoral s'ha dut a terme al grup de Cromatografia i Aplicacions Mediambientals de la Universitat Rovira i Virgili, que té una àmplia experiència en la determinació de compostos orgànics en matrius mediambientals.

En aquest capítol es descriuen la part experimental i els resultats obtinguts dels diferents estudis de recerca que s'han dut a terme al llarg d'aquesta tesi doctoral. Aquests resultats es presenten en forma d'articles científics que ja han estat publicats, o estan en procés de ser publicats, en revistes científiques de reconeixement internacional. El capítol està dividit en tres seccions i cadascuna d'elles inclou una breu introducció en la qual es descriu el context de la recerca, i una discussió dels resultats més rellevants de cada estudi. La llista dels articles resultants de la recerca duta a terme en el marc de la tesi doctoral està inclosa a l'Annex II.

Al primer apartat es presenta el desenvolupament d'un mètode analític basat en la SPE seguida per LC-(ESI)HRMS per la determinació de derivats de la benzotriazola, la benzotiazola i la benzosulfonamida en mostres d'aigua de riu i de mar.

Al segon apartat es presenten tres mètodes analítics per la determinació d'altres famílies de compostos en espècies de peix i marisc de consum habitual. Els dos primers mètodes utilitzen dues tècniques d'extracció diferents (PLE i QuEChERS) per la determinació simultània de dièsters i monoèsters de l'àcid ftàlic. El tercer mètode utilitza

l'extracció per QuEChERS per la determinació de dièsters de l'àcid fosfòric. Tots tres mètodes fan servir LC-(ESI)HRMS per la determinació dels compostos.

A l'últim apartat es presenten els resultats preliminars obtinguts del monitoratge de dièsters de l'àcid fosfòric en les espècies de peix i marisc més consumides per la població de Catalunya dut a terme al llarg d'un any utilitzant el mètode analític descrit al segon apartat d'aquesta tesi. Tot i això, cal tractar els resultats per poder escriure l'article i enviar-lo a una revista científiques de reconeixement internacional.

Aquesta tesi doctoral s'emmarca dins del projecte CTQ2017-84373-R titulat "*Exposición humana a contaminantes emergentes: vías de exposición, presencia y caracterización del riesgo*" finançat pel *Ministerio de Ciencia e Innovación* i el fons FEDER i del qual en són investigadors principals el Dr. Francesc Borrull i la Dra. Eva Pocurull.

3.1. Determinació de compostos orgànics d'alt volum de producció en aigües superficials

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

Aquest apartat se centra en la determinació de benzotriazoles, benzotiazoles i benzosulfonamides en mostres d'aigua de riu i de mar utilitzant SPE/LC-(ESI)HRMS.

Com ja hem comentat a la introducció, les benzotriazoles, les benzotiazoles i les benzosulfonamides són substàncies químiques amb moltes aplicacions industrials i que s'usen abundantment en productes quotidians com detergents, teixits o paper, entre d'altres. A més, moltes d'elles tenen propietats tòxiques que poden ser perilloses per al medi ambient i per als éssers vius. Com que s'alliberen amb facilitat al medi ambient, la presència dels compostos estudiats en aquest apartat s'ha determinat principalment en matrius aquoses com aigua de riu i llac [1,2], aigües residuals [1,3], però també en matrius sòlides com llots de depuradora [3], sediments [2] o biota [4]. Tot i això, la presència de molts d'aquests compostos en aigua de mar no ha estat gaire estudiada, ja que les concentracions trobades en aquest tipus de mostres solen ser més baixes i, generalment, els estudis que es poden trobar a la bibliografia són multiresidu i només inclouen algunes benzotriazoles en les anàlisis (com la BTR, la 4TTR o la 5TTR) [5]. Així doncs, l'objectiu principal d'aquest estudi és el de desenvolupar un mètode analític per la determinació dels compostos d'interès, no només en aigües de riu, sinó també en aigua de mar.

Per fer-ho, es va partir d'un mètode de SPE amb Oasis HLB prèviament desenvolupat al grup per l'extracció de benzotriazoles, benzotiazoles i bezosulfonamide de mostres d'aigua de riu [6] i es va adaptar per poder analitzar també mostres d'aigua d'aigua de mar. Primerament es va adaptar el mètode de detecció, prèviament desenvolupat per un analitzador QqQ (MS/MS), per utilitzar un analitzador Exactive Orbitrap (HRMS). Es van avaluar paràmetres crítics de la SPE com el pH i el volum de càrrega de la mostra, la quantitat de sorbent o el volum d'eluent. A més, el mètode cromatogràfic també es va modificar per incloure dos benzotriazoles (1OHBTR i 4OHBTR) que havien estat prèviament determinades per altres autors en mostres d'aigua de riu i aigües residuals [7,8]. Degut als efectes matriu observats en les mostres també es va avaluar una etapa de neteja afegint un cartutx de Florisil en línia amb el cartutx d'Oasis HLB.

Després de l'optimització, el mètode es va aplicar per la determinació dels anàlits d'interès en mostres del riu Ebre recollides a l'entrada de la planta potabilitzadora del Consorci d'Aigües de Tarragona, i en mostres d'aigua de mar recollides a dues platjes i dins del port de Tarragona.

Els resultats d'aquest estudi s'han publicat a la revista Separation *Science plus 2 (2019) 72–80*.

References

[1] J. Lu, M.M. Wang, Q. Wang, H.P. Li, Z.G. Yang, Determination of Benzotriazole and Its Derivatives in Aqueous Sample with Air-assisted Liquid-Liquid Microextraction Followed by High-performance Liquid Chromatography, Chinese J. Anal. Chem. 46 (2018) e1817–e1825.

[2] L.X. Hu, Y.X. Cheng, D. Wu, L. Fan, J.H. Zhao, Q. Xiong, Q. Le Chen, Y.S. Liu, G.G. Ying, Continuous input of organic ultraviolet filters and benzothiazoles threatens the surface water and sediment of two major rivers in the Pearl River Basin, Sci. Total Environ. 798 (2021) 149299.

[3] A.G. Asimakopoulos, A. Ajibola, K. Kannan, N.S. Thomaidis, Occurrence and removal efficiencies of benzotriazoles and benzothiazoles in a wastewater treatment plant in Greece, Sci. Total Environ. 452–453 (2013) 163–171.

[4] D. Álvarez-Muñoz, M. Rambla-Alegre, N. Carrasco, M. Lopez de Alda, D. Barceló, Fast analysis of relevant contaminants mixture in commercial shellfish, Talanta. 205 (2019) 119884.

[5] R. Loos, S. Tavazzi, B. Paracchini, E. Canuti, C. Weissteiner, Analysis of polar organic contaminants in surface water of the northern Adriatic Sea by solid-phase extraction followed by ultrahigh-pressure liquid chromatography-QTRAP® MS using a hybrid triple-quadrupole linear ion trap instrument, Anal. Bioanal. Chem. 405 (2013) 5875–5885.

[6] P. Herrero, F. Borrull, E. Pocurull, R.M. Marcé, Efficient tandem solid-phase extraction and liquid chromatography-triple quadrupole mass spectrometry method to determine polar benzotriazole, benzothiazole and benzenesulfonamide contaminants in environmental water samples, J. Chromatogr. A. 1309 (2013) 22–32.

[7] W. Xu, W. Yan, T. Licha, Simultaneous determination of trace benzotriazoles and benzothiazoles in water by large-volume injection/gas chromatography-mass spectrometry, J. Chromatogr. A. 1422 (2015) 270–276.

[8] M.T. Pena, X. Vecino-Bello, M.C. Casais, M.C. Mejuto, R. Cela, Optimization of a dispersive liquid-liquid microextraction method for the analysis of benzotriazoles and benzothiazoles in water samples, Anal. Bioanal. Chem. 402 (2012) 1679–1695.

3.1.1. Presence of benzotriazoles, benzothiazoles and benzenesulfonamides in surface water samples by liquid chromatography coupled to high-resolution mass spectrometry

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

PRESENCE OF BENZOTRIAZOLES, BENZOTHIAZOLES AND BENZENESULFONAMIDES IN SURFACE WATER SAMPLES BY LIQUID CHROMATOGRAPHY COUPLED TO HIGH-RESOLUTION MASS SPECTROMETRY

Míriam Hidalgo-Serrano, Francesc Borrull, Rosa M. Marcé, Eva Pocurull

Universitat Rovira i Virgili, Department of Analytical Chemistry and Organic Chemistry, Marcel·lí Domingo s/n, Tarragona 43007, Spain

---

Abstract

An analytical method has been developed for the simultaneous determination of seven benzotriazole, five benzothiazole and four benzenesulfonamide derivatives in surface water samples. The procedure is based on solid-phase extraction with Oasis HLB, followed by high-performance liquid chromatography coupled to high-resolution mass spectrometry. Apparent recoveries ranged from 40 to 101%, except for compounds the matrix effect of which was above -60%. The repeatability (*n = 5*) of the method was below 20% for all compounds except 4-hydroxybenzotriazole in river water. The method limits of detection for most compounds ranged from 1 to 12 ng/L for both river water and seawater. The method was then used for the determination of the studied compounds in several river water and seawater samples from harbour and coastal areas. 1-*H*-benzotriazole (up to 78 ng/L in river water and above the upper limit of the calibration curve in seawater) and 4-methyl-1-*H*-benzotriazole (up to 44 ng/L in river water and 338 ng/L in seawater) were the most abundant compounds quantified in the samples, even though 5-methyl-1-*H*-benzotriazole and 2-hydroxybenzothiazole were also found in most samples. Some compounds were frequently found in certain types of samples, such as 2-aminobenzothiazole in river water. Benzenesulfonamide derivatives could not be quantified in any sample.

Keywords: *Benzenesulfonamides, Benzothiazoles, Benzotriazoles, High-resolution mass spectrometry, Surface water*

---

1. Introduction

Benzotriazoles (BTRs), benzothiazoles (BTs) and benzenesulfonamides (BSAs) are high-production chemicals which can be commonly found in industrial processes. BTRs, for example, are mainly used as corrosion inhibitors, antifreeze fluids, aircraft de-icers and metal-cutting fluids but are also used in photography and the production of plastics [1,2]. BTs are used as biocides or as corrosion inhibitors in the production of rubber, while BSAs are used in the synthesis of dyes, photochemicals and disinfectants and in the treatment of animal diseases [3].

As their polarities make them highly soluble in water, all the above compounds are ubiquitous in the environment and may be resistant to biodegradation. More importantly, because some of them may have toxic effects on a wide range of organisms, they are becoming pollutants of increasing concern and are considered emerging organic contaminants. Studies suggest that some BTRs may be carcinogenic to humans and toxic to fish [4,5], aquatic plants and invertebrates [6], as well as being potential endocrine disruptors [7]. BTs are also associated with mutagenicity in microorganisms and carcinogenicity in humans and have been found to be dermal sensitisers [8]. Less information is available about the toxicity of BSAs. Studies exist only for *para*-toluenesulfonamide (*p*-TSA), which has shown moderate toxicity [9].

Bearing all this information in mind, it is not surprising that numerous methods have been developed for the determination of these compounds in environmental water samples. In regard to sample treatment, solid-phase extraction (SPE) using polymeric balanced polar/non-polar sorbents is the most common technique for extracting BTRs and BTs from tap water [10,11], river water [10–12], influent and effluent wastewater [10,12–14] and seawater [15,16]. More significantly, Herrero *et al.* [17] optimised a SPE method for the extraction of five BTRs, four BTs and five BSAs from river, influent and effluent wastewaters, obtaining recovery values between 75 and 106% with a matrix effect of less than 20%. However, results have also been satisfactory when mixed-mode anion [18] and cation [19] exchange sorbents have been used to extract BTRs, BTs and BSAs from river, influent and effluent wastewaters. Dispersive liquid-liquid microextraction [20,21], solid-phase microextraction [22], stir bar sorptive extraction [23] and dispersive solid-phase extraction [24] have also been used to extract these compounds from aqueous matrices such as tap water, river water, treated wastewater and raw wastewater.

Typically, BTRs, BTs and BSAs in water samples are determined by liquid chromatography mainly coupled to tandem mass spectrometry [11–18,24] even though high resolution mass spectrometry (HRMS) is also used in some studies [19,23]. In the past few years, the use of HRMS based on time-of flight or Orbitrap analysers for trace analysis has become more widely accepted and affordable. The heightened resolution allows the simultaneous confirmation of coeluting analytes from their exact mass and can help to avoid false positive results [25,26]. Moreover, analyses performed with an Orbitrap analyser have been known to allow for similar detection and quantification limits compared to tandem mass spectrometry instruments in SRM mode [27,28].

Some studies also use gas chromatography to separate the compounds [10,20,22], though this technique is less common than liquid chromatography.

The water matrices that have been most studied in recent years are influent and effluent wastewaters and river water. However, coastal areas are also affected by human activities such as urban and industrial wastewater discharges, agricultural activities and infrastructural development [15,29]. It would not be implausible, therefore, to find BTR, BT and BSA derivatives in seawater samples. However, these compounds have not been extensively determined in matrices in which they may be found at lower concentrations [16], since such studies tend to have a longer list of chemicals, such as pesticides, bisphenols, caffeine, organophosphorus flame retardants, musk fragrances and perfluoroalkyl substances. In fact, recent studies have only targeted a few BTR derivatives (i.e., 1-*H*-benzotriazole, 5-methyl-1-*H*-benzotriazole and 4-methyl-1-*H*-benzotriazole) [15,16].

The aim of this study is to develop an analytical method based on SPE followed by liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) for the simultaneous determination of seven BTR, six BT and five BSA derivatives in environmental water samples. The developed method will then be applied to the analysis of river water samples from the Ebre River in Catalonia as well as seawater samples from the coast and harbour of the city of Tarragona.

2. Materials and methods

2.1. Reagents and standards

The solid standards of 1-*H*-benzotriazole (BTR) and six of its derivatives: 1-hydroxybenzotriazole (1OHBTR), 4-hydroxybenzotriazole (4OHBTR), 4-methyl-1-*H*-

benzotriazole (4TTR), 5-methyl-1-*H*-benzotriazole (5TTR), 5,6-dimethyl-1-*H*-benzotriazole (XTR) and 5-chloro-1-*H*-benzotriazole (ClBTR); benzothiazole (BT) and five benzothiazole derivatives: 2-aminobenzothiazole (NH_2BT), 2-hydroxybenzothiazole (OHBT), 2-(methylthio)-benzothiazole (MeSBT), 2-mercaptobenzothiazole (MCBT) and 2-methylbenzothiazole (MeBT); and benzenesulfonamide (BSA) and four benzenesulfonamide derivates: *ortho*-toluenesulfonamide (*o*-TSA), *para*-toluenesulfonamide (*p*-TSA), N-methyl-*para*-toluenesulfonamide (Me-*p*-TSA), N-ethyl-*para*-toluenesulfonamide (Et-*p*-TSA), were purchased from Sigma-Aldrich (St. Louis, USA). Stock solutions of individual standards at 1000 mg/L were prepared in methanol and stored at -20 ^{o}C. A mixed working solution of 10 mg/L was prepared each week in water and stored in the dark at -20 ^{o}C.

Ultrapure water was obtained using an ultrapure water purification system from Veolia Water (Sant Cugat del Vallés, Spain). Acetonitrile (ACN) and methanol (MeOH) of HPLC grade and acetic acid (CH_3COOH) were provided by J.T. Baker (Deventer, The Netherlands). Hydrochloric acid (HCl) was purchased from Scharlab (Barcelona, Spain), and sodium hydroxide (NaOH) and N,N-dimethylformamide (DMF) were purchased from Sigma-Aldrich.

2.2. Sampling

River water samples were collected from the Ebre River in Catalonia at the intake of a drinking water treatment plant. Seawater samples were collected from six locations in the harbour and two locations along the coastline by the city of Tarragona, Spain. Water samples were collected in pre-cleaned bottles and stored at −20 ^{o}C. Prior to analysis, the samples were filtered through a 0.45 µm nylon filter (Whatman, Maidstone, UK).

2.3. Solid-phase extraction procedure

Sample volumes of 1 L for both river and sea water were loaded onto Oasis HLB (500 mg, 6 cc) cartridges from Waters (Milford, Massachusetts, USA) for SPE. The cartridges were preconditioned with 10 mL of MeOH followed by 10 mL of ultrapure water. The samples were adjusted to a pH of three with HCl and loaded onto the Oasis HLB cartridges. Each cartridge was then connected to the top of a 500 mg Florisil cartridge (Sigma-Aldrich) previously conditioned with 10 mL of MeOH. The analytes were then eluted from the first cartridge with 3 × 5 mL of MeOH and passed through the second cartridge. 500 µL of DMF were added to the extracts before being concentrated up to ∼200 µL with a miVac Duo sample concentrator (Genevac, Ipswich, UK). The final extract was taken up to 1 mL with

ultrapure water, filtered with a 0.22 µm PTFE syringe filter (Scharlab), and injected into the LC-(ESI)HRMS instrument.

2.4. Liquid chromatography coupled to high-resolution mass spectrometry

Chromatographic analysis was performed with an Accela 1250 UHPLC system (Thermo Scientific, Bremen, Germany) equipped with a quaternary pump and an Accela Autosampler. The LC system was coupled to an Exactive Orbitrap mass spectrometer (Thermo Scientific) equipped with a heated electrospray ionization source (HESI) and a HCD collision cell. An Ascentis Express C_{18} (100 mm x 2.1 mm i.d., 2.7 µm particle size) fused-core column (Sigma-Aldrich) was used for the chromatographic separation. The mobile phase was a mixture of 0.1% CH_3COOH in H2O (solvent A) and MeOH/ACN 98:2, v/v (solvent B). The gradient profile started with 0% of B and remained constant for 5.25 min before it was increased to 10% of B in 3 min and held again for 3.75 min. The gradient was then increased to 35% of B in 4 min and to 100% of B in 5 min. Finally, it was held at 100% of B for 3min before being returned to the initial conditions in 1 min. The flow rate was 800 µL/min and the injection volume was 20 µL. The column oven and sample tray temperatures were 50 and 10◦C, respectively.

Mass spectra were acquired in both positive and negative mode for the standards studied in order to optimize the HESI source parameters and obtain the highest sensitivity possible for all compounds. The optimal parameters were as follows: sheath gas flow rate, 55 A.U.; auxiliary gas flow rate, 20 A.U.; sweep gas flow rate, 0 A.U.; spray voltage, 4 kV in positive mode and −4 kV in negative mode; capillary voltage, 37.5 V in positive mode and –35.0 V in negative mode; tube lens voltage, 85 V in positive mode and –85 V in negative mode; skimmer voltage, 20 V in positive mode and –25 V in negative mode; capillary temperature, 350 ^{o}C; and heater temperature, 400 ^{o}C.

Four time windows alternating two scan events in each window were used for data acquisition, two of which were in negative mode (0.0–4.2 and 7.6–12.3 min) and two were in positive mode (4.2–7.6 and 12.3–27.0 min). In all windows, the first scan event was a full scan at 50 000 FWHM with 250 ms of injection time. The second scan event was a fragmentation scan at 10 000 FWHM with 50 ms of injection time using a collision voltage in the HCD cell of 25 eV. The quantification ions were measured with a mass extraction window of 5 ppm. The selected fragment ions were taken into account (**Table S1**) for confirmation purposes.

3. Results and discussion

3.1. Liquid chromatography coupled to high-resolution mass spectrometry

The chromatographic and spectrometric conditions were based on those of previous studies [17,19] in which most of the studied analytes were determined in wastewater samples.

The ionisation of the compounds was studied by directly injecting 1 mg/L individual standard solutions with a 50:50 mobile phase composition. For most compounds the highest response was obtained in positive ionisation mode as $[M+H]^+$, whereas the response for 1OHBTR, 4OHBTR, BSA, *o*-TSA and *p*-TSA was higher in negative ionisation mode as $[M-H]^-$. **Table S1** shows the structure of the analytes and the exact masses of the quantification ions and fragment ions selected for confirmation.

The chromatographic separation of some compounds was quite complex because of their similar structures and high polarity. For example, the separation of 4TTR and 5TTR was not complete even though in the previously mentioned studies it was improved by adding 2% ACN to the aqueous mobile phase [17,19]. The addition of this percentage of organic solvent at the start of the gradient caused the coelution of 4OHBTR and 1OHBTR almost on the solvent front. ACN was therefore switched to the methanolic phase and the gradient was set to begin with 100% aqueous phase in order to decrease the elution times and allow a better separation of the first eluting compounds. As the *o*-TSA/*p*-TSA separation was not possible, they were determined as a mixture named TSA.

In accordance with the referenced studies, the column temperature was initially set at 50◦C. However, different column temperatures (30 and 40°C) were also tested in an attempt to increase the elution times of the first eluting compounds and reduce the matrix effect by avoiding potential coelutions with interferences. Although no significant changes were observed when the temperature was changed from 50 to 40°C, retention times increased enough when the column temperature was set at 30 °C. The gradient elution was therefore modified in order to achieve the best separation for a column temperature of 30 °C. However, there were several coeluting peaks at that column temperature. Hence, as the best separation was still obtained with a column temperature of 50 °C, the other options were discarded.

Calibration curves were determined for each of the studied compounds taking into account the signal of the quantification ion. The instrumental limits of quantification (ILOQs) corresponded to the first point of the calibration curve. The ILOQ values ranged from 2.5 to 5 µg/L for BTR derivatives and from 2.5 to 25 µg/L for BT and BSA derivatives.

Linearity was satisfactory between the ILOQs and 5 mg/L. The instrumental limits of detection (ILODs) were the concentrations at which the signal-to-noise ratio was three for the response of the quantification ion under collision conditions. For ions for which the baseline was flat, the ILODs were the concentrations at which the response of the quantification ion was above 103. ILOD values ranged from 0.5 to 5 µg/L for the BTR derivatives, and from 2.5 to 25 µg/L for the BT and BSA derivatives. The values obtained can be found in **Table 1**. For some compounds, the ILOD and ILOQ values were the same because the criteria established for the value of both limits were satisfactorily met at the same concentration.

3.2. Solid-phase extraction procedure

BTR, BT and BSA derivatives have relatively high polarity. Based on previous studies [10,13,16,17,30], the most appropriate sorbents for their extraction from aqueous matrices are therefore polymeric phases with an hydrophilic-lipophilic balance. Although other studies used mixed-mode strong cation and anion-exchange sorbents for the extraction of these compounds, BSA derivatives were rinsed in the washing steps [19] and so these sorbents were disregarded in the present study.

In the first instance, Oasis HLB (250 mg) cartridges were conditioned and tested with standard solutions (250 mL of ultrapure water spiked at 2 µg/L) at four pHs (3, 5, 7 and 10) adjusted with HCl or NaOH to obtain the highest possible retention. The compounds were then eluted twice with 5 mL of MeOH and the extracts were taken to a volume of 25 mL with ultrapure water in order to minimise peak distortion in the chromatographic analysis. Since 1OHBTR was only retained in the sorbent when loaded at pH 3, the samples were loaded at that pH.

Since the first three eluting compounds presented peak deformation, the collected extracts had to be evaporated and redissolved with ultrapure water before injection. Total and partial evaporation were tested under N_2 flow and with a miVac sample concentrator. However, all procedures had low recoveries and unsatisfactory reproducibility values. For example, the evaporation recoveries for BT, MCBT, MeBT and MeSBT were all below 27%. To avoid their evaporation and obtain good reproducibility and recoveries, 500 µL of DMF were added to the extract [31] before the evaporation was performed up to ~200 µL and the extract taken to a final volume of 1 mL with ultrapure water. In these conditions, evaporation recoveries ranged from 87 to 101% for all compounds regardless of the evaporation method, except for MCBT, which was lost due

Table 1. Retention time (t_R), instrumental limit of detection (ILOD), instrumental limit of quantification (ILOQ), method limit of detection (MLOD), method limit of quantification (MLOQ), matrix effect (ME), apparent recovery (R_{app}) and repeatability (RSD, *n=5*).

Compound	t_R (min)	ILOD (µg/L)	ILOQ (µg/L)	River water					Seawater				
				MLOD (ng/L)	MLOQ (ng/L)	ME [a] (%)	R_{app} [a] (%)	RSD [a] (%)	MLOD (ng/L)	MLOQ (ng/L)	ME [a] (%)	R_{app} [b] (%)	RSD [b] (%)
4OHBTR	2.8	0.5	5	1	10	–67	47	26	1	6	–22	84	5
BSA	3.1	25	25	63	63	–41	40	6	30	30	–22	85	3
1OHBTR	3.1	0.5	2.5	5	20	–90	12	5	2	6	–60	42	7
NH_2BT	5.9	10	10	12	12	–25	83	3	12	12	–22	84	4
BTR	6.9	5	5	6	6	–11	97	6	5	5	–12	100	2
TSA	8.9	25	25	86	86	–78	29	5	120	120	–64	21	18
5TTR	13.1	2.5	2.5	3	3	–22	95	4	3	3	–18	86	8
4TTR	13.4	2.5	5	3	6	–27	95	3	3	6	–19	84	3
ClBTR	14.0	1	2.5	2	4	–34	70	3	1.5	4	–27	72	6
OHBT	14.5	5	10	10	20	–49	53	2	7.5	15	–40	67	4
BT	15.0	25	25	42	42	–34	60	11	48	48	–28	53	6
Me-*P*-TSA	15.6	2.5	5	4	8	–42	63	2	4	9	–37	59	2
XTR	16.2	0.5	5	1	6	–27	88	2	1	6	–24	82	2
Et-*P*-TSA	16.8	2.5	2.5	4	4	–41	75	2	4	4	–39	64	2
MeBT	17.5	2.5	2.5	9	9	–62	30	16	10	10	–29	27	10
MeSBT	19.0	5	10	8	16	–39	65	9	12	25	–33	40	10

[a] Samples spiked at 1,000 ng/L.

[b] Samples spiked at 250 ng/L, except for TSA which is spiked at 500 ng/L and 1OHBTR which is spiked at 1,000 ng/L.

to its volatility and thus discarded. Evaporation with the miVac sample concentrator was selected due to the fact that this method is less time consuming.

After the evaporation procedure was established, different sample volumes were tested with 500 mg Oasis HLB cartridges. When the loading volumes for the larger cartridge were increased to 500 and 1000 mL, the elution recoveries of some compounds were below 70%. The elution step was therefore modified so that the compounds were eluted three times with 5 mL of MeOH showing satisfactory recoveries overall (57-97%).

The procedure was then tested in river and seawater samples to evaluate apparent recoveries (R_{app}) and the matrix effect (ME). The apparent recoveries were calculated by comparing the concentration obtained when samples were spiked before the SPE with an external standard calibration. To that end, 1000 mL of river water spiked at 1 µg/L were analysed following the procedure described above. A non-spiked sample was also analysed in order to subtract the signals of the compounds already present in the sample. Due to the fact that the R_{app} values were lower than those obtained in ultrapure water due to the matrix effect, a clean-up step to retain interfering substances was tested during the elution step by connecting a lab-packed Florisil SPE cartridge (500 mg) to the bottom of the Oasis HLB cartridge [17]. Firstly, to evaluate the suitability of the clean-up strategy the ME was calculated for an equal volume of both river and seawater (500 mL) with and without this step. All ME values were calculated by comparing the response of the analytes obtained with a standard solution ($A_{standard}$) with the concentration obtained by spiking the sample extracts after the SPE procedures (A_{sample}) and subtracting the area of a non-spiked sample (A_{blank}) (($A_{sample} – A_{blank} – A_{standard}$) x $100/A_{standard}$) [17]. The results are shown in **Figure 1**. Looking at the plotted values, minor differences can be observed for most compounds (mainly seawater samples) with and without the clean-up step. However, as ME clearly decreased in river water samples for TSA (from -73 to -46%), the clean-up step was incorporated into the method. In addition, two different volumes (500 and 1000 mL) of river and seawater were tested (**Figure 2**). Although the ME values obtained for 1000 mL of sample were higher than those obtained for the 500 mL of sample, the differences were not as significant as expected. In other words, the ME did not decrease enough to compensate for the lower preconcentration obtained after SPE, hence 1000 mL was the preferred sample volume for the developed procedure.

3.3. Method validation

The method was validated for use in environmental waters by calculating R_{app}, repeatability and reproducibility (both of them expressed as relative standard deviation

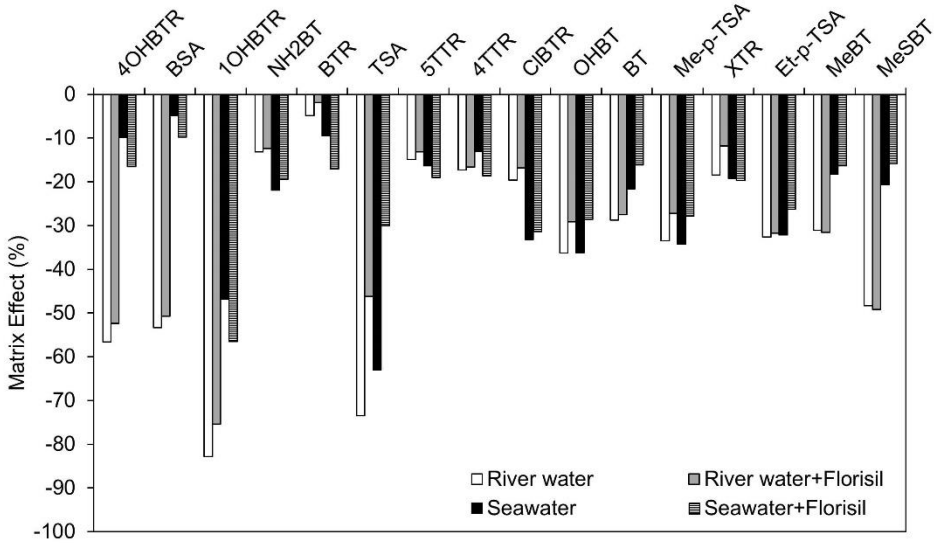


Figure 1. Matrix effect (%) obtained by preconcentrating 500 mL of river or seawater samples with or without a clean-up step with Florisil.

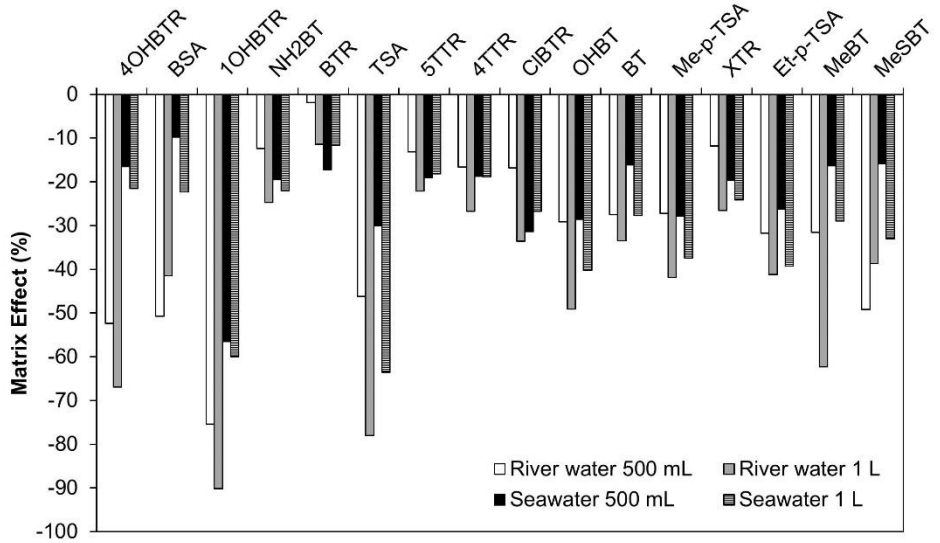


Figure 2. Matrix effect (%) obtained for each compound in river and seawater depending on the sample volume.

(RSD, *n = 5*)), method limits of detection (MLODs) and method limits of quantification (MLOQs) for both river water samples and seawater samples (**Table 1**).

As good repeatability and high recoveries were obtained for most compounds, quantification using external calibration was proposed.

To evaluate the R_{app} (which includes recovery yield and ME) and the repeatability of the method, five river water samples were spiked at 1 µg/L and five seawater samples were spiked at 250 ng/L except for 1OHBTR (which was spiked at 500 ng/L) and TSA (which was spiked at 1 µg/L). Non-spiked samples were also analysed in order to subtract the response of compounds already present in the matrices. R_{app} were found at a wide range of values for both matrices. In river water, most R_{app} ranged from 40 to 101% except for compounds with the highest ME. The apparent recoveries for 1OHBTR, TSA and MeBT, for example, were below 30%, probably because their ME were -90, -78 and -62%, respectively. Similar observations can be made for the low recoveries corresponding to certain compounds in seawater. Nevertheless, taking into account that ME values were sufficiently low and R_{app} values were high enough for the majority of the studied compounds in both samples, the use of external calibration curves was selected.

Repeatability for river water ranged from 2 to 16% for all compounds except 4OHBTR (26%), while for seawater it was below 10% for all compounds except TSA (18%). Moreover, reproducibility was below 20% for all compounds in both matrices except for 4OHBTR in river water (28%). In both cases these results were considered satisfactory.

The MLODs and MLOQs for each sample matrix were estimated from their instrumental parameters (i.e., R_{app}, ILODs and ILOQs) due to the fact that some of these compounds are ubiquitous water contaminants. For river water samples MLODs were found to be 1 – 86 ng/L. MLOQs ranged from 3 to 86 ng/L depending on the compound. For seawater samples, on the other hand, MLODs ranged from 1 to 120 ng/L and MLOQs ranged from 3 to 120 ng/L. For river water samples, despite their relatively high variability, MLOQ values were of a similar order of magnitude to those in the literature, which, depending on the compound, range from 5 to 155 ng/L [19]. The obtained limits can be considered low values for an Orbitrap analyser, although MLOQ values were found at 0.2 ng/L for BTR and at 0.1 ng/L for 5TTR for seawater matrices when these samples were analysed by SPE followed by UHPLC QTrap MS [15].

3.4. Application to environmental samples

Four samples from the Ebre river collected over the course of several months at the intake of a drinking water treatment plant, six samples from several locations selected to be equally distributed inside the harbour of the city of Tarragona, and four samples from two different beaches by that harbour were analysed in order to assess the applicability of the method. **Table 2** shows the concentrations found for all samples. The mass error for all compounds detected was below 5 ppm.

Overall, BTR, 4TTR, 5TTR and OHBT were found in most samples and the presence of some of the compounds was recurrent in certain groups of samples, such as NH_2BT in river water. BSA derivatives could not be quantified in any sample. **Figure 3** shows HRMS-extracted ion chromatograms of a harbour water sample and a river water sample, as well as the mass error.

To be more precise, in river water BTR, BT, OHBT, 4TTR, 5TTR and NH_2BT were found at the highest concentrations (between MLOD and 78 ng/L). However, other compounds which have been occasionally reported in other studies such as ClBTR, XTR or MeSBT could not be detected in the samples. Most of the obtained values seem to be in agreement with those in the literature regardless of the analytical method used. Casado *et al.* [20] for instance, reported BTR concentrations between 25 and 144 ng/L and 4TTR and 5TTR concentrations up to 102 ng/L in river water in Spain, while Salas *et al.* [19] found concentrations of BT, NH_2BT and OHBT up to 221, 43 and 101 ng/L, respectively. More importantly, Salas *et al.* considered their results for BT to be high in comparison with those of other authors, who reported values of up to 60 ng/L.

Regarding coastal samples, only BTR and OHBT could be quantified up to 27 and 32 ng/L, respectively, and 4TTR was found below MLOQ. In contrast, several compounds could be quantified in all four locations of the harbour, such as BTR (between 25 and 148 ng/L), 4TTR (between 7 and 28 ng/L), 5TTR (between 4 and 14 ng/L) and OHBT (between 20 and 33 ng/L). 4OHBTR, BT and MeBT were also detected and quantified in some of the samples. Little information is available about the concentration of the compounds of interest in seawater. Recent studies found BTR in the Mediterranean Sea at concentrations between 11 and 29 ng/L [16] and in the Adriatic Sea at concentrations between 3 and 9 ng/L [15]. These values can be considered comparable to the results obtained in this study.

Table 2. Concentrations (ng/L) of BTR, BT and BSA derivatives found in river and seawater samples.

Compound	Ebre River				Coast		Harbour				
	1	2	3	4	Location A	Location B	Location C	Location D	Location E		Location F	
									1	2	1	2
BTR	78	51	34	53	25	27	25	148	n.d.	26	30	>5000
4OHBTR	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	19
4TTR	44	26	17	25	<MLOQ	<MLOQ	8	18	7	10	28	338
5TTR	42	19	15	19	n.d.	n.d.	6	14	4	7	9	330
BT	55	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	51	87	n.d.
NH_2BT	<MLOQ	31	<MLOQ	<MLOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
OHNT	56	<MLOQ	22	22	32	27	33	25	25	20	28	n.d.
MeBT	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	19	n.d.	n.d.

<MLOQ: Below method limit of quantification.
n.d.: not detected (<MLOD).

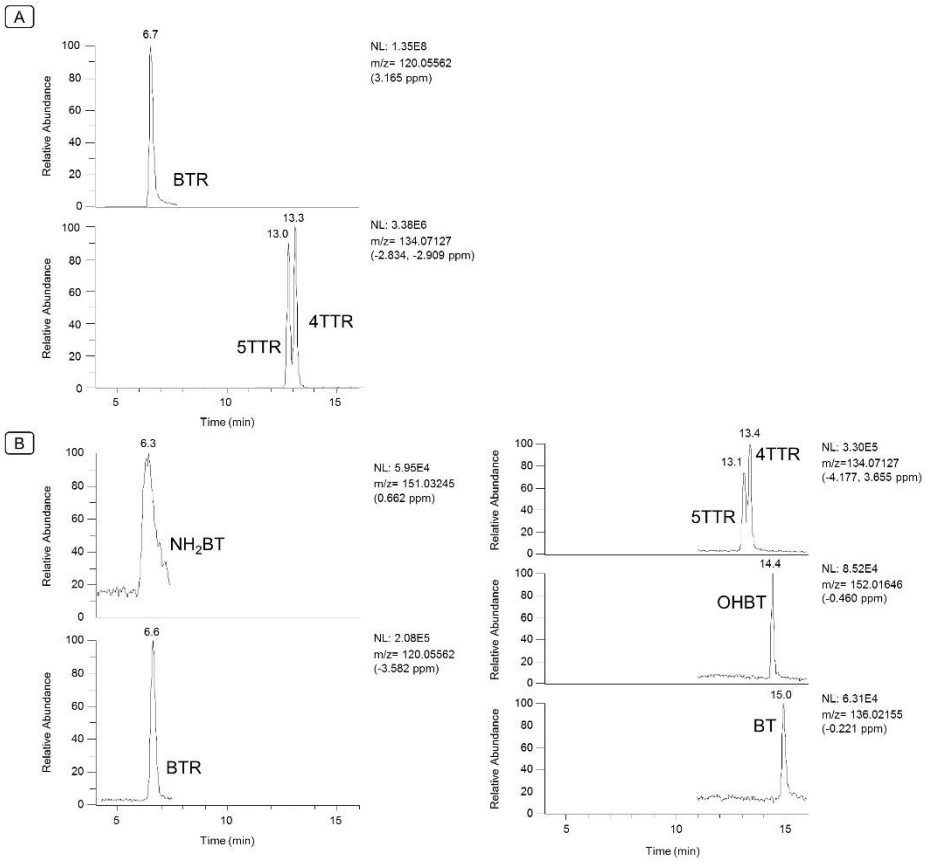


Figure 3. HRMS EIC and mass error in ppm of a harbour water sample (A) and a river water sample (B).

Additionally, it should be noted that the second sample collected at Location F showed much higher concentrations of the compounds than the river or seawater samples. More specifically, BTR, 5TTR and 4TTR were found above the upper limit of the calibration curve, at 300 and 338 ng/L, respectively. To understand this anomaly the source of the samples has to be taken into account. While Locations C and D are situated in the harbour near the open sea, Locations E and F are further inside the harbour. Moreover, samples at Location E were collected at a docking site for vessels with chemical shipments. Samples at Location F were collected next to what could be a wastewater treatment plant discharge site, so the results obtained for sample 2 at this location may be due to a sudden discharge. This interpretation would be in agreement with the values obtained for these compounds in analyses of effluent wastewater samples. Herrero *et al.* [17], for example, found concentrations of BTR up to 1440 ng/L, of 4TTR up to 641 ng/L and of 5TTR up to

1037 ng/L in secondary effluent wastewater collected from the same wastewater treatment plant. Other studies quantified at least 544 ng/L of BTR, 994 ng/L of 4TTR and 971 ng/L of 5TTR in effluent wastewater samples [19].

Considering that several compounds were successfully detected and quantified at low concentrations in the analysed samples, the applicability of the developed method can be confirmed, thus proving that LC-HRMS with an Orbitrap analyser can be a good alternative to tandem mass spectrometry for quantification.

Concluding remarks

An analytical method has been developed based on solid-phase extraction followed by high performance liquid chromatography coupled to high-resolution mass spectrometry for the simultaneous determination of seven benzotriazole, five benzothiazole and four benzenesulfonamide derivatives.

Most recoveries obtained by solid-phase extraction with Oasis HLB and a Florisil cartridge for clean-up ranged from 40 to 101%, depending on the compound and the matrix analysed due to the matrix effects. The repeatability (RSD, *n = 5*) of the method was below 20% for all compounds in all matrices except for 4OHBTR in river water.

When the method was applied for the determination of the compounds of interest in river and seawater samples, most of them were found below the limits of detection and quantification. However, some of them could be quantified at concentrations between 15 (5TTR) and 78 ng/L (BTR) in river water, and between 4 (5TTR) and 338 ng/L (4TTR) in seawater.

Acknowledgements

The authors would like to thank the Departament d'Empresa i Coneixement of the Generalitat de Catalunya for grant 2017FI_B_01116. This work (CTQ2017-84373-R) has been financed by the Spanish Ministry of Economy, Industry and Competitiveness and the European Regional Development Fund (AEI/FEDER/UE).

References

[1] Richardson, S. D., Kimura, S. Y., Water Analysis: Emerging Contaminants and Current Issues. Anal. Chem. 88 (2016) 546–582.

[2] Bruzzoniti, M. C., Sarzanini, C., Rivoira, L., Tumiatti, V., Maina, R., Simultaneous determination of five common additives in insulating mineral oils by high-performance liquid chromatography with ultraviolet and coulometric detection. J. Sep. Sci. 39 (2016) 2955–2962.

[3] Herrero, P., Borrull, F., Pocurull, E., Marcé, R. M., An overview of analytical methods and occurrence of benzotriazoles, benzothiazoles and benzenesulfonamides in the environment. Trends Anal. Chem. 62 (2014) 46–55.

[4] Durjava, M. K., Kolar, B., Arnus, L., Papa, E., Kovarich, S., Sahlin, U., Peijnenburg, W., Experimental assessment of the environmental fate and effects of triazoles and benzotriazole. Altern. Lab. Anim. 41 (2013) 65–75.

[5] Duan, Z., Xing, Y., Feng, Z., Zhang, H., Li, C., Gong, Z., Wang, L., Sun, H., Hepatotoxicity of benzotriazole and its effect on the cadmium induced toxicity in zebrafish Danio rerio. Environ. Pollut. 224 (2017) 706–713.

[6] Seeland, A., Oetken, M., Kiss, A., Fries, E., Oehlmann, J., Acute and chronic toxicity of benzotriazoles to aquatic organisms. Environ. Sci. Pollut. Res. 19 (2012) 1781–1790.

[7] Giraudo, M., Douville, M., Cottin, G., Houde, M., Transcriptomic, cellular and life-history responses of Daphnia magna chronically exposed to benzotriazoles: Endocrine-disrupting potential and molting effects. PLoS One. 12 (2017) e0171763.

[8] Herrero, P., Borrull, F., Marcé, R. M., Pocurull, E., A pressurised hot water extraction and liquid chromatography-high resolution mass spectrometry method to determine polar benzotriazole, benzothiazole and benzenesulfonamide derivates in sewage sludge. J. Chromatogr. A. 1355 (2014) 53–60.

[9] Meffe, R., Kohfahl, C., Hamann, E., Greskowiak, J., Massmann, G., Dünnbier, U., Pekdeger, A., Fate of para-toluenesulfonamide (p-TSA) in groundwater under anoxic conditions: Modelling results from a field site in Berlin (Germany). Environ. Sci. Pollut. Res. 21 (2014) 568–583.

[10] Xu, W., Yan, W., Licha, T., Simultaneous determination of trace benzotriazoles and benzothiazoles in water by large-volume injection/gas chromatography-mass spectrometry. J. Chromatogr. A. 1422 (2015) 270–276.

[11] Wang, L., Zhang, J., Sun, H., Zhou, Q., Widespread Occurrence of Benzotriazoles and Benzothiazoles in Tap Water: Influencing Factors and Contribution to Human Exposure. Environ. Sci. Technol. 50 (2016) 2709–2717.

[12] Wick, A., Fink, G., Ternes, T. A., Comparison of electrospray ionization and atmospheric pressure chemical ionization for multi-residue analysis of biocides, UV-filters and benzothiazoles in aqueous matrices and activated sludge by liquid chromatography-tandem mass spectrometry. J. Chromatogr. A. 1217 (2010) 2088–2103.

[13] Loi, C. H., Busetti, F., Linge, K. L., Joll, C. A., Development of a solid-phase extraction liquid chromatography tandem mass spectrometry method for benzotriazoles and benzothiazoles in wastewater and recycled water. J. Chromatogr. A. 1299 (2013) 48–57.

[14] Asimakopoulos, A. G., Ajibola, A., Kannan, K., Thomaidis, N. S., Occurrence and removal efficiencies of benzotriazoles and benzothiazoles in a wastewater treatment plant in Greece. Sci. Total Environ. 2013, 452–453, 163–171.

[15] Loos, R., Tavazzi, S., Paracchini, B., Canuti, E., Weissteiner, C., Analysis of polar organic contaminants in surface water of the northern Adriatic Sea by solid-phase extraction followed by ultrahigh-pressure liquid chromatography-QTRAP® MS using a hybrid triple-quadrupole linear ion trap instrument. Anal. Bioanal. Chem. 405 (2013) 5875–5885.

[16] Nödler, K., Voutsa, D., Licha, T., Polar organic micropollutants in the coastal environment of different marine systems. Mar. Pollut. Bull. 85 (2014) 50–59.

[17] Herrero, P., Borrull, F., Pocurull, E., Marcé, R. M., Efficient tandem solid-phase extraction and liquid chromatography-triple quadrupole mass spectrometry method to determine polar benzotriazole, benzothiazole and benzenesulfonamide contaminants in environmental water samples. J. Chromatogr. A. 1309 (2013) 22–32.

[18] Carpinteiro, I., Abuin, B., Ramil, M., Rodríguez, I., Cela, R., Simultaneous determination of benzotriazole and benzothiazole derivatives in aqueous matrices by mixed-mode solid-phase extraction followed by liquid chromatography-tandem mass spectrometry. Anal. Bioanal. Chem. 402 (2012) 2471–2478.

[19] Salas, D., Borrull, F., Marcé, R. M., Fontanals, N., Study of the retention of benzotriazoles, benzothiazoles and benzenesulfonamides in mixed-mode solid-phase extraction in environmental samples. J. Chromatogr. A. 1444 (2016) 21–31.

[20] Casado, J., Nescatelli, R., Rodríguez, I., Ramil, M., Marini, F., Cela, R., Determination of benzotriazoles in water samples by concurrent derivatization-dispersive liquid-liquid microextraction followed by gas chromatography-mass spectrometry. J. Chromatogr. A. 1336 (2014) 1–9.

[21] Pena, M. T., Vecino-Bello, X., Casais, M. C., Mejuto, M. C., Cela, R., Optimization of a dispersive liquid-liquid microextraction method for the analysis of benzotriazoles and benzothiazoles in water samples. Anal. Bioanal. Chem. 402 (2012) 1679–1695.

[22] Naccarato, A., Gionfriddo, E., Sindona, G., Tagarelli, A., Simultaneous determination of benzothiazoles, benzotriazoles and benzosulfonamides by solid phase microextraction-gas chromatography-triple quadrupole mass spectrometry in environmental aqueous matrices and human urine. J. Chromatogr. A. 1338 (2014) 164–173.

[23] Casado, J., Rodríguez, I., Ramil, M., Cela, R., Polyethersulfone solid-phase microextraction followed by liquid chromatography quadrupole time-of-flight mass spectrometry for benzotriazoles determination in water samples. J. Chromatogr. A. 1299 (2013) 40–47.

[24] Speltini, A., Maraschi, F., Sturini, M., Contini, M., Profumo, A., Dispersive multi-walled carbon nanotubes extraction of benzenesulfonamides, benzotriazoles, and benzothiazoles from environmental waters followed by microwave desorption and HPLC-HESI-MS/MS. Anal. Bioanal. Chem. 409 (2017) 6709–6718.

[25] Seitz, W., Schulz, W., Winzenbacher, R., Advantage of liquid chromatography with high-resolution mass spectrometry for the detection of the small and polar molecules trifluoroacetic acid and sulfamic acid. J. Sep. Sci. 41 (2018) 4437–4448.

[26] Zheng, K., Gong, J., Li, X., Jia, G., Wu, S., Zhang, H., Hu, D., Zhang, K., Stereoselective determination of dufulin in watermelon under field conditions using chiral ultra high performance liquid chromatography with high-resolution mass spectrometry. J. Sep. Sci. 40 (2017) 4142–4151.

[27] Prosen, H., Fontanals, N., Borrull, F., Marcé, R. M., Determination of seven drugs of abuse and their metabolites in surface and wastewater using solid-phase extraction coupled to liquid chromatography with high-resolution mass spectrometry. J. Sep. Sci. 40 (2017) 3621–3631.

[28] Herrero, P., Cortés-Francisco, N., Borrull, F., Caixach, J., Pocurull, E., Marcé, R. M., Comparison of triple quadrupole mass spectrometry and Orbitrap high-resolution mass spectrometry in ultrahigh performance liquid chromatography for the determination of veterinary drugs in sewage: Benefits and drawbacks. J. Mass Spectrom. 49 (2014) 585–596.

[29] Martí, N., Aguado, D., Segovia-Martínez, L., Bouzas, A., Seco, A., Occurrence of priority pollutants in WWTP effluents and Mediterranean coastal waters of Spain. Mar. Pollut. Bull. 62 (2011) 615–625.

[30] Huntscha, S., Hofstetter, T. B., Schymanski, E. L., Spahr, S., Hollender, J., Biotransformation of Benzotriazoles: Insights from Transformation Product Identification and Compound-Specific Isotope Analysis. Environ. Sci. Technol. 48 (2014) 4435–4443.

[31] Ramírez, N., Cuadras, A., Rovira, E., Marcé, R. M., Borrull, F., Risk assessment related to atmospheric polycyclic aromatic hydrocarbons in gas and particle phases near industrial sites. Environ. Health Perspect. 119 (2011) 1110–1116.

Supplementary data

Table S1. Chemical structure and exact masses of the analytes studied and their fragment ions.

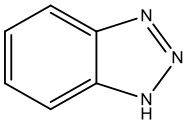
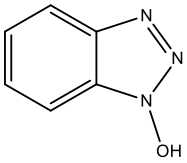
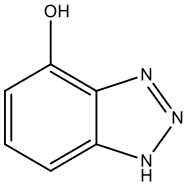
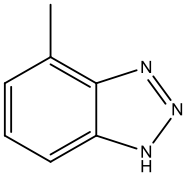
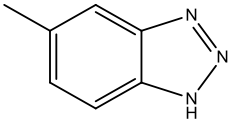
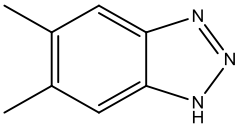
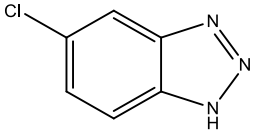
Compound	Structure	Quantification ion *(m/z)*	Fragment ions *(m/z)*
1-*H*-benzotriazole (BTR)		$[M+H]^+$ 120.05562	$C_5H_5^+$ (65.03858) $C_6H_6N^+$ (92.04948)
1-hydroxybenzotriazole (1OHBTR)		$[M-H]^-$ 134.03489	$C_6H_4ON^-$ (106.02874) $C_5H_4N^-$ (78.03383)
4-hydroxybenzotriazole (4OHBTR)		$[M-H]^-$ 134.03489	$C_5H_4N^-$ (78.03383) $C_6H_3ON^-$ (105.02092)
4-methyl-1-*H*-benzotriazole (4TTR)		$[M+H]^+$ 134.07127	$C_6H_7^+$ (79.05423) $C_6H_5N_2^+$ (105.04472)
5-methyl-1-*H*-benzotriazole (5TTR)		$[M+H]^+$ 134.07127	$C_6H_7^+$ (79.05423) $C_6H_5N_2^+$ (105.04472)
5,6-dimethyl-1-*H*-benzotriazole (XTR)		$[M+H]^+$ 148.08692	$C_7H_7^+$ (91.05423) $C_7H_9^+$ (93.06988)
5-chloro-1-*H*-benzotriazole (ClBTR)		$[M+H]^+$ 154.01665	$C_6H_4N^+$ (90.03382) $C_6H_5N_3Cl^+$ (126.01050)

Table S1. (Cont.)

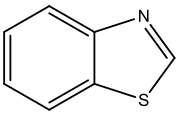
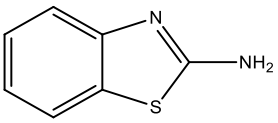
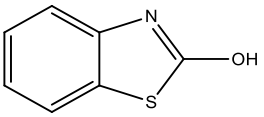
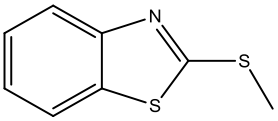
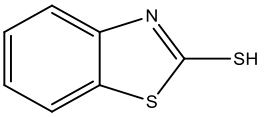
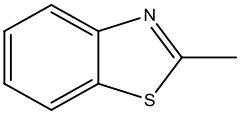
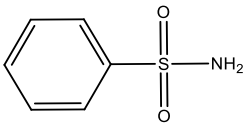
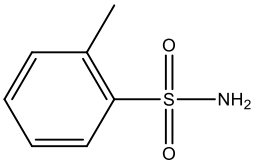
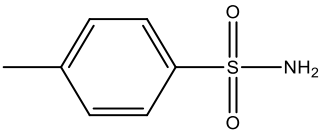
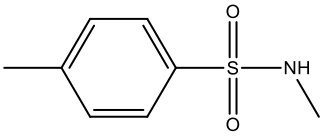
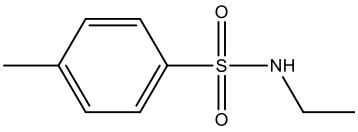
Compound	Structure	Quantification ion *(m/z)*	Fragment ions *(m/z)*
Benzothiazole (BT)		$[M+H]^+$ 136.02155	$C_5H_5S^+$ (109.01065) $C_5H_5^+$ (65.03858)
2-aminobenzothiazole (NH_2BT)		$[M+H]^+$ 151.03245	$C_6H_5S^+$ (109.01065) $C_6H_6NS^+$ (124.02155)
2-hydroxybenzothiazole (OHBT)		$[M+H]^+$ 152.01646	$C_6H_6NS^+$ (124.02155) $C_6H_6N^+$ (92.04948)
2-(methylthio)-benzothiazole (MeSBT)		$[M+H]^+$ 182.00927	$C_7H_5NS_2^+$ (166.98579) $C_6H_5S^+$ (109.01065)
2-mercaptobenzothiazole (MCBT)		$[M+H]^+$ 167.99362	$C_7H_5NS^+$ (135.01372) $C_6H_5S^+$ (109.01065)
2-methylbenzothiazole (MeBT)		$[M+H]^+$ 150.03720	$C_6H_5S^+$ (109.01065) $C_5H_5^+$ (65.03858)
Benzenesulfonamide (BSA)		$[M-H]^-$ 156.01138	$C_6H_6N^-$ (92.04948) SO_2NH^- (78.97225)
o-toluenesulfonamide (*o*-TSA)		$[M-H]^-$ 170.02703	SO_2NH^- (78.97225) $C_7H_8N^-$ (106.06513)
p-toluenesulfonamide (*p*-TSA)		$[M-H]^-$ 170.02703	SO_2NH^- (78.97225) $C_7H_8N^-$ (106.06513)

Table S1. (Cont.)

Compound	Structure	Quantification ion *(m/z)*	Fragment ions *(m/z)*
N-methyl-*p*-toluenesulfonamide (Me-*p*-TSA)		$[M+H]^+$ 186.05833	$C_7H_7^+$ (91.05423) SO_2H^+ (64.96918)
N-ethyl-*p*-toluenesulfonamide (Et-*p*-TSA)		$[M+H]^+$ 200.07398	$C_7H_7^+$ (91.05423) SO_2H^+ (64.96918)

3.1.2. Discussió de resultats

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

Tot i que els resultats obtinguts en l'estudi s'han discutit dins de l'article, aquesta secció en resumeix els aspectes més importants. En aquest estudi s'ha adaptat i optimitzat un mètode analític per la determinació de benzotriazoles, benzotiazoles i benzosulfonamides en aigua de riu i aigua de mar, el qual ha estat aplicat posteriorment a mostres recollides a l'àrea de Tarragona.

Inicialment es van optimitzar els paràmetres per la separació i detecció dels compostos mitjançant LC acoblada a HRMS amb un analitzador Exactive Orbitrap, ja que l'anterior mètode utilitzava LC acoblada a MS/MS amb un analitzador QqQ. Els dos compostos inclosos per primera vegada en aquest treball (1OHBTR i 4OHBTR) van donar una millor resposta amb el mode d'ionització en negatiu. La separació cromatogràfica dels compostos de l'article de referència també es va haver de modificar per incloure la 1OHBTR i la 4OHBTR, ja que utilitzant les fases mòbils originals tots dos compostos coeluien amb el front de dissolvent degut a la presència d'un 2% d'acetonitril a la fase mòbil aquosa. Aquesta quantitat d'acetonitril no es podia eliminar completament de les fases mòbils, ja que és el que fa que se separin els isòmers 4TTR i 5TTR [1], de manera que l'addició d'acetonitril es va canviar a la fase mòbil de metanol. Com que no es va aconseguir la separació dels isòmers *o*-TSA i *p*-TSA, tots dos es van determinar com un sol compost anomenat TSA.

Pel que fa a l'extracció de les mostres, la SPE es va dur a terme amb cartutxos Oasis HLB. Tot i que en algun treball s'han utilitzat sorbents d'intercanvi iònic de mode mixt per la determinació dels compostos d'interès, els derivats de la BSA sovint s'eliminen en etapes de neteja de manera que aquests sorbents es van descartar [2]. Per a l'optimització del procés d'extracció es van avaluar diversos paràmetres crítics per l'eficiència del procés. Per una banda es va estudiar la retenció dels compostos a diferents valors de pH (3, 5, 7, i 10). Es va triar l'extracció a pH 3, ja que la 1OHBTR només quedava retinguda als cartutxos a aquell pH. També es va optimitzar el volum de càrrega de mostra, que es va canviar de 250 mL a 1000 mL, augmentant així el factor d'enriquiment del mètode. Per poder utilitzar volums de mostra tan grans es va incrementar la quantitat de sorbent de 250 a 500 mg, i es va afegir una etapa de neteja amb un cartutx de Florisil en línia amb el d'extracció per mirar d'eliminar interferents i reduir l'efecte matriu.

El mètode es va validar per tots dos tipus de mostra i es va aplicar a l'anàlisi de mostres d'aigua del riu Ebre de quatre mesos diferents, mostres d'aigua de dues platges de Tarragona i mostres de quatre punts diferents dins del port de Tarragona. A les mostres es van poder determinar 8 del total de 16 anàlits inclosos a l'estudi, dels quals la BTR, la 4TTR, la 5TTR i la OHBT es van determinar en gairebé totes les mostres estudiades. Pel

que fa a les mostres d'aigua de riu, aquests compostos es van trobar a concentracions entre 15 i 78 ng/L. Els resultats són similars als obtinguts per altres autors en mostres de riu [2,3]. Cal destacar que altres compostos com la ClBTR, la XTR o la MeSBT, que en altres ocasions s'han determinat en aigua de riu, no es van detectar en les mostres del riu Ebre estudiades. A més, la NH_2BT només es va detectar en mostres d'aigua de riu.Pel que fa a les mostres d'aigua de mar, en les mostres recollides a la platja es van detectar menys anàlits que en les mostres recollides dins del port. En el cas de les mostres de la platja, només la BTR i la OHBT es van poder quantificar. En el cas de les mostres del port, a part de la BTR i la OHBT també es van poder quantificar la 4TTR i la 5TTR. És interessant destacar que la 4OHBTR, la BT i la MeBT només es van detectar i quantificar en mostres d'aigua recollides a la part interior del port, a prop dels molls on atraquen els vaixells que habitualment transporten substàncies químiques i al costat del que podria ser una canonada de descàrrega d'aigües residuals. Així doncs, les concentracions elevades observades en un dels mostrejos duts a terme en aquella zona podrien ser degudes a un abocament accidental. Aquesta hipòtesi estaria recolzada, ja que les concentracions determinades en aquest mostreig són similars a les que habitualment es troben en mostres d'aigües residuals efluents [2,4].

References

[1] P. Herrero, F. Borrull, E. Pocurull, R.M. Marcé, An overview of analytical methods and occurrence of benzotriazoles, benzothiazoles and benzenesulfonamides in the environment, Trends Anal. Chem. 62 (2014) 46–55.

[2] D. Salas, F. Borrull, R.M. Marcé, N. Fontanals, Study of the retention of benzotriazoles, benzothiazoles and benzenesulfonamides in mixed-mode solid-phase extraction in environmental samples, J. Chromatogr. A. 1444 (2016) 21–31.

[3] J. Casado, R. Nescatelli, I. Rodríguez, M. Ramil, F. Marini, R. Cela, Determination of benzotriazoles in water samples by concurrent derivatization-dispersive liquid-liquid microextraction followed by gas chromatography-mass spectrometry, J. Chromatogr. A. 1336 (2014) 1–9.

[4] P. Herrero, F. Borrull, E. Pocurull, R.M. Marcé, Efficient tandem solid-phase extraction and liquid chromatography-triple quadrupole mass spectrometry method to determine polar benzotriazole, benzothiazole and benzenesulfonamide contaminants in environmental water samples, J. Chromatogr. A. 1309 (2013) 22–3

3.2. Determinació de compostos orgànics d'alt volum de producció en peix i marisc

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

Aquest apartat descriu el desenvolupament de tres mètodes analítics per determinar compostos AVP en peix i marisc.

Els dos primers mètodes se centren en la determinació simultània de ftalats i els seus metabòlits. Els ftalats, que s'utilitzen a la indústria principalment com a plastificants, han estat molt estudiats durant els últims anys i s'ha detectat la seva presència en tots els compartiments mediambientals [1]. Aquests compostos se solen determinar amb GC acoblada a MS o MS/MS, tot i que en alguns casos també s'han determinat amb LC-MS/MS. Encara que en comparació amb els ftalats els seus metabòlits han estat molt menys estudiats, aquests també s'han detectat en tota mena de mostres i habitualment es determinen amb LC-MS/MS, ja que per determinar-los amb GC s'han de derivatitzar [2]. Hi ha molt pocs estudis que determinin simultàniament tant dièsters com monoèsters de l'àcid ftàlic i cap d'ells ho fa en mostres de peix o marisc destinat a l'alimentació.

És per això que l'objectiu principal dels dos primers estudis descrits en aquest apartat és desenvolupar mètodes analítics basats en la LC per la determinació simultània de sis dièsters i sis monoèsters de l'àcid ftàlic en mostres de peix i marisc. Es faran servir dues tècniques d'extracció diferents i es compararan els resultats obtinguts amb ambdós mètodes. Primerament, s'optimitzarà l'extracció de les mostres per PLE, ja que és una tècnica que només s'ha utilitzat amb anterioritat per l'extracció de monoèsters en mostres de biota i sediments [3]. L'altra tècnica d'extracció avaluada seran els QuEChERS, ja que es tracta d'una tècnica que utilitza volums més petits de dissolvents, requereix un temps d'extracció més petit i no necessita cap tipus d'equipament especial. Independentment de la tècnica d'extracció utilitzada, es provaran diferents estratègies de neteja com la LLE, la SPE o la dSPE, entre d'altres, per eliminar els lípids presents en els extractes i reduir l'efecte matriu present habitualment en aquest tipus de mostres, sobretot quan s'utilitza ESI en l'acoblament LC-MS. A més, al primer estudi també es compararan els resultats obtinguts amb dues fonts d'ionització diferents (ESI i ionització química a pressió atmosfèrica (APCI)), ja que a vegades l'ús de l'APCI en comptes de ESI ha resultat en millores pel que fa als límits de detecció i als efectes matriu observats [4].

El tercer estudi, en canvi, se centra en la determinació de metabòlits dels organofosfats utilitzant els QuEChERS com a tècnica d'extracció. Els organofosfats s'utilitzen a la indústria principalment com a retardants de flama i plastificants i, igual que en el cas dels ftalats, la seva presència al medi ambient ha estat àmpliament estudiada durant els últims anys [5]. En canvi, la informació sobre la determinació i presència dels seus metabòlits en mostres mediambientals, així com els seus efectes tòxics en éssers vius és molt limitada. Pocs estudis desenvolupen mètodes per la determinació d'aquests

compostos en mostres i els que ho fan utilitzen USAE o PLE com a tècniques d'extracció. Així doncs, l'objectiu principal d'aquest estudi és desenvolupar un mètode analític basat en l'extracció per QuEChERS per la determinació de sis dièsters de l'àcid fosfòric en mostres de peix i marisc. També s'avaluaran diversos processos de neteja per reduir l'efecte matriu.

La separació i detecció de tots els anàlits s'han dut a terme per LC-(ESI)HRMS i els tres mètodes proposats s'han aplicat per l'anàlisi de mostres de les deu espècies de peix i marisc més consumides a Catalunya.

Els resultats dels dos primers estudis s'han publicat a les revistes *Food Analytical Methods 13 (2020) 1442–1453* i *Food Chemistry 336 (2021) 127722* i els del tercer estan pendents d'enviar a revista per la seva publicació.

Referències

[1] D.W. Gao, Z.D. Wen, Phthalate esters in the environment: A critical review of their occurrence, biodegradation, and removal during wastewater treatment processes, Sci. Total Environ. 541 (2016) 986–1001.

[2] M. Del Bubba, C. Ancillotti, L. Checchini, D. Fibbi, D. Rossini, L. Ciofi, L. Rivoira, C. Profeti, S. Orlandini, S. Furlanetto, Determination of phthalate diesters and monoesters in human milk and infant formula by fat extraction, size-exclusion chromatography clean-up and gas chromatography-mass spectrometry detection, J. Pharm. Biomed. Anal. 148 (2018) 6–16.

[3] J.D. Blair, M.G. Ikonomou, B.C. Kelly, B. Surridge, F.A.P.C. Gobas, Ultra-trace determination of phthalate ester metabolites in seawater, sediments, and biota from an urbanized marine inlet by LC/ESI-MS/MS, Environ. Sci. Technol. 43 (2009) 6262–6268.

[6] S. Hagenhoff, H. Hayen, LC/MS analysis of vitamin D metabolites by dielectric barrier discharge ionization and a comparison with electrospray ionization and atmospheric pressure chemical ionization, Anal. Bioanal. Chem. 410 (2018) 4905–4911.

[5] G.L. Wei, D.Q. Li, M.N. Zhuo, Y.S. Liao, Z.Y. Xie, T.L. Guo, J.J. Li, S.Y. Zhang, Z.Q. Liang, Organophosphorus flame retardants and plasticizers: Sources, occurrence, toxicity and human exposure, Environ. Pollut. 196 (2015) 29–46.

3.2.1. Pressurised liquid extraction and liquid chromatography-high resolution mass spectrometry for the simultaneous determination of phthalate diesters and their metabolites in seafood species

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

PRESSURISED LIQUID EXTRACTION AND LIQUID CHROMATOGRAPHY–HIGH RESOLUTION MASS SPECTROMETRY FOR THE SIMULTANEOUS DETERMINATION OF PHTHALATE DIESTERS AND THEIR METABOLITES IN SEAFOOD SPECIES

Míriam Hidalgo-Serrano, Francesc Borrull, Eva Pocurull, Rosa M. Marcé

Universitat Rovira i Virgili, Department of Analytical Chemistry and Organic Chemistry, Marcel·lí Domingo s/n, Tarragona 43007, Spain

Abstract

We have developed an analytical method based on pressurised liquid extraction (PLE) followed by liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) for the simultaneous determination of five phthalate diesters and six phthalate monoesters in widely consumed seafood species. In order to obtain the highest extraction recoveries, as well as to reduce the matrix effect of such complex samples, the most important extraction parameters were optimised and several clean-up and preconcentration strategies were tested. This method provided limits of detection that ranged from 1 to 25 ng g^{-1} dry weight (d.w.) for most compounds in both fatty and low-fat species. Moreover, several target analytes were quantified in five seafood species (four phthalate diesters and three of their metabolites). The most abundant compounds were diethyl phthalate (DEP) and diethylhexyl phthalate (DEHP), which were quantified from 60 to 3393 ng g^{-1} (d.w.) and from 117 to 1562 ng g^{-1} (d.w.), respectively.

Keywords: *Seafood, Phthalate diesters, Phthalate monoesters, Pressurised liquid extraction, Liquid chromatography, High-resolution mass spectrometry*

1. Introduction

Phthalate diesters are a group of high-production chemicals used in plastic production mainly to increase the flexibility of plastics such as polyvinyl chloride. These compounds are found in consumer products such as food packaging and food supplements, personal care products, fabrics and medical equipment [1,2]. Since these compounds are not chemically bound to the plastic products, they can easily contaminate the environment by reaching the air and water [3]. Moreover, microplastic contamination is a growing environmental problem worldwide [4,5], hence it is probable that these compounds may be found in aquatic organisms. Taking into account that in Spain, for instance, the average household consumption of seafood was estimated at 23.07 kg per capita in 2018 [6], seafood is an important dietary route for human exposure to these contaminants.

The phthalate diesters found in the organisms we consume are rapidly hydrolysed into their corresponding monoesters, which are subsequently metabolised and excreted [7], even though these derivates can also occur due to abiotic and microbial degradation of the diesters in soil, sediment or water [2].

Phthalate diesters are known endocrine disruptors. Prolonged exposure to some of these compounds, even at low concentrations, can therefore have negative effects on the development of affected organisms and their reproductive system. For instance, phthalate diesters have been known to produce alterations in oxidative stress parameters, disturb sex hormone balances and alter gonad histology, which can increase the rate of reproductive defects and malformations and decrease fertility [8–10]. Although fewer studies exist on the toxicity of phthalate monoesters, some have pointed out possible alterations in the gene expression of antioxidant enzymes [8], thyroid endocrine disrupting effects [11], or disruptions in the balance of sex hormones [7]. Most studies call attention to the fact that the effects associated with phthalate diesters are induced by both the diester and its metabolites [10,12].

For these reasons, several environmental and food safety agencies have established limits and regulations on the use of some of these chemicals. In 2005, for instance, the European Food Safety Agency (EFSA) established tolerable daily intakes (TDI) of 0.01 mg/kg body weight (bw) for di-n-butyl phthalate (DBP) [13], 0.5 mg/kg bw for benzylbutyl phthalate (BzBP) [14] and 0.05 mg/kg bw for diethylhexyl phthalate (DEHP) [15]. Earlier in 2019, a group-TDI of 0.05 mg/kg bw was also proposed by EFSA for DBP, BzBP, DEHP and diisononyl phthalate [16]. The European Parliament has also considered these compounds to be of great concern and has restricted their use in toys and childcare

articles to 0.1% by weight of plasticised material. This restriction must also be enforced for di-n-octyl phthalate (DOP) in toys and childcare products that children may put in their mouths [17]. Similar restrictions have been established by the U.S. Consumer Product Safety Commission [18].

In view of the above, in the past few years numerous methods have been developed for determining phthalate diesters and, to a much lesser extent, their metabolites in matrices such as river and surface waters [3,19–21], soils and sediments [21–23], biological fluids [24–26], food and packaging [23,27–32] and biota [19,21,22,33,34]. For seafood, several extraction procedures have been used, including QuEChERS [33], ultrasound extraction [19,34] and pressurised liquid extraction (PLE) [21].

Gas chromatography (GC) coupled to mass spectrometry (MS) is the most common technique for determining phthalate diesters [24,32–34], even though in some cases they have also been determined by liquid chromatography (LC) [28–30]. Phthalate monoesters, on the other hand, are mainly determined by LC [19–21,31] because they have to be derivatised to be determined by GC [24]. That is the reason why when phthalate diesters and phthalate monoesters are included in the same study, two different chromatographic methods are usually developed [19,20,34]. There are some studies that use the same GC-MS method for the simultaneous analysis of several phthalate diesters and their corresponding metabolites in matrices such as fish bile [35] or human milk and infant formula samples [24]. However, to the best of our knowledge, no LC methods have been developed for the simultaneous determination of several phthalate diesters as well as their metabolites in complex food matrices such as seafood.

That is the reason why in this study we aim to develop a new analytical method for simultaneously determining six phthalate diesters and their six main metabolites in seafood using PLE followed by LC coupled to high resolution mass spectrometry (LC-HRMS). To do so, extraction parameters such as the extraction solvent, extraction temperature, static time and number of extraction cycles were optimised. When working with a matrix as complex as seafood, however, high matrix effect (ME) values usually hinder the correct identification and quantification of the target compounds. We therefore tested several clean-up steps to evaluate which ones provided lower matrix effect values. Moreover, since several studies have reported improvements in analyses of complex samples when using an atmospheric pressure chemical ionisation source (APCI) rather than a heated electrospray ionisation source (HESI) [36–39], we tested and compared both of these sources. It is also interesting to point out that, while most LC methods use tandem mass spectrometry (MS/MS) for the determination of the

compounds of interest [19–21,30,31], in this study HRMS was used instead. Finally, the method was validated and used to analyse five seafood species.

2. Materials and methods

2.1. Reagents and standards

The solid standards of monomethyl phthalate (MMP), monoethyl phthalate (MEP), monooctyl phthalate (MOP) and diethyl phthalate (DEP) were purchased from LGC (Teddington, Middlesex, UK) and those of monobutyl phthalate (MBP), monobenzyl phthalate (MBzP), mono(2-ethylhexyl) phthalate (MEHP), dimethyl phthalate (DMP), di-n-butyl phthalate (DBP), benzyl butyl phthalate (BzBP), bis(2-ethylhexyl) phthalate (DEHP), di-n-octyl phthalate (DOP) and bis(2-ethylhexyl) phthalate-3,4,5,6-d_4 (DEHP-d_4) were purchased from Sigma-Aldrich (St. Louis, USA). Stock solutions of individual standards at 1000 mg L^{-1} were prepared in methanol and stored at -23 ^{o}C.

Ultrapure water was obtained with an ultrapure water purification system from Veolia Water (Sant Cugat del Vallés, Spain). Acetonitrile (ACN) of MS grade was purchased from Chem-lab (Zedelgem, Belgium). Methanol (MeOH), ethyl acetate (EtOAc), hexane and acetone of HPLC grade, water of MS grade and acetic acid (CH_3COOH) were provided by J.T. Baker (Deventer, The Netherlands). Finally, hydrochloric acid (HCl) was purchased from Scharlab (Barcelona, Spain).

2.2. Sampling

Several seafood species such as mackerel (*Scomber scombrus*), salmon (*Salmo salar*), shrimp (*Aristeus antennatus*), sole (*Solea solea*) and squid (*Loligo vulgaris)* were purchased from local fish markets in Tarragona, Spain. All samples were immediately stored in the refrigerator before the fish were filleted and the shells of shrimps removed. Each sample was then homogenized, frozen and lyophilized with a miVac Duo sample concentrator with a SpeedTrap freeze-drying system (Genevac, Ipswich, UK). A coffee grinder from Moulinex (Aleçon, France) was used to grind the samples before they were sieved through a 500 µm mesh to homogenise the particle diameter.

2.3. Pressurised liquid extraction and clean-up

An ASE 350 Accelerated Solvent Extraction system from Dionex (Sunnyvale, California, USA) was used to perform the sample extractions. For this step, 11 mL extraction cells

were mounted as follows: a cellulose filter from Teknokroma (Sant Cugat del Vallès, Spain) was placed at the bottom of the extraction cell and 1 g (dry weight (d.w.)) of sample was mixed with 1.6 g of diatomaceous earth. The samples were extracted with MeOH at a temperature of 80 ºC for one extraction cycle of 10 min at 1500 psi, with a preheating time of 5 min, a flush volume of 60% and a purge time of 90 s.

The extract obtained (~18 mL) was concentrated to ~5 mL with a miVac Duo sample concentrator. Next, 2.5 mL of ultrapure water adjusted to pH 2.4 with HCl was added. The mixture was then vortexed and left overnight in the freezer at -23 ºC to prompt the precipitation of lipids and interferences. The extract was then filtered through filter paper and taken up to 25 mL with ultrapure water at pH 2.4 to carry out a solid-phase extraction (SPE) clean-up step. Briefly, Bond Elut Plexa (200 mg) cartridges from Agilent (Santa Clara, California, USA) were preconditioned with 5 mL of MeOH followed by 5 mL of ultrapure water at a pH of 2.4. The 25 mL extracts were then loaded onto the cartridges, the analytes were eluted with 5 mL of MeOH and the eluate was filtered with a 0.22 µm PTFE syringe filter (Scharlab). 1 mL of MeOH was also passed through the filter and added to the extract to ensure that none of the analytes of interest was retained. Finally, the extracts were concentrated up to ~200 µL with the miVac Duo sample concentrator, taken up to 1 mL with ultrapure water and injected into the LC-(HESI)HRMS instrument.

2.4. Liquid chromatography coupled to high-resolution mass spectrometry

The chromatographic analyses were performed with an Accela 1250 UHPLC system (Thermo Scientific, Bremen, Germany) equipped with a quaternary pump and an Accela Autosampler. The chromatographic system was coupled to an ExactiveOrbitrap™ mass spectrometer (Thermo Scientific) equipped with either a HESI or an APCI, and an HCD collision cell. An Ascentis Express C_{18} fused-core column (100 mm x 2.1 mm i.d., 2.7 µm particle size) was used for the separation (Sigma-Aldrich). The mobile phase was a mixture of 0.1% CH_3COOH in H_2O (solvent A) and MeOH (solvent B). The gradient profile began with 13% of B and held constant for 5 min before it was increased to 80% of B in 13 min. The gradient was then increased to 90% of B in 1 min and to 100% of B in 7 min. Finally, it remained constant at 100% of B for 3 min before returning to the initial conditions. The flow rate was 300 µL min^{-1} and the injection volume was 20 µL. The temperatures of the column oven and sample tray were 30 ºC and 10 ºC, respectively.

Positive and negative ionisation modes were both tested in order to optimize the HESI and APCI source parameters and obtain the highest response for all compounds. The optimal parameters for HESI were as follows: sheath gas flow rate, 50 A.U.; auxiliary gas

flow rate, 15 A.U.; sweep gas flow rate, 0 A.U.; spray voltage, ±4 kV; capillary voltage, ±20 V; tube lens voltage, ±55 V; skimmer voltage, ±16 V; heater temperature, 350 °C; and capillary temperature, 350 °C. The optimal parameters for APCI were as follows: sheath gas flow rate, 20 A.U.; auxiliary gas flow rate, 15 A.U.; sweep gas flow rate, 0 A.U.; ion current, ±8 µA; capillary voltage, ±20 V; tube lens voltage, ±55 V; skimmer voltage, ±16 V; heater temperature, 250 °C; and vaporiser temperature, 225 °C.

Six time windows with two scan events alternating in each window were used for data acquisition. Three of these windows were in negative mode (0.0-10.0, 11.5-12.8 and 15.6-17.1 min) and three were in positive mode (10.0-11.5, 12.8-15.6 and 17.1-33.0 min). In all windows, there was a full scan at 50,000 FWHM with 250 ms of injection time and a fragmentation scan at 10,000 FWHM with 50 ms of injection time using a collision voltage in the HCD cell of 10 eV. The quantification ions were measured with a mass extraction window of 5 ppm. The selected fragment ions (**Table 1**) were taken into account for confirmation purposes.

2.5. Control of blank concentrations

As phthalate diesters are ubiquitously found in the laboratory environment, samples can easily be contaminated. Several actions therefore had to be taken during the study in order to control the concentrations of the target analytes that were already present in the system.

Most of the equipment used was glassware that was rinsed twice with acetone and three times with MeOH and left to dry completely under a fume cupboard before use. When samples had to be spiked to optimise or validate the method, the lyophilised species was covered with acetone and the mixture was covered with a perforated piece of aluminium foil to avoid environmental contamination while still allowing the acetone to evaporate overnight.

However, full procedural blanks were regularly performed with diatomaceous earth instead of fish following the same procedure as described for the samples. Four of the studied compounds were successfully identified and quantified in the procedural blanks: MEHP and DBP at concentrations below 10 µg L^{-1}, and DEP and DEHP at concentrations below 50 µg L^{-1}. The relative standard deviation (RSD%, *n = 10*) values achieved were always lower than 5%. The corresponding blank values were thus subtracted from the analysed samples to ensure that environmental contamination did not lead to false positives.

Table 1. Chemical structure of the analytes studied and exact masses of their fragment ions.

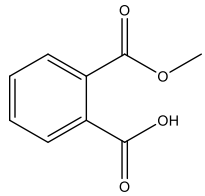
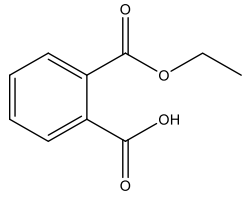
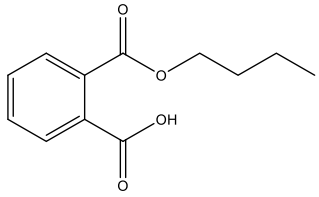
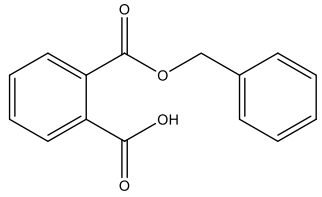
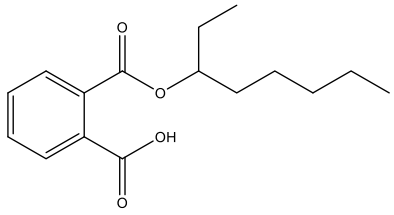
Compound and structure	Quantification ion (*m/z*)	Fragment ions (*m/z*)
Monomethyl phthalate (MMP) 	$[M-H]^-$ 179.03389	[a] $C_7H_7O^-$ (107.04914) $C_8H_7O_2^-$ (135.04406) [b] $C_7H_5O_2^-$ (121.02851)
Monoethyl phthalate (MEP) 	$[M-H]^-$ 193.04954	$C_7H_5O_2^-$ (121.02841) $C_9H_9O_2^-$ (149.05971)
Monobutyl phthalate (MBP) 	$[M-H]^-$ 221.08084	$C_{11}H_{13}O_2^-$ (177.09101) $C_7H_5O_2^-$ (121.02841)
Monobenzyl phthalate (MBzP) 	$[M-H]^-$ 255.06519	[a] $C_7H_7O^-$ (107.04914) $C_7H_5O_2^-$ (121.02841) [b] $C_{14}H_{11}O_2^-$ (211.07536)
Mono(2-ethylhexyl) phthalate (MEHP) 	$[M-H]^-$ 277.14344	$C_8H_6O_2^-$ (134.03623) $C_{15}H_{21}O_2^-$ (233.15361)

Table 1. (Cont.).

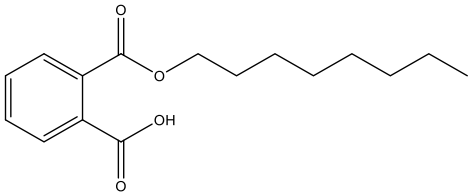
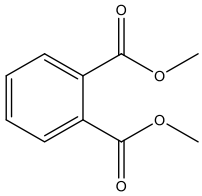
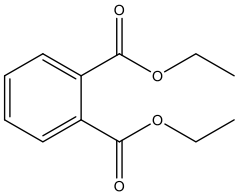
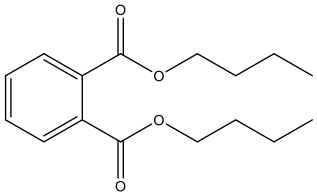
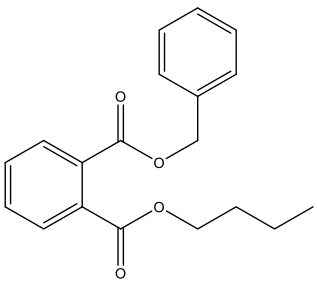
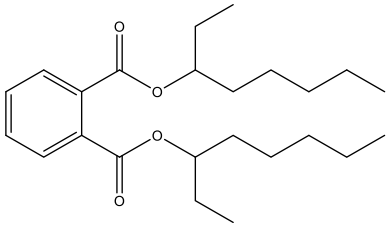
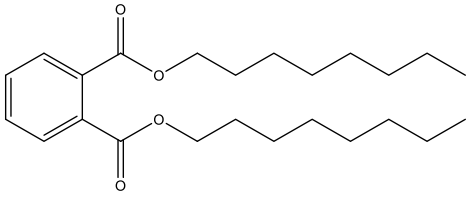
Compound and structure	Quantification ion (*m/z*)	Fragment ions (*m/z*)
Monooctyl phthalate (MOP) 	$[M-H]^-$ 277.14344	$C_{15}H_{21}O_2^-$ (233.15361) $C_8H_{15}O^-$ (127.11174)
Dimethyl phthalate (DMP) 	$[M+H]^+$ 195.06518	$C_9H_7O_3^+$ (163.03897) [b] $C_8H_7O_2^-$ (135.04406)
Diethyl phthalate (DEP) 	$[M+H]^+$ 223.09648	$C_8H_5O_3^+$ (149.02332) $C_{10}H_9O_3^+$ (177.05462)
Dibutyl phthalate (DBP) 	$[M+H]^+$ 279.15908	$C_8H_5O_3^+$ (149.02332) $C_{12}H_{13}O_3^+$ (205.08592)
Benzyl butyl phthalate (BzBP) 	$[M+H]^+$ 313.14343	$C_8H_5O_3^+$ (149.02332) $C_{12}H_{13}O_3^+$ (205.08592)

Table 1. (Cont.).

Compound and structure	Quantification ion (*m/z*)	Fragment ions (*m/z*)
Bis(2-ethylhexyl) phthalate (DEHP) 	$[M+H]^+$ 391.28428	$C_8H_5O_3^+$ (149.02332) $C_8H_7O_4^+$ (167.03389)
Di-n-octyl phthalate (DOP) 	$[M+H]^+$ 391.28428	$C_8H_5O_3^+$ (149.02332) $C_{16}H_{21}O_3^+$ (261.14852)

[a] Fragment ion only when using HESI

[b] Fragment ion only when using APCI

3. Results and Discussion

3.1. Liquid chromatography coupled to high-resolution mass spectrometry

The ionisation of the compounds was studied by direct injections of 1 mg L^{-1} individual standard solutions with a 50:50 (v:v) composition of solvent A:solvent B. The highest response for the phthalate diesters was obtained in positive ionisation mode as the protonated molecule $[M+H]^+$, whereas the highest response for the phthalate monoesters was obtained in negative ionisation mode as the deprotonated molecule $[M-H]^-$. **Table 1** shows the structure of the phthalate diesters and the phthalate monoesters, as well as the exact mass of the quantification ions and fragment ions selected for confirmation with both ionisation sources. For some compounds, the fragment ions selected for HESI and APCI are different.

With the optimised LC-HRMS method, the chromatographic separation obtained was highly satisfactory for all target analytes and instrumental quality parameters were calculated for both ionisation sources. Standard calibration curves were determined for each compound taking into account the response of the quantification ion. Instrumental

limits of detection (ILOD) correspond to a response of the quantification ion more than three times the signal-to-noise ratio. Instrumental limits of quantification (ILOQ) were established as the lowest point of the standard calibration curve for each compound. Two

linear ranges with satisfactory r^2 were established for each compound between ILOQ and 1,000 μg L^{-1}. The limits obtained with each ionisation source are shown in **Table 2**.

Both tested ionisation sources showed similar sensitivities for most compounds, though some differences were observed. For instance, ILOD values for phthalate diesters such as DMP or DEP were lower when APCI was used, whereas results were slightly better for phthalate monoesters such as MEHP or MOP when HESI was used. Since the instrumental limits were similar for both ionization sources, the matrix effect was evaluated to determine the suitability of HESI and APCI.

Table 2. Retention time (t_R), instrumental limit of detection (ILOD) and instrumental limit of quantification (ILOQ) obtained with each ionisation source.

Compound	t_R (min)	HESI		APCI	
		ILOD (μg L^{-1})	ILOQ (μg L^{-1})	ILOD (μg L^{-1})	ILOQ (μg L^{-1})
MMP	4.4	2.5	5	1	2.5
MEP	8.3	1	2.5	1	5
DMP	10.5	2.5	5	0.5	2.5
MBP	11.9	1	2.5	2.5	5
MBzP	12.3	0.5	1	1	5
DEP	13.3	10	25	0.5	1
MEHP	16.2	0.5	1	2.5	5
MOP	16.7	0.5	2.5	2.5	10
BzBP	17.8	0.5	1	0.5	1
DBP	18.1	1	2.5	5	10
DEHP	24.7	25	50	10	25
DOP	25.4	2.5	5	5	10

3.2. Pressurised liquid extraction

Based on our previous experience [40,41], the initial conditions for PLE were set as follows: 1 g of sample, an extraction temperature of 80 °C, a preheating time of 5 min, a static time of 10 min, one cycle, a flush volume of 60% and a purge time of 90 s. However,

to efficiently extract all the compounds of interest, several parameters were optimised. It is known that the efficiency of the extraction can depend on the lipidic content of the samples. Two types of seafood with different lipid percentages were therefore chosen for the optimisation: one species of seafood with high lipid content (salmon) and one species of seafood with low lipid content (sole). To evaluate the suitability of the changes in the extraction parameters, apparent recoveries (R_{app}), which take into account recovery yield and ME, were calculated with an external standard calibration curve. To do so, a stock mixture of standards was added to 1 g of lyophilised fish covered with acetone. Non-spiked fish samples were also analysed to subtract the signals of the compounds already present in the samples. All parameters were optimised using HESI as the ionisation source.

The extraction solvent was the first parameter optimised. Water, ACN, EtOAc, acetone, MeOH, a mixture of ACN:water (1:1, v:v) and a mixture of ACN:MeOH (1:1, v:v) were tested. To optimise the extraction process while avoiding the high ME values that result from the great complexity of the sample, extracts containing only organic solvent were evaporated to ~5 mL and taken up to 25 mL with ultrapure water, while water and ACN:water extracts (~18 mL) were diluted to 25 mL with ultrapure water. 1 mL of each extract was filtered with a 0.22 µm PTFE filter before injection. However, we observed that a portion of some of the less polar compounds in the samples (BzBP, DBP, BEHP and DOP) was slightly retained in the PTFE syringe filter. To ensure that none of the analytes of interest was retained in the filter, 1 mL of MeOH was passed through the same filter after extraction.

Extractions with water or ACN:water were discarded because the high lipidic content caused a foam to appear inside the collection vial that automatically aborted the extraction process. Extraction with acetone also resulted in R_{app} below 15% and was omitted. The R_{app} values obtained with salmon when the extraction was carried out with the remaining solvents are shown in **Figure 1**, which are comparable to those obtained with sole. Similar R_{app} values resulted from extraction with ACN or EtOAc. Although the extraction of most phthalate diesters was quite efficient (R_{app}>50%), the R_{app} values for all phthalate monoesters were below 40%. On the other hand, extraction of the samples with MeOH was the most efficient, with R_{app} for all compounds except BzBP and DBP ranging from 45 to 69%. Since BzBP and DBP were satisfactorily extracted with ACN (R_{app} of 51 and 71%, respectively), a mixture ACN:MeOH (1:1, v:v) was suggested as a way

to increase their recovery. However, the results did not improve and the extraction of other analytes worsened. We therefore selected MeOH as the extraction solvent.

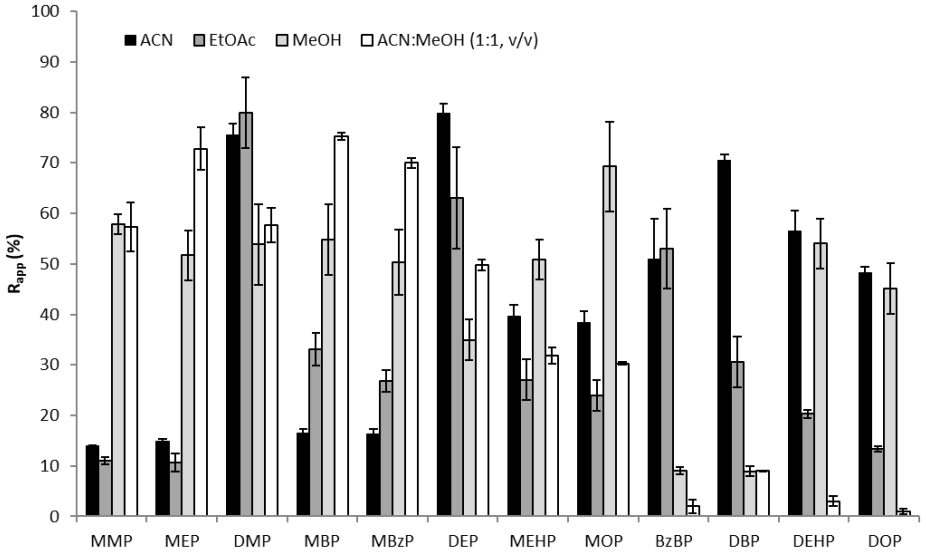


Figure 1. R_{app} (%) of salmon obtained after PLE with different organic solvents.

We also studied the extraction temperature, static time and number of cycles to enhance the efficiency of the extraction. Extraction temperatures of 60, 80 and 100 ^{o}C were tested. A temperature of 80 ^{o}C yielded higher recoveries for some of the target analytes while no significant differences between temperatures were observed for the remaining compounds. Static times of 10, 15 and 20 min were also tested. The results were best with 10 min since R_{app} decreased for most compounds when the static time increased. Finally, two and three cycles of extraction with a static time of 10 min were tested but the recoveries did not improve. The optimal parameters chosen for PLE extraction were therefore one cycle at 80 ^{o}C with a static time of 10 min.

Other parameters, such as preheating time, flush volume and purge time, are known to have no significant effect on extraction efficiency [42,43].

3.3. Clean-up and preconcentration strategies

The high ion suppression detected in the extracts and the complexity of the sample caused the R_{app} to decrease, thus making it necessary to add a clean-up step before

injecting the sample. Although several strategies were proposed, in- and on-cell clean-up steps were discarded after taking into account previous studies by our group that showed that they hardly improved the results when seafood samples were analysed [43]. Three clean-up and preconcentration strategies were therefore evaluated to decrease ME. All ME values were calculated by comparing the responses of the analytes in blank sample extracts spiked after the extraction and clean-up procedures (subtracting the response of the compounds present in the samples and the system) with the response of the analytes in a standard solution. Analyses for evaluating all procedures were carried out using HESI as the ionisation source.

The first clean-up procedure tested involved extracting lipidic interferences with hexane [40]. In brief, the PLE extract was evaporated to dryness, reconstituted with 4 mL of water and 2 mL of hexane, and shaken. The aqueous layer was taken to 5 mL with MeOH and filtered before injection. With this kind of clean-up the ME was greatly reduced for most compounds. However, as hexane led to low recoveries of some of the target analytes (specifically, DMP, DEP, BzBP, DBP and DOP), clean-up with hexane was discarded.

A SPE clean-up procedure was also proposed. Initially we tested the retention of possible interfering substances before evaporating the extract using strong cationic and anionic exchange cartridges from Waters (Milford, Massachusetts, USA) [43]. An Oasis MAX cartridge (150 mg, 6 cc) was then connected to the bottom of an Oasis MCX cartridge (150 mg, 6 cc). Both cartridges were conditioned with 5 mL of MeOH before the PLE extract was directly loaded. The load was then collected, evaporated to ~200 µL, taken up to 1 mL with ultrapure water and filtered before injection. As no significant improvements in ME were observed with this protocol, it was also rejected.

Another SPE procedure was evaluated to clean-up and preconcentrate the sample. Several commercial cartridges with different characteristics were tested, namely Oasis HLB (150 mg, 6 cc) from Waters, and Bond Elut Plexa (200 mg) and Bond Elut C_{18} (200 mg) from Agilent. To do so, the PLE extract was evaporated to ~5 mL of MeOH and 2.5 mL of ultrapure water at pH 2.4 were added before allowing it to remain in the freezer overnight at -23 ^{o}C. This procedure enabled the fatty precipitates to be filtrated the next day with paper filter to obtain a much cleaner extract that presented no turbidity. Moreover, adjusting the pH of the ultrapure water ensured that all the target phthalate monoesters were protonated since their pK_a values ranged from 3.32 to 3.38. Afterwards, the filtered extract was taken up to 25 mL with ultrapure water at pH 2.4 and loaded onto the cartridges, which had previously been conditioned with 5 mL of MeOH and 5 mL of

ultrapure water at pH 2.4. The target compounds were eluted with 5 mL of MeOH, filtered with a 0.22 µm PTFE syringe filter and concentrated to ~200 µL. The final extract was reconstituted to 1 mL with ultrapure water and injected into the LC-(HESI)HRMS.

After establishing this process, we evaluated the extraction recoveries obtained with the three SPE cartridges. First we tested the Bond Elut C_{18} cartridge. However, MMP, MEP and DMP were partially lost in the loading so these cartridges were discarded. Oasis HLB and Bond Elut Plexa cartridges were then compared, observing that DEHP and DOP were partially lost in the loading in both cases. However, the recoveries for DEHP and DOP were much higher with Bond Elut Plexa cartridge and extraction recoveries for all the other compounds ranged from 90 to 100%, hence this cartridge was selected as optimal for the clean-up and preconcentration steps.

3.4. Evaluation of ionisation source

Although the sample preparation procedures thoroughly cleaned the sample, high ion suppression was observed for some compounds when HESI was used. We therefore evaluated the ME obtained with HESI and APCI for both species of fish.

To do so, salmon and sole samples were spiked in triplicate at 100 ng g^{-1} (d.w.) except for DEP, DBP and DEHP, which were found in high concentrations in the system and spiked at 500 ng g^{-1}. Non-spiked fish samples were also analysed, as mentioned before.

The ME values obtained with HESI for the sole samples ranged from -50 to -80% for the phthalate monoesters and from -8 to -60% for the phthalate diesters. The ME values for the salmon samples ranged from -33 to -75% for the phthalate monoesters and from -24 to -43% for the phthalate diesters. However, most ME values obtained with APCI for both species were much higher than 100% of ion enhancement. As this could affect the accurate quantification of most target analytes, the use of the APCI source was dismissed.

3.5. Method validation

After all the parameters had been optimised, the method was validated. To do so, R_{app}, method limits of detection (MLOD), method limits of quantification (MLOQ), repeatability (intra-day) and reproducibility (day-to-day) were calculated for the sole and salmon samples. **Table 3** shows the validation parameters obtained.

Table 3. Apparent recovery (R_{app}), method limit of detection (MLOD), method limit of quantification (MLOQ), repeatability and reproducibility (expressed as RSD%, $n = 5$)

Compound	Sole (*Solea solea*)						Salmon (*Salmo salar*)					
	R_{app} (%)	MLOD (ng g^{-1})	MLOQ (ng g^{-1})	Repeatability (RSD%, $n = 5$)		Reproducibility (RSD%, $n = 5$)		R_{app} (%)	MLOD (ng g^{-1})	MLOQ (ng g^{-1})	Repeatability (RSD%, $n = 5$)		Reproducibility (RSD%, $n = 5$)	
				50 ng g^{-1}	250 ng g^{-1}	50 ng g^{-1}	250 ng g^{-1}				50 ng g^{-1}	250 ng g^{-1}	50 ng g^{-1}	250 ng g^{-1}
MMP	27	25	50	9	4	11	14	31	10	25	10	13	15	11
MEP	25	2.5	5	4	5	11	13	33	2.5	5	11	8	10	12
DMP	41	2.5	5	18	15	11	17	42	2.5	5	8	7	10	20
MBP	16	1	2.5	3	11	25	14	28	1	5	19	9	25	9
MBzP	10	2.5	5	9	10	17	11	19	2.5	5	7	6	23	9
DEP	76	25	50	16	8	13	20	47	25	50	11	11	16	13
MEHP	11	2.5	5	21	15	9	21	6	10	25	8	10	19	22
MOP	11	5	10	11	16	21	19	7	10	25	5	8	7	22
BzBP	61	2.5	10	23	5	7	4	44	5	10	8	21	22	25
DBP	60	25	50	-	10	-	12	52	100	250	-	23	-	21
DEHP	11	50	100	-	24	-	25	27	50	100	-	21	-	20

R_{app} was evaluated at 50 ng g^{-1} (d.w.) for all compounds except DBP, DEHP and DOP, which were evaluated at 250 ng g^{-1} (d.w.). R_{app} values in both types of species were as much as 76% for DEP and below 5% for DOP. Since the R_{app} values obtained for DOP were very low, this method did not correctly determine this compound in the samples and was not included in our analyses. The R_{app} values for the other compounds ranged from 10 to 76% for sole (with ME values up to -80%) and from 6 to 52% for salmon (with ME values up to -75%). We also tested using an isotopically labelled internal standard (DEHP-d_4) to correct the ME but this was discarded because the results did not improve for most of the studied compounds.

To account for the high ME values the use of matrix-matched calibration curves was proposed for quantification. These curves were obtained experimentally by spiking fish at ten different concentrations (from 1 to 1,000 ng g^{-1} (d.w.)). Two linear ranges were established for each compound with r^2 higher than 0.990. MLOQs were defined as the lowest point of the calibration curves, while MLODs were defined as the concentration at which the signal-to-noise ratio was equal to three for the response of the quantification ion. For most compounds, MLODs ranged from 1 to 25 ng g^{-1} (d.w.) and MLOQs ranged from 2.5 to 50 ng g^{-1} (d.w.) for both sole and salmon samples. For the salmon samples, some MLOD values were slightly higher due to the high lipidic content of the samples. These MLOD values seem to be in agreement with those in the literature, which for phthalate diesters ranged from 0.01 to 10 ng g^{-1} (fresh weight) [33] when the samples were extracted with QuEChERS and analysed by GC-MS/MS. Little information is available about MLODs for phthalate monoesters in fish samples. However, Blair *et al.* [21] obtained MLODs below 1 ng g^{-1} (fresh weight) for fish tissue by LC-MS/MS. No specific MLOD or MLOQ values could be found for studies that simultaneously determine phthalate diesters and their metabolites in fish muscle.

Finally, to evaluate repeatability (intra-day, *n = 5*) and reproducibility (day-to-day, *n = 5*), both of them expressed as relative standard deviation (RSD%), salmon and sole samples were spiked at two concentration levels (50 ng g^{-1} (d.w.) and 250 ng g^{-1} (d.w.)), except for DBP, DEHP and DOP, which were only evaluated at 250 ng g^{-1} since these compounds had MLOQs equal to or higher than 50 ng g^{-1} (d.w.). All repeatability and reproducibility values were equal to or lower than 25%.

3.6. Application to commercial seafood samples

The occurrence of the target compounds was then evaluated in several seafood species. Three species with low fat contents (shrimp (*Aristeus antennatus*), sole (*Solea*

solea) and squid (*Loligo vulgaris)*) and two fatty species (mackerel (*Scomber scombrus*) and salmon (*Salmo salar*)) were bought from local fish markets. Each sample was analysed by triplicate. It should be noted that ME and R_{app} values were calculated for shrimp, squid and mackerel species to confirm that the validated method could be applied to those seafood species. LC-HRMS-extracted ion chromatograms of a salmon and a shrimp sample are shown in **Figure 2**.

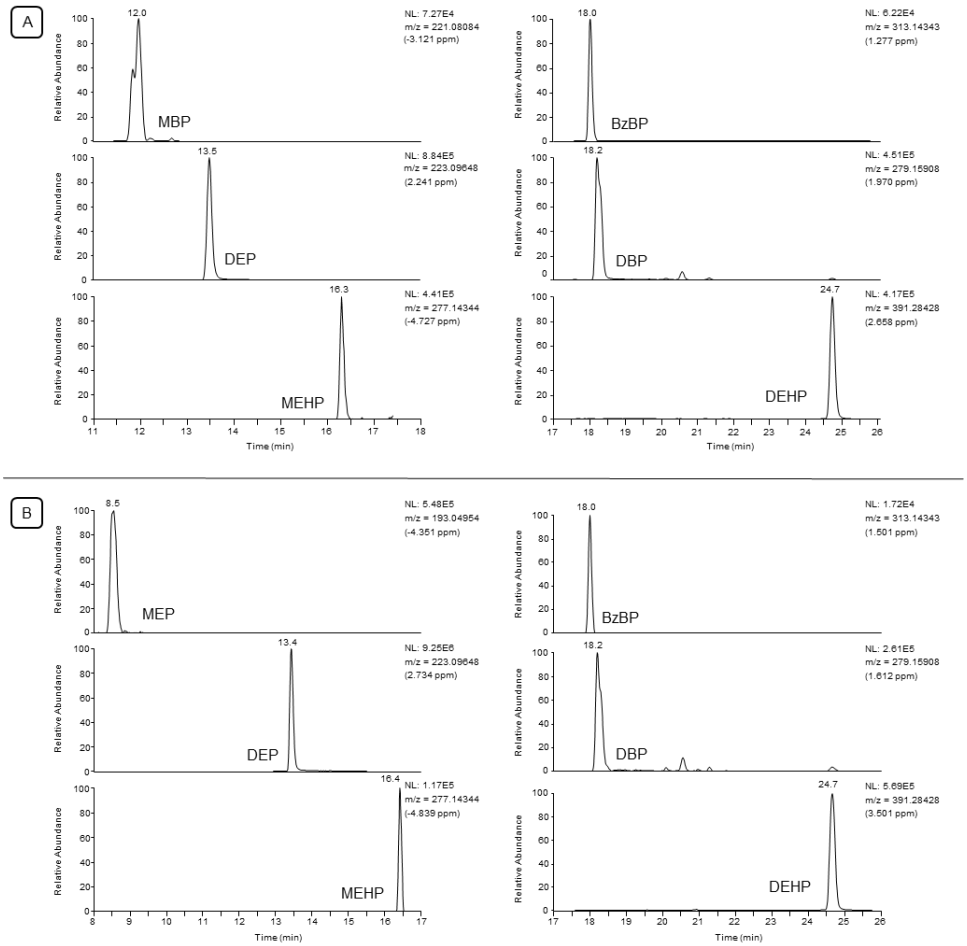


Figure 2. LC-HRMS extracted ion chromatogram and mass error in ppm of a salmon sample (A) and a shrimp sample (B).

Four phthalate diesters and three of their metabolites were detected and quantified with a mass error below 5 ppm. **Table 4** shows the concentration of the target compounds found in all samples. For two samples, the concentrations for DEP and DEHP were above the upper limit of the calibration curve. The extracts were therefore diluted in order to properly quantify both compounds.

Table 4. Concentrations (ng g^{-1} (d.w.)) and relative standard deviation (RSD%, *n = 3*) of phthalate diesters and phthalate monoesters found in different seafood species with low and high lipid content.

Compound	Seafood with low lipid content (<10%)			Seafood with high lipid content (>10%)	
	Shrimp (2% lipid) (*Aristeus antennatus*)	Sole (6% lipid) (*Solea solea*)	Squid (6% lipid) (*Loligo vulgaris)*	Mackerel (17% lipid) (*Scomber scombrus*)	Salmon (25% lipid) (*Salmo salar*)
Phthalate diesters					
DEP	3393 (4)	60 (9)	n.d.	412 (11)	202 (2)
BzBP	<MLOQ	n.d.	n.d.	46 (13)	161 (16)
DBP	<MLOQ	n.d.	n.d.	n.d.	373 (11)
DEHP	453 (11)	n.d.	117 (14)	225 (14)	1562 (13)
Phthalate monoesters					
MEP	260 (1)	n.d.	30 (5)	n.d.	n.d.
MBP	n.d.	n.d.	<MLOQ	6 (16)	10 (15)
MEHP	7 (14)	<MLOQ	47 (14)	144 (3)	162 (14)

n.d.: not detected (<MLOD)
<MLOQ: Below method limit of quantification

Briefly, DEP (from 60 to 3393 ng g^{-1} (d.w.)) and DEHP (from 117 to 1562 ng g^{-1} (d.w.)) were found at the highest concentrations in both fatty and low-fat species. These values seem to agree with those of other studies in the literature. Xu *et al.* [33] found DEHP concentrations between 66 and 763 ng g^{-1} in 60 random fish species bought from local markets in China, while Adeniyi *et al.* [22] reported DEP concentrations between 310 and 860 ng g^{-1} in *Tilapia*, *Chrysichthys* and *Synodontis*. BzBP and DBP were also determined at concentrations between MLOD and 161 ng g^{-1} (d.w.), and between MLOD and 373 ng g^{-1} (d.w.), respectively. These values for BzBP seem to be much higher than those found in previous studies. The concentrations reported usually only reach

1.6 ng g^{-1} [45] or 8 ng g^{-1} [27], though Valton *et al.* [19] reported concentrations of 155 ng g^{-1} (d.w.) in *Rutilus rutilus*.

With regard to phthalate monoesters in both fatty and low-fat species, MEP, MBP and MEHP were quantified up to 260, 10 and 162 ng g^{-1} (d.w.), respectively. Although MBP levels were similar to those obtained in other studies (up to 61 ng g^{-1} [21]), MEP and MEHP concentrations were higher than those reported (up to 25 ng g^{-1} [34]).

4. Conclusions

In this study, an analytical method consisting of PLE followed by LC-HRMS was developed for simultaneously determining five phthalate diesters and six phthalate monoesters in seafood. To do so, several extraction parameters were optimised. Moreover, three different clean-up approaches were tested to reduce the high ion suppression encountered in the samples and a SPE using Bond Elut Plexa cartridges was finally selected.

Two different ionisation sources (HESI and APCI) were also evaluated. It is interesting to point out that both sources yielded similar instrumental limits for most compounds. However, the ion enhancement obtained when using APCI was much higher than 100% for most compounds, hence its use was rejected.

The method was successfully validated for two kinds of seafood according to their lipid content, yielding good reproducibility and sensitivity values. MLOD were 1 – 25 ng g^{-1} (d.w.) for both types of seafood, which are comparable to those found in the literature. Repeatability and reproducibility values were all equal to or lower than 25%.

Then, five species of seafood were analysed. Four phthalate diesters and three phthalate monoesters were successfully quantified in several samples at concentrations between 7 ng g^{-1} (d.w.) (MEHP) and 3393 ng g^{-1} (d.w.) (DEP) in the low-fat content species and between 4 ng g^{-1} (d.w.) (MBP) and 1562 ng g^{-1} (d.w.) (DEHP) in the high-fat species. These results confirm that this new method is suitable for the simultaneous analysis of both phthalate diesters and monoesters, also proving the usefulness of LC and HRMS for that purpose.

Acknowledgements

The authors would like to thank the support of the Secretaria d'Universitats i Recerca del Departament d'Empresa i Coneixement de la Generalitat de Catalunya, the European Union (EU) and the European Social Fund (ESF) (2017FI_B_01116).

This work (CTQ2017-84373-R) has been financed by the Spanish Ministry of Economy and Competitiveness and the European Regional Development Fund (AEI/FEDER/UE).

References

[1] A.K. Sakhi, A. Sabaredzovic, E. Cequier, C. Thomsen, Phthalate metabolites in Norwegian mothers and children: Levels, diurnal variation and use of personal care products, Sci. Total Environ. 599–600 (2017) 1984–1992.

[2] S. Net, R. Sempéré, A. Delmont, A. Paluselli, B. Ouddane, Occurrence, fate, behavior and ecotoxicological state of phthalates in different environmental matrices, Environ. Sci. Technol. 49 (2015) 4019–4035.

[3] M. Clara, G. Windhofer, W. Hartl, K. Braun, M. Simon, O. Gans, C. Scheffknecht, A. Chovanec, Occurrence of phthalates in surface runoff, untreated and treated wastewater and fate during wastewater treatment, Chemosphere. 78 (2010) 1078–1084.

[4] D. Eerkes-Medrano, R.C. Thompson, D.C. Aldridge, Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritisation of research needs, Water Res. 75 (2015) 63–82.

[5] H.S. Auta, C.U. Emenike, S.H. Fauziah, Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions, Environ. Int. 102 (2017) 165–176.

[6] Ministerio de Agricultura Pesca y Alimentación, Informe del Consumo Alimentario en España 2018, (2019). https://www.mapa.gob.es/es/alimentacion/temas/consumo-y-comercializacion-y-distribucion-alimentaria/panel-de-consumo-alimentario/ultimos-datos/default.aspx (accessed September 16, 2021).

[7] P. Ventrice, D. Ventrice, E. Russo, G. de Sarro, Phthalates: European regulation, chemistry, pharmacokinetic and related toxicity, Environ. Toxicol. Pharmacol. 36 (2013) 88–96.

[8] M.H. Asghari, S. Saeidnia, M. Abdollahi, A review on the biochemical and molecular mechanisms of phthalate-induced toxicity in various organs with a focus on the reproductive system, Int. J. Pharmacol. 11 (2015) 95–105.

[9] J. Mathieu-Denoncourt, S.J. Wallace, S.R. de Solla, V.S. Langlois, Plasticizer endocrine disruption: Highlighting developmental and reproductive effects in mammals and non-mammalian aquatic species, Gen. Comp. Endocrinol. 219 (2015) 74–88.

[10] T. Ye, M. Kang, Q. Huang, C. Fang, Y. Chen, H. Shen, S. Dong, Exposure to DEHP and MEHP from hatching to adulthood causes reproductive dysfunction and endocrine disruption in marine medaka (Oryzias melastigma), Aquat. Toxicol. 146 (2014) 115–126.

[11] W. Zhai, Z. Huang, L. Chen, C. Feng, B. Li, T. Li, Thyroid endocrine disruption in zebrafish larvae after exposure to mono-(2-ethylhexyl) phthalate (MEHP), PLoS one. 9 (2014) 1–6.

[12] J. Mathieu-Denoncourt, S.J. Wallace, S.R. de Solla, V.S. Langlois, Influence of lipophilicity on the toxicity of bisphenol A and phthalates to aquatic organisms, Bull. Environ. Contam. Toxicol. 97 (2016) 4–10.

[13] European Food Safety Agency (EFSA), Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission related to di-butylphthalate (DBP) for use in food contact materials, EFSA J. 242 (2005) 1–17

[14] European Food Safety Agency (EFSA), Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission related to butylbenzylphthalate (BBP) for use in food contact materials, EFSA J. 241 (2005) 1–14.

[15] European Food Safety Agency (EFSA), Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission related to di(2-ethylhexyl) phthalate (DEHP) for use in food contact materials, EFSA J. 243 (2005) 1–20.

[16] European Food Safety Agency (EFSA), Draft update of the risk assessment of di-butylphthalate (DBP), butyl-benzyl-phthalate (BBP), bis(2-ethylhexyl)phthalate (DEHP), di-isononylphthalate (DINP) and di-isodecylphthalate (DIDP) for use in food contact materials, (2019). https://www.efsa.europa.eu/en/consultations/call/190221 (accessed September 16, 2019).

[17] European Parliament, Directive 2005/84/EC of the European Parliament and the Council, Off. J. Eur. Union 344 (2005) 40–43.

[18] Consumer Product Safety Comission (CPSC), CPSC Prohibits Certain Phthalates in Children's Toys and Child Care Products, (2017). https://www.cpsc.gov/content/cpsc-prohibits-certain-phthalates-in-children's-toys-and-child-care-products (accessed September 16, 2019).

[19] A.S. Valton, C. Serre-Dargnat, M. Blanchard, F. Alliot, M. Chevreuil, M.J. Teil, Determination of phthalates and their by-products in tissues of roach (Rutilus rutilus) from the Orge river (France), Environ. Sci. Pollut. Res. 21 (2014) 12723–12730.

[20] J. Jiang, D. Mu, M. Ding, S. Zhang, H. Zhang, J. Hu, Simultaneous determination of primary and secondary phthalate monoesters in the Taihu Lake: Exploration of sources, Chemosphere. 202 (2018) 17–24.

[21] J.D. Blair, M.G. Ikonomou, B.C. Kelly, B. Surridge, F.A.P.C. Gobas, Ultra-trace determination of phthalate ester metabolites in seawater, sediments, and biota from an urbanized marine inlet by LC/ESI-MS/MS, Environ. Sci. Technol. 43 (2009) 6262–6268.

[22] A.A. Adeniyi, O.O. Okedeyi, K.A. Yusuf, Flame ionization gas chromatographic determination of phthalate esters in water, surface sediments and fish species in the Ogun river catchments, Ketu, Lagos, Nigeria, Environ. Monit. Assess. 172 (2011) 561–569.

[23] J. Wang, G. Chen, P. Christie, M. Zhang, Y. Luo, Y. Teng, Occurrence and risk assessment of phthalate esters (PAEs) in vegetables and soils of suburban plastic film greenhouses, Sci. Total Environ. 523 (2015) 129–137.

[24] M. Del Bubba, C. Ancillotti, L. Checchini, D. Fibbi, D. Rossini, L. Ciofi, L. Rivoira, C. Profeti, S. Orlandini, S. Furlanetto, Determination of phthalate diesters and monoesters in human milk and infant formula by fat extraction, size-exclusion chromatography clean-

up and gas chromatography-mass spectrometry detection, J. Pharm. Biomed. Anal. 148 (2018) 6–16.

[25] G.M.B. Louis, M.M. Smarr, L. Sun, Z. Chen, M. Honda, W. Wang, R. Karthikraj, J. Weck, K. Kannan, Endocrine disrupting chemicals in seminal plasma and couple fecundity, Environ. Res. 163 (2018) 64–70.

[26] J. Li, H. Zhao, W. Xia, Y. Zhou, S. Xu, Z. Cai, Nine phthalate metabolites in human urine for the comparison of health risk between population groups with different water consumptions, Sci. Total Environ. 649 (2019) 1532–1540.

[27] T. Fierens, K. Servaes, M. Van Holderbeke, L. Geerts, S. De Henauw, I. Sioen, G. Vanermen, Analysis of phthalates in food products and packaging materials sold on the Belgian market, Food Chem. Toxicol. 50 (2012) 2575–2583.

[28] B. Aghvami, H. Faraji, H. Shahbaazi, Chemometrics-Assisted QuEChERS Method Coupled with HPLC-Diode Array Detection for the Determination of Phthalate Esters in Food-Grade Plastics, J. Anal. Chem. 73 (2018) 1111–1117.

[29] W. Jia, X. Chu, Y. Ling, J. Huang, J. Chang, Analysis of phthalates in milk and milk products by liquid chromatography coupled to quadrupole Orbitrap high-resolution mass spectrometry, J. Chromatogr. A. 1362 (2014) 110–118.

[30] D. Xu, X. Deng, E. Fang, X. Zheng, Y. Zhou, L. Lin, L. Chen, M. Wu, Z. Huang, Determination of 23 phthalic acid esters in food by liquid chromatography tandem mass spectrometry, J. Chromatogr. A. 1324 (2014) 49–56.

[31] C. Deng, C. Li, J. Zhou, Q. Wang, H. Shao, J. Wang, Y. Wu, H. Zhang, M. Gao, X. Xu, F. Jin, Simultaneous Determination of Eight Monoalkyl Phthalate Esters in Porcine Tissue by Solid-Phase Extraction and Liquid Chromatography-Tandem Mass Spectrometry, J. Agric. Food Chem. 67 (2019) 7167–7173.

[32] P. Liu, H. Chen, G. Gao, Z. Hao, C. Wang, G. Ma, Y. Chai, L. Zhang, X. Liu, Occurrence and Residue Pattern of Phthalate Esters in Fresh Tea Leaves and during Tea Manufacturing and Brewing, J. Agric. Food Chem. 64 (2016) 8909–8917.

[33] Y. Xu, R. Weng, Y. Lu, X. Wang, D. Zhang, Y. Li, J. Qiu, Y. Qian, Evaluation of phthalic acid esters in fish samples using gas chromatography tandem mass spectrometry with simplified QuEChERS technique, Food Anal. Methods. 11 (2018) 3293–3303.

[34] X. Hu, Y. Gu, W. Huang, D. Yin, Phthalate monoesters as markers of phthalate contamination in wild marine organisms, Environ. Pollut. 218 (2016) 410–418.

[35] O. Ros, J.K. Izaguirre, M. Olivares, C. Bizarro, M. Ortiz-Zarragoitia, M.P. Cajaraville, N. Etxebarria, A. Prieto, A. Vallejo, Determination of endocrine disrupting compounds and their metabolites in fish bile, Sci. Total Environ. 536 (2015) 261–267.

[36] L.M. Morrison, J.B. Renaud, L. Sabourin, M.W. Sumarah, K.K.C. Yeung, D.R. Lapen, High-throughput quantitation of neonicotinoids in lyophilized surface water by LC-APCI-MS/MS, J. AOAC Int. 101 (2018) 1940–1947.

[37] Y. Ohba, T. Nakajima, M. Kanda, H. Hayashi, Y. Matsushima, Y. Nakagawa, H. Koike, C. Nagano, K. Sekimura, K. Otsuka, T. Sasamoto, T. Hashimoto, Simultaneous determination of nine acaricides and two metabolites in comb honey by LC/MS/MS., Food Addit. Contam. Part A. 35 (2018) 2375–2386.

[38] S. Hagenhoff, H. Hayen, LC/MS analysis of vitamin D metabolites by dielectric barrier discharge ionization and a comparison with electrospray ionization and atmospheric pressure chemical ionization, Anal. Bioanal. Chem. 410 (2018) 4905–4911.

[39] C. Wang, P.R. Gardinali, Comparison of multiple API techniques for the simultaneous detection of microconstituents in water by on-line SPE-LC-MS/MS, J. Mass Spectrom. 47 (2012) 1255–1268.

[40] M. Núñez, F. Borrull, E. Pocurull, N. Fontanals, Pressurised liquid extraction and liquid chromatography-high resolution mass spectrometry to determine high-intensity sweeteners in fish samples, J. Chromatogr. A. 1479 (2017) 32–39.

[41] P. Arbeláez, F. Borrull, R.M. Marcé, E. Pocurull, Simultaneous determination of drugs of abuse and their main metabolites using pressurized liquid extraction and liquid chromatography-tandem mass spectrometry, Talanta. 125 (2014) 65–71.

[42] L. Vallecillos, E. Pocurull, F. Borrull, Influence of pre-treatment process on matrix effect for the determination of musk fragrances in fish and mussel, Talanta. 134 (2015) 690–698.

[43] M. Núñez, F. Borrull, N. Fontanals, E. Pocurull, Different sample treatments for the determination of ICM-XR in fish samples followed by LC-HRMS, Talanta. 163 (2017) 1–7.

[44] A. Schecter, M. Lorber, Y. Guo, Q. Wu, S.H. Yun, K. Kannan, M. Hommel, N. Imran, L.S. Hynan, D. Cheng, J.A. Colacino, L.S. Birnbaum, Phthalate concentrations and dietary exposure from food purchased in New York state, Environ. Health Perspect. 121 (2013) 473–479.

[45] A.K. Sakhi, I.T.L. Lillegaard, S. Voorspoels, M.H. Carlsen, E.B. Løken, A.L. Brantsæter, M. Haugen, H.M. Meltzer, C. Thomsen, Concentrations of phthalates and bisphenol A in Norwegian foods and beverages and estimated dietary exposure in adults, Environ. Int. 73 (2014) 259–269.

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

3.2.2. Simple method for determining phthalate diesters and their metabolites in seafood species using QuEChERS extraction and liquid chromatography-high resolution mass spectrometry

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

SIMPLE METHOD FOR DETERMINING PHTHALATE DIESTERS AND THEIR METABOLITES IN SEAFOOD SPECIES USING QUECHERS EXTRACTION AND LIQUID CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY

Míriam Hidalgo-Serrano, Francesc Borrull, Rosa M. Marcé, Eva Pocurull

Universitat Rovira i Virgili, Department of Analytical Chemistry and Organic Chemistry, Marcel·lí Domingo s/n, Tarragona 43007, Spain

---

Abstract

In this article we describe a new and simple analytical method based on the Quick, Easy, Cheap, Effective, Rugged and Safe technique followed by dispersive solid-phase extraction clean-up with C_{18} and Lipifiltr® and LC-HRMS for simultaneously extracting six phthalate diesters and six of their metabolites (phthalate monoesters) from highly consumed seafood species. The method was validated for seafood with high and low lipid contents. Apparent recoveries were up to 79% for all compounds. Matrix effect values ranged from -8 to -48% for all compounds in both types of matrices. Method limits of detection were 1–25 ng g^{-1} dry weight (d.w.) for most compounds. Five seafood species were analysed using this method, and several phthalate diesters and monoesters were successfully quantified. Phthalate diesters were found at concentrations of up to 982 ng g^{-1} (d.w.) and phthalate monoesters were found at concentrations of up to 178 ng g^{-1} (d.w.).

Keywords: *Seafood, Phthalate diesters, Phthalate monoesters, QuEChERS, Liquid chromatography, High-resolution mass spectrometry*

---

1. Introduction

The analysis of biological samples such as seafood is known to be highly complex due to the heterogeneous nature of the matrix. This usually leads to the need for tedious, time-consuming methods that require multiple steps and large amounts of solvents. For this reason, the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) technique has received significant recognition in the last few years [1,2]. QuEChERS methods are fast and simple extraction methods consisting of the initial extraction with acetonitrile of a sample with water content. This is followed by a salting-out step, which causes the partitioning of the two liquid phases. A clean-up step, which is usually dispersive solid-phase extraction (dSPE), is then added to remove interferences. Originally developed to extract pesticides from fruits and vegetables [3], this technique has broadened its range of application to various analytes and matrices [1]. In recent years, numerous QuEChERS-based methods have been developed for extracting pharmaceuticals, endocrine disruptors, pesticides, hormones, mycotoxins and polycyclic aromatic hydrocarbons, etc., mainly in food [4–6] but also in biological fluids [7,8] and environmental samples [9]. Specifically, several QuEChERS methods have focused on determining contaminants in fish and shellfish [10–12].

Among the contaminants easily found in seafood, phthalate diesters and monoesters are of increasing concern due to their widespread use in everyday products. Phthalate diesters are high production volume chemicals that are mainly used as plasticisers and are found, for example, in personal care products, food packaging and medical equipment [13]. These compounds are found ubiquitously in the environment since they are not covalently bound to the polymer structure of the products and can easily reach the air and water. Moreover, since phthalate diesters are known endocrine disruptors, prolonged exposure to them can damage the reproductive system of affected organisms and impair their development [14]. Phthalate diesters are therefore considered industrial contaminants of emerging concern and some of them have been subjected to numerous regulations and limitations [15–17].

Phthalate diesters eventually reach living organisms, where they are metabolised into phthalate monoesters [18]. Phthalate monoesters can also reach the environment through sources such as microbial or abiotic degradation in the soil, sediment or water of the corresponding phthalate diesters [19]. A small number of studies that evaluated the toxicity of phthalate monoesters have reported several endocrine-disrupting effects that may result in reproductive impairment [20].

Taking into account the widespread occurrence of phthalate diesters and monoesters in the environment, and more specifically in surface waters [21], these compounds are likely to be found in aquatic organisms. Several extraction techniques such as ultrasound extraction [22,23] and pressurised liquid extraction [24,25] have therefore been used in recent years, followed by either gas chromatography or liquid chromatography (LC), to control the levels of these compounds in seafood. Interestingly, and to the best of our knowledge, only a few QuEChERS methods have been developed for determining phthalate diesters or monoesters in seafood [26–28].

Taking all this information into account, the main objective of this study is to develop a new and simple analytical method based on the QuEChERS technique for simultaneously extracting six phthalate diesters and six phthalate monoesters in seafood. We also tested several clean-up strategies to reduce the high matrix effect (ME) these samples usually present. As well as more traditional clean-up methods, we also tested the use of a novel sorbent named Lipifiltr®. Lipifiltr® are push-through purification cartridges specifically developed to remove lipids from fatty samples following QuEChERS extraction [29] but which have not yet been tested for determining phthalate diesters or monoesters in seafood samples. Finally, the extracts were analysed by LC coupled to high resolution mass spectrometry (HRMS).

2. Materials and methods

2.1. Reagents and standards

Diethyl phthalate (DEP), monoethyl phthalate (MEP), monomethyl phthalate (MMP) and monooctyl phthalate (MOP) were purchased from LGC (Teddington, Middlesex, UK). Benzyl butyl phthalate (BzBP), bis(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), dimethyl phthalate (DMP), di-n-octyl phthalate (DOP), mono(2-ethylhexyl) phthalate (MEHP), monobenzyl phthalate (MBzP) and monobutyl phthalate (MBP) were purchased from Sigma-Aldrich (St. Louis, USA). Individual stock solutions of all standards were prepared in methanol at 1000 mg L^{-1} and stored at -23 ^{o}C.

Ultrapure water was produced with an ultrapure water purification system from Merck Millipore (Darmstadt, Germany). Acetonitrile (ACN) and water of MS grade for the mobile phase and hydrochloric acid (HCl) were provided by Scharlab (Barcelona, Spain). ACN, methanol and acetone of HPLC grade were purchased from J.T. Baker (Deventer, The Netherlands). Acetic acid (CH_3COOH) was purchased from Sigma-Aldrich.

For the clean-up strategies, Z-Sep$^+$ tubes as well as bulk primary and secondary amine (PSA), Florisil and ENVI-Carb were obtained from Sigma-Aldrich. EMR-lipid tubes and Bond Elut PPL cartridges were purchased from Agilent (Santa Clara, California, USA). Bulk C_{18} was purchased from Scharlab. LiChrolut EN cartridges were purchased from Merck Millipore, while Lipifiltr® push-through cartridges were purchased from UCT (Levittown, PA, USA).

2.2. Sampling

Specimens of five seafood species were purchased from several fish markets in Tarragona, Spain: *Aristeus antennatus* (shrimp), *Loligo vulgaris* (squid), *Salmo salar* (salmon), *Scomber scombrus* (mackerel), and *Solea solea* (sole). All the fish were immediately filleted and the shells of the shrimps were removed before the samples were frozen. The samples were then lyophilised using the Genevac miVac Duo sample concentrator with a SpeedTrap freeze-drying system (Ipswich, UK). Finally, the samples were ground and sieved (500 µm) to obtain a homogeneous powder. Since the efficiency of the extraction is known to vary depending on the lipidic content of the samples, two types of seafood were chosen to develop, optimise and validate the method: sole, to represent a species with a low lipid content (less than 10%); and salmon, to represent a species with a high lipid content (more than 10%).

2.3. QuEChERS extraction and clean-up

A total of 1 g (d.w.) of lyophilised sample was weighed into custom 50 mL glass centrifuge tubes from Serviquimia (Constantí, Spain) and 10 mL of ultrapure water at pH 2 was added to the tube before the mixture was vortexed for 1 min. Then, 10 mL of ACN was added and the tube was vortexed again for 1 min. An extraction salt packet (Scharlab) for European standard method EN 15662:2019 was then added to the mixture. This packet contained 4 g of anhydrous magnesium sulphate, 1 g of sodium chloride, 0.5 g of sodium citrate dibasic sesquihydrate, and 1 g of sodium citrate tribasic dihydrate. The sample tube was shaken vigorously by hand, vortexed for 5 min and finally centrifuged at 4000 rpm for 5 min. To determine the phthalate diesters, 5 mL of supernatant (ACN layer) was transferred to a custom 15 mL glass centrifuge tube (Serviquimia) containing the sorbent from one Lipifiltr® push-through cartridge for a dispersive solid-phase extraction (dSPE) clean-up. To determine the phthalate monoesters, the remaining 5 mL of the ACN layer was transferred to a 15 mL glass centrifuge tube containing 200 mg of bulk C_{18} sorbent (Scharlab) for dSPE clean-up. Both tubes were vortexed for 3 min and centrifuged under the same conditions as before. The

supernatants were evaporated to a final volume of approximately 200 µL with the miVac Duo sample concentrator and reconstituted to 1 mL with ultrapure water. Both extracts were analysed by LC-HRMS.

2.4. Liquid chromatography coupled to high resolution mass spectrometry

Chromatographic analyses were performed using an Accela 1250 UHPLC system with an Accela Autosampler and a quaternary pump coupled to an Exactive Orbitrap™ mass spectrometer equipped with a heated electrospray ionisation source (HESI) and an HCD collision cell (Thermo Scientific, Bremen, Germany). Separation was carried out with an Ascentis Express C_{18} fused-core column (100 mm x 2.1 mm i.d., 2.7 µm particle size) from Sigma-Aldrich at 30 ºC.

The LC-HRMS method was developed in a previous paper [25]. Briefly, ultrapure water 0.1% CH_3COOH (solvent A) and MeOH (solvent B) were the mobile phases, which started isocratic at 13% of B for 5 min. This increased to 80% in 13 min and then to 90% in 1 min. Finally, it increased to 100% in 7 min and was held constant for 3 min before returning to the initial conditions in 1 min. The flow rate was 300 µL min^{-1} and the injection volume 20 µL, respectively. For the HRMS measurements, six time windows were used: three in negative mode (0.0-9.5, 11.5-12.8 and 15.0-17.0 min) and three in positive mode (9.5-11.5, 12.8-15.0 and 17.0-33.0 min). Two scan events took place in each time window: a full scan (at 50,000 FWHM with 250 ms of injection time) and a fragmentation scan (at 10,000 FWHM with 50 ms of injection time) using a collision voltage of 10 eV in the HCD cell. Spray voltage was set at ±4 kV, capillary voltage was set at ±20 V, the tube lens voltage was set at ±55 V, and the skimmer voltage was set at ±16 V. The sheath gas flow rate was set at 50 A.U. and the auxiliary gas flow rate was set at 15 A.U.. The capillary and heater temperatures were set at 350 ºC. All ions were measured with a mass extraction window of 5 ppm. The fragment ions selected for confirmation purposes are shown in **Table S1**.

2.5. Blank quality control

To minimise sample contamination due to the presence of the target compounds in the laboratory environment, we performed several actions during the experimental process. The following protocol has already been described in a previous study [25].

Briefly, all the equipment used was, as far as possible, glassware, which was rinsed with acetone and methanol before use. The 50 and 15 mL glass centrifuge tubes were

custom made for the QuEChERS extraction and dSPE clean-up procedures, respectively. All the spiked samples were covered by a perforated piece of plastic-free aluminium foil and left to evaporate overnight under a fume cupboard.

Full procedural blanks were frequently performed without sample following the same analytical procedure as described for the seafood. In this case, six of the target compounds were quantified in the procedural blanks with relative standard deviation values (RSD%, *n = 10*) of less than 6%. Since the procedural blanks had good repeatability, the values were subtracted from the analysed samples.

3. Results and Discussion

3.1. QuEChERS extraction

To achieve the most efficient extraction, several QuEChERS parameters, such as the extraction salts, the vortex time after salt addition, the solvent volume and the pH of the water-extracting solution, were optimised. Moreover, it is known that the lipid percentage of the sample can affect the efficiency of the extraction. Two types of seafood with different lipidic content were therefore selected for the optimisation of the extraction: sole as a species of seafood with low lipid content, and salmon as a species of seafood with high lipid content.

For all tests, non-spiked fish samples were analysed to subtract the response of the compounds present in the samples. Apparent recoveries (R_{app}) were evaluated to select the best QuEChERS method by comparing the signal of the analytes after the extraction with the response of the analytes in an external standard calibration curve. To do so, ultrapure water was initially added to 1 g of sample spiked at 1000 ng g^{-1} (d.w.) in a glass centrifuge tube. The tube was vortexed for 1 min, ACN was added and the mixture was vortexed again. An extraction salt packet was added to the tube, which was then shaken by hand, vortexed again and centrifuged for 5 min at 4000 rpm. In the optimisation process, 1 mL of the ACN supernatant was transferred to a volumetric flask and taken up to 10 mL with ultrapure water before the injection in order to avoid high ME values in the final extracts.

Extraction salt mixtures for three extraction methods were evaluated: the European standard method [30], the original QuEChERS method [3], and the AOAC official method [31]. To test the extraction salts, other significant parameters were set based on our previous experiment [32] (the vortex time was set at 3 min, while the ultrapure water and

ACN volumes were 10 mL). The EN method yielded the highest R_{app} values. For sole samples, R_{app} ranged from 79 to 120% except for MEHP, MOP and BzBP, whose R_{app} were 57, 52 and 51%, respectively). For salmon samples, the R_{app} were lower (as we expected due to their high lipid content), ranging from 59 to 114% for most compounds. The lowest values (24–42%) were obtained for MBP, MBzP, MEHP and MOP. Since no significant differences were observed between the original method and the AOAC method, we selected the EN extraction salt packets. The R_{app} obtained for sole with the three extraction methods are shown in **Figure S1**.

Vortex times of 1, 3 and 5 min were then compared. Although no significant differences were observed for sole samples, the R_{app} of MEHP and MOP for salmon samples were above 40% only when the vortex time was 5 min. Ultrapure water and ACN volumes of 5, 10 and 15 mL were also evaluated. Note that the volume used for hydration was always the same as the volume used for extraction. The best overall results were obtained adding 10 mL of ultrapure water and extracting with 10 mL of ACN. For both types of sample, using 5 mL of each solvent not only made it difficult to separate the two phases after the salting-out step but also decreased the R_{app} of DEHP and DOP by more than 20–30%. Moreover, for salmon samples, using 15 mL of each solvent yielded R_{app} of less than 30% for both MEHP and MOP. The optimal solvent volume selected was therefore 10 mL. Taking into account other QuEChERS methods for extracting phthalate diesters from food matrices and the pK_a of the phthalate monoesters (from 3.32 to 3.38), we evaluated the ultrapure water at different pH values in order to improve the R_{app}. We therefore tested pH of 2 and 6 [33,34] and compared the results to those obtained with ultrapure water at a pH of 8. No significant improvements were observed when we used a pH of 6. However, as R_{app} for MEHP and MOP were slightly better with a pH of 2, this was the optimal pH chosen for QuEChERS extraction.

3.2. Clean-up strategies

Due to the complexity of the samples and the high ion suppression observed, R_{app} as low as 47% were obtained for some compounds even when the QuEChERS extract was diluted. Several clean-up strategies were therefore evaluated in order to decrease the ME after the extraction process, improve the R_{app} values, and preconcentrate the sample. To evaluate the efficiency of the clean-up, ME values were calculated by spiking blank samples after the clean-up procedure and comparing the responses of the analytes with those of a standard solution. Because of its higher lipidic content, salmon was chosen to optimise the clean-up process.

As we mentioned earlier, in QuEChERS methods extraction is usually followed by clean-up using dSPE. Again taking into account previous QuEChERS methods for extracting phthalate diesters from food matrices and methods for extracting other families of compounds from fish, we tested several sorbents separately: 200 mg of PSA [27,34], 200 mg of C_{18} [12,33], 200 mg of Florisil [32], Z-Sep+ tubes [35], EMR-lipid tubes [36] and 50 mg of ENVI-Carb [37]. Retention of the target compounds in the sorbents used to clean the extracts was calculated by comparing the response of the compounds in the final extract for ACN spiked before the clean-up step with the response obtained for ACN spiked after the clean-up step. To do so, 10 mL of ACN was transferred to a 15 mL glass centrifuge tube containing the dSPE sorbent. The mixture was vortexed for 3 min and centrifuged at 4000 rpm for 5 min. The extract was then evaporated to ~200 µL, reconstituted to 1 mL with ultrapure water, and injected into the LC-HRMS system. All retention values are shown in **Figure S2**.

Phthalate monoesters were almost completely retained in PSA, Florisil and Z-Sep$^+$ (98–100%) while retention in EMR-lipid ranged from 50 to 78%. We therefore rejected these sorbents. Retention (<30%) was low when either C_{18} or ENVI-Carb sorbents were used. The retention of phthalate diesters for C_{18} was below 5% for all compounds except BzBP and DBP, for which it was 54 and 48%, respectively. Similarly, only DOP was retained in the ENVI-Carb sorbent by more than 69%. We also carried out procedural blanks using both sorbents separately with ACN. When ENVI-Carb was used, the concentration of DEP found was higher than 1500 ng g^{-1}. Such high blank concentrations would render the procedure unrepeatable and could easily result in false positives or false negatives. Taking into consideration these results, we provisionally rejected the use of dSPE since none of the sorbents we tested was appropriate for both families of target compounds.

Other clean-up strategies such as freezing-lipid filtration, extraction of lipid interferences with hexane, and an SPE step with styrene-divinylbenzene based polymers (Bond Elut PPL (200 mg) and LiChrolut EN (200 mg)) were therefore evaluated. To do so, the ACN extract was evaporated and reconstituted with H_2O. However, none of these strategies provided satisfactory results. Finally, we tested the use of Lipifiltr® and found that all phthalate monoesters were retained in the Lipifiltr® cartridges but retention for the phthalate diesters was below 13%. The sorbent was removed from the plastic cartridge to be used as a sorbent for dSPE because the RSD% (*n = 10*) of the procedural blanks of these cartridges was above 30% for DEHP.

Since none of the clean-up steps we evaluated appeared to be suitable for simultaneously determining phthalate diesters and monoesters, we divided the ACN

extract equally and optimised dSPE procedures using different sorbents for each family of compounds. Extraction recoveries with the chosen sorbents were calculated for salmon extracts, which were very similar to those obtained without the matrix. For phthalate monoesters, 200 mg of C_{18}, 50 mg of ENVI-Carb and a mixture of both sorbents were evaluated (**Figure 1A**) since retention of the target compounds in the preliminary dSPE tests was less than 30%. The biggest differences in ME values were observed for MEHP and MOP, since they decreased from -91 and -86% (without a clean-up step), respectively, to -48 and -43% (when C_{18} was used), respectively. ME values for the other phthalate monoesters ranged from -21 to -43%. Similarly, for phthalate diesters we evaluated 200 mg of PSA, 200 mg of Florisil and the content of one Lipifiltr® cartridge (**Figure 1B**). In all cases, the retention of the phthalate diesters was below 30%, while the lowest ME values were obtained with Lipifiltr® (between -22 and -41%). C_{18} and Lipifiltr® were therefore chosen to clean up the sample for determining phthalate monoesters and phthalate diesters, respectively.

3.3. Method validation

Sole and salmon samples were used to evaluate the method's performance as species of seafood with low and high lipid content, respectively. To that end, linearity, method limits of detection (MLODs), method limits of quantification (MLOQs), repeatability (intra-day precision), reproducibility (day-to-day precision), R_{app} and ME were calculated for both types of fish. All validation results can be seen in **Table 1**.

First, we evaluated DEHP-d_4 as an internal standard to correct the ME even though it was considerably reduced by the clean-up steps. However, since the results only improved for DEHP and DOP, we discarded this procedure and selected matrix-matched calibration curves for the quantification. The linearity of the method was tested by spiking the fish at 12 concentrations ranging from 1 to 1,000 ng g^{-1} (d.w.). Linearity was good (r^2>0.992) for all compounds between the MLOQs and 1,000 ng g^{-1} (d.w.). MLOQs were defined as the lowest point of the calibration curves, while the MLODs were defined as the concentration with a signal-to-noise ratio equal to three for the response of the quantification ion. MLODs and MLOQs were similar for both sole and salmon samples. For phthalate monoesters, MLODs ranged from 1 to 10 ng g^{-1} (d.w.). For phthalate diesters, MLODs were 2.5–25 ng g^{-1} (d.w.) except for DEHP. This is due to the high background values of the procedural blanks, which increase the MLODs. These values, which are similar to those obtained by [38] (up to 25.8 ng g^{-1} for phthalate monoesters in eel) and [27] (up to 10 ng g^{-1} for phthalate diesters in fish), show that the sensitivity of our method is suitable.

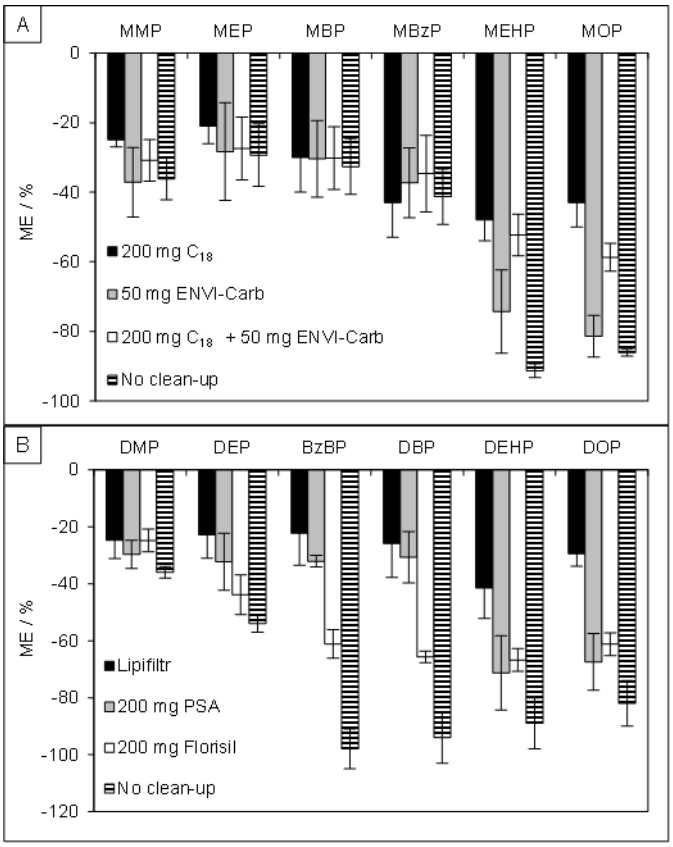


Figure 1. Matrix effect (ME) obtained for salmon samples (*n = 3*) after dispersive solid phase extraction with different sorbents (final extract volume of 1 mL) and without a clean-up step (final extract volume of 5 mL) for phthalate monoesters (A) and phthalate diesters (B).

To evaluate repeatability and reproducibility, both of which are expressed as RSD% (*n = 5*), sole and salmon samples were spiked at 50 ng g^{-1} (d.w.) and 250 ng g^{-1} (d.w.). DBP in salmon and DEHP in both sole and salmon were only evaluated at the higher concentration level since their MLOQs were over 50 ng g^{-1} (d.w.). All repeatability values were equal to or less than 17% and reproducibility values were below 20%. Finally, R_{app} and ME were evaluated in triplicate at 50 ng g^{-1} (d.w.) for all compounds except DBP and DEHP, which were evaluated at 250 ng g^{-1} (d.w.). R_{app} for phthalate monoesters were similar for both sole and salmon, ranging from 56 to 79% for all compounds except MEHP and MOP. The lower R_{app} values may be due to the higher retention observed for these compounds in C_{18} (23 and 27%, respectively). For phthalate diesters, R_{app} were slightly lower for salmon (14 – 48 %) than for sole (35 – 66 %) due to higher ME. Low recoveries

Table 1. Retention time (t_R), apparent recovery (R_{app}), matrix effect (ME), method limit of detection (MLOD), method limit of quantification (MLOQ) and repeatability (expressed as RSD%, $n = 5$).

Compound	t_R (min)	Sole (*Solea solea*)						Salmon (*Salmo salar*)					
		R_{app} (%)	ME (%)	MLOD (ng g^{-1})	MLOQ (ng g^{-1})	Repeatability (RSD%, $n = 5$)		R_{app} (%)	ME (%)	MLOD (ng g^{-1})	MLOQ (ng g^{-1})	Repeatability (RSD%, $n = 5$)	
						50 ng g^{-1}	250 ng g^{-1}					50 ng g^{-1}	250 ng g^{-1}
Phthalate monoesters													
MMP	4.1	77	-27	7.5	10	12	13	73	-25	7.5	10	13	8
MEP	8.2	79	-19	1.0	5.0	10	13	74	-21	1.0	5.0	7	11
MBP	11.9	59	-34	1.0	5.0	13	14	60	-30	1.0	5.0	11	11
MBzP	12.3	56	-47	2.5	5.0	6	13	57	-43	2.5	5.0	9	8
MEHP	16.1	20	-45	10	25	8	14	20	-48	10	25	9	5
MOP	16.6	13	-41	10	25	15	10	16	-43	10	25	15	14
Phthalate diesters													
DMP	10.5	44	-19	2.5	5.0	7	17	36	-25	2.5	5.0	17	6
DEP	13.3	66	-19	25	50	6	4	48	-23	25	50	4	6
BzBP	17.7	50	-21	5.0	7.5	11	8	36	-22	7.5	10	6	13
DBP	18.0	41	-24	10	50	17	1	47	-26	25	75	-	13
DEHP	24.5	35	-8	75	100	-	15	23	-41	100	250	-	12
DOP	25.2	37	-26	10	25	16	5	14	-29	25	50	15	11

are also found in the literature for several of our studied compounds due to high ME. The authors of one study [28] also proposed matrix-matched calibration curves to help compensate for ion suppression.

Interestingly, the R_{app} and ME obtained in the present study represent the biggest improvements compared to our previous method [25]. As **Figure 2** shows, the ion suppression of phthalate monoesters for sole decreased significantly, thus considerably increasing R_{app}. With regard to phthalate diesters, the most meaningful differences were observed for DEHP and DOP. For DOP, in particular, the increase in R_{app} enabled us to correctly determine this compound in the samples and include it in our analyses. Similar observations can be made for the results obtained with salmon.

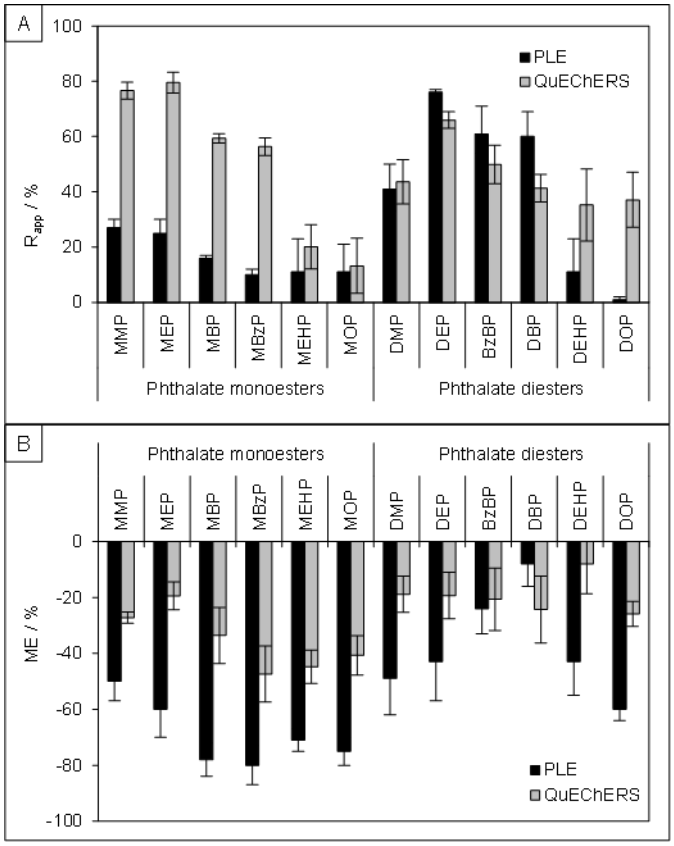


Figure 2. Apparent recoveries (A) and matrix effect (B) obtained for sole samples (*n = 3*) using our pressurised liquid extraction method (PLE) [25] and our new QuEChERS method.

3.4. Application to seafood samples

We bought three seafood species with a low-fat content (sole, squid and shrimp) and two seafood species with a high-fat content (salmon and mackerel) from different local fish markets in order to have three different samples (A, B and C) for each species. We then evaluated the presence of phthalate diesters and phthalate monoesters in triplicate for each sample. ME and R_{app} values were calculated for squid, shrimp and mackerel samples to confirm whether the calibration curves obtained for sole and salmon could be applied to those samples. However, since the values differed we constructed various calibration curves to quantify the target compounds in each seafood species. We also evaluated the repeatability and reproducibility for the three seafood matrices. The exact mass of the compounds and their ion ratios were used to confirm the presence of the detected compounds. **Table 2** shows the concentrations of these compounds in all samples.

Three phthalate monoesters and five phthalate diesters were detected and quantified in the samples with a mass error below 5 ppm. However, MMP, MBzP, MOP and DOP were not detected in any sample. **Figure 3** shows LC-HRMS extracted ion chromatograms of a 1 ppm quality control standard solution, a shrimp sample and a mackerel sample.

DEHP and DEP were found at the highest concentrations in the samples (978 and 982 ng g^{-1} (d.w.), respectively). BzBP and DBP were quantified up to 84 and 135 ng g^{-1} (d.w.), respectively. These values are in agreement with those previously reported. Xu *et al.* [27] reported DBP concentrations up to 384 ng g^{-1} and DEHP concentrations up to 763 ng g^{-1} in 60 random fish samples, while Valton *et al.* [23] found BzBP and DBP concentrations up to 185 and 451 ng g^{-1} (d.w.), respectively. For DEP, similar concentrations to those we have reported are also found in the literature (up to 860 ng g^{-1} in several fish species [39]). DMP was only detected in one mackerel sample.

With regard to phthalate monoesters, MEHP was detected in all samples at concentrations between MLOD and 117 ng g^{-1} (d.w.). MEP and MBP were also determined at concentrations between MLOD and 178 ng g^{-1} (d.w.), and between MLOD and 72 ng g^{-1} (d.w.), respectively. Some of these values are higher than those in the literature. For instance, Blair *et al.* [24] reported concentrations between 0.34 and 1.13 ng g^{-1} wet weight (w.w.) for MEHP and non-detectable values for MEP in *Hexogrammos stelleri*, while Hu *et al.* [22] found MEHP and MEP concentrations up to 24.8 and 4.70 ng g^{-1} (w.w.) in random fish samples, respectively. Both these studies determined MBP at similar concentrations to ours.

Table 2. Concentrations (ng g^{-1} (d.w.)) and relative standard deviation (RSD%, *n = 3*) of phthalate monoesters and phthalate diesters found in different seafood species with low and high lipid content.

Comp.	Seafood with low lipid content (<10%)									Seafood with high lipid content (>10%)					
	Sole (*Solea solea*)			Squid (*Loligo vulgaris*)			Shrimp (*Aristeus antennatus*)			Salmon (*Salmo salar*)			Mackerel (*Scomber scombrus*)		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Phthalate monoesters															
MEP	49 (1)	15 (11)	11 (12)	12 (0.1)	19 (8)	39 (8)	<MLOQ	<MLOQ	16 (7)	56 (16)	178 (1)	<MLOQ	10 (3)	22 (9)	31 (7)
MBP	n.d.	n.d.	<MLOQ	<MLOQ	n.d.	72 (2)	<MLOQ	n.d.	<MLOQ	n.d.	n.d.	<MLOQ	n.d.	n.d.	<MLOQ
MEHP	58 (15)	39 (10)	45 (1)	117 (5)	35 (5)	43 (8)	40 (9)	31 (3)	37 (2)	<MLOQ	43 (6)	57 (6)	62 (3)	45 (3)	42 (4)
Phthalate diesters															
DMP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	10 (3)	n.d.	n.d.
DEP	n.d.	n.d.	<MLOQ	n.d.	53 (9)	401 (8)	982 (17)	<MLOQ	<MLOQ	<MLOQ	492 (4)	n.d.	337 (14)	177 (3)	127 (11)
BzBP	n.d.	<MLOQ	<MLOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	84 (15)	n.d.	<MLOQ	n.d.
DBP	<MLOQ	<MLOQ	<MLOQ	n.d.	n.d.	135 (9)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<MLOQ	n.d.
DEHP	978 (12)	214 (5)	447 (8)	656 (11)	<MLOQ	<MLOQ	482 (13)	478 (15)	<MLOQ	<MLOQ	<MLOQ	944 (16)	n.d.	<MLOQ	n.d.

<MLOQ: Below limit of quantification
n.d.: not detected (<MLOD)

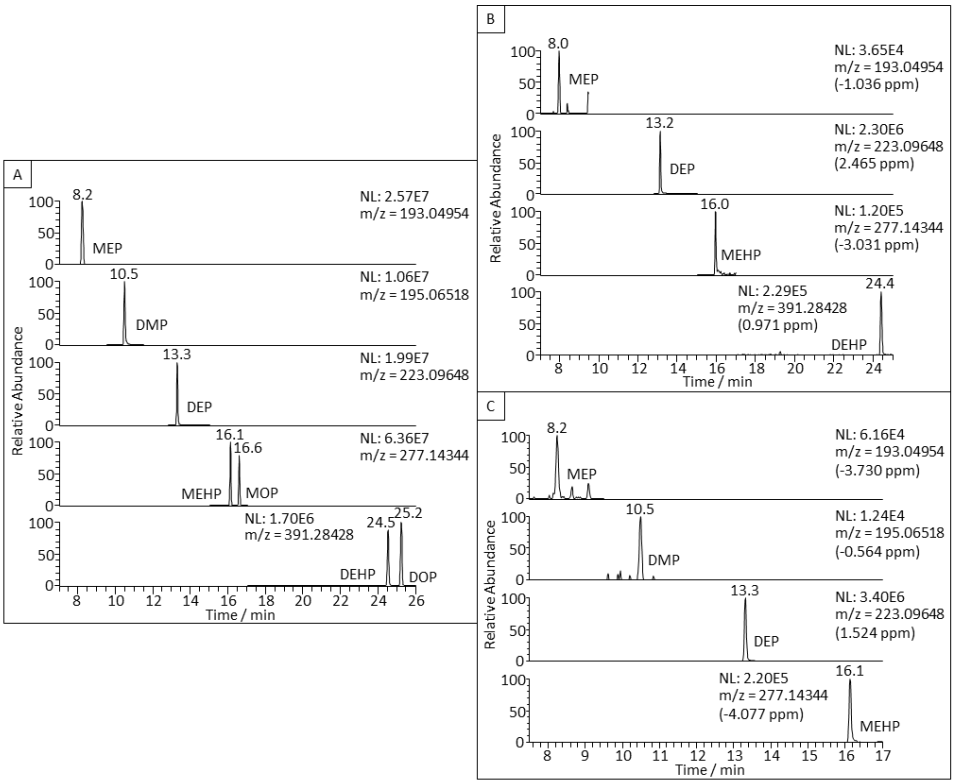


Figure 3. LC-HRMS extracted ion chromatogram and mass error in ppm of a 1 ppm standard solution (A), a shrimp sample (B) and a mackerel sample (C).

In general, we also obtained similar results in our previous work [25], where we reported the highest concentrations for DEP and DEHP (over 1000 ng g^{-1} (d.w.) for both compounds). Moreover, while MEHP was detected in all samples, we could not detect DMP in any of them. Note also that the detection frequency of MEP is much higher in the present study, probably due to the number of samples analysed.

There also appears to be a correlation between, on the one hand, the frequency of detection of the phthalate diesters found in the samples and their metabolites and, on the other, their production volume. For example, one of the most abundant phthalate diesters is DEHP [40], which we found at the highest concentrations in most samples. Moreover, we also detected its metabolite in all samples, also at some of the highest concentrations. The results obtained for DEP showed a similar tendency. Also interesting are the wide ranges of concentration for some compounds (mainly DEP and DEHP). This dispersion may be due to the different sources of the samples.

4. Conclusions

In this study, we have developed a simple analytical method for determining six phthalate monoesters and six phthalate diesters in seafood samples. The method is based on QuEChERS for the simultaneous extraction of both families of compounds, followed by dSPE and LC-HRMS. Several clean-up strategies were evaluated and two dispersants were chosen for the clean-up step, thus greatly reducing the high matrix effect inherent to such complex samples. For phthalate diesters, a novel extraction sorbent, Lipifiltr®, was successfully implemented by removing the sorbent from plastic cartridges to avoid sample contamination. C_{18} was chosen for determining phthalate monoesters.

The method was satisfactorily validated for seafood containing different lipid contents, providing good sensitivity and reproducibility values. The method was then applied to seafood samples purchased from different markets. Three phthalate monoesters, as well as five phthalate diesters, were quantified in several samples. Phthalate monoester concentrations ranged from 10 to 178 ng g^{-1} (d.w.), while phthalate diester concentrations ranged from 10 to 982 ng g^{-1} (d.w.). The analysis of these samples proved that our method is suitable for quickly and easily determining the pollutants of interest in seafood.

Acknowledgements

This study (CTQ2017-84373-R) was financed by the Spanish Ministry of Economy and Competitiveness and the European Regional Development Fund (AEI/FEDER/UE).

Míriam Hidalgo-Serrano would also like to thank the Departament d'Empresa i Coneixement of the Generalitat de Catalunya the European Union (EU) and the European Social Fund (ESF) for grant 2017FI_B_01116.

References

[1] Á. Santana-Mayor, B. Socas-Rodríguez, A. V. Herrera-Herrera, M.Á. Rodríguez-Delgado, Current trends in QuEChERS method. A versatile procedure for food, environmental and biological analysis, Trends Anal. Chem. 116 (2019) 214–235.

[2] R. Perestrelo, P. Silva, P. Porto-Figueira, J.A.M. Pereira, C. Silva, S. Medina, J.S. Câmara, QuEChERS - Fundamentals, relevant improvements, applications and future trends, Anal. Chim. Acta. 1070 (2019) 1–28.

[3] M. Anastassiades, S.J. Lehotay, D. Štajnbaher, F.J. Schenck, Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce, J. AOAC Int. 86 (2003) 412–431.

[4] N.E. Song, J.Y. Lee, A.R. Mansur, H.W. Jang, M.C. Lim, Y. Lee, M. Yoo, T.G. Nam, Determination of 60 pesticides in hen eggs using the QuEChERS procedure followed by LC-MS/MS and GC-MS/MS, Food Chem. 298 (2019) 125050.

[5] J.L. de O. Arias, C.B. Rocha, A.L.Q.S. Santos, L.C. Marube, L. Kupski, S.S. Caldas, E.G. Primel, Fast and simple method of simultaneous preservative determination in different processed foods by QuEChERS and HPLC-UV: Method development, survey and estimate of daily intake, Food Chem. 293 (2019) 112–119.

[6] J.L. Hidalgo-Ruiz, R. Romero-González, J.L. Martínez Vidal, A. Garrido Frenich, A rapid method for the determination of mycotoxins in edible vegetable oils by ultra-high performance liquid chromatography-tandem mass spectrometry, Food Chem. 288 (2019) 22–28.

[7] X. Jia, S. Yin, J. Xu, N. Li, M. Ren, Y. Qin, J. Zhou, Y. Wei, Y. Guo, M. Gao, Y. Yu, B. Wang, Z. Li, An efficient method to simultaneously analyze multi-class organic pollutants in human serum, Environ. Pollut. 251 (2019) 400–406.

[8] J.-C. Fan, R. Ren, Q. Jin, H.-L. He, S.-T. Wang, Detection of 20 phthalate esters in breast milk by GC-MS/MS using QuEChERS extraction method, Food Addit. Contam. Part A. 36 (2019) 1551–1558.

[9] M.J. Fernandes, P. Paíga, A. Silva, C.P. Llaguno, M. Carvalho, F.M. Vázquez, C. Delerue-Matos, Antibiotics and antidepressants occurrence in surface waters and sediments collected in the north of Portugal, Chemosphere. 239 (2020) 124729.

[10] D. Álvarez-Muñoz, M. Rambla-Alegre, N. Carrasco, M. Lopez de Alda, D. Barceló, Fast analysis of relevant contaminants mixture in commercial shellfish, Talanta. 205 (2019) 119884.

[11] E. López-García, C. Postigo, M. López de Alda, Psychoactive substances in mussels: Analysis and occurrence assessment, Mar. Pollut. Bull. 146 (2019) 985–992.

[12] M.V. Barbieri, C. Postigo, N. Guillem-Argiles, L.S. Monllor-Alcaraz, J.I. Simionato, E. Stella, D. Barceló, M. López de Alda, Analysis of 52 pesticides in fresh fish muscle by QuEChERS extraction followed by LC-MS/MS determination, Sci. Total Environ. 653 (2019) 958–967.

[13] A.K. Sakhi, A. Sabaredzovic, E. Cequier, C. Thomsen, Phthalate metabolites in Norwegian mothers and children: Levels, diurnal variation and use of personal care products, Sci. Total Environ. 599–600 (2017) 1984–1992.

[14] J. Mathieu-Denoncourt, S.J. Wallace, S.R. de Solla, V.S. Langlois, Plasticizer endocrine disruption: Highlighting developmental and reproductive effects in mammals and non-mammalian aquatic species, Gen. Comp. Endocrinol. 219 (2015) 74–88.

[15] European Parliament, Directive 2005/84/EC of the European Parliament and the Council, Off. J. Eur. Union 344 (2005) 40–43.

[16] U.S. Environmental Protection Agency, Phthalates action plan, US Environmental Protection Agency. (2012) 1–16.

[17] European Food Safety Agency (EFSA), Scientific opinion on the update of the risk assessment of di-butylphthalate (DBP), butyl-benzyl-phthalate (BBP), bis(2-ethylhexyl)phthalate (DEHP), di-isononylphthalate (DINP) and di-isodecylphthalate (DIDP) for use in food contact materials, EFSA J. 17 (2019) 1–85.

[18] P. Ventrice, D. Ventrice, E. Russo, G. de Sarro, Phthalates: European regulation, chemistry, pharmacokinetic and related toxicity, Environ. Toxicol. Pharmacol. 36 (2013) 88–96.

[19] S. Net, R. Sempéré, A. Delmont, A. Paluselli, B. Ouddane, Occurrence, fate, behavior and ecotoxicological state of phthalates in different environmental matrices, Environ. Sci. Technol. 49 (2015) 4019–4035.

[20] T. Ye, M. Kang, Q. Huang, C. Fang, Y. Chen, H. Shen, S. Dong, Exposure to DEHP and MEHP from hatching to adulthood causes reproductive dysfunction and endocrine disruption in marine medaka (Oryzias melastigma), Aquat. Toxicol. 146 (2014) 115–126.

[21] J. Jiang, D. Mu, M. Ding, S. Zhang, H. Zhang, J. Hu, Simultaneous determination of primary and secondary phthalate monoesters in the Taihu Lake: Exploration of sources, Chemosphere. 202 (2018) 17–24.

[22] X. Hu, Y. Gu, W. Huang, D. Yin, Phthalate monoesters as markers of phthalate contamination in wild marine organisms, Environ. Pollut. 218 (2016) 410–418.

[23] A.S. Valton, C. Serre-Dargnat, M. Blanchard, F. Alliot, M. Chevreuil, M.J. Teil, Determination of phthalates and their by-products in tissues of roach (Rutilus rutilus) from the Orge river (France), Environ. Sci. Pollut. Res. 21 (2014) 12723–12730.

[24] J.D. Blair, M.G. Ikonomou, B.C. Kelly, B. Surridge, F.A.P.C. Gobas, Ultra-trace determination of phthalate ester metabolites in seawater, sediments, and biota from an urbanized marine inlet by LC/ESI-MS/MS, Environ. Sci. Technol. 43 (2009) 6262–6268.

[25] M. Hidalgo-Serrano, F. Borrull, E. Pocurull, R.M. Marcé, Pressurised liquid extraction and liquid chromatography-high resolution mass spectrometry for the simultaneous determination of phthalate diesters and their metabolites in seafood species, Food Anal. Methods. 13 (2020) 1442–1453.

[26] E. Tsochatzis, P. Karayannakidis, S. Kalogiannis, Determination of selected dichloroanilines and phthalates in lyophilised mussels samples with ultra-high performance liquid chromatography-tandem mass spectrometry after QuEChERS clean-up, Food Addit. Contam. Part A. 36 (2019) 1253–1260.

[27] Y. Xu, R. Weng, Y. Lu, X. Wang, D. Zhang, Y. Li, J. Qiu, Y. Qian, Evaluation of phthalic acid esters in fish samples using gas chromatography tandem mass spectrometry with simplified QuEChERS technique, Food Anal. Methods. 11 (2018) 3293–3303.

[28] Y. Gu, X. Yu, J. Peng, S. Chen, Y. Zhong, D. Yin, X. Hu, Simultaneous solid phase extraction coupled with liquid chromatography tandem mass spectrometry and gas chromatography tandem mass spectrometry for the highly sensitive determination of 15 endocrine disrupting chemicals in seafood, J. Chromatogr. B. 965 (2014) 164–172.

[29] UCT, LipiFiltr®, (2019) 8. https://www.unitedchem.com/product/lipifiltr/ (accessed September 16, 2021).

[30] EN 15662:2019, Foods of plant origin. Multimethod for the determination of pesticide residues using GC- and LC-based analysis following acetonitrile extraction/partitioning and clean-up by dispersive SPE. Modular QuEChERS-method, (2019).

[31] S.J. Lehotay, Official Method 2007.01: Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate, J. AOAC Int.90 (2007) 485–520.

[32] L. Vallecillos, E. Pocurull, F. Borrull, Influence of pre-treatment process on matrix effect for the determination of musk fragrances in fish and mussel, Talanta. 134 (2015) 690–698.

[33] X. Sun, W. Dong, M. Liu, C. Shen, Y. Zhang, J. Sun, B. Sun, H. Li, F. Chen, Validation of a QuEChERS-Based bas chromatography-mass spectrometry (GC-MS) method for analysis of phthalate esters in grain sorghum, J. Food Sci. 83 (2018) 892–901.

[34] W. Dong, B. Sun, J. Sun, F. Zheng, X. Sun, M. Huang, H. Li, Matrix effects in detection of phthalate esters from wheat by a modified QuEChERS method with GC/MS, Food Anal. Methods. 10 (2017) 3166–3180.

[35] U.T. Sireli, A. Filazi, B. Yurdakok-Dikmen, G. Iplikcioglu-Cil, O. Kuzukiran, C.E. Orhan, Determination of phthalate residues in different types of yogurt by gas chromatography-mass spectrometry and estimation of yogurt-related intake of phthalates, Food Anal. Methods. 10 (2017) 3052–3062.

[36] R. Cruz, A. Marques, S. Casal, S.C. Cunha, Fast and environmental-friendly methods for the determination of polybrominated diphenyl ethers and their metabolites in fish tissues and feed, Sci. Total Environ. 646 (2019) 1503–1515.

[37] Y. Gao, X. Li, X. Li, Q. Zhang, H. Li, Simultaneous determination of 21 trace perfluoroalkyl substances in fish by isotope dilution ultrahigh performance liquid chromatography tandem mass spectrometry, J. Chromatogr. B. 1084 (2018) 45–52.

[38] C. Fourgous, M. Chevreuil, F. Alliot, E. Amilhat, E. Faliex, S. Paris-Palacios, M.J. Teil, A. Goutte, Phthalate metabolites in the European eel (Anguilla anguilla) from Mediterranean coastal lagoons, Sci. Total Environ. 569–570 (2016) 1053–1059.

[39] A.A. Adeniyi, O.O. Okedeyi, K.A. Yusuf, Flame ionization gas chromatographic determination of phthalate esters in water, surface sediments and fish species in the Ogun river catchments, Ketu, Lagos, Nigeria, Environ. Monit. Assess. 172 (2011) 561–569.

[40] D.W. Gao, Z.D. Wen, Phthalate esters in the environment: A critical review of their occurrence, biodegradation, and removal during wastewater treatment processes, Sci. Total Environ. 541 (2016) 986–1001.

Supplementary data

Table S1. Chemical structure of the analytes studied and exact masses of their quantification and fragment ions.

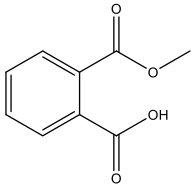
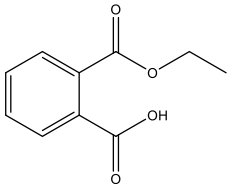
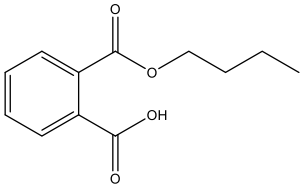
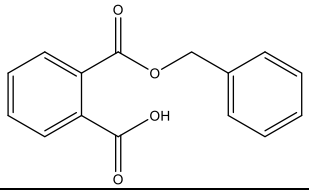
Compound and structure	Quantification ion (*m/z*)	Fragment ions (*m/z*)
Phthalate monoesters		
Monomethyl phthalate (MMP) 	$[M-H]^-$ 179.03389	$C_7H_7O^-$ (107.04914) $C_8H_7O_2^-$ (135.04406)
Monoethyl phthalate (MEP) 	$[M-H]^-$ 193.04954	$C_7H_5O_2^-$ (121.02841) $C_9H_9O_2^-$ (149.05971)
Monobutyl phthalate (MBP) 	$[M-H]^-$ 221.08084	$C_{11}H_{13}O_2^-$ (177.09101) $C_7H_5O_2^-$ (121.02841)
Monobenzyl phthalate (MBzP) 	$[M-H]^-$ 255.06519	$C_7H_7O^-$ (107.04914) $C_7H_5O_2^-$ (121.02841)

Table S1. (Cont.).

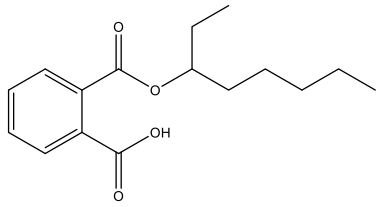
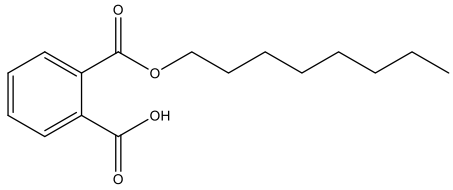
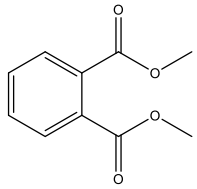
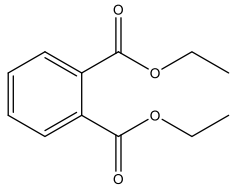
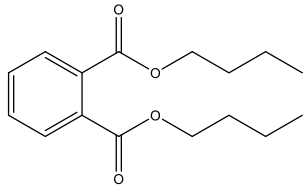
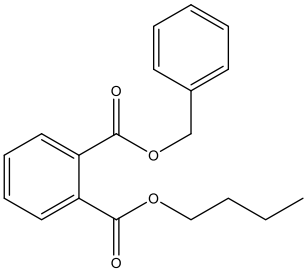
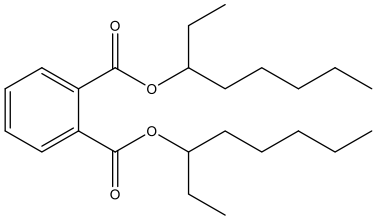
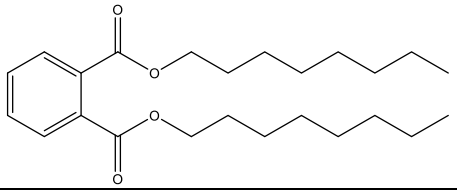
Compound and structure	Quantification ion (*m/z*)	Fragment ions (*m/z*)
Mono(2-ethylhexyl) phthalate (MEHP) 	$[M-H]^-$ 277.14344	$C_8H_6O_2^-$ (134.03623) $C_{15}H_{21}O_2^-$ (233.15361)
Monooctyl phthalate (MOP) 	$[M-H]^-$ 277.14344	$C_{15}H_{21}O_2^-$ (233.15361) $C_8H_{15}O^-$ (127.11174)
Phthalate diesters		
Dimethyl phthalate (DMP) 	$[M+H]^+$ 195.06518	$C_9H_7O_3^+$ (163.03897)
Diethyl phthalate (DEP) 	$[M+H]^+$ 223.09648	$C_8H_5O_3^+$ (149.02332) $C_{10}H_9O_3^+$ (177.05462)
Dibutyl phthalate (DBP) 	$[M+H]^+$ 279.15908	$C_8H_5O_3^+$ (149.02332) $C_{12}H_{13}O_3^+$ (205.08592)

Table S1. (Cont.).

Compound and structure	Quantification ion (*m/z*)	Fragment ions (*m/z*)
Benzyl butyl phthalate (BzBP) 	$[M+H]^+$ 313.14343	$C_8H_5O_3^+$ (149.02332) $C_{12}H_{13}O_3^+$ (205.08592)
Bis(2-ethylhexyl) phthalate (DEHP) 	$[M+H]^+$ 391.28428	$C_8H_5O_3^+$ (149.02332) $C_8H_7O_4^+$ (167.03389)
Di-n-octyl phthalate (DOP) 	$[M+H]^+$ 391.28428	$C_8H_5O_3^+$ (149.02332) $C_{16}H_{21}O_3^+$ (261.14852)

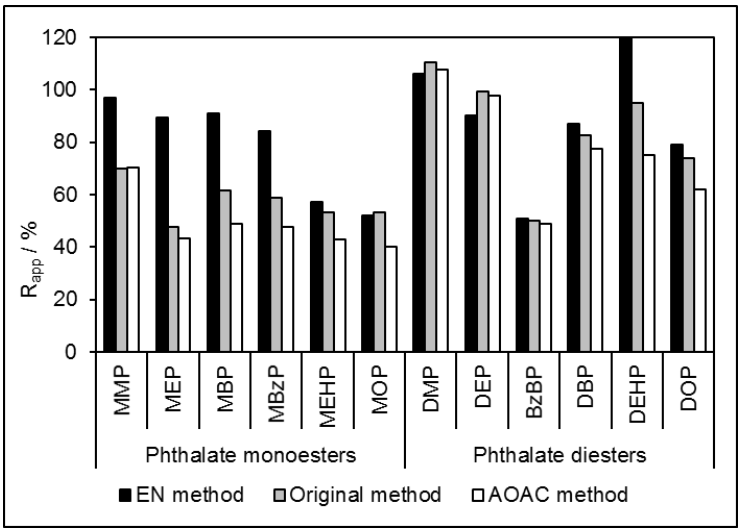


Figure S1. Apparent recoveries obtained for sole samples using different extraction methods.

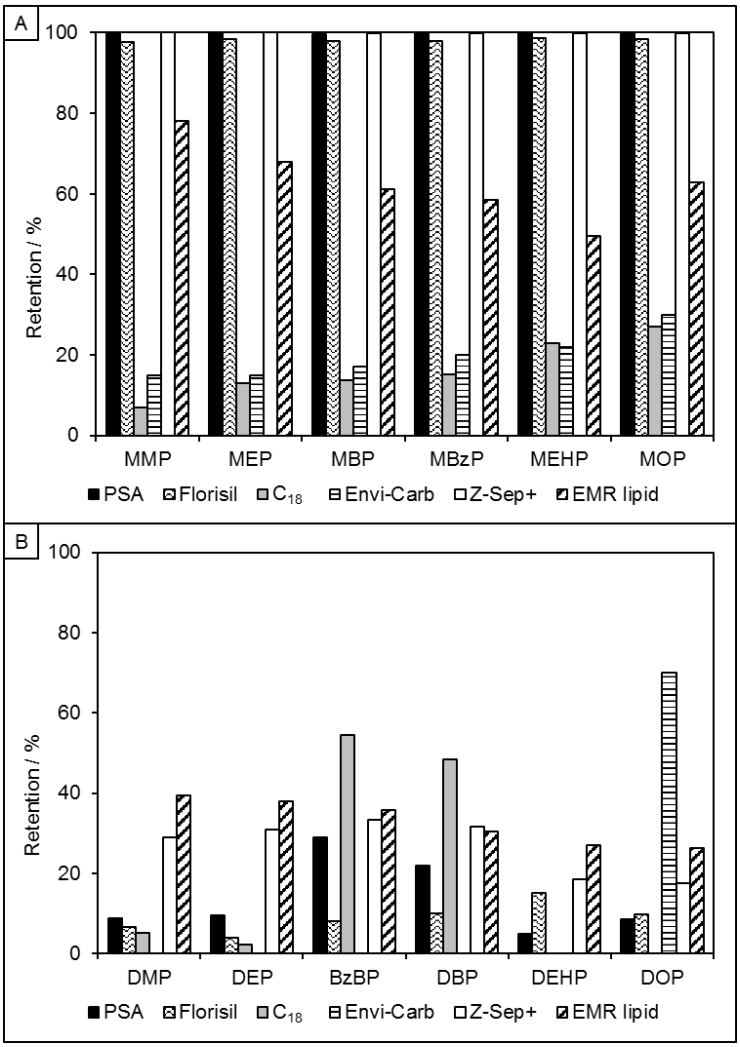


Figure S2. Retention of phthalate monoesters (A) and phthalate diesters (B) in several dSPE sorbents.

3.2.3. Determination of organophosphate ester metabolites in seafood species by QuEChERS-SPE followed by liquid chromatography-high-resolution mass spectrometry

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

DETERMINATION OF ORGANOPHOSPHATE ESTER METABOLITES IN SEAFOOD SPECIES BY QUECHERS-SPE FOLLOWED BY LIQUID CHROMATOGRAPHY-HIGH-RESOLUTION MASS SPECTROMETRY

Míriam Hidalgo-Serrano, Francesc Borrull, Eva Pocurull, Rosa M. Marcé

Universitat Rovira i Virgili, Department of Analytical Chemistry and Organic Chemistry, Marcel·lí Domingo s/n, Tarragona 43007, Spain

---

Abstract

Organophosphate triesters are compounds widely used as flame retardants and plasticiser additives in the industry and are ubiquitous in the environment. These compounds can be rapidly transformed into organophosphate diesters. Some organophosphate diesters are also used in the industry as plasticisers, catalysts and flame retardants. Several studies suggest these compounds can have hazardous and mutagenic properties, as well as toxic effects for reproduction. Due to the impact these compounds can have on marine biota and on human beings through intake, there is a need to study their presence in widely consumed seafood species.

To that end, we have developed a simple analytical method for the determination of six of the most common organophosphate diesters in seafood. The procedure is based on a Quick, Easy, Cheap, Effective, Rugged and Safe extraction method and a solid phase extraction clean-up, followed by liquid chromatography coupled to high-resolution mass spectrometry. The method has been optimised and validated for seafood with different lipid content, providing satisfactory relative recoveries (from 89 to 138% for most compounds), limits of detection (1.0–50 ng g^{-1} dry weight) and repeatability values (RSD% (*n = 5*, 100 ng g^{-1} (dry weight)) lower than 15%). Eight seafood species were analysed using this method and two organophosphate diesters were detected and quantified in all samples. Dibutyl phosphate was found at concentrations up to 79 ng g^{-1} (dry weight), and diphenyl phosphate was found at concentrations up to 100 ng g^{-1} (dry weight).

Keywords: *Seafood, Organophosphate diesters, Sold-phase extraction, QuEChERS, Liquid chromatography, High-resolution mass spectrometry*

---

1. Introduction

Organophosphate (OP) triesters are high-production volume chemicals, mainly used as flame retardant additives and plasticisers in consumer products such as upholstered furniture, textiles, electronic devices and plastics, among others [1]. These past few decades, the production of these chemicals has increased considerably after the use of brominated flame retardants was banned due to their persistence, toxicity and bioaccumulation potential [2]. OP triesters are typically applied to the products by physical mixing, not chemically bonded, so they easily leach into the environment during the lifetime of those products. As a result, OP triesters are ubiquitous and have been reported in a wide variety of matrices such as indoor dust [3,4], sediments [5,6], surface waters [2,7,8] and biota [2,9], which might be cause for concern considering that some of them are hazardous and suspected endocrine disruptors [2,10].

Several in vivo [11,12] and in vitro [13,14] studies report that OP triesters are rapidly hydrolysed by living organisms. OP diesters are some of the most commonly detected metabolites and could be used as biomarkers of exposure to these contaminants. Moreover, some OP diesters are also used as chemical additives in several industrial processes such as dibutyl phosphate (DNBP), bis(2-ethylhexyl) phosphate (BEHP) and diphenyl phosphate (DPHP), making industrial waste another source of environmental contamination for these compounds [15].

Similar to OP triesters, some OP diesters have shown potential to cause developmental cardiac defects [16], circulatory failures [17], general teratogenicity [18] and some have even demonstrated a stronger ability to alter gene expression than their parent products [19]. That is the reason why the European Chemicals Agency (ECHA) considered bis(2-chloroethyl) phosphate (BCEP), bis(2-butoxyethyl) phosphate (BBOEP) and DPHP as substances that can be cause for concern for the industry, indicating that they are suspected hazardous and persistent in aquatic environments as well as suspected mutagens or toxic for reproduction [20].

More specifically, marine environments are very delicate and sensitive to urban, agricultural and industrial sources of contamination. Taking into account that plastic and microplastic contamination is a growing problem worldwide [21], special attention should be paid to the effect that organic contaminants released from these materials (such as OP diesters) could have on marine organisms. However, information on the determination and occurrence of OP diesters in living organisms is very limited and should be expanded

considering that seafood is a very important dietary route of exposure to these contaminants for humans [22].

Only a few studies report analytical methods for the determination of OP diesters in fish. The extraction of the target compounds is usually carried out by ultrasound extraction [23,24], even though pressurised liquid extraction has also been used to that end [25]. The extraction procedures are frequently followed by solid phase extraction (SPE) clean-up with weak anion exchange cartridges [23, 25]. Then, the extracts are analysed by liquid chromatography (LC), usually coupled to tandem mass spectrometry (MS/MS), because they need to be derivatised in order to be analysed by gas chromatography [26].

Aiming to cover the lack of information regarding the presence of OP diesters in widely consumed seafood species, we developed a simple method for the determination of six OP diesters using a Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction method followed by LC coupled to high-resolution mass spectrometry (LC-HRMS). Moreover, we tested several strategies to clean-up the extracts, which usually present high matrix effects (ME) due to the complexity of the seafood matrices.

2. Materials and methods

2.1. Reagents and standards

The solid standards of bis(2-chloroethyl) phosphate (BCEP), diphenyl phosphate (DPHP), dibutyl phosphate (DNBP), bis(1,3-dichloropropyl) phosphate (BDCIPP), bis(2-butoxyethyl) phosphate (BBOEP), bis(2-ethylhexyl) phosphate (BEHP) and the surrogates d_8-BCEP, d_{10}-DPHP, d_{18}-DNBP, d_{10}-BDCIPP and d_8-BBOEP were purchased from LGC (Teddington, Middlesex, UK). Stock solutions of the individual standards were prepared in methanol (MeOH) and stored at -23 ^{o}C.

Acetonitrile (ACN), acetone and MeOH of HPLC grade were provided by J.T. Baker (Deventer, The Netherlands). Hydrochloric acid (HCl), water and MeOH of MS grade for the mobile phase were purchased from Scharlab (Barcelona, Spain). Ultrapure water was obtained with an ultrapure water purification system (Merck Millipore, Darmstadt, Germany). Acetic acid and ammonium acetate were purchased from Sigma-Aldrich (Saint Louis, Missouri, USA). The extraction salt packets for the QuEChERS original, EN and AOAC methods were supplied by Scharlab.

2.2. Sampling

Eight seafood species were obtained from several local fish markets in Tarragona, Spain: *Scomber scombrus* (mackerel), *Merluccius merluccius* (hake), *Solea solea* (sole), *Gadus morhua* (cod), *Loligo Vulgaris* (squid), *Sardina pilchardus* (sardine), *Thunnus thynnus* (tuna) and *Salmo salar* (salmon). All samples were cleaned, filleted and frozen. A Genevac miVac Duo sample concentrator with a SpeedTrap freeze-drying system (Ipswich, UK) was used to lyophilise the samples, which were homogenised and sieved (500 µm) afterwards.

2.3. QuEChERS extraction and clean-up

100 mg of freeze-dried sample spiked with 400 ng of surrogate standards was weighted into 50 mL polypropylene tubes (ThermoFisher Scientific, Waltham, USA) and 10 mL of ultrapure water was added to the tube. The mixture was vortexed for 1 min and 10 mL of acetone were added before the tube was vortexed again. Then, an extraction salt packet for the original QuEChERS method (Scharlab), containing 4 g of anhydrous magnesium sulphate and 1 g of sodium chloride, was added to the mixture. The tube was shaken by hand, vortexed again for 5 min and centrifuged at 7000 rpm for 5 min (Hettich Universal 32R, Tuttlingen, Germany). The extraction was repeated once more and the resulting extracts were combined.

The mixture was concentrated to ~5 mL with a miVac Duo sample concentrator. Then, 10 mL of ultrapure water were added and the mixture was vortexed to carry out an SPE clean-up step. Briefly, homemade 2 g PSA cartridges were conditioned with 5 mL of MeOH followed by 5 mL of ultrapure water/acetone 2:1 (v/v). The ~15 mL extracts were then loaded onto the cartridges and the analytes were eluted with 5 mL of MeOH 5% NH_4OH. Finally, the extracts were concentrated to ~200 µL with the miVac Duo sample concentrator, taken up to 1 mL with ultrapure water and injected into the LC-HRMS instrument.

2.4. Liquid chromatography coupled to high-resolution mass spectrometry

An Accela 1250 UHPLC system from Thermo Scientific (Bremen, Germany) equipped with an Accela Autosampler and a quaternary pump was used for the chromatographic analyses. The system was coupled to an ExactiveOrbitrap™ mass spectrometer (Thermo Scientific) equipped with a heated electrospray ionisation source (HESI) and an HCD collision cell. The chromatographic separation was carried out with an

Ascentis Express C_{18} fused-core column (100 mm x 2.1 mm i.d., 2.7 µm particle size) from Sigma-Aldrich. The initial mobile phase was 92% solvent A (2 mM ammonium acetate in water) and 8% solvent B (MeOH), with a flow rate of 200 µL min^{-1}, which was held constant for 4.5 min. The gradient was then increased to 90% of B in 9 min and to 100% of B in 0.5 min. It remained constant for 5 min before returning to initial conditions. The injection volume was 20 µL, and the sample tray and column oven temperatures were 10 ºC and 30 ºC, respectively.

Mass spectra were acquired in negative mode and the source parameters were optimised to obtain the highest response for all target compounds. Spray voltage was set at -2.5 kV, capillary voltage at -20 V, tube lens voltage at -70 V, skimmer voltage at -20 V. The sheath and auxiliary gas flow rates were set at 40 and 5 A.U., respectively, while the heater temperature was set at 425 ºC and the capillary temperature at 250 ºC. For HRMS measurements, two scan events took place in a single time window, a full scan at 50,000 FMHW with 250 ms of injection time, and a fragmentation scan at 10,000 FWHM with 50 ms of injection time using a collision voltage of 25 eV in the HCD cell.

3. Results and Discussion

3.1. Liquid chromatography coupled to high-resolution mass spectrometry

For the optimisation of HRMS conditions, the gas flow rates, voltages and temperature parameters were tested to obtain the highest response for all compounds. To do so, 1 mg L^{-1} individual standard solutions were directly infused into the system with a 1:1 (v/v) composition of water and MeOH. For all compounds, the highest response was obtained in negative ionisation mode for $[M-H]^-$ ion. Similarly, the optimum fragmentation energy was selected to obtain the highest response for all quantifier and qualifier ions, which are mostly in accordance with the literature except for those of BCEP and BDCIPP [24,25,27]. For those two compounds, the product ions selected when working with MS/MS are usually *m/z* 35 and 37. However, the Exactive high-resolution mass spectrometer only allows *m/z* values higher than 50, hence we selected just one qualifier ion for those compounds. The ions selected for quantification and confirmation purposes can be seen in **Table 1**.

Even though the chromatographic separation of organophosphate diesters can be carried out with HILIC [5] or biphenyl [28] columns, C_{18} is the most commonly used stationary phase [15, 24, 25]. Hence, we chose an Ascentis Express C_{18} fused-core column (100 mm x 2.1 mm i.d., 2.7 µm particle size) to separate the target compounds. Moreover,

Table 1. Chemical structure of the analytes studied and exact masses of their quantifier and qualifier ions

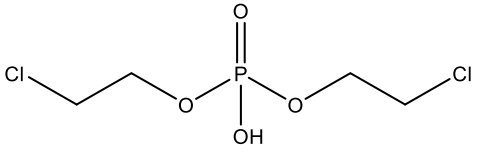
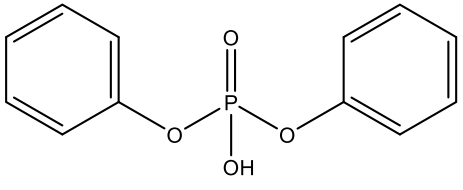
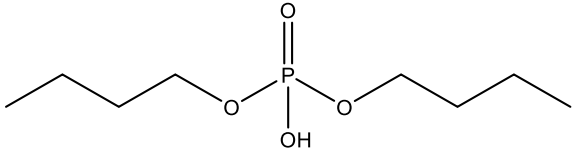
Compound and structure	Quantifier ions (*m/z*)	Qualifier ions (*m/z*)
Bis(2-chloroethyl) phosphate (BCEP) 	$[C_4H_8PO_4Cl_2]^-$ 220.95318	$[PO_3]^-$ 78.95796
Diphenyl phosphate (DPHP) 	$[C_{12}H_{10}PO_4]^-$ 249.03112	$[C_6H_5O]^-$ 93.03349 $[C_6H_4PO_3]^-$ 154.98926
Dibutyl phosphate (DNBP) 	$[C_8H_{18}PO_4]^-$ 209.09372	$[PO_3]^-$ 78.95796 $[C_4H_{10}PO_4]^-$ 153.03112

Table 1. (Cont.).

Compound and structure	Quantifier ions (*m/z*)	Qualifier ions (*m/z*)
Bis(1,3-dichloro-2-propyl) phosphate (BDCIPP)	$[C_6H_{11}PO_4Cl_3{}^{37}Cl]^-$ 318.90358	$[PO_3]^-$ 78.95796
Bis(2-butoxyethyl) phosphate (BBOEP)	$[C_{12}H_{26}PO_6]^-$ 297.14615	$[PO_3]^-$ 78.95796 $[C_6H_{14}PO_5]^-$ 197.05734
Bis(2-ethylhexyl) phosphate (BEHP)	$[C_{16}H_{34}PO_4]^-$ 321.21892	$[PO_3]^-$ 78.95796 $[C_8H_{18}PO_4]^-$ 209.09372

the separation of OP diesters on reversed-phase columns has usually been carried out either at acidic pH [29, 30] or with ammonium acetate [3, 9, 28]. In this study, we compared three different mobile phases to find the optimal conditions for the detection and separation of OP diesters: 0.1% formic acid in water, 0.1% acetic acid in water and 2 mM ammonium acetate in water. At first, we carried out the separation with a very simple gradient. The initial mobile phase was 92% solvent A (one of the three tested aqueous phases) and 8% of solvent B (MeOH) with a flow rate of 200 µL min^{-1}, which was held constant for 5 min. The gradient was then increased to 100% of B in 15 min and it remained constant for 5 min before returning to initial conditions.

As can be seen in **Figure 1**, the chromatogram using acetic acid as a modifier for solvent A resulted in overall broad peaks, especially for BEHP which presented pronounced peak fronting. The use of formic acid reduced that peak fronting but led to peak tailing, especially for BCEP. Using 2 mM ammonium acetate in water as a mobile phase (pH ~7) greatly reduced peak broadening and, even though it caused slight peak fronting, it yielded to better peak resolution, hence we selected this aqueous phase as optimal for the separation of the OP diesters. We also modified the gradient elution to reduce the time of analysis (finally choosing the gradient described in Section 2.4) and confirmed that the selected HRMS conditions still yielded the highest response possible for all compounds.

Then, instrumental quality parameters and standard calibration curves were calculated for each of the studied compounds taking into account the response of the quantifier ion. Instrumental limits of detection (ILOD) were determined as the concentration at which the response of the quantifier ion was three times the signal-to-noise ratio. Instrumental limits of quantification (ILOQ) corresponded to the lowest concentration of the standard calibration curve. Linear ranges were established for each compound between ILOQ and 1000 µg L^{-1}. ILOD and ILOQ ranged from 0.5 to 2.5 µg L^{-1} and from 1.0 to 5.0 µg L^{-1}, respectively, for all compounds except for BCEP, for which ILOD was 7.5 µg L^{-1} and ILOQ was 10 µg L^{-1}.

3.2. QuEChERS extraction

Extraction kits for the original QuEChERS method (containing 1 g sodium chloride and 4 g anhydrous magnesium sulphate), as well as the official EN method (containing 1 g sodium chloride, 1 g sodium citrate, 0.5 g sodium hydrogencitrate sesquihydrate and 4 g anhydrous magnesium sulphate) and AOAC method (containing 1.5 g sodium acetate

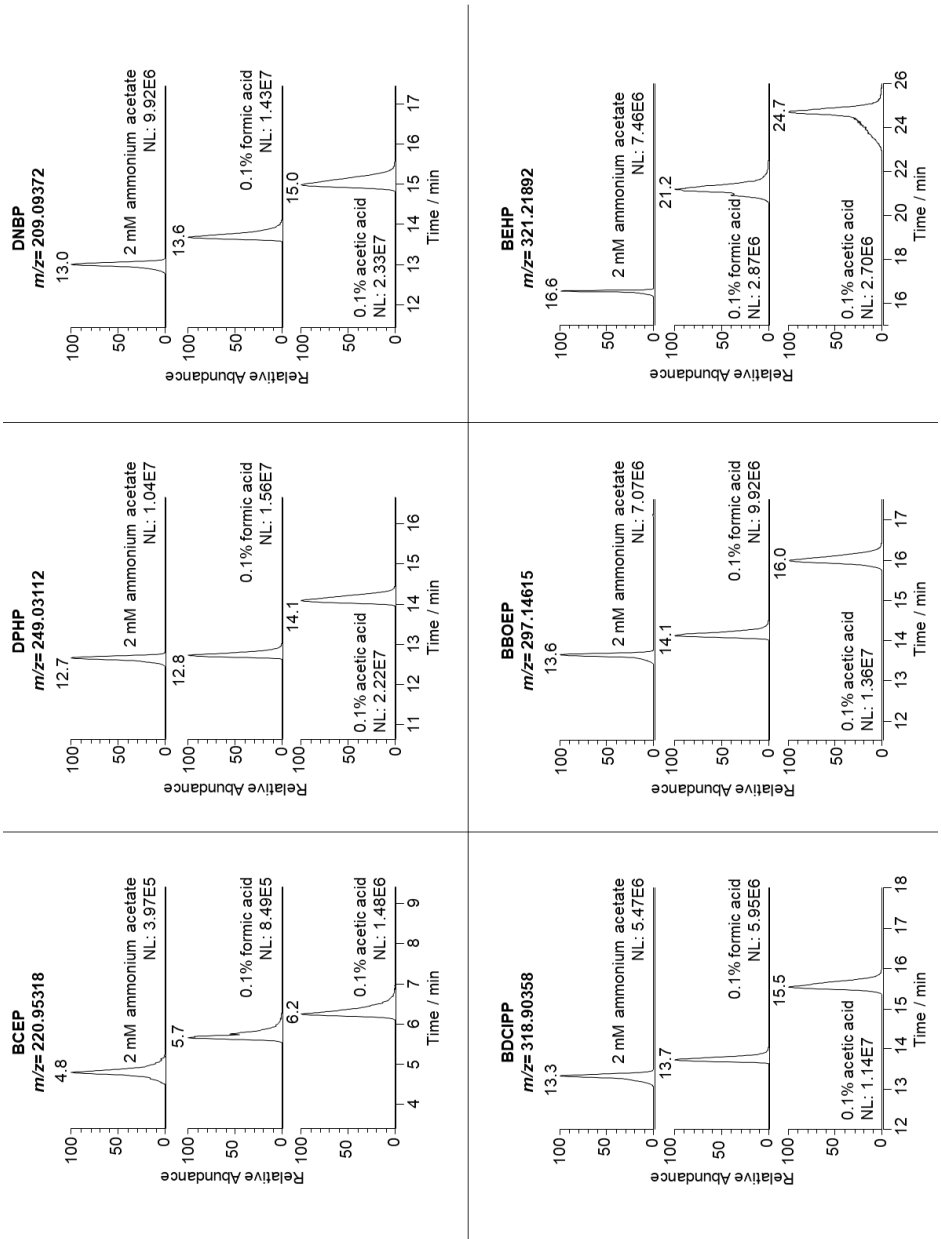


Figure 1. LC-HRMS extracted ion chromatograms of the injection of a standard mixture solution (1 mg L^{-1}) spiking the aqueous mobile phase with three different additives.

and 6 g anhydrous magnesium sulphate), were tested to achieve the most efficient extraction of the target compounds. Extraction recoveries (R_{ext}) were evaluated to select the best extraction method by comparing the response of the compounds in the final extract for samples spiked at 1000 ng g^{-1} dry weight (d.w.) before the extraction with the response obtained for extracts spiked at the same concentration after that step. To do so, 1 g of lyophilised sample was hydrated with 10 mL of ultrapure water and the mixture was vortexed for 1 min. Then, 10 mL of ACN was added to the tube, which was vortexed again before adding an extraction salt packet. The mixture was vigorously shaken by hand, vortexed for 5 min and centrifuged at 7000 rpm for 5 min. During the optimisation process, to avoid high ion suppression in the final extracts due to the lack of a clean-up step, and to prevent the loss by evaporation of the compounds, 1 mL of the ACN phase was transferred to a 10 mL volumetric flask and taken up to the final volume with ultrapure water, instead of preconcentrating the extracts before the injection. The solvent volume and vortex time conditions for the extraction were set based on our previous experience [31]. It should be noted that, since the lipidic content of the samples can affect the efficiency of the extraction, two seafood species with different lipid percentages were chosen for the optimisation: hake (low-lipid content) and mackerel (high-lipid content).

The R_{ext} obtained with all three extraction methods ranged from 10 to 65% for most compounds, so the use of other extraction solvents was tested. Based on a PLE method for the extraction of several OP triesters and diesters from fish [25], we decided to switch the extracting solvent to acetone. Following the same procedure as described above and only changing the 10 mL of ACN for the 10 mL of acetone, the R_{ext} of all compounds increased significantly (**Figure 2**). The EN extraction salts yielded the lowest R_{ext} for all compounds except BCEP in hake (42 – 69%). The original method yielded the highest R_{ext} values, ranging from 53 to 83% for both species, hence we selected the original salt packets for the extraction.

We also evaluated the change of R_{ext} with two and three consecutive extraction cycles. R_{ext} increased more than 20% for most compounds when carrying out two extraction cycles. Three extraction cycles yielded slightly better R_{ext}, but they also increased the ME by almost 20%. We therefore selected two consecutive extraction cycles for the present procedure yielding R_{ext} of 60–79% for both hake and mackerel.

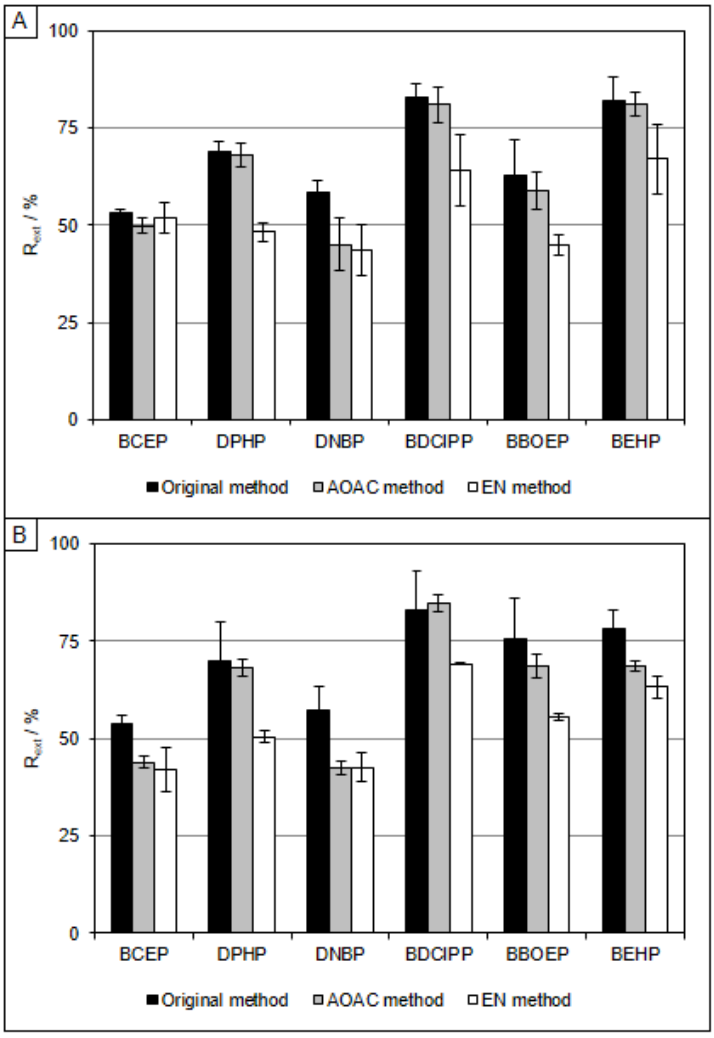


Figure 2. Extraction recoveries obtained for hake (A) and mackerel (B) samples using different extraction methods with acetone.

3.3. Clean-up strategies

Several clean-up strategies were tested to decrease the high ion suppression observed in the final extracts (up to -78%). To evaluate the performance of the different procedures, ME values were calculated by spiking samples after the clean-up step and comparing the signal of the analytes with the signal of a standard solution. We also

evaluated the retention of the target compounds in the different sorbents used to clean the extracts by comparing the response of the compounds for an extract spiked after the clean-up step with the response obtained for an extract spiked before the clean-up step. All procedures were tested on the same two seafood species chosen for QuEChERS optimisation.

For a quick and simple clean-up, we tested the use of Lipifiltr® push-thru purification cartridges which are specifically designed to remove lipids from fatty QuEChERS extracts [31,32]. Several clean-up strategies were tested to decrease the high ion suppression observed in the final extracts (up to -78%). To evaluate the performance of the different procedures, ME values were calculated by spiking samples after the clean-up step and comparing the signal of the analytes with the signal of a standard solution. We also evaluated the retention of the target compounds in the different sorbents used to clean the extracts by comparing the response of the compounds for an extract spiked after the clean-up step with the response obtained for an extract spiked before the clean-up step. All procedures were tested on the same two seafood species chosen for QuEChERS optimisation.

For a quick and simple clean-up, we tested the use of Lipifiltr® push-thru purification cartridges which are specifically designed to remove lipids from fatty QuEChERS extracts [33], so they were tested separately. To do so, 20 mL of acetone spiked with the analytes of interest was transferred to a tube containing 200 mg of sorbent. The tube was vortexed for 3 min and centrifuged at 7000 rpm for 5 min. The organic solvent was then evaporated to ~100 µL and reconstituted to 1 mL with ultrapure water before being injected into the LC-HRMS system. OP diesters were highly retained in PSA but low retention (<11%) was obtained when using C_{18}, making the latter an appropriate sorbent for dSPE. However, when we applied the procedure to sample extracts, we detected the formation of a surface lipid film during the evaporation step. That film hindered the evaporation of the organic solvent, making the process too time-consuming and evidencing the need to change the solvent before evaporation.

Taking into account the high retention of the target compounds in PSA, we proposed an SPE procedure with a lab-packed PSA cartridge to retain the analytes of interests and eliminate the interferences. First, we compared the retention of cartridges packed with different amounts of PSA (200 mg, 500 mg, 1 g and 2 g). To do so, the cartridges were conditioned with 5 mL of MeOH followed by 5 mL of acetone. Then, 20 mL of acetone spiked with the compounds of interest was loaded onto the cartridges and the analytes were eluted with 5 mL of 5% NH_4OH in MeOH. The extracts were concentrated to

~100 µL and reconstituted to 1 mL with ultrapure water before the injection. The best results were obtained with 2 g PSA cartridges.

Next, we carried out the same procedure with sample extracts to evaluate the ME obtained after the clean-up step, but the retention of the analytes dropped from ~100% to less than 20% for both hake and mackerel. That was due to the saturation of the sorbent, hence we reduced the quantity of sample extracted to 100 mg. Even though the results improved, the retention of the compounds was still low (<40%) so we tried to increase it by reducing the presence of organic solvent in the extracts. To do so, we evaporated the QuEChERS extract to ~5 mL and diluted it with 5, 10, 15 and 20 mL of ultrapure water. Overall, the best retention values for both hake and mackerel samples were obtained when diluting the extract with 10 mL of ultrapure water. The ME values obtained using either volume of water ranged from -2 to -70% for hake and from -1 to -51% for mackerel. We chose to dilute the extract with 10 mL of ultrapure water for the SPE clean-up.

Because of the high ME obtained for some analytes, two strategies were assessed to further clean up the SPE extract. On one hand, we tested a washing step with different volumes of ultrapure water/acetone (2:1, v/v) after loading the sample onto the cartridges. While no significant differences in ME were observed, some compounds were eluted in this washing step, so it was discarded. On the other hand, lab-packed Florisil and C_{18} cartridges were connected to the top of the PSA cartridge to retain interfering substances [30,34]. In both cases, the ME was either similar or just slightly better, so this strategy was also rejected and no further modifications were introduced to the method.

Little information can be found about the optimisation of the clean-up steps for seafood samples or about the matrix effects obtained in the literature. That makes the comparison of our results with previous studies infeasible.

3.4. Method validation

Hake and mackerel samples were used to evaluate the performance of the method with high- and low-lipid content seafood samples. To do so, we determined relative recoveries (R_{rel}), method limits of detection (MLOD), method limits of quantification (MLOQ), repeatability and reproducibility. The validation results are summarised in **Table 2**.

Table 2. Retention time (t_R), relative recovery (R_{rel}), matrix effect (ME), method limit of detection (MLOD) and method limit of quantification (MLOQ).

Compound	t_R (min)	Hake (*Merluccius merluccius*)				Mackerel (*Scomber scombrus*)			
		R_{rel} (%)	ME (%)	MLOD (ng g^{-1})	MLOQ (ng g^{-1})	R_{rel} (%)	ME (%)	MLOD (ng g^{-1})	MLOQ (ng g^{-1})
BCEP	4.8	113	-2	25	50	107	-1	50	75
DPHP	12.6	108	-40	1.0	7.5	99	-45	1.0	2.5
DNBP	13.0	138	-39	2.5	5.0	122	-35	1.0	2.5
BDCIPP	13.3	104	-24	2.5	5.0	100	-11	7.5	10
BBOEP	13.6	108	-28	25	50	89	-21	25	50
BEHP	16.6	38	-70	50	75	22	-56	50	75

We used d_8-BCEP, d_{10}-DPHP, d_{18}-DNBP, d_{10}-BDCIPP and d_8-BBOEP as surrogate standards for the validation of the method and the quantification of the samples to compensate for the ME and the low recoveries of some compounds. The surrogates for BCEP, DPHP, DNBP, BDCIPP and BBOEP were the corresponding deuterated analogues. Since no deuterated analogue was available for BEHP, calibration curves were calculated for that compounds with each of the surrogate standards. The use of d_8-BBOEP yielded the best linearity and R_{rel} values so it was selected as the surrogate for BEHP.Quantification was therefore carried out using instrumental calibration curves with surrogates.

R_{rel} was calculated in triplicate for hake and mackerel samples spiked at 100 ng g^{-1} (d.w.) before the extraction. Then, the concentration obtained using the instrumental calibration curves with surrogates was compared with the spiked concentration. As expected, BEHP was the only compound for which R_{rel} was much lower than 100%. R_{rel} was therefore applied to the results for BEHP, depending on the lipid content of the sample.

MLODs and MLOQs were evaluated by spiking the samples at several concentrations. MLOD was established as the concentrations with a signal-to-noise ratio of the quantification ion equal to three. MLOQ was defined as the lowest concentrations in the calibration curve. As can be seen in **Table 2**, the values obtained were similar for both hake and mackerel samples. MLODs ranged from 1.0 to 7.5 ng g^{-1} (d.w.) for DPHP, DNBP

and BDCIPP, and from 25 to 50 ng g^{-1} (d.w.) for BCEP, BBOEP and BEHP. MLOQs ranged from 2.5 to 10 ng g^{-1} (d.w.) for DPHP, DNBP and BDCIPP, and from 50 to 75 ng g^{-1} (d.w.) for BCEP, BBOEP and BEHP. The differences in the limits are due to lower R_{ext} values in the QuEChERS extraction and lower retention in the PSA cartridges for BCEP, BBOEP and BEHP. Overall, even though the values obtained are higher than those previously reported for fish samples using LC-MS/MS [24,25], they are similar to the MLODs and MLOQs obtained for house dust [3] and urine [5].

Repeatability (intra-day) and reproducibility (day-to-day), expressed as relative standard deviation (RSD%, *n = 5*, 100 ng g^{-1} (d.w.)), were below 15 and 21%, respectively, for all compounds except for BEHP. For that compound, repeatability and reproducibility were both above 30%.

Since repeatability and reproducibility were very high for BEHP, R_{rel} was very low, we can confirm that the surrogate chosen for quantification is inadequate. If BEHP is detected in any seafood samples the quantification should therefore be considered tentative or an estimation.

3.5. Application to seafood samples

The optimised QuEChERS/SPE/LC-HRMS method was applied to samples of eight seafood species to determine OP diesters. Four species with low-lipid content (hake, sole, cod and squid) and four species with a high-lipid content (sardine, tuna, mackerel and salmon) were bought from local markets. We evaluated the presence of OP diesters in triplicate for each sample, using the retention time, the exact mass of the analytes and the ratio between the quantifier and the qualifier ions to confirm the presence of the detected compounds. **Figure 3** shows LC-HRMS extracted ion chromatograms of hake and tuna samples.

DPHP and DNBP were determined in all the samples and their presence was confirmed with a mass error below 5 ppm and the expected ion ratios. The concentration of the compounds can be found in **Table 3**. The concentrations of DPHP ranged from 42 to 100 ng g^{-1} (d.w.). The concentrations of DNBP were similar and ranged from 30 to 79 ng g^{-1} (d.w.). No differences could be observed in the results between seafood with low- or high-lipid content, which is in line with previous reports [35]. Little information can be found regarding the occurrence of OP diesters in seafood samples, and the results are calculated depending on the lipid weight or the wet weight of the samples. That makes the comparison of our results with previous studies challenging.

Nevertheless, the concentrations found in this study are within the range of those reported by Hou *et al.* [25], which ranged from 8 to 284 ng g^{-1} (l.w.) for DPHP and from 9 to 161 ng g^{-1} (l.w.) for DNBP. Zheng *et al.* [23], in contrast, found concentrations much lower than those found in this study (up to 2.35 ng g^{-1} (w.w.) for both compounds).

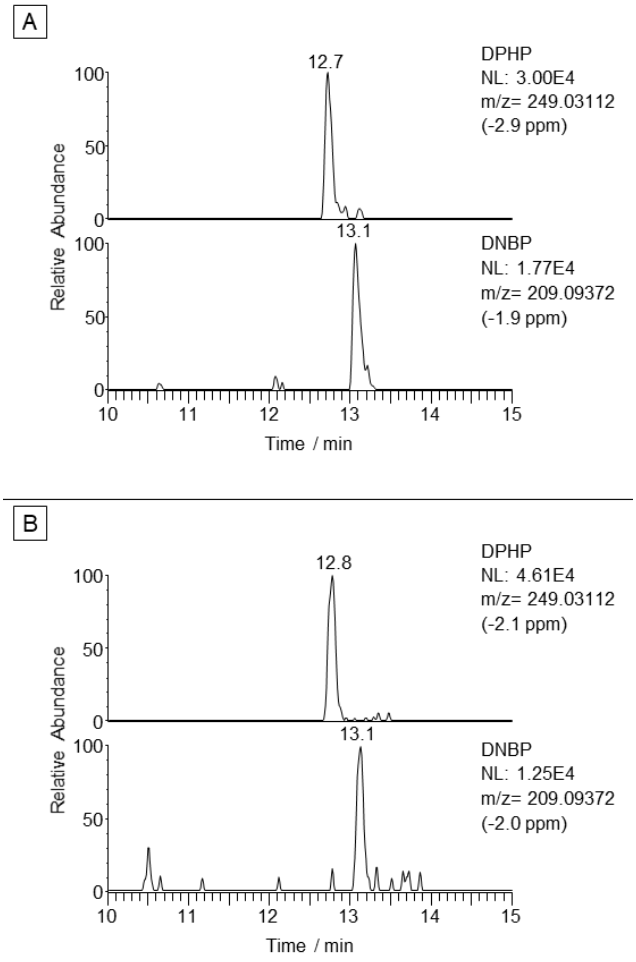


Figure 3. LC-HRMS extracted ion chromatogram of a hake (A) and a tuna (B) samples.

Table 3. Concentrations (ng g^{-1} (d.w.)) and relative standard deviation (RSD%, *n = 3*) of organophosphate diesters found in seafood species with different lipid content.

Compound	Seafood with low lipid content (<10%)				Seafood with high lipid content (>10%)			
	Hake	Sole	Cod	Squid	Sardine	Tuna	Mackerel	Salmon
DPHP	59 (7)	54 (4)	68 (4)	48 (8)	42 (1)	75 (4)	100 (1)	55 (10)
DNBP	67 (2)	36 (13)	54 (0.3)	32 (3)	33 (7)	50 (9)	79 (1)	30 (2)

BCEP, BDCIPP, BBOEP and BEHP were not detected in any sample. Similar results were obtained by Zheng *et al.* [23], ranging from non-detected to concentrations below 0.3 ng g^{-1} (w.w.). However, these results are not strange. Several studies demonstrate that when comparing the tissue-specific distribution of both OP triesters and diesters, muscle tissue usually contains these compounds at some of the lowest concentrations or even at undetectable levels, as OP triesters seem to accumulate and metabolise in metabolically active tissues like the liver and kidneys [24,25].

It should also be noted that a significant positive correlation between the concentration of DNBP and DPHP and the concentration of their parent compounds in several fish has been confirmed [23], making them a useful tool to evaluate the exposure of fish to OP triesters.

4. Conclusions

In this study, we have developed a simple method for the determination of six OP diesters in seafood. The method consists of a QuEChERS extraction, followed by an SPE clean-up and LC-HRMS. Several clean-up strategies were tested and the best results were obtained using PSA as the sorbent for SPE. The procedure was successfully validated for both low- and high-lipid content seafood, yielding good relative recoveries and providing satisfactory reproducibility and sensitivity. We then analysed eight widely consumed seafood species with different lipid contents using this method. DPHP and DNBP were detected and quantified in all eight samples, with concentrations ranging from 30 to 100 ng g^{-1} (d.w.). BCEP, BDCIPP, BBOEP and BEHP could not be detected in any samples. There were no significant differences in the concentrations of OP diesters depending on the lipid content of the sample. The results obtained prove that this method is suitable for the determination of OP diesters in seafood regardless of the lipid content of the samples.

Acknowledgements

This work has been financed by MCIN/AEI/10.13039/501100011033/ and FEDER (Project CTQ2017-84373-R).

Míriam Hidalgo-Serrano would also like to thank the Departament d'Empresa i Coneixement of the Generalitat de Catalunya for the grant 2017FI_B_01116.

References

[1] L. Han, Y. Sapozhnikova, A. Nuñez, Analysis and Occurrence of Organophosphate Esters in Meats and Fish Consumed in the United States, J. Agric. Food Chem. 67 (2019) 12652–12662.

[2] X. Wang, W. Zhong, B. Xiao, Q. Liu, L. Yang, A. Covaci, L. Zhu, Bioavailability and biomagnification of organophosphate esters in the food web of Taihu Lake, China: Impacts of chemical properties and metabolism, Environ. Int. 125 (2019) 25–32.

[3] H. Tan, L. Yang, Y. Yu, Q. Guan, X. Liu, L. Li, D. Chen, Co-Existence of Organophosphate Di- and Tri-Esters in House Dust from South China and Midwestern United States: Implications for Human Exposure, Environ. Sci. Technol. 53 (2019) 4784–4793.

[4] C. He, X. Wang, P. Thai, J.F. Mueller, C. Gallen, Y. Li, C. Baduel, Development and validation of a multi-residue method for the analysis of brominated and organophosphate flame retardants in indoor dust, Talanta. 164 (2017) 503–510.

[5] Y. Wang, W. Li, M.P. Martínez-Moral, H. Sun, K. Kannan, Metabolites of organophosphate esters in urine from the United States: Concentrations, temporal variability, and exposure assessment, Environ. Int. 122 (2019) 213–221.

[6] D. Cao, J. Guo, Y. Wang, Z. Li, K. Liang, M.B. Corcoran, S. Hosseini, S.M.C. Bonina, K.J. Rockne, N.C. Sturchio, J.P. Giesy, J. Liu, A. Li, G. Jiang, Organophosphate esters in sediment of the great lakes, Environ. Sci. Technol. 51 (2017) 1441–1449.

[7] L. Fu, L. Bin, J. Cui, D. Nyobe, P. Li, S. Huang, F. Fu, B. Tang, Tracing the occurrence of organophosphate ester along the river flow path and textile wastewater treatment

processes by using dissolved organic matters as an indicator, Sci. Total Environ. 722 (2020) 137895.

[8] Z. Zhang, H. Shao, M. Wu, J. Zhang, D. Li, J. Li, H. Wang, W. Shi, G. Xu, Occurrence, Distribution, and Potential Sources of Organophosphate Esters in Urban and Rural Surface Water in Shanghai, China, Arch. Environ. Contam. Toxicol. 77 (2019) 115–126.

[9] A. Strobel, W.G. Willmore, C. Sonne, R. Dietz, R.J. Letcher, Organophosphate esters in East Greenland polar bears and ringed seals: Adipose tissue concentrations and in vitro depletion and metabolite formation, Chemosphere. 196 (2018) 240–250.

[10] A.K. Greaves, R.J. Letcher, A Review of Organophosphate Esters in the Environment from Biological Effects to Distribution and Fate, Bull. Environ. Contam. Toxicol. 98 (2017) 2–7.

[11] G. Wang, Z. Du, H. Chen, Y. Su, S. Gao, L. Mao, Tissue-specific accumulation, depuration, and transformation of triphenyl phosphate (TPHP) in adult zebrafish (danio rerio), Environ. Sci. Technol. 50 (2016) 13555–13564.

[12] G. Wang, H. Chen, Z. Du, J. Li, Z. Wang, S. Gao, In vivo metabolism of organophosphate flame retardants and distribution of their main metabolites in adult zebrafish, Sci. Total Environ. 590–591 (2017) 50–59.

[13] R. Hou, C. Huang, K. Rao, Y. Xu, Z. Wang, Characterized in Vitro Metabolism Kinetics of Alkyl Organophosphate Esters in Fish Liver and Intestinal Microsomes, Environ. Sci. Technol. 52 (2018) 3202–3210.

[14] A.K. Greaves, G. Su, R.J. Letcher, Environmentally relevant organophosphate triesters in herring gulls: In vitro biotransformation and kinetics and diester metabolite formation using a hepatic microsomal assay, Toxicol. Appl. Pharmacol. 308 (2016) 59–65.

[15] L. Xu, Q. Hu, J. Liu, S. Liu, C. Liu, Q. Deng, X. Zeng, Z. Yu, Occurrence of organophosphate esters and their diesters degradation products in industrial wastewater treatment plants in China: Implication for the usage and potential degradation during production processing, Environ. Pollut. 250 (2019) 559–566.

[16] C.A. Mitchell, A. Reddam, S. Dasgupta, S. Zhang, H.M. Stapleton, D.C. Volz, Diphenyl Phosphate-Induced Toxicity during Embryonic Development, Environ. Sci. Technol. 53 (2019) 3908–3916.

[17] J.S. Lee, Y. Morita, Y.K. Kawai, A. Covaci, A. Kubota, Developmental circulatory failure caused by metabolites of organophosphorus flame retardants in zebrafish, Danio rerio, Chemosphere. 246 (2020) 125738.

[18] P.D. Noyes, D.E. Haggard, G.D. Gonnerman, R.L. Tanguay, Advanced morphological - behavioral test platform reveals neurodevelopmental defects in embryonic zebrafish exposed to comprehensive suite of halogenated and organophosphate flame retardants, Toxicol. Sci. 145 (2015) 177–195.

[19] G. Su, D. Crump, R.J. Letcher, S.W. Kennedy, Rapid in Vitro metabolism of the flame retardant Triphenyl Phosphate and effects on cytotoxicity and mRNA expression in chicken embryonic hepatocytes, Environ. Sci. Technol. 48 (2014) 13511–13519.

[20] ECHA, Annex III inventory, (2016). https://echa.europa.eu/information-on-chemicals/annex-iii-inventory (accessed September 10, 2021).

[21] H.S. Auta, C.U. Emenike, S.H. Fauziah, Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions, Environ. Int. 102 (2017) 165–176.

[22] J. Castro-Jiménez, N. Ratola, An innovative approach for the simultaneous quantitative screening of organic plastic additives in complex matrices in marine coastal areas, Environ. Sci. Pollut. Res. 27 (2020) 11450–11457.

[23] G. Zheng, P. Miller, F.A. von Hippel, C.L. Buck, D.O. Carpenter, A. Salamova, Legacy and emerging semi-volatile organic compounds in sentinel fish from an arctic formerly used defense site in Alaska, Environ. Pollut. 259 (2020) 113872.

[24] G. Wang, H. Shi, Z. Du, H. Chen, J. Peng, S. Gao, Bioaccumulation mechanism of organophosphate esters in adult zebrafish (Danio rerio), Environ. Pollut. 229 (2017) 177–187.

[25] R. Hou, C. Liu, X. Gao, Y. Xu, J. Zha, Z. Wang, Accumulation and distribution of organophosphate flame retardants (PFRs) and their di-alkyl phosphates (DAPs)

metabolites in different freshwater fish from locations around Beijing, China, Environ. Pollut. 229 (2017) 548–556.

[26] E. Cequier, R.M. Marcé, G. Becher, C. Thomsen, A high-throughput method for determination of metabolites of organophosphate flame retardants in urine by ultra performance liquid chromatography-high resolution mass spectrometry, Anal. Chim. Acta. 845 (2014) 98–104.

[27] Y. Wang, P. Kannan, R.U. Halden, K. Kannan, A nationwide survey of 31 organophosphate esters in sewage sludge from the United States, Sci. Total Environ. 655 (2019) 446–453.

[28] A. Alves, A. Covaci, S. Voorspoels, Method development for assessing the human exposure to organophosphate flame retardants in hair and nails, Chemosphere. 168 (2017) 692–698.

[29] Y. Choi, J. Jeon, Y. Choi, S.D. Kim, Characterizing biotransformation products and pathways of the flame retardant triphenyl phosphate in Daphnia magna using non-target screening, Sci. Total Environ. 708 (2020) 135106.

[30] L. Fu, B. Du, F. Wang, J.C.W. Lam, L. Zeng, E.Y. Zeng, Organophosphate Triesters and Diester Degradation Products in Municipal Sludge from Wastewater Treatment Plants in China: Spatial Patterns and Ecological Implications, Environ. Sci. Technol. 51 (2017) 13614–13623.

[31] M. Hidalgo-Serrano, F. Borrull, R.M. Marcé, E. Pocurull, Simple method for determining phthalate diesters and their metabolites in seafood species using QuEChERS extraction and liquid chromatography-high resolution mass spectrometry, Food Chem. 336 (2021) 127722.

[32] Ó. Castro, E. Pocurull, F. Borrull, Determination of organophosphate ester flame retardants and plasticisers in fish samples by QuEChERs followed by gas chromatography-tandem mass spectrometry. Exposure and risk assessment through fish consumption, J. Chromatogr. A. 1626 (2020).

[33] R. Perestrelo, P. Silva, P. Porto-Figueira, J.A.M. Pereira, C. Silva, S. Medina, J.S. Câmara, QuEChERS - Fundamentals, relevant improvements, applications and future trends, Anal. Chim. Acta. 1070 (2019) 1–28.

[34] B.K. Schindler, K. Förster, J. Angerer, Determination of human urinary organophosphate flame retardant metabolites by solid-phase extraction and gas chromatography-tandem mass spectrometry, J. Chromatogr. B. 877 (2009) 375–381.

[35] J.W. Kim, T. Isobe, K.H. Chang, A. Amano, R.H. Maneja, P.B. Zamora, F.P. Siringan, S. Tanabe, Levels and distribution of organophosphorus flame retardants and plasticizers in fishes from Manila Bay, the Philippines, Environ. Pollut. 159 (2011) 3653–3659.

3.2.4. Discussió de resultats

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

En aquesta secció es comenten els aspectes més importants dels resultats obtinguts en els estudis exposats en les seccions anteriors.

Al primer estudi s'ha optimitzat un mètode analític, utilitzant PLE com a mètode d'extracció, per la determinació de sis dièsters i sis monoèsters de l'àcid ftàlic en mostres de peix i marisc. Per optimitzar l'extracció dels compostos es van avaluar els paràmetres que normalment afecten més la seva eficiència: el dissolvent, la temperatura, el temps i els cicles d'extracció. Els dissolvents avaluats es van triar d'acord amb la bibliografia i les polaritats dels anàlits. Encara que la majoria de mètodes USAE duen a terme l'extracció dels ftalats amb acetona, les recuperacions obtingudes amb aquest dissolvent eren totes inferiors al 15% de manera que es va descartar. Els metabòlits dels ftalats, en canvi, s'extreuen amb aigua, però tot i que en estudis previs al grup de recerca s'havia utilitzat l'aigua com a dissolvent d'extracció de llots de depuradora i peixos en mètodes de PLE, en aquest cas va ser impossible fer-la servir degut a la formació d'una escuma dins dels vials de recol·lecció que interrompia el procés d'extracció automàticament. Així doncs, davant la impossibilitat d'extreure els anàlits amb els dissolvents habituals, es va proposar l'ús d'altres dissolvents amb polaritats similars. De tots els dissolvents que es van provar, el metanol va resultar en les millors recuperacions aparents per la majoria de compostos estudiats. Un gran inconvenient de l'extracció amb metanol, però, és que no és el dissolvent adequat per la majoria d'estratègies de neteja, de manera que cal evaporar els extractes a sequedat (o gairebé) i reconstituir-los amb aigua. El primer pas per reduir l'efecte matriu en mostres de peix i marisc va ser establir un procés de precipitació de lípids per congelació per poder eliminar el contingut lipídic de la mostra i treballar amb un extracte lleugerament més net. Cal destacar que perquè aquest procés funcionés adequadament, la proporció de metanol/aigua havia de ser aproximadament 2:1 (v/v). Aquest procés d'eliminació de lípids per congelació s'ha utilitzat amb anterioritat en mètodes per la determinació de ftalats i pesticides en mostres alimentàries i de biota [1,2]. Com que els efectes matriu encara eren alts es va triar la SPE com a mètode posterior de neteja. D'entre tots els sorbents que es van provar, els millors resultats es van obtenir amb cartutxos Bond Elut Plexa. En aquest estudi, a més, es va avaluar l'ús de dues fonts d'ionització diferents (ESI i APCI). A nivell instrumental la sensibilitat d'ambdues fonts era molt similar per tots els compostos. Tot i això, en analitzar extractes de mostres de peix els efectes matriu obtinguts amb APCI eren molt més elevats que els obtinguts amb ESI.

Al segon estudi s'ha optimitzat un mètode analític basat en l'extracció per QuEChERS per la determinació de sis dièsters i sis monoèsters de l'àcid ftàlic en mostres de peix i marisc. En aquest cas es van avaluar tres tipus de sals d'extracció i les que van donar millor

resultat van ser les sals del mètode estàndard europeu. Segons la nostra recerca, aquestes sals mai havien estat utilitzades per l'extracció de ftalats de mostres ambientals. Després de l'extracció es van avaluar diferents sorbents de dSPE pel procés de neteja, però cap dels sorbents triats era adequat per la neteja de la matriu per la determinació tant dels ftalats com dels seus metabòlits. Es van provar altres estratègies de neteja, però cap d'elles donava resultats acceptables. Així doncs es va decidir dividir l'extracte resultant dels QuEChERS en dos i optimitzar un procés de dSPE per cada tipus d'anàlits. Pels ftalats es va triar un sorbent de neteja que s'havia tret recentment al mercat anomenat Lipifiltr® i que no s'havia utilitzat amb anterioritat. Pels metabòlits dels ftalats es va triar C_{18} com a sorbent de neteja. Aquest segon mètode desenvolupat va millorar significativament els resultats obtinguts en el primer, especialment pel que fa a recuperacions aparents i efectes matriu. A més, en els dos estudis es va fer palesa la necessitat de crear un sistema de control de blancs per la facilitat amb què les mostres es poden contaminar a causa de la presència de ftalats a l'aire i en el material del laboratori.

Ambdós mètodes es van aplicar a l'anàlisi d'espècies de peix i marisc comprades en diferents mercats de Tarragona. Les espècies escollides van ser les deu espècies més consumides per la població de Catalunya segons l'ENCAT [3], i es van poder determinar 8 del total de 12 anàlits inclosos en els estudis. En general, els resultats obtinguts amb els dos mètodes van ser similars, tot i que les mostres analitzades no van ser les mateixes. Els ftalats detectats a concentracions més altes van ser el DEP i el DEHP, i els seus respectius metabòlits (MEP i MEHP) van ser també els monoèsters detectats a concentracions més elevades.

Al tercer estudi descrit en aquesta secció s'ha optimitzat per primera vegada un mètode analític basat en l'extracció per QuEChERS per la determinació de sis dièsters de l'àcid fosfòric en mostres de peix i marisc. A l'hora d'optimitzar la separació dels compostos es van comparar els cromatogrames obtinguts amb tres fases mòbils diferents escollides d'acord amb la bibliografia. Finalment, es va triar la fase mòbil que contenia acetat amònic, ja que amb aquesta fase mòbil s'obtenia una millor resolució de pic per la majoria d'anàlits. Pel que fa a l'extracció, tot i que el dissolvent orgànic utilitzat habitualment per QuEChERS és l'acetonitril, en aquest cas les recuperacions d'extracció obtingudes eren molt baixes. Així doncs, vam provar l'extracció amb acetona tenint en compte un mètode ja publicat en el que s'extreien quatre metabòlits dels organofosfats de mostres de peix per PLE amb aquest dissolvent [4]. Les recuperacions d'extracció van millorar considerablement. Després es van avaluar tres tipus de sals d'extracció i les que van donar millor resultat van ser les sals del mètode original de QuEChERS. Com que, un cop més, els efectes matriu obtinguts eren elevats es van avaluar diverses estratègies de

neteja. Primerament es va plantejar una neteja amb dSPE ja que és el procés de neteja habitual després dels QuEChERS. Tot i això, l'alt contingut lipídic encara present a les mostres després de la dSPE no permetia la concentració dels extractes i es va descartar. Finalment es va proposar una neteja per SPE amb 2 g PSA. Pel que fa a la validació, aquest és l'únic dels mètodes desenvolupats en aquesta tesi doctoral en el qual s'utilitzen patrons deuterats anàlegs per la quantificació dels compostos estudiats.

Aquest mètode també es va aplicar a l'anàlisi d'espècies de peix i marisc comprades en diferents mercats de Tarragona. Només es van poder determinar 2 del total de 6 anàlits inclosos en l'estudi. Tant el DNBP com el DPHP es van poder determinar en totes les mostres analitzades tot i que, a causa de la poca informació disponible sobre la presència d'aquests compostos en organismes marins i de les diferents unitats en les que es poden expressar els resultats, és difícil comparar els resultats obtinguts amb aquells de la bibliografia.

Referències

[1] R. Cariou, F. Larvor, F. Monteau, P. Marchand, E. Bichon, G. Dervilly-Pinel, J.P. Antignac, B. Le Bizec, Measurement of phthalates diesters in food using gas chromatography-tandem mass spectrometry, Food Chem. 196 (2016) 211–219.

[2] N.A.H. Ismail, S.Y. Wee, A.Z. Aris, Multi-class of endocrine disrupting compounds in aquaculture ecosystems and health impacts in exposed biota, Chemosphere. 188 (2017) 375–388.

[3] ENCAT, Avaluació de l'estat nutricional de la població catalana 2002-2003, Enquesta Nutricional de Catalunya. (2003). www.salutweb.gencat.cat.

[4] R. Hou, C. Liu, X. Gao, Y. Xu, J. Zha, Z. Wang, Accumulation and distribution of organophosphate flame retardants (PFRs) and their di-alkyl phosphates (DAPs) metabolites in different freshwater fish from locations around Beijing, China, Environ. Pollut. 229 (2017) 548–556.

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

3.3. Monitoratge de compostos orgànics d'alt volum de producció en peix i marisc

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

Tot i que la presència d'organofosfats en organismes marins ha sigut àmpliament estudiada, la informació sobre la presència dels seus metabòlits és extremadament limitada. Aquesta falta d'informació es fa encara més palesa quan parlem de peixos destinats al consum humà. Disposar d'aquest coneixement és interessant per diversos motius. Per una banda, la concentració de diversos dièsters de l'àcid fosfòric en peixos està correlacionada amb la concentració dels respectius trièsters [1]. Així doncs, es pot utilitzar la determinació dels metabòlits com una mesura de la contaminació d'organofosfats en peixos. A més, com ja hem esmentat a la introducció d'aquesta tesi, diversos dièsters poden tenir efectes disruptors endocrins i tòxics per la reproducció d'éssers vius, de manera que és important poder calcular l'exposició de la població a aquests compostos a través de la ingesta de peix i marisc.

Per mirar de cobrir aquest buit, en aquest apartat es presenten els resultats preliminars obtinguts durant el monitoratge de dièsters de l'àcid fosfòric en mostres recollides al llarg d'un any de les espècies de peix i marisc més consumides a Catalunya. Els mostrejos s'han dut a terme cada quatre mesos des de febrer de 2019 fins a febrer de 2020 (quatre mostrejos en total) i cada mes s'han escollit tres establiments diferents de la ciutat de Tarragona per tenir mostres representatives dels costums de compra de la població. Així doncs, en cada mostreig les mostres s'han adquirit en una peixateria del mercat municipal, una peixateria de barri i una peixateria de supermercat. Per a les anàlisis, només s'ha conservat les parts comestibles dels peixos i mariscos, que després s'han congelat, liofilitzat i homogeneïtzat. Posteriorment, per cada mostreig s'han fet mostres compostes per cada espècie afegint quantitats iguals de les mostres obtingudes en cadascun dels establiments. En total s'han analitzat quatre mostres de cada espècie i un total de 40 mostres.

En aquest apartat es presenten els resultats obtinguts a l'estudi. Tot i això, cal tractar-los per escriure un article científic i enviar-lo a una revista científica per a la seva publicació.

References

[1] X. Li, N. Zhao, J. Fu, Y. Liu, W. Zhang, S. Dong, P. Wang, X. Su, J. Fu, Organophosphate Diesters (Di-OPEs) Play a Critical Role in Understanding Global Organophosphate Esters (OPEs) in Fishmeal, Environ. Sci. Technol. 54 (2020) 12130–12141.

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

3.3.1. Resultats preliminars del monitoratge de dièsters de l'àcid fosfòric en peix i marisc

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

ORGANOPHOSPHATE ESTER METABOLITES IN SEAFOOD OF COMMON CONSUMPTION

Míriam Hidalgo-Serrano, Francesc Borrull, Eva Pocurull, Rosa M. Marcé

Universitat Rovira i Virgili, Department of Analytical Chemistry and Organic Chemistry, Marcel·lí Domingo s/n, Tarragona 43007, Spain

---

Abstract

Even though the occurrence of organophosphate triesters in different compartments of the environment has been widely studied, there is a lack of information on the occurrence of their metabolites (organophosphate (OP) diesters) especially in seafood species that can contribute to the intake of these compounds in humans. To cover that deficiency, in this study we carried out a monitoring program to evaluate the occurrence of OP diesters for one year in ten widely consumed seafood species in the Tarragona area. The highest total OP diester concentrations were determined in carnivore species (52–683 ng g^{-1} (dry weight (d.w.))). DNBP and DPHP were the predominant analytes in all the samples, ranging from 17 to 636 ng g^{-1} (d.w.)) and from 21 to 77 ng g^{-1} (d.w.)), respectively. The seasonal variation of OP diesters was also evaluated but no evident pattern was observed. Further research is needed in order to identify the sources of contamination of OP diesters and to be able to characterise the risk these compounds can have on humans.

Keywords: *Seafood, Organophosphate diesters, QuEChERS, Liquid chromatography, High-resolution mass spectrometry, Monitoring*

---

1. Introduction

Fish and shellfish are good sources of nutrients, including proteins, omega-3 fatty acids, vitamins and minerals, which is the reason why governments around the world recommend up to four portions of seafood a week to eat a balanced healthy diet [1–4]. However, aquatic organisms can be exposed to contaminants from the environment and can be one of the main sources of human exposure to these contaminants through intake.

For instance, since the ban of several classes of brominated flame retardants, the demand for alternative flame retardants such as organophosphate esters (OPEs) has gradually increased [5]. OPEs are not only used as flame retardants, but also as plasticisers, foaming and wetting agents, and as stabilisers in commercial products [6,7]. Since OPEs are not chemically bound to the materials they are added to, most of these compounds are released into the environment during the lifetime of the products [8]. Subsequently, OPEs have been widely reported in water [9–11], soil and sediments [12–15], indoor dust [16,17], biota [8,12,14,15,18–20] and human samples [21,22].

Several studies report that OPEs can be easily degraded to the corresponding organophosphate diesters (OP diesters) via degradation in humans [23], animals [24,25] or by other organisms [26] in the environment, as well as abiotic degradation via hydrolysis and photodegradation [27]. Moreover, some OP diesters are produced for their use in the industry as flame retardants, plasticisers, catalysts, wetting agents and as intermediates of pesticides, medicines and organic materials [5,28–30]. The annual production volume of OP diesters is estimated to be more than 17,050 metric tons worldwide, which makes industrial discharges an additional source of contamination of these compounds [5]. The presence of these compounds in the environment should be cause of concern, as some OP diesters have shown negative effects in animals, humans and other organisms. Reproductive and developmental toxicity [11,31,32], nephrotoxicity [33], and alteration of gene expression [11,34] are some of the adverse effects reported in the literature.

However, the presence of OP diesters in seafood and the role fish and shellfish can have on the dietary intake of these contaminants are still largely unexplored. Only a few studies can be found in the literature that determine the presence of OP diesters in fish [7,8,35]. With this information gap in mind, the present study investigates the occurrence of six OP diesters in ten widely consumed seafood species in samples purchased over a year. To the best of our knowledge, this is the first time the seasonal distribution of OP diesters has been evaluated in seafood samples.

2. Materials and methods

2.1. Reagents and standards

The solid standards of the six OP diesters studied, supplied by LGC Standards (Teddington, Middlesex, UK), were the following: bis(2-butoxyethyl) phosphate (BBOEP), bis(2-chloroethyl) phosphate (BCEP), bis(1,3-dichloropropyl) phosphate (BDCIPP), bis(2-ethylhexyl) phosphate (BEHP), diphenyl phosphate (DPHP) and dibutyl phosphate (DNBP). The five surrogate standards d_8-BBOEP, d_8-BCEP, d_{10}-BDCIPP, d_{10}-DPHP and d_{18}-DNBP were also provided by the same supplier. Individual standard solutions were prepared in methanol at concentrations of 1000 mg L^{-1} for the OP diesters and of 100 mg L^{-1} for the surrogate standards.

Acetone and methanol of HPLC grade for the extraction procedure and water and methanol of MS grade for the mobile phase were purchased from Scharlab (Barcelona, Spain). Ammonium acetate and ammonium hydroxide were provided by Sigma-Aldrich (Saint Louis, Missouri, USA). Ultrapure water was obtained with a water purification system from Merck Millipore (Darmstadt, Germany).

The QuEChERS extraction salt packets and the bulk PSA sorbent for the extraction procedure were supplied by Scharlab.

2.2. Sampling

Ten seafood species were selected among those most widely consumed by the population in Catalonia [36]. The chosen fish and shellfish species were cod (Gadus morhua), hake (Merluccius merluccius), mackerel (Scomber scombrus), mussel (Mytilus galloprovincialis), salmon (Salmo salar), sardine (Sardina pilchardus), shrimp (Aristeus antennatus), sole (Solea solea), squid (Loligo Vulgaris) and tuna (Thunnus thynnus). Samples of each species were purchased at three different local commercial establishments in Tarragona (fish market, local market and supermarket) every four months from February 2019 to February 2020. All samples were immediately cleaned, the fishes were filleted, and the shells of the shrimps and mussels were removed before the samples were stored in a freezer. The frozen samples were lyophilised separately using a Genevac miVac Duo sample concentrator with a freeze-drying system (Ipswich, UK), ground and sieved through a 500 µm mesh to obtain a homogenous sample. Then, four composite samples for each species were obtained by mixing equal amounts of the

samples obtained from the three different establishments each month. Four samples of each species and a total of 40 samples were therefore analysed.

2.3. Sample extraction

The following extraction procedure described was optimised and validated in a previous study of our group. Briefly, 0.1 g of lyophilised sample spiked with 400 ng of the surrogate standards was weighted in a 50 mL polypropylene tube (ThermoFisher Scientific, Waltham, USA). 10 mL of ultrapure water was added to the tube and the mixture was vortexed for 1 minute. Then, 10 mL of acetone was added to the mixture and the tube was vortexed again. An extraction salt packet for the original QuEChERS method (containing 1 g of sodium chloride and 4 g of anhydrous magnesium sulphate) was subsequently added. After the mixture was vortexed for 5 minutes and centrifuged at 4000 rpm for 5 minutes, the acetone layer was transferred to another tube and the extraction was repeated once more. The extracts of both extractions were combined and concentrated to 5 mL with the miVac Duo sample concentrator.

Then, 10 mL of ultrapure water were added to the extract and the mixture was loaded into 2 g homemade PSA cartridges previously conditioned with 5 mL of methanol and 5 mL of ultrapure water/acetone 2:1 (v/v). The analytes were eluted with 5 mL of methanol 5% ammonium hydroxide. The final extracts were concentrated to ~200 µL and taken up to 1 mL with ultrapure water for analysis.

2.4. Liquid chromatography-high resolution mass spectrometry analysis

The analysis of the samples was performed with an Accela UHPLC system (Thermo Scientific, Bremen, Germany) coupled to an ExactiveOrbitrap^{TM} mass spectrometer (Thermo Scientific). An Ascentis Express C18 fused-core column (100 mm x 2.1 mm i.d., 2.7 µm particle size) from Sigma-Aldrich was used for the chromatographic separation, which was performed in gradient elution with 2 mM ammonium acetate in water (solvent A) and MeOH (solvent B) as the mobile phase at a flow rate of 200 µL min^{-1}. The gradient started isocratic at 8% of B for 4.5 min. It was then increased to 90% of B in 9 min and to 100% of B in 0.5 min. It remained constant for 3 min and then returned to initial conditions. The column oven temperature was 30 ^{o}C, the sample tray temperature was 10 ^{o}C and the injection volume was 20 µL.

For HRMS measurements, a single window in negative mode was used with two scan events: a full scan (at 50,000 FWHM with 250 ms of injection time) and a fragmentation

scan (at 10,000 FWHM with 50 ms of injection time) using a collision voltage of 25 eV in the HCD cell. The capillary voltage was set at -20 V, spray voltage at -2.5 kV, skimmer voltage at -20 V and tube lens voltage at -70 V. The sheath gas flow rate was 40 A.U. and the auxiliary gas flow rate was 5 A.U. The heater and capillary temperature were set at 425 and 250 ^{o}C, respectively.

2.5. Quality control and quantification of samples

Quality control was performed to correctly determine the compounds of interest. Several blanks were performed to confirm there was no cross-contamination during the analyses. Procedural blanks were carried out following the complete analytical procedure described for the seafood without any sample (*n = 10*). For the LC-HRMS analyses, pure solvent blanks were analysed every nine seafood sample extracts. As expected, no target analytes were detected in any of the blanks carried out.

The validation of the procedure used was carried out in a previous study of our group. Briefly, deuterated analogues of BCEP, DPHP, DNBP, BDCIPP and BBOEP were used as surrogate standards for the quantification of these analytes in the samples. The deuterated analogue of BEHP was not available at the time of this study, hence this compound was quantified using d_8-BBOEP as a surrogate. Recovery experiments (*n = 3*) were calculated by spiking samples with high- and low-lipid content at 100 ng g^{-1} (d.w.) and comparing the ratio of the OP diesters and their surrogates with the one obtained in instrumental calibration curves. The relative recoveries for all compounds were approximately 100% except for BEHP, hence recoveries were applied to the results obtained for BEHP depending on the lipid content of the sample. Method limits of quantification (MLOQ) ranged from 2.5 to 10 ng g^{-1} (d.w.) for DPHP, DNBP and BDCIPP, and from 50 to 75 ng g^{-1} (d.w.) for BCEP, BBOEP and BEHP.

3. Results and Discussion

The detection frequencies (DFs), minimum, maximum and mean concentrations of OP diesters detected in the studied samples can be found in **Tables 1 and 2**. Some OP diesters were determined in all the fish and shellfish species studied. DPHP and DNBP were the most frequently determined OP diesters, with DFs ranging from 75 to 100%. BEHP and BDCIPP had DF of 25% and neither BCEP nor BBOEP were detected in any of the samples. Even though no large differences in DFs were observed between the studied species, BDCIPP was only determined in seafood species with high-lipid content (i.e.: tuna and mussels)

Table 1. Concentration of OP diesters (di-OPEs) in low-lipid content seafood samples (ng g^{-1} (d.w.)).

Compounds	Hake					
	February 2019	June 2019	October 2019	February 2020	DF (%)	mean (min-max)
DPHP	39	37	33	37	100%	37 (32-39)
DNBP	43	53	64	517	100%	144 (42-517)
BEHP	n.d.	n.d.	n.d.	107	25%	(n.d-107)[a]
∑di-OPEs	82	90	97	661	-	233 (82-661)
Compounds	Sole					
	February 2019	June 2019	October 2019	February 2020	DF (%)	mean (min-max)
DPHP	54	54	22	26	100%	41 (21-59)
DNBP	36	48	20	407	100%	80 (17-407)
BEHP	n.d.	n.d.	n.d.	81	25%	(n.d.-81) [a]
∑di-OPEs	90	102	42	514	-	187 (42-514)
Compounds	Cod					
	February 2019	June 2019	October 2019	February 2020	DF (%)	mean (min-max)
DPHP	68	56	37	68	100%	56 (36-68)
DNBP	54	36	53	532	100%	112 (34-532)
BEHP	n.d.	n.d.	n.d.	n.d.	-	n.d.
∑di-OPEs	122	92	90	600	-	226 (90-600)
Compounds	Shrimp					
	February 2019	June 2019	October 2019	February 2020	DF (%)	mean (min-max)
DPHP	41	44	23	64	100%	43 (23-64)
DNBP	37	37	20	41	100%	34 (20-41)
BEHP	n.d.	n.d.	n.d.	n.d.	-	n.d.
∑di-OPEs	78	81	43	105	-	77 (43-105)
Compounds	Squid					
	February 2019	June 2019	October 2019	February 2020	DF (%)	mean (min-max)
DPHP	48	40	22	47	100%	40 (22-51)
DNBP	32	38	30	636	100%	135 (28-636)
BEHP	n.d.	n.d.	<MLOQ	n.d.	25%	(n.d.-<MLOQ) [a]
∑di-OPEs	80	78	52	683	-	223 (52-683)

n.d.: not detected
<MLOQ: Concentration under method limits of quantification
[a] mean concentration not calculated because the compound was only detected in one sample

Table 2. Concentration of OP diesters (di-OPEs) in high-lipid content seafood samples (ng g^{-1} (d.w.)).

Compounds	Sardine					
	February 2019	June 2019	October 2019	February 2020	DF (%)	mean (min-max)
DPHP	42	38	29	33	100%	31 (29-42)
DNBP	33	106	38	42	100%	47 (30-106)
BDCIPP	n.d.	n.d.	n.d.	n.d.	-	-
BEHP	n.d.	<MLOQ	n.d.	n.d.	25%	(n.d.-<MLOQ) [a]
∑di-OPEs	75	144	67	75	-	90 (67-144)
Compounds	Tuna					
	February 2019	June 2019	October 2019	February 2020	DF (%)	mean (min-max)
DPHP	75	42	n.d.	36	75%	52 (35-77)
DNBP	50	347	199	59	100%	135 (47-347)
BDCIPP	n.d.	n.d.	18	n.d.	25%	(n.d.-18) [a]
BEHP	n.d.	n.d.	n.d.	n.d.	-	n.d.
∑di-OPEs	125	389	217	95	-	207 (95-389)
Compounds	Mackerel					
	February 2019	June 2019	October 2019	February 2020	DF (%)	mean (min-max)
DPHP	49	50	n.d.	38	75%	38 (50-46)
DNBP	40	46	24	45	100%	39 (24-46)
BDCIPP	n.d.	n.d.	n.d.	n.d.	-	n.d.
BEHP	n.d.	n.d.	n.d.	<MLOQ	25%	(n.d.-<MLOQ) [a]
∑di-OPEs	89	96	24	83	-	73 (24-96)
Compounds	Salmon					
	February 2019	June 2019	October 2019	February 2020	DF (%)	mean (min-max)
DPHP	55	33	27	43	100%	45 (27-61)
DNBP	30	42	39	42	100%	37(28-47)
BDCIPP	n.d.	n.d.	n.d.	n.d.	-	n.d.
BEHP	n.d.	n.d.	n.d.	n.d.	-	n.d.
∑di-OPEs	85	75	66	85	-	78 (66-85)
Compounds	Mussels					
	February 2019	June 2019	October 2019	February 2020	DF (%)	mean (min-max)
DPHP	47	42	23	35	100%	37 (23-47)
DNBP	61	100	32	35	100%	57 (32-100)
BDCIPP	n.d.	n.d.	18	n.d.	25%	(n.d.-18) [a]
BEHP	n.d.	n.d.	n.d.	n.d.	-	n.d.
∑di-OPEs	108	142	73	70	-	98 (70-142)

n.d.: not detected

<MLOQ: Concentration under method limits of quantification

[a] mean concentration not calculated because the compound was only detected in one sample

The sum of OP diester (∑di-OPEs) concentrations in the samples was in the range of 24 to 683 ng g^{-1} (d.w.). Among the species studied, hake presented the highest average ∑di-OPEs (233 ng g^{-1} (d.w.)), followed by cod (226 ng g^{-1} (d.w.)), squid (223 ng g^{-1} (d.w.)), and tuna (207 ng g^{-1} (d.w.)). These are all species of carnivorous fish and they feed primarily on smaller fishes, as well as crustaceans and molluscs [37–41], which might indicate food as one of the main sources of OPEs intake in these species.

As can be seen in Tables 1 and 2, DNBP was the predominant OP diester in most seafood species. It was detected in all the of samples, with average concentrations ranging from 34 to 144 ng g^{-1} (d.w.). The lowest concentration of DNBP was determined in sole samples (17 ng g^{-1} (d.w.)), while the highest concentration was determined in squid samples (636 ng g^{-1} (d.w.)). These results are consistent with those previously reported by Hou et al. [8], who found that mean concentrations of DNBP in freshwater fishes were significantly higher than the mean concentrations of other OP diesters like DPHP and BEHP. Moreover, the parent compound of DNBP, which is TNBP, has also been reported as the predominant OPE in seafood samples [8,35]. DNBP is also the OP diester with the highest annual production volume [5], indicating industrial pollution as a possible source of this compound besides metabolism.

DPHP had the second-highest average concentrations of OP diesters, ranging from 31 to 56 ng g^{-1} (d.w.) and was detected in 95% of the samples. The lowest concentration of this compound was determined in sole samples (21 ng g^{-1} (d.w.)) and the highest one in tuna samples (77 ng g^{-1} (d.w.)).

BDCIPP and BEHP were detected in only 5 and 12.5% of the samples, respectively. BDCIPP was determined at the same concentration in both tuna and mussel samples (18 ng g^{-1} (d.w.)). In contrast, BEHP was determined at high concentrations in hake and sole samples (107 and 81 ng g^{-1} (d.w.), respectively), and below method limits of quantification in squid, sardine and tuna samples.

There is very limited data on the occurrence of OP diesters in seafood samples which complicates the comparison of concentrations to find patterns and tendencies in the results obtained. However, not being able to detect or quantify some OP diesters in seafood muscle is common. Several studies report that the metabolic transformation of OPEs in fish is mainly carried out in the liver and kidneys [7,8]. Moreover, chlorinated OPEs are more stable and resistant to degradation than alkyl and aryl OPEs, hence it is common to find BCEP, BDCIPP and other chlorinated OP diesters and low concentrations or even non-detectable levels [42].

Overall, the lowest ∑di-OPEs concentrations were determined in October 2019 (24–90 ng g^{-1} (d.w.)), except for hake and tuna. In contrast, the highest ∑di-OPEs concentrations in low-lipid and in high-lipid seafood samples were determined in February 2020 (105–683 ng g^{-1} (d.w.)) and June 2019 (85–389 ng g^{-1} (d.w.)), respectively. The difference in these values is due to very high concentrations of DNBP, and in some cases BEHP, in the hake, sole, cod and squid samples of February 2020.

Regarding the individual concentrations of OP diesters, the sampling of October 2019 was the only one with samples in which DPHP could not be detected (in tuna and mackerel). In contrast, BDCIPP was determined only in tuna and mussel samples of October 2019.

Even though big differences are observed in all seafood species between the different samplings (Table 2), no obvious seasonal variation pattern can be discerned. One of the main reasons for this is that most fish and shellfish species bought in local markets are caught in different zones; some species like squid, sardines and mackerels are caught by local fishermen, but others like cod and salmon are not endemic and they are imported from abroad. The different origin of the seafood species makes it impossible to establish a clear seasonal pattern. It also hinders the identification of the sources of contamination of the samples.

References

[1] Agencia española de seguridad alimentaria y nutrición, Recomendaciones de consumo de pescado, (2021). http://www.aesan.gob.es/AECOSAN/web/seguridad_alimentaria/ampliacion/mercurio.html (accessed October 25, 2021).

[2] U.S. food and drug administration, Advice about eating fish, (2019). https://www.fda.gov/food/consumers/advice-about-eating-fish (accessed October 25, 2021).

[3] Victoria State Government, Fish, (2020). https://www.betterhealth.vic.gov.au/health/healthyliving/fish (accessed October 25, 2021).

[4] UK National Health Service, Fish and shellfish, (2018). https://www.nhs.uk/live-well/eat-well/fish-and-shellfish-nutrition/ (accessed October 25, 2021).

[5] Y. Liu, S. Gong, L. Ye, J. Li, C. Liu, D. Chen, M. Fang, R.J. Letcher, G. Su, Organophosphate (OP) diesters and a review of sources, chemical properties, environmental occurrence, adverse effects, and future directions, Environment International. 155 (2021) 106691.

[6] L. Han, Y. Sapozhnikova, A. Nuñez, Analysis and Occurrence of Organophosphate Esters in Meats and Fish Consumed in the United States, Journal of Agricultural and Food Chemistry. 67 (2019) 12652–12662.

[7] G. Wang, H. Shi, Z. Du, H. Chen, J. Peng, S. Gao, Bioaccumulation mechanism of organophosphate esters in adult zebrafish (Danio rerio), Environmental Pollution. 229 (2017) 177–187.

[8] R. Hou, C. Liu, X. Gao, Y. Xu, J. Zha, Z. Wang, Accumulation and distribution of organophosphate flame retardants (PFRs) and their di-alkyl phosphates (DAPs) metabolites in different freshwater fish from locations around Beijing, China, Environmental Pollution. 229 (2017) 548–556.

[9] L. Fu, L. Bin, J. Cui, D. Nyobe, P. Li, S. Huang, F. Fu, B. Tang, Tracing the occurrence of organophosphate ester along the river flow path and textile wastewater treatment processes by using dissolved organic matters as an indicator, Science of the Total Environment. 722 (2020) 137895.

[10] U.J. Kim, J.K. Oh, K. Kannan, Occurrence, Removal, and Environmental Emission of Organophosphate Flame Retardants/Plasticizers in a Wastewater Treatment Plant in New York State, Environmental Science and Technology. 51 (2017) 7872–7880.

[11] Y. Li, C. Yao, Q. Zheng, W. Yang, X. Niu, Y. Zhang, G. Lu, Occurrence and ecological implications of organophosphate triesters and diester degradation products in wastewater, river water, and tap water, Environmental Pollution. 259 (2020) 113810.

[12] S.H. Brandsma, P.E.G. Leonards, H.A. Leslie, J. de Boer, Tracing organophosphorus and brominated flame retardants and plasticizers in an estuarine food web, Science of the Total Environment. 505 (2015) 22–31.

[13] D. Cao, J. Guo, Y. Wang, Z. Li, K. Liang, M.B. Corcoran, S. Hosseini, S.M.C. Bonina, K.J. Rockne, N.C. Sturchio, J.P. Giesy, J. Liu, A. Li, G. Jiang, Organophosphate esters in

sediment of the great lakes, Environmental Science and Technology. 51 (2017) 1441–1449.

[14] J. Castro-Jiménez, N. Ratola, An innovative approach for the simultaneous quantitative screening of organic plastic additives in complex matrices in marine coastal areas, Environmental Science and Pollution Research. 27 (2020) 11450–11457.

[15] M. Giulivo, E. Capri, E. Kalogianni, R. Milacic, B. Majone, F. Ferrari, E. Eljarrat, D. Barceló, Occurrence of halogenated and organophosphate flame retardants in sediment and fish samples from three European river basins, Science of the Total Environment. 586 (2017) 782–791.

[16] S.H. Brandsma, J. de Boer, M.J.M. van Velzen, P.E.G. Leonards, Organophosphorus flame retardants (PFRs) and plasticizers in house and car dust and the influence of electronic equipment, Chemosphere. 116 (2014) 3–9.

[17] S. Brommer, S. Harrad, Sources and human exposure implications of concentrations of organophosphate flame retardants in dust from UK cars, classrooms, living rooms, and offices, Environment International. 83 (2015) 202–207.

[18] S. Chu, R.J. Letcher, Determination of organophosphate flame retardants and plasticizers in lipid-rich matrices using dispersive solid-phase extraction as a sample cleanup step and ultra-high performance liquid chromatography with atmospheric pressure chemical ionization mass, Analytica Chimica Acta. 885 (2015) 183–190.

[19] J.W. Kim, T. Isobe, K.H. Chang, A. Amano, R.H. Maneja, P.B. Zamora, F.P. Siringan, S. Tanabe, Levels and distribution of organophosphorus flame retardants and plasticizers in fishes from Manila Bay, the Philippines, Environmental Pollution. 159 (2011) 3653–3659.

[20] A. Strobel, W.G. Willmore, C. Sonne, R. Dietz, R.J. Letcher, Organophosphate esters in East Greenland polar bears and ringed seals: Adipose tissue concentrations and in vitro depletion and metabolite formation, Chemosphere. 196 (2018) 240–250.

[21] A. Alves, A. Covaci, S. Voorspoels, Method development for assessing the human exposure to organophosphate flame retardants in hair and nails, Chemosphere. 168 (2017) 692–698.

[22] E. Cequier, R.M. Marcé, G. Becher, C. Thomsen, A high-throughput method for determination of metabolites of organophosphate flame retardants in urine by ultra performance liquid chromatography-high resolution mass spectrometry, Analytica Chimica Acta. 845 (2014) 98–104.

[23] Y. Wang, W. Li, M.P. Martínez-Moral, H. Sun, K. Kannan, Metabolites of organophosphate esters in urine from the United States: Concentrations, temporal variability, and exposure assessment, Environment International. 122 (2019) 213–221.

[24] A.K. Greaves, G. Su, R.J. Letcher, Environmentally relevant organophosphate triesters in herring gulls: In vitro biotransformation and kinetics and diester metabolite formation using a hepatic microsomal assay, Toxicology and Applied Pharmacology. 308 (2016) 59–65.

[25] R. Hou, C. Huang, K. Rao, Y. Xu, Z. Wang, Characterized in Vitro Metabolism Kinetics of Alkyl Organophosphate Esters in Fish Liver and Intestinal Microsomes, Environmental Science and Technology. 52 (2018) 3202–3210.

[26] C. Yao, H. Yang, Y. Li, A review on organophosphate flame retardants in the environment: Occurrence, accumulation, metabolism and toxicity, Science of the Total Environment. 795 (2021) 148837.

[27] J. Li, L. Zhao, R.J. Letcher, Y. Zhang, K. Jian, J. Zhang, G. Su, A review on organophosphate Ester (OPE) flame retardants and plasticizers in foodstuffs: Levels, distribution, human dietary exposure, and future directions, Environment International. 127 (2019) 35–51.

[28] C.A. Mitchell, A. Reddam, S. Dasgupta, S. Zhang, H.M. Stapleton, D.C. Volz, Diphenyl Phosphate-Induced Toxicity during Embryonic Development, Environmental Science and Technology. 53 (2019) 3908–3916.

[29] L. Xu, B. Zhang, Q. Hu, Y. Liu, T. Shang, X. Zeng, Z. Yu, Occurrence and spatio-seasonal distribution of organophosphate tri- and di-esters in surface water from Dongting Lake and their potential biological risk, Environmental Pollution. 282 (2021) 117031.

[30] H. Tan, L. Yang, Y. Yu, Q. Guan, X. Liu, L. Li, D. Chen, Co-Existence of Organophosphate Di- and Tri-Esters in House Dust from South China and Midwestern

United States: Implications for Human Exposure, Environmental Science and Technology. 53 (2019) 4784–4793.

[31] J.S. Lee, Y. Morita, Y.K. Kawai, A. Covaci, A. Kubota, Developmental circulatory failure caused by metabolites of organophosphorus flame retardants in zebrafish, Danio rerio, Chemosphere. 246 (2020) 125738.

[32] Q. Zhang, C. Yu, L. Fu, S. Gu, C. Wang, New Insights in the Endocrine Disrupting Effects of Three Primary Metabolites of Organophosphate Flame Retardants, Environmental Science and Technology. 54 (2020) 4465–4474.

[33] H. Kang, J. Lee, J.P. Lee, K. Choi, Urinary metabolites of organophosphate esters (OPEs) are associated with chronic kidney disease in the general US population, NHANES 2013–2014, Environment International. 131 (2019) 105034.

[34] G. Su, D. Crump, R.J. Letcher, S.W. Kennedy, Rapid in Vitro metabolism of the flame retardant Triphenyl Phosphate and effects on cytotoxicity and mRNA expression in chicken embryonic hepatocytes, Environmental Science and Technology. 48 (2014) 13511–13519.

[35] G. Zheng, P. Miller, F.A. von Hippel, C.L. Buck, D.O. Carpenter, A. Salamova, Legacy and emerging semi-volatile organic compounds in sentinel fish from an arctic formerly used defense site in Alaska, Environmental Pollution. 259 (2020) 113872.

[36] ENCAT, Avaluació de l'estat nutricional de la població catalana 2002-2003, Enquesta Nutricional de Catalunya. (2003). www.salutweb.gencat.cat.

[37] O.A. Bergstad, Fish: Demersal fish (life histories, behavior, adaptations), 3rd ed., Elsevier, 2019.

[38] Atlantic Bluefin Tuna Status Review Team, Status review report of atlantic bluefin tuna (Thunnus thynnus), 2011.

[39] J. Casey, J. Pereiro, European hake (M. merluccius) in the North-east Atlantic, Hake: Biology, Fisheries and Markets, 1st Ed., Chapman & Hall, London, 1995.

[40] A. von der Decken, E. Lied, Metabolic effects on growth and muscle of soya-bean protein feeding in cod (Gadus morhua), British Journal of Nutrition. 69 (1993) 689–697.

[41] M. Coelho, P. Domingues, E. Balguerias, M. Fernandez, J.P. Andrade, A comparative study of the diet of Loligo vulgaris (Lamarck, 1799) (Mollusca:Cephalopoda) from the south coast of Portugal and the Saharan Bank (Central-East Atlantic), Fisheries Research. 29 (1997) 245–255.

[42] X. Li, N. Zhao, J. Fu, Y. Liu, W. Zhang, S. Dong, P. Wang, X. Su, J. Fu, Organophosphate Diesters (Di-OPEs) Play a Critical Role in Understanding Global Organophosphate Esters (OPEs) in Fishmeal, Environmental Science and Technology. 54 (2020) 12130–12141.

3.2.4. Discussió de resultats

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

En aquest estudi, el mètode analític desenvolupat per aquesta tesi i descrit a l'apartat 3.2.3. s'ha utilitzat per al monitoratge de sis dièsters de l'àcid fosfòric en les deu espècies de peix i marisc més consumides a Catalunya. S'han escollit les espècies més consumides per poder avaluar de manera representativa l'exposició de la població als dièsters de l'àcid fosfòric a través de la ingesta de peix i marisc. Només quatre dels sis dièsters estudiats es van determinar en com a mínim una mostra. El DNBP va ser el compost amb la major freqüència de detecció (100%) i aquell que va ser determinat a concentracions més altes. Aquests valors podrien estar relacionats amb el fet que el DNBP és el dièster que té un major volum de producció anual a la indústria [1], així com amb el fet que el compost pare també sol ser el predominant en mostres d'organismes marins [2]. El segon anàlit predominant en les mostres va ser el DPHP, seguit del BEHP i del BDCIPP. En general, sembla que les majors concentracions totals dels anàlits d'interès es determinen en espècies de peix carnívores que s'alimenten d'altres peixos petits, crustacis i mol·luscs. Tot i això, no es van observar grans diferències entre espècies pel que fa a la freqüència de detecció dels compostos, excepte pel BDCIPP que només es va poder determinar en espècies de peix i marisc d'alt contingut lipídic. Si s'observen els resultats, s'observa un augment inesperat de les concentracions de BEHP i DNBP en mostres de lluç, llenguado, bacallà i calamar del mostreig de febrer de 2020. És difícil trobar l'origen d'aquest augment. Com que al mostreig de febrer de 2019 no es va observar la mateixa tendència, no es pot dir que es tingui una causa estacional. A més, com que la majoria d'espècies de peix i marisc comprats en mercats locals es capturen en zones diferents d'arreu del món tampoc es pot afirmar que l'augment en les concentracions pugui ser degut a un pic de contaminació de la zona de captura.

La informació sobre la presència dels dièsters de l'àcid fosfòric en mostres d'organismes marins és extremadament limitada de manera que és molt complicat comparar els resultats obtinguts amb la bibliografia i, de fet, aquest és el primer estudi que n'avalua la presència en espècies de peix i marisc àmpliament consumides per la població.

Amb els resultats obtinguts en aquest estudi es realitzarà el càlcul de l'exposició de la població de Tarragona als dièsters de l'àcid fosfòric que per manca de temps no s'ha pogut incloure. Tot i això, com que per la majoria d'aquests compostos no s'han establert dosis de referència, valors d'ingesta diària tolerable o valors de NOAEL ("*non-observed-adverse-effect-level*", dosi sense afecte advers observable) no és possible caracteritzar el risc associat a la seva exposició. Aquest fet prova que hi ha una falta de recerca dels efectes toxicològics de l'exposició a llarg termini dels dièsters dels organofosfats i és necessari establir dosis de referència per aquests compostos.

Referències

[1] Y. Liu, S. Gong, L. Ye, J. Li, C. Liu, D. Chen, M. Fang, R.J. Letcher, G. Su, Organophosphate (OP) diesters and a review of sources, chemical properties, environmental occurrence, adverse effects, and future directions, Environ. Int. 155 (2021) 106691.

[2] R. Hou, C. Liu, X. Gao, Y. Xu, J. Zha, Z. Wang, Accumulation and distribution of organophosphate flame retardants (PFRs) and their di-alkyl phosphates (DAPs) metabolites in different freshwater fish from locations around Beijing, China, Environ. Pollut. 229 (2017) 548–556.

CAPÍTOL 4. CONCLUSIONS

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

Les conclusions més rellevants extretes dels estudis presentats en aquesta tesi doctoral es troben resumides en aquest apartat.

1. La LC-HRMS utilitzant un analitzador Exactive Orbitrap ha provat ser una eina sensible a nivell instrumental per la determinació dels anàlits d'interès, a més de tenir una alta capacitat de confirmació degut a l'alta resolució de l'espectròmetre. Tot i això, a causa dels elevats efectes matriu presents a les mostres ha estat necessari afegir etapes de neteja i preconcentració a tots els mètodes desenvolupats.

2. La SPE fent servir Florisil i Oasis HLB s'ha emprat amb èxit per a l'extracció dels derivats de la benzotriazola, la benzotiazola i la benzosulfonamida inclosos en aquesta tesi doctoral de mostres d'aigua del riu Ebre i mostres d'aigua de mar recollides a la costa de Tarragona.

3. No s'han trobat derivats de la benzosulfonamida en cap de les mostres d'aigua analitzades. Els derivats de la benzotriazola i la benzotiazola s'han determinat a nivells de ng L^{-1} en totes les mostres excepte en una localització on la BTR s'ha trobat a nivells de µg L^{-1}.

4. L'extracció amb PLE fent servir MeOH com a dissolvent d'extracció s'ha aplicat per primera vegada i de manera satisfactòria per a l'extracció simultània de sis dièsters i sis monoèsters de l'àcid ftàlic de mostres de peix i marisc.

5. El mètode basat en l'extracció amb QuEChERS desenvolupat per l'extracció de dièsters i monoèsters de l'àcid ftàlic, que utilitza acetonitril com a dissolvent d'extracció i les sals del mètode estàndard EN 15662, millora els valors de recuperació i efecte matriu obtinguts mitjançant l'extracció amb PLE. Per la majoria de compostos el percentatge de millora és igual o superior al 50%.

6. L'ús del sorbent de neteja Lipifiltr®, que s'ha emprat per primera vegada com a sorbent de dSPE, ha proporcionat bons resultats en la neteja d'extractes de QuEChERS de mostres de peix i marisc per la determinació de dièsters de

l'àcid ftàlic. A causa de la influència del contingut lipídic de les mostres tant en l'optimització com en la validació dels mètodes, aquestes s'han dut a terme de manera separada per espècies amb baix i alt contingut lipídic.

7. En la determinació de dièsters i monoèsters de l'àcid ftàlic, el DEP, el DEHP i els seus respectius metabòlits (el MEP i el MEHP) són els anàlits trobats a concentracions més altes en totes les mostres de peix i marisc analitzades. Tot i que la majoria de dièsters i monoèsters de l'àcid ftàlic s'han determinat a nivells de ng g^{-1}, en dues de les mostres analitzades el DEP i el DEHP s'han determinat a nivells de µg g^{-1}.

8. L'extracció amb QuEChERS s'ha aplicat de manera satisfactòria i per primera vegada a l'extracció de sis dièsters de l'àcid fosfòric de mostres de peix i marisc.

9. Tant la quantificació mitjançant calibratge en matriu, com la quantificació mitjançant calibratge amb patrons deuterats anàlegs han resultat útils per corregir els efectes matriu observats en les mostres de peix i marisc analitzades.

10. El monitoratge dut a terme per primera vegada de sis dièsters de l'àcid fosfòric en mostres recollides al llarg d'un any de les espècies de peix i marisc més consumides a Catalunya indica la presència de quatre dels sis anàlits estudiats en les mostres (DPHP, DNBP, BDCIPP i BEHP).

11. El lluç, el bacallà, el calamar i la tonyina han estat les espècies de peix i marisc on s'ha trobat una major concentració de dièsters de l'àcid fosfòric. Aquest fet podria estar relacionat amb la seva alimentació.

12. El DNBP i el DPHP són els dièsters de l'àcid fosfòric predominants a totes les mostres de peix i marisc i s'han determinat a nivells de ng g^{-1}.

ANNEXOS

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

Annex I. Abreviatures i acrònims

AA-DLLME	Microextracció líquid-líquid dispersiva assistida per aire
ACN	Acetonitril
APCI	Ionització química a pressió atmosfèrica
AVP	Alt volum de producció
BBOEP	Bis(2-butoxietil) fosfat
BCEP	Bis(2-cloroetil) fosfat
BDCIPP	Bis(1,3-dicloro-2-propil) fosfat
BEHP	Bis(2-etilhexil) fosfat
BSAs	Benzosulfonamides
BT	Benzotiazola
BTRs	Benzotriazoles
BTs	Benzotiazoles
BzBP	Benzilbutil ftalat
d.w.	Pes sec
d_{10}-BDCIPP	Bis(1,3-dicloro-2-propil) fosfat deuterat
d_{10}-DPHP	Difenil fosfat deuterat
d_{18}-DNBP	Di-n-butil fosfat deuterat
d_4-DEHP	Bis(2-etilhexil) ftalat deuterat
d_8-BBOEP	Bis(2-butoxietil) fosfat
d_8-BCEP	Bis(2-cloroetil) fosfat deuterat
DCM	Diclorometà
DEHP	Bis(2-etilhexil) ftalat
DEP	Dietil ftalat
DF	Freqüència de detecció
DiDP	Diisodecil ftalat
DinP	Diisononil ftalat
di-OPEs	Dièsters de l'àcid fosfòric
DLLME	Microextracció líquid-líquid dispersiva
DMF	N,N-dimetilformamida
DMP	Dimetil ftalat
DnBP	Di-n-butil ftalat
DNBP	Di-n-butil fosfat
DnOP	Di-n-octil ftalat
DPHP	Difenil fosfat
dSPE	Extracció en fase sòlida dispersiva
ECHA	Agència europea de productes químics

EFSA	Agència europea de seguretat alimentària
EI	Ionització electrònica
EPA	Agència per la protecció del medi ambient dels Estats Units
ESI	Ionització per electrospray
EtOAc	Acetat d'etil
FID	Detectors d'ionització de flama
GC	Cromatografia de gasos
GCB	Carbó negre grafititzat
GOs	Nanolàmines d'òxid de grafè
HF-LPME	Microextracció en fibra buida
HILIC	Cromatografia d'interacció hidrofílica
HRMS	Espectrometria de masses d'alta resolució
ILOD	Límit de detecció intrumental
ILOQ	Límit de quantificació intrumental
l.w.	Pes en lípids
LC	Cromatografia de líquids
LLE	Extracció líquid-líquid
log K_{ow}	Coeficient de partició octanol-aigua
LPME	Microextracció en fase líquida
MAE	Extracció amb microones
MBzP	Monobenzil ftalat
MCBT	2-mercaptobenzotiazola
m-dSPE	Extracció en fase sòlida dispersiva amb partícules magnètiques
ME	Efecte matriu
MeBT	2-metilbenzotiazola
MEHP	Mono(2-etilhexil) ftalat
MeOH	Metanol
MEP	Monoetil ftalat
MeSBT	2-(metiltio)-benzotiazola
MLOD	Límit de detecció de mètode
MLOQ	Límit de quantificació de mètode
MMP	Monometil ftalat
MnBP	Mono-n-butil ftalat
MnOP	Mono-n-octil ftalat
MPEs	Monoèsters de l'àcid ftàlic
MPs	Microplàstics
MS	Espectrometria de masses
MS/MS	Espectrometria de masses en tàndem

MWCNTs	Nanotubs de carboni multicapa
n.d.	No detectat
NH_2BT	2-aminobenzothiazola
NOAEL	Dosi sense afecte advers observable
OECD	Organització per la cooperació i el desenvolupament econòmic
OHBT	2-hidroxibenzotiazola
OP	Organofosfat
OPEs	Dièsters de l'àcid fosfòric
PAEs	Dièsters de l'àcid ftàlic
PCPs	Productes d'higiene i cura personal
PDMS	Polidimetilsiloxà
PLE	Extracció amb líquids pressuritzats
PSA	Amina primària i secundària
PVC	Clorur de polivinil
QqQ	Triple quadrupol
qToF	Quadrupol-temps de vol
QuEChERS	Ràpid, fàcil, barat, efectiu, robust i segur (*Quick, Easy, Cheap, Effective, Rugged and Safe*)
R_{app}	Recuperació aparent
REACH	Registre, avaluació i autorització de substàncies químiques
R_{ext}	Recuperació de l'extracció
R_{rel}	Recuperació relativa
RSD	Desviació estàndard relativa
SAX	Intercanvi aniònic fort
SBSE	Extracció mitjançant barres magnètiques agitadores
SCX	Intercanvi catiònic fort
SDME	Microextracció en gota
SPE	Extracció en fase sòlida
SPME	Microextracció en fase sòlida
TD	Desorció tèrmica
TDI	Ingesta diària tolerable
t_R	Temps de retenció
USAE	Extracció per ultrasons
w.w.	Pes humit
WAX	Intercanvi aniònic feble
WCX	Intercanvi catiònic feble

Annex II. Llistat de publicacions

Llistat de publicacions originades de la part experimental d'aquesta tesi doctoral:

1. Míriam Hidalgo-Serrano, Francesc Borrull, Rosa Maria Marcé, Eva Pocurull, Presence of benzotriazoles, benzothiazoles and benzenesulfonamides in surface water samples by liquid chromatography coupled to high-resolution mass spectrometry, Separation Science Plus. 2 (2019) 72–80.

2. Míriam Hidalgo-Serrano, Francesc Borrull, Eva Pocurull, Rosa Maria Marcé, Pressurised Liquid Extraction and Liquid Chromatography–High Resolution Mass Spectrometry for the Simultaneous Determination of Phthalate Diesters and Their Metabolites in Seafood Species, Food Analytical Methods. 13 (2020) 1442–1453.

3. Míriam Hidalgo-Serrano, Francesc Borrull, Rosa Maria Marcé, Eva Pocurull, Simple method for determining phthalate diesters and their metabolites in seafood species using QuEChERS extraction and liquid chromatography-high resolution mass spectrometry, Food Chemistry. 336 (2021) 127722.

4. Míriam Hidalgo-Serrano, Francesc Borrull, Rosa Maria Marcé, Eva Pocurull, Phthalate esters in marine ecosystems: analytical methods, occurrence and distribution, Trends in Analytical Chemistry (*Enviat*).

5. Míriam Hidalgo-Serrano, Francesc Borrull, Eva Pocurull, Rosa Maria Marcé, Determination of organophosphate ester metabolites in seafood species by QuEChERS-SPE followed by liquid chromatography-high-resolution mass spectrometry (*Pendent d'enviar*).

6. Míriam Hidalgo-Serrano, Francesc Borrull, Eva Pocurull, Rosa Maria Marcé, Organophosphate ester metabolites in seafood of common consumption (*Pendent d'enviar*).

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

UNIVERSITAT ROVIRA I VIRGILI
DETERMINACIÓ I PRESÈNCIA DE COMPOSTOS ORGÀNICS D'ALT VOLUM DE PRODUCCIÓ, ELS SEUS PRODUCTES DE TRANSFORMACIÓ I METABÒLITS EN AMBIENTS AQUÀTICS
Míriam Hidalgo Serrano

UNIVERSITAT
ROVIRA i VIRGILI