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Pressurised Liquid Extraction and Liquid Chromatography–High Resolution Mass Spectrometry for the Simultaneous Determination of Phthalate Diesters and Their Metabolites in Seafood Species

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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

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 (Net et al. 2015; Sakhi et al. 2017). Since these compounds are not chemically bound to the plastic products, they can easily contaminate the environment by reaching the air and water (Clara et al. 2010). Moreover, microplastic contamination is a growing environmental problem worldwide (Eerkes-Medrano et al. 2015; Auta et al. 2017), 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 (Ministerio de Agricultura Pesca y Alimentación 2019), 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 (Ventrice et al. 2013), even though these derivates can also occur due to abiotic and microbial degradation of the diesters in soil, sediment or water (Net et al. 2015).

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 (Ye et al. 2014; Asghari et al. 2015; Mathieu-Denoncourt et al. 2015). Although fewer studies exist on the toxicity of phthalate monoesters, some have pointed out possible alterations in the gene expression of antioxidant enzymes (Asghari et al. 2015), thyroid endocrine disrupting effects (Zhai et al. 2014), or disruptions in the balance of sex hormones (Ventrice et al. 2013). Most studies call attention to the

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fact that the effects associated with phthalate diesters are induced by both the diester and its metabolites (Ye et al. 2014; Mathieu-Denoncourt et al. 2016).

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) (European Food Safety Agency (EFSA) 2005a), 0.5 mg/kg bw for benzyl butyl phthalate (BzBP) (European Food Safety Agency (EFSA) 2005b) and 0.05 mg/kg bw for diethylhexyl phthalate (DEHP) (European Food Safety Agency (EFSA) 2005c). Earlier in 2019, a group-TDI of 0.05 mg/kg bw was also proposed by EFSA for DBP, BzBP, DEHP and diisononyl phthalate (European Food Safety Agency (EFSA) 2019). 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 (European Parliament 2005). Similar restrictions have been established by the U.S. Consumer Product Safety Commission (Consumer Product Safety Comission (CPSC) 2017).

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 (Blair et al. 2009; Clara et al. 2010; Valton et al. 2014; Jiang et al. 2018), soils and sediments (Blair et al. 2009; Adeniyi et al. 2011; Wang et al. 2015), biological fluids (Del Bubba et al. 2018; Louis et al. 2018; Li et al. 2019), food and packaging (Fierens et al. 2012; Jia et al. 2014; Xu et al. 2014; Wang et al. 2015; Liu et al. 2016; Aghvami et al. 2018; Deng et al. 2019) and biota (Blair et al. 2009; Adeniyi et al. 2011; Valton et al. 2014; Hu et al. 2016; Xu et al. 2018). For seafood, several extraction procedures have been used, including QuEChERS (Xu et al. 2018), ultrasound extraction (Valton et al. 2014; Hu et al. 2016) and pressurised liquid extraction (PLE) (Blair et al. 2009).

Gas chromatography (GC) coupled to mass spectrometry (MS) is the most common technique for determining phthalate diesters (Hu et al. 2016; Liu et al. 2016; Del Bubba et al. 2018; Xu et al. 2018), even though in some cases they have also been determined by liquid chromatography (LC) (Jia et al. 2014; Xu et al. 2014; Aghvami et al. 2018). Phthalate monoesters, on the other hand, are mainly determined by LC (Blair et al. 2009; Valton et al. 2014; Jiang et al. 2018; Deng et al. 2019) because they have to be derivatised to be determined by GC (Del Bubba et al. 2018). That is the reason why when phthalate diesters and phthalate monoesters are included in the same study, two different chromatographic methods are usually developed (Valton et al. 2014; Hu et al. 2016; Jiang et al. 2018). 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 (Ros et al. 2015) or human milk and infant formula samples (Del Bubba et al. 2018). 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) (Wang and Gardinali 2012; Hagenhoff and Hayen 2018; Morrison et al. 2018; Ohba et al. 2018), 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 (Blair et al. 2009; Valton et al. 2014; Xu et al. 2014; Jiang et al. 2018; Deng et al. 2019), in this study HRMS was used instead. Finally, the method was validated and used to analyse five seafood species.

Materials and Methods

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 °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).

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 homogenised, frozen and lyophilised 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.

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 °C 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 °C 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, CA, 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). One millilitre 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.

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 × 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 °C and 10 °C, respectively.

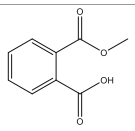
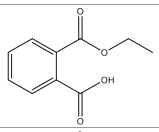
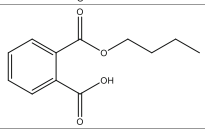
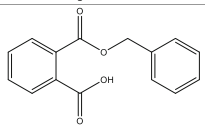
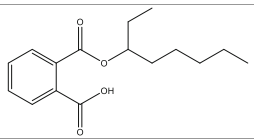
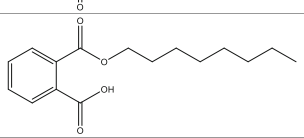
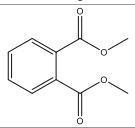
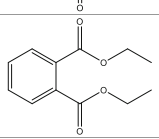
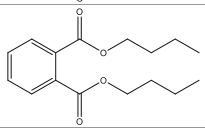
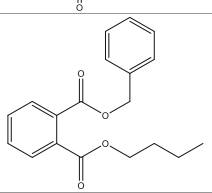
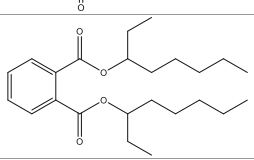
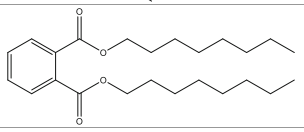
Positive and negative ionisation modes were both tested in order to optimise the HESI and APCI source parameters and obtain the highest response for all compounds. The optimal parameters for HESI were as follows: sheath gas flow rate, 50 A.U.; auxiliary gas flow rate, 15 A.U.; sweep gas flow rate, 0 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.

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.

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

Compound	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)
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)
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

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.

Results and Discussion

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 $[M\text{-}H]^-$. 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 1000 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 ionisation sources, the matrix effect was evaluated to determine the suitability of HESI and APCI.

Pressurised Liquid Extraction

Based on our previous experience (Arbeláez et al. 2014; Núñez et al. 2017b), 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, 1 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. One millilitre 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

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

solvents are shown in Fig. 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.

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 °C were tested. A temperature of 80 °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, 2 and 3 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 1 cycle at 80 °C with a static time of 10 min.

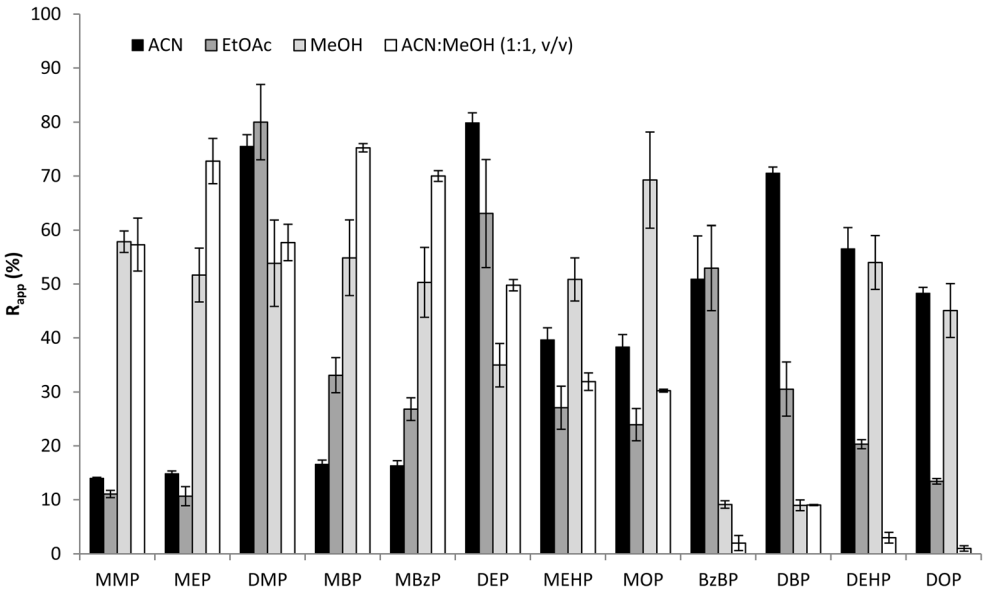


Fig. 1 R_{app} (%) of salmon obtained after PLE with different organic solvents

Other parameters, such as preheating time, flush volume and purge time, are known to have no significant effect on extraction efficiency (Vallecillos et al. 2015; Núñez et al. 2017a).

Clean-up and Preconcentration Strategies

The high ion suppression detected in the extracts and the complexity of the sample caused 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 (Núñez et al. 2017a). 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 (Núñez et al. 2017b). 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, MA, USA) (Núñez et al. 2017a). 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 C18 (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 °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 C18 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.

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.

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

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

sole and salmon samples. Table 3 shows the validation parameters obtained.

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 1000 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) (Xu et al. 2018) 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. (2009) 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%.

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*

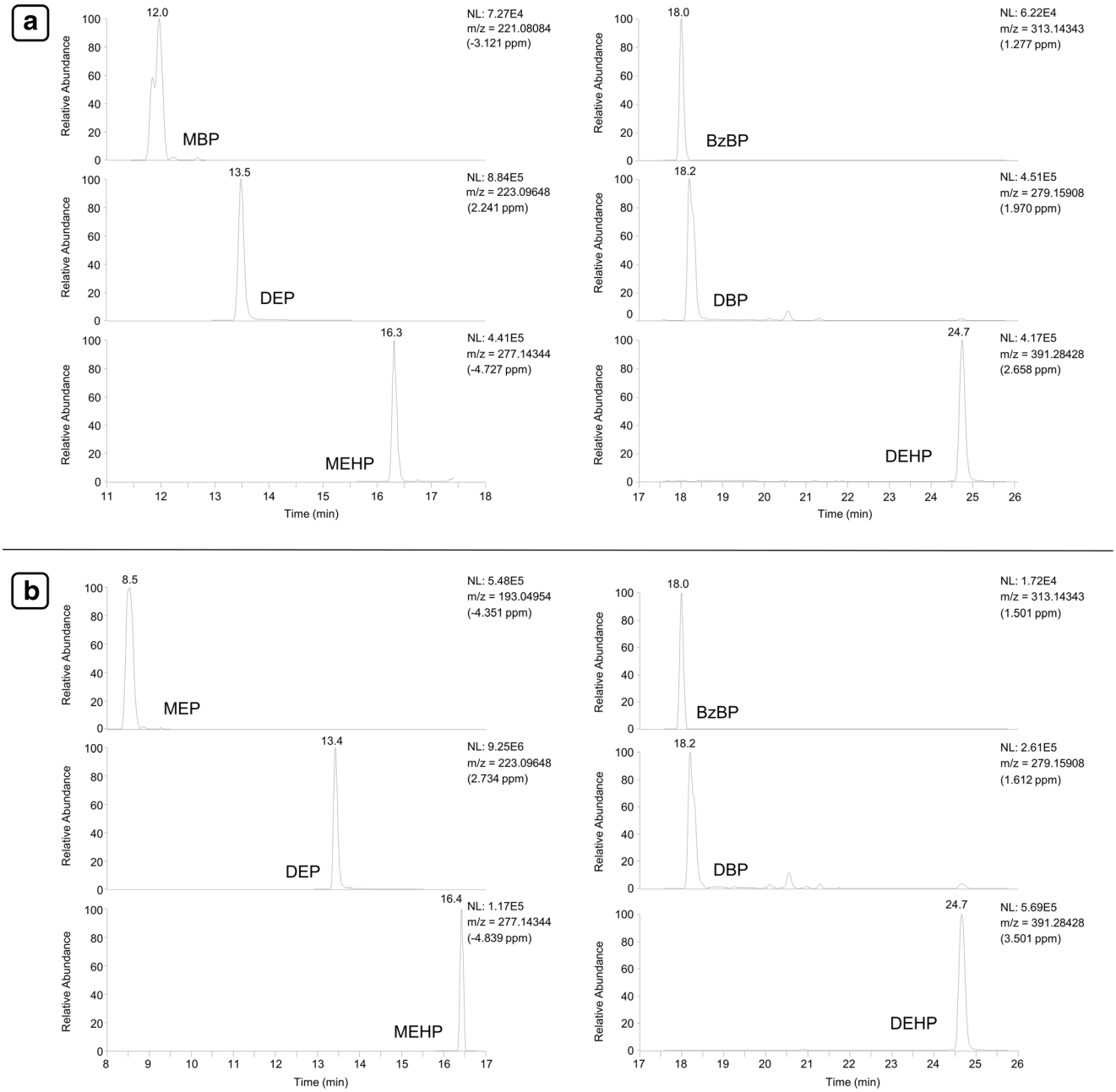


Fig. 2 LC-HRMS extracted ion chromatogram and mass error in ppm of a salmon sample (**a**) and a shrimp sample (**b**)

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 Fig. 2.

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.

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. (2018) 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. (2011) 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}

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)

<*MLOQ* below method limit of quantification, n.d. not detected (<MLOD)

(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} (Schecter et al. 2013), 7.5 ng g^{-1} (Sakhi et al. 2014) or 8 ng g^{-1} (Fierens et al. 2012), though Valton et al. (2014) 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} (Blair et al. 2009)), MEP and MEHP concentrations were higher than those reported (up to 25 ng g^{-1} (Hu et al. 2016)).

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 with 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.

Author Contributions All authors contributed to the conception and design of the study. Material preparation, data collection and analysis were performed by Míriam Hidalgo-Serrano. The first draft of the manuscript was written by Míriam Hidalgo-Serrano, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability Not applicable

Compliance with Ethical Standards

Conflict of Interest Míriam Hidalgo-Serrano declares that she has no conflict of interest. Francesc Borrull declares that he has no conflict of interest. Eva Pocurull declares that she has no conflict of interest. Rosa Maria Marcé declares that she has no conflict of interest.

Ethical Approval This article does not contain any studies with human or animal subjects.

Informed Consent Informed consent not applicable

Code Availability Not applicable

References

Adeniyi AA, Okedeyi OO, Yusuf KA (2011) 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 172:561–569. https://doi.org/10.1007/s10661-010-1354-2

Aghvami B, Faraji H, Shahbaazi H (2018) Chemometrics-assisted QuEChERS method coupled with HPLC-diode array detection for the determination of phthalate esters in food-grade plastics. J Anal Chem 73:1111–1117. https://doi.org/10.1134/S1061934818110035

Arbeláez P, Borrull F, Marcé RM, Pocurull E (2014) Simultaneous determination of drugs of abuse and their main metabolites using pressurized liquid extraction and liquid chromatography-tandem mass spectrometry. Talanta 125:65–71. https://doi.org/10.1016/j.talanta.2014.02.053

Asghari MH, Saeidnia S, Abdollahi M (2015) 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 11:95–105. https://doi.org/10.3923/ijp.2015.95.105

Auta HS, Emenike CU, Fauziah SH (2017) Distribution and importance of microplastics in the marine environment: a review of the sources, fate, effects, and potential solutions. Environ Int 102:165–176. https://doi.org/10.1016/j.envint.2017.02.013

Blair JD, Ikonomou MG, Kelly BC, Surridge B, Gobas FAPC (2009) 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 43:6262–6268. https://doi.org/10.1021/es9013135

Clara M, Windhofer G, Hartl W, Braun K, Simon M, Gans O, Scheffknecht C, Chovanec A (2010) Occurrence of phthalates in surface runoff, untreated and treated wastewater and fate during wastewater treatment. Chemosphere 78:1078–1084. https://doi.org/10.1016/j.chemosphere.2009.12.052

Consumer Product Safety Comission (CPSC) (2017) CPSC Prohibits Certain Phthalates in Children's Toys and Child Care Products. https://www.cpsc.gov/content/cpsc-prohibits-certain-phthalates-in-childrens-toys-and-child-care-products. Accessed 16 Sep 2019

Del Bubba M, Ancillotti C, Checchini L et al (2018) 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 148:6–16. https://doi.org/10.1016/j.jpba.2017.09.017

Deng C, Li C, Zhou J, Wang Q, Shao H, Wang J, Wu Y, Zhang H, Gao M, Xu X, Jin F (2019) Simultaneous determination of eight monoalkyl phthalate esters in porcine tissue by solid-phase extraction and liquid chromatography-tandem mass spectrometry. J Agric Food Chem 67:7167–7173. https://doi.org/10.1021/acs.jafc.9b01078

Eerkes-Medrano D, Thompson RC, Aldridge DC (2015) Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Res 75:63–82. https://doi.org/10.1016/j.watres.2015.02.012

European Food Safety Agency (EFSA) (2005a) 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 242:1–17. https://doi.org/10.2903/j.efsa.2005.242

European Food Safety Agency (EFSA) (2005b) 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 241:1–14. https://doi.org/10.2903/j.efsa.2005.241

European Food Safety Agency (EFSA) (2005c) 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 243:1–20. https://doi.org/10.2903/j.efsa.2005.243

European Food Safety Agency (EFSA) (2019) 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. https://www.efsa.europa.eu/en/consultations/call/190221. Accessed 16 Sep 2019

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

Fierens T, Servaes K, Van Holderbeke M et al (2012) Analysis of phthalates in food products and packaging materials sold on the Belgian market. Food Chem Toxicol 50:2575–2583. https://doi.org/10.1016/j.fct.2012.04.029

Hagenhoff S, Hayen H (2018) 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 410:4905–4911. https://doi.org/10.1007/s00216-018-1137-0

Hu X, Gu Y, Huang W, Yin D (2016) Phthalate monoesters as markers of phthalate contamination in wild marine organisms. Environ Pollut 218:410–418. https://doi.org/10.1016/j.envpol.2016.07.020

Jia W, Chu X, Ling Y, Huang J, Chang J (2014) Analysis of phthalates in milk and milk products by liquid chromatography coupled to quadrupole Orbitrap high-resolution mass spectrometry. J Chromatogr A 1362:110–118. https://doi.org/10.1016/j.chroma.2014.08.030

Jiang J, Mu D, Ding M, Zhang S, Zhang H, Hu J (2018) Simultaneous determination of primary and secondary phthalate monoesters in the Taihu Lake: exploration of sources. Chemosphere 202:17–24. https://doi.org/10.1016/j.chemosphere.2018.03.070

Li J, Zhao H, Xia W, Zhou Y, Xu S, Cai Z (2019) Nine phthalate metabolites in human urine for the comparison of health risk between population groups with different water consumptions. Sci Total Environ 649:1532–1540. https://doi.org/10.1016/j.scitotenv.2018.08.294

Liu P, Chen H, Gao G, Hao Z, Wang C, Ma G, Chai Y, Zhang L, Liu X (2016) Occurrence and residue pattern of phthalate esters in fresh tea leaves and during tea manufacturing and brewing. J Agric Food Chem 64:8909–8917. https://doi.org/10.1021/acs.jafc.6b03864

Louis GMB, Smarr MM, Sun L et al (2018) Endocrine disrupting chemicals in seminal plasma and couple fecundity. Environ Res 163:64–70. https://doi.org/10.1016/j.envres.2018.01.028

Mathieu-Denoncourt J, Wallace SJ, de Solla SR, Langlois VS (2015) Plasticizer endocrine disruption: highlighting developmental and reproductive effects in mammals and non-mammalian aquatic species. Gen Comp Endocrinol 219:74–88. https://doi.org/10.1016/j.ygcen.2014.11.003

Mathieu-Denoncourt J, Wallace SJ, de Solla SR, Langlois VS (2016) Influence of lipophilicity on the toxicity of bisphenol A and phthalates to aquatic organisms. Bull Environ Contam Toxicol 97:4–10. https://doi.org/10.1007/s00128-016-1812-9

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

Morrison LM, Renaud JB, Sabourin L, Sumarah MW, Yeung KKC, Lapen DR (2018) High-throughput quantitation of neonicotinoids in lyophilized surface water by LC-APCI-MS/MS. J AOAC Int 101:1940–1947. https://doi.org/10.5740/jaoacint.17-0486

Net S, Sempéré R, Delmont A, Paluselli A, Ouddane B (2015) Occurrence, fate, behavior and ecotoxicological state of phthalates in different environmental matrices. Environ Sci Technol 49:4019–4035. https://doi.org/10.1021/es505233b

Núñez M, Borrull F, Fontanals N, Pocurull E (2017a) Different sample treatments for the determination of ICM-XR in fish samples followed by LC-HRMS. Talanta 163:1–7. https://doi.org/10.1016/j.talanta.2016.11.004

Núñez M, Borrull F, Pocurull E, Fontanals N (2017b) Pressurised liquid extraction and liquid chromatography-high resolution mass spectrometry to determine high-intensity sweeteners in fish samples. J Chromatogr A 1479:32–39. https://doi.org/10.1016/j.chroma.2016.12.006

Ohba Y, Nakajima T, Kanda M, Hayashi H, Matsushima Y, Nakagawa Y, Koike H, Nagano C, Sekimura K, Otsuka K, Sasamoto T, Hashimoto T (2018) Simultaneous determination of nine acaricides

and two metabolites in comb honey by LC/MS/MS. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 35:2375–2386. https://doi.org/10.1080/19440049.2018.1539252

Ros O, Izaguirre JK, Olivares M, Bizarro C, Ortiz-Zarragoitia M, Cajaraville MP, Etxebarria N, Prieto A, Vallejo A (2015) Determination of endocrine disrupting compounds and their metabolites in fish bile. Sci Total Environ 536:261–267. https://doi.org/10.1016/j.scitotenv.2015.07.074

Sakhi AK, Lillegaard ITL, Voorspoels S, Carlsen MH, Løken EB, Brantsæter AL, Haugen M, Meltzer HM, Thomsen C (2014) Concentrations of phthalates and bisphenol A in Norwegian foods and beverages and estimated dietary exposure in adults. Environ Int 73:259–269. https://doi.org/10.1016/j.envint.2014.08.005

Sakhi AK, Sabaredzovic A, Cequier E, Thomsen C (2017) Phthalate metabolites in Norwegian mothers and children: levels, diurnal variation and use of personal care products. Sci Total Environ 599–600:1984–1992. https://doi.org/10.1016/j.scitotenv.2017.05.109

Schecter A, Lorber M, Guo Y, Wu Q, Yun SH, Kannan K, Hommel M, Imran N, Hynan LS, Cheng D, Colacino JA, Birnbaum LS (2013) Phthalate concentrations and dietary exposure from food purchased in New York state. Environ Health Perspect 121:473–479. https://doi.org/10.1289/ehp.1206367

Vallecillos L, Pocurull E, Borrull F (2015) Influence of pre-treatment process on matrix effect for the determination of musk fragrances in fish and mussel. Talanta 134:690–698. https://doi.org/10.1016/j.talanta.2014.12.010

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

Ventrice P, Ventrice D, Russo E, De Sarro G (2013) Phthalates: European regulation, chemistry, pharmacokinetic and related toxicity. Environ Toxicol Pharmacol 36:88–96. https://doi.org/10.1016/j.etap.2013.03.014

Wang C, Gardinali PR (2012) Comparison of multiple API techniques for the simultaneous detection of microconstituents in water by on-line SPE-LC-MS/MS. J Mass Spectrom 47:1255–1268. https://doi.org/10.1002/jms.3051

Wang J, Chen G, Christie P, Zhang M, Luo Y, Teng Y (2015) Occurrence and risk assessment of phthalate esters (PAEs) in vegetables and soils of suburban plastic film greenhouses. Sci Total Environ 523:129–137. https://doi.org/10.1016/j.scitotenv.2015.02.101

Xu D, Deng X, Fang E, Zheng X, Zhou Y, Lin L, Chen L, Wu M, Huang Z (2014) Determination of 23 phthalic acid esters in food by liquid chromatography tandem mass spectrometry. J Chromatogr A 1324:49–56. https://doi.org/10.1016/j.chroma.2013.11.017

Xu Y, Weng R, Lu Y, Wang X, Zhang D, Li Y, Qiu J, Qian Y (2018) Evaluation of phthalic acid esters in fish samples using gas chromatography tandem mass spectrometry with simplified QuEChERS technique. Food Anal Methods 11:3293–3303. https://doi.org/10.1007/s12161-018-1313-z

Ye T, Kang M, Huang Q, Fang C, Chen Y, Shen H, Dong S (2014) Exposure to DEHP and MEHP from hatching to adulthood causes reproductive dysfunction and endocrine disruption in marine medaka (Oryzias melastigma). Aquat Toxicol 146:115–126. https://doi.org/10.1016/j.aquatox.2013.10.025

Zhai W, Huang Z, Chen L, Feng C, Li B, Li T (2014) Thyroid endocrine disruption in zebrafish larvae after exposure to mono-(2-ethylhexyl) phthalate (MEHP). PLoS One 9:1–6. https://doi.org/10.1371/journal.pone.0092465