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 Maria Marcé*, Eva Pocurull

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

---

Email addresses:

Míriam Hidalgo-Serrano: miriam.hidalgo@urv.cat

Francesc Borrull: francesc.borrull@urv.cat

Rosa Maria Marcé: rosamaria.marce@urv.cat

Eva Pocurull: eva.pocurull@urv.cat

Corresponding author: Rosa Maria Marcé

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

E-mail address: rosamaria.marce@urv.cat

Tel. (+34) 977558170; Fax: (+34) 977558446

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 (Perestrelo et al., 2019; Santana-Mayor, Socas-Rodríguez, Herrera-Herrera, & Rodríguez-Delgado, 2019). 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 (Anastassiades, Lehotay, Štajnbaher, & Schenck, 2003), this technique has broadened its range of application to various analytes and matrices (Santana-Mayor et al., 2019). 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 (Arias et al., 2019; Hidalgo-Ruiz, Romero-González, Martinez Vidal, & Garrido Frenich, 2019; Song et al., 2019) but also in biological fluids (Fan, Ren, Jin, He, & Wang, 2019; Jia et al., 2019) and environmental samples (Fernandes et al., 2020). Specifically, several QuEChERS methods have focused on determining contaminants in fish and shellfish (Álvarez-Muñoz, Rambla-Alegre, Carrasco, Lopez de Alda, & Barceló, 2019; Barbieri et al., 2019; López-García, Postigo, & López de Alda, 2019).

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 (Sakhi, Sabaredzovic, Cequier, & Thomsen, 2017). 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 (Mathieu-Denoncourt, Wallace, de Solla, & Langlois, 2015). Phthalate diesters are therefore considered industrial contaminants of emerging concern and some of them have been subjected to numerous regulations and limitations (EFSA CEP Panel, 2019; European Parliament, 2005; U.S. Environmental Protection Agency, 2012).

Phthalate diesters eventually reach living organisms, where they are metabolised into phthalate monoesters (Ventrice, Ventrice, Russo, & De Sarro, 2013). 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 (Net, Sempéré, Delmont, Paluselli, & Ouddane, 2015). A small number of studies that evaluated the toxicity of phthalate monoesters have reported several endocrine-disrupting effects that may result in reproductive impairment (Ye et al., 2014).

Taking into account the widespread occurrence of phthalate diesters and monoesters in the environment, and more specifically in surface waters (Jiang, Mu, Ding, Zhang, Zhang, & Hu, 2018), these compounds are likely to be found in aquatic organisms. Several extraction techniques such as ultrasound extraction (Hu, Gu, Huang, & Yin, 2016; Valton, Serre-Dargnat, Blanchard, Alliot, Chevreuil, & Teil, 2014) and pressurised liquid extraction (Blair, Ikonomou, Kelly¸ Surridge, & Gobas, 2009; Hidalgo-Serrano, Borrull, Pocurull, & Marcé, *In Press*) 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 (Gu et al., 2014; Tsochatzis, Karayannakidis, & Kalogiannis, 2019; Xu et al., 2018).

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 (UCT, 2019) 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 ºC.

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 (Hidalgo-Serrano et al., *In Press*). 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 (Hidalgo-Serrano et al., *In Press*).

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 EN 15662:2019, the original QuEChERS method (Anastassiades et al., 2003), and the AOAC official method (Lehotay, 2007). To test the extraction salts, other significant parameters were

set based on our previous experiment (Vallecillos, Pocurull, & Borrull, 2015) (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 (Dong et al., 2017; Sun et al., 2018) 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 (Dong et al., 2017; Xu et al., 2018), 200 mg of C_{18} (Barbieri et al., 2019; Sun et al., 2018), 200 mg of Florisil (Vallecillos et al., 2015), Z-Sep$^+$ tubes (Sireli, Filazi, Yurdakok-Dikmen, Iplikcioglu-Cil, Kuzukiran, & Orhan, 2017), EMR-lipid tubes (Cruz, Marques, Casal, & Cunha, 2019) and 50 mg of ENVI-Carb (Gao, Li, Li, Zhang, & Li, 2018). 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-

244 Carb was used, the concentration of DEP found was higher than 1500 ng g^{-1}. Such high blank
245 concentrations would render the procedure unrepeatable and could easily result in false positives or
246 false negatives. Taking into consideration these results, we provisionally rejected the use of dSPE
247 since none of the sorbents we tested was appropriate for both families of target compounds.

248 Other clean-up strategies such as freezing-lipid filtration, extraction of lipid interferences with
249 hexane, and an SPE step with styrene-divinylbenzene based polymers (Bond Elut PPL (200 mg)
250 and LiChrolut EN (200 mg)) were therefore evaluated. To do so, the ACN extract was evaporated
251 and reconstituted with H_2O. However, none of these strategies provided satisfactory results. Finally,
252 we tested the use of Lipifiltr® and found that all phthalate monoesters were retained in the Lipifiltr®
253 cartridges but retention for the phthalate diesters was below 13%. The sorbent was removed from
254 the plastic cartridge to be used as a sorbent for dSPE because the RSD% (n = 10) of the procedural
255 blanks of these cartridges was above 30% for DEHP.

256 Since none of the clean-up steps we evaluated appeared to be suitable for simultaneously
257 determining phthalate diesters and monoesters, we divided the ACN extract equally and optimised
258 dSPE procedures using different sorbents for each family of compounds. Extraction recoveries with
259 the chosen sorbents were calculated for salmon extracts, which were very similar to those obtained
260 without the matrix. For phthalate monoesters, 200 mg of C_{18}, 50 mg of ENVI-Carb and a mixture of
261 both sorbents were evaluated (Figure 1A) since retention of the target compounds in the preliminary
262 dSPE tests was less than 30%. The biggest differences in ME values were observed for MEHP and
263 MOP, since they decreased from -91 and -86% (without a clean-up step), respectively, to -48 and -
264 43% (when C_{18} was used), respectively. ME values for the other phthalate monoesters ranged from
265 -21 to -43%. Similarly, for phthalate diesters we evaluated 200 mg of PSA, 200 mg of Florisil and the
266 content of one Lipifiltr® cartridge (Figure 1B). In all cases, the retention of the phthalate diesters was
267 below 30%, while the lowest ME values were obtained with Lipifiltr® (between -22 and -41%). C_{18}
268 and Lipifiltr® were therefore chosen to clean up the sample for determining phthalate monoesters
269 and phthalate diesters, respectively.

270 *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 Fourgous et al. (2016) (up to 25.8 ng g^{-1} for phthalate monoesters in eel) and Xu et al. (2018) (up to 10 ng g^{-1} for phthalate diesters in fish), show that the sensitivity of our method is suitable.

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

298 slightly lower for salmon (14 – 48 %) than for sole (35 – 66 %) due to higher ME. Low recoveries are
299 also found in the literature for several of our studied compounds due to high ME. The authors of one
300 study (Gu et al., 2014) also proposed matrix-matched calibration curves to help compensate for ion
301 suppression.

302 Interestingly, the R_{app} and ME obtained in the present study represent the biggest
303 improvements compared to our previous method (Hidalgo-Serrano et al., *In Press*). As Figure 2
304 shows, the ion suppression of phthalate monoesters for sole decreased significantly, thus
305 considerably increasing R_{app}. With regard to phthalate diesters, the most meaningful differences
306 were observed for DEHP and DOP. For DOP, in particular, the increase in R_{app} enabled us to
307 correctly determine this compound in the samples and include it in our analyses. Similar observations
308 can be made for the results obtained with salmon.

309 *3.4. Application to seafood samples*

310 We bought three seafood species with a low-fat content (sole, squid and shrimp) and two
311 seafood species with a high-fat content (salmon and mackerel) from different local fish markets in
312 order to have three different samples (A, B and C) for each species. We then evaluated the presence
313 of phthalate diesters and phthalate monoesters in triplicate for each sample. ME and R_{app} values
314 were calculated for squid, shrimp and mackerel samples to confirm whether the calibration curves
315 obtained for sole and salmon could be applied to those samples. However, since the values differed
316 we constructed various calibration curves to quantify the target compounds in each seafood species.
317 We also evaluated the repeatability and reproducibility for the three seafood matrices. The exact
318 mass of the compounds and their ion ratios were used to confirm the presence of the detected
319 compounds. Table 2 shows the concentrations of these compounds in all samples.

320 Three phthalate monoesters and five phthalate diesters were detected and quantified in the
321 samples with a mass error below 5 ppm. However, MMP, MBzP, MOP and DOP were not detected
322 in any sample. Figure 2 shows LC-HRMS extracted ion chromatograms of a 1 ppm quality control
323 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. (2018) 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. (2014) 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 (Adeniyi, Okedeyi, & Yusuf, 2011)). 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. (2009) 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. (2016) 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.

In general, we also obtained similar results in our previous work (Hidalgo-Serrano et al., *In Press*)**,** 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 (Gao & Wen, 2016), 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.

Declaration of Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Adeniyi, A. A., Okedeyi, O. O., & Yusuf, K. A. (2011). Flame ionization gas chromatographic determination of phthalate esters in water, surface sediments and fish species in the Ogun river catchments, Ketu, Lagos, Nigeria. *Environmental Monitoring and Assessment*, *172*(1–4), 561–569. https://doi.org/10.1007/s10661-010-1354-2

Álvarez-Muñoz, D., Rambla-Alegre, M., Carrasco, N., Lopez de Alda, M., & Barceló, D. (2019). Fast analysis of relevant contaminants mixture in commercial shellfish. *Talanta*, *205*(April), 119884. https://doi.org/10.1016/j.talanta.2019.04.085

Anastassiades, M., Lehotay, S. J., Štajnbaher, D., & Schenck, F. J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *Journal of AOAC International*, *86*(2), 412–431. https://doi.org/10.1021/jf00110a037

Arias, J. L. de O., Rocha, C. B., Santos, A. L. Q. S., Marube, L. C., Kupski, L., Caldas, S. S., & Primel, E. G. (2019). 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 Chemistry*, *293*, 112–119. https://doi.org/10.1016/j.foodchem.2019.04.094

Barbieri, M. V., Postigo, C., Guillem-Argiles, N., Monllor-Alcaraz, L. S., Simionato, J. I., Stella, E., Barceló, D., & López de Alda, M. (2019). Analysis of 52 pesticides in fresh fish muscle by QuEChERS extraction followed by LC-MS/MS determination. *Science of the Total Environment*, *653*, 958–967. https://doi.org/10.1016/j.scitotenv.2018.10.289

Blair, J. D., Ikonomou, M. G., Kelly, B. C., Surridge, B., & Gobas, F. A. P. C. (2009). Ultra-trace determination of phthalate ester metabolites in seawater, sediments, and biota from an urbanized marine inlet by LC/ESI-MS/MS. *Environmental Science and Technology*, *43*(16),

6262–6268. https://doi.org/10.1021/es9013135

Cruz, R., Marques, A., Casal, S., & Cunha, S. C. (2019). Fast and environmental-friendly methods for the determination of polybrominated diphenyl ethers and their metabolites in fish tissues and feed. *Science of the Total Environment Journal*, *646*, 1503–1515. https://doi.org/10.1016/j.scitotenv.2018.07.342

Dong, W., Sun, B., Sun, J., Zheng, F., Sun, X., Huang, M., & Li, H. (2017). Matrix effects in detection of phthalate esters from wheat by a modified QuEChERS method with GC/MS. *Food Analytical Methods*, *10*(9), 3166–3180. https://doi.org/10.1007/s12161-017-0892-4

EFSA CEP Panel, Baviera, M. B., Bolognesi, C., Chesson, A., Silano, V., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Steffensen, I., Tlustos, C., Loveren, H. Van, Mortensen, A., Rivi, G., Vernis, L., Zorn, H., Cravedi, J., Fortes, C., … Castle, L. (2019). 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 Journal*, *17*(12), 1–85. https://doi.org/10.2903/j.efsa.2019.5838

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).

European Parliament. (2005). Directive 2005/84/EC of the European Parliament and the Council. *Official Journal of the European Union*, *344*(40), 40–43.

Fan, J.-C., Ren, R., Jin, Q., He, H.-L., & Wang, S.-T. (2019). Detection of 20 phthalate esters in breast milk by GC-MS/MS using QuEChERS extraction method. *Food Additives & Contaminants: Part A*, *36*(10), 1551–1558. https://doi.org/10.1080/19440049.2019.1646435

Fernandes, M. J., Paíga, P., Silva, A., Llaguno, C. P., Carvalho, M., Vázquez, F. M., & Delerue-Matos, C. (2020). Antibiotics and antidepressants occurrence in surface waters and sediments collected in the north of Portugal. *Chemosphere*, *239*, 124729.

https://doi.org/10.1016/j.chemosphere.2019.124729

Fourgous, C., Chevreuil, M., Alliot, F., Amilhat, E., Faliex, E., Paris-Palacios, S., Teil, M. J., & Goutte, A. (2016). Phthalate metabolites in the European eel (Anguilla anguilla) from Mediterranean coastal lagoons. *Science of the Total Environment*, *569–570*, 1053–1059. https://doi.org/10.1016/j.scitotenv.2016.06.159

Gao, D. W., & Wen, Z. D. (2016). Phthalate esters in the environment: A critical review of their occurrence, biodegradation, and removal during wastewater treatment processes. *Science of the Total Environment*, *541*, 986–1001. https://doi.org/10.1016/j.scitotenv.2015.09.148

Gao, Y., Li, X., Li, X., Zhang, Q., & Li, H. (2018). Simultaneous determination of 21 trace perfluoroalkyl substances in fish by isotope dilution ultrahigh performance liquid chromatography tandem mass spectrometry. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *1084*, 45–52. https://doi.org/10.1016/j.jchromb.2018.03.008

Gu, Y., Yu, X., Peng, J., Chen, S., Zhong, Y., Yin, D., & Hu, X. (2014). 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. *Journal of Chromatography B*, *965*, 164–172. https://doi.org/10.1016/j.jchromb.2014.06.024

Hidalgo-Ruiz, J. L., Romero-González, R., Martínez Vidal, J. L., & Garrido Frenich, A. (2019). A rapid method for the determination of mycotoxins in edible vegetable oils by ultra-high performance liquid chromatography-tandem mass spectrometry. *Food Chemistry*, *288*(November 2018), 22–28. https://doi.org/10.1016/j.foodchem.2019.03.003

Hidalgo-Serrano, M., Borrull, F., Pocurull, E., & Marcé, R. M. (*In Press*). 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*.

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

457 Jia, X., Yin, S., Xu, J., Li, N., Ren, M., Qin, Y., Zhou, J., Wei, Y., Guo, Y., Gao, M., Yu, Y., Wang, B.,
458 & Li, Z. (2019). An efficient method to simultaneously analyze multi-class organic pollutants in
459 human serum. *Environmental Pollution*, *251*, 400–406.
460 https://doi.org/10.1016/j.envpol.2019.05.008

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

464 Lehotay, S. J. (2007). Official Method 2007.01: Pesticide Residues in Foods by Acetonitrile
465 Extraction and Partitioning with Magnesium Sulfate. *Journal of AOAC International*, *90*(2), 485–
466 520. http://lib3.dss.go.th/fulltext/E_content/1060-3271/2007v90n2.pdf

467 López-García, E., Postigo, C., & López de Alda, M. (2019). Psychoactive substances in mussels:
468 Analysis and occurrence assessment. *Marine Pollution Bulletin*, *146*(July), 985–992.
469 https://doi.org/10.1016/j.marpolbul.2019.07.042

470 Mathieu-Denoncourt, J., Wallace, S. J., de Solla, S. R., & Langlois, V. S. (2015). Plasticizer
471 endocrine disruption: Highlighting developmental and reproductive effects in mammals and
472 non-mammalian aquatic species. *General and Comparative Endocrinology*, *219*, 74–88.
473 https://doi.org/10.1016/j.ygcen.2014.11.003

474 Net, S., Sempéré, R., Delmont, A., Paluselli, A., & Ouddane, B. (2015). Occurrence, fate, behavior
475 and ecotoxicological state of phthalates in different environmental matrices. *Environmental*
476 *Science and Technology*, *49*(7), 4019–4035. https://doi.org/10.1021/es505233b

477 Perestrelo, R., Silva, P., Porto-Figueira, P., Pereira, J. A. M., Silva, C., Medina, S., & Câmara, J. S.
478 (2019). QuEChERS - Fundamentals, relevant improvements, applications and future trends.
479 *Analytica Chimica Acta*, *1070*, 1–28. https://doi.org/10.1016/j.aca.2019.02.036

Sakhi, A. K., Sabaredzovic, A., Cequier, E., & Thomsen, C. (2017). Phthalate metabolites in Norwegian mothers and children: Levels, diurnal variation and use of personal care products. *Science of the Total Environment*, *599–600*, 1984–1992. https://doi.org/10.1016/j.scitotenv.2017.05.109

Santana-Mayor, Á., Socas-Rodríguez, B., Herrera-Herrera, A. V., & Rodríguez-Delgado, M. Á. (2019). Current trends in QuEChERS method. A versatile procedure for food, environmental and biological analysis. *TrAC - Trends in Analytical Chemistry*, *116*, 214–235. https://doi.org/10.1016/j.trac.2019.04.018

Sireli, U. T., Filazi, A., Yurdakok-Dikmen, B., Iplikcioglu-Cil, G., Kuzukiran, O., & Orhan, C. E. (2017). Determination of phthalate residues in different types of yogurt by gas chromatography-mass spectrometry and estimation of yogurt-related intake of phthalates. *Food Analytical Methods*, *10*(9), 3052–3062. https://doi.org/10.1007/s12161-017-0854-x

Song, N. E., Lee, J. Y., Mansur, A. R., Jang, H. W., Lim, M. C., Lee, Y., Yoo, M., & Nam, T. G. (2019). Determination of 60 pesticides in hen eggs using the QuEChERS procedure followed by LC-MS/MS and GC-MS/MS. *Food Chemistry*, *298*(June), 125050. https://doi.org/10.1016/j.foodchem.2019.125050

Sun, X., Dong, W., Liu, M., Shen, C., Zhang, Y., Sun, J., Sun, B., Li, H., & Chen, F. (2018). Validation of a QuEChERS-Based bas chromatography-mass spectrometry (GC-MS) method for analysis of phthalate esters in grain sorghum. *Journal of Food Science*, *83*(4), 892–901. https://doi.org/10.1111/1750-3841.14063

Tsochatzis, E., Karayannakidis, P., & Kalogiannis, S. (2019). Determination of selected dichloroanilines and phthalates in lyophilised mussels samples with ultra-high performance liquid chromatography-tandem mass spectrometry after QuEChERS clean-up. *Food Additives & Contaminants: Part A*, *36*(8), 1253–1260. https://doi.org/10.1080/19440049.2019.1615642

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

UCT. *LipiFiltr*®. (2019). https://www.unitedchem.com/product/lipifiltr/ Accessed 16 March 2020.

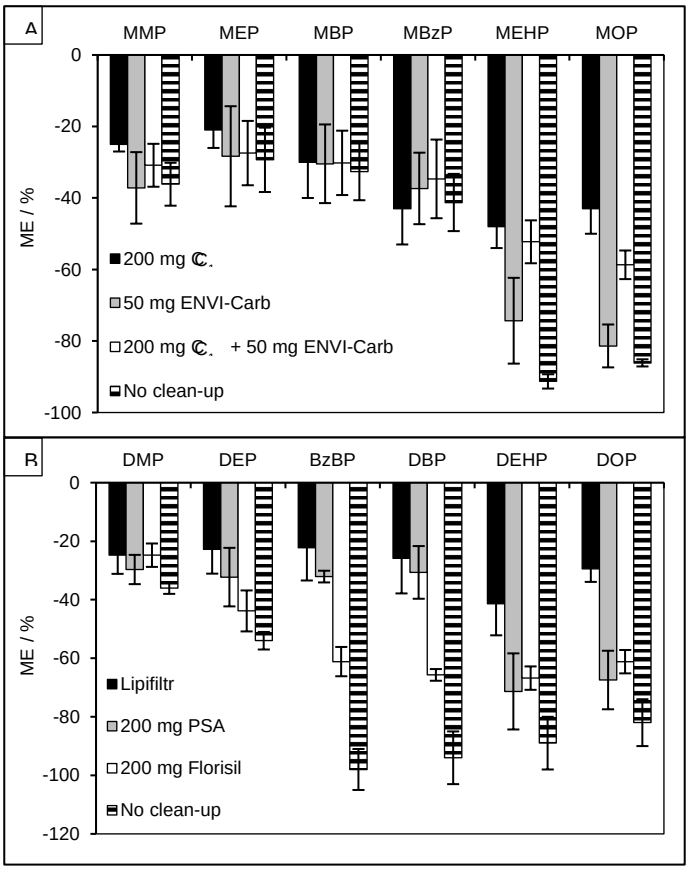
Vallecillos, L., Pocurull, E., & Borrull, F. (2015). Influence of pre-treatment process on matrix effect for the determination of musk fragrances in fish and mussel. *Talanta*, *134*, 690–698. https://doi.org/10.1016/j.talanta.2014.12.010

Valton, A. S., Serre-Dargnat, C., Blanchard, M., Alliot, F., Chevreuil, M., & Teil, M. J. (2014). Determination of phthalates and their by-products in tissues of roach (Rutilus rutilus) from the Orge river (France). *Environmental Science and Pollution Research*, *21*(22), 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. *Environmental Toxicology and Pharmacology*, *36*(1), 88–96. https://doi.org/10.1016/j.etap.2013.03.014

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 Analytical Methods*, *11*(12), 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). *Aquatic Toxicology*, *146*, 115–126. https://doi.org/10.1016/j.aquatox.2013.10.025



535

536 **Figure 1** Matrix effect (ME) obtained for salmon samples (*n = 3*) after dispersive solid phase
537 extraction with different sorbents (final extract volume of 1 mL) and without a clean-up step (final
538 extract volume of 5 mL) for phthalate monoesters (A) and phthalate diesters (B).

539

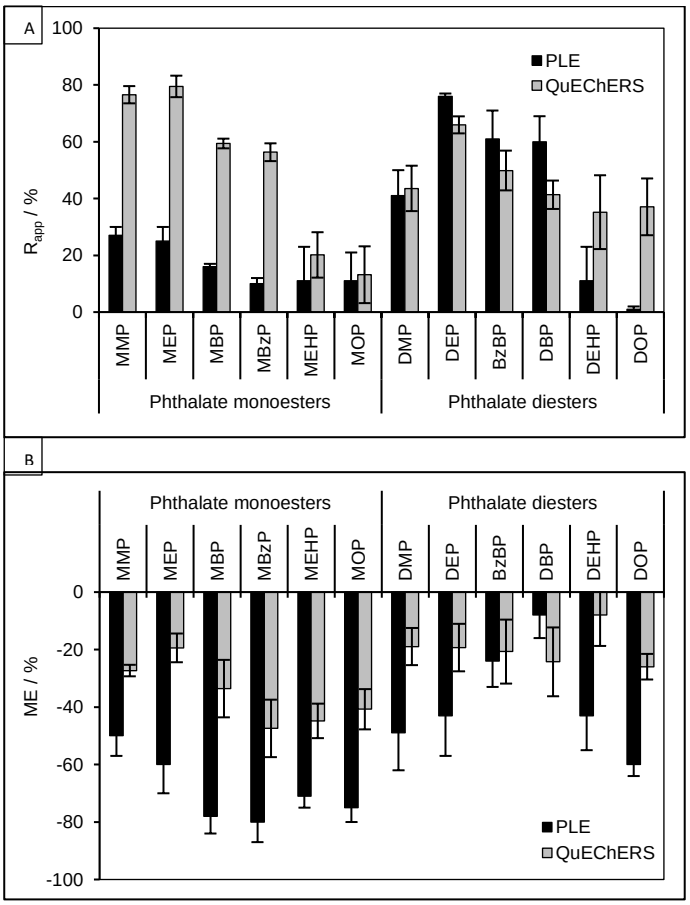
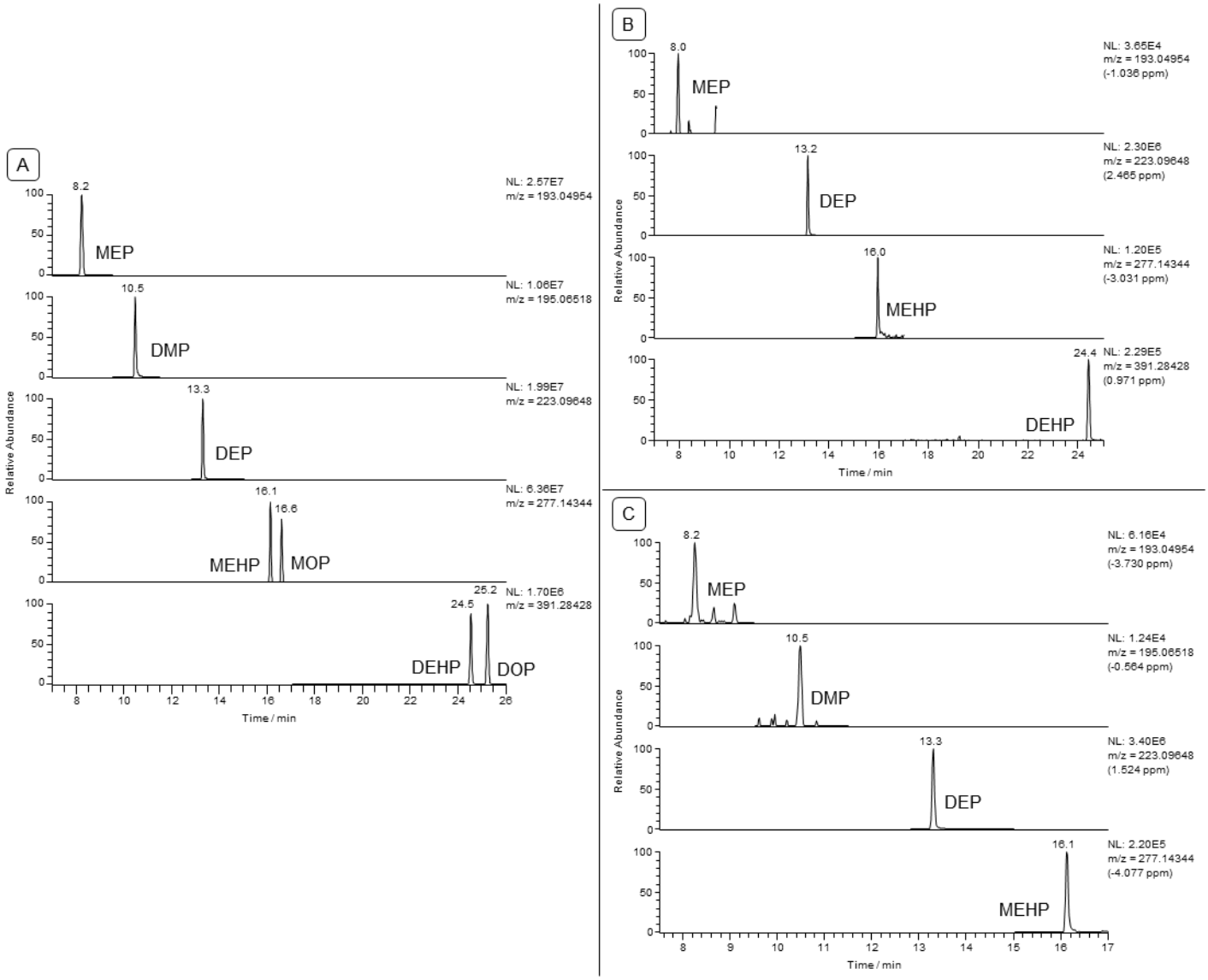


Figure 2 Apparent recoveries (A) and matrix effect (B) obtained for sole samples (*n = 3*) using our pressurised liquid extraction method (PLE) (Hidalgo-Serrano et al., *In Press*) and our new QuEChERS method.

548

549



550

551 **Figure 3** LC-HRMS extracted ion chromatogram and mass error in ppm of a 1 ppm standard solution

552 (A), a shrimp sample (B) and a mackerel sample (C).

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

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.

<MLOQ: Below method limit of quantification

Compounds	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.

n.d.: not detected (<MLOD)

562

563 Supplementary Material

564

565

566 Simple method for determining phthalate diesters and their metabolites 567 in seafood species using QuEChERS extraction and liquid 568 chromatography-high resolution mass spectrometry

569

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

571

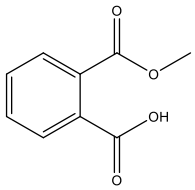
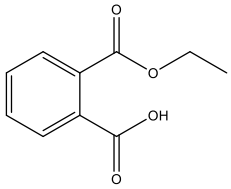
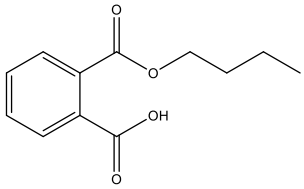
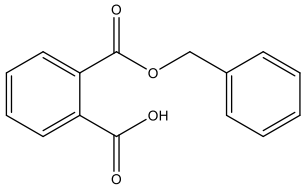
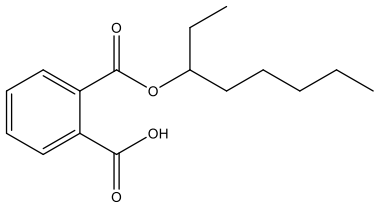
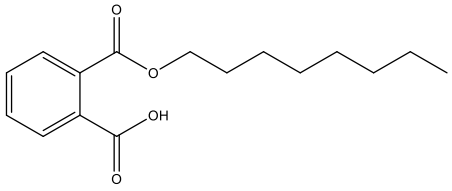
572

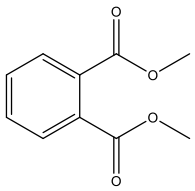
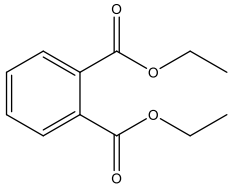
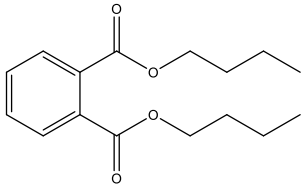
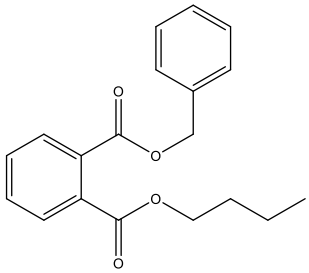
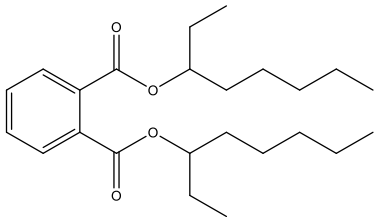
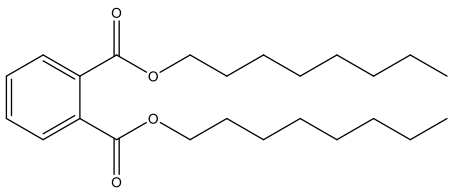
573

574

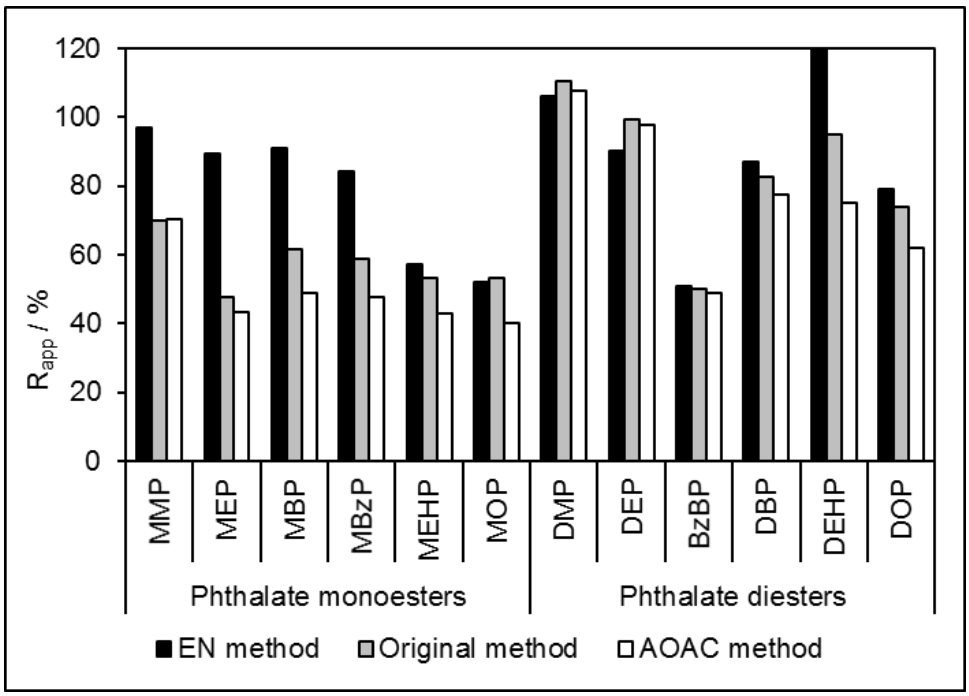
575 *Corresponding autor: Rosa Maria Marcé. Department of Analytical Chemistry and Organic
576 Chemistry, Universitat Rovira i Virgili, Marcel·lí Domingo s/n, Tarragona 43007, Spain.
577 Tel: (+34) 977559560 Fax: (+34) 977558446.
578 E-mail adress: rosamaria.marce@urv.cat
579

580 **Table S1** Chemical structure of the analytes studied and exact masses of their quantification and
581 fragment ions.

Compound	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)
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)
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)

582
583



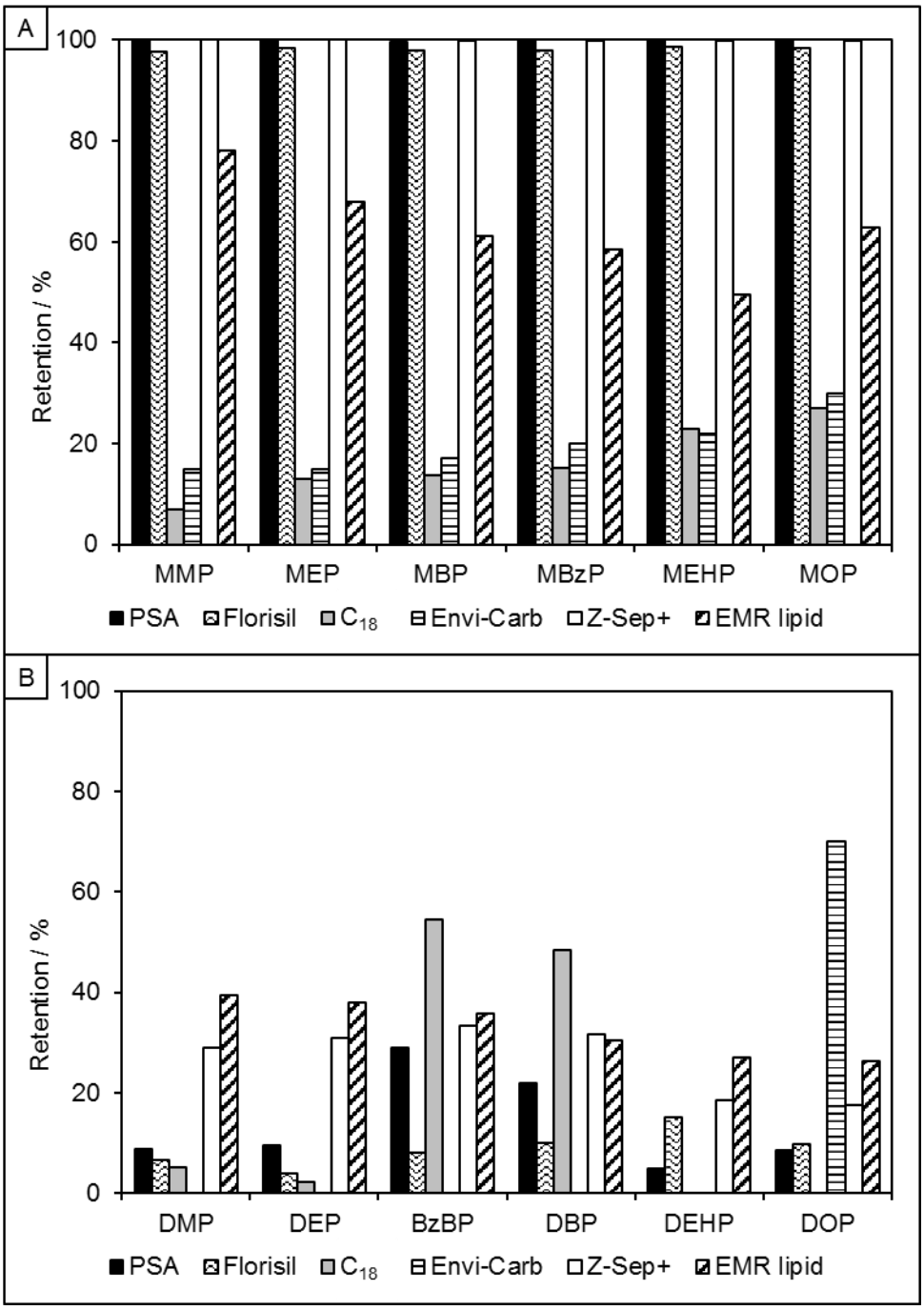
584

585 **Figure S1.** Apparent recoveries obtained for sole samples using different extraction methods.

586

587

588



589
590

591 **Figure S2** Retention of phthalate monoesters (A) and phthalate diesters (B) in several dSPE
592 sorbents.