Journal of Analytical Science and Technology

RESEARCH ARTICLE

Open Access

Fast determination of phthalates in mussel samples by micro-matrix solid-phase dispersion (micro-MSPD) coupled with GC–MS/MS

Nieves Carro*, Ana Mouteira, Isabel García, María Ignacio and Julio Cobas

Abstract

A fast, effective and low cost sample preparation method based on miniaturized matrix solid-phase dispersion (micro-MSPD) combined with gas chromatography coupled to tandem triple-quadrupole-mass spectrometry (GC–MS/MS) has been developed for the determination of six phthalate diesters (DMP, DEP, DBP, BzBP, DEHP and DnOP) in mussel samples. The six target compounds have been included in the list of priority pollutants by United States Environmental Protection Agency. The extraction step was optimized on real spiked mussel coming from Galician Rías by means of a factorial design. The final procedure involved the use of 0.45 g of sample, 0.5 g of dispersant agent (Florisil) and 3 mL of organic solvent (ethyl acetate). The optimized method was validated giving satisfactory analytical performance, low detection limits (0.09 to 6.73 ng g^{-1} dw) and high recoveries (93 and 114%). The validated method was applied to four real mussel samples coming from Galician Rías.

Keywords: Galician mussels, Factorial design, Micro-MSPD, GC–MS/MS

Introduction

Phthalates (diesters of *ortho*-phthalic acid) are ubiquitous chemicals easily released into environment during the production, use and disposal of plastics. They are widely used as plasticizers with the purpose of improving plastic properties as flexibility, durability and transparency. Phthalates are also part of the composition of cosmetics, personal care products, electronic equipments, insecticides, propellants and all kinds of household goods (Net et al. 2015). These compounds are not covalently bound to plastic polymers and can be liberated during their use reaching all environmental compartments, sediment and biota (Huang et al. 2008), seawater (Paluselli et al. 2018), freshwater fish (Teil et al. 2014) and air (He et al. 2020). A new study field of linking microplastics and phthalates in marine environment is currently being developed. A possible correlation between microplastics contamination and presence of phthalates in the marine compartments is explored and evaluated (Fred-Ahmadu, et al. 2020; Liu et al. 2020).

Although Giam et al. (1978) already considered phthalates as a new class of pollutants, the increase in their production, from 2.7 to 6 million tons per year from 2007 to 2017 (Gao et al. 2018), has caused important damage to environment, wildlife and human. These chemicals can act as endocrine disruptors, inducing toxics effect on kidney and liver, affecting the reproductive health and causing immunotoxicity (Meeker et al. 2009; Oehlmann et al. 2009; Liu et al. 2012). Due to their significant risks, six phthalate diesters (dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), benzyl butyl phthalate (BzBP), diethyl hexyl phthalate (DEHP) and di-n-octyl phthalate (DnOP)) have been included in the list of priority pollutants by United States Environmental Protection Agency (2012).

Phthalates were approved for use as food contact materials in the European Union market in 2011 (EC 10/2011).

*Correspondence: ncarro@intecmar.gal
Instituto Tecnolóxico para o Control do Medio Mariño de Galicia, INTECMAR, Consellería do Mar, Xunta de Galicia, Peirao de Vilaxoán s/n. 36611, Vilagarcía de Arousa, Spain

In order to reduce migration to food, both DEHP and DiBP (diisobutyl phthalate) were regulated by Europen Commission (EU 2018/2005) and their concentrations cannot exceed 0.1% by weight of plasticised material.

Phthalates are hydrophobic compounds with log octanol–water partition coefficients from 1.61 to 12.06 and with a high tendency to bioaccumulate both in terrestrial and marine living organisms (Net et al. 2015). The major source of phthalates contribution in humans is food intake and environmental exposure (Das et al. 2014; Ji et al. 2014). Many papers have been written focusing on the study of packaging material (plastic, cardboard, paper) and its transfer into food and mineral waters (Poças et al. 2010; Fierens, et al. 2012; Schecter et al. 2013) but little data about the presence of phthalates in fresh food and mainly in seafood whose content is related to the environmental levels (Valton et al. 2014; Hu et al. 2016). With a view to guarantee the seafood safety and to assess the distribution of these plasticizers in marine environment, the development of cheap and rapid analytical methods for the determination of pollutants in marine biota is required. There is an immediate need for monitoring of phthalates in the marine environment for being the plasticizers, good indicators of microplastic contamination. In addition to be highly prized seafood, mussels are used as sentinels in many marine environmental monitoring programs due to their chemical and biological characteristics (Mussel Watch Program). They are sessile, have easy culture and high filter-feeding power, accumulate the pollutants from sea water (integrative measure) (Grbin et al. 2019).

Several analytical methods have been optimized for determination of phthalates in food and environmental samples including different extraction techniques as solid-phase extraction (SPE) in fatty food packaged and liquid samples (Fan et al. 2012; Barciela-Alonso et al. 2017), microwave-assisted extraction (MAE) in soils and sediments (Liang et al. 2010; Zheng et al. 2014), accelerated solvent extraction (ASE) in soils (Hu et al. 2020a, b), ultrasound-vortex-assisted dispersant liquid–liquid micro-extraction (USVADLLME) in alcoholic beverages (Cinelli et al. 2013), single-drop-microextraction (SDME) in food (Battle and Nerin 2004), liquid–liquid extraction (LLE) in milk and cosmetics (Orssi et al. 2006), stir bar sorptive extraction (SBSE) in biosolid and sludge samples (Tan et al. 2008) and solid-phase microextraction (SPME) in water samples (Cortazar et al. 2002). In recent years, several QuEChERS-based procedures, initially known for pesticides analysis, have also been optimized for extraction of different pollutants including phthalates in seafood (Hidalgo-Serrano et al. 2021). Recently, liquid phase micro-extraction using hollow fibre (HF-LPME) has attracted attention for the phthalates analysis of liquid matrices due to its excellent clean-up efficiency, selectivity and simplicity (Sun et al. 2013; Wang et al. 2016). In general, the quantification of phthalates is based on chromatographic techniques, gas chromatography coupled to mass spectrometry (GC–MS) or to tandem MS/MS (GC–MS/MS) is the most interesting choice (Fierens et al. 2012; Russo et al. 2016) due to its high sensitivity and selectivity reducing the matrix interferences.

There are a lot of factors that make micro-extraction techniques attractive, the reduction of sample and reactive amounts and solvent volume, minimizing waste generation, and the easy handling, desirable from an environmental and economical point of view. Matrix solid-phase dispersion (MSPD) has been widely used in environmental and food samples providing low time-consuming and reducing cost extraction. The MSPD method was costumized and miniaturized (micro-MSPD) in order to reduce the risks of phthalate cross-contamination and to minimize the solvent residues and costs. The micro-MSPD does not need special material since extraction is carried out in glass Pasteur pipettes. It can be entered in any laboratory at low cost, minimizing even more the consumption of sample, solvents and sorbents. The micro-MSPD method has been applied for the first time for the analysis of plasticizers in cosmetics by Llompart et al. (2013). In 2014 Celeiro et al. developed a micro-MSPD method for determination of fragrance allergens and preservatives in personal care products. Carro et al. optimized a micro-MSPD procedure for PCBs analysis in 2017 and for OCPs analysis in 2021 (organochlorine pesticides) in mussel samples.

In this work, a miniaturized MSPD (micro-matrix solid-phase dispersion) combined with GC–MS/MS method for the determination of six phthalates (DMP, DEP, DBP, BzBP, DEHP and DnOP) in mussel samples was developed and optimized using a factorial design. These chemicals chosen are included in the list of priority substances. Both extraction and purification were carried out simultaneously in a single step and their miniaturization was investigated with the purpose of reducing the sample, solvents and reagents consumption. Phthalates extracts have been analyzed by gas chromatography coupled to tandem mass spectrometry GC-QqQ-MS/MS (EI) achieving high selectivity and sensitivity. The use of plastic material has also been avoided. The validated method has been applied to four mussel samples coming from the Galician Rías.

Material and methods

Materials, chemicals and samples

Standard of six phthalate esters mixture (2000 µg mL^{-1} in hexane) (dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), benzyl butyl phthalate

(BzBP), diethyl hexyl phthalate (DEHP) and di-n-octyl phthalate (DnOP)) and deuterated bis(2-ethylhexyl) phthalate-2,4,5,6-d4 (DEHP-d4, 98at.% D) (100.01 mg L^{-1} in cyclohexane) used as internal standard were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Calibration standard solutions were prepared in ethyl acetate by appropriate dilution of the stock standard solution and stored at − 20 °C.

Acetone and hexane were purchased from WR Prolabo (Fontenay-sous-Bois, France) and ethyl acetate from VWR chemicals (Barcelona, Spain). Florisil (60–100 mesh) was supplied by Supelco Analytical (Bellefonte, PA, USA). Sodium sulphate anhydrous (99%), silica gel and glass wool for laboratory were purchased from VWR chemicals (Barcelona, Spain).

Due to ubiquity of phthalates in the laboratory and in order to avoid the sample contamination, all the laboratory material, glassware, sorbents, and glass wool were baked at 250 °C overnight. Materials were cooled wrapped with aluminium foil and sorbents in desiccator. Solvents (1 L) were cleaned with Florisil (10 g) for 24 h in order to adsorb phthalates prior to use.

Mussel samples were collected in four Galician rías (Ría de Ferrol, Ría de A Coruña, Ría de Vigo and Ría de Ares-Betanzos). The flesh of each individual mussel was dissected from the shells. A pool of thirty individual was employed for each analysis. Mussel flesh was frozen (− 30 °C), freeze-dried and crushed in a Zirconium oxide mill.

micro-MSPD procedure

Irrespective of the working conditions given by the particular experiment of factorial design or proof performed outside the frame of design, all freeze dried mussel samples were prepared following the same procedure:

0.1–0.45 g of sample (raft mussel from Galicia coast, NW Spain) was spiked with an adequate volume of the standard stock solution containing the six phthalates in order to get the desired concentration (450 ng g^{-1} dw). Fortified mussel was blending with 0.1–0.4 g of Florisil (dispersant agent A) and 0.1 g of anhydrous sodium sulphate (drying agent) using a porcelain mortar. The mixture was transferred into a glass Pasteur pipette (1.8 mL) with a tiny amount of glass wool at the bottom, containing 0.1–0.4 g of Florisil (dispersant agent B), this later was used to improve of fractionation and as clean up agent. Finally, another small amount of glass wool was placed on top of the sample. Elution was performed by gravity with 1–3 mL of ethyl acetate and collected into a volumetric flask. Extracts were concentrated under nitrogen stream to dryness and redissolved in 1 mL of ethyl acetate, 10 µL of DEHP -d4 was added as an internal standard prior to analysis by GC–MS/MS.

The optimized micro-MSPD method, according to factorial design and further experiments, consisted of 0.45 g of freeze dried mussel, 0.2 g of Florisil (dispersant agent A), 0.1 g of anhydrous sodium sulphate, 0.3 g of Florisil (dispersant agent B) and 3 mL of ethyl acetate as elution solvent.

GC–MS/MS determination

The micro-MSPD extracts were determined by using an Agilent 7890B gas chromatograph coupled to an Agilent MS 7000D mass spectrometer with triple quadrupole (Agilent Technologies, Palo Alto, CA, USA). Optima 5 MS, 5% diphenyl 95% dimethyl siloxane (50 m × 0.20 mm i.d. × 0.35 µm phase thickness) (Machery-Nagel, Germany) was used as capillary column.

The conditions of GC–MS/MS system were, injector temperature at 260 °C and 35 psi, pulsed splitless mode at 50 psi until 1.15 min. Purge flow to split vent is 75 mL min^{-1} at 1 min. Helium was used as carrier gas at a constant flow rate of 1 $mLmin^{-1}$. The oven temperatures program was, 1 min isothermal at 60 °C, increased to 100 °C at 8 °C min^{-1}, to 150 °C at 20 °C min^{-1}, to 200 °C at 25 °C min^{-1} and held for 8 min, then increased to 220 °C at 8 °C min^{-1} and to 290 °C at 30 °C min^{-1}, held for 20 min. The mass spectrometer conditions were: Transfer line temperature at 280 °C, ion source and Q1 and Q3 temperatures at 280, 180 and 180 °C, respectively, emission current at 35 µA, electron energy at 70 eV and dwell time at 75 ms. Nitrogen was used as CID gas. Collision energy was in the range 5–35 V and was optimized for each phthalate in order to get the highest fragment ion response. The ionized samples were detected by multiple-reaction monitoring method (MRM). Depending on the compound, from one (for DEHP) to four (for DnOP) pairs of precursor-product ion transitions were set for qualification (qualifier transition), among them the most intense transition was selected for quantification (quantifier transition). In Table 1, the qualifier and quantifier transitions of the six phthalates are listed. Data analysis was performed by using MassHunter Workstation software (Agilent, Ver.B.07.00).

Statistical analysis

Factorial design and data variance analysis were performed using *Minitab 16* Statistical software (Minitab Inc., State College, PA, USA). A 2^{4-1} fractional factorial design with 3 central points (type IV resolution) was used for the micro-MSPD procedure optimization that involved the 11 experiments performing.

Table 1 Qualifier and quantifier transitions of the six phthalates in the mass spectrometry detector

Compound	Qualifier transition		Quantifier transitionQuantifier transition	
	Precursor ion	Product ion	Precursor ion	Product ion
DMP	163	135	163	77
	163	92		
DEP	177	149	149	65
	149	93		
	149	121		
DBP	149	121	149	65
	149	93		
BzBP	91	65	149	65
	149	93		
DEHP	167	149	149	65
DnOP	279	71	149	65
	261	149		
	279	149		
	149	121		

DMP dimethyl phthalate, *DEP* diethyl phthalate, *DBP* dibutyl phthalate, *BzBP* benzyl butyl phthalate, *DEHP* diethyl hexyl phthalate, *DnOP* di-n-octyl phthalate

Results and discussion

Preliminary experiments

Due to phthalates are ubiquitous in the laboratory environmental, the early proofs were aimed at reducing or eliminating contamination from materials, solvents and reactive that could interfere with the phthalates analysis. Only previously treated glass materials were used. Several solvents and reactive were checked performing the same micro-MSPD experiments as samples. Silica, acetone and hexane were discharged from procedure since they were not phthalates free.

All the proofs have been carried out using spiked lyophilized real sample (raft mussel from Galicia coast) at levels of 450 ng g^{-1} for the six analytes (DMP, DEP, DBP, BzBP, DEHP and DnOP).The first experimental conditions were selected according to optimized variables from previous studies (Celeiro et al. 2014; Carro et al. 2017, 2021). Florisil (magnesium silicate with basic properties) was chosen as dispersant agent instead of silica. Although mussel samples were lyophilized, their drying was very important in order to improve extraction efficiency, 0.1 g of anhydrous sodium sulphate was used as the drying agent. Ethyl acetate was considered as elution solvent. Although acetone-hexane mixture gave good recoveries, it presented very high interferences of phthalates.

Quality control blanks were periodically performed without sample following the same analytical procedure as mussel samples. For all proofs of optimization, non-spiked mussel samples were analysed to subtract the response of some compound such as DEHP, from spiked musses samples.

Optimization of micro-MSPD procedure

A 2^{4-1} fractional factorial design with 3 central points (type IV resolution) involving 11 experiments was applied in order to select the optimal extraction conditions of variable (factors). The degrees of freedom obtained were enough to assess the statistical significance of the interactions (the second-order factors) too. Four variables (factors) and their interactions were investigated, sample amount (factor A), Florisil as dispersant agent A (factor B), Florisil as dispersant and clean up agent B (factor C) and elution solvent volume (factor D). All experimental factors were studied at two levels that are shown in Table 2. All the optimization proofs have been carried out using spiked lyophilized real sample (450 ng g^{-1} dw of six analytes). Data analysis was carried out with statistical package Minitab 16. In Table 3 analysis of variance (ANOVA) for six compounds is shown. The p-values test the significance of each factor and interaction ($p < 0.05$). The results of design can also be exhibited using the Pareto charts and the interaction plots. The length of each bar in Pareto chart is proportional to the absolute value of its associated standardized effect (obtained by dividing the estimated effect by the standard error). The vertical line is the statistically significant bound at 95% confidence level ($p < 0.05$). In Fig. 1 the Pareto charts of

Table 2 Factors and levels considered in the experimental design

Factors	Key	Low level	High level	Optimum according to factorial design	Optimum of final procedure
Sample amount (g)	A	0.1	0.3	0.3	0.45
Dispersant agent A(g)	B	0.2	0.4	0.2	0.2
Dispersant agent B(g)	C	0.1	0.3	0.3	0.3
Elution volume (mL)	D	1	2	2	3

Optimal values for phthalates determination

Table 3 Analysis of variance

	Effect	Coefficient	p
DMP			
Sample amount	5.570	2.785	0.159
Dispersant agent A	1.120	0.560	0.702
Dispersant agent B	9.045	4.523	0.070
Elution solvent	5.585	2.793	0.158
Sample × dispersant A	−4.055	−2.027	0.251
Sample × dispersant B	5.570	2.785	0.159
Sample × volume	13.770	6.885	0.032
DEP			
Sample amount	−9.30	−4.65	0.206
Dispersant agent A	5.74	2.87	0.372
Dispersant agent B	11.90	5.95	0.142
Elution solvent	−1.86	−0.93	0.747
Sample × dispersant A	−2.19	−1.09	0.706
Sample × dispersant B	6.16	3.08	0.345
Sample × volume	27.36	13.68	0.032
DBP			
Sample amount	6.582	3.291	0.611
Dispersant agent A	6.883	3.441	0.596
Dispersant agent B	9.253	4.626	0.490
Elution solvent	12.973	6.486	0.360
Sample × dispersant A	−0.512	−0.256	0.967
Sample × dispersant B	15.398	7.699	0.297
Sample × volume	13.978	6.989	0.332
DEHP			
Sample amount	−4.552	−2.276	0.054
Dispersant agent A	−8.913	−4.456	0.015
Dispersant agent B	−10.443	−5.221	0.011
Elution solvent	7.807	3.904	0.019
Sample × dispersant A	1.572	0.786	0.290
Sample × Dispersant B	−9.617	−4.809	0.013
Sample × volume	−4.317	−2.159	0.059
BzBP			
Sample amount	11.385	5.692	0.010
Dispersant agent A	−8.820	−4.410	0.017
Dispersant agent B	0.800	0.400	0.562
Elution solvent	10.475	5.237	0.012
Sample × dispersant A	−9.585	−4.792	0.014
Sample × dispersant B	−0.285	−0.143	0.829
Sample × volume	6.510	3.255	0.030
DnOP			
Sample amount	8.980	4.490	0.125
Dispersant agent A	−2.720	−1.360	0.520
Dispersant agent B	8.050	4.025	0.149
Elution solvent	5.480	2.740	0.259
Sample × dispersant A	−8.665	−4.332	0.133
Sample × dispersant B	4.595	2.298	0.321
Sample × volume	3.905	1.953	0.382

Effects and estimated coefficients for six phthalates

DMP dimethyl phthalate, *DEP* diethyl phthalate, *DBP* dibutyl phthalate, *BzBP* benzyl butyl phthalate, *DEHP* diethyl hexyl phthalate, *DnOP* di-n-octyl phthalate

phthalates (DMP, DEP, DEHP and BzBP) are shown. The two factors interaction plots enable to study the effect of two factors simultaneously. In Fig. 2 the interaction plots of DMP, DEP and BzBp are shown.

Taking account data analysis of factorial design, the optimal extraction conditions were defined considering the p-values of each factor (Table 3). For sample amount (factor A), investigated at 0.1 and 0.3 g, p-values indicated that it was statistically significant for BzBP, and well, it had positive coefficient. This can also be seen in the Pareto chart of Fig. 1, factor A clearly exceeds the significant limit (vertical line) for BzBP. Three compounds also had positive coefficients for sample amount factor, DMP, DBP, and DnOP. In all factorial design experiments, DMP recoveries ranged from 42.0 to 76.8%, for that reason, sample amount was fixed at high level, 0.3 g, as optimal value in the experimental design.

In relation to the dispersant agent A (factor B), the half of compounds had positive coefficients (DMP, DEP, and DBP) and the other half had negative coefficients (DEHP and BzBP and DNOP). Furthermore, both coefficients of DEHP and coefficients of BzBP were statistically significant. See Fig. 1 where factor B clearly exceeded the vertical line for both compounds. Since the factor B was studied at 0.2 and 0.4 g levels, the lowest level, 0.2 g, was chosen as optimal value in the design.

With regard to the dispersant agent B (factor C), its coefficient was positive for all compounds except for DEHP (Fig. 1). The dispersant agent B amount significantly affected the extraction of DEHP, but since its recoveries were very good in all experiments of design, from 92 to 120%, it was intended to improve the recoveries of the other compounds with positive coefficients, mainly of DMP and DEP. Hence 0.3 g was considered as optimal value. Since sample is dispersed with the agent A at the first of procedure, the dispersant agent B (factor C) had a lower dispersive function. The function of factor C was purely cleaning.

About the elution volume (factor D), all the compounds had a positive coefficient, except the DEP. The factor D coefficients were statistically significant (p values < 0.05) for DEHP and BzBP, then, the elution volume of 2 mL contributed to higher recoveries (optimal value in the factorial design).

The plot of second-order factors (interactions) in Fig. 2 for DMP, DEP and BzBP showed the least squares means at all combinations of two factors in order to study the effects simultaneously. In regard to the AB interaction, its coefficients were negative for all compounds except for DEHP, compound with high recovery rates. The AB interaction was only statistically significant for BzBP (see Table 3), meaning the higher sample amount, lower quantity of dispersant agent A to obtain the highest

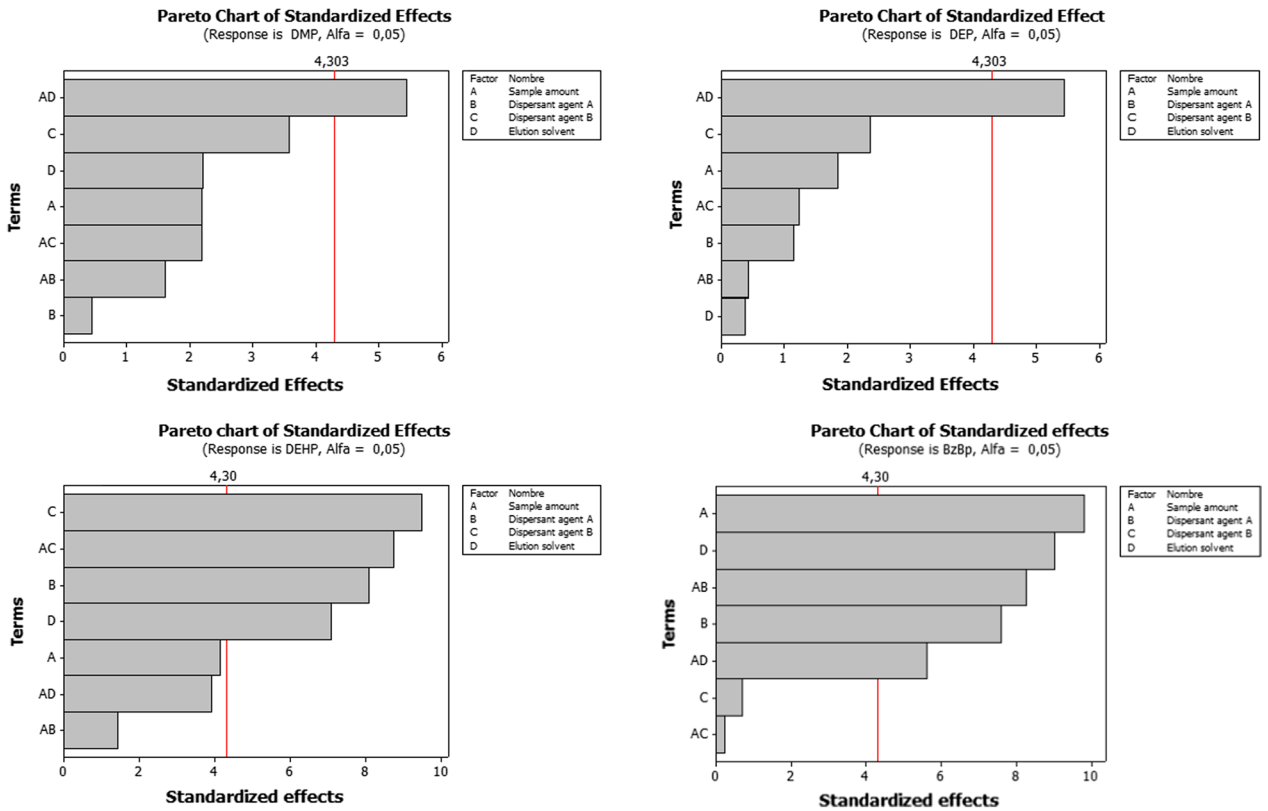

Fig. 1 Pareto chart of main effects for the experimental design, obtained by using the DMP (dimethyl phthalate), DEP (diethyl phthalate), DEHP (diethyl hexyl phthalate) and BzBP (benzyl butyl phthalate) recoveries. The vertical lines indicate the statistical significance bound for the effects

recovery (see Fig. 2). The reason for this response can be due to lack of space in Pasteur pipette (factor A ranged 0.1–0.3 g and factor B, 0.1–0.3 g) which did not allow a quantitative elution of analytes with ethyl acetate and led consequently to lower recovery efficiency. In the proposed procedure, sample was already homogenized and dispersed in a zirconium oxide mill prior to extraction step achieving a good homogenization and large specific surface area. The coefficients of AC interaction were positive for all compounds except for DEHP and BzBP, and significant for the first (compound with very high recovery rates). In this case, the higher sample amount (factor A), the lower proportion of purification agent (factor C) to achieve the highest recoveries. At low levels of sample amount, recoveries were quantitative no matter the amount of dispersant agent B, see Fig. 2. The AD interaction had positive coefficients for all analytes except for DEHP. This interaction was significant for analytes with the lowest recovery percentages, DMP and DEP, and also for BzBP. While for DMP and BzBP, the bigger sample amount, the bigger the elution volume, for DEP the opposite occurs, the lower sample amount, the lower the elution volume (see Fig. 2). This behaviour is logical, extraction solvent volume is usually attached to sample amount to extract and to solvent desorption power. The AD interaction had a negative coefficient for DEHP, however further clarification was required. When the highest volumes of ethyl acetate (3 mL) were used to extract the lowest amounts of sample (0.1 g), DEHP presented repeatedly recoveries around 120%. However, experiments using 0.3 g of sample and 3 mL of solvent, achieved quantitative recoveries close to 100%, which suggested less impurities and interferences.

As sample was dried (liophilized) and on the basis of our experience, anhydrous sodium sulphate amount (drying agent) were kept fixed at 0.1 g. The optimized values of factors according to factorial design are shown in Table 2.

Further experiments

In view of the factorial design results five experiments were performed, in the first one the conditions optimized in experimental design were applied (0.3 g sample, 0.2 g of dispersant agent A, 0.3 g of dispersant agent B and 2 mL of ethyl acetate, Experiment DF). In the following proofs, in order to improve recoveries of some analytes

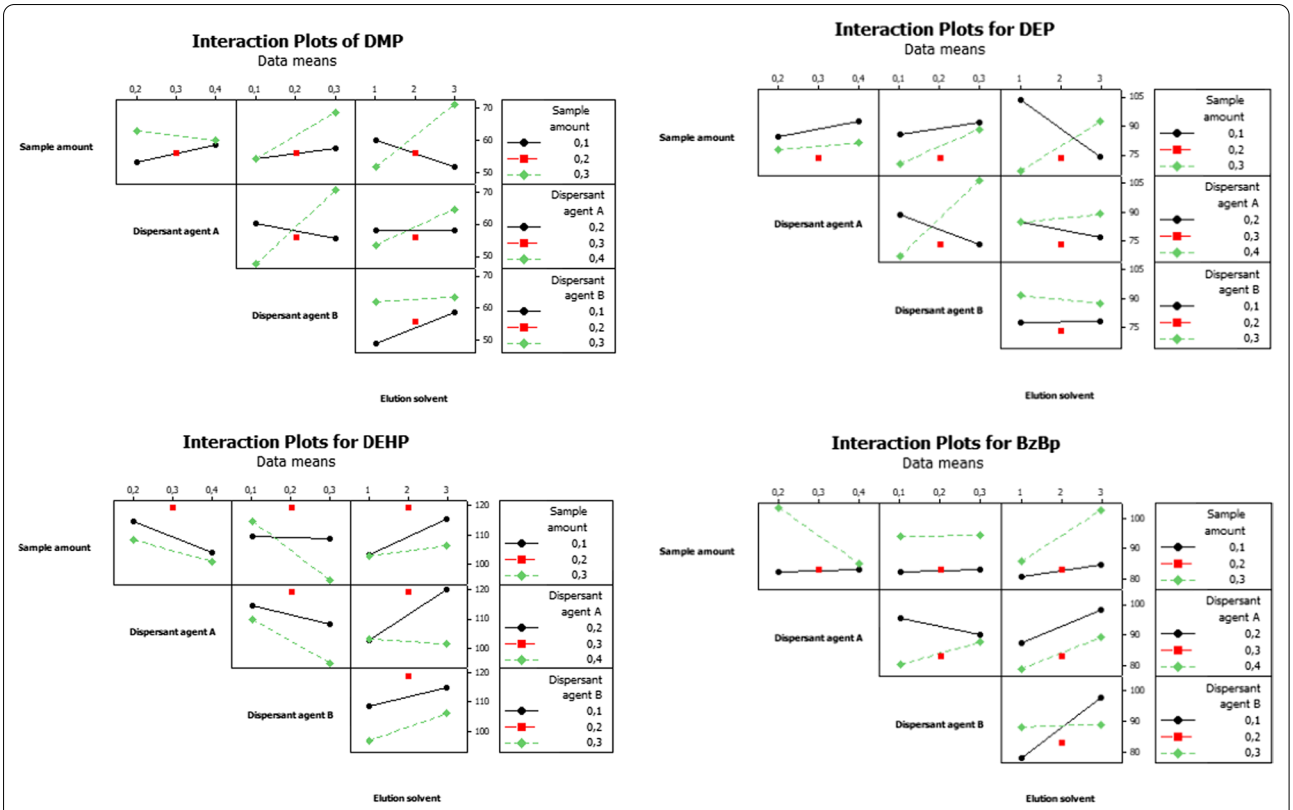

Fig. 2 Interaction plot of DMP (dimethyl phthalate), DEP (diethyl phthalate), DEHP (diethyl hexyl phthalate) and BzBP (benzyl butyl phthalate) for the four studied parameters (sample, dispersant agent A and dispersant agent B amounts, and solvent volume)

we conducted experimental conditions in the direction dictated by the design. Firstly we fixed the solvent volume at 3 mL keeping the other factors at the levels set by the experimental design (Experiment 1). In the 2 subsequent experiments we fixed the amounts of dispersant agent A at 0.1 g and the dispersant agent B at 0.4 g, respectively, keeping the other variables at the factorial design conditions (Experiments 2 and 3, respectively). The last experiment, taking account the AD interaction, varied the sample amount and ethyl acetate volume at 0.45 g and 3 mL, respectively (Experiment 4). In Fig. 3, the results are shown, the best recoveries for all compound, including DMP and DEP, were obtained when experimental conditions were set at 0.45 g of sample, 0.1 g of anhydrous sodium sulphate, 0.2 g of Florisil (dispersant agent A), 0.3 g Florisil (dispersant agent B) and 3 mL of ethyl acetate (Experiment 4).

In relation to dispersant agents (A and B), the further experiments produced the same results as the factorial design, the dispersant agent B had better effect on the extraction procedure than the dispersant agent A. In relation to sample and solvent volume, higher sample amount, higher extractant volume to recover analites from the matrix.

Method performance

The validation of analytical method was based on criteria defined in the Eurachen guide (Magnusson and Örnemark 2014). The proposed method performance parameters for the six phthalates are exhibited in Table 4. According to the instrumental linearity, there is a direct proportional relationship between the concentration of each analyte and the chromatographic response with correlation coefficients $R \geq 0.99$. Linear regression was taken by weighted. Concentrations of calibration standard solutions in ethyl acetate ranged from 1 to 500 ng mL^{-1}. The method precision was investigated among days ($n = 6$) at the studied concentration level. RSD values ranged from 5 to 20%. The limits of detection (LODs) and quantification (LOQs) of the overall method were determined as the concentration giving as signal-to-noise ratio of three (S/N = 3) and as signal-to-noise of ten (S/N = 10), respectively. The obtained values ranged from 0.09 to 6.73 ng g^{-1} dw for LODs and from 0.27 to 22.4 ng g^{-1} dw for LOQs. DEHP had the highest limit of detection due to the high background values of the procedural blanks. The source of DEHP has not been found, all glass material, even chromatographic vials, has been cleaned with different solvents and dried at 250 °C. The contamination

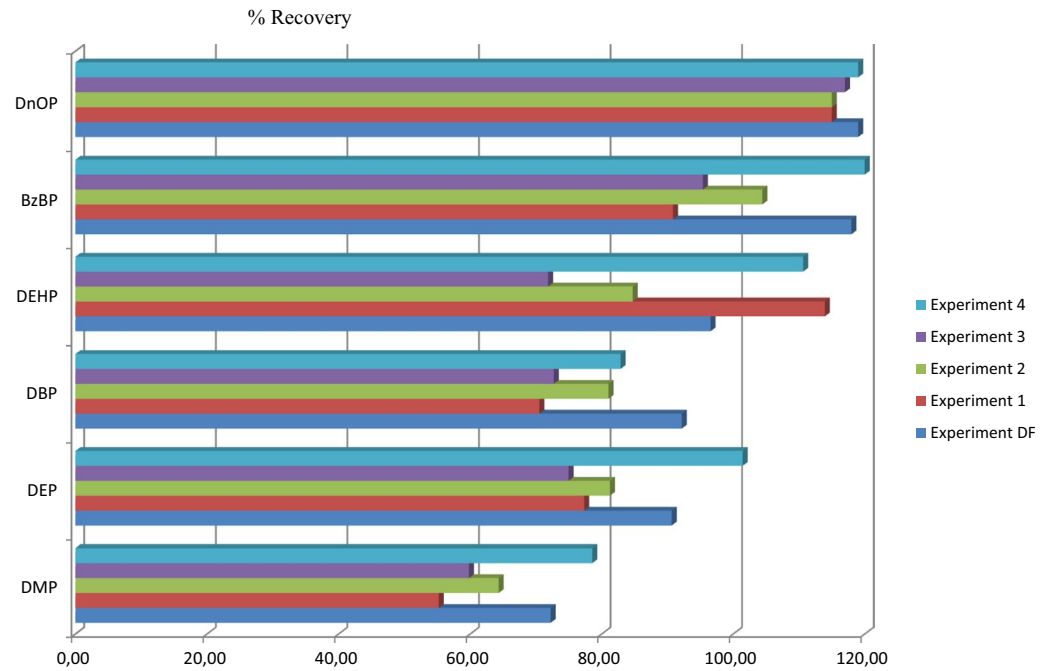

Fig. 3 Recoveries (%) of phthalates in further experiments

Table 4 Quality parameters of method: recoveries obtained for spiked mussel sample at studied level, $n = 6$ replicates

Compound	Reproducibility (%)	Recovery (%)	LODs (ng g^{-1})	LOQs (ng g^{-1})
DMP	15.5	93.2	0.09	0.29
DEP	12.5	108.2	2.36	7.82
DBP	9.5	103.9	0.91	3.00
DEHP	13.5	111.2	6.73	22.44
BzBP	20.5	104.2	0.09	0.27
DnOP	5.6	113.8	2.16	7.18

Method reproducibility, $n = 6$. LODs and LOQs of the whole method

by GC–MS/MS was also discarded since any signal was detected when solvent blank (ethyl acetate) was injected. The presence of phthalates in the laboratory environmental and material was the reason why we decided to develop the proposed method, a direct and simple method in which the number of sample preparation steps was reduced. The calculated LOQs were similar to those reported by other authors for seafood and fish samples (Xu et al. 2018; Hidalgo-Serrano et al. 2021). Cañadas et al. (2021) have optimized a method based on MSPD for determination of plastic additives in mussel samples with LOQs ranging of 0.25 to 16.20 ng g^{-1}, values similar to ours. The mean recoveries (accuracy) of the optimized method were estimated with mussel sample at the studied concentration level ($n = 6$). As can be seen in Table 4 in supplementary material, these values were 93 and 114%. In Additional file 1: Table S1, comparison of the proposed method with other methods of literature for phthalates analysis in marine organisms is shown.

Applications to real samples

The developed and validated method was applied to the analysis of four real samples from different sampling points in Galician Rías, FER1 coming from Ría de Ferrol, COR1 from Ría de A Coruña, VIG1 from Ría de Vigo and AR1 from Ría de Ares-Betanzos. All samples corresponded to wild mussel except sample coming from AR1 that corresponded to mussel cultivated in raft. Table 5 in supplementary material summarises the concentrations found in the four samples. Mussels from COR1 and VIG1 were the most polluted, both sampling points are located in highly populated and industrial zones. In these areas there are also high-activity ports. The concentrations obtained for DnOP were below the limit of

Table 5 Levels of six phthalates expressed in ng g^{-1} dry weight coming from four Galician Rías (Ría de Ferrol (FER1), Ría de A Coruña (COR1), Ría de Vigo (VIG1) and Ría de Ares-Betanzos (AR1))

Compound	FER1	COR1	VIG1	AR1
DMP	2.33	2.30	2.46	2.82
DEP	31.47	28.52	76.04	60.02
DBP	17.10	166.96	25.20	16.05
DEHP	82.62	186.32	141.44	135.83
BzBP	3.45	2.86	3.87	2.11
DnOP	< LOQ	< LOQ	< LOQ	< LOQ

LOQ limit of quantification of the whole method

quantification. DMP and BzBP were found in similar concentrations and at very quite levels in all samples. DEP, DBP and DEHP, were the most abundant, but in levels lower than those reported by some authors in several fish species (Teil et al. 2014; Valton et al. 2014; Xu et al. 2018). The predominance of DBP and DEHP in wild marine organism, included blue mussel, and other foods was in agree with previous studies (Huang et al. 2008; Fierens et al. 2012; Hu et al. 2016). However, variability found in several studies indicates that, possibly like other anthropogenic pollutants, phthalates distribution in biota is source-specific.

Conclusions

In this paper, an efficient and low cost method based on micro-MSPD combined with GC–MS/MS was optimized and validated to determine six phthalates in mussel samples for the first time. Various parameters of sample preparation, including extraction and clean up (sample and dispersant agent amounts and solvent volume), were optimized by using a factorial design. Extraction and clean up steps were carried out simultaneously without the need for special equipment in a glass Pasteur pipette. This has simplified the method and minimised the use of plastic ware preventing or reducing cross-contamination by phthalates. The proposed procedure would be easy to implement in routine and monitoring laboratories. The method performance parameters have been found to be satisfactory, mean recovery values ranged from 93 to 114% and RSD was below 20%. Finally, the validated method was applied to four real mussel samples coming from Galician Rías. DEHP, DEP and DBP were found as major compounds. Since not all laboratories have the infrastructure to be able to determine microplastics in environmental samples, the determination of phthalates in the marine organisms can be a good indicator of the magnitude and effects of the presence of microplastic in the marine environment.

Abbreviations

DMP: Dimethyl phthalate; DEP: Diethyl phthalate; DBP: Dibutyl phthalate; BzBP: Benzyl butyl phthalate; DEHP: Diethyl hexyl phthalate; DnOP: Di-n-octyl phthalate; Micro-MSPD: Micro-Matrix solid-phase dispersion; DEHP-d4, 98: Deuterated bis(2-ethylhexyl)phthalate-2,4,5,6-d4; SPE: Solid-phase extraction; MAE: Microwave assisted extraction; ASE: Accelerated solvent extraction; USVADLLME: Ultrasound-vortex-assisted dispersant liquid–liquid micro-extraction SDEMSingle-drop-microextraction (SDME) in food (Battle and Nerin 2004), liquid–liquid; LLE: Liquid–liquid extraction; SBSE: Stir bar sorptive extraction; SPME: Solid-phase microextraction.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40543-021-00303-4.

Additional file 1. Table S1 Comparison between the methodology proposed in this study and the ones reported in literature (LODs and LOQs expressed as ng g^{-1}; Recovery expressed as %; -, not reported).

Acknowledgements

Not applicable.

Authors' contributions

This study was designed by NC and JC. The experimental work was performed by AM, MI and IG. NC drafted the manuscript and interpreted the data. All authors read and approved the final manuscript.

Funding

This research was funded the Ministry of Economy and Competitiveness, Spain as part of the project number CTM2017-84763-C3-2-R and FEDER/ERDF. This research has also been co-financed by the European Regional Development Fund through the Interreg V-A Spain-Portugal Programme (POCTEP) 2014-2020.

Availability of data and materials

Not applicable.

Declarations

Competing interests

The authors declare that they have no competing interest.

Received: 22 July 2021 Accepted: 19 October 2021
Published online: 28 October 2021

References

Barciela-Alonso MC, Otero-Lavandeira N, Bermejo-Barrera P. Solid phase extraction using molecular imprinted polymers for phthalate determination in water and wine samples by HPLC-ESI-MS. Microchem J. 2017;132:233–7. https://doi.org/10.1016/j.microc.2017.02.007.

Battle R, Nerin C. Application of single-drop microextraction to the determination of dialkyl phthalate esters in food stimulants. J Chromatogr A. 2004;1045(1–2):29–35. https://doi.org/10.1016/j.chroma.2004.06.001.

Blair JD, Ikonomou MG, Kelly BC, Surridge B, Gobas FA. 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. 2009;43:6262–8. https://doi.org/10.1021/es9013135.

Cañadas R, Garrido-Gamarro E, Garcinuño-Martinez RM, Paniagua Gonzalez G, Fernandez-Hernando P. Occurrence of common plastic additives and contaminants in mussel samples: Validation of analytical method based on matrix solid-phase dispersión. Food Chem. 2021;349:129169. https://doi.org/10.1016/j.foodchem.2021.129169.

Carro N, Vilas L, García I, Ignacio M, Mouteira A. Optimization of a method based on micro-matrix solid-phase dispersion (micro-MSPD) for the determination of PCBs in mussel samples. Anal Chem Res. 2017;11:1–8. https://doi.org/10.1016/j.ancr.2016.11.003.

Carro N, Lopez A, Cobas J, García I, Ignacio M, Mouteira A. Development and optimization of a method for organochlorine pesticides determination in mussels based on miniaturized matrix solid-phase dispersion combined with gas chromatography-tandem mass spectrometry. J Anal Chem. 2021;76(5):603–12. https://doi.org/10.1134/S1061934821050099.

Celeiro M, Guerra E, Lamas JP, Lores M, Garcia-Jares C, Llompart M. Development of a multianalyte method based on micro-matrix-solid-phase dispersion for the analysis of fragrance allergens and preservatives in personal care products. J Chromatogr A. 2014;1344:1–14. https://doi.org/10.1016/j.chroma.2014.03.070.

Cinelli G, Avino P, Notardonato I, Centola A, Russo MV. Rapid analysis of six phthalate esters in wine by ultrasound-vortex-assisted dispersant liquid-liquid micro-extraction coupled with gas chromatography-flame ionization detector or gas chromatography-ion trap mass spectrometry. Anal Chim Acta. 2013;769:72–8. https://doi.org/10.1016/j.aca.2013.01.031.

Commission Regulation (EU). No. 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food.

Commission Regulation (EU). 2018/2005 of 17 December 2018 amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards bis(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP) and diisobutyl phthalate (DIBP)

Cortazar E, Zuloaga O, Sanz J, Raposo JC, Etxebarria N, Fernandez LA. Multi-Simplex optimisation of the solid-phase microextraction–gas chromatographic–mass spectrometric determination of polycyclic aromatic hydrocarbons, polychlorinated biphenyls and phthalates from water samples. J Chromatogr A. 2002;978:165–75. https://doi.org/10.1016/S0021-9673(02)01409-7.

Das MT, Ghosh P, Thakur IS. Intake estimates of phthalate esters for South Delhi population based on exposure media assessment. Environ Pollut. 2014;189:118–25. https://doi.org/10.1016/j.envpol.2014.02.021.

Fan J, Wu L, Wang X, Huang X, Jin Q, Wang S. Determination of the migration of 20 phthalate esters in fatty food packaged with different materials by solid-phase extraction and UHPLC–MS/MS. Anal Methods. 2012;4:4168–75. https://doi.org/10.1039/C2AY25916H.

Fierens T, Servaes K, Van Holderbeke M, Geerts L, De Henauw S, Sioen I, Vanermen G. Analysis of phthalates in food products and packaging materials sold on the Belgian market. Food Chem Toxicol. 2012;50:2575–83.

Fred-Ahmadu OH, Ayejuyo OO, Benson NU. Dataset on microplastics and associated trace metals and phthalate esters in sandy beaches of tropical Atlantic ecosystems. Nigeria Data in Brief. 2020;31: 105755. https://doi.org/10.1016/j.dib.2020.105755.

Gao DW, Li Z, Wang H, Liang H. An overview of phthalate acid ester pollution in China over the last decade: environmental occurrence and human exposure. Sci Total Environ. 2018;645:1400–9. https://doi.org/10.1016/j.scitotenv.2018.07.093.

Giam CS, Chan HS, Neff GS, Atlas EL. Phthalate esters plasticizers: a new class of marine pollutant. Science. 1978;199:419–21. https://doi.org/10.1126/science.413194.

Grbin D, Sabolić I, Klobučar G, Dennis SR, Šrut M, Bakarić R, Baković V, Brkanac SR, Nosil P, Štambuk A. Biomarker response of Mediterranean mussels *Mytilus galloprovincialis* regarding environmental conditions, pollution impact and seasonal effects. Sci Total Environ. 2019;694: 133470. https://doi.org/10.1016/j.scitotenv.2019.07.276.

He M-J, Lu J-F, Wang J, Wei S-Q, Hageman KJ. Phthalate esters in biota, air and water in an agricultural area of western China, with emphasis on bioaccumulation and human exposure. Sci Total Environ. 2020;698: 134264. https://doi.org/10.1016/j.scitotenv.2019.134264.

Hidalgo-Serrano M, Borrull F, Marcé RM, Pocurull E. Simple method for determining phthalate diesters and their metabolites in seafood species using QuEChERS extraction and liquid chromatography-high resolution mass spectrometry. Food Chem. 2021;336:127–722.

Hu X, Gu Y, Huang W, Yin D. Phthalate monoesters as markers of phthalate contamination in wild marine organisms. Environ Pollut. 2016;218:410–8. https://doi.org/10.1016/j.envpol.2016.07.020.

Hu A, Qiu M, Liu H, Xu Y, Tao Y, Yang G, He Y, Xu J, Lu Z. Simultaneous determination of phthalate diesters and monoesters in soil using accelerated solvent extraction and ultra-performance liquid chromatography coupled with tandem mass spectrometry. J Chromatogr A. 2020a. https://doi.org/10.1016/j.chroma.2020.461347.

Hu H, Mao L, Fang S, Xie J, Zhao M, Jin H. Occurrence of phthalic acid esters in marine organisms from Hangzhou Bay, China: implications for human exposure. Sci Total Environ. 2020b;721: 137605. https://doi.org/10.1016/j.scitotenv.2020.137605.

Huang PC, Tien CJ, Sun YM, Hsieh CY, Lee CC. Occurrence of phthalates in sediment and biota: relationship to aquatic factors and the biota-sediment accumulation factor. Chemosphere. 2008;73:539–44. https://doi.org/10.1016/j.chemosphere.2008.06.019.

Ji Y, Wang F, Zhang L, Shan C, Bai Z, Sun Z, Liu L, Shen B. A comprehensive assessment of human exposure to phthalates from environmental media and food in Tianjin, China. J Hazard Mater. 2014;279:133–40. https://doi.org/10.1016/j.jhazmat.2014.06.055.

Liang P, Zhang L, Peng L, Li Q, Zhao E. Determination of phthalate esters in soil samples by microwave assisted extraction and high performance liquid chromatography. Bull Environ Contam Tox. 2010;85(2):147–51. https://doi.org/10.1007/s00128-010-0078-x.

Liu L, Bao H, Liu F, Zhang J, Shen H. Phthalates exposure of Chinese reproductive age couples and its effect on male semen quality, a primary study. Environ Int. 2012;42:78–83. https://doi.org/10.1016/j.envint.2011.04.005.

Liu Y, Li Z, Jalon-Rojas I, Wang XH, Fredj E, Zhang D, Feng L, Li X. Assessing the potential risk and relationship between microplastics and phthalates in surface seawater of a heavily human-impacted metropolitan bay in northern China. Ecotoxicol Environ Saf. 2020;204: 111067. https://doi.org/10.1016/j.ecoenv.2020.111067.

Llompart M, Celeiro M, Pablo Lamas J, Sanchez-Prado L, Lores M, Garcia-Jares C. Analysis of plasticizers and synthetic musks in cosmetic and personal care products by matrix solid-phase dispersion gas chromatography–mass spectrometry. J Chromatogr A. 2013;1293:10–9. https://doi.org/10.1016/j.chroma.2013.03.067.

Lo Brutto S, Iaciofano D, Lo Turco V, Potorti AG, Rando R, Arizza V, Di Stefano V. First assessment of plasticizers in marine coastal Litter-Feeder Fauna in the Mediterraneam Sea. Toxics. 2021;9:31. https://doi.org/10.3390/toxics9020031.

Magnusson B, Örnemark U, eds. Eurachem guide: the fitness for purpose of analytical methods—a laboratory guide to method validation and related topics, 2nd ed; 2014. ISBN 978-91-87461-59-0. www.eurachem.org.

Meeker JD, Sathyanarayana S, Swan SH. Phthalates and other additives in plastics: human exposure and associated health outcomes. Philos Trans R Soc Lond. 2009;364:2097–113. https://doi.org/10.1098/rstb.2008.0268.

Net S, Sempéré R, Delmont A, Paluselli A, Ouddane B. Occurrence, fate, behavior and ecotoxicological state of phthalates in different environmental matrices. Environ Sci Technol. 2015;49(7):4019–35. https://doi.org/10.1021/es505233b.

Oehlmann J, Schulte-Oehlmann U, Kloas W, Jagnytsch O, Lutz I, Kusk KO, Wollenberger L, Santos EM, Paull GC, Van Look KJ, Tyler CR. A critical analysis of the biological impacts of plasticizers on wildlife. Phil Trans R Soc B. 2009;364:2047–62. https://doi.org/10.1098/rstb.2008.0242.

Orssi DD, Gagliardi L, Porrà R, Berri S, Climenti P, Granesse A, Carpani I, Tonelli D. A environmentally friendly reversed-phase liquid chromatography method for phthalates determination in nail cosmetics. Anal Chim Acta. 2006;555:238–41. https://doi.org/10.1016/j.aca.2005.09.029.

Paluselli A, Fauvelle V, Schmidt N, Galgani F, Net S, Sempéré R. Distribution of phthalates in Marseille Bay (NW Mediterranean Sea). Sci Total Environ. 2018;621:578–87. https://doi.org/10.1016/j.scitotenv.2017.11.306.

Panio A, Corsarini SF, Bruno A, Lasagni M, Labra M, Saliu F. Determination of phthalates in fish fillets by liquid chromatography tandem mass spectrometry (LC-MS/MS): a comparison of direct immersion solid phase microextraction (SPME) versus ultrasonic assisted solvent extraction (UASE). Chemosphere. 2020;255: 127034. https://doi.org/10.1016/j.chemosphere.2020.127034.

Poças MFF, Oliveira JC, Pereira J, Hogg T. Consumer exposure to phthalates from paper packaging: an integrated approach. Food Addit Contam. 2010;27:1451–9. https://doi.org/10.1080/19440049.2010.490790.

Russo MV, Avino P, Notardonato I. Fast analysis of phthalates in freeze-dried baby foods by ultrasound-vortex-assisted liquid-liquid microextraction

coupled with GC-IT/MS. J Chromatogr A. 2016;1474:1–7. https://doi.org/10.1016/j.chroma.2016.10.058.
Saliu F, Montano S, Lasagni M, Galli P. Biocompatible solid-phase microextraction coupled to liquid chromatography triple quadrupole mass spectrometry analysis for the determination of phthalates in marine invertebrate. J Chromatogr A. 2020;1618:460852. https://doi.org/10.1016/j.chroma.2020.460852.
Schecter A, Lorber M, Guo Y, Wu Q, Yun SH, Kannan K, Birnbaum LS. Phthalate concentrations and dietary exposure from food purchased in New York State. Environ Health Perspect. 2013;121:473–94. https://doi.org/10.1289/ehp.1206367.
Sun M, Tang RX, Wu QH, Wang C, Wang Z. Graphene reinforced hollow fiber liquid-phase microextraction for the determination of phthalates in water, juice and milk samples by HPLC. Anal Methods. 2013;5:5694–700. https://doi.org/10.1039/c3ay40966j.
Sun C, Chen L, Zhao S, Guo W, Luo Y, Wang L, Tang L, Li F, Zhang J. Seasonal distribution and ecological risk of phthalate esters in surface water and marine organisms of the Bohai Sea. Mar Pollut Bull. 2021;169: 112449. https://doi.org/10.1016/j.marpolbul.2021.112449.
Tan BLL, Hawker DW, Müller JF, Tremblay LA, Chapman HF. Stir bar sorptive extraction and trace analysis of selected endocrine disruptors in water, biosolids and sludge samples by thermal desorption with gas chromatography-mass spectrometry. Water Res. 2008;42(1–2):404–12. https://doi.org/10.1016/j.watres.2007.07.032.
Teil MJ, Tlili K, Blanchard M, Labadie P, Alliot F, Chevreuil M. Occurrence of polybrominated diphenyl ethers, polychlorinated biphenyls, and phthalates in freshwater fish from the Orge river (Ile-de France). Arch Environ Contam Toxicol. 2014;66:41–57. https://doi.org/10.1007/s00244-013-9955-8.
United States Environmental Protection Agency (U.S. EPA). Phthalates Action Plan. Rev. 2012;1.
Valton AS, Serre-Dargnat C, Blanchard M, Alliot F, Chevreuil M, Teil MJ. Determination of phthalates and their by-products in tissues of roach (Rutilusrutilus) from the Orge river (France). Environ Sci Pollut Res. 2014;21:12723–30. https://doi.org/10.1007/s11356-014-3213-0.
Van Holderbeke M, Geerts L, Vanermen G, Servaes K, Sioenb I, Henauw SD, Fierens T. Determination of contamination pathways of phthalates in food products sold on the Belgian market. Environ Res. 2014;134:345–52. https://doi.org/10.1016/j.envres.2014.08.012.
Vered G, Kaplan A, Avisar D, Shenkar N. Using solitary ascidians to assess microplastic and phthalate plasticizers pollution among marine biota: a case study of the Eastern Mediterranean and Red Sea. Mar Pollut Bull. 2019;138:618–25. https://doi.org/10.1016/j.marpolbul.2018.12.013.
Wang J, Huang S, Wangm P, Yang Y. Method development for the analysis of phthalate esters in tea beverages by ionic liquid hollow fibre liquid-phase microextraction and liquid chromatographic detection. Food Control. 2016;67:278–84. https://doi.org/10.1016/j.foodcont.2016.03.015.
Xu Y, Weng R, Lu Y, Wang X, Zhang D, Li Y, Qiu J, Qian Y. Evaluation of phthalic acid esters in fish samples using gas chromatography tandem mass spectrometry with simplied QuEChERs technique. Food Anal Methods. 2018;11(12):3293–303. https://doi.org/10.1007/s12161-018-1313-z.
Zheng X, Zhang B-T, Teng Y. Distribution of phthalate acid esters in lakes of Beijing and its relationship with anthropogenic activities. Sci Total Environ. 2014;476–477:107–13. https://doi.org/10.1016/j.scitotenv.2013.12.111.
Zhong Y-Y, Yin D-Q, Hu X-L. 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 sea food. J Chromatogr B. 2014;965:164–72. https://doi.org/10.1016/j.jchromb.2014.06.024.

Publisher's Note