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Phthalates and bisphenol-A residues in water samples: an innovative analytical approach

Ivan Notardonato[1] · Mario Vincenzo Russo[1] · Pasquale Avino[1]

Abstract

Phthalates (PAEs), a family of organic chemicals synthesized by double esterification of 1,2-benzenecarboxylic acid, and Bisphenol-A (BPA) are molecules widely used in packaging due to their malleability and workability of plastic polymers. Their large use during these last decades causes high presence in the environment. PAEs, which are not chemically bound to the polymer, show the tendency to migrate or dissociate, especially when they are in contact with lipophilic substances and/or in case of mechanical or thermal stress. The consequence is a contamination of water surface as well as food products, e.g., food products can be contaminated during packaging and storage by migration from polymers. PAE exposure can result from direct contact or through the transfer of such compounds from one product to another, as occurs for food or bottle packaging, whereas the main exposure routes can be ingestion, inhalation, intravenous and dermal. The food contamination by PAEs and/or BPA can also occur during the production process, handling, transport, packaging and preparation, even at domestic level. This paper would like to propose a novel, rapid and easy analytical approach for determining PAEs and BPA in water samples, specifically water surface. The determination is performed by GC-FID, common equipment present in every routinely chemical laboratory. The extraction is performed by means of Dispersed Liquid Liquid MicroExtraction (DLLME) method. All the analytical parameters are investigated and discussed. The method is really sensitive showing Limit of Detection (LOD) between 2 and 19 pg and Limit of Quantification (LOQ) between 4 and 48 pg. Furthermore, the method is reproducible with a variability of less than 9.7% and an $R^2 > 0.9718$ in a linear dynamic range between 0.5 and 500 ppm.

Keywords Phthalates · Bisphenol-A · Water · Dispersed liquid liquid microextraction · GC-FID

1 Introduction

Phthalates (PAEs) (Fig. 1a) are a family of organic chemicals synthesized by double esterification of 1,2-benzenecarboxylic acid with predominantly apolar, linear or branched

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substituents. Worldwide, up to 8 million tons of phthalic esters are produced every year, among them over 2 million are di-Ethyl-Esyl-Phthalate (DEHP), one of the most used compounds in the category. Very often this class of compounds is used in association with Bisphenol-A (BP-A) (Fig. 1b), which at room temperature is flakes or powder.

Both molecules are widely used in packaging because they increase the malleability of plastic polymers, but they are also used in the cosmetics industry and in the hospital disposal. Due to their properties, e.g., not being chemically bound to polymer and showing low polarity, PAEs and BP-A exhibit the tendency to migrate or dissociate from plastics, especially when they are in contact with lipophilic substances and/or in case of mechanical or thermal stress (Barlow 2009; Simoneau et al. 2012; Guart et al. 2014; Mertens et al. 2016).

Considering the increasing use of plastic materials used for transport, production and storage of food and their use in the human health, the PAE and BP-A amount released
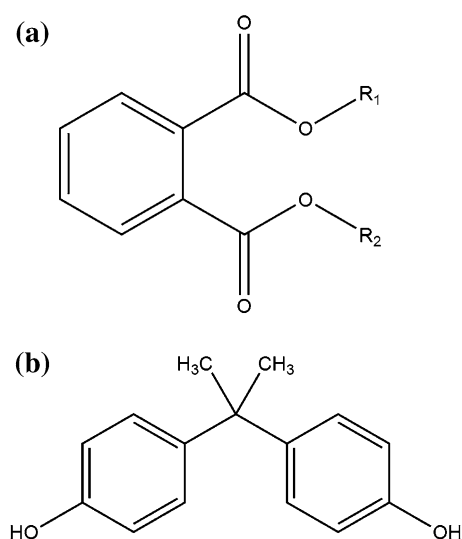
Fig. 1 Phthalic esters (PAEs) (**a**) and BisPhenol (BP-A) (**b**)

into the environment have exponentially grown and consequently also the human exposure. Recently, some International organizations, such as the World Health Organization (WHO) or the Environmental Protection Agency (EPA), studied the effects that these molecules could cause to the whole ecosystem, in particular to the human health: PAEs and BP-A have been related as molecules showing acute toxicity towards vertebrates and so there is the obligation to monitor them carefully (Wittassek et al. 2011; Braun et al. 2013; Rochester 2013; Serrano et al. 2014; Kasper-Sonnenberg et al. 2017).

Exposure to these molecules can result through four major routes, i.e., ingestion, inhalation, intravenous and dermal. The first is the main route of exposure; it concerns above all the plasticizing PAEs (Schettler 2006). The greatest source of exposure for ingestion is represented by contaminated food during production, process, or in packaging and storage (Latini 2005). Another important contamination source is the pharmaceutical preparation. Such formulations are often coated with polymer added with di-Ethyl-Phthalate (DEP) or di-Butyl-Phthalate (DBP): these influence the drug releasing timing and the location in the gastrointestinal tract, ensuring the resistance of the active principle to the acids present in the

stomach. Further, baby toys made by polymers softened with PAEs are a potential source of oral exposure in children. The inhalation exposure according to this path is more about DEP (Latini 2005) but also di-ethyl-hexyl-phthalate (DEHP) can be transferred in spite of the low volatility. The intravenous exposure originates from plasticized medical devices with DEHP, such as bags and/or pipes that convey intravenous fluids, nutritional formulas, blood. Finally, the dermal exposure directly depends on the contact with PAEs, especially DEP, through clothing, raincoats, boots, footwear, gloves, cosmetics, sunscreens, insecticides, waxes, hygiene products, paints, handling of toys from part of the children.

Table 1 shows the contributions of each PAE according to the exposure routes in relation to the human age. It is interesting to note how the exposure risk decreases with the individual age (Shea 2003; Hauser and Calafat 2005).

Recently, different researchers have begun to study the PAE and BP-A presence in marine waters. In fact, being considered ubiquitous pollutants, they can often be found in industrial waste and can, therefore, be found in sea water. The problem is really important: in fact, low estimates predict that plastics floating across the ocean weigh between 70,000 and 270,000 tons (Cózar et al. 2014; Eriksen et al. 2014; Van Sebille et al. 2015; Pietrelli et al. 2017; Sighicelli et al. 2018). Particularly, microplastics, i.e., plastics particles smaller than 5 mm (Arthur et al. 2009), are composed by polymerizing monomers and other substances (Lithner et al. 2011) in addition to plastic additives (Hermabessiere et al. 2017) like PAEs, BPA, nonylphenols, and Brominated Flame Retardants (BFR) (de Boer et al. 1998; Mackintosh et al. 2004; Xie et al. 2005, 2007; de los Ríos et al. 2012; Bergé et al. 2013; Net et al. 2015). These additives are released into the marine environment by numerous pathways (e.g., industrial and municipal wastewater, atmospheric deposition, sewage sludge in agriculture) as well as the plastic accumulation and degradation are other reasons of ingoing of such chemicals in oceans. For that, it is necessary to study plastic additives associated with microplastics, to find out analytical procedures for determining such compounds in sea water.

Even if the PAE/BP-A determination is well studied, their determination in marine waters is really difficult and

Table 1 Human exposure to the main PAEs (expressed as µg kg^{-1} of body weight per day) in relationship to the different ages

	PAE exposure (µg kg^{-1} b.w.day^{-1})					
	DEHP	DBP	DiNP	DiDP	DEP	Total
Newborn (6–12 month)	285.0	208.0	218.0	211.0	4.2	926.2
Children (1–6 years)	151.0	400.0	65.0	55.0	6.0	677.1
Children (7–14 years)	48.7	200.0	10.8	7.6	2.5	296.6
Adult	26.3	60.2	5.7	3.5	1.0	96.7

important as well: in the literature, there are few articles based on extraction by means of stir bar sorptive extraction or pre-concentration by Solid Phase Extraction (SPE) (Qingqing et al. 2016; Ze-Ming et al. 2017).

To examine the compliance with current regulations and to establish techniques for industrial quality control, analytical methods have been developed to quantify the presence of plasticizing additives in PVC in food packaging and food as a result of the migration process (Coltro et al. 2014; Lambertini et al. 2016; Vaclavikova et al. 2016; Mercogliano and Santonicola 2018).

For this aim, our research group, which deals the development of analytical methods for determining micro-pollutants present in different environmental and food matrices (Cinelli et al. 2014; Notardonato et al. 2014, 2018a, b; Russo et al. 2011, 2014, 2015, 2016a, 2017), would like to propose an innovative approach for PAE and BP-A determination in marine environment.

2 Materials and methods

2.1 Materials and chemicals

PAE standards investigated in this study, such as di-methyl phthalate (DMP; $C_{10}H_{10}O_4$; MW 194), di-ethyl-phthalate (DEP; $C_{12}H_{14}O_4$; MW 222), di-Butyl-Phthalate (DBP; $C_{16}H_{22}O_4$; MW 278), iso-butyl-cyclohexyl-phthalate (iBcEP; $C_{18}H_{24}O_4$; MW 304), benzyl-butyl-phthalate (BBP; $C_{19}H_{20}O_4$; MW 312), di-ethyl-hexyl-phthalate (DEHP; $C_{24}H_{38}O_4$; MW 390) and bisphenol-A (BP-A; $C_{15}H_{16}O_2$; MW 228), were obtained from Sigma-Aldrich (Milan, Italy). A mixture containing all the standards was prepared at 200 ppm and after diluted till to 50 ppb. The solutions are stored in 2 mL amber vials at -20 °C. n-Hexane, n-heptane, iso-octane and benzene were of pesticide grade (Carlo Erba, Milan, Italy), whereas sodium chloride (Carlo Erba) was of analytical reagent grade.

Anthracene (1 mg mL^{-1}) ($C_{14}H_{10}$; MW 178), purchased from LabService Analytica (Anzola Emilia, Bologna, Italy), was used as Internal Standard (I.S.): 5 µL was added to each sample before performing the analytical process.

For avoiding the cross-contamination due to reagents (expecially for minimizing the background contamination due to NaCl), materials and laboratory (e.g., glassware, tubing) equipment, which is still fundamental issue in PAE analysis, all the chemicals and instruments were undergone to severe cleaning procedure. Details are reported in previous papers (Cinelli et al. 2013; Russo et al. 2015). Briefly, the glassware was soaked and washed in acetone, dried at 140 °C for at least 4 h; NaCl was heated for 4 h at 140 °C and, after cooling, kept in a tightly sealed glass vial. For PAE standard solutions (0.1 mg mL^{-1} of each PAE), absolute

ethanol was used: each solution was further diluted by ethanol to obtain solutions at different PAE concentrations for spiking the samples.

2.2 Development of the extraction process using the DLLME methodology

The extraction method is based on the use of Dispersive Liquid–Liquid Microextraction (DLLME): it can be divided into three steps. The first phase allows determining the best extraction solvent and the relative ratio between the aqueous phase and solvent. Four solvents tested show a lower density than the water in order to carry out an extraction: n-hexane (0.66 g cm^{-3}), n-heptane (0.68 g cm^{-3}), iso-octane (0.69 g cm^{-3}) and benzene (0.88 g cm^{-3}). The tests were performed on blank water samples (ultra-pure water samples): 200 µL of extraction solvent was added without the use of the dispersive solvent. After, it has been investigated the best way to obtain an effective emulsification. Among the different methods used, the more stable and homogeneous emulsion has been obtained using ultrasounds: the solution, kept for 6 min in the ultrasound bath, allows the formation of a perfectly stable and homogeneous emulsion. This allows affirming that the DLLME extraction methodology can be performed with the only presence of the extraction solvent. A second phase, a very sensitive step of the procedure, regarded how to break the emulsion, trying simultaneously to keep short the mixing times and obtain quantitative and reproducible recovery rates. To break the emulsion, various tests have been carried out by adding NaCl at different concentrations. The third phase of the method developed has regarded the parameter evaluation of the PAEs and BP-A extraction. Starting from 1000 mL of water sample, different experiments have been performed by adding 100 µL of PAE and BPA mix solution (50 pg µL^{-1} of each analyte) and 50 µL of I.S. (1 µg µL^{-1}); different volumes of n-hexane, iso-octane and toluene were used for evaluating the best extraction solvents. The sample is subjected to ultrasounds for 6 min and, after NaCl addition, the solution is kept under agitation for 10 min in order to break the emulsion and allow the separation of the two phases. In this way, the drop is obtained: 1 µL withdrawn by syringe was injected in the GC-FID instrument.

2.3 GC-FID apparatus

The GC-FID system used for analysis was a MasterGC gas chromatograph coupled with a Flame Ionization Detector (FID) (Dany, Milano, Italy). Data acquisition and analysis were performed using standard software supplied by the manufacturer (CSW 32). A homemade-fused silica capillary column with chemically bonded phase SE-54 (5% phenyl-95% dimethylpolisiloxane) from Teknokroma model

TRB-Meta X5 (30 m × 0.25 mm × 0.25 μm) was used (Russo et al. 2012, 2016b; Notardonato et al. 2013; Avino et al. 2017). Hydrogen was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. Hydrogen is generated with hydrogen generator from Xi'an Heb Biotechnology model QL-500. 1 μL sample was injected into the Programmable Temperature Vaporization (PTV) injector in the splitless mode. 10 s after the injection, the vaporizer was heated from 110 to 290 °C at 14.5 °C min^{-1}; the splitter valve was opened after 120 s. The temperature of detector was 290 °C and the air/hydrogen flow ratio was 10:1 (300/30 mL min^{-1}). Furthermore, nitrogen is added to the detector as an auxiliary gas at 10 mL min^{-1}. The oven temperature program was as follows: 100 °C, held for 30 s, 10 °C min^{-1} up to 290 °C and held for 2 min. The quantitative analysis was performed by calibration graphs of the ratio area$_{(analytes)}$/area$_{(IS,anthracene)}$ plotted vs. each concentration (pg μL^{-1}). All the samples were determined in triplicate.

3 Results and discussion

In the studies aimed to the pollutant determination at (ultra-) trace levels, one of the most significant analytical problems regards the Limit of Detection (LOD) of the instrument, especially when clean-up and extraction procedures do not give sufficiently high pre-concentration factors (Avino and Russo 2018). This paper is oriented on a fundamental goal, i.e., the development of a easy method for simultaneous PAE and BP-A determination with high pre-concentration factors, so that the analytical measurements can be carried out even with insensitive and inexpensive instruments present in most of the routinely laboratories.

The method involves five steps: (1) preparation of the aqueous solution in a special 1 L flask (pH 5); (2) addition of 200 μL of heptane, extraction solvent; (3) dispersion formation with the aid of a magnetic plate (5 min) and an ultrasonic bath (6 min); (4) addition of 15 g of NaCl to promote the breakdown of the emulsion; (5) solvent recovery and injection of 1 μL to GC-FID. The method is summarized in the master scheme reported in Fig. 2.

In the development of the method, all experimental parameters are verified.

3.1 Evaluation of the extraction process

Percentage recoveries were studied by varying the pH of the solution. Recoveries were carried out both in the acidic environment and in the basic environment. Specifically, recoveries were made by buffering the solution at pH values between 4 and 9, in increments of a pH unit. Table 2 shows the recoveries obtained. The recovery percentages obtained with the respective relative errors are reported.
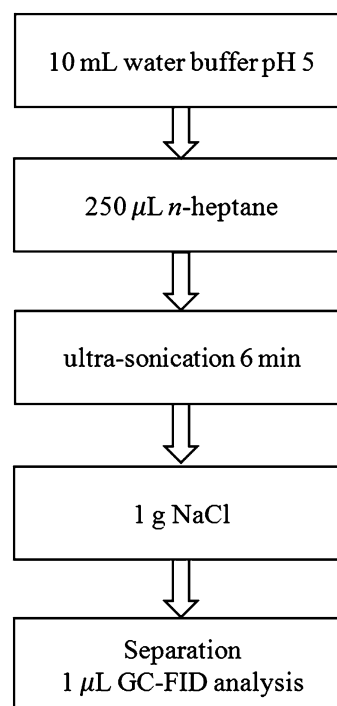

Fig. 2 Block diagram of the DLLME procedure

Table 2 Percentage recoveries obtained when pH changes

PAE	Recovery (%)			
	pH 4	pH 5	pH 6	pH 7
DMP	96.9 (5.2)	99.6 (3.4)	85.8 (4.6)	79.2 (6.8)
DEP	94.5 (8.3)	97.4 (4.6)	86.1 (6.3)	71.3 (6.0)
DBP	96.2 (9.1)	98.3 (2.1)	84.3 (5.1)	74.5 (7.3)
iBcEP	95.3 (7.8)	99.2 (3.0)	81.6 (3.9)	68.3 (5.7)
BP-A	97.5 (6.9)	97.9 (1.9)	72.5 (3.7)	66.2 (4.7)
BBP	97.9 (8.1)	98.4 (2.5)	84.5 (4.4)	70.4 (6.2)
DEHP	97.3 (5.6)	96.2 (3.3)	79.3 (5.1)	59.2 (5.9)

In brackets are reported the RSD

All the tests were carried out working at pH 5; at this pH value, the recovery is quantitative. The recoveries obtained at alkaline pH are not reported; at these pH values, the recoveries are no longer controlled. Probably, the alkaline pH value leads to an ionization of the internal standard and consequently the area$_{analyte}$/area$_{IS}$ ratio is completely altered.

In the SB-DLLME technique, the extraction solvent is dispersed into the solution. The function of the extraction solvent is to extract the analytes. The extraction solvent disperses in the form of microdroplets and makes the opalescent solution. The larger the droplets of the dispersion the greater the opalescence, but the smaller the contact surface between the dispersing solvent and the analytes. Moreover, the dispersion is quite stable and does not undergo alterations in a

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short time. Both the type and quantity of solvent to be added are tested. All solvents were selected with lower water density, so that they can be recovered with a Hamilton syringe directly on the surface of the solution. Specifically, *n*-hexane, *n*-heptane, *iso*-octane and benzene were tested. For all solvents it has been observed that by using volumes less than 150 μL it is impossible to recover the extraction solvent, since the solvent layer becomes very thin. On the contrary, volumes above 250 μL form a rather large drop, which then moves away from a microdrop extraction. Operating with a volume of between 150 and 250 μL, the solvent volume is between 120 and 210 μL. The recoveries obtained using the four solvents are summarized in Table 3 along with the Relative Standard Deviation (RSD): the best recoveries are obtained by means of 200 μL of *n*-hexane.

Both the mixing times on the magnetic stirring plate and the residence time inside the ultrasonic bath have been studied and optimized. Specifically, the mixing time on the agitator plate was studied between 5 and 25 min, at 5-min intervals. While the residence time inside the ultrasound bath was studied in a time interval between 6 and 30 min, at 6-min intervals. By varying the two parameters individually or simultaneously, no significant variations were found on the percentage recovery of the analytes; consequently, it was decided to operate in conditions sufficient to form a good dispersion and obtain a quantitative recovery of the analytes.

It is necessary to add a salt to break the dispersion. The salt increases the polarity of the solution, favoring the separation between the polar phase and the apolar phase.

Unfortunately, using 1 L of solution, it is impossible to use a centrifuge to separate the two phases. Therefore, it was decided to separate the two phases by modifying the ionic strength of the solution and using a stirring magnetic plate. The ionic strength is modified with the addition of a strongly dissociated salt. The addition of salt reduces the time required for separation. Several salts were tested at different concentrations, but in the end sodium chloride (NaCl) was used. Table 4 shows the recovery percentages according to the amount of salt added. The best results are obtained by adding a quantity equal to 15 g L^{-1}. By adding to the solution minor amounts of salt, the dispersion is not broken sufficiently, while adding a larger amount of salt the percentage recoveries remain close to the values obtained

Table 4 Percentage recoveries obtained in relation to the amount of NaCl added to break the dispersion. In brackets are reported the RSD

PAE	Recovery (%)			
	5 g L^{-1}	10 g L^{-1}	15 g L^{-1}	20 g L^{-1}
DMP	87.4 (2.3)	91.6 (2.4)	98.3 (1.1)	97.5 (2.5)
DEP	85.5 (4.2)	93.4 (3.9)	99.6 (3.2)	97.4 (2.9)
DBP	82.2 (3.5)	93.8 (3.2)	96.2 (2.7)	99.5 (3.2)
iBcEP	86.7 (2.9)	94.2 (4.0)	101.6 (4.2)	98.8 (5.2)
BP-A	81.4 (2.5)	90.5 (3.2)	97.0 (1.8)	102.4 (3.4)
BBP	84.0 (3.8)	94.1 (2.9)	101.7 (4.3)	101.3 (4.8)
DEHP	89.7 (3.1)	93.0 (2.7)	100.4 (2.6)	97.6 (4.8)

Table 3 Percentage recoveries obtained in relation to the volume and type of solvent used

PAE	*N*-Hexane			*N*-Heptane		
	150 μL	200 μL	250 μL	150 μL	200 μL	250 μL
DMP	74.3 (8.4)	98.7 (3.9)	89.0 (8.2)	65.6 (7.3)	73.5 (5.9)	59.6 (6.4)
DEP	69.2 (5.7)	92.4 (5.2)	85.4 (5.9)	62.0 (8.1)	69.5 (3.2)	64.1 (4.7)
DBP	58.6 (4.4)	96.8 (3.4)	89.3 (6.4)	58.3 (6.2)	65.7 (2.0)	58.0 (7.1)
iBcEP	78.4 (7.1)	99.5 (2.6)	82.6 (4.1)	66.3 (5.9)	83.6 (3.1)	79.2 (3.5)
BP-A	81.2 (4.8)	101.2 (3.1)	88.1 (2.5)	70.0 (3.5)	76.3 (5.1)	75.6 (4.7)
BBP	67.3 (6.4)	93.6 (2.5)	76.9 (7.0)	64.8 (9.1)	81.4 (4.7)	79.2 (8.1)
DEHP	71.9 (5.2)	97.1 (1.8)	83.7 (3.8)	55.6 (7.2)	68.9 (4.3)	60.1 (2.6)
PAE	*iso*-Octane			Benzene		
	150 μL	200 μL	250 μL	150 μL	200 μL	250 μL
DMP	54.2 (5.4)	62.6 (3.2)	60.1 (4.1)	58.3 (6.2)	62.5 (3.3)	67.7 (4.1)
DEP	48.2 (5.9)	57.2 (3.3)	59.1 (3.9)	48.5 (4.8)	71.2 (4.8)	58.5 (6.2)
DBP	46.5 (3.9)	49.2 (4.7)	50.3 (1.8)	56.3 (4.9)	51.7 (3.2)	51.5 (3.1)
iBcEP	62.5 (2.4)	55.6 (2.6)	57.9 (2.1)	58.1 (7.2)	51.7 (8.3)	61.8 (6.2)
BP-A	39.5 (6.2)	51.2 (4.8)	49.2 (3.7)	60.6 (5.2)	45.5 (9.1)	41.6 (7.4)
BBP	66.1 (5.4)	76.0 (5.2)	72.3 (2.4)	65.9 (8.1)	62.4 (6.2)	63.5 (5.4)
DEHP	58.2 (3.6)	65.4 (3.9)	61.6 (2.9)	40.9 (3.2)	49.6 (3.8)	67.7 (2.3)

In brackets are reported the RSD

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by adding 15 g L^{-1}; therefore, it is improper to use larger amounts of salt.

Once the two phases are separated, the recovery of the extraction solvent is done using appropriate glassware. The fitting, built and calibrated in our laboratory, allows to collect the apolar solvent in a glass tube with a very thin diameter. It is often made to go up into the tube by adding deionized water to the flask. The whole system is sealed and the junction between the flask and the fitting is made with ground glassware. The extraction solvent is taken directly from here and injected into the GC-FID.

The matrix effect was evaluated by comparing applying the developed method to a sample of tap water, both added with the same PAE and BP-A amount. The recoveries are analytically comparable, so the matrix effect can be considered negligible. Table 5 compares the obtained recoveries.

It is possible to note that recoveries between the real and standard matrix are comparable, respectively, between 96.9% and 99.0% and 97.2% and 101.4%. The relative standard deviation is less than 9.1 and 2.7, respectively. The matrix effect could be considered negligible.

3.2 Method development

The protocol developed involves the use of 1 L of water sample and 200 μL of heptane, extraction solvent. In this way,

it is possible to obtain a pre-concentration factor of about 5000. The extraction of the analytes from the aqueous solution is based on the Dispersive Liquid–Liquid Microextraction technique (DLLME). However, the developed protocol brings significant improvements to the traditional extraction method. In fact, the classic protocol of the DLLME provides for the use of a dispersive solvent, which facilitates the dispersion of the extraction solvent in the aqueous solution. In the developed method, the dispersive solvent is not used; it is replaced by means of an ultrasonic bath able to favor mechanical energy favoring the formation of the emulsion.

The linear dynamic range (LDR), the calibration curve (equation and R^2), and the sensitivity, expressed as limit of detection (LOD) and limit of quantification (LOQ), of each PAE are reported in Table 6 whereas the reproducibility, studied by adding different quantities to a sample of water, and the precision, as intraday and interday errors, are shown in Table 7.

It is possible to notice that the recoveries obtained by adding the real sample with two different concentrations of analytes are comparable with each other. In addition, intraday errors are between 3.1 and 6.6%, while interday errors are less than 9.7%.

Figure 3 shows the gas chromatograms of a standard of laboratory, prepared from the mix solution at a concentration of 20 ppm.

Table 5 Comparison of percentages obtained by applying the method to a solution of distilled water and to a tap water

PAE	Blank solution		Real sample	
	%	RSD	%	RSD
DMP	98.4	1.9	96.9	6.5
DEP	99.2	1.4	98.1	7.4
DBP	97.3	2.7	97.5	6.1
iBcEP	98.2	2.2	96.9	5.1
BP-A	101.4	1.8	98.1	4.3
BBP	97.2	2.7	99.0	9.1
DEHP	98.3	1.4	96.5	4.9

RSD relative standard deviation

Table 7 Recovery (%) at different PAE spiking and method precision investigated as intraday and interday measurements (%)

PAEs	Recovery		Intraday (RSD, %)	Interday (RSD, %)
	80 pg μL^{-1}	800 pg μL^{-1}		
DMP	95.7	97.6	4.9	9.7
DEP	98.2	99.1	3.5	8.6
DBP	96.1	102.5	6.4	9.3
iBcEP	97.1	96.5	5.5	8.1
BP-A	96.3	97.2	4.4	6.9
BBP	97.1	96.8	3.6	4.8
DEHP	99.4	98.1	3.1	6.7

Table 6 Linear dynamic range (LDR), calibration curve (equation and R^2) and sensitivity, expressed as limit of detection (LOD) and limit of quantification (LOQ), of each PAE investigated in this study

PAE	LDR (μg mL^{-1})	Calibration curves	R^2	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
DMP	0.05–150	$y = 0.0286x + 0.5158$	0.9944	3	9
DEP	0.05–150	$y = 0.0081x + 0.1298$	0.9974	6	14
DBP	0.05–150	$y = 0.0098x + 0.2266$	0.9936	3	6
iBcEP	0.05–150	$y = 0.0049x + 0.1006$	0.9816	19	48
BP-A	0.05–150	$y = 0.0102x + 0.2948$	0.9801	8	13
BBP	0.05–150	$y = 0.0092x + 0.2582$	0.9718	6	12
DEHP	0.05–150	$y = 0.0144x + 0.2529$	0.9792	2	4

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Fig. 3 Gas chromatograms of a standard of laboratory (20 ppm). Peaks: (1) DMP; (2) DEP; (IS) Anthracene; (3) DBP; (4) iBcEP; (5) BP-A; (6) BBP; (7) DEHP. For experimental conditions: see text
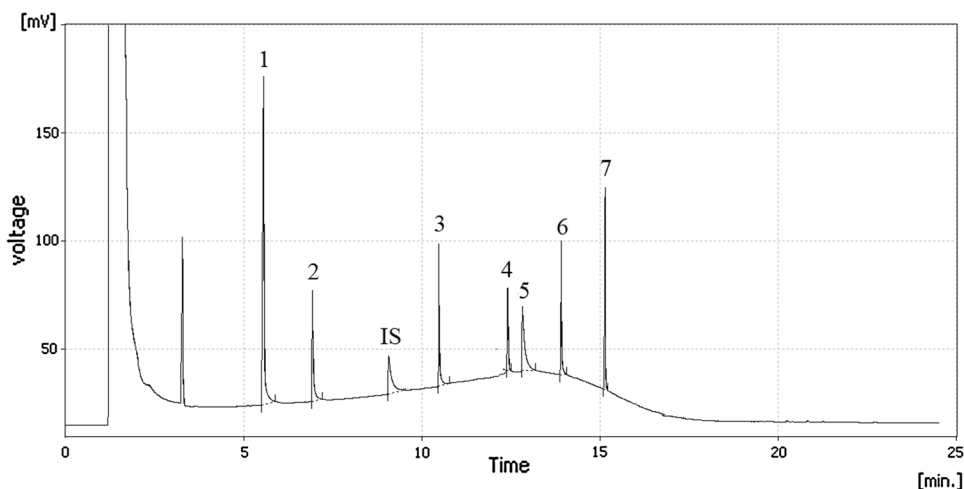

Figure 4 shows the gas chromatograms of a real water sample (Fig. 4a) and the same sample spiked with the mix solution of PAEs and BPA (20 ppm each one) (Fig. 4b). As it can be seen in Fig. 4b, the peaks are well solved and the chromatograms are clear meaning that the extraction procedure is effective.

3.3 Real sample application

Finally, four different samples of surface water are analyzed, particularly drinking water, irrigation water and two types of bottled water (Table 8).

The results show the presence of one phthalate, DEHP, the most widely used phthalate. However, whereas the LD_{50} of DEHP is equal to 0.05 mg kg^{-1}, the values determined should not cause concern, as the highest value is 25 ng mL^{-1}.

4 Conclusion

The method developed is easy to use and to apply and does not require the use of sophisticated equipment, the volume of water is extracted without performing any clean-up phase.

Also, the volume of solvent used is very small, so the problem of solvent consumption and its disposal are reduced. Finally, the use of non-toxic solvents protects the operator and allows the determination in any environment. There are many advantages of the proposed method, first of all the possibility of carrying out the analysis directly on the sample as such, without performing clean-up operations. In addition, the pre-concentration factor is very high, in fact we speak about 5000 times the initial concentration. Under this condition, four samples were tested. The values found in the tests show the presence only of the DHEP analyzed in the surface waters taken into consideration. Considering that the LD_{50} of DEHP is equal to 0.05 mg kg^{-1}, the determined DEHP values are not of concern, since the highest value is 25 ppb (drinking water).

The future studies will be oriented to the application of this procedure to determining PAEs and BP-A in sea water samples. The procedure will be the same: the focus will regard the ionic strength of the solution considering the NaCl presence in the real samples. The goal is to obtain a unique procedure for approaching whatever water samples.

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Fig. 4 Gas chromatograms of **a** a sample of drinking water without addition and **b** a sample of drinking water added with 20 ppm of phthalates and bisphenol-A. Peaks: (1) DMP; (2) DEP; (IS) Anthracene; (3) DBP; (4) iBcEP; (5) BP-A; (6) BBP; (7) DEHP. For experimental conditions: see text
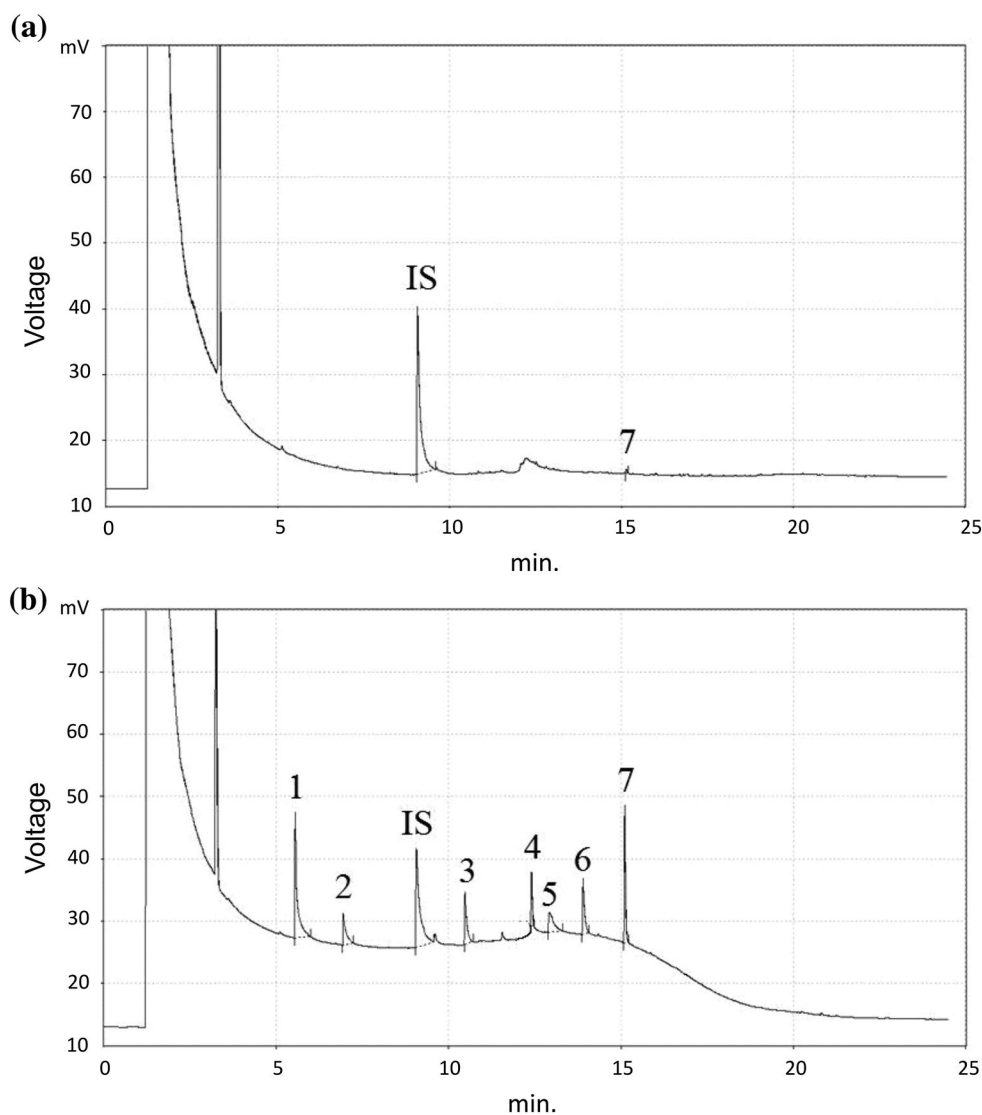

Table 8 PAE levels (ng mL^{-1}) determined in surface waters

PAE	Drinking water	Irrigation water	Bottled water #1	Bottled water #2
DMP	<LOD	<LOD	<LOD	<LOD
DEP	<LOD	<LOD	<LOD	<LOD
DBP	<LOD	<LOD	<LOD	<LOD
iBcEP	<LOD	<LOD	<LOD	<LOD
BP-A	<LOD	<LOD	<LOD	<LOD
BBP	<LOD	<LOD	<LOD	<LOD
DEHP	25.0	23.4	24.4	22.9

References

Arthur C, Baker J, Bamford H (2009) International research workshop on the occurrence, effects, and fate of microplastic marine debris. NOAA Technical Memorandum NOS-OR&R-30

Avino P, Russo MV (2018) A comprehensive review of analytical methods for determining persistent organic pollutants in air, soil, water and waste. Curr Org Chem 22:939–953. https://doi.org/10.2174/1385272822666180404144834

Avino P, Notardonato I, Perugini L, Russo MV (2017) New protocol based on high-volume sampling followed by DLLME-GC-IT/MS for determining PAHs at ultra-trace levels in surface water samples. Microchem J 133:251–257. https://doi.org/10.1016/j.microc.2017.03.052

Barlow SM (2009) Risk assessment of food-contact materials: past experience and future challenges. Food Addit Contam A 26:1526–1533. https://doi.org/10.1080/02652030903233231

Bergé A, Cladière M, Gasperi J, Coursimault A, Tassin B, Moilleron R (2013) Meta-analysis of environmental contamination by phthalates. Environ Sci Pollut Res 20:8057–8076. https://doi.org/10.1007/s11356-013-1982-5

Braun JM, Sathyanarayana S, Hauser R (2013) Phthalate exposure and children's health. Curr Opin Pediatr 25:247–254. https://doi.org/10.1097/MOP.0b013e32835e1eb6

Cinelli G, Avino P, Notardonato I, Centola A, Russo MV (2013) Rapid analysis of six phthalate ester in wine by ultrasound-vortex-assisted dispersive liquid-liquid micro-extraction coupled with gas chromatography-flame ionization detector or gas chromatography-ion trap mass spectrometry. Anal Chim Acta 769:72–78. https://doi.org/10.1016/j.aca.2013.01.031

Cinelli G, Avino P, Notardonato I, Centola A, Russo MV (2014) Study of XAD-2 adsorbent for the enrichment of trace levels of phthalate esters in hydroalcoholic food beverages and analysis by gas chromatography coupled with flame ionization and ion-trap mass spectrometry detectors. Food Chem 146:181–187. https://doi.org/10.1016/j.foodchem.2013.09.064

Coltro L, Pitta JB, da Costa PA, Favaro Perez MA, de Araújo VA, Rodrigues R (2014) Migration of conventional and new plasticizers from PVC films into food simulants: a comparative study. Food Control 44:118–129. https://doi.org/10.1016/j.foodcont.2014.03.058

Cózar A, Echevarría F, González-Gordillo JI, Irigoien X, Úbeda B, Hernández-León S, Palma ÁT, Navarro S, García-de-Lomas J, Ruiz A, Fernández-de-Puelles ML, Duarte CM (2014) Plastic debris in the open ocean. Proc Natl Acad Sci 111:10239–10244. https://doi.org/10.1073/pnas.1314705111

de Boer J, Wester PG, Klamer HJC, Lewis WE, Boon JP (1998) Do flame retardants threaten ocean life? Nature 394:28–29. https://doi.org/10.1038/27798

de los Ríos A, Juanes JA, Ortiz-Zarragoitia M, López de Alda M, Barceló D, Cajaraville MP (2012) Assessment of the effects of a marine urban outfall discharge on caged mussels using chemical and biomarker analysis. Mar Pollut Bull 64:563–573. https://doi.org/10.1016/j.marpolbul.2011.12.018

Eriksen M, Lebreton LC, Carson HS, Thiel M, Moore CJ, Borerro JC, Galgani F, Ryan PG, Reisser J (2014) Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLoS One 9:e111913. https://doi.org/10.1371/journal.pone.0111913

Guart A, Bono-Blay F, Borrell A, Lacorte S (2014) Effect of bottling and storage on the migration of plastic constituents in Spanish bottled waters. Food Chem 156:73–80. https://doi.org/10.1016/j.foodchem.2014.01.075

Hauser R, Calafat AM (2005) Phthalates and human health. Occup Environ Med 62:806–818. https://doi.org/10.1136/oem.2004.017590

Hermabessiere L, Dehaut A, Paul-Pont I, Lacroix C, Jezequel R, Soudant P, Duflos G (2017) Occurrence and effects of plastic additives on marine environments and organisms: a review. Chemosphere 182:781–793. https://doi.org/10.1016/j.chemosphere.2017.05.096

Kasper-Sonnenberg M, Wittsiepe J, Wald K, Koch HM, Wilhelm M (2017) Pre-pubertal exposure with phthalates and bisphenol A and pubertal development. PLoS One 12:e0187922. https://doi.org/10.1371/journal.pone.0187922

Lambertini F, Catellani D, Vindigni M, Suman M (2016) Multiresidual LC-MS analysis of plasticizers used in PVC gaskets of lids and assessment of their migration into food sauces. J Mass Spectrom 51:805–813. https://doi.org/10.1002/jms.3815

Latini G (2005) Monitoring phthalate exposure in humans. Clin Chim Acta 361:20–29. https://doi.org/10.1016/j.cccn.2005.05.003

Lithner D, Larsson Å, Dave G (2011) Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Sci Total Environ 409:3309–3324. https://doi.org/10.1016/j.scitotenv.2011.04.038

Mackintosh CE, Maldonado J, Hongwu J, Hoover N, Chong A, Ikonomou MG, Gobas FAPC (2004) Distribution of phthalate esters in a marine aquatic food web: comparison to polychlorinated biphenyls. Environ Sci Technol 38:2011–2020. https://doi.org/10.1021/es034745r

Mercogliano R, Santonicola S (2018) Investigation on bisphenol A levels in human milk and dairy supply chain: a review. Food Chem Toxicol 114:98–107. https://doi.org/10.1016/j.fct.2018.02.021

Mertens B, Van Hoeck E, Blaude M-N, Simon C, Onghena M, Vandermarken T, Van Langenhove K, Demaegdt H, Vandermeiren K, Covaci A, Scippo M-L, Elskens M, Van Loco J (2016) Evaluation of the potential health risks of substances migrating from polycarbonate replacement baby bottles. Food Chem Toxicol 97:108–119. https://doi.org/10.1016/j.fct.2016.08.019

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

Notardonato I, Avino P, Centola A, Cinelli G, Russo MV (2013) Validation of a novel derivatization method for GC-ECD determination of acrylamide in food. Anal Bioanal Chem 405:6137–6141. https://doi.org/10.1007/s00216-013-7001-3

Notardonato I, Avino P, Cinelli G, Russo MV (2014) Fast determination of phthalate ester residues in soft drinks and light alcoholic beverages by ultrasound/vortex assisted dispersive liquid-liquid microextraction followed by gas chromatography-ion trap mass spectrometry. RSC Adv 4:59655–59663. https://doi.org/10.1039/c4ra08574d

Notardonato I, Salimei E, Russo MV, Avino P (2018a) Simultaneous determination of organophosphorus pesticides and phthalates in baby food samples by ultrasound–vortex-assisted liquid–liquid microextraction and GC–IT/MS. Anal Bioanal Chem 410:3285–3296. https://doi.org/10.1007/s00216-018-0986-x

Notardonato I, Russo MV, Vitali M, Protano C, Avino P (2018b) Analytical method validation for determining organophosphorus pesticides in baby foods by a modified liquid–liquid microextraction method and gas chromatography-ion trap/mass spectrometry analysis. Food Anal methods. https://doi.org/10.1007/s12161-018-1335-6

Pietrelli L, Di Gennaro A, Menegoni P, Lecce F, Poeta G, Acosta ATR, Battisti C, Iannilli V (2017) Pervasive plastisphere: first record of plastics in egagropiles (Posidonia spheroids). Environ Pollut 229:1032–1036. https://doi.org/10.1016/j.envpol.2017.07.098

Qingqing S, Fengmin L, Chenchen G, Cong W, Zhenyu W, Jian Z (2016) Detection of phthalate esters in seawater by stir bar sorptive extraction and gas chromatography–mass spectrometry. Mar Pollut Bull 108:163–170. https://doi.org/10.1016/j.marpolbul.2016.04.034

Rochester JR (2013) Bisphenol A and human health: a review of the literature. Reprod Toxicol 42:132–155. https://doi.org/10.1016/j.reprotox.2013.08.008

Russo MV, Notardonato I, Cinelli G, Avino P (2011) Evaluation of an analytical method for determining phthalate esters in wine samples by solid-phase extraction and gas chromatography coupled with ion-trap mass spectrometer detector. Anal Bioanal Chem 402:1373–1381. https://doi.org/10.1007/s00216-011-5551-9

Russo MV, Avino P, Cinelli G, Notardonato I (2012) Sampling of organophosphorus pesticides at trace levels in the atmosphere using XAD-2 adsorbent and analysis by gas chromatography

coupled with nitrogen–phosphorus and ion-trap mass spectrometry detectors. Anal Bioanal Chem 404:1517–1527. https://doi.org/10.1007/s00216-012-6205-2

Russo MV, Notardonato I, Avino P, Cinelli G (2014) Determination of phthalate esters at trace levels in light alcoholic drinks and soft drinks by XAD-2 adsorbent and gas chromatography coupled with ion trap-mass spectrometry detection. Anal Method 6:7030–7037. https://doi.org/10.1039/c4ay00926f

Russo MV, Avino P, Perugini L, Notardonato I (2015) Extraction and GC-MS analysis of phthalate esters in food matrices: a review. RSC Adv 5:37023–37043. https://doi.org/10.1039/c5ra01916h

Russo MV, Avino P, Notardonato I (2016a) Fast analysis of phthalates in freeze–dried baby foods by ultrasound–vortex-assisted liquid–liquid microextraction coupled with gas chromatography-ion trap/mass spectrometry. J Chromatogr A 1474:1–7. https://doi.org/10.1016/j.chroma.2016.10.058

Russo MV, Avino P, Perugini L, Notardonato I (2016b) Fast analysis of nine PAHs in beer by ultrasound–vortex-assisted dispersive liquid–liquid micro-extraction coupled with gas chromatography-ion trap mass spectrometry. RSC Adv 6:13920–13927. https://doi.org/10.1039/c5ra24873f

Russo MV, Avino P, Notardonato I (2017) PAH residues in honey by ultrasound–vortex-assisted liquid–liquid micro-extraction followed by GC-FID/IT-MS. Food Anal Methods 10:2132–2142. https://doi.org/10.1007/s12161-016-0783-0

Schettler T (2006) Human exposure to phthalates via consumer products. Int J Androl 29:134–139. https://doi.org/10.1111/j.1365-2605.2005.00567.x

Serrano SE, Braun J, Trasande L, Dills R, Sathyanarayana S (2014) Phthalates and diet: a review of the food monitoring and epidemiology data. Environ Health A. https://doi.org/10.1186/1476-069X-13-43

Shea KM (2003) Pediatric exposure and potential toxicity of phthalate plasticizers. Pediatrics 111:1467–1474

Sighicelli M, Pietrelli L, Lecce F, Iannilli V, Falconieri M, Coscia L, Di Vito S, Nuglio S, Zampetti G (2018) Microplastic pollution in the surface waters of Italian Subalpine Lakes. Environ Pollut 236:645–651. https://doi.org/10.1016/j.envpol.2018.02.008

Simoneau C, Van den Eede L, Valzacchi S (2012) Identification and quantification of the migration of chemicals from plastic baby bottles used as substitutes for polycarbonate. Food Addit Contam A 29:469–480. https://doi.org/10.1080/19440049.2011.644588

Vaclavikova M, Paseiro-Cerrato R, Vaclavik L, Noonan G, DeVries J, Begley TH (2016) Target and non-target analysis of migrants from PVC-coated cans using UHPLC-Q-Orbitrap MS: evaluation of long-term migration testing. Food Addit Contam 33:352–363. https://doi.org/10.1080/19440049.2015.1128564

Van Sebille E, Wilcox C, Lebreton L, Maximenko N, Hardesty BD, Van Franeker JA, Eriksen M, Siegel D, Galgani F, Law KL (2015) A global inventory of small floating plastic debris. Environ Res Lett 10:124006. https://doi.org/10.1088/1748-9326/10/12/124006

Wittassek M, Koch HM, Angerer J, Brüning T (2011) Assessing exposure to phthalates—the human biomonitoring approach. Mol Nutr Food Res 55:7–31. https://doi.org/10.1002/mnfr.201000121

Xie Z, Ebinghaus R, Temme C, Caba A, Ruck W (2005) Atmospheric concentrations and air–sea exchanges of phthalates in the North Sea (German Bight). Atmos Environ 39:3209–3219. https://doi.org/10.1016/j.atmosenv.2005.02.021

Xie Z, Ebinghaus R, Temme C, Lohmann R, Caba A, Ruck W (2007) Occurrence and air-sea exchange of phthalates in the Arctic. Environ Sci Technol 41:4555–4560. https://doi.org/10.1021/es0630240

Ze-Ming Z, Hong-Hai Z, Jian-Long L, Gui-Peng Y (2017) Determination of phthalic acid esters in seawater and sediment by solid-phase microextraction and gas chromatography-mass spectrometry. Chin J Anal Chem 45:348–356. https://doi.org/10.1016/S1872-2040(17)60999-X