# Phthalates and Bisphenol-A Determination and Release from Different Beverage Plastic Containers by Dispersive Liquid-Liquid Microextraction and GC-IT/MS Analysis

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## Abstract

Phthalates and bisphenol-A are molecules widely used in packaging because they increase the plastic malleability and workability but they show the tendency to migrate or dissociate from plastics. Recently, researchers raised the alarm to have found their traces in water samples of plastic bottles left at high outdoor temperatures for a long time. The paper would like to show a simple, sensitive, and reproducible method for the simultaneous determination of phthalates and bisphenol-A in drinking water, based on the dispersive liquid-liquid microextraction followed by gas chromatography-mass spectrometry analysis. The dispersion is favored by means of ultrasonic bath and stirring magnetic plate, without dispersive solvent. The method presents an average $R^2$ 0.993 in the range 10–5000 ng mL$^{-1}$, a limit of detection below 1.2 ng mL$^{-1}$, and a limit of quantification below 7.7 ng mL$^{-1}$. The release of such compounds from different beverage containers (6 plastic bottles, 6 canteens, and 3 newborn feeding bottles) has been analyzed. The release kinetics from the bottles are studied over 2 months, whereas over 6 h for the other containers. Only 2 compounds have been found in a plastic bottle and in a canteen bottle, with concentrations ranging between 24 and 117 ng mL$^{-1}$.

**Keywords** Phthalates · Bisphenol-A · plastics · Analytical method · Extraction procedure · GC-MS · Water · Bottles · Exposure · Release

## Introduction

Nowadays phthalates (PAEs), bisphenol-A (BP-A), and microplastics are found almost everywhere, e.g., in plastic bottles, cosmetics, children's toys, newborn feeding bottles, hair shampoos, and perfumes (Net et al. 2015). Very often large supermarkets, small local retailers, and food stores during logistics leave unsuitable plastic containers of various sizes containing water or other food and then place them inside after a long time. The unsuitable conditions are all those factors that can favor a modification of the polymeric structure constituting the plastic, basically due to the release of molecules such as PAE and BP-A

(Barlow 2009; Simoneau et al. 2012; Mertens et al. 2016). For example, since in the summertime high temperatures are reached (30–35 °C) as well as long exposures to sunlight, plastic bottles can release PAEs and BP-A more easily. The high temperatures accelerate the migration kinetics from the polymer (i.e., container) to the beverage, due to a greater agitation of these molecules whereas the solar rays stress the molecules to migrate in the liquid (aqueous) matrix. Another factor contributing to the PAE migration, which however concerns food containing fat, is to be apolar molecules (they are formed by benzene and hydrocarbon chains) (Erythropel et al. 2014): if they put in contact with food containing fats, they migrate into. In any case, this release from the plastic causes contamination of the food with which the plastic is in contact.

PAEs are industrially obtained by double esterification of phthalic acid with different compounds, mainly alcohols: for example, the reaction with methanol, ethanol, or butanol leads to the formation of di-methyl phthalate (DMP), di-ethyl phthalate (DEP), di-butyl phthalate (DBP), and di-ethylhexyl phthalate (DEHP), the most used in the food industry as plastic constituents. On the other hand, BP-A is a synthetic organic

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compound also used in plastics: it is synthesized according to a condensation reaction between acetone and excess phenol in the presence of strong acid, e.g., HCl, which acts as a catalyst.

Their main role as plasticizing agents is to insert themselves between the plastic polymer chains weakening their intermolecular bonds: in this way, the plastics, mainly polyvinyl chloride (PVC) and PET, become malleable and workable (Wilkes et al. 2008). Since they are apolar and not covalently bound to the polymer molecules, PAEs tend to migrate or dissociate from plastics especially when they are subjected to mechanical and thermal stresses or in contact with lipophilic substances. B-PA plays the same role but in addition gives transparency to the polymers making the transparent bottles: for example, B-PA is used in the polycarbonate, which is a transparent and high-performance thermoplastic polymer (Al-Shukri et al. 2011).

From a toxicological point of view, a PAE/BP-A accumulation in the human body can give different consequences (Heudorf et al. 2007): the main effects are due to be endocrine disruptors (Rudel and Perovich 2008) and to favor the human sterility (Zamkowska et al. 2018). BP-A has also been related to different health problems, particularly in pregnant women, fetus, and young children (Carlstedt et al. 2013; Braun 2017). According to the literature (Shea 2003; Hauser and Calafat 2005), it should be underlined that the accumulation of each PAE decreases in relationship to the age (high in the newborn, almost 10 times lower in adult age) through the exposure routes and consequently also the exposure risk.

As far as legislation is concerned, PAEs and BP-A fall within the category of additives (article 3, paragraph 7) of the EU regulation 10/2011 (Commission Regulation 2011): in the regulation, the specific migration limit (SML, expressed in mg substance per kg food) applicable for each substance is also defined.

In this paper, the attention has focused on the plastic water bottles because they are the most recurrent to be used as well as on canteen bottles, largely used by cyclists or sports in general, and newborn feeding bottles, which represent a large slice of the market for babies. In literature, there are several studies on different matrices, e.g., wines,

children's toys, environmental samples, and paints (Russo et al. 2015; Avino and Russo 2018). There is considerable concern for the population, especially for infants and children who are almost always in contact during the day with plastic toys and objects. The scope of this paper is to study the effective release of such hazard compounds, PAEs and BP-A, in water beverages from containers stored in unsuitable conditions by means of a recent analytical method, i.e., the dispersive liquid-liquid microextraction (DLLME) (Rezaee et al. 2006; Zgoła-Grześkowiak and Grześkowiak 2011; Cinelli et al. 2013).

## Materials and Methods

### Materials

PAES and BP-A standards were furnished from Sigma-Aldrich (Milan, Italy): Table 1 reports the phthalates investigated in this study. Anthracene, used as Internal Standard (I.S.), was from LabService Analytical (Anzola Emilia, Bologna, Italy) whereas methanol, heptane, iso-octane, n-hexane, benzene, and toluene were of pesticide grade (Carlo Erba, Milan, Italy), and sodium chloride was of analytical grade (Carlo Erba).

An important issue in the PAE analysis is the cross-contamination from chemicals, materials, and laboratory equipment. Organic reagents such as heptane, iso-octane, hexane, and toluene were subjected to distillation whereas NaCl (a particular focus to this reagent for minimizing background contamination) was heated for 4 h at 140 °C in a shallow tray and, after cooling, it was kept in a tightly sealed glass vial. Further, the laboratory equipment was cleaned by a strict procedure: the glassware was soaked and washed in acetone and dried at 140 °C for at least 4 h. Before used, all reagents and glassware were checked for potential phthalate contamination by GC-IT/MS analysis.

Each PAE standard solution (0.1 mg mL$^{-1}$ each) was obtained by dissolving each compound in iso-octane whereas BP-A was in methanol; the mixed PAEs/BP-A solution used for spiking real samples was obtained by acetone dilution, a solvent with intermediate polarity. This point is important

Table 1 Determinants (PAEs and BP-A) investigated in this study, with their corresponding acronym, chemical formula, CAS number, molecular weight (MW), selected ion monitoring (SIM), and specific migration limit (SML, expressed as mg kg$^{-1}$)

| Determinant | Acronym | Formula | # CAS | MW | SIM | SML |
|---|---|---|---|---|---|---|
| Di-methyl-phthalate | DMP | $C_{10}H_{10}O_4$ | 131-113 | 194 | 163 | |
| Di-ethyl-phthalate | DEP | $C_{12}H_{14}O_4$ | 84-66-2 | 222 | 149,177 | |
| Di-iso-butyl phthalate | DiBP | $C_{16}H_{22}O_4$ | 84-69-5 | 278 | 149,205 | |
| Di-butyl phthalate | DBP | $C_{16}H_{22}O_4$ | 84-74-2 | 278 | 149,205 | 0.3 |
| Bisphenol-A | BP-A | $C_{15}H_{16}O_2$ | 80-05-7 | 228 | 213 | |
| Di-ethyl-exyl-phthalate | DEHP | $C_{24}H_{38}O_4$ | 117-81-7 | 390 | 149,167 | 1.5 |
| Di-octyl-phthalate | DOP | $C_{24}H_{38}O_4$ | 117-84-0 | 390 | 149,261 | |

because it allows PAEs and BPA to solubilize easily for the further analytical steps.

## DLLME Protocol

An aliquot of 10 mL of sample (standard solution or real/spiked beverage) was transferred in a 10-mL screw cap glass tube with conical bottom and a mixture of NaCl, 10 g L$^{-1}$, anthracene (as Internal Standard, I.S.; 10 µg mL$^{-1}$) and 40 µL of extraction solvent was added. The disperser solvent was not used but the dispersion was favored by mechanical energy: the whole solution was vortexed for 5 min and after, it was kept in an ultrasound bath for 6 min. The sample was centrifuged for 30 min at 4000 rpm. The amount, collected into a vial, was sufficient for analyzing PAEs and BPA by means of GC-IT/MS.

In Fig. 1, the different steps of the UVADLLME procedure are resumed.

## GC Analysis and Quantification

The GC-IT/MS system used for analysis was a Trace GC coupled with a PolarisQ mass spectrometer (Thermo Finnigan, Bremen, Germany) whereas data acquisition and analysis were performed using Xcalibur 1.4.1 software. The home-made fused-silica capillary column with chemically bonded phase (SE-54, 5 % phenyl-95 % dimethylpolysiloxane; analytical characteristics are reported in Russo et al. 2016a, b, 2017) used in this study is very similar to commercial ones: it shows very good chromatographic efficiency and is cheaper than the others. A sample of 1 µL was injected into the PTV in splitless mode: 10 s after injection, the vaporizer was heated from 110 to 280 °C at 800 °C min$^{-1}$ and cooled after 300 s. The splitter valve was opened after 120 s. The temperature of the GC/IT-MS transfer line was 270 °C; the temperature of the ion source was 250 °C. Helium (IP 5.5) was used as carrier gas at a flow rate of 1.0 mL. The oven temperature program was 100 °C, held for 1 min, 10 °C min$^{-1}$ up to 280 °C, and for 3 min. The ion-trap mass spectrometer was operated in the electron ionization mode (70 eV) and the determinants were recorded in full-scan mode (m/z 45–400).

Glass tight syringes at different volumes (Hamilton, NV, USA) were employed for the measurements of the extraction organic solvent volumes and for the DLLME procedure.

In both cases, the PAE concentrations were obtained by calibration graphs of the ratio Area$_{(PAE/BPA)}$/Area$_{(I.S.,anthracene)}$ plotted versus each PAE/BPA concentration (pg µL$^{-1}$). All the samples were quantified in triplicate.

## Results and Discussion

Starting from a method already known and developed, i.e., the DLLME (Russo et al. 2014a, b, 2016a), preliminarily the extraction method optimization has been performed: this has allowed to determine all the best experimental conditions regarding the enrichment and analysis process. Particularly, this
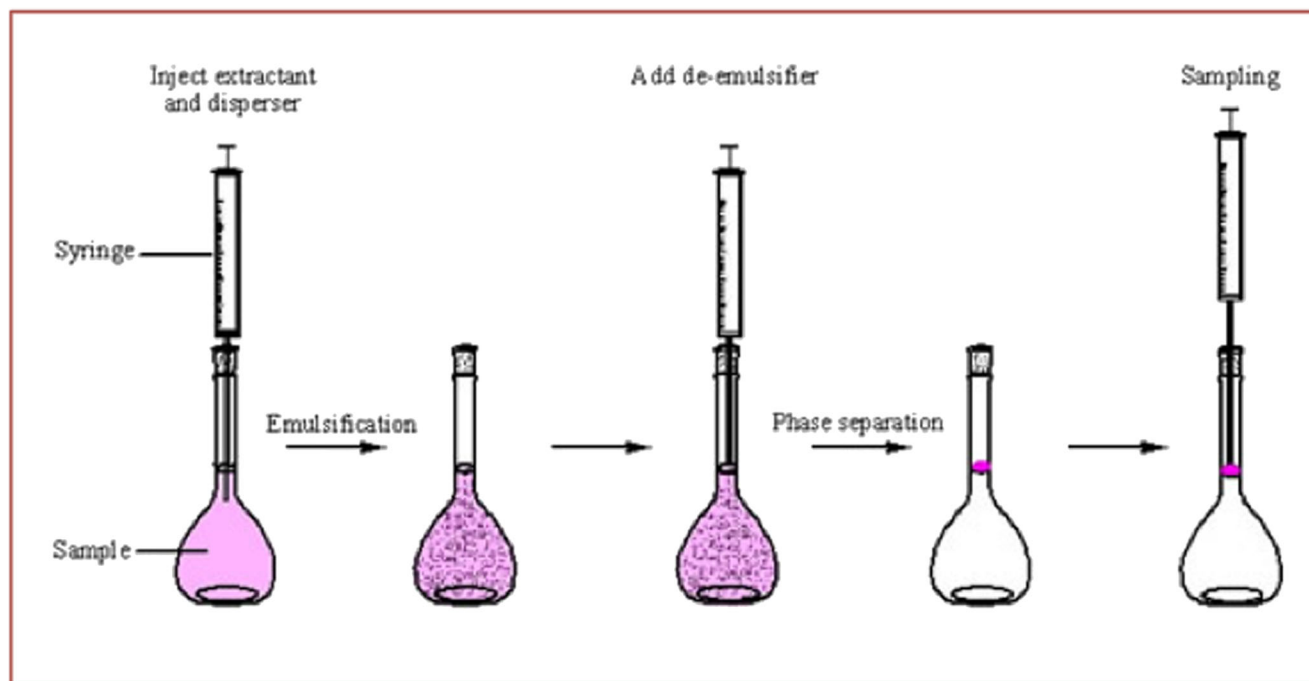


**Fig. 1** Master scheme of the DLLME protocol set up for analyzing PAEs/BP-A in plastic water bottle samples

paper is focused on the development of an easy method for simultaneous determination of PAEs and BP-A in aqueous samples with high pre-concentration factors.

All the steps of the developed methods have been largely investigated. First, the analytical parameter influence on the extraction methodology for determining PAEs and BP-A was studied, such as solution pH, extraction solvent, sonication and centrifugation times, and NaCl amount. After, the entire methodology was validated along with the matrix effect and applied to water samples collected from different plastic containers (i.e., mineral water bottles, canteen bottles, newborn feeding bottles).

## Parameter Optimization

All the analytical steps were investigated and optimized for achieving the best possible recoveries. First, the pH influence was studied: the attention was focused on pH ranging between 3 and 10.

Figure 2 shows the effect of different pH on mixed standard solutions: it can be seen that at pH 5, there are the best PAE recoveries ranging between 96 and 102 %, except BP-A, 94 %. The plot also reports the errors, which are below 5 %, except BP-A, 9 %. The decrease of pH confirms the finding in previous papers (Notardonato et al. 2018; Russo et al. 2011), especially at high-alkaline pH where it is not recommended to perform the analytical procedure, maybe due to the internal standard ionization, and therefore, the $area_{determinant}/area_{IS}$ ratio is totally altered. For further

experiments, the extraction process will then take place at acid pH, with a value around 5.

The extraction solvent plays an important role; i.e., it is used for extracting the determinants. Basically, it is dispersed as microdroplets; the solution becomes opalescent. The opalescence depends on the droplet dimension (larger droplet, greater opalescence) but this affects the interaction between dispersing solvent and determinants (i.e., contact surface). Different solvents different volumes were tested for identifying the best conditions: specifically, n-hexane, n-heptane, iso-octane, benzene, and toluene were tested. First, toluene was discharged as an extraction solvent because its recoveries were below 30 %. Similar situation for benzene, even if the recoveries (< 71 %) using this solvent were better than those obtained by toluene, they were not satisfactory for these analyses. The extractions by means of the other three solvents, i.e., extractions with n-hexane, n-heptane, and iso-octane, were investigated. About the volume, it should be underlined that it is not possible to achieve the extraction with extraction solvent volumes less than 150 μL because the solvent layer becomes very thin and the recoveries are not effective. On the other hand, if the procedure uses volumes larger than 250 μL, it is no longer a micro-droplet extraction. So, the authors decided to perform experiments using different extraction solvent volumes ranging between 150 and 250 μL. Figure 3, which shows the results obtained with the four solvents (except toluene) and using 200 μL of each solvent, evidences that 200 μL of n-hexane is the best extraction condition for obtaining almost quantitative recoveries.
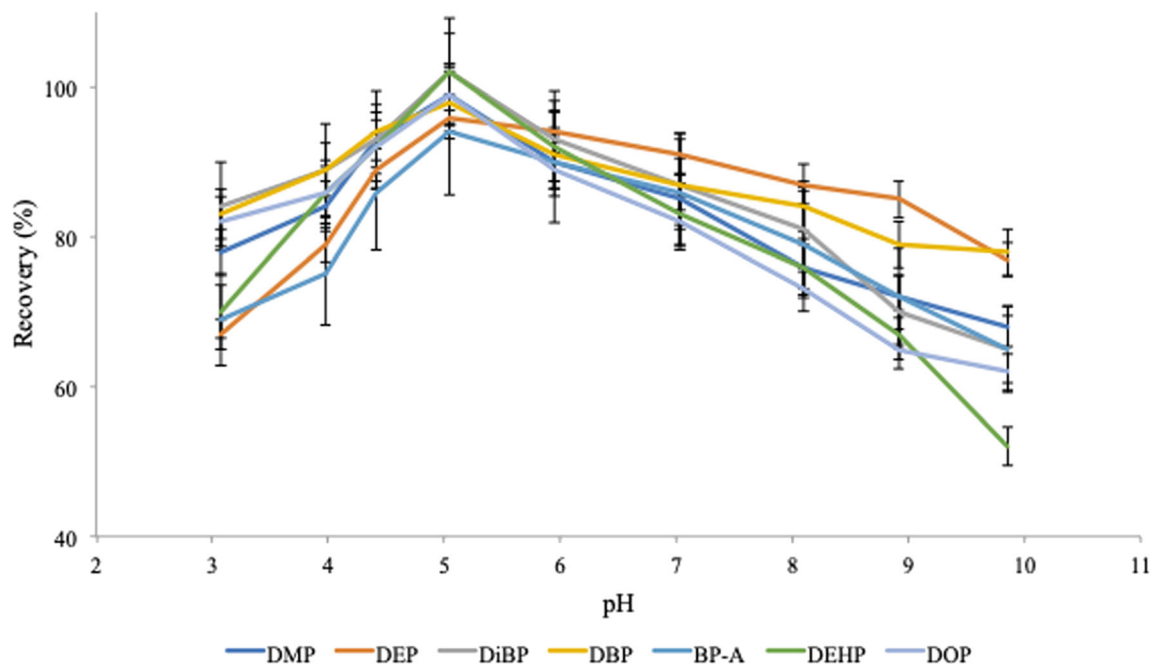


**Fig. 2** Average recoveries (%) with relative error bars, related to the effect of pH on water samples spiked with a mixed PAE/BP-A standard solution (50 ng mL$^{-1}$)
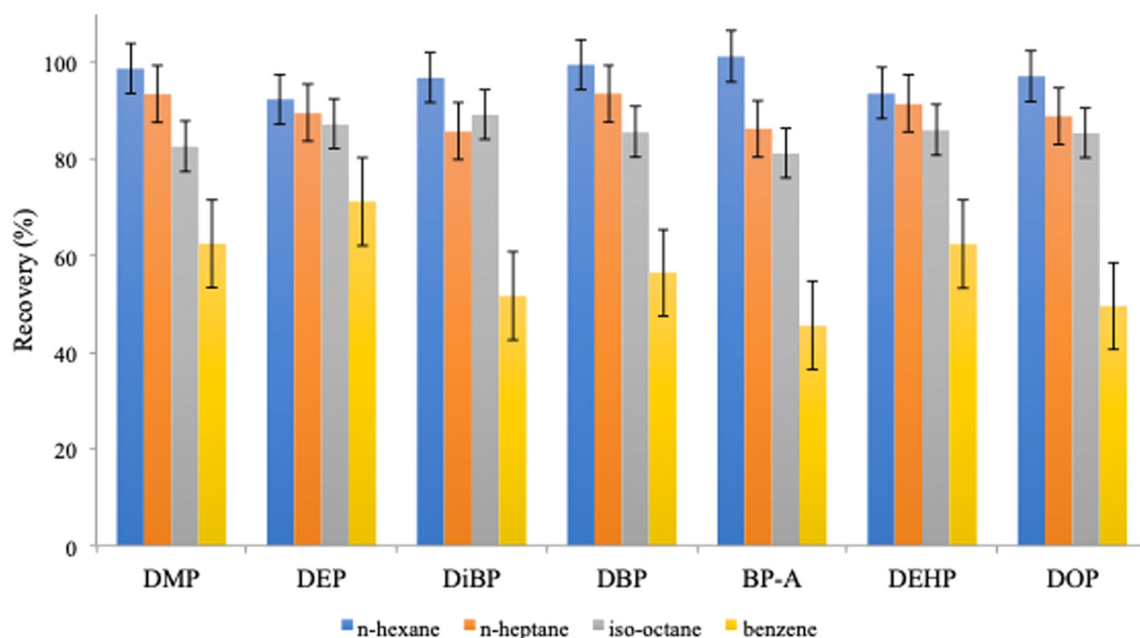
**Fig. 3** Average recoveries (%) with relative error bars, related to the effect of different solvents on water samples spiked with a mixed PAE/BP-A standard solution. The conditions were as follows: each PAE/BP-A at 50 ng mL^{-1}, solution pH 5, and 200 μL of extraction solvent

After it was evaluated if different vortex mixing times could improve the percentage recovery of the determinants, the hypothesis is that by keeping the solution containing the heptane more agitated, the dispersion could be better, thus increasing the determinant recoveries. Although experiments were carried out for a long time, the recovery did not show any particular changes. Therefore, as a standard time, a not too long time was chosen, but sufficient to disperse the extraction solvent, equal to 5 min.

Another parameter evaluated is the sample residence time in the ultrasonic bath. After a first dispersion of the extraction solvent by vortex, a real dispersion is achieved by means of ultrasound waves. The phenomenon that allows the extraction solvent dissolution is the cavitation: propagating in the liquid, ultrasounds at a certain frequency "translate" into alternating cycles of high and low pressure, creating steam bubbles at a high temperature which then quickly collapse (Riesz et al. 1985) and favor the solvent dispersion. DLLME extractions were carried out on spiked samples (50 ppb of each determinant) undergoing to ultrasounds for 6, 10, and 14 min. Table 2 shows the average recoveries obtained.

All the three conditions considered show comparable recoveries between them; the shortest time, equal to 6 min, was chosen as the residence time in the ultrasound bath.

Other important parameters affecting the extraction procedure, such as the centrifuge time and the rotor revolutions, were evaluated: in fact, the right combination between time and speed (i.e., rotor revolutions, expressed as rpm) of the centrifuge gives the conditions for achieving the best determinant recovery. For this scope, two rotor speed values were evaluated, i.e., 3500 and 4000 rpm, and not beyond to avoid

centrifugal instability problems. For these two rotor revolutions, different centrifuge duration times were carried out for 10, 20, and 30 min. The values obtained are shown in Table 3.

It is possible to note that the greatest recoveries occur using the combinations of 3500 rpm for 20 min and 4000 rpm for 30 min. Evaluating the data, the second combination was chosen as the optimal one to be used for all the determinants, also because as it has a greater recovery as regards the DEP.

The last investigated parameter is the sodium chloride amount to be added after the emulsion formation to favor the breaking. DLLME experiments with spiked PAE/BP-A solutions were performed, adding different salt concentrations from 1 g L^{-1} to 250 g L^{-1}: in this way, after the extraction solvent dispersion, the sodium chloride can

**Table 2** Average recoveries (%) at different ultrasound time. The reported coefficient of variations are in bracket (cv%). The conditions were as follows: each PAE/BP-A at 50 ng mL^{-1}, solution pH 5 and 200 μL of *n*-hexane

| Determinant | Recovery (%) | | |
|---|---|---|---|
| | 6 min | 10 min | 14 min |
| DMP | 87.4 (4.4) | 89.2 (3.5) | 88.3 (3.7) |
| DEP | 98.4 (2.5) | 96.5 (2.8) | 96.4 (3.4) |
| DiBP | 100.6 (3.1) | 107.9 (4.0) | 108.1 (3.1) |
| DBP | 102.4 (4.1) | 105.7 (3.2) | 106.2 (2.6) |
| BP-A | 85.6 (6.1) | 81.5 (7.1) | 84.6 (5.7) |
| DEHP | 98.7 (1.2) | 102.6 (3.8) | 103.4 (1.9) |
| DOP | 97.5 (4.6) | 108.3 (3.5) | 109.8 (2.9) |

**Table 3** Average recoveries (%) at different centrifugal times and the rotor revolutions (average cv% below 8 %) on water samples spiked with a mixed PAE/BP-A standard solution. The conditions were as follows: each PAE/BP-A at 50 ng mL$^{-1}$, solution pH 5, 200 μL of $n$-hexane, and ultrasounds 6 min

| Determinant | Recovery (%) | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| | 3500 rpm | | | 4000 rpm | | |
| | 10 min | 20 min | 30 min | 10 min | 20 min | 30 min |
| DMP | 44.7 | 62.4 | 77.1 | 58.5 | 74.3 | 89.5 |
| DEP | 87.9 | 88.4 | 90.3 | 87.3 | 91.2 | 96.1 |
| DiBP | 93.9 | 100.6 | 89.9 | 92.0 | 92.9 | 101.7 |
| DBP | 109.2 | 112.4 | 100.3 | 103.0 | 98.2 | 102.0 |
| BP-A | 42.8 | 58.3 | 72.6 | 53.6 | 71.6 | 86.8 |
| DEHP | 112.2 | 118.7 | 98.8 | 92.8 | 98.5 | 99.5 |
| DOP | 115.9 | 107.5 | 97.6 | 91.7 | 97.4 | 103.6 |

dissolve in water, increase the ionic strength of the solution, and decrease the solubility of the apolar solvent. In Figure 4, the results are shown.

As it can be seen that the salinity of the solution helps the PAE extraction, except for DMP and bisphenol-A that are poorly extracted (max recovery 57 % and 61 %, respectively) because of their low partition coefficient. As regards DEP, recovery increases with increasing salt concentration with a maximum value of 200 g L$^{-1}$. The other PAEs also show a considerable recovery at 200 g L$^{-1}$. For practical operational reasons, the value of 100 g L$^{-1}$ was chosen, a value in which

the PAE recoveries are analytically significant. So resuming, it can be affirmed that the salinity of the solution favors the PAE extraction but does not allow good recoveries of DMP and BP-A.

The entire developed procedure (resuming the best analytical conditions, pH 5, 200 μL of $n$-hexane as extraction solvent, ultrasonication for 6 min, centrifugation for 30 min at 4000 rpm, and NaCl at 10 g L$^{-1}$) was applied to validate the analytical parameters for determining PAEs/BP-A in different bottle water samples as well as in distilled water samples considered for studying the matrix effect.

## Chromatographic Profiles

Figure 5 reports the GC-IT/MS chromatograms of water samples analyzed according to the procedure reported above. Figure 5a shows the chromatogram of the PAE/BP-A standard solution whereas Fig. 5b the blank run of a real water sample not containing the determinants; particularly, this last one evidences no peak where PAEs and BP-A should come out. Figure 5c shows the chromatogram obtained applying the entire procedure to a real water sample in plastic canteen bottle whereas Fig. 5d the same sample spiked with 80 ng mL$^{-1}$ of each determinant. Further, Fig. 6 shows a typical chromatogram of a water sample collected from a newborn feeding bottle. As it can be seen in all chromatograms, the peaks are well solved and the matrix does not affect the chromatograms meaning that the extraction procedure is efficacious for such determinants.
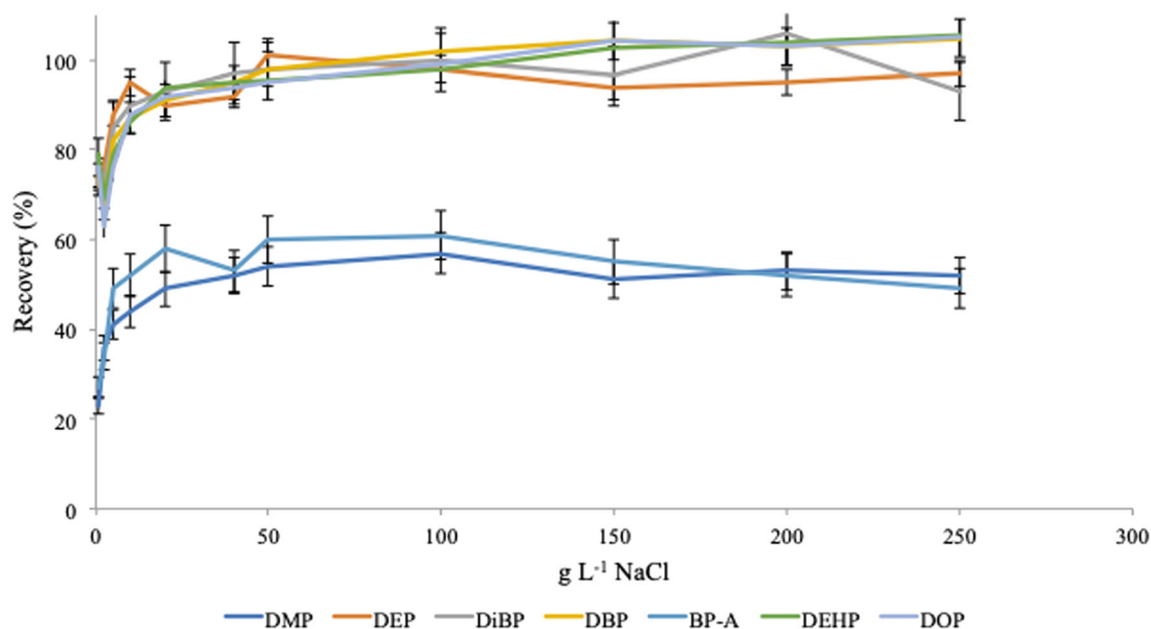


**Fig. 4** Average recoveries (%) with relative error bars, related to the effect of NaCl salt on water samples spiked with a mixed PAE/BP-A standard solution. The conditions were as follows: each PAE/BP-A at 50 ng mL$^{-1}$, solution pH 5, 200 μL of $n$-hexane, solvent ultrasounds 6 min, and centrifugation for 30 min at 4000 rpm
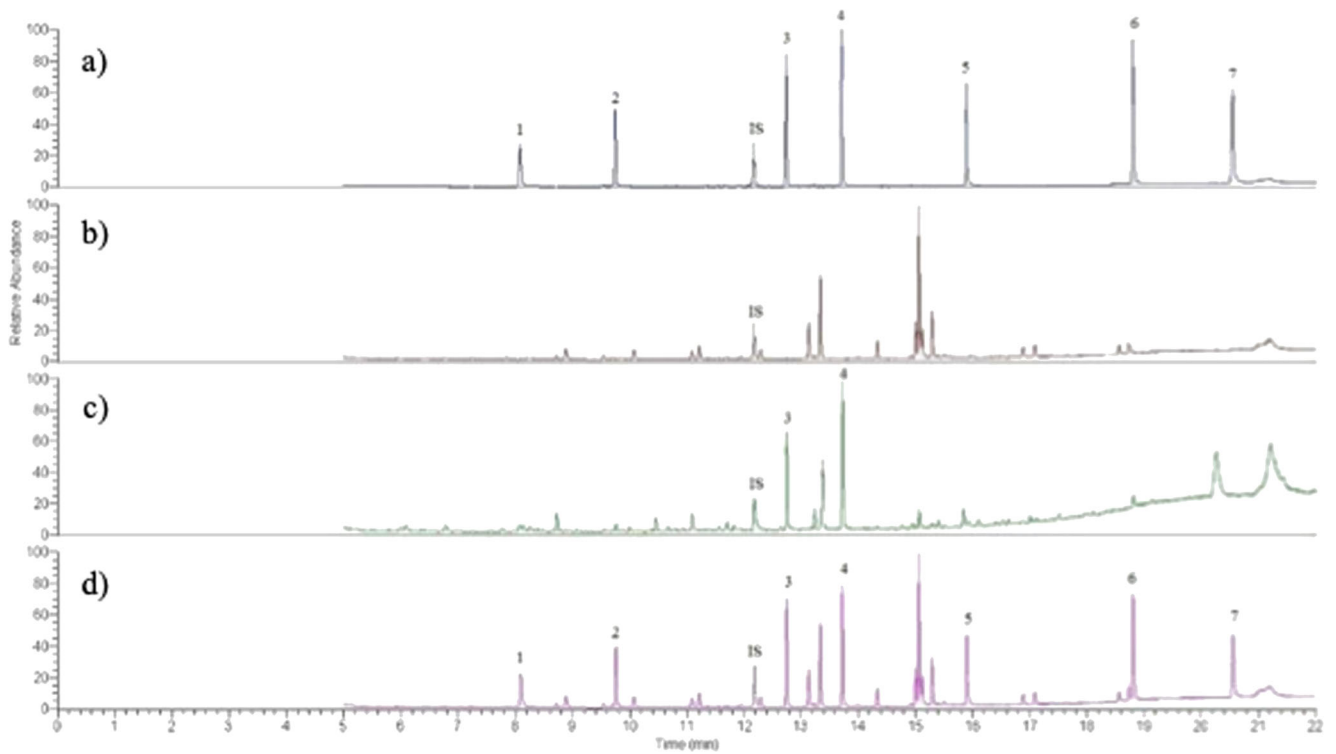
**Fig. 5** GC-IT/MS chromatograms of PAE/BP-A (50 ng mL$^{-1}$ of each one) standard solution (**a**), blank sample (i.e., real water sample in plastic bottle) (**b**), real water sample from plastic canteen bottle (**c**), and the same sample spiked with 80 ng mL$^{-1}$ of each determinant (**d**). For experimental conditions, see text. Peak list: 1 DMP; 2 DEP; IS Anthracene; 3 DiBP; 4 DBP; 6 BP-A; 6 DEHP; 7 DOP
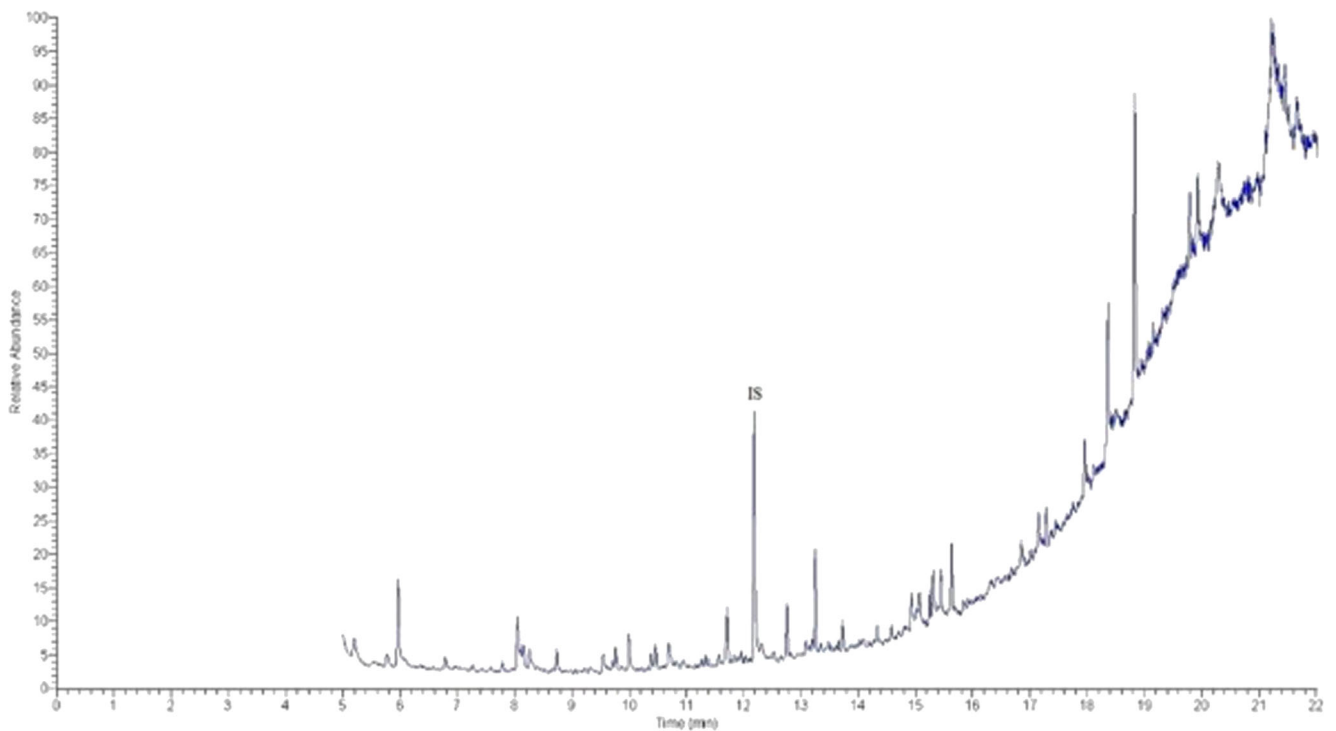


**Fig. 6** GC-IT/MS chromatogram of a water sample collected from a newborn feeding bottle. For experimental conditions, see text. Peak list: 1 DMP; 2 DEP; IS Anthracene; 3 DiBP; 4 DBP; 6 BP-A; 6 DEHP; 7 DOP

The analytical conditions investigated for optimizing the DLLME procedure have been applied to blank (distilled water) and real (tap water) samples both for determining the method validation and for evidencing the matrix effects (if present).

## Method Development

The method developed in this study, based on DLLME extraction with *n*-hexane, makes possible to achieve a preconcentration factor of about 5000. For this goal, a significant change in the extraction protocol has been investigated: the dispersive solvent, which helps the extraction solvent dispersion in the solution, is not used and is substituted by means of an ultrasonic bath. The mechanical energy produced during this operation manages to favor the emulsion formation.

Table 4 shows the calibration curves (regression equation and $R^2$) in the concentration range from 10 to 5000 ng mL$^{-1}$, the limit of detections (LODs) and limit of quantifications (LOQs) (calculated according to Knoll's definition; Knoll 1985), the recoveries (%) in blank (distilled water spiked with 20 ng mL$^{-1}$ of each determinant), and real (low, 20 ng mL$^{-1}$, and high, 500 ng mL$^{-1}$, concentrations) samples and the inter-day and intra-day precisions of each determinant investigated in this study.

It should be noted the very low LODs and LOQs were achieved: those results are possible using the mass spectrometry as the detection system. In fact, LODs and LOQs in water samples were also determined by the same authors in a previous paper using the GC-FID analysis: in that case, the LODs and LOQs ranged between 0.1–1.2 ng mL$^{-1}$ and 2.1–7.5 ng mL$^{-1}$, respectively. The improvement can be immediately noted as well as the better inter-day and intra-day precisions, except for DMP and BP-A, than those reported in the cited paper (LODs 2–19 ng mL$^{-1}$; LOQs 4–48 ng mL$^{-1}$; intra-day < 6.4; inter-day < 9.7). Such LODs and LOQs are suitable for determining PAEs/BP-A at ultra-trace levels in such matrices.

Further, they are lower than those found by other authors in similar or different beverage (Simoneau et al. 2012; Petersen and Breindahl 2000; Tsumura et al. 2002; Yano et al. 2005; Gärtner et al. 2009; Guo et al. 2012; Chang et al. 2014; Celeiro et al. 2015; Cirillo et al. 2015), i.e., LODs ranging between 1–100 ng mL$^{-1}$ and LOQs 2–350 ng mL$^{-1}$, except for González-Castro et al. (2011), Bradley et al. (2013), and Schecter et al. (2013) that show better LODs and LOQs (0.01–50 ng g$^{-1}$ and 0.02–60 ng g$^{-1}$, respectively) calculated both in similar matrices (baby bottles) and in different matrices containing orange juice, high protein and carbohydrate levels, and food homogenate. Further, it should be noted that the percentage recoveries of the PAEs and BP-A in blank samples and real samples at low concentrations are quite similar; it means that the matrix effect does not affect the measurements. Similarly, the percentage recoveries studied at two different concentrations are quite similar: particularly, it is to be underlined that the DMP and BP-A determinants increase their recoveries and they are almost quantitative at such levels. In addition, intra-day errors are between 1.8 and 3.2 %, except DMP and BP-A, 7.2 % and 8.1 %, respectively, whereas inter-day errors are less than 6.5 %, except for DMP and BP-A, 10.3 % and 12.1, respectively.

## Application to Real Samples

Fifteen water beverage containers have been analyzed by means of the analytical procedure developed: in particular, six water plastic bottles by 500 mL, all bottles are made of poly(ethylene terephthalate) (commonly abbreviated PET) but with different consistency and coloration; three newborn feeding bottles including two at low PET density and one in PET; and six canteen bottles for cyclists. After collected sample at zero-time exposure, which is "blank" situation, each bottle was left on the window sill and after 7, 15, 30, and 60 days, other 4 samples were collected: all the samples were analyzed using the method developed. Similar treatment was performed for water samples collected from the other two types of

**Table 4** Calibration curve (regression equation and $R^2$) in the concentration ranging from 10 to 5000 ng mL$^{-1}$, LOD and LOQ, recoveries (%) in blank (distilled water spiked with 20 ng mL$^{-1}$ of each determinant) and real (low and high concentrations) samples and inter-day and intra-day precision of each determinant investigated in this study

| Determinant | Regression eq. | $R^2$ | LOD | LOQ | Recovery | | | Intra-day | Inter-day |
|---|---|---|---|---|---|---|---|---|---|
| | | | (ng mL$^{-1}$) | (ng mL$^{-1}$) | Blank | Low | High | (RSD, %) | (RSD, %) |
| DMP | $y = 1.0992x\text{-}0.1491$ | 0.9941 | 0.7 | 2.8 | 85.2 | 76.3 | 94.9 | 7.2 | 10.3 |
| DEP | $y = 1.2201x\text{-}0.2004$ | 0.9949 | 0.1 | 7.5 | 98.2 | 96.1 | 99.5 | 2.4 | 5.1 |
| DiBP | $y = 2.6544x\text{-}0.5049$ | 0.9946 | 0.8 | 4.7 | 99.6 | 95.2 | 98.4 | 2.6 | 4.9 |
| DBP | $y = 2.9584x\text{-}0.3506$ | 0.9932 | 0.4 | 3.6 | 97.9 | 96.4 | 101.0 | 3.2 | 6.5 |
| BP-A | $y = 1.1453x\text{-}0.6973$ | 0.9843 | 1.1 | 6.2 | 90.3 | 82.5 | 93.8 | 8.1 | 12.1 |
| DEHP | $y = 2.5049x\text{-}1.1373$ | 0.9922 | 0.6 | 2.1 | 99.4 | 98.7 | 99.6 | 1.8 | 4.9 |
| DOP | $y = 3.1221x\text{-}1.9445$ | 0.9983 | 1.2 | 4.9 | 98.7 | 97.4 | 101.6 | 2.4 | 4.8 |

**Table 5** PAE concentration levels (ng mL$^{-1}$) in the two plastic containers at different sampling time

| Determinant | Exposure time | | | | | | |
|---|---|---|---|---|---|---|---|
| Water bottle | 0 days | 7 days | 15 days | 30 days | 60 days | | |
| DEHP | 56.6 | 59.6 | 68.9 | 78.7 | 91.2 | | |
| DOP | 72.1 | 62.3 | 65.0 | 76.0 | 101.6 | | |
| Canteen bottle | 0 h | 1 h | 2 h | 3 h | 4 h | 5 h | 6 h |
| DiBP | 55.1 | 52.4 | 69.5 | 76.8 | 77.1 | 84.8 | 114.0 |
| DBP | 34.1 | 24.5 | 51.4 | 63.0 | 63.9 | 75.3 | 117.7 |

containers. In both cases, a kinetic study based on few hours was followed because their use is limited in time. Substantially, after having filled the containers with water at zero-time, they were left under the hot airflow, and the samples were collected after every hour up to a time of 6 h and processed by DLLME-GC-IT/MS procedure. Each time before collecting the sample, the water temperature was measured using a mercury thermometer: it ranged between 28 and 44 °C. In this way, the sun exposure was simulated (for instance, when the plastic bottle is left under the sun or in the car for some hour).

Only in two of the 15 samples, the PAE release from the vessel appeared relevant. The release of the di-2-ethylhexyl phthalate (DEHP) and di-octyl phthalate (DOP) in a half-liter bottle and of di-isobutyl phthalate (DiBP) and di-*n*-butyl phthalate (DBP) in a canteen bottle, respectively, was found.

Looking at Table 5, a release increase over time could be noted whereas from the other 13 samples analyzed, no PAE/BP-A release was detected.

## Conclusions

In this paper, an analytical method for determining PAEs and BP-A released from plastic food container has been set up. The extraction method is good for all the determinants, even if for DMP, further technical insights (e.g., different recoveries respect other PAEs) are necessary due to its different water/octanol partition coefficient ($K_{w/o}$) whereas the BP-A extraction needs a deep revision because significant recoveries are achieved at high concentration levels. Basically, LODs, LOQs, and precision/reproducibility data make this method easy, reliable, sensitive, and reproducible as well as to be used routinely.

The study on the various containers showed an analytically significant release of some PAEs only and exclusively in two cases. It should be reported that this plastic green water bottle which presented the major release was tested immediately after its purchase. The authors would like to suggest that it is a good behavior to rinse well and for many times a new plastic bottle before its first use. It is also advisable not to expose it to heat sources or thermal changes. These small precautions may limit the PAE release and therefore the exposure. As regards the green plastic bottle that has shown an appreciable release, it should be underlined that it is good to avoid exposure to light for a long time and, if this happens, to try to consume it in a period of no more than 3 months.

## Compliance with Ethical Standards

**Conflict of Interest** Ivan Notardonato declares that he has no conflict of interest. Carmela Protano declares that she has no conflict of interest. Matteo Vitali declares that he has no conflict of interest. Pasquale Avino declares that he has no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

**Informed consent** Not applicable.

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