Trends in Chromatography
Vol. 11, 2017

Original Communication

Determination of phthalic acid esters in drinking water and olive oil by ultra-high performance liquid chromatography-electrospray-tandem mass spectrometry: Study of phthalate migration from plastic bottles to drinking water at different domestic exposure conditions

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ABSTRACT

A UHPLC-ESI-MS/MS method for the determination of ten phthalates in mineral water and olive oil samples was developed. A hold-back column placed between the pump and the injection valve allowed preventing the instrument background contamination by the phthalates. Good instrumental limits of detection (1-9 μg/L), precisions (RSD <23.9%), and trueness (relative error <20%) were achieved. A study of phthalate migration from PET bottles to mineral water at different domestic exposure conditions revealed that only diethyl phthalate migrated when exposed at 40 °C for six months, although at concentration levels below the method limit of quantitation. No matrix effect was found for mineral water samples and only dicyclohexyl phthalate was detected. Matrix-matched calibration was proposed for the analysis of olive oil samples due to the huge matrix effect encountered with the employed liquid-liquid extraction method. Among the olive oil samples where phthalates were detected, dibutyl phthalate was found only in one sample at the established EU specific migration level (0.3 mg/kg).

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KEYWORDS: food packaging contaminants, phthalic acid esters, UHPLC, tandem mass spectrometry, migration studies, olive oils, mineral water.

INTRODUCTION

Diesters of 1,2-benzenedicarboxylic acid (phthalic acid), commonly referred to as phthalic acid esters (PAEs) or simply as phthalates, are a group of anthropogenic chemicals of increasing public importance due to their potential toxicity [1]. PAEs are mainly polymeric additives widely used as plasticizers in various domestic and industrial products to increase flexibility, plasticity, elasticity, durability and transparency of plastics such as polyvinyl chloride (PVC), polyethylene terephthalate (PET) and cellulose acetate, among others. Low-molecular weight PAEs such as dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), benzyl butyl phthalate (BBP) and diisobutyl phthalate (DIBP) are widely used as solvents in personal care products, paints and adhesives [2, 3]. In contrast, high-molecular weight PAEs such as di(2-ethylhexyl) phthalate (DEHP), diisononyl phthalate (DINP), di-n-octyl phthalate (DNOP) and dipropylheptyl phthalate (DPHP) are employed mainly as plasticizers

in order to soften PVC, and in several building and construction materials [4]. An important characteristic of these compounds is that they are rather non-volatile substances that are not chemically bounded to the polymer plastic structures. Because of these non-covalent bonding properties, their high production volume and their widespread use, PAEs are released extensively in the environment where they show some persistence due to their relatively non-polar properties [5]. All this may result in the direct contamination of feed and food products, in their bioaccumulation in animal tissues and, consequently, in their transfer through the food chain. Additionally, phthalates can leach, migrate or evaporate into foodstuffs and beverages from packaging materials containing plastic components, and hence human exposure to PAEs is widespread [1, 6].

Based on some toxicological evidences, several PAEs have been classified by their impact strength. For example, DEHP is in Class B2 and has been shown to be embryotoxic and teratogenic, while BBP is in Class C (possible human carcinogen). Others such as DEP and DBP are not yet classified as human carcinogens [7, 8]. Besides, PAEs have long been considered to be endocrine disrupting chemicals and some of them have shown reproductive effects in animals. Considering these evidences, since 14 December 2005, the EU Directive 2005/84/EC [9] has banned the use of several phthalates in PVC and other plasticized materials including all toys and child-care articles. Nevertheless, it seems that the major route of phthalate exposure is through the diet, especially by migration from food-contact packaging materials. Thus, EU Commission Regulation No. 10/2011 established specific migration limits (SMLs) for some phthalates such as DBP, BBP, DEHP and C_8 to C_{11}-dialkyl phthalates at 0.30, 30, 1.5 and 9 mg/kg, respectively [10]. Special attention should be taken in the case of fatty foodstuffs because they are more susceptible to phthalate migration from food-contact packaging materials due to the lipophilic properties of these compounds.

The detection and determination of PAEs is usually carried out by gas chromatography (GC) that includes the EPA 606-phthalate ester method for organic chemical analysis of municipal and industrial wastewaters [11]. Gas chromatography with flame ionization detector (GC-FID), mass spectrometry (GC-MS) and tandem mass spectrometry (GC-MS/MS) are among the most employed methods for the determination of phthalates in food and beverage samples [4, 12, 13]. Liquid chromatography (LC) techniques have also been employed for this purpose as they have several advantages over GC, i.e. less sample clean-up steps are typically required, and more relatively polar and high-molecular-weight PAE compounds can be identified. Thus, today, LC coupled with mass spectrometry (LC-MS) or tandem mass spectrometry (LC-MS/MS) is becoming a popular technique for the analysis of PAEs in a great variety of samples [4, 12, 13]. High-resolution mass spectrometry (HRMS) and accurate mass measurements by employing either time-of-flight (TOF) or Orbitrap mass analyzers have recently gained great popularity in the determination of PAEs because of their capability to provide more comprehensive information concerning the exact molecular mass, the elemental composition and detailed molecular structure of a compound in comparison to low resolution MS instruments [14, 15]. The extraction of PAEs from food samples is one of the most important steps in the reliable determination of these compounds. Currently, a high number of sample preparation methods are widely employed for PAEs determination, such as solvent extraction (SE) [16, 17], solid-phase extraction (SPE) [18, 19], QuEChERS procedures [14, 20], as well as microextraction techniques such as solid-phase microextraction (SPME) [21, 22], or dispersive liquid-liquid microextraction (DLLME) [23], among others. However, it should be taken into consideration that the determination of PAEs is not an easy task, especially in food matrices. These compounds are found in the laboratory environment, including in the air, due to their widespread use and they can be absorbed into glass and other materials. Besides, they can also be found in several laboratory products used for sample preparation and analysis such as sorbents or even organic solvents, and hence false positive outputs can be produced during their analysis [24-26]. Therefore, the risk of contamination is present in the entire analytical scheme, including sampling, sample preparation and analysis.

This problem can be diminished using different methods proposed in the literature to prevent phthalate contamination problems, and by reducing the number of steps necessary to prepare the sample [17, 25, 27]. However, the use of instrumental and procedural blanks is recommended.

The present work is aimed to develop an ultra-high performance liquid chromatography-electrospray-tandem mass spectrometry (UHPLC-ESI-MS/MS) method for the determination of ten phthalic acid esters in mineral water and olive oil samples packed in plastic bottles at the concentration levels specified by EU legislation. In order to prevent the background contamination of the UHPLC instrument by the phthalates, a C18 hold-back column was placed between the UHPLC pump and the injection valve. Mineral water samples were directly analyzed without any sample treatment apart from filtration, while for olive oil samples a simple LLE using acetonitrile as extracting solvent was employed. Special attention was given while performing procedural blanks to control phthalate contamination of the laboratory during sample treatment. Instrumental and method validation was also performed. Sample recoveries, method limits of detection and quantitation, method trueness, and matrix effects were evaluated. For olive oil samples, where an important matrix effect was observed, matrix-matched calibration was proposed for a reliable phthalate quantitation. Besides, a study of phthalate migration from PET bottles to mineral water at different domestic exposure conditions was performed. Finally, the applicability of the proposed UHPLC-ESI-MS/MS method was assessed by analyzing 15 mineral water samples and 30 olive oil samples packed in plastic bottles.

MATERIALS AND METHODS

Chemicals and standard solutions

Unless otherwise stated, all chemicals and reagents were of analytical grade. Dimethyl phthalate (DMP, CAS 131-11-3), diethyl phthalate (DEP, CAS 84-66-2), diallyl phthalate (DAP, CAS 131-17-9), di-n-propyl phthalate (DPrP, CAS 131-16-8), diisobutyl phthalate (DIBP, CAS 84-69-5), benzyl butyl phthalate (BBP, CAS 85-68-7), di-n-butyl phthalate (DBP, CAS 84-74-2), dicyclohexyl phthalate (DCP, CAS 84-61-7), di-n-hexyl phthalate (DNHP, CAS 84-75-3), and di(2-ethylhexyl) phthalate (DEHP, CAS 117-81-7) were purchased from Sigma-Aldrich (Steinheim, Germany).

Water, methanol and acetonitrile (LC-MS Chromasolv® grade) and formic acid (≥98%) were also obtained from Sigma-Aldrich. All chemicals were handled in accordance with the most current material safety data sheets.

Instrumentation

An ultrahigh pressure liquid chromatography (UHPLC) instrument (Open Accela system, Thermo Fisher Scientific, San José, CA, USA), equipped with a quaternary pump and a CTC autosampler, was employed. Chromatographic separation was performed in a Syncronis C18 reversed-phase (100 × 2.1 mm, 1.7 µm particle size) UHPLC column (Thermo Fisher Scientific) under gradient elution using 0.1% formic acid aqueous solution (solvent A) and methanol (solvent B) as mobile phase. The gradient elution program consisted of a linear gradient from 60% to 75% solvent B in 0.5 min followed by a linear gradient up to 80% solvent B in 5.5 min, then linear gradient up to 90% B in 4 min, and a linear gradient up to 100% solvent B in 2 min. Mobile phase was then kept under isocratic conditions at 100% solvent B for 7 min, and then back to initial conditions (60% solvent B) in 1.5 min, and kept under these conditions for 5.5 min for column re-equilibration. The column was kept at room temperature and the mobile phase flow-rate was 200 µL/min. A sample injection volume of 10 µL was employed. In order to control and remove UHPLC instrumental phthalate background levels a hold-back column, Kinetex C18 (50 × 2.1 mm, 2.6 µm particle size) porous-shell column (Phenomenex, California, USA), was set between the UHPLC pump and the injection valve as indicated in Fig. 1S (supplementary information). Injector post clean and valve clean with methanol and water was performed thrice each to prevent injector valve phthalate contamination.

The UHPLC system was coupled with a triple quadrupole (QqQ) mass spectrometer (TSQ Quantum Ultra AM, Thermo Fisher Scientific), equipped with an electrospray ionization (ESI) source. Nitrogen (purity 99.98%) was used as the sheath gas, ion sweep gas and auxiliary gas at a flow rate of 40, 0, and 25 a.u. (arbitrary units),

respectively. ESI vaporizer temperature and the ion transfer tube temperature were set at 350 °C and 270 °C, respectively. Capillary electrospray voltage at 4.0 kV in positive ionization mode was employed. Multiple reaction monitoring (MRM) acquisition mode was used by recording two or three (depending on the phthalate compound) selected reaction monitoring (SRM) transitions. For this purpose, a mass resolution of 0.7 *m/z* full width at half maximum (FWHM) on both Q1 and Q3 and a scan width of 0.05 *m/z* were employed. Argon was used as the collision gas at 1.5 mtorr and the optimum collision energy (CE) for each SRM transition monitored (quantifier and qualifier) is shown in Table 1. The chromatogram was segmented into eight windows, and two SRM transitions for each compound with a dwell time of 100 ms and 3 μ scans were monitored (Table 1). For DBP and DiBP an additional third SRM transition (used also as qualifier ion) was monitored due to a common first qualifier SRM transition. Tube lens offset voltages employed for each phthalate are also indicated in Table 1. Xcalibur software version 2.1 (Thermo Fisher Scientific) was used to control the UHPLC-ESI-MS/MS system and to process data.

Table 1. MRM acquisition parameters.

Segment	Time (min)	Phthalate	Precursor ions	Product ion assignment (Quantifier/Qualifier)	Collision energy (CE, V)	Tube lens offset voltage (V)
1	0-4.2	DMP	195.0 $[M+H]^+$	163.0 $[M+H-CH_4O]^+$	11	50
				77.0 $[M+H-C_4H_6O_4]^+$	33	50
2	4.2-5.4	DEP	223.0 $[M+H]^+$	149.0 $[M+H-C_4H_{10}O]^+$	18	70
				177.0 $[M+H-C_2H_6O]^+$	5	70
3	5.4-6.7	DAP	247.0 $[M+H]^+$	189.0 $[M+H-C_3H_6O]^+$	5	60
				41.0 $[M+H-C_{11}H_{10}O_4]^+$	16	60
4	6.7-9.2	DPrP	251.0 $[M+H]^+$	149.0 $[M+H-C_6H_{14}O]^+$	15	70
				65.0 $[M+H-C_9H_{14}O_4]^+$	42	70
5	9.2-12.7	DIBP	279.0 $[M+H]^+$	149.0 $[M+H-C_8H_{18}O]^+$	16	70
				223.0 $[M+H-C_4H_8]^+$	10	60
				65.0 $[M+H-C_{11}H_{18}O_4]^+$	42	70
		BBP	313.0 $[M+H]^+$	149.0 $[M+H-C_{11}H_{16}O]^+$	14	90
				91.0 $[M+H-C_{12}H_{14}O_4]^+$	33	90
		DBP	279.0 $[M+H]^+$	149.0 $[M+H-C_8H_{18}O]^+$	16	60
				205.0 $[M+H-C_4H_{10}O]^+$	8	60
				121.0 $[M+H-C_9H_{18}O_2]^+$	34	60
6	12.7-15.0	DCP	331.0 $[M+H]^+$	149.0 $[M+H-C_{12}H_{22}O]^+$	26	60
				167.0 $[M+H-C_{12}H_{20}]^+$	12	60
7	15.0-17.3	DNHP	335.0 $[M+H]^+$	149.0 $[M+H-C_{12}H_{26}O]^+$	16	40
				121.0 $[M+H-C_{13}H_{26}O_2]^+$	38	40
8	17.3-26.0	DEHP	391.0 $[M+H]^+$	149.0 $[M+H-C_{16}H_{34}O]^+$	28	70
				167.0 $[M+H-C_{16}H_{32}]^+$	34	70

The optimization of both ESI source and tandem mass spectrometry working conditions was performed by the infusion of stock standard solutions of each phthalate (5 mg/L in water) at a flow-rate of 5 µL/min using the syringe pump integrated in the TSQ instrument, and mixed with the mobile phase (200 µL/min, 0.1% formic acid aqueous solution:methanol 50:50 *v/v*) by means of a Valco zero dead volume tee piece (Supelco, Bellefonte, PA, USA).

Samples and sample treatment

Fifteen mineral water samples packed in plastic bottles (PET, different volumes) and thirty olive oil samples also packed in plastic bottles (PET, 1 L), obtained from local supermarkets (Barcelona, Spain), were analyzed in triplicate. The use of plastic material was prevented as much as possible during sample treatment. Nevertheless, due to the ubiquitous presence of phthalates in the laboratory environment [25, 26], procedural blanks were also obtained and analyzed with the proposed UHPLC-ESI-MS/MS method.

Mineral water was directly injected into the UHPLC-ESI-MS/MS system without any sample treatment. For this purpose, three glass injection vials were each filled with water samples by employing glass Pasteur pipettes. LC-MS Chromasolv® water (Sigma-Aldrich) was employed in the procedural blanks.

Olive oil samples were treated by a simple liquid-liquid extraction method. Briefly, 2.0 g of olive oil was weighed in a 12 mL glass vial (by using glass Pasteur pipettes) and extracted with 5 mL acetonitrile under vortex (1 min) and sonication (10 min) conditions. Then, the samples were centrifuged for 5 min at 4000 rpm and the supernatant was transferred into a new 12 mL glass vial (using glass Pasteur pipettes). The extraction was repeated and both acetonitrile supernatants (total volume ~10 mL) were collected together into the same vial, evaporated to dryness under gentle nitrogen stream, and reconstituted in 500 µL methanol. Then, the extract was frozen at -4 °C for 2 h to remove any possible fat residue, centrifuged for 3 min at 6000 rpm, and the methanolic extract was transferred into a glass injection vial (using glass Pasteur pipettes), and directly injected into the proposed UHPLC-ESI-MS/MS system. Procedural blanks without the olive oil were carried out in triplicate with each batch of samples analyzed.

Phthalate migration study in a mineral water sample

A study of the migration of phthalates from the bottle plastic material to a mineral water sample under four different storage conditions was performed. For this purpose, 40 mineral water samples packaged in plastic bottles (PET, 33 cL) obtained from the same producer and within the same batch were purchased from a local supermarket (Barcelona, Spain). All the mineral water bottles were opened and 1 mL of sample (collected with 1 mL plastic pipettes) was transferred into glass injection vials, and the vials were stored at -4 °C until analyzed (time zero of the storage exposure study). Procedural blanks were performed by collecting 1 mL of LC-MS Chromasolv® water (Sigma-Aldrich) using the same kind of 1 mL plastic pipettes and storing them in the corresponding glass injection vials at -4 °C.

Then, the samples were stored at the four different exposure conditions for six months (10 water bottles per condition): (i) at room temperature and protected from light, (ii) under climate-not-controlled environment conditions (bottles exposed to light and climate variations in a balcony), (iii) at 40 °C (in an oven), and (iv) at -4 °C (in a freezer). For the migration study, water sampling was performed once a month. For this purpose, water bottles were shaken (frozen ones were initially allowed to unfroze), and 1 mL of water (measured with 1 mL plastic pipettes) each was transferred into glass injection vials and stored at -4 °C until analyzed. Each month, procedural blanks were performed as previously commented.

RESULTS AND DISCUSSION

UHPLC-ESI-MS/MS optimization

UHPLC separation of the ten studied phthalates was easily attainable by reversed-phase chromatography using a sub-2 µm C18 column under gradient elution and 0.1% formic acid aqueous solution and methanol as the mobile phase. The optimized gradient program started

with an isocratic step at 60% methanol followed by linear gradients up to 80 and 90% methanol (see 'Instrumentation' section). Initially, a mobile phase flow rate of 300 µL/min was employed. Under these conditions, baseline chromatographic separation of all analyzed phthalates was obtained in less than 16 min, with a column backpressure of ~833 bars. As an example, Fig. 1 shows the separation of a standard mixture of 500 µg/L registered in selected ion monitoring mode. As can be seen, only three phthalates (DIBP, DBP and BBP) eluted within a short time window. An ion suppression study was performed with these three compounds to verify if their ionization could be affected by the co-elution among them. For this purpose, individual standards and mixtures (under co-elution conditions) of these three compounds at the same concentration levels were analyzed, and the comparison of the signals obtained revealed an ion suppression effect of 15, 20 and 27% for DBP, DIBP and BBP, respectively, showing the importance of achieving an acceptable separation.

The UHPLC system was coupled to a triple quadrupole mass spectrometer by using a heated-electrospray (H-ESI) source in positive mode. ESI source conditions were optimized by the infusion of stock standard solutions of each phthalate (5 mg/L in water) at a flow-rate of 5 µL/min, mixed with the mobile phase (200 µL/min, 0.1% formic acid aqueous solution:methanol 50:50 *v/v*) by means of a Valco zero dead volume tee piece. Optimal ESI parameters were selected taking into consideration the highest vaporization efficiency to achieve the highest signal for the molecular ion for each compound (values given in 'Instrumentation' section). Furthermore, the optimal tube lens offset voltage was employed for each compound (Table 1).

ESI (positive) full scan MS spectra of phthalates were characterized by the presence of the protonated molecule $[M+H]^+$ as the base peak, and no in-source fragmentation was observed. See, as an example, the full scan MS spectrum for DEP in Fig. 2Sa (supplementary information). The protonated molecules were selected as the precursor ion (Table 1) for the tandem mass spectrometry experiments. The fragmentation of these compounds under tandem mass spectrometry conditions in the triple quadrupole was studied and the most intense and characteristic transitions

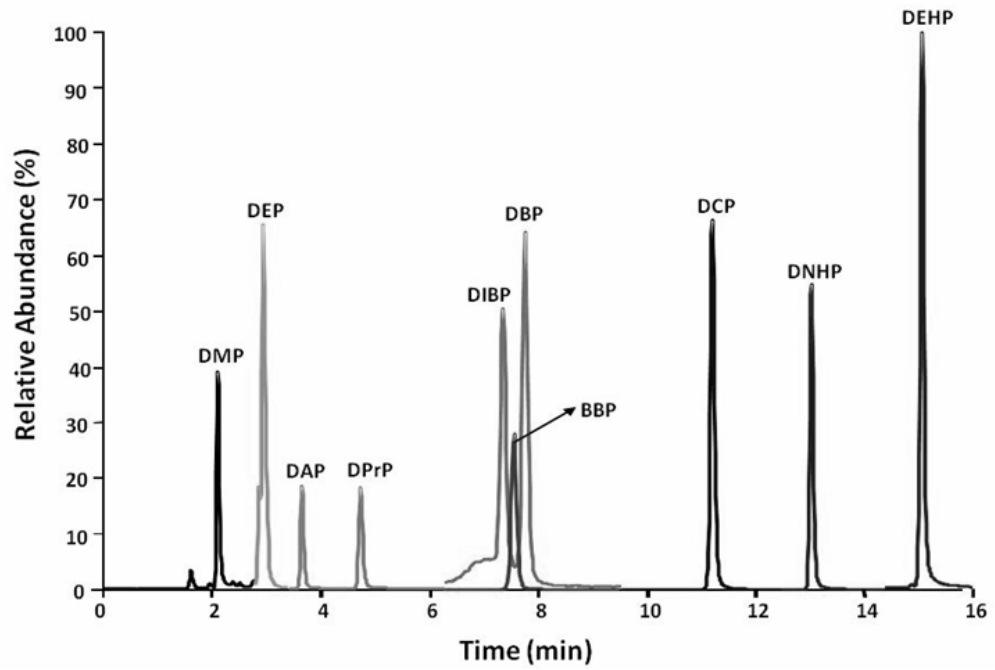

Fig. 1. UHPLC-ESI-MS chromatographic separation of a standard mixture of phthalates (500 µg/L) in selected ion monitoring mode ($[M+H]^+$ ion for each compound indicated in Table 1).

were selected for both quantitative and confirmation purposes. For the accurate product ion assignment, collision energy curves (5-80 V) were studied. The assignments for both precursor and monitored product ions for each selected reaction monitoring (SRM) transition are given in Table 1, together with the optimal collision energies. Fig. 2Sb shows, as an example, the MS/MS spectrum obtained for DEP. Phthalate fragmentation is characterized by the corresponding losses of the alkyl side chains. All phthalates evaluated, with the exception of DMP, give the protonated phthalic anhydride ion at *m/z* 149 as a characteristic product ion, which, in most of the cases, is also the base peak ion. In the case of DMP a methoxyl group was removed by α-cleavage, which formed the base peak at *m/z* 163. Then, under collision activation, the fragment ion m/z 163 loses a methoxyl group and undergoes further fragmentation. In order to comply with EU Directive 2002/657/EC [28], the most intense SRM transition was selected as the quantifier ion, and the second most intense transition as the qualifier ion. Nevertheless, two phthalic acids, DIBP and DBP, showed a common SRM transition (*m/z* 279.0>149.0, used for quantitation); thus to achieve further confirmatory power with the triple quadrupole, a third SRM transition was also registered (see Table 1).

As previously commented in the introduction, phthalates are ubiquitously present in the laboratory and in the LC instruments. Thus, instrumental blanks were evaluated, showing the presence of three phthalic acids (DIBP, DBP and DEHP), as can be seen in Fig. 2a. The presence of this phthalate background level was attributed to the solvents employed in the mobile phase that leaches phthalic acids from the plastic components in the UHPLC instrument. Under these conditions,

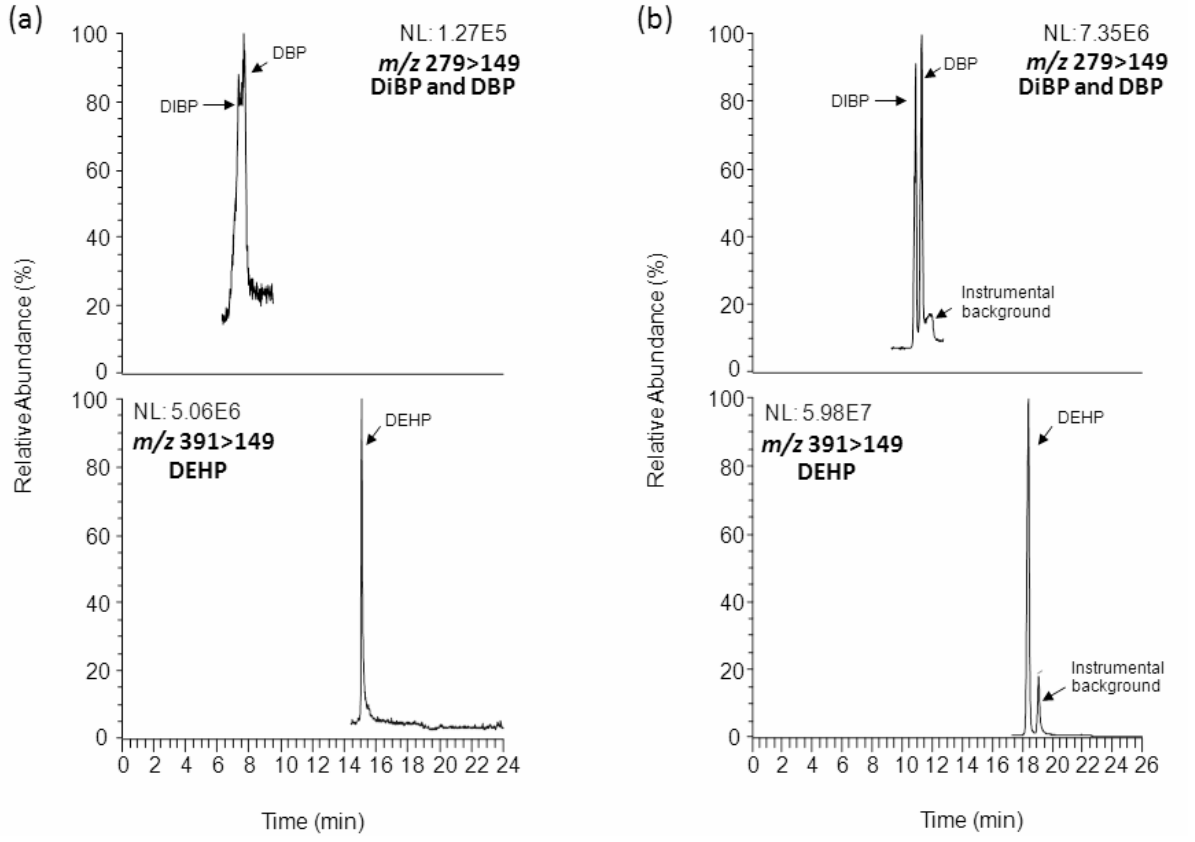

Fig. 2. Comparison of the chromatographic signals (quantifier SRM transition) for DiBP, DBP and DEHP (a) without and (b) with the C18 hold-back column placed between the UHPLC instrument pump and injection valve. Standard concentration: 500 µg/L.

instrumental limits of detection (ILODs) for these three compounds, calculated as the concentration providing a signal-to-noise ratio of 3:1, were in the range 25-30 µg/L, one order of magnitude greater than the values achieved for the other phthalates (1-5 µg/L). This fact do not allow to propose this method for the quantitative determination of some of these compounds, especially DEHP, at the SML levels established by legislation [10], because instrumental limits of quantitation increased up to 82-100 µg/L. Several approaches have been reported in the literature to avoid phthalate contamination of the LC instrumental, such as the employment of hold-back columns [27]. Thus, a Kinetex C18 (50 × 2.1 mm, 2.6 µm particle size) porous-shell column was placed between the UHPLC pump and the injection valve in order to retain phthalic acids coming from the UHPLC instrument and to separate them from the ones coming from the samples. The first consequence of working with both the hold-back and the separation columns was a huge increase in the system backpressure that prevented their use, which was solved by decreasing the mobile phase flow-rate to 200 µL/min. This reduced the system backpressure to ~845 bars, and only produced an increase in the analysis time of ~3 min while keeping the same selectivity and even better chromatographic resolution. Under these conditions, the background contamination of the UHPLC instrument by the phthalates was avoided as can be seen in Fig. 2b, and ILOD values for DIBP, DBP and DEHP decreased to the same level as the values obtained for the other compounds.

As an example, Fig. 3 shows the UHPLC-ESI-MS/MS chromatographic separation for a 500 µg/L phthalate standard under optimal conditions when using the hold-back column and also shows all the SRM monitored transitions. Separation within an analysis time of 19 min was achieved.

Instrumental quality parameters

In order to verify the performance of the proposed UHPLC-ESI-MS/MS method, several quality parameters such as instrumental limits of detection (ILODs) and limits of quantitation (ILOQs), linearity,

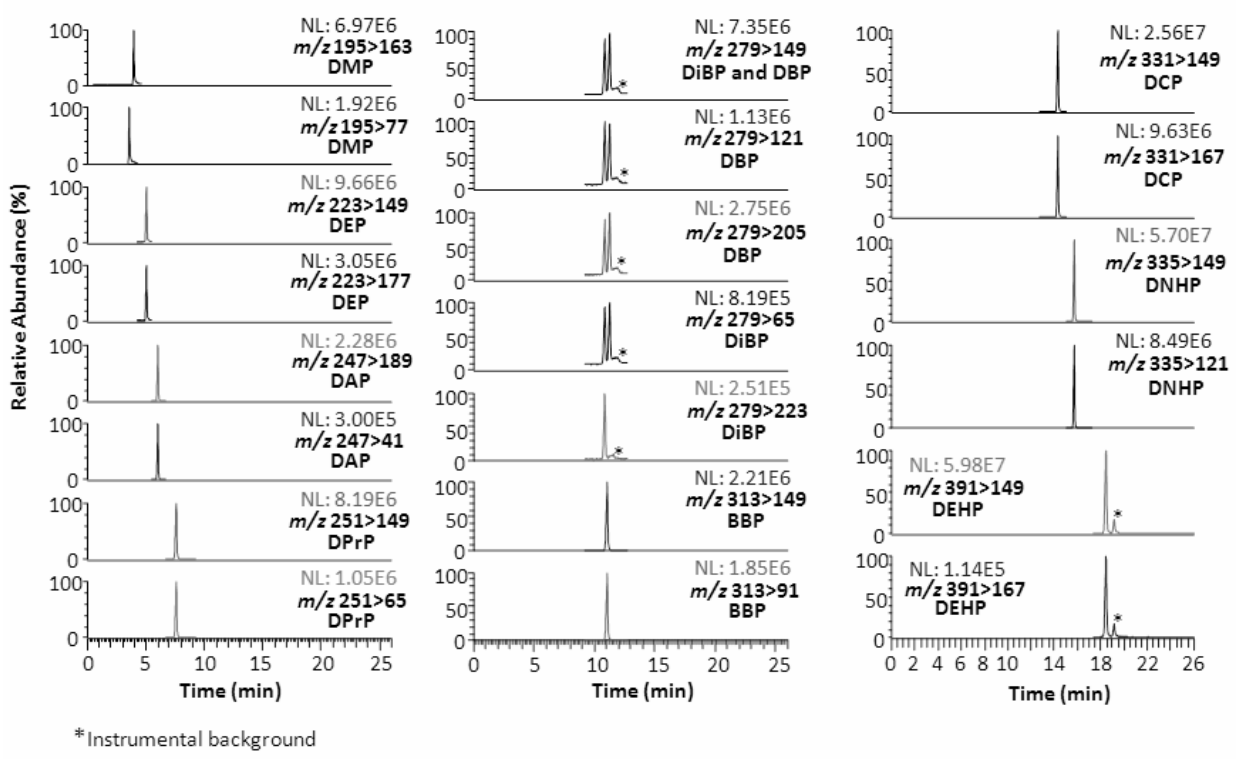

Fig. 3. UHPLC-ESI-MS/MS chromatogram of a standard mixture of phthalates (500 µg/L) in SRM acquisition mode showing all the SRM transitions monitored (Table 1).

Table 2. UHPLC-ESI-MS/MS instrumental quality parameters.

Parameter	**DMP**	**DEP**	**DAP**	**DPrP**	**DIBP**	**BBP**	**DBP**	**DCP**	**DNHP**	**DEHP**
ILOD (µg/L)	4	5	1	1	9	4	9	2	4	5
ILOQ (µg/L)	13	17	4	4	30	13	30	7	12	17
Linearity (r^2)	0.998	0.997	0.998	0.998	0.998	0.998	0.997	0.997	0.999	0.997
Concentration level 1 (~300 µg/L)										
run-to-run precision (n = 5, %RSD)	10.7	4.5	2.5	2.3	2.8	5.2	3.6	6.7	8.3	13.2
day-to-day precision (n = 3 x 5, %RSD)	9.3	10.0	19.4	21.7	19.8	23.1	14.9	16.3	6.3	13.4
Spiked concentration (µg/L)	300	304	300	301	302	297	300	302	302	300
Calculated concentration (µg/L)	294	321	312	311	308	325	310	301	269	302
Trueness (% relative error)	2.0	5.6	4.0	3.3	1.9	9.4	3.3	0.3	10.9	0.6
Concentration level 2 (~50 µg/L)										
run-to-run precision (n = 5, %RSD)	11.6	9.9	9.8	7.7	7.0	3.4	0.5	2.9	3.1	1.7
day-to-day precision (n = 3 x 5, %RSD)	17.9	12.5	16.2	6.7	21.9	18.7	4.1	10.9	7.0	14.2
Spiked concentration (µg/L)	50	51	50	50	50	50	50	50	50	50
Calculated concentration (µg/L)	43	46	48	50	47	51	53	54	52	46
Trueness (% relative error)	14.0	9.8	4.0	0	6.0	2.0	6.0	8.0	4.0	8.0
Concentration level 3 (~20 µg/L)										
run-to-run precision (n = 5, %RSD)	7.9	6.7	3.4	2.1	-	0.3	-	6.2	12.7	6.5
day-to-day precision (n = 3 x 5, %RSD)	15.2	8.7	13.2	23.1	-	23.9	-	6.7	12.7	12.5

Table 2 continued..

Parameter	DMP	DEP	DAP	DPrP	DIBP	BBP	DBP	DCP	DNHP	DEHP
Spiked concentration (µg/L)	20	20	20	20	-	20	-	20	20	20
Calculated concentration (µg/L)	17	18	20	21	-	21	-	23	17	22
Trueness (% relative error)	15.0	10.0	0	5.0	-	5.0	-	15.0	15.0	10.0
Concentration level 4 (~5 µg/L)										
run-to-run precision (n = 5, %RSD)	-	-	13.4	6.5	-	-	-	-	-	-
day-to-day precision (n = 3 x 5, %RSD)	-	-	12.8	6.8	-	-	-	-	-	-
Spiked concentration (µg/L)	-	-	5	5	-	-	-	-	-	-
Calculated concentration (µg/L)	-	-	4	6	-	-	-	-	-	-
Trueness (% relative error)	-	-	20.0	20.0	-	-	-	-	-	-

run-to-run and day-to-day precisions, and trueness under optimal conditions were established, and the figures of merit are given in Table 2. ILODs, based on a signal-to-noise ratio of 3:1, were calculated by analyzing phthalate standard solutions at decreasing concentration levels, and values in the range 1 to 9 µg/L, with DIBP and DBP being the two phthalates with the higher ILOD values, were achieved. As can be seen, for those phthalic acid esters showing UHPLC background levels (DIBP, DBP, and DEHP) the use of the C18 hold-back column allowed the decrease of ILOD values to the same levels as those obtained for the other PAEs, allowing the perfect detection of all the analyzed compounds at the levels established by legislation. ILOQ values, based on a signal-to-noise ratio of 10:1, between 4 and 30 µg/L were obtained. These values are similar to those described in the literature with the same kind of instrumentation [29-31].

External calibration curves based on peak area at concentrations between LOQs and 1000 µg/L were established and good linearity within the evaluated working range was observed with correlation coefficients (r^2) higher than 0.997.

Run-to-run and day-to-day precisions for PAE quantitation using external calibration were calculated at several concentration levels depending on the compound (see Table 2). In order to determine run-to-run precision, five replicate determinations for each concentration level were performed. Similarly, day-to-day precision was calculated by performing 15 replicate determinations for each concentration level on three non-consecutive days (five replicates each day). Overall, relative standard deviation (%RSD) values obtained for the run-to-run precision were in the range 0.3-13.4%, increasing to 4.1-23.9% for the day-to-day precision. The precisions obtained were quite acceptable taking

into consideration the methodology employed and the concentration levels analyzed.

Instrumental method trueness was also established. In general, very good results, with relative error (%) values lower than 10.9% were obtained, and only in very few cases values increased up to 14-20% at, in general, low concentration levels (for instance, DAP and DPrP at 5 µg/L). Thus, overall the proposed method showed acceptable trueness.

The good results obtained in the instrumental method validation in terms of detection, precision and trueness show that the proposed UHPLC-ESI-MS/MS method, using a hold-back column to prevent the effect of background contamination of the instrument by PAEs, is a satisfactory methodology for the quantitative analysis of the 10 studied PAEs.

Analysis of mineral water samples

The suitability of the developed UHPLC-ESI-MS/MS method for the determination of the ten studied phthalates in plastic-packed mineral water samples was studied. First, a matrix study effect at a low concentration level was performed. For this purpose, a blank mineral water sample (packed in glass) fortified at 50 µg/L was analyzed, and the phthalate signal was compared to those obtained with a phthalate standard prepared in Chromasolv® LC-MS water at the same concentration level. Because sample fortification was performed by employing plastic pipettes, procedural blanks were also analyzed, which showed no detectable signal for any of the analyzed phthalates (see example in Fig. 3S in the supplementary information). A statistical paired-sample comparison analysis was carried out. For a 95% confidence level, the phthalate peak areas obtained with both mineral and Chromasolv® LC-MS water samples were not significantly different, with a p value (0.83) higher than 0.05 (probability at the confidence level). Therefore, no matrix effect was observed for this kind of samples and quantitation by external calibration can be proposed for their analysis. Method limits of detection and of quantitation (MLODs and MLOQs) in the mineral water matrix were then determined and the same values reported for ILODs and ILOQs were obtained (Table 2).

Method trueness was also determined. For this purpose, a blank mineral water sample spiked at 50 µg/L with each phthalate was analyzed (triplicate), and the phthalate concentration levels were quantified by external calibration. The results obtained are given in Table 3. Very good method trueness, with relative errors lower than 7.4%, was achieved.

Once the suitability of the UHPLC-ESI-MS/MS method for the determination of phthalates in mineral water samples was assessed, it was applied for the analysis (in triplicate) of fifteen PET-packed mineral water samples (PET bottles of different volumes and plastic colors), and the obtained PAE levels are reported in Table 1S (supplementary information). Fig. 4 shows the chromatogram obtained for mineral water sample 1. Only one phthalate (DCP) was detected in four of the analyzed samples but at concentrations bellow the MLOQ (7 µg/L). These results show that PET plastic mineral water bottles are normally safe for human consumption and at most only phthalate concentrations below µg/L level are attainable, which is in agreement with the levels typically reported in the literature [4, 16].

Phthalate migration study in water samples

A study of migration of phthalates from PET plastic bottles to the mineral water they hold was performed. For this purpose, mineral water samples were kept for six months (October 2016 to April 2017) under four different exposure conditions (10 water bottles per condition): (i) at room temperature and protected from light, (ii) under climate-not-controlled environment conditions (bottles exposed to light and climate variations in a balcony), (iii) at 40 °C (temperature controlled in an oven), and (iv) at -4 °C (temperature controlled in a freezer). These exposure conditions were selected taking into consideration that they were easily achievable in a domestic setting, although at extreme temperature conditions (for instance higher temperatures) higher phthalate migration may happen due to plastic degradation.

All samples were homogenized by shaking them and were opened on the same day. Then, 1 mL of mineral water samples were measured with a plastic pipette and transferred from each bottle to injection glass vials that were kept at -4 °C until analysis. In order to control possible phthalate

Table 3. Method trueness in the UHPLC-ESI-MS/MS analysis of a mineral water sample.

Compound	Spiked concentration (µg/L)	Calculated concentration (µg/L)	Trueness (% relative error)
DMP	50.0	46.3 ± 4.5	7.4
DEP	51.0	51.3 ± 2.9	2.6
DAP	50.0	49.0 ± 1.7	2.0
DPrP	50.0	48.3 ± 3.2	3.4
DIBP	50.0	47.0 ± 4.4	6.0
BBP	50.0	47.3 ± 4.0	5.4
DBP	50.0	49.7 ± 5.8	0.6
DCP	50.0	49.0 ± 6.9	2.0
DNHP	50.0	51.0 ± 1.0	2.0
DEHP	50.0	46.3 ± 0.6	7.4

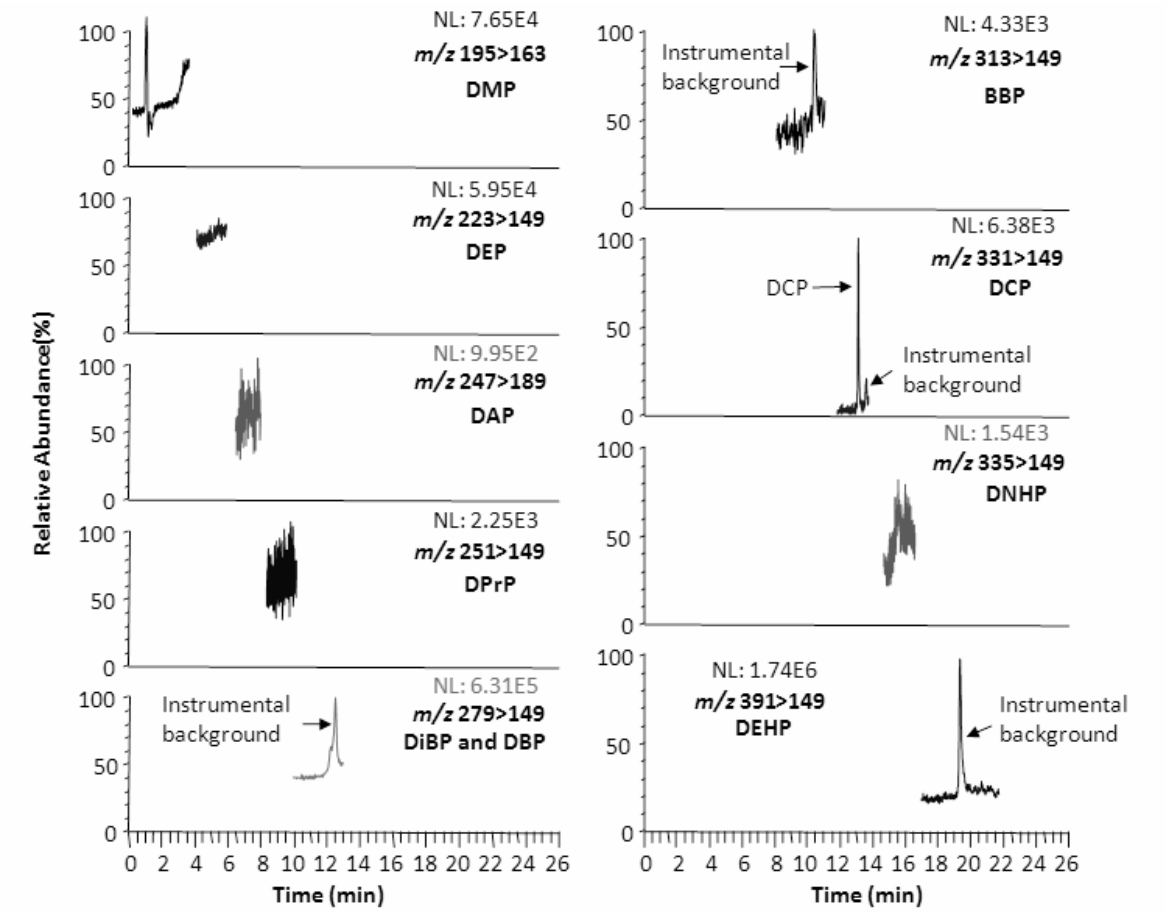

Fig. 4. UHPLC-ESI-MS/MS chromatogram (quantifier SRM transition) for the mineral water sample number 1.

contamination occurring from the plastic pipettes employed or from the vials during their time of storage in the freezer, procedural blanks (in triplicate) were performed employing Chromasolv® LC-MS water and they were also kept in the freezer during the same period. Every month, mineral water sampling and procedural blanks were carried-out. All samples and procedural blanks were randomly analyzed in the same sequence using the proposed UHPLC-ESI-MS/MS method.

Regarding the results, it should be mentioned that no phthalate signal background was observed in any of the procedural blanks performed. Of the four exposure conditions evaluated, only when the bottles were kept at 40 °C the migration of DEP was observed. Fig. 5a shows the variation of DEP signal during the studied period for one of the mineral water samples under 40 °C, and Fig. 5b shows the chromatogram for DEP (quantifier transition) in one of these samples at the third month of study. As can be seen, DEP was not detected before the beginning of the migration study (time zero in October 2016), and the highest migration was observed during the first month. Then migration slightly increased through the second and third month of exposure, but then a constant signal was observed. However, it should be mentioned that the observed signal for DEP was in all the samples bellow the established MLOQ for mineral water.

These study shows that mineral water samples packed in PET bottles are perfectly safe for human consumption from the point of view of phthalate migration at common exposure conditions in domestic settings for, at least, a half a year period.

Analysis of olive oil samples

The applicability of the proposed UHPLC-ESI-MS/MS method for the determination of phthalates in plastic-packaged olive oil was also evaluated. Olive oil samples were extracted by employing a simple sample preparation method (see 'Samples and sample treatment' section) consisting of a liquid-liquid extraction with acetonitrile, followed by evaporation and reconstitution in methanol. As previously commented, due to the ubiquitous presence of phthalates in the laboratory environment, procedural blanks, in triplicate, were also performed each time the olive oil samples were processed. As an example, Fig. 6 shows the extracted ion chromatogram of a procedural blank. Only DIBP, DBP, DCP and DEHP provided a detectable background contamination signal originating from

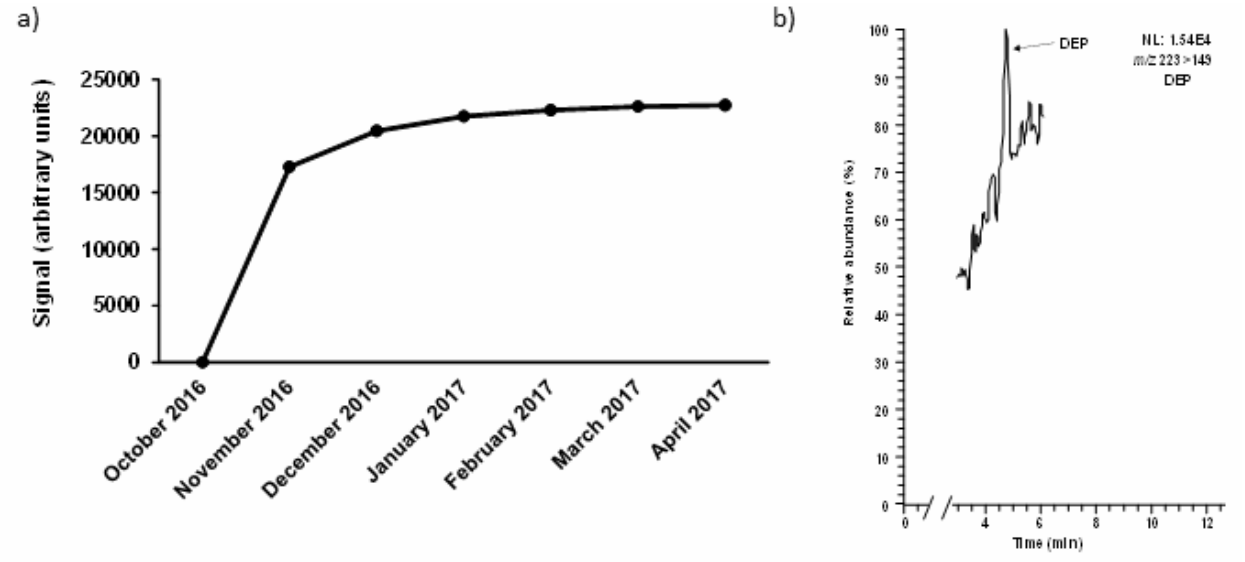

Fig. 5. Results of the study of migration of phthalates from PET bottles to mineral water when exposed at 40 °C. (a) Variation of DEP signals during a six-month period. (b) Chromatogram of DEP (quantifier SRM transition) for one of the mineral water samples during the third month of exposure.

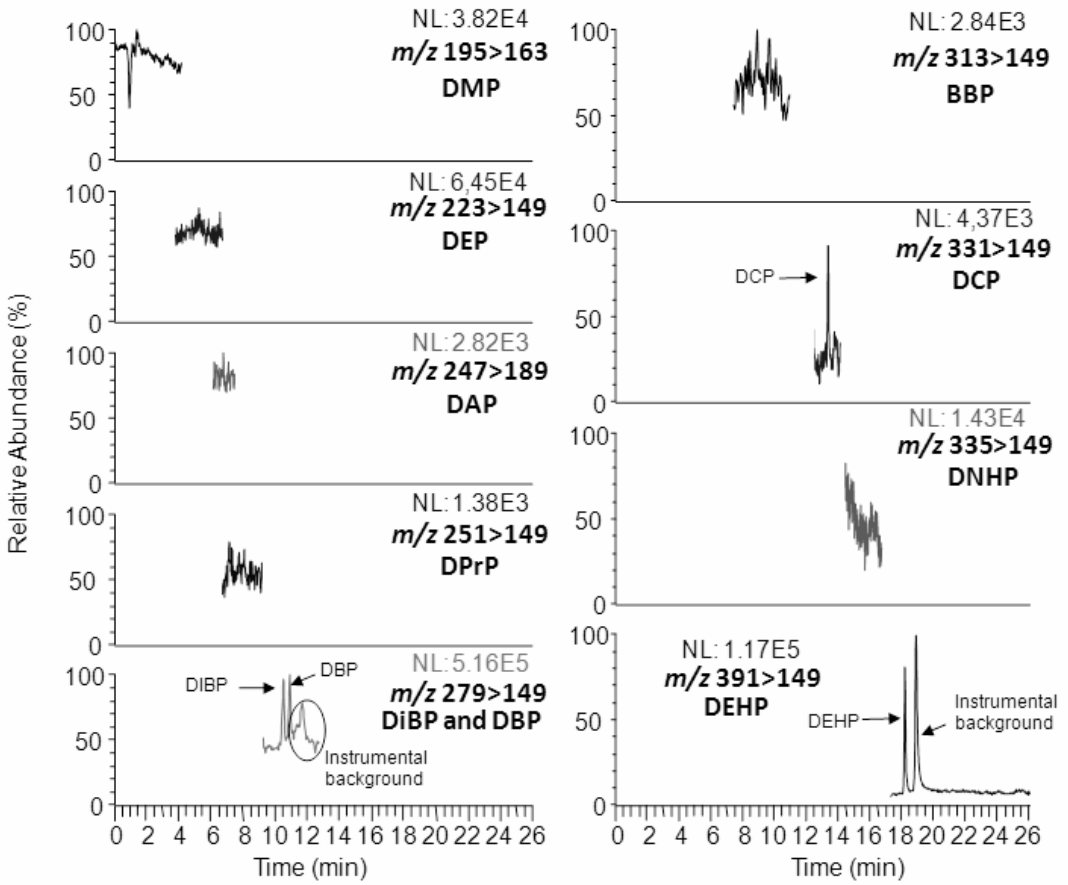

Fig. 6. UHPLC-ESI-MS/MS chromatogram (quantifier SRM transition) obtained with the procedural blank sample of the liquid-liquid extraction procedure employed for the analysis of olive oil samples.

the laboratory environment and the material employed in the olive oil sample treatment used. Thus, the obtained responses for each phthalate were employed to correct the signals in any olive oil sample analyzed.

First, a study of the matrix effect was carried out by employing a blank olive oil packed in a glass bottle. For this purpose, blank samples were processed and the final extracts were fortified with the studied phthalates at four different concentration levels (1000, 500, 100 and 50 µg/kg). The extracts were then analyzed with the proposed analytical method and the signal response obtained for each compound was compared to the one observed when analyzing phthalate standards prepared in LC-MS water matrix at the same concentration levels. The results obtained are shown in Fig. 4S (supplementary information). In contrast to the behavior observed when analyzing mineral water samples, an important matrix effect based on ion suppression was obtained for all the phthalate compounds at the four evaluated concentration levels. These effects were particularly important for some phthalates such as DMP, DCP, DNHP and DEHP, and more noticeable with the increase in the phthalate concentration level. As a consequence of this considerable matrix effect, external calibration cannot be employed, and standard addition would be the ideal calibration method in this application in order to correct the observed matrix effect. However, standard addition is a laborious and time-consuming quantitation method when dealing with the analysis of a high number of samples. Thus, in the present work, quantitation by matrix-matched calibration wherein the calibration standards are prepared using a blank olive oil sample packed in a glass container was proposed for the determination and quantitation of phthalates in plastic-packed olive oil.

Method performance of the analysis of olive oil was evaluated by determining method limits of detection (MLODs) and limits of quantitation (MLOQs), as well as the recoveries and the method trueness at the four concentration levels previously commented, and the figures of merit are shown in Table 4. MLODs, based on a signal-to-noise ratio of 3:1, were in the range 0.1-1.0 µg/kg, while MLOQs, based on a signal-to-noise ratio of 10:1, were between 0.3 and 3.3 µg/kg. For those phthalates showing responses in the procedural blanks (DIBP, DBP, DCP and DEHP) MLODs were calculated based on a 3:1 signal with respect to the one obtained as background level in the procedural blanks. This fact explains that for these compounds, slightly higher MLOD and MLOQ values were observed in comparison to the other phthalates. Nevertheless, the values obtained were acceptable for the determination of this family of compounds at the required legislated levels.

In order to evaluate method recoveries, blank olive oil samples were fortified with phthalates at the four evaluated concentration levels, and kept at room temperature and protected from light for 24 h before being processed in order to better simulate the extraction of phthalates from a real olive oil sample. At the same time, non-spiked blank olive oil samples were processed and the final extracts fortified with phthalates at concentrations corresponding to a 100% recovery. Then, recovery values were calculated by comparing the response observed for each compound in the fortified blank olive oil samples with those observed when fortifying the non-spiked blank olive oil extracts. Overall, all the studied phthalates showed high recovery values (between 85.5 and 95.5%) at the four evaluated concentration levels (Table 4). Only slight differences between concentration levels for each specific phthalate were observed. The obtained recoveries are in agreement with those described in the literature [17]. These results showed that the simple liquid-liquid extraction method proposed is appropriate for the quantitative extraction of phthalates from olive oils.

Method trueness was calculated by quantifying spiked blank olive oil samples at different concentration levels (see Table 4) using matrix-matched calibration (standards from MLOQ to 1000 µg/kg). In general, relative errors (%) below 6.0% were observed for all compounds and concentration levels, and only in some specific cases such as BBP and DEHP at 100 µg/kg and

Table 4. UHPLC-ESI-MS/MS method performance in the analysis of olive oil samples.

Phthalate	MLOD (µg/kg)	MLOQ (µg/kg)	Method recovery (%)				Method trueness (% relative error)			
			1 mg/kg level	500 µg/kg level	100 µg/kg level	50 µg/kg level	1 mg/kg level	500 µg/kg level	100 µg/kg level	50 µg/kg level
DMP	0.8	2.8	91.0	89.5	90.0	90.1	1.3	0.6	1.0	4.0
DEP	0.7	2.2	93.1	92.1	90.8	90.0	0.1	0.8	1.0	2.0
DAP	0.4	1.3	91.5	91.2	90.9	90.0	3.3	1.8	1.0	6.0
DPrP	0.2	0.7	94.9	95.2	92.1	91.1	1.2	0.1	1.0	0.1
DIBP	1.0	3.3	89.5	87.5	88.0	88.6	0.9	0.2	0.1	4.0
BBP	0.1	0.3	88.4	89.5	86.2	85.5	0.9	1.8	11.1	12.0
DBP	1.0	3.3	92.5	94.2	90.5	89.4	2.1	1.6	0.1	2.0
DCP	1.0	3.3	95.5	94.5	91.2	90.5	1.8	1.0	3.0	0.2
DNHP	0.3	1.1	87.4	86.2	85.1	87.9	1.1	0.1	4.0	6.0
DEHP	1.0	3.3	88.6	88.0	87.2	86.8	1.2	0.4	16.0	18.0

Table 5. Plastic-packaged olive oil samples analyzed using the proposed UHPLC-ESI-MS/MS method (concentrations in µg/kg).

Sample	DMP	DEP	DAP	DPrP	DIBP	BBP	DBP	DCP	DNHP	DEHP
Arbequina EVOO 1	< MLOQ	5.0 ± 0.24	n.d.	n.d.	10.0 ± 0.8	30 ± 1	14.0 ± 0.8	< MLOQ	n.d.	275 ± 30
Arbequina EVOO 2	3.0 ± 0.4	n.d.	n.d.	n.d.	11 ± 3	92 ± 9	173 ± 15	< MLOQ	n.d.	< MLOQ
Arbequina EVOO 3	n.d.	6.9 ± 0.2	n.d.	n.d.	8.6 ± 0.3	15 ± 1	15 ± 1	< MLOQ	n.d.	< MLOQ
Picual EVOO 1	< MLOQ	< MLOQ	n.d.	n.d.	< MLOQ	11.0 ± 0.4	< MLOQ	< MLOQ	n.d.	< MLOQ
Picual EVOO 2	< MLOQ	n.d.	n.d.	n.d.	6.9 ± 0.7	10.0 ± 0.7	14 ± 2	< MLOQ	n.d.	< MLOQ
Picual EVOO 3	3.69 ± 0.06	< MLOQ	n.d.	n.d.	17.7 ± 0.3	5.2 ± 0.8	73 ± 4	< MLOQ	n.d.	< MLOQ
Picual EVOO 4	17.7 ± 0.5	< MLOQ	n.d.	n.d.	49 ± 4	44 ± 2	309 ± 16	< MLOQ	n.d.	< MLOQ
Picual EVOO 5	< MLOQ	< MLOQ	n.d.	n.d.	< MLOQ	< MLOQ	< MLOQ	< MLOQ	n.d.	< MLOQ
Cornicabra EVOO 1	10.3 ± 0.4	< MLOQ	n.d.	n.d.	14.7 ± 0.8	< MLOQ	32 ± 2	< MLOQ	n.d.	< MLOQ
Cornicabra EVOO 2	< MLOQ	n.d.	n.d.	n.d.	5 ± 1	122 ± 11	15 ± 2	< MLOQ	n.d.	< MLOQ
VOO 1	< MLOQ	< MLOQ	n.d.	n.d.	< MLOQ	< MLOQ	< MLOQ	< MLOQ	n.d.	16 ± 1
VOO 2	< MLOQ	< MLOQ	n.d.	n.d.	5 ± 1	< MLOQ	5 ± 1	< MLOQ	n.d.	< MLOQ
VOO 3	3.5 ± 0.3	4.51 ± 0.07	n.d.	n.d.	17.5 ± 0.2	41 ± 4	18 ± 1	< MLOQ	n.d.	< MLOQ
VOO 4	< MLOQ	< MLOQ	n.d.	n.d.	7.1 ± 0.9	24 ± 2	129 ± 5	< MLOQ	n.d.	< MLOQ
EVOO 1	< MLOQ	n.d.	n.d.	n.d.	< MLOQ	< LOQ	4.7 ± 0.2	< MLOQ	n.d.	< MLOQ
EVOO 2	< MLOQ	n.d.	n.d.	n.d.	7.1 ± 0.7	69 ± 2	59 ± 3	< MLOQ	n.d.	< MLOQ
EVOO 3	n.d.	n.d.	n.d.	n.d.	< MLOQ	< MLOQ	< MLOQ	< MLOQ	n.d.	< MLOQ
EVOO 4	< MLOQ	< MLOQ	n.d.	n.d.	3.2 ± 0.8	42 ± 2	< MLOQ	< MLOQ	n.d.	< MLOQ
EVOO 5	5.3 ± 0.3	n.d.	n.d.	n.d.	21.4 ± 0.9	39 ± 3	254 ± 12	< MLOQ	n.d.	< MLOQ
EVOO 6	< MLOQ	n.d.	n.d.	n.d.	7 ± 2	40 ± 4	21 ± 1	< MLOQ	n.d.	15 ± 2
EVOO 7	7.7 ± 1.0	< LOQ	n.d.	n.d.	15.6 ± 0.2	24 ± 2	269 ± 13	< MLOQ	n.d.	< MLOQ
EVOO 8	< MLOQ	8.1 ± 0.2	n.d.	n.d.	20.0 ± 0.2	< MLOQ	21 ± 1	< MLOQ	n.d.	< MLOQ

Table 5 continued..

Sample	DMP	DEP	DAP	DPrP	DIBP	BBP	DBP	DCP	DNHP	DEHP
EVOO 9	< MLOQ	< MLOQ	n.d.	n.d.	8.1 ± 0.8	16.0 ± 0.9	8.8 ± 0.7	< MLOQ	n.d.	< MLOQ
EVOO 10	< MLOQ	6.6 ± 0.4	n.d.	n.d.	10 ± 2	51.7 ± 0.2	9 ± 1	< MLOQ	n.d.	< MLOQ
EVOO 11	5.3 ± 0.4	5.1 ± 0.3	n.d.	n.d.	3.5 ± 0.2	5.0 ± 0.4	123 ± 5	< MLOQ	n.d.	64 ± 3
EVOO 12	n.d.	12.5 ± 0.1	n.d.	n.d.	8.0 ± 0.2	91 ± 8	19 ± 2	< MLOQ	n.d.	< MLOQ
ROO 1	5.2 ± 0.3	6.40 ± 0.5	n.d.	n.d.	27 ± 3	9 ± 1	47 ± 3	< MLOQ	n.d.	15.1 ± 0.7
ROO 2	5.4 ± 0.3	6.0 ± 0.4	n.d.	n.d.	7.7 ± 0.2	125 ± 4	10.1 ± 0.6	< MLOQ	n.d.	< MLOQ
ROO 3	< MLOQ	8.3 ± 0.3	n.d.	n.d.	29.9 ± 0.5	< MLOQ	45 ± 4	< MLOQ	n.d.	< MLOQ
ROO 4	< MLOQ	17.8 ± 1.0	n.d.	n.d.	8 ± 1	11.9 ± 0.3	6.94 ± 0.09	< MLOQ	n.d.	< MLOQ

All concentrations are given as the Mean value ± Standard deviation.
n.d: not detected.

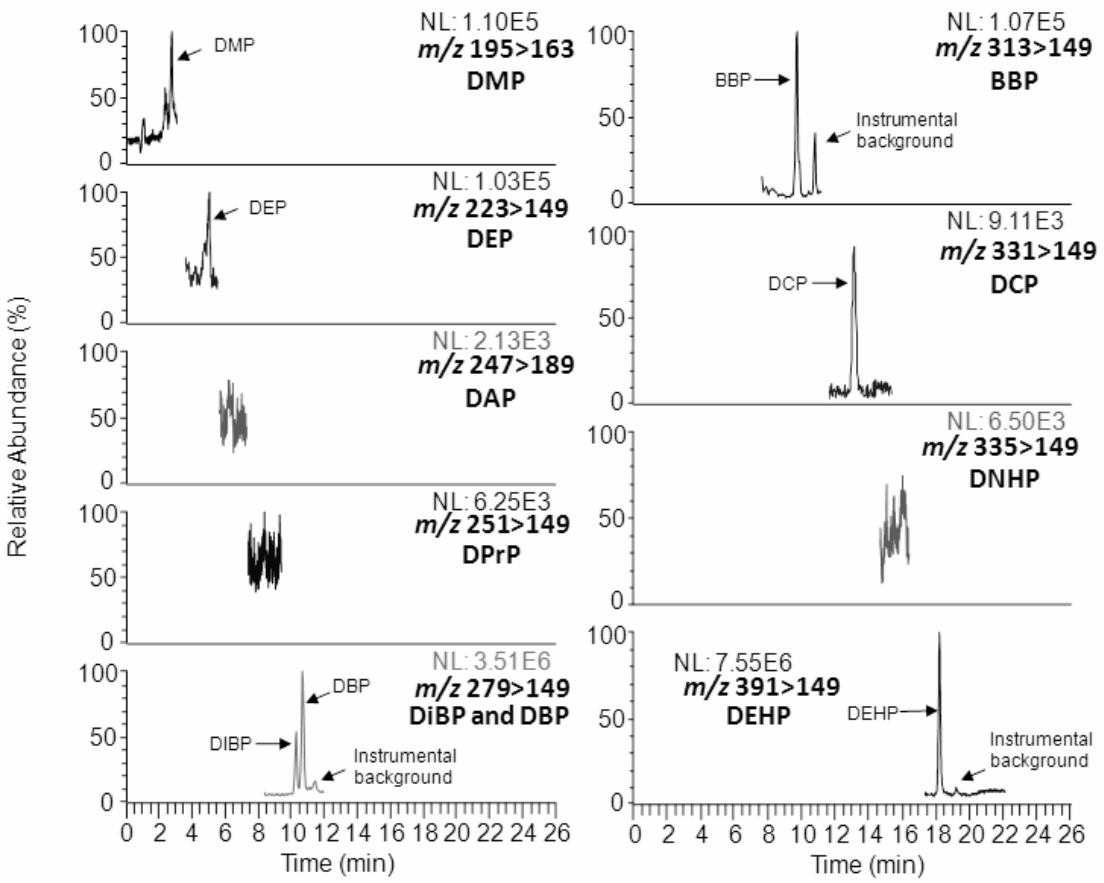

Fig. 7. UHPLC-ESI-MS/MS chromatogram (quantifier SRM transition) for the refined olive oil sample number 1.

50 μg/kg concentration levels trueness worsened up to 11.1-18.0%, although these values were acceptable taking into consideration the methodology employed, the analyzed samples, and the concentration levels.

The results obtained showed that the proposed UHPLC-ESI-MS/MS method is suitable for the quantitative analysis of phthalates in olive oil samples. The method was then applied for the analysis in triplicate of 30 plastic-packed olive oil samples, and the concentration levels found (given as the mean value ± standard deviation) are depicted in Table 5. As an example, Fig. 7 shows the chromatogram obtained for one of the analyzed samples (refined olive oil sample 1). As can be seen in Table 5, DAP, DPrP and DNHP were not detected in any of the analyzed samples. In contrast, DIBP, BBP, DBP and DEHP were detected in all the samples, although DCP and DEHP levels were always below the LOQ value, except for the DEHP in five samples (in one of them, Arbequina EVOO sample 1, a relatively high concentration level, 275 ± 30 μg/kg, was observed). DMP and DEP were detected and quantified in most of the analyzed samples. The highest concentration levels observed were for DBP in five samples (concentration between 123 ± 5 and 309 ± 16 μg/kg) followed by DEHP in one sample (275 ± 30 μg/kg) and BBP in two samples (122 ± 11 and 125 ± 4 μg/kg, respectively), but in most of the cases low μg/kg concentration values were found. These results are generally in agreement with the phthalate concentration levels reported in the literature in the same kind of samples, [30, 32] and although the presence of several phthalates was found in the analyzed Spanish olive oils, the majority of them are within the legislated accepted levels. Only one sample, Picual EVOO 4 showed the presence of DBP at a concentration equal to the EU established SML value (0.3 mg/kg) [10].

CONCLUSION

The results obtained in this work showed that the proposed UHPLC-ESI-MS/MS method using a C18 hold-back column was selective and reliable for the quantitative determination of phthalates in mineral water samples and olive oil samples packed in plastic bottles at the concentration levels established by EU legislation.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support received from Spanish Ministry of Economy and Competitiveness under the project CTQ2015-63968-C2-1-P and from the Agency for Administration of University and Research Grants (Generalitat de Catalunya, Spain) under the project 2017 SGR310.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ABBREVIATIONS

BBP, benzyl butyl phthalate; DAP, diallyl phthalate; DBP, di-n-butyl phthalate DCP, dicyclohexyl phthalate; DEHP, di(2-ethylhexyl) phthalate; DEP, diethyl phthalate; DIBP, diisobutyl phthalate; DLLME, dispersive liquid-liquid microextraction; DMP, dimethyl phthalate; DNHP, di-n-hexyl phthalate; DPrP, di-n-propyl phthalate; ESI, electrospray; EVOO, extra-virgin olive oil; FID, flame ionization detector; FWHM, full width at half maximum; GC, gas chromatography; HRMS, high resolution mass spectrometry; ILOD, instrumental limit of detection; ILOQ, instrumental limit of quantitation; LC, liquid chromatography; LLE, liquid-liquid extraction; MLOD, method limit of detection; MLOQ, method limit of quantitation; MRM, multiple reaction monitoring; MS, mass spectrometry; PAE, phthalic acid ester; PET, polyethylene terephthalate; PVC, polyvinyl chloride; ROO, refined olive oil; RSD, relative standard deviation; SE, solvent extraction; SIM, selected ion monitoring; SML, specific migration limit; SPE, solid-phase extraction; SPME, solid-phase microextraction; TOF, time-of-flight; UHPLC, ultra-high pressure liquid chromatography; VOO, virgin olive oil.

SUPPLEMENTARY INFORMATION

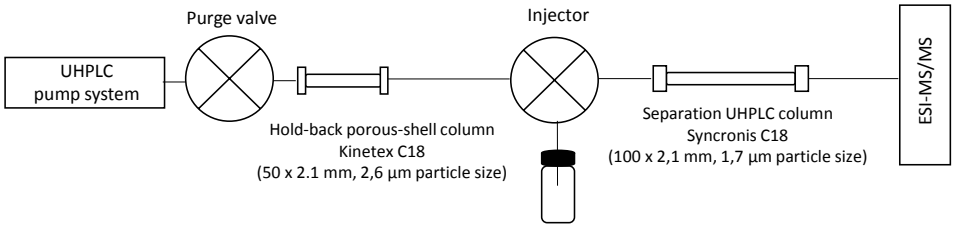

Fig. 1S. Scheme of the UHPLC system employed, showing the positions of the holdback and separation columns.

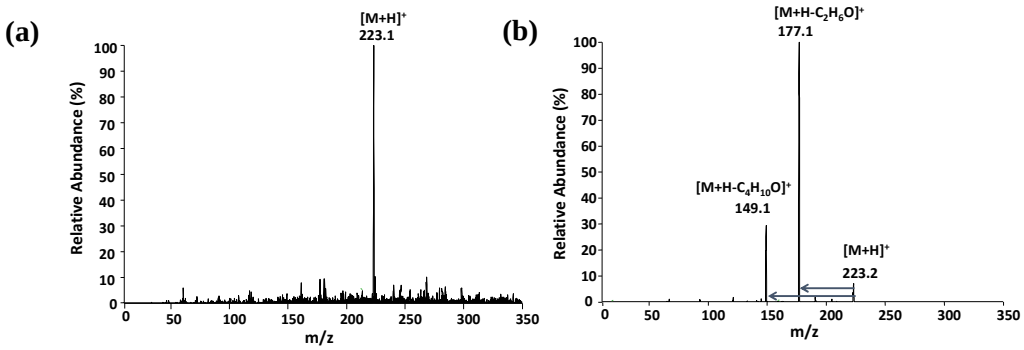

Fig. 2S. (a) Full-scan MS and (b) MS/MS spectra of DEP.

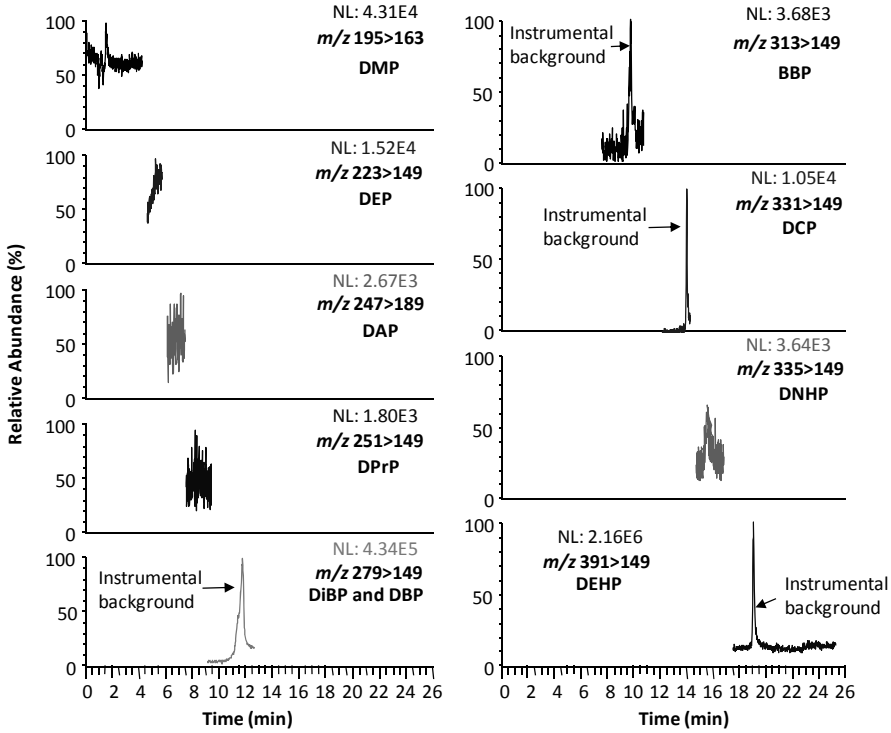

Fig. 3S. Procedural blank (plastic pipettes) employed in the analysis of mineral water samples. Only SRM quantifier transitions are shown.

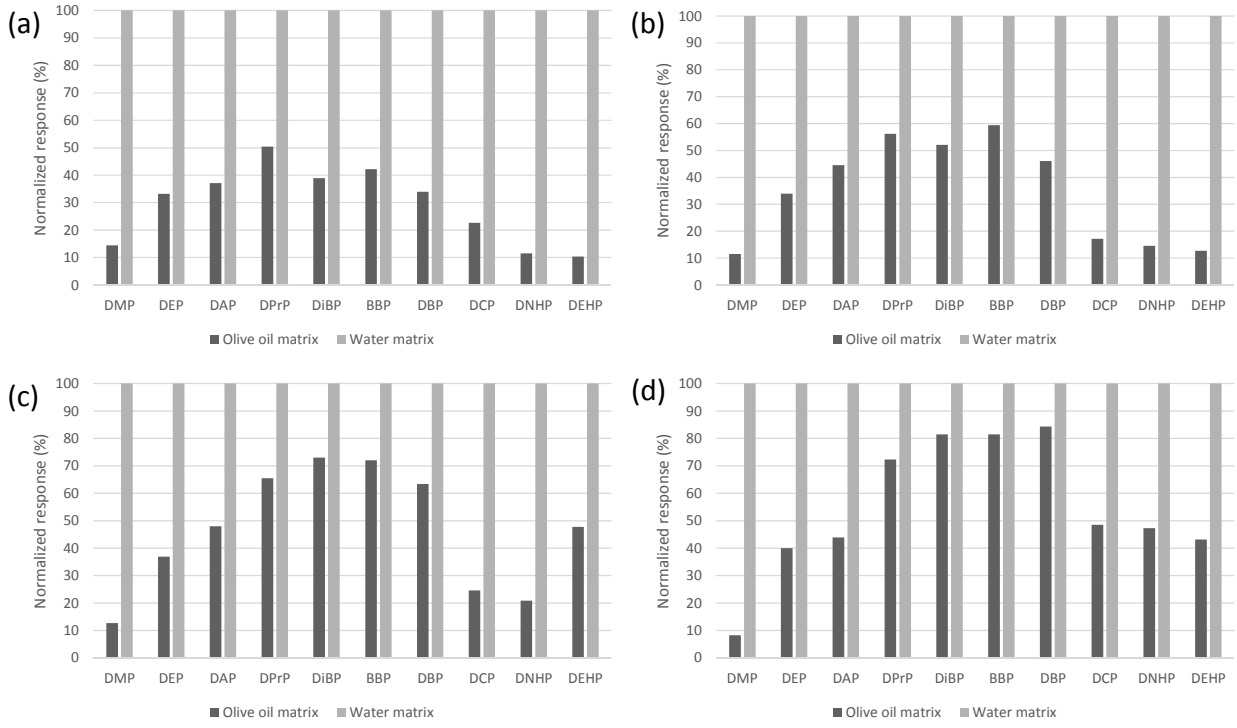

Fig. 4S. Study of matrix effect. Comparison of normalized phthalate signal response in water matrix and in an olive oil matrix at different phthalate concentration levels: (a) 1000 µg/kg, (b) 500 µg/kg, (c) 100 µg/kg and (d) 50 µg/kg.

Table 1S. Plastic-packaged mineral water samples analyzed using the proposed UHPLC-ESI-MS/MS method (µg/L).

Sample	Container	DMP	DEP	DAP	DPrP	DIBP	BBP	DBP	DCP	DNHP	DEHP
Mineral water 1	PET (blue), 330 mL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< MLOQ	n.d.	n.d.
Mineral water 2	PET, 500 mL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< MLOQ	n.d.	n.d.
Mineral water 3	PET, 500 mL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Mineral water 4	PET, 250 mL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< MLOQ	n.d.	n.d.
Mineral water 5	PET, 501 mL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< MLOQ	n.d.	n.d.
Mineral water 6	PET, 500 mL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Mineral water 7	PET (red), 500 mL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Mineral water 8	PET, 330 mL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Mineral water 9	PET, 8000 mL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Mineral water 10	PET, 500 mL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Mineral water 11	PET, 1500 mL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Mineral water 12	PET, 330 mL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Mineral water 13	PET, 330 mL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Mineral water 14	PET, 1500 mL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Mineral water 15	PET, 500 mL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not detected.

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