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Methodology for Determining Phthalate Residues by Ultrasound–Vortex-Assisted Dispersive Liquid–Liquid Microextraction and GC-IT/MS in Hot Drink Samples by Vending Machines

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Abstract: In this study, a simple, fast, and effective methodology has been developed for the detection and quantification of seven phthalates potentially released in hot drinks from disposable containers used in vending machines. The authors determined the optimal conditions to be applied during the various steps of extraction of seven phthalates (DMP, DEP, DBP, DiBP, DEHP, DNOP, and DDP) from hot beverages using a model solution. The extraction and preconcentration technique used was ultrasound–vortex-assisted dispersive liquid–liquid microextraction (UVA-DLLME) followed by gas chromatographic analysis obtaining recoveries from 66.7% to 101.2% with precision and reproducibility <6.3% and <11.1%, respectively. The influence of waiting time, from the dispensing of the drink to its actual consumption, for the extraction of molecules was investigated, obtaining a temporal release profile slightly shifted towards the PAEs with higher molecular weight and vice versa for those with low molecular weight. In addition, the best instrumental parameters to be applied during the analysis of the extracts obtained were established. This optimization was carried out using GC-FID, whereas the analysis of real samples was carried out by means of GC-IT/MS for ultra-trace analysis purposes; limits of detection (LODs) ranging between 0.8 ng mL^{-1} and 15.4 ng mL^{-1} and limits of quantification (LOQs) from 1.6 ng mL^{-1} to 35.8 ng mL^{-1}, both of them lower than those found by FID, were obtained.

Keywords: UVA-DLLME; residuals; phthalates; GC-FID; GC-IT/MS; coffee; tea; espresso; vending machine

1. Introduction

Food products supplied by vending machines represent the main source of nourishment available in indoor public environments such as universities, schools, and offices [1]. The purchase of foodstuffs, whatever they are, through these distributors involves considerable saving in terms of time for the consumer. It is, therefore, evident that in order to obtain speed of service, food and drinks are dispensed using containers made of more or less resistant plastic material.

The main materials used for the construction of plastic containers are polyethylene terephthalate (PET), polythene (PE), polypropylene (PP), polyvinyl chloride (PVC), and polystyrene (PS) [2]. The polymeric molecular structure of plastic containers makes them

incredibly versatile, but at the same time, generates possible risks for the health of the consumer [3]. In other words, plastic containers have countless functional properties such as protection against microorganisms and exogenous insects, prevention of loss of flavor and humidity, but at the same time, there is risk that monomers, oligomers, and plasticizing additives migrate towards the food, contaminating it [4]. In fact, the industrial production process of plastics inevitably requires the use of specific catalysts and other additives, e.g., phthalic acid esters [5].

Phthalic acid esters, or better known as phthalates (PAEs), are added to the polymer molecular structure with the aim of increasing its flexibility, plasticity, and strength [6]. Their role is to insert themselves between the chains of plastic polymers, weakening their intermolecular bonds. This allows the polymer molecules to flow and move mutually, transforming the plastic material from rigid to flexible, soft, and moldable even at room temperature [7]. Although PAEs are able to improve the structural and functional characteristics of plastics, the latter are not chemically bonded to the polymeric structure through covalent bonds, and for this reason, they can undergo a leaching process that makes them migrate from the polymeric structure to the food, resulting in chemical contamination [8]. It is, therefore, clear that plastic containers, or more generally plastic packaging, interact with liquid and solid foodstuffs through dynamic migration processes that mainly depend on the chemical–physical characteristics of the packaging, the contact time, the temperature of the system, and finally, the percentage of fat that characterizes the food [9]. Indeed, the quantities of plasticizing additives released by leaching into the food increase as the fat content increases [10]. This is justifiable thanks to the clearly apolar character of most of the plasticizers used, especially for PAEs, which are, therefore, able to dissolve inside the apolar matrices.

The main phthalates used to transform hard PVC resins into flexible and workable plastics are benzyl butyl phthalate (BBP), dimethyl phthalate (DMP), dibutyl phthalate (DBP), di-2-(ethylhexyl) phthalate (DEHP), and di-isobutyl phthalate (DIBP) [11]. For some time now, it has been shown that these molecules alter the endocrine system causing damage to the reproductive and cardiovascular systems, and finally, favor the onset of asthma and childhood obesity [12]. In fact, there is evidence that the role of phthalates in inducing an interruption of estrogenic activity and causing reproductive and hepatic toxicity in both laboratory animals and humans [13]. The population can be exposed to phthalates through food sources (solid and liquid foods) and environmental sources, for instance, the atmosphere, the indoor environment, and plastic objects. However, ingestion of food and drink represents one of the main routes of human PAE exposure [14]. Food contamination with PAEs can occur not only during storage but also during the manufacturing and packaging processes if the production lines have plastic components [15]. In this regard, the European legislative bodies together with the European Food Safety Authority (EFSA) have established the maximum quantities of phthalates that can migrate from the packaging consisting of plastic material to the food. These quantities are defined by Regulation (EU) n.10/2011 as specific migration limits (SMLs), i.e., the maximum permitted quantities of substances released from a material or object in food products or simulants [16]. The SML in food for DMP, DEP, and DIBP is 60 mg kg^{-1}, whereas for DBP and DEHP it is 0.3 and 1.5 mg kg^{-1}, respectively. Consequently, the need to determine the presence and quantity of PAEs in food and beverages has become increasingly important over time [17].

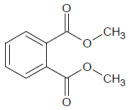
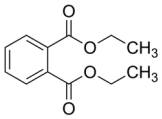
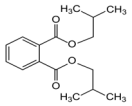
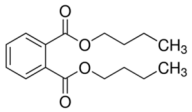
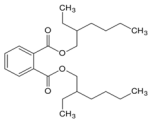
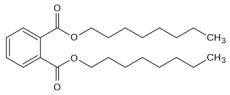
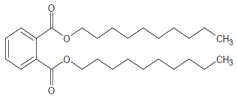
The aim of this work is to propose an easy-to-perform analytical method that allows the qualitative and quantitative determination of the phthalates eventually released by the polymeric structure of plastic containers that contain hot drinks dispensed by automated dispensers. In particular, all the differences in the analytical data obtained from gas chromatographic (GC) analysis coupled with two different detectors are highlighted; in one case, detection through a flame ionization detector (FID) and in the other, through the use of a mass spectrometer (MS). The proposed method suggests the microextraction of the analytes through the use of an extracting solvent suitably dispersed in solution (DLLME) [18,19].

2. Materials and Methods

2.1. Chemicals

The PAE standards used to carry out the qualitative–quantitative analysis were purchased from Sigma-Aldrich (Milan, Italy) and are as follows: Dimethyl phthalate (DMP); Diethyl phthalate (DEP); Diisobutyl phthalate (DiBP); Dibutyl phthalate (DBP); Di-2-ethylhexyl phthalate (DEHP); Dioctyl phthalate (DOP); Didecyl phthalate (DDP) (Table 1) [20,21].

Table 1. Name, chemical structure, acronym, CAS number, molecular weight, lethal dose 50 (DL_{50}), acceptable daily intake (ADI), solubility, octanol–water partition coefficient (K_{ow}), and boiling point of each phthalate (PAE) investigated in this study.

PAE	Symbol	CAS #	MW	DL_{50} [1] (g kg^{-1})	ADI [2] (ng kg^{-1})	Solubility [3] (mg L^{-1})	K_{ow} [4] (log k_{ow})	Boiling Point [5] (°C)
Dimethyl phthalate 	DMP	131-11-3	194.18	8–10	79.1	4000	1.6	283.7
Diethyl phthalate 	DEP	84-66-2	222.24	8–10	1.4-28.2	1080	2.47	295.0
Diisobutyl phthalate 	DiBP	84-69-5	278.34	8–10	105	6.2	4.11	327.0
Dibutyl phthalate 	DBP	84-74-2	278.35	8–10	191.8	11.2	4.5	340.0
Di-(2-ethylhexyl) phthalate 	DEHP	117-81-7	390.56	14	1458	0.27	7.6	386.9
Di-*n*-octyl phthalate 	DNOP	117-84-0	390.56	13	37×10^6	0.022	8.1	384.0
Di-*n*-decyl phthalate 	DDP	84-77-5	446.7	17	N/A	0.00022	9.05	268.0 [6]

[1] calculated related to rat; [2] expressed as body weight; [3] solubility in water; [4] octanol–water partition coefficient; [5] N/A data not available; [5] ref. [21]; [6] calculated at 5 mm Hg.

To create calibration curves, phenanthrene (Merks, Darmstadt, Germany) was used as an internal standard (I.S.). For preparing the I.S. and PAE standard solutions at known concentrations, dimethyl ketone was used as solvent. For the extraction process, n-heptane, purchased from Carlo Erba (Milan, Italy), was used as extraction solvent.

2.2. Extraction Procedure, Dispersive Liquid–Liquid Microextraction (DLLME)

For the extraction procedure, a modified procedure of DLLME, namely UVA-DLLME [7], was carried out followed by GC-FID analysis and confirmation by GC-IT/MS. All extraction conditions were experimentally optimized, carrying out extractions in which a known quantity of PAEs equal to 0.1 μg mL^{-1} was added to water samples and evaluating the recoveries after the entire extraction process using UVA-DLLME and subsequent analysis in GC-FID. The conditions studied were the following: choice of extraction solvent, influence of mechanical stirring times by vortexing, sonication time of the microemulsion, quantity of NaCl suitable for breaking the emulsion, centrifugation time and speed for the separation of the extraction solvent from the polar phase.

First, the extraction solvent was studied for identifying the best performance. To evaluate whether there could be a substantial difference in terms of analyte recovery using different organic solvents, the authors performed phthalate extraction tests using the following five different solvents: *n*-heptane, *iso*-octane, benzene, xylene, and cyclohexane. Extractions by UVA-DLLME were performed on distilled water samples enriched with the analytes of interest at a concentration of 0.1 μg mL^{-1} and brought to a temperature of 61 °C to simulate the real matrix. The boiling temperatures (T_{eb}) of all the solvents used for the extraction are higher than the average temperature of 61 °C at which the "hot drinks" are dispensed. This does not exclude any losses due to evaporation of the solvents during the whole extraction process. In any case, the tests show objective evidence that the solvents used do not suffer losses and that, therefore, they can be used for this analytical procedure.

To extract and preconcentrate the phthalates of interest from the real samples, the DLLME extraction procedure was applied, supported by the shaking treatment by vortexing and final ultrasonication (UVA-DLLME). This phase allows the PAEs passage from the aqueous matrix that characterizes the sample to the extraction solvent which is subsequently injected into the GC, allowing the extraction and concentration of the phthalates at the same time [22,23]. The applied procedure comes as follows: 10 mL of sample was transferred to a 10 mL Pyrex tube for centrifuge and 4 μL of I.S. (80 mgL^{-1}) and 250 μL of *n*-heptane were rapidly added. The latter represents the most suitable solvent for the PAEs extraction. The solution was subjected to vigorous stirring for 5 min using a vortex (ZX3, VELP Scientific, Usmate, Italy). This allows an efficient dispersion of the *n*-heptane by creating a microemulsion which increases the contact surface between the extraction solvent and the aqueous solution containing the PAEs and I.S. The obtained microemulsion was placed for 6 min in an ultrasonic bath (Strasonic, 18–35, Liarres.r.l., Casalfiumanese, Italy). The treatment with ultrasonic sound waves markedly favors dispersion and extraction through the phenomenon of cavitation [24]. Finally, the last step involved breaking the microemulsion and separating the apolar phase containing the PAEs and I.S. from the aqueous phase. The breaking of the microemulsion was favored by the addition of 10 g L^{-1} of NaCl, which increases the ionic strength of the solution. Subsequently, with the use of a centrifuge (Neya 8, Giorgio Bolmac s.r.l., Carpi, Italy) set at 4000 rpm for a duration of 30 min, the two phases were separated. A total of 1μL of supernatant was withdrawn and injected into GC-FID and GC-IT/MS.

2.3. Real Sample Analysis

For the analysis of real samples, different types of hot drinks dispensed from vending machines were chosen. The extraction procedure was applied to six real samples—espresso, long espresso, coffee, ginseng coffee, and tea. All samples were taken from a vending machine (Necta, Canto, Milan, Italy), dispensing exclusively hot drinks, present in public offices (specifically, authors' university), and constantly supplied by the company Sogeda vending machines. The samples were dispensed in polystyrene (PS) containers. PAE extraction from the real samples was carried out after 15 s from dispensing by measuring the temperature of each single sample. The temperature was 59 °C with very small variations between the various samples analyzed. After the extraction phase, the extracts were ready for GC analysis.

2.4. GC Analysis

2.4.1. GC-FID Analysis

The selective separation and subsequent detection of the analytes present in the sample were carried out through a gas chromatography technique, using a Master GC DANI gas chromatograph (Monza, Italy) equipped with a universal detector, the flame ionization detector (FID). The instrumental data obtained were processed using the Clarity software v.2.6.3 (Data Apex 2007, Prague, Czech Republic). The GC injector used is a programmed temperature vaporizer (PTV), which allows the analytes to pass through the vapor phase before entering the column. The injector temperature program is the following: from 110 °C to 280 °C at 800 °C min^{-1} after 8 s from injection and kept for 5 min at 280 °C. Two minutes after the injection, the splitter, adjusted in splittless mode, was opened making all the vapors flow exactly. Hydrogen was used as carrier gas at constant and linear velocity ($\bar{u}$) of 38 cm s^{-1}. A fused-silica capillary chromatographic column was used for the analyte separation with the following stationary phase: SE-54, 5% phenyl (polar part), and 95% dimethylsiloxane (apolar part) (Teknokroma, Rome, Italy). The column parameters were 30 m × 250 µm i.d. The film thickness (d_f) was 0.25 µm, the number of theoretical plates, *N*, that marks the column for *n*-dodecane at 90 °C was 120,000 [25]. The oven temperature was programmed from 100 °C to 280 °C at 10 °C min^{-1}, whereas the FID temperature was 310 °C.

2.4.2. GC-IT/MS Analysis

The analyses were subsequently carried out my means of a gas chromatograph equipped with a mass spectrometer as a detection system. The instrument used is composed of a Trace GC gas chromatograph (ThermoFischer, Milan, Italy) combined with a PolarisQ mass spectrometry (Thermo Finnigan, Bremen, Germany) equipped with an Ion Trap (IT/MS). The software used for the management of the instrument, the acquisition, and analysis of the data is Xcalibur 1.4.1. The chromatographic column used for the separation of the analytes is the same used in the analysis in GC-FID, namely SE-54 (Teknokroma), reporting the same analytical characteristics. A total of 1 µL of sample was injected into the GC-IT/MS. The injector used for introducing the sample into the chromatographic system was a PTV programmed in the following way in splitless mode: from 110 °C to 310 °C in 30 s at 800 °C min^{-1}, after 5 min at 310 °C. The separation valve was opened after 2 min. Helium (IP 5.5) was used as carrier gas at a flow of 1.0 mL min^{-1}. The transfer line temperature was set at 270 °C. The source that allows ionization by positive electronic impact of the analytes was set at a temperature of 250 °C and energy of 70 eV. All analytes were detected and recorded in Selected Ion Monitoring (SIM) mode. PAEs were identified according to their ion target and main fragment (m/z) as follows: DMP 163; DEP 149 and 177; DIBP 149 and 205; DBP 149 and 205; DEHP 149 and 167; DOP 149 and 167; DDP 149 and 177.

3. Results and Discussion

All analytical methodology was evaluated by means of GC-FID analysis. After, the confirmation was performed by means of GC-IT/MS analysis. Some real samples collected from vending machines were analyzed by this procedure.

3.1. Optimization of PAE Extraction Conditions

In this paper, each step of the entire extraction protocol and subsequent analysis was studied, along with the main analytical parameters. The whole procedure was optimized using blank samples (i.e., distilled water) brought to 61 °C for simulating any "hot drinks" dispensed by the vending machines. Evaluation of a possible matrix effect on the analyte recoveries was carried out after the optimization of the extraction method.

The water samples (10 mL) were spiked with each PAE until reaching a concentration equal to 0.1 µg mL^{-1} and 4 µL of I.S. at a concentration of 80 µg mL^{-1}. The volume of solvent used for the extraction was fixed at 250 µL. To facilitate the breaking of the emulsion, 1 g of NaCl was added. Three extractions were carried out for each solvent and

Table 2 shows the average values of the percentage recoveries of all the PAEs along with the standard deviations.

Table 2. PAE recoveries (%) related to different extraction solvents tested in this study.

PAE [1]	Recovery (%)				
	***n*-Heptane**	***iso*-Octane**	**Benzene**	**Xylene**	**Cyclohexane**
DMP	59.6 ± 5.8	33.5 ± 10.1	40.5 ± 8.1	42.8 ± 6.9	11.9 ± 6.2
DEP	66.7 ± 4.1	38.6 ± 7.6	60.3 ± 6.6	50.3 ± 2.5	39.9 ± 2.6
DiBP	108.9 ± 6.1	92.4 ± 4.5	109.4 ± 3.1	107.4 ± 6.1	62.3 ± 3.9
DBP	104.2 ± 2.6	90.4 ± 6.7	102.1± 7.7	98.9 ± 8.9	75.4 ± 7.9
DEHP	102.4 ± 6.0	89.2 ± 5.5	100.5 ± 9.2	101.2 ± 10.6	66.0 ± 5.7
DNOP	107.0 ± 5.7	101.7 ± 10.8	95.9 ± 9.1	111.0 ± 4.7	59.2 ± 8.2
DDP	100.0 ± 1.0	89.8 ± 11.9	97.0 ± 12.0	103.5 ± 1.6	74.4 ± 7.0

[1] For PAE acronyms, see Table 1.

The solvents showing the best recoveries are benzene and *n*-heptane. In particular, *n*-heptane allows a greater recovery of the two lower molecular weight phthalates, namely DMP and DEP. In any case, DMP and DEP always show significantly lower recoveries than other phthalates, regardless of the type of solvent used. This is essentially due to their molecular structure that gives a boiling temperature (T_{eb}) which favors their volatilization during the extraction process. Also, in terms of standard deviation (s.d.), the use of *n*-heptane shows better values than all the other solvents used. Benzene also shows very low standard deviation values and high recoveries, but on the other hand, it is a solvent with a high risk for the health of the operator who handles it. The International Agency for Research on Cancer (IARC) has placed benzene in group 1, which is a known human carcinogen.

As regards the mechanical stirring times, although prolonged time tests were carried out to allow a better operation of the extracting solvent in the solution, the recovery of the analytes did not show significant variations over 5 min (Table 3). Consequently, a time of 5 min was chosen as the standard time, which is not too long but sufficient for reaching the maximum recovery ratios.

Table 3. Recovery (%) of each phthalate as the mechanical stirring times vary.

PAE [1]	Recovery (%)			
	1 min	**5 min**	**7 min**	**9 min**
DMP	12.5 ± 1.0	22.4 ± 1.6	23.7 ± 1.4	29.2 ± 1.8
DEP	20.4 ± 0.8	88.6 ± 4.4	80.7 ± 4.0	83.6 ± 5.0
DiBP	10.1 ± 0.7	95.7 ± 3.7	94.1 ± 6.6	95.2 ± 5.7
DBP	12.7 ± 0.6	96.2 ± 4.8	100.1 ± 4.0	101.9 ± 6.1
DEHP	15.7 ± 0.9	99.6 ± 5.0	95.9 ± 5.7	92.3 ± 3.7
DNOP	11.1 ± 0.6	98.8 ± 4.9	97.2 ± 4.7	90.9 ± 5.4

[1] For PAE acronyms, see Table 1.

Regarding the sonication times in the ultrasonic bath, the enriched samples were kept inside the bath for various times (in particular: 6 min, 10 min, 14 min, and 18 min). All data of the recoveries showed no relevant variations except for the time at 18 min where the PAEs showed recoveries in a fairly wide range, between 81.4 and 133.4% (Table 4).

To favor the emulsion breaking, extractions were carried out by adding different quantities of sodium chloride (NaCl) after sonication; the ionic solution strength was increased and, consequently, the solubility of the extraction solvent decreased. Experiments with NaCl at different concentrations ranging between 1 g L^{-1} and 50 g L^{-1} were performed; the greater PAE recoveries were recorded by addition of NaCl 10 g L^{-1}.

Table 4. Recoveries (%) of each phthalate based on the sonication times in the ultrasonic bath.

PAE [1]	Recovery (%)			
	6 min	**10 min**	**14 min**	**18 min**
DMP	45.2 ± 3.2	41.7 ± 2.9	42.9 ± 3.0	39.2 ± 2.7
DEP	78.4 ± 4.7	69.6 ± 4.2	80.1 ± 4.8	81.4 ± 4.9
DiBP	96.6 ± 3.9	99.9 ± 4.0	99.2 ± 4.9	118.1 ± 4.7
DBP	97.4 ± 7.8	101.7 ± 8.1	101.9 ± 8.1	116.9 ± 9.3
DEHP	98.7 ± 6.9	102.6 ± 7.2	103.3 ± 7.2	133.4 ± 9.3
DNOP	99.5 ± 5.9	101.3 ± 6.1	102.2 ± 6.1	129.8 ± 7.8

[1] For PAE acronyms, see Table 1.

After evaluating the effect of NaCl addition, the authors moved on to determining the spin-time combination of the centrifuge that shows the best percentage recovery of the analytes. It was considered appropriate to evaluate only two rotor rpm values, in particular, 3500 and 4000 rpm, to avoid problems of centrifuge instability. For these rotor speed values, centrifugations of the microemulsions were carried out at different durations (10, 20, and 30 min). The values obtained are shown in Table 5. Looking at the data, it was considered appropriate to choose a speed of 4000 rpm for a duration of 30 min as operating values for centrifugal separation.

Table 5. Recoveries (%) obtained at 3500 and 4000 rpm at different times.

PAE [1]	Recovery (%)		
	10 min	**20 min**	**30 min**
3000 rpm			
DEP	77.9	78.4	70.3
DiBP	93.9	100.6	99.9
DBP	89.2	96.4	100.3
DEHP	92.2	95.7	97.8
DNOP	95.9	97.5	97.6
DDP	94.6	95.9	98.5
4000 rpm			
DEP	67.3	71.2	82.1
DiBP	92.0	92.9	103.7
DBP	103.0	98.2	102.0
DEHP	92.8	98.5	99.5
DNOP	91.7	97.4	103.6
DDP	94.7	96.4	101.2

[1] For PAE acronyms, see Table 1.

3.2. Influence of Time and Temperature on the PAE Extraction from Hot Drinks

This paper would like to focus the attention on the impact of the contact time between container and hot drinks on PAE release. PAEs at concentration 0.1 μg mL^{-1} were added to the samples (e.g., coffee, etc.) and, subsequently, analyzed by UVA-DLLME-GC-FID at different times, 0, 15, 40 and 120 s. Additionally, as time elapsed since dispensing (time zero) increases, the temperature of the samples decreases. In this regard, the influence of the sample temperature on the extraction of phthalates was evaluated. Figure 1 shows the recoveries obtained at different pickup times from the vending machine and at different temperatures (i.e., pick time 0 s, temperature 61 °C; 15 s, 59 °C; 40 s, 53 °C; 120 s, 35 °C).

The recovery levels obtained from the extraction tests indicate an optimal recovery in all time–temperature combinations. As regards the DEP extraction, the latter is greater than time zero, with a recovery value of 69.5%. All other PAEs show very similar recoveries in all tests performed, except for DBP. The latter shows recovery values close to 100% in the tests at 0, 15, 40 s and a value of 81% in the extraction after 120 s. In conclusion, the contact time and the temperature of the medium do not significantly affect the quantitative

extraction of PAEs from hot drinks, except for DEP. In this regard, the authors chose 15 s as the average waiting time to carry out the analyses on the real samples.

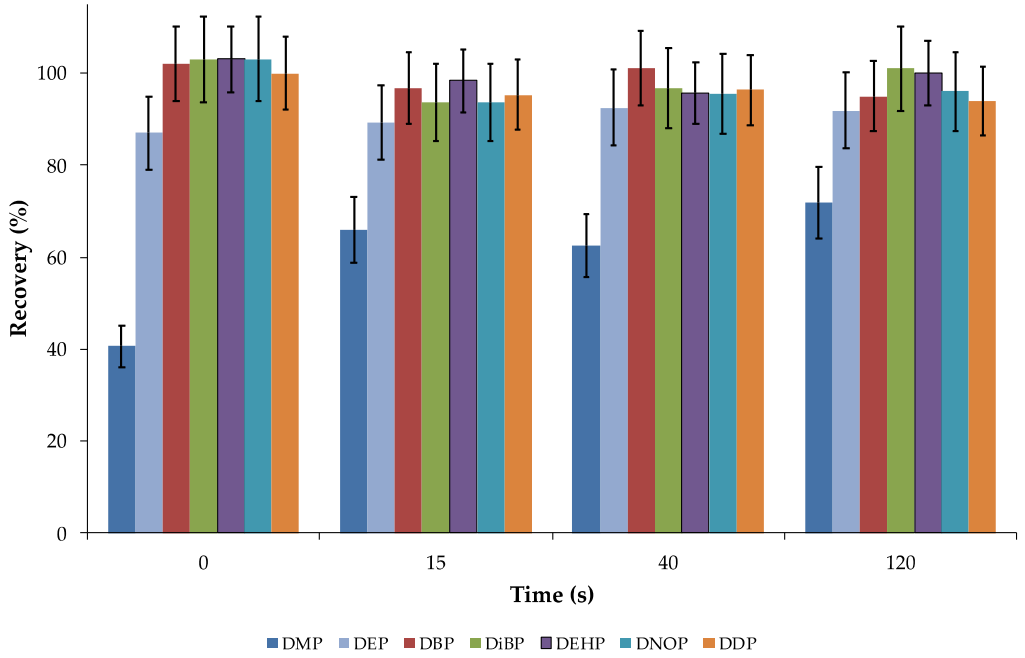


Figure 1. PAE recovery (%) in relation to the pick times from the vending machine and at different temperatures (i.e., pick up time 0 s, temperature 61 °C; 15 s, 59 °C; 40 s, 53 °C; 120 s, 35 °C). The bars represent the standard deviation of each measure. For PAE acronyms: see Table 1.

3.3. Matrix Effect

The last parameter that was evaluated concerned a possible matrix effect on the influence of extraction and subsequent analysis of phthalates from hot drinks dispensed by vending machines. As it is well known, the chemical composition of the sample can be quite complex and influence the instrumental response. The variation in the analytical signal caused by everything in the sample excluding the analyte is referred to as the matrix effect. Hot drinks (i.e., coffee, espresso, ginseng coffee, chocolate drink, tea) have a chemical composition characterized by a very high percentage of water (except the chocolate matrix which is more complex than the others), which makes the matrix free of important quantities of other molecules or classes of molecules that can interfere in the analysis. To verify the influence of the matrix effect, extractions and analyzes were carried out by adding to multiple real samples (short coffee, long coffee, tea, ginseng coffee, chocolate drink, short espresso and long espresso) a known quantity of PAEs up to when the concentration of 1 μg mL^{-1} is reached. The results obtained from the tests are shown in Figure 2.

DEP is mostly recovered (78.1%) in the ginseng coffee matrix, whereas in the other matrices it shows percentage recovery values lower than 70%. In the long coffee, coffee, and tea matrices, DEP, DBP, DiBP, and DEHP tend to show increasing recoveries, respectively, in the matrices listed above. It should be noted that in ginseng coffee, DEPH, DOP, and DDP undergo a drastic decline in terms of recovery, passing at levels of 70.5%, 56.4% and 55.6%, respectively. As far as espresso and long espresso are concerned, the recoveries obtained are in any case greater than that of ginseng coffee and are around 100%. To conclude, from the data obtained it is possible to state that there is no particular matrix effect. Consequently, the matrices to be examined do not need any kind of pretreatment or clean-up.

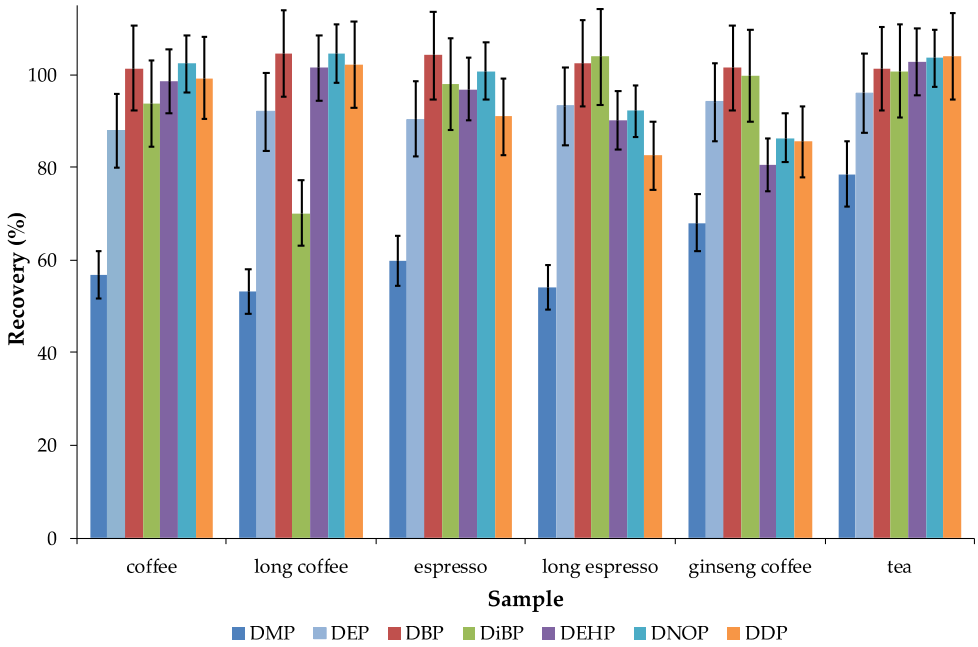


Figure 2. Recovery (%) of each PAE in relation to the matrix effect. The bars represent the standard deviation of each measure. For PAE acronyms: see Table 1.

3.4. Analytical Parameters by GC-IT/MS

Briefly resuming the procedure based on the synergic effect both of vortexing and ultrasonication for the emulsion breaking and of the extraction solvent is the following: sample 10 mL; extraction solvent 250 µL *n*-heptane; 5 min of vortexing; 6 min of ultrasound bath at 25 °C; NaCl 10 g L^{-1}, and 30 min of stirring at 4000 rpm. Under these previously described optimal experimental conditions, all the analytical parameters were investigated. Figure 3 shows chromatographic profiles of a PAE standard solution (0.1 µg mL^{-1}) (Figure 3a,b), a coffee sample (Figure 3c,d), and the same coffee sample spiked with a PAE standard solution 0.1 µg mL^{-1} (Figure 3e,f) in Total Ion Chromatogram (TIC) and Selected Ion Monitoring (SIM), respectively, using phenanthrene as I.S. As it can be seen, the peaks are well-separated and well-solved.

The analyses aimed at optimization of the operational parameters of the UVA-DLLME, the choice of the optimal solvent for the extraction, the influence of time–temperature, and the evaluation of the matrix effect were carried out using the FID detector. On the other hand, GC-IT/MS was used as confirmation analysis, as well as for reaching lower limit of detection (LOD) and limit of quantification (LOQ). Table 6 reports all the analytical parameters obtained by means of the UVA-DLLME–GC-IT/MS procedure. In particular, the following data were reported for each PAE investigated in this study: the linear equation calculated at five different concentration levels along with the related correlation coefficients (R^2), LOD, LOQ, the recovery (%), and the precision values of the method studied, in particular, the intra- and inter-day values.

The linear dynamic range (LDR) in which the PAEs were analyzed ranged from 10 to 100 ng mL^{-1} except for DMP, whose LDR ranged from 30 to 100 ng mL^{-1}. As regards the values of the correlation coefficients (R^2), the latter all have values greater than 0.996 with the exception of DMP, whose value is 0.978. Furthermore, the authors determined LOD and LOQ values for all seven analytes as follows: LODs were derived from the instrumental intensity signal equal to three times the standard deviation of the background noise, whereas LOQs were derived from the instrumental intensity signal equal to seven times the standard deviation of the background noise [26]. The method used showed fairly high LOD and LOQ values for DMP (15.4 and 35.8 ng mL^{-1}, respectively), which attests to good sensitivity of the proposed method to the detection and quantification of

this phthalate in relation to the SMLs reported by regulation [16]. For all other analytes the values are sensitive in relation to the instrumentation used. Recoveries are quite good, ranging between 91.7 and 101.2% for all PAEs except for DMP (66.7%). This occurrence could be due to the high temperature reached by the system during the preparation in the machine. It should be underlined that DMP shows the lowest boiling points among the PAEs investigated (Table 1). DEP and DDP are the other PAEs with boiling temperature below 300 °C and they show no quantitative recoveries (91.7% and 93.1%, respectively).

Finally, for evaluating the precision of the method, the authors carried out extraction/analysis tests close to each other over time (repeatability as intra-day error) and in different days (intermediate precision as inter-day error) (Table 6), obtaining values $\leq$6.3% and $\leq$11.1%, respectively.

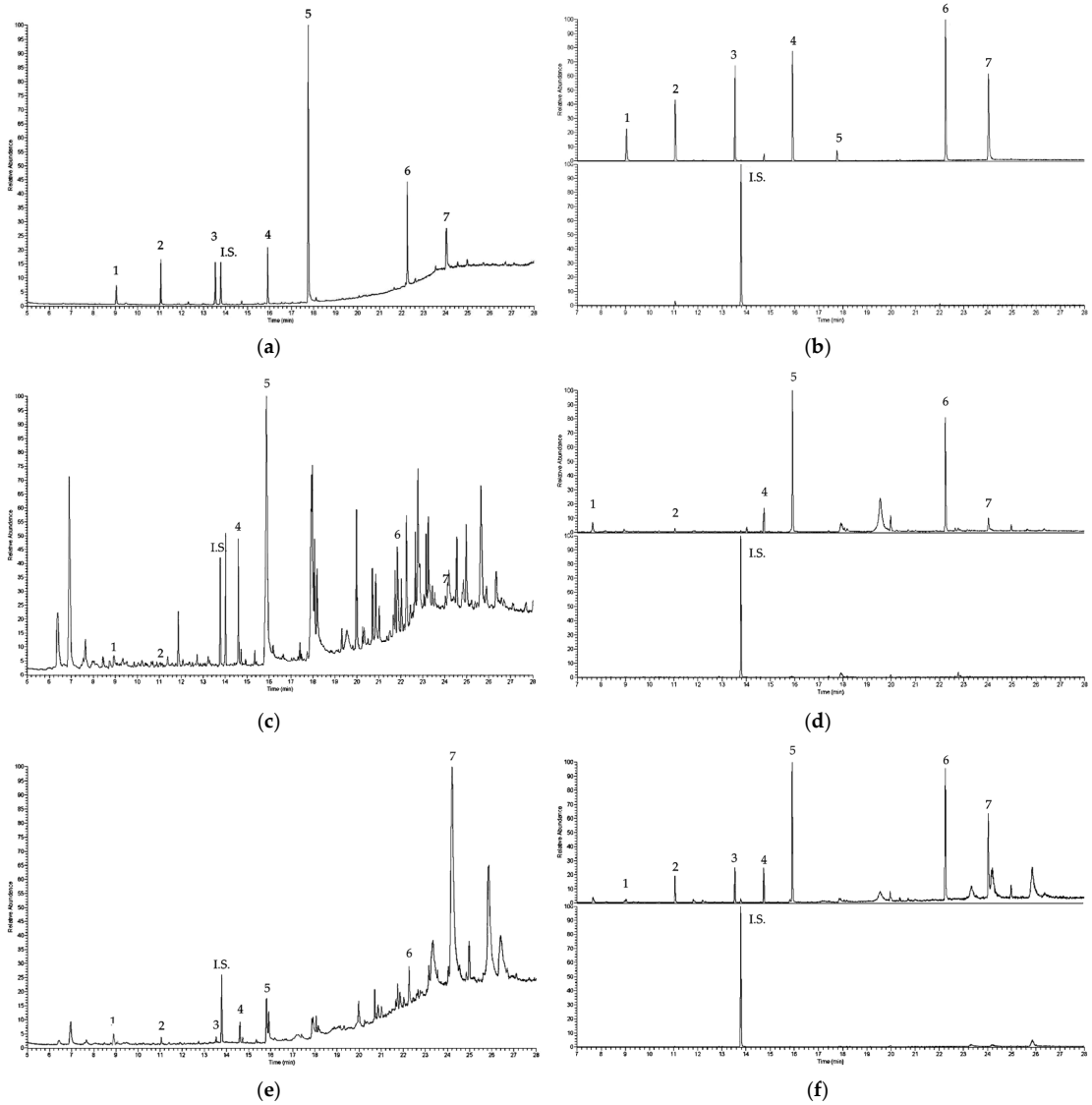


Figure 3. GC-IT/MS profiles a PAE standard solution (0.1 µg mL^{-1}) (**a**,**b**), a coffee sample (**c**,**d**), and the same coffee sample spiked with a PAE standard solution 0.1 µg mL^{-1} (**e**,**f**) in Total Ion Chromatogram (TIC) and Selected Ion Monitoring (SIM), respectively. For peak identification: 1. DMP (t_r 8.99 min); 2. DEP (11.03 min); 3. DiBP (13.5 min); 3. I.S. (phenanthrene) (13.81 min); 4. DBP (14.72 min); 5. DEHP (15.89 min); 6. DNOP (22.23 min); 7. DDP (24.00 min). For acronyms: see Table 1. For experimental conditions: see text.

Table 6. Regression equation (expressed as y = mx + q) and related correlation coefficient, limit of detection (LOD, ng mL^{-1}) and limit of quantification (LOQ, ng mL^{-1}), recovery (% ± s.d.), and precision and reproducibility as inter-day and intra-day (% ± s.d.) errors for each PAE investigated in this study by UVA-DLLME–GC-IT/MS.

PAE [1]	y = mx + q	R^2	LOD	LOQ	Recovery	Intra-day	Inter-day
DMP	y = 0.0065x − 0.0563	0.9782	15.4	35.8	66.7 ± 3.7	5.7	10.7
DEP	y = 0.0692x − 0.0065	0.9993	3.8	9.0	91.7 ± 5.5	6.1	9.2
DiBP	y = 0.0131x + 0.0035	0.9964	0.8	1.9	98.9 ± 3.9	4.2	11.1
DBP	y = 0.0164x − 0.0053	0.9972	1.2	2.8	101.2 ± 4.0	3.5	10.6
DEHP	y = 0.0192x + 0.0004	0.9973	0.7	1.6	99.4 ± 3.9	3.8	7.3
DNOP	y = 0.0186x + 0.0107	0.9971	2.2	5.0	100.7 ± 5.8	6.3	10.1
DDP	y = 0.0189x + 0.0375	0.9972	10.7	24.9	93.1 ± 5.5	5.2	9.8

[1] For PAE acronyms, see Table 1.

3.5. LODs and LOQs: A Comparison among Different Procedures

Two important issues of this paper were the LOD/LOQ comparison between two different chromatographic detections and the same comparison with studies present in literature involving such matrices.

First, the comparison between GC-FID and GC-IT/MS analysis was carried out comparing the data obtained in this study with the data obtained in a previous study of the same research group where both two extraction methods were compared, and the analyses were carried out by means of GC-FID [27]. Table 7 resumes both data. First, it can be seen that the lowest levels were reached by IT/MS detection. In similar study where toxicological effects on human health can be due to the presence of compounds at (ultra-)trace levels, the mass spectrometry contribution is really important for detecting them. On the other hand, GC-FID still plays a fundamental role in the method development.

Table 7. Comparison between limit of detection (LOD, ng mL^{-1}) and limit of quantification (LOQ, ng mL^{-1}) of each PAE investigated in this study by UVA-DLLME followed by GC-FID and GC-IT/MS analysis.

PAE [1]	LOD		LOQ	
	FID	IT/MS	FID	IT/MS
DMP	1200	15.4	2800	35.8
DEP	600	3.8	2400	9.0
DiBP	600	0.8	1300	1.9
DBP	400	1.2	1300	2.8
DEHP	700	0.7	1200	1.6
DNOP	1600	2.2	2800	5.0
DDP	500	10.7	2400	24.9

[1] For PAE acronyms, see Table 1.

The second important issue regarded the comparison on the analytical parameters between this study and others present in the literature. The authors focused their attention on analytical methodologies developed for tea, coffee, and infusion samples that are matrices similar to those investigated in this paper. It should be necessary to underline that, although the matrices are the same (i.e., coffee and tea), the coffee and tea compositions along with the grain origins are different and this can justify differences in both the recoveries and the matrix effect and, consequently, in the LOD and LOQ values [28–30]. Table 8 shows the comparison based on analytical parameters such as LODs/LOQs (expressed as ng mL^{-1}), recoveries (%), and RSD (%) between data in the literature [31–41] and those determined in this paper.

Table 8. Comparison between the method developed in this paper and some reported in literature on the same matrices (-: data not reported).

Analytes	Matrix	Extraction Method	LOD/LOQ (ng mL^{-1})	Recovery (%)	RSD (%)	Ref.
7 PAEs	coffee, tea	SPE [1]	3–4/10	83–105	8–15	[31]
10 PAEs	tea leaves	LLE	-/1–120	85.6–114.1	<20	[32]
6 PAEs	tea, infusion	QuEChERS [2]	9–18/27–58	70.1–101.3	0.6–1.5	[33]
6 PAEs	coffee	DI-SPME [3]	5–30/-	87.6–100.7	3.1–9.2	[34]
11 PAEs	tea	m-SPE	-/0.03–0.18	80–114	0–16	[35]
8 PAEs	coffee	LLE [4]	0.18–0.67/0.6–2.1	80.3–105.1	0.7–3.1	[36]
6 PAEs	coffee	PATC-HS-SPME [5]	0.04–0.10/-	75.5–105.3	1.8–12.0	[37]
8 PAEs	coffee	MSPE [6]	30–200/10–500	77.3–119.4	0.8–15	[38]
8 PAEs	iced-tea	VA-DLLME	-/17.2–59.4	84–120	1–11	[39]
11 PAEs	tea, infusion	NADES [7]	-/4.3–51.1	71–1215	1–22	[40]
14 PAEs	tea, infusion	VA-DLLME	-/25–1250	63–124	1–19	[41]
7 PAEs	coffee, ginseng, tea	UVA-DLLME	0.8–15.4/1.6–35.8	66.7–101.2	3.5–11.1	This study

[1] Solid-phase extraction; [2] Quick, easy, cheap, effective, rugged, and safe; [3] Direct immersion-SPME; [4] Liquid–liquid extraction; [5] Purge-assisted and temperature-controlled headspace solid-phase microextraction; [6] Magnetic solid-phase extraction; [7] Natural deep eutectic solvent.

As it can be seen, the extraction methods are different. In particular, five papers are based on solid-phase extraction (SPE) or similar extraction methods [31,34,35,37,38], two papers on liquid–liquid extraction (LLE) [32,36], two papers on quick, easy, cheap, effective, rugged, safe (QuEChERS) [33] and natural deep eutectic solvent (NADES) [40], and two papers based on DLLME [39,41] plus this study.

Looking at the data, the good analytical parameters reached by using the procedure developed in this study demonstrate the potential of this new proposed method. The only problem remains the low recovery of DMP (66.7%), even if the other papers show lower recoveries that are the same or quite similar (63–87%).

3.6. Application to Real Samples

The entire analytical procedure was applied to some real samples, i.e., espresso, long espresso, coffee, long coffee, ginseng coffee, and tea. The samples were withdrawn by a vending machine present in a public office. This machine is subject to routine maintenance (weekly frequency), and it is largely used daily.

For peak quantification, the peak areas in SIM mode were used using the internal standard method. The results are reported in Table 9. Among the investigated PAEs, DEHP, and DNOP are present in all the samples, whereas DiBP is always absent. Basically, all the real samples investigated show PAE contamination at different levels. Espresso sample shows the most complete profile (i.e., DMP, DEP, DEHP, DNOP, and DDP), whereas coffee and tea samples the poor one (i.e., DEHP and DNOP). The DEHP levels range between 0.21–0.73 μg mL^{-1}, DNOP between 0.07–0.23 μg mL^{-1}, whereas DMP, DEP, and DDP are at very low levels.

Table 9. PAEs concentration levels (μg mL^{-1}) determined in samples withdrawn by vending machine.

Sample	PAE [1]					
	DMP	DEP	DiBP	DEHP	DNOP	DDP
SML [2]	60	60	60	1.5	60	60
Hazard	N/A [3]	2 [4]	125 [5]	4.8 [5]	N/A	N/A
Espresso	0.021	0.083	<LOQ	0.210	0.072	0.042
Long espresso	<LOQ	0.091	<LOQ	0.314	0.104	<LOQ
Coffee	<LOQ	<LOQ	<LOQ	0.447	0.173	<LOQ
Long coffee	<LOQ	<LOQ	<LOQ	0.353	0.148	0.054
Ginseng coffee	<LOQ	<LOQ	<LOQ	0.536	0.122	0.049
Tea	<LOQ	<LOQ	<LOQ	0.731	0.237	<LOQ

[1] For PAE acronyms, see Table 1; [2] SML specific migration limit (expressed as mg kg^{-1}); [3] not applicable; [4] LOAEL lowest observed adverse effect level (expressed as mg kg^{-1} d^{-1}) [42]; [5] NOAEL no observed adverse effect levels (expressed as mg kg^{-1} d^{-1}) [42].

As regards the comparison with similar studies which are not many, there is substantial agreement about the PAEs released and the relative amount. All papers detect presence of DEHP, also at levels up to 32 μg mL^{-1} [31,43,44].

The authors agree with the other papers that such contamination is due to the PAE migration from the plastics to the beverages and it is favored by the hot temperature. Further, the authors suggest the "plastic pipelines" present in the vending machine as another possible source of contamination; each step, for instance, the grinding of coffee beans (it occurs in such vending machine) is able to contaminate the beverage. So, it is really difficult to identify a reasonable cause of contamination. In any case, the PAE levels detected in these samples are below the levels ruled by EU regulation [16] and do not represent a danger to the consumer public health.

4. Conclusions

This study reported an optimized method based on an UVA-DLLME–GC-IT/MS procedure for determining PAEs released by plastics in hot beverages withdrawn from a vending machine. The task is important due to the large distribution of such machines in all public offices; many people daily use such equipment. This paper would like to propose a methodology for investigating such release by means of a modified DLLME extraction procedure followed by GC-IT/MS analysis. The developed method allows to reach low LODs and LOQs and good recoveries which are sufficient for analyzing such compounds at low levels. The procedure has been applied to real samples; a general contamination by DEHP and DNOP has been detected, whereas DMP and DiBP are (almost) absent. In all cases the levels are far below the SMLs reported by legislation and, therefore, they do not represent a risk for the consumers.

Finally, it should be noted that this method was applied to these plastic (PS) containers in particular conditions (high temperature). These authors are studying the possibility to extend this approach to other plastic food containers, but other studies are necessary for confirming it in other situations.

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