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Development of an elevated temperature–dispersive liquid–liquid microextraction method combined with gas chromatography–flame ionization detection as a sensitive method for determining phthalate esters

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Running Head: ET-DLLME for PEs determination

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Abstract

In the present study, an elevated temperature–dispersive liquid–liquid microextraction method combined with gas chromatography–flame ionization detection has been developed for the extraction, preconcentration and determination of trace concentration of phthalate esters in aqueous samples. Effect of different variables on the extraction efficiency was studied, in details. The variables of interest in the proposed method were extraction solvent volume and type, disperser solvent volume and type, temperature, salt effect, pH, extraction time, and centrifugation time and speed. An appropriate mixture of dimethyl sulfoxide (disperser solvent) and 1,2–dibromoethane (extraction solvent) was rapidly injected into heated aqueous solution of the analytes. Then the heated solution allowed cooling at room temperature and phase separation was accelerated by centrifuging. Figures of merit such as linearity (r^2 > 0.996), enrichment factors (1440–2460), limits of detection (0.25–1.00 ng mL^{-1}) and quantification (0.84–3.64 ng mL^{-1}), extraction recoveries (57–98 %), and relative standard deviations (4–8 %) for |intra–day (n = 6) and inter–days (n = 4) precisions (C=10 ng mL^{-1} of each analyte) for the proposed method were satisfactory for determination of the selected phthalate esters.

Keywords: Elevated temperature–dispersive liquid–liquid microextraction; Gas chromatography; Phthalate esters; Aqueous sample

1 Introduction

Phthalate esters (PEs) are used as plasticizers to make materials more flexible and elastic.[1] Many consumer products and food packaging products contain PEs.[2] Because PEs are not chemically bound to the plastics, they can be released easily from products and migrate into the surround. Because of the potential health impact on humans,[3,4] the European Commission is proposing a ban on the use of PEs in soft poly vinyl Chloride (PVC) materials.[5] Due to these reasons, there is a need to determine PEs in trace levels in different samples. Several analytical methods were developed for the determination of these materials, such as gas chromatography (GC),[6-8] high–performance liquid chromatography (HPLC),[9,10] capillary electrophoresis (CE),[11] and micellar electrokinetic chromatography (MEKC).[12] However a sample preparation step is normally required to isolate and concentrate the compounds of interest from the sample matrix, before analysis. Several sample preparation methods such as liquid–liquid extraction (LLE),[13] solid phase extraction (SPE),[14-16] solid phase microextraction (SPME),[17-19] headspace solid phase microextraction (HS–SPME), [20] stir bar sorptive extraction (SBSE), [21,22] and liquid phase microextraction (LPME) [23] such as air–assisted liquid–liquid phase microextraction (AALLME), [24] ultrasound–assisted dispersive liquid–liquid microextraction (USA–DLLME) [25], ultrasound/vortex assisted dispersive liquid-liquid microextraction (US/VA–DLLME) [26] and magnetic stirring-assisted dispersive liquid-liquid microextraction (MSA-DLLME) [27] have been used for this purpose. MSA-DLLME method is based on the fast injection of an extracting solvent into an aqueous solution, which is being stirred by a magnetic stirrer, to form a cloudy binary component solvent. The main difficulties of LLE include the use of large amounts of toxic organic solvents, formation of emulsion and low enrichment factors (EF). SPE has higher EFs than LLE but requires column conditioning and elution with organic solvent. Therefore, analytical researchers are looking for approaches with low consumption of toxic organic solvents to minimize the disadvantages of LLE and SPE. SPME is based on equilibrium of analytes concentration between the sample matrix and a fused silica fiber coated with an extractive phase.[28, 29] Despite the advantages provided by this method, most commercial extractive fibers used in SPME are relatively expensive, fragile and sample carry–over is also a problem.[30] In SBSE, based on sorptive extraction,

whereby the solutes are extracted into a polymer coating on a magnetic stirring rod. [31] The basic principles of SBSE are identical to SPME but the volume of extraction phase is larger. LPME as a miniaturized sample preparation approach emerged in the mid–to–late 1990s.[32] LPME is a solvent–minimized sample pretreatment procedure in which only several microliters of an extracting solvent is required. In 2006, a novel LPME method named dispersive liquid–liquid microextraction (DLLME) was developed by Assadi and co–workers. [33] In DLLME, a mixture of an extraction solvent and a dispersive solvent are rapidly injected into an aqueous solution by a syringe. A cloudy solution containing fine droplets of the extraction solvent dispersed entirely into the aqueous phase is formed. Analytes in the sample are extracted into the fine droplets, which are further separated by centrifuging, and the enriched analytes in the organic phase are determined by either chromatographic or spectrometric methods. The advantages of DLLME method are short extraction time, low cost, simplicity of operation, and high EFs. In this study a new mode of DLLME is reported in which an aqueous solution containing the compounds of interest is heated, and then a mixture of an extraction solvent and a disperser are rapidly injected into the heated solution and allows cooling at room temperature. By injecting the above mixture, a partially cloudy solution is obtained. During cooling, turbidity of the solution is increased by forming new droplets of the extraction solvent which leads to an efficient extraction from high volume of aqueous phase. In 2008, a novel ionic liquid (IL)–LPME method termed temperature-controlled ionic liquid dispersive LPME has been developed. The method was based on the dispersion of IL into aqueous phase by changing the temperature. In this method IL was dissolved in aqueous phase completely and by cooling the new droplets of IL were produced.[34-37] A traditional DLLME is usually performed using 5 mL aqueous sample solution and finally about 10 µL collected organic phase is obtained. The highest EF can be (5000/10) =500. Whereas an elevated temperature (ET)–DLLME method can be carried out using 50 mL or more aqueous phase and about 20 µL final organic phase. In this method the highest EF will be 2500 (50000/20). Also owing to gradually formation of organic phase droplets during cooling period an extractive mode similar to continuous extraction method is performed in which high ERs are obtainable in spite of high volume ratio of aqueous phase to organic phase. The main disadvantage of the developed

technique lies in its extractant solvent which is usually a halogenated solvent of highly toxic nature that is difficult to handle in laboratory. Furthermore, 1,2-dibromoethane (1,2-DBE) (extraction solvent used in this study) has considerable hepatotoxicity and is classified by International Agency for Research on Cancer (IARC) as Group 2A, suspected carcinogen to humans with evidence of carcinogenicity in animals. [38]

The aim of this study was to develop an ET–DLLME method followed by GC determination for monitoring some PEs in aqueous samples. To the best of our knowledge this is the first report regarding application of ET–DLLME method for the extraction and preconcentration of PEs from aqueous samples. Different parameters affecting microextraction efficiency are completely investigated.

2 Materials and methods

2.1 Reagents and solutions

The target PEs including dimethyl phthalate (DMP), diethyl phthalate (DEP), di–*n*–butyl phthalate (DNBP), di–*iso*–butyl phthalate (DIBP), di–*iso*–octyl phthalate (DIOP) as well as bis–2–ethyl hexyl adipate (DEHA) had a purity of >98 % and were purchased from Sigma–Aldrich (Louis, USA). 1,1,2,2–Tetrachloroethane (1,1,2,2–TCE), and 1,1,2–trichloroethane (1,1,2–TCE) were purchased from Janssen Chimica (Beerse, Belgium), and 1,2–DBE was from Merck (Darmstadt, Germany). Dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), and *n*–propanol tested as disperser solvents and the other chemicals such as sodium chloride, hydrochloric acid, and sodium hydroxide were obtained from Merck. De–ionized water (Ghazi Company, Tabriz, Iran) was refluxed and distillated in the presence of 0.1 M NaOH. By this action PEs content of de-ionized water was hydrolyzed and PEs-free water was obtained to prepare aqueous solutions. A mixture stock solution of PEs (1000 mg L^{-1}, each PE) was prepared by dissolving an appropriate amount of each PE in acetone. Working solutions were prepared daily by appropriate dilutions of the stock solution with distilled de–ionized water. Another standard solution of the analytes (250 mg L^{-1}, each PE) prepared in 1,2–DBE was directly injected into the separation system

each day (three times) for quality control and the obtained peak areas were used in calculation of EFs and ERs.

2.2 Samples

Mineral water and beverage (cola) were purchased from local supermarkets (Tabriz, Iran) and sodium chloride (0.9 %) and dextrose (5 %) injection solutions purchased from a drug store to be tested as real samples. All samples were packed in polymeric packages. Beverage was diluted with distilled de–ionized water at a ratio of 1:4 before analysis. Other samples were used without any treatment or dilution.

2.3 Microextraction procedure

A 50 mL sample or standard solution was placed into a 70–mL glass centrifuge tube with conical bottom. Sodium chloride (2.5 g) was added and manually shacked to dissolve. The tube was placed into a water bath for 5 min at 80 ˚C. A mixture of 1.5 mL DMSO (as a disperser solvent) and 104 μL 1,2–DBE (as an extractant) was rapidly injected into the solution using a 5–mL glass syringe. In this step, 1,2–DBE was dispersed completely in all parts of the aqueous solution and a partially turbid solution was produced. The solution is cooled to room temperature with tap water. In this step turbidity of the solution was completely increased. Then the solution was centrifuged at a rate of 4000 rpm for 4 min. Volume of the sedimented phase was 20 ± 1 μL. Finally, 1 μL of the sedimented phase was removed and injected into the GC system for analysis.

2.4 Instrumentation

Chromatographic analysis was performed on a Shimadzu 2014 gas chromatograph (Kyoto, Japan) equipped with a split/splitless injector operated at 300 ˚C in a splitless mode (sampling time 1 min) and a flame ionization detector (FID). Hydrogen gas was generated with a hydrogen generator (OPGU–1500S, Shimadzu, Japan) for FID at a flow rate of 30 mL min^{-1}. Separation was carried out on an OPTIMA delta–3 capillary column (30 m × 0.25 mm i.d., and film thickness 0.25 μm), (Macherey–Nagel,

Germany). The column oven temperature was initially held at 50 °C for 1 min, then raised to 300 °C at a rate of 15 °C min^{-1}, and held at 300 °C for 2 min. The FID temperature was maintained at 300 °C. Helium (99.999%) (Gulf Cryo, United Arab Emirates) was used as a carrier gas at a linear velocity of 30 cm s^{-1} and make up gas (30 mL min^{-1}). The flow rate of air for FID was 300 mL min^{-1}. Gas chromatography–mass spectrometry (GC–MS) analysis was carried out on an Agilent 7890A gas chromatograph equipped with a 5975C mass–selective detector (Agilent Technologies, CA, USA) and a split/splitless injector operated at 300 °C in a splitless mode (sampling time 1 min). The column oven temperature programming was the same as GC–FID analysis mentioned above. Separation was performed on an HP–5 MS capillary column (30 m × 0.25 mm i.d., and film thickness of 0.25 μm) (Hewlett–Packard, Santa Clara, USA). The carrier gas was helium with a flow rate of 1.0 mL min^{-1}. pH measurements were performed with a Metrohm pH meter model 654 (Herisau, Switzerland). A ROTOFIX 32A centrifuge from Hettich (Germany) was used in microextraction procedure.

2.5 Calculation of EF and ER

EF is defined as the ratio between analyte concentration in the sedimented phase (C_{sed}) and the initial concentration of analyte (C_0) within the sample.

$$EF = C_{sed} / C_0 \quad (1)$$

C_{sed} is obtained by comparing the obtained peak areas after performing the proposed method with those obtained by direct injection of the standard solution of PEs prepared in 1,2– DBE.

ER is defined as the percentage of the total analyte amount (n_0) which is extracted into the sedimented phase (n_{sed}).

$$ER = (n_{sed}/n_0) \times 100 = [(C_{sed} \times V_{sed}) / (C_0 \times V_{aq})] \times 100$$

$$ER = (V_{sed}/V_{aq}) \times EF \times 100 \quad (2)$$

where V_{sed} and V_{aq} are the volumes of sedimented phase and aqueous solution, respectively.

3 Results and discussion

Several factors such as disperser and extraction solvents kind and volume, salt addition, pH, temperature, and centrifugation time and speed affect the process. So all of these parameters are optimized in order to obtain good performance and discussed in details in the following sections.

3.1 Selection of extraction solvent

Selecting an appropriate extractant is essential in an ET–DLLME method. By considering this fact that the ET–DLLME method is performed at a relatively high temperature, some solvents with high boiling points including 1,2–DBE (b.p. 132 °C), 1,1,2–TCE (b.p. 113.8 °C), and 1,1,2,2–TCE (b.p. 146.5 °C) were tested as the extraction solvent. The used volumes were 140 μL of 1,1,2,2–TCE, 120 μL of 1,1,2–TCE, and 110 μL of 1,2–DBE to obtain 20 ± 1 μL for the sedimented phase volume. Figure 1 depicts extraction recovery *versus* extraction solvent type. As can be seen 1,2–DBE extract all analytes more than other solvents used. Therefore 1,2–DBE was selected as the extraction solvent for the further studies which had some preferences over the others such as its relatively good extraction efficiency and its low volume used.

Fig. 1

3.2 Selection of disperser solvent

Dispersive solvent should be miscible with both water and the extraction solvent. Also in this study, disperser solvent should be a relatively high boiling point solvent. Therefore, DMF, DMSO, and *n*–propanol were tested as disperser solvents and the effect of these solvents on performance of the developed method was investigated. A 2 mL of each disperser along with different volumes of 1,2– DBE was used to obtain a similar volume for the sedimented phase (20 ± 1 μL). The volume of 1,2–DBE was 110 μL for DMF, 117 μL for DMSO, and 125 μL for *n*–propanol. According to the obtained results,

DMSO was selected as a suitable disperser because of forming a cloudy state with very fine droplets and having the high ERs for most analytes.

3.3 Selection of extraction solvent volume

In order to investigate effect of extraction solvent volume on the extraction efficiency, different volumes of 1,2–DBE (117 to 170 μL) and a constant volume of DMSO (2 mL) were tested. ERs increased from 117 to 130 μL of 1,2–DBE, and then remained constant up to 170 μL. It is noted that by increasing the volume of 1,2–DBE from 117 to 170 μL, volume of the sedimented phase increased from 10 to 60 μL, accordingly. When the volume of 1,2–DBE was 117 μL, volume of the sedimented phase was about 10 μL that was difficult to remove by microsyringe. According to the results, 130 μL of 1,2–DBE was selected to obtain 20 μL for the sedimented phase volume, and having higher ERs.

3.4 Selection of dispersive solvent volume

For optimizing the volume of disperser, different volumes of DMSO (0.5, 1.0, 1.5, 2.0, and 3.0 mL) containing 100, 115, 119, 130, and 155 μL of 1,2–DBE, respectively, were examined. It is necessary to change the volume of 1,2–DBE simultaneously by changing the volume of DMSO to reach a constant volume for the sedimented phase (20 μL). The results (not shown here) indicated that 1.5 mL is better than other tested volumes for DMSO. At low volumes of disperser, it cannot disperse 1,2–DBE properly into aqueous phase and the solution cannot get a cloudy state very well. On the other hand, at high volumes of DMSO, solubility of PEs in aqueous phase increases owing to decreased polarity of aqueous phase by dissolving disperser solvent in it. So 1.5 mL DMSO was chosen as the optimum volume for the further studies.

3.5 Salt addition

A salt is often added to aqueous sample in order to decrease the solubility of analytes in aqueous phase and increase amount of the extracted analytes into organic phase. Therefore, the effect of salt addition on extraction efficiency was investigated at different amounts of NaCl from 0 to 15 % (*w/v*). In order to obtain a constant volume for the sedimented phase, the experiments were performed using different volumes of the extraction solvent to achieve 20 μL of the sedimented phase volume. It was 119, 110, 104, 97, 90, and 80 μL for 0, 2.5, 5.0, 7.5, 10, and 15 %, *w/v,* NaCl, respectively. The results (Fig. 2) indicate that extraction efficiency increases up to 5 %, *w/v*, NaCl and then decreases gradually at high concentrations of NaCl. It could be due to increase in viscosity of the aqueous phase by adding NaCl which leads to decrease in diffusion coefficients of the analytes. Therefore, 5 %, *w/v*, NaCl was selected for the further experiments in which 104 μL 1,2–DBE was used as the extraction solvent.

Fig. 2

3.6 Effect of temperature

Temperature is a very important parameter in an ET–DLLME method. High temperatures lead to fast extraction that reduce extraction time. Also partition coefficient of the analytes can be different at high temperatures with respect to room temperature which may be alters ERs. However the main effect of an elevated temperature which is allowed to reach room temperature is formation of new droplets of the extractants that induce an efficient extraction. To determine the influence of temperature, the microextraction procedure was performed at different temperatures ranging from 23 (room temperature) to 90 °C. When the temperature is increased, solubility of the extraction solvent into aqueous phase is also increased. Hence, during the cooling period as mentioned above, more tiny droplets of the extraction solvent are formed and move thorough the solution and extract the analytes. The results show that by increasing the temperature from 23 to 80 °C extraction efficiency is improved and then decreased gradually at high temperatures, probably due to loss of analytes, disperser or extraction solvent (Fig. 3). Finally 80 °C was adopted as the optimum microextraction temperature.

Fig. 3

3.7 Effect of pH

Aqueous solution pH is another important factor that may influence extraction of the analytes. In this study, the aqueous phase pH was investigated between 2–12 adjusted using 1 M HCl or NaOH solutions. The results indicated that extraction efficiency of the analytes increased with pH increasing from 2 to 5 and remained constant up to pH 8. At pH 9 and higher, ERs decreased noticeably. Decreasing in extraction efficiencies of the target compounds can be attributed to their hydrolysis at highly acidic or alkaline pHs. The samples used in this study had a pH between 5.5 and 6.7, except beverage, thus there was no need to adjust pH in analysis of them by the proposed ET–DLLME method. In the case of beverage, pH was 2.6 after dilution and adjusted at a pH between 5 and 8 using 1 M NaOH solution before analysis.

3.8 Optimization of centrifugation time and speed

Centrifugation is a mandatory process to collect the extractant droplets. The effect of time and speed of centrifugation were examined in the ranges of 1 to 10 min and 2000 to 6000 rpm, respectively. The obtained results showed that these parameters were less effective at high centrifugation time and speed and so 4000 rpm and 4 min were selected as the centrifuging rate and time, respectively.

3.9 Quantitative features of the method

The optimum experimental conditions were used to assess applicability of the proposed method in determination of the target analytes by GC–FID. Some analytical features of the method such as linear range (LR), coefficient of determination (r^2), relative standard deviation (RSD %), EF, ER, and limits of detection (LOD) and quantification (LOQ) were investigated. Table 1 summarizes these analytical characteristics of the method. The results obtained demonstrate a good linearity for all analytes with r^2 higher than 0.996. Repeatability of the proposed method, expressed as RSD %, was evaluated by performing the method on six repeated samples (for intra–day) and four repeated samples (for inter–days) at a concentration of 10 ng mL^{-1} (each PE) and ranged from 4 – 5 % and 4 – 8 %, respectively. High EFs and ERs ranging from 1440 to 2460 and 57 to 98 % were obtained, respectively. LODs and LOQs were in

the ranges 0.25 – 1.00 and 0.84 – 3.64 ng mL^{-1}, respectively. Low LODs and LOQs, high EFs and ERs, and good repeatability are the main advantages of the method.

Table 1

3.10 Samples analysis

To evaluate performance of the presented method, extraction and determination of the selected PEs in different samples including, NaCl injection solution (0.9 %), dextrose injection solution (5 %), beverage, and mineral water were carried out under the optimum conditions established above. As mentioned in Section 2.2 in all samples except beverage the proposed method was directly performed on them without dilution or other pretreatments. The beverage diluted at a ratio of 1:4 with de–ionized water and its pH is adjusted between 5 and 8. Analytes' contents of the samples obtained by GC–FID after performing the proposed ET–DLLME method on them are summarized in Table 2. DMP, DEP, and DIBP were not detected in any of samples. However in all samples one or two PEs were determined at ng mL^{-1} level. Typical GC–FID chromatograms for samples and standard solution are shown in Fig. 4. To verify the results obtained from GC–FID all samples were injected into GC–MS after performing the proposed extraction/preconcentration method. In the case of beverage a typical total ions current (TIC) chromatogram along with mass data are given in Fig. 5. The presence of two PEs (DEHA and DNBP) in beverage was verified by comparison of mass data for scans 1004 and 2870 (retention times 15.90 and 20.01 min, respectively) with those of the studied PEs. Also the presence of DIOP in mineral water and DNBP and DIOP in dextrose and NaCl injection solutions were verified by GC-MS data. It is noted that GC-MS is an efficient method in identification of the unknown samples but, it is expensive and most researchers have no access to this apparatus.

Table 2

Fig. 4

Fig. 5

In order to evaluate matrix effect, the samples were spiked with the analytes at three levels (25, 50, and 100 ng mL^{-1} of each PE) and the proposed method was applied on them (three times for each concentration). The recoveries obtained for the analytes in samples in comparison with those of the distilled de–ionized water spiked at the same three concentration levels are listed in Table 3. According to the obtained results, matrices of the samples have no significant effect on performance of the presented technique. It is noted that relatively strong matrix effect was observed in beverage without dilution. Therefore it was diluted at a ratio of 1:4 with distilled de–ionized water before analysis to reduce its matrix effect.

Table 3

3.11 Comparison of the proposed method with other approaches

Efficiency of the presented method was compared with those of the other reported methods used in analysis of the target analytes considering some aspects such as LOD, LR, RSD, and EF in Table 4. In comparison with the other methods, the proposed method shows high EFs, broad LRs, relatively low LODs, and good precision.

Table 4

4. Conclusion

In this study, an ET–DLLME method has been reported for the extraction and preconcentration of six plasticizers from different aqueous samples followed by GC–FID determination. The developed method has numerous advantages such as simplicity, low cost, and good repeatability. Also, the ratio of sample volume to extraction solvent volume is high using 50 mL as sample size which leads to obtaining high EFs (1440–2460). The results revealed that the developed method is suitable for determination of the selected PEs at ng mL^{-1} level in aqueous samples.

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Figure captions:

Fig.1. Effect of chemical identity of the extraction solvent on the ET–DLLME performance.

Extraction conditions: aqueous sample, 50 mL de–ionized water spiked with 25 ng mL^{-1} of each PE; extraction solvent, 1,2–DBE (110 μL), 1,1,2,2–TCE (140 μL), and 1,1,2–TCE (120 μL); disperser solvent, DMF; disperser solvent volume, 2 mL; temperature, 75 °C, heating time, 5 min; centrifuge rate, 4000 rpm; and centrifuge time, 4 min. The error bars indicate the minimum and maximum of three independent determinations.

Fig. 2. Effect of NaCl concentration on the extraction efficiency of ET–DLLME method.

Extraction conditions: extraction solvent, 1,2-DBE; and disperser solvent, DMSO (1.5 mL). Other conditions are the same in Fig. 1. The error bars indicate the minimum and maximum of three determinations.

Fig. 3. Effect of temperature on the extraction efficiency.

Extraction conditions: the same as in Fig. 2, except 5 %, *w/v*, NaCl along with 104 μL 1,2–DBE was used. The error bars indicate the minimum and maximum of three determinations.

Fig. 4. Typical GC–FID chromatograms of: (A) distilled de–ionized water, (B) mineral water, (C) beverage, (D) NaCl injection solution (0.9 %), (E) dextrose injection solution (5 %), and (F) standard solution prepared in 1,2–DBE (150 mg L^{-1} of each analyte). In all cases except chromatogram (F) the proposed method is performed and 1 μL of the sedimented phase was injected into the separation system. Peak identification: 1, DMP; 2, DEP; 3, DIBP; 4, DNBP; 5, DEHA; and 6, DIOP.

Fig. 5. Typical GC–TIC–MS chromatogram of beverage and mass spectra of DNBP and scan 1004, retention time 15.90 min, and mass spectra of DEHA and scan 2870, retention time 20.01 min.

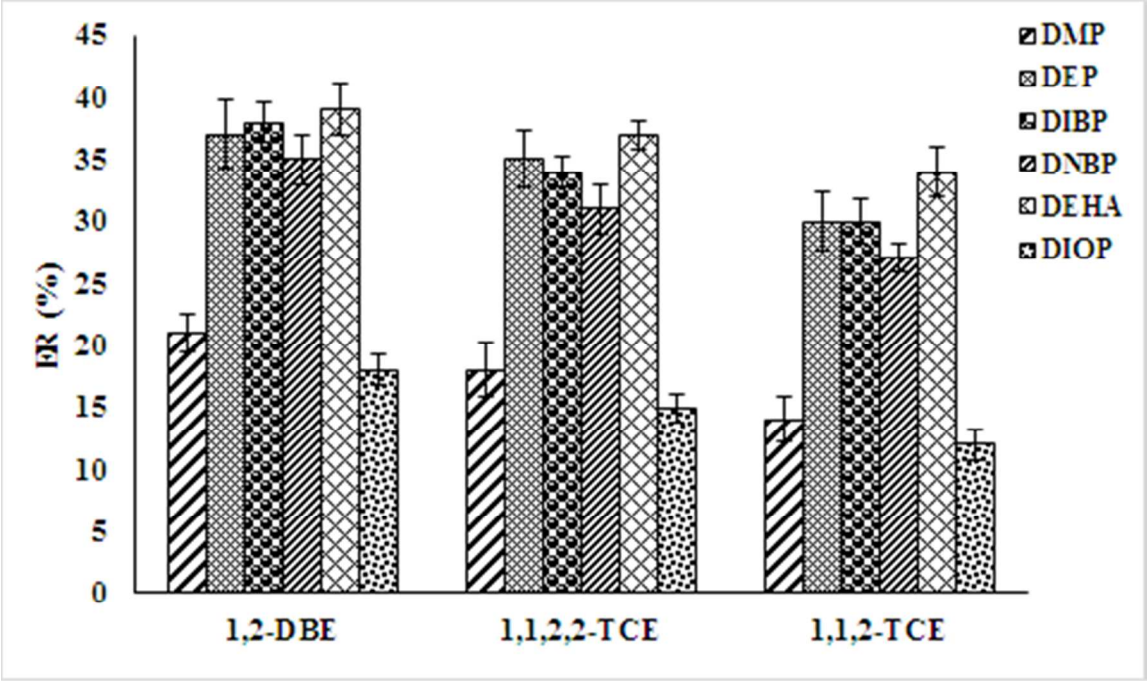
45
40
35
30
25
20
15
10
5
0
ER (%)
DMP
DEP
DIBP
DNBP
DEHA
DIOP
1,2-DBE
1,1,2,2-TCE
1,1,2-TCE

Fig. 1

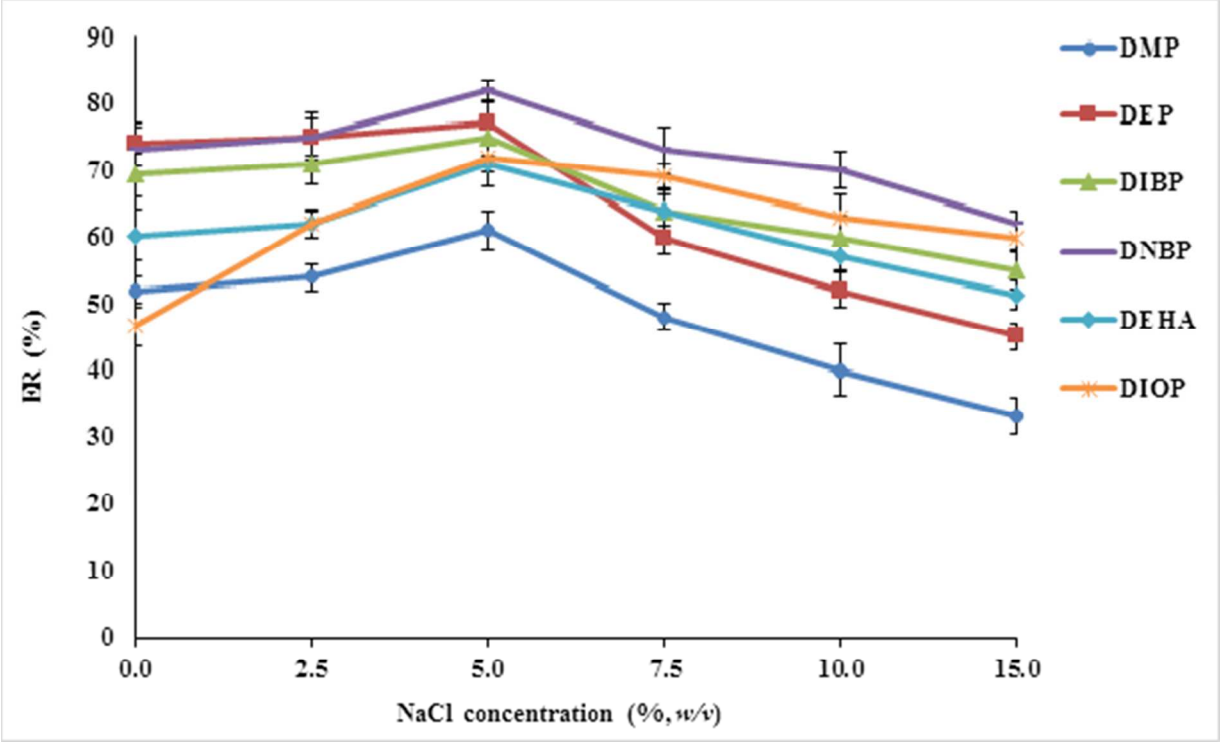
90
80
70
60
50
40
30
20
10
0
ER (%)
0.0
2.5
5.0
7.5
10.0
15.0
NaCl concentration (%, w/v)
DMP
DEP
DIBP
DNBP
DEHA
DIOP

Fig. 2

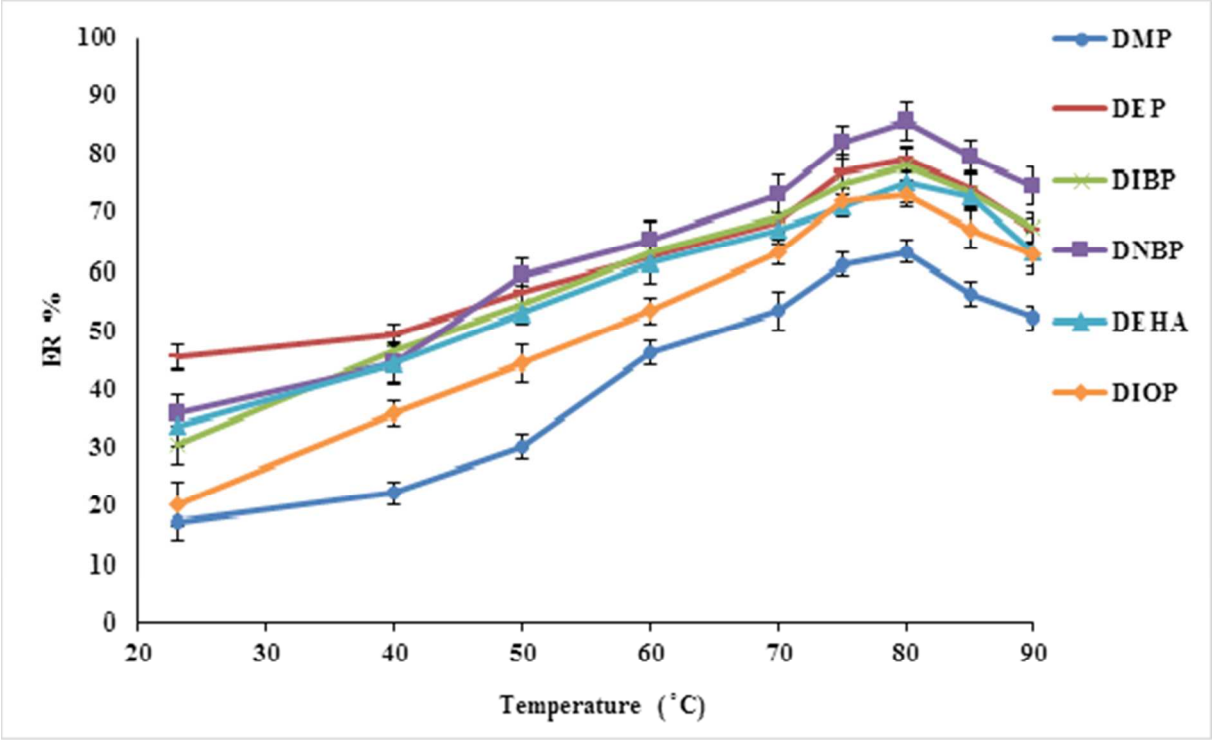
100
90
80
70
60
50
40
30
20
10
0
ER %
20
30
40
50
60
70
80
90
Temperature (˚C)
DMP
DEP
DIBP
DNBP
DEHA
DIOP

Fig. 3

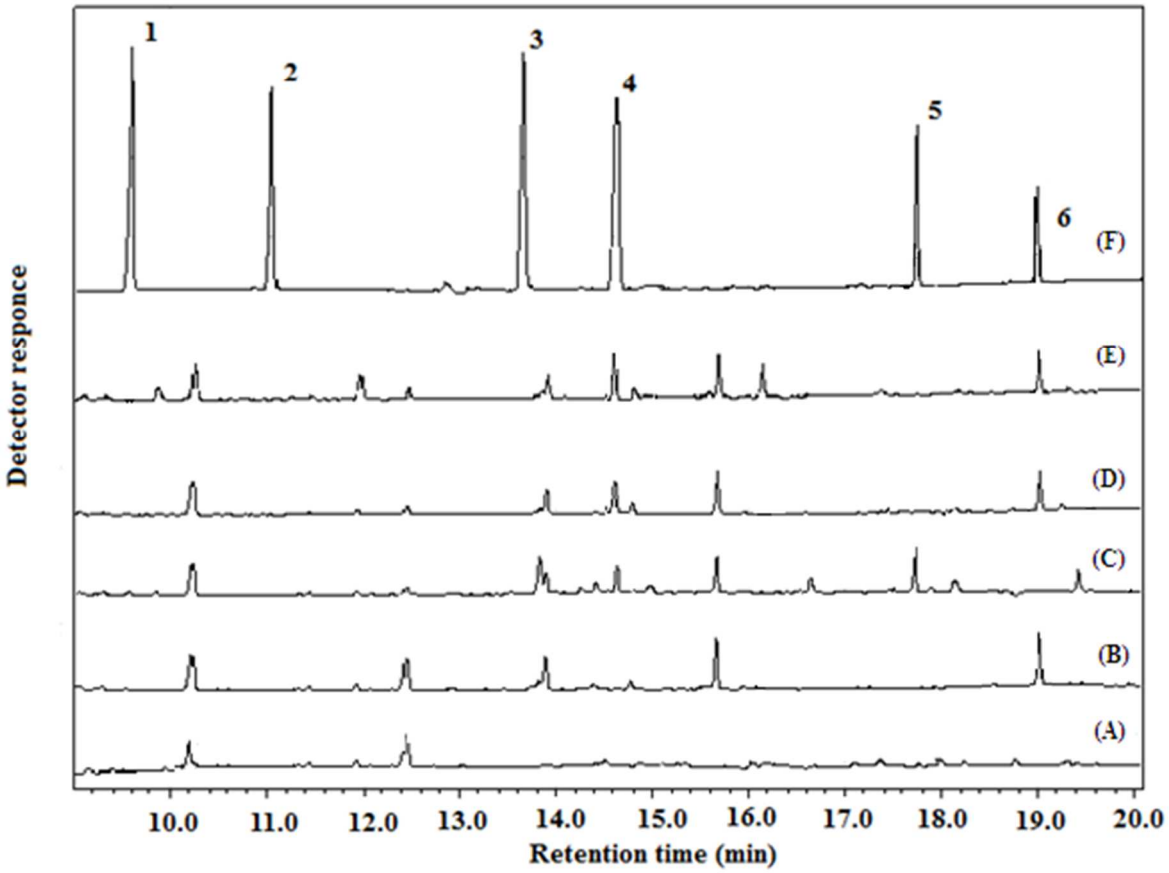
1
2
3
4
5
6
(F)
(E)
(D)
(C)
(B)
(A)
Detector responce
10.0
11.0
12.0
13.0
14.0
15.0
16.0
17.0
18.0
19.0
20.0
Retention time (min)

Fig. 4

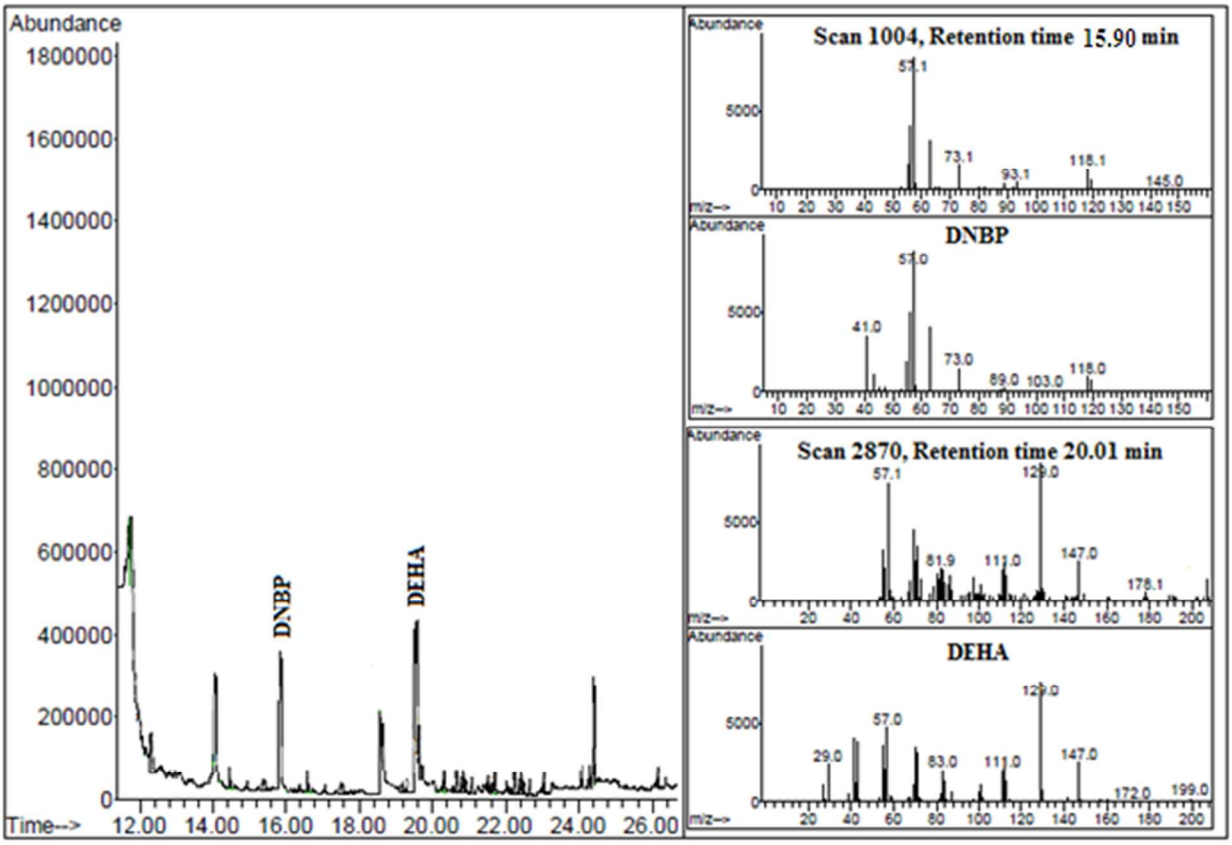
Abundance
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
Time-->
12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00
DNBP
DEHA
Scan 1004, Retention time 15.90 min
DNBP
Scan 2870, Retention time 20.01 min
DEHA
m/z-->

Fig. 5

Table 1. Quantitative features of the method for the selected PEs.

Analyte	LOD [a] (ng mL^{-1})	LOQ [b] (ng mL^{-1})	LR [c] (ng mL^{-1})	r^2 [d]	RSD % [e]		EF ± SD [f]	ER ± SD [g]
					Intra–day	Inter-days		
DMP	0.25	0.84	1 – 1000	0.998	5	6	1440 ± 76	57 ± 3
DEP	0.36	1.21	1 – 1000	0.998	4	7	1717 ± 68	68 ± 3
DIBP	0.28	0.96	1 – 1000	0.996	4	7	2460 ± 77	98 ± 3
DNBP	0.57	1.91	2 – 1000	0.999	4	6	2408 ± 69	96 ± 3
DEHA	0.61	2.04	2 – 1000	0.998	4	8	2328 ± 58	93 ± 2
DIOP	1.00	3.64	4 – 1000	0.998	5	4	2282 ± 90	91 ± 4

a) Limit of detection (S/N=3).

b) Limit of quantification (S/N=10).

c) Linear range.

d) Coefficient of determination.

e) Relative standard deviation (C =10 ng mL^{-1} of each PE) for intra–day (n = 6) and inter–days (n = 4).

f) Enrichment factor ± standard deviation (n=3).

g) Extraction recovery ± standard deviation (n= 3).

Table 2. Analytes'contents of the samples obtained by the proposed ET–DLLME–GC–FID method.

Sample	DMP	DEP	DIBP	DNBP	DEHA	DIOP
Mineral water	ND[a)]	ND	ND	ND	ND	32 ± 2[b)]
NaCl injection solution	ND	ND	ND	19 ± 2	ND	17 ± 2
Dextrose injection solution	ND	ND	ND	27 ± 3	ND	23 ± 1
Bevarage (cola)	ND	ND	ND	40 ± 3	80 ± 5	ND

a) Not detected

b) Mean concentration (ng mL^{-1}) ± standard deviation (n=3)

Table 3. Study of matrix effect in the samples spiked at different concentrations. Beverage was diluted with de-ionized water at a ratio of 1:4 and its pH was adjusted between pH 5 and 8. Other samples were used without dilution.

Analyte	Relative recovery			
	Mineral water	Beverage	NaCl (0.9 %)	Dextrose (5 %)
All samples were spiked with each analyte at a concentration of 25 ng mL^{-1}.				
DMP	83, 89, 86	96, 100, 98	94, 97, 100	96, 101, 99
DEP	85, 89, 87	82, 88, 84	93, 96, 99	94, 100, 97
DNBP	98, 97, 97	97, 94, 97	95, 97, 95	93, 85, 89
DIBP	101, 96, 99	98, 96, 100	101, 93, 97	95, 91, 93
DEHA	98, 95, 101	99, 95, 103	99, 97, 98	97, 93, 101
DIOP	91, 97, 94	82, 76, 79	101, 97, 99	87, 83, 85
All samples were spiked with each analyte at a concentration of 50 ng mL^{-1}.				
DMP	98, 95, 101	96, 90, 93	95, 98, 101	96, 99, 102
DEP	88, 94, 91	100, 94, 97	92, 100, 96	98, 95, 92
DNBP	94, 100, 97	98, 94, 102	94, 100, 97	100, 96, 98
DIBP	86, 92, 89	97, 94, 100	94, 102, 98	101, 97, 93
DEHA	101, 101, 98	102, 98, 102	102, 101, 99	96, 100, 101
DIOP	95, 103, 99	91, 87, 89	98, 95, 101	102, 96, 99
All samples were spiked with each analyte at a concentration of 100 ng mL^{-1}.				
DMP	94, 100, 97	97, 94, 100	97, 100, 94	91, 87, 95
DEP	95, 98, 101	101, 95, 98	95, 98, 101	96, 100, 98
DNBP	98, 95, 101	96, 98, 100	97, 95, 99	97, 94, 100
DIBP	94, 98, 102	97, 93, 101	101, 95, 98	98, 92, 95
DEHA	96, 102, 99	85, 85, 88	101, 93, 97	103, 95, 99
DIOP	95, 103, 99	98, 100, 96	99, 95, 97	93, 100, 96

Table 4. Comparison of the presented method with the other methods used in preconcentration and determination of the target analytes.

Method	Analytes	sample	LR [a] (ng mL^{-1})	LOD [b] (ng mL^{-1})	RSD [c] %	EF [d]	Ref.
GDSPE–GC–MS [e]	DMP	Environmental	5–100	2	9	–	[39]
	DEP	water	5–100	2	9	–	
	DIBP		5–100	2	6	–	
	DBP		5–100	2	9	–	
VSBME–GC–MS [f]	DMP	Bottled mineral	0.5–10	0.076	5.3	8	[40]
	DEP	water	0.1–10	0.035	6.4	57	
	DBP		0.1–10	0.010	4.7	1214	
SB–DLLME–GC–FID [g]	DMP	Aqueous sample	1–2000	0.20	3.8	406	[41]
	DEP		1–2000	0.25	2.5	556	
	DIBP		0.5–2000	0.10	3.6	430	
	DNBP		0.5–2000	0.13	3.3	443	
	DEHA		0.5–2000	0.90	4.5	266	
	DEHP		0.5–2000	0.15	4.4	286	
ESy–GC–FID [h]	DMP	Reagent water	–	3	1.7	–	[6]
	DEP		–	1	1.8	–	
ET–DLLME–GC–FID [i]	DMP	Aqueous sample	1–1000	0.25	5	1440	This method
	DEP		1–1000	0.36	4	1717	
	DIBP		1–1000	0.28	4	2460	
	DNBP		2–1000	0.57	4	2408	
	DEHA		2–1000	0.61	4	2328	
	DIOP		4–1000	1.00	5	2282	

a) Linear range.
b) Limit of detection.
c) Relative standard deviation.
d) Enrichment factor.
e) Graphen dispersive solid–phase extraction– gas chromatography–mass spectrometry.
f) Vortex solvent bar microextraction– gas chromatography–mass spectrometry.
g) Solid based dispersive liquid–liquid microextraction– gas chromatography– flame ionization detector.
h) Extracting syringe– gas chromatography–flame ionization detector.
i) Elevated temperature–dispersive liquid– liquid microextraction–gas chromatography–flame ionization detector.