Simultaneous determination of four phthalate esters in bottled water using ultrasound-assisted dispersive liquid–liquid microextraction followed by GC-FID detection

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A simple and rapid sample pretreatment procedure based on ultrasound-assisted dispersive liquid–liquid microextraction is proposed for the determination of four phthalate esters (dibutyl phthalate, butyl benzyl phthalate, diisooctyl phthalate, dioctyl phthalate) in bottled water samples using gas chromatography-flame ionization detection. An ultrasound-assisted process was applied to accelerate the formation of the fine cloudy solution using less disperser solvent, which markedly increased the extraction efficiency and reduced the equilibrium time. Under optimum conditions, the enrichment factor of the four analytes ranged from 490- to 530-fold and the recovery ranged from 84.8%~104.7%.

Introduction

Phthalate esters (PAEs) are used widely as plasticizers in polymeric materials to increase flexibility through weak secondary molecular interactions with polymer chains. Since PAEs are physically bound to the polymer chains, they can be easily leached into food or water from the packaging materials. PAEs, as well as metabolites and degradation products, can cause adverse effects on human health (particularly to liver, kidney and testicles). Potential endocrine disrupting properties of PAEs were also reported recently, possibly associated with known estrogenic and antiandrogenic activity. Food products contaminated with PAEs have been reported due to the use of plastics as food containers and packaging. Especially, penetration of PAEs from plastic packaging into water is commonly occurring and has become a matter of public concern in recent years. Therefore, the development of sensitive and reliable analytical methods to analyze trace amounts of plasticizers in different water samples is desirable.

Owing to the complexity of sample matrices and the relative low concentration of PAEs, sample pretreatment and enrichment processes are a crucial step in the analytical procedure to obtain accurate and sensitive results. Traditional pretreatment methods, such as liquid–liquid extraction (LLE) and solid-phase extraction (SPE), are time-consuming and need a large amount of organic solvents, which are dangerous for human health and environment. Recent research has been oriented towards the development of simple, economical, and miniaturized pretreatment methods, mainly solid-phase microextraction (SPME) and liquid-phase microextraction (LPME). SPME is a solvent free process that includes simultaneous extraction and preconcentration of analytes from aqueous samples or the headspace of the samples. However, the equipment is relative expensive, especially fibers which are fragile and have limited life-time, and sample carry-over can also be a problem. LPME was developed as a solvent-minimized pretreatment procedure that is inexpensive, simple to use, and requires minimal exposure to toxic organic solvents. However, LPME suffers from several disadvantages such as the small contact surface, it is time-consuming and non-equilibrium procedures in most cases.

Recently, a novel microextraction technique termed as dispersive liquid–liquid microextraction (DLLME) was developed by Assadi and co-workers. It is based on a ternary solvent system like homogeneous liquid–liquid extraction and cloud point extraction. In this method, the appropriate mixture of extraction solvent and dispersive solvent is injected rapidly into an aqueous sample by syringe to form a cloudy solution, which markedly increases the contact surface between phases and reduces the extraction times with increasing enrichment factors. After extraction, phase separation is performed by centrifugation and analytes in the sediment phase are determined by chromatography or spectrometry methods. The advantages of DLLME are its simplicity, rapidity, low cost, high recovery and enrichment factors. DLLME has been successfully applied for the determination of trace organic pollutants and metal ions in several actual samples. Yamini et al. compared floating drop microextraction and homogenous liquid–liquid microextraction (HLLLE) for the extraction of two plasticizers from water samples. The results show HLLLE provided higher preconcentration factors (472.5- and 551.2-fold) with shorter extraction time as well as better RSDs (4.5–6.9%). Liang et al. determined three phthalate esters in water samples using DLLME and the enrichment factors ranged from 45–196 with a RSD of...
Its main drawbacks are low reproducibility, difficulty to automate and the necessity of using a third component (disperser solvent), which usually decreases the partition coefficient of analytes into the extractant solvent.

The aim of the present work is to develop a simple and sensitive method based on ultrasound-generated emulsions and DLLME for the determination of four PAEs in bottled water samples. The ultrasound-assisted process was applied to accelerate the formation of fine cloudy solution with less disperser solvent, which is increased the extraction efficiency and reduced the equilibrium time. Under the optimum condition, enrichment factors could reach 490–530-fold for PAEs with lower consumption of organic solvent and reduced equilibrium time.

**Experimental**

**Reagents and standards**

Dibutyl phthalate (DBP), butyl benzyl ester (BBP), diisooctyl phthalate (DIOP), dioctyl phthalate (DNOP) were obtained from Huaxin Chemical Reagent Co. (Baoding, China). The structures of the four PAEs are shown in Fig.1. Chloroform (CHCl₃), dichloromethane (CH₂Cl₂), 1,2-dichloroethane (C₂H₄Cl₂), 1,1,2,2-tetrachloroethane (C₂H₄Cl₄), chlorobenzene (C₆H₅Cl), tetrachloroethylene (C₂Cl₄), and carbon tetrachloride (CCl₄) were purchased from Tianyi Chemical Co., Ltd. (Tianjin, China). All the other reagents used in the experiment were of the highest grade commercially available. The stock solutions were prepared in acetone at a concentration of 1.0 mg mL⁻¹. The working standard solutions were prepared by diluting the stock solution with ultrapure water to get different concentrations in a range of 6.9–444 μg L⁻¹. All the glassware used in the study was previously washed with acetone and finally dried in an oven at 250 °C.

**Instrumentation**

A gas chromatograph (Shimadzu GC-2014) equipped with a split/splitless injector and a flame ionization detector (FID) (Shimadzu, Japan). High-purity nitrogen (99.999%) was used as the carrier gas and a GH-300 high-purity hydrogen generator and GA-2000A air pump (Beijing ZXHL Technology Development Co., Ltd.) were used to supply hydrogen and oxygen at the rate of 40 mL min⁻¹ and 400 mL min⁻¹, respectively. The capillary column was KB-1 (30 m × 0.25 mm × 0.25 μm, 100% dimethylpolysiloxane as stationary phase, Kromat Co., Delran, NJ, USA) and its column flow rate was set at 1.5 mL min⁻¹ with a split ratio of 10. N-2000 chromatography data workstation (Zhide Zheneng Co. Ltd., Hangzhou, China) was applied as the data acquisition system. The column temperature programming was as follows: the initial oven temperature was held at 185 °C for 5.0 min, and then increased to 285 °C at a rate of 25 °C min⁻¹ and held at 285 °C for 5.0 min. The injection port and FID temperature were maintained at 290 °C and 300 °C, respectively.

**DLLME procedure**

5.0 mL sample solution with 5% NaCl (m/v) was placed in a 10.0 mL conical centrifuge tube. 0.8 mL methanol and 20 μL CCl₄ was injected rapidly into the sample solution and then the mixture was gently shaken for several seconds. The cloudy solution was further emulsified by ultrasound for 2.0 min to make the fine droplets of extractant disperse entirely into the aqueous phase. Then phase separation was performed by a rapid centrifugation at 4000 rpm for 5.0 min and 2.0 μL of sediment phase was injected into GC for further analysis.

**Results and discussion**

**Effect of types of extraction solvent**

The extraction solvent of DLLME should be a higher density than water and have an extraction capability of the interested compounds and low solubility in water. Therefore, CHCl₃,
CH$_2$Cl$_2$, C$_2$H$_5$Cl, C$_2$H$_4$Cl$_2$, C$_2$Cl$_4$, C$_6$H$_5$Cl, and CCl$_4$ were investigated by spiking different volumes (40 $\mu$L and 140 $\mu$L) of each extraction solvent and 0.5 mL acetone into 5.0 mL water sample to achieve the sediment phase at the bottom of conical tube. The enrichment factors (EFs) in Fig. 3 indicated that CCl$_4$ was the best extraction solvent for the extraction of PAEs from water. Moreover, due to the different solubility of the extraction solvent in water and dispersive solvent, the volume of sediment phases varied significantly when a small amount of extraction solvent (40 $\mu$L) was applied. No sediment phase was observed after centrifugation when CHCl$_3$, C$_2$H$_5$Cl, and C$_2$H$_4$Cl$_2$ were used as the extraction solvent. Other extraction solvents (Fig. 3) could form stable cloudy solution and less consumption volumes, while the highest EF was obtained with CCl$_4$.

**Effect of types of disperser solvent**

The basic criterion in the selection of a disperser solvent is that it should form a cloudy state when injected with the organic extraction solvent into water. The main point for selection of disperser solvent is that it should be miscible in both the organic phase (extraction solvent) and the aqueous phase. This decreases the interfacial tension between the two phases and accelerates the droplets of extraction solvent into the aqueous phase. Thereby, methanol, ethanol, acetonitrile, THF, acetone and isopropanol were studied by applying 0.5 mL of each disperser solvent containing 40 $\mu$L CCl$_4$ into 5.0 mL water sample. Fig. 4 shows that the best EF was obtained using methanol as the disperser solvent, which may be due to its higher solubility for PAEs. Therefore, methanol was selected as the dispersive solvent for further work.

**Effect of extraction solvent volume**

In order to study the effect of extraction solvent volume on extraction efficiency, different volumes of CCl$_4$ (10–70 $\mu$L at 10 $\mu$L intervals) contained in 0.5 mL methanol were applied to the DLLME procedure (Fig. 5). The volume of sediment phase increased from 1.6 to 41 $\mu$L with the volume of extraction solvent increasing from 10 to 70 $\mu$L. However, the EF of PAEs decreases, obviously due to the increasing volume of sediment phase. When the volume of extraction solvent is 10 $\mu$L, the sediment phase was only 1.6 $\mu$L, which was hard to remove by microsyringe. Moreover, the reproducibility and extraction recoveries were reduced drastically with the reducing the volume of sediment phase. Considering the enrichment factor, droplet volume, reproducibility, and extraction recovery, 20 $\mu$L of extraction solvent was used in subsequent experiments.
Effect of disperser solvent volume

The volume of disperser solvent is a crucial parameter that has an important effect on extraction efficiency. Commonly, it is expected that as little as possible is used to achieve the highest EF and the lowest toxicity for environment; on the other hand, volumes should be sufficient to extract analyte and ensure enough sediment phases for further chromatographic analysis. Therefore, various volumes of disperser solvent (0.2, 0.4, 0.5, 0.6, 0.8 and 1.2 mL) respectively were used with 5.0 mL water samples. The results (Fig. 6) show that the extraction recoveries (ERs) increased with the increasing volume of disperser solvent up to 0.8 mL. However, due to the solubility of PAEs in water–methanol solution increasing, the recoveries decreased when the volume of disperser solvent was further increased. Considering the extraction efficiency, the enrichment factor and volume of sediment phase, 0.8 mL methanol was chosen for further work.

Effect of ultrasonic time

An ultrasound-assisted process can promote fine droplets of extraction solvent and accelerate the formation of cloudy solution. However, the volume of sediment phase decreased with the extension of ultrasonic time. This was caused by the mechanical effect and thermal effect of ultrasound which resulted in the volatile loss of analytes and extraction solvent. So, it is essential to optimize the ultrasonic time to ensure both enough sediment phases for injection and the best extraction performance. For this purpose, different ultrasonic times ranging from 0 to 4.0 min were investigated. The results (Fig. 7) show that the EFs were obviously increased from 0 to 2.0 min and then only slightly changed when further prolonging the ultrasonic time over 2.0 min. This was due to the finer droplets of extraction solvent being formed by ultrasonic vibration in the first 2.0 min and then these fine droplets keeping constant even at further increased ultrasonic time. Therefore, to enable a rapid extraction procedure and stable cloudy solution, 2.0 min was used as the optimum ultrasonic time.

Effect of ionic strength and pH

Different amounts of sodium chloride (0~25% (m/v)) were used to investigate the influence of ionic strength on extraction performance of DLLME. With increasing volumes of sodium chloride from 0 to 25%, the volumes of sediment phase increased from 8.0 to 11.0 μL due to the decreased solubility of extraction solvent in water. Increasing the sodium chloride (0~3%, m/v) led to an obviously increase in ERs for DIOP and DNOP from 41% to 100% and 37% to 96%, respectively. Then ERs were nearly constant even with further increases in the amount of sodium chloride from 5% to 25%. For DBP and BBP, the ERs were only slightly changed under the ranged salt concentration. The presence of salt increases the ionic strength of the sample solution and decreases the solubility of the extraction solvent in water, which leads to increased volume of sediment phase. On the other hand, a salting-out effect decreased the solubility of the analytes in water and therefore increased the concentration of analytes in the sediment phase (Fig. 8). By considering the volume of sediment phase, enrichment factor and extraction recovery, 5.0% sodium chloride was chosen for this work. The effect of pH was evaluated in the range 3.0~9.0 and the results demonstrated that the extraction efficiencies were nearly constant within the selected pH (Fig. 9).
Evaluation of DLLME method

To evaluate the proposed UA-DLLME-GC method, the linearity, precision, recovery, LOD and LOQ were investigated under optimum conditions. Calibration curves were constructed using the areas of the chromatographic peaks measured at seven increasing concentrations, in the range 6.9–444 μg L⁻¹. Good linearity was observed for all analytes throughout the concentration range, and the regression equations are shown in Table 1. The limits of detection (LOD) based on a signal-to-noise ratio of 3 ranged from 1.0 to 1.2 μg L⁻¹ and the limits of quantification (LOQ) ranged from 3.4 to 4.5 μg L⁻¹. Precision and accuracy were determined by analyzing five replicates of spiked samples at three-concentration levels on the same day and on five different days. The relative standard deviations (RSDs) were in the range 1.4–2.0% for intra-day precision and 3.0–3.7% for inter-day precision, respectively. The enrichment factors for the four PAEs ranged from 490- to 530-fold.

Real water samples analysis

Bottled water samples were filtered through 0.45 μm micropore membranes and 10 mL of the filtrated solution was divided into two tubes and extracted using the proposed UA-DLLME method. Three of PAEs were observed in water samples at levels of 9.1–11.3 μg L⁻¹ respectively. To investigate the effect of sample matrix and the accuracy of the proposed UA-DLLME method for real samples analysis, recovery testing was carried out by spiking three different concentrations of standard analytes into the samples and the results are shown in Table 2 and Fig. 10. The recoveries ranged from 84.8%–104.7% with a RSD less than 5.0%. These results demonstrate that the feasibility of the UA-DLLME method for the determining of trace phthalate esters in bottled water.

Table 1 Features of the UA-DLLME method

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Linear equation</th>
<th>r²</th>
<th>LOD/µg L⁻¹</th>
<th>LOQ/µg L⁻¹ (%)</th>
<th>RSD (%)</th>
<th>EF</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBP</td>
<td>y = 1.33 x 10⁶ x – 1.33 x 10⁵</td>
<td>0.9994</td>
<td>1.0</td>
<td>3.5</td>
<td>3.0</td>
<td>490</td>
</tr>
<tr>
<td>BBP</td>
<td>y = 1.59 x 10⁶ x – 1.80 x 10⁴</td>
<td>0.9994</td>
<td>1.0</td>
<td>4.5</td>
<td>3.4</td>
<td>530</td>
</tr>
<tr>
<td>DIOP</td>
<td>y = 1.52 x 10⁶ x – 1.45 x 10⁴</td>
<td>0.9992</td>
<td>1.0</td>
<td>3.4</td>
<td>3.0</td>
<td>517</td>
</tr>
<tr>
<td>DNOP</td>
<td>y = 1.49 x 10⁶ x + 1.58 x 10⁴</td>
<td>0.9992</td>
<td>1.1</td>
<td>3.9</td>
<td>3.7</td>
<td>515</td>
</tr>
</tbody>
</table>

Table 2 Analytical results of PAEs in bottled water sample (n = 3)

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Sample/µg L⁻¹</th>
<th>Spiked amount/µg L⁻¹</th>
<th>Detected amount/µg L⁻¹</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
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<tbody>
<tr>
<td>DBP</td>
<td>11.3</td>
<td>5.6</td>
<td>16.3</td>
<td>89.8</td>
<td>1.6</td>
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<td></td>
<td>11.2</td>
<td>21.1</td>
<td>32.3</td>
<td>87.8</td>
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<tr>
<td></td>
<td>22.4</td>
<td>30.8</td>
<td>61.2</td>
<td>87.0</td>
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</tr>
<tr>
<td>BBP</td>
<td>nd</td>
<td>7.6</td>
<td>24.9</td>
<td>98.6</td>
<td>2.2</td>
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<tr>
<td></td>
<td>15.2</td>
<td>21.1</td>
<td>36.3</td>
<td>95.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30.4</td>
<td>28.8</td>
<td>59.2</td>
<td>94.8</td>
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</tr>
<tr>
<td>DIOP</td>
<td>10.3</td>
<td>5.4</td>
<td>14.9</td>
<td>84.8</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>10.8</td>
<td>20.4</td>
<td>31.2</td>
<td>93.5</td>
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</tr>
<tr>
<td></td>
<td>21.6</td>
<td>29.9</td>
<td>51.5</td>
<td>90.9</td>
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<tr>
<td>DNOP</td>
<td>9.1</td>
<td>6.0</td>
<td>15.0</td>
<td>98.3</td>
<td>4.9</td>
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<td></td>
<td>12.0</td>
<td>20.5</td>
<td>32.5</td>
<td>94.9</td>
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<td></td>
<td>24.0</td>
<td>34.2</td>
<td>58.2</td>
<td>104.7</td>
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* nd, not detected.
Conclusion

A simple UA-DLLME method was developed for rapid concentration and determination of trace levels of phthalate esters in bottled water samples. An ultrasound-assisted process was applied to accelerate the formation of the fine cloudy solution using less disperser solvent, which markedly increased the extraction efficiency and reduced the equilibrium time. Under the optimum conditions, the enrichment factors for the PAEs ranged from 490- to 530-fold. The recoveries of four PAEs at three spiked levels were in the range 84.8%–104.7% with a RSD less than 5.0%. This method offers a good alternative to conventional techniques for routine analysis due to its simplicity and reliability.

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References