

# Slow-Injection Ultrasound-Assisted Emulsification–Microextraction for Determination of Phthalate Esters in Water<sup>†</sup>

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<sup>†</sup>Dedicated to Professor Jean'ne M. Shreeve on the occasion of her 80th birthday.

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**A type of ultrasound-assisted emulsification–microextraction (USAEME) was applied for the extraction and determination of four phthalate esters (e.g., dimethyl phthalate, diethyl phthalate, dibutyl phthalate and benzyl butyl phthalate) in aqueous samples prior to quantification using HPLC-UV. The simultaneous injection of the extraction solvent and ultrasonication of the mixture results in an efficient extraction with the extractant well-dispersed in the sample. The parameters affecting the experimental results were analyzed and optimized through the design of the experiment. Using a central composite face-centered design, the results of 28 experimental data points were analyzed and validated. An optimal set of operating conditions was obtained using 40  $\mu\text{L}$  of 1,1,2,2-tetrachloroethane and 1% NaCl subjected to 2.0 min of ultrasonication under natural pH. Under optimized conditions, the extractions resulted in analyte recoveries of 75–87% and high enrichment factors of 356–415. The calibration curves were linear, and the correlation coefficients ranged from 0.9992 to 0.9997 at concentrations of 5 to 300  $\mu\text{g L}^{-1}$ . The RSDs ( $n = 5$ ) were 5.6–7.9%. The limits of detection for the four phthalate esters ranged from 0.26 to 1.46  $\mu\text{g L}^{-1}$ .**

## Introduction

Phthalate esters (PAEs) are one of the more widely used man-made organic compounds. These compounds are largely used as plasticizers in polymeric materials to enhance flexibility, workability and general handling properties. The production and use of PAEs have increased worldwide significantly. However, PAEs and their metabolites and degradation products can cause adverse effects on human health, especially in the liver, kidney and testicles (1). Previous studies have shown that PAEs may cause hormone-disrupting activities, possibly associated with known estrogenic and antiandrogenic activities (2–4). Considering that PAEs and polymer chains combine through weak secondary molecular interactions, PAEs can be gradually released into food or water from the packaging materials and into the environment during production and incineration (3, 5). Water, soil, air and food products contaminated with PAEs have been reported (6–10), and the pollution they cause has become a matter of public concern. Therefore, the development of reliable analytical methods for the determination of PAEs in environmental matrices, especially in water samples, is necessary.

In recent years, interest in the development of simplified and miniaturized methods for sample pretreatment techniques has grown. Various microextraction (ME) techniques have been

explored as alternatives to conventional extraction procedures. Aside from the traditional liquid–liquid extraction (7, 11) and solid-phase extraction (12, 13), solid-phase microextraction (14, 15), single-drop microextraction (16) and hollow fiber liquid–phase microextraction (17) have also been applied to pre-concentrate PAEs in environmental, food and biological matrices.

Recently, a novel ME technique called dispersive liquid–liquid microextraction (DLLME) was developed by Rezaee *et al.* (18). The method is based on a ternary component solvent system after injecting the appropriate mixture of high-density extractant and water-miscible dispersive solvent into an aqueous sample. The mixture system reaches equilibrium rapidly, which considerably shortens the operation time. DLLME has been widely applied for the determination of pesticides (19), environmental contaminants (20) and pharmaceuticals (21) in different matrices. Ultrasonication can produce very fine emulsions from immiscible liquids, which result in very large interfacial contact areas and a corresponding great increase in mass transfer between two immiscible phases. This technique enhanced the extraction efficiency of the procedure in minimal time (22, 23). Hence, the method called ultrasound-assisted dispersive liquid–liquid microextraction (USA-DLLME) was applied (24–26) and became one of the most important steps in DLLME. Regueiro *et al.* developed an ultrasound-assisted emulsification–microextraction (USAEME) to analyze PAEs in water samples in 2008 (27), in which only ultrasonication was employed to form an emulsion. USAEME was used successfully in the determination of pesticides, sulfur compounds, bisphenol, heavy metals and Brett character responsible compounds, among others (28–32). Considering the lower efficiency of ultrasonic bath in energy transmission compared with the ultrasonic probe (33), direct ultrasound-assisted DLLME (DUSA-DLLME) (34) was recently developed by Cortada *et al.*, in which an efficient ultrasonic probe (i.e., sonotrode) was introduced directly into a sample to emulsify the mixture. With the aid of a high-energy transmission instrument, the extraction could be accomplished efficiently in DUSA-DLLME.

In the present study, a type of USAEME (present USAEME, i.e., P-USAEME) is applied to determine PAEs in water samples. Only a microsyringe and an ordinary ultrasonic bath are required in the method. Unlike the typical USAEME (T-USAEME), a small amount of extraction solvent in the microsyringe is slowly injected into the aqueous sample, while ultrasonication of the mixture is being carried out. The simultaneous injection of the solvent and ultrasonication of the mixture causes the extractant

to disperse throughout the sample immediately. Compared with USAEME and DUSA-DLLME, the proposed method does not require the aid of a high-energy transmission instrument and accomplishes the extraction in a short time. Although a dispersive solvent or an ultrasonic probe were not used in the process, the emulsification was sufficient, and therefore, the enrichment factors (EFs) were relatively high. Good performance and high efficiency can be achieved using a microsyringe and an ordinary ultrasonic bath. It could be a fast, simple, inexpensive and efficient microextraction method for the future use. The extraction parameters, including the volume of the extractant, ultrasonication time, pH and salt concentration, were evaluated and optimized using a response surface methodology (RSM) based on statistical design of experiments (DOE). The optimum USAEME followed by analysis using HPLC-UV was performed to determine the four PAEs [dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP) and benzyl butyl phthalate (BBP)] in real water samples (river water, lake water, reservoir water and tap water).

## Experimental

### Reagents

PAE standards (DMP, DEP, DBP and BBP) were obtained from Aladdin Chemistry Co., Ltd. (Shanghai, China). HPLC-grade methanol was purchased from Dikma Technologies, Inc. (Lake Forest, CA, USA). Analytical-grade chlorobenzene, carbon tetrachloride, 1,1,2,2-tetrachloroethane and sodium chloride were obtained from the Beijing Chemical Factory (Beijing, China).

Stock solutions ( $1 \text{ g L}^{-1}$ ) were prepared by dissolving PAE standards in HPLC-grade methanol and were stored in a refrigerator. Mixed standard solutions were also prepared in methanol. Fresh working standard solutions were prepared daily by diluting the mixed standard solutions to different concentrations using ultrapure water. River and lake water used for method validation were collected from Yangtze River and East Lake in Wuhan, Hubei Province, China; reservoir water was collected from Beijing, China; and tap water was collected from the laboratory. The water samples were collected in glass bottles and stored in the refrigerator, protected from light and filtered through a  $0.22\text{-}\mu\text{m}$  mixed cellulose membrane (Agela, Wilmington, DE, USA) before use.

### Instrumentation and software

Chromatographic analysis was carried out using an Agilent 1200 HPLC system equipped with a variable wavelength detector system (Agilent, CA, USA). The injection volume was  $10 \mu\text{L}$  in all cases. Separation of the analytes was carried out on an Agilent Eclipse Plus C18 column ( $5 \mu\text{m}$ ,  $4.6 \times 250 \text{ mm}$ ) using methanol–water (77:23, v/v) as the mobile phase. The flow rate was kept at  $1 \text{ mL min}^{-1}$  and the absorbance was measured at  $280 \text{ nm}$ . Emulsification of the sample system was carried out at  $40 \text{ kHz}$  and  $50 \text{ W}$  using a KQ-50DE ultrasonic water bath purchased from Kunshan Ultrasonic Instruments Co., Ltd (Kunshan, China). The pH of the water samples was adjusted using a Hanna bench pH 211 meter from HANNA Instruments, Inc. (Woonsocket, RI, USA). Centrifugation was performed in a 52a centrifuge from the Baiyang Centrifuge Factory (Xin'an, China).

Optimization of the parameters affecting the extraction performance of USAEME was performed using a central composite face-centered design (CCF) via the MINITAB<sup>®</sup> Release 16 statistical software (35). The range of variables, including the volume of the extraction solvent, ultrasonication time, pH and the concentration of salt that affect the ER% and EF, is represented in Supplementary data, Table S1. The volume of the extraction solvent ranged from  $40$  to  $70 \mu\text{L}$ , the ultrasonication time ranged from  $0$  to  $3 \text{ min}$ , pH ranged from  $6$  to  $8$  and the concentration of salt ranged from  $0$  to  $10\%$ . Experiments were carried out using working solutions containing  $50 \mu\text{g L}^{-1}$  of each PAE in all cases.

### Extraction procedure

Each standard solution ( $10 \text{ mL}$ ) or water sample ( $10 \text{ mL}$ ) with  $1\%$  NaCl (m/v) and under natural pH was placed into a  $15\text{-mL}$  screw-cap polytetrafluoroethylene centrifuge tube. While the sample was being ultrasonicated,  $40 \mu\text{L}$  of 1,1,2,2-tetrachloroethane was slowly injected into the sample solution using a  $100\text{-}\mu\text{L}$  microsyringe within  $\sim 1 \text{ min}$ . During the injection process, a cloudy solution was formed caused by the dispersion of fine extractant droplets into the sample. The resulting solution was ultrasonicated for  $2.0 \text{ min}$ . After ultrasonication, the turbid mixture was centrifuged at  $4000 \text{ rpm}$  for  $10 \text{ min}$ . The upper aqueous phase was removed using a syringe. The extract was removed and injected directly into the HPLC system for analysis, the inject volume was  $10 \mu\text{L}$ .

## Results

### Optimization of the SI-USAEME method

EF and extraction recovery (ER%) were used to evaluate the extraction efficiency under different conditions to obtain the optimized extraction procedure. EF is defined as the concentration ratio of the analyte between the sedimented phase and the initial water sample. The equations for EF and ER% are shown in Equations (1) and (2):

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

where  $C_{\text{sed}}$  and  $C_0$  are the analyte concentration in the sediment phase and the initial analyte concentration in the aqueous sample, respectively.

ER% is computed as follows:

$$\text{ER}\% = \frac{C_{\text{sed}} \cdot V_{\text{sed}}}{C_0 \cdot V_0} \times 100 = \text{EF} \times \frac{V_{\text{sed}}}{V_0} \times 100 \quad (2)$$

where  $V_{\text{sed}}$  is the volume of the sediment phase and  $V_0$  is the volume of the aqueous sample.

### Selection of the extraction solvents

Considering that various chlorinated hydrocarbons, such as chlorobenzene, carbon tetrachloride and 1,1,2,2-tetrachloroethane, have been successfully applied as extraction solvents (36–38), these compounds were selected for optimization. A series of experiments were studied using  $40 \mu\text{L}$  of different extraction solvents, and the recoveries are shown in Figure 1.

During injection, all the three extractants could disperse well in the sample. Thus, the operation can be used widely in the preconcentration of various analytes when combined with appropriate extraction solvents. For the present study, the extraction efficiency of  $\text{CCl}_4$  for DMP was extremely low (9.3%). Chlorobenzene showed an average performance in the extraction (44.4–72.6%); hence, it could not be the ideal choice. 1,1,2,2-Tetrachloroethane achieved good results in extracting all

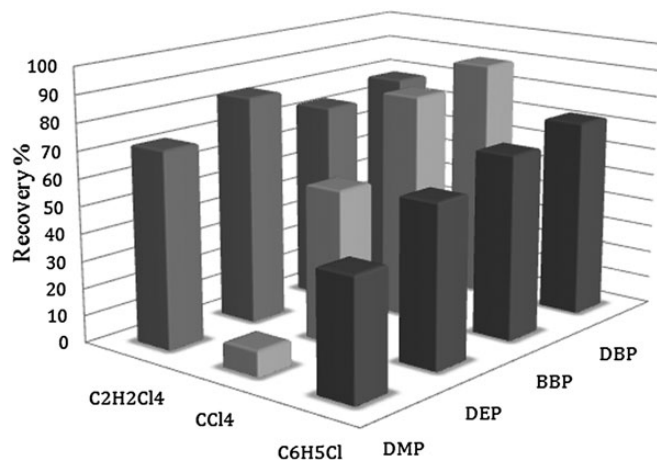
the PAEs being studied and presented the best extraction efficiency (72.2–84.2%) compared with the other two solvents. Consequently, 1,1,2,2-tetrachloroethane was chosen as the best extraction solvent in the presented USAEME method.

#### Optimization of the injection speed

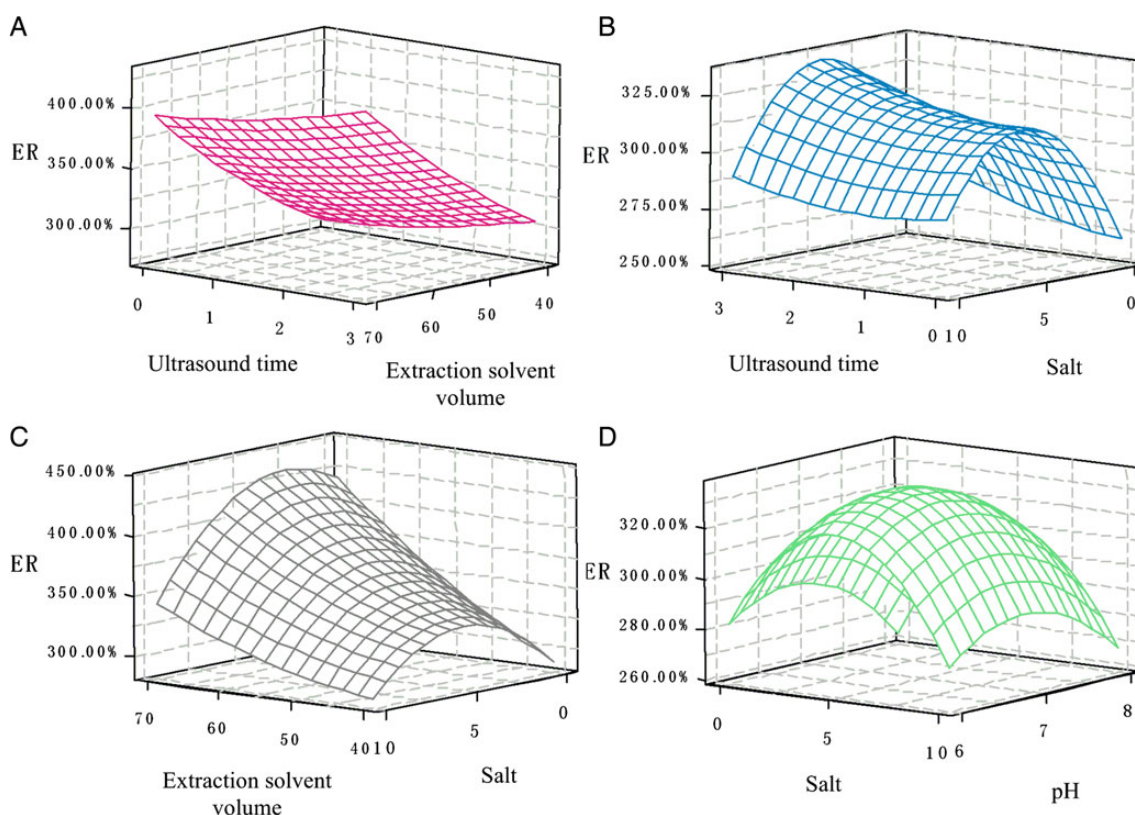
The injection speed was optimized through comparison of dispersion of extractant. The extractant was poorly dispersed in the aqueous phase when the speed was high (injection time < 1 min), the aggregates of extractant directly sink in solution to the bottom of the test tube. However, it was time consuming if the injection was excessively slow. In our research, the highest injection speed was selected when it could make the extractant dispersed well in the samples (no extractant congregated and dropped to the bottom of vessel), i.e., 1 min of injection time.

#### Optimization of the procedure using the RSM approach

After performing some preliminary experiments, optimization of the extraction conditions was conducted using a CCF design. The sums of the four ER% and EF ( $\sum \text{ER}\%$  and  $\sum \text{EF}$ ) were taken as the responses, and ER and EF were applied to the rest for convenience. Multiple regression results obtained from CCF, including  $t$  and  $P$ -values, along with the constants and coefficients (estimated in coded units), are given in Supplementary data, Table SII. Coefficient terms that have a large  $t$ -value and a smaller  $P$ -value were considered more significant compared with the

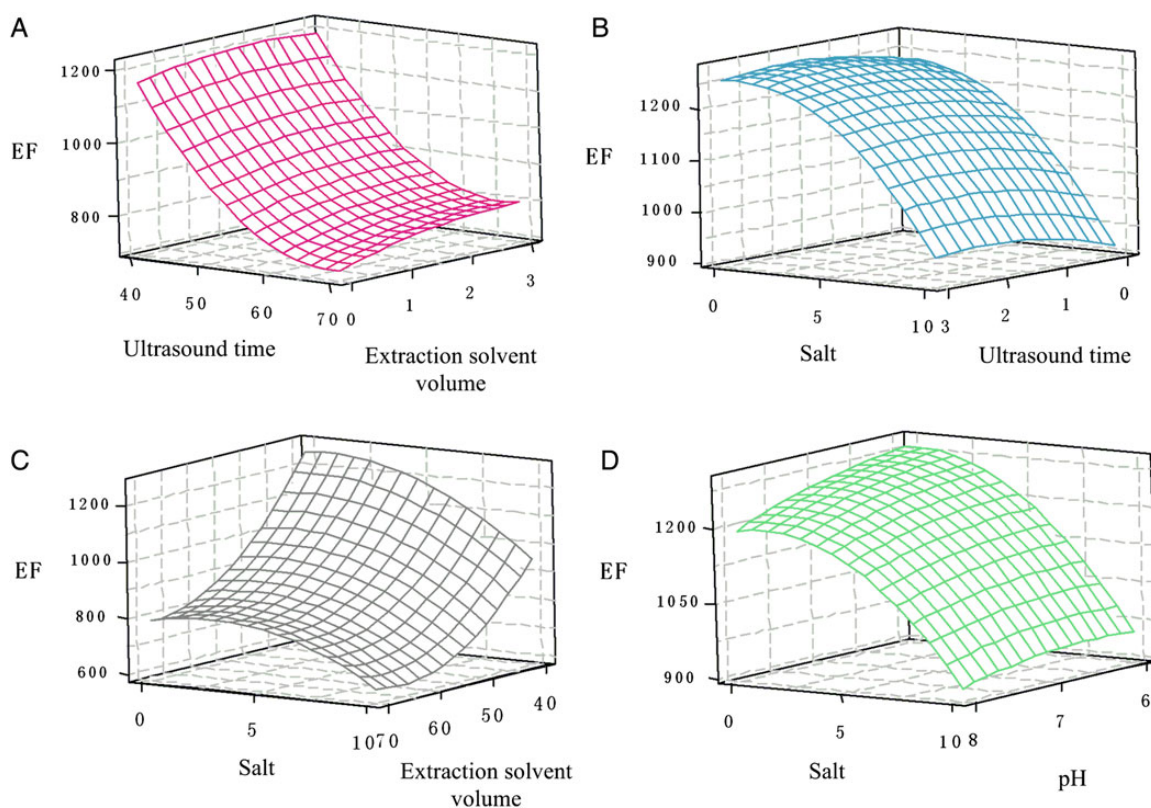


**Figure 1.** Comparison of the extraction recoveries using different extractants in SI-USAEME (sample volume of 10 mL, extractant volume of 40  $\mu\text{L}$ , ultrasound time of



**Figure 2.** Response surface plots for the sum of extraction recoveries as a function of the factors: (A) extraction solvent volume and ultrasound time, (B) ultrasound time and concentration of NaCl, (C) extraction solvent volume and concentration of NaCl and (D) concentration of NaCl and pH.





**Figure 3.** Response surface plots for the sum of EFs as a function of the factors: (A) extraction solvent volume and ultrasound time, (B) ultrasound time and concentration of NaCl, (C) extraction solvent volume and concentration of NaCl and (D) concentration of NaCl and pH.

PAEs	Linear range ( $\mu\text{g L}^{-1}$ )	Linearity	$r$	LODs ( $\mu\text{g L}^{-1}$ ) <sup>a</sup>	LOQs ( $\mu\text{g L}^{-1}$ ) <sup>b</sup>	RSD (%) <sup>c</sup>	EF	Recovery (%) <sup>d</sup>
DMP	5–300	$y = 1.04x + 4.30$	0.9992	0.28	0.87	7.91	356	75
DEP	5–300	$y = 1.01x + 4.07$	0.9995	0.26	0.87	7.82	415	87
BBP	10–300	$y = 0.71x + 2.22$	0.9997	1.46	4.87	5.64	360	76
DBP	10–300	$y = 0.72x + 2.28$	0.9995	1.36	4.53	6.29	384	81

<sup>a</sup>LODs are calculated from spiked water sample with  $10 \mu\text{g L}^{-1}$  of each phthalate ester,  $S/N = 3$ .

<sup>b</sup>LOQs are calculated from spiked water sample with  $10 \mu\text{g L}^{-1}$  of each phthalate ester,  $S/N = 10$ .

<sup>c</sup>RSD values are calculated by five extraction reduplicates ( $n = 5$ ) of the studied PAEs.

<sup>d</sup>The ER and EF are obtained at the spiked level of  $50 \mu\text{g L}^{-1}$ .

others. Terms with  $P$ -values of  $<0.05$  were considered significant to the response when the confidence level was set to 95%. The  $R^2$  statistics indicated that the models displayed variations of 97.31 and 97.48%. The adjusted  $R^2$  statistic ranged from 94.40 to 94.76%, which indicates high dependence and coefficient of estimation between the experimental and the predicted response values. Standard deviations of the residues were found to be 0.115 and 47.908 for the variables. The values confirm that the regression adequately describes the relationships between the experimental responses and the variables.

The results for the analysis of variance (ANOVA) are presented in Supplementary data, Table SIII. In the ANOVA study, a  $P$ -value of  $<0.05$  indicates the statistical significance of an effect at a 95% confidence level, implying that the model is statistically significant (39). Besides, the normality of each data was checked by

plotting the normal probability plot of the residuals. Thus, the applicability of the predicted model is confirmed through this ANOVA study.

The three-dimensional response surfaces were drawn to visualize the relationship between the responses and the significant experimental factors (40, 41). The response surfaces of ER and EF versus the significant parameters, including extractant volume, ultrasound time, and salt concentration as well as versus non-significant pH are shown in Figures 2 and 3, respectively. While two parameters were taken as the  $X$  and  $Y$  values, the others were held constant at their central levels. The curvatures of these plots indicate the interaction between the variables.

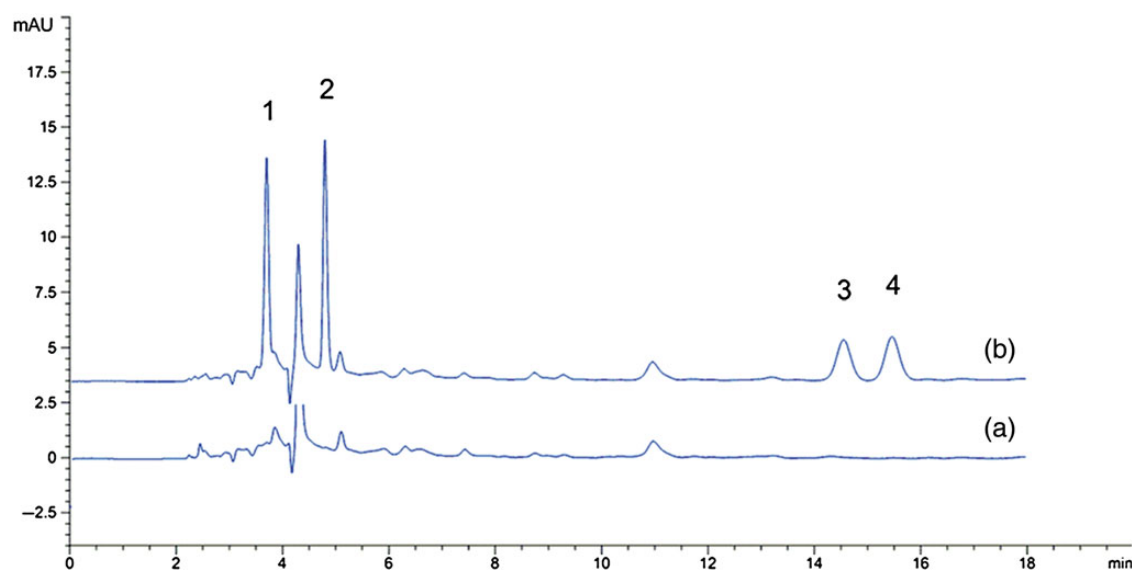
Final optimization of the experimental conditions was performed using optimization plots With the aim of finding the maximum of ER and EF at reasonable operating conditions, the

**Table II**

Extraction recoveries and relative standard deviations in four blank and spiked water samples by the USAEME–HPLC method

PAEs	Spiked level ( $\mu\text{g L}^{-1}$ )	River water		Lake water		Reservoir water		Tap water	
		ER (%)	RSD (%)	ER (%)	RSD (%)	ER (%)	RSD (%)	ER (%)	RSD (%)
DMP	Blank	ND		ND		ND		ND	
	25	73.9	5.9	75.4	7.3	72.5	5.1	74.3	8.3
	50	74.3	5.2	73.3	6.0	72.6	5.0	75.0	4.8
DEP	Blank	ND		ND		ND		ND	
	25	80.1	6.5	83.9	4.9	83.0	1.5	85.8	7.1
	50	80.6	3.9	82.0	6.7	82.4	3.1	86.6	6.1
BBP	Blank	ND		ND		ND		ND	
	25	76.2	1.5	75.3	5.8	72.6	5.2	73.1	5.3
	50	74.5	4.8	72.9	4.0	74.3	4.4	79.3	9.3
DBP	Blank	ND		ND		ND		ND	
	25	80.5	5.7	80.6	3.9	79.3	2.2	70.6	8.9
	50	74.5	3.6	74.6	5.0	76.2	3.4	81.3	9.3

ER, extraction recovery; ND, not detected.

RSD values are calculated by performing three extraction reduplicates ( $n = 3$ ) of the studied PAEs.**Figure 4.** Chromatograms of phthalate esters in (a) blank and (b) spiked (at the concentration level of  $50 \mu\text{g L}^{-1}$ ) Yangtze River water samples obtained through SI-DLLME. Peaks: (1) DMP, (2) DEP, (3) BBP and (4) DBP.

experimental factors were optimized using the following conditions:  $40 \mu\text{L}$  of 1,1,2,2-tetrachloroethane, 2.0 min of ultrasound, pH 7, and 1% NaCl concentration, with a desirability score of 1.0000. Natural pH was maintained because of its minimum effect on the final results.

#### Evaluation of method performance

The USAEME method was evaluated according to linearity, limits of detection (LODs), limits of quantification (LOQs), precision, EFs and recoveries under the optimized conditions. Linearity was observed within the ranges  $5\text{--}300 \mu\text{g L}^{-1}$  of DMP and DEP and  $10\text{--}300 \mu\text{g L}^{-1}$  of BBP and DBP. Correlation coefficients ranged from 0.9992 to 0.9997. Precision was obtained by conducting five replicates of water samples spiked with  $50 \mu\text{g L}^{-1}$  of PAEs. The RSDs of the PAEs ranged from 5.6 to 7.9%. The

LODs and LOQs were in the ranges  $0.26\text{--}1.46 \mu\text{g L}^{-1}$  and  $0.87\text{--}4.87 \mu\text{g L}^{-1}$ , respectively. The values were determined as the analyte concentrations that gave signal-to-noise ratios of 3 and 10, as calculated by the instrument software at a spiked level of  $10 \mu\text{g L}^{-1}$ . Calculated LODs and LOQs were experimentally tested. EF reached a high level ranging from 356 to 415 and ER% ranged from 75 to 87%. The validated results are shown in Table I.

#### Real water sample analysis

The applicability of the USAEME method was validated by performing extractions in four real water samples, including river water, lake water, reservoir water and tap water at spiked levels of 25 and  $50 \mu\text{g L}^{-1}$ . The recoveries and RSDs are shown in Table II. The results show that the extraction recoveries were

Table III

Comparison of the USAEME–HPLC-UV method with other methods for the determination of PAEs in aqueous samples

Method	Sample volume (mL)	Extraction time (min)	LOD ( $\mu\text{g L}^{-1}$ )	Linear range ( $\mu\text{g L}^{-1}$ )	EF	References
DLLME–HPLC-UV <sup>a</sup>	5	1	0.64–1.8	5–5000	44–196	(36)
IL-CIA-DLLME-HPLC-UV <sup>b</sup>	5	>3	0.68–1.36	2–100	174–212	(42)
USAEME–GC/MS	10	10	0.54–2.88	5–500	>100	(27)
USA-DLLME-GC-FID <sup>c</sup>	5	3	1.0–1.1	6.9–444	490–530	(24)
The presented method	10	3	0.26–1.46	5–300	356–415	The presented method

<sup>a</sup>Conventional dispersive liquid–liquid microextraction; extraction solvent:  $\text{CCl}_4$ .<sup>b</sup>Ionic liquid cold-induced aggregation dispersive liquid–liquid microextraction; extraction solvent:  $[\text{C}_8\text{MIM}][\text{PF}_6]$ .<sup>c</sup>USA-DLLE; extraction solvent:  $\text{CCl}_4$ .

between 70.6 and 86.6%, and the RSDs were between 1.5 and 9.3% in the four water samples. These results indicate that the matrices of the real water samples have little effect on the proposed USAEME method in the preconcentration of PAEs from water samples. The typical chromatograms of the non-spiked and spiked Yangtze River samples obtained using the USAEME method are shown in Figure 4.

## Discussion

As a novel USAEME method, the extraction efficiency of P-USAEME was compared with that of T-USAEME. In T-USAEME, the extractant was injected before the ultrasonication. Even though the extractant began to disperse when the ultrasonication was carried out, the dispersion is poor and not sufficient. It could be attributed to the high surface tension of 1,1,2,2-tetrachloroethane. By means of intensive hand shaking (several times during ultrasound), the extractant dispersed into the sample. The comparison of the extraction performance is listed in Supplementary data, Table SIV. As can be seen from the Supplementary data, Table SIV, the total recovery of P-USAEME was 19% higher than T-USAEME and the dispersion and extraction were more efficient with injecting the extractant during ultrasonication. Simultaneous injection and ultrasound is necessary for the ME procedure.

Extraction and determination of PAEs using the proposed method were compared with those of the other methods for evaluation (25, 28, 36, 42), and the results are shown in Table III. Although no dispersive solvent was used in the presented method, USAEME still achieved good performance in the analysis and obtained higher EF than other methods using HPLC-UV detection. Higher EF could be attributed to: (1) good dispersion of the extractant into the aqueous solution with simultaneous injection and ultrasonication (2) absence of organic dispersive solvent which increased the partition coefficient of the analytes into the extractant, lowering the extraction difficulty. The analytical results of the proposed method could even be comparable with those of some methods using GC or GC/MS detection which used less extractant to achieve high EFs and low LODs. Compared with DUSA-DLLME, the proposed method does not require a sonotrode instrument and accomplishes the extraction in a short time. Good performance and high efficiency could be achieved using a microsyringe and ordinary ultrasonic bath. Thus, the method is expected to be applied widely in the analysis of different analytes (target compounds) when combined with appropriate extractants in the future.

## Conclusion

In the current study, a type of USAEME coupled with HPLC-UV was applied to the analysis of PAEs in water samples. Parameters affecting the experimental results were analyzed and optimized with the aid of DOE. Simultaneous injection and ultrasonication results in an efficient extraction because the extractant is dispersed well in the sample. The method showed higher EF than the other reported methods. Good performance was achieved in analyzing the PAEs from Yangtze River water, East Lake water, reservoir water and tap water using the proposed method. Thus, the method has been proved to be fast, simple, inexpensive, efficient and environmental friendly, and is expected to be widely applied in the analysis of different target compounds when combined with appropriate extractants in the future.

## Supplementary data

Supplementary data are available at *Journal of Chromatographic Science* online.

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