

Determination of Trace Benzene Derivatives in Aqueous Samples by Ultrasonic Enhanced Hollow Fiber Liquid-Phase Microextraction Prior to Gas Chromatography

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Abstract

A new method was developed for the determination of trace compounds in water samples using ultrasonic-enhanced hollow fiber liquid-phase microextraction (U-HF-LPME). The ultrasonic radiation, which produces mechanical vibration and ultrasonic cavitation, could be used for accelerating the diffusion mass transfer process. Thus, ultrasonic was introduced into the HF-LPME procedure to enhance the mass-transfer rate during the aqueous and extraction solvent phases. Experimental parameters such as the extraction solvent, the extraction time, the ultrasonic frequency and power, the extractant volume, and ionic strength of the sample were assessed and optimized. Under optimal conditions, HF-LPME was achieved within 10 min. The high enrichment factor in the range of 120–666 and a good relative recovery in the range of 97–103% were evaluated with the relative standard deviations (RSDs, $n = 5$) of 0.3–7.0%. The limit of detection was in the range of 0.8–3.0 $\mu\text{g/L}$. The method was applied to the analysis of groundwater, lake water, and seawater. The results showed that the method can determine trace benzene derivatives in real samples with RSD values of 1.1–4.2%. The results demonstrated that U-HF-LPME is a rapid, accurate, and effective sample preparation method, and could be successfully applied for the determination of trace compounds in analytical chemistry.

Introduction

The method of liquid-phase extraction (LPE) prior to gas chromatography with a flame ionization detector (GC–FID) is usually recommended to detect benzene derivatives present in aqueous samples. However, traditional liquid-phase extraction uses large sample and solvent volumes and requires long equilibrium times (1,2). Thus, various forms of liquid-phase microextraction methods (LPME) have been developed since the drop-in-drop LPME system has been reported by Dasgupta in 1996 as a sample

pre-concentration method, and involved the used of a droplet of organic solvent hanging at the end of a micro-syringe needle (3–6). This method was a solvent-minimized pretreatment technique, and a high enrichment factor (EF) may be achieved for the analyte with high partition coefficients because they are transferred by diffusion from a relatively large sample volume (1–5 mL) into a micro-extract of typically 5–50 μL . However, the droplet in LPME may be lost from the needle tip of the syringe during extraction (7). Hollow fiber liquid-phase microextraction (HF-LPME) redressed these limitations successfully (8–13). A porous HF, which had a favorable mass flux and was served as an interface between the donor and acceptor phases, could protect the organic phase. The possibility of less volumes in the acceptor solvent phase resulted in increased sensitivity. Therefore, HF-LPME represents an attractive alternative with the advantages of being simple, inexpensive, sensitive, and virtually solvent-free. It has been successfully applied to the extraction of drugs from a variety of biological fluids or to the pre-concentration of pollutants from several environmental matrices (11,14). Even though HF-LPME is a very simple and effective sample preparation method, it is a relatively time-consuming technique, and one extraction usually takes typically 25–45 min. Recently, electromembrane extraction (EME) (15,16) using an electrical potential difference as the driving force shortened the extraction time to typically 5 min per extraction (17–19). But the electric field can only drive charged particles. The EME technique is not suitable for un-charged compounds. Therefore, a method was developed for the determination of compounds in water samples using ultrasound-assisted dispersive liquid-liquid microextraction (20–22). Even if the whole extraction process was completed within 10 min (23), it is a relatively complicated operation and low-stability technique.

It's well known that some process can be improved effectively by the use of ultrasound energy (24–26). Thus, the ultrasound-accelerated procedure was introduced into the HF-LPME system to increase the rate of analyte mass transfer between the two immiscible phases. The system can be called as ultrasonic

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enhanced hollow-fiber liquid phase microextraction (U-HF-LPME). In the study, the U-HF-LPME system was evaluated by the determination of trace benzene derivatives in water samples. Several experimental variables on the extraction efficiency, such as extraction solvent, extraction time, ultrasonic power and frequency, extractant volume, and the ionic strength of the sample, were investigated and optimized.

Experimental

Reagents and chemicals

All of the reagents used [*n*-octanol, chloroform, carbon tetrachloride, *n*-hexane, *n*-heptane, benzene, toluene, ethylbenzene, styrene, (1-methylethyl)benzene, dimethylsulfoxide, ethanol, and sodium chloride] were of analytical grade or higher and were purchased from Kemi'ou Reagent Co., Ltd. (Tianjin, China). The poly(vinylidene difluoride) HF was purchased from Tianjin Motimo Membrane Engineering and Technology Co., Ltd. (Tianjin, China). The dimensions were 600 μm (i.d.), the pore size was 0.2 μm , and a pore ratio of 60%. The HF membrane tubes were ultrasonically cleaned in ethanol at least three times and dried before use. Segments of 3 cm lengths were used for extractions.

The stock standard solutions were prepared in deionized water with the concentration level of 20 mg/L for benzene, toluene, ethylbenzene, styrene, and (1-methylethyl)benzene, and stored in a freezer at about 4°C. Then, the working standard solutions were freshly prepared by diluting the mixed standard solution with deionized water to the required concentration.

Apparatus

Ultrasonic radiation washers (DL-360E and DL-400A, Shanghai Zhixin Co., Ltd., Shanghai, China) were used for all experiments. The aqueous solution in the HF-LPME system was stirred by a magnetic stirrer using a 2.0 \times 0.5 cm stirring bar at 600 rpm. The separation and quantification of the benzene derivatives were performed using a SP-6800A gas chromatograph, equipped with a flame ionization detector (Lunan Ruihong, Shandong, China). An SE-54 (5% phenyl and 95% methylpolysiloxane) fused-silica capillary column (30 m \times 0.20 mm i.d., 0.25 μm) was used and obtained from Lanzhou Atech Technology Co., Ltd. (Gansu, China). The injector and detector temperatures were set at 220°C, and the column temperature was set as a programmed temperature according to the properties of the analyte.

Procedure for HF-LPME

HF-LPME was performed according to the following procedure: the 50 mL aqueous sample solution was placed into the 100 mL glass bottle. The required amount of extraction solvent was then injected using a 100 μL syringe and two ends of the HF segment were closed by mechanical pressure. The filled HF segment was then placed in the glass bottle for immediate extraction. During microextraction, the aqueous solution was continuously stirred at room temperature with a magnetic stirrer at 600 rpm. After extraction, the extraction phase was collected with a microsyringe and analyzed by GC directly.

Procedure for U-HF-LPME

All operation steps of U-HF-LPME were the same as with HF-LPME before extraction. Particularly, the LPME was performed using ultrasonic agitation (see Figure 1), instead of magnetic stirring during the microextraction.

Calculation of EF and relative recovery

The EF was defined as the ratio between the analyte concentration in the extraction solvent phase (c_{esp}) and the initial concentration of analyte (c_i) within the sample:

$$EF = c_{esp}/c_i \quad \text{Eq. 1}$$

The relative recovery (RR) was obtained as the following equation:

$$RR = (c_{found} - c_{real} / 100) / c_{added} \times 100 \quad \text{Eq. 2}$$

where c_{found} , c_{real} , and c_{added} are the concentrations of the analyte after the addition of a known amount of the standard in the real sample, the concentration of the analyte in the real sample, and the concentration of a known amount of the standard which was spiked to the real sample, respectively.

Results and Discussion

Selection of organic solvent

The selection of an appropriate extraction solvent is of great importance for the optimization of the U-HF-LPME process. In the present study, *n*-octanol, chloroform, carbon tetrachloride, *n*-hexane, and *n*-heptane were tested as extraction solvents. The results showed that chloroform, carbon tetrachloride, *n*-hexane, and *n*-heptane were discarded due to their relative solubility in water, instability, or high volatility. The volatilization rate (expressed as % residue) in a HF is 99% in *n*-octanol, 27% in

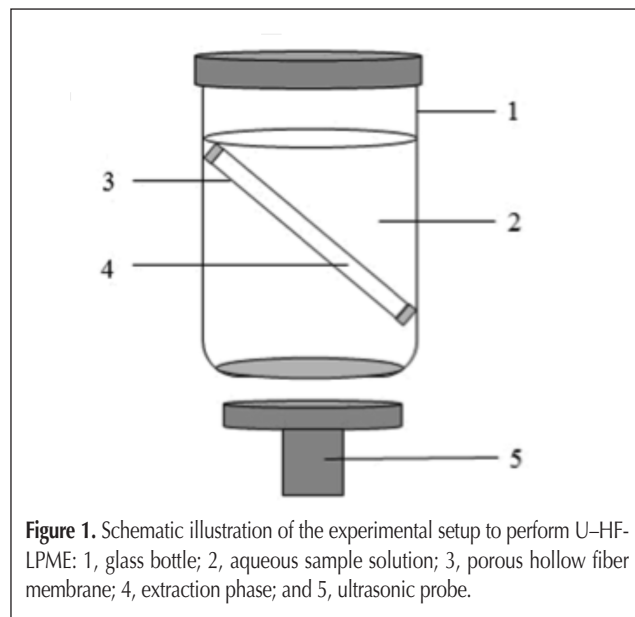


Figure 1. Schematic illustration of the experimental setup to perform U-HF-LPME: 1, glass bottle; 2, aqueous sample solution; 3, porous hollow fiber membrane; 4, extraction phase; and 5, ultrasonic probe.

chloroform, 30% in carbon tetrachloride, 25% in *n*-hexane, and 33% in *n*-heptane. *n*-Octanol was the most preferred selected solvent for these extractions, while its chromatographic peak was easily separated from the sample peaks. Therefore, *n*-octanol was observed to be the preferred extraction solvent.

Extraction time

Extraction time is an important influencing factor for the liquid film diffusion mass transfer under HF-LPME conditions. The analyte mass transfer in each of the two immiscible phases also has an influence on the extraction time. An electromagnetic stirring or shake flask is usually employed in traditional HF-LPME in order to facilitate this mass transfer. However, it has no obvious effect on the diffusion mass transfer of the target analyte in the extraction solvent phase.

Ultrasonic radiation, which produces mechanical vibration, ultrasonic cavitation, and vibration of the liquid might have contributed to an accelerating diffusion mass transfer process. Furthermore, the thickness of the liquid film between *n*-octanol and the aqueous phase might have been reduced, which resulted in a lower mass transfer resistance and a higher extraction efficiency. Thus, the effect of the extraction time on the EF of styrene was evaluated by conducting extractions between 4–14 min when using U-HF-LPME. As indicated in Figure 2, the EF did not increase above 10 min, confirming an accelerated extraction time when compared to HF-LPME.

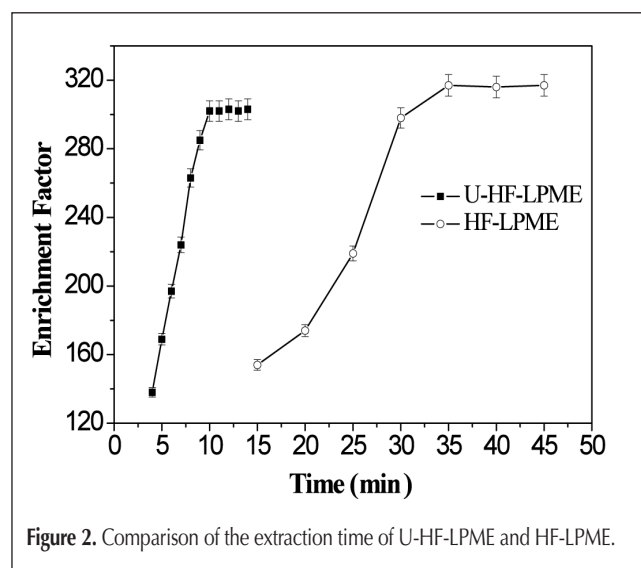


Figure 2. Comparison of the extraction time of U-HF-LPME and HF-LPME.

Power/Frequency	25 kHz	40 kHz	60 kHz
15 W	12.0 min	14.2 min	18.0 min
27 W	10.4 min	12.0 min	16.0 min
39 W	—*	10.5 min	14.5 min
50 W	—*	10.0 min	13.0 min
60 W	—*	—	12.0 min

*Extraction solvent was dispersed and dissolved into the sample phase.

Effect of ultrasonic power and frequency

The influence of the ultrasonic power and frequency were also investigated. The ultrasonic power settings were evaluated at 15, 27, 39, 50, and 60 W using an ultrasonic frequency of 25, 40, and 60 kHz. Table I shows that the extraction time decreased with lower ultrasonic frequency and higher ultrasonic power. The optimum conditions were an ultrasonic power of 50 W, and a frequency of 40 kHz. The extraction process required 10 min.

Effect of extractant volume

The volume of the extraction solvent has an effect on the extraction efficiency. A variety of extractant volumes have been evaluated and ranged from 15 to 30 μ L using aqueous samples using a styrene solution at a concentration of 5 mg/L. The results showed that the EF increased with the decreasing extractant volume. An extractant volume of 20 μ L was selected since a further volume reduction affected sample stability.

Effect of salt

Previous data (27) showed that the addition of salt could lead to an increase of ionic strength in the solution and then decrease the solubility of the target analytes in the aqueous phase. Therefore, the EF of benzene derivatives was enhanced accordingly. The salting-out effect has been commonly used in SPME and LPME (28,29). In the present study, the effect of ionic strength on the extraction efficiency was evaluated by increased NaCl concentration in the range of 0–6 mol/L using the same aqueous samples. Extraction time was set at 10 min. Figure 3 shows that increased extraction efficiencies have been observed for styrene with NaCl concentrations above 4 mol/L. However, NaCl was not fully dissolved in aqueous samples above 4 mol/L and further extractions were performed under these conditions.

Evaluation of method performance

To evaluate the U-HF-LPME method for determining benzene derivatives in the water samples, GC-FID was adopted under optimal experimental conditions. The results of the chromato-

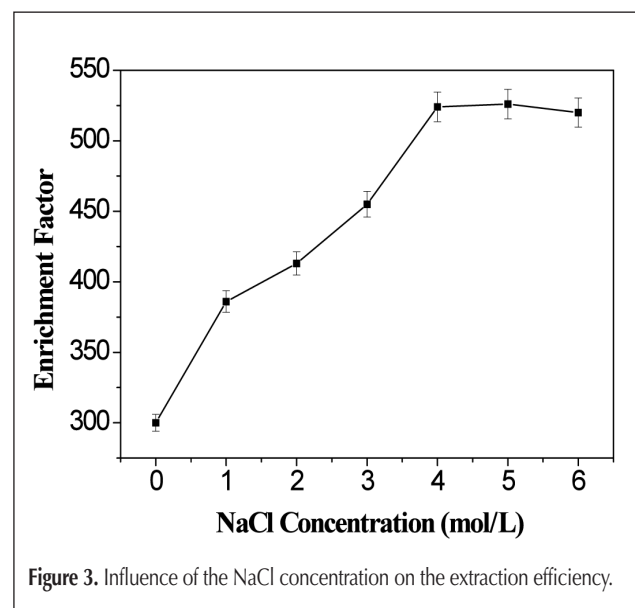
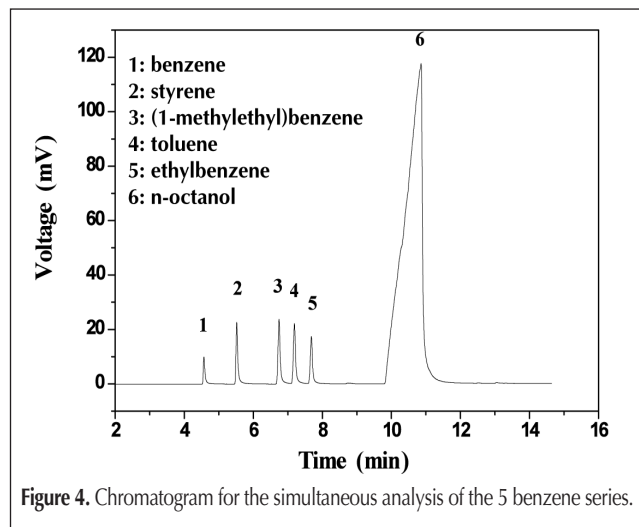


Figure 3. Influence of the NaCl concentration on the extraction efficiency.

Table II. LODs, Regression Equations, Correlation Coefficients, Linear Range, RSDs, RR, and EF Suing U-HF-LPME Analysis

Analyte	LODs ($\mu\text{g/L}$)	R	LR (mg/L)	Regression equation	RSDs (%)	RR (%)	EF
Benzene	3.0	0.9981	0.36–50	$C = 0.0120x - 0.0073$	7.0	98.6	120
Toluene	2.4	0.9992	0.55–50	$C = 0.0119x - 0.0060$	6.5	99.3	232
Ethylbenzene	1.1	0.9953	0.56–50	$C = 0.0122x - 0.0197$	4.6	103.0	512
Styrene	1.2	0.9994	0.63–50	$C = 0.0129x - 0.0232$	3.0	97.4	524
(1-methylethyl) benzene	0.8	0.9998	0.53–50	$C = 0.013x + 0.0007$	0.3	97.0	666

**Figure 4.** Chromatogram for the simultaneous analysis of the 5 benzene series.

graphic peaks for the microextractions are shown in Figure 4. The corresponding regression equations, correlation coefficients, and the linear range are listed in Table II. The limits of detection (LODs) were calculated based on the signal that differed three times from the blank average signal. The obtained LODs were in the range of 0.8–3.0 $\mu\text{g/L}$ (see Table II).

In order to investigate the pre-concentration efficiency of each compound, the extractions were performed at optimal conditions from each of the compound solutions. The EF was in the range of 120–666.

Finally, the method revealed a good reproducibility with the relative standard deviation values (RSDs) in the range of 0.3–7.0%. The analytical results together with the RR for the spiked samples are given in Table II. As shown, the relative recoveries for the spiked samples are in the acceptable range of 97–103.0%.

Analysis of the real samples

Three water samples, including groundwater, lake water, and seawater were analyzed to validate the method. All of the real samples were without any pretreatment because the porous HF were able to purify water samples from solid contaminations effectively. The GC injector and detector temperatures were all at 220°C, the column oven was initially held at 60°C for 1 min, raised to 160°C at 10°C/min, and held for 3 min. The result showed that the concentrations of the target compounds in the groundwater and seawater were all under the detection limits. The benzene and toluene were determined in lake water with a

concentration of 12.4 $\mu\text{g/L}$ and 15.2 $\mu\text{g/L}$. The method can determine trace benzene derivatives in real samples with RSD values of 1.1–4.2%.

All the real water samples were fortified with a level of 5 mg/L for each of compounds, and the recoveries were determined. The recoveries examined for all target compounds from fortified real water samples were in the range of 93.4–100.7%, with the RSDs in the range of 1.2–4.5%. These results demonstrated that no significant matrix effects of the real water samples on U-HF-LPME efficiency were found.

Conclusions

A novel sample pre-concentration method termed ultrasound-enhanced U-HF-LPME has been developed for the determination of benzene derivatives in aqueous samples in the study. In the method, ultrasonic radiation was introduced into the HF-LPME procedure to enhance the mass transfer rate of the analyte during the immiscible phases. Experimental parameters, such as the extraction solvent, the extraction time, ultrasonic power and frequency, the extractant volume, and the ionic strength of the sample were assessed and optimized. Good relative recovery and reproducibility were obtained in 10 min. This method should be useful for pre-concentration studies used for pharmaceutical analysis, bioanalysis, and food analysis.

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