Talanta 85 (2011) 2581–2586

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Determination of phenols in environmental water samples by two-step liquid-phase microextraction coupled with high performance liquid chromatography

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ARTICLE INFO

Article history:
Received 8 May 2011
Received in revised form 5 August 2011
Accepted 9 August 2011
Available online 16 August 2011

Keywords:
LPME
Phenols
HPLC

ABSTRACT

In this work, a two-step liquid-phase microextraction (LPME) method was presented for the extraction of phenols in environmental water samples. Firstly, the polar phenol in water samples (donor phase) was transferred to 1-octanol (extraction mesophase) by magnetic stirring-assisted LPME. Subsequently, target analytes in the 1-octanol was back extracted into 0.1 mol/L sodium hydroxide solution (acceptor phase) by vortex-assisted LPME. By combination of the two-step LPME, the enrichment factors were multiplied. The main features of this two-step LPME for phenols lie in the following aspects. Firstly, the extraction can be accomplished within relatively short time (ca. 20 min). Secondly, it was compatible with HPLC analysis, avoiding derivatization step that is generally necessary for GC analysis. Thirdly, high enrichment factors (296–954 fold) could be obtained for these analytes. Under the optimized conditions, the linearities were 10–1000, 1–500, 1–500, 5–500 and 1–500 ng/mL for different phenols with all regression coefficients higher than 0.9985. The limits of detection were in the range from 0.3 to 3.0 ng/mL for these analytes. Intra-and inter-day relative standard deviations were below 7.6%, indicating a good precision of the proposed method.

1. Introduction

Phenols are ubiquitous in environment as a result of pharmaceutical, chemical and petrochemical activities. Owing to the toxicity of phenols, they are included in the list of priority pollutants by the US Environmental Protect Agency and the European Union (EU) [1,2]. The EU has classified several phenols as priority contaminants and the 80/778/EC directive states a maximum concentration for phenols in drinking water is 0.5 μg/L. Developing simple and sensitive analytical methods is of great importance for the rapid determination of phenols in environment.

Currently, gas chromatography (GC) [3–7] and high performance liquid chromatography (HPLC) [8–13] have been the most commonly used techniques for the quantitative determination of phenols. Since phenols are weakly volatile or non-volatile compounds, a derivatization step is usually involved prior to GC analysis [14,15], which may entail tedious operation and bring about uncertain errors as a result of low derivatization reaction yield, the chromatographic interference of the derivatization reagent to the analytes and so on [12]. One of the advantages of HPLC over GC lies in that phenols can be detected directly without derivatization. Since phenols are commonly present in environment at low concentrations (ng/mL level) with complex sample matrices, suitable cleanup and enrichment prior to instrumental analysis is necessary.

Currently, various extraction methods have been established for these compounds. For example, in EPA method 528, SPE is used for the preconcentration and purification of phenols in drinking water. Using GC–MS analysis, method detection limits are from 0.02 to 0.58 ng/mL. With the popularity of miniaturized sample pretreatment, microextraction based on solid-phase microextraction [16–21] and liquid-phase microextraction (LPME) [22–30], have been proposed for the enrichment of phenols. Among these methods, LPME, which is characterized by low cost, has drawn much attention. However, LPME can be coupled with GC analysis much easier than HPLC as the extraction phases are generally organic solvents. To be amenable to HPLC analysis, the solvent exchange is necessary [22,23]; or multiphase LPME such as liquid–liquid–liquid microextraction (LLLME) [24] or liquid–gas–liquid microextraction (LGLME) [25] was adopted. Although sample preparation methods to the determination of phenols are quite available, a simple and easy-to-handle method is still desired.

In this work, a two-step LPME method was proposed for the enrichment of phenols in environmental water samples. A special device (Fig. 1), which may facilitate the extraction, was designed to transfer the analytes from aqueous to 1-octanol by magnetic stirring-assisted LPME. Subsequently, the enriched analytes in

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doi:10.1016/j.talanta.2011.08.021

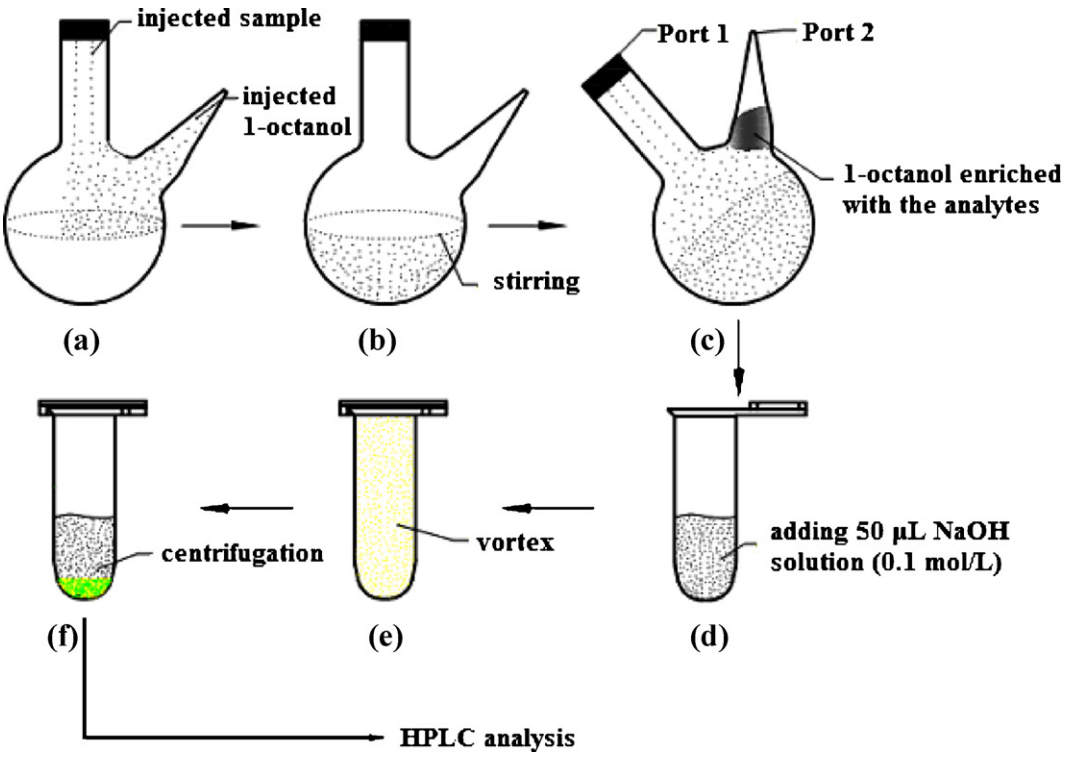

Fig. 1. Schematics of the two-step LPME.

1-octanol were back extracted into 0.1 mol/L NaOH solution (acceptor phase) by vortex-assisted LPME. By adopting this two-step extraction, the enrichment factors for phenols can be multiplied.

2. Experimental

2.1. Chemicals and reagents

Analytical reagent grade of methanol, sodium hydroxide, hydrochloric acid, acetic acid, 1-octanol and ammonia (25%, w/v) were purchased from Shanghai General Chemical Reagent Factory (Shanghai, China). Pure water was obtained on an Aike apparatus (Chengdu, China). Phenol, *o*-nitrophenol, *m*-nitrophenol, *p*-nitrophenol and *p*-chlorophenol were bought from Acros (New Jersey, USA).

2.2. Sample preparation

A stock solution (containing 1000 mg/L of each analyte) was prepared by dissolving the standard with methanol and was stored at 4 °C in the refrigerator. Water samples were prepared daily by diluting the stock solution with deionized water at a known concentration (100 ng/mL for each analyte) to study the extraction performance under different conditions.

Real water samples collected from a tap and the East Lake (Wuhan, China) were filtrated through 0.22-μm disposable membranes. The conductivity and TOC of them were 106 us/m, 3.10 mg/L (tap water) and 335 us/m, 6.74 mg/L (lake water), respectively. The filtered water was adjusted to pH 2.0 with 12 mol/L HCl by a Delta 320 pH-meter (Mettler Toledo, Switzerland) before extraction.

2.3. Extraction procedure

100 mL of water (acidified with hydrochloric acid at different pHs) spiked with an appropriate amount of the compounds was placed in the homemade apparatus (as shown in Fig. 1). 800 μL of 1-octanol was rapidly added into the apparatus with a pipette. The mixture was agitated by a magnetic stirrer for 10 min. After that, the stirrer was stopped and the 1-octanol was observed to float above the aqueous solution within 5 min. By tilting the flask and adding pure water into the apparatus through Port 1, the liquid level was elevated, and the 1-octanol, which enriched the target analytes, was concentrated in the narrow branch tip of the flask (Port 2). The 1-octanol was transferred into a 1.5-mL Eppendorf tube by a pipettor, followed by injecting 50 μL of 0.1 mol/L NaOH solution into it. The mixture was vigorously vortexed for 3 min. Then it was centrifugated at 2000 rpm for another 3 min. As NaOH solution is heavier than 1-octanol, it settled at the bottom of the tube, which was collected by a microsyringe for HPLC injection. The schematic extraction procedures were depicted in Fig. 1.

2.4. Instrumentation

A centrifuge from Gongyi Yuhua Instrument Co., Ltd (Gongyi, China), a magnetic stirrer from Sile Company (Shanghai, China) and a vortex from Qilinbeier Instrument Manufacturing Company (Jiangsu, China) were used for the two-step LPME.

The HPLC analysis was carried out on a separation system from Waters (Milford, MA, USA), which was equipped with a 1525 binary pump and a 996 photodiode array detector. An Xterra C_{18} column (150 mm × 3.9 mm i.d., 5 μm) from Waters was used for the separation of the target analytes. The mobile phase was a mixture of methanol and 1% acetic acid (30:70, v/v), which was filtered and degassed prior to use. The flow rate was 0.75 mL/min. The injection volume was 10 μL and the detection wavelength was 274 nm.

3. Results and discussion

The concentrations of phenols in environmental samples are generally present at low concentrations. To determine these analytes effectively and precisely, processing of a large volume of the sample solutions would be suitable, as it may increase the

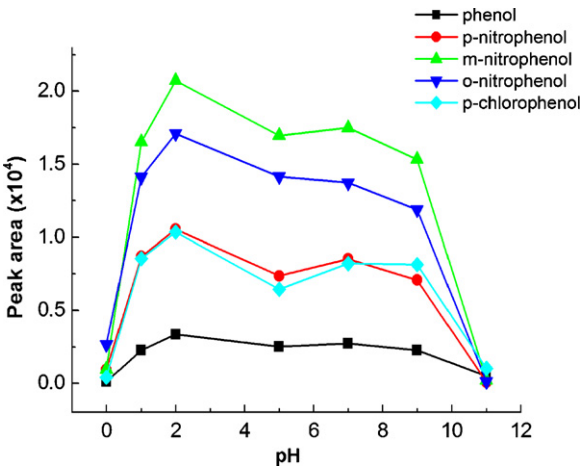

Fig. 2. Optimization the pH values of the sample solution. The samples (100 mL) were spiked with each analyte at 100 ng/mL. Extraction conditions: 1-octanol (500 μL); NaOH solution (0.1 mol/L, 50 μL); stirring time, 10 min; vortex time, 3 min; centrifugation time, 3 min.

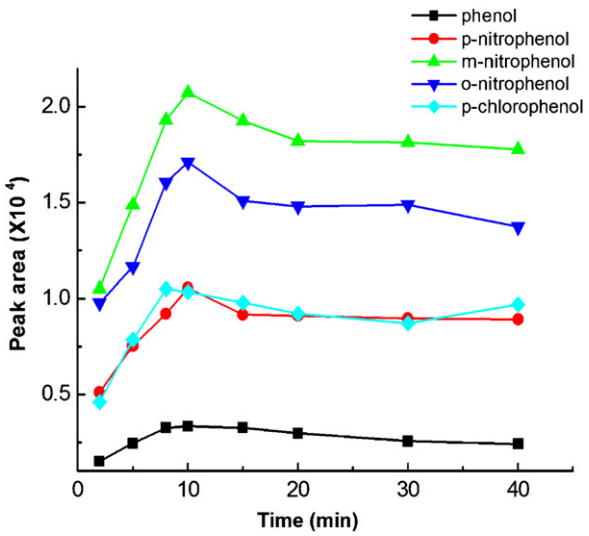

Fig. 3. Effect of stirring time on the extraction efficiency. The samples (100 mL, pH = 2) were spiked with each analyte at 100 ng/mL. Extraction conditions: 1-octanol (500 μL); NaOH solution (0.1 mol/L, 50 μL); vortex time, 3 min; centrifugation time, 3 min.

enrichment for analytes and also minimize the possible sampling errors. However, in traditional LPME methods, such as dispersive liquid–liquid microextraction (DLLME) [22,23], it is not suitable to process samples with large volumes. Thus, design of appropriate LPME device would be necessary for such application.

In this study, a homemade extraction device (flask), which has two narrow open necks with one of them having a capillary tip, was proposed for LPME procedure. The volume of the device can be easily altered by selecting flasks with approximate size. To accelerate the extraction, a magnetic stirrer was used to agitate the sample solutions. Moreover, since there is no presence of disperser solvent (generally used in DLLME), the extraction phase can be conveniently separated from the aqueous samples by standing statically for a while.

The extraction carried out in the homemade device was the first step of the whole LPME process. The parameters, including extraction time, pH values of sample solutions, extraction solvent volume and ionic strength, which may influence the extraction in this step were optimized as follows.

3.1. Study of experimental variables involved in the magnetic stirring-assisted LPME

3.1.1. The pH values of sample solutions

LPME is based on the partition of analytes between two immiscible liquid phases. Generally, a kind of non-polar or weak polar organic solvent is used to extract the target analytes from aqueous solution. However, extraction of polar organic compounds, i.e. phenols, is rather difficult, as they tend to stay in aqueous media. In order to extract these analytes efficiently, the pH of the sample solutions should be controlled to keep the analytes in their deprotonated status, which is beneficial for extraction. In this study, different pH values, ranging from 0 to 11, were evaluated. As shown in Fig. 2, the extraction efficiency was low at pH > 7.0 and pH < 2.0, but increased slightly with pH from 7.0 to 2.0. With the decreasing pH from 7.0 to 2.0, deprotonated phenols may increase correspondingly. The extraction of these target analytes from aqueous to 1-octanol may thus be increased. When the pH is higher than 7.0, they are apt to ionize, which is detrimental for them to be transferred into the organic phase, resulting in a decreased extraction efficiency. However, when pH was lowered to less than 2.0, a decrease in signal was observed. This observation may be explained by the fact that more hydrochloric acid was necessary to lower the pH (pH from 2.0 to 0). Hence, ionic strength of the solution was increased, which may have a negative effect on extraction efficiency (also demonstrated in Section 3.1.4). According to the investigation, pH of 2.0 was chosen as the suitable pH for extraction.

3.1.2. Stirring time

It is known that agitation is an effective way to accelerate mass transfer between the sample solution and the extraction phase. In this study, the stirring speed was set to the maximum of the stirrer (1000 rpm) and the stirring time was investigated from 2 to 40 min. From Fig. 3 it can be observed that the chromatographic peak areas of the phenols increased as the stirring time increased from 2 to 10 min, and then decreased slightly after that. Therefore, 10 min was chosen as the optimized extraction time.

3.1.3. Extraction solvent volume

1-octanol is one of the most widely used organic extraction solvents in LPME, which has shown satisfactory extraction performance for various analytes. In this study, it was adopted as the extraction solvent for the first LPME step. Its volume ranging from 100 to 1200 μL was studied to examine the influence on the extraction. The results are shown in Fig. 4. It is obvious that the extraction performance for most of the compounds improved with increasing volume of the extractant up to 800 μL. Based on this result, 800 μL of 1-octanol was used in the study.

3.1.4. Ionic strength

Usually the presence of salt in aqueous sample solution would affect the solubility of organic solutes. To investigate the influence of ionic strength on the extraction, experiments were performed by adding different amounts of NaCl (0–100 mmol/L) into the sample solutions. The results are shown in Fig. 5. It was observed that, by increasing the concentration of NaCl, there was a slight decrease in the extraction efficiency. Therefore, no salt addition would be suitable for the extraction.

3.2. Study of experimental variables involved in the vortex-assisted LPME

3.2.1. pH value of acceptor phase

Since the solubility of phenols in basic solutions is quite high, they may be back extracted into basic aqueous solution from

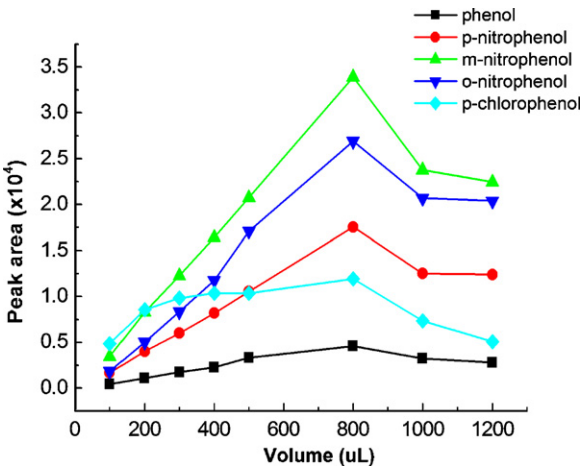

Fig. 4. Effect of 1-octanol volume on extraction efficiency. The samples (100 mL, pH = 2) were spiked with each analyte at 100 ng/mL. Extraction conditions: NaOH solution (0.1 mol/L, 50 μL); stirring time, 10 min; vortex time, 3 min; centrifugation time, 3 min.

1-octanol. Several kinds of aqueous extractants including water, ammonia and NaOH solutions were investigated as the acceptor phase. The result is shown in Fig. 6. It demonstrates that the extraction efficiency improved with the increasing pH of the acceptor phase. Therefore, 0.1 mol/L NaOH solution was selected as suitable acceptor phase.

3.2.2. Vortex time

High frequent vortex can break an organic extractant into very fine droplets, which disperse in the sample solution to accelerate extraction. In this study, the vortex apparatus was set to the maximum speed (2800 rpm) and the effect of vortex time on the

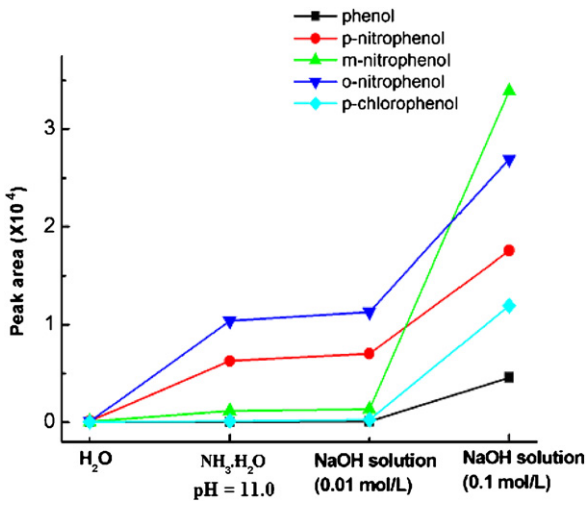

Fig. 6. Comparison of different acceptor phase (50 μL) on extraction efficiency. The samples (100 mL, pH = 2) were spiked with 100 ng/mL of each analyte. Extraction conditions: 1-octanol (800 μL); stirring time, 10 min; vortex time, 3 min; centrifugation time, 3 min.

extraction efficiency was investigated. Fig. 7 shows that the highest extraction performance was attained within 3 min for all phenols. For this reason, 3 min was chosen as the suitable vortex time.

3.3. Evaluation of the two-step LPME method

Based on the above experiments, the optimized conditions were 100 mL of sample solution acidified with hydrochloric acid at pH 2.0, 800 μL of 1-octanol as the extraction mesophase, stirring at 1000 rpm for 10 min, 50 μL of 0.1 mol/L NaOH solution as the final

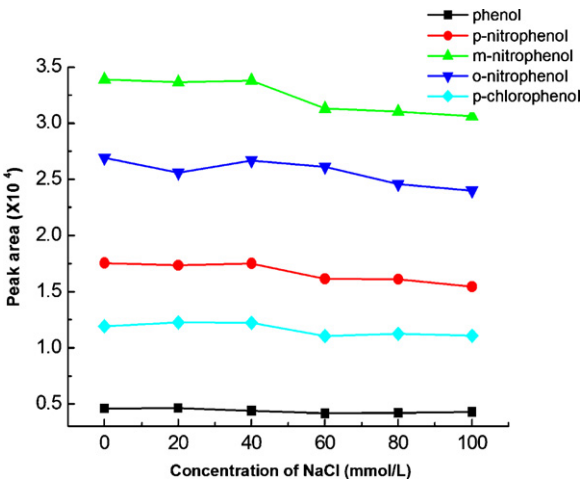

Fig. 5. Effect of ionic strength of sample solutions on extraction efficiency. The samples (100 mL, pH = 2) were spiked with each analyte at 100 ng/mL. Extraction conditions: 1-octanol (800 μL); NaOH solution (0.1 mol/L, 50 μL); stirring time, 10 min; vortex time, 3 min; centrifugation time, 3 min.

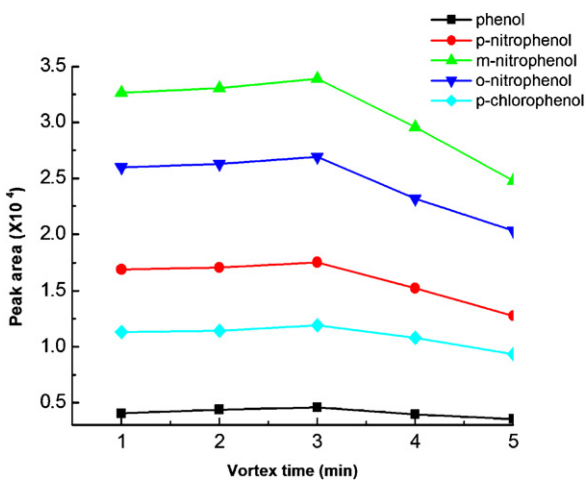

Fig. 7. Effect of vortex time on extraction efficiency. The samples (100 mL, pH = 2) were spiked with 100 ng/mL of each analyte. Extraction conditions: 1-octanol (800 μL); NaOH solution (0.1 mol/L, 50 μL); stirring time, 10 min; centrifugation time, 3 min.

Table 1
Analytical data for the five phenolic compounds by the two-step LPME method.

Analytes	Enrichment factor	Linear range (ng/mL)	R^2	LOD (ng/mL)	LOQ (ng/mL)
Phenol	296	10–1000	0.9986	3.0	10.1
p-Nitrophenol	883	1–500	0.9992	0.5	1.6
m-Nitrophenol	856	1–500	0.9997	0.3	1.1
o-Nitrophenol	678	5–500	0.9998	1.3	5.0
p-Chlorophenol	954	1–500	0.9985	0.3	1.0

Table 2
Reproducibility of the two-step LLME method.

Analytes	Intra-day precision (RSD%, $n = 3$)		Inter-day precision (RSD%, $n = 3$)	
	10 ng/mL	100 ng/mL	10 ng/mL	100 ng/mL
Phenol	7.2	3.3	5.6	3.6
p-Nitrophenol	4.9	3.1	4.5	4.1
m-Nitrophenol	5.2	3.8	4.9	4.3
o-Nitrophenol	4.7	2.9	3.7	2.8
p-Chlorophenol	5.0	4.5	7.6	4.0

Table 3
Comparison of different analytical methods applied for the determination of phenols in water samples.

Analytical technique	LR (ng/mL)	LOD (ng/mL)	Extraction time (min)	EF	References
SPME-GC (electrospun fibers)	1–1000 (phenol)	0.01			
	1–1000 (*p*-chlorophenol)	0.1	60	–	[19]
	1–400 (*p*-nitrophenol)	0.03			
LGLME[a]-CE	10–4000 (phenol)	2.0		15	[25]
	10–4000 (*o*-nitrophenol)	1.0	10	113	
DLLME-HPLC	5–200 (phenol)	1.3		30	
	0.5–200 (*p*-nitrophenol)	0.3		37	[22]
	0.5–500 (*o*-nitrophenol)	0.4	>4	97	
LPME-BE[b]-HPLC	1–1000 (o-chlorophenol)	0.5		190	[10]
	1–1000 (m-chlorophenol)	0.5	30	72	
Two-step LLME-HPLC	10–1000 (phenol)	3.0		296	
	1–500 (*p*-nitrophenol)	0.5		883	This method
	1–500 (*m*-nitrophenol)	0.3		856	
	5–500 (*o*-nitrophenol)	1.3	<21	678	
	1–500 (*p*-chlorophenol)	0.3		954	

[a] Liquid–gas–liquid microextraction.
[b] Liquid phase microextraction-back extraction.

Table 4
Five phenolic compounds determined in environmental aqueous samples by the two-step LLME and the recovery of the method.

Samples	Analytes	Found (ng/mL)	Added (ng/mL)	Concentration determined (ng/mL)	Recovery (%)	RSD (%)
Tap water	Phenol	N.D.[a]	100	96.4	96.4	3.2
	p-Nitrophenol	N.D.	100	95.3	95.3	2.9
	m-Nitrophenol	N.D.	100	95.4	95.4	5.1
	o-Nitrophenol	N.D.	100	102.7	102.7	6.3
	p-Chlorophenol	N.D.	100	93.0	93.0	3.7
East Lake water	Phenol	35.0	100	135.2	100.2	4.1
	p-Nitrophenol	N.D.	100	101.0	101.0	2.3
	m-Nitrophenol	N.D.	100	98.4	98.4	5.6
	o-Nitrophenol	N.D.	100	99.3	99.3	2.8
	p-Chlorophenol	N.D.	100	98.0	98.0	7.1

[a] N.D. Not detected.

acceptor phase, vortex at 2800 rpm for 3 min, centrifugation at 2000 rpm for 3 min and no salt addition.

Under the optimized conditions, the linearity of the method was investigated by using water samples spiked with the five phenols at different concentration levels varying from 1 to 1000 ng/mL. Linear relationships were observed with linear correlation coefficients higher than 0.9985 for the phenols. The limits of detection (LODs), calculated at a signal-to-noise of 3, were found to be in the range from 0.3 to 3.0 ng/mL. Enrichment factors, calculated as the ratio of the final concentration of analytes in the acceptor phase after extraction to the initial concentration of analytes in the aqueous solution, were found in the range from 296 to 954 fold. The calibration linear ranges, LODs, enrichment factors of the proposed method are listed in Table 1.

Intra- and inter-day precisions were evaluated for the reproducibility by extracting aqueous samples spiked with each target analyte at 10 or 100 ng/mL under the optimal conditions. The results are summarized in Table 2. It was observed that, the relative standard deviation values were below 7.6%, indicating a good precision of the method.

The proposed two-step LPME was compared with previous sample preparation methods for analysis of phenols. The results are

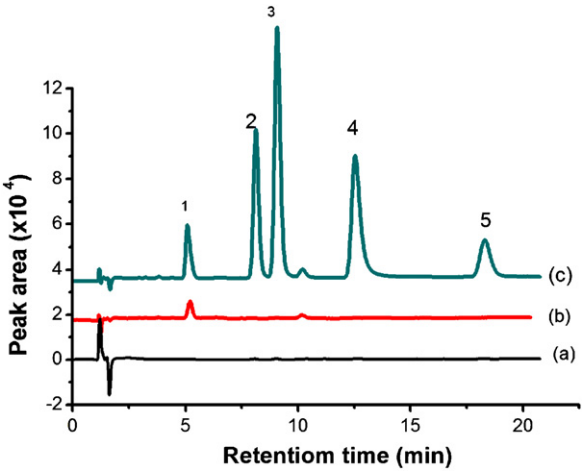

Fig. 8. Chromatograms for five phenolic compounds (a) direct analysis of the lake water without extraction; (b) analysis of the lake water after the two-step LPME; (c) analysis of the spiked lake water (each analyte at 100 ng/mL) after the two-step LLME. Peak identification: 1. phenol; 2. *p*-nitrophenol; 3. *m*-nitrophenol; 4. *o*-nitrophenol; 5. *p*-chlorophenol.

listed in Table 3. It can be observed that, though the LODs by different methods are quite similar, the enrichment factors obtained in this method is far higher than those achieved by previous ones.

3.4. Real water sample analysis

Real water samples collected from a local region (tap water and lake water) were used to validate the feasibility and reliability of the proposed method. Prior to extraction, the real samples were adjusted to pH 2.0 with 12 mol/L HCl solution. The extraction and analysis results were shown in Table 4. It indicated that phenol was found in the East Lake water. The spiked recoveries were satisfactory in the range from 93 to 102.7% by investigating two real water samples at a spiked concentration of 100 ng/mL. The typical chromatograms of the phenols with or without the two-step LPME were depicted in Fig. 8. Apparently, the two-step LPME can effectively enrich the target analytes in environmental water samples.

4. Conclusion

A two-step LPME method was successfully applied to the determination of phenols in environmental water samples. The results demonstrate that the proposed method is simple, convenient and timesaving for sample preparation. This technique is an effective and efficient approach for enrichment of polar analytes like phenols in aqueous environmental samples.

Acknowledgements

The support of National Nature Science Funds of China (No.21075091, 20605015) and the Fundamental Research Funds for the Central Universities (No. 203274618) are gratefully acknowledged.

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