ELSEVIER

Journal of Chromatography A, 931 (2001) 95–105

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Determination of phenols in water using liquid phase microextraction with back extraction combined with high-performance liquid chromatography

Limian Zhao, Hian Kee Lee*

Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore

Received 21 June 2001; received in revised form 10 August 2001; accepted 10 August 2001

Abstract

Liquid phase microextraction with back extraction (LPME/BE) combined with high-performance liquid chromatography (HPLC) was studied for the determination of a variety of phenols in water samples. The target compounds were extracted from 2-ml aqueous sample adjusted to pH 1 (donor solution) through a microliter-size organic solvent phase (400-μl *n*-hexane), confined inside a small PTFE ring, and finally into a 1-μl basic aqueous acceptor microdrop suspended in the aforementioned solvent phase from the tip of a microsyringe needle. After extracting for a prescribed time, the microdrop was taken back into the syringe and directly injected into an HPLC for detection. Factors relevant to the extraction procedure were studied. At the optimized extraction conditions, a large enrichment factor (more than 100-fold) can be achieved for most of the phenols within 35 min. The detection limit range was 0.5–2.5 μg/l for different analytes in aqueous samples. The results demonstrate the suitability of the LPME/BE approach to the analysis of polar compounds in aqueous samples.

Keywords: Liquid phase microextraction with back extraction; Extraction methods; Water analysis; Phenols

1. Introduction

Phenolic compounds are present in the aquatic environment due to their widespread use in industrial applications. These compounds are generated in the production of plastics, dyes, drugs, pesticides, antioxidants and paper, and by the petrochemical industry [1]. Because most phenolic compounds exhibit a high degree of toxicity, although each class follows different metabolic pathways and toxicokinetic patterns, they have been included in the US Environmental Protection Agency (EPA) (Methods 604, 625 and 8041) list of priority pollutants [2–4].

Gas chromatography (GC) has been widely used for the analysis of phenols, usually with a derivatization step [5–7]. However, derivatization increases the sample preparation time and introduces a possible source of errors. For these reasons, alternative analytical techniques used in the determination of phenols are mainly high-performance liquid chromatography (HPLC) with different detections such as ultraviolet (UV) detection [8], photodiode array UV detection [9,10], mass spectrometry [11,12], and fluorescence detection [13]. In addition,

*Corresponding author. Tel.: +65-874-2995; fax: +65-779-1691.

E-mail address: chmleehk@nus.edu.sg (H.K. Lee).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)01199-2

capillary liquid chromatography was used for determination of pollutant phenols too [14]. However a preconcentration step is necessary in trace analysis due to the relatively high detection limits of HPLC.

Liquid–liquid extraction (LLE) [2–4] and solid-phase extraction (SPE) [15,16] are the most commonly used techniques for preconcentration and cleanup of phenols prior to HPLC. Normally the solvents used for LLE and SPE, such as trichloromethane, are not suitable for reverse phase HPLC injection because these solvents are immiscible with the mobile phase, therefore both techniques require solvent evaporation to concentrate the sample and reconstitution for the subsequent HPLC analysis. These steps are tedious and time-consuming, and some analytes may be lost during this procedure. Solid-phase microextraction (SPME) has been applied to the preconcentration of phenols [17,18]. SPME is normally combined with GC-flame ionization detection (FID) or GC–mass spectrometry (MS) for analysis; however, derivatization is usually still required in this technique. When SPME is coupled to HPLC or CE, a solvent desorption step is required to recover all sorbed analytes and to avoid carry-over. Owing to these reasons, most current applications of SPME are limited to non-polar or medium polar compounds [19].

For ionizable analytes, Ma and Cantwell developed a solvent microextraction procedure with simultaneous back-extraction (SME/BE), otherwise more descriptively referred to as liquid phase microextraction with back extraction (LPME/BE) here, to preconcentrate and purify their target compounds [20,21]. An LPME/BE system consists of three liquid phases, the donor solution where pH is adjusted to deionize the compounds, the organic solvent phase and the acceptor solution, the pH of which is adjusted to ionize the compounds. The unsupported liquid organic phase is held within a Teflon ring to develop an organic solvent layer, and the microdrop of acceptor phase is suspended in the organic phase directly from the tip of the syringe needle. With the help of stirring, the analytes are extracted from the donor solution into the solvent phase and back-extracted simultaneously into the acceptor phase.

In this work, we investigated the application of LPME/BE combined with HPLC to the determination of phenols in aqueous sample. Parameters affecting the extraction efficiency (solvent selection, solvent size, phase ratio between donor solution and acceptor phase, extraction time, composition of donor and acceptor solutions) were investigated. Under the optimal extraction conditions, high enrichment factors were achieved in a relatively short time. In addition, LPME/BE was validated for quantitative analysis, and applications to reservoir water were illustrated.

2. Experimental

2.1. Extraction apparatus

The LPME/BE device is shown in Fig. 1. A 4-ml glass sample vial (Supelco, Bellefonte, PA, USA) (14 mm O.D.×13 mm I.D.×44 mm height) was used as the container of the three-liquid phases. The PTFE ring, 13 mm O.D.×10 mm I.D.×3 mm height (manually cut from a PTFE tube), was positioned in the middle of the vial so that the surface of the donor solution would just touch the bottom of the ring. Once the position of the ring was fixed, it was not adjusted again throughout the subsequent extractions. The sample vial was placed on a magnetic stirrer (Heidolph MR3001K, Germany) and clamped to fix its position. Aluminum foil was used to cover the glass vial during extraction to prevent the evaporation of organic solvent. A 10-μl HPLC syringe (SGE, Australia) was used for suspending the microdrop of the acceptor solution in the organic solvent phase and also for injection into the HPLC for analysis. In order to avoid any carryover, the sample vial together with the PTFE ring was washed by detergent, pure water and acetone, respectively, and subsequently dried before the next extraction.

2.2. Reagents and chemicals

Phenol standards were purchased from various sources; the purity was more than 98%. All the phenolic compounds used in the present work are listed in Table 1, along with their pK_a and log $K_{o/w}$ values ($K_{o/w}$=octanol–water partition coefficient). The toluene, xylene, dichloromethane and ethyl acetate (all from J.T. Baker, NJ, USA), *n*-hexane and

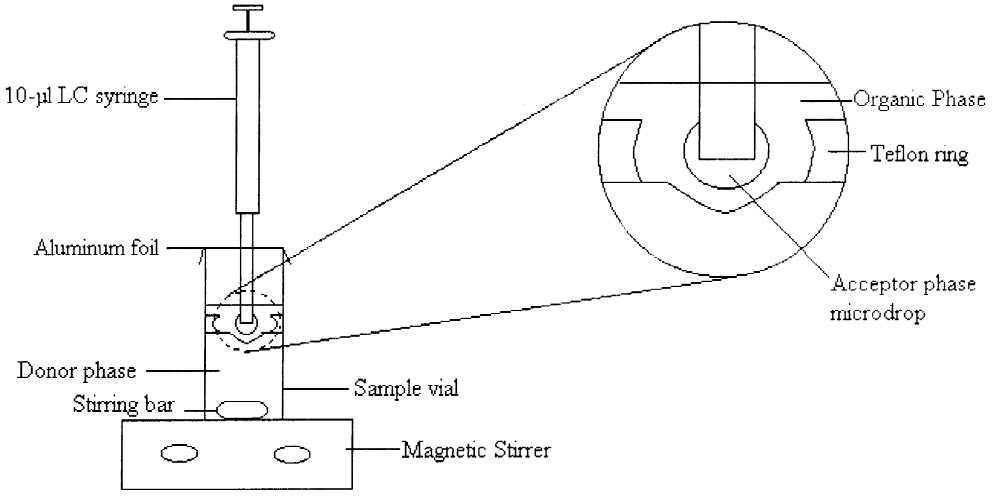

Fig. 1. Schematic diagram of the LPME/BE system.

isooctane (both from Fisher, NJ, USA), and methanol (Mallinckrodt, KY, USA) were all of HPLC-grade. The HPLC-grade acetonitrile was from EM science (Darmstadt, Germany) and the water used was purified on a Milli-Q Ultrapure water purification system (Millipore, Bedford, MA, USA). Trifluoroacetic acid (about 100%) was from BDH (Poole, UK).

Each phenol was dissolved in methanol to obtain a standard solution with a concentration of 1.00 mg/ml. They were stored at 4°C. A new 10.0 mg/l (10 ppm) standard solution containing six phenols was prepared in methanol once every 2 weeks and also stored at 4°C.

Water samples from a reservoir were collected for this work. The water was filtered through a Whatman (UK) filter paper and a 0.45-μm membrane (Millipore) to eliminate particulate matter before analysis. It was stored at 4°C.

Tap water samples (directly potable) were collected from a laboratory. It was freshly collected after allowing the water to flow for about 3–4 min.

2.3. Instrumentation

The HPLC system consisted of a Shimadzu (Kyoto, Japan) LC-6A pump, a Rheodyne 7010 injector equipped with 20-μl sample loop, a Shimadzu SPD-6AV UV–VIS detector and a Shimadzu C-R6A integrator. A 4-μm Nova-Pak C_{18} (3.9×150 mm, Waters) column was used for separations. The mobile phase was a mixture of acetonitrile–trifluoroacetic acid (0.05%, pH 3.2) solution

Table 1
Physical properties of target phenols

Target compounds	pK_a[a]	Log $K_{o/w}$[a]	Retention time (min)
m-Cresol (c-M)	10.09	1.96	5.02
2-Chlorophenol (2-CP)	8.56	2.15	6.05
3-Chlorophenol (3-CP)	9.12	2.50	8.32
2,4-Dimethylphenol (2,4-DMP)	10.61	1.30	9.78
2,3,5-Trimethylphenol (2,3,5-TMP)	10.67	3.15	17.58
2,4-Dichlorophenol (2,4-DCP)	7.89	3.06	20.30

[a] Values take from Ref. [22].

(20:80). The mobile phase flow-rate was 0.8 ml/min and UV detection was at 220 nm.

2.4. Microextraction procedure

The working solutions, consisting of 200.0 μg/l of each phenol, were freshly prepared as donor phase in 0.1 *M* HCl–20% NaCl buffer solutions (pH 1). Before extraction, the donor solution was degassed by a sonicator for about 5 min. The acceptor solution (0.5 *M* NaOH buffer solution) was prepared every week.

Extractions were performed according to the following procedure: the PTFE ring was put into a dry and clean sample vial, followed by the stirring bar. A 2.0-ml volume of donor solution was transferred into the vial. The position of the PTFE ring was adjusted carefully so that the surface of the donor solution just touched the bottom of the ring. Once the position of the ring was fixed, no further adjustment was necessary for future extractions. The organic solvent (400-μl *n*-hexane) was carefully pipetted along the inner wall on the top of the donor solution to form the solvent layer. The vial was placed at the fixed position on the stirring plate and then clamped steady. The stirrer was switched on (400 rpm) to facilitate the extraction of analytes from the donor solution to the organic phase and a vortex was produced at the bottom of the organic solvent layer.

After 30 min of extraction of analytes from the donor phase to the organic phase, an HPLC syringe, rinsed and filled with 1.0 μl of the acceptor phase, was placed above the sample vial such that its needle penetrated the aluminum foil; the syringe was then clamped to hold it in a stable position. The tip of the syringe needle was positioned slightly below the surface of the solvent layer and in the middle of the vortex. The plunger was depressed completely to suspend a microdrop of acceptor phase (1.0 μl) at the needle tip and to expose it to the organic solvent phase.

After a short period of time to allow mass transfer of analytes from the organic phase to the microdrop of acceptor phase, the latter was retracted slowly while the sample solution was still being agitated. The syringe was removed from the clamp and the magnetic stirrer was switched off. An aliquot (4 μl) of 0.1 *M* HCl solutions was withdrawn into the former syringe to combine with the 1 μl of acceptor phase in the syringe, and the entire solution in the needle was injected into the HPLC system for analysis.

3. Results and discussion

3.1. Basic principle

Since the analytes in the donor phase are first extracted into the organic phase and then back-extracted into the acceptor phase, it is necessary to convert the analytes by some reactions, such as protonation, complexation, so that the converted analytes have slight affinity for the organic phase and are easy to be back-extracted into the acceptor phase [20].

In this study, protonation was used to promote the extraction. Owing to the acidic condition of the donor solution, the target phenols were deionized as their neutral forms; thus their solubility in the donor solution was decreased. Under stirring, the target compounds were easily extracted into the solvent phase, which was layered at the top of the donor solution. After a period of time (ca. 30 min), a microdrop of acceptor phase was introduced into the organic phase, at which point the extraction of analytes from solvent to the acceptor phase began. Since the acceptor phase was adjusted to a strongly basic condition, the phenols were ionized when extracted by this phase, which promoted the back extraction as well as prevented analytes from returning into the solvent phase again. This was a fast procedure with the analytes being extracted into it from the organic phase in a short period of time.

3.2. Optimization of LPME/BE

The extraction yield was evaluated by HPLC signals (peak areas) and the enrichment factor (EF), defined as ratio of the peak of the analytes obtained after and before LPME/BE extraction. At each operating condition, the mean values of peak area or EF were obtained by three replicate analyses of pure water spiked with 200 μg/l of each target analyte.

3.2.1. Extraction solvent

The choice of organic solvent used in LPME/BE was a major consideration. In order to promote analyte transferring from the donor solution through the organic phase to the acceptor microdrop, the solubility of the neutral analytes in the organic solvent should be higher than that in the donor solution and simultaneously the solubility of ionic analytes should be lower than that in the acceptor phase. Furthermore, the solvent should be immiscible with water to avoid dissolution during extraction and to serve as a barrier between the donor and acceptor phases.

In conventional liquid–liquid extraction, solvents of high polarity are usually used, such as trichloromethane [23] and dichloromethane [24], for phenols because these compounds are relatively highly polar. In our work, the extraction efficiency of various solvents with different polarities was studied. Six solvents were chosen for this purpose: isooctane (polarity index, 0.1), *n*-hexane (0.1), toluene (2.4), xylene (2.5), ethyl acetate (4.4), and dichloromethane (3.1) [25]. When ethyl acetate and dichloromethane were used, the last two compounds (2,3,5-trimethylphenol and 2,4-dichlorophenol) could not be extracted; although toluene and xylene could extract all the six compounds, their extraction efficiency was much less than those of isooctane and *n*-hexane. It indicated that solvents of relatively high polarity were unsuitable. This is due to the high solubility of the analytes in such solvents. Consequently, it was difficult to further extract the analytes from the organic solvents to the acceptor phase, especially for the previously mentioned two analytes because of their relatively higher log $K_{o/w}$ values (Table 1). On the other hand, isooctane and *n*-hexane performed well because of their lower polarity, and the analytes could be extracted back into the acceptor phase easily. *n*-Hexane could provide higher extraction efficiencies than isooctane, and furthermore, it has low solubility in water (1.4×10^{-4}, w/w) [26]. Hence, *n*-hexane was selected for subsequent experiments.

3.2.2. Volume of organic solvent

We investigated the influence on extraction of different volumes of *n*-hexane in the range of 300–600 μl. The results, shown in Fig. 2, indicated that the extraction efficiencies were better when lower volumes of *n*-hexane were used. Except for *m*-cresol

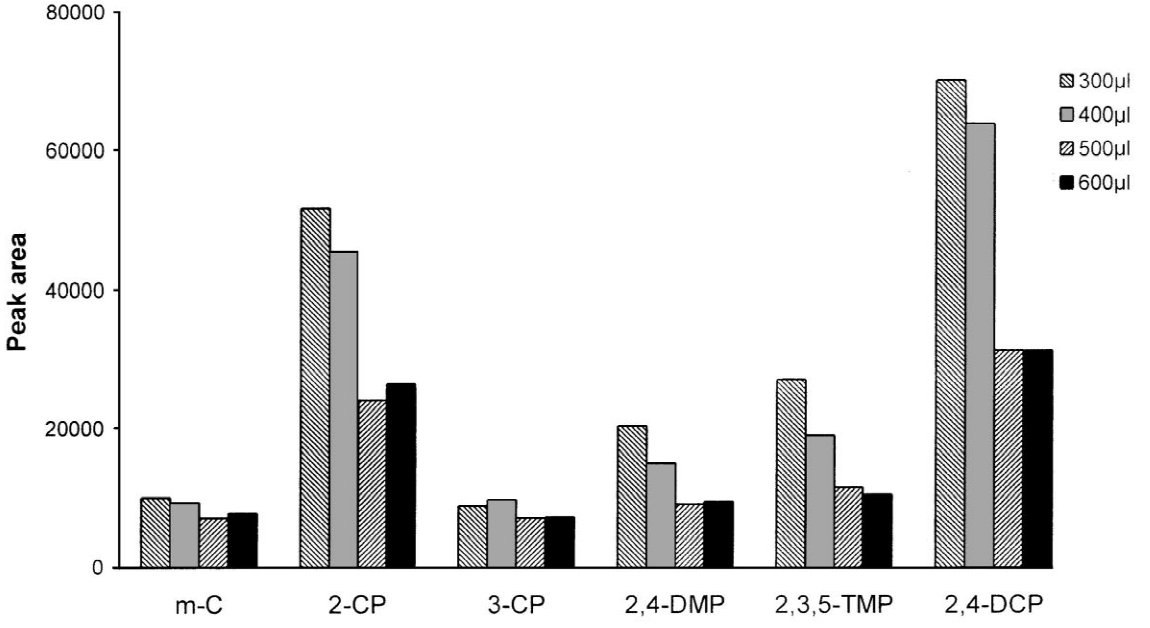

Fig. 2. Effect of organic phase volume on extraction efficiency. The volumes of organic phase varied in the range of 300–600 μl.

and 3-chlorophenol, the analytical signals obtained with 500 and 600 μl of *n*-hexane were only half of those obtained with 300 μl. However, when 300 μl of *n*-hexane was used, the organic layer was so thin that the operation of introducing the microdrop of acceptor phase into the organic phase was problematic such that the precision of the extraction was consequently affected. Hence, to compromise, we selected 400 μl of *n*-hexane as the organic phase volume for subsequent experiments.

3.2.3. Volume ratio between donor solution and receiving phase

LPME/BE is a type of equilibrium extraction [20,21], and there exist two equilibria in this system: the equilibrium of an analyte in the donor solution (a1) and the organic solvent phase (o), and the equilibrium of the analyte in the organic phase and the acceptor solution (a2). Suppose the initial concentration of the analyte in the donor solution be $C_{\mathrm{a1,initial}}$ and at the equilibrium, the concentration of that analyte in acceptor solution be $C_{\mathrm{a2,eq}}$, and if complete extraction of analyte from the donor phase to acceptor phase at equilibrium, it can be written as [20]:

$$C_{\mathrm{a2,eq}} = \frac{V_{\mathrm{a1}}}{V_{\mathrm{a2}}} \cdot C_{\mathrm{a1,initial}}$$

Therefore the extraction efficiency can be improved by the increase in the volume ratio of donor solution and acceptor phase. In the present work, the volume ratio of donor solution and acceptor phase was changed in the range of 1000:1 to 3000:1. Since the organic film was so thin that a larger acceptor drop was prone to dissolve into the donor phase caused by agitation during the extraction procedure, the phase ratio was varied by changing the volume of the donor solution while the acceptor solution volume was kept constant (1 μl). The results, shown in Fig. 3, indicate that the enrichment factor increased significantly from a volume ratio of 1000:1 to 2000:1, but no obvious increase was obtained for a volume ratio of 3000:1. Hence, a volume ratio of 2000:1, that is 2 ml donor solution and 1 μl receiving solution, was applied to subsequent experiments.

3.2.4. Extraction time

As described above, LPME/BE is a type of

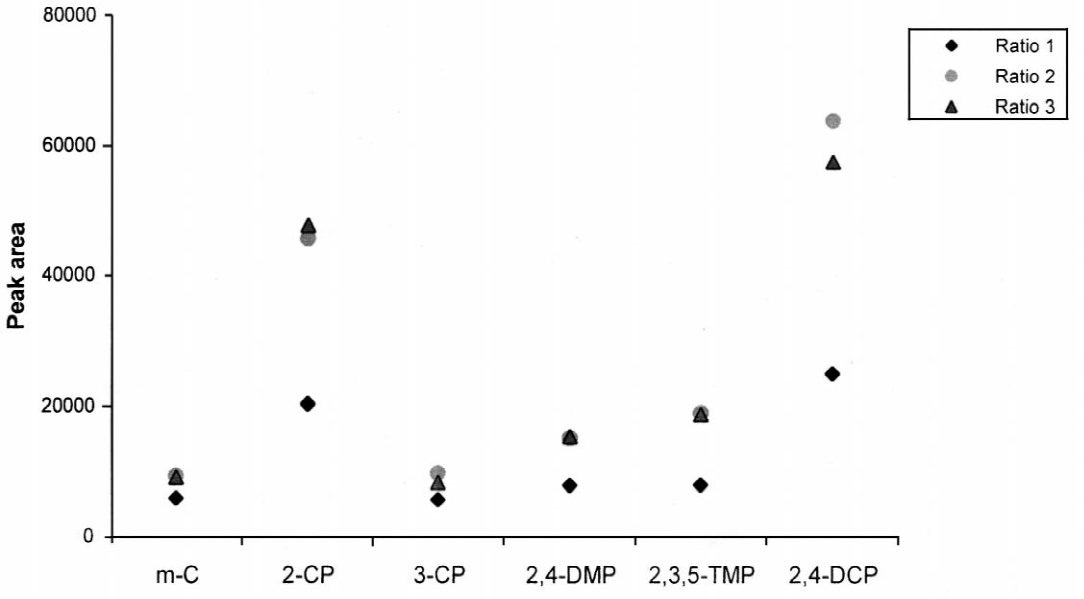

Fig. 3. Effect of volume ratio of the donor solution and the acceptor solution on the extraction efficiency. Ratio 1, 1000:1; ratio 2, 2000:1; ratio 3, 3000:1.

equilibrium extraction, and the optimal extraction efficiency is obtained when equilibrium is established. Therefore, the extraction time plays a very essential role in the whole process. Since there exist two equilibria, we should consider the extraction in great detail with respect to the extraction times involved.

3.2.4.1. Extraction time: donor phase–organic phase

The extraction of phenols from the donor solution into the organic phase can be described as a slow equilibrium procedure. The range of extraction times investigated was 5–50 min with other extraction conditions left constant. Fig. 4 shows the behavior of these analytes under varying extraction times. It is obvious that the HPLC signals increase over an extraction time of 30 min, after which steady states were reached. No dramatic increase was obtained over extraction time longer than 30 min, hence, the optimal extraction time for the first step was set at 30 min.

3.2.4.2. Extraction time: organic phase–acceptor solution

The effect of back extraction time was examined in the range of 1–9 min with other extraction conditions kept constant. The results are shown in Fig. 5, which indicates that the equilibrium of most analytes in the organic phase and the acceptor phase was reached in 5 min except 2-chlorophenol and 3-chlorophenol. Even for these two compounds, their equilibrium states were attained after 7 min. It is obvious that the back extraction of analytes from the organic phase to acceptor phase is a fast procedure by comparing Figs. 4 and 5. It also confirmed that in order to get a fast back extraction procedure, it is imperative to convert the back-extracted analytes by reactions [20,21]. However, a lengthy back-extraction time caused the microdrop of acceptor phase to be unstable. Therefore, an exposure time of 5 min was selected as a reasonable compromise that gave good extraction efficiencies for all the analytes.

3.2.5. Donor and acceptor solutions

Experiments were conducted to optimize the com-

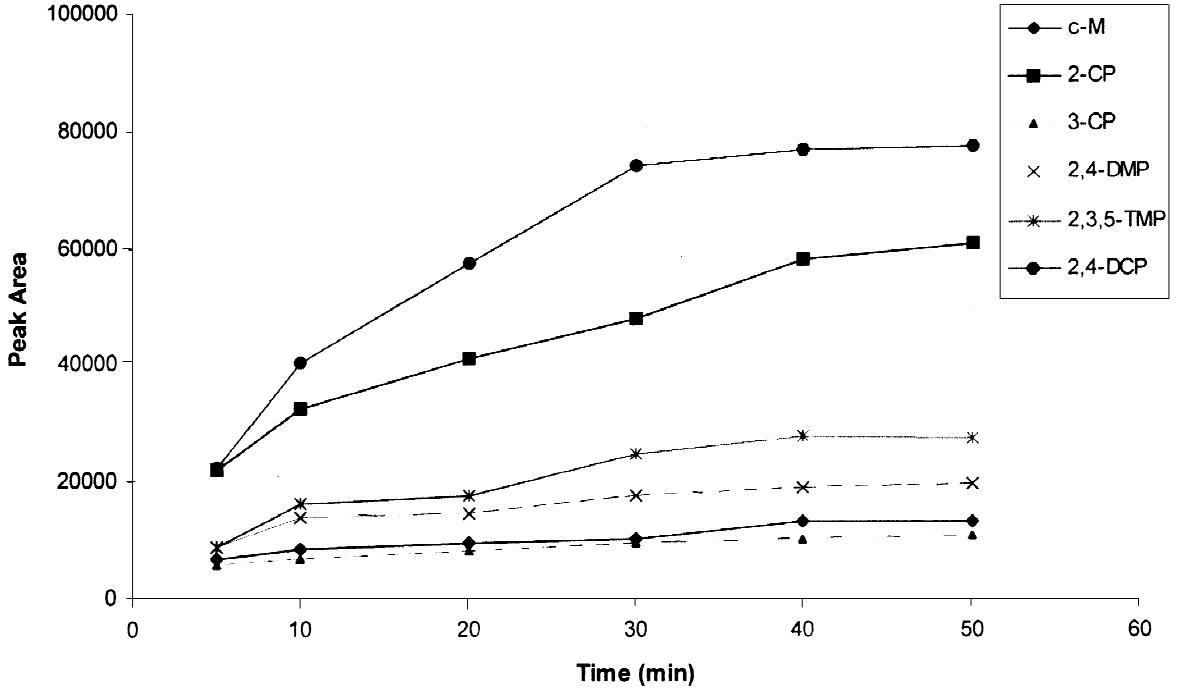

Fig. 4. Time dependence for the equilibrium of phenols between donor phase and solvent phase.

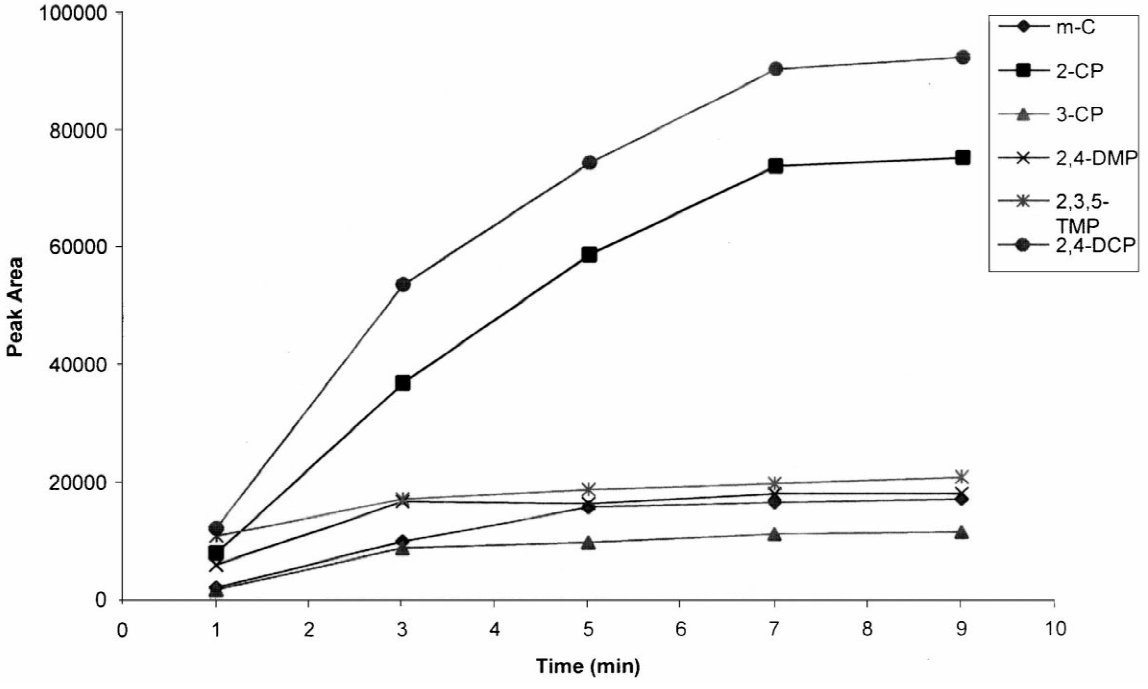

Fig. 5. Time dependence for the equilibrium of phenols between solvent phase and acceptor phase.

position of the respective donor and acceptor solutions. For all experiments, LPME/BE was carried out over 35 min with HCl in the donor solution (aqueous sample) and NaOH in the acceptor solution. For the donor solution, the HCl concentration was varied from 0.001 to 1 *M*. As shown in Table 2, the enrichment factor of analytes increased with increasing concentration of HCl. By comparing the EF of analytes extracted under different HCl concentrations, it is obvious that while 1 *M* HCl provided the highest EF for 2-chlorophenol, that of 2,4-dichlorophenol decreased due to its low pK_a (lowest of the

Table 2
Effect on composition of donor and acceptor solutions[a]

Target compounds	0.1 *M* NaOH, no salt (acceptor solution)[b]				0.1 *M* HCl, no salt (donor solution)[c]					0.1 *M* HCl (donor) 0.5 *M* NaOH (acceptor)[d]		
	0.001 M HCl	0.01 M HCl	0.1 M HCl	1 M HCl	0.001 M NaOH	0.01 M NaOH	0.1 M NaOH	0.5 M NaOH	1 M NaOH	10% NaCl	20% NaCl	Saturated NaCl
m-C	16	22	40	37	2	10	40	45	54	119	127	123
2-CP	73	96	174	272	3	83	174	178	230	180	190	167
3-CP	24	29	49	52	3	29	46	48	47	61	72	77
2,4-DMP	31	43	78	80	8	10	78	131	117	178	183	154
2,3,5-TMP	16	19	47	51	ND[e]	1.5	47	126	119	117	135	102
2,4-DCP	137	165	305	273	7	133	305	278	247	257	302	234

[a] Enrichment factor varied within 11% RSD ($n=3$) for working solution at a concentration of 200 μg/l of each analyte.
[b] The concentration of NaOH in the acceptor solution is fixed, and the concentration of HCl in the donor solution is varied.
[c] The concentration of HCl in the donor solution is fixed, and the concentration of NaOH in the acceptor solution is varied.
[d] The concentrations of HCl in the donor solution and NaOH in the acceptor solution are fixed, and the concentration NaCl in the donor solution is varied.
[e] Not detectable.

pK_a's of all the six phenols, Table 1) and there was no significant difference in extraction efficiencies for the other analytes. Therefore, 0.1 *M* HCl was selected as a reasonable compromise for every analyte for the subsequent experiments.

While the concentration of HCl of the donor solution was not so critical, the extraction efficiency was more sensitive to NaOH concentration in the acceptor solution. As illustrated in Table 2, the concentration of NaOH was studied in the range of 0.001 to 1 *M*. The EF of analytes increased dramatically with increasing NaOH concentration up to 0.5 *M* because the higher concentration NaOH has bigger absolute buffer capacity within the small volume of the acceptor solution. However, the EF of analytes in 1 *M* of NaOH was less than those of most analytes (except 2-CP) in 0.5 *M* of NaOH, thus, 0.5 *M* NaOH was selected as the optimized acceptor solution.

The effect of salt in the donor was investigated, too, and results are also shown in Table 2. NaCl was added at the concentration levels of 10%, 20% and saturated in our experiments. All in all, the addition of NaCl improved the extraction efficiency of analytes, especially for 2-chlorophenol and 2,4-dimethylphenol. However, the EF for the analytes in saturated NaCl was lower than those in 20% NaCl. This behavior can be explained by considering two simultaneously occurring processes. When salt was added into the solution, water molecules could form hydration spheres around the ionic salt molecules. These hydration spheres reduce the amount of water available to dissolve analyte molecules; thus it drove additional analytes into the organic solvent [27]. On the contrary, phenol molecules may participate in electrostatic interactions with the salt ions in solution [28], thereby decreasing their ability to move into the extraction phase. Initially, the predominant process would be the interaction of the salt molecules with water molecules; as the salt concentration increased further, salt molecules would interact with analyte molecules. It therefore seemed reasonable to add 20% NaCl to the donor phase since it contributed to the best extraction efficiency.

A practical demonstration of the enrichment that is attainable with LPME/BE is presented in Fig. 6. Chromatogram (a) is an injection into the HPLC of 1.0-μl of aqueous standard solution containing 1 mg/l (1 ppm) of a mixture of six phenols. Chromatogram (b) was obtained by injecting 1.0-μl of acceptor solution after microextraction at the optimized extraction conditions. The concentration in the donor solution of the analytes was 20 μg/l (20 ppb) each. By comparing peak heights in the chromatograms, it can be seen that most of the target compounds have been preconcentrated more than 100-fold in the microdrop of the acceptor solution.

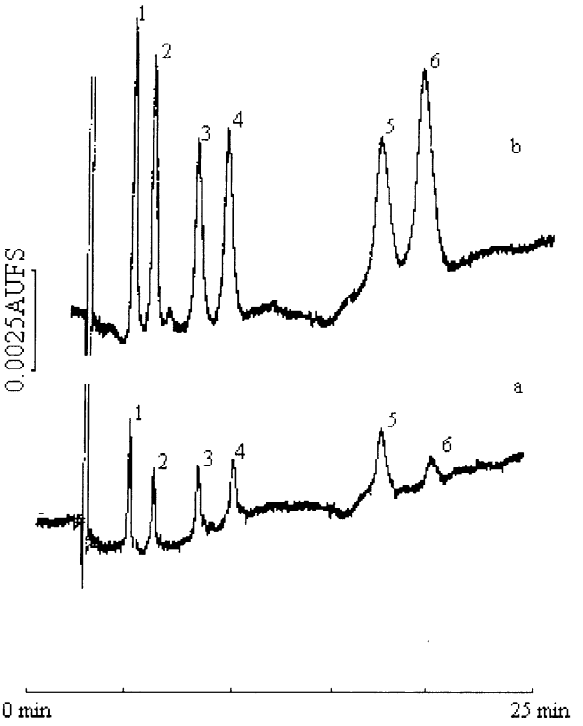

Fig. 6. Chromatograms of a mixture of *m*-cresol (1), 2-chlorophenol (2), 3-chlorophenol (3), 2,4-dimethylphenol (4), 2,3,5-trimethylphenol (5), 2,4-dichlorophenol (6) in the solutions: (a) 1.0 μl of 1.0 mg/l standard solution without extraction; (b) 1.0 μl of receiving solution after extraction from 20.0 μg/l working solution. Chromatographic conditions are given in the text.

3.3. Quantitative analysis

On the basis of the experiments discussed above, the optimal LPME/BE conditions were 400 μl *n*-hexane as organic phase, 1 μl of 0.5 *M* NaOH solution as acceptor phase, 2 ml donor solution containing 0.1 *M* HCl and 20% NaCl, and an

extraction time of 35 min, of which 30 min was for the first step and 5 min was for the back-extraction.

In order to evaluate the practical applicability of the LPME/BE technique, repeatability, linearity and the limits of detection under the optimal extraction conditions were investigated. The repeatability in peak areas was studied for six replicate experiments. The relative standard deviations (RSDs) were lower than 7% except for 2,3,5-trimethylphenol (11.5%) and 2,4-dichlorophenol (10.6%). To obtain reasonable RSD values, the selected extraction conditions must be strictly maintained and the microdrop of the acceptor phase should be held stable at the tip of the microsyringe needle during the whole procedure.

The calibration curves were obtained by plotting peak areas vs. concentration of analytes in the donor solution. All the phenols exhibited good linearity over the range studied under the optimal conditions. Coefficients of correlation (r^2) better than 0.994 were obtained. The limits of detection (LODs), based on a signal-to-noise ratio of 3, ranged from 0.5 to 2.5 μg/l. These values are better than those that can be obtained by EPA method 625, which uses GC–MS detection [3]. Most of the LODs are higher than those of SPME with GC–MS (0.02–0.4 μg/l); however, the sensitivity of GC–MS is generally higher than that of UV detection [18]. Moreover, since the acceptor phase used in LPME/BE is aqueous, after extraction it can be directly introduced to a reversed-phase HPLC system. This is more convenient than the combination of SPME with HPLC. Analytes extracted by SPME must be desorbed into a suitable receiving solvent prior to HPLC analysis. The SPME–HPLC interface requires a desorption chamber as part of a switching valve and is thus not as convenient to use [29]. Therefore the present method does offer an alternative and simpler approach to coupling microextraction with HPLC. Table 3 summarizes the analytical data obtained.

3.4. Real water analysis

Natural water samples from a reservoir and tap water from a laboratory were studied using the method developed. Despite the low detection limits, no target analytes were detected. Therefore, the tap water and reservoir water were spiked with analyte standards at various concentrations to assess matrix effects. The relative recovery, defined as the peak area ratio of a real sample and ultra pure water sample spiked with analytes at the same level, was applied. Table 4 lists the relative recoveries from the respective water sample spiked at 20.0 μg/l and 200.0 μg/l levels. More than 85% relative recoveries were obtained for most of the analytes in the two samples, indicating that the influence of matrix was not significant for environment analysis. However, owing to the influence of sample matrix, especially for the reservoir water, the precision of LPME/BE (RSD values) in real water samples was not as good as that of LPME/BE in pure water samples.

4. Conclusion

The proposed solvent microextraction technique, LPME/BE, is attractive owing to its simplicity, analytical precision, short sample preparation time, low cost and minimization of organic solvent used. This technique is designed specifically for analytes like phenols that are ionized in aqueous solution.

Table 3
Quantitative results of LPME/BE

Target compounds	RSD (%)[a] ($n=6$)	Linearity range (μg/l)	Coefficient of correlation (r^2)	Limit of detection (μg/l)
m-C	6.6	1.0–1000.0	0.994	0.5
2-CP	5.6	1.0–1000.0	0.9982	0.5
3-CP	6.6	1.0–1000.0	0.994	0.5
2,4-DMP	5.4	2.0–1000.0	0.9991	1.0
2,3,5-TMP	11.5	5.0–1000.0	0.9996	2.0
2,4-DCP	10.6	5.0–1000.0	0.9978	2.5

[a] Repeatability was investigated at a concentration of 200 μg/l for each analyte.

Table 4
Summary of results from analysis of phenols in spiked tap water and reservoir water

Target compounds	The relative recovery of tap water[a] (%)		The relative recovery of reservoir water[a] (%)	
	200 μg/l	20 μg/l	200 μg/l	20 μg/l
m-C	95.8	89.8	88.9	97.0
2-CP	100.7	87.4	94.9	73.7
3-CP	93.4	92.9	91.2	94.7
2,4-DMP	79.1	95.3	81.6	87.3
2,3,5-TMP	90.5	88.9	95.0	97.3
2,4-DCP	102.0	83.0	105.3	92.7

[a] Results varied within 15% RSD.

From the results of our experiments, LPME/BE method combined with HPLC has been illustrated to be viable, easy to use, and rapid for qualitative and quantitative analysis of phenols in aqueous samples.

Acknowledgements

This work was financially supported by the National University of Singapore.

References

[1] D. Martínez, E. Pocurull, R.M. Marcé, M. Calull, J. Chromatogr. A 734 (1996) 367.
[2] Federal Register, EPA Method 604, Phenols, Part VIII, 40 CFR part 136, Environmental Protection Agency, Washington, DC, 26, October 1984, p. 58.
[3] EPA Method 625, Basic/Neutral and Acids, Part VIII, 40 CFR Part 136, Environmental Protection Agency, Washington DC, 1984, p. 153.
[4] EPA Method 8041, Phenols by Gas Chromatography: Capillary Column Technique, Environmental Protection Agency, Washington, DC, 1995, p. 1.
[5] L. Renberg, K.J. Lindström, J. Chromatogr. A 214 (1981) 327.
[6] J. Hajslová, V. Kocourek, I. Iemanová, F. Pudil, J. Chromatogr. 439 (1988) 307.
[7] I. Turnes, I. Rodríguez, C.M. Garciá, R. Cela, J. Chromatogr. A 743 (1996) 283.
[8] D. Puig, D. Barceló, Trends Anal. Chem. 15 (1996) 362.
[9] E.R. Brouwer, U.A.Th. Brinkman, J. Chromatogr. A 678 (1994) 223.
[10] R. Belloli, B. Barletta, E. Bolzacchini, S. Meinardi, M. Orlandi, B. Rindone, J. Chromatogr. A 846 (1999) 277.
[11] O. Jáuregui, E. Moyano, M.T. Galceran, J. Chromatogr. A 787 (1997) 79.
[12] S. Pérez-Magariño, I. Revilla, M.L. González-SanJosé, S. Beltrán, J. Chromatogr. A 847 (1999) 75.
[13] M. Wada, S. Kinoshita, Y. Itayama, N. Kuroda, K. Nakashima, J. Chromatogr. B 721 (1999) 179.
[14] S.A. María Paz, L.G. María Eugenia, P.A. Luis Vicente, P.D. Luis María, J. High Resol. Chromatogr. 23 (5) (2000) 367.
[15] M.T. Galceran, O. Jáuregui, Anal. Chim. Acta 304 (1995) 75.
[16] J. Cheung, R.J. Wells, J. Chromatogr. A 771 (1997) 203.
[17] K.D. Buchholz, J. Pawliszyn, Environ. Sci. Technol. 27 (1993) 2844.
[18] K.D. Buchholz, J. Pawliszyn, Anal. Chem. 66 (1994) 160.
[19] T. Gorecki, P. Martos, J. Pawliszyn, Anal. Chem. 70 (1998) 19.
[20] M. Ma, F.F. Cantwell, Anal. Chem. 70 (1998) 3912.
[21] M. Ma, F.F. Cantwell, Anal. Chem. 71 (1999) 388.
[22] P.H. Howard, W.M. Meylan (Eds.), Handbook of Physical Properties of Organic Chemicals, CRC Press/Lewis Publ., Boca Raton, FL, 1997.
[23] O. Busto, J.C. Olucha, F. Borrull, Chromatographia 32 (1991) 556.
[24] A. Gonzales-Casado, N. Navas, M. del Olmo, J.L. Vilchez, J. Chromatogr. Sci. 36 (1998) 565.
[25] V.J. Barwick, Trends Anal. Chem. 16 (1997) 293.
[26] I.M. Smallwood (Ed.), Handbook of Organic Solvent Properties, Halsted Press, New York, USA, 1996, p. 7.
[27] A. A Boyd-Boland, J. Pawliszyn, J. Chromatogr. A 704 (1995) 163.
[28] D.D. Perrin, B. Dempsey, Buffers for pH and Metal Ion Control, Chapman and Hall, London, 1994.
[29] H. Lord, J. Pawliszyn, J. Chromatogr. A 902 (2000) 17.