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One-step extraction and derivatization liquid-phase microextraction for the determination of chlorophenols by gas chromatography–mass spectrometry

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ABSTRACT

A sample pretreatment method for the determination of 18 chlorophenols (CPs) in aqueous samples by derivatization liquid-phase microextraction (LPME) was investigated using gas chromatography–mass spectrometry. Derivatization reagent was spiked into the extraction solvent to combine derivatization and extraction into one step. High sensitivity of 18 CPs derivatives could be achieved after optimization of several parameters such as extraction solvent, percentage of derivatization reagent, extraction time, pH, and ionic strength. The results from the optimal method showed that calibration ranging from 0.5 to 500 $\mu g\,L^{-1}$ could be achieved with the RSDs between 1.75% and 9.39%, and the limits of detection (LOD) are ranging from 0.01 to 0.12 $\mu g\,L^{-1}$ for the CPs. Moreover, the proposed LPME method was compared with solid-phase microextraction (SPME) coupled with on-fiber derivatization technique. The results suggested that using both methods are quite agreeable. Furthermore, the recoveries of LPME evaluated by spiked environmental samples ranged from 87.9% (3,5-DCP) to 114.7% (2,3,5,6-TeCP), and environmental water samples collected from the Pearl River were analyzed with the optimized LPME method, the concentrations of 18 CPs ranged from 0.0237 $\mu g\,L^{-1}$ (3,5-DCP) to 0.3623 $\mu g\,L^{-1}$ (2,3,6-TCP).

1. Introduction

Chlorophenols (CPs) are high-priority pollutants and several of them are classified as carcinogens. They are introduced into the environment mainly during the manufacturing of various products, including wood preservatives, leather impregnants, pesticides and herbicides [1,2], the chlorination of municipal waters and the degradation of other chemicals such as triclosan (2-(2,4-dichlorophenoxy)-5-chlorophenol) [3]. Due to their resistance to usual biological treatments and to their slow or ineffective removal from contaminated water streams using physical methods, several CPs belong to a class of toxic organic compounds that listed by EPA as priority pollutants. Chlorophenols can be accumulated in river sediment, ground water, tissue of organism and food chain [4]. Moreover, it was proved that polychlorinated dibenzo-*p*-dioxinsand/dibenzofurans (PCDD/Fs) can be formed by the increasing of chlorophenols especially pentachlorophenol and more toxicity to organism [5]. Therefore, a fast, accurate and inexpensive method for chlorophenols determination is usually necessary.

In recent years, despite the great advances in technology, most analytical instruments cannot handle sample matrices directly, and sample-preparation steps are commonly introduced to transfer the analytes into a form that is pre-purified, concentrated, and compatible [6]. Liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are conventional sample pretreatment techniques, which have been prescribed in many standard analytical methods [7]. However, the use of toxic organic solvents poses a health hazard to laboratory personnel and results in the production of hazardous laboratory waste, thus adding extra operational costs for waste treatment [8]. As a result, the development of faster, simpler, less inexpensive and more environmentally friendly sample pretreatment techniques becomes a very important issue in chemical analysis [9].

Equilibrium sample pretreatment methods such as solid-phase microextraction (SPME), membrane extraction and liquid-phase microextraction (LPME) are simple, solvent-less, and usually result in lower losses of analytes than those conventional sample

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pretreatment methods [10,11]. LPME was first introduced by Jeannot and Cantwell in 1996, which was based on a droplet of organic solvent as the extraction phase hanging at the end of a micro-syringe needle [12]. For years development, by greatly reducing the amount of organic solvent, leading to the development of solvent microextraction methods, LPME is now becoming one of the most common methods of microextraction, particularly for organic compounds from aqueous matrices [13]. Single drop LPME could provide high-extraction efficiency, but it had some drawbacks such as instability of microdrop, operational difficulties and bubble formation during extraction. In order to prevent such drawbacks, micropore hollow fiber was chosen for LPME [14]. For years development, LPME technique had provided several different kinds of operation mode, including two or three phase extraction [15,16], directly immerging or headspace extraction [17,6], static or dynamic extraction [18]. Moreover, different organic or inorganic (e.g. ion liquid) extraction solvents were chosen representing excellent selectivity of this method both for organic and inorganic compounds determination [19–21].

Analysis of chlorophenols by LPME combined with high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE) was reported, including single drop LPME with back extraction, headspace LPME with ultrasonic-assisted, and hollow fiber-protected LPME (HF-LPME) via gaseous diffusion [22–24]. However, for gas chromatography (GC) analysis, several reasons may necessitate a derivatization step, namely improving the thermal stability, ameliorating the volatility of analytes with polar functional groups, and introducing a detector-oriented marker on the molecule [25,26]. Thus, derivatization is a very useful tool for detecting compounds in complex matrix, and it was widely used in forensic, medical and environment chemistry [27]. There are many different derivatization methods developed for LPME. A two-step derivatization type was introduced to determine hydroxyl carbonyls in rain samples by LPME [28]. The extraction and first derivatization (carboxylic functional group) took place in the rain samples by adding pentafluorobenzylhydroxylamine (PFBHA), then the syringe was held in the head-space of a vial containing bis-(trimethylsilyl)trifluoroacetamide (BSTFA). Besides this, an on-column derivatization was also used to analyze carbamate pesticide which was used to prevent insects and fungi. Analytes were extracted by LPME, and the derivatization reagent was also withdrawn to the syringe after extraction. Finally, the whole mixture was injected into GC [29]. Moreover, derivatization of chlorophenols can take place in aqueous phase by using acetic anhydride, and the derivates were extracted by dispersed liquid–liquid microextraction (DLLME), which was another type of solvent microextraction technique [30,31].

Generally, HF-LPME was mainly used to prevent the drawbacks of single drop LPME and provide three phases LPME, but there are also some problems of HF-LPME: (1) mass transfer rate of HF-LPME was significantly decreased by the hollow fiber; (2) not all solvent was injected into instruments, which cause the lower sensitivity. Thus, single drop LPME could provide high-extraction efficiency to enhance the sensitivity of analysis and shorten the extraction time. In this paper, a simple, fast extraction and derivatization method for LPME coupled with GC/MS was developed for the determination of 18 chlorophenols in aqueous samples. The derivatization reagent was dissolved into the organic extraction solvent, so that the extraction and derivatization took place simultaneously in one step. Several aspects affecting LPME behavior were evaluated, and the optimal method was compared with SPME method. Finally, chlorophenols in the Pearl River water samples were determined by the proposed LPME methods.

2. Experiment

2.1. Material and reagents

The following standards (98+%) were purchased from Sigma–Aldrich (St. Louis. MO, USA): 2-chlorophenol (2-CP), 3-chlorophenol (3-CP), 4-chlorophenol (4-CP), 2,5-dichlorophenol (2,5-DCP), 2,6-dichlorophenol (2,6-DCP), 3,5-dichlorophenol (3,5-DCP), 2,4-dichlorophenol (2,4-DCP), 2,3-dichlorophenol (2,3-DCP), 3,4-dichlorophenol (3,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP), 2,3,5-trichlorophenol (2,3,5-TCP), 2,4,5-trichlorophenol (2,4,5-TCP), 2,3,6-trichlorophenol (2,3,6-TCP), 2,3,4-trichlorophenol (2,3,4-TCP), 2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP), 2,3,4,5-tetrachlorophenol (2,3,4,5-TeCP), 2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP) and pentachlorophenol (PCP). The internal standards, 2,6-dibromophenol (2,6-DBrP) and 2,4,6-tribromophenol (2,4,6-TBrP) (98 + %), were also from Sigma–Aldrich. The silylating reagents, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (98+%) and *N*-(*tert*-butyldimethylsilyl)-*N*-methyl- trifluoroacetamide (MTBSTFA) (97+%) were obtained from Arcros (New Jersey, USA) and used without further purification. Sodium chloride (NaCl, AR), hydrochloric acid (HCl, AR), acetone (AR), hexane (AR) and toluene (AR) were from Guangzhou Chemical Reagents Factory (Guangzhou, China). Furthermore, the SPME device, consisting of the holder and the fiber (85 μm, polyacrylate) assembly for manual sampling, was purchased from Supelco (Bellefonte, PA, USA).

Stock solutions of individual standards at a concentration of 1.0 g L^{-1} were obtained by dissolving 0.100 g of each standard compound into 100 mL acetone. From these standards, a mixture of 18 standards were prepared (10 mg L^{-1}) and stored in amber glass vials with Teflon-lined caps at 4 °C. Fresh working solutions were prepared by the dilution with appropriate amount of pure water or river water and spiked with internal standard of 100 μg L^{-1}. NaCl and HCl were used to adjust ionic strength and pH value, respectively.

River water samples collected from the Pearl River (Guangzhou, China) was used as practical samples and stored at 4 °C before used. The Pearl River is one of the largest rivers in China, where the explosive increases in industrial and agricultural productivities and growing population in its deltaic region have led to great concerns [4]. The water samples were filtered with membrane (pore size 0.45 μm) first and adjusted according to the optimized matrix condition (salt addition and pH adjustment).

2.2. One-step extraction and derivatization procedure of LPME

The extraction solvent was prepared by adding derivatization reagent into the selected organic solvent, which allowed that the extraction and derivatization took place simultaneously. For LPME sampling, a 3 μL extraction solvent was withdrawn into a 10 μL micro-syringe, which was clamped so that the needle of the syringe was immersed into the 4 mL sample solution hold in a vial. Subsequently, the plunge was depressed to expose a 2 μL droplet to the sample solution and then the magnetic was turned on. After extraction, the droplet was withdrawn. Finally, the all 3 μL extraction organic solvent was injected into GC/MS for instrumental analysis.

2.3. SPME and derivatization procedure

The SPME procedure for comparing with LPME extraction was performed according to our previous work [32]. Briefly, each new fiber was conditioned in GC inlet under 300 °C for 2 h as described in the Supelco's conditioning instruction. The detailed procedures of extraction and derivatization were as follows: the fiber was inserted and immersed directly into 4 mL sample solution (pH 3, 15% NaCl) in a vial with an agitator, after 60 min extraction the fiber was

inserted into another vial containing 100 μL silylating reagent for headspace derivatization (10 min), afterwards, the fiber was immediately inserted into the GC injector for thermal desorption (280 °C, 3 min).

2.4. GC/MSD analysis

GC–MS analysis was performed on an HP 6890 GC (Agilent technologies, USA) interfaced to a 5973 MSD (Agilent technologies, USA). A HP-5MS fused silica capillary column coated with 5% phenylmethyl polysiloxane (30 m length, 0.25 mm i.d., 0.25 mm film thickness; J&W Scientific, Folsom, CA) was used. Helium (99.999%) was used as carrier gas, and the flow rate was set at 1.0 mL min^{-1}. The GC column was operated at an initial temperature at 70 °C, held for 1 min, raised to 115 °C at 15 °C min^{-1} and to 155 °C at 3 °C min^{-1}, finally raised to 300 °C at 20 °C min^{-1} for 5 min. The inlet temperature was set to 280 °C. Mass spectrometric measurement was performed with electron ionization (EI) at 70 eV. The time for solvent delay was set to 5 min. The total GC–MS analysis time was about 30 min and this procedure was used both for LPME and SPME injection.

3. Results and discussion

3.1. Optimization of one-step extraction and derivatization LPME

Generally, silylation is difficult because silylmethylated reagents and the derivatives will hydrolyze in aqueous solutions. To overcome this problem, the silylation of LPME was performed by adding derivatization reagent into the solvent drop, which allowed that the extraction and derivatization took place simultaneously in organic phase. Fig. 1 shows the total ion chromatography (TIC) of *tert*-BDMS derivatives of 18 chlorophenols. It could be seen that most of CPs were separated by the proposed silylation LPME method with high sensitivity. Table 1 showed the molecular weights and most intense mass spectra fragments of the *tert*-BDMS derivatives of chlorophenols obtained in the electron impact mode (70 eV) and the retention times as well.

Some factors affecting the derivatization process in LPME drop should be considered such as extraction solvent, type and concentration of the silylmethylated reagent. Moreover, in order to achieve higher sensitivity and selectivity, some parameters of extraction

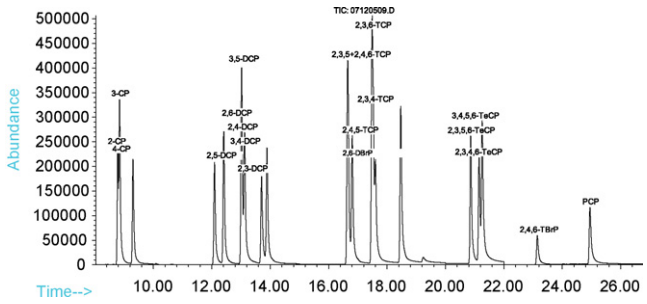

Fig. 1. Total ion mass chromatogram (TIC) of 18 CPs and internal standards (2,6-DBrP and 2,4,6-DBrP) by GC/MSD at 100 μg L^{-1} in aqueous solution after silylated derivatization using LPME.

were also studied such as reaction time, influence of sample pH, and amount of sodium chloride added to the sample solution.

3.1.1. Effects of extraction solvents

In conventional LLE, polar solvents such as chloroform and dichloromethane have been used for extracting phenols from aqueous samples. Suitable extraction solvents used in LPME are limited since they should be immiscible and preferably insoluble in water, and are of low volatility to reduce the loss of solvent during the extraction time. Based on the above considerations, toluene, hexane (volalite solvent, by considering its high-extraction efficiency of CPs), and hexane/toluene mixture (1:1 v:v) were investigated as the extraction solvent, respectively. It had been proved that organic solvent including aromatic structures such as benzene or toluene, can cause peak tailing, peak boarding or double peak formation [33,34]. In our study, when toluene was used as the extraction solvent, the phenomenon of double peak appeared for each chlorophenol. Compared to hexane, the mixture of hexane and toluene exhibited better extraction efficiency for most of the CPs, except 3,4,5,6-TeCP and PCP (Fig. 2). Thus, the mixture of hexane and toluene was selected as the extraction solvent.

3.1.2. Effects of derivatization reagents

The influence of derivatization of 18 CPs for LPME by two commonly used silylation reagents, MEBSTFA and BSTFA, was investigated respectively. *tert*-BDMS derivatives are more resis-

Table 1
Molecular weights, CAS numbers, retention time and most intense mass spectra fragments of the *tert*-BDMS derivatives of chlorophenols obtained in the electron impact mode (70 eV).

Compound	MW (amu)[a]	CAS number	*m/z* ratio for qualitative and quantitative[b] ion	Retention time (min)
2-Chlorophenol	242	95-57-8	185 [M-57]$^+$, 242 [M]$^+$	10.929
3-Chlorophenol		108-43-0		10.995
4-Chlorophenol		106-48-9		11.400
2,5-Dichlorophenol	276	583-78-8	219 [M-57]$^+$, 276 [M]$^+$	14.345
2,6-Dichlorophenol		87-65-0		14.632
3,5-Dichlorophenol		591-35-5		15.229
2,4-Dichlorophenol		120-83-2		15.322
2,3-Dichlorophenol		576-24-9		15.934
3,4-Dichlorophenol		95-77-2		16.141
2,3,5-Trichlorophenol	310	933-78-8	255 [M-57]$^+$, 310 [M]$^+$	18.932
2,4,6-Trichlorophenol		88-06-2		
2,4,5-Trichlorophenol		95-95-4		19.140
2,3,6-Trichlorophenol		933-75-5		19.805
2,3,4-Trichlorophenol		15950-66-0		20.812
2,3,4,5-Tetrachlorophenol	344	4901-51-3	289 [M-57]$^+$, 344 [M]$^+$	23.205
2,3,4,6-Tetrachlorophenol		58-90-2		23.539
2,3,5,6-Tetrachlorophenol		935-95-5		23.642
Pentachlorophenol	378	87-86-5	323 [M-57]$^+$, 378 [M]$^+$	26.665

[a] Molecular weights of the *tert*-BDMS derivatives of CPs.
[b] Underline means quantitative ion of *tert*-BDMS derivatives of CPs.

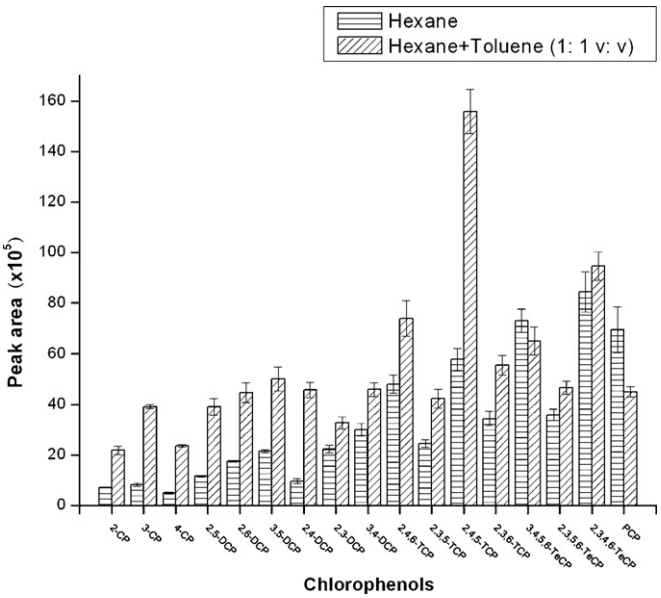

Fig. 2. Effect of extraction solvent for 18 CPs determinations by LPME. Other conditions: 5% MTBSTFA was spiked into extraction solvent, and 100 μg L^{-1} 18 CPs in 4 mL pH 2, 15% salinity (NaCl) pure water, extraction time was 30 min.

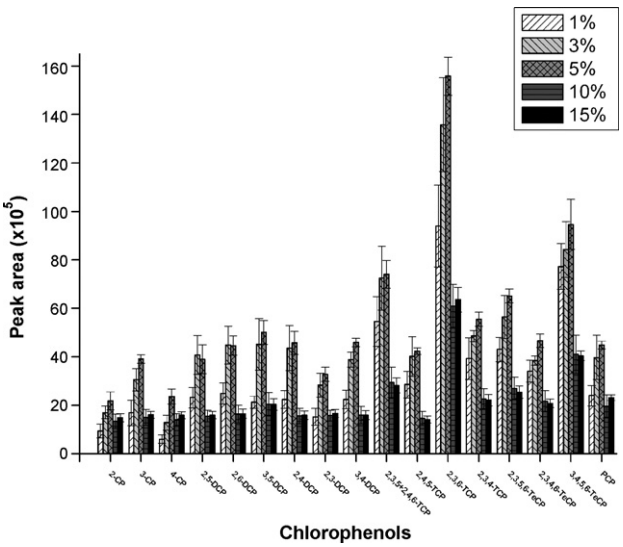

Fig. 3. Effect of percentage of the derivatization reagent added in extraction solvent for 18 CPs determinations by LPME. Other LPME conditions: sample, 100 μg L^{-1} 18 CPs in 4 mL pH 2, 15% salinity (NaCl) pure water; extraction time, 30 min.

tant to hydrolysis and more stable than TMS derivatives [35], and its parent derivatization reagent MTBSTFA is more stable than BSTFA in water. So that MTBSTFA was chosen as derivatization reagent in this study. When using MTBSTFA as the derivatization reagent, the resulting *tert*-BDMS derivatives produce very characteristic mass spectra with electron impact-mass spectrometry (EI-MS). For derivatization of 18 chlorophenols by MTBSTFA, most molecular ion were very weak or absent, but the spectra are dominated by base peaks formed by the loss of the *t*-butyl moiety $[M\text{-}57]^+$ [36,37]. However, we found that trimethyl silyl (TMS) derivates of 2,6-DCP, 3,5-DCP by using BSTFA could not be separated under the chromatographic conditions

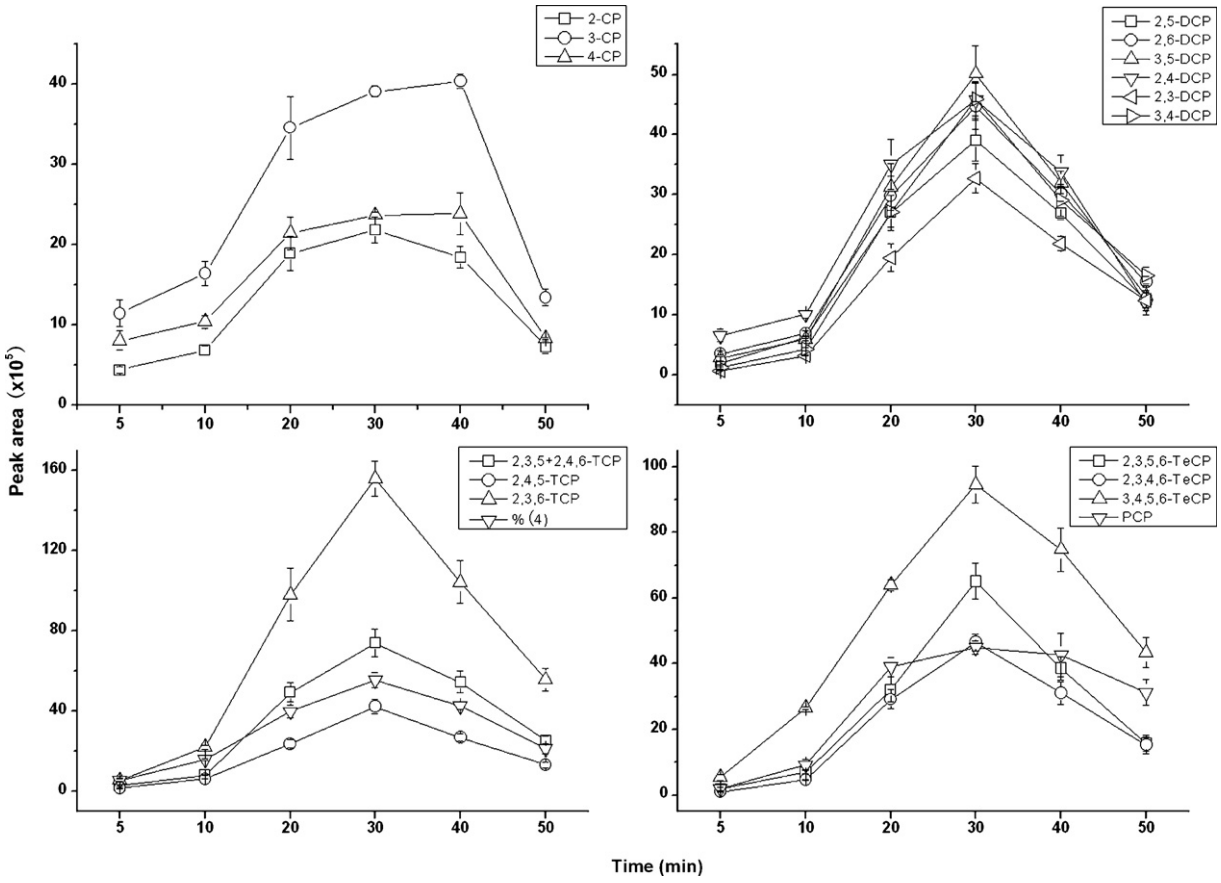

Fig. 4. One-step extraction time optimization of LPME procedures for 18 CPs determinations. Other LPME conditions: sample, 100 μg L^{-1} 18 CPs in pure water with pH 2 and 15% NaCl; extraction phase, toluene/hexane mixture (1:1 v:v) containing 5% MTBSTFA.

in this study, and the same phenomenon happened to *tert*-BDMS derivates of 2,4,6-TCP, 2,3,5-TCP by using MTBSTFA.

3.1.3. Percentage of derivatization reagent in solvents

Additional percentage (1%, 3%, 5%, 10% and 15%) of the selected derivatization reagent into the extraction solvent was also optimized (shown in Fig. 3). The results indicated that more sensitivity and stabilization could be achieved when 5% MTBSTFA was added. Excessive amount (>5%) of MTBSTFA caused poor GC resolution of the analytes and low sensitivity and precision of the analysis.

3.1.4. Effects of extraction time

Since the extraction of analytes in LPME is based on an equilibrium distribution process, the amount of analytes extracted at a given time depends on the mass transfer of the analytes from the aqueous phase into the organic phase of microdrop. The extraction time of proposed method was optimized. Fig. 4 shows that high response of the derivates was attained for most of the analytes after 30 min. Although longer extraction time may increase the extraction amount of some analytes, in our study the amount of most target analytes decreased after 30 min, which might be caused by the lost of the solvent. Therefore, 30 min was used for further study.

3.1.5. Effects of pH

Generally, in order to increase the extraction efficiencies of the target compounds in aqueous solution, the solution is generally weakly acidified to prevent them from dissociating during extraction [32]. The effects of the pH of the sample solution were examined at pH 2, 3, and 4, respectively (Fig. 5). The highest extraction yield was observed at pH 2. The optimum value of pH 2 was chosen for subsequent analysis.

3.1.6. Effects of ionic strength

It is common practice to add salt to aqueous samples in order to enhance the partition of polar analytes into the organic phase. The effect of decreasing solubility of organic compounds by the addition of salt is known as salting out. In this study, varying amount of NaCl (0%, 15%, 30%) was added to decrease the solubility of chlorophenols and increase the partition of the analytes into the organic solvent by the microdrop. The results in Fig. 6 showed that, 15% of NaCl appeared to be optimum, especially for low chloric CPs. Therefore, 15% of NaCl was added for subsequent extractions.

3.2. Evaluation of optimal LPME method

The optimized LPME procedures were evaluated with respect to linear range, correlation coefficient (R^2), precision, limit of detection (LOD) and limit of quantitation (LOQ). Pure water under optimal conditions (pH 2, 15% NaCl) and spiked with a series of various concentration levels of 18 CPs were analyzed. The LOD values were calculated by applying the 3σ criterion, while LOQ was 10σ criterion. Furthermore, analytical performances of LPME and DI-SPME were compared, the results were shown in Table 2.

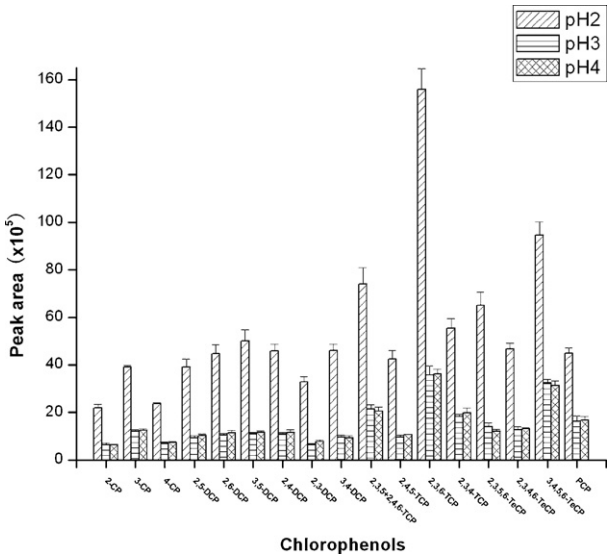

Fig. 5. Effect of sample pH for 18 CPs determinations by LPME. Other LPME conditions: sample, 100 ng L^{-1} 18 CPs in 4 mL 15% NaCl pure water, extraction phase, toluene/hexane mixture (1:1 v:v) containing 5% MTBSTFA, extraction time, 30 min.

It could be seen from Table 2 that the calibration ranges of 18 CPs using LPME were 0.5–500 μg L^{-1}, whereas those for SPME were 0.1–100 μg L^{-1}, and the linearity of calibration curve both for LPME and SPME for 18 CPs were all acceptable. The LOD and LOQ of LPME ranged from 0.0011 to 0.0097 μg L^{-1} and 0.0193 to 0.0323 μg L^{-1}, respectively, whereas the respective ranges for SPME were 0.0010–0.0040 μg L^{-1} and 0.0032–0.0131 μg L^{-1}. Overall, the detection and quantification limits of LPME method were in the same magnitude to those of SPME method, and the RSDs of both LPME and SPME were less than 10% for all CPs determination. However, LPME (30 min) was less time-consuming while comparing to SPME on-fiber derivatization method (60 min + 10 min). Moreover, the SPME fiber was much more expensive and friable. So that LPME by combining extraction and derivatization was a good replacement of SPME for CPs determination. Thus, single drop LPME could pro-

Table 2
Comparison of LPME and SPME techniques for the determination of 18 CPs in water.

Compounds	Correlation coefficient (r^2)		Calibration range (μg L^{-1})		RSD (%, $n = 3$)		LODs (μg L^{-1})		LOQs (μg L^{-1})	
	LPME	SPME	LPME	SPME	LPME	SPME	LPME	SPME	LPME	SPME
2-CP	0.9979	0.9985			7.39	2.76	0.0049	0.0011	0.0164	0.0036
3-CP	0.9993	0.9933			1.83	3.41	0.0087	0.0026	0.0291	0.0087
4-CP	0.9985	0.9971			1.75	6.34	0.0042	0.0028	0.0141	0.0094
2,5-DCP	0.9993	0.9999			8.60	6.35	0.0035	0.0029	0.0115	0.0098
2,6-DCP	0.9993	0.9997			8.53	3.92	0.0035	0.0015	0.0116	0.0050
3,5-DCP	0.9991	0.9979			9.39	6.38	0.0044	0.0028	0.0146	0.0094
2,4-DCP	0.9986	0.9992			6.64	5.35	0.0060	0.0017	0.0201	0.0057
2,3-DCP	0.9988	0.9999			7.29	5.78	0.0045	0.0029	0.0150	0.0097
3,4-DCP	0.9972	0.9992	0.5	0.1	6.08	4.88	0.0046	0.0017	0.0152	0.0058
2,3,5 + 2,4,6-TCP	0.9987	0.9999	–500	–50	9.30	2.84	0.0011	0.0034	0.0193	0.0037
2,4,5-TCP	0.9993	0.9999			8.68	7.55	0.0070	0.0024	0.0232	0.0080
2,3,6-TCP	0.9999	0.9995			2.63	4.87	0.0075	0.0024	0.0251	0.0081
2,3,4-TCP	0.9990	0.9994			7.03	7.86	0.0013	0.0026	0.0044	0.0088
2,3,5,6-TeCP	0.9997	0.9999			8.45	3.13	0.0015	0.0014	0.0048	0.0048
2,3,4,6-TeCP	0.9995	0.9991			5.54	1.92	0.0038	0.0010	0.0126	0.0032
3,4,5,6-TeCP	0.9981	0.9999			5.94	4.85	0.0067	0.0027	0.0222	0.0090
PCP	0.9961	0.9963			4.61	7.21	0.0097	0.0040	0.0323	0.0131

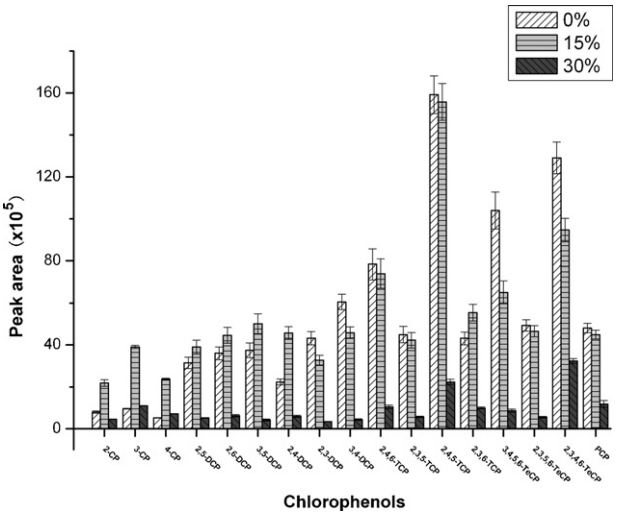

Fig. 6. Effect of sample ionic strength for 18 CPs determinations by LPME. Other LPME conditions: sample, 100 ng L^{-1} 18 CPs in 4 mL pure water with pH 2, extraction phase, toluene/hexane mixture (1:1 v:v) containing 5% MTBSTFA, extraction time, 30 min.

Table 3
Concentration of 18 CPs in real environmental samples which were collected from the Pearl River in South China. Recovery of LPME was also evaluated by spiked 100 μg L^{-1} standard into the river water samples.

Compounds	Concentration of river water samples (μg L^{-1}, $n = 3$)	Recovery of CPs by LPME in river water matrix (%)
2-CP	0.082 ± 0.020	104.4
3-CP	0.079 ± 0.032	108.0
4-CP	0.170 ± 0.057	101.8
2,5-DCP	0.145 ± 0.057	101.5
2,6-DCP	0.027 ± 0.007	98.1
3,5-DCP	0.024 ± 0.003	87.9
2,4-DCP	0.066 ± 0.018	91.5
2,3-DCP	0.182 ± 0.070	93.7
3,4-DCP	0.027 ± 0.002	92.1
2,3,5 + 2,4,6-TCP	0.059 ± 0.014	97.2
2,4,5-TCP	0.065 ± 0.025	100.0
2,3,6-TCP	0.362 ± 0.025	104.7
2,3,4-TCP	0.072 ± 0.025	93.1
2,3,5,6-TeCP	0.065 ± 0.011	114.7
2,3,4,6-TeCP	0.053 ± 0.006	112.1
3,4,5,6-TeCP	0.122 ± 0.025	107.6
PCP	0.137 ± 0.040	94.7

vide high-extraction efficiency to enhance the sensitivity of analysis and shorten the extraction time. Compared to the reference of determination of chlorophenols by HF-LPME [24,38], the LODs of proposed method for all compounds are much lower with higher sensitivity.

3.3. Analysis of real water samples

The present method was also applied to analyze real environmental samples, which were collected from the Pearl River in South China, to investigate the effectiveness of the developed method. The internal standards, 2,6-dibromophenol (2,6-DBrP) and 2,4,6-tribromophenol (2,4,6-TBrP) (98+%) were added into the water sample for quantitative analysis. The concentrations of CPs in river water samples ranged from 0.024 μg L^{-1} (3,5-DCP) to 0.362 μg L^{-1} (2,3,6-TCP) calculated by internal standard method (Table 3). Recoveries of CPs from sample matrix were evaluated by adding standard solution to the river water samples at the concentrations of 100 μg L^{-1}. As shown in Table 3, the results illustrated that LPME presented a good recovery ranging from 87.9% (3,5-DCP) to 114.7% (2,3,5,6-TeCP). It is clear that the optimized LPME coupled with GC/MSD could be used to quantitatively analyze concentrations of 18 CPs in river water samples.

4. Conclusions

A simple, fast one-step derivatization and extraction LPME combined with GC/MSD were evaluated for determining 18 chlorophenols in aqueous samples. The method was optimized and the optimal conditions could be obtained as follows: 1:1 (v:v) hexane/toluene mixture spiked with 5% MTBSTFA was used as extraction and derivatization solvent, LPME sampling time was 30 min, sample matrix was adjusted to NaCl addition of 15% and pH at 2. The total duration with instrumental analysis of each sample was about 30 min. The *tert*-BDMS derivatives of CPs were complete and stable. In comparison with SPME procedure, the method developed was proved to be precise and reliable to determine chlorophenols especially by its less time-consuming and inexpensive. Eighteen CPs in the river water could be detected by such promising method. Moreover, the automation of single-drop LPME has been reported recently [39,40], which means that the mechanical and operational difficulties can be overcome by autosampler, and the precision of the technique can be improved. The further study will be approach in automation process.

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