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Research Article

Dispersive liquid–liquid microextraction of five chlorophenols in water samples followed by determination using capillary electrophoresis

Dispersive liquid–liquid microextraction (DLLME) coupled with CE was developed for simultaneous determination of five types of chlorophenols (CPs), namely 2-chlorophenol (2-CP), 4-chlorophenol (4-CP), 2,4-dichlorophenol (2,4-DCP), 2,6-dichlorophenol (2,6-DCP), and 2,4,6-trichlorophenol (2,4,6-TCP) in water samples. Several parameters affecting DLLME and CE conditions were systematically investigated. Under the optimized DLLME-CE conditions, the five CPs were separated completely within 7.5 min and good enrichment factors were obtained of 40, 193, 102, 15, and 107 for 4-CP, 2,4,6-TCP, 2,4-DCP, 2-CP, and 2,6-DCP, respectively. Good linearity was attained in the range of 1–200 μg/L for 2,4,6-TCP, 2,4-DCP, 2−300 μg/L for 4-CP and 2-CP, and 1−300 μg/L for 2,6-DCP, with correlation coefficients (r) over 0.99. The LOD (S/N = 3) and the LOQ (S/N = 10) were 0.31−0.75 μg/L and 1.01−2.43 μg/L, respectively. Recoveries ranging from 60.85 to 112.36% were obtained with tap, lake, and river water spiked at three concentration levels and the RSDs (for $n = 3$) were 1.31–11.38%. With the characteristics of simplicity, cost-saving, and environmental friendliness, the developed DLLME-CE method proved to be potentially applicable for the rapid, sensitive, and simultaneous determination of trace CPs in complicated water samples.

Keywords:
Capillary electrophoresis / Chlorophenols / Dispersive liquid–liquid microextraction / Water samples
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Additional supporting information may be found online in the Supporting Information section at the end of the article.

1 Introduction

Chlorophenols (CPs), a general name for chlorine-substituted phenolic compounds, are widely used in industry as intermediates in the production of dyes, pharmaceuticals, and plastics [1]. CPs are commonly found in aquatic environments and their main sources are industrial wastewater and waste leachate, pesticides, disinfectants, and preservatives [1, 2]. The chlorination of tap water produces chlorophenol from phenol, which is responsible for the unfavorable smell in the air [3]. It can be released directly or indirectly through industrial waste water, natural and synthetic chemical by-products, which can seriously affect human health and environmental quality [4]. The United States Environmental Protection Agency (EPA) has listed 11 phenolic compounds as priority pollutants, including 2-chlorophenol (2-CP), 2,4-dichlorophenol (2,4-DCP) and 2,4,6-trichlorophenol (2,4,6-TCP) and so on [5]. The World Health Organization (WHO) has stipulated the maximum allowable concentration of CPs in drinking water: 300 μg/L for 2,4,6-TCP, 40 μg/L for 2,4-DCP, and 10 μg/L for 2-CP. European Union (EU) legislation stipulates that the maximum permissible concentration of phenolic compounds is 0.5 μg/L in tap water [6]. In China's surface water environmental quality standards of specific project standards for centralized drinking water,

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Abbreviations: **2,4-DCP**, 2,4-dichlorophenol; **2,6-DCP**, 2,6-dichlorophenol; **2,4,6-TCP**, 2,4,6-trichlorophenol; **CP**, chlorophenol; **CPE**, cloud point extraction; **DLLME**, dispersive liquid–liquid microextraction; **DSPE**, dispersive solid-phase extraction; **EF**, enrichment factor; **MIPs**, molecularly imprinted polymers

Color Online: See the article online to view Fig. 1 in color.

urban wastewater reuse landscape water, and integrated wastewater discharge standards, CPs are regulated in the control range [7–9]. Hence, it is necessary and important to develop reliable, sensitive, and efficient analytical methods for the determination of trace CPs in water environments.

Many analytical approaches have been used for the trace-level analysis of CPs, and CE [9, 10], GC [8], and HPLC [11–13] are of more practical interest. For GC analysis, CPs usually require to derive with suitable derivatization reagents to increase their volatility before injection into GC. HPLC-UV has the disadvantages of consuming organic solvents and lower detection sensitivity. CE is an ideal alternative with high resolution, short analysis time, low solvent consumption, and flexible separation modes [14, 15]. However, CE-UV often faces a severe problem of poor sensitivity for use at trace and ultratrace analysis owing to small injection volume and narrow optical path length [16]. Therefore, it is imperative to develop high efficiency sample enrichment technology to improve the analysis sensitivity of CE-UV [17].

Various pretreatment and preconcentration procedures for CPs have been utilized, mainly including SPE [10, 18, 19], solid-phase microextraction (SPME) [18, 19], cloud point extraction (CPE) [13, 20], liquid–liquid–liquid microextraction [21], and hollow fiber supported liquid–liquid–liquid membrane microextraction [22], dispersive liquid–liquid microextraction (DLLME) [8, 9, 12, 23]. In DLLME, the amount of extractant used is small, and the contact area between the extractant and the sample solution is maximized by the dispersant, so high enrichment factors (EFs) can be obtained. Moreover, DLLME has the advantages of simple device, easy operation, short extraction time, and high reproducibility [9, 24, 25], and is popular in water sample analysis. DLLME has demonstrated broad application prospects in the analysis of organic compounds and metal ions, even in the species analysis of trace elements [26–30]. Recently, in the aspect of CPs analysis, the DLLME combined with HPLC-UV [31] and HPLC–MS-MS [32] methods have been reported. Molecularly imprinted magnetic nanoparticle (Fe_3O_4@MIP)-based extraction coupled with CE has been developed to determine trace CPs [33]. Our group has developed dispersive solid-phase extraction (DSPE) along with CE for CPs analysis [10, 34]. These studies provide higher analysis efficiency than other methods, but the preparation of molecularly imprinted polymers (MIPs) and SPE procedure is relatively labor-intensive and time/reagents-consuming. Therefore, we propose to develop a method of DLLME coupled to CE for the separation and detection of CPs in water samples.

In this study, DLLME coupled with CE was introduced to determine five CPs including 4-chlorophenol (4-CP), 2,4,6-TCP, 2,4-DCP, 2-CP, and 2,6-dichlorophenol (2,6-DCP) in water samples. The DLLME-CE method was optimized and validated, and then applied for the simultaneous separation and determination of five CPs in river, lake and tap water samples. It was expected to provide an alternative for simultaneous determination of trace CPs in water samples.

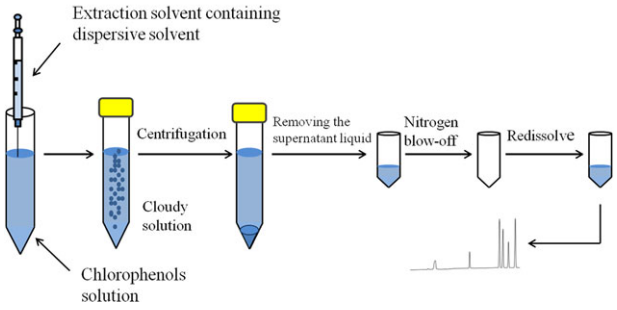

Figure 1. Schematic illustration of the DLLME-CE procedure.

2 Materials and methods

2.1 Reagents and samples

HPLC grade reagents of 2-CP, 4-CP, 2,4-DCP, and 2,4,6-TCP were obtained from Sigma-Aldrich (Shanghai, China) and the standard stock solutions were prepared by dissolving them in MeOH with a concentration of 10 g/L. The standard solution of 2,6-DCP in MeOH with a concentration of 10 g/L was purchased from Dr. Ehrenstorfer (Augsburg, Germany). The structures of CPs are shown in Supporting Information Fig. 1. All the standard solutions were stored at 4°C in a refrigerator and used for CE separation. Chromatographic grade acetonitrile (ACN), methanol (MeOH), ethanol (EtOH), and chlorobenzene (C_6H_5Cl) were all purchased from J&K Chemical (Beijing, China). The other chemicals, such as sodium hydroxide (NaOH), sodium tetraborate decahydrate ($Na_2B_4O_7{\cdot}10H_2O$), acetone, carbon dichloride (C_2Cl_2), and chloroform ($CHCl_3$) were of analytical grade and were obtained from Sinopharm Chemical Reagent (Shanghai, China). Carbon tetrachloride was purchased from Aladdin (Shanghai, China). Working solutions were obtained by appropriate dilution of the stock standard solution and all standard solutions were stored in a refrigerator at 4°C for use. The water used throughout the work was produced by a Milli-Q ultrapure water system (Millipore, Bedford, MA, USA).

Tap water was taken from the laboratory and collected after 5 min of self-flow. The lake water was taken from an artificial lake on the campus of the Yantai University. The river water was taken from the Wandering River in Laishan District, Yantai City. During the collection, the sampler and the glass container were rinsed with tap, river, and lake water respectively for three times. All water samples were filtered through microporous nylon filters with a pore diameter of 0.45 μm before use. The filtered samples were stored in a refrigerator at 4°C.

2.2 Instruments

A Beckman-Coulter P/ACE MDQ CE system (Fullerton, CA, USA) equipped with a diode-array detector and bare

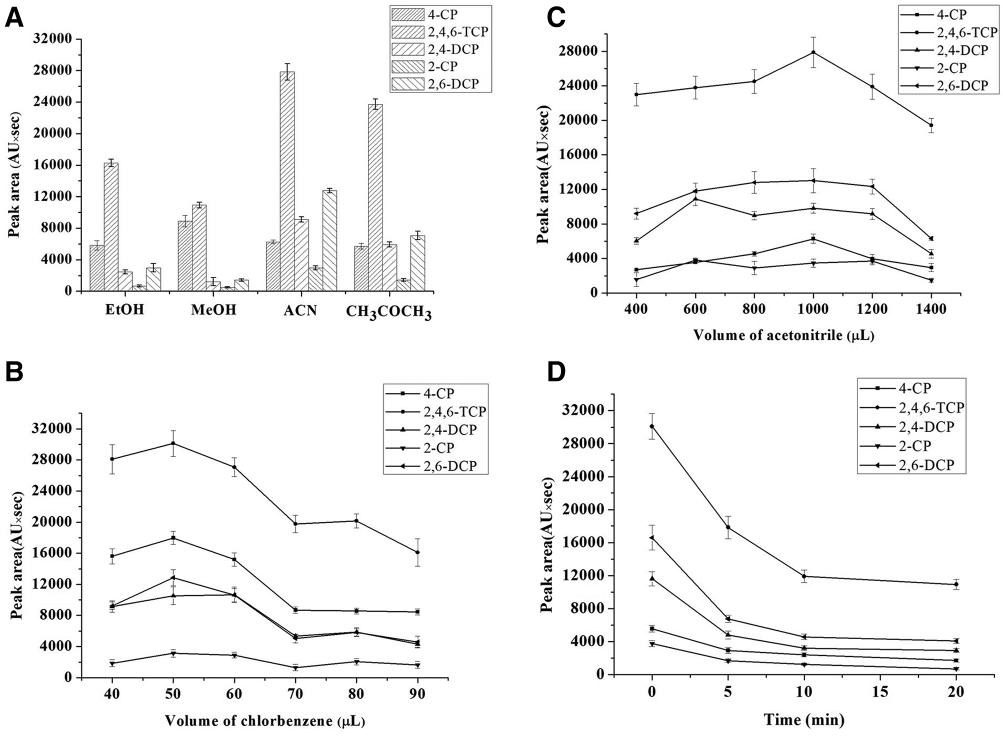

Figure 2. Effect of (A) the kinds of dispersive solvent, (B) extraction solvent volume, (C) dispersive solvent volume, and (D) extraction time on the peak area of the five CPs. Extraction conditions: (A) sample volume, 10 mL; extraction solvent, C_6H_5Cl; (B) sample volume, 10 mL; dispersive solvent, ACN; extraction solvent, C_6H_5Cl; (C) sample volume, 10 mL; dispersive solvent, ACN; extraction solvent, 50 μL C_6H_5Cl; (D) sample volume, 10 mL; dispersive solvent, 1000 μL ACN; extraction solvent, 50 μL C_6H_5Cl. CE conditions: 20 mmol/L $Na_2B_4O_7{\cdot}10H_2O$ containing 10% v/v ACN at pH = 9.8, injection 5 s with 0.5 psi, +20 kV applied voltage.

fused-silica capillary (Yongnian Photoconductive Fiber Factory, Hebei, China) with 75 μm id, 375 μm od, total length of 50.2 cm, and effective length of 40 cm was utilized in all the experiments. The pH value measurements were made with a Rex pH meter (Shanghai Precision Scientific Instrument Corporation, Shanghai, China). Data acquisition was performed using Karat 32 software (Beckman-Coulter, Fullerton, CA, USA).

2.3 CE conditions

Before the first usage, new capillary was conditioned by rinsing in order, with MeOH (5 min), water (5 min), 1 mol/L NaOH (20 min), water (10 min), and running buffer (30 min). The capillary was conditioned daily by flushing with 1 mol/L NaOH, water, and running buffer for 5, 5, and 10 min, respectively. Between the two separation analyses, it should be rinsed with running buffer for 5 min. All solutions were filtered through microporous nylon filters with a pore diameter of 0.22 μm before use. The detection wavelength was set at 195 nm for 4-CP and 2-CP, and 214 nm for 2,4,6-TCP, 2,4-DCP, and 2,6-DCP. The capillary temperature was maintained at 25°C and the applied voltage was +20 kV and pressure injection was performed using 0.5 psi for 5 s (1 psi = 6894.76 Pa). The running buffer consisted of 20 mM $Na_2B_4O_7{\cdot}10H_2O$ and 10% v/v ACN, and then the solution pH was adjusted with 1 mol/L NaOH after addition of ACN, as a result, the apparent pH namely "pH*" 9.80 was used.

2.4 DLLME procedure

For the DLLME, 10.00 mL aqueous sample containing the analytes was placed in a 10 mL centrifuge tube with conical bottom, in which the five spiked CPs were 50 μg/L individually. ACN of 1.0 mL (disperser solvent) containing 50 μL C_6H_5Cl (extraction solvent) was injected rapidly into the aqueous solution with 1 μL syringe. By injecting the above mentioned mixture in water sample, dispersed fine droplets of C_6H_5Cl formed a cloudy solution, and then the analytes were extracted into the fine droplets. After centrifugation for 5 min at 1737 *g*, fine droplets of extraction solvent were sedimented at the bottom of the centrifuge tube with conical bottom. Then, the sedimentated solvent was removed with a syringe and dried under a gentle flow of nitrogen. At last, the evaporation residue was redissolved using 10 μL ACN and H_2O (v/v = 1:1) for further CE analysis. The schematic illustration of the DLLME-CE procedure is shown in Fig. 1.

The extraction capability was evaluated by the EF, which was calculated as follows:

$$\mathrm{EF} = \frac{C_{\mathrm{sed}}}{C_0},$$

where C_{sed} and C_0 are the concentration of analyte in the sedimented phase and the initial concentration of analyte in the aqueous solution.

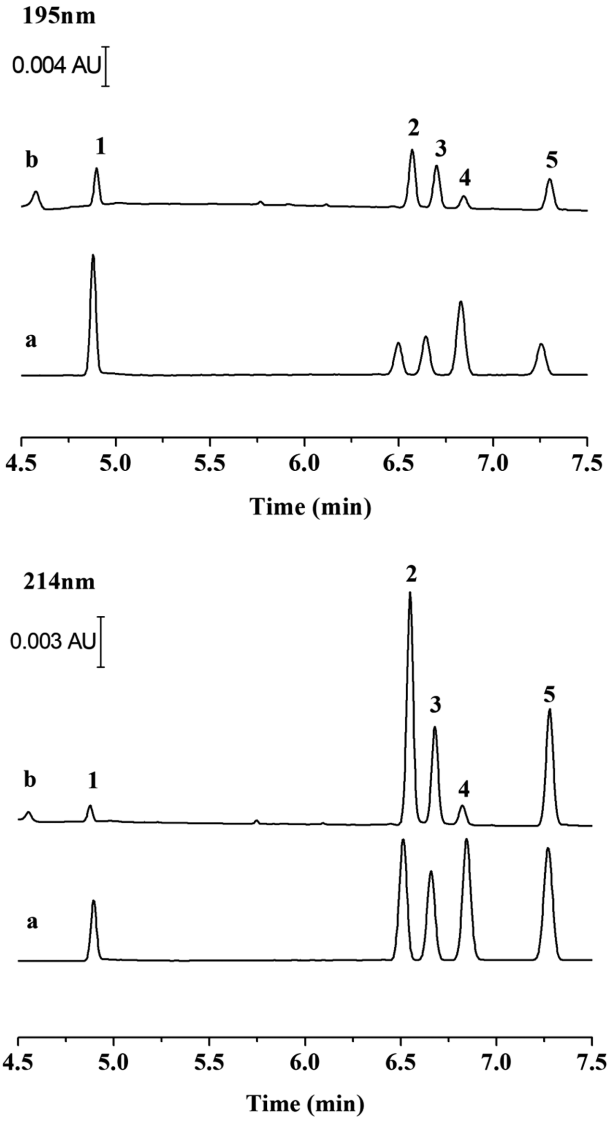

Figure 3. Electropherograms of five CPs. Standard solution monitored at 195 and 214 nm with the concentration of 5 μg/mL (a) and 50 μg/L after DLLME (b). Peak identification: (1) 4-CP; (2) 2,4,6-TCP; (3) 2,4-DCP; (4) 2-CP; (5) 2,6-DCP. CE conditions: 20 mmol/L $Na_2B_4O_7{\cdot}10H_2O$ containing 10% v/v ACN at pH = 9.8, injection 5 s with 0.5 psi, +20 kV applied voltage.

3 Results and discussion

3.1 Optimization of CE conditions

Initially, we intended to use capillary zone electrophoresis mode for the separation of the five CPs by referring to our previous study [34]. The separation of the five analytes was greatly influenced by the running buffer, pH of the buffer solution, and the organic solvent as well as the applied voltage. In order to optimize the separation conditions, the factors were individually studied. Selection of the buffer used as background electrolyte has a great influence on the migration behavior. For this system, two kinds of buffer including NaH_2PO_4 and $Na_2B_4O_7{\cdot}10H_2O$ were tested. Finally, $Na_2B_4O_7{\cdot}10H_2O$ presented better peak shape and resolution. The buffer concentration directly affects the Zeta potential on the inner wall of the capillary [35]. Four levels (5, 10, 20, 30, 40 mmol/L) of $Na_2B_4O_7{\cdot}10H_2O$ solution were optimized. As shown in Supporting Information Fig. 2, 20 mmol/L $Na_2B_4O_7{\cdot}10H_2O$ obtained better resolution and larger peak area than 10 mmol/L. Low buffer concentration can lead to a high Zeta potential and thereby high electroosmotic flow (EOF), easily resulting in an incomplete separation [35]. When the concentration of $Na_2B_4O_7{\cdot}10H_2O$ was greater than 20 mmol/L, the peaks of the five analytes could not be separated and the separation time was prolonged. Therefore, 20 mmol/L borax was selected as the experimental buffer.

The pH of the buffer solution has a close relationship with the effective charge of the analyte, which in turn affects effective mobility of the analyte in the buffer [36]. To determine the effect of buffer pH on migration behavior, experiments were performed by using a background electrolyte solution consisting of 20 mmol/L $Na_2B_4O_7{\cdot}10H_2O$. The pH values of 9.20, 9.50, 9.80, and 10.00 were investigated, which were adjusted by 1.0 mol/L of NaOH. As shown in Supporting Information Fig. 3, the resolution of the suite of CPs was best achieved at a pH of 9.50, whereas 4-CP showed peak-broadening. At pH of 9.20 and 10.0, a complete peak overlapping was observed. Happily, at pH of 9.8, baseline separation was achieved for the CPs except a slight overlap between 2-CP and 2,6-DCP. Therefore, the buffer pH was adjusted to 9.80 for the following study.

Table 1. Analytical performances of the DLLME-CE method for the determination of five CPs

CPs	Calibration curve[a)]		Correlation coefficient (r)	Linear range (μg/L)	LOD (μg/L)	LOQ (μg/L)	EF
	k (mean ± SD[b)])	b (mean ± SD)					
4-CP	74.97 ± 2.547	739.8 ± 361.0	0.9983	2–300	0.66	2.11	40
2,4,6-TCP	278.1 ± 7.804	837.4 ± 660.9	0.9984	1–200	0.44	1.31	193
2,4-DCP	128.5 ± 6.043	1017 ± 621.6	0.9967	1–200	0.31	1.03	102
2-CP	27.53 ± 1.531	613.9 ± 198.2	0.9939	2–300	0.75	2.43	15
2,6-DCP	159.7 ± 7.404	526.8 ± 1250	0.9968	1–300	0.32	1.01	107

a) $y = kx+b$; y and x stand for the peak area and the concentration (μg/L) of all the analytes, respectively.
b) SD, $n = 6$.

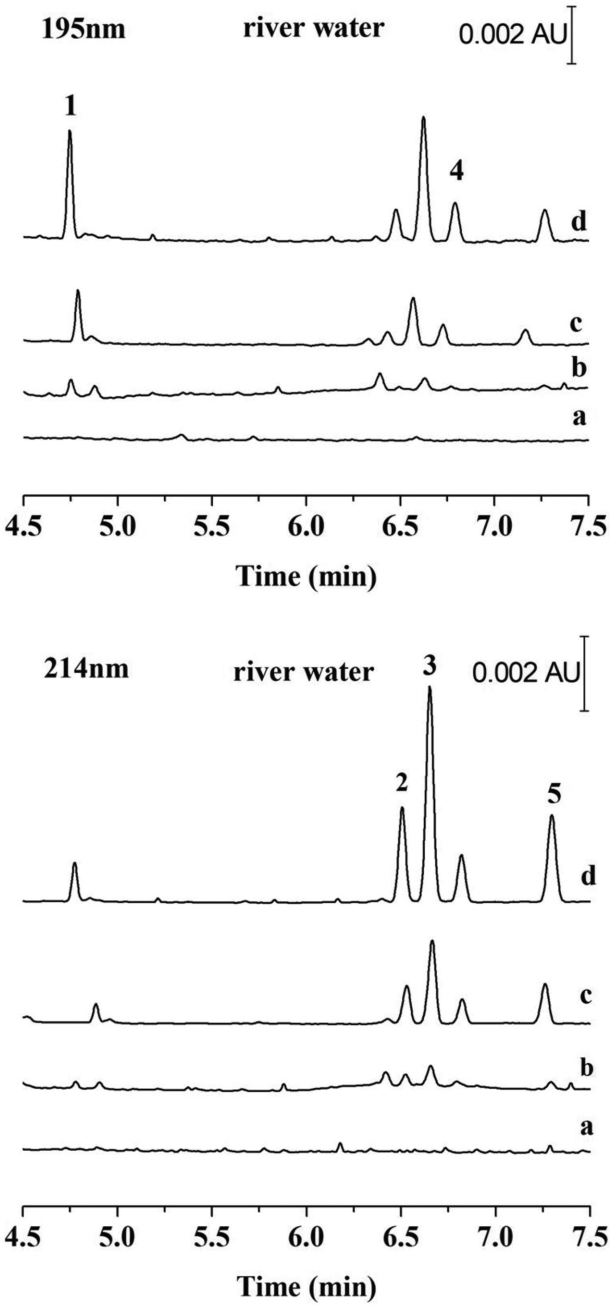

Figure 4. Electropherograms of five CPs monitored at 195 and 214 nm in river water samples using DLLME-CE method under the optimal conditions. Unspiked (a), spiked with 5 μg/L (b), spiked with 20 μg/L (c), and spiked with 50 μg/L (d) phenolic compounds, respectively. Peak identification: (1) 4-CP, (2) 2,4,6-TCP, (3) 2,4-DCP, (4) 2-CP, and (5) 2,6-DCP.

In electrophoresis analysis, the buffer is generally formulated with water, and the organic additive can effectively improve the degree of separation or separation selectivity [37]. For investigating the effect of organic modifier on the separation efficiency of the analyte, various experiments were performed by adding MeOH and ACN. The results show that the five CPs can be better separated with ACN as an organic modifier. Therefore, we chose ACN as an organic modifier and the proportion of ACN (5, 10, 20, and 30 v/v) was investigated. As shown in Supporting Information Fig. 4, the best separation performance was obtained when the ratio of ACN was 10% v/v. Even though the proportion of ACN was 5% v/v, the latter four analytes were able to separate, but the resolution and peak area are inferior to that of 10% v/v. When the ACN ratio is 30% v/v, the time required for the separation of the five CPs is too long. Therefore, 10% v/v ACN was used as an organic modifier.

In CE, the separation voltage is also an important parameter for controlling electro-osmosis [38]. The separation voltage of 15, 17, 19, 20, and 22 kV was checked by using a background eletrolyte solution consisting of 20 mmol/L $Na_2B_4O_7{\cdot}10H_2O$, and 10% v/v ACN at a pH value of 9.80. With the increase of voltage, the analysis time is shortened and the separation degree is increased; however, the high voltage will widen the band width and reduce the separation efficiency. Therefore, 20 kV was selected as the optimal separation voltage.

From the results described above, the optimized CE conditions were confirmed as follows: 20 mmol/L $Na_2B_4O_7{\cdot}10H_2O$, 10% v/v ACN, pH 9.80, and an applied voltage of 20 kV at 25°C.

3.2 Optimization of DLLME conditions

Peak area is the best indicator of DLLME method and the peak area of CPs in DLLME process are mainly subjected to several factors such as type and volume of extraction and disperser solvents, extraction time, and salt addition [25]. In this study, these six major factors were investigated using a spiked ultrapure water sample (50 μg/L), and all the optimization experiments were conducted three times.

In the DLLME, suitable extractants are essential for improving the extraction efficiency of the target compound [16]. Some characteristics of the extractant determine the formation of a three-phase equilibrium system [39]: (1) the extraction solvent must not be mixed with the water sample; (2) the target has a certain solubility in the extractant; (3) the density of the extractant should be greater than the mixed solution of water sample and dispersant. Based on the above consideration, four organic solvents, CH_2Cl_2, $CHCl_3$, CCl_4, and C_6H_5Cl were selected as the extractants to investigate their extraction effects. The experimental results show that when CH_2Cl_2 and $CHCl_3$ are used as extractants, no sedimentary phase is generated after centrifugation. When CCl_4 and C_6H_5Cl are used as extractants, they have a certain ability to extract the target, and a clear delamination phenomenon occurs after centrifugation. However, the extraction efficiency of C_6H_5Cl to 2-CP and 4-CP was significantly higher than that of CCl_4. Therefore, C_6H_5Cl was finally selected as the extractant.

Dispersants are also an important factor in the formation of three-phase equilibrium systems in DLLME. With the use of C_6H_5Cl as extracting solvent, four kinds of organic

Table 2. Recoveries obtained for the determination of five CPs in spiked tap, lake and river water samples ($n = 5$)

CPs		Tap water			Lake water			River water		
	Spiked (μg/L)	Found ± SD (μg/L)	Recovery (%)	RSD (%)	Found ± SD (μg/L)	Recovery (%)	RSD (%)	Found ± SD (μg/L)	Recovery (%)	RSD (%)
4-CP	5	4.45 ± 0.27	88.98	6.07	5.33 ± 0.24	106.58	4.50	4.31 ± 0.34	96.22	7.89
	20	19.65 ± 0.26	98.27	1.32	22.47 ± 0.46	112.36	2.05	17.74 ± 0.64	88.72	3.58
	50	50.34 ± 0.66	100.68	1.31	44.62 ± 3.28	89.25	7.35	55.02 ± 2.17	110.03	3.94
2,4,6-TCP	5	3.33 ± 0.25	66.64	7.51	3.30 ± 0.25	65.95	7.58	3.11 ± 0.26	62.13	8.36
	20	12.17 ± 0.21	60.85	1.73	12.64 ± 0.84	63.18	6.68	13.48 ± 0.98	67.42	7.28
	50	44.22 ± 0.78	88.44	1.76	34.12 ± 1.35	68.24	3.96	32.18 ± 0.71	64.36	2.21
2,4-DCP	5	5.36 ± 0.61	107.13	11.38	4.97 ± 0.15	99.40	3.02	4.48 ± 0.21	89.64	4.69
	20	21.88 ± 0.36	109.34	1.65	20.26 ± 0.36	101.30	1.78	19.92 ± 1.41	99.60	7.08
	50	54.71 ± 4.16	109.43	7.60	38.41 ± 2.95	76.82	7.68	53.22 ± 1.72	106.43	3.23
2-CP	5	3.59 ± 0.055	71.75	1.53	5.00 ± 0.47	100.08	9.40	4.33 ± 0.14	86.69	3.23
	20	19.40 ± 1.52	97.00	7.83	19.14 ± 0.86	95.73	4.49	15.95 ± 0.73	79.75	4.55
	50	42.30 ± 0.80	84.59	1.89	51.67 ± 3.08	103.33	5.96	51.35 ± 0.70	102.71	1.36
2,6-DCP	5	3.67 ± 0.34	73.33	9.26	5.38 ± 0.25	107.69	4.64	4.53 ± 0.21	90.60	4.64
	20	16.18 ± 1.07	80.91	6.61	18.10 ± 1.20	90.48	6.63	19.93 ± 0.57	99.66	2.85
	50	40.73 ± 2.15	81.46	5.28	43.58 ± 2.65	87.17	6.08	38.79 ± 3.56	77.58	3.56

solvents, MeOH, EtOH, ACN, and acetone were selected for experiments. The effects of these four dispersing solvents on peak area are presented in Fig. 2A. The results showed that when ACN was used as a dispersant, the extraction of the analytes was better compared with the other three reagents. Therefore, ACN was chosen as the dispersing solvent for this study.

In order to evaluate the effect of extractant volume on extraction efficiency, different volumes of C_6H_5Cl (40, 50, 60, 70, 80, and 90 μL) were used for the extraction. The peak areas obtained with different extractant dosages are shown in Fig. 2B. It can be seen from the figure that 50 μL and 60 μL volumes of the extraction solvent are similar. However, 50 μL of extractant has a better extraction effect on 2-CP. Thus, 50 μL of extractant is a better choice.

For obtaining optimized volume of ACN, various experiments were performed by using different volumes of ACN (400, 600, 800, 1000, and 1200 μL) containing 50 μL C_6H_5Cl. As shown in Fig. 2C, the highest extraction efficiency was obtained for each analyte at a dispersant volume of 1000 μL. When the dispersant volume is small, the extractant cannot be well dispersed in the solution, resulting in a decrease in the extraction efficiency, when the dispersant volume is too high, the solubility of the analyte in the water increases, and the extraction efficiency decreases. Therefore, we chose 1000 μL as the optimal amount of dispersant.

In the DLLME process, the extraction time refers to the time between the injection of the extractant and the dispersant and the start of centrifugation [40]. The effect of extraction time on the extraction efficiency within 0–20 min (in intervals of 5 min) was examined. Figure 2D shows that the prolonged extraction time decreased the extraction efficiency. This may be because of the large contact surface of the extractant with the solution under the dispersion of the dispersant, which can quickly extract the analyte from the aqueous phase. With the increase of the extraction time, the analyte may be redissolved in the aqueous solution and the extraction efficiency may be reduced. Therefore, it should be centrifuged quickly after the extraction. The short extraction time (a few seconds) is also one of the main advantages of the dispersion liquid microextraction technology.

There may be some effects on the extraction efficiency created by salt addition. To investigate the impact of the ionic strength on the performance of DLLME, different NaCl contents (0–5%, w/v) were tested to find the optimum amount of salt addition. As shown in Supporting Information Fig. 5, the addition of salt caused the extraction efficiency to decrease gradually. Therefore, no salt was added in the following experiment.

Consequently, the DLLME conditions were optimized, i.e. C_6H_5Cl as extraction solvent with 50 μL, ACN as dispersive solvent with 1000 μL, without additon of salt.

3.3 Method performance

Under the optimal CE and DLLME conditions, two typical electropherograms of standard solutions of five CPs at 5 μg/mL individual without DLLME (curve a) and 50 μg/L with DLLME (curve b) were attained, as shown in Fig. 3. The calculated EFs within the range of 15–193 showed relatively high pretreatment capability. Linear correlation coefficients (r) assessed at six different concentrations were obtained between the peak area and the corresponding concentrations of the CPs within the corresponding linear range as shown in Table 1. Good linearity was attained in the range of 2–300 μg/L for 4-CP and 2-CP, and 1–200 μg/L for 2,4,6-TCP, 2,4-DCP, and 2,6-DCP, with correlation coefficients (r) over 0.99. The LODs were obtained based on the peak height as

three times of background noise ($S/N = 3$), in the range of 0.31–0.75 μg/L. The LOQs calculated based on a signal-to-noise ratio ($S/N = 10$) were in the range of 1.01–2.43 μg/L. The values are much lower than the maximum allowed concentrations for CPs, i.e. 300 μg/L for 2,4,6-TCP, 40 μg/L for 2,4-DCP, and 10 μg/L for 2-CP in drinking water regulated by the WHO, and meet the requirement of trace analysis. Therefore, the developed DLLME-CE held great application potential for determination of trace CPs in real water samples.

Furthermore, the intra and interday precisions (RSD) were investigated in terms of migration time and peak area obtained. From Supporting Information Table 1, it can be seen that the the intraday precisions of the migration time were in the range of 0.27–0.67% and the interday precisions were 0.65–0.93%, the intraday precisions of the peak area were 2.97–5.66%, and the interday precisions were 3.57–6.10%. Thus, the method was proved to be robust and reliable, and was capable of quantifying CPs accurately.

3.4 Application of the DLLME-CE to real water samples

To further assess the applicability of the DLLME-CE, the detection of CPs in real water samples including tap, lake, and river water was demonstrated by recovery tests. Figure 4 shows that the endogenous CPs were not detected (curve a). These samples were spiked with the standards of CPs at different concentration levels (5, 20, and 50 μg/L) to assess matrix effects. The averaged spike recovery obtained based on five triplicate measurements for each concentration was used to evaluate the feasibility of the DLLME-CE method. As listed in Table 2, satisfactory recoveries for the four CPs (4-CP, 2,4-DCP, 2-CP, and 2,6-DCP) in tap, lake, and river water were between 71.75 and 109.43% with RSDs of 1.31–11.38%, 76.82–112.36% with RSDs of 1.78–9.40%, and 77.58–110.03% with RSDs of 1.36–7.89%, respectively. It was noticed that the recoveries for 2,4,6-TCP in three water samples were lower, i.e. 60.85–88.44% for tap water, 63.18–68.24% for lake water, and 62.13–67.42% for river water, possibly owing to the matrix effect of the real samples. Nevertheless, on the whole, the developed DLLME-CE was suitable for the determination of CPs in real water samples.

3.5 Performance comparison with other methods for CPs

Analytical performance of the developed DLLME-CE was compared with other reported HPLC and GC methods for determination of CPs. As shown in Supporting Information Table 2, our method presents lower LODs (0.27–0.66 μg/L) for five CPs, in comparison with that reported MIPs-SPE (0.57–1.08 μg/L) [11], CPE-based HPLC (3.00–5.00 μg/L) [13], and DLLME-MEEKC (1.40–3.00) methods [23]. The LOQs of our study (1.01–2.43) are lower than that of DLLME-MEEKC (4.50–10.20) [23]. Although our LODs and LOQs are higher than that of MIPs-DSPE [10] and MIPs-SPE [34] methods, they require synthetic materials and thereby the processes are complicated and time/reagents-consuming [10]. Higher EFs are obtained in DLLME-GC [8] and DLLME-HPLC [12]; however, more organic reagents and higher analysis costs are needed than our method. Furthermore, our DLLME-CE only takes 7.5 min for chromatographic separation of all the analyzed CPs, much shorter than that by SPE-HPLC (20 min) [11], CPE-HPLC (28 min) [13], and MIPs-DSPE-CE (30 min) [33], DLLME-CE (16 min) [9], and DLLME-GC (14 min) [8]. Therefore, in general, our developed DLLME coupled with CE-UV method has the advantages of simple device, easy operation, short extraction/separation time and cost-effectiveness, and it is practically feasible for trace CPs determination in water samples.

4 Concluding remarks

In this study, we developed and evaluated a simple, fast, and efficient DLLME sample pretreatment and CE separation method for simultaneous separation and determination of five CPs in real water samples. The DLLME-CE offered low LODs from 0.31 to 0.75 μg/L, satisfactory linearity, and high precision. The DLLME-CE method has proven to be a simple, rapid, effective and environmentally friendly application for the simultaneous separation and determination of trace CPs in aqueous matrices. Furthermore, more work needs to be done to further improve the detection sensitivity and promote the development of the DLLME-CE method such as combining sample preparation with on-line enrichment strategies. Further explorations of a wide range of multifunctional off-line/on-line enrichment technologies are currently being conducted in our laboratories using CE.

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5 References

[1] Jin, M. C., Zhu, Y., *J. Chromatogr. A* 2006, *1118*, 111–117.

[2] Alonso, M. C., Puig, D., Silgoner, I., Grasserbauer, M., Barceló, D., *J. Chromatogr. A* 1998, *823*, 231–239.

[3] Khairy, M. A., *Environ. Monit. Assess.* 2013, *185*, 441–455.

[4] Migaku, K., Yumiko, I., Norihiro, S., Noriya, O., Rie, I., Koichi, S., Hiroyuki, N., *Anal. Chim. Acta* 2005, *533*, 57–65.

[5] Keith, L. H., US Environmental Protection Agency, Boca Raton, FL, 1991, 389–486.

[6] Rodriguez, I., Turnes, M. I., Mejuto, M. C., Cela, R., *J. Chromatogr. A* 1996, *721*, 297–304.

[7] Lin, C. Y., Huang, S. D., *J. Chromatogr. A* 2008, *1193*, 79–84.

[8] Fattahi, N., Assadi, Y., Reza, M., Hosseini, M., Jahromi, E. Z., *J. Chromatogr. A* 2007, *1157*, 23–29.

[9] He, H., Liu, S. H., Meng, Z. F., Hu, S. B., *J. Chromatogr. A* 2014, *1361*, 291–298.

[10] Lu, W. H., Ming, W. N., Zhang, X. S., Chen, L. X., *Electrophoresis* 2016, *37*, 2487–2495.

[11] Feng, Q. Z., Zhao, L. X., Yan, W., Li, J. M., Zheng, Z. X., *J. Hazard. Mater.* 2009, *167*, 282–288.

[12] Moradi, M., Yamini, Y., Esrafili, A., Seidi, S., *Talanta* 2010, *82*, 1864–1869.

[13] Seronero, L. C., Fernández Laespada, M. E., Pavón, J. L., Moreno, C.B., *J. Chromatogr. A* 2000, *897*, 171–176.

[14] Liang, T. T., Lv, Z. H., Jiang, T. F., Wang, Y. H., *Electrophoresis* 2013, *34*, 345–352.

[15] Xu, Z. Q., Li, A. M., Wang, Y. L., Chen, Z. L., Hirokawa, T., *J. Chromatogr. A* 2014, *1355*, 284–290.

[16] Yang, F. F., Li, J. H., Lu, W. H., Wen, Y. Y., Cai, X. Q., You, J. M., Ma, J. P., Ding, Y. J., Chen, L. X., *Electrophoresis* 2014, *35*, 474–481.

[17] Wen, Y. Y., Li, J. H., Ma, J. P., Chen, L. X., *Electrophoresis* 2012, *33*, 2933–2952.

[18] Gołębiowski, M., Stepnowski, P., Leszczyńska, D., *Chem. Pap.* 2017, *71*, 831–839.

[19] Wang, X. W., Chen, R. H., Luan, T. G., Lin, L., Zou, S. C., Yang, Q. S., *J. Sep. Sci.* 2012, *35*, 1017–1026.

[20] Wen, Y. Y., Li, J. H., Liu, J. Y., Lu, W. H., Ma J. P., Chen, L. X., *Anal. Bioanal. Chem.* 2013, *405*, 5843–5852.

[21] Li, P. J., Zhang, X., Hu, B., *J. Chromatogr. A* 2011, *1218*, 9414–9421.

[22] Chen, C., Peng, M. T., Hou, X. D., Zheng, C. B., Long, Z., *Anal. Methods* 2013, *5*, 1185–1191.

[23] Shi, L. D., Wang, J., Feng, J., Zhao, S. H., Wang, Z. M., Tao, H., Liu, S. H., *J. Sep. Sci.* 2017, *40*, 2662–2670.

[24] Bigus, P., Namieśnik, J., Tobiszewski, M., *J. Chromatogr. A* 2018, *1553*, 25–31.

[25] Bai, X. Z., Zhang, T., Li, H. P., Yang, Z. G., *J. Sep. Sci.* 2016, *39*, 2146–2155.

[26] Liu, J. Y., Lu, W. H., Liu, H. T., Wu, X. Q., Li, J. H., Chen, L. X., *Electrophoresis* 2016, *37*, 2502–2508.

[27] Jia, X. Y., Han, Y., Liu, X. L., Duan, T. C., Chen, H. T., *J. Anal. At. Spectrom.* 2011, *66*, 88–92.

[28] Arghavani-Beydokhti, S., Rajabi, M., Bazregar, M., Asghari, A., *Anal. Methods* 2017, *9*, 1732–1740.

[29] Alexovi, M., Horstkotte, B., Sramkov, I., Solich, P., Sabo, J., *Trends Anal. Chem.* 2016, *86*, 39–55.

[30] Song, X. L., Li, J. H., Liao, C. Y., Chen, L. X., *Chromatographia* 2011, *74*, 89–98.

[31] Yang, P., Li, H. Q., Wang, H., Han, F. L., Jing, S. Y., Yuan, C. L., Guo, A. Q., Zhang, Y.L., Xu, Z. M., *Food Anal. Methods* 2017, *10*, 2383–2397.

[32] Asati, A., Satyanarayana, G. N. V., Patel, D. K., *J. Chromatogr. A* 2017, *1513*, 157–171.

[33] Qi, S. D., Zhang, H. G., Zhu, Q., Chen, H. L., Dong, Y. L., Zhou, L., Ren, C. L., Chen, X. G., *Anal. Methods* 2014, *6*, 1219–1226.

[34] Lu, W. H., Wang, X. Y., Wu, X. Q., Liu, D. Y., Li, J. H., Chen, L. X., Zhang, X. S., *J. Chromatogr. A* 2017, *1483*, 30–39.

[35] Ghosal, S., *Anal. Chem.* 2002, *74*, 4198–4203.

[36] Friedl, W., Kenndler, E., *Anal. Chem.* 1993, *65*, 2003–2009.

[37] Woźniakiewicz, M., Gładysz, M., Nowak, P. M., Kędzior, J., Kościelniak, P., *Talanta* 2017, *167*, 714–724.

[38] Moreira, B. J., Borges, K. B., de Oliveira, A. R. M., de Gaitani, C. M., *Anal. Methods* 2015, *7*, 8763–8770.

[39] Ma, J. P., Lu, W. H., Chen, L. X., *Trends Anal. Chem.* 2012, *8*, 78–90.

[40] Ma, Y. L., Wen, Y. Y., Li, J. H., Wang, H., Ding, Y. J., Chen, L. X., *Cent. Eur. J. Chem.* 2013, *11*, 394–403.

