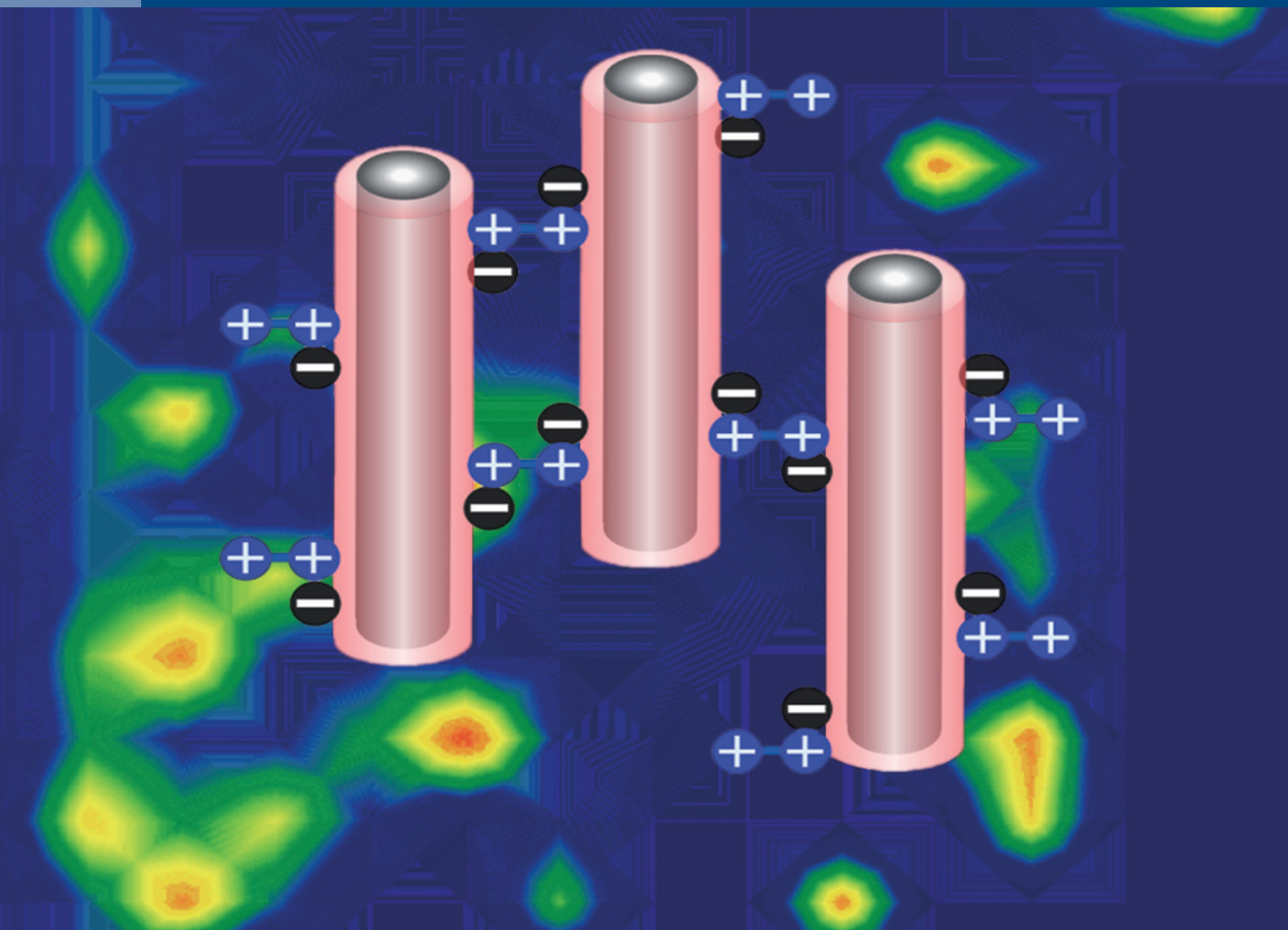


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## RESEARCH ARTICLE

# Dispersive liquid-liquid microextraction coupled with pressure-assisted electrokinetic injection for simultaneous enrichment of seven phenolic compounds in water samples followed by determination using capillary electrophoresis

Suya Ma<sup>1,2</sup> | Fangfang Gao<sup>2</sup> | Wenhui Lu<sup>3</sup> | Na Zhou<sup>2</sup> | Huiyan You<sup>1</sup> | Jinhua Li<sup>2</sup> |  
Lingxin Chen<sup>2\*</sup> 

<sup>1</sup>School of Environment and Chemical Engineering, Dalian University, Dalian, P. R. China

<sup>2</sup>CAS Key Laboratory of Coastal Environmental Processes and Ecological Remediation, Research Center for Coastal Environmental Engineering and Technology, Chinese Academy of Sciences, Yantai Institute of Coastal Zone Research, Yantai, P. R. China

<sup>3</sup>School of Light Industry and Engineering, Qilu University of Technology (Shandong Academy of Sciences), Jinan, P. R. China

## Correspondence

Professor Huiyan You, School of Environment and Chemical Engineering, Dalian University, Dalian, 116622, P. R. China.  
Email: dlyhy@dicp.ac.cn

\*Additional corresponding author

Professor Lingxin Chen  
Email: lxchen@yic.ac.cn

Offline dispersive liquid-liquid microextraction combined with online pressure-assisted electrokinetic injection was developed to simultaneously enrich seven phenolic compounds in water samples, followed by determination using capillary electrophoresis, namely phenol, 4-chlorophenol, pentachlorophenol, 2,4,6-trichlorophenol, 2,4-dichlorophenol, 2-chlorophenol, and 2,6-dichlorophenol. Several parameters affecting separation performance of capillary electrophoresis and the enrichment efficiency of pressure-assisted electrokinetic injection and dispersive liquid-liquid microextraction were systematically investigated. Under the optimal conditions, seven phenolic compounds were completely separated within 14 min and good enrichment factors were obtained of 61, 236, 3705, 3288, 920, 86, and 1807 for phenol, 4-chlorophenol, pentachlorophenol, 2,4,6-trichlorophenol, 2,4-dichlorophenol, 2-chlorophenol, and 2,6-dichlorophenol, respectively. Good linearity was attained in the range of 0.1–200 µg/L for 2,4-dichlorophenol, 0.5–200 µg/L for 4-chlorophenol, pentachlorophenol, 2,4,6-trichlorophenol, 2-chlorophenol, and 2,6-dichlorophenol, as well as 1–200 µg/L for phenol, with correlation coefficients (*r*) over 0.9905. The limits of detection and quantification ranging from 0.03–0.28 and 0.07–0.94 µg/L were attained. This two step enrichment method was potentially applicable for the rapid and simultaneous determination of phenolic compounds in water samples.

## KEYWORDS

capillary electrophoresis, dispersive liquid-liquid microextraction, phenolic compounds, pressure-assisted electrokinetic injection, water samples

Article Related Abbreviations: 2,4,6-TCP, 2,4,6-trichlorophenol; 2,4-DCP, 2,4-dichlorophenol; 2,6-DCP, 2,6-dichlorophenol; 2-CP, 2-chlorophenol; 4-CP, 4-chlorophenol; DLLME, dispersive liquid-liquid microextraction; EF, enrichment factor; PAEKI, pressure-assisted electrokinetic injection; PCP, pentachlorophenol.

## 1 | INTRODUCTION

Phenolic compounds especially chlorinated ones are widely used in industry as intermediates in the production of dyes, plastics, and pharmaceuticals, and are commonly found in paper and pulp mill wastewater, which might pollute the groundwater sources without treatment [1,2]. They can represent serious health hazards due to their moderate

bioaccumulation and high toxicity, which increase with the increment of chlorination [3]. Once CPs enter the food chain, human health is threatened by biological concentration, causing metabolic disorders, physiological imbalance, and cancer [4,5]. The European community has stipulated the maximum allowed phenol concentration in drinking water to be 0.5 µg/L [6]. The United States Environmental Protection Agency (EPA) has also listed eleven phenolic compounds as priority pollutants, including three kinds of CPs, namely 2-chlorophenol (2-CP), 2,4-dichlorophenol (2,4-DCP), and 2,4,6-trichlorophenol (2,4,6-TCP) [7]. Nowadays, phenolic compounds have been detected in some water samples as a result of environmental contamination. Hence, it is necessary and urgent to develop simple, effective, and sensitive methods for monitoring the concentration of phenolic compounds.

Considering their low concentration presented in complicated matrices, a number of pretreatment approaches have been developed to concentrate phenolic compounds, such as hollow fiber supported liquid-liquid liquid membrane microextraction [8], SPE [9,10], dispersive solid phase extraction [11], SPME [12,13], dispersive liquid-liquid microextraction (DLLME) [14,15] and cloud point extraction [16]. Moreover, various detection technologies have been applied for analysis of phenolic compounds including HPLC [17], CE [18], and GC [19] coupled with UV, fluorescence, and MS detectors. Amongst them, DLLME was first and was introduced in 2006 [20] which has advantages of rapidity, simplicity, and high enrichment ability, and have been widely applied to extract low concentration of organic compounds and metal ions [21]. For the analysis of phenolic compounds, DLLME combined with HPLC-UV [22], HPLC-MS-MS [23] and GC-MS [24] methods have been reported. Our group has also used DLLME coupled with CE-UV for simultaneous determination of five CPs in water samples, demonstrating good enrichment factor [25]. However, CE with absorbance detection showed relatively poor sensitivity, because of the limited optical pathway of on-capillary photometry and the small sample injection volume. In order to enhance detection sensitivity of CE-UV, several online enrichment technologies have been established, such as stacking [26], sweeping [27], solid-phase packing [28], and transient trapping technique [29]. Pressure-assisted electrokinetic injection (PAEKI) as an online enhancement technique has been proposed to provide a powerful enhancement capability without compromising the separation efficiency [30]. Until now, PAEKI technique has been successfully applied to online enrichment and analyze kinds of analytes, such as, flotation reagents in tin mining [31], nucleotides [32], oligonucleotides, and monophthalates in various samples [33].

The offline and online combined enrichment strategies can greatly improve the detectability of CE-UV. For example, Xu et al. developed LLE combined with PAEKI stacking for verteporfin drug to achieve highly sensitive enantioseparation

and detection in artificial urine by CE [34]. Sun's group developed a method of DLLME and reversed phase liquid-liquid microextraction (RP-LLME) procedure coupled with sweeping-micellar electrokinetic chromatography (sweeping-MEKC) to extract and determine three CPs [35]. Wen et al. utilized dual cloud point extraction offline enrichment coupled with a hydrodynamic-electrokinetic two-step injection sample online enrichment followed by micellar electrokinetic chromatography for the determination of HS, DS, and DES [36].

Inspired by these researches, high sensitive analysis method for phenolic compounds by CE-UV, two steps including preconcentration method of offline DLLME combined with online PAEKI was proposed to simultaneously enrich, separate, and determine seven phenolic compounds in water samples, namely phenol, 4-chlorophenol (4-CP), pentachlorophenol (PCP), 2,4,6-TCP, 2,4-DCP, 2-CP, and 2,6-dichlorophenol (2,6-DCP). The main parameters influencing CE separation and the enrichment efficiency of PAEKI and DLLME were systematically investigated, including property of running buffer, organic modifier, and applied voltage for CE separation, injection voltage, time, and water plug for PAEKI, as well as property of extraction, disperser solvent, extraction time, and salt addition for DLLME. The validated DLLME-PAEKI-CE method showed high enrichment factors and wide linear range in ultrapure water samples, which was potentially applicable to determine phenolic compounds in real water samples.

## 2 | MATERIALS AND METHODS

### 2.1 | Reagents and samples

HPLC grade reagents of phenol, 2-CP, 4-CP, 2,4-DCP and 2,4,6-TCP were obtained from Sigma-Aldrich (Shanghai, China) and the standard stock solutions was dissolved into methanol (MeOH) at the concentration of 1000 mg/L. The standard solution of PCP and 2,6-DCP in MeOH with concentration of 1000 mg/L was purchased from Dr. Ehrenstorfer (Augsburg, Germany). The chemical structures of seven phenolic compounds were shown in Supporting Information Figure S1. Sodium tetraborate decahydrate ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ) and sodium hydroxide (NaOH) were supplied from Sinopharm Chemical Reagent (Shanghai, China). Chromatographic grade ACN, MeOH, ethanol (EtOH), and chlorobenzene ( $\text{C}_6\text{H}_5\text{Cl}$ ) were all purchased from J & K Chemical (Beijing, China). The other analytical grade chemicals, such as acetone, dichloromethane ( $\text{CH}_2\text{Cl}_2$ ), and chloroform ( $\text{CHCl}_3$ ) were purchased from Sinopharm Chemical Reagent (Shanghai). Carbon tetrachloride ( $\text{CCl}_4$ ) was obtained from Aladdin (Shanghai). Working solutions were prepared by dilution of the stock standard solution and all standard solutions were stored at 4°C. Ultrapure water

with the specific resistances of 18.2 M $\Omega$  cm was produced by Pall cascada<sup>TM</sup> lab water purification system (Pall, USA) for aqueous solution preparation throughout the study.

Tap water sample was collected from our laboratory after 5 min of self-flow, filtered using 0.22  $\mu$ m microporous nylon filters and stored in a refrigerator at 4°C before use.

## 2.2 | Instrumentation

A Beckman P/ACET<sup>TM</sup> MDQ CE system (Fullerton, CA, USA) equipped with a diode-array detector (DAD) and bare fused-silica capillary (Yongnian Photoconductive Fiber Factory, Hebei, China) with 75  $\mu$ m i.d., 375  $\mu$ m o.d., total length of 50.2 cm and effective length of 40 cm were utilized in all the experiments. The pH value was measured with a Rex pH meter (Shanghai Precision Scientific Instrument, Shanghai). Data acquisition was performed using Karat 32 software (Beckman-Coulter, Fullerton). All CE experiments were carried out at 25°C.

## 2.3 | Capillary electrophoresis conditions

New capillary was conditioned by rinsing with MeOH for 5 min, water for 5 min, 1 mol/L NaOH for 20 min, water for 10 min, and running buffer for 30 min. The running buffer consisted of 20 mmol/L Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O containing 10% v/v MeOH with the pH of 9.80. Each day, the capillary was orderly conditioned with 1 mol/L NaOH, water, and running buffer for 5, 5, and 10 min. Between the two separation analyses, running buffer was rinsed for 5 min. All solutions were filtered through 0.22  $\mu$ m microporous nylon filters. The detection wavelength was set at 195 nm for phenol, 4-CP, and 2-CP, 214 nm for 2,4,6-TCP, 2,4-DCP, and 2,6-DCP, 228 nm for PCP. The capillary temperature, applied voltage, and pressure injection was set as 25°C, +20 kV and 0.5 psi for 5 s (1 psi = 6894.76 Pa).

## 2.4 | Two-step enrichment procedure

Ten mL aqueous solution containing seven phenolic compounds was placed in a 10 mL centrifuge tube and the spiked concentration were 50  $\mu$ g/L for phenol, 4-CP, PCP, 2,4,6-TCP, 2,4-DCP, 2,6-DCP, and 150  $\mu$ g/L for 2-CP. The mixture of 1 mL ACN (disperser solvent) and 80  $\mu$ L C<sub>6</sub>H<sub>5</sub>Cl (extraction solvent) was injected rapidly into the aqueous solution with 1 mL syringe. Then, dispersed fine droplets of C<sub>6</sub>H<sub>5</sub>Cl containing the analytes formed a cloudy solution, which was delaminated by centrifugation for 5 min at 8000 rpm. After removing the supernatant, the sedimented solvent was dried under a gentle flow of nitrogen. The evaporation residue was redissolved using 20  $\mu$ L ACN and H<sub>2</sub>O (1:1, v/v) for injection into capillary. The capillary was first filled with BGE and then a water plug was introduced hydrodynamically (3.45 kPa

for 6 s, 1.014 cm water plug injection) before PAEKI. Then, electrokinetic injection of the evaporation residue sample during 0.40 min at a voltage of −10 kV was performed at an assisted pressure of 3.45 kPa, simultaneously. Once the injection was complete, a voltage of +20 kV was applied in order to separate the compounds. The schematic illustration of the DLLME-PAEKI-CE procedure was shown in Figure 1.

Enrichment factor (EF) was used to evaluate the enrichment capability of DLLME and PAEKI, which was calculated as follows:

$$EF = \frac{C_1}{C_0} \quad (1)$$

where  $C_0$  was the initial concentration of seven phenolic compounds in aqueous solution, and  $C_1$  was the concentration of analytes in the sediment phase of DLLME or after PAEKI.

## 3 | RESULTS AND DISCUSSION

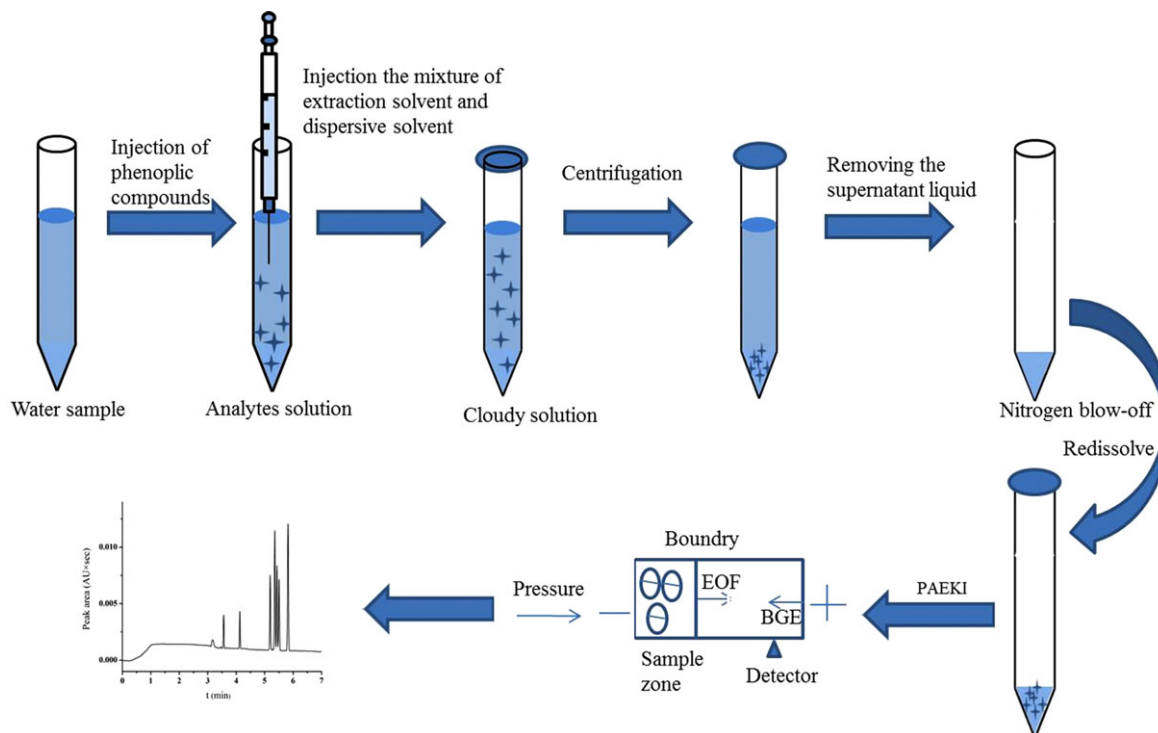
### 3.1 | Optimization of capillary electrophoresis separation conditions

In this study, CZE mode was used for the separation of seven phenolic compounds referring to our previous work [25]. Factors affecting CZE separation were optimized including the type, concentration, and pH of running buffer; the type, concentration of organic modifier, and voltage applied.

Selection of the buffer used as BGE has a great influence on the migration behavior. Two types of commonly used separation buffer solutions were investigated, i.e., Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O and NaH<sub>2</sub>PO<sub>4</sub>. Result showed that better peak shape and resolution was obtained by separation with Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O buffer, compared to NaH<sub>2</sub>PO<sub>4</sub> buffer. Moreover, the buffer concentration is well known to directly affect the Zeta potential on the inner wall of the capillary. As shown in Supporting Information Figure S2, different concentrations of Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O buffer (10, 20, 30, 40 mmol/L) were optimized and 20 mmol/L Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O buffer achieved excellent separation resolution. The possible reason is that low buffer concentration (10 mmol/L) can lead to high Zeta potential and thereby high EOF, easily resulting in incomplete separation. When the buffer concentration was higher than 20 mmol/L, peaks were widened and peak heights did not increase. Besides, the pH of 20 mmol/L Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O buffer was also investigated with values of 9.30, 9.50, 9.80, and 10.00. As shown in Supporting Information Figure S3, the best separation resolution of seven phenolic compounds was achieved at pH 9.80. Therefore, 20 mmol/L Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O with pH 9.80 was used for further study.

The addition of organic modifier to separation buffer can effectively improve separation selectivity, efficiency, and resolution. ACN and MeOH were investigated as the organic





**FIGURE 1** Schematic illustration of the DLLME-PAEKI-CE procedure

modifiers, and they both showed good separation ability for seven phenolic compounds. Considering relatively low toxicity, MeOH was chosen as modifier. The concentration of MeOH was also investigated with the volume ratio of 5, 10, and 15% v/v. As can be seen in Supporting Information Figure S4, the best separation performance was obtained when the ratio of MeOH was 10% v/v, considering the separation time, the peak resolution, and area.

Separation voltage is also important to control EOF. The applied voltage of +15, +20, and +25 kV were tested by using separation buffer consisting of 20 mmol/L  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$  containing 10% v/v MeOH at pH of 9.80. The results indicated that the analysis time was shortened with the increase of separation voltage and higher voltage over +20 kV showed poor peak shape. So, +20 kV was selected.

Based on the above results, the optimized CE conditions were confirmed: 20 mmol/L  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$  containing 10% v/v MeOH at pH of 9.80 as separation buffer and +20 kV as applied voltage.

### 3.2 | Optimization of dispersive liquid-liquid microextraction conditions

In this study, the value of peak area was used as an indicator to evaluate the extraction efficiency of DLLME. The main parameters of DLLME, including the type and volume of dispersive solvent and extraction solvent, extraction time, and the amount of salt added, were studied systematically. The dispersive solvent should meet the requirement of the good

miscibility in both aqueous phase and extraction solvent, and then it should enable the dispersion of fine droplets of the extractant into the aqueous phase containing the analytes [25]. Based on the above considerations, MeOH and ACN were examined. The results showed that ACN had the better enrichment effect. By further referring to our previous work [25], 1 mL ACN was selected as the dispersive solvent, and then the remaining conditions were examined. The whole optimal experiments were performed in six times using spiked seven phenolic compounds concentration at 50  $\mu\text{g/L}$  for phenol, 4-CP, PCP, 2,4,6-TCP, 2,4-DCP, 2,6-DCP, and 150  $\mu\text{g/L}$  for 2-CP.

For DLLME, extraction solvent should have specific property of dissolving disperser solvent, target analytes, and insolubilization in aqueous sample solution. Based on that, four kinds of extraction solvent were investigated including  $\text{CH}_2\text{Cl}_2$ ,  $\text{CHCl}_3$ ,  $\text{CCl}_4$ , and  $\text{C}_6\text{H}_5\text{Cl}$ . As shown in Supporting Information Figure S5A, not all seven analytes can be extracted by  $\text{CH}_2\text{Cl}_2$  and obviously different extraction efficiency was attained of  $\text{CHCl}_3$ ,  $\text{CCl}_4$ , and  $\text{C}_6\text{H}_5\text{Cl}$ . Compared with  $\text{CHCl}_3$  and  $\text{CCl}_4$ ,  $\text{C}_6\text{H}_5\text{Cl}$  showed more outstanding extraction solvent for target analytes. Besides, different volume of  $\text{C}_6\text{H}_5\text{Cl}$  (40, 60, 80, 100, 120, and 140  $\mu\text{L}$ ) used to evaluate the effect of extractant volume on extraction efficiency and the results were shown in Supporting Information Figure S5B. The optimum extraction efficiency was achieved at 80  $\mu\text{L}$ , since almost all of the seven phenolic compounds in aqueous solution could be extracted.

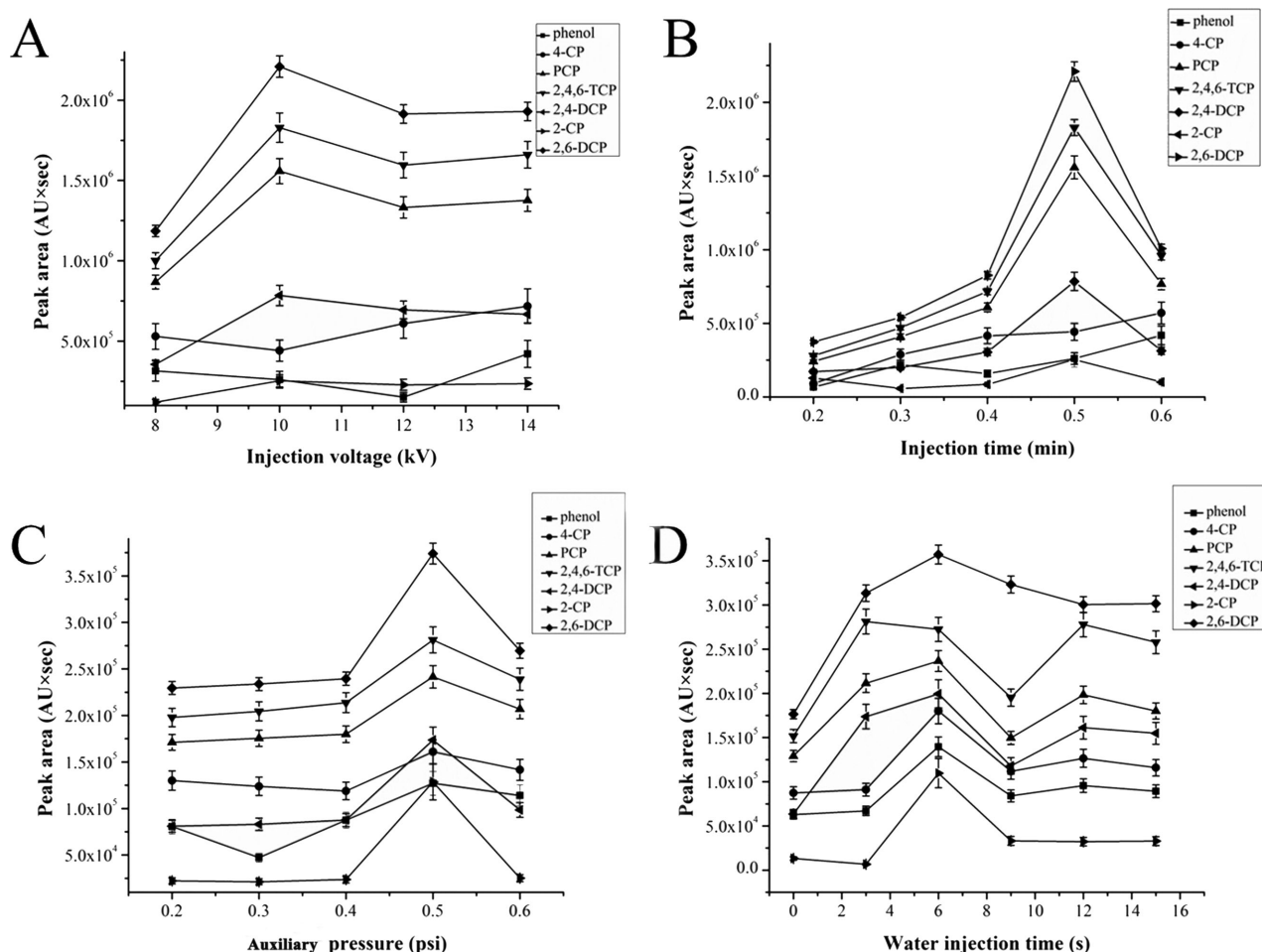
Therefore, the DLLME procedure was performed by using the mixture of 1 mL ACN and 80  $\mu$ L  $C_6H_5Cl$  to inject rapidly into aqueous sample solution for the extraction of seven phenolic compounds.

### 3.3 | Optimization of pressure-assisted electrokinetic injection conditions

In the process of sample injection, the phenolic compounds ion migration direction is opposite to EOF, which leads to the depletion of the analytes at the inlet. So a constant pressure was applied at the capillary inlet during PAEKI to counterbalance the EOF, the anionic sample was stacked by PAEKI at the boundary of sample zone and BGE. In PAEKI, it has been proved that preinjection of a short water plug before sampling can improve the stacking efficiency. When the analytes enter the capillary through the water plug and cross the interface of the water plug and BGE at high speed, they encounter a lower electric field and would slow and focus at this interface. Figure 1 schematically illustrates the process. The PAEKI

sampling stacking method was used to online enrich seven phenolic compounds, and the parameters affecting the stacking efficiency were optimized, including auxiliary pressure, injection voltage, injection time, and the length of water plug.

Under the condition of auxiliary pressure of 0.50 psi and fixed injection time of 0.40 min, the injection voltage was investigated. The electrokinetic injection voltage of  $-8$ ,  $-10$ ,  $-12$ , and  $-14$  kV was investigated. When the injection voltage was  $-10$  kV, it had the best peak shape and stacking effect. As shown in Figure 2A, the enrichment efficiency increased with the increase of the injection voltage. It can be seen that higher the voltage was, more analytes entered the capillary, and better was the accumulation effect. However, when the voltage was too high, the peak shape began to deteriorate and the peak height began to decrease, which may be because the excessive electro percolation pushed the analytes out of the capillary orifice, making the accumulation effect worse. In addition, it was also found that, when the voltage was high, the electrophoresis separation was prone to flow interruption, repeatability was poor, and also showed a great



**FIGURE 2** Effect of (A) injection voltage, (B) injection time, (C) auxiliary pressure, (D) water injection time on the peak area of the seven phenolic compounds. Sample conditions: 10 mg/L standard seven phenolic compounds solution. PAEKI conditions: the auxiliary pressure of electric injection is 0.50 psi (ca.3.45 kpa), 1.014 cm water plug injection, pressure injection at  $-10$  kV voltage, and injection time of 0.40 min

relationship with the use of capillary column, and washing state. Therefore, the injection voltage was selected as  $-10$  kV.

Under the conditions of  $-10$  kV injection voltage and  $0.5$  psi auxiliary pressure, the injection time was investigated. As can be seen from Figure 2B, the stacking effect increased with the increase of injection time within  $0.20$ – $0.50$  min. However, when the injection time was below  $0.40$  min, the peak height did not increase significantly, and the peak heights of 2,6-DCP, 2,4-DCP, 2,4,6-TCP, 2-CP, and PCP decreased. When the injection time was over  $0.40$  min, the peak shape widened and the separation degree decreased, which may be the reason for the excessive injection amount. Therefore,  $0.40$  min was selected as the injection time.

At the injection voltage of  $-10$  kV and the injection time of  $0.40$  min, the auxiliary pressure of electric injection was investigated, as shown in Figure 2C. For PAEKI, the application of pressure can balance the force of reverse EOF, prevent the sample from being pushed out of the capillary by reverse electroosmosis, and extend the injection time as much as possible. Experimental results showed that  $0.50$  psi pressure was the best condition. When the pressure was below  $0.50$  psi, the sample would be pushed out of the entrance of the capillary by reverse electroosmosis, which would reduce the amount of analytes entering the capillary. When the pressure was greater than  $0.50$  psi, the peak area of seven phenolic compounds decreased, and the separation degree also decreased correspondingly. Therefore,  $0.50$  psi was selected as the optimal auxiliary pressure.

The water plug in PAEKI procedure is a key factor in the effective accumulation of analytes, because injecting a certain length of water plug can enhance the electric field inside the capillary tube, which is used as a highway for transporting charged analytes. When the analytes enters the capillary tube through the water plug and passes through the interface between the water plug and the background buffer at a high speed, the electric field intensity suddenly decreases,

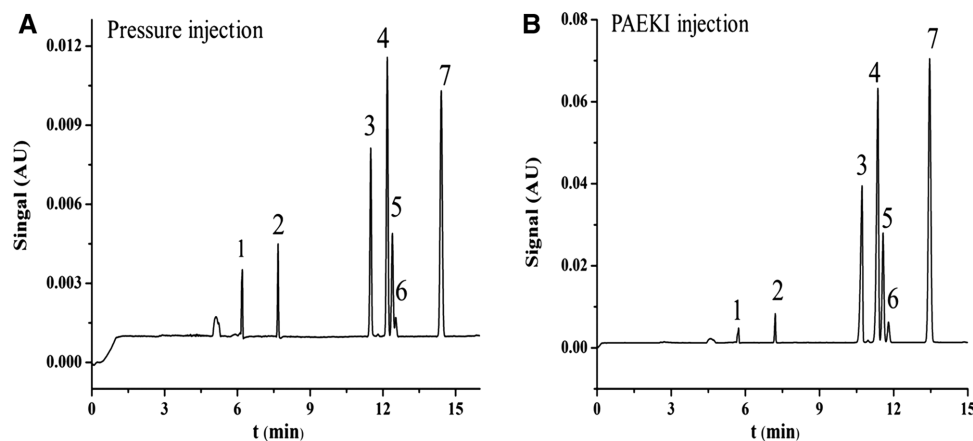
which slows the migration speed of the analytes and thus accumulates at the interface, and the concentration of the analytes increases. Therefore, at the conditions of electrokinetic injection voltage of  $-10$  kV, injection time of  $0.40$  min, and auxiliary pressure of  $0.50$  psi, the effect of the length of the water plug on the enrichment of seven phenolic compounds was investigated. As shown in Figure 2D, when the water plug length was  $1.014$  cm ( $3.45$  kPa  $\times$   $6$  s), the enrichment efficiency of seven phenolic compounds reached the maximum. When the water injection time was less than  $6$  s, the analytes were rapidly pushed out of the capillary tube by electric seepage, and the detection sensitivity thereby could not be improved. When the water injection time was longer than  $6$  s, serious peak broadening and overlapping occurred. Therefore, the optimum length of water plug was set at  $1.014$  cm.

From the results above, the optimized PAEKI conditions were confirmed, i.e.,  $6$  s hydrodynamic injection of water for  $1.014$  cm plug,  $0.40$  min of electrokinetic injection ( $-10$  kV) of the sample, at an assisted pressure of  $0.50$  psi were applied simultaneously.

Figure 3 shows the electropherograms of  $10$  mg/L seven phenolic compounds standard solution by pressure hydrodynamic injection and PAEKI injection after DLLME enrichment procedure. As can be seen, PAEKI had a significant superposition effect with the EF as high as  $35$  times than that of hydrodynamic injection.

### 3.4 | Method performance

Under the optimal DLLME and PAEKI conditions, the analytical performance of the developed DLLME-PAEKI-CE method was assessed by EFs, linearity, sensitivity, and repeatability, and the results were listed in Supporting Information Table S1 and Table S2. As seen, relatively high values of EFs within  $236$ – $3705$  for five CPs showed excellent



**FIGURE 3** Electropherograms of pressure injection (A) and PAEKI injection (B) of seven phenolic compounds at the concentration of  $10$  mg/L after DLLME in ultrapure water. Peak identification: (1) Phenol; (2) 4-CP; (3) PCP; (4) 2,4,6-TCP; (5) 2,4-DCP; (6) 2-CP; (7) 2,6-DCP

enrichment capability of the off/online combined technology. More chlorine substitution and/or ortho-position effects in the CPs facilitated the ionization of hydroxy and thereby led to more charge numbers of the CPs, which is more suitable for PAEKI. So, amongst the five CPs, the EF for PCP was the highest while 4-CP the lowest. For phenol, the decrease of EF after PAEKI (EF = 61) from 93 (DLLME) was possibly owing to the low charge number; For 2-CP, the EF of 86 was mainly because of the low enrichment of DLLME (EF = 3). Good linearity was obtained with correlation coefficients ( $r$ ) over 0.9905. The LODs and LOQs calculated based  $S/N = 3$  and 10, respectively, were in the range of 0.03–0.28 and 0.07–0.94  $\mu\text{g/L}$ , respectively, which could satisfy the requirement of trace analysis. As presented in Supporting Information Table S1, the intra- and interday precisions ranged from 0.25–0.67 and 0.46–0.83% for migration time, respectively, as well as 2.97–5.06 and 3.57–6.10% for peak area, respectively, showing high accuracy and reliability. Consequently, the DLLME-PAEKI-CE method was validated to be sensitive, reliable, and accurate for the simultaneous enrichment and determination of seven phenolic compounds in ultrapure water.

### 3.5 | Application to real water samples

The practical applicability of the DLLME-PAEKI-CE method was evaluated by spiking standards of seven phenolic compounds into tap water samples. It can be seen from Supporting Information Figure S6, after the DLLME-PAEKI procedure, endogenous phenolic compounds were not found in the tap water sample (A), however all the seven phenolic compounds were detected in the spiked water sample (B). Furthermore, for the comparison of spiked tap water samples with low concentrations (50  $\mu\text{g/L}$  individual for the other six phenolic compounds and 100  $\mu\text{g/L}$  for 2-CP) (A) and high concentrations (300  $\mu\text{g/L}$  individual for the six ones and 500  $\mu\text{g/L}$  for 2-CP) (B), phenol and 4-CP were detected at high concentration as well as other five CPs exhibited better peaks (B), as shown in Supporting Information Figure S7. Obviously, the enrichment effects were different for them individual, possibly due to the different number/position and chlorine substitution influencing PAEKI efficiency [34].

It should be noted that, PAEKI is easily affected by subtle changes in sample composition which results in biased method efficiency and accuracy [37]. Two common methods can be utilized to mitigate this effect, i.e., using internal standards and using the corresponding real sample matrices for linearity. On the other hand, the analytes for PAEKI should be appropriately selected; more charged analytes are preferred. For simultaneous enrichment, multiple analytes with similar charged number and similar structures should be considered. Moreover, offline enrichment procedure is an effective way to eliminate the interference of matrix [34].

It is highly desirable to combine such offline enrichment procedure as well as eliminate the matrix interference in favor of PAEKI. Also, analyte loss during the enrichment should be reduced to great extent. More exploration will be carried out to attain the good feasibility of the PAEKI combined enrichment method in real samples.

## 4 | CONCLUDING REMARKS

In this study, offline DLLME combined with online PAEKI was developed to simultaneously enrich seven phenolic compounds in water samples. This two-step enrichment method with high enrichment efficiency followed by CE-UV was potentially applicable for the rapid and simultaneous determination of phenolic compounds in water samples. PAEKI was proven to be an effective preconcentration method to improve the detection sensitivity in this study. Although the LODs were significantly decreased and approached the request in pure water, the analytical performances and practical applicability in complicated matrices are required to be continuously investigated in order to expand utilization of PAEKI, since PAEKI performances are greatly dependent on targeted analytes, charges, and sample matrices. Further exploration of wide range of multifunctional offline/online combined enrichment technologies would provide promising potential to enhance the detection sensitivity of CE-UV.

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## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

## ORCID

Lingxin Chen  <https://orcid.org/0000-0002-3764-3515>

## REFERENCES

1. Jin, M., Zhu, Y., Ion chromatography-atmospheric pressure chemical ionization mass spectrometry for the determination of trace chlorophenols in clam tissues. *J. Chromatogr. A* 2006, 1118, 111–117.
2. Levshina, S., Petroleum products and phenols in snow cover in khabarovsk, southern Russian far east. *Water, Air, Soil Pollut.* 2012, 223, 3553–3563.
3. Quintana, M. C., Ramos, L., Sample preparation for the determination of chlorophenols. *Trend. Anal. Chem.* 2008, 27, 418–436.



4. Alizadeh, N., Zarabadipour, H., Mohammadi, A., Headspace solid-phase microextraction using an electrochemically deposited dodecylsulfate-doped polypyrrole film to determine of phenolic compounds in water. *Anal. Chim. Acta* 2007, **605**, 159–165.
5. Karimiyan, H., Hadjmohammadi, M., Ultrasound-assisted supramolecular-solvent-based microextraction combined with high-performance liquid chromatography for the analysis of chlorophenols in environmental water samples. *J. Sep. Sci.* 2016, **39**, 4740–4747.
6. Rodríguez, I., Turnes, M. I., Mejuto, M. C., Cela, R., Determination of chlorophenols at the sub-ppb level in tap water using derivatization, solid-phase extraction and gas chromatography with plasma atomic emission detection. *J. Chromatogr. A* 1996, **721**, 297–304.
7. Ladavière, C., Lacroixdesmazes, P., Delolme, F., First systematic MALDI/ESI mass spectrometry comparison to characterize polystyrene synthesized by different controlled radical polymerizations. *Macromolecules* 2009, **42**, 70–84.
8. Chen, C., Peng, M., Hou, X., Chen, B. Z., Improved hollow fiber supported liquid-liquid-liquid membrane microextraction for speciation of inorganic and organic mercury by capillary electrophoresis. *Anal. Methods* 2013, **5**, 1185–1191.
9. Lu, W., Wang, X., Wu, X., Liu, D., Li, J., Chen, L., Zhang, X., Multi-template imprinted polymers for simultaneous selective solid-phase extraction of six phenolic compounds in water samples followed by determination using capillary electrophoresis. *J. Chromatogr. A* 2017, **1483**, 30–39.
10. Chen, M. L., Min, J. Q., Cai, M. Q., Pan, S. D., Jin, M. C., Rapid analysis of six trace trichlorophenols in seawater by magnetic micro-solid-phase extraction and liquid chromatography with tandem mass spectrometry. *J. Sep. Sci.* 2016, **39**, 2396–2405.
11. Lu, W., Ming, W., Zhang, X., Chen, L., Molecularly imprinted polymers for dispersive solid-phase extraction of phenolic compounds in aqueous samples coupled with capillary electrophoresis. *Electrophoresis* 2016, **37**, 2487–2495.
12. Wu, T., Wang, X. H., Chang, M. T., Wang, Q. Y., Wu, C., Wang, Q. H., Covalent organic framework as fiber coating for solid-phase microextraction of chlorophenols followed by quantification with gas chromatography-mass spectrometry. *J. Agr. Food. Chem.* 2018, **66**, 11158–11165.
13. Wang, C., Zou, P., Zhang, T., Li, H., Yang, Z., Simultaneous determination of haloanisoles and halophenols in water using in situ acylation combined with solid phase microextraction with gas chromatography and mass spectrometry. *J. Sep. Sci.* 2016, **40**, 514–523.
14. He, H., Liu, S., Meng, Z., Dispersive liquid-liquid microextraction for the determination of phenols by acetonitrile stacking coupled with sweeping-micellar electrokinetic chromatography with large-volume injection. *J. Chromatogr. A* 2014, **1361**, 291–298.
15. Shi, L., Wang, J., Feng, J., Zhao, S.H., Wang, Z. M., Tao, H., Liu, S. H., Determination of chlorophenols in water using dispersive liquid-liquid microextraction coupled with water-in-oil microemulsion electrokinetic chromatography in normal stacking mode. *J. Sep. Sci.* 2017, **40**, 2523–2682.
16. Zain, N. N. M., Abu Bakar, N. K., Mohamad, S., Cloud point extraction of 2,4-dichlorophenol from aqueous samples employing  $\beta$ -cyclodextrin. *Sep. Sci. Technol.* 2016, **51**, 653–663.
17. Ye, Q., Liu, L., Chen, Z., Hong, L., Analysis of chlorophenols in environmental water using polydopamine-coated magnetic graphene as an extraction material coupled with high-performance liquid chromatography. *J. Sep. Sci.* 2016, **39**, 1684–1690.
18. Gao, F., Liu, J., Lu, W., Li, J., Liu, H., Determination of five chlorophenols in water samples by pressure-assisted electrokinetic injection coupled with capillary electrophoresis. *Chin. J. Chem.* 2018, **36**, 573–577.
19. Pokryshkin, S. A., Kosyakov, D. S., Kozhevnikov, A. Y., Lakhmanov, D. E., Ul'yanovskii, N. V., Highly sensitive determination of chlorophenols in sea water by gas chromatography-tandem Mass spectrometry. *J. Anal. Chem.* 2018, **73**, 991–998.
20. Rezaee, M., Assadi, Y., Milani Hosseini, M. R., Aghae, E., Ahmadi, F., Berijani, S., Determination of organic compounds in water using dispersive liquid-liquid microextraction. *J. Chromatogr. A* 2006, **1116**, 1–9.
21. Ma, J., Lu, W., Chen, L., Recent advances in dispersive liquid-liquid microextraction for organic compounds analysis in environmental water: A Review. *Curr. Anal. Chem.* 2012, **8**, 78–90.
22. Suh, J. H., Lee, Y. Y., Lee, H. J., Kang, M., Hur, Y., Lee, S. N., Yang, D. H., Han, S. B., Dispersive liquid-liquid microextraction based on solidification of floating organic droplets followed by high performance liquid chromatography for the determination of duloxetine in human plasma. *J. Pharm. Biomed. Anal.* 2013, **75**, 214–219.
23. Caldas, S. S., Rombaldi, C., Arias, J. L., Marube, L. C., Primel, E. G., Multi-residue method for determination of 58 pesticides, pharmaceuticals and personal care products in water using solvent demulsification dispersive liquid-liquid microextraction combined with liquid chromatography-tandem mass spectrometry. *Talanta* 2016, **146**, 676–688.
24. Saraji, M., Ghambari, H., Suitability of dispersive liquid-liquid microextraction for the in situ silylation of chlorophenols in water samples before gas chromatography with mass spectrometry. *J. Sep. Sci.* 2015, **38**, 3552–3559.
25. Gao, F., Lu, W., Liu, H., Li, J., Chen, L., Dispersive liquid-liquid microextraction of five chlorophenols in water samples followed by determination using capillary electrophoresis. *Electrophoresis* 2018, **39**, 2431–2438.
26. Liu, M., Zhao, H., Zhang, Z., Li, X., Mao, L., Zhang, R., Wang, Y., Derivatization reagent-assisted enantioseparation of 3-hydroxyaspartate with two chiral centers in rat cerebrospinal fluid by capillary electrophoresis-mass spectrometry. *Anal. Chim. Acta* 2019, **1047**, 257–266.
27. Yao, Y., Zhang, B., Li, S., Yu, J., Guo, X., Enantioselective analysis of pheniramine in rat using large volume sample stacking or cation-selective exhaustive injection and sweeping coupled with cyclodextrin modified electrokinetic chromatography. *Talanta* 2019, **192**, 226–232.
28. Quirino, J. P., Terabe, S., Approaching a million-fold sensitivity increase in capillary electrophoresis with direct ultraviolet detection: cation-selective exhaustive injection and sweeping. *Anal. Chem.* 2000, **72**, 1023–1030.
29. Kohl, F. J., Sánchez-Hernández, L., Neusüß, C., Capillary electrophoresis in two-dimensional separation systems: techniques and applications. *Electrophoresis* 2015, **36**, 144–158.
30. Zhang, H., Zhu, J., Feng, Y. L., On-line enrichment and measurement of four halogenated phenols in water samples using pressure-assisted electrokinetic injection-tandem mass spectrometry. *Anal. Sci.* 2010, **26**, 1157–1162.
31. Feng, Y. L., Zhu, J., On-line enhancement technique for the analysis of nucleotides using capillary zone electrophoresis/mass spectrometry. *Anal. Chem.* 2006, **78**, 6608–6613.

32. Feng, Y. L., Lian, H., Zhu, J., Application of pressure assisted electrokinetic injection technique in the measurements of DNA oligonucleotides and their adducts using capillary electrophoresis-mass spectrometry. *J. Chromatogr. A* 2007, 1148, 244–249.
33. Zhu, Y., Wang, B., Phillips, J., Zhang, Z. N., Du, H., Xu, T., Huang, L. C., Zhang, X. F., Xu, G. H., Li, W. L., Wang, Z., Wang, L., Liu, Y. X., Deng, X., Global transcriptome analysis reveals acclimation-primed processes involved in the acquisition of desiccation tolerance in *boea hygrometrica*. *Plant Cell Physiol.* 2015, 56, 1429–1441.
34. Xu, Z., Li, A., Wang, Y., Chen, Z. L., Hirokawa, T., Pressure-assisted electrokinetic injection stacking for verteporfin drug to achieve highly sensitive enantioseparation and detection in artificial urine by capillary electrophoresis. *J. Chromatogr. A* 2014, 1355, 284–290.
35. Sun, J. Z., He, H., Liu, S. H., Determination of three chlorophenols in red wine by sweeping-micellar electrokinetic chromatography coupled with dispersive liquid-liquid microextraction and reversed phase liquid-liquid microextraction, *Chin. J. Chromatogr.* 2014, 32, 256–262.
36. Wen, Y., Li, J., Liu, J., Lu, W. H., Ma, J. P., Chen, L. X., Dual cloud point extraction coupled with hydrodynamic-electrokinetic two-step injection followed by micellar electrokinetic chromatography for simultaneous determination of trace phenolic estrogens in water samples. *Anal Bioanal Chem.* 2013, 405, 5843–5852.
37. Huang, X. H., Gordon M. J., Zare, R. N., Bias in quantitative capillary zone electrophoresis caused by electrokinetic sample injection. *Anal. Chem.* 1988, 60, 375–377.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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