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Research Article

Field-amplified sample injection combined with capillary electrophoresis for the simultaneous determination of five chlorophenols in water samples

A sensitive method of CZE-ultraviolet (UV) detection based on the on-line preconcentration strategy of field-amplified sample injection (FASI) was developed for the simultaneous determination of five kinds of chlorophenols (CPs) namely 4-chlorophenol (4-CP), 2-chlorophenol (2-CP), 2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP), and 2,6-dichlorophenol (2,6-DCP) in water samples. Several parameters affecting CZE and FASI conditions were systematically investigated. Under the optimal conditions, sensitivity enhancement factors for 4-CP, 2-CP, 2,4-DCP, 2,4,6-TCP, and 2,6-DCP were 9, 27, 35, 43, and 43 folds, respectively, compared with the direct CZE, and the baseline separation was achieved within 5 min. Then, the developed FASI-CZE-UV method was applied to tap and lake water samples for the five CPs determination. The LODs ($S/N = 3$) were 0.0018–0.019 $\mu\text{g/mL}$ and 0.0089–0.029 $\mu\text{g/mL}$ in tap water and lake water, respectively. The values of LOQs in tap water (0.006–0.0074 $\mu\text{g/mL}$) were much lower than the maximum permissible concentrations of 2,4,6-TCP, 2,4-DCP, and 2-CP in drinking water stipulated by World Health Organization (WHO) namely 0.3, 0.04, and 0.01 $\mu\text{g/mL}$, respectively, and thereby the method was suitable to detect the CPs according to WHO guidelines. Furthermore, the method attained high recoveries in the range of 83.0–119.0% at three spiking levels of five CPs in the two types of water samples, with relative standard deviations of 0.37–8.58%. The developed method was proved to be a simple, sensitive, highly automated, and efficient alternative to CPs determination in real water samples.

Keywords:

Capillary electrophoresis / Chlorophenols / Field-amplified sample injection / 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) are industrially important chemicals which are widely used as raw materials in the production of

plastics and pharmaceutical production intermediates, which have a variety of applications such as pesticides, medicines, and dyes [1–3]. In addition, the chlorination process of tap water produces chlorophenol from phenol, responsible for the unfavorable smell in the air [4]. CPs can enter the body through mouth and skin contact, and long-term contact or ingestion can cause skin pruritus, rash, dizziness, anemia, and various neurological diseases [5]. Moreover, CPs in water are easily accumulated in the organism, and excessive intake can cause acute poisoning symptoms [6]. Many countries and regions have made strict regulations on the residues of CPs in the environment, listing 2,4-dichlorophenol (2,4-DCP) and

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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; CPs, chlorophenols; FASI, field-amplified sample injection; SEF, sensitivity enhancement factor; UV, ultraviolet

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

2,4,6-trichlorophenol (2,4,6-TCP) as priority controlled pollutants. For example, the United States has listed the above two CPs among the priority controlled 129 pollutants [7–9]. The maximum permissible concentrations of 2,4,6-TCP, 2,4-DCP, and 2-chlorophenol (2-CP) in drinking water stipulated by The World Health Organization (WHO) are 0.3, 0.04, and 0.01 $\mu\text{g}/\text{mL}$, respectively. The maximum allowable emission concentrations of 2,4-DCP and 2,4,6-TCP are also specified in “Comprehensive Wastewater Discharge Standards” of China, and the primary standard is 0.6 $\mu\text{g}/\text{mL}$. As well as, the “Urban Water Supply Standard” of China requires that the total content of CPs (including 2-CP, 2,4-DCP, and 2,4,6-TCP) detected is less than 20 $\mu\text{g}/\text{mL}$. The determination of CPs present in environmental waters is becoming an urgent task due to their toxic or carcinogenic characteristics. Following this, developing rapid and sensitive analytical methods to detect the levels of CPs in actual water samples is urgently required.

At present, HPLC-ultraviolet (HPLC-UV) [10–13], LC-MS [14], GC-flame ionization detector (GC-FID) [15], GC with electron-capture detection (GC-ECD) [16], and CE-UV [17, 18] have been used to analyze CPs. Although good chromatographic separation and peak shape can be obtained for CPs by GC [15, 16], the derivatization procedures of the CPs are time-consuming, multi-step, and tedious; due to adsorption problems and tailed peaks, the detectability, separation, and quantification of CPs by GC are problematic. Compared with GC, HPLC not only avoids tedious treatment and easy blockage of chromatographic column, but also has higher reproducibility and separation efficiency [19, 20]. However, HPLC-UV also has the disadvantages of consuming lots of organic solvents and low detection sensitivity, since the purchase of a mass spectrometer is not always an affordable expense for some laboratories. It is necessary to develop cheap, fast, and sensitive alternative methods. Interestingly, CE can represent a real alternative to current methods since it can provide high separation efficiencies similar to GC and lower-cost separations compared with HPLC because it requires only a small amount of solvents.

CE has become a very important technique for the separation of analytes since it has many advantages such as high separation efficiency, small sample size, low solvent consumption, and rapid analysis [21, 22]. Unfortunately, an important drawback for CE-UV is the relatively low sensitivity due to the low amount of CE samples and the short optical path [23, 24]. The determination of trace analytes in samples by CE usually requires on-line/off-line enrichment [25, 26]. The on-line (on-capillary) enrichment of CE can be readily realized by simply adjusting the BGE composition, the sample matrix composition, and the sample injection procedure. The on-line enrichment modes mainly include stacking (large-volume sample stacking and field-amplified sample stacking (FASI)), dynamic pH junction, sweeping, and transient isotachopheresis [27–29]. Amongst them, FASI is the combination of electrophoresis and EOF that allows ions to enter the capillary under the action of an electric field to achieve stacking [30–33]. The samples are prepared in a low

conductivity solution and electrokinetically injected into a high conductivity BGE, where the ions stack at the interface between the low conductivity sample zone and the high conductivity BGE zone [34, 35]. The faster is the electromigration rate, better is the ion stacking effect [36, 37]. In order to prevent the loss of ions from the injection end, a small length of water plug is usually injected into the capillary before the sample electrokinetic injection, which not only improves the enrichment factor, but also improves the reproducibility [26, 38–40]. For example, Liu et al. [31] utilized FASI to achieve on-line preconcentration of folic acid and nicotinic acid. The enrichment factors of FASI-water plug for FA and NA were 172 and 134, respectively, higher than that without using water plug for folic acid (145) and nicotinic acid (82). The RSDs for the migration time and peak area were less than 5.1% suggesting high reproducibility. Moreno-Gonzalez et al. [40] developed a FASI-CZE-UV method for the rapid determination of tetracyclines in human urine. A high-resistivity water plug was injected prior to sample injection to improve the FASI procedure. Accordingly, the high enrichment factors were attained in a range of 450–800 for the studied tetracyclines, as well as the improved reproducibility with RSDs below 14%.

Inspired by these studies, herein, we purpose to adopt FASI coupled with CE for the simultaneous separation and determination of five kinds of CPs in water samples, including 2-CP, 4-chlorophenol (4-CP), 2,4-DCP, 2,6-dichlorophenol (2,6-DCP), and 2,4,6-TCP. The conditions affecting the enrichment effect were optimized, including injection voltage, injection time, assisted pressure, and injection water plug. Compared with ordinary pressure injection, FASI is a very simple preconcentration technique that only requires the electrokinetic injection of the sample after the introduction of a short water plug, achieving high enhancement factors. The FASI combined with CE-UV was proved potentially applicable for efficient preconcentration and high sensitive determination of CPs in tap water and lake water samples.

2 Materials and methods

2.1 Reagents and samples

HPLC grade CPs standards, which included 4-CP, 2-CP, 2,4,6-TCP, 2,4-DCP, and 2,6-DCP, were purchased from Sigma-Aldrich (Shanghai, China) and the structures of CPs are shown in Supporting Information Fig. 1. Stock solutions of the standards were prepared as 1 g/L in MeOH and stored at 4°C in brown bottles. They were stable for at least 1 month. Working standard solutions containing all the CPs were freshly prepared by proper dilution of the stock standard solutions with ultrapure water. Chromatographic grade acetonitrile (ACN) and methanol (MeOH) were both purchased from J&K Chemical (Beijing, China). Other chemicals such as sodium hydroxide (NaOH) and sodium tetraborate

decahydrate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) were of analytical grade and were obtained from Sinopharm Chemical Reagent (Shanghai, China). The water used throughout the work was produced by a Milli-Q ultrapure water system (Millipore, Bedford, MA, USA).

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

2.2 Instrumentation

All electrophoresis experiments were performed on a SCIEX P/ACE™ MDQ plus CE system (Fullerton, CA, USA) equipped with a DAD. Data were collected using Beckman P/ACE workstation version 32 Karat software. The pH value measurements were made with a STARTER 3100 (Shanghai OHAUS Instrument Corporation, Shanghai, China).

2.3 CE conditions

Separations were carried out in a fused-silica capillary (Hebei Yongnian Ruifeng Chromatogram Equipment Company, China) with 75 μm i.d., 375 μm o.d., total length of 50.2 cm, and effective length of 40 cm. Each separation was rinsed for 3 min with separation buffer to ensure run-to-run reproducibility. New capillaries were first rinsed with MeOH (5 min), water (5 min), 1 mol/L NaOH (20 min), water (10 min), and separation buffer (30 min). The capillary was conditioned daily by flushing with NaOH (1 mol/L), water, and separation buffer for 5, 5, and 10 min, respectively. All solutions used in electrophoresis experiments were filtered through microporous nylon filters with a pore diameter of 0.45 μm before use.

The electrophoretic separation was achieved using a voltage of 20 kV (normal mode). The BGE was an aqueous solution of 20 mmol/L $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$, adjusted to pH 8.90 with 1 mol/L NaOH. The temperature of the capillary was kept constant at 25°C. CPs were monitored at 214 nm and an ordinary pressure injection namely hydrodynamic injection (HDI) was 3.45 kPa \times 5 s. On the other hand, injection of the sample occurred using the following FASI procedure.

2.4 FASI conditions

Ultrapure water was used as the sample solvent. A water plug was introduced into the capillary with hydrodynamic injection for 9 s at 3.45 kPa. A high voltage (−10 kV) was then applied to electrokinetically introduce the sample into the capillary for 30 s with an assisted pressure of 3.45 kPa.

3 Results and discussion

3.1 Optimization of CE conditions

CZE is the most basic and widely used CE mode for the analysis of charged solutes. Under alkaline conditions, these five CPs are all easily ionizable compounds. In our previous researches [2, 17, 18], CZE was chosen as separation mode to analyze CPs compounds in water samples. Therefore, we proposed to use the CZE mode for the separation of the five CPs and the separation conditions referred to that reported [18]. Experimental results showed that the peaks of 2,4,6-TCP, 2,4-DCP, and 2-CP were partially overlapped after FASI. It may be that FASI injection method leads to slight broadening of the peaks. Therefore, the separation conditions of these five CPs were re-optimized, that is, the pH values of 20 mM $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ (the same buffer as that reported [18]) were re-optimized to obtain baseline separation for all the five CPs. The pH of the BGE would affect the charges of the analytes, which would in turn affect the migration rate of the analytes in the BGE. The pK_a values of 4-CP, 2-CP, 2,4-DCP, 2,6-DCP, and 2,4,6-TCP are 9.18, 5.45, 7.89, 6.78, and 5.99, respectively, so in order to have these compounds in their ionic form for their proper separation, the pH value of the BGE was tested in alkaline conditions. In particular, the pH values of 8.5, 8.9, 9.2, and 9.5 of borax buffers were investigated for the effects on CPs separation. As seen from Supporting Information Fig. 2, a well-defined separation of these five CPs was completely achieved within 5.5 min at pH of 8.9. Although better sensitivity of four CPs could be obtained with the pH of 8.5, 4-CP was not completely separated from EOF. At the pH of 9.2, the chromatographic peaks of the two CPs overlapped completely. When pH was greater than 8.9, complete baseline separation of the five CPs was not achieved. A BGE pH of 8.9 was used for further experiments.

From the results above, the optimized CE conditions were confirmed: 20 mmol/L $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$, pH 8.9, an applied separation voltage of 20 kV at 25°C and HDI of 3.45 kPa \times 5 s.

3.2 FASI procedure

Figure 1 depicts the schematic diagram of FASI mode that was applied for CPs injection in this study. For negatively charged CPs, the FASI at negative polarity is displayed in Fig. 1A. The ion migration was in the opposite direction as the EOF resulted in the analytes depletion at the inlet. So a constant pressure was applied at the capillary inlet during FASI to counterbalance the EOF force, and therefore, the anionic sample was stacked by FASI at the boundary of sample zone and BGE (Fig. 1A). In FASI, it has been proved that pre-injection of a short water plug before sampling can improve the stacking efficiency [41, 42]. When the analytes entered the capillary through the water plug and crossed the interface of the water plug and BGE at high speed, they encountered a

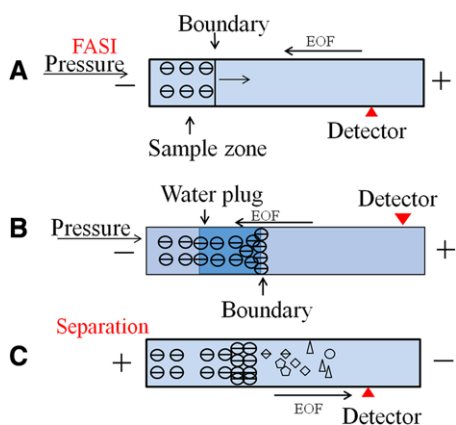


Figure 1. Schematic diagram of field amplified sample injection (FASI) prior to CE separation.

lower electric field and would slow and focus at this interface, as seen in Fig. 1B.

The capillary was first filled with the BGE and then a water plug was hydrodynamically introduced (3.45 kPa for 9 s). Then, electrokinetic injection of sample at a voltage of -10 kV during 30 s was performed and an assisted pressure of 3.45 kPa were simultaneously performed. Once the injection was completed, a voltage of 20 kV was applied in order to separate the compounds, as shown in Fig. 1C.

3.3 Optimization of FASI conditions

In order to achieve the maximum amount of target analytes that entered the capillary with FASI, several important parameters such as electrokinetic injection voltage, sample injection time, assisted pressure, and water plug should be optimized. The conditions were optimized using ultrapure water as the water sample matrix as follows:

In FASI, analytes are driven into the capillary by electrokinetic injection. The electrokinetic injection voltage and injection time are the crucial factors that affect the sensitivity enhancement in FASI. Under the conditions of 3.45 kPa assisted pressure and 24 s of injection time, the voltage of the injection from -4 to -12 kV (in intervals of -2 kV) were investigated. As shown in Fig. 2A, when the injection voltage increased, the peak area of the CPs increased gradually. Meanwhile, a minor peak broadening and a major peak area declining were observed from Supporting Information Fig. 3A, when the injection voltage exceeded -10 kV. The peak broadening problem will lead to a peak overlap problem in a real sample assay. Thus, an injection voltage of -10 kV was chosen in the experiment. Optimization of sample injection time was carried out in the range of 12 to 36 s with 6 s increments at -10 kV, and the results are displayed in Fig. 2B. As the sample injection time increased, a marked increase of peak intensity was observed. However, it can be seen from Supporting Information Fig. 3B that long sample injection time would also cause the peak broadening

problem. Therefore, an injection time of 30 s was chosen as the optimum injection time.

By using the voltage of -10 kV and the injection time of 30 s, assisted pressure of the electric injection was investigated, as shown in Fig. 2C. For FASI, the application of an assisted pressure can balance the force of the reverse EOF and prevent the sample from being pushed out of the capillary by the EOF. The results showed that a pressure of 0.5 psi (ca. 3.45 kPa) at -10 kV was the optimum condition. The sample would be pushed out of the capillary inlet by reverse EOF, reducing the amount of analytes entering the capillary when the pressure was below 3.45 kPa. When the pressure was greater than 3.45 kPa, the peak area of three CPs did not increase significantly, while that of 2,4-DCP and 2,4,6-TCP decreased. Meanwhile, the resolution of the five CPs decreases accordingly. Therefore, 3.45 kPa was selected as the optimum assisted pressure.

Since the sensitivity enhancement factor (SEF) of FASI is proportional to the conductivity ratio of BGE to sample, in this research the conductivity of the ultrapure water sample is lower than that of the BGE, which helps to improve the SEF. In addition, the hydrodynamic injection of a low conductivity ratio solvent plug prior to sample injection can improve the FASI procedure, avoiding possible disturbances of the stacking process on the boundary between the BGE and the sample. In order to enhance SEF for the analysis of five CPs, a MeOH, ACN, and water plug as low conductivity ratio in FASI was respectively studied. However, the enrichment effect was low and severe current interruption occurred when using MeOH and ACN as low conductivity solvent before the electrokinetic injection. Moreno-González et al. [40] also failed in the experiments with MeOH and ACN as low conductivity solvent. Other related researches [41, 42] eventually chose a water plug as low conductivity solvent before the electrokinetic injection. Water plug provides an enhanced electric field at the injection end of the capillary and acts like a fast freeway to carry the charged analytes. Therefore, at the conditions of electrokinetic injection voltage of -10 kV, sample injection time of 30 s, and assisted pressure of 3.45 kPa, the effect of the length of the water plug on the enrichment of five CPs was investigated. As shown in Fig. 2D, when the length of the water plug was 15 mm (3.45 kPa \times 9 s), as seen in Supporting Information Eq. 1, the enrichment efficiency of the four CPs reached a maximum, while that of 2,4,6-DCP slightly reduced compared to that at 10 mm (3.45 kPa \times 6 s). When the time of the water plug injection was less than 9 s, the analytes would easily be quickly pushed out of the capillary by the EOF. When the time of the water plug injection was longer than 9 s, peak broadening and peak overlap were most likely to occur. Therefore, the optimal water plug injection was 9 s, i.e. the optimal water plug length was 15 mm.

As a summary, the optimal FASI conditions were obtained, i.e. 9 s hydrodynamic injection (3.45 kPa) of water plug for 15 mm, 30 s of electrokinetic injection (-10 kV) of the sample and an assisted pressure of 3.45 kPa were simultaneously performed.

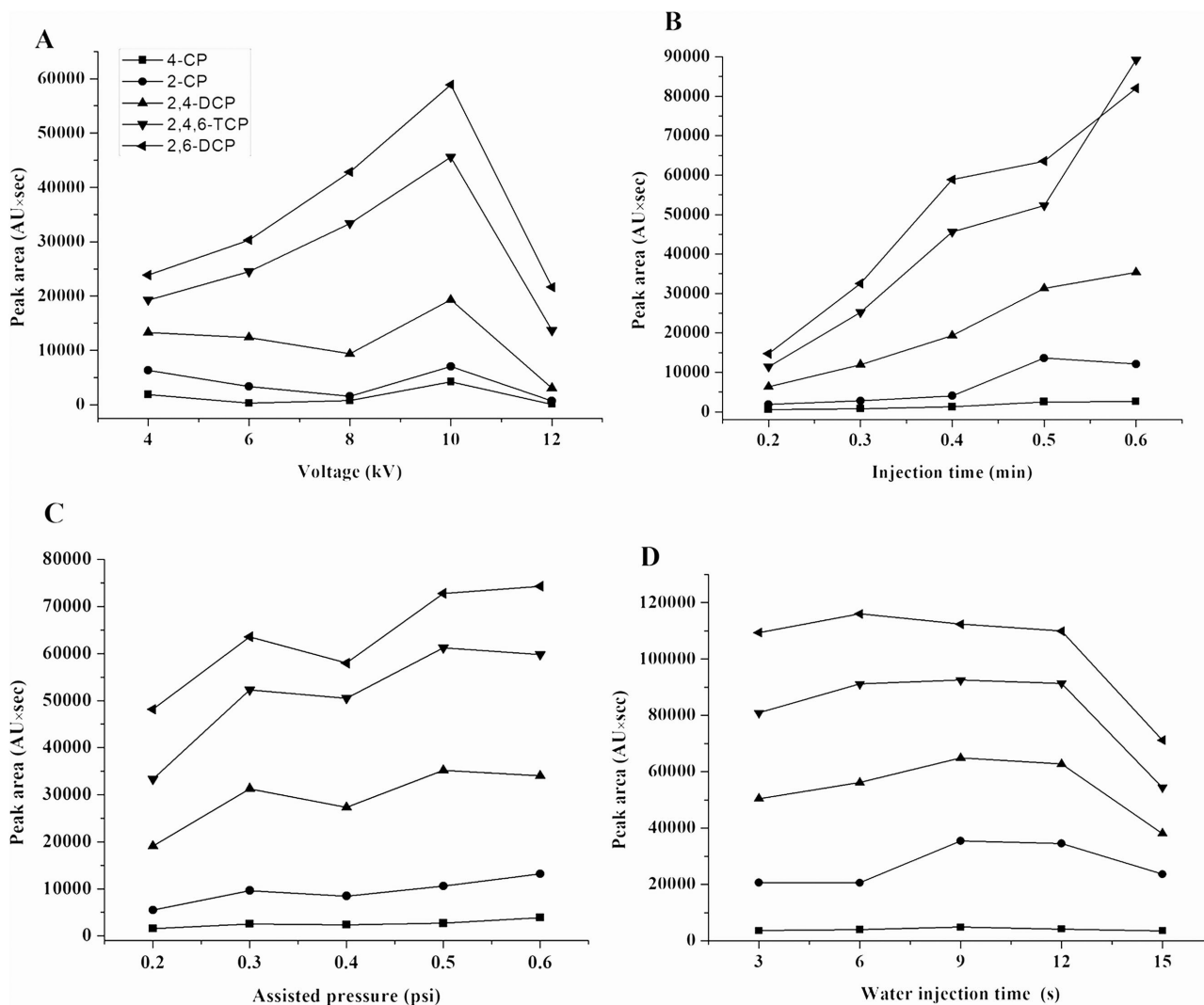


Figure 2. (A) Effect of electrokinetic injection voltage on the peak area of the five CPs. FASI conditions: assisted pressure, 3.45 kPa; sample injection time, 24 s. (B) Effect of sample injection time on the peak area of the five CPs. FASI conditions: electrokinetic injection voltage: -10 kV; assisted pressure, 3.45 kPa. (C) Effect of assisted pressure on the peak area of the five CPs. FASI conditions: electrokinetic injection voltage: -10 kV; sample injection time, 30 s. (D) Effect of the length of the water plug (water plug injection time) on the peak area of five CPs. FASI conditions: electrokinetic injection voltage: -10 kV; assisted pressure, 3.45 kPa; the length of the water plug, 15 mm (water plug injection time, 9 s); sample injection time, 30 s.

In order to estimate the sensitivity increase achieved in FASI, the SEF based on peak area was estimated from this equation:

$$SEF = \frac{C_F}{C_0}$$

where C_F and C_0 are the concentrations of analyte after FASI and the initial concentration of analyte in the aqueous solution.

The electropherograms from HDI (a) and FASI (b) are shown in Fig. 3. As seen, the FASI offered an effective stacking within 5 min, and the SEFs for the five CPs were 9 (4-CP), 27 (2-CP), 35 (2,4-DCP), 43 (2,4,6-TCP), and 43 (2,6-DCP), respectively.

3.4 Method performance

Under the optimal FASI-CE conditions, the five CPs were not detected in tap and lake water without any spiking, as shown in Fig. 4A. It should be noted that FASI is affected by subtle changes in sample composition which can bias method efficiency and accuracy [43]. Two common methods can be adopted to mitigate this effect, one by using internal standards and the other by using the corresponding real sample matrices for linearity. Herein, tap water and lake water samples were used as sample matrices, respectively, to mitigate this effect. A series of CPs solutions with the concentrations ranging from 0.01 to 10 $\mu\text{g/mL}$ were used to determine the calibration parameters. As listed from Table 1, good linearity was attained in the range of 0.1–10 $\mu\text{g/mL}$

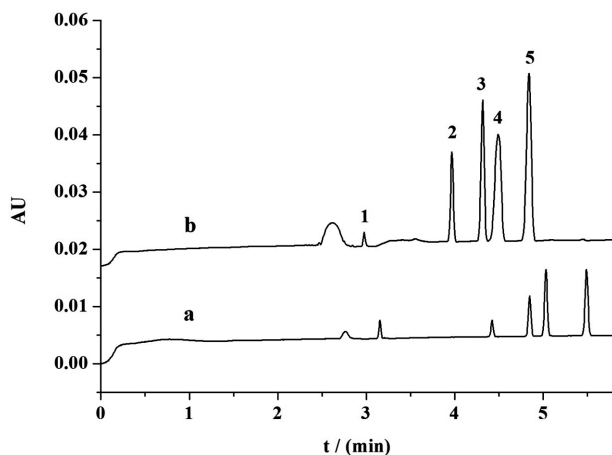


Figure 3. Electrophoregrams from the HDI (a) and FASI (b). (a) HDI: 3.45 kPa \times 5 s; the concentrations of five CPs: 10 $\mu\text{g}/\text{mL}$. (b) FASI: electrokinetic injection voltage -10 kV \times 30 s, 15 mm water plug and assisted pressure of 3.45 kPa; the concentrations of five CPs: 1 $\mu\text{g}/\text{mL}$. CE conditions: 20 mmol/L $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ at pH = 8.9, an applied separation voltage of 20 kV. Peak identification: (1) 4-CP, (2) 2-CP, (3) 2,4-DCP, (4) 2,4,6-TCP and (5) 2,6-DCP.

for 4-CP and 0.01–10 $\mu\text{g}/\text{mL}$ for other four kinds of CPs in tap water, and in the range of 0.1–10 $\mu\text{g}/\text{mL}$ for 4-CP and 0.05–10 $\mu\text{g}/\text{mL}$ for other four CPs in lake water, with correlation coefficients (r) all over 0.9973. The LODs were obtained based on the peak height as three times of background noise ($S/N = 3$), in the range of 0.0018–0.019 $\mu\text{g}/\text{mL}$ in tap water and 0.0089–0.029 $\mu\text{g}/\text{mL}$ in lake water. The values of LODs in tap water were much lower than the maximum permissible concentrations of 2,4,6-TCP, 2,4-DCP, and 2-CP in drink-

ing water stipulated by World Health Organization (WHO) namely 0.3, 0.04 and 0.01 $\mu\text{g}/\text{mL}$, respectively. The LOQs calculated based on a S/N ($S/N = 10$) were in the range of 0.0060–0.063 $\mu\text{g}/\text{mL}$ in tap water and 0.030–0.095 $\mu\text{g}/\text{mL}$ in lake water. The values were much lower than the primary standards of 2,4-DCP and 2,4,6-TCP in “Sewage Discharge Standards” of China, i.e., 0.6 $\mu\text{g}/\text{mL}$. The data meet the requirements of trace analysis, but the stacking efficiency still needs to be further enhanced to pursue higher sensitivity, such as combining two or more enrichment methods. To use this FASI method, researchers would only need to regulate the composition of BGE and the sample injection procedure to perform on-line enrichment of analytes, without using other reagents or procedures; this demonstrates the simplicity, rapidity, environment friendliness, and highly automated nature of this method. In addition, for samples with complex matrices in the environment, FASI can also be used with other sample pretreatment technologies, such as liquid-liquid extraction and SPE, to further purify and enrich the samples, increasing the enrichment factor and improving the detection sensitivity.

A mixed standard solution with a concentration of 1 $\mu\text{g}/\text{mL}$ was used to investigate the precision of the FASI-CE in the determination of five CPs. As shown in Supporting Information Table 1, intraday precisions (relative standard deviation (RSD), $n = 5$) for the migration time and peak area were 0.17–0.69% and 1.45–2.82%, respectively. Interday precisions ($n = 5$) for the migration time and peak area were 1.02–2.40% and 4.34–5.26%, respectively. The results showed that the method was stable, reliable, and suitable for accurate determination.

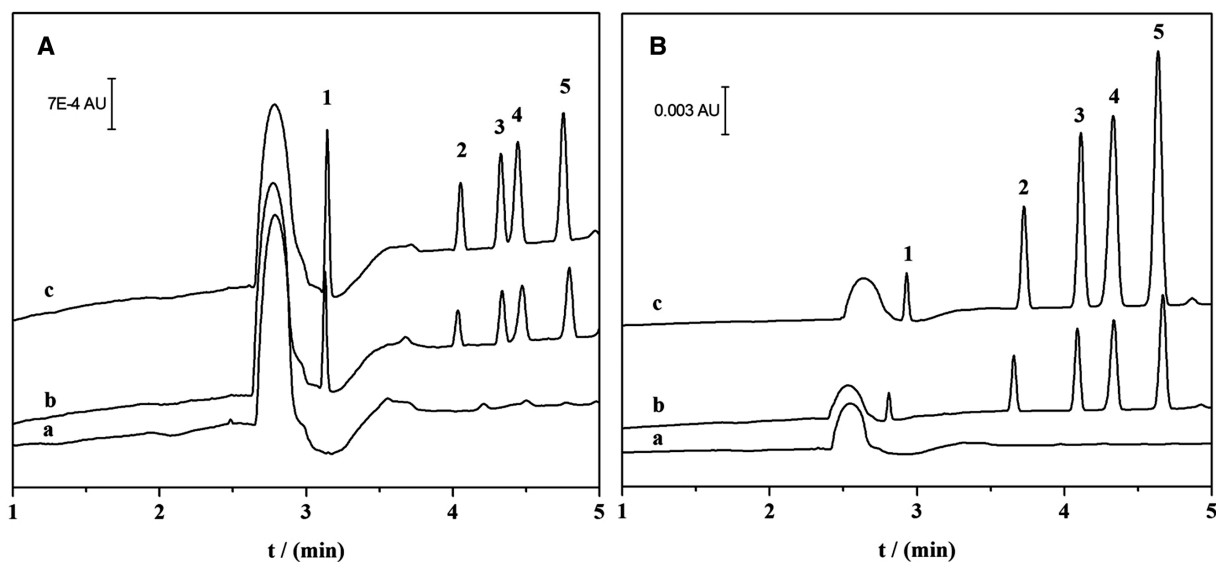


Figure 4. FASI electrophoregrams of the five CPs in blank and spiked samples of tap water (A) and lake water (B). (A) (a) without spiking; (b) spiked with 4-CP at 1.2 $\mu\text{g}/\text{mL}$ and other four CPs individual at 0.12 $\mu\text{g}/\text{mL}$; (c) spiked with 4-CP at 2 $\mu\text{g}/\text{mL}$, and other four CPs individual at 0.2 $\mu\text{g}/\text{mL}$. (B) (a) without spiking; (b) spiked with 4-CP at 1.2 $\mu\text{g}/\text{mL}$, and other four CPs individual at 0.6 $\mu\text{g}/\text{mL}$; (c) spiked with 4-CP at 2 $\mu\text{g}/\text{mL}$, and other four CPs individual at 1 $\mu\text{g}/\text{mL}$. FASI-CE conditions was the same as described in Fig. 3. Peak identification: (1) 4-CP, (2) 2-CP, (3) 2,4-DCP, (4) 2,4,6-TCP, and (5) 2,6-DCP.

Table 1. Analytical performances of the FASI-CZE-UV method for the determination of five CPs in water samples

Water sample	CPs	Regression equation ^{a)}	<i>r</i>	Linear range (µg/mL)	LOD (µg/mL) ^{b)}	LOQ (µg/mL) ^{b)}
Tap water	4-CP	$y = 1691.4x + 433.52$	0.9982	0.1–10	0.019	0.063
	2-CP	$y = 10846x + 185.65$	0.9998	0.01–10	0.0021	0.0070
	2,4-DCP	$y = 20437x - 938.91$	0.9997	0.01–10	0.0018	0.0060
	2,4,6-TCP	$y = 31061x - 812.79$	0.9999	0.01–10	0.0022	0.0074
	2,6-DCP	$y = 35705x - 903.75$	0.9997	0.01–10	0.0023	0.0079
Lake water	4-CP	$y = 1885.2x - 82.308$	0.9992	0.1–10	0.029	0.095
	2-CP	$y = 8800.7x + 2231.8$	0.9982	0.05–10	0.012	0.039
	2,4-DCP	$y = 19649x + 2851.3$	0.9973	0.05–10	0.0089	0.030
	2,4,6-TCP	$y = 36975x + 2584.9$	0.9999	0.05–10	0.0098	0.033
	2,6-DCP	$y = 43412x - 1875.9$	0.9999	0.05–10	0.010	0.033

a) Based on peak area; *y*: peak area; *x*: concentration, µg/mL.

b) Based on peak height.

3.5 Analysis of water samples

To further evaluate the practical application of the method, the developed FASI-CE method was applied for the analysis of five CPs in tap water and lake water samples. Figure 4 shows the electropherograms of five CPs in water samples. Standard recovery experiment was carried out, three standard addition level of five CPs in tap water and lake water samples were 0.12, 0.2 and 1.2 µg/mL and 0.6, 1.2 and 2 µg/mL, respectively. The averaged spike recovery obtained based on three triplicate measurements for each concentration was used to evaluate the feasibility of the FASI-CZE-UV method. As listed in Table S2, the recoveries of five CPs in tap water and lake water samples were 83.0–119.0% with RSDs of 2.09–8.58% and 91.0–115.8% with RSDs of 0.57–7.29%, respectively, indicating the developed method was practically applicable.

3.6 Analytical performance comparison with other CE-UV methods for CPs

Analytical performance of our developed FASI-CZE for CPs in water sample was compared with that reported by other CE-UV methods [2, 18, 44–46], as listed in Supporting Information Table 3. As can be seen from the table, although the present method doesn't have significant advantages over other methods in terms of LODs and SEF, the migration time of FASI-CZE (5 min) is much shorter than that of MEKC (16, 30 min) [44, 46], MEEKC (14 min) [45], and CZE (13, 7.5 min) [2, 18]. Moreover, it takes some time to carry out the off-line enrichment procedures [2, 18, 44–46], so the present FASI-CE method has the obvious advantage of being fast. But FASI also has some limitations, e.g. it is mainly applicable to samples of low conductivity and minimal sample matrix complexity. The method of molecularly imprinted polymers based dispersive solid phase extraction (MIPs-DSPE) [2, 46] requires synthetic materials and thereby their processes are complicated and time/reagents consuming. Dispersive liquid-liquid microextraction (DLLME) [18, 44, 45] also uses certain amounts of toxic organic reagents. However, interestingly, FASI is easily conducted by simply controlling the BGE composition and in-

jection procedure. Furthermore, compared with that reported for the same five CPs [18], the present method provides advantages of (1) simpler and faster on-line sample enrichment, (2) requiring lower amounts of methanol, and (3) improving separation efficiencies. This on-line enrichment technique not only gives full play to the advantages of CE, but also meets the requirements of current trace analysis. Therefore, in general, our developed FASI-CZE-UV method demonstrates the superiority of simplicity, rapidity, low-consumption, and eco-friendliness.

4 Concluding remarks

A new FASI-CZE-UV method was developed, providing a useful alternative for the rapid determination of CPs in actual water samples. The FASI preconcentration technique could overcome the main drawback of low concentration sensitivity in CE-UV. The strategy of FASI resulted in the sensitivity enhancement of CZE by 9, 27, 35, 43, and 43 folds in determination of 4-CP, 2-CP, 2,4-DCP, 2,4,6-TCP, and 2,6-DCP, respectively. So, sensitivity was considerably enhanced by applying FASI as on-line preconcentration method, simplifying sample treatment. In order to accurately quantify the actual water samples, standard curves were made in tap water and lake water, respectively. The recoveries of 83.0–119.0% with RSDs of 0.57–8.58% indicated the FASI-CE method was applicable. On the other hand, the use of pressure assistance under counter-EOF conditions requires carefully balancing the pressure and accurately determining the EOF velocity. There are alternatives to pressure assistance, for instance, capillary coatings [47, 48] and hydrogels [49] could be used to modify the EOF and assist FASI in counter-EOF of CZE. So, FASI should be continuously developed by using various strategies for wider applications. Overall, the FASI was simple, highly automated and environment-friendly, and can be used in combination with other sample preparation technologies, further elevating the detectability of CE-UV.

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