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RESEARCH PAPER

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

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Abstract A dual cloud point extraction (dCPE) off-line enrichment procedure coupled with a hydrodynamic–electrokinetic two-step injection online enrichment technique was successfully developed for simultaneous preconcentration of trace phenolic estrogens (hexestrol, dienestrol, and diethylstilbestrol) in water samples followed by micellar electrokinetic chromatography (MEKC) analysis. Several parameters affecting the extraction and online injection conditions were optimized. Under optimal dCPE–two-step injection–MEKC conditions, detection limits of 7.9–8.9 ng/mL and good linearity in the range from 0.05 to 5 μg/mL with correlation coefficients $R^2 \geq 0.9990$ were achieved. Satisfactory recoveries ranging from 83 to 108 % were obtained with lake and tap water spiked at 0.1 and 0.5 μg/mL, respectively, with relative standard deviations ($n=6$) of 1.3–3.1 %. This method was demonstrated to be convenient, rapid, cost-effective, and environmentally benign, and could be used as an alternative to existing methods for analyzing trace residues of phenolic estrogens in water samples.

Keywords Dual-cloud-point extraction · Micellar electrokinetic chromatography · Phenolic estrogen · Water samples

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Introduction

Endocrine-disrupting chemicals (EDCs) are exogenous compounds with the potential to elicit adverse effects on the endocrine system, and consequently have received general attention as a major global issue [1]. Among the EDCs identified so far, hexestrol (HS), dienestrol (DS), and diethylstilbestrol (DES) are often studied as a group of phenolic estrogens (PEEs) because of their structural and estrogenic similarities [2]. They have been used to treat various symptoms and have been used as growth promoters or oral contraceptives [2]. However, the illegal addition and abuse of PEEs has caused increasingly serious effects on the health of organisms because of their potential carcinogenic properties and other adverse effects [3]. Also, many studies have confirmed the presence of PEEs at levels of toxicological concern in aquatic environments [4]. Therefore, it is imperative to urgently develop simple, fast, and effective methods for monitoring the presence of and determining the levels of PEEs.

Nowadays, various methods are available for the determination of PEEs, such as solid-phase extraction (SPE)–ultraperformance liquid chromatography [5–7], solid-phase microextraction–high performance liquid chromatography–(HPLC) mass spectrometry–tandem mass spectrometry [8], SPE–HPLC [9–11], and capillary electrophoresis (CE) [12, 13]. In SPE, sorbents are required, such as hydrophilic–lipophilic balance sorbents [5, 6, 8], 17β-estradiol molecularly imprinted polymer [7], bamboo charcoal [9], and functionalized magnetic nanoparticles (Fe_3O_4–SiO_2/β-cyclodextrin core/shell) [10, 11]. Besides, in methods such as SPE–HPLC and solid-phase microextraction–HPLC, complex enrichment procedures [5–7, 9–11], time-consuming procedures [9–12], rigorous detection methods [5–7], and high cost [5–7, 9–11] are also involved. Although HPLC has often been used for the determination of PEEs, CE is also used. An improved technique, pressurized capillary electrochromatography coupled with end-column amperometric detection, was developed for the separation and determination of estrogens [12]. Microemulsion electrokinetic chromatography was used for the separation of several priority EDCs [13]. However, as far as we are aware, there is no report on cloud point extraction (CPE) along with CE analysis for PEEs.

CPE, based on the clouding phenomena of nonionic surfactants, has become an alternative to conventional solvent extraction because of possible advantages such as low cost, environmental safety, and high capacity to concentrate a wide variety of analytes with high recoveries and concentration factors [14]. A number of reports have concerned the use of the CPE approach as a means by which to extract and enrich inorganic, organic, and biological analytes prior to spectroscopic, chromatographic, or CE analysis [14–20]. In this work, dual CPE (dCPE) was firstly developed for extraction of PEEs from water samples. As illustrated schematically in Fig. 1, HS, DS, and DES were firstly transferred into the surfactant-rich phase in the form of monovalent ions after the first CPE, and then divalent ions entered the aqueous phase from the surfactant-rich phase after the second CPE. Notably, the proposed extraction theory is different from that previously reported [19, 20]. We intended to validate the feasibility of dCPE using a pH-mediated method to control the fractional composition (α) of PEEs.

Despite the attempt to increase the detection sensitivity by dCPE, the main limitation of the CE technique lies in its extremely small injection volume, typically in the nanoliter range, three orders of magnitude smaller than the usual injection amount in HPLC [21]. Fortunately, a number of strategies have been developed to improve the sensitivity of CE through online enrichment, which is collectively known as stacking, such as field amplified sample stacking, transient isotachophoresis, dynamic pH junction, and sweeping [22, 23]. We adopted a simpler and more direct online strategy, namely, hydrodynamic–electrokinetic two-step injection, to increase the amounts of sample injected and thereby improve the CE sensitivity. This technique, being a pressure injection followed by a voltage injection, is different from pressure-assisted electrokinetic injection, which involves the simultaneous use of both voltage and pressure for injection [21, 24], but there are still some similarities between them. For example, the present two-

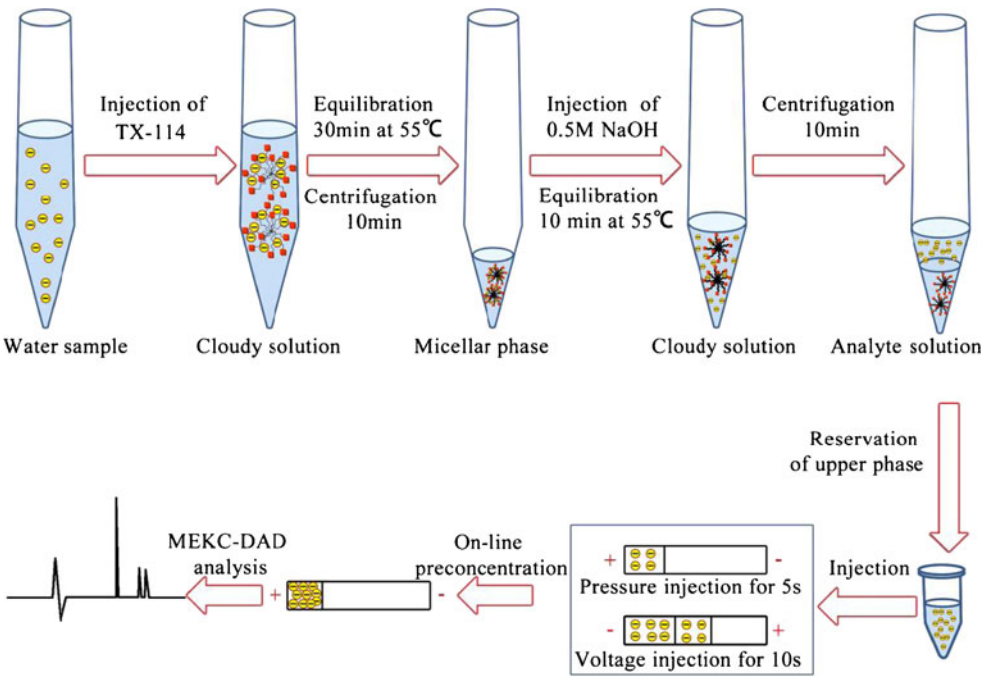

Fig. 1 The proposed dual cloud point extraction (dCPE)–two-step injection–micellar electrokinetic chromatography (*MEKC*) method. *DAD* diode-array detection, *TX-114* Triton X-114

step injection is also based on the concept that the movement of running buffer in the capillary column due to electroosmotic flow (EOF) can be balanced during sample injection by a pressure applied in the opposite direction to the EOF under a given electric field, and thereby a stationary state of the running buffer inside the column can be achieved [21, 24].

In this work, we intended to develop a method of dCPE off-line enrichment coupled with two-step injection sample online enrichment followed by micellar electrokinetic chromatography (MEKC) for the simultaneous preconcentration, separation, and determination of HS, DS, and DES. Figure 1 shows the entire procedure. This work was expected to offer a powerful, simple, fast, and eco-friendly analytical strategy for simultaneous determination of trace PEEs in complicated samples.

Experimental

Chemicals, standard solutions, and water samples

Three PEE standards of HS, DS, and DES were purchased from Sigma-Aldrich (Shanghai, China), and their chemical structures are shown in Fig. S1. Triton X-114 (TX-114) was also obtained from Sigma-Aldrich (Shanghai, China), and 5.00 g was dissolved in 100 mL water for use. Sodium dodecyl sulfate (SDS) was purchased from Aladdin (Shanghai, China). Chromatographic grade acetonitrile (ACN) and methanol were purchased from J&K Chemical (Beijing, China). All other chemicals, such as sodium hydroxide (NaOH), sodium tetraborate decahydrate ($Na_2B_4O_7{\cdot}10H_2O$), sodium dihydrogen phosphate (NaH_2PO_4), and phosphoric acid (H_3PO_4), were of analytical grade and were obtained from Sinopharm Chemical Reagent (Shanghai, China). The water used throughout this work was produced by a Milli-Q Ultrapure water system (Millipore, Bedford, MA, USA).

Stock solutions containing 1,000 μg of each PEE per milliliter were prepared by dissolving the required amounts of the standards in methanol. Working solutions were prepared by diluting the stock solutions with appropriate amounts of water. They were stored in a refrigerator at 4 °C before use.

Lake water was collected from an artificial lake located in Laishan District of Yantai City (China) and was stored in the dark at 4 °C before use. Tap water was collected after it had flowed for about 5 min in the laboratory when needed. Before use, the samples were passed through microporous nylon filters with a pore diameter of 0.45 μm. Several aliquots from 10 mL of filtered water samples were spiked with the PEE standards of different concentrations, followed by the CPE procedure.

pH-mediated dCPE procedure

For the first CPE, 10.0 mL of standard solutions or real sample solutions containing analytes, which were adjusted to pH 8.8 with 1 M phosphoric acid and NaOH, were poured into a 15-mL screw-capped centrifuge tube, and then 205 μL of TX-114 at 5.00 % (w/v) in aqueous solution was added and mixed by shaking by hand for a few minutes. The mixture was then placed in an ultrasonic bath for 5 min and immersed in a thermostatic bath at 55 °C for 30 min. In this step, a cloudy solution was formed and the PEEs in the water phase were extracted into the surfactant-rich phase. After the mixture had been centrifuged for 10 min at 748 *g* and cooled in an ice bath for 5 min to increase the viscosity of the surfactant-rich phase, the supernatant aqueous phase was removed carefully with a pipette.

For the second CPE, the sticky micellar phase at the bottom was mixed with 100 μL of 0.5 M NaOH (pH 13.7). The mixture was placed in an ultrasonic bath for 5 min and then immersed in a thermostatic bath at 55 °C for 10 min. It was centrifuged for 10 min at 748 *g*, the supernatant aqueous phase was passed through microporous nylon filters with a pore diameter of 0.45 μm, and was analyzed by MEKC. The dCPE procedure is illustrated in Fig. 1.

The extraction efficiency was evaluated by the enrichment factor (EF), which was calculated as follows:

$$EF = \frac{A_2}{A_1} \times 10,$$

where A_2 is the area of the PEEs (with a concentration of 1 μg/mL) after dCPE and A_1 is the area of the PEEs (with a concentration of 10 μg/mL) before dCPE. The standard deviation (SD; %) was calculated as follows:

$$SD = \sqrt{\sum \left(EF_i - \overline{EF}\right)^2/(N-1)},$$

where EF_i is the EF of each extraction, and $\overline{EF}$ is the average value of three extractions.

Sample injection and MEKC conditions

All experiments were performed with a P/ACE MDQ CE system (Beckman Coulter, Fullerton, CA, USA) in conjunction with a diode-array detector. The detection wavelength was at set at the maximum absorption wavelength of 228 nm for HS and DS and 240 nm for DES. The optimal conditions of sample introduction when using two-step injection were as follows: injection pressure of 1 psi for 5 s and injection voltage of -10 kV for 10 s (Fig. 1). Once the injection was complete, the voltage was switched to +28 kV for the MEKC separation. The running buffer was prepared by freshly mixing 10 mM $Na_2B_4O_7{\cdot}10H_2O$, 20 mM SDS, and 40 % (v/v) ACN adjusted to pH 10.6 with 1 M NaOH.

Bare fused-silica capillaries (inner diameter of 75 μm, outer diameter of 375 μm, total length of 50.2 cm, and effective length of 40 cm; Yongnian Photoconductive Fiber Factory, Hebei, China) were used for PEE separations. An Ion 510 pH meter (Ayer Rajah Crescent, Singapore) was used to monitor the pH adjustment. New capillaries were initialized by flushing them with water (10 min), 0.5 M NaOH (40 min), water (10 min), and running buffer (30 min) before use. Between analyses the capillary was rinsed with running buffer (2 min). All solutions were passed through microporous nylon filters with a pore diameter of 0.45 μm.

Results and discussion

Optimization of MEKC conditions

As for CE methods for these three specific PEEs, a search using Scopus revealed that there are no articles reporting their separation by capillary zone electrophoresis (CZE), MEKC, or microemulsion electrokinetic chromatography, and there is an excellent article on separation by pressurized capillary electrochromatography [12]; however, the migration time is more than 25 min, which is a long time for a CE analysis. We also investigated CZE mode, but the PEEs could not be separated. MEKC mode provided good separation of the PEEs within 7 min. The MEKC conditions were optimized as follows.

In general, the MEKC process is mainly subject to parameters such as running buffer, surfactant, organic modifier, and applied voltage. We firstly selected 10 mM $Na_2B_4O_7{\cdot}10H_2O$ and 20 mM SDS as the running buffer and surfactant, respectively, according to our research experience and some publications [25–27]. Then the content of ACN as a modifier, the pH of the running buffer, and the separation voltage were investigated for optimization of the MEKC conditions, and all test runs together with the parameters varied and fixed are listed in Table S1.

The addition of organic modifier to the MEKC buffer can alter the partition coefficient of the analytes, and this is an effective way of improving separation selectivity, efficiency, and resolution [28]. ACN was selected as the organic modifier in MEKC because of its superior EOF-promoting ability [29, 30]. The results showed higher contents of ACN enhanced the separation performance, albeit at the cost of an increase in separation time with an increase of ACN content from 10 to 40 % (v/v). At 50 % ACN, three individual peaks were observed in the extended migration time of more than 10 min. Therefore, 40 % ACN was selected for the following work, offering high separation efficiency and resolution within a short time, although the critical micelle concentration of SDS is above 30 mM for a volume fraction of ACN of 40 % [31]. However, if there was no SDS, all three analytes migrated with the EOF, and they could not be separated. On the other hand, a higher concentration of SDS resulted in a longer migration time as well as lower column efficiency, and the peaks were distorted. Overall, 20 mM SDS and 40 % ACN were selected in order to simultaneously separate the three PEEs within a short time.

To optimize the buffer pH, different pHs (9.7, 10, 10.3, 10.6, and 11) were investigated. As the effective charge of the analyte was dependent on the buffer pH, variation of the pH of the background electrolyte resulted in variation of the effective electrophoretic mobility. When the pH of buffer was 10.6 and 11, respectively, there was better separation between the three PEEs. This was very likely because of the greater charges of the PEEs and therefore the more favorable electrophoretic mobility [32]. From comprehensive consideration of the resolution and peak shape, 10.6 was finally selected as the pH of the buffer.

Th applied voltage is also very important for MEKC separation. Voltages of 25, 28, and 30 kV were used. Baseline separation was achieved for the three PEEs within 7 min at an applied voltage of 28 kV.

Optimization of pH-mediated dCPE conditions

The pH-mediated dCPE procedure is shown schematically in Fig. 1. In the first step, after adjustment of the pH of the sample solution to 8.8, HS, DS, and DES became monovalent species and were extracted into the surfactant-rich phase after the addition of TX-114. After 100 μL of 0.5 M NaOH solution (pH 13.7) had been added to the surfactant-rich phase, the three PEEs formed stable divalent ions and transferred to the aqueous phase during the second procedure.

The factors affecting dCPE efficiency mainly included acidity–alkalinity during the two CPE procedures, the concentration of TX-114, and the equilibration temperature and time. Systematic investigations of the factors were conducted by MEKC, and the varied and fixed parameters are listed in Table S2. The EF was used as the index of dCPE efficiency, and was defined as the ratio of the analyte's peak area after and before dCPE.

Effect of pH of the sample and the extraction solution

As shown in Fig. 2a, the EFs of HS, DS, and DES increased with increase in pH in the range from 6.5 to 8.8. However, a decrease of the EF was observed when the pH was increased from 8.8 to 11. So pH 8.8 was selected as the optimum pH of the sample solution.

HS, DS, and DES are weakly diprotic acidic compounds, and their $pK_{a,1}$ and $pK_{a,2}$ values are 7.23 and 10.14, 7.43 and 10.47, and 7.34 and 10.21, respectively (see Fig. S2). So the pH of the extraction solution has a great impact on their degree of ionization. The species of a diprotic system

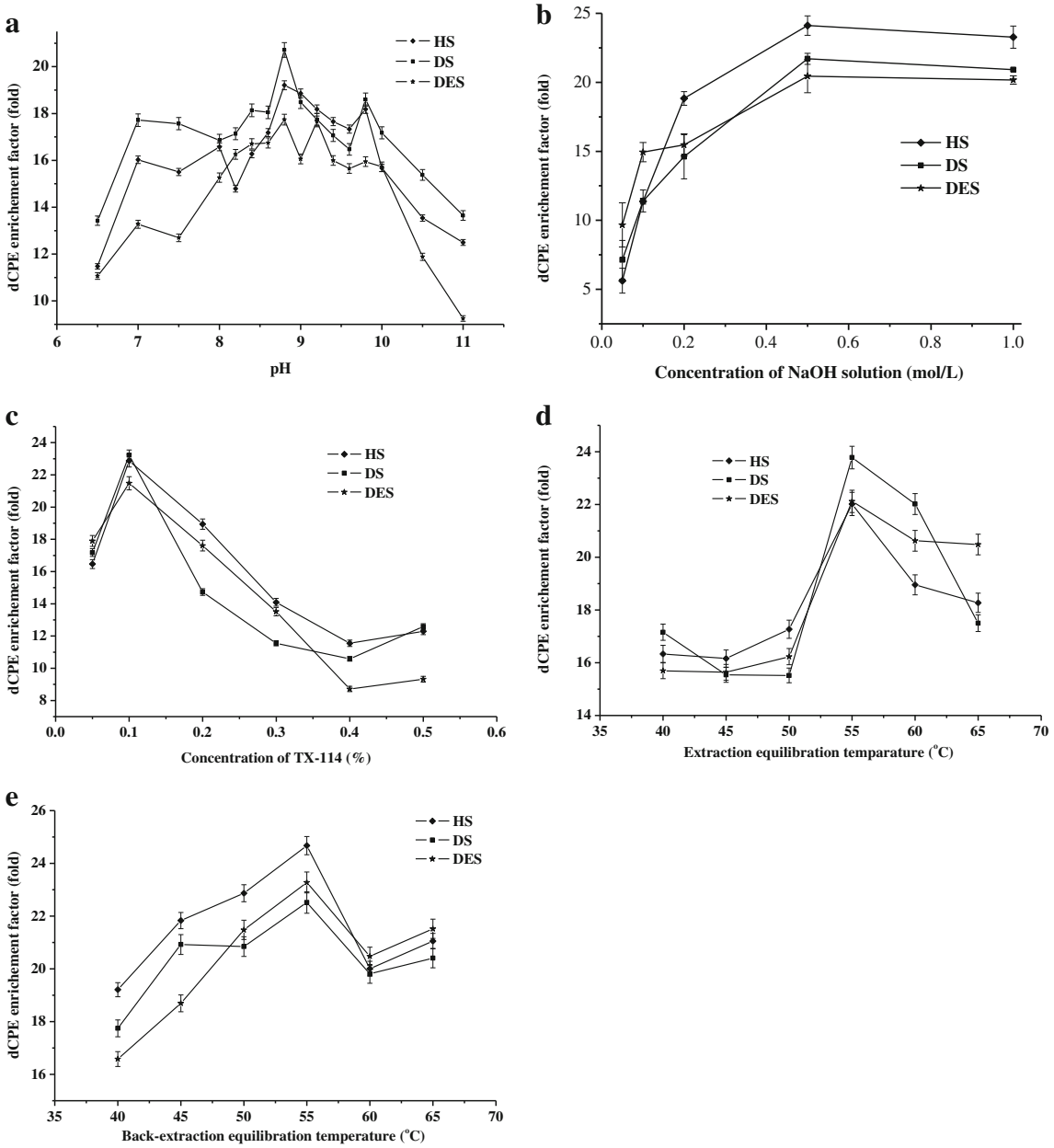

Fig. 2 Effects of **a** pH, **b** NaOH concentration, **c** TX-114 concentration, **d** extraction equilibration temperature, and **e** back-extraction equilibration temperature on enrichment factors (EFs) of dCPE for 1 μg of each PEE per milliliter. For every EF value, three parallel measurements were made. *HS* hexestrol, *DS* dienestrol, *DES* diethylstilbestrol

include H_2A, HA^-, and A^{2-}. Figure S2 shows the fraction composition (α) diagrams for various forms of the PEEs, namely, for α_{H_2A}, α_{HA^-}, and $\alpha_{A^{2-}}$, in the pH range from 6.5 to 12, and the theoretical/calculated curves obtained according to Eq. S1. As can be seen from the figure, below pH 7.5, H_2A was the dominant form because the neutral species of the analytes are so nonpolar that their solubility in water is extremely low. With increasing pH, α_{HA-} increased and the amount of H_2A species decreased. Because there was a hydrophilic group at one end of HA^-, the solubility of each PEE in solution increased. This can be testified by the UV spectrum (see Fig. S3). As seen, the UV absorbance of DES increased with increase of the pH from 5 to 12, which may be caused by the increasing solubility of DES. Therefore, it can be concluded that when the pH was low, the analytes formed a dispersed pseudo-solid phase within a liquid dispersant. Extraction of dissolved chemical component X from liquid phase A is accomplished by bringing the liquid solution of X into contact with a second phase, B [33]. In extractions, the analyte should be dissolved in the liquid medium. Because the neutral species were not completely dissolved in the sample solution, the extraction efficiency

was quite low at low pH. However, the extraction into micelles of the dissolved monovalent analytes in the sample solution was easier and more rapid than for the neutral species. Consequently, the extraction efficiency increased. So, the larger α_{HA-} was, the greater the EF was. When the pH was 8.8, α_{HA-} was almost the greatest (more than 0.9) for each PEE. Therefore, the largest EF was obtained at pH 8.8. This was consistent with the observed pH effect. Also, from Fig. S2, as the pH increased from 8.8 to 11, $\alpha_{A^{2-}}$ increased and α_{HA^-} decreased. When the pH increased above 9.5, the A^{2-} form dominated and the EF decreased, because it was not solubilized well in the micelle. In short, we can conclude that at pH<7.5 neutral species dominated, at pH 8.8 monovalent species dominated, and at pH>9.5 the divalent form dominated; Fig. 2 shows that the optimum extraction is when the PEEs are monovalent.

Although $K_A{}^{2-}<K_{HA}{}^-$ at high pH is adverse for extraction, it is advantageous for back-extraction. Therefore, basic solution was used for back-extraction. The influence of the back-extraction solution on the second CPE was investigated by using 0.05–1 M NaOH (pH 12.7–14.0); the results are shown in Fig. 2b. The EF of the three PEEs increased with increase in NaOH solution concentration up to 0.5 M (pH 13.7). Then when the concentration of NaOH was 1 M, there was no significant change in the EF. This indicated that with increasing pH, the concentration of divalent species increased, and the back-extraction efficiency increased. When the pH was 13.7, a satisfactory back-extraction efficiency was obtained; When the pH was higher than 13.7, the back-extraction efficiency changed little. Additionally, the migration time of the PEEs was prolonged with an NaOH solution of higher concentration, which was unfavorable for separation. So, 0.5 M NaOH (pH 13.7) was selected as the optimum solution for back-extraction.

Effect of TX-114 concentration

As TX-114 has a critical micellar concentration of 0.01 % (w/v) [34], its concentration was optimized in the range from 0.05 to 0.5 % (w/v). The EFs of HS, DS, and DES increased with TX-114 concentration increasing in the range from 0.05 to 0.1 % (w/v) and then decreased, as shown in Fig. 2c. Increasing the amounts of TX-114 also increased the volume of the bottom phase and therefore resulted in lower concentrations of the PEEs in the micelle phase. This resulted in a larger amount of micelles being present during back-extraction, which led to a reduction in recovery and hence a lower EF. So, 0.1 % (w/v) was adopted in the following studies.

Effect of equilibration temperature and time

Both the equilibration temperature and the equilibration time play important roles in the dCPE. With the equilibration temperature increased, the surfactant-rich volume decreased, but the concentration of the PEEs in the surfactant phase increased. Generally, the optimum equilibration temperature of CPE is 15–20 °C higher than the cloud point temperature [35]. Considering the low cloud point temperature (22–30 °C) of TX-114 [14], we tested the effect of the equilibration temperature in the range from 40 to 65 °C. The EF increased with increase in temperature in the range from 40 to 55 °C and then decreased above 55 °C (Fig. 2d). This might be attributed to the heat instability of phenolic hydroxyl groups in PEEs. This could result in the PEEs being changed into a quinone or another compound at high temperature. Therefore, the PEEs might be transformed into other compounds which could not be extracted into the micelles when the temperature was above 55 °C [36]. So the equilibration temperature was set at 55 °C. Interestingly, for the back-extraction, the trend of the equilibration temperature was similar to that for extraction (Fig. 2e),

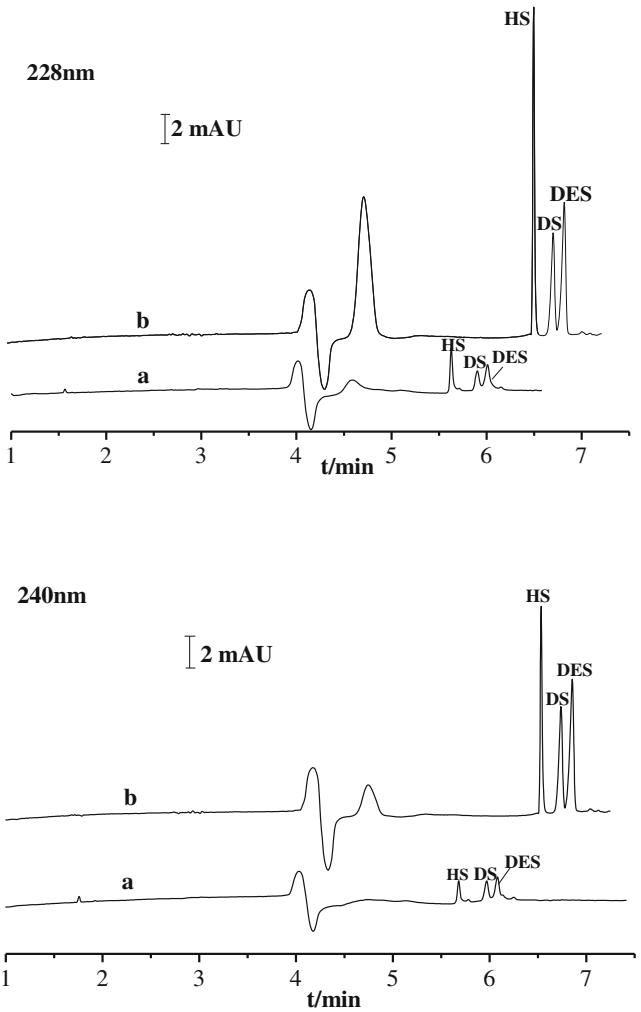

Fig. 3 MEKC electropherograms of the PEE standards monitored at 228 and 240 nm without (*a*) and with (*b*) the dCPE–two-step injection procedure. *a* standard PEE solution, 10 μg/mL, common pressure injection for 3 s; *b* standard PEE solution, 1 μg/mL. The MEKC conditions were as follows: 10 mM $Na_2B_4O_7{\cdot}10H_2O$, 20 mM sodium dodecyl sulfate, 40 % (v/v) acetonitrile, pH 10.6, and applied voltage of 28 kV

Table 1 Linear range, regression data, and concentration limits for the phenolic estrogens (*PEEs*)

PEEs	Linear range (μg/mL)	*a* (mean ± SD[a])	*b* (mean ± SD[a])	R^2	LOD (ng/mL)	LOQ (ng/mL)
HS	0.05–5	46,630±236	−2,359±56	0.9996	7.9	26.3
DS	0.05–5	24,133±10	2,807±98	0.9992	8.2	27.4
DES	0.05–5	41,527±298	−2,189±68	0.9990	8.9	29.7

Calibration equation: $y=ax+b$

SD standard deviation, *LOD* limit of detection, *LOQ* limit of quantification, *HS* hexestrol, *DS* dienestrol, *DES* diethylstilbestrol

[a] $n=6$

and the equilibrium temperature was also chosen as 55 °C. As mentioned above, the optimum equilibration temperature of CPE is 15–20 °C higher than the cloud point temperature of TX-114 (22–30 °C), so 55 °C was consistent with the temperature reported in [14, 35]. The effect of the equilibration time was studied in the range from 10 to 60 min at 55 °C. Consequently, the optimum equilibration times of extraction and back-extraction were 30 and 10 min, respectively.

Optimization of sample injection conditions

After dCPE, the PEEs were negatively charged. As shown schematically in Fig. 1, they were firstly injected into the capillary under hydrodynamic conditions, and then were electrokinetically injected under a negative voltage; the reverse EOF caused the bulk buffer to move toward the injection end. The sample solution moved toward the injection end because of the reverse EOF, which is usually undesired, and PEEs were focused at the boundary layer of the sample solution and running buffer in the capillary [21, 24]. So the EOF was compensated by an external pressure in the opposite direction and the running buffer in the capillary was stationary during sample injection. The balance of EOF can be achieved by adjusting either the pressure under a given voltage or the voltage under a given pressure. Since the maximum injection voltage of the CE instrument used is limited to 10 kV, the injection voltage was fixed at -10 kV. The optimum external pressure was chosen as 1 psi, where both sharp peaks and good resolution of the PEEs were achieved.

Table 2 Method precision of migration time and peak area for PEEs (spiked at 1 μg/mL)

PEEs	RSD (%)			
	Intraday ($n=6$)		Interday ($n=6$)	
	Migration time	Peak area	Migration time	Peak area
HS	0.0	1.6	1.2	5.4
DS	0.2	2.5	1.0	3.9
DES	0.3	1.2	1.5	6.0

RSD relative standard deviation

The injection time was also investigated by using injection times of 5 s and 10 s. As for hydrodynamic injection, it was observed that the peaks overlapped for 10 s, whereas baseline separation was attained for 5 s. So 5 s was selected as the hydrodynamic injection time. In the case of electrokinetic injection, the peak areas were larger for 10 s than for 5 s. So 10 s was selected as the electrokinetic injection time.

Combined EFs

Under the optimized conditions, Fig. 3 shows the electropherograms of PEEs in MEKC with the detection wavelengths at 228 and 240 nm. Compared with the common hydrodynamic injection for 3 s without an enrichment procedure (Fig. 3, electropherograms a), a remarkable increase of peak height/area was obtained for each PEE with dCPE–two-step injection (Fig. 3, electropherograms b). The migration time was longer when using the two-step injection. This indicated that the two-step injection reduced the sample zone length, narrowed the sample band, and extended the effective column length [21], and thereby resulted in a longer migration time, but with a better peak shape and still rapid separation within 7 min.

The combined EF of dCPE and two-step injection was defined as the ratio of the analyte's peak area with and without dCPE–two-step injection. For all three PEEs, the combined EFs were about 50-fold to 150-fold at various concentrations from 0.05 to 5 μg/mL.

Analytical performance of the dCPE–two-step injection–MEKC method

The performance of the dCPE–two-step injection–MEKC method under optimal conditions was investigated. Linear correlation coefficients (R^2) assessed at six different concentrations were obtained between the peak area and the corresponding concentrations of the PEEs in the range from 0.05 to 5 μg/mL, as shown in Table 1. The limits of detection for all three PEEs, calculated as the analyte concentration for which the peak height was three times the background noise (signal-to-noise ratio of 3), were 7.9, 8.2, and 8.9 ng/mL, respectively (Table 1). This method

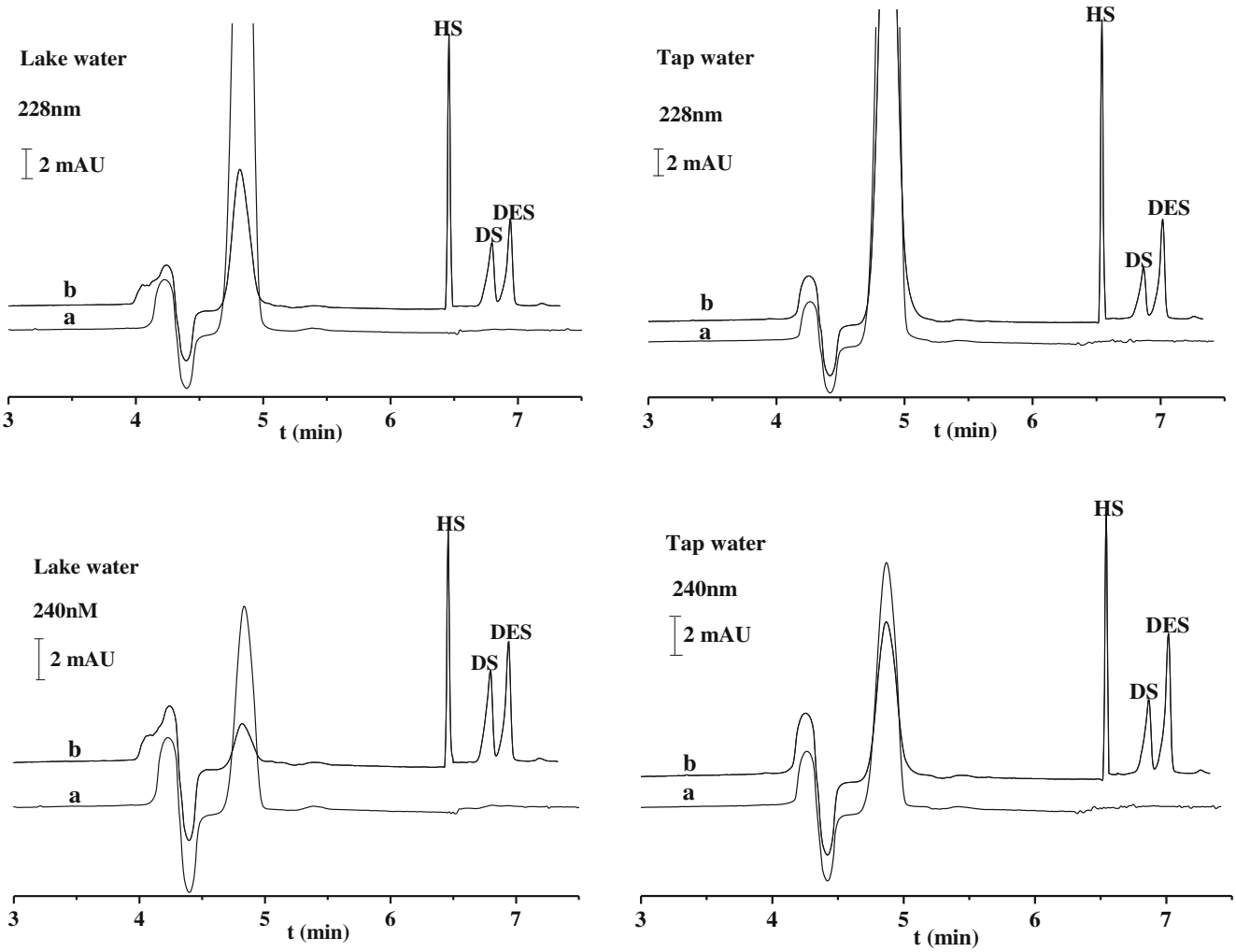

Fig. 4 MEKC electropherograms of water samples monitored at 228 and 240 nm after the dCPE–two-step injection procedure. The water samples were not spiked (*a*) and were spiked (*b*) with 1 μg of HS, DS, and DES per milliliter. The MEKC conditions were the same as those described in the legend for Fig. 3

Table 3 Method recoveries for PEEs in tap and lake water samples

PEEs	Lake water				Tap water			
	Added (μg/mL)	Detected (μg/mL)	Recovery (%)	RSD[a] (%)	Added (μg/mL)	Detected (μg/mL)	Recovery (%)	RSD[a] (%)
HS	0.1	0.10	100	1.9	0.1	0.09	90	2.0
	0.5	0.44	88	1.5	0.5	0.44	88	1.6
	1.0	0.94	94	1.7	1.0	0.97	97	1.8
DS	0.1	0.10	100	3.1	0.1	0.10	103	2.1
	0.5	0.42	84	2.6	0.5	0.35	70	1.6
	1.0	0.85	85	2.4	1.0	0.64	64	3.3
DES	0.1	0.11	110	2.0	0.1	0.11	110	1.3
	0.5	0.46	92	2.5	0.5	0.44	88	2.1
	1.0	0.75	75	1.8	1.0	0.83	83	2.0

[a] $n=6$

has achieved the requirement for trace analysis [23]. Given that many of the EDCs identified have the potential to cause an estrogenic response at very low concentrations (parts per billion to parts per trillion levels), it is cause for concern that measurable concentrations of many of the chemicals mentioned herein have been found in wastewater, surface waters, sediments, groundwater, and even drinking water [37]. Therefore, the limits of detection of this method can reach the required levels for the three analytes in environmental water investigations to some extent.

On the other hand, the relative SDs (RSDs) obtained under repeatability (intraday precision, six samples of the same concentration were treated with the optimum conditions including extraction and injection in 1 day) conditions in terms of migration time and peak area were less than 0.3 % and 2.5 %, respectively, whereas under reproducibility (interday precision, samples of the same concentration were treated with the optimum extraction and injection conditions on 6 days) conditions they remained under 1.5 % and 6.0 % (Table 2), respectively. The underlying migration time and peak area data for the three PEEs are given in Table S3. The method was capable of accurately quantify PEEs.

Determination of PEEs in water samples

The dCPE–two-step injection–MEKC method was further applied to lake and tap water samples in order to check its practicality. Figure 4 shows the MEKC electropherograms of tap and lake water samples after dCPE–two-step injection. The targeted PEEs were not detected in the water samples (Fig. 4, electropherograms a).

On the other hand, analysis of the water sample spiked with the PEE standards at 1 μg/mL under the same experimental conditions showed three resolved peaks with marked peak height corresponding to HD, DS, and DES, respectively (Fig. 4, electropherograms b). The MEKC–diode-array detection analysis of the PEEs did not appear to suffer from interference from the water matrix, which was probably caused by the cleaning effect of dCPE. The results obtained suggest that by coupling the dCPE off-line enrichment procedure with the two-step injection online enrichment technique followed by MEKC analysis, good separation and detection of the PEEs in a real water matrix could be achieved. Table 3 gives the recoveries in the two water samples. High recoveries of HS, DS, and DES of 88–99 %, 64–103 %, and 75–110 % were obtained at three concentrations, with the RSD ranging from 1.5 to 2.0 %, 1.6 to 3.3 %, and 1.3 to 2.5 %, respectively. It is also noticeable that there is a "scattering of data" which is not reflected in the low RSDs. Again, it seems that the scattering of data associated with the recovery results reflects the true precision of the overall method, whereas the excellent RSDs reflect only the excellent precision of the second step of the method. We will try our best to give priority to obtaining better precision of the overall method in future research work. Still, the method developed is potentially applicable for the simultaneous separation and determination of trace PEEs in water samples.

Conclusions

In conclusion, a good, easy, and efficient method for the determination of PEEs in water samples was developed. An off-line preconcentration method (dCPE) and an online enrichment method (two-step injection) were combined. The dCPE with TX-114 and NaOH was validated as being easy, practical, and effective for the preconcentration of PEEs. The two-step injection provided a rapid and remarkable increase in the injected amount of analytes in CE. The combined EFs (50-fold to 150-fold) indicated a potentially tremendous sample enhancement power. The dCPE–two-step injection–CE method with simple UV detection offered good quantitative ability, high precision, and a wide linear range, and it was demonstrated to be a simple, fast, cost-effective, and eco-friendly option for simultaneous determination of PEEs in water samples. Given the advantages, further research focusing on various combined enrichment methods will be promising for trace analysis of various estrogens in complicated samples by using CE.

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References

1. Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacob DR, Lee DH, Shioda T, Soto AM, vom Saal FS, Welshons WV, Zoeller RT, Myers JP (2012) Endocr Rev 33:378–455
2. Xu Q, Wang M, Yu SQ, Tao Q, Tang M (2011) Analyst 136:5030–5037
3. Liu MH, Li MJ, Qiu B, Chen X, Chen GN (2010) Anal Chim Acta 663:33–38
4. Kuster M, Alda MJL, Barceló D (2004) TrAC Trends Anal Chem 23:10–11
5. Tan LC, Ge F, Shan ZJ, Wang Y (2011) Chin J Ecol Rural Environ 27:86–92
6. Chen XB, Wu YL, Yang T (2011) J Chromatogr B Anal Technol Biomed Life Sci 879:799–803
7. Lucci P, Núñez O, Galceran MT (2011) J Chromatogr A 1218:4828–4833
8. Sapozhnikova Y, Hedgespeth M, Wirth E, Fulton M (2011) Anal Methods 3:1079–1086

9. Zhou JB, Hu C, Zhao RS (2011) Anal Methods 3:2568–2572
10. Ji YS, Liu XY, Guan M, Zhao CD, Huang HY, Zhang HX, Wang CM (2009) J Sep Sci 32:2139–2145
11. Xu ZX, Zhang J, Cong L, Meng L, Song JM, Zhou J, Qiao XG (2011) J Sep Sci 34:46–52
12. Liu SF, Wu XP, Xie ZH, Lin XC, Guo LQ, Yan C, Chen GN (2005) Electrophoresis 26:2342–2350
13. Fogarty B, Dempsey E, Regan F (2003) J Chromatogr A 1014:129–139
14. Wang LL, Wang JQ, Zheng ZX, Xiao P (2010) J Hazard Mater 177:114–118
15. Wetterhall M, Shevchenko G, Artemenko K, Sjödin MOD, Bergquist J (2011) Anal Bioanal Chem 400:2827–2836
16. Seebunrueng K, Santaladchaiyakit Y, Soisungnoen P, Srijaranai S (2011) Anal Bioanal Chem 401:1703–1712
17. Seebunrueng K, Santaladchaiyakit Y, Srijaranai S (2012) Anal Bioanal Chem 404:1539–1548
18. Kukusamude C, Santalad A, Boonchiangm S, Burakham R, Srijaranai S, Chailapakul O (2010) Talanta 81:486–492
19. Wei W, Yin XB, He XW (2008) J Chromatogr A 1202:212–216
20. Yin XB, Guo JM, Wei W (2010) J Chromatogr A 1217:1399–1406
21. Feng YL, Zhu JP (2006) Anal Chem 78:6608–6613
22. Breadmore MC, Quirino JP (2008) Anal Chem 80:6373–6381
23. Wen YY, Li JH, Ma JP, Chen LX (2012) Electrophoresis 33:2933–2952
24. Zhang HJ, Gavina J, Feng YL (2011) J Chromatogr A 1218:3095–3104
25. Liu HT, Wen YY, Luan F, Gao Y (2008) Acta Chromatogr 20:239–246
26. Iqbal J, Müller CE (2011) J Chromatogr A 1218:4764–4771
27. Wen YY, Chen LX, Li JH, Ma YL, Xu SF, Zhang Z, Niu ZL, Choo J (2012) Electrophoresis 33:2454–2463
28. Robert W, Ira SL (1991) Anal Chem 63:823–827
29. Cikalo MG, Bartle KD, Myers P (1999) J Chromatogr A 836:35–51
30. Li JH, Chan W, Cai ZW (2009) Electrophoresis 30:1790–1797
31. López-Grío S, Baeza-Baeza JJ, García-Alvarez-Coque MC (1998) Chromatographia 48:655–663
32. Chen LX, Lee S, Choo J, Lee EK (2008) J Micromech Microeng 18:013001
33. Mitra S (2003) In: Winefordner (ed) Sample preparation techniques in analytical chemistry. Wiley, Hoboken
34. Liu JF, Chao JB, Liu R, Tan ZQ, Yin YG, Wu Y, Jiang GB (2009) Anal Chem 81:6496–6502
35. Wu YJ, Fu XW, Yang H (2011) Arch Environ Contam Toxicol 61:359–367
36. Chang HT, Su YC, Chang ST (2006) Polym Degrad Stab 91:816–822
37. Campbell CG, Borglin SE, Green FB, Grayson A, Wozei E, Stringfellow WT (2006) Chemosphere 65:1265–1280