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A sweeping-micellar electrokinetic chromatography method for the detection of three chlorophenols in cosmetic samples

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ABSTRACT

A sweeping micellar electrokinetic chromatography (sweeping-MEKC) enrichment model was established for the determination of three chlorophenols (CPs) in cosmetics, namely, bithionol, pentachlorophenol (PCP), and 2,4,6-trichlorophenol (2,4,6-TCP). The optimum electrophoretic conditions were 20 mM NaH₂PO₄-80 mM sodium dodecyl sulfate (SDS) and 30% (v/v) acetonitrile (pH 2.3). The optimum on-line concentration conditions were as follows: sample matrix, 100 mM NaH₂PO₄; pressure injection at 20.67 kPa (3 psi) for 25 s. The linear range of bithionol, PCP, and 2,4,6-TCP are 0.20–4.00 µg mL⁻¹, 0.10–2.00 µg mL⁻¹, and 0.05–2.00 µg mL⁻¹ respectively, with correlation coefficient (*r*) over 0.9972. The limits of detection (LOD) based on three times the signal-to-noise ratio (*S/N* = 3) are in the range of 0.0061–0.024 µg mL⁻¹. Recoveries for the three CPs in powder and lotion samples are between 79.7 and 110.2% with relative standard deviation (RSD) of 1.38–5.54% and 92.2–121.3% with RSD of 0.72–6.09%, respectively. The proposed method can provide reference for the determination of trace CPs in different sample matrix.

KEYWORDS

sweeping micellar electrokinetic chromatography, bithionol, pentachlorophenol, 2,4,6-trichlorophenol, cosmetics

INTRODUCTION

Halogenated phenols have strong bactericidal action against gram-positive bacteria. They are generally used as preservative in toothpaste, cream, and lotion cosmetics, and the most commonly used are chlorophenols (CPs) [1]. Cosmetics are a common component of our daily lives and usually contain preservatives that are added to prevent microbial growth [2]. Bithionol has been used as preservatives in cosmetics production, but it has been banned by the United States (US) Food and Drug Administration due to photosensitivity and mild urticaria reaction [3]. Studies show that the toxicity of halogenated phenol is higher than that of phenol, and it increases with the increase of the number of halogen atoms. Long-term exposure can cause damage to multiple organs of the human body, and its high toxicity and non-degradability have been widely concerned [4–7]. China's cosmetics hygiene regulations (2007 version) clearly stipulate that bithionol, pentachlorophenol (PCP), 2,4,6-trichlorophenol (2,4,6-TCP) are banned substance in cosmetics, and should not be used as a cosmetic raw material [8]. Therefore, the detection of bithionol, PCP, and 2,4,6-TCP residues in cosmetics is of great significance to ensure the quality of cosmetics products, which can protect the health of consumers and improve the cosmetics inspection technology system.

Several methods have been developed to detect and quantify CPs in different samples. CPs in water samples and cosmetics have been analyzed by high performance liquid chromatography with ultraviolet detection (HPLC-UV) [9, 10] or photodiode array detection (HPLC-DAD) [11, 12]. Gas chromatography (GC) with either flame ionization detection (FID) [13], or mass spectrometric (MS) [14] detection also are used to detect phenolic compounds in urine samples.

Na_2HPO_4 and filtered through a 0.45 μm filter for further CE analysis.

CE conditions

Before the first usage, new capillary was conditioned by the procedure referring to our previous work [7], and the rinsing pressure was 20 psi (1 psi = 6894.76 Pa). The background solution (BGS) consisted of 20 mM NaH_2PO_4 -80 mM SDS and 30% (v/v) ACN, and the solution pH was adjusted with 3 mol L^{-1} H_3PO_4 after addition of ACN to pH 2.3. Sample matrix was 100 mM Na_2HPO_4 , and samples were injected by pressure at 3 psi $\times$ 25 s. The applied separation voltage was -15 kV and the detection wavelength was set at 214 nm. BGS with different concentrations was prepared from 200 mM Na_2HPO_4 and 400 mM SDS stock solutions. All electrolytes and samples were filtered through 0.45 μm membrane filters and degassed by ultrasonic prior to analysis.

Sweeping-MEKC

When preparing the sample, the working stock solution was diluted with sample matrix containing no SDS (non-micelle BGS) and a conductivity value similar to that of the micellar BGS. In sweeping-MEKC, in order to suppress the electro-osmotic flow (EOF) and make the electrophoretic speed of SDS larger than the EOF rate, an acidic phosphate buffer solution (pH 2.3) was selected. Much longer than the normal sample plug sample was then hydrodynamically injected into a capillary. The PSP with negative charge has a higher electrophoretic velocity than that of the CPs without charge under low pH conditions. It made sure that the PSP penetrates into the sample plug and the analytes in the sample zone are concentrated into a relatively narrow zone under the sweeping of the micelle. The introduction of a large sample zone is integrated into a narrow sample zone to improve the sensitivity of the test and then the voltage was applied at negative polarity (positive at outlet) until all peaks were detected.

RESULTS AND DISCUSSION

Optimization of sweeping-MEKC

Fig. 2 gives a brief description for the sweeping-MEKC process of CPs in the presence of very low (nearly zero) EOF. In the beginning (Fig. 2A), CPs solution has similar conductivity as that of the injected BGS. The length of the CPs zone injected is given as L_{inj} , which is much longer than that of normal injection. Because the pK_a values of bithionol, PCP, and 2,4,6-TCP are 4.82, 4.93, and 5.99, respectively, the CPs are nearly uncharged molecules in the BGS with pH value lower than their pK_a , upon application of voltage at negative polarity (Fig. 2B), the electrophoretic velocity are nearly zero. The PSP with negative charge has a higher electrophoretic velocity thus sweep the sample plug toward the anode (detection window). After a certain period of time, all CPs molecules are picked and accumulated by PSP

(Fig. 2C), the accumulated CPs are then separated by MEKC.

In the sweeping preconcentration mode, the relationship between the length of the swept zone (L_{swept}) and that of the injected zone (L_{inj}) is described by the following equation [29]:

$$L_{\text{swept}} = L_{\text{inj}} \frac{1}{1+k} \quad (1)$$

where k is the retention factor of the analyte. The sweeping phenomenon initially proposed by Quirino and Terabe [28] had shown that L_{swept} is solely dependent on the retention factor (k) and the length of the initial zone (L_{inj}).

SDS concentration

According to the equation above, narrower bands of the swept zone are obtained with larger k values, which in turn can be obtained with higher concentrations of SDS. The concentration of SDS from 60 to 100 mM was investigated. The peak grew higher from 60 to 80 mM (Fig. 3A). The migration time of analytes decreased with the increase of SDS concentration because more and more of the analytes were incorporated into the micellar phase. However, a higher concentration at 100 mM did not further increase the peak height and the stability of the peak is deteriorated due to Joule heating. From the standpoint of higher sweeping efficiency and shorter analytical time 80 mM SDS was selected and used in the micellar BGE for sweeping-MEKC.

Kinds and content of organic solvent

The addition of organic solvent in the buffer solution could adjust the distribution capacity of the analytes in the two phases of the micelle and buffer system to improve the separation degree and peak shape of the analytes. In the experiment, 25 mM NaH_2PO_4 and 80 mM SDS were used as buffer, and 30% MeOH and ACN were added respectively to investigate the influence on the peak of the analytes. The results showed that both of the two kinds of organic solvent

Fig. 2. Schematic illustration of sweeping-MEKC. (A) Starting situation, a longer than a typical injection of CPs prepared in a matrix having a conductivity similar to that of the BGS; (B) application of voltage at negative polarity, micelles emanating from the cathodic side sweeping CPs; (C) the injected CPs are assumed to be completely swept

Fig. 3. Effect of SDS concentration (A) and ACN concentration (B) on peak area of the three CPs in sweeping-MEKC system. Other sweeping-MEKC conditions: BGE, 25 mM NaH_2PO_4 with SDS, and ACN concentration indicated in the figure (pH 2.3); samples: bithionol, PCP, and 2,4,6-TCP; 2, 1, 1 $\mu\text{g mL}^{-1}$ dissolved in 80 mM NaH_2PO_4 ; injection, 3 psi $\times$ 25 s; separation voltage, -15 kV

could improve the peak shape of the analytes. Compared with MeOH, ACN as an additive can get better peak shape and larger peak area. Therefore the content of ACN (20, 25, 30, 35, 40% (v/v)) was investigated and the results were shown in Fig. 3B. As the ACN content increased, the separation time of the analytes were prolonged. Considering both peak shape and peak area, 30% ACN was selected.

Running buffer concentration and sample matrix composition

The effect of the concentration of running buffer on the separation of the three CPs in the range of 10–40 mM is illustrated in Fig. 4A. With the increase of NaH_2PO_4 concentration, the separation time of the analytes was prolonged, but the peak shape and resolution did not change significantly. When the concentration of NaH_2PO_4 was 20 mM, the sensitivity enhancement of the three CPs reaches the maximum. Therefore, 20 mM NaH_2PO_4 was used. Sample matrix is important for sweeping-MEKC since it could decide the dissociation of the analytes, which affect the

degree of sample stacking. The effect of sodium dihydrogen phosphate solution with concentrations of 60–120 mM on sweeping was investigated. The results in Fig. 4B indicate that the highest peak intensity of CPs was achieved using the sample matrix solution of 100 mM NaH_2PO_4 .

Sample injection time and separation voltage selection

For sweeping technique, the injected length of an analyte zone could be limited by the sweeping capacity of the micelles, which is dependent on the retention factor (k) of the analytes with the micelles. When k goes to infinity, only the column length restricts the injection lengths. If the k value of the analyte is not high enough, it is suggested that the length of the injected sample must be optimized. In this study, the sample solution was injected at 3 psi for 10, 20, 25, and 30 s into the capillary column. From Fig. 5 we can see that the peak height of the analytes increased when the injection time increased from 10 to 30 s. When the injection time was increased to 25 s, the three CPs can get better resolution and detection sensitivity. When the injection time is 30 s,

Fig. 4. Effect of buffer concentration (A) and sample matrix composition (B) on the sweeping efficiency for the three CPs. Peak identification: (1) bithionol, (2) PCP, and (3) 2,4,6-TCP; BGE: the concentration of NaH_2PO_4 indicated in Fig. 4A with 80 mM SDS and 30% ACN (pH = 2.3); Other conditions same to Fig. 3

Fig. 5. Effect of injection time on the sweeping-MEKC efficiency for the three CPs. BGE: 20 mM NaH_2PO_4 , 80 mM SDS, and 30% (v/v) ACN (pH 2.3); Sample matrix: 100 mM NaH_2PO_4 ; Other conditions the same as Fig. 3. Peak identification: the same as Fig. 4

although the sensitivity of the analyte is higher, the resolution is decreased. Hence, 25 s was used as the optimal injection time. The effect of separation voltage (–15, –17, and –20 kV) on separation was also investigated. The results show that the peak area of the analytes decreases with the increasing voltage. Further increase of separation voltage resulted in current disruption due to Joule heating. Therefore, –15 kV was chosen as the separation voltage.

To sum up, the optimum conditions are as follows: BGS composed of 20 mM NaH_2PO_4 , 80 mM SDS, and 30% (v/v) ACN (pH 2.3); sample matrix composed of 100 mM NaH_2PO_4 ; sample was injected by pressure injection (3 psi) for 25 s.

Fig. 6. Electropherograms of the sweeping-MEKC (a) and CZE (b). (a) sweeping-MEKC: BGE: 20 mM NaH_2PO_4 , 80 mM SDS, and 30% (v/v) ACN (pH 2.3); sample matrix: 100 mM NaH_2PO_4 ; pressure injection: 3 psi $\times$ 25 s; separation voltage: –15 kV; 214 nm. bithionol ($2 \mu\text{g mL}^{-1}$), PCP ($1 \mu\text{g mL}^{-1}$), 2,4,6-TCP ($1 \mu\text{g mL}^{-1}$). (b) CZE: BGE: 15 mM $\text{Na}_2\text{B}_4\text{O}_7$ and 15% (v/v) MeOH (pH 9.8); pressure injection: 0.5 psi $\times$ 5 s; separation voltage: +15 kV; 214 nm. Bithionol ($20 \mu\text{g mL}^{-1}$), PCP ($10 \mu\text{g mL}^{-1}$), 2,4,6-TCP ($10 \mu\text{g mL}^{-1}$).

Peak identification: (1) bithionol, (2) PCP, and (3) 2,4,6-TCP

In order to estimate the sensitivity increase achieved in sweeping-MEKC, sensitivity enhancement factors (EFs) based on peak area were estimated from the equation:

$$EF = \frac{A_{\text{swept}}}{A_{\text{CZE}}} \quad (2)$$

where A_{swept} and A_{CZE} are the peak areas of the analytes after sweeping-MEKC and conventional capillary zone electrophoresis (CZE) [32].

Method performance

Under the optimal conditions, Fig. 6 shows the electropherograms of CPs obtained by sweeping-MEKC (a) and normal CZE (b). The EFs for the three CPs were 57 (bithionol), 76 (PCP) and 57 (2,4,6-TCP), respectively. The detection limits, linearity, and repeatability of the three CPs under optimal conditions are shown in Table 1. The linearity of the sweeping-MEKC method was evaluated by calibration curves in the range of $0.20\text{--}4.00 \mu\text{g mL}^{-1}$ for bithionol, $0.10\text{--}2.00 \mu\text{g mL}^{-1}$ for PCP, and $0.05\text{--}2.00 \mu\text{g mL}^{-1}$ for 2,4,6-TCP at five concentration levels. Linear relationship was found between peak area and the concentration of the analytes with correlation coefficient >0.99 , indicating a good linearity of this method. The LOD were obtained based on the peak height as three times of background noise ($S/N = 3$), in the range of $0.0061\text{--}0.024 \mu\text{g mL}^{-1}$ and the limits of quantification (LOQ) were obtained based on the peak height as ten times of background noise ($S/N = 10$), in the range of $0.020\text{--}0.080 \mu\text{g mL}^{-1}$, which meet the requirements of trace analysis. From Table 2 we can see that the intra-day precision of migration time and peak area were $0.17\text{--}1.64$ and $2.61\text{--}3.90\%$, respectively. The inter-day precision of migration time and peak area were $2.44\text{--}3.57$ and $3.54\text{--}6.55\%$, respectively. The repeatability of this method is satisfactory. All these results indicated that the developed sweeping-MEKC method could be a reliable approach for the analysis of CPs with high sensitivity.

Sample analysis

To demonstrate how the sweeping-MEKC method can be applied for routine analysis of real cosmetic samples, the developed sweeping-MEKC method was applied for analyzing the three CPs levels in two cosmetic samples including loose powder and body milk. Fig. 7 (curve a) showed the electropherograms of blank cosmetic samples. It can be seen that none of the three CPs were found in blank cosmetic samples. As mentioned in the introduction, China's cosmetics hygiene regulations (2007 version) clearly stipulate that bithionol, PCP, 2,4,6-TCP are banned substance in cosmetics, and should not be used as a cosmetic raw material [8]. To further evaluate the performance of the sweeping-MEKC method in complex matrices and verify the specificity and accuracy of the proposed method, three replicates of the cosmetic samples spiked with 0.50 (Fig. 7 curve b) and $1.00 \mu\text{g mL}^{-1}$ (Fig. 7 curve c) of the three CPs were analyzed. From Fig. 7 we can see that the three CPs can be detected and well separated with other components in the samples. As listed in Table 3, recoveries for bithionol,

Table 1. Parameters of the analytical performance of sweeping-MEKC for the three CPs

CPs	Calibration curve ^a	Correlation coefficient (<i>r</i>)	Linear range (μg mL ⁻¹)	LOD (μg mL ⁻¹)	LOQ (μg mL ⁻¹)	EF
bithionol	$y = 120350x - 29797$	0.9975	0.20–4.00	0.024	0.080	57
PCP	$y = 186590x - 9670.9$	0.9972	0.10–2.00	0.015	0.048	76
2,4,6-TCP	$y = 180935x - 3677.8$	0.9998	0.05–2.00	0.0061	0.020	57

^a *y* and *x* stand for the peak area and the concentration (μg mL⁻¹) of the three CPs, respectively.

Table 2. Method precision of sweeping-MEKC for three CPs

CPs	RSD ^a (% , <i>n</i> = 5)			
	Intra-day		Inter-day	
	Migration time	Peak area	Migration time	Peak area
Bithionol	1.64	3.90	3.57	6.55
PCP	1.41	2.61	3.54	3.54
2,4,6-TCP	0.17	2.72	2.44	5.86

^a The concentrations of 3 CPs are 2, 1, 1 μg mL⁻¹ for bithionol, PCP and 2,4,6-TCP, respectively were chosen for the assays of intra-day precision and inter-day precision.

PCP, 2,4,6-TCP in loose powder and body lotion were between 79.7 and 110.2% with relative standard deviations (RSDs) of 1.38–5.54% and 92.2–121.3% with RSDs of 0.72–6.09%, respectively. All of these results demonstrate that the sweeping-MEKC method developed in this work is an alternative approach for analyzing CPs levels in cosmetic samples.

CONCLUSION

In this experiment, the electrophoretic separation mode of MEKC, combined with its unique sweeping technology, was selected to establish a method for simultaneous enrichment, separation and detection of bithionol, PCP, and 2,4,6-TCP.

Fig. 7. Sweeping-MEKC analysis of three CPs in blank and spiked samples of loose powder (A) and body lotion (B). (a) without spiking; (b) 3CPs, 0.50 μg mL⁻¹, (c) 3CPs, 1.00 μg mL⁻¹. Peak identification: (1) bithionol, (2) PCP, and (3) 2,4,6-TCP; BGE: 20 mM NaH₂PO₄, 80 mM SDS, and 30% (v/v) ACN (pH 2.3); Sample matrix: 100 mM NaH₂PO₄; Other conditions the same as Fig. 3

Table 3. Recoveries of the three CPs in cosmetic samples by sweeping-MEKC (*n* = 3)

CPs	Background (μg mL ⁻¹)	Added (μg mL ⁻¹)	Loose powder			Body lotion		
			Found (μg mL ⁻¹)	Recovery (%)	RSD (%)	Found (μg mL ⁻¹)	Recovery (%)	RSD (%)
Bithionol	ND	0.50	0.55 ± 0.025	110.2	4.64	0.61 ± 0.035	121.3	5.74
		1.00	0.80 ± 0.020	79.7	2.51	0.92 ± 0.056	92.2	6.09
PCP	ND	0.50	0.45 ± 0.011	89.2	2.46	0.52 ± 0.021	104.6	4.04
		1.00	1.01 ± 0.014	101.2	1.38	0.99 ± 0.007	100.0	0.72
2,4,6-TCP	ND	0.50	0.48 ± 0.025	96.0	5.21	0.55 ± 0.045	110.0	5.18
		1.00	0.81 ± 0.061	80.9	5.54	1.13 ± 0.042	99.9	3.71

ND: not detected.

The influencing factors for separation and enrichment in the sweeping-MEKC were systematically investigated. The method has the advantages of high enrichment factor, high sensitivity, simple operation, good repeatability and low cost, and has been successfully applied to the determination of the three CPs in cosmetic samples. It provides an effective way for the detection of CP fungicides in cosmetics.

Conflict of interest: There are no conflicts to declare.

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