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Comparative study for the analysis of parabens by micellar electrokinetic capillary chromatography with and without large-volume sample stacking technique

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Abstract

The separation and determination of four parabens (methyl, ethyl, propyl, and butyl *p*-hydroxybenzoate) which are commonly used as preservatives in cosmetic products, by micellar electrokinetic capillary chromatography (MEKC) with and without large-volume sample stacking (LVSS) technique were compared. As an effective on-line concentration technique, LVSS was successfully combined with MEKC to determine neutral parabens in an acidic media. The effects of some typical parameters such as sample volume, buffer pH, temperature, and concentration of surfactant were examined. The detection limits for this LVSS-MEKC method were found to be 3.0×10^{-7} M for each of the parabens based on the signal-to-noise ratio of 3, which were around 300 times lower than normal MEKC technique. The curves of peak response versus concentration were linear from 1.0×10^{-6} to 5.0×10^{-5} M with regression coefficients of 0.9987, 0.9960, 0.9925 and 0.9864, respectively. A simple and easy-manipulative sample preparation method was developed and validated by analyzing commercially available cosmetic samples. It was found that with current sample preparation process and instrumentation system, 0.5 g of sample is enough for the analysis of parabens preservatives in cosmetic product with satisfactory results.

Keywords: Parabens; Cosmetic; Large-volume sample stacking; Micellar electrokinetic capillary chromatography

1. Introduction

In order to prolong products' shelf life, parabens, a group of closely related chemicals which are esters of *p*-hydroxybenzoic acid, are widely used as preservatives in underarm deodorants, antiperspirants and other cosmetic products for inhibiting the development of micro-organisms. It is well known that most of the preservatives may be harmful to the consumers due to their potency to induce allergic contact dermatitis and some other diseases [1–5]. Moreover, the parabens also have inherent oestrogenic and other hormone related activity (increased progesterone receptor gene expression). As oestrogen is a major aetiological factor in the growth and development of the majority of human breast cancers, parabens in underarm cosmetics may contribute to the rising incidence of breast cancer [6]. Four main parabens are in use: methyl, ethyl, propyl and butylparabens; many products will have two or more of these chemicals as part of a preservative system. Therefore, the highest concentration of single parabens allowed in a cosmetic product is 0.4% (w/w). For mixtures of parabens the highest total parabens concentration allowed is 0.8% (w/w) [7]. Obviously, the significance of developing a method for the assay of these substances in cosmetic products with high precision and accuracy is highly topical. Fig. 1 shows the chemical structures of such four parabens analyzed in this paper.

Published methods for the analysis of parabens in cosmetic products are mainly based on high-performance liquid chromatography (HPLC) [8–11]. Nowadays, compared with HPLC, capillary electrophoresis (CE) has become a more attractive separation technique in preservative analysis due to its many advantages including high efficiency, low waste production, and fast separation [10,12–15]. However, the usage of HPLC and CE for a particular application is very dependent upon the relative merits of each technique to the individual assay.

Micellar electrokinetic chromatography (MEKC) is a powerful CE separation approach that augments the usage

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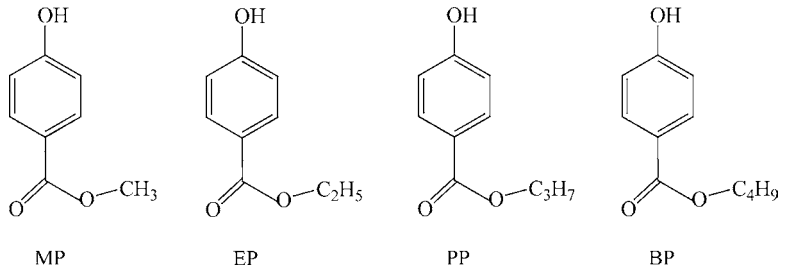

Fig. 1. Chemical structures of methyl *p*-hydroxybenzoate (MP), ethyl *p*-hydroxybenzoate (EP), propyl *p*-hydroxybenzoate (PP) and butyl *p*-hydroxybenzoate (BP).

of electrokinetic phenomena for the separation of nonionic compounds [16]. MEKC separations are based on the partitioning of neutral solutes between the electrokinetically moved micellar phase and the electroosmotically pumped bulk aqueous phase. One restraint of the technique is the low concentration sensitivity as a consequence of the limited optical path length for on-line UV detection and the limited volume of sample solution that can be injected. This hinders the application of MEKC in the analysis of real samples.

Sample stacking is an inherent and exclusive feature of CE [17], taking place when the sample compounds encounter isotachophoretic concentration at the interface between sample zone and buffer (isotachophoretic sample stacking, ITPSS) [18,19] or when the conductivity of the sample is smaller than that of the buffer (field-amplified sample stacking, FASS) [20,21]. Additionally, large-volume sample stacking (LVSS) has been demonstrated to improve detection limits of charged analytes by more than 1000-fold and to be easily automated and controlled by software [22–28]. Sweeping is another on-line sample concentration method for either charged or neutral analytes, the sample concentration effect relies on how the pseudostationary phase enters the sample solution zone (nonmicelle buffer) and sweeps the analytes [29–34]. Prof. Terabe has reviewed the on-line concentration of neutral analytes for MEKC [35–39]. To our knowledge, no paper regarding the analysis of neutral parabens by MEKC combined with LVSS technique are published.

The aim of this work was to develop an alternative for the analysis of trace parabens based on MEKC, with improved resolution and better sensitivity by combining LVSS technique. The developed method has been successfully applied in the separation and determination of low levels of parabens in cosmetic samples with reliable results.

2. Experimental

2.1. Apparatus

All experiments were performed with an HP^{3D} CE system with air-cooling and a diode-array detector (Agilent Technologies, Palo Alto, CA, USA). Separations were performed in 58.5 cm (50.0 cm to detector) × 50 μm i.d. uncoated fused-silica capillaries (Yongnian Optical Fiber Factory, Hebei, China). UV detection was carried out at 210 nm. Prior to separation, the new capillary was flushed with 1.0 M sodium hydroxide for 10 min and then with deionized water for 10 min. The capillary was conditioned daily by purging with 1.0 M sodium hydroxide for 5 min. Between consecutive analysis, the capillary was flushed with 1.0 M sodium hydroxide (1 min), then with methanol (1 min), water (2 min), and finally with the running buffer (2 min) in order to improve repeatability.

2.2. Reagents and solutions

All chemicals used were of analytical-reagent grade. Sodium dodecyl sulfate (SDS) was purchased from Fluka (Buchs, Switzerland). Methylparaben, ethylparaben, propylparaben and butylparaben were obtained from Beijing Chemical Reagent Company (Beijing, China).

2.3. Procedure

Methanol/water mixing solvent (3:2 v/v) was used to dissolve the samples. Phosphoric acid buffer (50 mM, pH 2.28, containing 100 mM SDS and 1% v/v methanol) was selected as the running buffer. All electrolyte solutions were filtered through a 0.45 μm polytetrafluoroethylene membrane filter. All measurements were carried out at least three times.

3. Results and discussion

3.1. Parabens separation by MEKC

In the absence of SDS, neutral parabens could not be separated under capillary zone electrophoresis mode (CZE). With the application of negative voltage, the electrophoretic mobility of SDS-parabens was in the direction opposite to that of the electroosmotic flow (EOF). If the former was greater than the latter, parabens might be separated.

A series of injection time at 50 mbar were investigated by using a phosphoric acid buffer (50 mM, pH 2.28) containing 100 mM SDS and 1% methanol (v/v). With the voltage of −20 kV and the injection time of 1 s, all four parabens were separated completely in better peak shapes and the repeatability was also good for each of the compounds.

When the concentration of SDS surfactant in the above running buffer was changed from 50 to 150 mM, migration behaviors for most analytes were markedly influenced. As the concentration of SDS increased in the running buffer, the migration velocities of MP, EP and PP increased, while the migration velocity of BP decreased at first and then got back. A good separation was obtained with the running buffer containing 100 mM SDS.

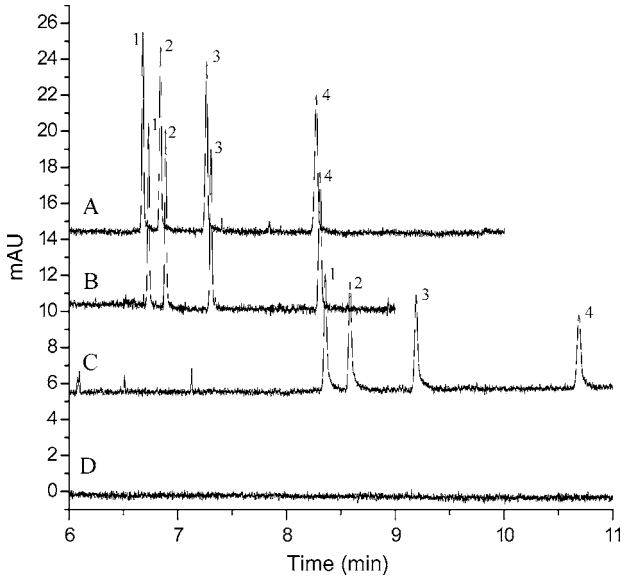

Fig. 2. Effect of buffer pH on the separation of MP, EP, PP and BP (5.0×10^{-4} M each) by normal MEKC. Separation conditions: 50 mM pH 2.28 phosphoric acid buffer containing 100 mM SDS and 1% v/v methanol, −20 kV applied voltage, 25 °C capillary temperature, injection at 50 mbar for 1 s, buffer pH: (A) 2.28, (B) 3.14, (C) 3.81, (D) 4.55; peak identity: (1) BP, (2) PP, (3) EP, (4) MP.

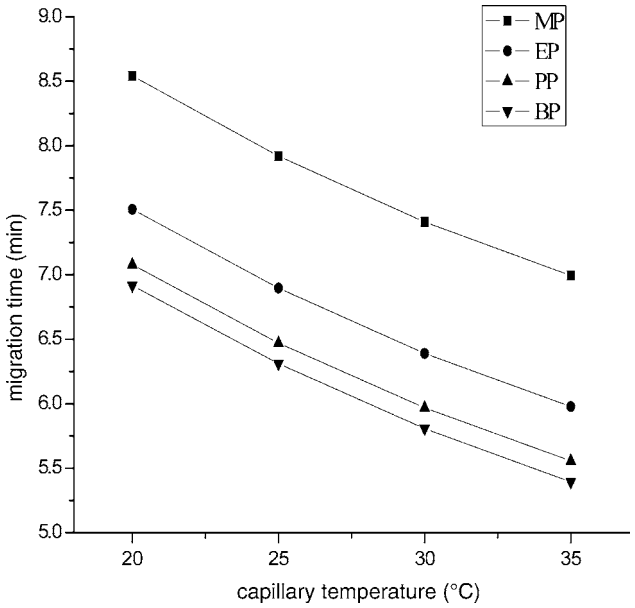

Fig. 3. Effect of capillary temperature on the separation by normal MEKC. Other conditions are the same as in Fig. 2A.

The addition of methanol in buffer resulted in the decrease of EOF, which advantaged the shortening of analysis time. Moreover, the presence of 1% methanol (v/v) could obviously improve the peak shapes.

The effect of buffer pH was also carefully investigated. As shown in Fig. 2, no obvious differences were observed for the separation between curves A and B, which indicated that the EOF had been decreased to approach zero as long as pH was below 3. As buffer pH increased more, the EOF was gradually increased, which resulted in longer migration time. For instance, no analyte peak emerged in 11 min when pH was above 4.5 (see curves C and D in Fig. 2).

The migration time was shortened when either the applied voltage of capillary temperature was enhanced (Fig. 3). Under −30 kV applied voltage, all the analytes can be separated completely within 5 min (Fig. 4).

In a word, the optimal conditions for the baseline separation of four analytes by normal MEKC method are as follows: 50 mM phosphoric acid buffer containing 100 mM SDS and 1% v/v methanol (pH 2.28) as the running buffer, 50 mbar as injection pressure and 1 s as injection time, −30 kV as applied voltage and 35 °C as capillary temperature (Fig. 4). A series of calibration solutions were prepared with parabens concentrations ranging from 1.0×10^{-4} to 1.0×10^{-3} M. Based on a signal-to-noise ratio of 3, the detection limit was as low as 8.0×10^{-5} M for each of the parabens. Other quantification data is shown in Table 1. It can be found that such a normal MEKC method is not available for the accurate determination of parabens.

3.2. Large-volume sample stacking procedure

In order to improve the sensitivity of this MEKC method for determining parabens, large-volume sample stacking was applied. Generally, in LVSS the electrophoretic mobility of the target ions must be in the direction opposite to that of the EOF in order that cations or anions could be focused at the rear or front of the sample zone. During the separation procedure, the electrophoretic velocities of interested ions must be greater than the EOF velocities so that the two procedures can proceed consecutively under the same voltage [25]. In our system, the electrophoretic velocities of SDS-parabens (V) must be greater than the EOF velocity (V_{EOF}). This is required for both removing the sample matrix from the capillary using EOF pump and the subsequent separation. When negative voltage is applied from

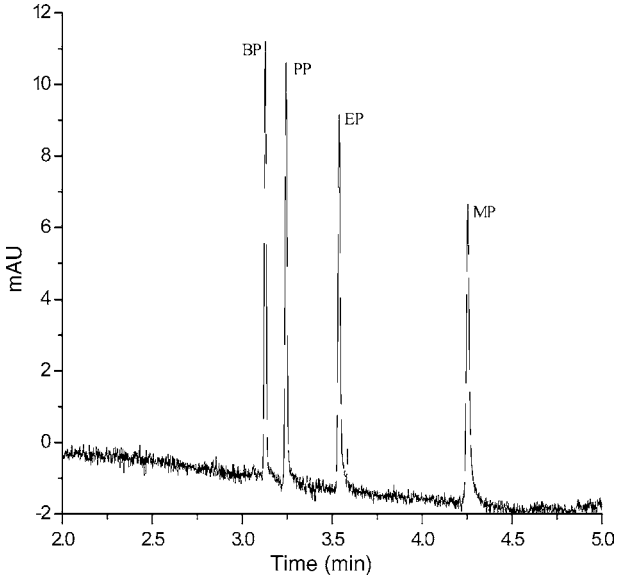

Fig. 4. The separation of MP, EP, PP and BP (5.0×10^{-4} M each) in 50 mM phosphoric acid buffer containing 100 mM SDS and 1% v/v methanol (pH 2.28) by normal MEKC. Separation conditions: injection at 50 mbar for 1 s, −30 kV applied voltage, 35 °C capillary temperature.

Table 1
Quantification data of MEKC

Parameter	Regression equation ($y = ax + b$)		r	Detection limit (M)	Linear range (M)
	a	b			
MP	15	1.1	0.9742	8.0×10^{-5}	1.0×10^{-4} to 1.0×10^{-3}
EP	13	0.76	0.9841		
PP	13	0.81	0.9849		
BP	13	0.62	0.9890		

y, peak area (Au min); x, concentration of parabens ($\times 10^{-3}$ M); r, regression coefficient.

the cathodic inlet toward the anodic outlet, the anions will move to the outlet. Thus, LVSS and water removal can be carried out under the same voltage. On the basis of this principle, a scheme was designed for the stacking and separation of parabens, as illustrated in Fig. 5.

Firstly, after large volumes sample were introduced into the capillary by pressure injection, the whole capillary was separated into two zones: the sample zone and the running buffer zone. Then, a negative voltage was applied and the sample stacking began. Due to its poor conductivity, the electric field strength was mainly applied in the sample zone. Because of the applied negative electric field, the negatively charged SDS molecules would combine with parabens and move towards the anodic end in the sample zone until they encountered the electrolyte boundary where they experienced a lower applied field and their migration rate slowed down. During this process, these SDS-parabens were stacked at the interface between the low-conductivity sample zone and the running buffer zone. In the meantime, the EOF pump pushed out the water in the sample zone. The final result was that the stacked SDS-parabens were focused at the start point of capillary. Once the water in the sample zone was totally pushed out, the subsequent separation began. If the EOF in the running buffer zone could be suppressed, for instance, an acidic buffer was chosen as the running buffer, these concentrated SDS-parabens would move towards the anodic outlet until they were detected when passing through the capillary window. Therefore,

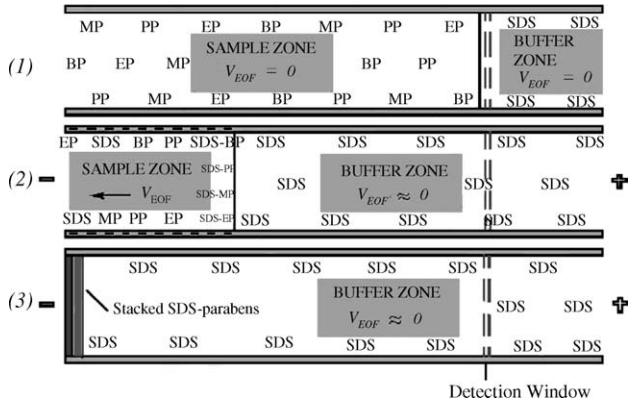

Fig. 5. Schematic illustration of the matrix removal and stacking of parabens. (1) Sample injection; (2) sample stacking; (3) ready to separate.

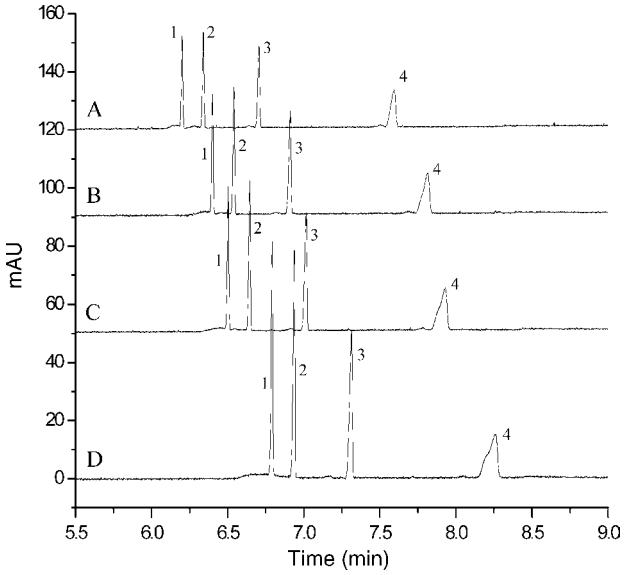

Fig. 6. Effect of injection time on the separation of MP, EP, PP and BP (2.5×10^{-5} M each) by LVSS-MEKC. Separation conditions: 50 mM phosphoric acid buffer containing 150 mM SDS and 1% v/v methanol, −20 kV applied voltage, 25 °C capillary temperature, injection time: (A) 60 s, (B) 80 s, (C) 90 s, (D) 120 s; peak identity: (1) BP, (2) PP, (3) EP, (4) MP.

this stacking would result in an improved sensitivity and high peak efficiency.

In addition, since the volume of the capillary is very small and there is almost no analytes pushed out during the sample stacking, there is no need to change the inlet running buffer between each runs. All measurements were carried out at least three times with good reproducibility (R.S.D. ≤3%, $n = 6$).

3.3. Effect of sample volume

Sample solutions containing four parabens (2.5×10^{-5} M each) were injected into the capillary at different time interval at an inlet pressure of 50 mbar. The peak areas were proportional to the injection time between 30 and 120 s as shown in Figs. 6 and 7. The longer injection time was, the longer time it took to stack the samples. In addition, the MP peak got worse with longer injection time. Thus, an injection time of 30 s at 50 mbar is a compromise between high stacking efficiency and good peak shape in our following experiment (Fig. 7). Compared with normal MEKC, the separation shows some leading peaks, which may be due to an incomplete focusing during sample stacking. However, it has little effect on quantitative determination.

3.4. Effects of running buffer and instrumental parameters

In LVSS, the roles of the EOF in the sample zone and the buffer zone are completely different. As a positive role, the former is used to remove the sample matrix. In contrast, as a negative role, the latter has to be suppressed or reversed. Both of them are for achieving the sample stacking without sacrificing the high resolution of CE and without causing loss of the stacked sample prior to separation. In our system, the sample was diluted in methanol/water (3:2 v/v) where pH was around 7. The EOF

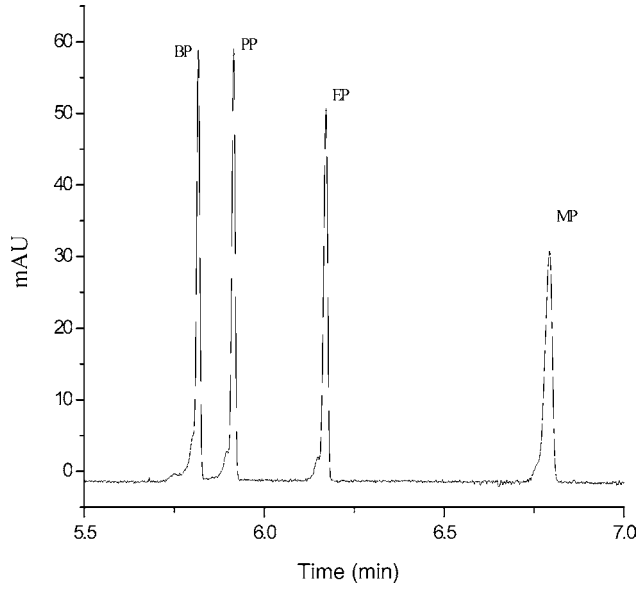

Fig. 7. The separation of MP, EP, PP and BP (5.0×10^{-5} M each) in 50 mM phosphoric acid buffer containing 150 mM SDS and 1% v/v methanol (pH 2.28) by LVSS-MEKC. Separation conditions: injection at 50 mbar for 30 s, −20 kV applied voltage, 25 °C capillary temperature.

in the sample zone was enough to pump out the sample matrix due to neutral environment. For the buffer zone, a 50 mM pH 2.28 phosphoric acid was chosen, and both sample stacking and subsequent separation proceeded consecutively under the same voltage.

The concentration of SDS surfactant showed similar effect comparing with in normal MEKC procedure. A phosphoric acid buffer containing 150 mM SDS and 1% v/v methanol was still chosen due to its better separation and symmetric peak shapes.

Essentially, increased differences in ion strength between sample zone and buffer zone should improve the stacking efficiency, due to a much higher electric field being distributed in the sample zone. A small quantity of NaCl was tried to be added in the running buffer. However, no better separation was achieved.

On the other hand, in LVSS the application of a higher voltage for both stacking and separation usually caused permanently physical damage of the capillary, which would obstruct the following CE process. This is because during the stacking process, Joule heating generated was mainly distributed in the sample zone. As the EOF drove the sample zone moving back to remove water, the sample zone became narrower and narrower, which resulted in Joule heating being focused on a small area close to the capillary inlet in the sample zone. If a high voltage was applied, a high electric current would be produced, which could generate great Joule heating in this narrow area and could embrittle and destroy the capillary itself. In our experiment, −30 kV would easily cause damage to capillary, which would obstruct the capillary and the following CE process. For this reason, a mild negative voltage (−20 kV) was applied for both sample stacking and subsequent separation. Based on similar reason, 25 °C was chosen as capillary temperature

Consequently, the optimum conditions for our system are as follows: 50 mbar as injection pressure and 30 s as injection time; 50 mM pH 2.28 phosphoric acid buffer containing 150 mM SDS and 1% v/v methanol as the running buffer; 25 °C capillary temperature, −20 kV as stacking and separation voltage.

Table 2
Quantification data of LVSS-MEKC

Parameter	Regression equation ($y = ax + b$)		r	Detection limit (M)	Linear range (M)
	a	b			
MP	7.2×10^2	−0.13	0.9987	3.0×10^{-7}	1.0×10^{-6} to 5.0×10^{-5}
EP	6.5×10^2	−0.27	0.9960		
PP	6.9×10^2	−0.33	0.9925		
BP	7.3×10^2	−0.50	0.9864		

y, peak area (Au min); x, concentration of parabens ($\times 10^{-6}$ M); r, regression coefficient.

3.5. Calibration

To test the working range of the present method, calibration curves were carried out. A series of calibration solutions were prepared with parabens concentrations ranging from 1.0×10^{-6} to 5.0×10^{-5} M. Based on a signal-to-noise ratio of 3, the detection limit was as low as 3.0×10^{-7} M for each of the parabens. Other quantification data was shown in Table 2.

3.6. Analytical application

A simple and effective method was developed to prepare cosmetic sample for the present LVSS-MEKC analysis. Parabens residues were extracted with methanol. The procedure was described in detail as follows.

Two different cosmetic samples purchased in a marketplace in Beijing were analyzed. 0.5 g of cosmetic sample was placed

Table 3
Determination of parabens in cosmetic samples

Parameter/analyte	Sample 1		Sample 2	
	Detected concentration (M)	Percentage (% w/w)	Detected concentration (M)	Percentage (% w/w)
MP	5.0×10^{-6}	0.34	–	–
EP	4.8×10^{-6}	0.33	2.9×10^{-6}	0.20
PP	1.0×10^{-5}	0.69	2.8×10^{-6}	0.20
BP	1.4×10^{-5}	0.99	3.5×10^{-6}	0.24
Total	3.4×10^{-5}	2.4	9.2×10^{-6}	0.64

in a vial and extracted with methanol 20–30 min by sonication. The organic layer was transferred to another vial after centrifuging for 10 min. This procedure was repeated three times, and then all the extraction was mixed together and diluted to 25 mL with methanol. After another 100 times of dilution, it was ready for LVSS-MEKC analyses. Table 3 lists the analysis results of cosmetic samples based on our developed method. Most of the parabens (except for MP in sample 2) had been detected, and the total concentration of sample 1 was out of the standard. These results show that the present LVSS-MEKC method can be applied in the determination of parabens in cosmetic samples with both ease and high sensitivity.

4. Conclusions

Capillary electrophoresis proved to be a fast and efficient method for the determination of parabens including methyl *p*-hydroxybenzoate, ethyl *p*-hydroxybenzoate, propyl *p*-hydroxybenzoate, and butyl *p*-hydroxybenzoate. Complete resolutions were achieved in the optimal background electrolyte system with both MEKC and LVSS-MEKC. Large-volume sample stacking was successfully applied in their determinations to improve the sensitivity. A simple and easy-manipulative sample preparation method was developed and validated by analyzing cosmetic samples. It was found that with current sample preparation process and instrumentation system, 0.5 g of cosmetic sample is sufficient for the analysis of parabens, where the EOF was successfully suppressed by using an acidic buffer at pH 2.28. In conclusion, it was experimentally proved that such a sample stacking technique represents a promising alternative to achieve higher sensitivity and resolution for preservative analysis.

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