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ORIGINAL RESEARCH PAPER

Determination of hexachlorophene in cosmetics by capillary electrophoresis compared with high performance liquid chromatography

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ABSTRACT

A simple capillary electrophoresis (CE) method with ultraviolet (UV) detection was developed for the determination of hexachlorophene (HCP) in cosmetics. Separation conditions were obtained in 20 mM $Na_2B_4O_7$, 10% MeOH (pH 9.20), with 25 kV applied voltage and UV detection at 208 nm. Under the selected conditions, electrophoretic analysis was completed in about 4 min, with limit of detection (LOD) of 0.06 $\mu g \cdot mL^{-1}$ for HCP. The method was successfully applied to determine HCP in three kinds of cosmetics with relative standard deviations (RSD) of 0.52–3.02% and recoveries from 90.0 to 96.4% for the spiked samples. The results indicated that the proposed method was reliable. Comparative experiments were also carried out with high-performance liquid chromatography (HPLC)-UV method described in National Standards of People's Republic of China. The validation results of the two methods are comparable, but the proposed CE method is simple, rapid, which makes separation and analyte quantification in shorter time with much less reagent consumption.

KEYWORDS

capillary electrophoresis, high-performance liquid chromatography, hexachlorophene, cosmetics

INTRODUCTION

Hexachlorophene (HCP) is an antimicrobial agent, which was probably used in soaps, liquid detergents, cosmetics, and plant fungicides and pesticides [1]. However, when HCP is absorbed too much, it can lead to nervous system disturbance, central system inhibition, symptoms such as loss of consciousness, respiratory inhibition and so on [2–4]. HCP may be found in cosmetics due to the production conditions of cosmetic raw materials, the production process, and improper storage of cosmetics. The presence of HCP in cosmetics poses a potential threat to human health [5]. Chinese cosmetics hygiene standards, cosmetics hygiene code and European Union (EU) cosmetics regulations had banned the use of HCP in cosmetic components [6, 7]. Therefore, it is important to develop a proper method to determine the levels of HCP in cosmetics in order to ensure the quality of cosmetics and public health.

Several analytical methods including paper and plate chromatography [8], gas chromatography-electron capture detection (GC-ECD) [9], gas chromatography-mass spectrometry (GC-MS) [10], gas-liquid chromatography (GLC) [11], high-performance liquid chromatography (HPLC) [12–14], and ultra-high-performance liquid chromatography-quadrupole time of flight mass spectrometry (UPLC-QTOF-MS) [15], have been used to determine HCP. For GC analysis, HCP usually requires suitable derivatization, although it can get higher sensitivity, the procedure is complicated and time consuming. Among them, HPLC with tandem MS is the most selective and sensitive technique for identification and quantification of HCP. But compared with capillary electrophoresis (CE), HPLC has the disadvantages of consuming a large amount of organic solvent, long analysis time and more sample usage [16, 17]. As a

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result, CE has become the preferred alternative method with high resolution, rapid analysis, low sample and reagent consumption, and flexible separation modes [18–21].

CE methods especially capillary zone electrophoresis (CZE) have found their place in modern separation science as versatile and efficient techniques for the analysis of a wide range of analytes in a variety of sample matrices, such as the analytes of organic compound [22], inorganic ions [23, 24], proteins [25], and vitamins [26], in a series of samples. CE based methods have been described in literatures for the quantification of chlorophenols in some matrices [27–29]. However, only one research has been reported regarding the determination of HCP in water samples by CE with electrochemical detection [30]. The determination of HCP by CE in cosmetics has not been reported.

In this study, a novel, simple and low-cost method for the determination of HCP was established by CZE with UV detection. Applicability of the method was discussed in comparison with HPLC according to the National Standards of the People's Republic of China (GB/T 29673-2013). The results show that the CZE method described in this paper is a good alternative to HPLC for the rapid analysis of HCP in cosmetics.

EXPERIMENTAL

Apparatus

SCIEX P/ACE™ MDQ plus CE system (Fullerton, CA, USA) equipped with a diode-array detector (DAD) and bare fused-silica capillary (Yongnian Photoconductive Fiber Factory, Hebei, China) with 75 μm id, 375 μm od, total length of 50.2 cm, and effective length of 40 cm were utilized in all the experiments. The pH value measurements were made with a STARTER 3100 (Shanghai OHAUS Instrument Corporation, Shanghai, China). Data acquisition was performed using Karat 32 software (Beckman-Coulter, Fullerton, CA, USA).

CE conditions

Before the first usage, new capillary was conditioned by rinsing in order, with MeOH (5 min), water (5 min), 1 $mol \cdot L^{-1}$ NaOH (20 min), water (10 min), and running buffer (30 min). The capillary was conditioned daily by flushing with 1 $mol \cdot L^{-1}$ NaOH, water and running buffer for 5, 5, and 10 min, respectively. Between the two separation analyses, it should be rinsed with running buffer for 3 min. All solutions were filtered through microporous nylon filters with a pore diameter of 0.45 μm before use. The detection wavelength was set at 208 nm for HCP. The capillary temperature was maintained at 25 °C, the applied voltage was +25 kV and pressure injection was performed using 0.5 psi for 7 s (1 psi = 6,894.76 Pa). The running buffer consisted of 20 mM $Na_2B_4O_7 \cdot 10\ H_2O$ and 10% (v/v) CH_3OH, and then the solution pH was adjusted with 1 $mol \cdot L^{-1}$ NaOH after addition of CH_3OH. As a result, the apparent pH namely "pH*" 9.20 was used.

HPLC conditions

A HPLC system consisted of a Waters e2695 pump, a Waters 2998 PDA detector, a Empower chromatography management system (Waters, Milford Massachusetts, USA) and a C18 reversed-phase column (Tianyuan Science and Technology Co., Ltd., Tianjin, China; Aces aq C18, 5 μm, 250 × 4.6 mm) was used. Acetonitrile and 0.5% phosphoric acid solution (80:20 v/v) was used as the mobile phase and the flow rate was set at 1.0 $mL \cdot min^{-1}$. PDA detection wavelength was set at 205 nm. All analyses were performed at room temperature with sample injection volume of 10 μL according to the National Standards of the People's Republic of China (GB/T 29673-2013).

Reagents and samples

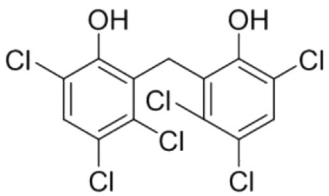
HPLC grade reagents of HCP (purity > 99.0%) was purchased from Dr. Ehrenstorfer (Augsburg, Germany) and the standard stock solutions were prepared by dissolving them in methanol (MeOH) with a concentration of 10 $g \cdot L^{-1}$. The structure of HCP is shown in Fig. 1. Sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$) and sodium hydroxide (NaOH) of analytical grade were supplied from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Chromatographic grade acetonitrile (ACN) and MeOH were purchased from J&K Chemical (Beijing, China). Working solutions were obtained by appropriate dilution of the stock standard solution and all standard solutions were stored in a refrigerator at 4 °C. The water used throughout the work was produced by a Milli-Q ultrapure water system (Millipore, Bedford, MA, USA).

Loose powder, emulsion, and toner samples were purchased from a local supermarket. For CE and HPLC methods, 1 g of each sample were accurately weighed and placed into a 10 mL polypropylene centrifuge tube. Then five mL MeOH were added to the weighed sample and mixed well. The sample was ultrasonically extracted for 15 min and centrifuged at 8,110×g for five min. The supernatant was filtered through microporous nylon filters with a pore diameter of 0.45 μm before use.

RESULTS AND DISCUSSION

CE method

The pKa values of HCP are 5.15 and 7.00. So CZE mode was used under alkaline separation conditions. The



Hexachlorophene (HCP)

Figure 1. The chemical structure of HCP

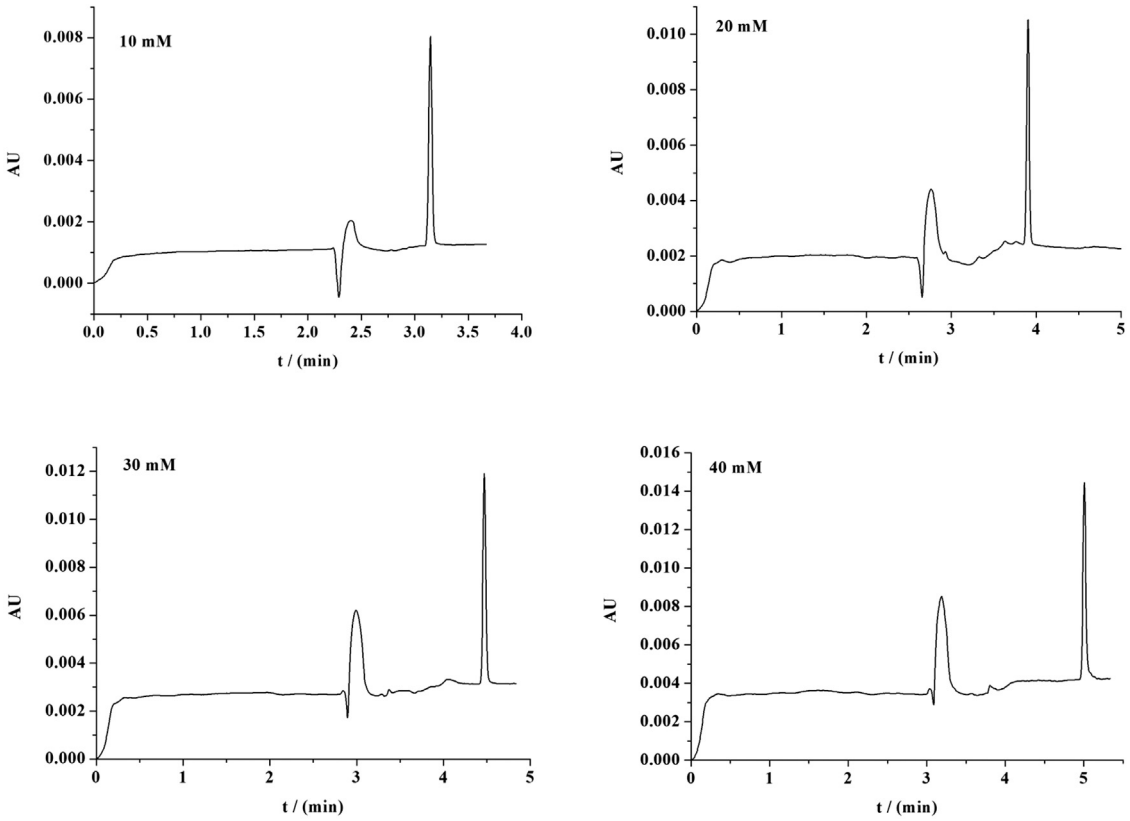


Figure 2. Effect of $Na_2B_4O_7$ concentration on the analysis of HCP

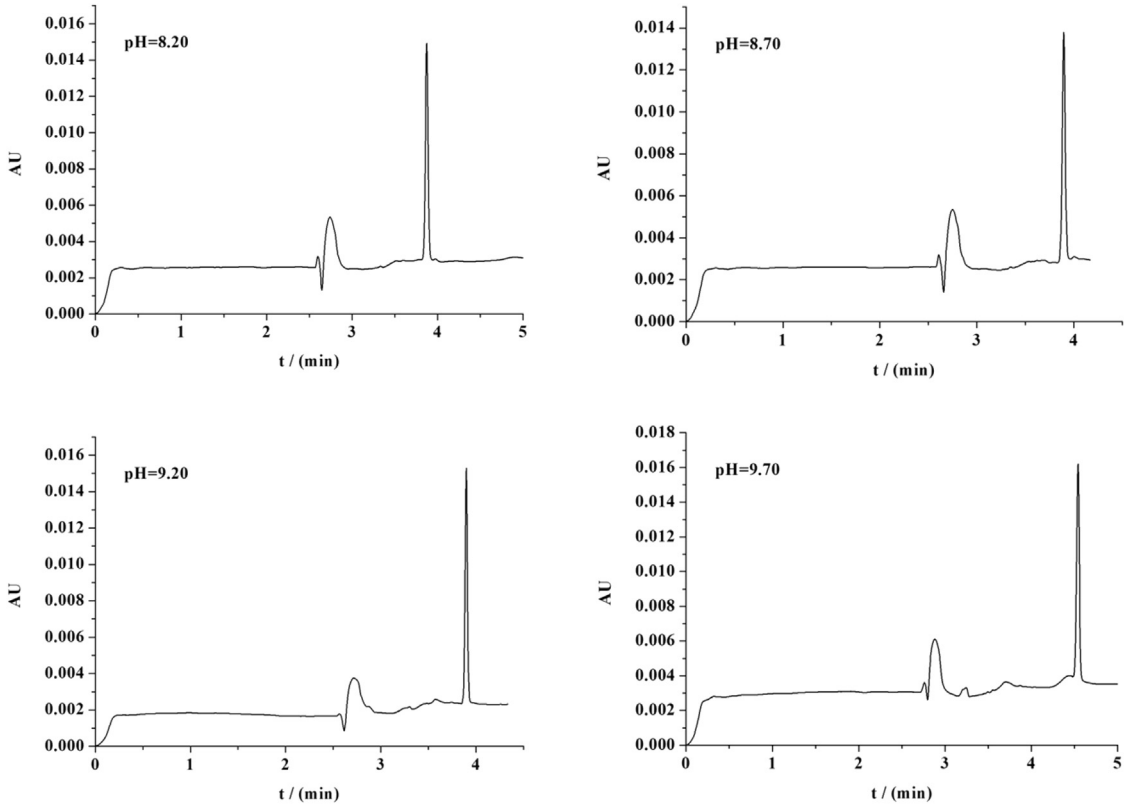


Figure 3. Effect of buffer pH on the analysis of HCP

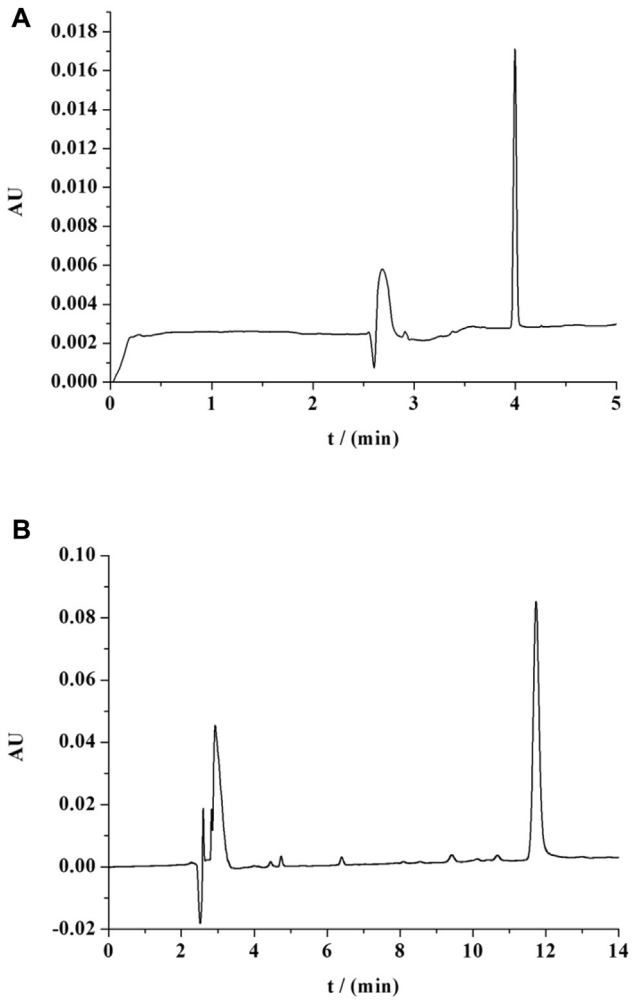


Figure 4. Typical (A) electropherogram and (B) chromatogram of 10 μg/mL individual for HCP. CE conditions: 20 mM $Na_2B_4O_7$, 10% (v/v) MeOH, pH = 9.20, 7 s pressure injection at 0.5 psi, 25 kV applied voltage, UV detection at 208 nm HPLC conditions: mobile phase, 80:20 (v/v) ACN/0.2% phosphoric acid aqueous solution, 1.0 mL·min^{-1} flow rate, UV detection at 205 nm

concentration of running buffer solution can affect the ionic strength, thus affect the electroosmotic flow (EOF), and then influence the separation efficiency. So four levels (10, 20, 30, 40 mM) of $Na_2B_4O_7$ solution for the determination of HCP was studied. As shown in Fig. 2, with the increasing of $Na_2B_4O_7$ concentration, the migration time of HCP prolonged, but the peak area increased. Considering both the migration time and sensitivity, 20 mM $Na_2B_4O_7$ (pH 9.20) was selected for subsequent experiments.

The pH of the buffer solution has a close relationship with EOF and the effective charge of the analyte, which in turn affects effective mobility of the analyte in the buffer. To determine the effect of buffer pH on migration behavior, experiments were performed by using a background electrolyte solution consisting of 20 mM $Na_2B_4O_7$. The pH values of 8.20, 8.70, 9.20, and 9.70 were investigated, which were adjusted by 1.0 mol·L^{-1} NaOH. As shown in Fig. 3, the migration time of HCP at pH 9.70 was longer than others. At pH 9.20 shorter migration time and better peak shape as well as sensitivity were achieved. Therefore pH 9.20 was used.

The injection time influences the volume of sample and the sensitivity. The effect of injection time (5–10 s) at 0.5 psi on sensitivity was investigated. The results showed that with the increase of the injection time (5–7 s), the peak area of HCP were increasing. However, with further increase of the injection time, the peak zone became wider. Therefore, 7 s of injection time was selected.

Separation voltage is also an important parameter in CE. Thus, separation voltage of 20, 22, 25, and 27 kV were checked using a background eletrolyte solution consisting of 20 mM $Na_2B_4O_7$, at a pH value of 9.20. When the applied voltage was 25 kV, not only was the peak height the highest, the peak width the narrowest and the peak shape the best, but also the current was moderate. Therefore, 25 kV was selected as the optimal separation voltage.

In electrophoresis analysis, buffer is generally formulated with water, and the organic additive can effectively improve the degree of separation or separation selectivity. Although only one component is analyzed, considering the complexity of the sample matrix (loose powder, emulsion, and toner), preliminary experiments for real sample analysis were carried out under the above CE conditions. The results were not satisfactory. HCP could not completely separated with other components coexisted in the sample. Therefore organic modifier was used to improve separation. For investigating the effect of organic modifier on the separation efficiency of the analyte, experiments were performed by adding 10% MeOH or ACN. The results show that HCP and other components in the sample can be

Table 1. Results of system suitability tests (n = 5)[ab] and method precision for analysis of HCP

				RSD[b] (%, n = 5)			
				Intra-day		Inter-day	
Method	Migration/retention time, (min)	Tailing factor	Theoretical plates	Migration/retention time	Peak area	Migration/retention time	Peak area
CZE	3.962 ± 0.012	1.15 ± 0.04	25116 ± 94	2.11	2.16	2.69	3.06
HPLC	11.736 ± 0.01	1.75 ± 0.02	4,420 ± 37	1.18	1.20	1.55	2.67

[a]Values are means of five measurements ± SD. [b]Concentration of 10 μg/mL individual for HCP was chosen for the assays of system suitability and method precision tests.

better separated with 10% MeOH rather than 10% ACN as organic modifier. So, 10% MeOH were added to the buffer to continue the following work.

Above all the selected CE conditions were as follows: 20 mM $Na_2B_4O_7$, 10% MeOH (pH 9.20), UV detection at 208 nm, injection time of 7 s at 0.5 psi, working voltage at 25 kV, and temperature at 25 °C. The corresponding electropherogram of the HCP standard solution obtained under the optimized conditions is shown in Fig. 4(a).

HPLC method

HPLC experiments were carried out according to the method described in National Standards of People's

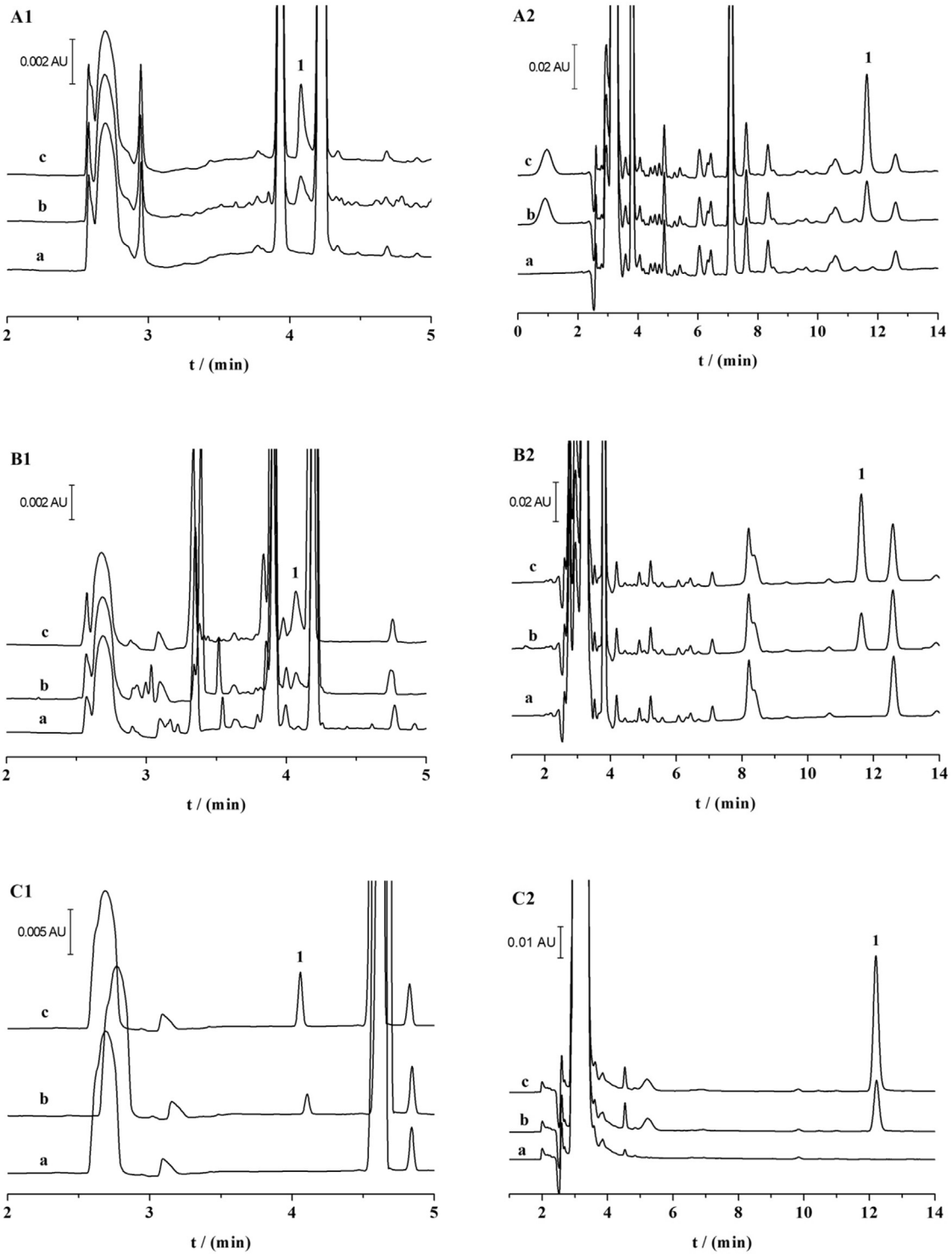


Figure 5. Electropherograms (A1, B1, C1) and chromatograms (A2, B2, C2) of cosmetic samples: none-spiked (a), spiked with 2 $\mu g \cdot mL^{-1}$ (b), spiked with 5 $\mu g \cdot mL^{-1}$ (c); (A1, A2) Loose powder, (B1, B2) Emulsion, (C1, C2) Toner, other conditions are same as those in Fig. 4

Table 2. The recoveries of HCP in sample by CE and HPLC methods (n = 3)

Method	Sample	Original amount ($\mu g \cdot mL^{-1}$)	Added ($\mu g \cdot mL^{-1}$)	Found ± SD ($\mu g \cdot mL^{-1}$)	Recovery (%)	RSD (%)
CZE	Loose powder	–[a]	2.0	1.83 ± 0.033	91.5	1.80
			5.0	3.96 ± 0.056	90.0	1.42
	Emulsion	–	2.0	1.64 ± 0.049	92.0	3.02
			5.0	4.03 ± 0.021	90.6	0.52
	Toner	–	2.0	1.87 ± 0.028	93.5	1.51
			5.0	4.82 ± 0.081	96.4	1.68
HPLC	Loose powder	–	2.0	2.18 ± 0.011	109.0	0.50
			5.0	4.84 ± 0.0071	96.8	0.15
	Emulsion	–	2.0	2.11 ± 0.0071	105.5	0.15
			5.0	4.86 ± 0.021	97.2	0.44
	Toner	–	2.0	2.06 ± 0.010	103.0	0.49
			5.0	5.07 ± 0.010	101.4	0.20

[a]Not detected.

Republic of China (GB/T 29673–2013) for comparison with the results obtained from the CE method (see HPLC conditions). A typical chromatogram of the HCP standard was shown in Fig. 4(b).

Validation of CE and HPLC methods

China's cosmetics hygiene standards and EU cosmetics regulations ban the use of HCP in cosmetic components. In this study (CE) the concentration range employed of the calibration curve was consulted the National Standards of the People's Republic of China (GB/T 29673-2013), in which the employed concentration range is 0.5–30 μg/mL. Calibration curves were prepared between 0.2 and 30 $\mu g \cdot mL^{-1}$ for HCP with the following concentrations: 0.2, 0.5, 1, 5, 10, and 30 $\mu g \cdot mL^{-1}$ using the developed CE method. Linear relationships between the concentration of HCP and (x, μg mL^{-1}) and the corresponding peak area (y) are y = 2,800.2x−426.04 (r = 0.9999, $F = 1.76 < F_{0.05\ (4,\ 6)} = 4.53$) for CE and y = 101963x−25917 (r = 0.9999, $F = 1.21 < F_{0.05\ (4,\ 6)} = 4.53$) for HPLC. The correlation coefficient (r) of 0.9999 over the concentration range indicate good correlations between concentrations and their peak areas. Limit of detection (LOD) and limit of quantification (LOQ) were determined at the signal to noise ratio (S/N) of 3 and 10. The LODs obtained were 0.06 $\mu g \cdot mL^{-1}$ by CE and 0.05 $\mu g \cdot mL^{-1}$ by HPLC. The LOQs obtained were 0.19 $\mu g \cdot mL^{-1}$ by CE and 0.15 $\mu g \cdot mL^{-1}$ by HPLC.

According to the U.S. Pharmacopeia, system suitability tests are an integral part of chromatographic methods [31]. The parameters, migration/retention time (t), tailing factor (T), and theoretical plates (N), were evaluated using five replicate injections of a standard solution of the analytes. As shown in Table 1, CE analysis only spends 3.962 min for chromatographic separation of the HCP, much shorter than that by HPLC (11.736 min). Tailing factor of the CE method (1.20) is smaller than that of the HPLC method (1.87), which shows that the peak shape of HCP in CE is better than that in HPLC. Furthermore, the higher theoretical plate number of HCP in CE (25116) than that in HPLC (4420) demonstrated much better column efficiency of CE.

Precision of the two methods was determined by means of an intra-assay experiment and an inter-assay experiment using 10 μg/mL HCP. Intra-day variability was determined by repeated (n = 5) injections of a standard of HCP under the optimum conditions within one day, while for inter-day variability test, the samples were examined in triplicate for a consecutive five days. The relative standard deviation (RSD) were calculated and summarized in Table 1. The results indicated that both the CE and HPLC methods were with satisfactory precision and were suitable for the determination of HCP.

Sample analysis

To demonstrate the applicability of the developed CE and HPLC method for the analysis of real samples three kinds of cosmetics samples (loose powder, emulsion, and toner) were selected (marked as A, B, and C). Typical electropherograms and chromatograms of the samples are shown in Fig. 5. All samples were pretreated according to Chinese standard stated above. From Fig. 5 we can see that HCP was not found in the samples by both two methods (curve a). In Fig. 5B1(a), it seems like a small peak appearing at the elution time of HCP. However, when looking at Fig. 5B2(a) no peak appeared at the elution time of HCP. So we state that there is no HCP in the analyzed sample. The same is for Fig. 5A2(a). The use of HCP in cosmetic components has been banned in China. That's why no HCP was found in any of the real samples. However the following recovery experiments demonstrated the applicability of the method.

To verify the accuracy of the CE and HPLC methods, recovery experiments were performed under the optimum conditions by spiking the standards of different levels to real samples. Usually the spiked concentration of the analyte depends on the concentration of the analyte found in the sample. However in this study, no HCP was found in the samples. So only the lower region was explored. Electropherogram and chromatogram for the spiked sample are also shown in Fig. 5. From Table 2 we can see that the recoveries of HCP was between 90.0 and 96.4% with RSD of 0.52–3.02% and 96.8–109.0% with RSD of 0.15–0.49% for

the method of CZE and HPLC, respectively. On the whole, the developed CE method was suitable for HCP determination in cosmetic samples.

CONCLUSION

In this work, a reliable analytical method was developed to determine HCP in cosmetics based on CE-UV system. The developed method was validated with respect to linearity, limit of detection, precision, and accuracy. The adequate analytical performance of the method makes it suitable for HCP analysis in different samples. The CE method is simple, rapid, and low-cost in comparison with HPLC and can be a good environmental friendly alternative to HPLC for the routine analysis of HCP.

Conflict of interest: There are no conflicts to declare.

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