Analytical Methods

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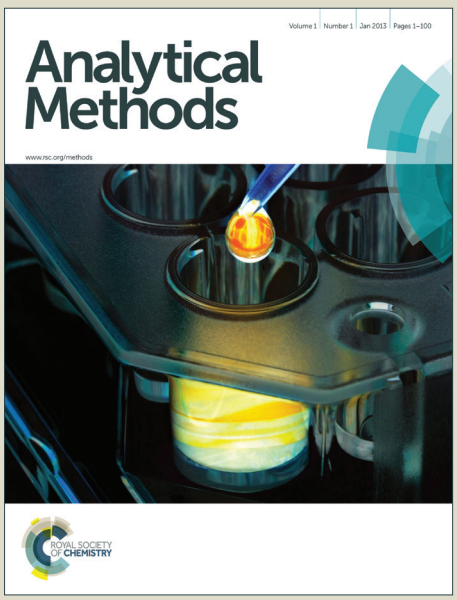

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ARTICLE

Simultaneous determination of whitening agents and parabens in cosmetic products by capillary electrophoresis with on-line sweeping enhancement

I-Chi Tsai, Chia-Yu Su, Cho-Chun Hu and Tai-Chia Chiu*

Cite this: DOI: 10.1039/x0xx00000x

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/x0xx00000x

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In this study, a simple and effective method for determining whitening agents and parabens, using on-line focusing with sweeping–micellar electrokinetic chromatography (MEKC), was developed. The optimum conditions for the on-line concentration and separation of arbutin, kojic acid, resorcinol, salicylic acid, and methyl-, ethyl-, propyl-, and butyl-parabens were investigated in detail. Sweeping–MEKC was performed at 15 kV using a background electrolyte containing 15 mM tetraborate buffer (pH 8.5), 40 mM sodium dodecyl sulfate, and 0.100% (w/v) poly(ethylene oxide). The analyte detection limits (S/N = 3) were 8–162 nM (1.1–21.0 ng mL^{-1}), and these were 46–279 times lower than those achieved using conventional sample injections (10 s, no polymer in the buffer solution). Baseline separation of all of the analytes was achieved within 10 min. The method was validated and then used to determine whitening agents and parabens in five commercial cosmetic products. The average recoveries from the commercial products were 85.2–118.0%. The proposed method is a powerful alternative approach for identifying and determining whitening agents and parabens in commercial cosmetic samples.

Introduction

Melanin, which is a unique, pigmented biopolymer that is secreted by melanocyte cells in the basal layer of the epidermis, is the major component that determines human skin color.[1] Melanin is also important in protecting the skin from harmful ultraviolet radiation. Cosmetics include commercial skin-care and beauty products, and routine treatments for skin diseases. Skin-whitening cosmetic products are used to lighten the skin color and eliminate unaesthetic marks by inhibiting, through a number of mechanisms, the biosynthesis of melanin.[2,3] Besides their applications in cosmetics, whitening agents are also used in pharmaceuticals to treat skin disorders, including age spots, freckles, melasma, and inflammatory hyperpigmentation.[4,5] The most popular skin-whitening chemicals are hydroquinone, arbutin (AR), ascorbic acid, azelaic acid, kojic acid (KA), and phytic acid. However, these can cause unwanted local effects and systemic toxicity if they are used repeatedly and for a long period.[6,7]

The parabens are alkyl esters of 4-hydroxybenzoic acid. Methyl (MP)-, ethyl (EP)-, propyl (PP)-, and butyl (BP)-parabens have been used widely as preservatives in cosmetics products because they each have a broad antimicrobial activity spectrum, relatively low toxicity, and good stability, and because they are non-volatile and efficient over a wide pH range.[8] In practice, shorter alkyl chain esters are commonly used because they have higher aqueous solubilities than the longer alkyl chain esters.[9] Two or more parabens are often used together because of their synergistic effects.[10] Some recent studies have shown toxic effects from parabens in daily-use cosmetic products.[11,12] The European Economic Community has, therefore, restricted the concentrations of individual parabens in cosmetic products to a maximum of 0.4% (w/w) and restricted the total parabens concentration to a maximum of 0.8% (w/w), expressed as the p-hydroxybenzoic acid concentration.[13] An effective and convenient quantitative method for the simultaneous determination of whitening agents and parabens in cosmetics would be valuable for ensuring that the cosmetics comply with government regulations.

Recently, capillary electrophoresis (CE) has been seen as an attractive separation technique for whitening agents and parabens analysis because of its high efficiency, low reagent consumption, and high speed.[14–18] However, poor sensitivity due to the short optical path length for UV detection and the low injection volumes of the sample solution are the major disadvantages of CE. Many on-line focusing methods, such as large volume sample stacking, field amplified sample stacking, transient isotachophoresis, dynamic pH junctions, and sweeping, have, therefore, been developed to overcome the sensitivity limitations of CE. This topic has attracted a huge amount of attention and has already been reviewed a number of times.[19–24] Sweeping is one of the most efficient on-line focusing methods because of its versatility and effectiveness in stacking both neutral and charged analytes. Sweeping involves interactions between a pseudostationary phase or a complexing agent in the separation buffer and the analytes in a matrix that does not contain additives (pseudostationary phase or a complexing agent).[22] Accumulation of the analyte is caused by chromatographic partitioning, complexation, or any interaction between the analytes and additives during electrophoresis. Analytes must have a strong affinity for the pseudostationary phase or the complexing agent to achieve optimum preconcentration using the sweeping technique. Cheng *et al.*[25] recently combined large volume sample stacking with electroosmotic flow (EOF) and sweeping to determine ten preservatives (including five parabens) in cosmetic products. The LODs were down to 0.005–2 μg mL^{-1}. Different capillary electrophoretic preconcentration strategies (large volume sample

stacking, field-amplified sample injection, sweeping, and in-line solid phase microextraction) for parabens have been demonstrated by Maijó *et al.*[26] The LODs obtained for parabens were in the range 0.01–0.02 ng mL^{-1}. Wu *et al.*[27] incorporated sweeping and the use of polymer solutions to achieve the on-line focusing of seven parabens and used the method to analyze four cosmetic products. The LODs were in the range from 0.9 to 1.5 ng mL^{-1}.

Here, we describe a simple, cost effective, and fast analytical method for the quantitative determination of whitening agents and parabens by CE after on-line focusing using the sweeping technique. The performance of the method was evaluated in terms of its ability to give accurate and precise qualitative and quantitative data over a relevant concentration range. The method was then used to analyze whitening agents and parabens in cosmetic products with satisfactory results.

Experimental

Chemicals and sample handling

MP, EP, PP, and BP were purchased from AccuStandard (New Haven, CT, USA). Sodium tetraborate was purchased from Acros Organics (Geel, Belgium). Resorcinol (R) and salicylic acid (SA) were purchased from Chem Service (West Chester, PA, USA). Sodium hydroxide was purchased from Riedel-de Haën (Seelze, Germany). AR, KA, sodium dodecyl sulfate (SDS), sodium hydroxide, and poly(ethylene oxide) (PEO) (M_v = 8.0 × 10^6 g mol^{-1}) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethanol was purchased from Shimakyu's Pure Chemicals (Osaka, Japan). Stock 10 mM standards were made by separately dissolving each analyte in ethanol, except for the stock standards of AR and KA, which were prepared in deionized water. The stock standards were stored in the dark at 4°C. Fresh working standard solutions were prepared each day by diluting the stock solutions with deionized water. Deionized water was collected from a Barnstead Nanopure Ultrafiltration Unit (Boston, MA, USA). The tetraborate buffer was adjusted to the desired pH for each experiment using 5 M HCl. PEO (0.025–0.0625 g) was gradually added to a tetraborate solution (50 mL), and the mixture was continuously stirred using a magnetic stirring rod to produce a homogeneous suspension. This suspension was stirred for at least 8 h after all of the PEO had been added, and then degassed using a vacuum system in an ultrasonic tank before being used for CE separations.

Apparatus

All experiments were performed using a laboratory-made unit, which was similar to a system that has been described previously.[28] The unit consisted of a 20 kV high-voltage power supply (ES20P-20W/DAM; Gamma High Voltage Research, Ormond Beach, FL, USA) and a UV–Vis detector (SAPPHIRE 600 detector; ECOM, Prague, Czech Republic). Electropherograms were recorded and processed using DataApex Software (DataApex, Prague, Czech Republic). Fused silica capillaries (75 μm I.D., 365 μm O.D.) were purchased from Polymicro Technologies (Phoenix, AZ, USA). The total length of each capillary was 60 cm and effective length was 50 cm. All measurements were repeated five times.

Electrophoretic conditions

New separation capillaries were conditioned before use by rinsing them with 0.5 M NaOH for 30 min and then with deionized water for 1 min. A capillary was equilibrated with the separation buffer for 3 min before a run, then rinsed with 0.5 M NaOH for 10 min, and rinsed with the separation buffer for 3 min after the run. Pre- and post-run conditioning steps were important for achieving a reproducible EOF. Samples were hydrodynamically injected for 30–150 s (approximately 55–276 nL), with a 20 cm height difference between the capillary inlet and the outlet. MEKC was performed using a UV–Vis detector, with a wavelength of 200 nm and separation voltage of 15 kV. The solution used to fill the capillary was 15 mM tetraborate buffer (pH 8.5) containing 40 mM SDS. The separation buffer was the same solution with 0.100% PEO added.

Analysis of commercial samples

Commercial cosmetic samples, including a mask, two lotions, golden essence, and facial washing milk, were purchased from local shops. An accurate amount of each sample (1.0 g) was extracted with 10.0 mL ethanol for 10 min in an ultrasonic bath. The sample was then centrifuged for 10 min at 6,000 rpm. The upper, clear liquid was diluted by a suitable factor (2000-fold for the facial washing milk, 500-fold for the lotions and golden essence, and 100-fold (for EP and PP analysis) and 500-fold (for MP analysis) for the mask) with deionized water before MEKC analysis. The analytical recoveries were determined by spiking standard solutions into real samples before analyzing them.

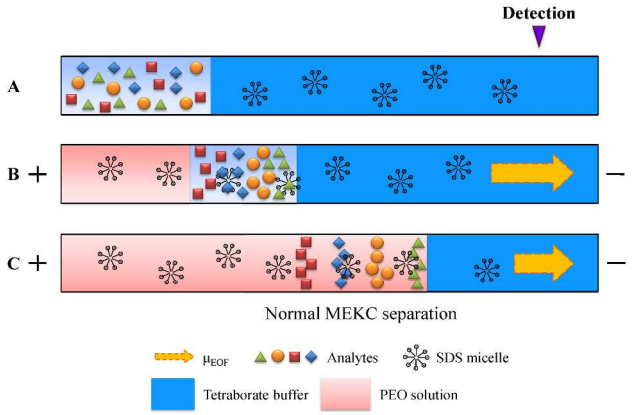

Fig. 1 Schematic of the sweeping–MEKC method using a polymer solution. (A) The samples (in deionized water) are hydrodynamically injected for 90 s once the capillary is filled with tetraborate buffer containing SDS, (B) the SDS micelles sweep the analytes present in the sample zone once a positive high voltage is applied, and both the SDS micelles and analytes migrate against EOF and enter the PEO zone during stacking, and (C) the analytes are stacked in a narrow band, migrate into the PEO zone and are separated by MEKC.

Results and discussion

Method development

An on-line focusing strategy using sweeping–MEKC with polymer solutions was illustrated in Fig. 1. The capillary was filled with 15 mM tetraborate solution (pH 8.5) containing 40 mM SDS (Fig. 1A), then the analytes, in deionized water were hydrodynamically injected into the anodic end of the capillary for 90 s. Once a high voltage is applied, the SDS micelles from the 15 mM tetraborate buffer migrating toward the anodic side and interacted with the analytes in the sample zone (Fig. 1B). The SDS micelles migrated against the EOF[29] and they would have swept analytes into a narrow zone in the sample zone. Meanwhile, the PEO also entered the capillary from the anodic

end because of EOF. The analytes and aggregates of the SDS micelles and analytes would have been stacked as they migrated into the PEO zone because of the increase in the viscosity at that point (Fig. 1C). The PEO solution also contained SDS micelles, so the analytes would have interacted with both the SDS and PEO molecules. The analytes were finally separated according to the normal MEKC operating mode.[27]

Effect of buffer pH, SDS, and PEO

The buffer pH was important in controlling the resolution of the analytes. Different amounts of 5 M HCl were added to a 15 mM tetraborate, 40 mM SDS, and 0.100% PEO buffer to adjust its pH to between 8.0 and 9.3. The resolution between MP and KA was improved when the pH was increased from 8.0 to 8.5, whereas the resolution deteriorated when the pH was increased from 8.5 to 9.3, as illustrated in Fig. 2. All of the analytes were well separated at pH 8.5; hence, this value was selected as the optimum buffer pH.

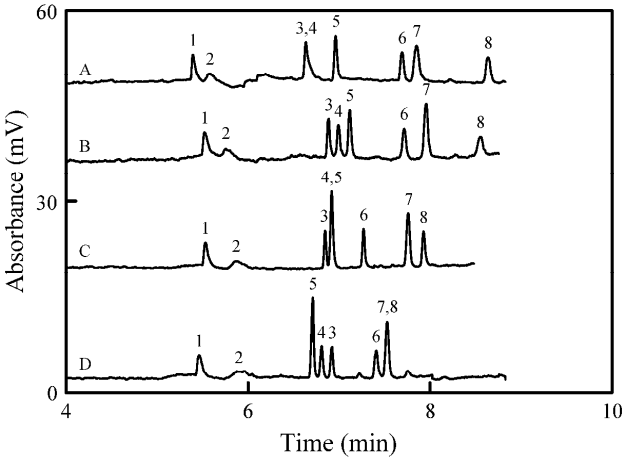

Fig. 2 Effect of tetraborate pH on analyte preconcentration. The sample (1.0 μM) was hydrodynamically injected for 90 s. The 60 cm capillary (50 cm effective length) was filled with 15 mM tetraborate buffer at a pH of (A) 8.0, (B) 8.5, (C) 9.0, and (D) 9.3 containing 40 mM SDS before the sample was injected. Peak identities: (1) AR, (2) R, (3) MP, (4) KA, (5) EP, (6) PP, (7) SA, and (8) BP. A 0.100 % PEO solution in 15 mM tetraborate buffer containing 40 mM SDS was used at the same pH values as listed above. The separation was conducted at 15 kV.

We also assessed the effects of varying the SDS concentration in the buffer between 0 and 50 mM to find the optimum concentration for the concentration effect (Fig. 3A). We found that the analytes could not be satisfactorily separated at the optimum buffer pH unless SDS was added to the buffer. Increasing the SDS concentration to 50 mM caused the MP and KA to stop being resolved. It also caused the migration time to increase; therefore, as a compromise, an SDS concentration of 40 mM was selected for subsequent experiments.

In previous studies, the separation efficiency and resolution were improved because of a decrease in analyte adsorption onto the capillary wall when a PEO solution with a concentration higher than 0.6% was used;[29,30] however, the high viscosity of the PEO solution lead to a long analysis time. In an attempt to decrease the analysis time, we assessed the effects of using PEO concentrations of 0–0.125% in 15 mM tetraborate buffer (pH 8.5) containing 40 mM SDS (Fig. 3B). The addition of 0.100% PEO has shown improved separation efficiency of the most peaks when comparing to no PEO in the buffer solution. Because of the higher viscosity of the buffer

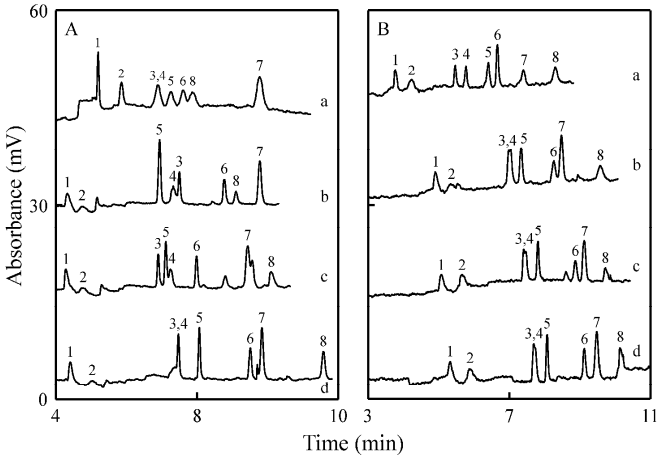

Fig. 3 (A) Electropherograms showing the impact of SDS in 0.100% PEO solutions: SDS of (a) 0 mM, (b) 20 mM, (c) 30 mM, and (d) 50 mM. (B) Effect of the PEO concentration on the stacking and separation of the analytes: PEO solutions of (a) 0%, (b) 0.050%, (c) 0.075%, and (d) 0.125% were prepared in 15 mM tetraborate buffer containing 40 mM SDS. Other conditions were same as those in Fig. 2B.

solution the sweeping efficiency was improved. This causes the migration velocity of the analytes to be significantly lower, concentrating them into a narrow zone. The analysis time was longer using a PEO concentration of 0.125% than while using a PEO concentration of 0.100%; hence, a PEO concentration of 0.100% was chosen for use in the subsequent experiments. Based on our experimental results, the optimum on-line focusing conditions are as follows: a solution of 15 mM tetraborate buffer (pH 8.5) and 40 mM SDS was used for filling the capillary, the analytes were prepared in deionized water, and a 0.100% PEO in 15 mM tetraborate buffer (pH 8.5) containing 40 mM SDS was used as the separation buffer. The separation current was 45 μA under these conditions.

Method performance

We analyzed whitening agent and parabens standard solutions using the optimized operating parameters and determined the linearity of the method (expressed as the correlation coefficient) and its reproducibility, which was measured using replicate injections of standard solutions. The results are shown in Table 1. Signal to noise ratios (S/Ns) of 3 and 10 were used to define the limit of detection (LOD) and limit of quantification (LOQ), respectively. The enhancement factor (EF) was calculated as the ratio of the LOD obtained using the stacking method (90 s) to that obtained using a normal injection (10 s) without any polymer in the separation buffer. There were excellent linear relationships between the peak heights and analyte concentrations with correlation coefficients higher than 0.990. The LODs were between 8 and 162 nM (1.1 and 21.0 ng mL^{-1}). The sensitivity was improved by 46–279 times (RSD of peak height < 10%, n =5) in terms of the LODs using the stacking method compared to those using the normal injection method. The run-to-run and day-to-day precision was tested using 0.5 μM standards, and the relative standard deviations were lower than 3.93% (repeatability) and 5.36% (reproducibility) (n = 5). Table 2 which summarizes a comparison of the analytical characteristics of on-line concentration methods reported in the recent literatures, indicates that the proposed method showed

shorter analysis time (10 min vs. 13 min), variety of analytes (3 whitening agents and 5 preservatives vs. 7 preservatives), and real samples (4 kinds vs. 1 kind) when comparing to the sweeping-MEKC methods.

Table 1 Linearities, precisions, LOQs, LODs, and EFs for the proposed method

Analyte	Liner range (μM)	R^2	Repeatability (%)[a]	Reproducibility (%)[a]	LOQ[b] (nM)	LOD[b] (nM)	LOD[b] (ng mL^{-1})	LOD[c] (μM)	EF
AR	0.2-300	0.991	1.82	5.36	255	77	21.0	3.8	49
R	0.5-50	0.997	1.61	4.94	534	162	17.8	7.5	46
MP	0.2-50	0.990	2.75	4.57	120	36	5.5	2.6	72
KA	0.2-100	0.991	3.01	4.36	215	65	9.2	5.2	80
EP	0.1-50	0.998	2.90	4.72	53	16	2.7	2.9	181
PP	0.1-50	0.994	3.30	4.85	47	14	2.5	3.9	279
SA	0.05-30	0.997	3.72	4.10	27	8	1.1	1.7	213
BP	0.1-50	0.998	3.93	5.17	57	17	3.3	2.7	159

[a] RSD for 5 replicate injections on the same day (repeatability) or over successive days (reproducibility) of migration time. [b] Sample was injected for 90 s. [c] Sample was injected for 10 s and no polymer in the buffer solution.

Table 2 Comparison of on-line focusing CE methods for detecting parabens

Mode	Compound	Time (min)	LOD (ng mL^{-1})	EF	Reference
LVSS-EP-MEKC	MP, EP, PP, BP, iBP, PHBA, DAH, SA, SO, BA	< 35	5–2000	N.P.	25
Sweeping-MEKC	MP, EP, iPP, PP, BP, BzP	< 21	18–27	17–29	26
LVSS-CZE	MP, EP, PP, BP	< 27	3–4	53–77	26
FASI-CZE	MP, EP, PP, BP	< 20	2	105–120	26
In-line-SPE-CZE	MP, EP, PP, BP	< 86	0.01–0.02	12600–18400	26
Sweeping-MEKC	MP, EP, iPP, PP, iBP, BP, BzP	< 13	0.9–1.5	930–2200	27
Sweeping-MEKC	MP, EP, PP, BP, SA, KA, R, AR	< 10	1.1–21.0	46–279	This work

AR, arbutin; BA, benzoic acid; BP, butyl paraben; BzP, benzyl paraben; CZE: capillary zone electrophoresis; DAH, dehydroacetic acid, EP: ethyl paraben, FASI: field-amplified sample injection, iBP, isobutyl paraben; iPP, isopropyl paraben; KA, kojic acid; LVSS, large volume sample stacking; LVSS-EP, large volume sample stacking with an electroosmotic flow pump; MEKC, micellar electrokinetic chromatography; MP, methyl paraben; N.P.: not provided; PHBA, *p*-hydroxybenzoic acid; PP, propyl paraben; R, resorcinol; SA, salicylic acid; SPE, solid phase extraction; SO, sorbic acid.

Analytical applications

The optimized sweeping-MEKC method was used to determine the whitening agent and parabens concentrations in five commercial cosmetics and the results are summarized in Fig. 4 and Table 3. AR, EP, MP, PP, and SA were detected in the cosmetic products. The recoveries of the analytes from the products, determined using spiking experiments, were 85.2–118.0%. The results showed that the whitening agent and parabens concentrations in all of the cosmetic products that were examined were significantly lower than the allowed limits by Ministry of Health and Welfare from Taiwan.[31–33]

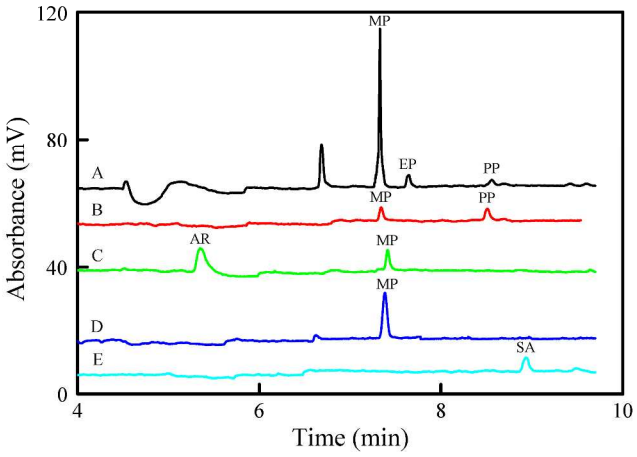

Fig. 4 Electropherograms of whitening agents and parabens in cosmetic products: (A) Mask, (B) Golden essence, (C) Lotion 1, (D) Lotion 2, and (E) facial washing milk. Other conditions were the same as those in Fig. 2B.

Table 3 Recoveries of the analytes from the cosmetic products (n = 5)

Analyte	Allowed limits	Mask		Golden essence		Lotion 1		Lotion 2		Facial washing milk	
		Original (μM)	Recovery[a] (%)	Original (μM)	Recovery (%)	Original (μM)	Recovery (%)	Original (μM)	Recovery (%)	Original (μM)	Recovery (%)
AR	7.0%[b]	N.D.[c]	88.0%	N.D.	98.1%	15.3 (2.08)[d]	95.5%	N.D.	96.1%	N.D.	85.2%
R	0.1%[e]	N.D.	103.0%	N.D.	107.4%	N.D.	106.1%	N.D.	95.5%	N.D.	91.4%
KA	2.0%[b]	N.D.	92.2%	N.D.	115.6%	N.D.	109.4%	N.D.	90.8%	N.D.	109.3%
SA	0.2–2.0%[f]	N.D.	104.9%	N.D.	95.5%	N.D.	106.8%	N.D.	110.9%	2.66 (0.73)[d]	110.6%
MP	Total amount < 1.0%[e]	5.38 (0.41)[d]	98.4%	4.52 (0.34)[d]	104.1%	6.54 (0.50)[d]	93.2%	11.8 (0.90)[d]	97.1%	N.D.	108.7%
EP		2.34 (0.04)[d]	110.9%	N.D.	95.3%	N.D.	109.2%	N.D.	110.0%	N.D.	108.6%
PP		1.62 (0.03)[d]	107.8%	2.65 (0.24)[d]	92.5%	N.D.	99.5%	N.D.	101.4%	N.D.	116.5%
BP		N.D.	112.4%	N.D.	98.0%	N.D.	118.0%	N.D.	107.4%	N.D.	96.4%

[a] The recovery results were calculated by spiking 3.0 μM standards to each sample. [b] Data from Ref. 31. [c] N.D.: not detected. [d] Data in parentheses were measured values (% w/w). [e] Data from Ref. 32. The total amount of parabens in cosmetic products < 1.0%. [f] Data from Ref. 33.

Conclusions

A sweeping-MEKC method, using a polymer solution, for the on-line focusing and analysis of whitening agents and parabens was developed. The method gave LODs of 8–162 nM (1.1-21.0 ng mL^{-1}) for the analytes, and these were 46–279 times lower than the LODs obtained using conventional sample injection (10 s, no polymer in the buffer solution). The method is simple and rapid, and it was used to determine whitening agent and parabens concentrations in commercial cosmetic products with satisfactory results. In conclusion, we experimentally proved that the proposed on-line sweeping-MEKC method promises improvements in sensitivity and resolution for the CE analysis of whitening agents and parabens.

Abbreviations

AR Arbutin
BP Butyl paraben
EF Enhancement factor
EOP Electroosmotic flow
EP Ethyl paraben
KA Kojic acid
MP Methyl paraben
PEO Poly(ethylene oxide)
PP Propyl paraben
R Resorcinol

Acknowledgements

This study was supported by the Ministry of Science and Technology of Taiwan under contract number MOST 103-2113-M-143-002.

Notes and references

Department of Applied Science, National Taitung University, 684, Section 1, Chunghua Road, Taitung 95002, Taiwan. E-mail: tcchiu@nttu.edu.tw; Tel.: +886 89 353840; Fax: +886 89 342539

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