Journal of Food and Drug Analysis, Vol. 16, No. 6, 2008, Pages 22-28 藥物食品分析 第十六卷 第六期

Simultaneous Identification of Eight Sunscreen Compounds in Cosmetic Products Using High-Performance Liquid Chromatography and Capillary Electrophoresis

LIH-JENG JUANG[1], BOR-SEN WANG[2], HUO-MU TAI[3], WEI-JING HUNG[4] AND MING-HSING HUANG[4]*

[1.] Department of Applied Cosmetic Science, Ching Kuo Institute of Management and Health, Keelung, Taiwan (R.O.C.)
[2.] Department of Food Science & Technology, Chia Nan University of Pharmacy and Science, Tainan, Taiwan (R.O.C.)
[3.] Department of Application Chemistry, Chia Nan University of Pharmacy and Science, Tainan, Taiwan (R.O.C.)
[4.] Department of Cosmetic Science, Chia Nan University of Pharmacy and Science, Tainan, Taiwan (R.O.C.)

(Received: January 7, 2008; Accepted: May 7, 2008)

ABSTRACT

p-Aminobenzoates (PABA) and benzophenones in cosmetic products absorb UV radiation. We have developed two simple methods to simultaneously determine whether cosmetic products contain any of eight sunscreen compounds. The first method used high-performance liquid chromatography (HPLC) and a Cosmosil 5C18-MS column with an isocratic system consisting of acetonitrile-H_2O solution (60/40, *v/v*) acidified by 0.1% phosphoric acid (v/v); the analysis was monitored by absorbance at 254 nm with a constant flow-rate 0.8 mL/min. This method could easily determine the presence of these compounds in non-pretreated sunscreen products in 30 min. The second method employed capillary electrophoresis (CE) in buffer solution (pH 10.0) containing 20 mM borate; the same analysis was made within 10 min. Reproducibility (relative standard deviation) of the proposed methods, on the basis of the peak-area ratios in six replicate injections, was good with only a slight deviation of 0.31 - 0.75% (for HPLC system) and 1.65 - 3.55% (for CE system). The detection limit (S/N = 3) of the individual marker substances varied from 0.08 to 0.87 μg/mL (for HPLC system) and 0.23 to 1.86 μg/mL (for CE system). Although the HPLC method was superior to the CE method in both reproducibility and resolution, it required longer retention time. The HPLC and CE methods developed were both successfully applied to the assay of 8 UV-absorbing agents in 11 commercial sun protection products.

Key words: sunscreen, benzophenones, high-performance liquid chromatography, capillary electrophoresis

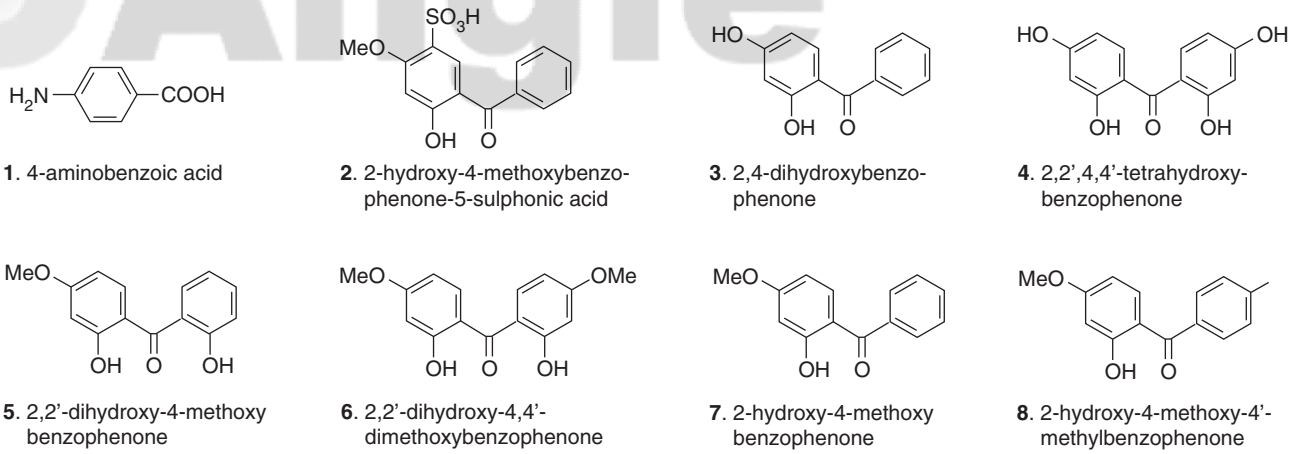
INTRODUCTION

Sunscreen agents are sun protection agents which consist of two major categories of chemical compounds. One category is the powders such as titanium dioxide and zinc oxide to physically reflect and scatter sun light. The other one comprises the chemical compounds of p-aminobenzoates (PABA), benzophenones and others. The latter compounds are chemicals intended to selectively absorb ultraviolet (UV) light; some are effective for preventing photobiological damage to the human skin, which can virtually lead to cutaneous disorders such as skin cancer and premature aging[(1)]. It has become essential to add sunscreen agents to cosmetic products in recent years. Among many putative sunscreen compounds, PABA and benzophenones are the most commonly used because of their high efficiency in absorbing UV light. Although the use of PABA has diminished in the market, benzophenones are popularly used for enhancing Sun Protection Factor (SPF) values in recent years. However, both PABA and benzophenones may produce photoallergic contact urticaria[(2,3)]. The benzophenone family has recently been listed among "chemicals suspected of having endocrine disrupting effects" by the World Wildlife Fund since it may contaminate the environment through sewage disposal channel when the products are dissolved in tap water after uses. The 4-hydroxyl group on the phenyl ring of benzophenone derivatives is believed to be essential for the high hormonal activities. Therefore, it is beneficial to the end users of these products that analytical methods are available to easily and properly identify these sunscreening chemicals.

In this study, two optimal methods were established to analyze eight UV-absorbing agents including 4-aminobenzoic acid **[1]**, 2-hydroxy-4-methoxybenophenone-5-sulphonic acid **[2]**, 2-hydroxy-4-methoxybenzophenone **[3]**, 2,2'-4,4'-tetrahydroxybenzophenone **[4]**, 2,2'-dihydroxy-4-methoxybenzophenone **[5]**, 2,2'-

* Author for correspondence. Tel: +81-75-712-9402; Fax: +81-75-753-6233; Email: zakirh1000@yahoo.com

Journal of Food and Drug Analysis, Vol. 16, No. 6, 2008



1. 4-aminobenzoic acid

2. 2-hydroxy-4-methoxybenzophenone-5-sulphonic acid

3. 2,4-dihydroxybenzophenone

4. 2,2',4,4'-tetrahydroxybenzophenone

5. 2,2'-dihydroxy-4-methoxy benzophenone

6. 2,2'-dihydroxy-4,4'-dimethoxybenzophenone

7. 2-hydroxy-4-methoxy benzophenone

8. 2-hydroxy-4-methoxy-4'-methylbenzophenone

Figure 1. Chemical structures of the eight sunscreen agents.

dihydroxy-4,4'-dimethoxy- benzophenone **[6]**, 2,4-Dihydroxybenzophenone **[7]** and 2-hydroxy-4-methoxy-4'-methylbenzo-phenone **[8]**. Their chemical structures are depicted in Figure 1.

Among these UV absorbing agents, component **[7]** and **[8]** are not included in the official Regulation Table for Medicated Cosmetics provided by the Department of Health, Executive Yuan, yet they are often found in some commercial products. Therefore, these two ingredients are also used as marker substance in this study. Several methods for analyzing multiple sunscreen components in cosmetic products are described in previous literatures including ultraviolet-visible spectrometry (UV-VIS)[4,5], gas chromatography-mass spectrometry (GC-MS)[6-8], high-performance liquid chromatography (HPLC)[9-19], and capillary electrophoresis (CE)[20-22]. UV-VIS is very accurate in analyzing pure compound, but not efficient for analyzing mixtures of multiple components. HPLC can simultaneously analyze up to seven components, but accompanied by some peak broadening and severe tailing phenomena (unpublished observations), and the partial overlapping of component **[5]** and **[6]** resulted in inaccurate quantization[12]. To analyze the metabolites of the benzophenones and PABA derivatives effectively[17,19], the elution mode of the mobile phase in HPLC was adopted since it is the main stream of contemporary HPLC technology[15-19]. The characterization of PABA using micellar electrokinetic capillary chromatography (MECC), a CE technique, was proposed, but it was not easy to establish a stable baseline[20]. When analyzing benzophenones using capillary zone electrophoresis (CZE), the peak of 2-hydroxy-4-octyloxybenzophenone overlapped with the electroendoosmotic flow (EOF)[22]. In general, HPLC is the most popular tool for this type of analysis. On the other hand, CE is a widely applied technique in separation science because of its high separation efficiency, and small sample requirement which is highly attractive as far as the green industry is concerned nowadays. Hence, we developed two simple and direct methods for simultaneous identification of eight marker compounds **[1-8]** in cosmetic products. The feasibility and efficiency of these two methods were then compared.

MATERIALS AND METHODS

I. *Materials*

Compounds [2], [3], [4], [5], [6], [7] and [8] were purchased from Aldrich (Milwaukee, WI, USA). Phosphoric acid and compound [1] were from Merck (Darmstadt, Germany). Sodium borate, sodium hydroxide, potassium dihydrogenphosphate, and cinnamic acid were obtained from Sigma (St. Louis, MO, USA). Methanol and acetonitrile were of LC grade (Fison, Loughborough, England). Deionized water was from Milli-Q system (Millipore, Bedford, MA, USA). Cosmetic products were purchased from different retail sources in Taipei, Taiwan.

II. *Preparing Extracts from Cosmetic Products*

One-gram samples of cosmetic products were extracted using 70% methanol (20 mL) by stirring at room temperature for 20 min and then centrifuging at 5000 rpm for 20 min. The extraction was repeated three times. The extracts were then combined and filtered through No. 1 filter-paper. The filtrate was diluted to 100 mL with 70% methanol. This solution was passed through a 0.45-μm PVDF-filter and injected into the HPLC (10 μL) or CE system (8.5 nL).

III. *Calibration Curve*

For each compound, stock solutions were prepared by dissolving 10 to 22 mg of marker substances (**1**, 11 mg; **2**, 12 mg; **3**, 11 mg; **4**, 22 mg; **5**, 14 mg; **6**, 10 mg; **7**, 11 mg; **8**, 10 mg) in 100 mL of 70% methanol. Stock solutions (0.1, 0.5, 1, 2.5, 5, and 8 mL) and 1 mL of inter-

nal standard (cinnamic acid, 500 μg/mL) were diluted to 10 mL in a flask. The linearity of the plot of peak-area ratio vs. concentration (μg/mL) for each of the marker substances in HPLC and CE was determined.

IV. *HPLC System*

The HPLC system was equipped with a pump (model 510; Waters Corp., Milford, MA USA), and a photodiode array detector (SPD-M10AVP; Shimadzu Co., Kyoto, Japan). The separations were achieved using a reversed-phase column (Cosmosil 5C_{18}-MS, 5 μm, 25 cm × 4.6 mm I.D.; Nacalai Tesque, Inc., Kyoto, Japan). An isocratic mode of 60% acetonitrile-water solution (v/v), which is acidified by phosphoric acid (0.1%, v/v), was used in this study. The flow-rates were kept constant at 0.8 mL/min and the elution peaks were monitored at 254 nm. A guard column of μ-Bondapak^{TM} C_{18} (Millipore), was attached to the analytical column.

V. *CE System*

All CE analyses were done on a Quanta 4000 CE system (Waters Corp., Milford, MA, USA) equipped with a UV detector set at 254 nm and a 60 cm × 50 μm I.D. uncoated capillary (Polymicro Technologies, Phoenix, AZ, USA) with the detection window placed at 52.5 cm. The conditions were as follows: sampling time, 5 s hydrostatic (injection volume, 8.5 nL); run time, 15 min; applied voltage, 25 KV (constant voltage, positive-to-negative polarity); and temperature, 25.0-26.0°C. The electrolyte was a buffer solution (pH 10.0) containing 20 mM borate.

VI. *Suitability*

To prepare the test solution, the standard stock solution (2 mL) and internal standard (IS; 1 mL) were spiked into a 10-mL volumetric flask, and 70% methanol was then added to the volume. Intra-day and inter-day analyses were done six times separately, using the optimum HPLC or CE condition. Series of dilutions were injected into an HPLC or CE system and the detection limit was determined based on the signal-to-noise ratio (S/N ratio) of 3.

RESULTS AND DISCUSSION

I. *Analytical Conditions for the HPLC Method*

Seven of the eight marker substances, **2**, **3**, **4**, **5**, **6**, **7**, and **8**, were successfully determined using acetic acid, methanol, and acetontrile, respectively, as the mobile phase [12]. However, under this analytical condition, compounds **3** and **5** partially overlapped and the theoretical plate numbers of most peaks were quite low. The resolution between **3** and **5** was dramatically improved by using acetontrile instead of methanol. Phosphoric acid (1%, v/v) was added to the mobile phase to keep the eluent in mild acidic condition. Variations of the acetonitrile/water ratio (80/20, 70/30, 60/40 and 50/50) in the mobile phase showed a positive relationship between the organic solvent ratio and K' value. In general, the greater the amount of acetonitrile, the shorter the retention time of all compounds. Because there were serious peak overlapping using the ratios of 80/20 and 70/30 for the carrier solvents, and prolonged retention time (> 40 minutes) for 50/50, the ratio of 60/40 was finally chosen as the standard for this study.

The effect of the concentrations of potassium dihydrogen phosphate salt (0, 5, 10, and 15 mM) on the eight marker substances was examined with the above standard solution. Phosphate precipitated when the concentration was greater than 20 mM. Since no significant difference in either the K' value or the theoretical plates were found, the phosphate salt was then excluded. To verify the effect of pH on retention, experiments were carried out by adding 1% phosphoric acid to achieve different pH values (range: 2.5-5.5). A solution with pH 3.0 was observed to separate all the constituents well. At lower pH values, the peaks of compounds **1**, **2**, and the internal standard were too narrow; at higher pH values, those peaks were broader. The running time for the separation of all eight compounds was about 30 min (Figure 2A). When methanol-water extract of the cosmetic product was directly injected and analyzed, the results were as good as those obtained using pure chemical samples (Figure 2B).

II. *Analytical Conditions for the CE Method*

All eight compounds and the Internal Standard (IS)

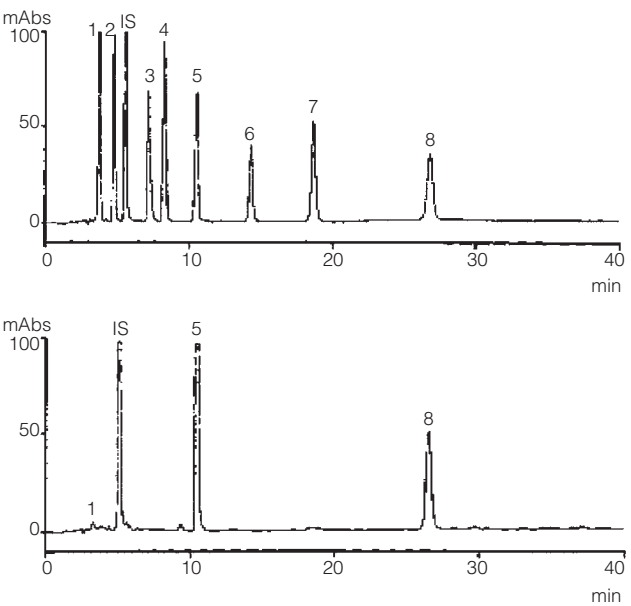


Figure 2. (A) HPLC graph of the eight compounds of sunscreen agents. (B) HPLC graph of a cream product (cream 1).

Journal of Food and Drug Analysis, Vol. 16, No. 6, 2008

were successfully analyzed in a single run by capillary zone electrophoresis (CZE) under proper conditions. The separation was achieved by optimizing the pH of the buffer and the concentrations of borate. The borate concentrations used were 5, 10, 15, 20, 25, and 30 mM. Increasing borate concentration greatly improved the resolution for compounds **5**/**7** and **3**/**8** (Table 1). In particular, at 20 mM of borate, the **3**/**8** pair showed a resolution value of 1.4 and baseline separation. At concentrations higher than 20 mM, there were no change in efficiency, but the analysis required longer run time. Finally, the composition of the mobile phase was optimized to 20 mM of borate.

Several borate solutions of 20 mM with various pH values between 9.0 and 11.0 (filtrated by adding 1% NaOH or HCl) were used to demonstrate the effect on separation of the marker compounds (Figure 3A & 3B). At pH 10.0, a better resolution for **3**/**8** (Rs = 1.7) was obtained than that at any other pH. This result was achieved with a 20 mM borate solution (pH 10.0).

Table 1. Comparisons of resolution by HPLC for 2 compound pairs

	Resolution	
Borate (mM)	5/7	3/8
5	×	×
10	1.1	1.0
15	1.3	1.2
20	1.5	1.4
25	1.6	1.4
30	1.6	1.4

×: peak overlapping.

III. *Method Validation*

We calculated the linearity of the plot of the peak-area (y) vs. concentration (x, μg/mL) for each of the marker substances in HPLC and CE. The linear ranges and the correlation coefficients for these compounds are showed in Table 2. In the HPLC system, the regression equations of the constituents were as follows: **1**, y = 0.0110x – 0.0026; **2**, y = 0.0211x – 0.0111; **3**, y = 0.0184x – 0.0381; **4**, y = 0.0193x – 0.0161; **5**, y = 0.2555x – 0.2048; **6**, y = 0.0255x – 0.0418; **7**, y = 0.3981x – 0.7746; **8**, y = 0.3823x – 1.0508; and in the CE system, the regression equations of the constituents were as follows: **1**, y =

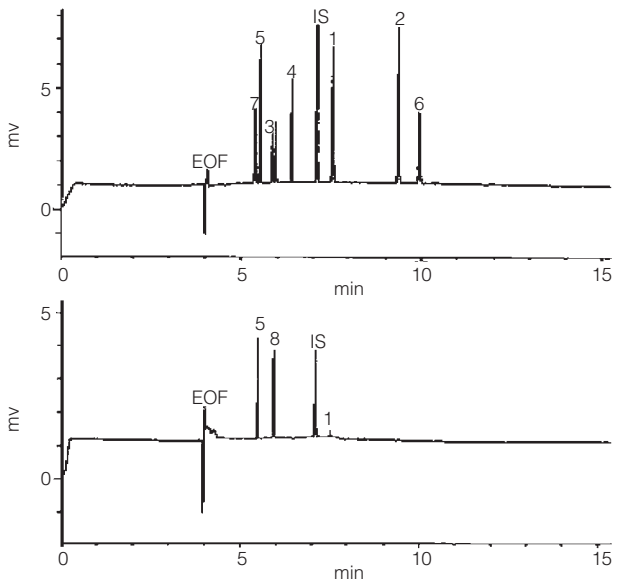


Figure 3. (A) CE graph of the eight compounds of sunscreen agents. (B) CE graph of a cream product (cream 1).

Table 2. Linear range, correlation coefficient (R^2), recovery, detection limit on 8 marker compounds in HPLC and CE system

	HPLC system				CE system			
Compounds	Linear range (μg/mL)	R^2	Recovery (%)*	Detection limit (μg/mL)	Linear range (μg/mL)	R^2	Recovery (%)*	Detection limit (μg/mL)
1	1.1-110	0.9991	94.8	0.87	2.2-110	0.9985	90.2	1.48
2	1.2-120	0.9991	95.2	0.51	1.2-120	0.9991	92.3	0.95
3	1.1-110	0.9995	96.5	0.76	2.2-110	0.9951	92.8	1.33
4	2.2-220	0.9999	100.5	0.49	2.2-220	0.9948	93.4	1.02
5	1.4-140	0.9997	101.8	0.11	1.4-140	0.9914	95.0	0.25
6	1.0-100	0.9993	100.2	0.81	2.0-100	0.9935	91.0	1.86
7	1.1-110	0.9993	98.1	0.08	1.1-110	0.9921	90.2	0.23
8	1.0-100	0.9992	95.6	0.10	1.0-100	0.9908	93.1	0.23

*Concentration of each compound for recovery test: Stock solution: **1**, 55; **2**, 60; **3**, 551; **4**, 110; **5**, 70; **6**, 50; **7**, 55; **8**, 50 (μg/mL); Added amount: **1**, 30; **2**, 30; **3**, 30; **4**, 50; **5**, 30; **6**, 30; **7**, 30; **8**, 25 (μg/mL).

Table 3. Comparisons of reproducibility on 8 marker compounds

Compound	HPLC R.S.D. (%) (n = 6)				CE R.S.D. (%) (n = 6)			
	Retention Time		PAR		Migration Time		PAR	
	Intraday	Interday	Intraday	Interday	Intraday	Interday	Intraday	Interday
1	0.18	0.32	0.52	0.31	0.55	0.95	1.85	1.81
2	0.32	0.35	0.63	0.48	0.64	3.89	2.05	1.95
3	0.28	0.28	0.55	0.56	0.62	1.02	1.65	2.56
4	0.28	0.41	0.50	0.75	0.48	1.24	3.88	3.12
5	0.34	0.30	0.45	0.48	0.57	0.95	3.75	2.85
6	0.22	0.28	0.54	0.59	0.79	0.84	2.76	2.04
7	0.26	0.42	0.44	0.55	0.61	1.20	2.98	2.81
8	0.31	0.31	0.32	0.38	0.73	1.03	3.55	2.15

HPLC, high-performance liquid chromatography; CE, capillary electrophoresis; R.S.D., relative standard deviation; PAR: Peak-area ratio with respect to the internal standard (IS).

0.0168x + 0.0252; **2**, y = 0.0185x – 0.0314; **3**, y = 0.0184x – 0.0381; **4**, y = 0.0037x + 0.0109; **5**, y = 0.0139x – 0.0319; **6**, y = 0.0106x – 0.0121; **7**, y = 0.0132x – 0.0365; **8**, y = 0.0124x – 0.1065. The linearity of calibration curves is good over 2-3 orders, and the correlation coefficients (R^2) exceed 0.999 for all compounds for HPLC, and 0.99 for all compounds for CE

IV. *Precision*

Reproducibility (relative standard deviation) of the proposed methods, on the basis of the peak-area ratios in six replicate injections, was 0.31-0.75% (for the HPLC system) and 1.65-3.55% (for the CE system). The relative standard deviation of the retention time of each peak for six replicate injections was less than 0.42% for the HPLC system and 3.89% for the CE system, respectively. Detailed data for individual constituents are given in Table 3.

V. *Recovery*

Suitable amounts (25.0-100.0 μg) of compounds **1**, **5**, and **8** were spiked to a pretreated sample of Cream 1. The recoveries of **1**, **5**, and **8** determined by either method were around 92.12-103.13% (n = 3) (Table 4). The tailing factors of all peaks were very close to unity. The detection limit (S/N = 3) of the individual marker substances varied from 0.08 to 0.87 μg/mL in the HPLC system and from 0.23 to 1.86 μg/mL in the CE system (Table 2).

VI. *Determining the Marker Substances in Cosmetic Products*

When test solutions were analyzed by HPLC and CE under the selected conditions, those data from Figures 2B and 3B and the contents of constituents in a cosmetic products extract were calculated (Table 5). While most of the samples contained 3 marker substances, cream 3 had only 2 markers, and cream 5 had 5 markers. The elution time for each compound in HPLC and CE, respectively, was remarkably different mainly due to the different separation mechanism. Within a detection time frame of 10 minutes as demonstrated in this study, compounds 1-5 would be preferably analyzed by HPLC and compounds 3-8 effectively by CE. Moreover, the substantial reduction in solvent consumption by CE consists of additional advantage over HPLC as far as the environment protection or green chemistry is concerned.

Table 4. Summary of recovery of compounds **1**, **5**, and **8** (n = 3) in cream 1

Constituent	Added (μg)	HPLC Recovery (%)	CE Recovery (%)
1	25.0	98.65	93.38
	50.0	97.29	92.12
	100.0	98.05	92.66
5	25.0	103.13	96.99
	50.0	101.27	95.34
	100.0	102.42	92.61
8	25.0	98.13	94.97
	50.0	100.27	98.84
	100.0	98.42	93.69

Journal of Food and Drug Analysis, Vol. 16, No. 6, 2008

Table 5. Analytical results from the HPLC and CE determinations of marker substances in commercial sunscreen products (%, *w/w*)

Cosmetics	HPLC								CE							
	compound								compound							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Cream 1	0.5	-	-	-	2.8	-	-	1.5	0.5	-	-	-	2.5	-	-	1.5
Cream 2	-	1.8	-	1.5	-	-	2.6	1.0	-	1.6	-	1.7	-	-	2.5	0.8
Cream 3	-	3.3	-	-	1.6	-	-	-	-	3.1	-	-	1.4	-	-	-
Cream 4	-	1.0	-	-	2.6	-	-	0.8	-	1.0	-	-	2.3	-	-	0.7
Cream 5	1.1	1.2	0.6	-	1.4	-	2.0	-	1.0	1.0	0.5	-	1.4	-	1.8	-
Cream 6	-	1.1	-	-	0.8	-	0.8	-	-	1.0	-	-	0.8	-	0.8	-
Cream 7	-	0.8	-	-	1.0	-	1.8	-	-	0.8	-	-	0.9	-	1.7	-
Cream 8	0.8	-	-	-	1.6	-	-	0.5	0.7	-	-	-	1.4	-	-	0.5
Lotion 1	-	0.8	-	-	0.8	-	1.6	-	-	0.7	-	-	0.8	-	1.6	-
Lotion 2	0.6	-	-	-	0.8	-	-	0.8	0.5	-	-	-	0.8	-	-	0.7
Lotion 3	-	0.8	-	-	0.7	-	0.5	-	-	0.7	-	-	0.7	-	0.5	-

CONCLUSIONS

By optimizing the pH, buffer composition, and concentration of the eluent or carrier, eight components in the extracts of cosmetic products were determined within 30 min by HPLC and within 10 min by CE. The two proposed methods showed acceptable reproducibility, high accuracy, and good linear relationships between the peak-area ratios and concentrations. Although the HPLC method was superior to the CE method in both reproducibility and resolution, it required longer retention time. Results from this study also demonstrated that multiple sunscreen compounds were discernibly present in various commercial products.

ACKNOWLEDGEMENTS

Financial support from the Chia Nan University of Pharmacy and Science, Taiwan, is gratefully acknowledged.

REFERENCES

1. Carpenter, T., O'Connor, A., Orfanelli, J. and Siegfried, R. 1996. Protection from Sun Protectors. Drug and Cosmetic Industry. 158: 56-58.
2. Gasparro, F. P., Mitchcnick, M. and Nash, J. F. 1998. A review of sunscreen safety and efficacy. Photochem. Photo-Biol. 68: 243-256.
3. Lowe, N. J., Shaath, N. A. and Pathak, M. A. 1997. In "Sunscreens: Development, Evaluation and Regulatory Aspects". pp. 201-260. Marcel Dekker. New York, U.S.A.
4. Michaud, P., Soto, P., Le Roy, T. and Rodriguez, F. 1994. First-derivative spectroscopic determination of sunscreens in cosmetic formulations. Int. J. Cosm. Sci. 16: 93-104.
5. Chisvert, A., Salvador, A. and Pascual-Marti, M. C. 2001. Simultaneous determination of oxybenzone and 2-ethylhexyl 4-methoxycinnamate in sunscreen formulations by flow injection-isodifferential derivative ultraviolet spectrometry. Anal. Chim. Acta 428: 183-190.
6. Ikeda, K., Suzuki, S. and Watanabe, Y. 1990. Determination of sunscreen agents in cosmetic products by gas chromatography and gas chromatography-mass spectrometry. J. Chromatogr. 513: 321-326.
7. Ro, K. W., Choi, J. B., Lee, M. H. and Kim, J. W. 1994. Determination of salicylate- and benzophenone-type sunscreen agents in cosmetic products by gas chromatography -mass spectrometry. J. Chromatogr. A 688: 375- 382.
8. Lambropoulou, D. A., Giokas, D. L., Sakkas, V. A., Albanis, T. A. and Karayannis, M. I. 2002. Gas chromatographic determination of 2-hydroxy-4-methoxybenzophenone and octyldimethyl-p-aminobenzoic acid sunscreen agents in swimming pool and bathing waters by solid-phase microextraction. J. Chromatogr. A 967: 243-253.
9. Gagliardi, L., Amato, A., Basili, A., Cavazzutti, G. and Tonelli, D. 1987. Determination of sun-screen agents in cosmetic products by reversed-phase high-performance liquid chromatography. J. Chromatogr. 408: 409-415.

10. Gagliardi, L., Cavazzutti, G., Montanarella, L. and Tonelli, D. 1989. Determination of sunscreen agents in cosmetic products by reversed-phase high-performance liquid chromatography. Part II. J. Chromatogr. 464: 428-433.
11. Vanquerp, V., Rodriguez, C., Coiffard, C., Coiffard, L. J. and De Roeck-Holtzhauer, Y. High-performance liquid chromatographic method for the comparison of the photostability of five sunscreen agents. J. Chromatogr. A 832: 273-277.
12. Wang, L. H. 1999. Simultaneous determination of seven sunscreen benzophenones in cosmetic products by high-performance liquid chromatography. Chromatographia 50: 565-570.
13. Scalia, S. 2000. Determination of sunscreen agents in cosmetic products bysupercritical fluid extraction and high-performance liquid chromatography. J. Chromatogr. A 870: 199-205.
14. Mazonakis, N. E., Karathanassi, P. H., Panagiotopoulos, D. P., Hamosfakidi, P. G. and Melissos, D. A. 2002. Cleaning validation in the toiletries industry. Anal. Chimi. Acta 467: 261-266.
15. Schakel, D. J., Kalsbeek, D. and Boer, K. 2004. Determination of sixteen UV filters in suncare formulations by high-performance liquid chromatography. J. Chromatogr. A 1049: 127-130.
16. Simeoni, S., Tursilli, R., Bianchi, A. and Scalia, S. 2005. Assay of common sunscreen agents in suncare products by high-performance liquid chromatography on a cyanopropyl-bonded silica column. J. Pharm. Biomed. Anal. 38: 250-255.
17. Kasichayanula, S., House, J. D., Wang, T. and Gua, X. 2005. Simultaneous analysis of insect repellent DEET, sunscreen oxybenzone and five relevant metabolites by reversed-phase HPLC with UV detection. J. Chromatogr. B 822: 271-277.
18. Kedor-Hackmann, E. R. M. and Santoro, M. I. R. M. 2006. Validation of a HPLC method for simultaneous determination of five sunscreens in lotion preparation. Int. J. Cosmet. Sci. 28: 219-224.
19. Wang, L. H., Huang, W. S. and Tai, H. M. 2007. Simultaneous determination of p-aminobenzoic acid and its metabolites in the urine of volunteers treated with p-aminobenzoic acid sunscreen formulation. J. Pharm. Biomed. Anal. 43: 1430-1436.
20. Wang, S. P. and Chen, W. J. 2000. Determination of p-aminobenzoates and cinnamate in cosmetic matrix by supercritical fluid extraction and micellar electrokinetic capillary chromatography. Anal. Chimi. Acta 416: 157-167.
21. Lin, C. E. and Chen, M. J. 2001. Separation and selectivity of benzophenones in micellar electrokinetic chromatography using sodium dodecyl sulfate micelles or sodium cholate modified mixed micelles. J. Chromatogr. A 923: 241-248.
22. Wang, S. P. and Lee, W. T. 2003. Determination of benzophenones in a cosmetic matrix by supercritical fluid extraction and capillary electrophoresis. J. Chromatogr. A 987: 269-275.