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行政院國家科學委員會專題研究計畫成果報告

化妝品中防曬劑二苯甲酮系列化合物測定方法之研究

Determination of Sunscreen Benzophenones in Cosmetic Products

計畫編號：NSC 88- 2113- M- 041- 001
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利用高效率液相層析法，同時測定市售化妝品中防曬劑二苯甲酮系列化合物的含量。以乙睛-甲醇-水(acetonitrile-methanol-water,30:30:40, *v/v/v*)爲移動相，在 Hypersil ODS (25 cm x 4.6 mm i.d.)管柱中，於流速 1.0 mL/min 及 286 nm 偵測條件下，同時測定七種二苯甲酮系列化合物。將分析結果與微分脈波伏安法比較發現很一致。

關鍵詞：液相層析，防曬劑，二苯甲酮系列化合物，化妝品。

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Simultaneous Determination of Seven Sunscreen Benzophenones in Cosmetic Products by High-Performance Liquid Chromatography

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Key Words

Column liquid chromatography
Sunscreens
Benzophenones
Cosmetic products

Summary

A HPLC method for the simultaneous determination of benzophenones is described. The seven compounds studied were separated on a Hypersil ODS C_{18} (25 cm x 4.6 mm i.d.) using acetonitrile-methanol-water (30:30:40, *v/v/v*) as mobile phase at 1.0 mL min^{-1} and detected at 286 nm. The results obtained were in good agreement with those obtained by differential pulse voltammetry.

Introduction

Twelve different benzophenones, designated Benzophenones 1 ~ 312, are substituted derivatives of 2-hydroxybenzophenone (see Reagents and Materials). They are used as photostabilizers in cosmetics and have a photoprotective effect on skin [1-2]. Benzophenones -2, 3, 4, 6, 8 and 9 are used in suntan lotion and hair sprays. These active ingredients also photostabilize cosmetic dyes, lipstick, creams and lotions. Most benzophenones are solid at room temperature and water insoluble, the presence of a sulfonic acid group in Benzophenones 4, 5 and 9 makes them soluble in water. Benzophenones are frequently incorporated in plastics, textiles and films imparting protection and color fastness to UV and antioxidants [3]. According to the Food and Drug Ad ministration (FAD) in 1976, benzophenones are used in over 1,000 cosmetic formulation. The following are the maximum reported product concentrations for each compound: Benzophenone -1, 1 % (*w/w*); Benzophenone-2, 5 % (*w/w*); Benzophenone-3, 1 % (*w/w*); Benzophenone-4, 10 % (*w/w*) ; Benzophenone-5, ≦0.1 % (*w/w*); Benzophenone-6, 1 % (*w/w*) (*w/w*); Benzophenone-8, 1 % (*w/w*); Benzophenone-9; Benzophenone-11, 5 % (*w/w*). Benzophenones -7, 10 and 12 have no current cosmetic use. Benzophenones have been studied for absorption, metabolism and excretion in rats [4-6]. They are practically nontoxic when chronically administered orally to rats, and Benzophenones-3 and 4 are nontoxic when applied to the skin of rabbits at doses *mG 5 $g.kg^{-1}$. However, Benzophenone-3 is metabolized to three compounds, namely: 2, 4-dihydroxy benzophenone, 2, 3, 4-trihydroxybenzophene and 2, 2′-dihydroxy-4-methoxybenzophenone which is a bacterial mutagen. Toxicity studies using animal models have also indicated the potential of Benzophenone-3 and its congeners to cause harmful effects with prolonged usage. There have been 12 reported cases of photocontact allergy and two cases of contact allergy to Benzophenone-3 [7].

Several methods have been proposed for the identification and determination of sunscreen agents, based on thin-layer chromatography [8], gas chromatography-mass spectrometry [9,10] and high-performance liquid chromatography [11-19]. The peaks of the sunscreen agents in practical GC analysis sometimes overlap those of other ingredients contained in cosmetic products. In such cases, MS is essential to confirm whether particular sunscreen agents are present or not. Since most benzophenones have low sensitivity and volatility for GC purposes, derivatization such as silylation must be used. These procedures are relatively time consuming and laborious because of the complex sample pretreatment and therefore are not suitable for the routine analysis of cosmetic products. Reversed-phase, high-performance liquid chromatography (HPLC) is ideally suited for the analysis of sunscreen agents. However, the above papers reported determination of octyldimethyl PABA, octyl methoxycinnamate, octyl salicylate and Benzophenone-3. A few papers describe the determination of benzophenones in cosmetics by HPLC [13,15]. Separations were carried out by gradient elution using diode array detection. In this study we describe a HPLC method for the rapid and simultaneous determination of seven benzophenones using isocratic elution.

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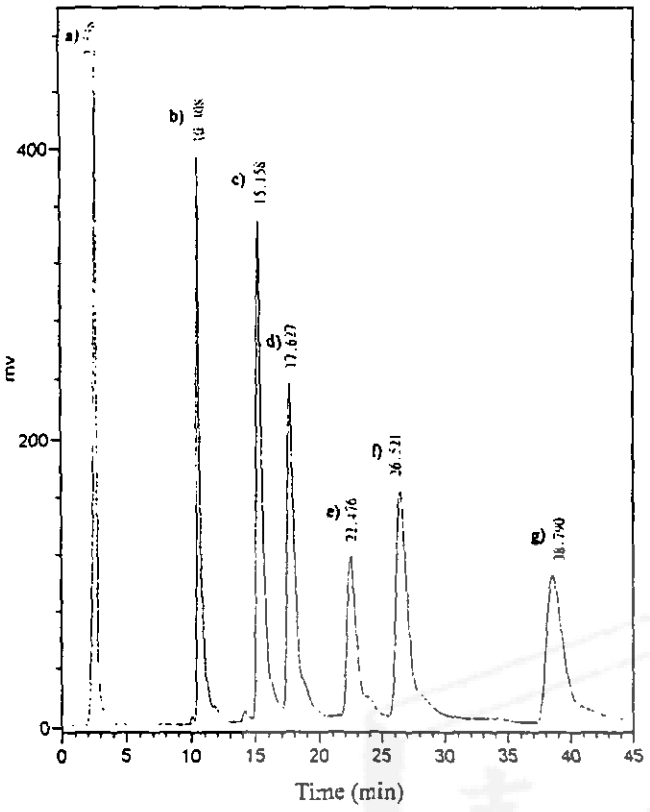

Figure 1 A

Separation of mixture (160 mg L^{-1}) of a = Benzophenone-4, b = Benzophenone-2, c = Benzophenone-1, d = Benzophenone-8, e = Benzophenone-6, f = Benzophenone-3, g = Benzophenone-10. Stationary phase, Hypersil ODS (25 cm x 4.6 mm i. d.); mobile phase; organic (acetonitrile- methanol) linear gradient with water 40 %–60 % in 40 min.; flow rate, 1.0 mL.min^{-1}; injection, 25μL; detection 286 nm.

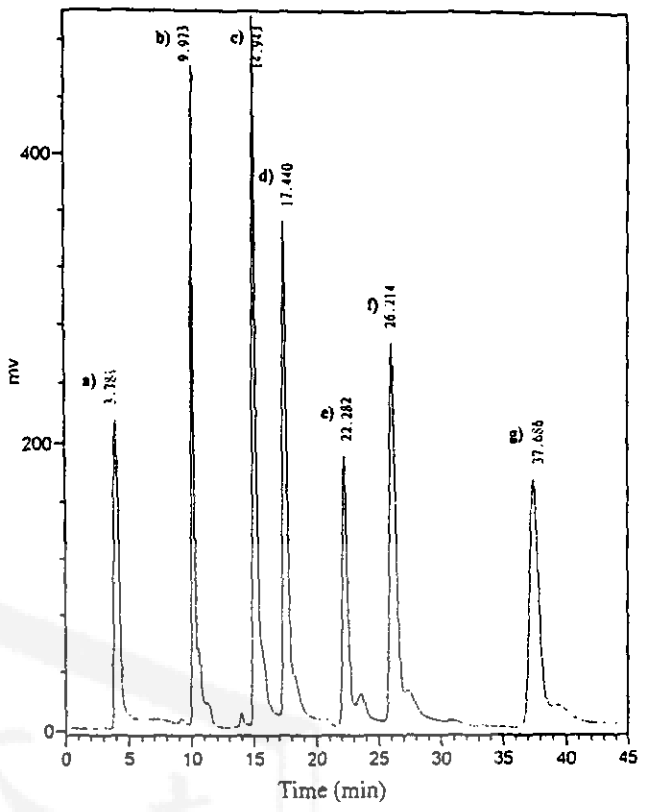

Figure 1 B

Separation of (160 mg L^{-1}) a = Benzophenone-4, b = Benzophenone-2, c = Benzophenone-1, d = Benzophenone-8, e = Benzophenone-6, f = Benzophenone-3, g = Benzophenone-10. Stationary phase, Hypersil ODS (25 cm x 4.6 mm i. d.); mobile phase , organic (acetonitrile- methanol) linear gradient with water containing 1 % acetic acid 40 %–60 % in 40 min.; flow rate, 1.0 mL.min^{-1}; injection, 25μL; detection 286 nm.

Experimental

Reagents and Materials

The sunscreen agents are tested: 2,4-dihydroxybenzophenone (Benzophenone-1), (TCI); 2, 2′-4, 4′-tetrahydroxybenzophenones (Benzophenone-2), (Aldrich, Milwaukee, W. I. U.S.A.); 2-hydroxy-4-methoxybenzophenone (Benzophenone-3), (TCI, Tokyo Kasei Kogyo Co.,Japan); 2-hydroxy-4-methoxybenzophenone-5-sulphonic acid (Benzophenone-4), (TCI); 2-2′-dehydroxy-4,4′-dimethoxybenzophenone (Benzophenone-6), (Lancaster, Eastgate, White Lund, Morecambe, U.K.);2-2′-dihydroxy-4-methoxybenzophenone (Benzophenone-8), (Aldrich); 2-hydroxy-4-methoxy-4′-methylbenzophenone (Benzophenone-10), (TCI). The supporting electrolyte was 0.1 M tetrabutylammonium hydroxide (pH 12). Sample of sun cream, refresh lotion, antibacterial body lotion and lipstick were from a number of retail outlets in the south of Taiwan.

Apparatus

The HPLC system consisted of a Model 576 pump (Gasukuro Kogyo, Japan) , a Model 7125 injector equipped with 20μL sample loop and a Model 502 U spectro detector. Chromatograms and peak areas were obtained with a SISC Chromatogram Data Integrator. Absorbance measurements were on a Cary UV-visible spectrophotometer (Varian Australia Pty Ltd). Matched quartz cells 1 cm path length were used to hold all solution for measurement. An EG & G Princeton applied Research (Princeton, NJ. USA) Model 254 Versatat was connected to a Metrohm 628 disk glassy carbon electrode (3 mm diameter). The three-electrode system consisted of the glassy carbon electrode, a platinum counter electrode and a saturated calomel electrode (SCE) reference.

Determination by liquid chromatography

Stock solutions of standards were prepared by dissolving the appropriate amount of sunscreen agents in ethanol and deionized water (1:1,*v/v*). A set of standard solutions was produced by diluting aliquots of stock solutions with ethanol and deionized water to 10 mL in volumetric flasks. Taking into account about the Benzophenone content of the sun cream, refresh lotion, antibacterial body lotion and lipstick, samples (approx.

0.01–1.00 g) of the latter were weighed accurately in a 15 mL beaker, diluted to about 5 mL with methanol and deionized water, dissolved and transferred into a 10 mL volumetric flask. The beaker was rinsed twice with 3 mL portions of methanol and deionized water and the rinsings were combined in the volumetric flask The solution was diluted to volume with methanol and deionized water. Benzophenones were separated from the treatment lipstick using liquid-liquid extraction .The lipstick (approx. 0.01 ~ 0.05 g) was accurately weighed and dissolved with 3 mL of chloroform and 5 ~ 6 mL methanol. After centrifugation, the supernate was transferred to a 10 mL volumetric flask and made up to volume with methanol. The final solution was filtered through 0.45 μm and 0.20 μm membrane filters before LC analysis. Reverse-phase LC was on a Hypersil ODS C_{18} column (Shandon southern, Runcorn, U. K.; particle size 5 μm , 25 cm x 4.6 mm i.d) column. The separation was performed using two mobile phases, organic and aqueous. The organic phase was acetonitrile- methanol; it was followed by a linear gradient to 60 % organic phase in 40 min. The aqueous phase was water either containing 1 % (*w/w*) acetic acid or not, respectively. The isocratic mobile phases were 15: 15: 70 (*v/v/v*), 20: 20: 60 (*v/v/v*), and 30:30:40 (*v/v/v*) , acetonitrile -methanol -water ; the mobile phase flow rate was1.0 mL min^{-1} and the UV-detector operated at 286nm. Injections (25 μL) of sample and standard solution were by the injection valve. Quantitation was based on compound peak area.

Determination by Differential Pulse Voltammetry (DPV)

About 0.50 g cosmetic (sun cream, refresh lotion, antibacterial body lotion) was accurately weighed, dissolved in 5 mL methanol and de-ionized water, and mixed ultrasonically for 5 min. The mixture was centrifuged and transferred into a 10 mL volumetric flask and made up to volume with methanol. The lipstick (approx. 0.10 ~ 0.50 g) was accurately weighed and dissolved in 5 ~ 8 mL dimethylformamide. After centrifugation, the supernate was transferred into a 10 mL volumetric flask and made up to volume with dimethylformamide. To obtain calibration graphs for the benzophenones, 10 mL supporting electrolyte were pipetted into a voltammetric cell and de-aerated with nitrogen for 4 min. before voltammetric measurement. By micropipette, aliquots of 1.0 g L^{-1} benzophenone solution were added. After each addition voltammograms were obtained ; the solution was de-aerated for 1 min. after each addition before obtaining the voltammogram. Quantitative analyses were performed in the differential pulse mode with the potential at –1.0 to –2.0 V *versus* SCE. The pulse height was 50 mV, and the E_{step} 4 mV had a drop time of 1.0 s. For sample solution analysis, 0.1 mL of the solution was pipetted into a 10 mL voltammetric cell and analysed by DPV using the same conditions as for the calibration graph.

Results and Discussion

Optimization of HPLC Separation

Figures 1A and 1B show the chromatograms of the aqueous phase without or with 1 % (*w/w*) acetic acid, respectively, obtained at 286 nm using linear gradient elution. The type of stationary phase used influenced the extent of peak tailing of benzophenones. Peak tailings of Benzophenone-8, 6, 3 and 10 are probably due to differences in surface coverage of the silica packing by the bonded phases. Separation improvement during chromatography is achieved by addition of acetic acid to the eluent. Acetic acid does not affect the selectivity for the sunscreens, but reduces tailing of benzophenone. The retention times of benzophenones were still the same, except Benzophenone-4, and well separated. However, the solvent gradient method is unsuitable for routine analysis because it is time consuming and requires expensive apparatus. Therefore, we tried to separate the seven benzophenones using an isocratic mobile phase15: 15: 70 (*v/v/v*), 20: 20: 60 (*v/v/v*), and 30:30:40 (*v/v/v*) , acetonitrile -methanol -water. After various studies of the retention behavior of the benzophenones, we achieved baseline separation. A typical HPLC trace under optimum conditions is shown in Figure 2. Acetonitrile-methanol-water (30:30:40, *v/v/v*) was used as mobile phase and Hypersil ODS (5μm) packed in column(25 cm x 4.6 mm i.d.) was the stationary phase. Flow rate and measurement wavelength were adjusted to 1.0 mL min^{-1} and 286nm, respectively.

In order to optimize the HPLC conditions described above, the following parameters were examined: (1) effect of acetic acid (2) proportion of acetonitrile and methanol and (3) proportions of organic solvents and aqueous solution. The retention time of Benzophenone-4 increases with increasing concentration of acetic acid, whereas the other benzophenones were independent of concentration. As a satisfactory separation could not be obtained when we used methanol or concentration alone as an organic solvent in the mobile phase, we tried mixtures of them and the effect of the proportions of the compounds was investigated. Acetonitrile and methanol gave similar selectivity. However, the retention of ben-

Table 1. Retention times, correlation coefficients (*r*) and detection limits of Benzophenones chromatographed on Hypersil ODS with acetonitril-methanol-water(30: 30: 40, *v/v/v*) mobile phase.

Benzophenones	Retention time (min)	*r*	Detection limit (mg L^{-1})
Benzophenone-1	8.785	0.9999	0.82
Benzophenone-2	4.849	0.9997	0.82
Benzophenone-3	19.305	0.9995	0.34
Benzophenone-4	2.161	0.9999	0.89
Benzophenone-6	15.166	0.9999	0.51
Benzophenone-8	10.946	0.9999	0.91
Benzophenone-10	29.224	0.9999	2.03

Table II. Recovery and reproducibility of the analysis of cosmetic samples by HPLC

Cosmetics	Benzophenone-1			Benzophenone-3			Benzophenone-4		
	Added ($mg\,L^{-1}$)	Found ($mg\,L^{-1}$)	Recovery (%)	Added ($mg\,L^{-1}$)	Found ($mg\,L^{-1}$)	Recovery (%)	Added ($mg\,L^{-1}$)	Found ($mg\,L^{-1}$)	Recovery (%)
Whiteness cleanser	2.00	2.10	105 (4.8 %)[a]	----	----	----	----	----	----
Suncream	----	----	----	5.00	5.18	104 (5.0 %)	----	----	----
Lipstick	----	----	----	10.00	9.91	99 (1.8 %)	----	----	----
Shampoo	----	----	----	----	----	----	5.00	4.95	99 (1.6 %)
Refresh lotion	----	----	----	----	----	----	5.00	4.98	99 (2.5 %)

[a] Relative standard deviation (n=3)

Table III. Analytical results from the HPLC and differential pulse voltammetry (DPV) determination of Benzophenones in commercial cosmetic products

Cosmetics	Concentration (%, *w/w*)					
	Benzophenone-1		Benzophenone-3		Benzophenone-4	
	HPLC	DPV	HPLC	DPV	HPLC	DPV
Whiteness cleanser	2.663×10^{-3} (1.1 %)	----[b]	----	----	----	----
Whitener washing foam 1	6.510×10^{-3} (4.3 %)	----[b]	----	----	----	----
Whitener washing foam 2	6.259×10^{-3} (1.5 %)	----[b]	----	----	----	----
Lightening facial foam	5.639×10^{-3} (4.6 %)	----[b]	----	----	----	----
Shampoo	----	----	----	----	0.134 (2.4 %)	0.143 (5.0 %)
Shower and both gel 1	----	----	----	----	0.159 (3.5 %)	0.158 (4.5 %)
Shower and both gel 2	----	----	----	----	0.054 (4.8 %)	----[b]
Shower and both gel 3	----	----	----	----	0.065 (1.2 %)	----[b]
Refresh lotion	----	----	----	----	0.0555 (5.0 %)	----[b]
Suncream 1	----	----	3.097 (2.6 %)	2.987 (5.0 %)	----	----
Suncream 2	----	----	2.881 (2.6 %)	2.774 (0.2 %)	----	----
Suncream 3	----	----	3.321 (1.6 %)	3.355 (0.8 %)	----	----
Suncream 4	----	----	3.040 (1.4 %)	2.723 (0.8 %)	----	----
Suncream 5	----	----	4.657 (1.7 %)	4.770 (0.6 %)	----	----
Suncream 6	----	----	2.028 (2.2 %)	2.070 (2.9 %)	----	----
Antibacterial hand lotion	----	----	0.0607 0.0725 (2.9 %)	---- (2.9 %)	----	
Lipstick 1	----	----	5.647 (2.4 %)	5.695 (2.2 %)	----	----
Lipstick 2	----	----	3.511 (2.5 %)	3.448 (2.6 %)	----	----
Lipstick 3	----	----	4.026 (4.8 %)	4.157 (1.0 %)	----	----[b]

[a]: Relative standard deviation (n=6) [b]: Not detected by the DPV method

zophenones increased with increasing proportions of acetonitrile, we concluded that acetonitrile acts as a kind of controller for suitable separation. Seven benzophenones showed the greatest effect with acetonitrile, a ratio of acetonitrile to methanol of 1:1 was adopted based mainly on the separation of benzophenones. The effect of the proportions of organic solvents and aqueous solution was investigated. We chose a 4 ratio of aqueous solution in the mobile phase, because baseline separation of the seven benzophenones was achieved.

Table I reports retention times and limits of detection at 286 nm for the benzophenones investigated. Calibration graphs were linear for all compounds considered with correlation coefficients ranging from 0.9995 to 0.9999.

Precision and Recovery

Recovery tests were carried out on cosmetic products for evaluation of the reproducibility and accuracy of the proposed method. Five commercial products were spiked with the amounts of the agents reported in Table II and subjected to the full extraction procedure. As is seen, excellent recoveries and precision were observed.

Application

The proposed method was applied to the determination of benzophenones in 19 samples of commercial cosmetic products. Figure 3 shows typical chromatograms obtained after analysis of a commercial whitener washing foam (A), refresh lotion (B), antibacterial hand lotion (C), and lipstick (D). The results are shown in Table III. Comparison with results from differential pulse voltammetry (DPV) (Table III) show good agrement.

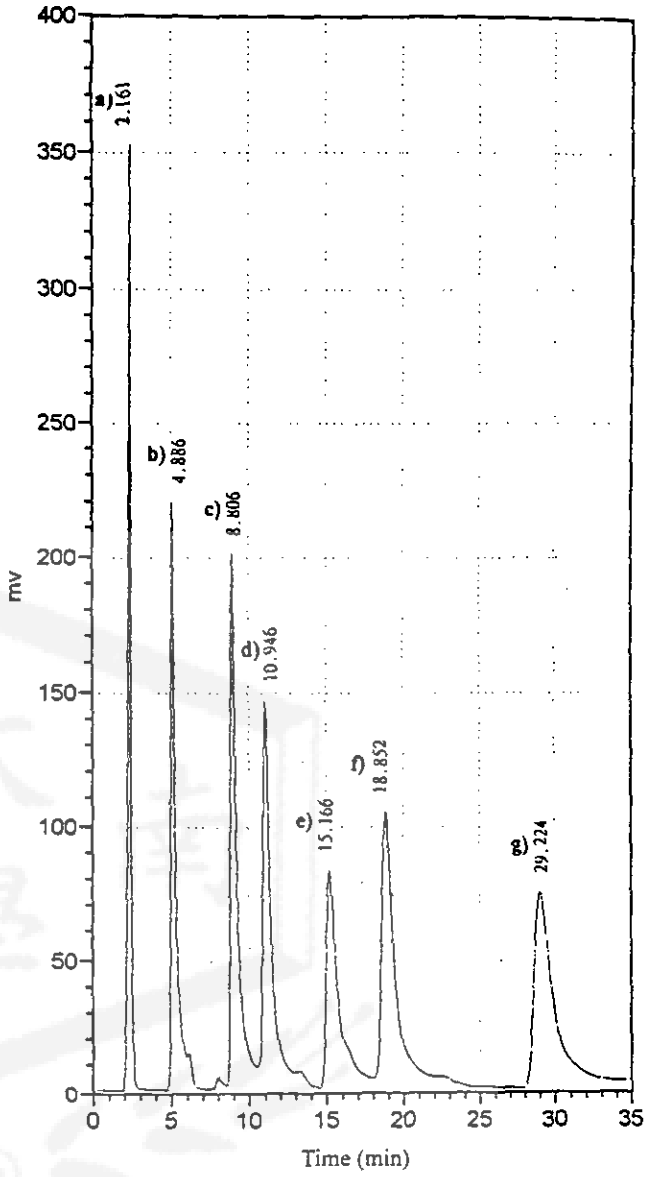

Figure 2

Separation of (80 mg L^{-1}) a = Benzophenone-4, b = Benzophenone-2, c = Benzophenone-1, d = Benzophenone-8, e = Benzophenone-6, f = Benzophenone-3, g = Benzophenone-10. Stationary phase, Hypersil ODS (25 cm x 4.6 mm i. d.); mobile phase , acetonitrile- methanol-water, 30: 30: 40 (*v/v/v*); flow rate, 1.0 mL.min^{-1}; injection, 25μL; detection 286 nm.

Conclusion

A RP-HPLC system for the simultaneous determination of Benzophenone -1, 2, 3, 4, 6, 8 and 10 was established. Separation of seven sunscreen agents was performed on a Hypersil ODS (5 μm) column(250 mm x 4.6 mm i.d.) using acetonitrile-methanol-water (30:30:40, *v/v/v*) as the mobile phase , with detection at 286 nm. Using this system, the seven sunscreen agents were successfully separated within 30 min, and their calibration graphs were linear between 10 mg L^{-1} and 200mg L^{-1}. Therefore, this HPLC system is recommended for routine analysis of sunscreen agents.

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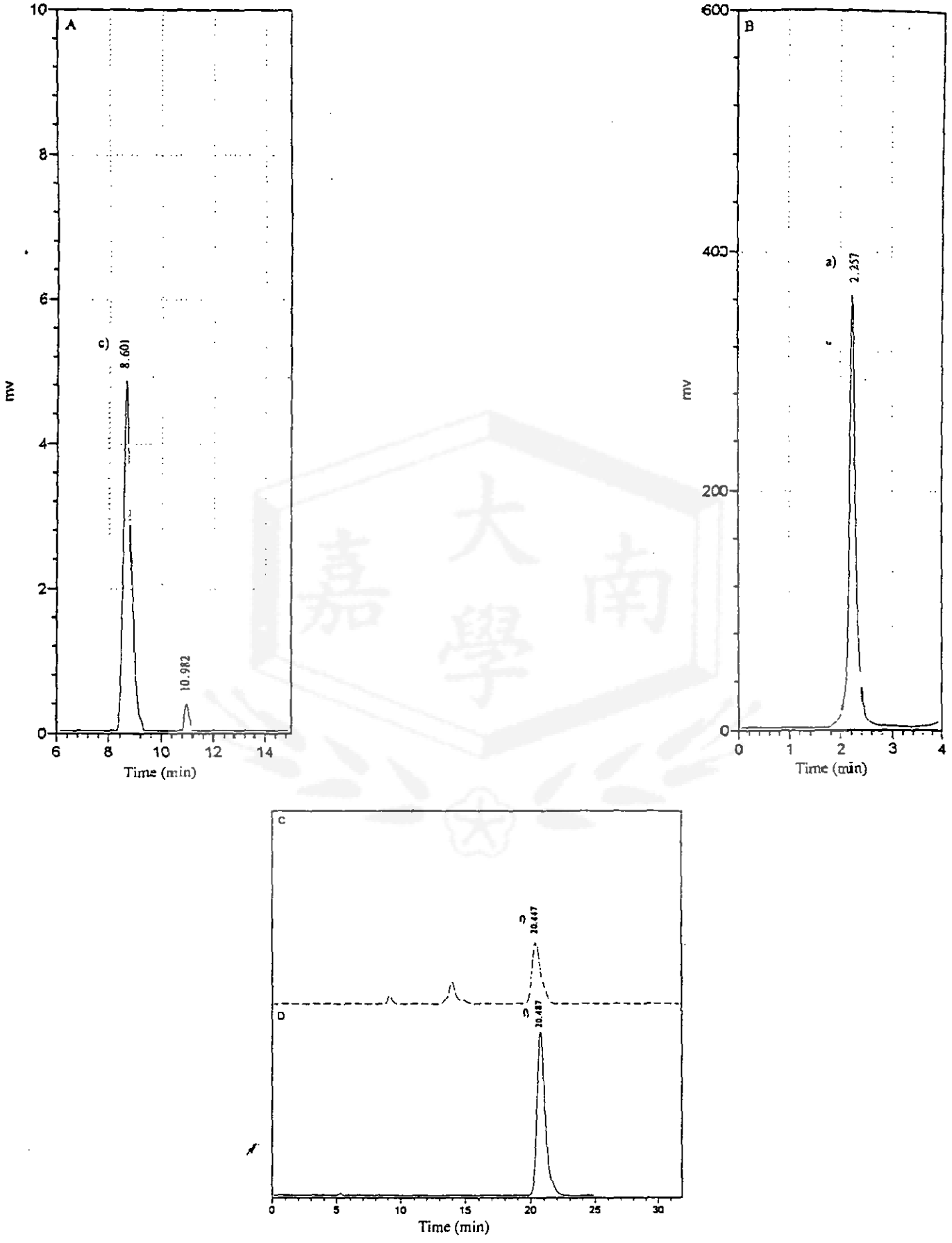

Figure 3

Chromatograms obtained from commercial (A) whitener washing foam (13.9 mg L^{-1}); (B) refresh lotion (50.8 mg L^{-1}); (C) antibacterial hand lotion (60.0 mg L^{-1});(D) lipstick (285 mg L^{-1}). Stationary phase, Hypersil ODS (25 cm x 4.6 mm i. d.); mobile phase , acetonitrile- methanol-water, 30: 30: 40 (*v/v/v/*); flow rate, 1.0 mL.min^{-1}; injection, 25μL; detection 286 nm.

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