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SIMULTANEOUS DETERMINATION OF MULTIPLE ADDITIVES IN COSMETICS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

An high-performance liquid chromatographic method for the simultaneous determination of multiple additives in cosmetics was studied by using a methanol gradient with a multi-wavelength detector capable of scanning a range of wavelengths. For accurate determinations, it was important to use the same solvent composition both for the sample and standard, and to minimize the injection volume as well as to employ peak-area integration in the construction of the calibration curve. The simultaneous determination of twelve additives in cosmetics was carried out and satisfactory results were obtained both for the recovery and the coefficient of variation. The method was also applied to the determination of these additives in commercial cosmetics and the results showed a good agreement with the conventional method. This method would be suitable for routine, quality control analysis of cosmetic products.

INTRODUCTION

The use of an elution gradient and a multi-wavelength detector in high-performance liquid chromatography (HPLC) seems most effective in the simultaneous determination of multiple additives in cosmetics. Gradient elution has the following advantages in overcoming general elution problems in HPLC[1]: (1) the total analysis time can be significantly reduced; (2) the effective sensitivity is very high because of a negligible variation in the peak shape; (3) elution and separation of multiple components having a wide range in polarity can be accomplished simultaneously.

There are two types of multi-wavelength detector, the variable wavelength scanning detector and the photodiode array detector. The use of this type of detector overcomes the most difficult problems arising from extreme differences in the composition or the responses of multiple additives to be determined simultaneously.

This paper presents the simultaneous determination of multiple additives in cosmetics by a methanol gradient in conjunction with a multi-wavelength detector. The optimum wavelength for each component may be selected by this detector during an experiment. This method has been successfully utilized for routine analysis and quality control analysis of micro amounts of additives in cosmetics.

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EXPERIMENTAL

Reagents

Table I shows the twelve additives which are frequently present in the cosmetics used in this work. The samples were all cosmetic grade, chromatographically pure and, therefore, were used without further purification. Methanol for HPLC and phosphoric acid were obtained from Wako Pure Chemical (Osaka, Japan). Deionized water was used for the mobile phase, which was degassed by sparging it with helium gas[2].

TABLE I

ADDITIVES FREQUENTLY PRESENT IN COSMETICS

A	Pantothenyl ethyl ether
B	Methyl *p*-hydroxybenzoate
C	Salicylic acid
D	Benzyl nicotinate
E	4-Isopropyl-3-methylphenol
F	Butyl *p*-hydroxybenzoate
G	Monoammonium glycyrrhizinate
H	Trichlorocarbanilide
I	2,6-Di-*tert.*-butyl-4-methylphenol
J	Pyridoxine dioctanoate
K	Tocopheryl acetate
L	Stearyl glycyrrhetinate

Apparatus

The HPLC equipment used was an LC4A chromatograph (Shimadzu, Kyoto, Japan), a Shimadzu SPD2AS variable wavelength scanning detector and a Shimadzu SPDM1A photodiode array detector. The column (150 mm × 4 mm I.D.) was slurry-packed by using methanol with Nucleosil $5C_{18}$ (5 μm) from Macherey-Nagel (Düren, F.R.G).

Procedures

For the gradient from 35 to 99.9% methanol, a mixture of water and methanol (65:35), adjusted to pH 2.5 with phosphoric acid, was prepared as the initial eluent. From $t = 0$ to $t = 26$ min the eluent composition was linearly changed to methanol–phosphoric acid (1000:1). This composition was maintained for an additional 12 min. Throughout the flow-rate was set at 1.0 ml/min and the column temperature was maintained at 40°C. After the gradient had been started, it took 5 min to detect the eluent passed through the column and after completion of an experiment the initial eluent composition was restored in 17 min. Other HPLC conditions were based on our previous work[3]. All the samples were dissolved in methanol at concentrations from 10 to 80 μg/ml, and 20 μl of the sample solution were injected.

RESULTS AND DISCUSSION

Choice of detector for quantitative analysis

In general, small amounts of various additives with differing solubilities are present in cosmetics. Some materials are soluble in water and others are soluble in oil. Fig. 1 shows the gradient elution profile of the twelve additives selected for this study. Two detectors, SPD2AS and SPDM1A, having different detection characteristics, but giving identical chromatograms were used. The wavelength for the detection was selected so that the peak response of each component is nearly equal. The coefficients of variation of the retention time of each component were below 0.3% in five repeated analyses. Table II shows the reproducibility of the peak heights for the quantitative analysis.

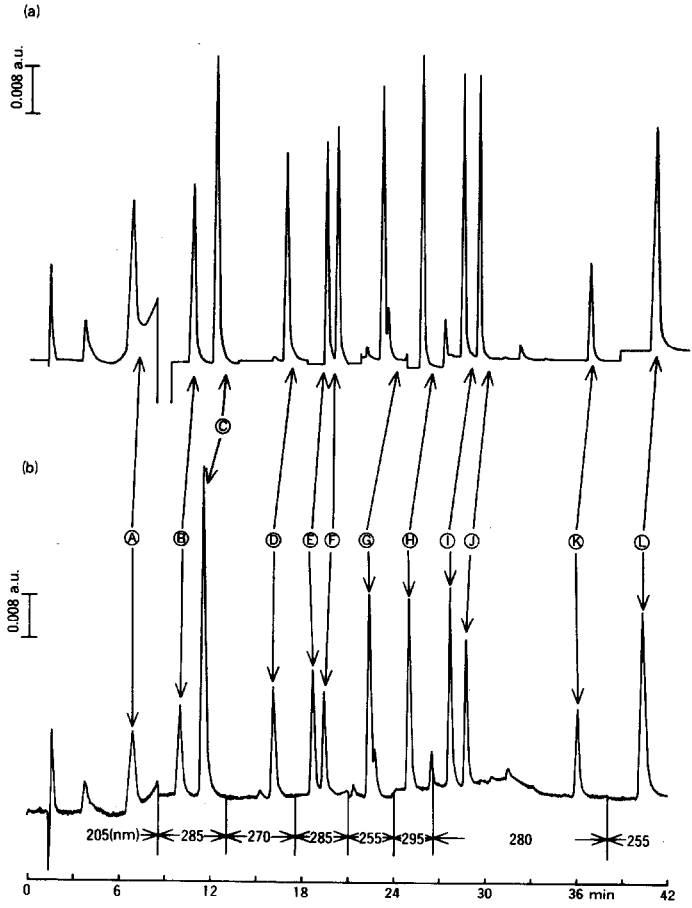

Fig. 1. Gradient elution profile of typical cosmetic additives. Mobile phase: 26-min linear gradient starting with water–methanol (65:35), adjusted to pH 2.5 by phosphoric acid, and ending with methanol–phosphoric acid (1000:1) adjusted to pH 2.5 with phosphoric acid followed by 12 min of the final eluent. Sample size: 20 μl, 50 μg/ml of each of component. Detectors: (a) SPD2AS (scanning); (b) SPDM1A (diode array).

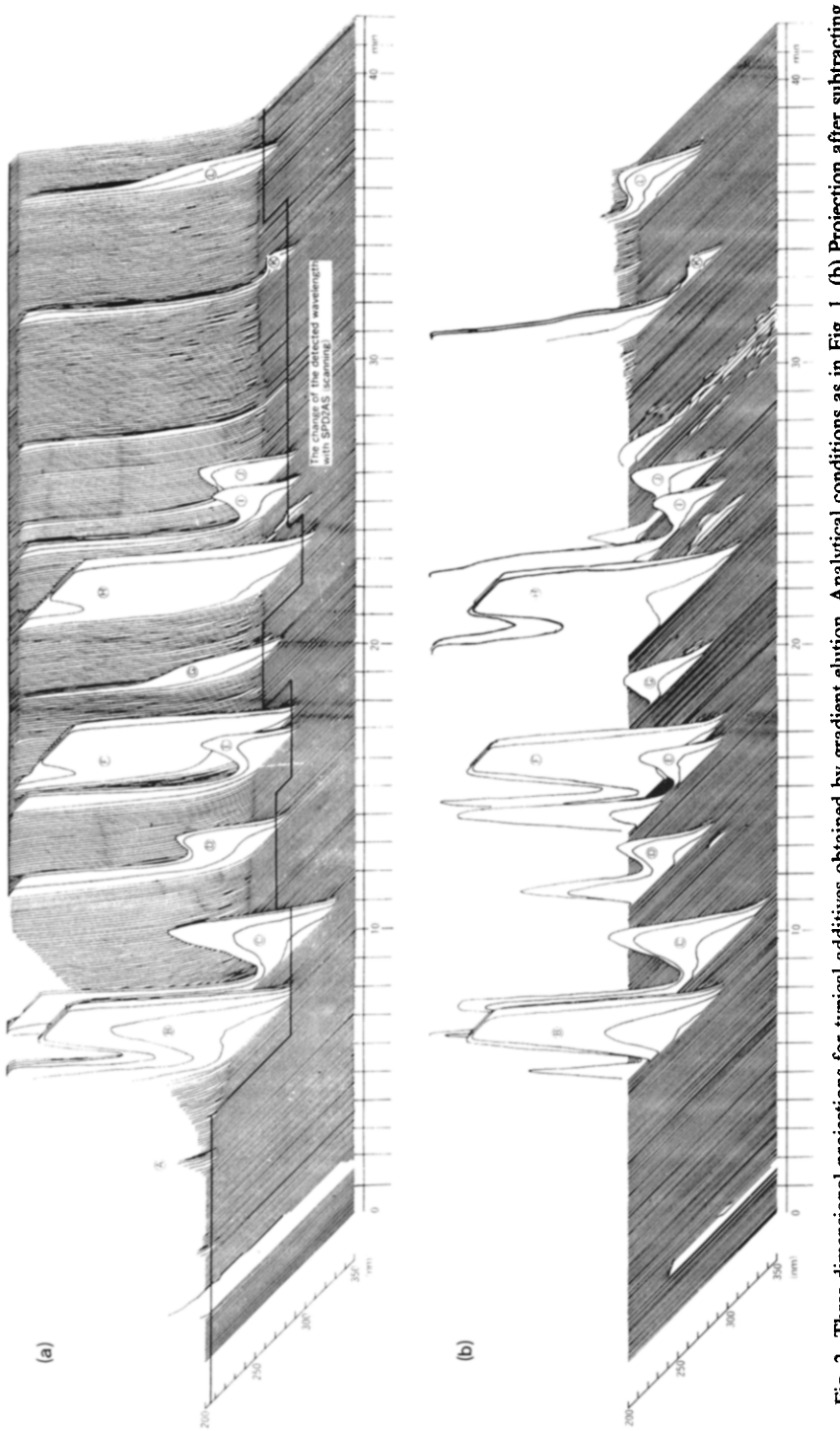

Fig. 2. Three-dimensional projections for typical additives obtained by gradient elution. Analytical conditions as in Fig. 1. (b) Projection after subtracting the methanol spectrum from (a).

TABLE II

COEFFICIENTS OF VARIATION (%) FOR THE PEAK HEIGHTS OF ADDITIVES OBTAINED BY TWO MULTI-WAVELENGTH DETECTORS

Analytical conditions as in Fig. 1. The reproducibility test was effected on five replicates.

	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*
SPD2AS	1.2	0.8	0.8	0.9	1.5	1.3	1.4	1.5	1.9	2.1	1.7	1.8
SPDM1A	2.7	4.9	0.7	3.1	4.1	4.8	1.9	2.6	1.7	2.1	1.0	2.0

Since the coefficients of variation with the SPDM1A were higher than those obtained with the SPD2AS, probably due to the long observation time required for the measurement and the low sensitivity of the SPDM1A, the SPD2AS detector was chosen for simultaneous determination. However, this conclusion is not always applicable, as it seems that the properties of diode array detectors differ according to the equipment suppliers[4,5]. Nevertheless, there is no doubt about the advantages of this type of detector which gives much information and has wide application.

Fig. 2 shows the three-dimensional projections of the UV spectra of the twelve additives upon gradient elution. The changes in the detection wavelength with time with the SPD2AS are shown in Fig. 2a. The UV absorption of methanol in the mobile phase is significant at lower wavelengths and interferes with the UV absorption of the compounds. The spectrum of the ghost peak which often appears at methanol concentrations near 100% during a gradient elution could also be obtained by this projection[6]. Fig. 2b shows the projection after subtracting the spectrum of methanol from Fig. 2a. The spectrum of pantothenyl ethyl ether which is similar to that of methanol cannot be distinguished in Fig. 2b, but those of the other components become much clearer than in Fig. 2a.

Effect of the water content in the sample solution on the determination

Table III shows the calibration curves and the correlation coefficients for the additives obtained through six measurements from 10 to 80 μg/ml. Each plot was linear and the correlation coefficients were greater than 0.9995. The recoveries of the additives were also studied by adding each compound to a known lotion at a concentration of 500 μg/g. Table IV shows the results. The coefficients of variation were relatively low, but the recoveries of pantothenyl ethyl ether, methyl *p*-hydroxybenzoate and salicylic acid were found to be over 100%. In this experiment the sample preparation was carried out by 10-fold dilution in methanol, however, the water content of the lotion was about 85%. Therefore, the effect of the water content in the sample solution on the peak height was investigated further under isocratic condition for nine representative additives. The results are shown in Fig. 3. The peak height of each component was greatly affected by the water content in the sample solution, and this phenomenon was more marked when the injection volume was increased. In case of 40-μl injections, a change in the peak height of pantothenyl ethyl ether became apparent and an asymmetric peak similar to those obtained in overloading was obtained when the sample was diluted in methanol: however, the retention time and the peak area were not affected by the water content of the sample

TABLE III

CALIBRATION CURVES FOR THE ADDITIVES

Y = Peak height (mm); X = concentration (μg/ml).

Component	*Calibration curve*	*Correlation coefficient*
A	$Y = 1.02X - 0.6$	1.0000
B	$Y = 1.13X + 1.3$	0.9999
C	$Y = 2.34X - 3.1$	1.0000
D	$Y = 1.28X + 0.1$	0.9999
E	$Y = 1.36X + 1.2$	0.9997
F	$Y = 1.31X + 2.0$	0.9995
G	$Y = 1.82X - 2.5$	1.0000
H	$Y = 1.93X + 0.2$	0.9998
I	$Y = 1.89X + 0.2$	1.0000
J	$Y = 1.71X + 0.9$	0.9999
K	$Y = 0.67X - 0.4$	0.9998
L	$Y = 1.49X + 0.1$	0.9997

solution. This band-broadening phenomenon is probably due to the increasing diffusion of the solutes caused by introduction of a solution with lower polarity than that of the mobile phase, while the sample had a reasonable retention time[7,8]. Therefore, the recoveries over 100% obtained for some of the components are due to the fact that the sample solution contained 8.5% water, while the standard solution only contained methanol. Table V shows the results of a recovery test which was performed after adjusting the difference in water contents of the sample and standard solution. Satisfactory results were obtained for both the recoveries and the coefficients of variation.

Fig. 4 shows the effect of the column diameter on the peak height for two components. It was found that the effect of the solvent composition of the sample solution could be reduced by using a wider column. This effect was independent of the flow-rate. From these results, several conclusions can be drawn for the accurate determination by HPLC, when the sample was diluted in solvent: (1) the sample solution and the standard solution should be in the same solvent; (2) the injection volume should be as low as possible, less than 10 μl for a 4 mm I.D. column, and below 20 μl for a 6 mm I.D. column; (3) the peak-area method should be chosen for the calibration.

TABLE IV

RECOVERIES OF ADDITIVES ADDED TO A KNOWN LOTION AT 500 μg/g

C.V. = Coefficient of variation. The recoveries test was effected on five replicates.

	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*
Recovery (%)	111.4	107.2	110.4	97.4	99.6	97.1	100.6	96.7	98.6	96.8	100.8	101.0
C.V. (%)	1.1	2.9	1.1	1.5	0.9	2.2	0.6	0.9	0.9	1.0	1.1	0.3

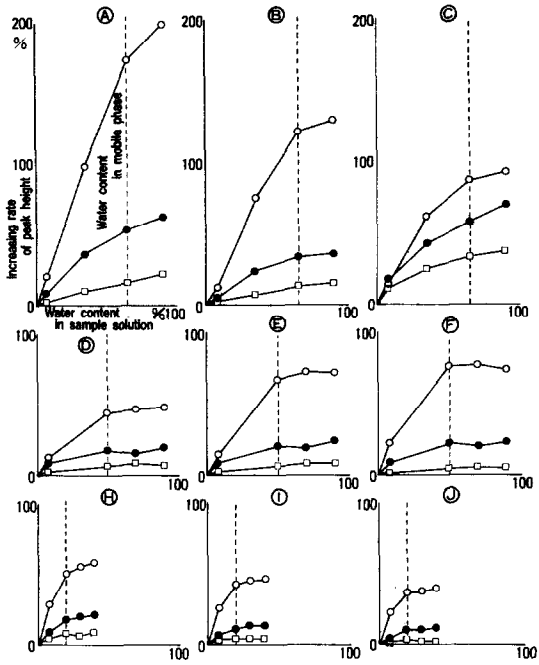

Fig. 3. Effect of the water content in the sample solution on the peak height. Injection volumes: ○, 40 μl; ●, 20 μl; □, 10 μl.

Application for routine or quality control analyses

The results of the investigation described above were applied to the simultaneous determination of micro amounts of additives in commercial cosmetics. Table VI shows the results of these simultaneous determinations and those obtained by conventional isocratic HPLC methods established independently for each component. The two sets of results agreed with each other and the fluctuation of the measurements obtained by each method are small enough. Therefore, it is concluded that this method is applicable to routine or quality control analyses. A problem remaining unresolved was the employment of the same pretreatment procedure for all samples, which is an important factor for the simultaneous determination of multiple components. However, this still depends upon the sample matrix. This method has the

TABLE V

RECOVERY TEST PERFORMED AFTER ADJUSTING THE DIFFERENCE IN WATER CONTENTS OF THE SAMPLE AND THE STANDARD SOLUTION

Conditions as in Table IV.

	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*
Recovery (%)	100.0	100.5	100.3	99.4	99.4	99.3	100.7	99.4	98.9	99.7	101.3	100.7
C.V. (%)	2.2	3.1	0.9	1.6	1.5	2.9	0.5	1.6	0.3	1.5	0.7	0.5

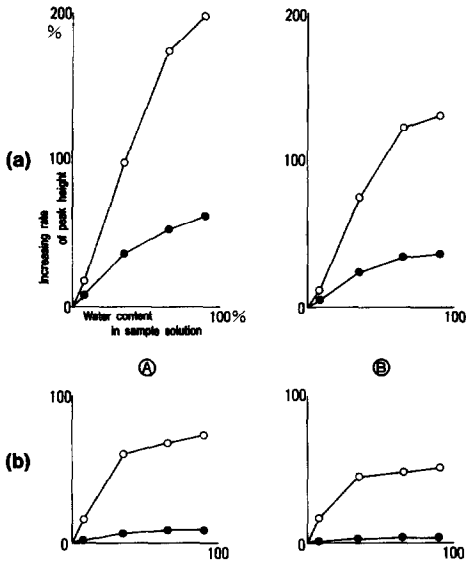

Fig. 4. Effect of the column diameter on the peak height. Column diameters: (a) 4 mm; (b) 6 mm. Other symbols as in Fig. 3.

following advantages for the quality control of cosmetic products, even though an universally effective pretreatment procedure cannot be proposed: (1) it may be possible to design an analyzer to meet the requirement, and avoid the need to change the HPLC conditions; (2) the effectiveness of the autosampler for HPLC can be improved. It is concluded that this method is suitable for quality control by the cosmetic industries which manufacture many kind of products in small quantities.

TABLE VI

DETERMINATION OF ADDITIVES IN THE COMMERCIAL COSMETICS

$\bar{X}$ = Average of two replicate analyses; R = the difference between the two measurements.

	Component	*Simultaneous determination (%, w/w)*		*Conventional method*[9–11] *(%, w/w)*	
		$\bar{X}$	R	$\bar{X}$	R
Lotion 1	A	0.0502	0.0006	0.0502	0.0005
	G	0.0488	0.0006	0.0487	0.0003
	K	0.0497	0.0004	0.0490	0.0005
Lotion 2	A	0.0502	0.0006	0.0535	0.0008
	G	0.0516	0.0006	0.0527	0.0008
	K	0.0494	0.0004	0.0492	0.0001
Shampoo	H	0.291	0.003	0.298	0.001
Hair tonic	E	0.1007	0.0007	0.1040	0.0020
	F	0.0514	0.0012	0.0521	0.0015

CONCLUSION

A method for the simultaneous determination of multiple additives in cosmetics by using gradient HPLC with a multi-wavelength detector was described. It was found that this method could be utilized for the routine, quality control analyses of multiple additives in cosmetics. In Japan, it is required under Good Manufacturing Practice (GMP) regulation that all manufactured batches of medicated cosmetics (Quasi-Drugs) be guaranteed for the content of active ingredients approved by the Ministry of Health and Welfare. The accuracy of the proposed method was adequate enough for this purpose and may be considered as a useful means for the routine analysis of active ingredients and additives.

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