Journal of Chromatography, 396 (1987) 404–409
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 493

Note

Simultaneous determination of multiple additives in cosmetics by high-performance liquid chromatography

II. Pre-treatment procedure for oil-rich cosmetics

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(First received December 29th, 1986; revised manuscript received February 9th, 1987)

We have previously studied methods for the effective quality control analysis of cosmetic products[1,2]. In a previous paper[2], a method for the simultaneous determination of twelve additives in cosmetics was proposed, involving the use of a methanol gradient high-performance liquid chromatographic (HPLC) system with a multi-wavelength detector. This method could be successfully utilized for routine analysis and quality control analysis of micro amounts of additives in cosmetics. A problem remaining unresolved is the employment of the same pre-treatment procedure for all samples, which is an important factor for our proposed method. In previous work[2], the samples were lotion, hair tonic and shampoo, which could be diluted with methanol because they contain mainly water and alcohol. This paper presents a modified sample preparation procedure for oil-rich cosmetics (milky lotion and cream) and its extension to the simultaneous determination of multiple additives.

EXPERIMENTAL

Reagents

Twelve additives that are frequently present in cosmetics were studied, as in Part I[2], *viz.*, pantothenyl ethyl ether, methyl *p*-hydroxybenzoate, salicylic acid, benzyl nicotinate, 4-isopropyl-3-methylphenol, butyl *p*-hydroxybenzoate, monoammomium glycyrrhizinate, trichlorocarbanilide, 2,6-di-*tert.*-butyl-4-methylphenol, pyridoxine dioctanoate, tocopheryl acetate and stearyl glycyrrhetinate. Special-grade methanol and chloroform for the preparation of sample solutions, methanol for HPLC and special-grade phosphoric acid were purchased from Wako (Osaka, Japan). Water for the mobile phase was obtained from a Milli-Q II system (Millipore, Bedford, MA, U.S.A.).

Apparatus

The HPLC equipment was an LC4A chromatograph (Shimadzu, Kyoto, Japan) with a SIL2AS autosampler and an SPD2AS variable-wavelength scanning detector. A column (100 mm × 6 mm I.D.) packed with ODS-2101-L (Senshu, Tokyo, Japan) was used.

0021-9673/87/$03.50

Procedures

The HPLC conditions were as in our previous work[2], but in order to reduce the analysis time a programme for changing the flow-rate during an experiment was employed. In gradient elution, a long analysis time may result from the recovery stage from the initial to the final eluent composition. This time is reduced to 16 min per injection in the present method compared with our previous HPLC conditions, as the flow-rate is changed by using a short wide column. An experiment consequently requires a total of 39 min, including an 8 min recovery time. All the standard samples were dissolved in methanol–chloroform (1:1) at concentrations from 5 to 50 μg/ml, and 20 μl of the sample solution were injected.

RESULTS AND DISCUSSION

Examination of solvents for effective pre-treatment procedure

In general, the oil which consists of hydrocarbons, fatty alcohols, fatty acids, wax and glycerides is present to a maximum of about 25% in milky lotions and about 70% in creams. Small amounts of various additives are usually extracted from these cosmetics, and the sample solution must be cleaned up. However, in the simultaneous determination of multiple components, effective extraction of all components is impossible and a pre-treatment procedure involving solvent dispersion and dilution is required. Further, the extent of dilution must be as small as possible for the determination of micro-scale components, and it is important to use a good solvent.

The following points seem to be required of such a solvent: first, good solubility for the initial eluent (water–methanol, 65:35; pH 2.5) and oil-rich cosmetics; second, the solvent itself and its impurities should not be retained on the column under the HPLC conditions or should not interfere with the solute peaks at the detection wavelengths, and third, the diffusion of the components that are eluted early in the gradient should be as low as possible because a solvent of lower polarity than that of the initial eluent is chosen, considering the solubility of the samples. Taking into account these requirements, methanol–chloroform solvents were chosen for the sample preparation. Dichloromethane, methyl acetate, ethyl Cellosolve, butyl Cellosolve, N,N-dimethylformamide, 1,4-dioxane, methyl ethyl ketone and tetrahydrofuran were examined, but none satisfied all three requirements. In particular, tetrahydrofuran had a good solubility and did not interfere with any solute peaks, but it caused substantial band broadening. Methanol–chloroform and methanol–acetone were examined. The solubility test was carried out by 10-fold dilution, and the sample was a typical oil–water cream containing about 30% of oil. Satisfactory results for the sample solubility were obtained in both solvent systems when the methanol concentration was less than 50%.

Fig. 1 shows the effects of the solvent composition on the peak intensity in order to elucidate the third requirement mentioned above. The sample was pantothenyl ethyl ether, and the experiment was carried out under isocratic conditions and with the initial eluent using a 4 mm I.D. column. The peak height was greatly affected by the solvent composition, peak compression and band broadening occurring[3,4]. The results in Fig. 1 indicate that increasing diffusion of the solutes was caused by the introduction of a solution with a lower polarity than that of the mobile phase for the water–methanol–acetone system.

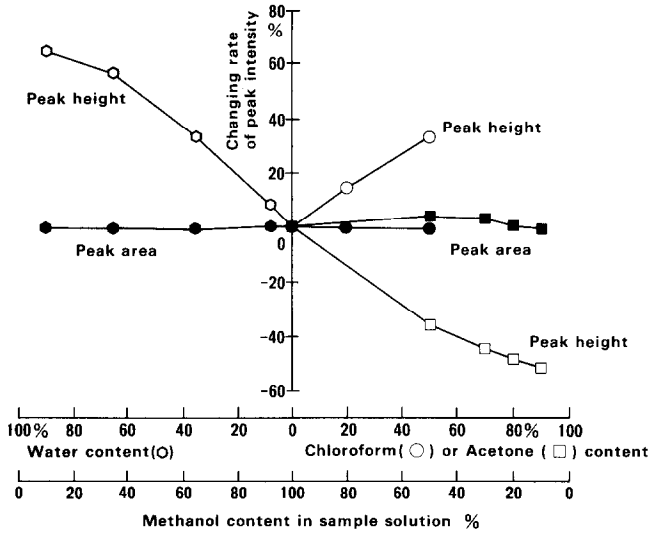

Fig. 1. Effect of sample solvent composition on the peak intensity with water–methanol (65:35) (pH 2.5) as the mobile phase. Column I.D.: 4 mm. Injection volume: 20 μl of pantothenyl ethyl ether solution.

Peak splitting was observed on using methanol–acetone (1:1) and the peak height decreased by one third when methanol–acetone (1:1) was used instead water–methanol (65:35). On the other hand, the peak heights were greater when methanol–chloroform solutions were used than when methanol was used, although the polarity of the mixed solutions is lower than that of methanol. This is probably due to the decreasing diffusion of the solutes caused by insolubility between chloroform in the sample solution and water in the mobile phase.

Fig. 2 shows the same effect for stearyl glycyrrhetinate. Elution was carried out under isocratic conditions and with the final methanol eluent. The peak heights

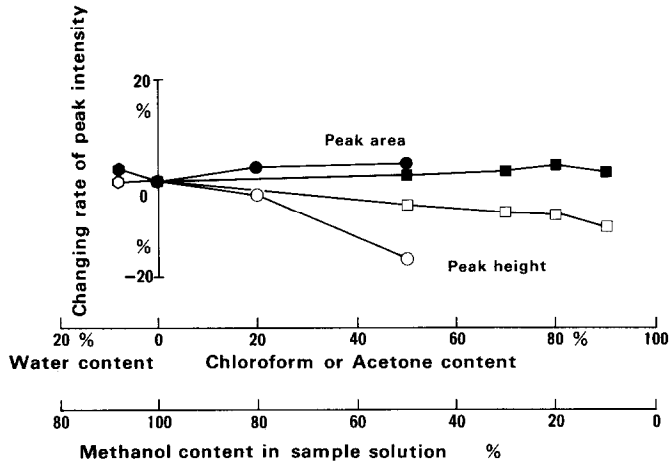

Fig. 2. Effect of sample solvent composition on the peak intensity with methanol as the mobile phase. Injection volume: 20 μl of stearyl glycyrrhetinate solution. Other condition and symbols as in Fig. 1.

on using methanol–chloroform were smaller than that with either methanol or methanol–acetone. It was found that the peak area was not affected by the sample solvent composition (Figs. 1 and 2).

From these results, methanol–chloroform (1:1) was chosen as the pre-treatment solvent in order to suppress the diffusion of the solutes, especially in the initial eluent. A major peak due to chloroform in the sample solution was observed after a few minutes near the elution of methyl *p*-hydroxybenzoate and salicylic acid with detection at below 250 nm, but did not interfere with these two peaks.

Recovery test

All of the relationships between peak area and amount of each additive were linear in the range 5–50 μg/ml, with the correlation coefficients of over 0.9992 (n = 6). The recoveries of the additives were also studied by adding 500 μg/g of each compound to known cosmetics containing 10–60% of oil.

The standard pre-treatment procedure was as follows. Approximately 0.5 g of sample was accurately weighed into a small beaker, and about 10 ml of methanol–chloroform (1:1) were added. Dispersion was effected using ultrasonic waves. The suspension was compressively filtered using a small disposable syringe packed with about 0.5 ml of glass-wool. The same operation was repeated with the washings in the beaker and the test solution for HPLC was prepared by dilution with the mixed solvent until the total volume was 20 ml.

In general, the residual components are water-soluble polymers and inorganic compounds in the cosmetics. Table I shows the results of the recovery test for twelve components added to five cosmetics. Satisfactory results were obtained for both the recoveries and the coefficients of variation.

The standard pre-treatment involved a 40-fold dilution. The preparation of samples that contain less than 30% of oil required a 20-fold dilution because the extent of dilution depends on the oil content of the sample.

CONCLUSION

A method for the simultaneous determination of multiple additives in oil-rich cosmetics has been developed. Methanol–chloroform (1:1) is used for the preparation of the sample because of the good solubility of the sample components and the low diffusion of the solutes for HPLC analysis. The preparation of the sample solution involves simple dilution and filtration steps. The simultaneous determination of multiple micro-scale components in cosmetics is possible whatever the sample matrix. It is concluded that this effective pre-treatment procedure is suitable for the rapid quality control-analysis of cosmetic products.

ACKNOWLEDGEMENT

We thank Toshio Uehara for allowing us to publish this paper.

TABLE I

RECOVERIES OF COMPOUNDS ADDED TO KNOWN COSMETICS AT 500 μg/g

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

Compound	*Milky lotion I (10% oil)*		*Milky lotion II (15% oil)*		*Cream I (30% oil)*		*Cream II (50% oil)*		*Cream III (60% oil)*	
	Recovery (%)	*C.V. (%)*	*Recovery (%)*	*C.V. (%)*	*Recovery (%)*	*C.V. (%)*	*Recovery (%)*	*C.V. (%)*	*Recovery (%)*	*C.V. (%)*
Pantothenyl ethyl ether	101.5	0.7	104.9	0.4	105.0	0.9	98.3	1.4	97.6	0.6
Methyl *p*-hydroxybenzoate	99.5	0.7	99.3	0.7	101.4	1.1	95.5	0.8	94.5	0.6
Salicylic acid	100.2	0.4	101.1	0.7	99.8	1.5	95.4	2.0	94.6	1.2
Benzyl nicotinate	98.7	1.0	100.7	1.0	100.6	1.4	96.1	1.7	96.6	3.2
4-Isopropyl-3-methylphenol	100.8	0.5	101.3	0.7	101.0	0.8	96.6	0.8	95.7	0.7
Butyl *p*-hydroxybenzoate	100.5	1.0	104.9	2.7	101.5	0.7	96.8	1.4	96.0	0.4
Monoammonium glycyrrhizinate	97.2	3.1	96.5	2.8	94.3	2.6	97.9	3.4	103.8	1.2
Trichlorocarbanilide	101.1	1.1	99.4	1.3	101.2	4.5	98.1	2.8	99.8	0.7
2,6-Di-*tert.*-butyl-4-methylphenol	98.1	8.3	93.3	2.2	92.3	1.9	93.0	5.6	99.4	1.9
Pyridoxine dioctanoate	98.9	1.6	97.3	3.8	100.2	3.2	97.7	1.3	92.2	2.6
Tocopheryl acetate	99.7	0.8	100.4	1.0	99.9	1.3	93.4	0.8	94.4	1.7
Stearyl glycyrrhetinate	99.9	0.8	100.2	0.7	101.0	3.0	95.1	0.8	94.8	0.7

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