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Note

Determination of preservatives in cosmetic products by reversed-phase high-performance liquid chromatography

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Some organic acids and their esters are commonly used singly or in combination as antimicrobial agents in cosmetic toiletries and pharmaceutical products[1]. The analysis of each individual additive is an important quality assurance step in many commercial products. European Economic Community (EEC) Instruction No. 76/768 (first part, enclosure VI) states the preservatives authorized as cosmetic additives and their tolerated concentrations. Sorbic, benzoic and *p*-hydroxybenzoic acids and their esters are the antimicrobials mostly used in the cosmetic industry.

Numerous assay methods have been reported for quantitation of these preservatives in pharmaceuticals, foods and cosmetics, and include colorimetry and UV spectrophotometry[2,3], thin-layer chromatography (TLC)[4–6], gas–liquid chromatography (GLC)[7,8] and high-performance liquid chromatography (HPLC)[1,9–14]. Colorimetric and spectrophotometric methods usually require lengthy extraction procedures and are not specific. TLC is suitable for qualitative detection, in particular when silica gel plates are used, and has recently been used successfully for the quantitation of parabens in liquid samples without any kind of isolation step[6]. GLC methods are specific and sensitive and allow the assay of complex mixtures; however, they require derivatization prior to injection and this step is inherently time-consuming. In the last few years HPLC has been applied to the determination of preservatives in food, pharmaceutical and cosmetic samples. Its major advantages over GLC are the simple isolation procedures and the lack of derivatization steps. In reversed-phase and ion-exchange HPLC, aqueous samples can often be injected directly onto the column without prior partitioning into an organic solvent. This capability together with recent advances in column technology makes HPLC an extremely powerful technique in the field of cosmetic preservatives analysis.

This paper reports an application of reversed-phase HPLC for the separation and simultaneous quantitation of some preservatives in skin cosmetics, shampoos and bath preparations. The compounds investigated, together with their maximum limits in compliance with the EEC legislation, were as follows: sorbic acid (0.6% as free acid); benzoic acid and its esters (0.5% as free acid); *p*-hydroxybenzoic acid and its esters (0.4 or 0.8% as free acid depending on whether the paraben is used singly or in combination).

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EXPERIMENTAL

Reagents

Benzoic acid (I), methyl benzoate (II), ethyl benzoate (III), benzyl benzoate (IV), sorbic acid (V), *p*-hydroxybenzoic acid (VI), methyl *p*-hydroxybenzoate (VII), ethyl *p*-hydroxybenzoate (VIII) and *n*-propyl *p*-hydroxybenzoate (IX) were obtained from Merck (Darmstadt, F.R.G.). Sulphuric and acetic acids and sodium acetate were purchased from Farmitalia-Carlo Erba (Milan, Italy). All chemicals used were of analytical grade used without further purification. Methanol and acetonitrile were of special HPLC grade (Farmitalia-Carlo Erba). Water was deionized and doubly distilled from glass. All solvents and solutions for HPLC analysis were filtered through a Millipore filter, pore size 0.5 μm, and vacuum degassed by sonication before use.

Apparatus

A Varian Model 5000 liquid chromatograph equipped with a variable-wavelength UV detector (Varichrom UV 50), a Valco AH 60 injection valve and a Waters Assoc. Model 730 integrator-recorder were used. The analytical column was a 5-μm Erbasil C_{18} (150 × 4.6 mm I.D.) (Farmitalia-Carlo Erba). Peak areas were determined by electronic integration (Varian Model CDS 111).

Chromatographic conditions

The HPLC conditions were as follows: mobile phase, acetonitrile–0.005 *M* sodium acetate (pH 4.4 adjusted with acetic acid), 5:100 for 5 min, then a linear gradient up to 10% acetonitrile in 5 min and up to 65% acetonitrile in the next 40 min; flow-rate, 1.6 ml/min; column temperature, 35°C; injection volume, 10 μl; detector wavelengths, 232 and 258 nm; detector sensitivity,. 0.32 a.u.f.s.

Calibration curves

Five standard solutions were prepared by dissolving weighed amounts of com-

TABLE I

RECOVERY OF PRESERVATIVES IN COSMETIC PRODUCTS

Compound	*Night cream*		*Day cream*		*Cleansing lotion*		*Bath foam*		*Shampoo*	
	*A**	*B***	*A**	*B***	*A**	*B***	*A**	*B***	*A**	*B***
I	0.50	96.3 ± 1.7	0.50	96.0 ± 1.8	0.50	95.9 ± 1.6	0.50	97.1 ± 1.3	0.50	98.2 ± 1.3
II	0.50	95.4 ± 1.6	0.50	95.9 ± 1.9	0.50	98.2 ± 0.9	0.50	96.4 ± 1.4	0.50	99.0 ± 1.9
III	0.50	97.8 ± 1.2	0.50	94.3 ± 1.5	0.50	97.4 ± 1.4	0.50	95.9 ± 1.7	0.50	98.1 ± 1.7
IV	0.50	94.3 ± 1.7	0.50	98.7 ± 1.6	0.50	95.7 ± 1.3	0.50	96.0 ± 1.7	0.50	96.9 ± 1.9
V	0.60	95.0 ± 1.5	0.60	97.0 ± 1.6	0.60	97.1 ± 1.3	0.60	99.0 ± 1.9	0.60	97.0 ± 1.3
VI	0.40	93.0 ± 1.3	0.40	94.2 ± 1.7	0.40	93.6 ± 1.2	0.40	93.1 ± 1.3	0.40	94.5 ± 1.6
VII	0.40	97.3 ± 1.5	0.40	101.0 ± 1.1	0.40	98.1 ± 1.4	0.40	97.0 ± 1.9	0.40	98.3 ± 1.9
VIII	0.40	97.0 ± 1.6	0.40	100.0 ± 1.7	0.40	99.0 ± 1.8	0.40	98.3 ± 1.2	0.40	97.9 ± 1.7
IX	0.40	97.2 ± 1.5	0.40	102.3 ± 1.6	0.40	101.1 ± 1.9	0.40	98.3 ± 1.4	0.40	99.6 ± 1.8

* Grams added to 100 g of cosmetic sample.

** Recovery (%) ± S.D. (each value is the mean from five determinations).

pounds I–IX in acetonitrile–0.005 *M* sodium acetate, pH 4.4 (60:40). The concentration of each preservative ranged from 12.5 to 200 μg/ml. The solutions were processed using the HPLC conditions described above. Peak areas were used to calculate calibration graphs.

Assay of preservatives in cosmetic samples

The mixture of preservatives was added to five cosmetic products to yield the concentrations given in Table I. A 1-g amount of cosmetic product was accurately weighed into a glass centrifuge-tube; 0.25 ml of 2 *M* sulphuric acid and 3 ml of methanol were added and the tube was immersed in an ultrasonic bath for 30 min. When the cosmetic sample contained fat-soluble excipients, the mixture was heated at 60°C for 10 min to break up the emulsion, followed by ultrasonic treatment. Then the tube was centrifuged for 10 min at 900 *g*. The extraction procedure was repeated and the combined extracts were made up to volume (10 ml) with methanol. A 10-μl aliquot of the solution was injected into the liquid chromatograph.

RESULTS AND DISCUSSION

Figs. 1 and 2 show typical chromatograms of a standard mixture of compounds I–IX obtained by setting the detector at 232 and 258 nm, respectively. As is seen,

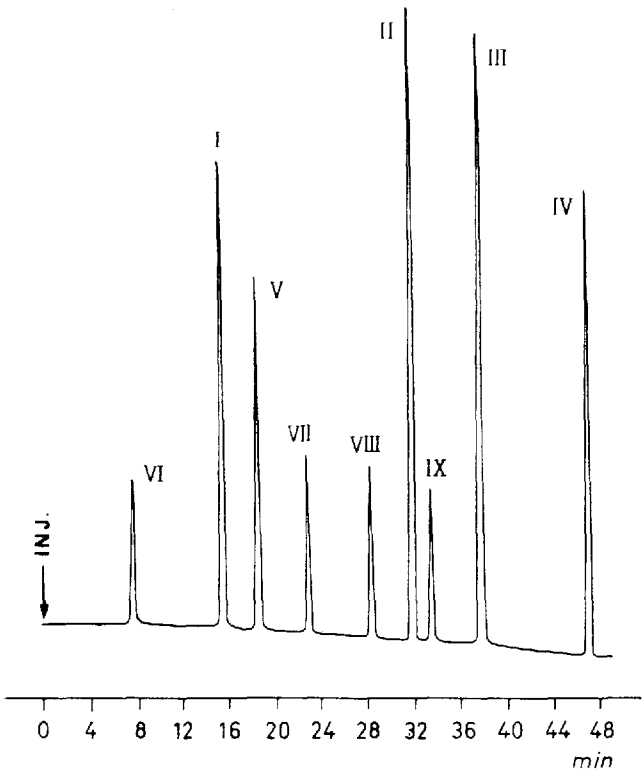

Fig. 1. Typical chromatogram of a standard mixture of preservatives obtained by setting the detector at 232 nm. Chromatographic conditions as in the text.

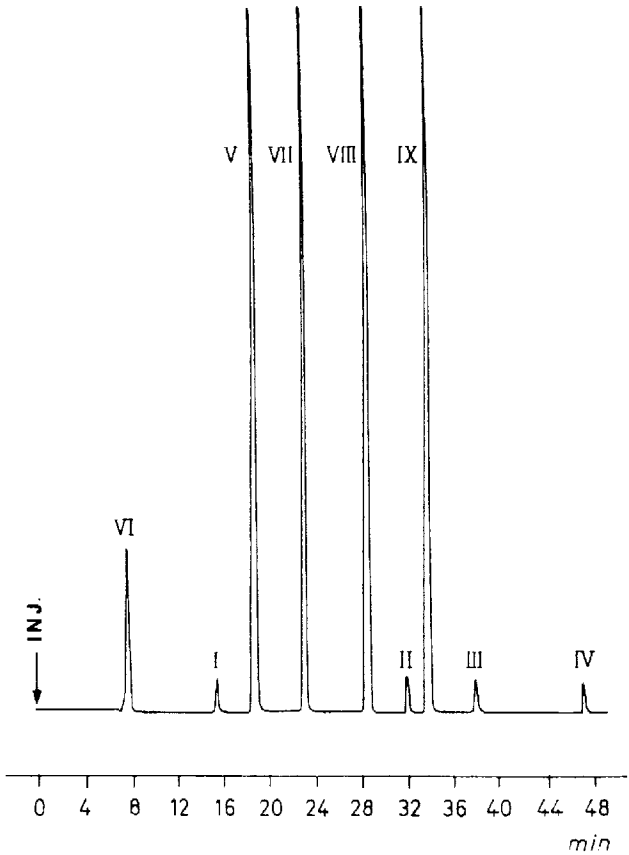

Fig. 2. Typical chromatogram of a standard mixture of preservatives obtained by setting the detector at 258 nm. Chromatographic conditions as in the text.

good resolution of all preservatives examined has been achieved. Table II gives the chromatographic properties of I–IX. Under the conditions used, all retention times were quite reproducible. Also shown are the peak-areas ratios at 232 and 258 nm for each preservative, which are very important both in confirming the identification of the preservative and in estimating possible interferences from other compounds when complex samples are assayed.

Calibration graphs for compounds I–IX were constructed from five consecutive injections and were linear over the range of concentrations used, with regression coefficients ranging from 0.9932 to 0.9982.

The detection limits for all nine compounds were at least ten times below the levels normally used in the cosmetic chemistry. The reproducibility of the assay was very satisfactory, the average coefficient of variation being less than 1.8%.

The applicability of the HPLC method was demonstrated by assaying compounds I–IX in five cosmetic products spiked with the standard mixture of preservatives. The results obtained are shown in Table I. Excellent recoveries and good precision were observed. The recoveries of all the compounds indicate that the proposed extraction procedure is highly specific with essentially no interference from the

TABLE II

RETENTION TIMES, CAPACITY FACTORS AND PEAK-AREA RATIOS AT 232 AND 258 nm

Each value is the mean from five determinations.

Compound	*Retention time (min)*	*Capacity factor*	$Area_{232}/Area_{258}$
I	15.08	8.81	14.38
II	31.15	19.21	13.00
III	37.06	22.93	13.10
IV	46.05	28.73	12.98
V	18.12	10.80	0.18
VI	7.30	3.94	0.07
VII	22.30	13.55	0.07
VIII	27.48	16.96	0.08
IX	32.58	20.25	0.06

cosmetic sample. In comparison with the extraction procedure described by Fitzpatrick *et al.*[14], which is often used in assaying parabens in cosmetics[11,13], our extraction method, though requiring more time, allows more quantitative recoveries. Further, the dual-wavelength UV detection can be very useful in monitoring the stability of the preservative during the manufacture and later during storage of the cosmetic product.

The HPLC method described is specific and sensitive and allows the simultaneous assay of sorbic, benzoic, *p*-hydroxybenzoic acids and their esters, commonly added as preservatives to a great variety of cosmetic formulations. Further, the simple extraction procedure which permits quantitative recoveries makes it suitable for routine analyses of cosmetic products, particularly in respect of their compliance with EEC regulations.

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