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Note

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

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An increasing number of analytical techniques has been published in the last few years for the identification and quantitation of the preservatives present in cosmetic preparations, especially because of the requirements of efficacy, safety, stability and compliance with regulations. Since the use of combinations of preservatives is already common practice, tracing every one of them in the finished products is extremely difficult considering that sometimes very low amounts are involved. Among the available analytical techniques, high-performance liquid chromatography (HPLC) seems to be the most appropriate for this purpose. We have previously reported two applications of HPLC for the simultaneous determination of some aromatic alcohols and organic acids, respectively, from cosmetic products[1,2].

In the present paper we describe the separation and quantitation of ten phenolic and/or halogenated compounds, commonly used as antimicrobial agents in cosmetic formulations. The compounds investigated, together with their maximum limits under the European Economic Community legislation (Instruction No. 76/768, first and second parts of enclosure VI) are as follows: glycerol-*p*-chlorophenylether (chlorphenesin) (I), 0.5%; 4-chloro-3-methylphenol (II), 0.2%; 3,5-dimethyl-4-chlorophenol (III), 0.5%; 5,5′-dichloro-2,2′-dihydroxydiphenylmethane (dichlorophene) (IV), 0.2%; 3,4,4′-trichlorocarbanilide (triclocarban) (V), 0.2%; 2,4,4′-trichloro-2′-hydroxydiphenyl ether (Irgasan DP 300) (VI), 0.2%; 4,4′-dichloro-3-trifluoromethylcarbanilide (Irgasan CF_3) (VII), 0.3%; 3,3′-dibromo-5,5′-dichloro-2,2′-dihydroxydiphenylmethane (Brophen) (VIII), 0.1%; hexachlorophene (IX), 0.1%; usnic acid (X), 0.2%. Various methods have been proposed for the extraction and determination of some of these compounds in cosmetic products, including colorimetry and UV spectrophotometry[3,4], thin-layer chromatography (TLC)[4–6], gas–liquid chromatography (GLC)[7,8] and HPLC[8–10]. The present method involves a simple extraction from the cosmetic sample and a reversed-phase HPLC separation by gradient elution using UV detection at three wavelengths. The method is suitable for adaptation to routine analysis.

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EXPERIMENTAL

Reagents

Compounds II, III and VI–VIII were obtained from Bode (The Netherlands), IX from Givaudan (Switzerland), I and IV from BDH (U.K.), X from Fluka (Switzerland) and V from Monsanto (St. Louis, MO, U.S.A.). The reagents used were potassium dihydrogen phosphate, 2 and 4 *M* sulphuric acid, 80% phosphoric acid, sodium hydroxide, diethyl ether and HPLC-grade acetonitrile (Farmitalia-Carlo Erba, Italy). All chemicals were of analytical grade and used without further purification. Water was deionized and doubly distilled from glass apparatus. 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 UV50), a Valco AH60 injection valve and a Waters Assoc. Model 730 integrator-recorder were used. The analytical column was a 10-μm Erbasil C_{18} (Farmitalia-Carlo Erba) (250 × 4.6 mm I.D.). Peak areas were determined by electronic integration (Varian Model CDS 111).

HPLC conditions

The operating conditions were as follows: mobile phase, acetonitrile–$5 \cdot 10^{-3}$ *M* potassium dihydrogen phosphate (pH 2.8, adjusted with phosphoric acid) with a linear gradient from 20 to 50% acetonitrile in 30 min; held constant for 15 min, another linear gradient up to 90% acetonitrile in 10 min and subsequently held constant; flow-rate, 2.5 ml/min; column temperature, 25°C; injection volume, 10 μl; detector wavelengths, 240, 260 and 280 nm; detector sensitivity, 0.32 a.u.f.s.

Calibration curves

Five standard solutions were prepared by dissolving weighed amounts of compounds I–X in acetonitrile so as to obtain the concentrations reported in Table I. Calibration curves were constructed by use of the peak areas measured at the various amounts injected.

TABLE I

COMPOSITIONS OF THE STANDARD SOLUTIONS OF THE PRESERVATIVES TESTED

Reference solution	*Concentration (μg/ml)*									
	I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*	*X*
1	40	24	28	20	8	32	16	40	80	64
2	80	48	56	40	16	64	32	80	160	128
3	160	96	112	80	32	128	64	160	320	256
4	320	192	224	160	64	256	128	320	640	512
5	640	384	448	320	128	512	256	640	1280	1024

Assay of preservatives in cosmetic samples

A cosmetic sample (1 g), spiked with the mixture of preservatives under investigation, 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 tubes immersed in an ultrasonic bath for 30 min. When the cosmetic sample contained fat-soluble excipients, the mixture had to be heated in a water-bath at 60°C for 10 min to break up the emulsion, followed by ultrasonic treatment. After centrifugation for 10 min at 900 *g* the supernatant was transferred into another clean glass tube and the extraction procedure repeated. The combined extracts were made up to volume (10 ml) with methanol (solution A).

Since it was not possible to separate compound V from VI under the chromatographic conditions described above, it was necessary to carry out a fractionation of the acidic from the neutral compounds. The methanolic solution was added to 20 ml of diethyl ether and extracted three times with 15 ml of aqueous 1 *M* sodium hydroxide. The organic phase was washed with water, dried with anhydrous sodium sulphate and evaporated under reduced pressure. The residue was redissolved in methanol and made up to 10 ml (solution B). The mixed alkaline aqueous phases were acidified with 4 *M* sulphuric acid in an ice-bath, and extracted three times with diethyl ether. The ether extracts, washed with water and dried, were evaporated and the residue redissolved in methanol to a final volume of 10 ml (solution C). Aliquots (10 μl) of these solutions were injected into the chromatograph.

RESULTS AND DISCUSSION

The most important chromatographic parameters of compounds I–X are summarized in Table II which reports retention times, capacity factors and peak-area ratios at the three detection wavelengths. All the retention times were reproducible under the experimental conditions used. Calibration graphs for I–X were constructed from five consecutive injections and were linear in the range of concentrations used. Their slopes were used in the quantitation of the preservatives in some cosmetic

TABLE II

CHROMATOGRAPHIC PROPERTIES OF COMPOUNDS I–X

Each value is the mean of five determinations.

Compound	*Retention time (min)*	*Capacity factor*	*Peak area ratios*		
			280 nm/240 nm	*280 nm/260 nm*	*260 nm/240 nm*
I	6.27	3.82	1.46	4.75	0.30
II	14.49	10.15	1.81	7.25	0.25
III	19.52	14.02	1.04	3.20	0.33
IV	26.26	19.20	1.32	5.10	0.26
V	34.32	25.40	1.28	0.29	4.33
VI	35.17	26.05	0.46	3.81	0.12
VII	37.59	27.92	1.63	0.33	4.91
VIII	40.89	30.45	0.46	1.33	0.35
IX	45.82	34.25	0.22	0.54	0.41
X	63.58	47.91	1.31	1.38	0.95

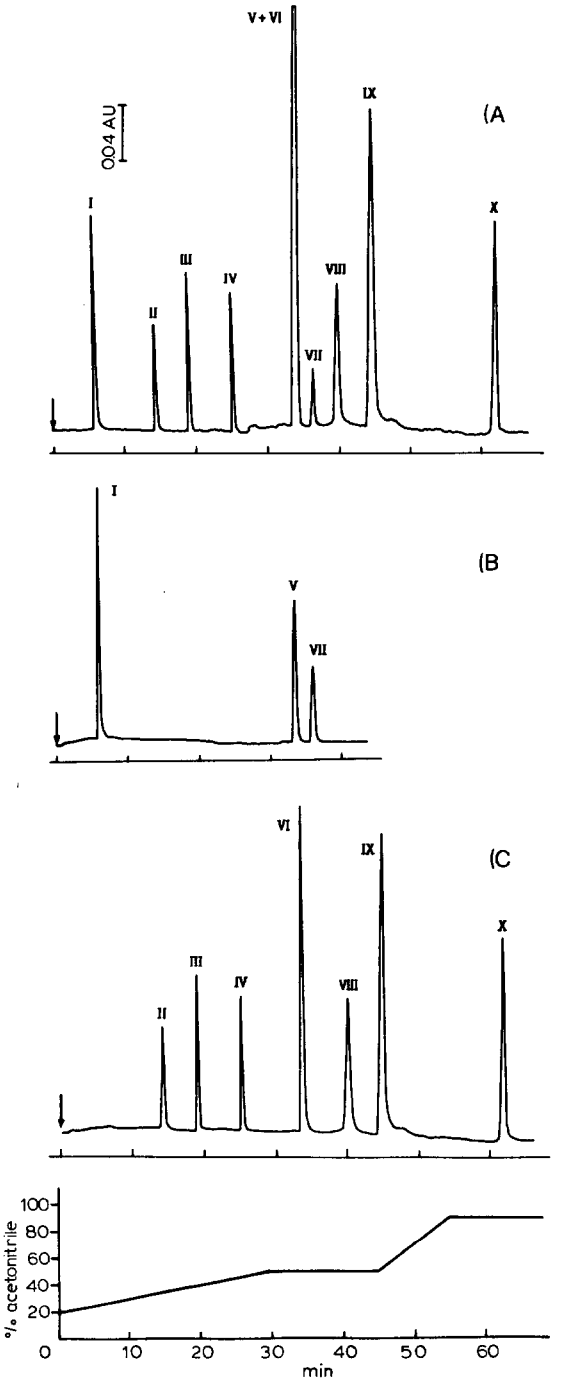

Fig. 1. Typical chromatograms, recorded at 280 nm, of a mixture of preservatives submitted to the whole extraction procedure. Details of sections A, B and C are given in the Results and discussion section.

products. The correlation coefficients were within the range 0.9910–0.9981. The reproducibility of the determination was very satisfactory, the average coefficient of variation being less than 2.4%. The minimum detectable concentrations of all compounds were at least five times below the values reported in the first line of Table I, when injecting 10-μl samples.

The applicability of the HPLC method was demonstrated by assaying compounds I–X in three cosmetic samples spiked with a standard mixture of preservatives. In Fig. 1A is shown the chromatogram obtained by injecting 10 μl of solution A, at the detection wavelength of 280 nm. As is seen, peak VI overlaps peak V. Hence, it was necessary to carry out a further extraction in order to separate Irgasan DP 300 from triclocarban. In Fig. 1B and C are reported the chromatograms obtained after the alkaline extraction, the aim of which was to separate the neutral (I, V, and VII) from the phenolic compounds.

The recoveries obtained after performing the whole extraction procedure are reported in Table III. As is seen, good results and excellent precision are obtained. The identification of compounds I–X on the basis of their retention times can be confirmed by measurement of the peak area ratios at 240, 260 and 280 nm.

TABLE III

RECOVERIES OF THE PRESERVATIVES FROM COSMETIC SAMPLES

Each value is the mean of five determinations.

Compound	*Amount added**	*Recoveries (% ± S.D.)*		
		Cleansing lotion	*Bath foam*	*Day cream*
I	0.5	98.4 ± 2.6	97.8 ± 2.5	97.1 ± 1.9
II	0.2	97.1 ± 1.9	97.0 ± 2.0	96.2 ± 1.8
III	0.4	95.1 ± 3.2	96.2 ± 1.9	95.9 ± 2.7
IV	0.2	93.2 ± 2.1	95.0 ± 2.1	93.0 ± 2.5
V	0.1	92.0 ± 2.9	91.8 ± 2.8	89.9 ± 2.1
VI	0.2	91.1 ± 2.8	90.7 ± 3.2	88.7 ± 1.9
VII	0.2	92.6 ± 3.4	92.8 ± 3.1	94.0 ± 1.8
VIII	0.1	95.2 ± 2.7	96.1 ± 2.0	98.3 ± 2.1
IX	0.1	96.4 ± 3.2	95.9 ± 3.0	97.0 ± 1.9
X	0.2	94.9 ± 3.4	94.7 ± 1.9	97.0 ± 1.6

* Grams added to 100 g of cosmetic sample.

Consideration of all the results presented leads to the conclusion that the HPLC method proposed is suitable for the routine analysis in cosmetic products of all the preservatives examined.

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