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Note

Determination of preservatives in cosmetic products by ion-pair reversed-phase high-performance liquid chromatography. III.

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As part of a study concerning the determination by high-performance liquid chromatography (HPLC) of several kinds of preservative in cosmetics[1–3] in order to verify their adherence to the legislation of the European Economic Community (EEC), we have extended our investigation to four compounds containing an amidino group. The compounds investigated, together with their maximum limits under the EEC Directive No 76/768 (enclosure VI, second part) are as follows: dibromopropamidine (I), 0.1%; hexamidine (II), 0.1%; dibrohexamidine (III); 0.1%; and chlorhexidine (IV), 0.3%.

Since chlorhexidine and hexamidine were first described[4,5], they have been used as antiseptics in pharmaceutical products for their good antibacterial and antifungal properties. Being diamidines they have a strong basic character and readily form salts, from which a range of pharmaceutical preparations have been formulated. Chlorhexidine is generally employed as the digluconate, dihydrochloride, or diacetate, and hexamidine as diisethionate. Recently, they have found wide application, alone or in combination with other bases, as preservatives in cosmetic products[6,7]. To date dibromopropamidine and dibromohexamidine have been employed to a smaller extent, and to our knowledge no quantitative determination has so far been described in literature, although something has been reported concerning the identification and the mode of antibacterial action of dibromopropamidine[8,9].

Various analytical techniques have been used for the quantitative analysis of compound IV, including non-chromatographic methods: spectrophotometry, colorimetry, polarography and gravimetry. Other methods using thin-layer chromatography, gas–liquid chromatography (GLC) and HPLC have also been described (ref. 10 and references cited therein). Recently, ion-pair chromatography has been shown to be valuable for HPLC of ionizable compounds[11]. As for chlorhexidine, reversed-phase separations on bonded supports, such as C_{18} (ref. 12) or CN (ref. 13), have been proposed.

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For the determination of compound II, spectrophotometric, GLC, and HPLC techniques have been used (ref. 14 and references cited therein; also refs. 6 and 7).

Owing to the lack of reports on the determination of the basic preservatives II and III, we have developed a rapid HPLC method that has also proved successful for the simultaneous determination of compounds I and IV. The method is based on ion-pair reversed-phase HPLC using an Erbasil C_{18} column, gradient elution, and UV detection. The chromatographic separation has been used to provide a specific analysis of compounds I–IV in samples of creams developed in-house.

EXPERIMENTAL

Chemicals

Compounds I–III as diisethionates, and IV as dihydrochloride, were kindly supplied by Dr. J. W. Weijland (Keuringsdienst van Waren, Enschede, The Netherlands) and were used as received. Throughout this note the symbols I, II, III, and IV will be used to designate the actual substances used. Sodium perchlorate and *n*-propyl *p*-hydroxybenzoate (internal standard 1, IS_1) were obtained from Merck (Darmstadt, F.R.G.), dichlorophene (IS_2) from BDH (U.K.) and tetramethylammonium bromide (TMAB) from Eastman-Kodak (Rochester, NY, U.S.A.). Perchloric acid (60% aqueous solution) and HPLC-grade acetonitrile were purchased from Farmitalia-Carlo Erba (Milan, Italy). All chemicals were analytical grade and were used without further purification. Water was deionized and doubly distilled in glass. All solvents and solutions for HPLC analysis were filtered through a Millipore (Milford, MA, U.S.A.) 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 Model 730 integrator–recorder were used. The analytical column was a 10-μm Erbasil C_{18} (250 × 4.6 mm I.D.) (Farmitalia-Carlo Erba). Peak areas were determined by electronic integration (Varian Model CDS 111).

Chromatographic conditions

Optimized chromatographic parameters were: mobile phase, acetonitrile–water containing $5 \cdot 10^{-2}$ *M* sodium perchlorate and $5 \cdot 10^{-3}$ *M* TMAB (pH 3.0 adjusted with perchloric acid) (40:60, v/v), for 2 min, then a linear gradient up to 60% acetonitrile in 8 min, and a 10-min time-purge at this composition; flow-rate, 2.5 ml/min; column temperature, 40°C; injection volume, 10 μl; detector wavelength, 264 nm (unless otherwise stated); detector sensitivity, 0.64 a.u.f.s.; chart speed, 0.5 cm/min.

Calibration curves

Stock solutions were prepared by dissolving weighed amounts of compounds I–IV in the initial mobile phase containing 83 μg/ml of IS_1 and 767 μg/ml of IS_2. A set of standard solutions produced daily by serial dilution was used. These solutions were processed using the HPLC conditions described above. The ratios of the peak areas of compounds I–IV relative to the peak area of IS_1 or IS_2 were used to calculate the calibration graphs, the slopes of which were used for the quantitation of com-

TABLE I

CHROMATOGRAPHIC PROPERTIES OF THE COMPOUNDS TESTED

Each value is the mean of six determinations.

Compound	*Retention time (min)*	*Capacity factor*	*Response factor relative to IS_1*	*Response factor relative to IS_2*	*Peak-area ratios*	
					264 nm:254 nm	*264 nm:280 nm*
I	2.36	1.36	7.57	0.12	1.29	1.63
II	2.79	1.79	5.56	0.09	1.29	1.31
III	4.74	3.74	8.28	0.13	1.72	2.06
IV	6.25	5.25	4.70	0.08	1.00	2.23
IS_1	3.75	2.79				
IS_2	7.39	6.39				

pounds I–IV in three samples of creams. The response factors relative to both internal standards and calculated from the weight ratio are reported in Table I.

Assay of preservatives in creams

A sample of cream (1.0 g), spiked with the mixture of preservatives investigated, was accurately weighed into a glass centrifuge tube, 4 ml of the mobile phase containing both internal standards were added, and the tube was immersed in an ultrasonic bath for 30 min. The mixture was then heated in a water-bath at 60°C for 10 min and centrifuged. After centrifugation the supernatant was transferred to a 10-ml volumetric flask, and the extraction procedure was repeated with a second 4-ml aliquot of the mobile phase. The combined extracts were diluted to volume with the mobile phase. A 10-μl aliquot of the solutions was injected into the liquid chromatograph.

RESULTS AND DISCUSSION

The chromatography of ionizable substances such as amines and organic acids often presents problems with respect to retention and peak symmetry. These considerations led us to the selection of a reversed-phase C_{18} column and to the use of an ion-pairing agent. Perchlorate ion proved successful in retaining on the column the compounds investigated, but the peak shape (and thus column efficiency) was poor. The problem was solved by adding TMAB to the mobile phase. TMAB is effective in deactivating residual negatively-charged silanol sites of the bonded-phase support, which might interact with the ionic samples so causing tailing peaks.

Fig. 1 shows the chromatogram of a standard mixture of compounds I–IV (each at a concentration of 0.05%, w/v) and IS_1 and IS_2, obtained setting the detector at 264 nm. As can be seen, good resolution of all preservatives examined has been achieved. Chromatograms were also recorded with the detector set at 254 and 280 nm, respectively. HPLC detection was performed at three wavelengths in order to estimate the values of the peak-area ratios for each preservative, listed in Table I.

This measurement is very important both in confirming the identification of

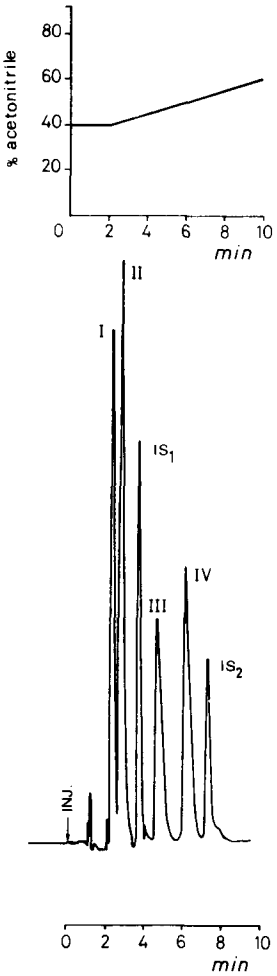

Fig. 1. Typical chromatogram of a standard mixture of preservatives (0.05%, w/v) obtained with the detector set at 264 nm. Chromatographic conditions reported in the text.

the preservative, made on the basis of its retention time, and in estimating possible interferences from other compounds when complex cosmetic samples are assayed.

The most important chromatographic parameters of compounds I–IV are summarized in Table I. All retention times were reproducible under the experimental conditions used. Calibration graphs of peak-area ratios (compounds I–IV to IS_1 or to IS_2) *versus* amounts injected were constructed from six consecutive injections. Linearity was observed from 0.02 up to 6 μg injected for each preservative. Correlation coefficients obtained by linear regression analysis were in the range 0.9947–0.9992. The detection limits for all preservatives were at least twenty times below the levels normally used in cosmetic chemistry. The reproducibility of the assay was very satisfactory, the average coefficient of variation being less than 2.1%.

The applicability of the proposed method for the determination of compounds

I–IV in cosmetic samples was demonstrated by studying their analytical recoveries from three different samples of creams developed in-house and spiked with a standard mixture of the compounds. The results obtained after performing the whole procedure are shown in Table II. As can be seen, good recoveries and excellent precision were obtained. The use of either IS_1 or IS_2 is linked to the kind of cream under investigation. *n*-Propyl paraben is one of the most common preservatives added to a cosmetic product. Therefore, if this is the case or in the event of other components eluting next to the peak of IS_1, it is preferable to perform the quantitative determination with the aid of the internal standard IS_2, whose peak occurs in a region of the chromatogram free from interferences.

TABLE II

RECOVERY OF PRESERVATIVES FROM SAMPLES OF CREAMS

Each value is the mean of six determinations.

Compound	*Amount added**	*Recoveries (% ± S.D.)*		
		Cream A	*Cream B*	*Cream C*
I	0.1	94.2 ± 1.6	92.6 ± 1.8	98.2 ± 1.7
II	0.1	96.3 ± 1.9	94.5 ± 1.9	100.7 ± 2.2
III	0.1	93.3 ± 1.5	98.6 ± 1.6	92.3 ± 2.0
IV	0.3	91.4 ± 1.5	93.2 ± 2.0	91.7 ± 1.4

In conclusion, the method described provides a sensitive and quantitative assay for compounds I–IV in cosmetic formulations. The extraction step is short and simple and the analysis time is 10 min. Recoveries, precision and reproducibility are good. Because of its simplicity the HPLC assay reported here is suitable for the routine analysis of cosmetic creams, especially in order to verify their adherence to ECC legislation.

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