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Note

Determination of sun-screen agents of the *p*-aminobenzoic acid type in cosmetic products by reversed-phase high-performance liquid chromatography

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As part of its programme to harmonize legislation on cosmetics, the Council of the European Community has approved a list of UV absorbers for use in cosmetic products. The European Directive 76/768 (enclosure VI) states which agents are provisionally authorized in sun-screen preparations and their concentrations. We have started a study on the identification and determination of UV absorbers in cosmetic products to test their compliance with EEC regulations, taking into account, first of all, agents of the *p*-aminobenzoic acid (PABA) type.

PABA and some of its esters are among the most common agents in sun-screen preparations. Several reports have shown the effectiveness of these substances[1] and some methods have been proposed for their identification and determination in cosmetics[1–5].

PABA and monoglyceryl *p*-aminobenzoate (glyceryl PABA) are so widely used in sun-screens that about 10% of such products marketed in Italy is believed to contain at least one of them; their levels vary from 0.4 to 5%[3]. Obviously this is linked to the "sun protection factor"[6] claimed by the manufacturer. It is possible for a product to contain more than one such agent, frequently two, since it is believed that in this way the overall sun-protection properties are improved.

The EEC Directive (enclosure II) forbids the presence of the local anaesthetic benzocaine (ethyl *p*-aminobenzoate) in cosmetic products containing glyceryl PABA. Benzocaine is not intentionally used in sun-screens but may occur as a contaminant in glyceryl PABA the synthesis of which may be based on benzocaine[6]. Photocontact allergy to benzocaine in a sun-screen has been reported[7]. The benzocaine contamination is also important in that it may elicit or maintaining dermatitis in previously benzocaine-sensitized individuals. Therefore, there is an obvious need for methods of detecting PABA and its esters in sun-screen preparations.

We report here a simple analytical method based on reversed-phase high-performance liquid chromatography (HPLC) which allows a qualitative and quantitative determination of PABA, glyceryl PABA and benzocaine in sun-creams with the aid of an internal standard.

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MATERIALS AND METHODS

Materials

PABA(II), benzocaine(III), methyl *p*-hydroxybenzoate as internal standard (I.S.), perchloric acid (60% aqueous solution), sodium perchlorate monohydrate and HPLC-grade acetonitrile were purchased from Farmitalia-Carlo Erba (Milan, Italy). Tetramethylammonium chloride was obtained from Eastman Kodak (Rochester, NY, U.S.A.). All chemicals were of analytical grade and were used without further purification. Glyceryl PABA (I) was prepared by reaction of 2,2'-dimethyl-4-hydroxymethyl-1,3-dioxolane with 4-nitrobenzoyl chloride in pyridine[8]. The nitro group of the resulting ester was reduced to the corresponding amine by hydrogenation with Raney nickel as catalyst and the adduct hydrolyzed with dilute acetic acid. Water was deionized and doubly distilled from glass apparatus. All solvents and solutions for HPLC analysis were filtered through a Millipore filter, pore size 0.45 μm, and vacuum degassed by sonication before use.

Apparatus

A Model 5000 liquid chromatograph (Varian, Zug, Switzerland) equipped with a variable-wavelength UV detector (Varichrom UV50), a Valco AH60 injection valve and a Model 730 integrator-recorder (Waters Assoc., Milford, MA, U.S.A.) were used. The analytical column was a 10-μm Erbasil C_{18} (250 mm × 4.6 mm I.D.) (Farmitalia-Carlo Erba). Peak areas were determined by electronic integration (Varian Model CDS 111).

HPLC conditions

The operating conditions were as follows: mobile phase, acetonitrile–water (18:82, v/v) containing 10^{-2} *M* sodium perchlorate and $5 \cdot 10^{-3}$ *M* tetramethylammonium chloride (pH 3.0, adjusted with perchloric acid) for 5 min, then a linear gradient to 50% acetonitrile in 10 min and a 10-min purge at this composition; flow-rate, 2.0 ml/min; column temperature, 35°C; injection volume, 10 μl; detector wavelengths, 274 and 295 nm; detector sensitivity, 0.64 a.u.f.s.; chart speed, 0.5 cm/min.

Sample preparation

A 1–2 g amount of a sample of cream, known not to contain any sun-screen agent, was weighed in a 25-ml, hermetically sealed, calibrated flask and spiked with small amounts of compounds I–III. An equal amount of sodium chloride, 1 ml of 2 *N* sulphuric acid and 15 ml of methanol containing the internal standard (60 μg/ml) were added and the mixture heated in a water-bath at 50°C for 1 h. After cooling, the mixture was made up to volume with methanol (+ I.S.) and filtered through filter-paper (Schleicher & Schüll, Type 589[1]). A 5-ml volume of the filtrate was diluted to 25 ml in methanol (+ I.S.). Aliquots of 10 μl were subjected to HPLC.

RESULTS AND DISCUSSION

Fig. 1A and B shows typical chromatograms of a standard mixture of compounds I–III and I.S., obtained by detection at 274 and 295 nm, respectively. As is

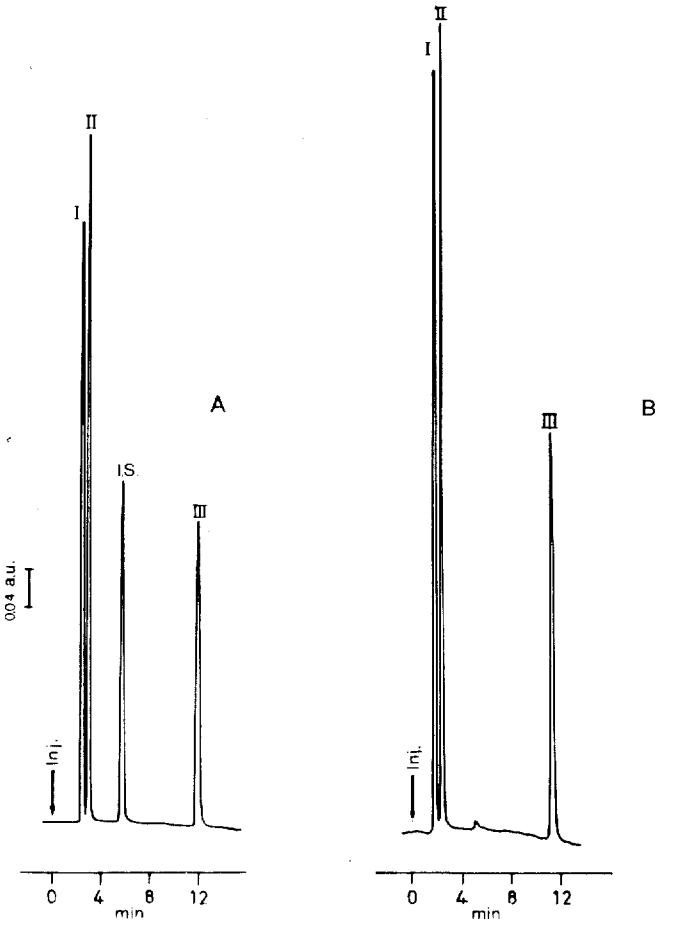

Fig. 1. Chromatograms of a standard mixture of I (98 μg/ml), II (165 μg/ml), III (60 μg/ml) and I.S. (60 μg/ml) obtained by detection at 274 (A) and 295 (B) nm, respectively. Chromatographic conditions as in the text.

seen, a good resolution of the compounds investigated and I.S. was obtained. The wavelength of 295 nm was chosen for the detection since PABA and its esters have absorption maxima around this value. Since the I.S. was not detectable at 295 nm, the detection was performed also at 274 nm.

The most important chromatographic parameters of the compounds considered are summarized in Table I. Retention times were reproducible under the experimental conditions used. The response factors relative to I.S. were calculated from the weight ratio. The Table also shows the values of the peak-area ratio at 295 and 274 nm for each sun-screen agent, which can be very useful for evaluating the purity of the chromatographic peak and for confirming the identity of the compound, on the basis of its retention time or capacity factor.

Calibration curves were constructed from six consecutive injections. Stock solutions were prepared by dissolving weighed amounts of compounds I–III in meth-

TABLE I

CHROMATOGRAPHIC PROPERTIES OF THE COMPOUNDS TESTED

Each value is the mean of six determinations.

Compound	*Retention time (min)*	*Capacity factor*	*Relative response (at 274 nm)*	$\frac{Area_{295}}{Area_{274}}$
I	2.41	0.55	1.05	1.29
II	3.06	0.97	0.77	1.23
III	11.45	6.39	0.88	1.25
I.S.	6.60	3.26	1.00	–

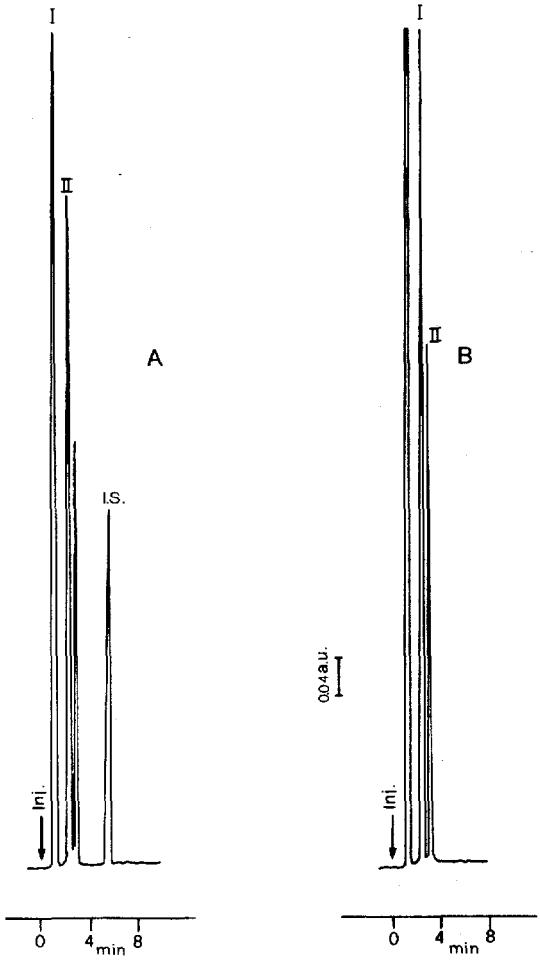

Fig. 2. HPLC chromatograms of a sample of sun-cream spiked with 1.25% of I and II recorded at 274 (A) and 295 nm (B).

anol containing 60 μg/ml of I.S. A set of standard solutions was produced by serial dilutions and processed using the HPLC conditions described above. The ratio of the peak areas of compounds I–III at 274 nm relative to the peak area of I.S. were plotted *versus* the amounts of I–III injected. Linearity was observed up to 40 μg. The correlation coefficients ranged from 0.991 to 0.997. The reproducibility of the assay was good, the average coefficient of variation being less than 2.3%. The detection limits, calculated as twice the noise level, were approximately 5 and 10 ng at 295 and 274 nm, respectively.

The applicability of the proposed HPLC assay was demonstrated by determining PABA and glyceryl PABA in two samples of sun-creams. Small amounts of I and II were added to a cream known not to contain any sun-screen agent and the recoveries were determined. Fig. 2A and B shows the chromatograms obtained at the two detection wavelengths for a sample of cream. Peak identities were confirmed by measurement of the peak area ratios at 295 and 274 nm, which were in good agreement with the values reported in Table I. The results of the analysis are summarized in Table II. As is seen, good recoveries and precision were obtained.

TABLE II

RECOVERIES OF COMPOUNDS I AND II FROM SUN-CREAMS

Each value is the mean of six determinations.

Compound	*Amount added (%, w/w)*	*Recovery (%) ± S.D.*	
		Cream A	*Cream B*
I	1.25	96.3 ± 2.4	96.4 ± 2.2
I	3.00	97.0 ± 2.5	98.2 ± 2.7
II	1.25	94.5 ± 2.1	97.0 ± 2.6
II	3.00	96.7 ± 2.4	97.7 ± 2.4

Because of its simplicity, the HPLC assay reported here is suitable for the routine analysis of sun-screen agents of the PABA type in cosmetic products, particularly to verify their compliance with the EEC regulations.

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