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### Determination of sun-screen agents in cosmetic products by reversed-phase high-performance liquid chromatography

#### Part II.

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As part of a study concerning the determination by high-performance liquid chromatography (HPLC) of sun-screen agents in cosmetics<sup>1,2</sup> in order to verify their adherence to legislation within the European Economic Community (EEC), we have extended our investigation to a series of compounds commonly employed in suntan preparations as UV absorbers. Table I lists the compounds investigated with the code system proposed by Liem and Hilderink<sup>3</sup>, and reports the maximum limits established under Italian legislation (law 713/86 and subsequent adjournments) in compliance with EEC Directive 76/768 on cosmetics.

The sun-screen agents BENZ 1, BENZ 6, BENZ 8, PABA 8 and CINA 11, already investigated<sup>2</sup>, were considered again because of their wide application in cosmetic products. BENZ 1, BENZ 2, BENZ 4 and BENZ 5, which are not permitted as sun-screen agents for the protection of the skin, were examined owing to their frequent use in commercial products to protect them from UV radiation. This paper reports the results of an analysis performed on 85 samples of commercial suntan cosmetics.

#### EXPERIMENTAL

##### *Apparatus*

A model 5000 liquid chromatograph (Varian, Zug, Switzerland), equipped with a Valco AH 60 injection valve, a Varian Polychrom 9060 photodiode array detector and a Varian 4290 integrator, was used. The analytical column was made of stainless steel (250 mm × 4.6 mm I.D.), packed with 10- $\mu$ m LiChrosorb RP-18 (Merck, Darmstadt, F.R.G.).

### Reagents

All reagents were of analytical-reagent grade, except methanol and acetonitrile (solvent for liquid chromatography grade). 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  $\mu\text{m}$ ) and vacuum degassed by sonication before use. The sun-screen agents, available commercially, were obtained as listed in Table I.

### Chromatographic conditions

The HPLC conditions were as follows: initial mobile phase, acetonitrile–water (5:95, v/v) containing  $10^{-3}$  M perchloric acid and  $5 \cdot 10^{-2}$  M sodium perchlorate; a linear gradient to 35% acetonitrile at 20 min; this composition for 5 min, and then another gradient to 80% acetonitrile at 40 min; flow-rate, 2.5 ml/min; column temperature, 30°C; injection volume, 10  $\mu\text{l}$ ; detector wavelength, 311 nm; detector sensitivity, 0.32 a.u.f.s.; and chart speed, 0.5 cm/min.

### Calibration graphs

Stock solutions were prepared by dissolving the appropriate amount of the sun-screen agent in methanol. A set of standard solutions were produced by diluting aliquots of the stock solutions with methanol to give concentrations of each compound ranging from 5 to 100  $\mu\text{g/ml}$ . The methanol solution was evaporated under reduced pressure and the residue extracted twice with 5 ml of 2 M sulphuric acid. The pooled acidic supernatants, containing BENZ 4, BENZ 8, SSCR 1 and PABA 8 (solution A) were submitted to HPLC. The residue containing the remaining sun-screen agents was dissolved in 10 ml of methanol (solution B) and injected into the chromatograph. Calibration graphs of peak area *versus* amount injected were constructed.

### Extraction of sun-screen agents from cosmetics

The mixture of the compounds under investigation was added to samples of commercial cosmetic samples, known not to contain any sun-screen agent, to yield the concentrations given in Table III. About 1 g of cosmetic sample was weighed exactly into a centrifuge tube, 0.25 ml of 2 M sulphuric acid and 10 ml of methanol were added and the tube was immersed in an ultrasonic bath thermostated at 30°C for 30 min, then centrifuged at 900 g for 10 min. The extraction procedure was repeated and the pooled supernatants were diluted to volume (25 ml) with methanol. If PABA 8 and CINA 11 were both present, it was necessary to perform another extraction in order to separate the two sun-screen agents; 10 ml of the methanol solutions were withdrawn, evaporated under reduced pressure, and submitted to the extraction procedure described above to give solutions A and B.

## RESULTS AND DISCUSSION

Table II reports the retention times, the purity parameter format values<sup>4</sup> and the limits of detection for the sun-screen agents investigated. All retention times were reproducible under the experimental conditions used, the average coefficient of variation being less than 3%. The UV spectral characteristics of the compounds were studied using a photodiode-array detector and the spectral data were interpreted by determining the purity parameter (Varian) format. This parameter, which utilizes an

TABLE I  
SUN-SCREEN AGENTS STUDIED AND THEIR MAXIMUM LIMITS ACCORDING TO ITALIAN LAW 713/86 (ANNEX V, SECTION II)

Code name <sup>a</sup>	Chemical name	Maximum admissible concentration (%)	Supplier
BENZ 1 <sup>b</sup>	2,4-Dihydroxybenzophenone	—	Sigma (I)
BENZ 2 <sup>b</sup>	2,2'-Dihydroxy-4-methoxybenzophenone	—	Saipo (I)
BENZ 4 <sup>b</sup>	2,2'-Dihydroxy-4,4'-dimethoxybenzophenone-5-sulphonic acid (sodium salt)	—	Saipo (I)
BENZ 5 <sup>b</sup>	2,2',4,4'-Tetrahydroxybenzophenone	—	Saipo (I)
BENZ 6	2-Hydroxy-4-methoxybenzophenone	10	Sigma (I)
BENZ 8	2-Hydroxy-4-methoxybenzophenone-5-sulphonic acid (sodium salt)	5 (expressed as free acid)	Sigma (I)
CAMP 4	3-(4-Methylbenzylidene)-4,4'-camphor	6	Merck (D)
SSCR 1	2-Phenylbenzimidazole-5-sulphonic acid	8 (expressed as free acid)	Saipo (I)
CINA 7	Diethanolammonium 4-methoxycinnamate	8 (expressed as free acid)	Saipo (I)
CINA 10	Isoamyl 4-methoxycinnamate	8 (expressed as free acid)	Saipo (I)
CINA 11	2-Ethylhexyl 4-methoxycinnamate	10	Haarman & Reimer (NL)
SALA 5	Homomenthyl salicylate	10	Givaudan (CH)
SALA 9	Isopropylbenzyl salicylate	4	Quest International (NL)
PABA 8	2-Ethylhexyl 4-dimethylaminobenzoate	8	Vevy (I) Van Dyk (NL)

<sup>a</sup> According to ref. 3.

<sup>b</sup> Not covered by Italian law 713/86.

TABLE II

RETENTION TIMES, PURITY PARAMETER FORMAT VALUES ( $\lambda_m$ ) AND LIMITS OF DETECTION

Compound	Retention time (min)	$\lambda_m$ (nm)	Detection limit ( $\mu\text{g/ml}$ )
BENZ 1	27.05	284.75	0.20
BENZ 2	29.85	284.39	0.30
BENZ 4	3.27	263.06	0.40
BENZ 5	17.05	292.07	0.20
BENZ 6	33.84	284.00	0.20
BENZ 8	9.91	266.82	0.30
CAMP 4	38.61	294.02	0.10
SSCR 1	6.89	278.29	0.10
CINA 7	18.49	297.24	0.10
CINA 10	37.73	294.94	0.05
CINA 11	42.29	292.78	0.05
SALA 5	43.22	250.61	0.05
SALA 9	39.59	240.75	1.00
PABA 8	42.35	304.20	0.10

absorbance-weighted mean wavelength of a spectrum to reduce spectral data to a single value, can be used to confirm chromatographic peak identity and purity and to establish the class to which a compound belongs (e.g. to identify a compound as an amine). The detection limits were calculated as a response three times the noise level.

Fig. 1 shows the chromatogram of a standard mixture of sun-screen agents. PABA 8 and CINA 11 coelute with the mobile phase used, and to overcome this drawback a rapid screening of the cosmetic sample was carried out by thin-layer chromatography<sup>5,6</sup> to establish their presence. In this instance it was necessary to perform an extraction procedure capable of separating the compounds soluble in an aqueous solvent acidified with sulphuric acid, such as PABA 8, from the neutral and acidic compounds extractable into methanol, such as CINA 11. Fig. 2 shows the chromatograms of the aqueous and methanolic phases obtained after the extraction procedure described under Experimental.

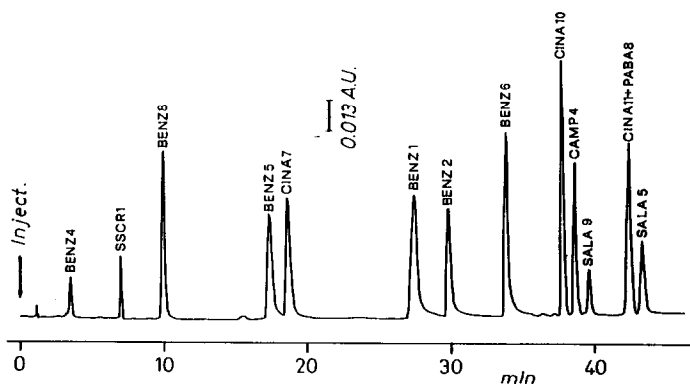


Fig. 1. Typical chromatogram of a standard mixture of sun-screen agents.

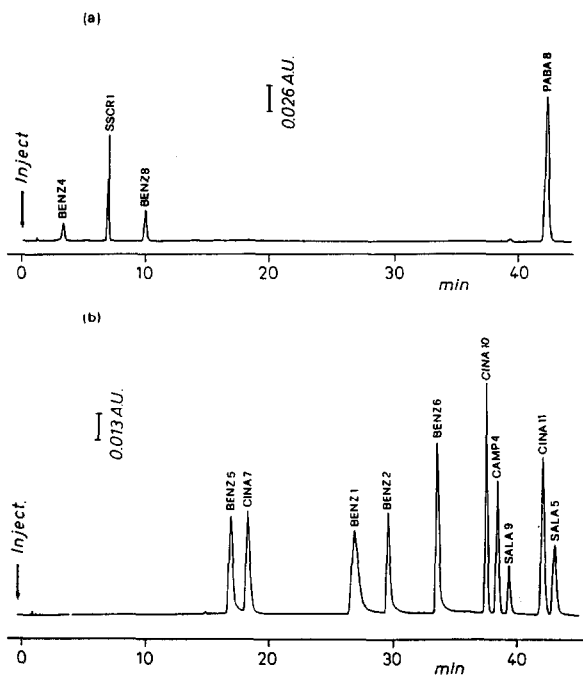


Fig. 2. Typical chromatograms of (a) solution A and (b) solution B obtained from a mixture of sun-screen agents subjected to the whole extraction procedure.

TABLE III

RECOVERIES OF SUN-SCREEN AGENTS FROM COSMETIC SAMPLES

Each value is the mean of five determinations.

Compound	Amount added (%, w/w)	Recovery (%)	S.D. (%)
BENZ 1	1.25	98.4	0.9
BENZ 2	1.25	98.2	1.6
BENZ 4	1.00	98.7	1.9
BENZ 5	1.25	99.6	1.4
BENZ 6	1.25	99.0	1.5
BENZ 8	1.00	97.9	2.0
CAMP 4	0.50	99.6	1.4
SSCR 1	0.50	97.6	1.6
CINA 7	0.50	98.1	1.6
CINA 10	0.37	97.6	1.8
CINA 11	0.37	98.7	1.2
SALA 5	2.00	97.9	1.0
SALA 9	1.75	97.6	1.9
PABA 8	0.50	99.1	0.9

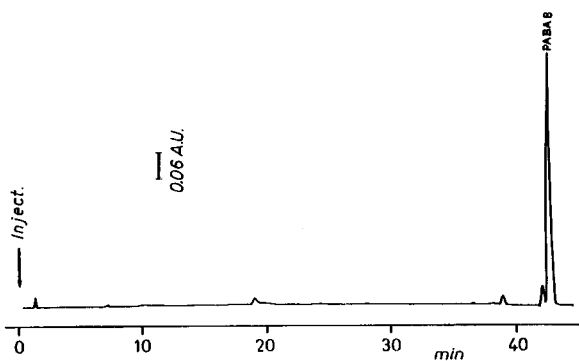


Fig. 3. Chromatogram obtained for a commercial suncream containing PABA 8.

Calibration graphs were constructed from six consecutive injections. Correlation coefficients obtained by linear regression analyses were in the range 0.9922–0.9999. Linearity was observed up to 1  $\mu\text{g}$  of each sun-screen agent injected.

The applicability of the proposed method was demonstrated by determining the analytical recoveries of sun-screen agents added to some cosmetic samples known not to contain any of the compounds under investigation. Four proprietary suncreams were spiked with various amounts of the agents and submitted to the extraction procedure, and the recoveries obtained are shown in Table III. Good recoveries and precision were observed.

Fig. 3 shows the chromatogram obtained for a commercial suncream containing PABA 8. The minor peaks correspond to the excipients present in the cosmetic sample. Eighty-five commercial products were analysed for the presence of the sun-screen agents considered. In all instances the levels found were lower than the limits imposed by Italian law 713/86. Among the sun-screen agents examined the six most frequently used by the industry were found to be CINA 11 (47%), CAMP 4 (28%), SSCR 1 (25%), PABA 8 (18%), CINA 10 (13%) and BENZ 6 (10%). Frequently the cosmetic products contained a combination of two sun-screen agents, the most common being CINA 11 + CAMP 4 and CAMP 4 + SSCR 1.

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