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Assay of common sunscreen agents in suncare products by high-performance liquid chromatography on a cyanopropyl-bonded silica column

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Abstract

A rapid high-performance liquid chromatographic method was developed for the simultaneous assay of eight of the most common sunscreen agents (octyl-methoxycinnamate, oxybenzone, butyl-methoxydibenzoylmethane, octyl-salicilate, methylbenzylidene camphor, octyl-dimethylamminobenzoate, phenylbenzimidazole sulphonic acid and octocrylene) in sun protection products. Evaluation of the influence of different stationary phases and eluents on the separation selectivity showed that optimal resolution was obtained on a cyanopropyl-silica column eluted with methanol–acetonitrile–tetrahydrofuran–aqueous acetic acid. A small adjustment of the proposed chromatographic system (reduction in the aqueous content of the mobile phase) permitted also the determination of the extremely hydrophobic UV filter, methylene bis-benzotriazolyl tetramethylbutylphenol along with three other sunscreen agents, octyl-methoxycinnamate, oxybenzone, butyl-methoxydibenzoylmethane. Recoveries of the UV filters from the spiked formulation were between 95.7 and 103.7% and the precision of the method was better than 6.1% relative standard deviation. The developed HPLC procedure is suitable for quality control and photostability analyses of commercial suncare products.

Keywords: Sunscreen agents; Column liquid Chromatography; Suncare products; Photodegradation

1. Introduction

The expanding knowledge of the deleterious effects of the UV radiation from the sun (290–400 nm) has fuelled the widespread use of topical sunscreening preparations as a measure to protect human skin against the sunlight-induced damages [1–4]. The active constituents in these products are classified as inorganic sunscreens that act mainly by reflecting or scattering the UV radiation and organic sunscreens which attenuate the transmission of the solar UV rays to the skin by absorbing the radiation, the latter being used most commonly [4]. The trend toward products with higher protective effect and screening efficiency against both UV-B (290–320 nm) and UV-A (320–400 nm) wavelengths has led to the extensive development of preparations containing combinations of various organic UV filters at different concentrations [2,5–7]. Moreover, regulatory authorities in Europe [8], USA [9], Japan and Australia [2] have set lists of the authorized sunscreen agents with their maximum allowed concentrations. Therefore, rapid and reliable methods for the determination of UV filters in commercial cosmetics are required to check whether the products conform to the existing legislation and also for quality control purposes and for evaluation of the sunscreen stability in the finished formulation.

Several techniques have been reported, including UV spectroscopy [10], gas chromatography [11] and high-performance liquid chromatography (HPLC), the latter being the method of choice for the simultaneous analysis of several UV absorbers in cosmetic products [5,6]. Despite the large number of chromatographic systems described in the literature [5–7,12–16], the HPLC assay of the foregoing

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compounds has been carried out almost exclusively on octadecyl- or octyl-silica reversed-phase packings. While the HPLC determination of several sunscreen agents has been reported, complete resolution of certain UV filters (e.g., butyl-methoxydibenzoylmethane, octyl-methoxycinnamate, octyl-salicilate, octyl-dimethylamminobenzoate) presents difficulties for proper quantification [6,13,14], or the separation deals only with few compounds [5,7,12,15,16]. Moreover, some of the methods are not really suitable for routine analyses because of lengthy gradient elution procedures [6,13].

These problems prompted a study of the performance of a series of chemically bonded reversed-phase supports with different selectivity for the isocratic HPLC of seven of the most commonly used [2,17] sunscreen compounds (see Fig. 1): octyl-methoxycinnamate (OMC), oxybenzone (OB), butyl-methoxydibenzoylmethane (BMDBM), octyl-salicilate (OS), octyl-dimethylamminobenzoate (ODAB),

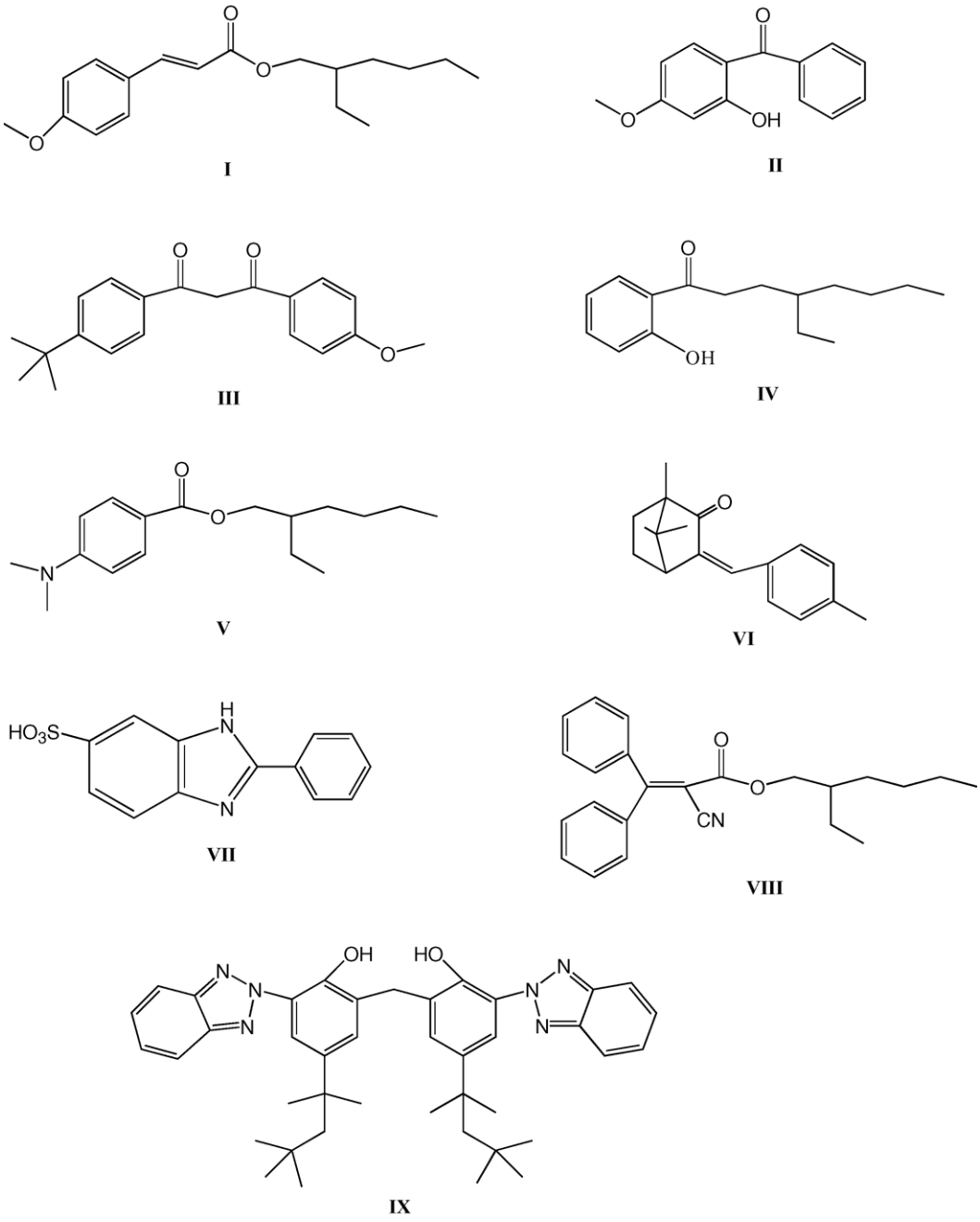

Fig. 1. Chemical structures of the investigated sunscreen agents: (I) octyl-methoxycinnamate, (II) oxybenzone, (III) butyl-methoxydibenzoylmethane, (IV) octyl-salicilate, (V) octyl-dimethylamminobenzoate, (VI) methylbenzylidene camphor, (VII) phenylbenzimidazole sulphonic acid, (VIII) octocrylene, (IX) methylene bis-benzotriazolyl tetramethylbutylphenol.

methylbenzylidene camphor (MBC) and phenylbenzimidazole sulphonic acid (PBSA). In addition, the applicability of the optimized chromatographic system to the analysis of the two sunscreens, octocrylene (Fig. 1) and methylene bis-benzotriazolyl tetramethylbutylphenol (Tinosorb M; Fig. 1) is also demonstrated.

2. Materials and methods

2.1. Materials

PBSA, OB, ODAB and MBC were provided by Merck (Darmstat, Germany). BMDBM and OMC were supplied by Roche Ltd. (Basel, Switzerland). OS and octocrylene were obtained by Haarmann & Reimer (Holzminden, Germany). Tinosorb M was from Ciba (High Point, NC, USA). Methanol, acetonitrile, water and tetrahydrofuran of HPLC grade were obtained by Merck. All other chemicals were of analytical grade (Sigma, St. Louis, MO, USA). Suncare products were kindly donated by General Topics (San Felice del Benaco, Italy), Symrise (Hamburg, Germany) and Roche.

2.2. High-performance liquid chromatography

The HPLC apparatus comprised a Model LabFlow 3000 pump (LabService Analytica, Bologna, Italy), a Model 7125 injection valve with a 20 μl sample loop (Rheodyne, Cotati, CA, USA) and a Model 975-UV variable wavelength UV–vis detector (Jasco, Tokyo, Japan) set at 320 nm, which is a compromise absorption wavelength to obtain satisfactory UV responses for all analytes. Data acquisition and processing were accomplished with a personal computer using Borwin software (JBMS Developpements, Le Fontanil, France). Sample injections were effected with a Model 701 syringe (10 μl; Hamilton, Bonaduz, Switzerland). Separations were performed on a 5-μm Zorbax SB-CN column (150 × 4.6 mm i.d.; Agilent Technologies, Waldbronn, Germany) fitted with a guard column (5-μm particles, 4 × 2 mm i.d.) and eluted isocratically, at a flow-rate of 1.0 ml/min, with methanol–acetonitrile–tetrahydrofuran–water (40:10:10:40, v/v/v/v) containing 0.5% (v/v) acetic acid. Chromatography was performed at ambient temperature. Other columns used in this study included a Zorbax SB-C_{18}, a Zorbax SB-Phenyl (5-μm particles, 150 × 4.6 mm i.d.) and a Hypersyl BDS C_{18} (5-μm particles, 150 × 4.6 mm i.d.; Hypersil, Runcorn, UK). The identity of the separated peaks was assigned by co-chromatography with the authentic standards. Quantification was carried out by integration of the peak areas using the external standardization method.

2.3. Sample preparation

The cosmetic product (ca. 100 mg) was accurately weighed into a 50-ml volumetric flask and dispersed in methanol or 20% (v/v) acetonitrile in tetrahydrofuran (for product containing Tinosorb M) by ultrasonication. After dilution to volume, the sample was filtered through 0.45-μm membrane filters (Whatman, Clifton, NJ, USA) and analysed by HPLC.

2.4. Assay validation

A cream (oil-in-water emulsion) test sample was prepared in the laboratory by adding known concentrations of each sunscreen agent (PBSA was first neutralized with NaOH) to the formulation components (sorbitan monostearate, polyoxyethylene sorbitan monostearate, butylated hydroxyanisole, isopropyl isostearate, cetearyl isononanoate, cetearyl alcohol, sodium benzoate, glycerin, dehydroacetic acid, EDTA, water). The cream was prepared according to the common procedure used in compounding practice [18]. The percentage recoveries were calculated by comparing the peak areas of the sunscreen agents extracted from the test sample with those obtained by direct injections of an equivalent concentration of the analytes dissolved in methanol.

The chromatographic precision was evaluated by repeated analyses ($n = 6$) of the same sample solution from a cream. The method precision was calculated by extraction and HPLC assay of independent samples ($n = 6$) from the same cream formulation.

Calibration curves of peak area versus concentration were generated with placebo extracts spiked with known amounts of the examined UV filters in the concentration range 0.002–0.1 mg/ml.

2.5. Photodegradation studies

A portion (100–120 mg) of the sunscreen product was spread by means of a syringe onto the bottom of a beaker and then irradiated for 1 h with a solar simulator (Suntest CPS+; Atlas, Linsengericht, Germany) equipped with a Xenon lamp, an optical filter to cut off wavelengths shorter than 290 nm and an IR-block filter to avoid thermal effects. The solar simulator emission was maintained at 250 W/m^2 [19]. After the exposure interval, the beaker was removed and its content quantitatively transferred into a 50-ml calibrated flask with methanol or 20% (v/v) acetonitrile in tetrahydrofuran (for product containing Tinosorb M). The resulting sample was dispersed under sonication, diluted to volume and subjected to HPLC assay, as outlined above. All samples were protected from light both before and after irradiation. The degree of photodegradation was evaluated by comparing the peak areas of the sunscreen agents from the irradiated samples, with those obtained by analysis of an equivalent amount of the unirradiated preparations.

Data were analyzed for significance by using the Student's paired *t*-test (Instat, Graphpad Software, San Diego, CA). *P*-values <0.05 were considered significant.

3. Results and discussion

3.1. Chromatography

The objectives of this study were to determine optimum conditions for the HPLC separation of seven major sunscreen agents used in cosmetic products [2,17]. Preliminary experiments were performed on an octadecyl-silica column (Zorbax SB-C18) with binary eluents (methanol–water, acetonitrile–water or tetrahydrofuran–water) as the mobile phase, since this represents the most commonly used chromatographic system for the analysis of the examined UV filters [5,12,14,15]. Under these conditions, partial overlapping of some component peaks (BMDBM, OMC) and co-elution of ODAB and OS were observed. In the course of the study, it was found that the use of a quaternary solvent system including methanol, acetonitrile and tetrahydrofuran as the organic modifiers and acetic acid as additive in the aqueous portion of the mobile phase (60:10:10:20, v/v/v/v), produced a more efficient resolution of the foregoing compounds, although satisfactory separation of OS, BMDBM and OMC was not achieved. In addition, PBSA was weakly retained, eluting in the void volume region where reduced resolution and increased interference from unretained matrix constituents are drawbacks. A C_{18} packing from a different manufacturer (Hypersil ODS) and a phenyl-bonded Zorbax phase (Zorbax SB-Phenyl) were also tested in conjunction with the methanol–acetonitrile–tetrahydrofuran–aqueous acetic acid mobile phase. However, satisfactory baseline separation of all component peaks and in particular of BMDBM and OMC was not achieved. Interestingly, improved separation selectivity for the sunscreen agents was observed on a cyanopropyl packing (Zorbax SB-CN). Complete resolution of the seven UV filters was attained by this stationary phase (Fig. 2) with the solvent system optimized for the C_{18} column. However, for chromatography on the less hydrophobic cyano support, the concentration of the aqueous portion of the mobile phase had to be increased (from 20 to 40%, v/v) to obtain retention factors similar to those produced by the C_{18} sorbent. The use of a cyanopropyl column for the analysis of sunscreen compounds and the simultaneous baseline separation of the examined UV filters have not been reported before. Under the conditions outlined above, satisfactory retention was achieved for PBSA. This represents an advantage compared with chromatography on C_{18} packings producing elution of the UV filter close to the dead time [20] or requiring gradient analysis starting with high water content mobile phases [6]. Although the proposed chromatographic system was specifically optimized for the separation of the seven foregoing compounds, octocrylene, a frequently used UV filter [2,4], was also completely resolved from the other sunscreen agents (retention time, 14.2 min) on the cyano column. Because of the recent approval by the regulatory authorities of Europe [21] of Tinosorb M as UV absorber, simple analytical procedures for the determination of this compound in suncare products are desirable. This UV filter exhibits extremely hydrophobic characteristics [16]. Consequently, in the only paper found in the literature on the chromatographic determination of Tinosorb M [16], a totally organic eluent (non-aqueous reversed-phase HPLC) was required in order to achieve its elution on a C_{18} column. In this study, it was found that the less hydrophobic cyano phase in conjunction with an aqueous eluent (methanol–acetonitrile–tetrahydrofuran–aqueous acetic acid, 55:15:10:20, v/v/v/v) provided satisfactory chromatography of this sunscreen agent (see Fig. 3). Moreover, under the same conditions, the three major UV filters OB, OMC and BMDBM [2,4,12] can be determined along with Tinosorb M in a single chromatographic run (Fig. 3). Also MBC can be analysed with this system, although it is not completely resolved from OB.

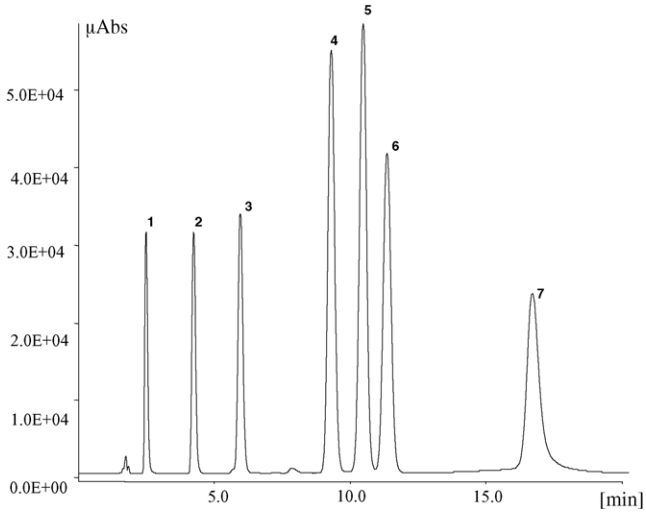

Fig. 2. Typical HPLC separation of a standard mixture of sunscreen agents. Column, Zorbax SB-CN; mobile phase, methanol–acetonitrile– tetrahydrofuran–water (40:10:10:40, v/v/v/v) containing 0.5% (v/v) acetic acid. Other operating conditions as described in Section 2. Peaks: 1, PBSA; 2, OB; 3, MBC; 4, ODAB; 5, OS; 6, OMC; 7, BMDBM.

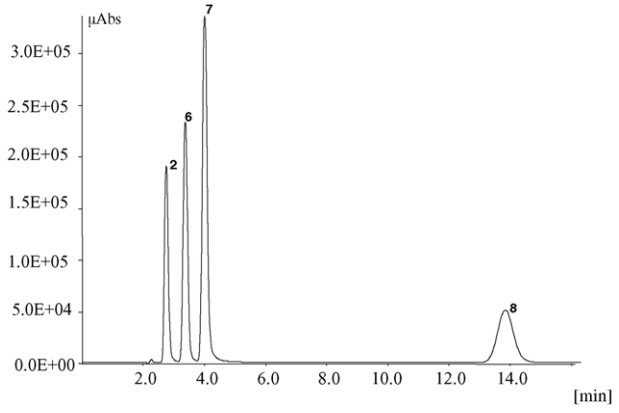

Fig. 3. HPLC trace of a cream product. Column, Zorbax SB-CN; mobile phase, methanol–acetonitrile–tetrahydrofuran–water (55:15:10:20, v/v/v/v) containing 0.5% (v/v) acetic acid. Other operating conditions are as described in Section 2. Peaks: 2, OB; 6, OMC; 7, BMDBM; 8, Tinosorb M.

Table 1
Recovery studies of the seven UV filters added to the test formulation

UV filter	Spiked concentration (%, w/w)	Recovery %[a]
PBSA	1.5	103.7 (2.7)
OB	1.0	96.2 (2.3)
MBC	1.0	96.4 (3.1)
ODAB	0.5	97.7 (4.4)
OS	2.5	102.4 (6.1)
OMC	1.0	98.6 (5.2)
BMDBM	2.5	95.7 (1.6)

[a] Each value is the mean (R.S.D.) of six determinations.

The accuracy of the developed method was examined by recovery experiments using a spiked cream (oil-in-water emulsion) as a model formulation since this vehicle represents the most common type of sunscreen preparation [22]. Average recoveries of more than 95.7% were obtained for each of the UV filters incorporated into the cream placebo (Table 1).

Applying the proposed HPLC method to the test cream formulation, as described in Section 2.4, the sunscreens were determined with relative standard deviation (R.S.D.) values ranging from 0.7 to 3.1% ($n = 6$) for the chromatographic precision and from 2.9 to 6.1% ($n = 6$) for the method precision. Calibration curves for each sunscreen agent were linear over the range 0.002–0.004 and 0.07–0.1 mg/ml, with correlation coefficients greater than 0.998. The intercepts with the *y*-axis were not significantly different from zero ($P > 0.05$).

3.2. Application

Four sunscreen products, all commercially available, and containing various combinations and concentrations of UV filters were assayed using the HPLC method developed in this study. The data obtained (Table 2) show compliance with the label claim and indicate that the sunscreen contents do not exceed the maximum authorized levels established by the European legislation [8].

Table 2
Levels of sunscreen agents in commercial suncare products determined by HPLC

Product	UV filter	Label claim (%, w/w)	% Found[a]
Cream 1[b]	OB	3.0	97.1 (0.6)
	OMC	6.0	103.5 (1.0)
	BMDBM	4.0	102.2 (4.5)
Lotion[b]	PBSA	1.0	92.3 (2.9)
	BMDBM	0.6	98.5 (5.2)
Cream 2[b]	MBC	2.0	103.2 (2.1)
	OMC	8.5	96.8 (1.7)
	BMDBM	3.5	98.8 (1.9)
Cream 3[c]	MBC	2.5	104.0 (2.3)
	OMC	5.0	95.6 (2.3)
	BMDBM	3.5	102.8 (1.4)
	Tinosorb M	2.0	99.4 (6.2)

[a] Each value is the mean (R.S.D.) of three determinations.
[b] Chromatographic conditions: Zorbax SB-CN column; mobile phase, methanol–acetonitrile–tetrahydrofuran–water (40:10:10:40, v/v/v/v) containing 0.5% (v/v) acetic acid.
[c] Chromatographic conditions: Zorbax SB-CN column; mobile phase, methanol–acetonitrile–tetrahydrofuran–water (55:15:10:20, v/v/v/v) containing 0.5% (v/v) acetic acid.

Table 3
Photodegradation data for UV filters in two sunscreen products after 1h irradiation with the solar simulator

Product	UV filter	% Sunscreen loss[a]
Cream 2[b]	MBC	6.1 ± 1.5
	OMC	8.7 ± 1.0
	BMDBM	6.7 ± 1.3
Cream 3[c]	MBC	3.6 ± 1.7
	OMC	3.9 ± 2.3
	BMDBM	1.2 ± 0.9
	Tinosorb M	0

[a] Each value is the mean ± S.D. of three determinations.
[b] Chromatographic conditions: Zorbax SB-CN column; mobile phase, methanol–acetonitrile–tetrahydrofuran–water (40:10:10:40, v/v/v/v) containing 0.5% (v/v) acetic acid.
[c] Chromatographic conditions: Zorbax SB-CN column; mobile phase, methanol–acetonitrile–tetrahydrofuran–water (55:15:10:20, v/v/v/v) containing 0.5% (v/v) acetic acid.

Photostability is a prime requirement for the effectiveness of sunscreen products, since the decomposition of the UV filters under sunlight exposure reduces their expected screening capacity. Therefore, in order to ensure adequate photoprotection during usage, the photochemical behaviour of sunscreen agents needs to be determined under conditions that parallel those encountered in the finished suncare preparation. Following irradiation of two sunscreen products with a solar simulator, the extent of UV filter degradation was measured by the newly developed HPLC method and the results are reported in Table 3. In the formulation containing MBC, OMC and BMDBM (Cream 2), the percentage loss of the sunscreen agents varied between 6.1 and 8.7%, the observed reduction in UV filter concentration being statistically significant ($P < 0.05$). On the other hand, the product (Cream 3) containing a combination of MBC, OMC and BMDBM with Tinosorb M was photochemically stable. In fact, the decrease in sunscreen levels measured upon illumination with simulated sunlight (Table 3) was not significantly different ($P > 0.1$) from the recovery data.

In conclusions, the proposed HPLC method allows fast and efficient separation of the examined UV filters suitable for quality control assays of sunscreen agents in cosmetics.

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