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Journal of Chromatography A, 828 (1998) 311–316

JOURNAL OF CHROMATOGRAPHY A

Identification of UV filters in sunscreen products by high-performance liquid chromatography–diode-array detection

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Abstract

A HPLC method for the identification of twenty UV filters has been developed in the present study. The method employs an analytical column with polymer packing, ion pairing and gradient elution followed by diode-array detection of UV filters. No problems were encountered for the analysis of UV filters in 24 sunscreen products by the present method.

Keywords: UV filters; Sunscreen products; Cosmetics; Pharmaceutical analysis

1. Introduction

Several high-performance liquid chromatography (HPLC) methods have been described for the analysis of UV filters in sunscreen products [1–6]. However, only some selected UV filters can be analysed by these methods. To check the compliance of sunscreen products with the European Union's Cosmetic Directive, a method for the analysis of all permitted UV filters ($n=20$) was required. In the present study, we have developed a HPLC method for the identification of 18 permitted UV filters and two non-permitted UV filters, which could be obtained as reference materials. The method has been applied for the analysis of UV filters in 24 sunscreen products. The target UV filters are described in Table 1.

2. Experimental

2.1. Chemicals

Standard UV filters EC 1.2, EC 1.4, EC 1.5, EC 1.6, EC 1.8, EC 1.9, EC 2.12, EC 2.13, EC 2.17, EC 2.2, EC 2.25, EC 2.26, EC 2.28, EC 2.29, EC 2.32 and EC 2.5 (Table 1) were obtained through The European Cosmetic Toiletry and Perfumery Association (COLIPA), Brussels, Belgium; UV filter EC 2.6 was from TCI, Japan; and the UV filters EC 1.10, EC 1.3, and EC 1.7 were kindly provided by Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin (BgVV), Berlin, Germany. All other chemicals were of analytical grade, and suitable for HPLC where appropriate.

2.2. Liquid chromatography

A HPLC system consisting of a solvent delivery pump (Waters 616), an autosampler (Waters 717), a photodiode array detector (Waters 996) and chromatography software Millennium version 2.10 were from Waters, Milford, MA, USA. The analytical

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0021-9673/98/$ – see front matter
PII: S0021-9673(98)00784-5

Table 1
Target UV filters in the present study

UV Filter	CAS Reg. No.	EC No.
Octocrylene	6197-30-4	1.10
Camphor benzalkonium methosulfate	52793-97-2	1.2
Homosalate	118-56-9	1.3
Benzophenone-3	131-57-7	1.4
Urocanic acid	104-98-3	1.5[a]
Phenylbenzimidazole sulfonic acid	27503-81-7	1.6
Terephthalydiene dicamphor sulfonic acid	90457-82-2	1.7
Butyl methoxydibenzoylmetane	70356-09-1	1.8
Benzylidene camphor sulfonic acid	56039-58-8	1.9
Isoamyl *p*-methoxycinnamate	71617-10-2	2.12
Octyl methoxycinnamate	5466-77-3	2.13
Benzophenone-4	4065-45-6	2.17
Benzophenone-5	6628-37-1	
PEG-25 *p*-aminobenzoic acid	113010-52-9	2.2
	116242-27-4	
3-(4′-Ethylbenzylidene)-*d*,*l*-camphor	38102-62-4	2.25
3-Benzylidene-*d*,*l*-camphor	15087-24-8	2.26
Isopropyl dibenzoylmethane	63250-25-9	2.28[a]
Megasol	94134-93-7	2.29
Octyl triazone	88122-99-0	2.32
Octyl dimethyl PABA	21245-02-3	2.5
Octyl salicylate	118-60-5	2.6

[a] Not permitted at the time of sample collection.

HPLC column PLRP-S, 100 Å, 5 μm, 150 mm×4.6 mm and guard cartridge PLRP-S were from Polymer Labs., Shropshire, UK. HPLC analysis was performed employing gradient elution as described in Table 2. Column temperature was set at 25°C, and the data collection across the 240–400 nm wavelength range was performed as follows: one spectrum per second, resolution 4.8 nm and no smoothing.

2.3. Analysis of standard UV filters

Stock solutions of UV filters: 40 mg of all UV filters, except EC 1.5, EC 1.6 and EC 2.32, were dissolved in 10 ml methanol (UV filter EC 1.8 was dissolved by ultrasonication). 40 mg of UV filters EC 1.5 and EC 1.6 were first dissolved in approximately 500 μl of 2 *M* aqueous sodium hydroxide and then made up to 10 ml with methanol. Forty mg of UV filter EC 2.32 was dissolved in 10 ml ethyl acetate.

Standard solutions of UV filters for HPLC: The stock solutions of UV filters were diluted in HPLC solvent (buffer–acetonitrile–tetrahydrofuran, 80:10:10, v/v/v) as follows: 0.1 ml to 100 ml for EC 1.5 and EC 2.12; 0.1 ml to 5 ml for EC 2.13, EC.17

Table 2
Gradient time table

Time (min)	Flow (ml/min)	Solvent A (%)	Solvent B (%)	Solvent C (%)
0	0.8	10	10	80
2.5	0.8	10	10	80
25	0.6	45	45	10
35	0.6	45	45	10
40	0.8	10	10	80
45	0.8	10	10	80

Solvent A: acetonitrile, Solvent B: tetrahydrofuran, solvent C: buffer (aqueous solution containing 1.4 g citric acid monohydrate and 6.8 g tetrabutylammonium hydroxide per litre, pH adjusted to 9.0 with conc. ammonia).

and EC 2.5; 0.2 ml to 5 ml for EC 1.6, EC 1.7, EC 1.9 and EC 2.26; 0.3 ml to 5 ml for EC 2.32; 0.5 ml to 5 ml for EC 1.2, EC 1.3, EC 1.4, EC 1.10, EC 1.8, EC 2.25, EC 2.28, EC 2.29 and EC 2.6; 1.0 ml to 5 ml for EC 2.2.

The solutions were stored in dark at 4°C. An appropriate volume of each standard UV filter solution (5–50 μl, so that the absorbance at peak maxima was 0.1–0.8 AU) was analysed by HPLC for 45 min. The data was processed to create a max-plot chromatogram and a spectrum index. A spectral library SOLFILTER consisting of HPLC t_R and spectrum of each of the standard UV filters (as well as of the significant impurities in these) was then built. The spectral library was used for the identification of UV filters in sunscreen products.

2.4. Analysis of UV filters in sunscreen products

Sample preparation: Approximately 2 g sample was accurately weighed in a 60-ml dark glass bottle with screw cap. Forty ml methanol and 0.25 ml 2 *M* sulphuric acid were transferred into the bottle. The bottle was capped and heated at 60°C for approximately 5 min, until a homogeneous suspension/solution was obtained. After cooling to room temperature, the solution/suspension was transferred into a 50-ml volumetric flask, the bottle was rinsed twice with approximately 4 ml methanol and the washings were mixed with the solution/suspension in the volumetric flask, which was then filled up to the mark with methanol (very inhomogeneous solutions were centrifuged at this stage). One ml of the clear sample solution was diluted to 5 ml with HPLC solvent. The diluted sample solution was stored in a closed glass vial and analysed within 24 h.

2.5. Identification

Depending upon the content of UV filters in a sample, 2–30 μl of the sample solution was analysed by HPLC. The data were processed to create a max-plot chromatogram and a spectrum index of the eluted peaks. The UV filters present in the sample were then identified by matching the t_R and spectrum of each peak in the max-plot chromatogram of the sample solution with the t_R and spectra of standard UV filters in the spectral library SOLFILTER. The following match parameters were used for automatic library search: retention time window 5%, spectrum window 10 nm.

3. Results and discussion

For routine analysis of UV filters in sunscreen products, solutions of individual reference UV filters were analysed every day. Relative standard deviation as well as day-to-day variation of HPLC t_R of the UV filters were ≤5%. The detection limits of the target UV filters present in sunscreen products were 50 ppm to 500 ppm, depending upon the HPLC response factors of the UV filters. The HPLC t_R and λ_{max} of the target UV filters by the optimized HPLC method are described in Table 3.

The identification of UV filters in a sample was performed by matching both their HPLC t_R and their 240–400 nm spectra with the t_R and spectra of the reference substances in the spectral library SOLFILTER. As an example, identification of UV filters in

Table 3
HPLC retention times (t_R) and λ_{max} of target UV filters

UV filter (EC No.)	t_R (min)	λ_{max} (nm)
1.5	2.468	278.4
1.2	6.178	287.9
1.6	8.898	302.1
2.2	10.788	306.8
2.17	12.468	240.7[c], 287.9[d], 321.1[e]
1.7	13.180	340.1
1.9	15.770	297.4
1.4	25.928	287.9
2.26	26.470	292.6
2.12	26.933	306.8
2.25	27.300	297.4
2.5	28.828	311.6
1.10	29.130	302.1
2.29[a]	29.172	240.7[c], 306.8[d]
2.29[b]	36.472	325.9
2.13	29.428	306.8
1.8	29.707	358.7
2.28	29.857	349.7
2.6	30.033	240.7[c], 306.8[d]
1.7	30.317	240.7, 306.8[d]
2.32	33.720	311.6

[a] Major component.
[b] Minor component.
[c] Primary λ_{max}; for the identification of the substance.
[d] Secondary λ_{max} (and [e], where applicable) must also be present.

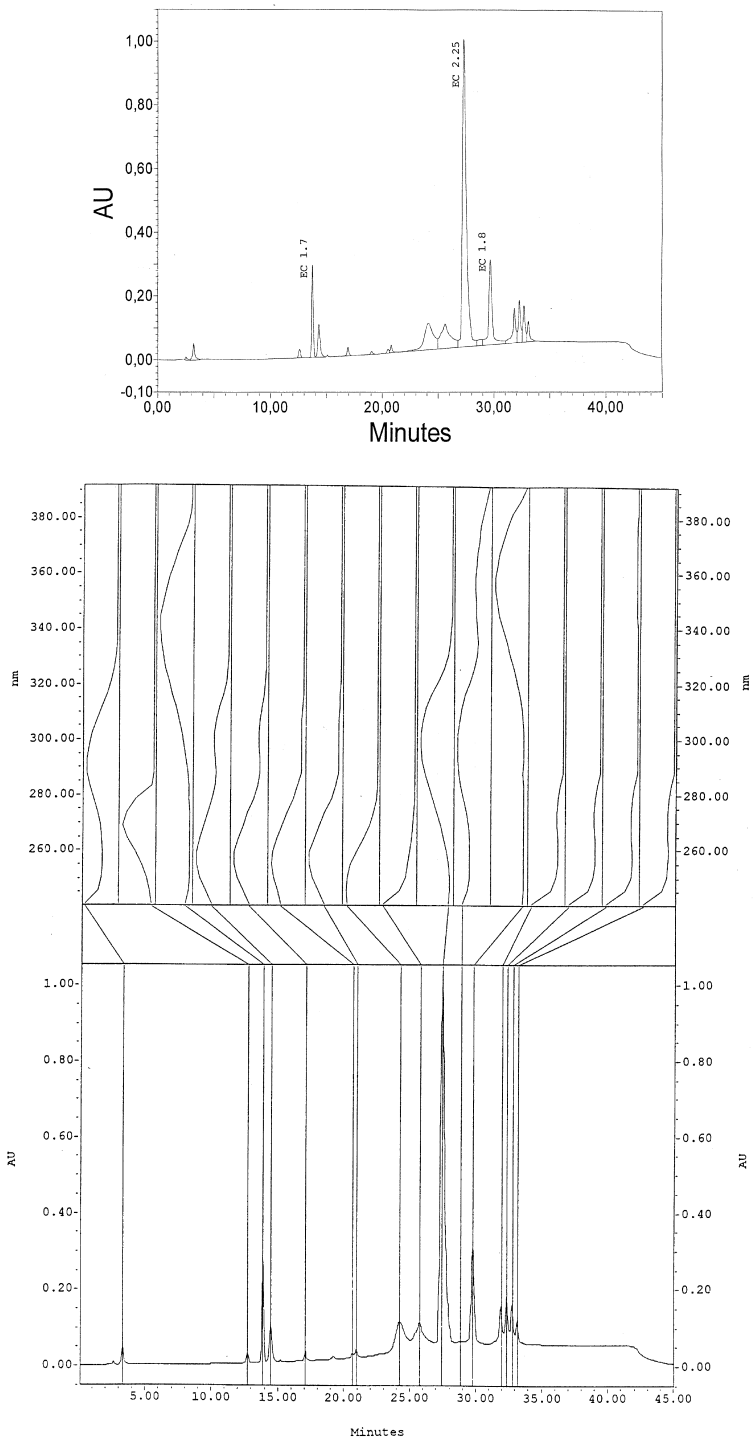

Fig. 1. (Top) Max-plot chromatogram of sample 712. Peaks marked with the UV filter (EC No.) were identified by automatic library search. (Bottom) Spectrum index of the max-plot chromatogram of sample 712.

Table 4
Identification of UV filters in sample 712 by automatic library search

Sample peak		Library SOLFILTER: Match 1				
Retention time (min)	λ_{max} (nm)	Retention time (min)	λ_{max} (nm)	Match threshold	Match angle	Spectrum name
3.337						
12.753						
13.887	340.1	13.180	340.1	1.01	0.27	EC 1.7
14.487						
17.087						
20.653						
20.937						
24.253						
24.753						
27.437	297.4	27.437	297.4	1.00	0.26	EC 2.25
28.870						
29.787	358.7	29.707	358.7	1.02	21.4	EC 1.8
31.953						
32.403						
32.803						
33.187						

sample 712 by an automatic library search revealed that the sample contained three UV filters EC 1.7, EC 1.8 and EC 2.25 (Fig. 1 (top) Table 4). A look at spectrum index of the max-plot chromatogram (Fig. 1 (bottom)) revealed that the sample also contained some other compounds which have spectra similar to some of the spectra in the library SOLFILTER. However, a manual review of t_R and spectrum match of all the peaks revealed that no other target UV filters, except the three mentioned before, were present in the sample.

The reason for choosing the UV spectra >240 nm, but not >220 nm, was the presence of two ghost peaks (source not yet identified) in the max-plot chromatogram of a blank (HPLC solvent) run: a large peak (absorbance >2 AU) with t_R 24.3 min and λ_{max} 229 nm; and a relatively small peak (absorbance ≥0.1 AU) with t_R 25.8 min and λ_{max} 247 nm. To minimize the influence of the large ghost peak on the normalized max-plot chromatograms, it was considered to acquire data at wavelengths >240 nm. The absorbance of both of the ghost peaks under these conditions were ≥0.1 AU. As shown in the max-plot chromatogram of sample 712 (Fig. 1 (top)), the two ghost peaks (t_R 24.3 min and 25.8 min) do not have any significant effect on the analysis of UV filters in a sunscreen product. The ghost peak with t_R 25.8 min may, however, be superimposed with the peak of UV filter EC 1.4 (t_R 25.9 min) when the latter is present in relatively large amounts in a sample, for example in sample 711 (Fig. 2). The ghost peak (t_R 25.8 min) is probably merged with the peak of UV filter EC 1.4, but it has no significant

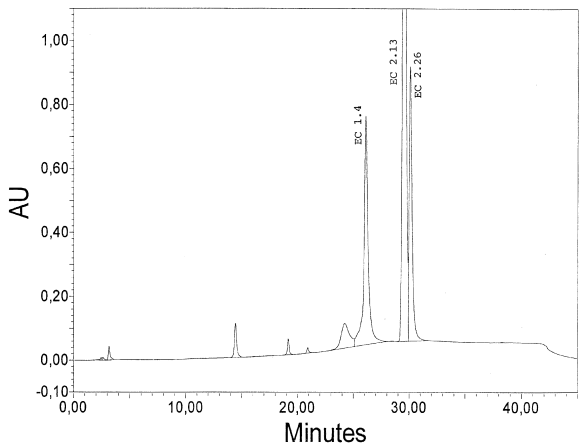

Fig. 2. Max-plot chromatogram of sample 711. Peaks marked with the UV filter (EC No.) were identified by automatic library search. The peak marked with an asterisk is one of the two ghost peaks. The second ghost peak with t_R 25.8 min appears to be merged with the peak of UV filter EC 1.4, t_R 26.0 min.

Table 5
Match indices for the identification of EC 1.4 in sample 711

Sample peak		Library SOLFILTER: Match 1				
Retention time (min)	λ_{max} (nm)	Retention time (min)	λ_{max} (nm)	Match threshold	Match angle	Spectrum name
26.087	287.9	25.928	287.9	1.07	1.78	EC 1.4

influence in the spectrum match of this compound (Table 5).

When two or more of the UV filters with t_R very close to each other are present in relatively large amounts in a sample, the peaks of UV filters may not resolve properly and an additive spectrum of the UV filters may be observed. In such cases, manual library search and subtraction (employing Millenium software) of a known spectrum (from the library) may be necessary for the identification. It is though recommended that appropriate volumes (μl) of the sample extract should be analysed in such cases.

Two samples (sample Nos. 714 and 718, Table 6) with known contents of UV filters were analysed to check the suitability of the present method for the identification of UV filters. All of the UV filters present in these products were identified by the HPLC method reported here. Thus, the HPLC method developed in the present study appeared to be suitable for the identification of UV filters in sunscreen products. The method was then applied for the identification of UV filters in a series of products (creams and lotions, Table 6). No problems were encountered for the identification of UV filters in these products. The investigated products were found to contain one to four of the permitted UV filters (Table 6). The HPLC method developed in the present study will be validated for quantification of UV filters in sunscreen products, in future studies.

Table 6
UV filters identified in the sunscreen products investigated

Sample No.	UV filters identified (EC No.)
700	2.25
701	2.25
703	1.4, 1.8, 2.13
704	1.4, 1.8, 2.13
705	1.8, 2.13
706	1.3, 2.25, 2.26
707	1.6, 1.8, 2.25
708	1.8, 2.13
709	1.4, 2.13
710	1.8, 2.13, 2.6
711	1.4, 2.13, 2.6
712	1.7, 1.8, 2.25
713	1.10, 1.7, 1.8
714	1.10, 1.7, 1.8, 2.25
715	1.10, 1.7, 1.8, 2.25
716	1.10, 1.7, 1.8
717	1.8, 2.13
718	1.4, 1.6, 2.13, 2.6
719	1.4, 1.8, 2.13
720	1.4, 1.8, 2.13, 2.6
721	1.4, 1.8, 2.13
722	1.4, 1.8, 2.13
723	1.8, 2.25
724	1.8, 2.25

Acknowledgements

We are grateful to Dr. Siegfried Dalchau, BgVV, Berlin for providing reference materials of three of the target UV filters. The sunscreen products investigated in the present study were provided by the Danish Environmental Protection Agency.

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