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Determination of the UV filters worldwide authorised in sunscreens by high-performance liquid chromatography Use of cyclodextrins as mobile phase modifier

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Abstract

Simultaneous determination of organic UV filters worldwide authorised in sunscreen formulations was performed by HPLC with UV spectrophotometric detection. The filters determined were: benzophenone-4, benzophenone-3, butyl methoxydibenzoylmethane, octyl dimethyl PABA, octyl methoxycinnamate, homosalate and octyl salicylate. A C_{18} stationary phase and an isocratic mobile phase of ethanol–water–acetic acid (70:29.5:0.5) containing 65.4 m*M* of hydroxypropyl-β-cyclodextrin, were used with a flow-rate of 0.6 ml/min. UV measurements were carried out at 313 nm. The time required for the analysis was 20 min and the limits of detection were between 1.5 and 2.3 mg/l. The procedure proposed provides a green analytical method with a basic instrumental configuration, it is fast and accurate and does not involve highly toxic organic solvents.

Keywords: Sunscreens; UV filters; Mobile phase composition; Cyclodextrins

1. Introduction

Progressive and continuous damage of the stratospheric ozone layer of the atmosphere has been the cause of an increase in skin cancer, cutaneous photoageing and damage to the skin's immunological system in recent years.

UV filters are chemical compounds that mitigate the deleterious effects of sunlight and they are used in variety of cosmetics. The specific cosmetics used for protection from the sun are sunscreen creams, lotions and sprays. The use of sunscreens products can help to prevent or minimise the harmful effects of solar radiation on the skin [1].

The efficacy of sunscreens can be estimated by the sun protection factor (SPF), which depends on the UVfilters contents of the formulation. High SPF and screening efficiency against both UV-A (320–400 nm) and UV-B (290–320 nm) wavelengths has led to the development of sunscreen preparations containing different UV filter combinations. However, in the case of organic filters, some dermatological reactions have been described [2]. Therefore, the maximum content of such filters in sunscreens has been legislated, and their composition must therefore be analysed.

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A list of approved UV filters and their maximum allowed concentrations in commercial products have been draw up by various regulatory authorities in Europe, the USA, or Japan. Of the nearly 40 substances permitted in some countries only 10 are accepted by Europe [3], the USA [4] and Japan [5]. Two of these 10 are inorganic filters (TiO_2 and ZnO), while *p*-aminobenzoic acid is not employed at present because it can cause dermatological problems [6]. The other seven are the subject of the present study: benzophenone-4, benzophenone-3, butyl methoxydibenzoylmethane, octyl dimethyl PABA, octyl methoxycinnamate, homosalate and octyl salicylate.

Table 1 gives the maximum concentration of the seven UV filters allowed by the EU, US and Japanese laws.

Different instrumental techniques have been used to determine UV filters in sunscreen products. Inorganic filters have been determined by FAAS or ICP-AES [7], while organic filters have been determined by NMR spectroscopy [8], Raman spectroscopy [9], UV–Vis absorption spectroscopy [10,11], gas chromatography (GC) [12,13], high-performance thin-layer chromatography (HPTLC) [14,15], and especially high-performance liquid chromatography (HPLC) [16–23].

Reversed-phase HPLC is the most common option, with C_{18} or C_8 as the stationary phase and acetonitrile [18], tetrahydrofuran [19] or methanol [17]–water as the binary mobile phase. A ternary mixture of acetonitrile–tetrahydrofuran–water [20] or methanol–tetrahydrofuran–water [23] has also been used [20], as have quaternary mixtures [22,23].

Due to the similar structure of some of the UV filters the complete resolution of the chromatographic peaks presents certain difficulties, even when gradient elution and a diode array detector are employed. Ones of the most difficult to resolve are butyl methoxydibenzoylmethane (BDM), octyl methoxycinnamate (OMC), octyl dimethyl PABA (ODP), octyl salicylate (OS) and homosalate (HS) which present similar retention times. HS is specially problematic because presents two peaks corresponding at two isomeric forms.

Table 1
Maximum contents of UV filters authorised

UV filters	Authorised concentration (g/100 g)		
	EU	USA	Japan
Benzophenone-4 (BZ4)	5	10	10
Benzophenone-3 (BZ3)	10	6	5
Butyl methoxydibenzoylmethane (BDM)	5	3	10
Octyl dimethyl PABA (ODP)	8	8	10
Octyl methoxycinnamate (OMC)	10	7.5	10
Homosalate (HS)	10	15	10
Octyl salicylate (OS)	5	5	10

DiNunzio et al. [19] studied the separation of BZ3, OMC and OS in different mobile phases (acetonitrile–0.2% acetic acid and THF–0.2% acetic acid, with different proportion) and separation of BZ3, ODP, OMC and OS was achieved in THF–acetic acid–water (55:0.09:44.91).

Fourteen UV filters were chromatographed in 50 min by Gagliardi et al. [18], including BZ3, BZ4, OMC, HS and ODP, using acetonitrile–water (with 0.001 *M* perchloric acid and 0.05 *M* sodium perchlorate) elution gradient; however, OMC and ODP peaks were overlapped in these conditions.

Ikeda et al. [20] separated six UV filters, including BZ3, OMC, BDM and ODP, using methanol–THF–water (4:6:6) but ODP was partially overlapped with isopropyl dibenzoylmethane and HS was not present.

Eleven UV filters were determined by Schneider et al. [23] using diode array detection and gradient elution. Elution gradient of 0.1% aq. trifluoracetic acid–methanol–acetonitrile (65:28:7) to (13:80:7) phase does not resolve the first peak of HS from BDM. The elution gradient of 0.1% aq. trifluoracetic acid–methanol–acetonitrile (61:33:5) to (14:71:15) phase definitely achieves separation of both peaks, but ODP, OMC and BDM are almost, but not completely, baseline separated.

Five sunscreen agents were isolated from cosmetics by supercritical fluid extraction and determined by HPLC under isocratic conditions with methanol–acetonitrile–THF–water (45:10:10:35), BZ3, ODP and BDM were well separated [22], but HS, OS and OMC were not present.

Rastogi and Jensen [17] developed a HPLC method for the identification of 20 UV filters using diode-array detection, with gradient elution of buffer (pH 9)–acetonitrile–THF. The obtained resolution of the peaks permitted identification but it was not suitable for determination.

Studies have been performed on the use of cyclo-

dextrins to encapsulate UV filters in order to increase their aqueous solubility and photostability. Cyclodextrins are cyclic oligosacharides which can interact with appropriately sized organic compounds by forming non-covalent inclusion complexes [24]. Cyclodextrins have a rigid structure with a relatively hydrophobic cavity and a hydrophilic exterior that is responsible for the increase in aqueous solubility. Photochemical behaviour under UVA radiation of butyl methoxydibenzoylmethane encapsulated in β-cyclodextrin was performed by Biloti et al. [25], while inclusion complexation with hydroxypropyl-β-cyclodextrin of octyl dimethyl PABA [26] and butyl methoxydibenzoylmethane [27] filters were done by Scalia and co-workers.

In the present study hydroxypropyl-β-cyclodextrin is used as a modifier of the mobile phase in order to perform the HPLC separation of the seven aforementioned UV filters, using a C_{18} stationary phase and ethanol–water mobile phase. Detection is carried out at 313 nm by a variable wavelength UV–Vis detector. The method is simple and fast, with a basic liquid chromatograph configuration and low toxicity solvents.

2. Experimental

2.1. Apparatus

A Hitachi liquid chromatograph equipped with a Hitachi L-7100 high pressure pump, a 20-μl loop injector and a Hitachi L-7420 UV–Vis detector were employed, using a LiChrospher® 100 RP-18 (12.5 cm length, 4 mm I.D., 5 μm particle size) (Merck) column.

2.2. Reagents

Benzophenone-4 (BZ4) (2-hydroxy-4-methoxybenzophenone-5-sulfonic acid), octyl dimethyl PABA (ODP) (2-ethylhexyl 4-(*N*,*N*-dimethylamino)-benzoate), butyl methoxydibenzoylmethane (BDM) (4-*tert*.-butyl-4′-methoxydibenzoylmethane) and octyl methoxycinnamate (OMC) (2-ethylhexyl-4-methoxycinnamate) were from Roig Farma, Tarrasa (Spain); benzophenone-3 (BZ3) (2-hydroxy-4-methoxybenzophenone) and octyl salicylate (OS) (2-ethylhexyl salicylate) from Aldrich (Barcelona, Spain); homosalate (HS) (3,3,5-trimethylcyclohexyl salicylate) from Chemir (Barcelona, Spain); ethanol (EtOH) HPLC grade from Scharlab (Barcelona, Spain); acetic acid (AcH) analytical grade from Panreac (Barcelona, Spain). β-Cyclodextrin (1135 molecular mass) (β-CD) and hydroxypropyl-β-cyclodextrin (1309 molecular mass) (HP-β-CD) from Acros Organics (Geel, Belgium).

Other reagents were of analytical grade except those used to prepare the home-made sunscreen.

2.3. Samples

Commercial samples were purchased in local shops: a sun milk from Laboratorios Vigmar (Valencia, Spain) (sample A); an oil-free sun block from Clinique Laboratories (London, UK) (sample B); a fresh suntan lotion from Laboratorios Vigmar (Valencia, Spain) (sample C); a sun cream from Parfums Christian Dior (Paris, France) (sample D); a sun milk from Laboratorios Berioska (Valencia, Spain) (sample E); and a sun milk from Laboratorios Isdin (Barcelona, Spain) (sample F).

A UV filters-free sunscreen cream was provided by Berioska.

A home-made sunscreen sample containing known concentrations of benzophenone-4, benzophenone-3, butyl methoxydibenzoylmethane, octyl dimethyl PABA, octyl methoxycinnamate, octyl salicylate and homosalate was prepared in the laboratory according to a common procedure followed in the cosmetic industry (provided by Guinama Laboratories (Valencia, Spain) and using Guinama reagents. This formulation also contained other ingredients such as: a Base PFC o/w (base cream with myristyl myristate, cetyl alcohol, glyceryl laurate, cetearyl octanoate, isopropyl myristate and other lipophilic components), avocado oil, dimethicone 350, propylene glycol, vitamin E, hydroviton, and phenonip.

2.4. Method

Samples of 0.2–1.0 g were dissolved with 25 ml ethanol, and 2 ml of this solution (previously filtered when the sample contained TiO_2 or other non-soluble components) were transferred to a 10-ml volumetric flask and diluted with ethanol.

Multicomponent solutions of the seven UV filters in ethanol were used as standards (25–150 mg/l).

Twenty μl of standard and sample solutions were injected into the liquid chromatograph and eluted, using H_2O–AcH–EtOH (29.5:0.5:70, v/v/v) containing 65.4 m*M* of hydroxypropyl-β-cyclodextrin as mobile phase, at a flow-rate of 0.6 ml/min. The UV–Vis detection was carried out at 313 nm.

3. Results and discussion

3.1. Study of the chromatographic variables

Ethanol–water–acetic acid was chosen as the mobile phase because of the good solubility of the samples in ethanol and the low toxicity and cost of this solvent. Acetic acid was used to decrease the peak tailing of benzophenone-3 [19]. Moreover, in preliminary work [28] the H_2O–HAc–EtOH mobile phase gave good results in separating some UV filters.

β-Cyclodextrin (β-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD) were tested to find the most suitable cyclodextrin. β-Cyclodextrin was rejected in the preliminary experiments because of its low solubility in hydroalcoholic solutions, and the study was performed with hydroxypropyl-β-cyclodextrin. It has been reported [29] that depending on the solvent BDM requires an equilibration time before injection to get reproducible areas, but in the present conditions this delay time is not necessary.

Fig. 1 gives the variations in the retention time of the analytes with the variation in HP-β-CD concentration. As can be seen when the HP-β-CD concentration increases the BDM retention time decreases sharply and even elutes before ODP. Homosalate presents two isomeric forms (HS-1 and HS-2), which have different retention times. HS-1 is not influenced by the HP-β-CD concentration and its retention time remains unaltered, but HS-2 is influenced by the HP-β-CD concentration and its retention time decreases when the HP-β-CD concentration increases, this effect, however, is not as strong as in the case of BDM.

The BZ4, BZ3, ODP, OMC and OS retention times are not influenced by the HP-β-CD concentration.

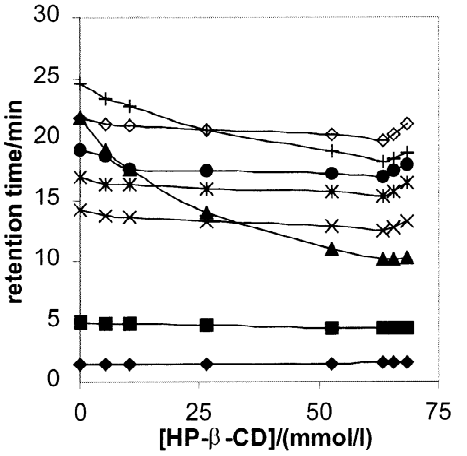

Fig. 1. Influence of hydroxypropyl β-cyclodextrin concentration on the elution time. Mobile phase, H_2O–HAc–EtOH (29.5:0.5:70, v/v/v). Flow rate, 0.5 ml/min. Injection volume, 20 μl. Detection at 313 nm. (♦) BZ4, (■) BZ3, (▲) BDM, (×) ODP, (*) OMC, (●) HS-1, (+) HS-2 and (◊) OS.

The most suitable HP-β-CD concentration was considered to be 65.4 m*M*. This concentration permits good resolution of the filters in less time than in absence of HP-β-CD. A higher concentration decreases OMC-HS-1 and HS-2 peaks resolution.

To select the most suitable mobile phase flow-rate two mobile phases were prepared containing different HP-β-CD concentrations (63.3 and 65.4 m*M*) (Fig. 2). The flow-rates assayed were: 0.4, 0.5, and 0.6 ml/min. As can be seen in the figure, an increase in the flow-rate does not significantly affect the resolution but causes an important decrease in the analysis time. The experiments confirmed the 65.4 m*M* as the most suitable concentration and a flow-rate of 0.6 ml/min as the most favourable because it permits good resolution with an analysis time of 20 min.

The composition of the mobile phase (ethanol–water ratio) was tested for the aforesaid optimised conditions (65.4 m*M* of HP-β-CD and 0.6 ml/min flow-rate). Fig. 3 shows the chromatograms obtained for three different ethanol–water ratios. As can be seen, a mobile phase with 65% of ethanol does not resolve OMC and HS peaks. Seventy-five percent of ethanol provides bad resolution of BDM, ODP, OMC HS and OS peaks, and 70% (v/v) of ethanol gives

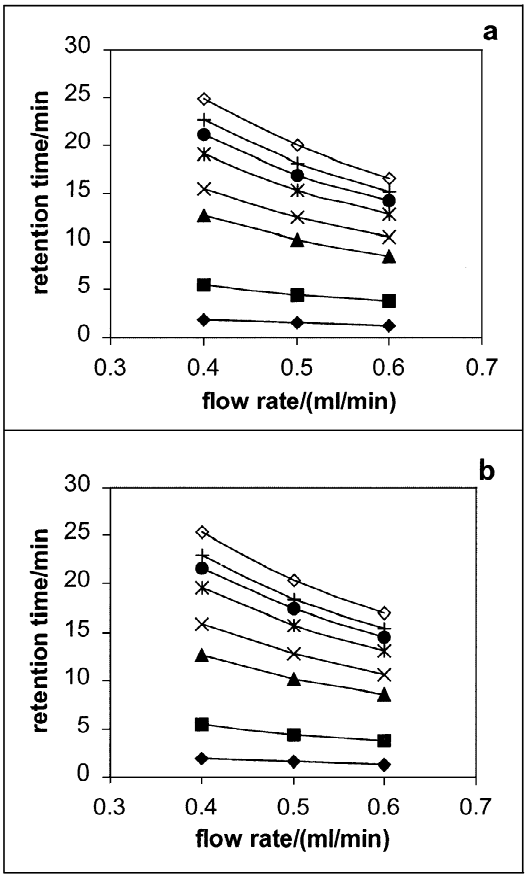

Fig. 2. Influence of flow-rate on the elution time. Injection volume, 20 μl. Detection at 313 nm. Mobile phase, H_2O–HAc–EtOH (29.5:0.5:70, v/v/v) containing different concentration of hydroxypropyl-β-cyclodextrin. (a) 63.3 m*M* (b) 65.4 m*M*. (♦) BZ4, (■) BZ3, (▲) BDM, (×) ODP, (*) OMC, (●) HS-1, (+) HS-2 and (◊) OS.

good resolution of all the peaks and it was therefore selected for the analysis of the UV filters.

3.2. Analytical figures of merit

The studies of linearity, reproducibility, sensitivity and detection limits were performed using multi-component standard solutions of the seven UV filters. Due to its higher sensitivity, quantification of homosalate (HS) in samples was performed using the HS-2 peak, and the studied parameters, therefore, correspond to this peak.

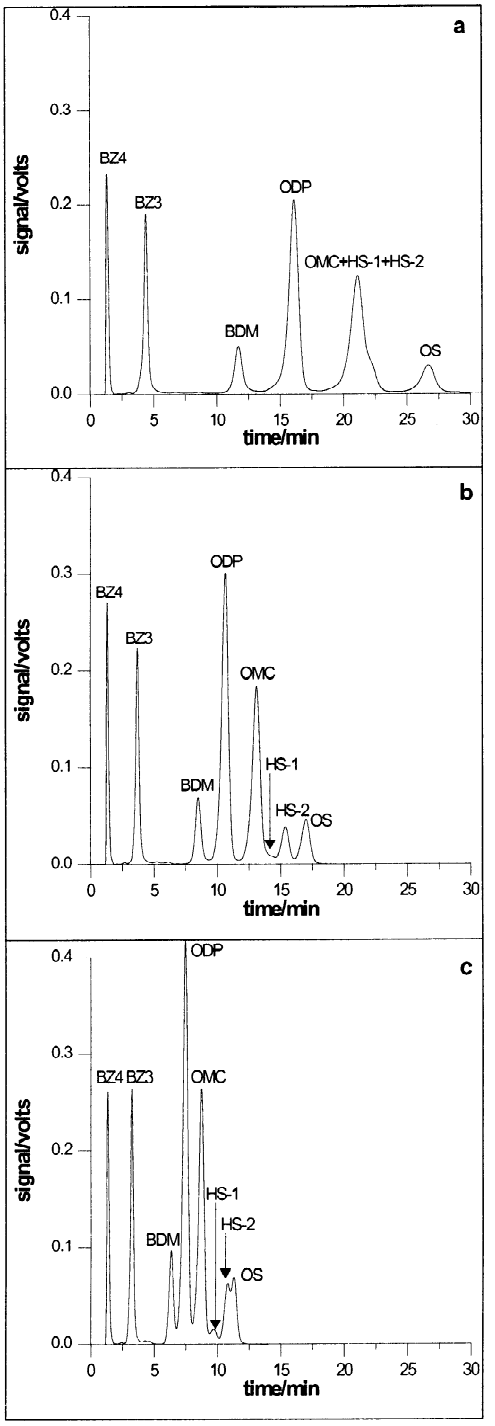

Fig. 3. Influence of ethanol–water ratio. Mobile phase containing 65.4 m*M* of hydroxypropyl β-cyclodextrin and different ethanol percentage. Flow rate, 0.6 ml/min. Injection volume, 20 μl. Detection at 313 nm. (a) 65%, (b) 70%, (c) 75%.

Table 2
Analytical figures of merit

UV filter	Retention time (min)	Working range (mg/l)	Variation coefficient in area (%)	Sensitivity[b] ($l/mg \times 10^2$)	Detection limit[c] (mg/l)
BZ4	1.31±0.01	25–250	1.5	608±4	2.1
BZ3	3.71±0.01	25–280[a]	0.3	923±5	1.7
BDM	8.50±0.03	25–300[a]	2.5	481±3	2.3
ODP	10.71±0.04	25–270	0.6	2080±10	1.6
OMC	13.23±0.05	25–225	0.6	1790±10	2.2
HS	15.35±0.03	25–330[a]	0.6	181±1	2.3
OS	17.09±0.05	25–330[a]	0.6	256±1	1.5

Mobile phase, H_2O–HAc–EtOH (29.5:0.5:70, v/v/v) containing 65.4 m*M* of hydroxypropyl-β-cyclodextrin. Flow-rate, 0.6 ml/min; injection volume, 20 μl; detection at 313 nm.

[a] Maximum concentration tested.

[b] Slope of the calibration curve.

[c] Calculated as $3S_{y/x}/b$ (where $S_{y/x}$ is standard deviation and b is slope of the straight line calibration).

Table 2 shows the analytical parameters calculated. As can be seen, all the analytical parameters give values adequate to the contents of the analytes in the sunscreen samples, with detection limits lower than 2.3 mg/l and RSD of areas lower than 2.5%.

3.3. Analysis of samples

The samples were analysed in two steps. In a first step the home-made and the sample free of UV filters (spiked with known amount of the seven filters) were analysed, in triplicate, following the proposed procedure specified in the experimental section, in order to test the accuracy of the method. Then the commercial samples were analysed, in triplicate, following the proposed procedure.

Table 3 shows the results obtained in the analysis of the spiked and home-made samples. As can be seen, the contents obtained were consistent with the real contents in all cases, which means that the accuracy of the proposed method is good. The reproducibility of the analysis was, in all cases, adequate to the objectives of the analysis.

The triplicate analysis of the commercial samples was done as follows: the sample was weighed and diluted as specified in the proposed procedure in Section 2 and was injected into the chromatograph. One other portion of the sample was spiked with a known amount of the UV filter present in the sample

Table 3
Analysis of home-made and spiked samples

Sample		UV filters content (g/100 g)						
		BZ4	BZ3	BDM	ODP	OMC	HS	OS
Spiked sample	Present content	2.81	2.97	2.69	3.50	3.28	3.19	3.34
	Found content	2.78±0.03	3.03±0.01	2.7±0.1	3.59±0.03	3.34±0.03	3.31±0.09	3.47±0.03
Home-made sample	Present content	2.49	4.98	2.51	4.17	4.97	5.13	2.52
	Found content	2.40±0.02	5.16±0.02	2.49±0.01	4.19±0.01	4.90±0.05	5.56±0.04	2.45±0.02

Mobile phase, H_2O–HAc–EtOH (29.5:0.5:70, v/v/v) containing 65.4 m*M* of hydroxypropyl-β-cyclodextrin. Flow-rate, 0.6 ml/min; injection volume, 20 μl; detection at 313 nm; number of replicates 3.

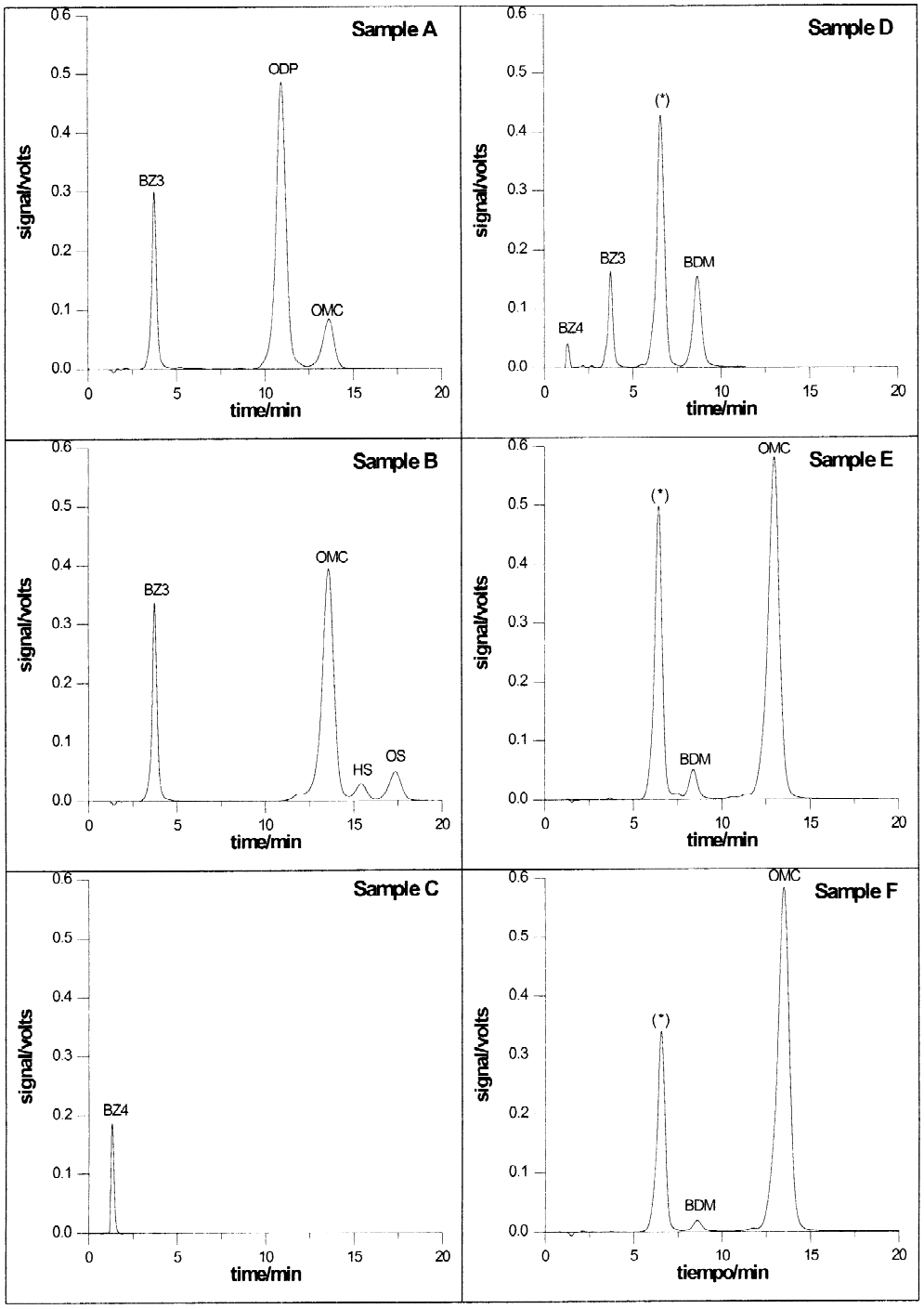

Fig. 4. Sample chromatograms. The peak marked with * can be attributed to 4-methylbenzylidene camphor present in some samples.

Table 4
Analysis of commercial samples

UV filter	Contents±*S* (g/100 g) % Recovery±*S*					
	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
BZ4	–	–	0.436±0.006	0.139±0.006	–	–
	–	–	97±2	100±2	–	–
BZ3	1.56±0.01	5.19±0.04	–	0.74±0.02	–	–
	103±1	98±4	–	101±1	–	–
BDM	–	–	–	2.21±0.02	1.597±0.008	0.76±0.02
	–	–	–	102±1	97±1	103±1
ODP	2.29±0.02	–	–	–	–	–
	101±2	–	–	–	–	–
OMC	0.50±0.02	7.3±0.3	–	–	7.62±0.07	8.0±0.1
	102±3	103±4	–	–	99±3	102±3
HS	–	3.58±0.05	–	–	–	–
	–	99±2	–	–	–	–
OS	–	5.13±0.07	–	–	–	–
	–	95±2	–	–	–	–

Mobile phase, H_2O–HAc–EtOH (29.5:0.5:70, v/v/v) containing 65.4 m*M* of hydroxypropyl-β-cyclodextrin. Flow-rate, 0.6 ml/min; injection volume, 20 μl; detection at 313 nm; number of replicates 3.

and injected into the chromatograph in order to calculated the recovery percentage.

Fig. 4 shows the chromatograms obtained from the commercial samples.

Table 4 shows the contents obtained and the percent recovery of the UV filters present in the samples. The percent recoveries are between 95 and 103%, in all cases, which means that there are no proportional errors.

4. Conclusions

As has been commented in Section 1 the most difficult to resolve are butyl methoxydibenzoylmethane (BDM), octyl methoxycinamate (OMC), octyl dimethyl PABA (ODP), octyl salicylate (OS) and homosalate (HS) peaks. These five UV filters together with BZ3 and BZ4 are worldwide authorised sunscreen agents in cosmetic products; however, at the present they have not been determined together.

The minor toxicity of ethanol instead methanol and its similar selectivity make this solvent preferable as mobile phase.

Separation of the BDM from OS in ethanol–water cannot be achieved, as can see in Fig. 1 where BDM and OS are completely overlapped. Suitable isolation of the seven UV filters were achieved by using hydroxypropyl-β-cyclodextrin as modifier of the mobile phase ethanol–water–acetic acid (70:29.5:0.5).

OMC and HS-1 are almost, but not completely, baseline separated; however, HS-1 has very low sensitivity and does not affect to OMC determination. HS-1 and HS-2 are isomeric forms of homosalate present in raw materials; in this study homosalate is determined only by the HS-2 peak with good results.

From the present study it can be concluded that the use of hydroxypropyl-β-cyclodextrin as mobile phase modifier makes possible the rapid and efficient isolation of the seven UV filters using a basic instrumental configuration.

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