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Journal of Chromatography A, 1049 (2004) 127–130
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Determination of sixteen UV filters in suncare formulations by high-performance liquid chromatography

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Received 22 June 2004; received in revised form 9 July 2004; accepted 28 July 2004

Abstract

Reversed-phase liquid chromatography (RP-LC) with UV detection was developed for the simultaneous determination of 16 organic UV filters worldwide authorised in suncare products. The filters determined were: 4-aminobenzoic acid, homosalate, benzophenone-3,2-phenylbenzimidazole-5-sulfonic acid, terephthalidene dicamphor sulfonic acid, 4-*tert*-butyl-4′-methoxy-dibenzoylmethane, octocrylene, 2-ethylhexyl-4-methoxycinnamate, isoamyl-*p*-methoxycinnamate, ethylhexyltriazone, drometrizole trisiloxane, diethylhexyl butamido triazone, 3-(4-methylbenzyliden) camphor, 2-ethylhexylsalicylate, 2-ethylhexyl-4-dimethylaminobenzoate and benzophenone-4. A C_{18} stationary phase and a gradient of ethanol-aqueous acetate buffer containing 0.2 mM of EDTA, was used with a flow-rate of 1.0 ml/min. UV detection was carried out at 313 and 360 nm. The analysis required 32 min and the limits of detection were between 30 and 4130 mg/kg in the original suncare product. Tween 80 was used to break down the different emulsions in order to procure a proper extraction of the UV filters. The method was validated for UV filters in three matrices, oil, water-in-oil emulsion and oil-in-water emulsion. Recoveries from spiked samples were 86–113% depending on the matrix used.

Keywords: Suncare products; UV filters; Tween 80; EDTA

1. Introduction

The growing publicity about the damaging effects of UV radiation, the hole in the ozone layer and protection against UV radiation receive great public interest. The protection consists predominantly of the ability of a sunscreen to filter out UVB rays (290–320 nm). UVB rays are responsible for sunburn. Suncare products do in some cases claim a protection against UVA (320–400 nm). Exposure to UVA causes skin ageing. In contrast to UVB, UVA does not cause sunburn.

A list of approved UV filters and their maximum allowed concentrations in commercial products have been drawn up by the regulatory authority in Europe in Annex VII of Directive 76/768/EEC (Table 1).

Many methods are reported to quantify UV filters in cosmetics. UV filters are easy to determine with liquid chromatography (LC) in combination on different types of stationary phase and with a great variety of mobile phases [1–4]. Isocratic as well as gradient elution has been used. Often the methods are appropriate to determine four to six UV filters. In some cases different mobile phase-column combination are used within a method. Due to the similar structure of some of the UV filters the baseline separation gave difficulties, even when gradient elution was used. The most difficult UV filters to separate are BMDBM, EMC, ED-PABA, ES and HMS. HMS especially is problematic because this UV filter presents two peaks corresponding two isomeric forms [2]. Together with BMDBM and ES migration takes place in the same time window and even the spectra are similar. In these cases the method is only for identification purposes [1].

The extraction consists of breaking the usually very complex emulsions. Besides water-in-oil (W/O) and oil-in-water

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doi:10.1016/j.chroma.2004.07.068

Table 1
Maximum allowed concentrations stated in Directive 76/768/EEC

UV filter	Concentration (%)
4-Aminobenzoic acid (PABA)	5
Homosalate (HMS)	10
Benzophenone-3 (BENZ-3)	10
2-Phenylbenzimidazole-5-sulfonic acid (PBSA)	8
Terephthalidene dicamphor sulfonic acid (TDSA)	10
4-*tert*-Butyl-4′-methoxy-dibenzoylmethane (BMDBM)	5
Octocrylene (OC)	10
2-Ethylhexyl-4-methoxycinnamate (EMC)	10
Isoamyl-*p*-methoxycinnamate (IMC)	10
Ethylhexyltriazone (ET)	5
Drometrizole trisiloxane (DTS)	15
Diethylhexyl butamido triazone (DBT)	10
3-(4-Methylbenzyliden) camphor (MBC)	4
2-Ethylhexylsalicylate (ES)	5
2-Ethylhexyl-4-dimethylaminobenzoate (ED-PABA)	8
Benzophenone-4 (BENZ-4)	5

(O/W) emulsions ternary systems are found often. In most cases extraction of cosmetics is performed with ethanol or methanol, if necessary in combination with low pH and/or high temperature (60 °C). It seems that vigorous shaking is necessary to break the emulsion. If not, the result will not be reproducible. Another way to break an emulsion system is to add a surfactant. In cosmetic microbiology the use of surfactants is well accepted in those cases the product is immiscible with water. Tween 80 is preferred most of the time.

In the present study extraction took place in a waterbath at 60 °C, followed by vigorous shaking and ultrasonication at ambient temperature with ethanol as extraction solvent and Tween 80 for breaking down the emulsion. Ethylene diaminotetraacetic acid (EDTA) was used as a modifier of the mobile phase to perform a HPLC separation of sixteen UV filters, using a C_{18} stationary phase and ethanol-aqueous acetate buffer mobile phase with gradient elution. UV detection was carried out at 313 and 360 nm. The method was tested for robustness and validated for 12 UV filters.

2. Experimental

2.1. Reagents

4-Aminobenzoic acid, benzophenone-3,2-phenylbenzimidazole-5-sulfonic acid and 2-ethylhexyl-4-methoxycinnamate were obtained from Across (Landsmeer, The Netherlands), homosalate, 4-*tert*-butyl-4′-methoxy-dibenzoylmethane, octocrylene, 3-(4-methylbenzyliden) camphor, 2-ethylhexylsalicylate and 2-ethylhexyl-4-dimethylaminobenzoate from Merck obtained supplied by Boom (Meppel, The Netherlands), terephthalidene dicamphor sulfonic acid and drometrizole trisiloxane from L' Oreal (Paris, France), isoamyl-*p*-methoxycinnamate from Geyer (Friederichsthal, Germany), ethylhexyltriazone from BASF (Ludwigshafen, Germany), diethylhexyl butamido triazone from Beiersdorf (Hamburg, Germany) and benzophenone-4 from ICN (Zoetermeer, The Netherlands), were used to prepare the standards. The solvents used, ethanol 96% and glacial acetic acid from Merck, were supplied by Boom. Polyoxyethylene sorbitan monooleate from Merck, used to disrupt the emulsion system, was supplied by Boom. Ethylenediaminetetraacetic acid dipotassium salt dihydrate from Merck, used as modifier for the mobile phase, was supplied by Boom.

Table 2
Gradient time table

Time (min)	Solvent A (%)	Solvent B (%)
0	60	40
4	60	40
5	75	25
18	75	25
19	100	0
27	100	0
28	60	40

Solvent A: ethanol (containing 80.0 mg EDTA dipotassium salt dihydrate dissolved in 5 ml water per litre), solvent B: buffer (aqueous solution containing 56.7 mg sodiumacetate and 80.0 mg EDTA dipotassium salt dihydrate per litre, pH adjusted to 2.5 with glacial acetic acid).

2.2. Chromatography

An Agilent liquid chromatographic system equipped with a binary pump, an injector with variable loop and a DAD was used. The above system was controlled using ChemStation software (Agilent Technologies). A 5-μm LiChrospher 100 RP-C_{18} column (125 mm × 4.6 mm) was used. The mobile phase was a gradient of ethanol-aqueous acetate buffer containing EDTA. LC was performed at 28 °C with gradient elution at 1.0 ml/min as described in Table 2. Injection volume was 20 μl. UV absorption was done at 313 and 360 nm. The run time was 32 min. Fig. 1 shows chromatograms obtained under these conditions.

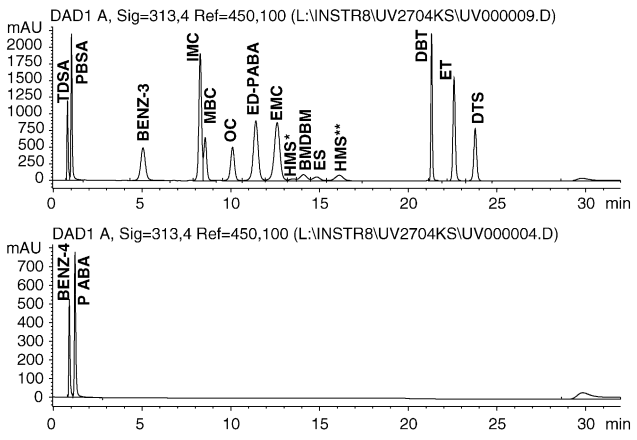

Fig. 1. Chromatograms of 16 UV filters. Conditions as mentioned in the text.

2.3. Standard solutions

Stock standard solutions of the UV filters were prepared daily. The individual solutions of all UV filters except for PBSA, TDSA and ET were prepared in ethanol and their concentration was about 4 mg/ml. Stock solution of PBSA with concentration of 4 mg/ml was prepared by dissolving 200 mg in 2 ml 2 M sodium hydroxide and then diluted to 50 ml with ethanol. Stock solution of TDSA with concentration of 4 mg/ml was prepared by dissolving 200 mg in 2 ml 25% acetic acid and then diluted to 50 ml with ethanol. Stock solution of ET with concentration of 4 mg/ml was prepared by dissolving 200 mg in 2 ml ethyl acetate and then diluted to 50 ml with ethanol.

Keeping the allowed concentrations of the UV filters in mind, different concentration ranges were prepared for the working standard solutions. If for any reason TDSA is not present in the working standard solutions check the pH being 7.0 due to the instability of HMS, OC and ES at high pH. Two working standard solutions were prepared because PABA, PBSA, TDSA and BENZ-4 migrated in the same time window. The concentration ranges for the separate UV filters are typically between 10 and 200 mg/l.

2.4. Sample preparation

Commercial samples were purchased in local shops. Samples of 0.5 g were dissolved in 25 ml ethanol in the presence of 0.5 ml Tween 80. Extraction was performed in a waterbath at 60 °C for 10 min followed by vigorous shaking during 30 s and ultrasonication at ambient temperature for 10 min. Each step homogenisation was performed. After cooling to ambient temperature the solution was transferred to a 50 ml volumetric flask and diluted with ethanol. This solution was diluted 10 times in ethanol by transferring 1 ml in a 10 ml volumetric flask. If PABA is present in the sample, another dilution was performed with ethanol–acetate buffer (60:40, v/v) parallel with the ethanol dilution. Prepared solutions were then injected into the HPLC system.

3. Results and discussion

3.1. Robustness

Robustness of the method was determined with an internal procedure [5]. With this procedure a number of potential critical parameters in the analytical method were varied in order to test whether the results remained constant. These experiments were performed for an O/W-emulsion and a W/O-emulsion.

Labels showed that BENZ-3, BMDBM, EMC and MBC were most used as UV filters in sun protection products. EMC alone or the combination of EMC with BENZ-3 and BMDBM was the most used filter in sun protection products. Therefore, the robustness test included only these filters, extended with PABA, PBSA and ET due to the presence of these UV filters in the investigated matrix or due to deviating molecule structure.

It seemed that the column temperature is a critical parameter. Experimental work showed that the temperature of the column should be 28 ± 1 °C in order to keep the resolution optimised.

3.2. Mobile phase

The method was developed using a gradient elution as described in Table 2 without using EDTA. During analysing commercial samples the performance of the column changed. The cosmetic matrix altered the properties of the stationary phase of the chromatographic column. The chromatographic behaviour of BMDBM changed. Due to the tailing peak of BMDBM quantitation of BMDBM and ES, which elutes in the same time window, became difficult. Sometimes compounds that are of no interest can interact with residual silanols. Retention times can shift and tailing can occur [6]. A chelating reagent as EDTA can be flushed through the column to overcome this problem. Inactivation of metaloproteins could be realised with 0.1–1 mM EDTA.

Addition of EDTA resulted in a good chromatographic peak shape. The higher the concentration of EDTA, the better the peak shape of BMDBM became; moreover, resolution of HMS, BMDBM and ES was influenced positively. Since a high concentration of EDTA will contaminate the pump, due to the insolubility of EDTA in ethanol, 0.2 mM EDTA was chosen.

3.3. Analytical performance

The LOD was defined as the analyte concentration that gives a signal equal to $3y_b$, where y_b is the baseline noise at the retention time the analyte was expected to migrate. Similarly, the LOQ was defined as $9y_b$. Based on the above-mentioned equations, the calculated LOQ values of the original sample are calculated (Table 3).

Based on structure and frequency of UV filters in 407 commercial samples, the accuracy of the method is tested for 12 UV filters. The recovery was studied by standard addition of the UV filters at three different levels. Addition was performed in duplicate at half the legal limit, the legal limit and one and a half the legal limit mentioned in Table 1. The study included a W/O-emulsion, an O/W-emulsion and an oil sample. The six recoveries for an UV filter were checked for outliers with the single and double Grubbs test [7]. From the remaining data, the mean recovery of each UV filter was calculated (Table 3).

The recoveries, which varied between 86 and 113%, do not comply with the limits according to the AOAC [8]. For the maximum allowed concentrations stated in Directive 76/768/EEC (Table 1) the recovery should be between 98 and 102%. Also the maximum allowed relative standard deviation (RSD) is in some cases too high. In accordance with Pocklington [9], the RSD should not exceed 2.5%. Due to the complexity of the determination of cosmetics, a moderately large imprecision of the results must be accepted.

Table 3
Recovery study for 12 UV filters

UV filter	LOQ (mg/kg)	W/O-emulsion		O/W-emulsion		Oil	
		Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)
PABA	2490	111.6	3.4	110.5	5.8	113.0	3.7
HMS	420	94.6	2.9	93.2	1.1	96.3	2.3
BENZ-3	110	96.6	1.0	97.7	3.0	96.4	2.1
PBSA	4130	103.3	1.1	103.3	2.6	102.5	2.4
BMDBM	70	105.4	7.1	85.9	12.4	106.8	4.2
OC	120	93.6	1.8	93.0	1.4	95.0	0.9
EMC	70	96.9	5.5	93.8	5.3	93.4	3.4
ET	1520	99.7	1.0	98.6	1.7	94.2	2.4
DTS	1290	95.7	1.0	91.9	2.8	88.8	3.1
DBT	1050	98.6	5.3	98.9	6.0	99.5	2.9
MBC	40	90.8	5.3	94.4	5.3	92.9	2.2
ES	420	97.3	1.7	96.2	1.5	97.8	1.6
TDSA	70						
IMC	30						
ED-PABA	50						
BENZ-4	70						

4. Conclusions

From the present study it can be concluded that with the proposed method 16 UV filters can be determined in all kind of suncare products. The use of Tween 80 as an afforcement to ethanol in combination with heat and ultrasonication is a good alternative to break the emulsions that are present in cosmetic products. EDTA as mobile phase modifier makes it possible to create a good chromatographic peak for BMDBM. As a consequence the resolution of HMS, BMDBM and ES improved. The migration of PABA, PBSA, TDSA and BENZ-4 in the same time window is no problem. From 407 different commercial samples can be concluded that combinations of these UV filters rarely occurred. In one sample PBSA and TDSA were both present.

At presence of PABA the sample should also be diluted with ethanol–acetate buffer to determine PABA. It seemed that peak splitting occurred for PABA when diluted with ethanol. Of the 407 commercial suncare products only one contained PABA.

Recoveries from spiked samples were 86–113% depending on the used matrix. Due to the complex matrix of cosmetics a moderate great deviation in the results must be accepted.

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