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Determination of Organic Sunscreen Filters in Cosmetics with HPLC/DAD

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Introduction

Sun protection cosmetics have been produced for over 50 years now. Compared with the former, current products show better sun protection factors, higher photo stability, better water resistance and a combined protection against UVA and UVB rays. To achieve these features, new organic sun screen filters were introduced in the last years and have been approved by the European authorities. Recently, concern was raised that several sunscreen filters could have an estrogenic potential (1). Published methods (2–4) only allow the determination of a few of these substances or have relatively long chromatographic run times and poor resolution (4). For the filters anisotriazine, methylene bis-benzotriazole tetramethylbutylphenol, diethyl hexyl butamido triazone and drometrizol trisiloxane no method has been described so far.

We report here a new HPLC method which permits to check if legal restrictions concerning sunscreen filters are met. It allows the simultaneous screening of 21 organic sunscreen filters of which two, menthyl anthranilate and benzophenone 2, are not approved as such in Switzerland. Quantitative determination needs different extraction methods for terephthalylidene dicamphor sulfonic acid, anisotriazine, methylene bis-benzotriazolyl tetramethylbutylphenol and diethylhexylbutamido-triazone.

Experimental

Materials and instruments

Analytical balance (AT 200, Mettler Toledo, Greifensee), ultrasonic bath (Branson 3510, Merck, Zürich), centrifuge (Heräus Biofuge Primo, BGB, Anwil), mixer (Polytron PT 3100), vortex (Genie, Bender & Hobein, Zürich), water bath (IKA RTM 5, Huber, Reinach), Quaternary gradient HPLC system consisting of a low pressure mixing quaternary gradient pump (P4000, narrow bore configuration), an

autosampler (AS 3000), a photo diode array detector (UV 6000LP fitted with 2 µl 10 mm flowcell) and a data station (ChromQuest), all from Thermo Finnigan, Allschwil.

Analytical column: Kromasil C18, 3.5 µm, 125×4 mm (Macherey-Nagel, Oensingen), precolumn: Kromasil C18, 3.5 µm, 8×4 mm (Macherey-Nagel, Oensingen), nylon syringe filters for HPLC, 13 mm diameter, 0.45 µm pore size (Titan Filtration Systems, Schmidlin, Neuheim).

Chemicals

Acetic acid p.a. (Merck), acetonitrile gradient grade for HPLC (Merck), methanol gradient grade for HPLC (SDS), tetrahydrofuran (THF) for HPLC (Merck), demineralised water for HPLC, methanol p.a. (Merck), acetone p.a. (Merck), sodium hydroxide p.a. (Merck), ammonium acetate p.a. (Merck).

Reagents

Methanolic sodium hydroxide solution (12.5 mM)

20 g of sodium hydroxide pellets are weighed into a 100 ml flask and dissolved in demineralised water. 2.5 ml of this solution are transferred to a 1000 ml flask and filled up to the mark with methanol.

Procedures

Calibration

Stock solutions are prepared by weighing 100 mg of UV filters into a 10 ml measuring flask, dissolving them with approximately 2 ml of methanol and filling up to the mark with methanol/acetone 1:1 (v/v). For terephthalylidene dicamphor sulfonic acid 1 ml of water and for phenylbenzimidazole sulfonic acid a few drops of ammonia (25 %) have to be added before filling to the mark. Anisotriazine has to be dissolved with 1 ml of toluene instead of methanol. Methylene bis-benzotriazolyl tetramethylbutylphenol has to be dissolved in 5 ml of toluene and filled up to the mark with acetone/THF 1:1 (v/v). It must not be mixed with the other stock solutions, because otherwise precipitation can occur. Stock solutions are stable for at least one month if stored in the dark at 4°C. Before use, they have to be brought to room temperature in order to redissolve precipitated substances.

Calibration solutions of 5 to 500 ng/µl are prepared by dilution with methanol/acetone 1:1 (v/v). For methylene bis-benzotriazolyl tetramethylbutylphenol diluted solutions have to be prepared with acetone/THF 1:1 (v/v).

Sample preparations

Screening (all substances) and quantitative determination (substances 1–5, 7–10, 14–17, 20–21).

Table 1

Reference substances

Nr.	INCI Name	EC Nr. ¹⁾	CAS Nr.	Producer
1	PABA (para-aminobenzoic acid)	1.1	150-13-0	Fluka
2	Camphor benzalkonium methosulfate	1.2	52793-97-2	Chimex
3	Homosalate	1.3	118-56-9	Aldrich
4	Oxybenzone	1.4	131-57-7	Fluka
5	Phenylbenzimidazole sulfonic acid	1.6	27503-81-7	Aldrich
6	Terephthalydene dicamphor sulfonic acid	1.7	90457-82-2	Chimex
7	Butyl methoxydibenzoylmethane	1.8	70356-09-1	Merck
8	Octocrylene	1.10	6197-30-4	Aldrich
9	Octyl methoxycinnamate	1.12	5466-77-3	Merck
10	Isoamyl methoxycinnamate	1.14	71617-10-2	Haarmann & Reimer
11	Octyl triazone	1.15	88122-99-0	BASF
12	Drometrizole trisiloxane	1.16	155633-54-8	Chimex
13	Diethylhexylbutamidotriazone	1.17	154702-15-5	Sigma 3V
14	Methyl benzylidene camphor	1.18	36861-47-9	Merck
15	Octyl salicylate	1.20	118-60-5	Aldrich
16	Octyl dimethyl PABA	1.21	21245-02-3	Aldrich
17	Sulisobenzone	1.22	4065-45-6	Fluka
18	Methylene bis-benzotriazolyl tetramethylbutylphenol	1.23	103597-45-1	CIBA
19	Anisotriazine	1.25	187393-00-6	CIBA
20	Menthyl anthranilate	²⁾	134-09-8	Haarmann & Reimer
21	Benzophenone-2	²⁾	131-55-5	BASF

¹ Classification according to the European Cosmetic Directive 76/768/EC Annex VII part 1 and 2

² not approved as sunscreen agent in Europe

Weigh 500 mg of the sample into a stoppered 50 ml Erlenmeyer flask and add 20 ml acetone/methanol 1:1 (v/v). Vortex sample solution for a minute. Creams and sticks that can not be homogenized by this procedure have to be homogenized with a rod mixer. Transfer flasks to an ultrasonic bath at 60°C for 15 minutes.

Centrifuge suspension at 4000 U/min for 5 minutes and filter the supernatant liquid through an HPLC micro filter (nylon, 0.45 µm).

Dilute the filtered solution by a factor of 1:5 (v/v) with methanol/acetone 1:1 (v/v).

Quantitative extraction of terephthalydene dicamphor sulfonic acid

12.5 mM methanolic sodium hydroxide solution is used instead of the acetone/methanol 1:1 (v/v) and the extraction is performed as above. Solutions usually don't have to be diluted, because terephthalydene dicamphor sulfonic acid is used in the range of only 0.5 to 2%.

Quantitative extraction of the non polar substances 11–13 and 18–19

Extract the mentioned substances with acetone/THF 1:1 (v/v) and dilute 1:5 before analysis with acetone/THF 1:1 (v/v). Depending on the matrix, other low

polarity substances as octyl methoxycinnamate, octocrylene or butyl methoxy-dibenzoyl methane, can also show better recoveries with this extractant.

HPLC parameters

Perform HPLC analysis with gradient elution as described in table 2. Run time is 30 minutes and column temperature 30°C. The usual injection volume is 1 µl.

Note: If only terephthalylidene dicamphor sulfonic acid or the non polar analytes 11–13, 18 and 19 have to be analysed, the gradient can be shortened to speed up the analysis.

Spectra are recorded between 220 and 400 nm with a resolution and a bandwidth of 1.2 nm and a sampling rate of 1 Hz. Discrete channels are recorded at 300 and 350 nm with a bandwidth of 5 nm and a sampling rate of 5 Hz.

Table 2
Gradient time table

Time (min)	Flow (ml/min)	Sodium Acetate	Methanol	Acetonitrile	THF
0.0	1.00	90 %	10 %	0 %	0 %
5.0	1.00	15 %	55 %	30 %	0 %
6.0	1.00	15 %	85 %	0 %	0 %
11.0	1.00	15 %	85 %	0 %	0 %
11.2	1.30	15 %	85 %	0 %	0 %
16.5	1.30	15 %	85 %	0 %	0 %
17.0	1.30	0 %	100 %	0 %	0 %
18.0	1.30	0 %	100 %	0 %	0 %
18.2	2.00	0 %	100 %	0 %	0 %
22.0	2.00	0 %	100 %	0 %	0 %
23.0	2.00	0 %	70 %	0 %	30 %
25.0	2.00	0 %	70 %	0 %	30 %
26.0	1.00	90 %	10 %	0 %	0 %
30.0	1.00	90 %	10 %	0 %	0 %

Ammonium acetate buffer (5 mM)

Place 380 mg sodium acetate in an Erlenmeyer flask and add 900 ml of water for HPLC. Add acetic acid until the pH of the solution is 3.85 (approximately 2 ml). Transfer this solution into a 1000 ml flask and fill up to the mark with HPLC grade water.

Results and discussion

Chromatography

The method allows the simultaneous analysis of all sunscreen filters that were used in suntan products on the Swiss market. Other filters that are approved in Switzerland like isopropylbenzyl salicylate, 3-benzylidenecamphor (EC VII-1.19) and benzylidene camphor sulfonic acid (EC VII-1.9) don't seem to be used anymore

and were not available to us. Because of their chemical structure, their determination with the present method should be possible, although interferences with other sunscreen filters are not known. In addition, the method allows the analysis of benzophenone-2 and menthyl anthranilate which are only allowed in some countries outside Europe. In Europe benzophenone-2 is only used as a UV stabiliser. The polymeric sunscreen filter N-((2 and 4)-[(2-oxoborn-3-yliden)-methyl]-benzyl)-acrylamid polymer (EC VII-1.11) was designed for hair application and was not available to us. Its usage is not to be expected in suncreams and we don't know if a determination would be possible with the present method. The other polymeric filter PEG25-PABA (e.g. Uvinul P25, EC VII-1.13) gives several closely eluting peaks which could interfere with the determination of benzophenone-3. For PEG25-PABA only a qualitative analysis is possible.

The present method is selective and relatively short regarding the fact that 21 analytes are well separated in one run. Due to the selective detection at the wavelengths of 300 and 350 nm and the high concentrations of sunscreen filters used in cosmetics, we observed virtually no interferences with coeluting cosmetic ingredients. Several preservatives for cosmetics which show absorption at 300 nm, like o-phenylphenol, salicylic acid, dehydro acetic acid and phenyl salicylate were tested but did not interfere. Chromatograms of a reference solution, detected at the two

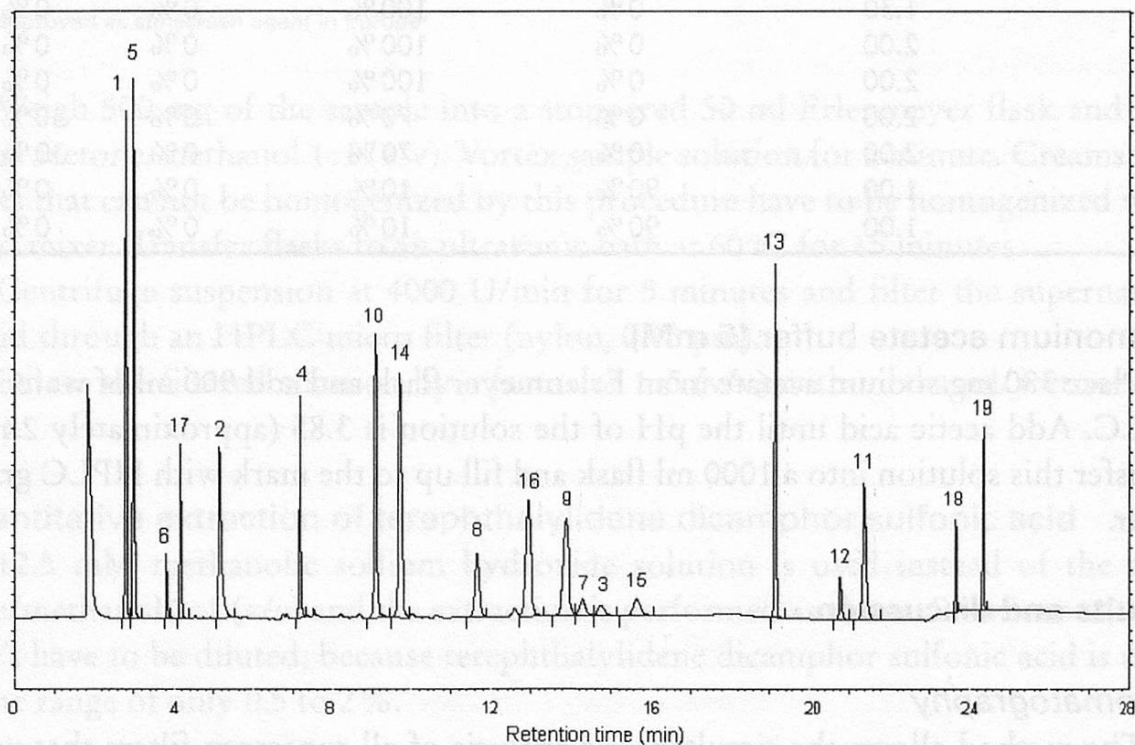


Figure 1 **Chromatogram of a reference solution with detection at 300 nm (for peak identification, see table 1)**

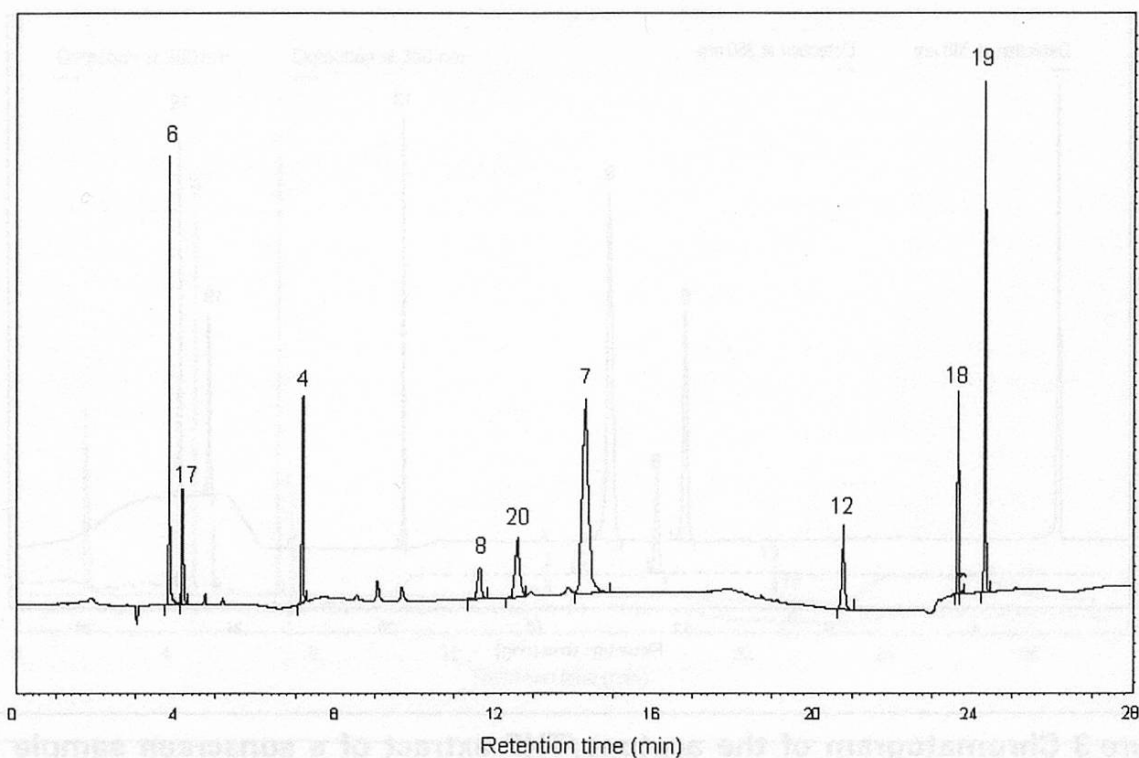


Figure 2 Chromatogram of a reference solution with detection at 350 nm (for peak identification, see table 1)

wavelengths, are shown in figure 1 and figure 2. Different extracts (THF/acetone, methanol/acetone and methanol/0.1 mol/l NaOH) of three samples are shown in figures 3 to 5. We believe that the method is also suitable for screening products that may contain other unknown unapproved sunscreen filters because the wavelengths for the detection of sunscreen filters are selective and the concentrations are usually in the g/100 g range. Unknown sunscreen filters are identified by comparing retention times and UV spectra with libraries.

Although the use of multilinear quaternary solvent gradients and changing flow rates is not common in HPLC and can cause problems with the transfer of the method to other laboratories, we decided to choose this approach to optimize resolution and run time. The method has been performed successfully on another HPLC equipment with much higher dwell volume. The method proved to be rugged, if differences in the design of the HPLC equipments are known and taken into account.

Separation is also possible without addition of tetrahydrofuran and without changing the flow rates, but the run time is higher, especially because of the late eluting anisotriazine and methylene bis-benzotriazole tetramethylbutylphenol. Nevertheless flow rates should not be changed near an eluting peak because shifting retention times would lead to varying dilution and hence to poor reproducibility of the peak areas.

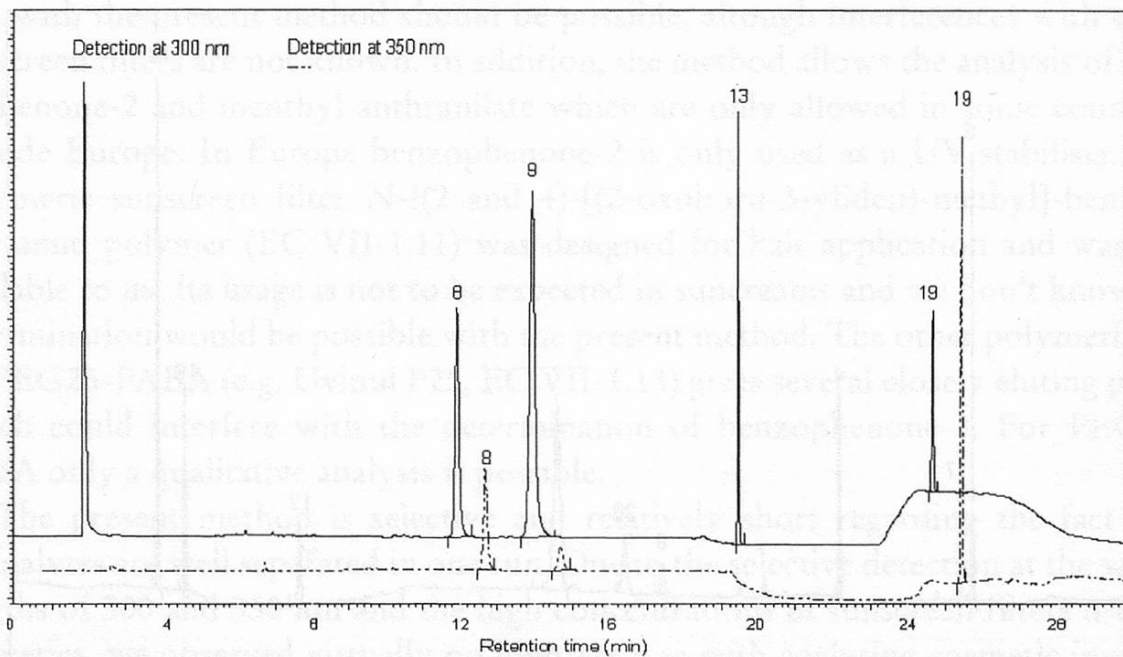


Figure 3 Chromatogram of the acetone/THF extract of a sunscreen sample (for peak identification, see table 1)

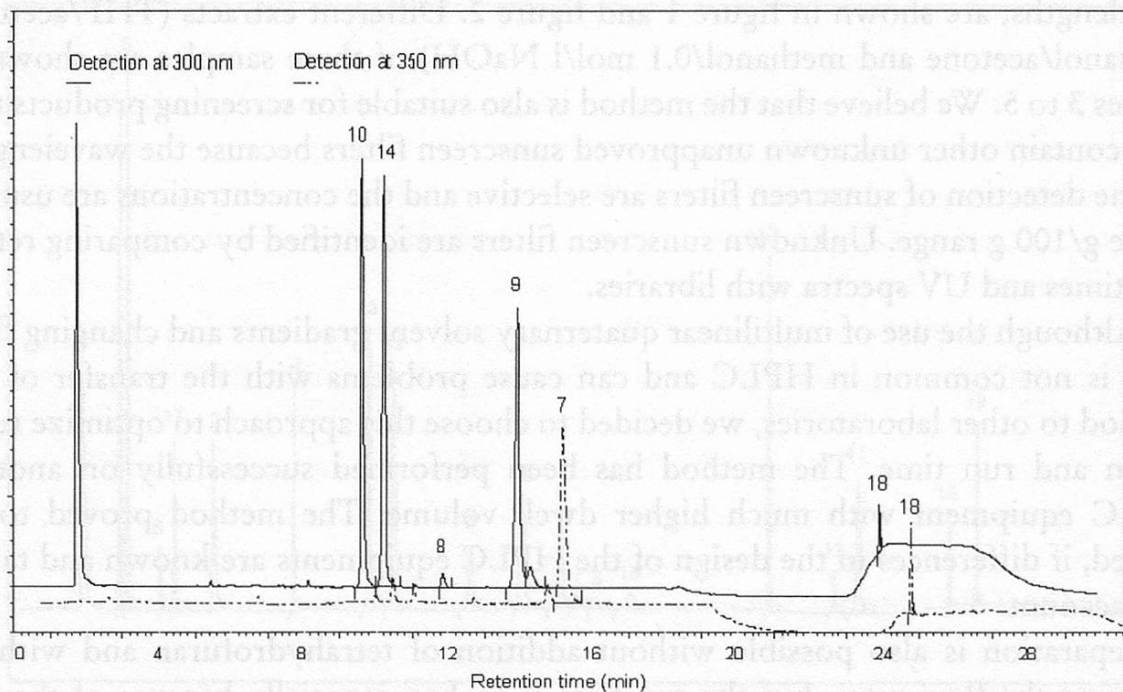


Figure 4 Chromatogram of the acetone/methanol extract of a sunscreen sample (for peak identification, see table 1)

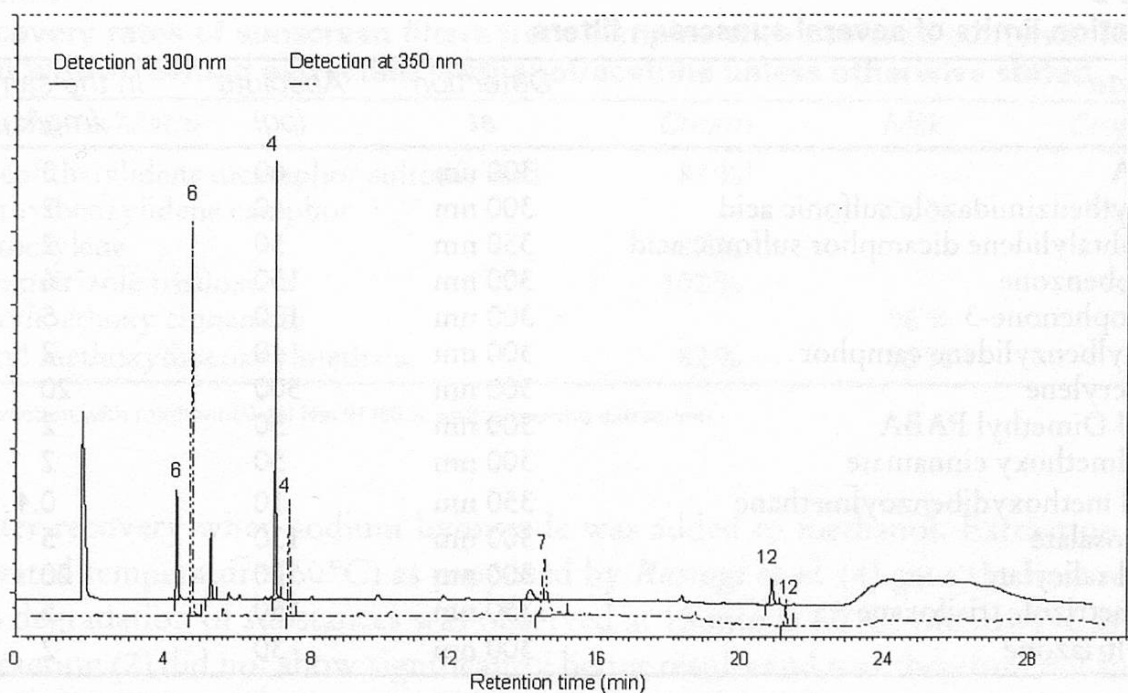


Figure 5 **Chromatogram of the methanol/NaOH extract of a sunscreen sample (for peak identification, see table 1)**

The chosen stationary phase (Kromasil C18) gave the best overall results regarding resolution and peak shape in comparison with other C18 or with C8 and phenyl-phases. It proved to be rugged under these conditions: far over 200 cosmetic sample injections did not alter the column performance significantly.

The addition of acetate buffer was necessary to improve the peak shape of terephthalylidene dicamphor sulfonic acid and proved to be better than the addition of acetic acid, phosphate buffer or methane sulfonic acid, which was chosen as a competing agent.

With this selective separation, comparison of the retention times in combination with uv-spectra allows a reliable interpretation of the chromatograms.

Because sunscreen filter concentrations are in the range of 0.1 to 10%, detection limits (LOD) are not a problem. The detection limits are shown in table 3 for several analytes. The detection limits in samples were calculated under the assumption that no interfering substances are present and the sample solution is not diluted prior to analysis. LOD's are far below the found concentrations and legal limits. Therefore it was not necessary to analyse the substances at the wavelengths of their UV-maximum which makes analysis much more convenient.

Most of the filters show linear correlations between signal and concentration in the range of 5 to 500 ng injected with the exception of butyl methoxydibenzoylmethane (50 to 500 ng) and octyl salicylate and homosalate (10 to 500 ng). Butyl methoxydibenzoylmethane response is non linear over the whole range and the

Table 3
Detection limits of several sunscreen filters

Analyte	Detection at	Absolute (pg)	In the sample (mg/kg)
PABA	300 nm	60	3
Phenylbenzimidazole sulfonic acid	300 nm	40	2
Terephthalylidene dicamphor sulfonic acid	350 nm	50	2
Sulisobenzone	300 nm	150	6
Benzophenone-3	300 nm	130	5
Methylbenzylidene camphor	300 nm	40	2
Octocrylene	300 nm	500	20
Octyl Dimethyl PABA	300 nm	50	2
Octylmethoxy cinnamate	300 nm	50	2
Butyl methoxydibenzoylmethane	350 nm	10	0.4
Homosalate	300 nm	130	5
Octyl salicylate	300 nm	500	20
Drometrizole trisiloxane	350 nm	150	5
Octyltriazone	300 nm	30	2

response of the salicylates is too weak for a reproducible integration at 5 ng. For most analytes this gives a determination range of 0.1 to 10% sunscreen filter in the sample, if sample solutions are diluted 1:5 before analysis. This range covered all analysed products.

Extraction

The extreme polarity differences of the sunscreen filters made it impossible to find one single procedure which allowed a quantitative extraction of all substances. On the other hand, the large difference between detection limit and concentration range of the samples allowed to develop a screening method suitable for all compounds of interest.

The analysis of spiked samples with different extractants often gave too optimistic recovery rates, whereas the same unspiked samples showed very different assays with different extractants. This was especially true for the most polar and non polar substances in combination with fatty matrices.

We therefore used several samples with various sunscreen filters and compared the results of different extraction methods. In addition we used a stick and a cream with known amounts of filters to establish the best overall extraction conditions. Buffers (basic and acid), methanol, n-propanol, acetone, ethyl acetate and mixtures of them were evaluated as extraction solvents. Methanol gave best results for the polar substances whereas n-propanol and acetone provided better recovery of the non polar substances, but none of the solvents gave satisfactory results for all of them. The influence of the addition of sulfuric acid (4), acetic acid or sodium hydroxide to methanol was also investigated but results were not better with the exception of terephthalylidene dicamphor sulfonic acid. This compound showed

Table 4

Recovery rates of sunscreen filters from samples with declared contents. Extraction with screening extractant methanol/acetone unless otherwise stated

<i>Substance/Matrix</i>	<i>Cream</i>	<i>Milk</i>	<i>Cream</i>
Terephthalylidene dicamphor sulfonic acid	87% ¹		
Methylbenzylidene camphor		100%	98%
Octocrylene	97%		
Drometrizole trisiloxane	102%		
Octylmethoxy cinnamate		96%	
Butyl methoxydibenzoylmethane	82%	95%	

¹ extraction with methanol/0.1N NaOH (69% with screening extractant)

better recovery when sodium hydroxide was added to methanol. Extraction at an elevated temperature (60°C) as proposed by *Rastogi et al.* (4) gave the best results. No degradation of substances was observed at 15 min at 60°C. Microwave assisted extraction (2) did not show significantly better results and was therefore not further pursued in order to keep analysis as simple as possible.

The analysis of samples with known content of sunscreen filters (table 4) showed that for most of the analytes, methanol/acetone is a good extractant, although in some cases, e.g. very fatty creams, the extraction rate may be below 100%. For terephthalylidene dicamphor sulfonic acid and the non polar analytes diethylhexylbutamidotriazone, octyltriazone, drometrizole trisiloxane and especially anisotriazine and methylene bis-benzotriazolyl tetramethylbutylphenol, methanol/acetone is usually not suitable for a quantitative determination. Methanol/acetone can nevertheless also be used for these analytes as a screening extractant because the recovery rates are seldom below 70% even in these cases. The exception is methylene bis-benzotriazolyl tetramethylbutylphenol where only 30% have been found compared to tetrahydrofuran/acetone extraction. In spite of this low recovery, undeclared methylene bis-benzotriazolyl tetramethylbutylphenol could still be found because the detection limit is far below the used concentrations. Specific extraction as described above must therefore only be done if the mentioned substances are found in the screening process. For compounds in the polarity range of octyl methoxycinnamate or octocrylene, the extraction with tetrahydrofuran/acetone can also give better recoveries (10 to 20% improvement). Usually this is only observed for thick creams. It is advisable to use both extraction solvents if an accurate determination is needed.

The precision of the method, expressed as relative standard deviation, is usually below five per cent but depends on the concentration as well as on the substance.

Market survey

In order to get an overview of organic sunscreen filters currently used, we analysed 47 sunscreen products sold on the Swiss market. Forty-two of these

Table 5

Market overview of the concentration of sunscreen filters*Number of samples with sunscreen filter concentrations (in %):*

Substance	9-10	8-9	7-8	6-7	5-6	4-5	3-4	2-3	1-2	0,5-1
Octyl methoxycinnamate	1		17	2	2	2	5	3	6	
Octocrylene	5	2			1			1	1	2
Methylbenzylidene camphor						2	12	5	4	1
Phenylbenzimidazole sulfonic acid							5		3	
Butyl methoxydibenzoyl-methane							1	10	15	6
Octyl salicylate						2	2	2		
Benzophenone-3					1	1		2		
Octyl triazone								1	8	
Terephthalylidene dicamphor sulfonic acid								2	3	1
Drometrizole trisiloxane								4	1	1
Isoamyl methoxycinnamate							1	1		1
Diethylhexylbutamidotriazone									1	
Anisotriazine									1	

products claimed to have sun protection factors of 5 and higher. All declared sunscreen filters were available as references and could be determined with the present method. Table 5 gives an overview of the concentration ranges and frequencies of the used organic filters.

The results show that for several filters, e.g. octocrylene and methylbenzylidene camphor, concentrations are near the legal limit and therefore need an accurate determination whereas the concentrations for the other filters are usually far below the limits. One product contained too much methyl benzylidene camphor (4.4%, limit 4%). The producer admitted using 4.5% which was allowed until 2001. One product contained 0.1% of undeclared isoamyl methoxycinnamate and methylbenzylidene camphor. This was explained by the producer as a cross-contamination with another sunscreen during the production. A third product claimed to contain isoamyl methoxycinnamate but did not, which was explained by a change of formulation without adapting the declaration on the package.

We were interested in the correlation between total content of organic sunscreen filters and claimed sun protection factor (SPF). Unfortunately we were not able to determine the inorganic sunscreen filters that were present in 32 of the 47 products. Therefore only 15 products which contained no inorganic filters could be evaluated. As suspected, large differences between claimed sun protection factors and measured total concentration of organic sunscreen filters were found (fig. 6). It is well known that the sun protection factor is not only determined by the extinction factor and concentration of the filters but also by the product formulation and the combination of the used filters (5). The total concentration of organic sun screen filters

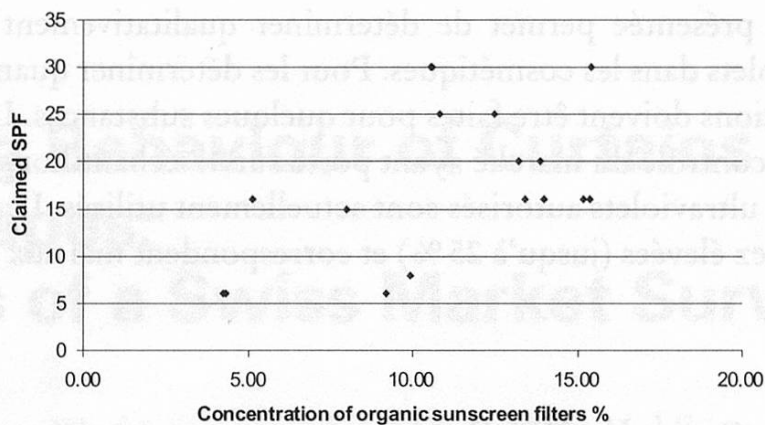


Figure 6 Comparison of measured contents of organic sunscreen filters vs. claimed sun protection factor (SPF)

ranged between 5 and 25 %. Given the amount of sunscreen products used, several grams of sunscreen filters may be applied onto the skin during one day. The development of creams which need less sunscreen filters with equal sun protection as well as photo stable products should therefore be sought after.

Acknowledgements

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Summary

An HPLC method for the screening of 21 sunscreen filters in cosmetics is presented. For quantitation small adaptations of the extraction must be made for some of these substances. A market survey on 47 products revealed that only 14 of over 25 approved organic sunscreen filters are currently used. Total organic sunscreen concentrations are very high (until 25 %) in some products and do not correlate well with claimed sun protection factors.

Zusammenfassung

Es wurde eine Analysenmethode entwickelt, welche das Screening auf 21 UV-Filter ermöglicht. Einzelne Substanzen benötigen kleine Anpassungen bezüglich der Extraktion für eine quantitative Bestimmung. Eine Marktuntersuchung an 47 Produkten ergab, dass im Moment nur 14 von über 25 erlaubten organischen UV-Filtern eingesetzt wurden. Die Einsatzkonzentration sind zum Teil sehr hoch (bis 25 %) und korrelieren nur bedingt mit dem angegebenen Sonnenschutzfaktor.

Résumé

La méthode présentée permet de déterminer qualitativement la présence de 21 filtres ultraviolets dans les cosmétiques. Pour les déterminer quantitativement, de petites modifications doivent être faites pour quelques substances. La méthode a été utilisée pour un contrôle du marché ayant porté sur 47 échantillons. Seuls 14 parmi plus de 25 filtres ultraviolets autorisés sont actuellement utilisés. Les concentrations utilisées sont assez élevées (jusqu'à 25 %) et correspondent mal aux facteurs de protection indiqués.

Key words

Sunscreen filters, Cosmetics, HPLC

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