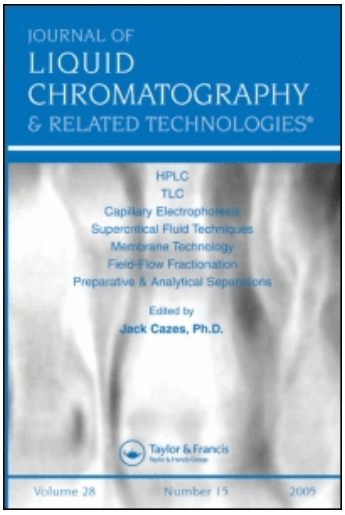

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STABILITY ANALYSIS OF THREE UV-FILTERS USING HPLC

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ABSTRACT

UV-absorbing substances are widely used in sunscreens to filter out ultraviolet radiation in sunlight. These substances need to be stable in sunscreens in order to obtain the expected protection during the shelf-life of the products.

A conveniant HPLC method for the separation and quantification of three common UV-filters in a sunscreen emulsion is presented. Using this method the stability of benzophenone-3, butylmethoxy dibenzoylmethane and octyl methoxycinnamate was determined. Calibration curves for the three UV-filters showed linear response in the interval 0.03 to 30 μg. The absolute recovery of the spiked sample was close to 100 %. The shelf life data indicate good stability of the filters.

INTRODUCTION

Sunlight have both beneficial and harmful effects on human skin. While being necessary for the initial steps in the formation of vitamin D [1], it may also cause various damages to the skin, such as skin cancer and photoaging [2,3]. The increase in the incidence of skin cancer in western countries appears to be related to excessive exposure to sunlight [2,3]. These harmful effects are especially due to the light of low wavelengths in the UV-portion of the solar radiation spectra.

Skin has several barriers of protection against the adverse effects of solar radiation [4]. This defense system may however not be adequate for all individuals and situations. In order to prevent photodamage, caucasians are recommended to use sunscreens for

outdoor activities in sunny climates if clothing or other sun protection cannot be used [5].

Sunscreens contain UV-filters which prevent the transmission of deleterious UV-radiation into the skin, either by absorbing the energy or reflecting it [4]. There are a number of formulations on the market. These contain various combinations and concentrations of UV-filters, emollients, emulsifiers, preservatives etc in order to provide different sun protection factors and cosmetic properties [4]. Some products adhere more efficiently to skin and hence give protection also after swimming or sweating.

In Europe sunscreens are classified as cosmetics, whereas in the USA they are OTC drugs. Cosmetic having a durability of less than 30 months should be labelled with a date stamp according to directives in the EC. In order to ensure adequate protection after storage the stability of the UV-filters in the product need to be determined. The aim of this study was to investigate experimental conditions on HPLC for the analysis of three common UV-filters in a sunscreen and to determine the stability of the filters in an ordinary sun lotion.

MATERIALS AND METHODS

Materials

Benzophenone-3 (Merck AG, Germany) and butylmethoxy dibenzoylmethane and octyl methoxycinnamate (Givaudan, Switzerland) was used without any further purification (see Table I for their full names). The compounds were weighed (accuracy of 0.01 mg) and dissolved in ethanol (99.5 % from Kemetyl AB, Sweden). A Shimadzu UV-260 double beam spectrophotometer was used to record the absorbance spectra of the test compounds. The main ingredients in the lotion were silicones, acrylic acid copolymer, PVP/triacontene copolymer, cetostearyl alcohol, sorbitan oleate, water and preservatives (imidazolinid urea) with a final pH of 5.

Equipment

An Hitachi L-6200 ternary solvent pump was used for the generation of gradients while samples were injected with a Hitachi AS-4000 auto sampler. The separation was achieved on a C8 column (4.6 x 200 mm, 5 µm, Beckman Ultrasphere). The column was termostated to 25 ^{o}C in a Hitachi T-6300 column oven. The samples were detected using a Hewlett Packard 1040A diode-array detector coupled to a Hewlett Packard 9000 series 300 computer with a Hewlett Packard 9144 tape unit for back-up. The detector performance is checked with a reference filter containing holmium oxide. Primary

TABLE I.
UV-filters Investigated.

Trade name	Chemical name	CTFA name	Stated purity (min %)
Eusolex 4360	2-hydroxy-4-methoxybenzophenone	Benzophenone-3	99.5
Parsol 1789	4-tertbutyl-4´-methoxy-dibenzoylmethane	Butylmethoxy dibenzoylmethane	95
Parsol MCX	2-ethylhexyl-p-methoxycinnamate	Octyl methoxycinnamate	98

analysis of the data was achieved on a Hewlett Packard 9000 computer using the Hewlett Packard software HP 79997 A Rev 3.21. Water equivalent to HPLC quality was obtained from a Millipore water purification unit. Methanol of HPLC-quality ("Lichrosolv") and acetic acid (p.a.) were from Merck AG, Germany.

Extraction of UV-filters and stability test

The above mentioned lotion without UV-filters (vehicle) was used to test the extractability of the UV-filters. Since all UV-filters are freely soluble in alcohols we used ethanol as a solvent for their extraction. The vehicle was weighed into measuring flasks and UV-filters (dissolved in ethanol) and 20 ml of ethanol (heated to 60 ^{o}C) added. The contents were mixed on a magnetic stirrer for 30 min at room temperature. The solution was made up to 25 ml and stirred for a further five minutes. An aliquot was centrifuged at 14,500 g for five minutes and the supernatant used for HPLC analysis.

The stability of the UV-filters was tested both in ethanol and in the sun lotion. UV-filters were dissolved in ethanol and kept in capped test tubes under air and was stored in the dark at room temperature, cold (approx. 4 ^{o}C) and in a freezer (approx - 20 ^{o}C). One additional sample was stored at room temperature under normal light conditions on a lab bench (i.e. dark at night). Aliquots were analyzed on HPLC after one, two, seven and twenty eight days. Two batches of the sun lotion were stored at 40^{o}C. After 30 and 90 days the concentration of the UV-filters was determined.

RESULTS AND DISCUSSION

Absorbance spectra and method of analyses of the UV-filters

The absorbance spectra of the three UV-filters were analyzed between 200 and 420 nm (Figure 1). The spectrum of benzophenone-3 showed minima at 230, 263 and 309 nm and maxima at 207, 242, 287 and 326 nm. Butylmethoxy dibenzoylmethane had minima at 217, 249 and 301 nm and maxima at 206, 233, 267, 286 and 358 nm while octyl methoxycinnamate had minima at 215 and 247 nm and maxima at 212, 227 and 309 nm. Since at 325 nm, the three compounds had nearly equal absorbance, on weight basis, this wavelength was choosen subsequently for routine monitoring of the compounds on HPLC. The sensitivity at this wavelength is lower, (benzophenone-3 - 33 %; butylmethoxy dibenzoylmethane - 52 %; octyl methoxycinnamate - 35 % lower) compared to measurement at the wavelength with maximal absorbance in each case.

The standard separation system used a C8 column with a flow rate of 1.0 ml/min with a gradient from 80 % to 100 % methanol during 10 min (the other phase consisting of 1

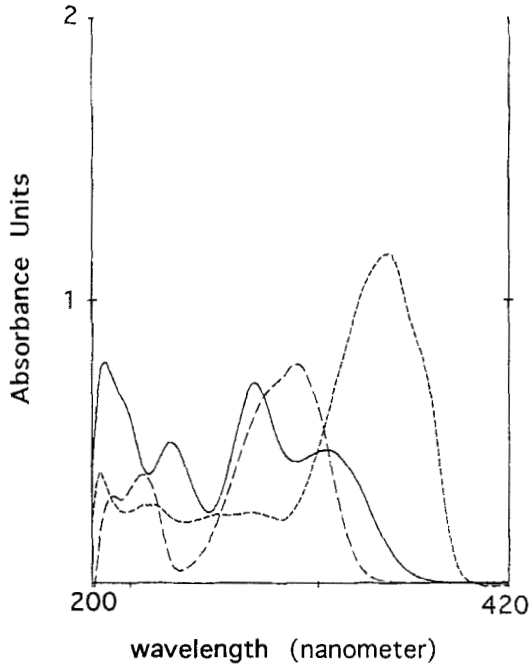

FIGURE 1. Absorbance spectras of UV-filters. Benzophenone-3 (——), butylmethoxy dibenzoylmethane (- - - -) and octyl methoxycinnamate (– – –) were dissolved in ethanol and analyzed at concentrations of 10 mg m^{-3}.

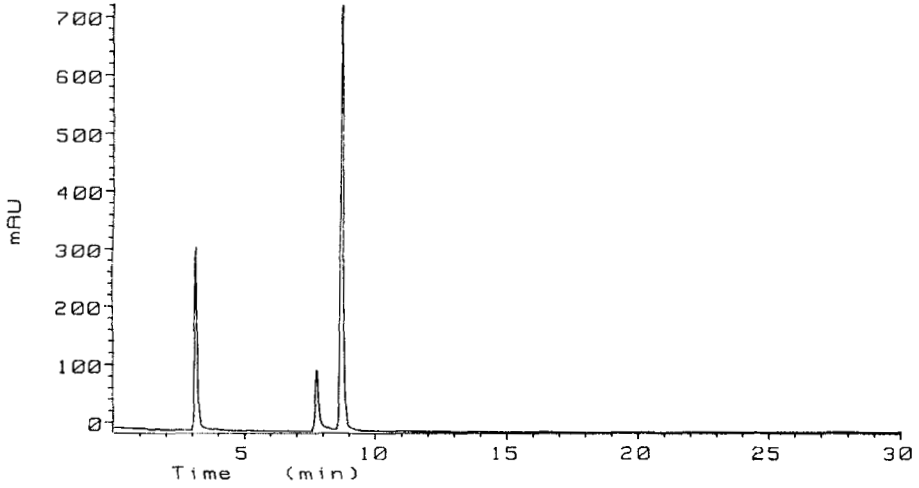

FIGURE 2. Chromatogram of UV-filters on HPLC. A mixture of UV-filters equivalent to 1 μg of each compound was analyzed by HPLC. Benzophenone-3 (3.1 min), butylmethoxy dibenzoylmethane (7.7 min) and octyl methoxycinnamate (8.7 min) are eluting with increasing amounts of methanol in the gradient.

% acetic acid in water). The column is washed for 2 min with methanol and then equilibrated with 80 % methanol for at least 4 min. A typical chromatogram is shown in Figure 2. The reproducibility of the system is high as shown in the small day-to-day drift in retention time (Table II). The carry-over of the compounds were tested at several different concentrations. At high levels of UV-filters ($\geq$ 5 µg) this effect was at a level of 0.18 % or less. At levels used for routine analysis, however, no carry-over was detected in our system.

Purity and standard curves of UV-filters for quantitative analysis

When high amounts of the UV-filters ($\geq$ 30 µg) were analyzed by HPLC some new peaks were found in addition to the main peak in each case. Furthermore, impurities in the main peak of butylmethoxy dibenzoylmethane was found as judged from spectral analysis. The purities were determined to be 98 - 99 % (Table III), which is in accordance with that specified (Table I).

Different amounts of UV-filters (0.03 - 30 µg) were dissolved in ethanol and injected on the HPLC where the area of the peaks at 325 nm were measured. A linear relationship was found throughout the concentration interval analyzed (Figure 3). The detector could not record accurate spectra of the peak maximum at the highest concentrations used of UV-filters due to the high optical density. No useful spectra could be recorded at the lowest concentrations analyzed although the peaks could be clearly distinguished in the chromatograms. To obtain accurate peak purity tests of the peaks one must compensate for base line drift in the case of the butylmethoxy dibenzoylmethane peak to compensate for its tailing (probably due to a tautomeric shift). In the routine analysis vials containing a standard concentration of the three UV-filters were used to test the accuracy of the instruments.

Stability of UV-filters

In ethanol benzophenone-3 and butylmethoxy dibenzoylmethane were stable throughout the whole time period (about one month) as judged from the amounts recovered and spectral analysis of the peak (Figure 4). In the case of octyl methoxycinnamate, however, a new peak with slighly lower retention was found in all samples upon storage. A dramatic increase in amount of this new peak was found in the sample stored in the presence of light at room temperature (Figures 4). Analysis of the spectra for the new peak and octyl methoxycinnamate showed a high degree of similarity. The absolute and relative intensity of the maxima at short wavelengths increased and a

TABLE II.

Reproducibility in the Analysis of UV-filters.

UV-filter	Retention time (min)					
	A	C.V. (%)	B	C.V. (%)	C	C.V. (%)
Benzophenone 3	3.0467 ± 0.0211	0.69	3.0227 ± 0.0238	0.79	3.0084 ± 0.0133	0.44
Butylmethoxy dibenzoylmethane	7.7178 ± 0.0498	0.65	7.6092 ± 0.0282	0.37	7.5838 ± 0.0286	0.38
Octyl methoxycinnamate	8.6992 ± 0.0436	0.50	8.6072 ± 0.0265	0.31	8.5672 ± 0.0428	0.50

Values shown (means ± standard deviations) from six different samples analyzed in duplicate in three independent experiments (A - C).

TABLE III.

Purity of UV-filters Analyzed by HPLC.

Approximately 30 µg of each compound were analyzed by HPLC. The relative quantitation is made relative to the peak area at 325 nm.

UV-filter	retention time (min)	area	%	comment#
Benzophenone 3	2.992	29,458	98.4	
	3.710	488.47	1.6	> 1 compound
	7.4			not integrated
			Σ 100.0	
	3.508	45.16	0.14	
Butylmethoxy	3.859	302.80	0.92	> 1 compound
dibenzoylmethane	7.845	32,438	98.4	impurity in the tail ?
	11.145	185.63	0.56	
			Σ 100.02	
	3.0			not integrated
	3.597	50.27	0.16	
Octyl methoxy-	7.755	199.93	0.66	
cinnamate	8.229	30,161	99.1	
	9.641	28.08	0.09	
			Σ 100.01	

Some of the peaks seem to contain more than one peak judging from the symmetry of the peak and/or spektral/isogram analysis. Non-integrated peaks contained only a few area units.

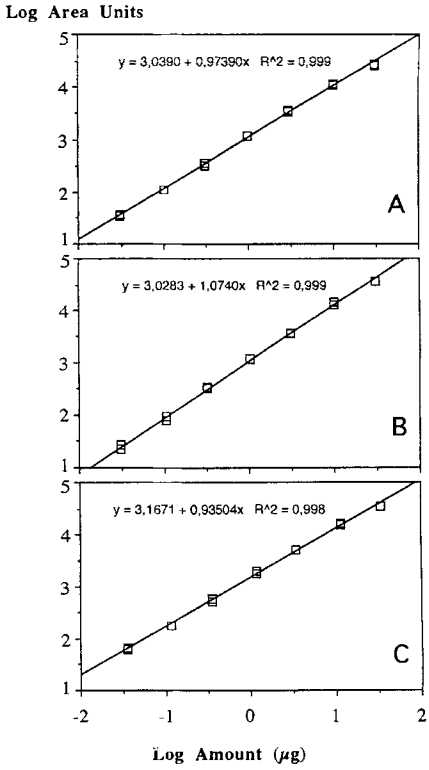

FIGURE 3. Calibration curves for UV-filters. Different amounts of Benzophenone-3 (A), butylmethoxy dibenzoylmethane (B) and octyl methoxycinnamate (C) (0.03 - 30 μg) dissolved in ethanol were analyzed in duplicate by HPLC. Quantitation was performed based on area counts at 325 nm.

blue shift in wave length maximum around 309 nm was found in the new peak. This new compound is most probably due to cis/trans isomerization of octyl methoxycinnamate. Such an isomerization has been demonstrated upon irradiation of cinnamate sun screens [6]. Similar results have been reported after irradiation of UV-filters with ultraviolet light up to five minutes [7]. In that study a blue shift of octyl methoxycinnamate of 2.3 nm was found in the peak maximum at 309 nm which probably may be explained by the dual contribution of octyl methoxycinnamate and the new peak detected in our analysis. This fact points out the advantage of analyzing the effects on the UV-filters through a combination of separation and physico-chemical characterization. No significant effects

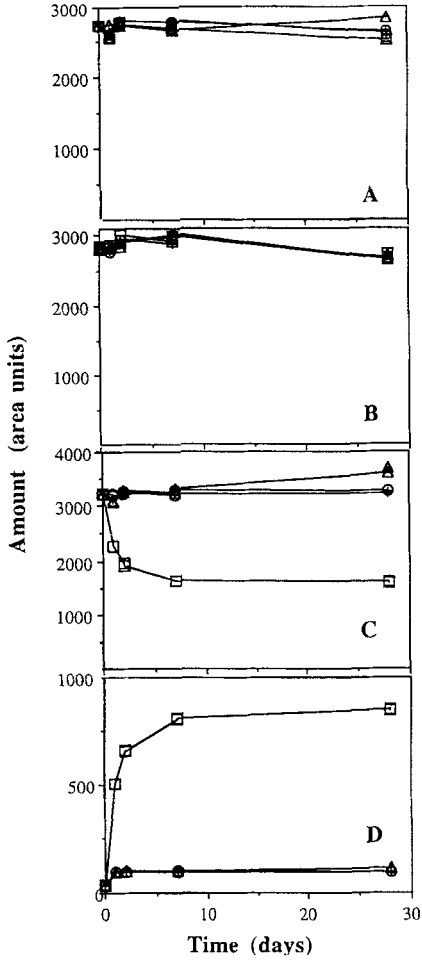

FIGURE 4. Stability of UV-filters. UV-filters were dissolved in ethanol and stored dark at - 20°C (O), at 4 °C (+) and at room temperature (18 - 20°C)(Δ) or in day light at room temperature ( ). Benzophenone-3 (A), butylmethoxy dibenzoylmethane (B), octyl methoxycinnamate (C) and the new peak derived from octyl methoxycinnamate (D).

TABLE IV.

Recovery of UV-filters From Vehicle Lotion.

A lotion without UV-filters was used. Results from four different experiments with duplicate samples (concentrations of UV-filters around 0.25, 0.5 and 1 % on a weight basis) are shown as means ± standard deviations.

UV-filter	% recovery		
	0.25 %	0.5 %	1 %
Benzophenone 3	102 ±4.6	103 ± 1.3	104 ± 1.1
Butylmethoxy dibenzoylmethane	100 ± 4.2	102 ± 0.4	100 ± 1.0
Octyl methoxycinnamate	102 ± 4.7	102 ± 1.1	100 ± 4.6

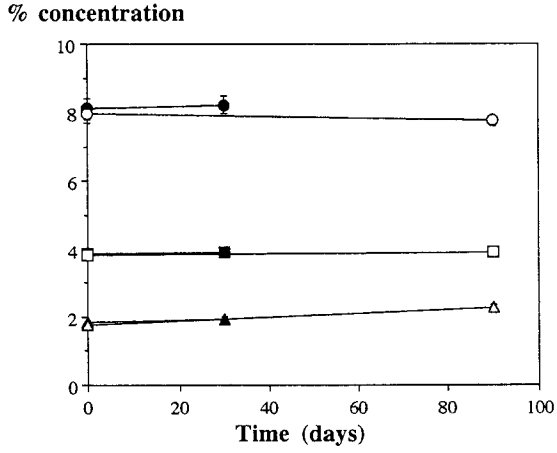

FIGURE 5. Concentration of UV-filters in a sun lotion after storage at 40°C for different times. Different batches of a sun lotion containing Benzophenone-3 ( ), butylmethoxy dibenzoylmethane (Δ) and octyl methoxycinnamate (O) were stored at 40°C for 30 (filled symbols) or 90 (open symbols) days. Extraction and analysis of UV-filters were performed as described in Materials and Methods. Values are shown as means and S.D. of six analysis of each batch and time pooint (C.V. 1.9-4.4 %).

were reported in the UV-spectra of benzophenone-3 and butylmethoxy dibenzoylmethane after irradiation [6], which is in accordance with the results in the present study.

Extractions of the UV-filters with ethanol from sun lotion was found to be effective and the absolute recovery was close to 100 % at several different concentrations (Table IV). No other compounds interfered with the analysis of the UV-filters (neither at 325 nm nor using spectral analysis from 200 - 420 nm). No significant change in concentration of the UV-filters in the lotion was found during storage for 30 or 90 days at 40°C (Figure 5).

CONCLUSIONS

A simple and convenient method for the separation of three UV-filters on reversed phase HPLC is described. The peak areas of the UV-filters have linear responses over a wide concentration range (10^4). The sensitivity in detection can be increased with 30 - 50 % if time programmed wave length changes are performed instead of detection at 325 nm. One should be careful with exposure to light in the case of octyl methoxycinnamate. The UV-filters were found to be stable for at least three months at 40°C in the tested sun lotion. This indicate adequate sunscreening properties of the lotion during storage for 2-3 years at room temperatture.

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