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Determination of sunscreen compounds in topical sunscreen products

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ABSTRACT

A method for the determination of sunscreen compounds in over-the-counter topical sunscreen products is described. The method uses reversed-phase liquid chromatography with an eluent of tetrahydrofuran and dilute acetic acid. The analytes are detected using an ultraviolet detector at 313 nm. The selectivity of methanol, acetonitrile, and tetrahydrofuran was investigated using selected test sunscreen compounds. The best selectivity for the separation of the analytes was obtained using tetrahydrofuran. The specificity of the method was demonstrated by showing that the analytes could be separated from several potential degradation products. Quantitative recoveries were obtained for all six sunscreen compounds determined in a variety of formulations.

INTRODUCTION

Sunscreen compounds are UV absorbers which act to mitigate the deleterious effects of sunlight. The use of these compounds has become increasingly widespread as the general public recognizes the serious skin-related problems associated with overexposure to the sun. The large number of commercial sunscreen products is a testament to their extensive use [1].

In the last few years the trend toward the development of topical sunscreen products possessing very high sunscreen protection factors (SPFs), along with the desire to remove *p*-aminobenzoic acid (PABA) from topicals, has resulted in the development of products containing different sunscreen compound combinations. In particular, the combination of octyl methoxycinnamate and octyl salicylate is being used in many commercially available topical sunscreen products to address these trends [2]. These can be used in combination with other sunscreen compounds to give a waterproof, broad-spectrum, high-SPF product.

Because topically applied sunscreen products usually consist of a combination of sunscreen compounds in a water-resistant base, reversed-phase high-performance liquid chromatography (HPLC) is ideally suited for the analysis of these products. However, only a few papers have been published describing the determination of sunscreen compounds in cosmetics by HPLC [3–8]. While some of these papers show the separation of a variety of sunscreen compounds, no reversed-phase method for the separation and determination of octyl methoxycinnamate and octyl salicylate in topical products have been reported. Also, the majority of papers on the determina-

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tion of sunscreen compounds in cosmetics are not suitable for routine analysis because of the tedious sample preparation procedures employed [8].

This paper describes the development of an analytical method for the routine determination of a variety of sunscreen compounds in topical sunscreen products. In particular, optimization of the separation of octyl methoxycinnamate and octyl salicylate is described.

EXPERIMENTAL

Apparatus

A Hewlett-Packard Model 1090 HPLC system with a diode-array detector was used for method development. Routine analyses were performed on a Waters Assoc. Model 204 HPLC system with a Model M6000 pump, a Model 440 absorbance detector, and a Model 710B WISP autoinjector. Data collection was performed on a Hewlett-Packard HP-3350A LAS computer. A Waters μBondapak C_{18} column (30 cm × 3.9 mm I.D., 10-μm packing) a Waters μBondapak Phenyl column (30 cm × 3.9 mm I.D., 10-μm packing), and a DuPont Instruments Zorbax Phenyl column (15 cm × 4.6 mm, 6-μm packing) were used.

The column was conditioned for at least 1 h with the eluent at a flow-rate of 1.0 ml/min prior to analysis. Analyses were performed at the same flow-rate by injecting 5–15 μl of the sample and standard solutions prepared as described below. The eluent was monitored at 313 nm with a detector sensitivity of 0.1 a.u.f.s.

Reagents

The sources of sunscreen compounds are as follows: benzophenone-3, GAF (Wayne, NJ, U.S.A.); octyl dimethyl PABA (octyl dimethyl *p*-aminobenzoate), Van Dyk & Co. (Belleville, NJ, U.S.A.); octyl methoxycinnamate, Givaudan (Clifton, NJ, U.S.A.); octyl salicylate and menthyl anthranilate, Felton International (Brooklyn, NY, U.S.A.); octocrylene (2-ethylhexyl 2-cyano-3,3-diphenylacrylate), BASF Wyandotte (Parsippany, NJ, U.S.A.). The internal standard, 2-chloroanthracene, as well as salicylic acid, anthranilic acid, and p-methoxycinnamic acid were obtained from Aldrich (Milwaukee, WI, U.S.A.). The internal standard was recrystallized before use. The sunscreen compounds were used as received. HPLC-grade acetonitrile, methanol and tetrahydrofuran (THF) and reagent-grade glacial acetic acid were obtained from Fisher Scientific (Springfield, NJ, U.S.A.).

Sample preparation

Standard and internal standard stock solutions were prepared by accurately weighing known amounts of the reagents and dissolving in THF. Typical concentrations used were: benzophenone-3, 0.6 mg/ml; octyl dimethyl PABA, 0.8 mg/ml; octyl methoxycinnamate, 0.7 mg/ml; menthyl anthranilate, 0.5 mg/ml; octyl salicylate, 0.5 mg/ml; octocrylene, 1 mg/ml; 2-chloroanthracene, 1.6 mg/ml. Working standard solutions were prepared for analysis by pipetting 5 ml of the appropriate solutions into a 50-ml volumetric flask, adding 20 ml of water, and diluting to volume with THF.

Samples were prepared by accurately weighing 1 ± 0.2 g of sample into a 100-ml volumetric flask. This was dissolved in 10 ml of THF, and about 65 ml of acetonitrile were added. The sample was mixed well and diluted to volume with

acetonitrile. An aliquot of the sample was filtered through Whatman No. 2 filter paper, and 5 ml of this solution and 5 ml of the internal standard stock solution were pipetted into a 50-ml volumetric flask. Water (20 ml) was added and the sample was diluted to volume with THF.

RESULTS AND DISCUSSION

Benzophenone-3, octyl methoxycinnamate, and octyl salicycate were used to evaluate the effect of the mobile phase organic modifier on the chromatographic selectivity. Three organic modifiers were examined: methanol, acetonitrile, and THF. Methanol and acetonitrile gave similar selectivity, however, THF produced significant differences in selectivity.

Figs. 1 and 2 show the effect of variation of the amount of organic modifier in the mobile phase on the retention of the test sunscreen compounds. Only partial

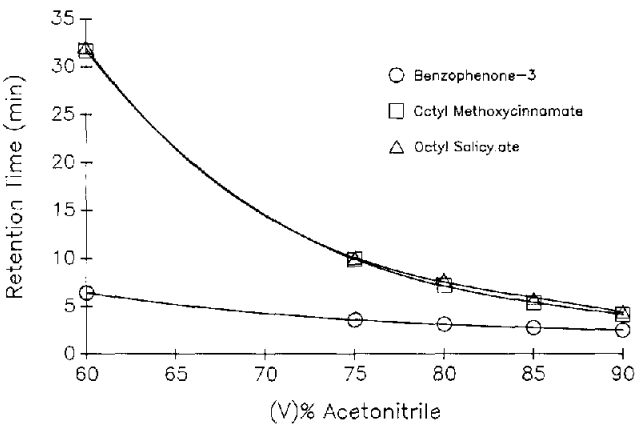

Fig. 1. Effect of acetonitrile on the retention of selected sunscreen compounds. Column, μBondapak C_{18}; eluent, acetonitrile–0.2% (v/v) acetic acid.

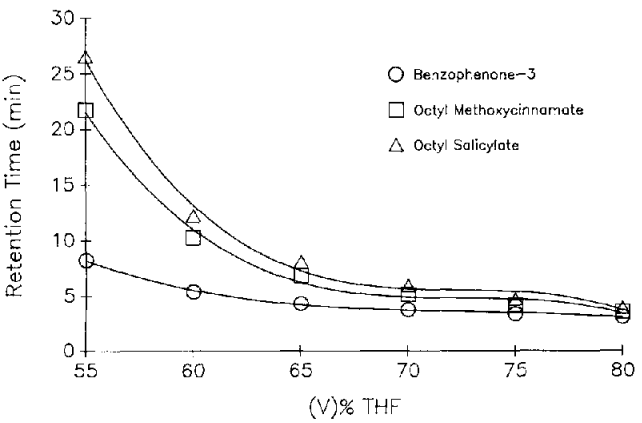

Fig. 2. Effect of THF on the retention of selected sunscreen compounds. Column, μBondapak C_{18}; eluent, THF–0.2% (v/v) acetic acid.

separation of octyl salicylate from octyl methoxycinnamate was achieved when acetonitrile was used in the mobile phase. This was also observed when methanol was used. When THF was used as the organic modifier complete separation of octyl salicylate from octyl methoxycinnamate was obtained. The unique selectivity obtained when THF was used in the mobile phase [9–11] allowed separation of these closely related sunscreen compounds.

Another improvement in the chromatography was achieved by the addition of acetic acid to the eluent. Acetic acid did not affect the selectivity for the sunscreens, but reduced the tailing of benzophenone-3. Table I shows the effect of acetic acid on the tailing factor [12] of benzophenone-3. A dramatic decrease in the tailing factor for benzophenone-3 is observed with the initial addition of acetic acid. With an acetic acid concentration of 0.03% (v/v) acceptable peak symmetry was obtained. Above 0.25% (v/v) acetic acid in the mobile phase the tailing of benzophenone-3 in this system is eliminated.

The type of stationary phase used influenced the extent of peak tailing of benzophenone-3. The data shown in Table I was obtained on a Waters μBondapak C_{18} column. Separations performed on a phenyl column (DuPont Zorbax Phenyl) required significantly higher concentrations of acetic acid. However, another phenyl column (Waters μBondapak Phenyl) gave results comparable to C_{18}. Therefore, peak tailing of benzophenone-3 is not related to the nature of the bonded phase, but is probably due to differences in surface coverage of the silica packing material by the bonded phases.

Based on the above information an eluent consisting of THF and dilute acetic acid was selected for use. Figs. 3–5 show typical separations obtained for a variety of sunscreen combinations in topical formulations using various chromatographic conditions. Baseline resolution was obtained for these compounds using the THF–dilute acetic acid eluent.

The majority of sunscreen compounds used in topical products are esters. Some compounds, for example octyl dimethyl PABA, contain functional groups besides the ester group. Although these can undergo degradation by other mechanisms [13], ester hydrolysis is the main mechanism of degradation of sunscreen compounds within the topical formulation. Therefore, in order to demonstrate the selectivity of the method and its applicability to product stability monitoring, potential hydrolysis degradation

TABLE I

EFFECT OF ACID ON THE PEAK SYMMETRY OF BENZOPHENONE-3

Column, μBondapak C_{18}; eluent, 65% (v/v) THF–acetic acid–water.

Acetic acid (%, v/v)	Tailing factor
0.00	5.34
0.03	1.64
0.12	1.08
0.25	1.00
0.37	1.00
0.49	1.00
0.61	1.00

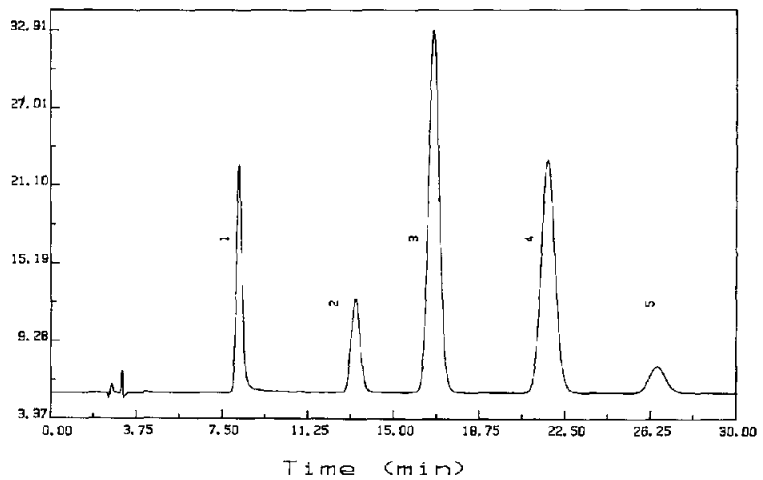

Fig. 3. Chromatogram of topical sunscreen product. Column μBondapak C_{18}; eluent, THF–acetic acid water (55:0.09:44.91, v/v). Peaks: 1 = Benzophenone-3; 2 = 2-chloroanthracene; 3 = octyl dimethyl PABA; 4 = octyl methoxycinnamate; 5 = octyl salicylate.

products were separated from the sunscreen compounds using the conditions described above. Table II shows the relative retention values for the five sunscreen compounds, internal standard, and potential degradation products. All the sunscreen compounds are separated from the degradation products and each other using this eluent system.

The response linearity for the test sunscreen compounds was determined, and the data are shown in Table III. Excellent linearity was obtained for all of the test

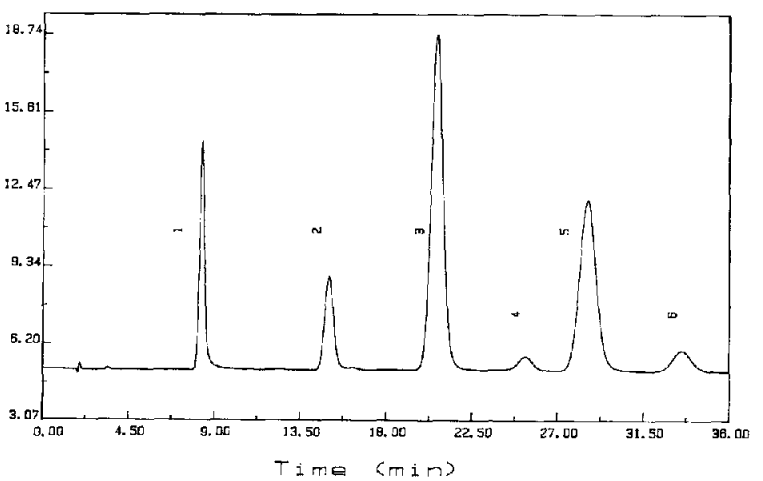

Fig. 4. Chromatogram of topical sunscreen product. Column, μBondapak C_{18}; eluent, THF–acetic acid–water (50:0.1:49.9, v/v). Peaks: 1 = benzophenone-3; 2 = 2-chloroantracene; 3 = octyl dimethyl PABA; 4 = menthyl anthranilate; 5 = octyl methoxycinnamate; 6 = octyl salicylate.

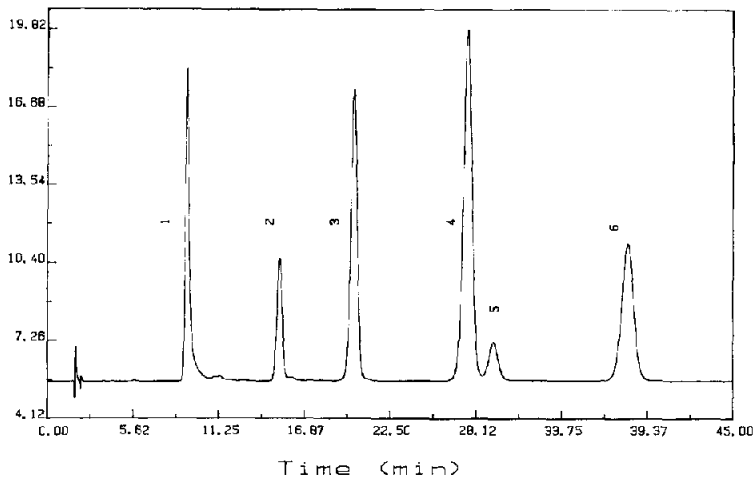

Fig. 5. Chromatogram of topical sunscreen product. Column, Zorbax Phenyl; eluent, THF–acetic acid–water (45:1:54, v/v). Peaks: 1 = benzophenone-3; 2 = 2-chloroanthracene; 3 = octyl dimethyl PABA; 4 = octyl methoxycinnamate; 5 = octyl salicylate; 6 = octocrylene.

compounds over the normal working range expected to be used for sunscreen product analysis.

The precision of the method was determined by analysis of replicate samples of sunscreen products. The results for this study are shown in Table IV. Excellent reproducibility is obtained for most of the test sunscreen compounds. Somewhat poorer precision is observed for menthyl anthranilate. This is due to the poor sensitivity for this compound at the wavelength used for analysis. However, acceptable precision is obtained for this compound as well.

The accuracy for the analysis of topical sunscreen products using the method described was determined by the analysis of synthetic samples prepared by spiking

TABLE II

RELATIVE RETENTION OF SUNSCREEN COMPOUNDS AND DEGRADATION PRODUCTS

Column, Waters μBondapak C_{18}; eluent: THF–acetic acid–water (55:0.09:44.91, v/v).

Compound	Type[a]	Relative retention
Anthranilic acid	D	0.30
p-Methoxycinnamic acid	D	0.31
4-(Dimethylamino)benzoic acid	D	0.35
Salicylic acid	D	0.41
Benzophenone-3	S	0.60
2-Chloroanthracene	I	1.00
Octyl dimethyl PABA	S	1.32
Menthyl anthranilate	S	1.57
Octyl methoxycinnamate	S	1.74
Octyl salicylate	S	2.11

[a] D = Degradation product; I = internal standard; S = sunscreen compound.

TABLE III

RESPONSE LINEARITY OF SUNSCREEN ASSAY METHOD

Compound	Range[a] (ng)	r
Benzophenone-3	237– 714	1.000
Octyl dimethyl PABA	41–1235	1.000
Menthyl anthranilate	42–1279	0.997
Octyl methoxycinnamate	286– 861	1.000
Octyl salicylate	195– 587	1.000
Octocrylene	435–1307	1.000

[a] Amount of sunscreen injected.

TABLE IV

PRECISION OF SUNSCREEN ASSAY METHOD

Compound	R.S.D. (%)	N
Benzophenone-3	0.8	16
Octyl dimethyl PABA	1.1	9
Menthyl anthranilate	3.4	16
Octyl methoxycinnamate	0.9	16
Octocrylene	1.0	9
Octyl salicylate	0.7	9

product placebos with solutions of the analytes. The recoveries ranged from 98.4 ± 1.5 to 100.7 ± 0.7%, confirming that quantitative and reproducible results can be obtained with this method.

The method described above is precise and accurate, and has been used in our laboratory for several years to monitor the stability of many different topical sunscreen formulations. Because the sample preparation is simple, the method is suitable for routine analysis. The selectivity of the eluent system allows the determination of the most commonly used sunscreen compounds in high-SPF topical sunscreen products.

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