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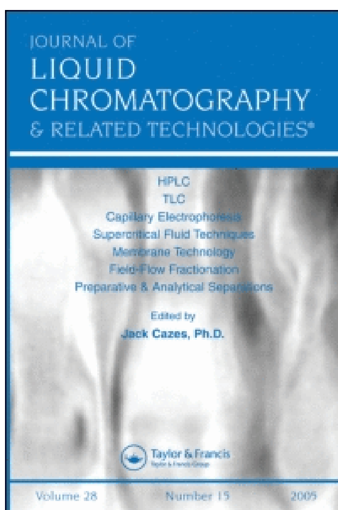

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E. Westgate[a]; J. Sherma[a]
[a] Department of Chemistry, Lafayette College, Easton, PA, U.S.A.

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DETERMINATION OF THE SUNSCREEN OXYBENZONE IN LOTIONS BY REVERSED-PHASE HPTLC WITH ULTRAVIOLET ABSORPTION DENSITOMETRY

E. Westgate, J. Sherma

Department of Chemistry
Lafayette College
Easton, PA 18042

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ABSTRACT

A quantitative method using preadsorbent chemically bonded C_{18} reversed-phase silica gel plates with fluorescent indicator, automated sample application, and computer-controlled UV absorption densitometry has been developed for the determination of the sunscreen ingredient oxybenzone (OB) in lotions. Nine sunscreen lotions were analyzed to test the proposed method and assess the relationship between OB content and the SPF value stated on the labels. It was found that the amount of OB in the lotions tested ranged from 0.796-6.03% (SPF 4-45). Precision (RSD) was 2.2% for replicate analysis of four sample solutions of one lotion on one plate and 4.6% for four analyses of one sample solution on different plates. Errors from spiked blanks and standard addition analyses to validate accuracy were 0 and 0.67% for the two blanks and 1.3% for the standard addition validation.

INTRODUCTION

In previous papers, new quantitative high performance thin layer chromatography (HPTLC) methods were described for determining the sunscreen ingredients 2-ethylhexyl-p-methoxycinnamate (octyl methoxy-cinnamate)[1] and 2-ethylhexyl-p-dimethylaminobenzoate (octyl dimethyl PABA)[2] in cosmetics.

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The purpose of this research was to extend these earlier HPTLC methods to the analysis of oxybenzone (OB), another widely used sunscreen ingredient in a variety of lotions.

Most previously published methods for the quantitative determination of OB in cosmetic products involve the use of reversed-phase[3-8] or micellar[9] high performance column liquid chromatography (HPLC) using a UV-absorption detector. A computer-based search of Chemical Abstracts for references on OB analysis by TLC located only one paper, in which TLC was used in combination with microcolumn HPLC and FT-IR spectrometry to obtain a spectrum of OB and other model compounds for qualitative analysis.[10] No quantitative TLC-densitometry methods were found.

This paper describes a simple and sensitive HPTLC method with high sample throughput and good accuracy and precision. Chemically bonded C-18 layers containing a fluorescent phosphor to facilitate detection of the UV-absorbing zones were used, but unlike the previous methods,[1,2] instrumental application of samples and standards in the form of bands onto the preadsorbent zone of the plate and computer-controlled automated densitometric scanning of chromatograms were carried out rather than manual application and scanning.

The new method was applied to a variety of commercial products to obtain their OB content and the relationship to the “sun protection factor” (SPF) number stated on the label. To validate accuracy, recovery experiments were performed by spiking two commercial skin lotions that do not contain sunscreen agents but have other ingredients present in common with the OB-containing products (blanks) and by standard addition of OB to a pre-analyzed sample. Precision was confirmed by replicate analysis of a sample solution on multiple plates, analysis of multiple samples of a single product on one plate, and scanning duplicate sample zones on chromatograms.

EXPERIMENTAL

Preparation of Standard Solutions

A 10.0 mg mL^{-1} stock solution, used for spiking the blanks, was prepared by dissolving oxybenzone (2-hydroxy-4-methoxy-benzophenone, CAS #131-57-7, Acros Organics, Fairlawn, NJ, 98% purity) reference standard in absolute ethanol. A 1.00 mg mL^{-1} standard, used for standard addition spiking, was prepared by 1:10 dilution of the stock solution with absolute ethanol. A TLC standard solution was prepared by further 1:10 dilution of the 1.00 mg mL^{-1} standard with absolute ethanol to a concentration of 0.100 mg mL^{-1}.

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Preparation of Sample Solutions

Sunscreen lotions containing OB active ingredient were purchased from local drug stores. SPF numbers ranging from 4 to 45 were designated on the labels, but the concentrations of OB were not stated. A 1.00-g sample of each lotion was added to approximately 75 mL of absolute ethanol in a 100-mL volumetric flask. The solution was magnetically stirred for 60 min to dissolve the active ingredient, the stirbar was removed using a strong magnet, and the flask was filled to the line with absolute ethanol. Undissolved ingredients were allowed to settle to the bottom of the volumetric flask during a 20 min standing period. The flask was wrapped with aluminum foil to protect the solution from possible decomposition as a result of exposure to light.

HPTLC Analysis

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Analyses were performed on 10 x 20 cm HPTLC RP-18 F_{254}s plates (EM Science, Gibbstown, NJ) coated with a layer of chemically bonded C_{18} silica gel with a preadsorbent or concentrating zone and fluorescent indicator. Layers were prewashed by development with dichloromethane-methanol (1:1). Sample and standard solutions were applied to the preadsorbent zone by means of a Camag (Wilmington, NC) Linomat IV automated spray-on band applicator equipped with a 100-µL syringe and operated with the following settings: band length 6 mm, application rate 4 sec/µL, table speed 10 mm/sec, and distances of 4 mm between bands, 20.7 cm from the plate edge, and 1.5 cm from the bottom of the plate. For analysis of the lotions, 2.00-µL, duplicate 4.00-µL, and 8.00-µL volumes of the TLC standard (equivalent to 0.200-0.800 µg) and duplicate sample solution aliquots were spotted. Preliminary experiments were performed for each sample to determine the amount of dilution and volume of sample aliquots needed for the sample zone scan areas to be bracketed by the scan areas of the standards and as close as possible to the areas of the duplicate middle standard. These dilutions ranged from 1:5 (SPF 8) to 1:10 (SPF 45), and the sample volumes spotted were 4.00-12.0 µL.

Plates were developed for a distance of 6 cm beyond the preadsorbent-C_{18} layer interface using a mobile phase consisting of methanol-tetrahydrofuran-deionized water (50:35:15) in a Whatman No. 1 chromatography paper-lined Camag twin-trough HPTLC chamber that had been equilibrated with the mobile phase for 15 min. Approximately 40 mL of solvent were used for each development, which required 25-30 min.

After development, the plates were air dried for 5 min, and sample and standard zones were quantified by linear scanning at 300 nm by use of a Camag TLC scanner II using the deuterium source, slit dimension settings of length 4 and width 4, and a scanning rate of 4.0 mm/sec. The wavelength of maximum

absorption was determined to be 300 nm by measurement of the in situ UV absorption spectrum of a standard OB zone using the spectral mode of the densitometer. The CATS-3 software program controlling the densitometer produced a calibration curve by linear regression of the weights and peak areas of the standard zones and interpolated the weights of the sample zones from the curve based on their areas. The calibration curves produced on each plate by linear regression of the four standards (scan area count vs micrograms spotted) had correlation coefficient (R) values ranging from 0.982 to 0.997, with an average of 0.993. For each tablet analysis, percent OB was calculated by dividing the ratio [interpolated average weight of oxybenzone (μg) in the duplicate samples/ volume (μL) of sample solution spotted] by the weight (μg) of sample in the spotted aliquot and multiplying the quotient by 100 and the dilution factor.

The accuracy of the method was validated by standard addition analysis in which a sample solution was prepared as described above, and an aliquot of the 1.00 mg mL^{-1} stock standard solution of OB was added to 1.00 mL of the pre-analyzed sample solution to increase the concentration to a known value. The spiked solution was reanalyzed, and recovery was calculated by comparing the weight obtained in the analysis to the theoretical added weight.

The accuracy of the method was also validated by determining the recovery from samples prepared by fortifying two blanks, a dry skin lotion and a body lotion, with aliquots of the 10.0 mg mL^{-1} stock standard OB solution. Although the blank samples contained no OB active sunscreen ingredient, they contained many of the same inactive ingredients as the analyzed lotions, such as sorbitol, stearic acid, triethanolamine, methylparaben, and propylparaben for the dry skin lotion, and stearic acid, propylene glycol, tocopheryl acetate, and cetyl alcohol for the body lotion.

To prepare the fortified samples, aliquots of the stock solution were added to 1.00-g blank samples in 100-mL volumetric flasks, and the solutions were prepared as described above. The recovery analysis was performed by comparing the experimental percents of OB to the theoretical fortification percents.

RESULTS AND DISCUSSION

Development on the C_{18} layers using the mobile phase described above produced flat, compact zones of OB with an R_F value 0.58 when viewed under 254 nm UV light. The number of additional UV-absorbing zones detected in sample chromatograms varied from one to four, with R_F values of 0.18 (homosalate), 0.26 (2-ethylhexyl salicylate), 0.35 (ethylhexyl p-methoxy-cinnamate), and 0.43 (octocrylene). These compounds were identified in lotions by matching R_F values of sample zones to standards chromatographed on the same plate

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Table 1

Percent Oxybenzone in Lotions with Known SPF Values

Lotion*	SPV Values	% OB
1 (a, b, c)	45	5.50
2 (b, c, d)	45	5.79
3 (b, c)	30	6.03
4 (a, b, c)	30	3.53
5 (a, b, c)	25	5.72
6 (b, c)	15	3.00
7 (c)	8	1.68
8 (c)	4	0.826
9 (c)	4	0.796

* Additional active sunsreeen ingredients are shown in parentheses according to the following key: a, homosalate; b, 2-ethylhexyl salicylate; c, ethylhexyl p-methoxycinnamate; d, octocrylene.

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(Table 1). None of these additional zones interfered with scanning of the OB zones.

Nine lotions with OB listed as an active ingredient and SPF values from 4 to 45 were analyzed by the new TLC method. Table 1 shows that the OB concentrations ranged from 0.796 to 6.03% and that there was a general increase in the percent of OB with increasing SPF value. However, the relationship is not completely direct because the other active sunscreen ingredients in each product contribute to the SPF value. Our results are similar to those obtained in an earlier study[8] in which sunscreen products with SPF values between 4 and 15 were analyzed using micellar column liquid chromatography.

Precision of the new method was confirmed by replicate analysis of a sample solution of one lotion on four separate plates. Percent OB ranged from 3.36-3.76% with an average of 3.57% and relative standard deviation (RSD) of 4.6%. As another evaluation of precision, four replicate sample solutions were prepared from a different lotion and analyzed on one plate. Percent OB ranged from 5.37-5.64% for this sample with an average of 5.47% and an RSD of 2.2%. As a final evaluation of precision, the percentage differences between the scanned areas of the duplicate sample aliquots in each analysis averaged 1.8% with a range of 0.11-4.1%.

The accuracy of the proposed method was validated by performing recovery analyses of blank dry skin and body lotions fortified with OB at 1.98 and 2.97%. The analytical results were 1.98 and 2.99%, respectively, which represent errors of 0 and 0.67% or recoveries of 100 and 101%. Duplicate samples of the unspiked blanks were chromatographed and scanned simultaneously. No zones were detected at the R_F value of OB in the spiked samples, proving that these samples were valid blanks. The accuracy of the proposed method was validated by a second method, standard addition of OB to a preanalyzed sample. In the preanalysis of a sample for standard addition validation, a weight of 0.445 µg of OB was obtained (equivalent to 0.843% of OB). A 1.00-mL aliquot was spiked with 30.0 µL of the 1.00 mg mL^{-1} standard, and reanalysis gave a result of 0.451 µg after calculations. This represents a 1.3% error or 101% recovery.

The described HPTLC method involves extremely simple sample preparation because filtration of the test solution or addition of an internal standard is not required. Accuracy and precision values that we obtained are comparable to those in papers published on the HPLC analysis of OB and other sunscreen agents. Examples of previously-reported respective values for recovery (%) and standard deviation (%) for HPLC analysis of various products are 94-96% and 0.9-2.8%,[3] 97.6-99.6% and 0.9-2.0%,[4] 98.1-100.9% and 0.8-3.2%,[5] 98.4-100.7% and 0.8-3.4%,[6] 94.1-101% and 1-5%,[7] and 97.7-99.3% and 2.2-2.5%.[8] In another HPLC study,[9] the mean RSD value for sunscreen analyses was reported as 7.67%. The high-quality results were obtained by our HPTLC method because a unique calibration curve is calculated on each plate from the four standards, and samples are analyzed under identical conditions on the same plate (in-system calibration). The practice of analyzing multiple samples on each plate leads to very high sample throughput and low consumption of solvent per sample, reducing the cost of purchase and disposal.

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