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Determination of seven sunscreen agents and two ultraviolet stabilizers in skin care products using ultra-performance liquid chromatography

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Synopsis

Ultraviolet radiation (UVR) is a well-known environmental carcinogen. Protection against UVR exposure has resulted in an increasing number of sunscreen agents being incorporated into a greater variety of cosmetic formulations including moisturizing lotions, color cosmetics, and skin care creams. Meanwhile, global regulation of sun care products is changing. New guidelines for sunscreen efficacy have resulted in a shift in product formulation that requires sunscreen products to provide broad spectrum UV protection. Since not all sunscreen ingredients protect against both UVA and UVB radiation, most sun care products require a combination of sunscreen agents. This article describes a new method for simultaneous separation and quantitation of seven organic sunscreens and two UV stabilizers using ultra-performance liquid chromatography. This method is capable of resolving all nine analytes, and has been validated for selectivity, precision, and accuracy. Because of the use of core-shell column technology, the separation is also achieved at back pressures compatible with conventional high-performance liquid chromatography instrumentation.

INTRODUCTION

Ultraviolet radiation (UVR) from sunlight is known to be an environmental human carcinogen. The primary negative effects of UV irradiation of normal human skin are sunburn, immunosuppression, photoaging, and skin cancer (1). UVR damages collagen fibers and accelerates the appearance of aging in skin. It also disrupts vitamin A supply (2). UVR is also the main cause of all three types of skin cancer (3,4). Because of these negative effects on human health, sun care products have become very important for the prevention of overexposure to UVR.

The use of sun care products is advised for the prevention of sunburn, photoaging, and skin cancer (5). Studies have also shown that daily use of a skin care product with a sun protection factor (SPF) of 16 results in 40% fewer squamous-cell carcinoma lesions of the skin (6). As a result of the proven skin protection of sunscreen products, sunscreen agents

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are being incorporated into a greater number of cosmetic formulations, and the global market for these products has grown to over US$9 billion in 2014 (7).

Meanwhile, global regulation of sun care products is changing; new guidelines for sunscreen efficacy are requiring a shift in sun care product formulation (8). Food and Drug Administration (FDA) labelling guidelines require sunscreen products to indicate if they are broad spectrum, meaning that they provide proportional protection against both UVA (320–400 nm) and UVB (290–320 nm) radiation. Since most sunscreen agents do not provide equal protection against both UVA and UVB radiations, most sun care products require a combination of sunscreen agents (8). In addition to FDA regulations, other global regions and countries have regulations outlining the use of organic sunscreens (9). For example, the European Union Cosmetic Directive requires sunscreen products to protect against all dangerous UVR and the product labels and claims should provide sufficient guidance to assist in selecting an appropriate skin care product (10). The global increase in sunscreen use and the regulatory push for products with broad-spectrum UV protection containing multiple sunscreen agents has resulted in the need for a simple, convenient, and rapid method to quantitate multiple sunscreen analytes in a single sun care product.

The United States Pharmacopeia and European Pharmacopeia cite monograph methods for quantitating individual sunscreen agents; however, the monograph methods are not validated for analyzing sunscreen agents in finished sun care products and the methods only quantitate one sunscreen agent at a time (10,11). Methods for analyzing multiple sunscreen agents are available (12–16), but these methods tend to be labor intensive, have lengthy analysis times, poor peak resolution, and only analyze a limited combination of active ingredients. One promising method from an application note was shown to separate a combination of six organic sunscreens, but upon validation, the method failed to resolve the stereoisomers of homosalate from octisalate (12). Another published method

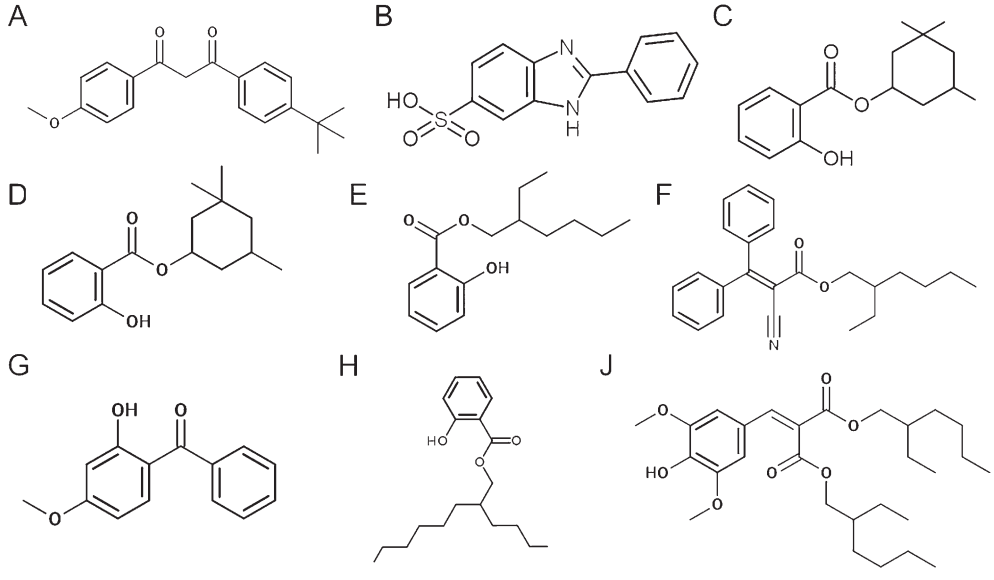

Figure 1. Chemical structures of the seven sunscreen agents and the two UV stabilizers: (A) avobenzone, (B) ensulizole, (C) homosalate, (D) octinoxate, (E) octisalate, (F) octocrylene, (G) oxybenzone, (H) butyloctyl salicylate, and (J) DESM.

Table I
UPLC Column Elution Gradient for Sunscreen Agents and UV Stabilizers

Time (min)	0.1% TFA[a] in water	0.1% TFA in methanol
0.0	75%	25%
0.9	75%	25%
1.0	25%	75%
1.5	25%	75%
7.0	20%	80%
10.0	10%	90%
10.1	0%	100%
11.2	0%	100%
11.3	75%	25%
12.5	75%	25%

[a]Trifluoroacetic acid.

for the determination of six organic sunscreens analyzes some active ingredients not yet approved for use in the United States and does not analyze for some commonly used sunscreens including octocrylene, oxybenzone, homosalate, and ensulizole (15). An application note exists for a method to separate 10 sunscreen actives in a single injection; however, it requires dual-wavelength detection and may not resolve both isomers of homosalate (16).

In this article, we describe a new and validated method for the simultaneous separation and quantification of seven organic sunscreens (avobenzone, ensulizole, homosalate, octinoxate, octisalate, octocrylene, and oxybenzone) and two UV stabilizers [butyloctyl salicylate and diethylhexyl syringylidene malonate (DESM)] using ultra-performance liquid chromatography (UPLC). We selected this combination of sunscreen agents, whose structures can be seen in Figure 1, because they are currently some of the most commonly used, and globally approved, organic sunscreen agents (17). In this study, we demonstrate that this UPLC method is capable of resolving all nine analytes including the separation of the two stereoisomers of homosalate. This method employs core-shell column technology. Core-shell columns are packed with particles consisting of a solid core and a porous outer

Table II
Linearity of Detection for Analytes Using UPLC Analysis Method

Analyte	Least-squares regression equation	R^2 value
Avobenzone	$y = 1179.5x - 1305.3$	0.9997
Avobenzone	$y = 488.85x - 755.29$	0.9997
Butyloctyl salicylate	$y = 1059.3x - 429.09$	0.9998
DESM	$y = 1059.3x - 429.09$	0.9998
Ensulizole	$y = 2337.1x - 1988.7$	0.9998
Octinoxate	$y = 3485.4x - 8347$	0.9998
Octisalate	$y = 3485.4x - 8347$	0.9998
Octocrylene	$y = 1412.2x - 1465.8$	0.9998
Oxybenzone	$y = 1834.5x - 24.582$	0.9997
Homosalate	$y = 579.24x - 3641.9$	0.9998

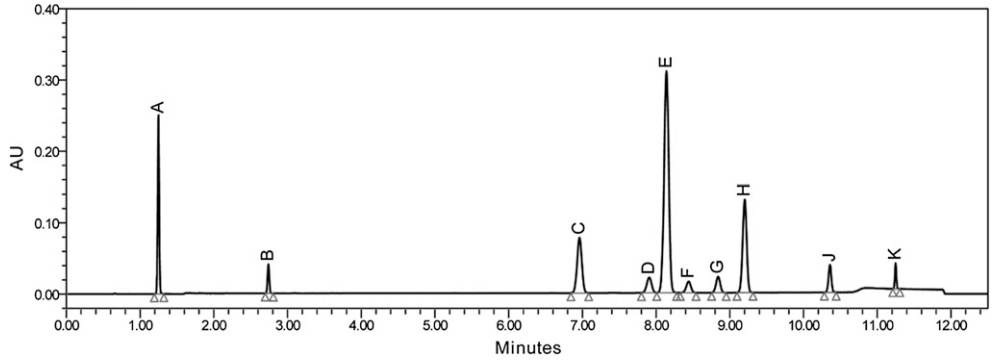

Figure 2. Representative chromatogram of UPLC separation of mid-concentration standard solution: (A) ensulizole, (B) oxybenzone, (C) octocrylene, (D) avobenzone, (E) octinoxate, (F) homosalate A, (G) octisalate, (H) homosalate B, (J) DESM, and (K) butyloctyl salicylate.

shell. This design limits diffusion of analytes within the particle giving high-capacity factors at reduced back pressures (18,19). Although the method was developed on an UPLC instrument, the core-shell column technology results in back pressures that are compatible with conventional high-performance liquid chromatography (HPLC) instrumentation.

MATERIALS AND METHODS

REAGENTS

The organic sunscreen agents and UV stabilizers, avobenzone (lot: P500073, purity: 99.9%), oxybenzone (lot: STBC0800V, purity: 99.9%), ensulizole (lot: MKBP8062V, purity: 99.1%), octisalate (lot: MKBK1099B, purity: 99.7%), octocrylene (lot: MKBP5033V, purity: 99.5%), and homosalate (lot: P500085, purity: 99.9%), were purchased from Sigma-Aldrich (St. Louis, MO); octinoxate (lot: A0299866, purity: 99.8%) from Acros Organics (Fisher Scientific, Pittsburgh, PA); DESM (lot: 2166E1MR, purity: 100%) from EMD Chemicals (Gibbstown, NJ); and butyloctyl salicylate (lot: 5032970,

Table III
Target Sunscreen Agent Concentrations for Accuracy Analysis

Analyte	BB Cream (% w/w)	UV Shield (% w/w)
Avobenzone	–	3.0
Butyloctyl Salicylate	–	2.5
DESM	–	4.0
Ensulizole	2.0	–
Octinoxate	5.5	2.5
Octisalate	–	5.0
Octocrylene	–	0.75
Oxybenzone	–	4.5
Homosalate	–	9.0

Table IV
Percent Recovery of Sunscreen and UV Stabilizers in a BB Cream Matrix Blank

Analyte	50% Spike level (%)	100% Spike level (%)	160% Spike level (%)
Avobenzone	96.6	98.1	98.0
Butyloctyl Salicylate	100.1	97.5	96.0
DESM	97.5	97.0	96.5
Ensulizole	99.2	98.1	98.1
Octinoxate	98.4	97.5	97.2
Octisalate	98.4	97.5	97.2
Octocrylene	98.1	97.4	96.9
Oxybenzone	99.9	98.2	97.7
Homosalate	98.5	97.6	97.2

BB Cream, Artistry Exact Fit™ Beauty Balm Perfecting Primer Fit™ Beauty Balm Perfecting Primer.

purity: 100%) from HallStar (Chicago, IL). Reagent grade trifluoroacetic acid (TFA) and tetrahydrofuran (THF) were purchased from Fisher Scientific, and pyridine from Sigma-Aldrich. HPLC grade methanol was obtained from OmniSolv, Inc. (Charlotte, NC).

The cosmetic formulations tested in this method were Artistry Exact Fit™ Beauty Balm Perfecting Primer (BB Cream), an oil-in-water color cosmetic emulsion, and Artistry™ UV Shield SPF 50+ PA+++ (UV Shield) for men, an oil-in-water cream emulsion, supplied by Amway Corporation (Ada, MI) both as complete formulations and as formulations without sunscreen agents and UV stabilizers to use as blank matrices.

STANDARD AND SAMPLE PREPARATION

A stock standard solution of avobenzone (1.65 mg/ml), ensulizole (3 mg/ml), octinoxate (8 mg/ml), oxybenzone (0.75 mg/ml), octisalate (5 mg/ml), octocrylene (3.1 mg/ml), and homosalate (20 mg/ml) was prepared in pyridine/methanol (25/75 v/v) solution. Standard solutions for linearity were prepared by pipetting 2.00, 3.00, 5.00, 7.00, and 10.00 ml stock

Table V
Percent Recovery of Sunscreen and UV Stabilizers in the UV Shield Matrix Blank

Analyte	**50% Spike level (%)**	**100% Spike level (%)**	**160% Spike level (%)**
Avobenzone	98.8	98.4	100.2
Butyl octyl Salicylate	101.8	98.6	99.4
DESM	99.5	98.8	100.2
Ensulizole	99.8	98.6	100.0
Octinoxate	99.7	98.8	100.0
Octisalate	99.9	98.4	99.9
Octocrylene	99.9	98.8	100.0
Oxybenzone	101.1	98.8	99.8
Homosalate	99.7	98.6	99.9

UV Shield, Artistry™ UV Shield SPF 50+ PA+++.

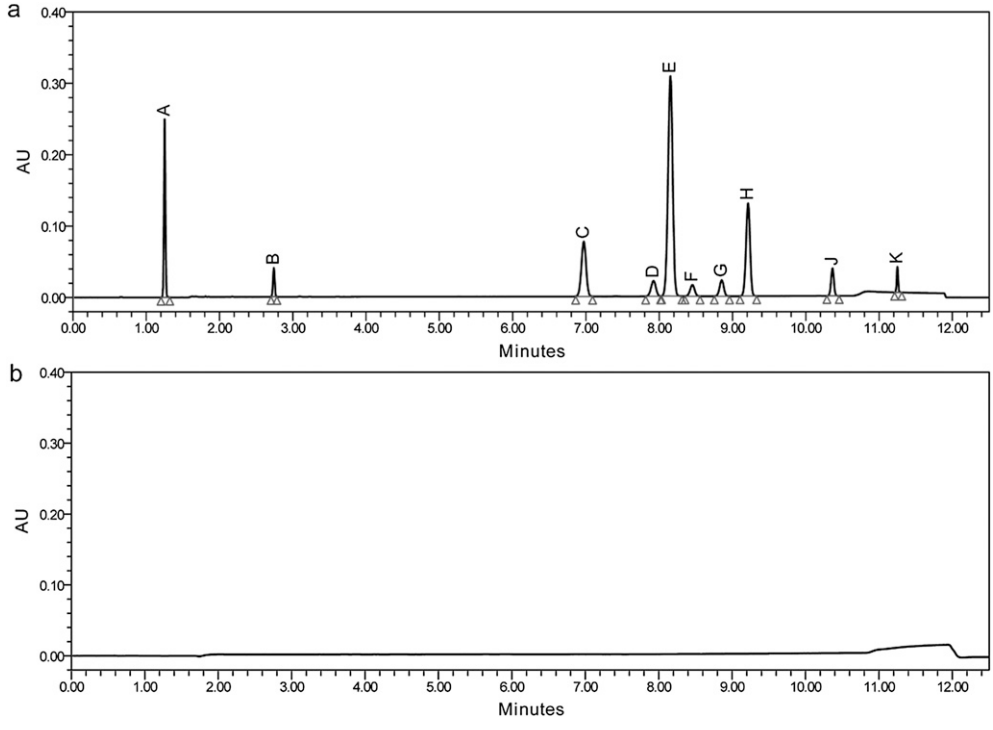

Figure 3. Representative chromatograms of blank color cosmetic (BB Cream) (A) matrix spiked with standard solution of sunscreen agents and UV stabilizers and (B) blank BB Cream matrix. Standard compounds: (A) ensulizole, (B) oxybenzone, (C) octocrylene, (D) avobenzone, (E) octinoxate, (F) homosalate A, (G) octisalate, (H) homosalate B, (J) DESM, and (K) butyloctyl salicylate.

standard solution into a series of 100-ml volumetric flasks and diluting in THF/methanol (5/95 v/v) solution. A single point standard was similarly prepared by diluting 5.00 ml of the stock solution in a 100-ml volumetric flask with a THF/methanol (5/95 v/v) solution.

Samples of the BB Cream and UV Shield formulations were weighed (0.75 g) into 100-ml volumetric flasks, 5 ml THF was added, the samples were briefly vortexed to break the emulsions, and then, diluted to volume with methanol. The samples were well mixed and an aliquot filtered through a Whatman 25 mm GD/X syringe filter with a PVDF with GMF membrane and 0.2-µm pore size (Catalogue Number 6872-2502) into an autosampler vial for analysis.

Spiked matrix samples were prepared by weighing 0.75 g of each blank matrix into individual 100-ml volumetric flasks. The stock standard was used to spike the matrix blanks with 50%, 100%, and 160% of the nominal concentration of the sunscreen analytes. The samples were dispersed in 5 ml THF, diluted to volume with methanol, well mixed, and an aliquot filtered through a Whatman PVDF with GMF 0.2-µm syringe filter into an autosampler vial for analysis.

UPLC ANALYSIS

Chromatographic separation was performed on a Waters Acquity UHPLC chromatograph with UV detection and an Agilent Poroshell EC-C18 (2.7 µm, 4.6 mm × 100 mm)

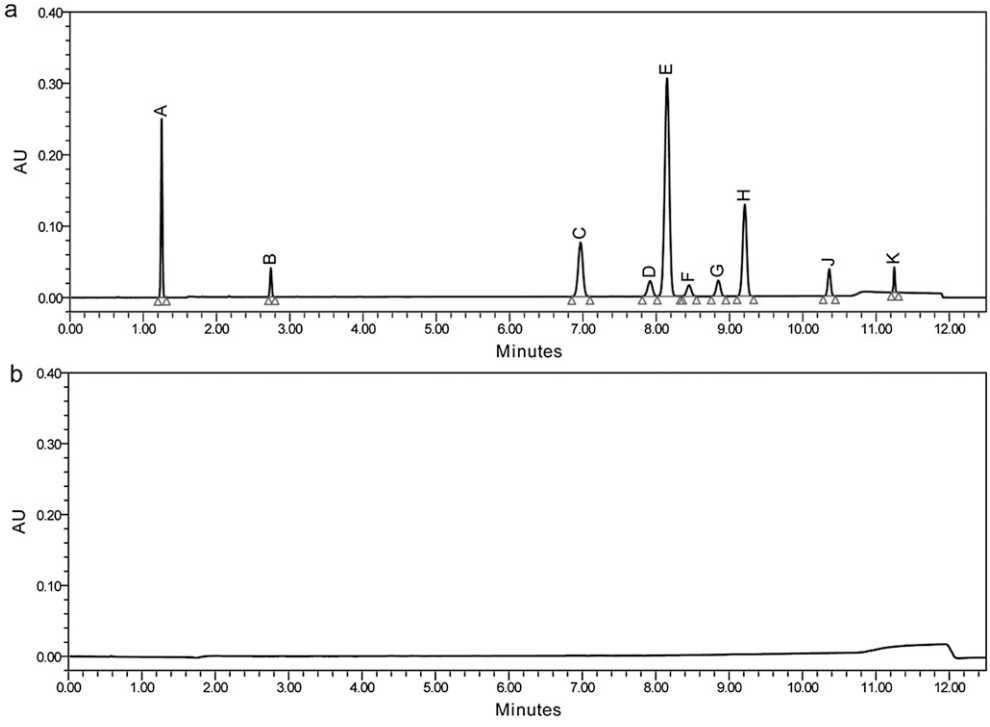

Figure 4. Representative chromatograms of blank cream/lotion (UV Shield) (A) matrix spiked with standard solution of sunscreen agents and UV stabilizers and (B) blank UV Shield matrix. Standard compounds: (A) ensulizole, (B) oxybenzone, (C) octocrylene, (D) avobenzone, (E) octinoxate, (F) homosalate A, (G) octisalate, (H) homosalate B, (J) DESM, and (K) butyloctyl salicylate.

column. The mobile phase solutions used for gradient separation were 0.1% TFA in water and 0.1% TFA in methanol. The solutions were degassed using sonication under vacuum, and the step-wise gradient separation was run at 50°C with a flow rate of 1.5 ml/min as described in Table I. The injection volume was 1 µL for both samples and standards and the chromatograms were recorded at a wavelength of 315 nm.

METHOD VALIDATION

For validation of the method, dilutions of the nine standard analyte mixtures were separated as outlined and the least-squares regression equations and correlation coefficients (R^2) calculated for each analyte using Microsoft Excel software. The accuracy of the method was determined using blank matrices spiked with standard mixtures at 50%, 100%, and 160% of the formulation target content. Three accuracy spike solutions were prepared at each level and analyzed for all of the sunscreen agents and UV stabilizers. Injection precision was determined by analyzing the mid-concentration standard solution five times and calculating the relative standard deviations (RSD) for each analyte using Waters Empower 3 software (Milford, MA). Assay robustness was evaluated by varying the chromatographic parameters of flow rate by ±0.1 ml/min, detection wavelength by ±5 nm, and mobile phase acid modifier by ±20%, and assaying the sunscreen content of BB Cream samples.

The validated method was then tested on the two finished product formulations, Artistry Men UV Shield SPF 50+ PA+++ (UV Shield), containing six sunscreen agents and two UV stabilizers, and Artistry Exact Fit Beauty Balm Perfecting Primer (BB Cream), containing two sunscreen agents.

RESULTS AND DISCUSSION

To demonstrate assay linearity, the standard curves of each analyte were analyzed and the results plotted as peak area counts against standard concentration. The least-squares regression equations and R^2 values for the linearity plots are presented in Table II. The R^2 value for each analyte was greater than 0.9995 and the *y*-intercept was below 2% of the area at the nominal concentration. Figure 2 is a representative chromatogram of the analyte separation of a mid-concentration standard solution.

Accuracy of the method was determined using matrix blank formulations spiked with the stock standard solution at 50%, 100%, and 160% of the target analyte level as listed in Table III. The results of three experiments were averaged and are presented in Table IV for the BB Cream and Table V for the UV Shield matrices. Not all nine analytes are present in the original formulas; however, a stock standard containing all of the analytes was used for the accuracy validation studies so that the recovery of all the sunscreen agents and UV stabilizers were determined for both spiked blank matrices.

Figures 3 and 4 are representative chromatograms of the BB Cream and UV Shield matrix blanks spiked with the standard solution at the 100% target level along with chromatograms of the unspiked blank matrix. The chromatograms of the blank matrices demonstrate that no interfering peaks (>1% of nominal peak area) were observed at the retention times of the analytes in either the BB Cream or UV Shield matrix blanks, confirming that this method is specific for the seven organic sunscreens and two UV stabilizers analyzed.

Table VI
Robustness of Chromatographic Parameters as Shown with BB Cream Analysis

Method conditions	Ensulizole results (%w/w)	Octinoxate results (% w/w)	Ensulizole % difference from nominal conditions	Octinoxate % difference from nominal conditions
Nominal conditions	2.070	5.010	–	–
Flow rate: 1.4 mL/min	2.042	5.180	1.362	3.337
Flow rate: 1.6 mL/min	2.008	5.105	3.041	1.878
Detection wavelength: 310 nm	2.072	5.082	0.097	1.427
Detection wavelength: 320 nm	2.071	5.098	0.048	1.741
Acid modifier: 0.08% TFA	2.090	5.144	0.962	2.639
Acid modifier: 0.12% TFA	2.069	5.100	0.048	1.780
Column temperature: 45°C	2.016	5.146	2.643	2.678
Column temperature: 55°C	2.143	5.456	3.465	8.523

BB Cream, Artistry Exact Fit™ Beauty Balm Perfecting Primer Fit™ Beauty Balm Perfecting Primer.

Analyte recovery from both sample matrices at all three concentrations (50%, 100%, and 160% of target concentration) were between 96% and 102%, demonstrating the accuracy of the method for all analytes in both a color cosmetic (BB Cream) and a cream/lotion (UV Shield) matrix.

Injection precision was measured by analyzing the mid-concentration standard solution five times. The %RSD for the injection precision for each analyte was below 1.00. The robustness of the chromatographic parameters was evaluated by varying the flow rate by ±0.1 ml/min, the detection wavelength by ±5 nm, the mobile phase acid modifier by ±20%, and column temperature by ±5°C and assaying the sunscreen content of the BB Cream formulation. The analysis results from these assay modification are presented in Table VI. For each condition tested with the exception of column temperature, the assay value of each analyte was within 5% (relative) of the values determined using the nominal method conditions demonstrating that the method is robust for moderate changes in flow rate, wavelength, and amount of acid modifier in the mobile phase. It is indicated in the method parameters to not exceed 50°C for the column temperature as the resolution is sensitive to column temperature and did not meet robustness acceptance criteria when the column temperature was increased to 55°C.

Representative assay chromatograms for the two finished product formulations, BB Cream, containing two sunscreen agents, and UV Shield, containing six sunscreen agents and two UV stabilizers are presented in Figure 5.

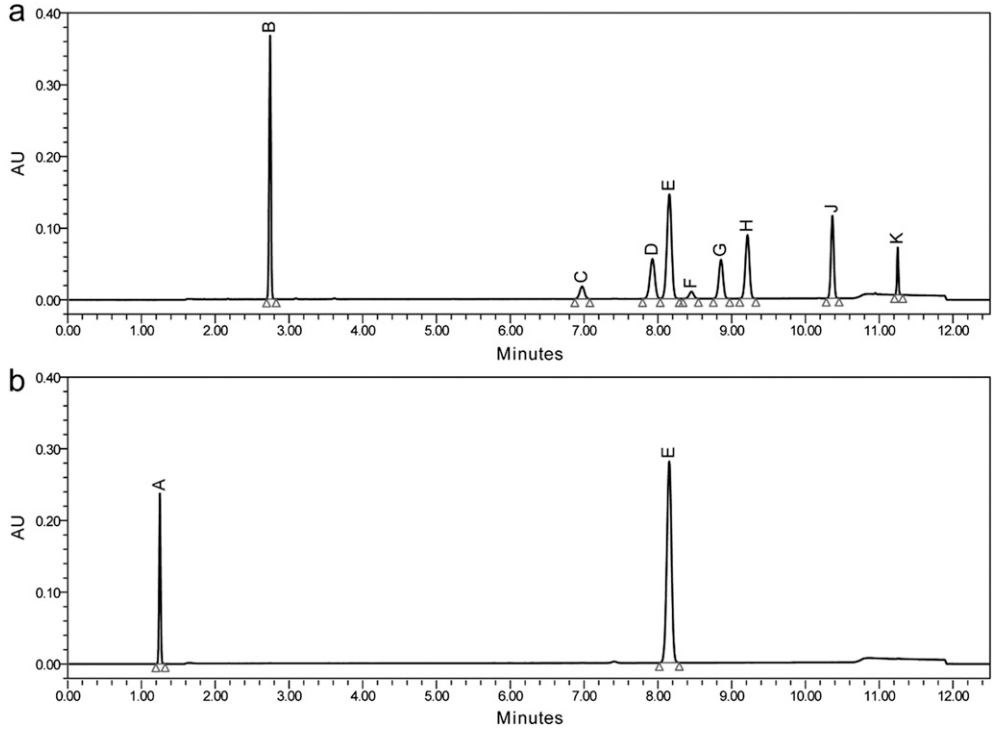

Figure 5. Representative chromatograms of UPLC separation of sunscreens and UV stabilizers in (A) color cosmetic (BB Cream) and (B) cream/lotion (UV Shield) formulations. Analytes: (A) ensulizole, (B) oxybenzone, (C) octocrylene, (D) avobenzone, (E) octinoxate, (F) homosalate A, (G) octisalate, (H) homosalate B, (J) DESM, and (K) butyloctyl salicylate.

CONCLUSIONS

We have demonstrated the validation of a UPLC method for the identification and quantification of seven organic sunscreens (avobenzone, ensulizole, homosalate, octinoxate, octisalate, octocrylene, and octinoxate) and two UV stabilizers (butyloctyl salicylate and DESM). Our results show that this method is selective, precise, accurate, and suitable for measurement of all nine analytes on a UPLC system. The method utilizes core-shell column technology so it may also be used on conventional HPLC instrumentation.

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