Identification and Quantitation of Oxybenzone, Octocrylene, Avobenzone, Octinoxate, Homosalate and Octisalate in Sunscreen Products by High Performance Liquid Chromatography-Diode Array Detection (HPLC-DAD)

(CARTS No. IR01925)

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ABSTRACT

A high-performance liquid chromatography (HPLC) method was developed and validated for the simultaneous analysis of the six most common organic active pharmaceutical ingredients (APIs) in sunscreen: oxybenzone, octocrylene, avobenzone, octinoxate, homosalate and octisalate. The APIs were extracted from sunscreen lotion and spray products using a 0.1% acetic acid in methanol solution. The samples were separated using a Luna C18(2) liquid chromatography column and then analyzed with a diode array detector (DAD) at 313 nm. This method was validated using both sunscreen lotion and spray samples with a label claim of avobenzone (3%), homosalate (15%), octisalate (5%), octocrylene (10%). Oxybenzone and octinoxate were included in the standard preparations at target sample concentrations of 3% and 4%, respectively, for validation purposes. The linearity ranges from 10% to 250% of the target sample concentrations had correlation coefficients (R^2) > 0.9999 for each API. The average spike recovery for all APIs at spike levels of 80%, 100% and 120% was between 97.2% – 100.5% in the spray sample and 99.5% – 100.8% in the lotion sample. The measure of precision for all APIs at spike levels of 80%, 100% and 120% displayed %RSDs between 0.23% – 0.96% in the spray sample and 0.16% – 1.34% in the lotion sample. This validated method was immediately used to analyze six commercial sunscreen products.

Key Words

sunscreen, lotion, spray, oxybenzone, octocrylene, avobenzone, octinoxate, homosalate, octisalate, high performance liquid chromatography, HPLC, diode array detection, DAD

INTRODUCTION

Sunscreens are considered drugs and are regulated by the U.S. Food and Drug Administration as they characteristically contain a combination of several UVA and UVB-absorbing compounds:[1] oxybenzone, octocrylene, avobenzone, octinoxate, homosalate and octisalate. A current trend toward the use of more "natural" cosmetics and sun care products has given rise to many "oxybenzone-free" product label claims.

In May 2021, a **Citizen Petition on Benzene in Sunscreen and After-sun Care Products** was filed with the U.S. FDA when benzene levels exceeding 2 ppm were detected in several non-prescription sunscreen drug products. Out of concern for public health and safety, the Center for Drug Evaluation and Research (CDER) issued a mission critical, high-priority, for-cause sample collection assignment. This impending sample collection for benzene analysis also prompted U.S. FDA laboratories' response in developing a separate method for the analysis of the active pharmaceutical ingredients (APIs) in sunscreen.

This bulletin details the validation, performed according to USP <1225>,[2] of a high-performance liquid chromatography method with simultaneous diode array detection of six (6) of the most common organic APIs in sunscreen: oxybenzone, octocrylene, avobenzone, octinoxate, homosalate and octisalate. A wavelength of 313nm was selected as the optimum wavelength for detection of all six analytes. The method set the following concentrations in sunscreen as 100% of the target level: avobenzone, 3%; homosalate, 15%; octisalate, 5%; octocrylene, 10%; oxybenzone, 3%; octinoxate, 4%.

METHOD AND MATERIALS

Equipment

a) Agilent Technologies 1260 Infinity® HPLC with Binary Pump, HiP Degasser, Diode Array Detector HS, thermostatted column compartment, vial sampler, OpenLab CDS ChemStation® software
b) Phenomenex® Luna 5 µmC18(2) 100 Å Column, 150 mm x 4.00 mm, part number: 00F-4252-D0
c) Mettler Toledo® XS204 analytical balance
d) Mettler Toledo® XP6 microbalance
e) Branson 5510 Sonicator™
f) Thermo Scientific™ Choice™ 0.2 µm nylon membrane syringe filters
g) Class A pipettes and amber volumetric flasks

Samples Used for Validation

a) Sunscreen spray
Active ingredients: avobenzone (3%), homosalate (15%), octisalate (5%), octocrylene (10%)
Inactive ingredients: alcohol denat., *iso*butane, butyloctyl salicylate, dicaprylyl carbonate, diethylhexyl 2,6-naphthalate, polyester-7, acrylates/octylacrylamide copolymer, *neo*pentyl glycol diheptanoate, fragrance, tocopheryl acetate

b) Sunscreen lotion
Active ingredients: avobenzone (3%), homosalate (15%), octisalate (5%), octocrylene (10%)
Inactive ingredients: water, butyloctyl salicylate, styrene/acrylates copolymer, silica, dimethicone, potassium cetyl phosphate, benzyl alcohol, beeswax, caprylyl methicone, aluminum starch octenylsuccinate, glyceryl stearate, PEG-100 stearate, cetyl dimethicone, caprylyl glycol, ethylhexylglycerin, behenyl alcohol, acrylates/dimethicone copolymer, xanthan gum, chlorphenesin, dimethicone/PEG-10/15 crosspolymer, sodium polyacrylate, hydrolyzed jojoba esthers, fragrance, disodium EDTA, ethylhexyl stearate, tocopheryl acetate, BHT, jojoba esters, trideceth-6

Reagents and Standards

a) Methanol (MeOH), Fisher Scientific, Catalog No. A456
b) Glacial acetic acid, Fisher Scientific, Catalog No. A38SI
c) DI water (resistivity NLT 18 MΩ·cm)
d) Oxybenzone, USP reference standard, Catalog No. 1485001
e) Octocrylene, USP reference standard, Catalog No. 1477411
f) Avobenzone, USP reference standard, Catalog No. 1045337
g) Octinoxate, USP reference standard, Catalog No. 1477900
h) Octisalate, USP reference standard, Catalog No. 1477943
i) Homosalate, USP reference standard, Catalog No. 1311408

Solutions

a) Extraction Solution (Diluent)
Pipette 1 mL of glacial acetic acid and dilute to 1 L with Methanol
b) 0.5% Acetic Acid Solution
Pipette 5 mL of glacial acetic acid and dilute to 1 L with DI Water
c) Mobile Phase A (80:20)
Combine 800 mL Methanol with 200 mL 0.5% Acetic Acid Solution
d) Mobile Phase B (100% MeOH)
e) Mixed Stock Standard Solution
Weigh each USP reference standard, to approximately the value given in Table 1, into a single 50 mL volumetric flask and dilute to volume with diluent — Table 1.

Table 1. Approximate Mass of USP Reference Standards in Mixed Stock Solution

USP ref. std.	~ Mass (mg)
Oxybenzone	37.5
Octocrylene	125.0
Avobenzone	37.5
Octinoxate	50.0
Octisalate	62.5
Homosalate	187.5

f) Mixed Standard Solution
Pipette 10 mL of mixed stock standard solution into a 100 mL volumetric flask and dilute to volume with diluent.

g) Sample Solution
Weigh approximately 250 mg of sample in a tared 100 mL volumetric flask, record the weight of the sample, and dilute to volume with diluent.

h) Sample and Spike Sample Solutions —– Table 2.

Table 2. Preparation of Sample and Spike Sample

Solution Name	Mass of Sample (mg)	Mixed Stock Std Soln. (mL)	Sample Soln. (mL)	Final Vol. (mL)
Sample	250	0	0	100
80% Spike Sample		2	25	50
100% Spike Sample		1	10	20
120% Spike Sample		3	25	50

i) Validation Solutions for Linearity and LOQ Solutions —–Table 3.

Table 3. Preparation of Linearity and LOQ Solutions for Validation

Solution Name	Mixed Stock Std (mL)	Final Vol. (mL)	Target Sample Conc. (%)
Linearity 1 (LOQ)	1	100	10
Linearity 2	1	20	50
Linearity 3	2	25	80
Linearity 4	5	50	100
Linearity 5	3	25	120
Linearity 6	3	20	150
Linearity 7	5	20	250

Instrumentation

An Agilent Technologies 1260 Infinity HPLC-DAD with Phenomenex C18(2) column is used in the method validation and sample analysis — see Table 4 for instrument parameters.

Table 4. HPLC-DAD Parameters

Column Temp.	40 °C
Wavelength	313 nm
Injection Volume	4 µL
Flow Rate	1.0 mL/min

Gradient

Time (min)	Mobile Phase A(%)	Mobile Phase B (%)
0	100	0
20.0	100	0
20.1	0	100
27.0	0	100
27.1	100	0
30.0	100	0

Run Time	~ 30 min

Quantitation of APIs is determined by single point calibration using the peak area. Homosalate consists of two isomers that elute as separate peaks; the sum of the isomer peak areas must be used for quantification. Concentrations of each API are calculated according to the formula shown below:

$$\% \text{ Result} = \left(\frac{C \times R_U}{R_S \times \text{Spl Wt}} \times 100 \text{ mL} \times 100\% \right)$$

C = Concentration of Standard Solution (mg/mL)

R_U = Peak response obtained from the Sample Solution preparation

R_S = Peak response obtained from the Standard Solution preparation (average of 5 consecutive system suitability injections)

Spl Wt = Sample weight (mg)

100 mL = Sample dilution factor

RESULTS AND DISCUSSION

Analysis of Sample Matrices Used for Validation

A commercially available sunscreen lotion and sunscreen spray were purchased from a national retailer to use for the validation. Six preparations of both the lotion and spray were analyzed according to the method, see Table 5 and Table 6 for results. General USP assay acceptance criterion is 90 – 110% of label claim for active pharmaceutical ingredients,[3] and all preparations are within this criterion. Figures 1 – 3 display chromatograms of mixed standard solution, sample (spray) solution and sample (lotion) solution. Figure 4 displays UV spectral data of each compound in mixed standard solution.

Table 5. Sunscreen Spray results (n = 6)

Label Claim	***10%***	***3%***	***5%***	***15%***
90 – 110% of Label Claim	9.0 – 11.0%	2.7 – 3.3%	4.5 – 5.5%	13.5 – 16.5%
Spray Prep	% Octocrylene	% Avobenzone	% Octisalate	% Homosalate
1	10.09	3.02	5.07	15.16
2	10.10	3.01	5.06	15.15
3	10.14	3.03	5.08	15.21
4	10.21	3.04	5.12	15.32
5	10.18	3.04	5.10	15.26
6	10.12	3.01	5.08	15.20
% RSD	0.48	0.46	0.41	0.42

Table 6. Sunscreen Lotion results (n = 6)

Label Claim	***10%***	***3%***	***5%***	***15%***
90 – 110% of Label Claim	9.0 – 11.0%	2.7 – 3.3%	4.5 – 5.5%	13.5 – 16.5%
Lotion Prep	Octocrylene (%)	Avobenzone (%)	Octisalate (%)	Homosalate (%)
1	9.99	3.02	5.02	15.09
2	10.05	3.04	5.05	15.19
3	10.01	3.04	5.02	15.13
4	10.08	3.05	5.04	15.23
5	10.00	3.03	5.01	15.12
6	10.12	3.07	5.06	15.30
% RSD	0.52	0.56	0.40	0.51

Figure 1. HPLC-DAD Chromatogram Mixed Standard Solution

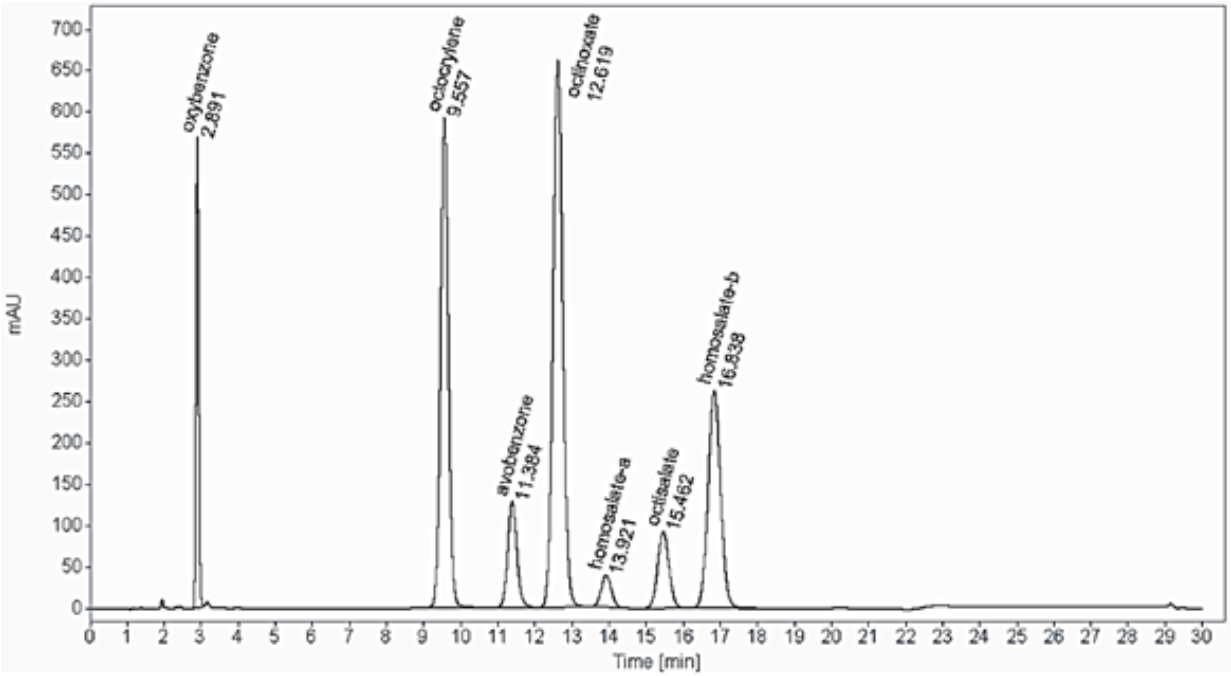


Figure 2. HPLC-DAD Chromatogram Sample (Spray) Solution

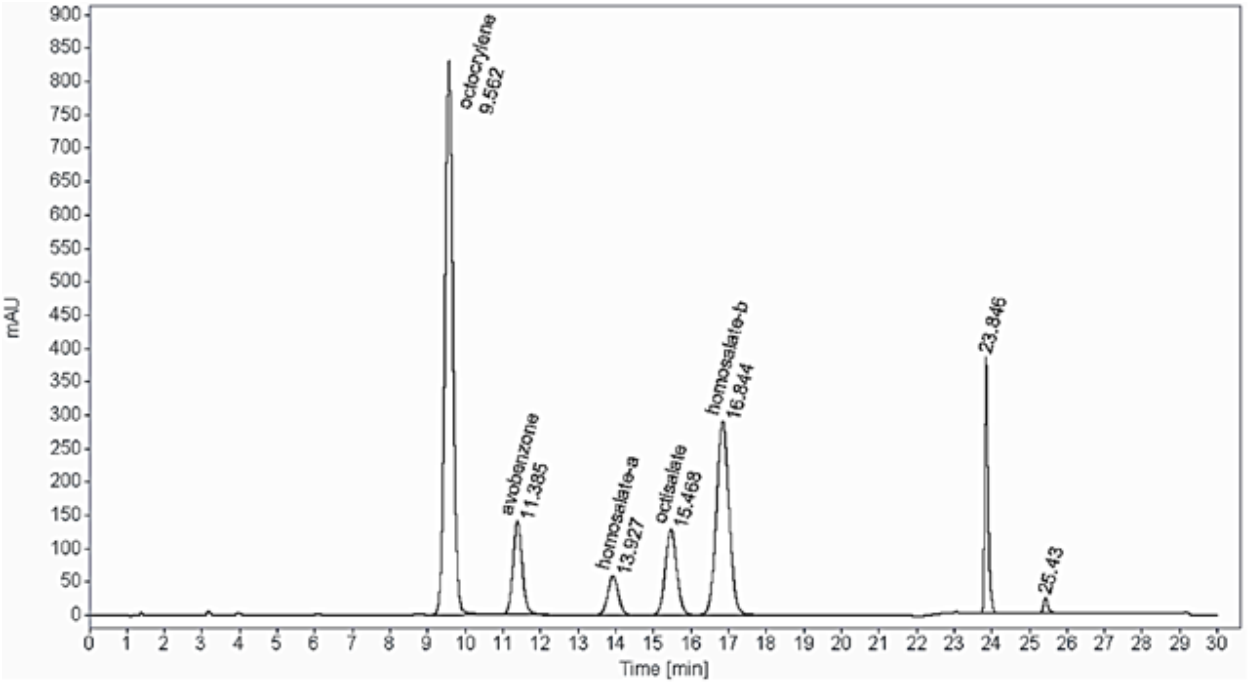


Figure 3. HPLC-DAD Chromatogram Sample (Lotion) Solution

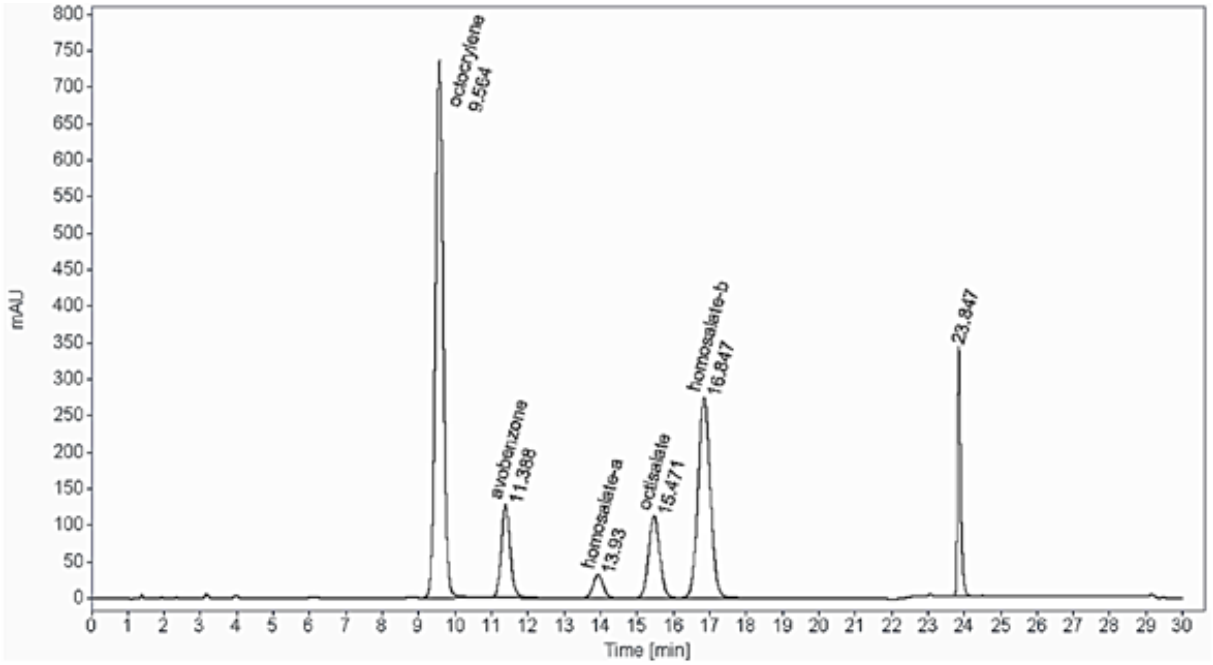
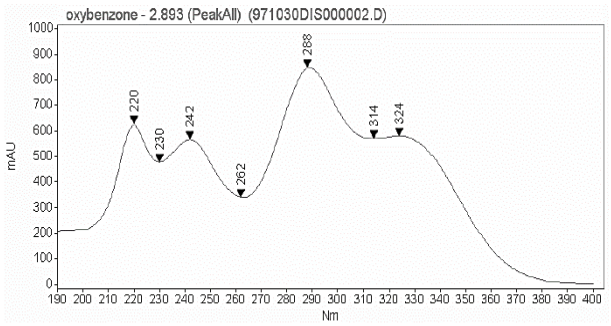
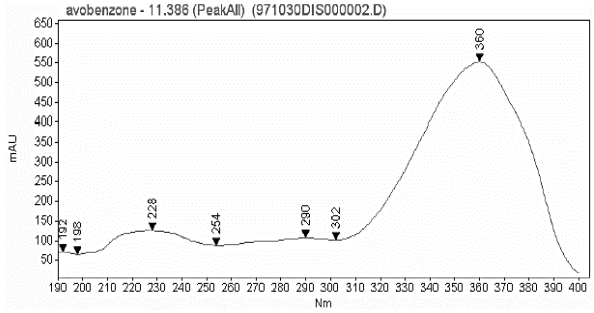
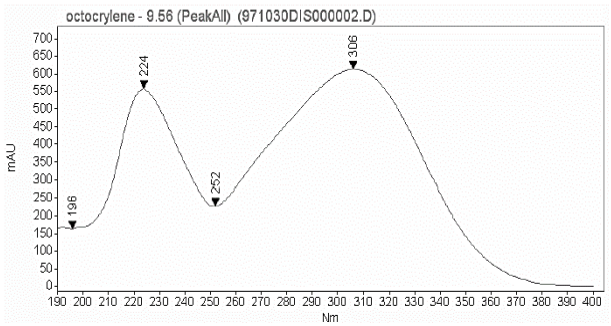
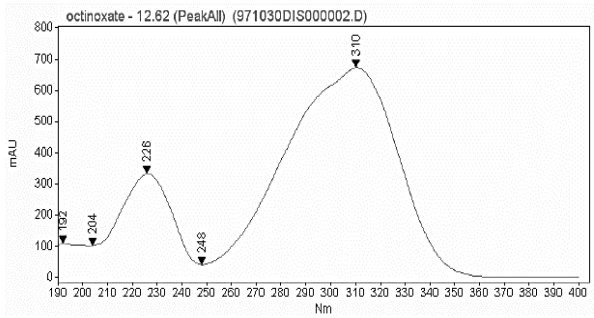


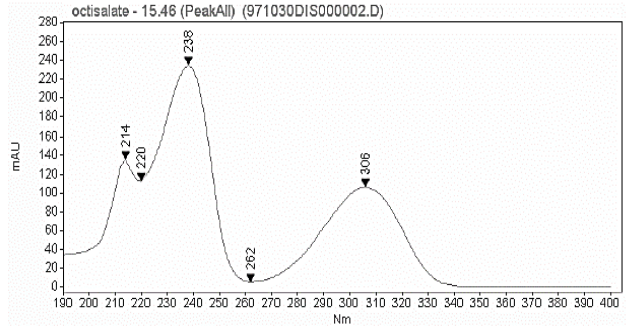
Figure 4. UV Spectral Data for Target Analytes Present in the Mixed Standard Solution

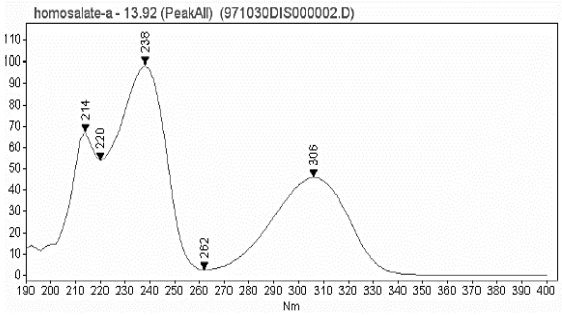


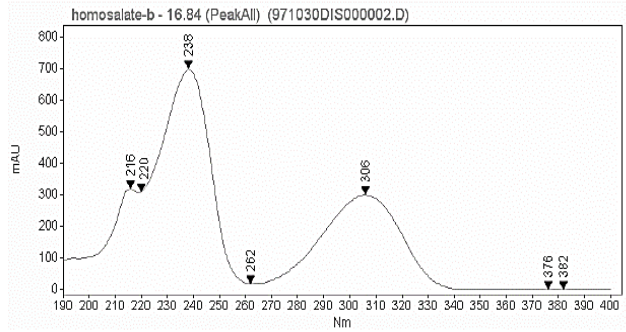












Method Validation

The method was validated using commercially purchased sunscreen lotion and sunscreen spray. System suitability was established using five consecutive injections of Mixed Standard Solution; results and acceptance criteria are noted in Table 7. Linearity was established using seven concentration levels, ranging from 10% – 250% of a target sample concentration. The linearity range was equivalent to 0.3 – 7.5% oxybenzone, 1 – 20% octocrylene, 0.3 – 7.5% avobenzone, 0.4 – 10% octinoxate, 0.5 – 12.5% octisalate, and 1.5 – 37.5% homosalate. LOQ was established at 0.3% oxybenzone, 0.8% octocrylene, 0.3% avobenzone, 0.4% octinoxate, 0.5% octisalate, and 1.5% homosalate, by calculating the %RSD of three replicate injections and verifying signal-to-noise (S/N) ratio ≥ 10 for each API. Precision and accuracy were evaluated using the spike recoveries of triplicate spiked matrix preparations at three different concentrations and calculating the %RSD. Specificity was determined by the absence of interfering peaks in the blank solution.

Table 7: System Suitability Results

%RSD peak area (n=5)

Sequence Date		Oxy.	Octo.	Avo.	Octinox.	Octis.	Homo.A	Homo.B	Criteria	Result
7/21/21	*%RSD*	0.08	0.08	0.11	0.08	0.09	0.12	0.10	≤ 2.0%	
	Tailing Factor	1.1	1.1	1.1	1.1	1.1	1.0	1.0	≤ 2.0	Pass
	*Resolution**	–	25.9	4.4	2.7	2.9	2.6	2.3	≥ 1.5	
7/23/21	*%RSD*	0.07	0.12	0.07	0.14	0.14	0.17	0.14	≤ 2.0%	
	Tailing Factor	1.1	1.0	1.1	1.1	1.1	1.0	1.0	≤ 2.0	Pass
	*Resolution**	–	25.8	4.4	2.7	2.9	2.6	2.3	≥ 1.5	
7/28/21	*% RSD*	0.09	0.08	0.10	0.08	0.08	0.07	0.08	≤ 2.0%	
	Tailing Factor	1.1	1.0	1.1	1.1	1.1	1.0	1.0	≤ 2.0	Pass
	*Resolution**	–	25.7	4.4	2.7	2.9	2.6	2.3	≥ 1.5	
8/12/21	*%RSD*	0.19	0.20	0.17	0.18	0.15	0.17	0.14	≤ 2.0%	
	Tailing Factor	1.1	1.0	1.1	1.0	1.0	1.0	1.0	≤ 2.0	Pass
	*Resolution**	–	26	4.5	2.7	2.9	2.7	2.4	≥ 1.5	

*Resolution to the preceding analyte peak in first mixed standard solution injection

Table 8: Linearity Results; coefficient of determination (R^2)

	Oxy.	Octo.	Avo.	Octinox.	Octis.	Homo.*	Criteria	Result
Eq. Conc. Range in Sample (%)	0.3 – 7.5	0.8 – 20	0.3 – 7.5	0.4 – 10	0.5 – 12.5	1.5 – 37.5		
Slope	55.4	45.6	31.0	109	20.1	19.7		
y-intercept	33.8	36.8	-15.1	-54.9	5.18	24.4		
R^2	0.999	0.999	0.999	0.999	0.999	0.999	≥ 0.995	Pass

*Sum of homosalate isomer peaks areas used for calculation

Table 9. Signal-to-Noise (S/N) of LOQ solution (10% of target sample concentration)

	Oxy.	Octo.	Avo.	Octinox.	Octis.	Homo.A	Homo.B	Criteria	Result
Eq.Conc. in Sample (%)	0.3	0.8	0.3	0.4	0.5	1.5			
Inj. #1	192.6	1637.5	361.9	1761.4	852.2	304.3	2035.4		
Inj. #2	186.6	1430.2	316.0	1538.2	817.6	291.8	1951.4	≥ 10	Pass
Inj. #3	188.3	1517.3	335.5	1632.0	667.8	238.0	1594.9		
Average	189.2	1528.3	337.8	1643.9	779.2	278.0	1860.6		

Table 10. %RSD for LOQ solution

	Oxy.	Octo.	Avo.	Octinox.	Octis.	Homo.A	Homo.B	Criteria	Result
Inj. #1	423.61	884.89	233.99	1176.47	254.76	83.13	676.29		
Inj. #2	423.08	884.54	233.69	1174.74	254.40	82.87	672.59		
Inj. #3	423.42	885.26	233.64	1175.32	254.31	82.82	673.82		
Ave	423.37	884.90	233.77	1175.51	254.49	82.94	674.24		
%RSD	0.06	0.04	0.08	0.08	0.09	0.20	0.28	≤ 2.0%	Pass

Table 11. Precision and Accuracy, % Recovery for Sunscreen Spray

Spike Level		Oxy.	Octo.	Avo.	Octinox.	Octis.	Homo.*	Criteria	Result
80%	Prep 1	100.4	100.8	98.1	99.7	100.7	100.6	95 – 105%	Pass
	Prep 2	99.7	99.2	96.5	99.0	99.2	99.3		
	Prep 3	100.1	100.1	97.1	99.3	100.1	100.0		
	Average	100.1	100.1	97.2	99.3	100.0	100.0		
	%RSD	0.33	0.78	0.82	0.37	0.73	0.67	≤ 3.0%	Pass
100%	Prep 1	100.0	99.8	98.2	99.4	99.8	99.8	95 – 105%	Pass
	Prep 2	101.0	101.2	99.9	100.4	101.3	101.2		
	Prep 3	100.3	100.3	98.4	99.7	100.4	100.4		
	Average	100.4	100.4	98.8	99.8	100.5	100.5		
	%RSD	0.55	0.73	0.96	0.54	0.77	0.71	≤ 3.0%	Pass
120%	Prep 1	100.1	100.4	98.9	99.7	100.5	100.4	95 – 105%	Pass
	Prep 2	100.5	100.7	98.6	100.1	100.6	100.5		
	Prep 3	100.1	99.2	98.4	99.6	99.7	99.6		
	Average	100.3	100.1	98.6	99.8	100.3	100.2		
	%RSD	0.23	0.80	0.25	0.25	0.47	0.48	≤ 3.0%	Pass

*Sum of homosalate isomer peaks areas used for calculation

Table 12. Precision and Accuracy, % Recovery for Sunscreen Lotion

Spike Level		Oxy.	Octo.	Avo.	Octinox.	Octis.	Homo.*	Criteria	Result
80%	Prep 1	99.9	100.9	100.3	99.3	100.7	100.7	95 – 105%	Pass
	Prep 2	100.4	100.2	100.2	99.8	100.5	100.5		
	Prep 3	100.2	100.5	100.6	99.7	100.9	100.8		
	Average	100.2	100.5	100.4	99.6	100.7	100.7		
	%RSD	0.23	0.34	0.16	0.24	0.22	0.18	≤ 3.0%	Pass
100%	Prep 1	99.3	98.2	98.9	98.8	98.8	98.8	95 – 105%	Pass
	Prep 2	101.0	100.8	101.6	100.6	101.2	101.2		
	Prep 3	99.7	99.4	100.1	99.3	100.0	99.9		
	Average	100.0	99.5	100.2	99.6	100.0	100.0		
	%RSD	0.93	1.30	1.34	0.96	1.22	1.21	≤ 3.0%	Pass
120%	Prep 1	100.3	100.6	101.4	99.9	101.0	100.8	95 – 105%	Pass
	Prep 2	99.7	99.4	100.0	99.4	99.6	99.6		
	Prep 3	100.0	99.8	100.9	99.7	100.5	100.3		
	Average	100.0	99.9	100.8	99.7	100.3	100.3		
	%RSD	0.27	0.57	0.69	0.26	0.69	0.61	≤ 3.0%	Pass

*Sum of homosalate isomer peaks areas usedin calculation

Analysis of Domestic Samples Using HPLC-DAD Validated Method

Using the validated method, analysis of six for-cause samples collected in a mission-critical assignment and sent to the Pacific Southwest Medical Products Laboratory (PSMPL) was conducted. Each sample was prepared in duplicate and tested according to the method to quantify the APIs. Accuracy was verified by spiking a portion of the sample preparations at 100% level and calculating the percent recovery.

Quantitative determination of APIs listed on the label yielded percent label claims between 96.0 – 102.3% (Table 13), and percent spike recovery between 99.5 – 102.3% for the six sunscreen samples. Percent label claims, and percent spike recoveries were within acceptable criteria ranges.

Table 13. Results of Domestic Samples Tested at PSMPL

Sample	**Oxybenzone**		**Octocrylene**		**Avobenzone**		**Octisalate**		**Homosalate**	
	Label Claim	% of Label Claim	Label Claim	% of Label Claim	Label Claim	% of Label Claim	Label Claim	% of Label Claim	Label Claim	% of Label Claim
Spray # 1	6 %	98.0	10 %	97.5	3 %	96.3	5 %	97.3	15 %	98.0
Spray # 2	6 %	98.2	4 %	97.2	3 %	97.3	5 %	96.5	15 %	97.9
Spray # 3	6 %	97.5	10 %	96.7	3 %	96.0	5 %	97.6	15 %	97.5
Spray # 4	-- *	-- *	10 %	100.8	3 %	100.5	5 %	100.8	10 %	100.9
Spray # 5	6 %	102.3	10 %	101.2	3 %	100.2	5 %	99.8	15 %	101.6
Lotion # 6	-- *	-- *	10 %	100.1	3 %	100.1	5 %	100.9	10 %	101.1

* Not listed as an active ingredient

CONCLUSION

The results of the method validation study demonstrate that the HPLC-DAD method for the analysis of oxybenzone, octocrylene, avobenzone, octinoxate, octisalate, and homosalate in sunscreen lotion and spray samples is specific, accurate, precise, and linear. LOQ was established at 0.3% oxybenzone, 0.8% octocrylene, 0.3% avobenzone, 0.4% octinoxate, 0.5% octisalate, and 1.5% homosalate.

The validated method was successfully used to quantify API in six sunscreen samples.

REFERENCES

1. Code of Federal Regulations Title 21, Volume 5, 21CFR352, “Sunscreen Drug Products for Over-the-Counter Human Use”. (online copy) https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=352&showFR=1
2. USP43-NF38 <1225> “VALIDATION OF COMPENDIAL PROCEDURES”
3. USP43-NF38 <2> section “ASSAY”