DIONEX

Application Note 223

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Determination of Ten Active Ingredients in Sunscreen-Containing Products in a Single Injection

INTRODUCTION

To prevent skin damage from the sun's radiation, many skin care products, such as lipstick, makeup, and lotions contain one or more compounds to block UV radiation. The lotions containing these compounds are commonly referred to as sunscreens and other products that contain one or more of these compounds are said to contain sunscreen. The active ingredients in sunscreens are usually aromatic compounds conjugated with carbonyl groups (e.g. homosalate) and rather than literally blocking the UV radiation, they absorb it and release it as lower-energy UV radiation. The FDA allows over 15 different compounds to be used as the active ingredient in sunscreens and sunscreen-containing products. Additional compounds have been approved for use in the European Union and other parts of the world.

In this Application Note (AN) we developed a separation of the following 10 compounds used in sunscreen: 2-phenyl-benzimidazole-5-sulfonic acid, benzophenone-3, diethylamino-hydroxybenzoylhexyl benzoate, 4-methylbenzylidine-camphor, octocrylene, methylanthranilate, octyl-methoxycinnamate, butyl-methoxydibenzoylmethane, octyl-salicylate, and homosalate. A manufacturer of sunscreen products chose these 10 compounds and requested baseline resolution of all 10 in a single injection.

Using a 3-µm Acclaim® 120 C18 column with an ethanol-containing mobile phase we were able to baseline resolve all 10 compounds in less than 12 min. This method successfully determined subsets of these 10 compounds in a lipstick, a cosmetic powder, and a lotion provided by the manufacturer. The Acclaim 120 C18 column paired with a Dionex UltiMate® 3000 system is an ideal platform for developing methods to determine sunscreen ingredients in a variety of products.

EQUIPMENT

Dionex UltiMate 3000RS chromatography system consisting of:

- SRD-3600 Solvent Rack with integrated vacuum degasser
- HPG-3400RS Binary gradient pump with 200 µL static mixer kit (P/N 6040.5150)
- WPS-3000RS split loop sampler with 100 µL sample loop
- TCC-3000RS Thermostatted column compartment
- DAD-3000RS Diode array detector

Chromeleon® Chromatography Data System, Version 6.80 SP5

REAGENTS AND STANDARDS

Deionized water (DI), Type I reagent grade, 18 MΩ-cm resistivity or better

Absolute ethanol (C_2H_5OH), AR grade (LAB-SCAN)

Methanol (CH_3OH), HPLC grade (LAB-SCAN)

Glacial acetic acid (CH_3COOH), AR grade (LAB-SCAN)

2-Phenyl-Benzimidazole-5-sulfonic acid (PHS)

Benzophenone-3 (B-3)

Diethylamino-hydroxybenzoylhexyl benzoate (DHHB)

4-Methylbenzylidine-camphor (4-MBC)

Octocrylene (OCR)

Methylanthranilate (MA)

Octyl-methoxycinnamate (OMC)

Butyl-methoxydibenzoylmethane (BMDM)

Octyl-salicylate (OS)

Homosalate (HMS)

CHROMATOGRAPHIC CONDITIONS

Column:	Acclaim 120 C18 3 µm, 4.6 × 100 mm (P/N 059132)
Eluent:	A: 0.8% Acetic acid B: Ethanol
Eluent Gradient:	25% B from -5 to 1 min, 25 to 80% from 1 to 1.5 min, and 80% B from 1.5 to 11.5 min
Flow rate:	0.7 mL/min
Column Temp.:	25 ºC
Inj. Volume:	5 µL
Detection:	UV, 310 and 354 nm, Wavelength scanning 250–600 nm
Backpressure:	2600–2900 psi

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent A

0.8% Acetic acid

Add approximately 100 mL deionized water to a 1000 mL volumetric flask, pipet 8 mL of glacial acetic acid to the same volumetric flask, bring to volume with deionized water, and mix.

Standards

Stock Standard Solutions

To prepare a 1000 mg/L stock standard for each of ten compounds, weigh 0.1 g of the compound, add to a 100 mL breaker, add 70 mL of methanol, and place in an ultrasonic bath for 10 min to ensure dissolution. Move this solution to a 100 mL volumetric flask and bring it to volume with methanol.

Working Standard Solutions

To prepare the five mixed standard solutions with analyte concentrations of 10, 25, 35, 50, and 75 mg/L, pipet 100, 250, 350, 500, and 750 µL of the individual stock standards into 100 mL volumetric flasks and bring to volume with methanol. Filter each standard with a 0.2 µm nylon filter prior to analysis.

Sample Preparation

Three products containing sunscreen compounds were provided by a customer. These products were a lotion, a lip balm, and a cosmetic powder. The customer also provided versions of these products without sunscreen compounds and, in this note, we refer to these as placebo products. Accurately weigh 0.1 g of sample and place in a 100 mL breaker. Add 70 mL of methanol, and place in an ultrasonic bath for 10 min to ensure dissolution. Move the sample solution to a 100 mL volumetric flask and bring to volume with methanol. Filter this sample solution with a 0.2 µm nylon filter prior to analysis.

RESULTS AND DISCUSSION

Separation

The 10 compounds in this study are all ideal candidates for reversed-phase chromatography with UV detection. A spectral scan of the ten compounds revealed that eight of them would be ideally detected at 310 nm and the other two at 354 nm. We chose the Acclaim 120 C18 column because it contains small-pore, high-purity, low-metal content silica with high C18 surface coverage (i.e. high carbon load), ideal for developing high resolution separations of compounds typically determined by reversed-phase chromatography. Using a methanol/acetic acid mobile phase we were unable to achieve a separation with all resolution factors 2.0 or greater.

Table 1. Resolution and Peak Purity of the Ten Sunscreen Ingredient Standards in an Injection of a Mixed Standard (35 mg/L) with Detection at 310 nm (Wavelength Scanning 250–600 nm for Peak Purity)

Compound	Resolution* (USP)	Match	RSD Match	PPI (nm)	RSD PPI
2-Phenyl-benzimidazole -5-sulfonic acid (PHS)	20.53	1000	0.02	279.6	0.01
Benzophenone-3 (B-3)	7.17	1000	0.11	284.4	0.04
Diethylamino-hydroxybenzoylhexyl-benzoate (DHHB)	3.64	1000	0.39	335.6	0.11
4-Methylbenzylidine-camphor (4-MBC)	3.07	1000	0.21	281.0	0.07
Octocrylene (OCR)	2.57	999	0.74	285.0	0.25
Methylanthranilate (MA)	2.93	976	5.56	313.5	0.88
Octyl-methoxycinnamate (OMC)	2.52	999	0.38	284.7	0.12
Butyl-methoxydibenzoylmethane (BMDM)	4.38	1000	0.20	332.9	0.06
Octyl-salicylate (OS)	3.32	986	3.37	283.8	0.59
Homosalate (HMS)	n.a.	995	1.22	285.5	0.20

* All values in this table were calculated by Chromeleon.

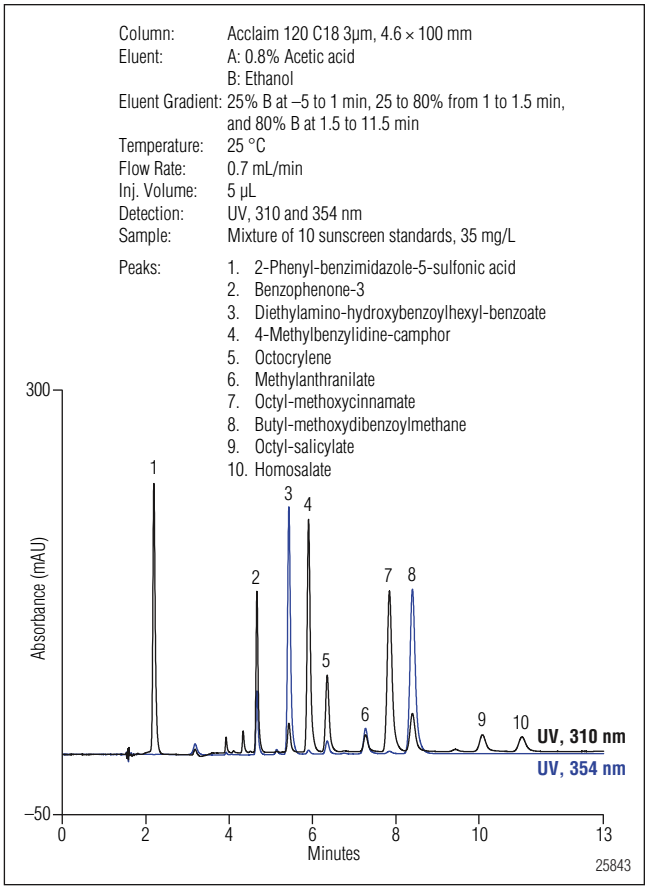

Figure 1. Chromatogram of a mix of 10 sunscreen ingredient standards with detection at 310 nm (Black) and 354 nm (Blue).

Switching to an ethanol/acetic acid mobile phase yielded the required separation (Figure 1). The resolution of all 10 components is greater than 2.5. Spectral matching of each peak compared to the spectral library (loaded by making single injections of a each of the standards) showed high purity of all ten peaks (Table 1). The low RSD of the peak purity index (PPI) of all ten peaks also indicates peak purity.

Table 2. Calibration Data from Chromeleon for 10 Sunscreen Ingredient Standards at 310 nm, Unless Otherwise Noted

Compound	Cal.Type	Points	R-Squared	Slope
PHS	Lin	5	0.9994	0.4408
B-3	Lin	5	0.9994	0.2347
DHHB	Lin	5	0.9983	0.0578
DHHB – 354 nm	Lin	5	0.9993	0.4991
4-MBC	Lin	5	0.9992	0.5007
OCR	Lin	5	0.9992	0.1876
MA	Lin	5	0.9994	0.0517
OMC	Lin	5	0.9992	0.5248
BMDM	Lin	5	0.9989	0.1352
BMDM- 354 nm	Lin	5	0.9993	0.6049
OS	Lin	5	0.9986	0.0678
HMS	Lin	5	0.9989	0.0681

Method Calibration

Before sample analysis, the 10 sunscreen compounds was separated at 5 concentrations: 10, 25, 35, 50, and 75 mg/L, and the data used to prepare a calibration curve that was forced through the origin. Table 2 displays the calibration data and shows a good linear fit for all ten compounds between 0 and 75 mg/L.

Sample Analysis

The manufacturer provided three products containing sunscreen compounds, a lipstick, a cosmetic powder, and a lotion. They also supplied the same products without added sunscreen compounds, referred to here as placebo products. We analyzed each of the placebo products after sample preparation to determine if there were any peaks from the sample that would interfere with sample analysis. None of the three products contained interfering compounds. Figure 2 shows the chromatogram of the cosmetic powder placebo. Chromatography of the lipstick and lotion placebos was indistinguishable from Figure 2.

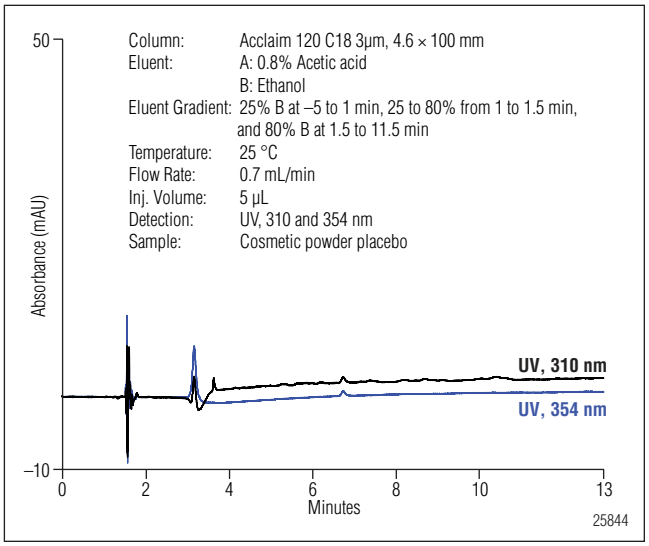

Figure 2. Chromatography of a cosmetic powder placebo with detection at 310 nm (Black) and 354 nm (Blue). The other 2 placebos yielded the same result.

To evaluate recovery, we spiked each placebo product with the 35 mg/L mixed standard. Table 3 shows that there was excellent recovery of all 10 compounds from each of the 3 samples, suggesting that this method is accurate for the determination of these compounds in the three products. Figure 3 shows chromatography of the lotion placebo product spiked with the 35 mg/L mixed standard. Chromatography of the spiked lipstick and cosmetic powder placebos was nearly identical to Figure 3.

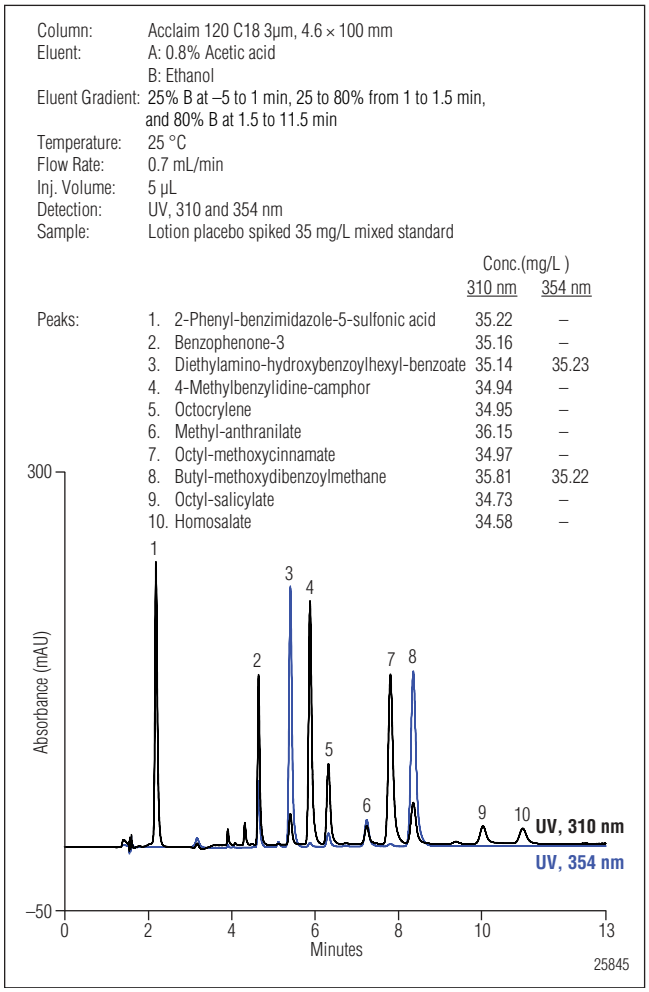

Figure 3. Overlay of three injections of a lotion placebo spiked with a 35 mg/L mixed standard with detection at 310 nm (Black) and 354 nm (Blue)

Finally we determined the amounts of the 10 sunscreen ingredients in 3 injections of each of the 3 products. The cosmetic powder sample was found to contain MA, OMC, and OS (Figure 4), the lotion sample contained PHS, B-3, 4-MBC, OMC, OS, and HMS (Figure 5), and the lipstick sample contained PHS, B-3 DHHB, 4-MBC, OMC, BMDM, OS, and HMS (Figure 6). Table 4 summarizes the amount of each sunscreen compound found in each sample.

Table 3. Recovery Results for the Spiked (35 mg/L) Cosmetic Powder, Lotion, and Lipstick Placebo Samples													
		Concentration (mg/L) Determined at 310 nm and 354 nm When Noted											
		PHS	B-3	DHHB	DHHB (354 nm)	4-MBC	OCR	MA	OMC	BMDM	BMDM (354 nm)	OS	HMS
Cosmetic Powder Placebo	Average*	35.07	36.05	35.80	36.00	35.76	35.82	37.25	35.89	34.52	33.94	37.45	35.46
	RSD	0.27	0.23	0.74	0.17	0.23	0.37	1.06	0.24	0.25	0.34	0.39	0.34
	%Recovery	100.2	103.0	102.3	102.9	102.2	102.3	106.4	102.5	98.60	97.00	107.0	101.3
Lotion Placebo	Average*	35.22	35.16	35.14	35.23	34.94	34.95	36.15	34.97	35.81	35.22	34.73	34.58
	RSD	0.11	0.02	0.12	0.12	0.01	0.03	0.60	0.03	0.09	0.02	0.22	0.14
	%Recovery	100.6	100.5	100.4	100.7	99.83	99.86	103.3	99.90	102.3	100.63	99.23	98.80
Lipstick Placebo	Average*	37.09	35.55	35.71	35.54	35.22	35.30	35.63	35.27	35.95	35.45	35.89	34.91
	RSD	0.07	0.08	0.12	0.13	0.12	0.12	0.74	0.14	0.26	0.30	0.04	0.39
	%Recovery	106.0	101.6	102.0	101.5	100.6	100.9	101.8	100.8	102.7	101.3	102.5	99.74

*Three injections were made of each sample.

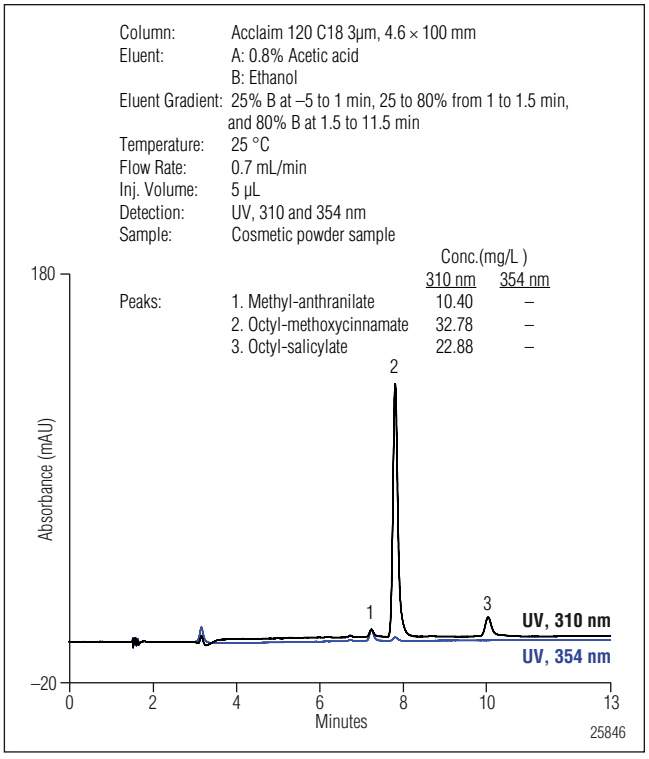

Figure 4. Overlay of three injections of the cosmetic powder sample with detection at 310 nm (Black) and 354 nm (Blue).

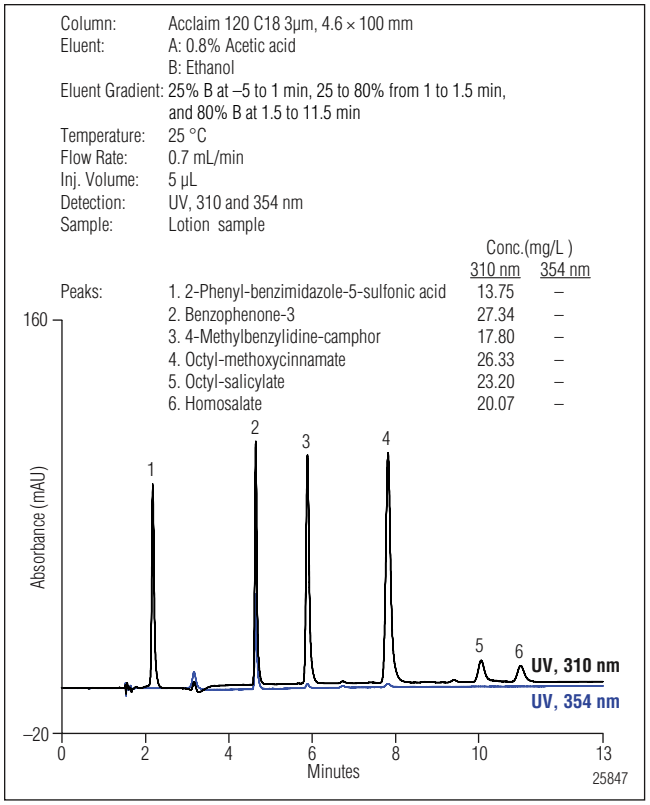

Figure 5. Overlay of three injections of the lotion sample with detection at 310 nm (Black) and 354 nm (Blue).

Table 4. Determination of Sunscreen Ingredients in Cosmetic Powder, Lotion, and Lipstick Samples													
		Concentration (mg/L) Determined at 310 nm and 354 nm When Noted											
		PHS	B-3	DHHB	DHHB (354 nm)	4-MBC	OCR	MA	OMC	BMDM	BMDM (354 nm)	OS	HMS
Cosmetic Powder Sample	Average*	N.A	N.A	N.A	N.A	N.A	N.A	10.40	32.78	N.A	N.A	22.88	N.A
	RSD	N.A	N.A	N.A	N.A	N.A	N.A	3.30	0.12	N.A	N.A	0.21	N.A
	%W/W	N.A	N.A	N.A	N.A	N.A	N.A	1.04	3.28	N.A	N.A	2.29	N.A
Lotion Sample	Average*	13.75	27.34	N.A	N.A	17.80	N.A	N.A	26.33	N.A	N.A	23.20	20.07
	RSD	0.74	0.20	N.A	N.A	0.08	N.A	N.A	0.08	N.A	N.A	0.17	0.19
	%W/W	1.38	2.73	N.A	N.A	1.78	N.A	N.A	2.63	N.A	N.A	2.32	2.01
Lipstick Sample	Average*	26.87	27.37	N.A	0.32	26.99	N.A	N.A	24.11	27.39	26.99	34.00	30.39
	RSD	0.33	0.32	N.A	2.15	0.15	N.A	N.A	0.31	0.50	0.28	0.68	0.56
	%W/W	2.69	2.74	N.A	0.03	2.70	N.A	N.A	2.41	2.74	2.70	3.40	3.04

*Three injections were made of each sample.

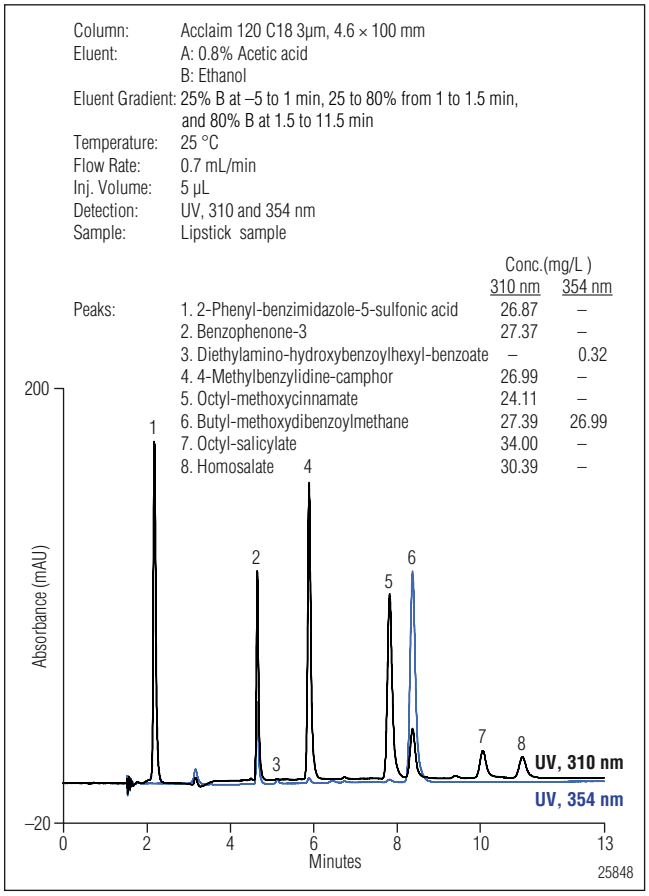

Figure 6. Overlay of three injections of the lipstick sample with detection at 310 nm (Black) and 354 nm (Blue).

CONCLUSION

This application note shows that 10 sunscreen compounds are baseline resolved in less than 12 min using an Acclaim 120 C18 column on an UltiMate 3000 system. This method accurately determines these compounds in a cosmetic power, a lotion and a lipstick.

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