Application Note

Pharma & Biopharma

Determination of UV Filters in Sunscreens Using Agilent Captiva EMR—Lipid Cleanup by HPLC

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Abstract

Chemical sunscreens are formulated with ultraviolet (UV)-filtering compounds that protect the skin from sunburn and DNA damage. This Application Note develops and validates a robust method to prepare samples followed by quantitative analysis of the active UV filter ingredients in suncare products. Active ingredients were extracted from sunscreen lotions by organic extraction followed by membrane filtration before HPLC analysis. While the retention time reproducibility of the reference standard analytes was excellent (% RSD ≤0.11), severe retention time drift, baseline shifting, and irregular peak shapes were observed among consecutive injections of sunscreen samples on the column. The chromatographic issues were resolved using Agilent Captiva EMR—Lipid cartridges to remove matrix lipids from the extracted samples. Acceptable levels of quantitative accuracy and recovery (95 to 103.5%) were achieved, with significantly improved retention time consistency for consecutive injections of all the UV filters present in seven over-the-counter suncare products.

Introduction

The active ingredients in chemical suncare products are FDA-approved aromatic compounds with high molar absorptivity in the UV range. These UV-filtering compounds are integrated with moisturizing agents, emulsifiers, and thickeners to produce a stable formula in which the active ingredients can be applied to and protect the skin from UV radiation.

Quantitative analytical testing is required to ensure that over-the-counter (OTC) suncare products provide broad spectrum protection and comply with federal regulations.[1] The complex matrix of sunscreen formulations and UV absorptivity of the active ingredients makes HPLC the current method of choice to ensure product consistency and quality. However, the matrix lipids present in these products contribute to poor chromatographic reproducibility, resulting in column contamination and unreliable method accuracy and precision. This Application Note discusses a simple sample preparation technique to improve chromatographic reproducibility and column longevity in the analysis of suncare products using Captiva EMR—Lipid cartridges.

Experimental

Chemicals and standards

HPLC grade water, HPLC grade isopropyl alcohol (IPA), and USP reference standards for five FDA-approved UV filters commonly used in the formulation of sunscreens[2] were purchased from Sigma-Aldrich (Table 1). A standard mix stock solution was prepared by combining 10 mg of each reference standard and dissolving in IPA to achieve a final concentration of 2 mg/mL per compound. This stock solution was used as the most concentrated calibration level (level 5), and also to prepare four serial dilutions of the stock to serve as intermediate calibration levels containing 1 (Level 4), 0.5 (Level 3), 0.25 (Level 2), and 0.125 mg/mL (level 1) per compound.

Consumables

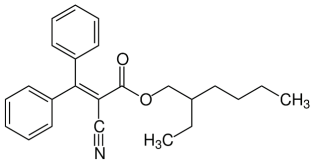
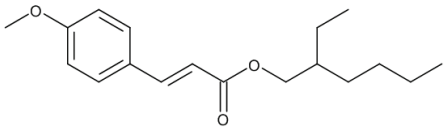
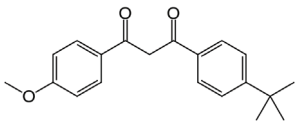
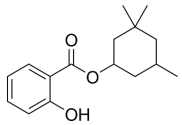
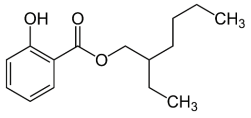
The following consumables were used for sample preparation:

- 15 mL centrifuge tubes (Celltreat p/n 229412)
- Agilent 6 mL Captiva EMR—Lipid cartridges (p/n 5190-1004)
- 10 mL NORM-JECT luer slip syringe (Henke Sass Wolf p/n 4100-000V0)
- Agilent Adapters: 1,3, and 6 mL (p/n 12131001)
- Agilent Premium 0.2 µm glass fiber/nylon syringe filters (p/n 5190-5132)
- Agilent 2 mL amber screw cap vials (p/n 5182-0716)
- Agilent Screw cap PTFE/red silicone septa (p/n 5190-7024)

Extraction and cleanup of UV filters from personal care products

An analytical scale was used to weigh 100 mg of sunscreen or lip balm directly into a 15 mL centrifuge tube. To begin the extraction process, 2 mL of hot water (85 to 95 °C) was added to the tube, and the sample was shaken vigorously and vortexed for two minutes. An aliquot of 10 mL of IPA was added to the tube, which was then vortexed for another two minutes and sonicated for 10 minutes. To promote further solvation and dispersion, the sample was vortexed and sonicated a second time in the same manner.

Table 1. Chemical properties of UV filter reference standards.

UV Filter	Structure
Name: Octocrylene Molecular formula: $C_{24}H_{27}NO_2$ Molecular wt. (g/mol): 361.48	
Name: Octinoxate Molecular formula: $C_{18}H_{26}O_3$ Molecular wt. (g/mol): 290.40	
Name: Avobenzone Molecular formula: $C_{20}H_{22}O_3$ Molecular wt. (g/mol): 310.39	
Name: Homosalate Molecular formula: $C_{16}H_{22}O_3$ Molecular wt. (g/mol): 262.34	
Name: Octisalate Molecular formula: $C_{15}H_{22}O_3$ Molecular wt. (g/mol): 250.33	

To facilitate separation of the liquid and solid phases, the sample was centrifuged for 15 minutes at 2,500 rpm. The pellet was discarded and approximately half of the supernatant was carefully transferred into a 6 mL Agilent Captiva EMR—Lipid cartridge. An adapter cap was secured to the top of the cartridge, and a 10 mL luer slip syringe was used to apply positive pressure to the sample. The sample mixture was passed through the cartridge with low pressure at a flow rate of three to five seconds per drop. The eluent was collected into a clean 15 mL conical centrifuge tube. The rest of the sample was transferred to the EMR cartridge after the first half flowed through. Particles were removed by filtering the sample through an Agilent premium 0.2 µm glass fiber/nylon syringe filter directly into a 2 mL amber glass vial.

Instrument configuration

An Agilent 1260 Infinity II LC system controlled by Agilent ChemStation software (rev. C.01.09) and equipped with a quaternary pump, a vacuum degasser, an autosampler, a column heat exchanger, and a diode array detector (DAD) was used for the analysis. An Agilent InfinityLab Poroshell 120 EC-C18 column (3.0 mm × 50 mm, 2.7 µm) and InfinityLab Poroshell 120 EC-C18 guard column (2.1 mm × 5 mm, 2.7 µm) were used for chromatographic separation. The injection volume was 1 µL, and the needle was washed with IPA three times between injections. The isocratic mobile phase, flow rate, and column compartment temperature used for analyte separation are proprietary. The DAD was used at 238 nm wavelength for data collection. The analysis stop time was 11 minutes. A blank run without injection was performed after each sample analysis during which the column was washed with 95% IPA and re-equilibrated with the mobile phase before the next injection.

Results and discussion

System suitability of standards

The method provided excellent baseline resolution of the reference standard mix, shown in Figure 1. The negative peak observed at 0.78 minutes is an artifact of the injection. The peak observed at 1.92 minutes is an impurity in the avobenzone standard, as reported previously.[3] The six prominent peaks observed between 4.92 to 9.87 minutes are the standard analytes identified in the order of elution in the figure:

The retention time, peak width (half height), peak symmetry, USP tailing factor, and resolution values confirm the excellent baseline separation of the six components of the reference standard mix (Table 2). Figure 2 displays a chromatographic overlay of six replicate injections of the standard mix. The method provided outstanding reproducibility for retention time, with % RSD of 0.06 to 0.11, and peak area, with % RSD <0.41 for all six reference standard peaks.

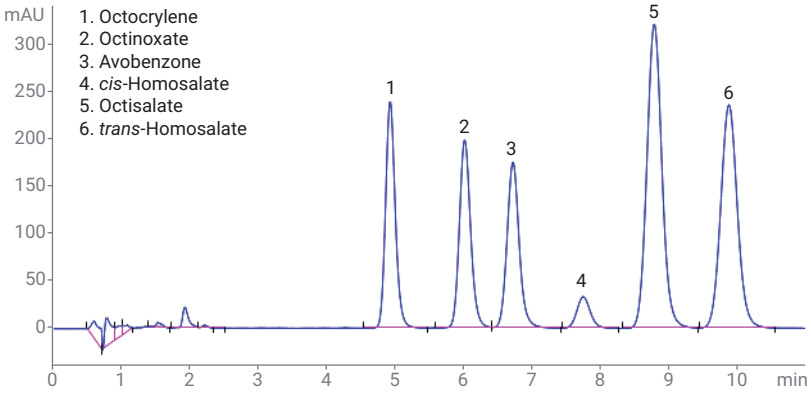


Figure 1. Chromatographic elution profile of UV filters in the reference standard mix.

Table 2. Retention time (minutes), peak width (half height), peak symmetry, USP tailing factor, and resolution values of the six analytes in an injection of the reference standard mix.

Peak No.	Analyte Name	Retention Time	Half Peak Width	Symmetry	USP Tail	Resolution
1	Octocrylene	4.922	0.150	0.85	1.158	NA
2	Octinoxate	6.011	0.174	0.85	1.154	3.97
3	Avobenzone	6.716	0.186	0.90	1.121	2.31
4	*cis*-Homosalate	7.742	0.210	0.86	1.105	3.06
5	Octisalate	8.779	0.234	0.93	1.082	2.76
6	*trans*-Homosalate	9.870	0.260	0.94	1.074	2.61

Linearity

A linearity calibration curve was generated for each UV filter by plotting peak area against the corresponding concentration of the reference standard mix from 0.125 to 2 mg/mL. Figure 3 shows the linearity curves and corresponding correlation coefficients (R^2) of the five UV filters. The R^2 values of all five linearity curves were >0.999, indicating a linear relationship between peak area and concentration of each reference standard within the calibration range.

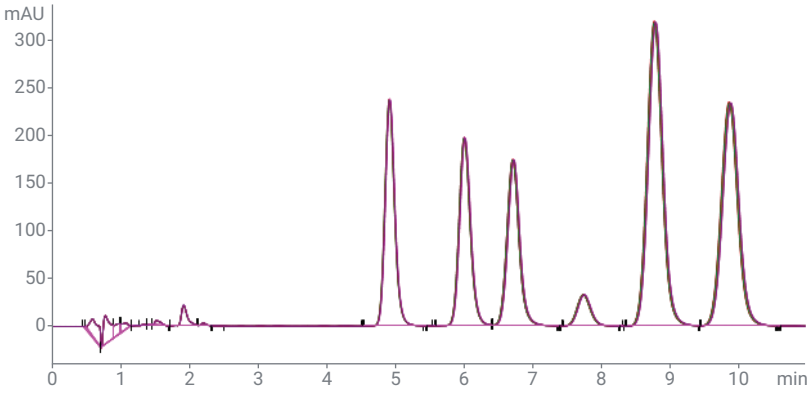


Figure 2. Chromatographic overlay of six replicate injections of the reference standard mix.

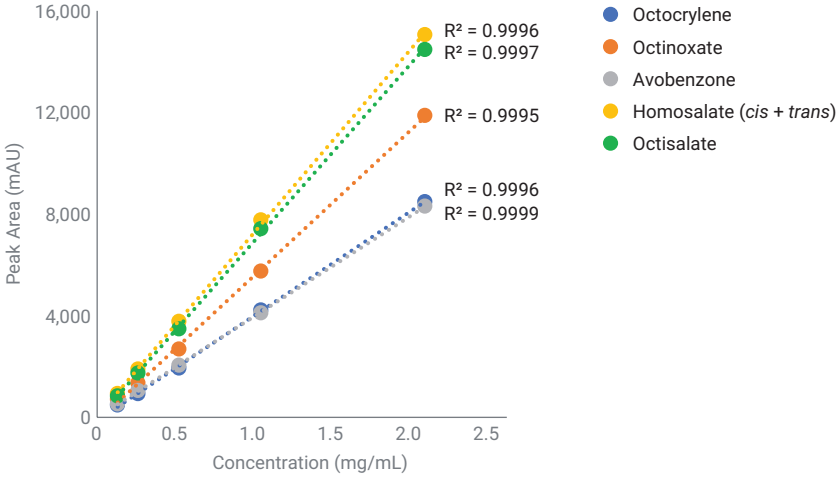


Figure 3. Calibration curves linearity and correlation coefficients of UV filter reference standards.

OTC sun care product sample chromatography without Captiva EMR—Lipid cleanup

The active ingredients were extracted from two commercially available OTC sunscreen lotions (sunscreens A and B), filtered through 0.45 µm nylon membrane syringe filters (Sigma-Aldrich), and injected onto the column without further cleanup. The sunscreen samples are overlaid with the reference standard mix in Figures 4 and 5. The sunscreen A extracted sample without cleanup in the sequence displayed all six expected peaks at the same retention times as the reference standard mix. However, with more consecutive injections of sunscreen A extracted samples without cleanup run on the system, the chromatograms started to exhibit more obvious retention time drift (Figure 4), suggesting accumulated column contamination even with the high organic washes between injections.

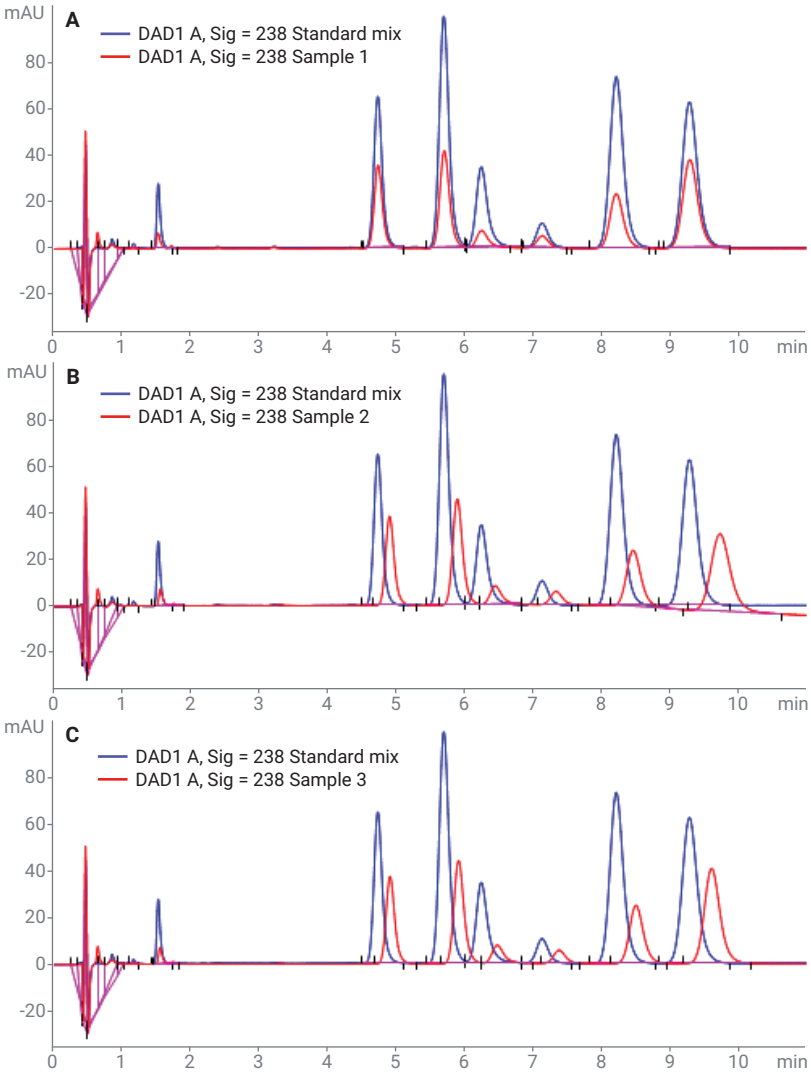


Figure 4. Sequential injections of three sunscreen A extracted samples without cleanup overlaid with the reference standard mix.

The same problem was observed among the three sunscreen B extracted samples without cleanup that were injected immediately after the sunscreen A samples (Figure 5). Exchanging the guard column and backwashing the analytical column slightly corrected the retention time shifting at first, but other severe chromatographic issues, including irregular peak shapes and baseline shifting, became more pronounced with each subsequent injection of the standard mix (Figure 6). The column became unusable after just 200 injections. Interestingly, the majority (97%) of samples injected on the column were neat standards, indicating that only six injections of matrix sample without cleanup (Figures 4 and 5) had completely damaged the analytical column.

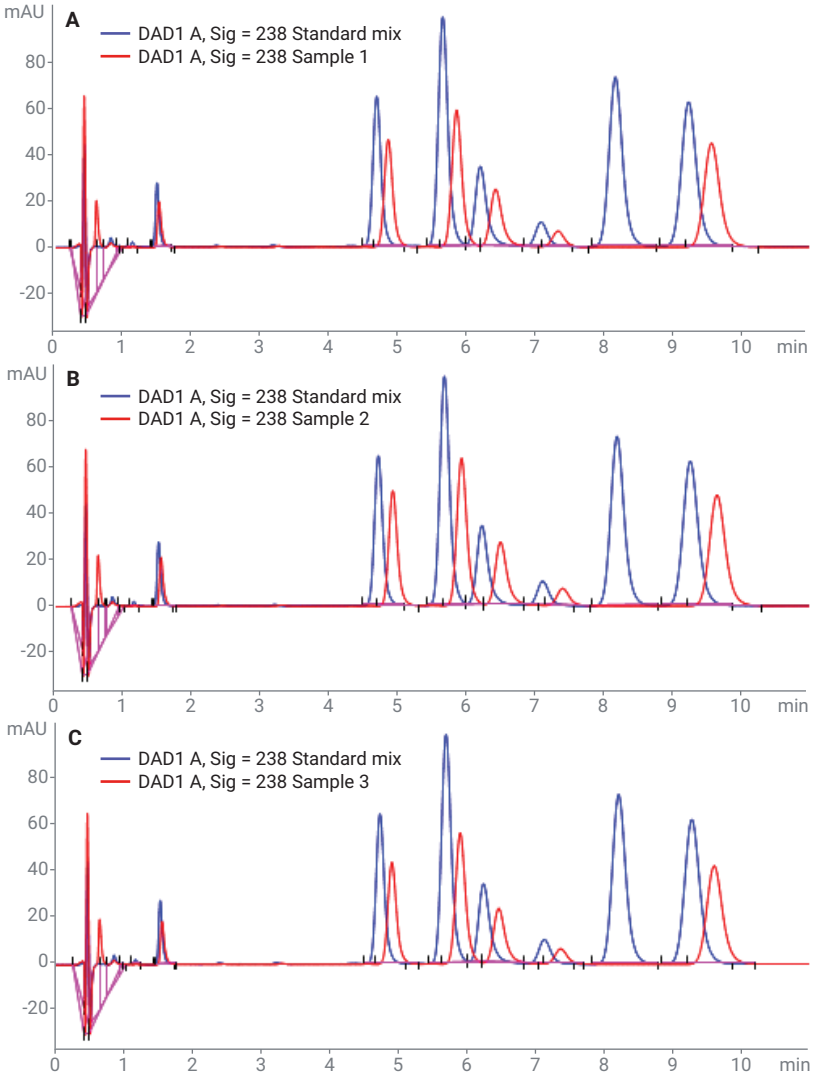


Figure 5. Sequential injections of three sunscreen B extracted samples without cleanup overlaid with the reference standard mix.

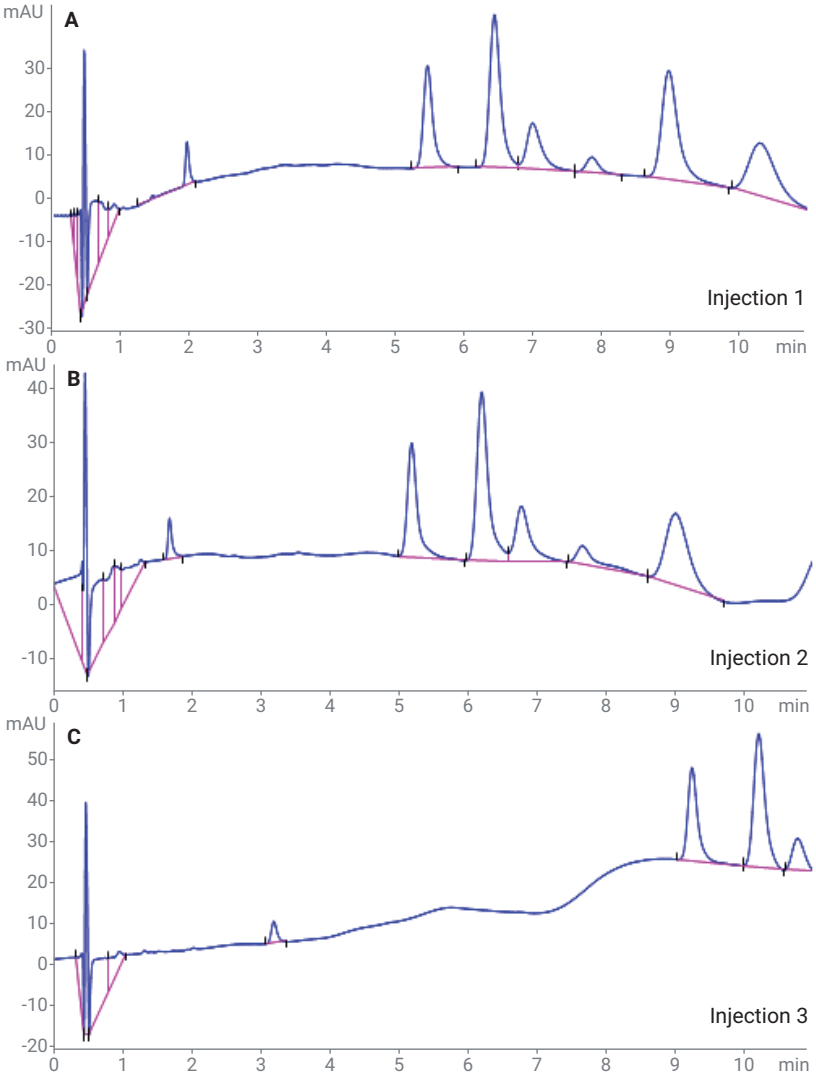


Figure 6. Sequential injections of the reference standard mix after six sunscreen sample injections.

Chromatography improvement provided by Captiva EMR—Lipid cleanup after extraction

To resolve the significant chromatographic deterioration resulting from complex sample matrix, Captiva EMR—Lipid cleanup was used after sample extraction. The active ingredients were extracted from six commercially available OTC sunscreen lotions and an OTC lip balm. Each product was formulated with two to five active UV filters. A Captiva EMR—Lipid cartridge was used to further clean the crude sample extract to remove the matrix lipids. A new analytical column and guard column were installed, and each product was extracted in triplicate and analyzed with duplicate injections. Figure 7 shows representative chromatograms of samples extracted from each product (A to G) with Captiva EMR—Lipid cleanup. Based on the active ingredients present in each OTC formula, all the samples displayed the expected peaks with retention times consistent with the respective standards and peak areas within the calibration range. Neither retention time drift nor other chromatographic issues were observed throughout six consecutive injections of each replicate from the seven different product samples injected onto the column.

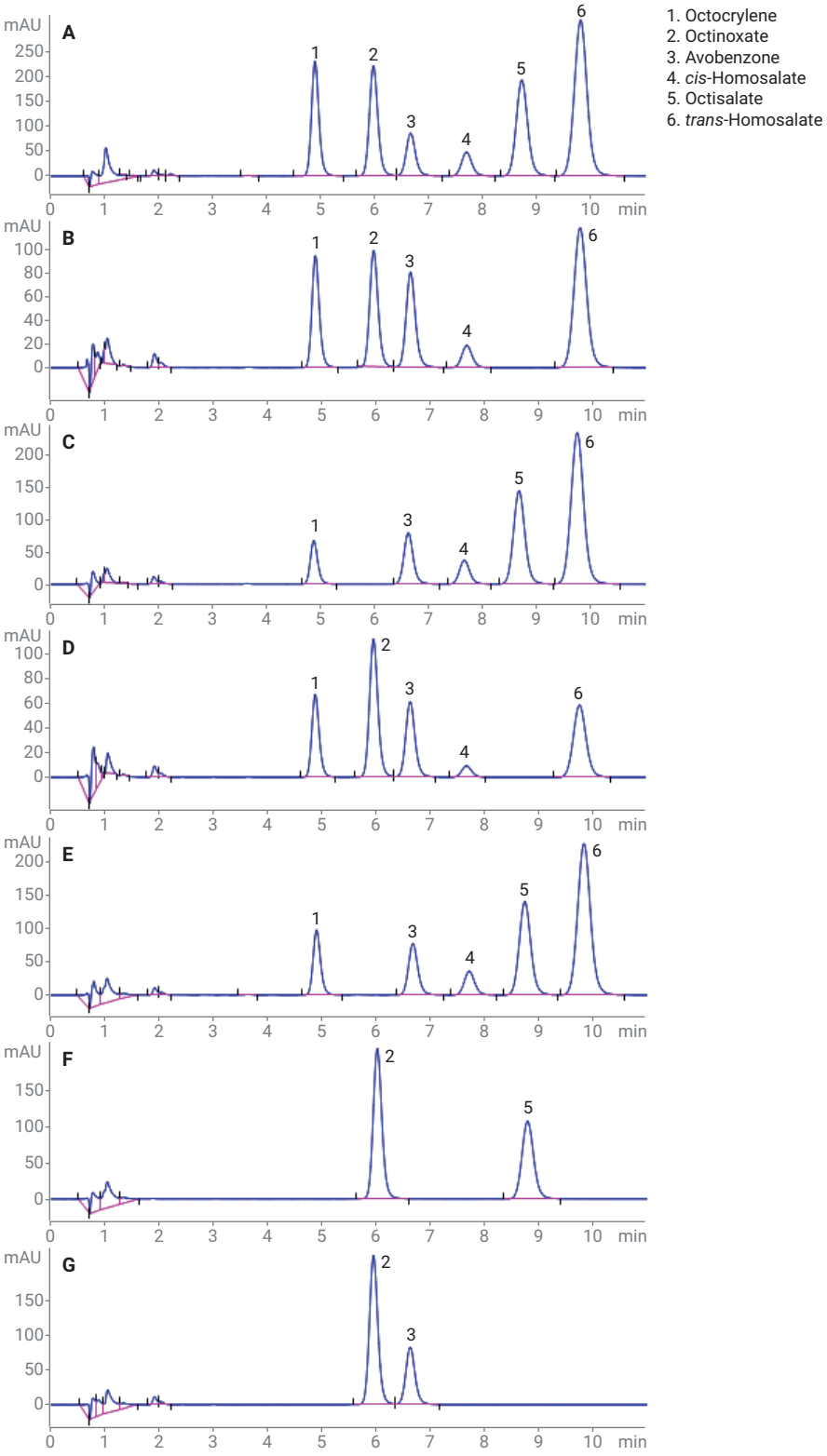


Figure 7. Representative six overlapped chromatograms of OTC sunscreen lotion (A, B, C, D, E, and F) and lip balm (G) samples.

Table 3 shows the quantitative results of the OTC sample analysis. The % recovery of active UV filter ingredients in all 42 sample injections ranged from 95.0 to 103.5%, and the RSD for recovery was ≤2.19%. The average recovery of each UV filter among the seven products ranged from 95.1 to 101.1%. Since the quantitative results for all product sample replicates were within the acceptable confidence range (95 to 105% recovery), this analytical method meets the acceptance criteria for accuracy and recovery for all products tested. The same column has been used to perform over 600 injections and to measure acceptable levels of UV filter recovery in 150 OTC product samples, maintaining high-quality chromatography.

Table 3. Average recovery (%), standard deviation, and % RSD of UV filters extracted from seven different OTC product samples after Captiva EMR—Lipid cleanup (n = 6 per product).

Sunscreen lotion A	**Octocrylene**	**Octinoxate**	**Avobenzone**	**Homosalate**	**Octisalate**
Average Recovery (%)	98.67	97.63	100.22	98.92	97.63
Standard Deviation	2.16	1.94	2.20	2.12	1.97
% RSD	2.18	1.99	2.19	2.15	2.02
Sunscreen lotion B	**Octocrylene**	**Octinoxate**	**Avobenzone**	**Homosalate**	
Average Recovery (%)	97.25	97.71	99.39	100.46	
Standard Deviation	1.58	1.47	1.48	1.62	
% RSD	1.63	1.50	1.49	1.62	
Sunscreen lotion C	**Octocrylene**	**Avobenzone**	**Homosalate**	**Octisalate**	
Average Recovery (%)	98.55	97.5	95.58	100.13	
Standard Deviation	0.60	0.62	0.58	0.49	
% RSD	0.60	0.64	0.61	0.49	
Sunscreen lotion D	**Octocrylene**	**Octinoxate**	**Avobenzone**	**Homosalate**	
Average Recovery (%)	99.46	98.76	100.14	101.01	
Standard Deviation	0.72	1.26	1.35	1.48	
% RSD	0.73	1.28	1.34	1.46	
Sunscreen lotion E	**Octocrylene**	**Avobenzone**	**Homosalate**	**Octisalate**	
Average Recovery (%)	100.46	100.28	99.04	100.63	
Standard Deviation	1.52	1.82	1.17	0.95	
% RSD	1.51	1.81	1.19	0.94	
Sunscreen lotion F	**Octinoxate**	**Octisalate**			
Average Recovery (%)	98.18	95.25			
Standard Deviation	0.26	0.20			
% RSD	0.26	0.21			
Lip Balm	**Octinoxate**	**Avobenzone**			
Average Recovery (%)	95.68	99.52			
Standard Deviation	0.70	0.71			
% RSD	0.73	0.72			

Conclusion

This Application Note demonstrates the challenges of matrix effects on the chromatography in analytical testing of sunscreen products. Agilent Captiva EMR—Lipid cleanup selectively removed the matrix lipids from the crude extracts of six different sunscreen lotions and a lip balm without interfering with the recovery of UV-filtering agents. Acceptable levels of quantitative accuracy and recovery were achieved for the active ingredients in all product samples tested. Adding Captiva EMR—Lipid cleanup to the sample preparation process enabled the sequential, robust analysis of multiple sunscreen and lip balm samples on the same column and increased the total number of effective injections by >200%. This method can be used to improve experimental efficiency, data reproducibility, and cost-effectiveness of OTC testing in cosmetic research, product development, and quality control.

References

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