APPLICATION NOTE

Liquid Chromatography

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The Analysis of Sunscreen-Active Ingredients and Parabens in Lotions and Lip Balms by UHPLC with PDA Detection

Introduction

Individuals typically use 5-20 cosmetics per day, many of which contain sunscreen to prevent skin damage from the sun's radiation, and antimicrobial preservatives called parabens.

Although sunscreen-active ingredients are designed to block UV radiation, some cell damage may be caused when these ingredients are illuminated by sunlight after absorption into the skin.[1,2] For example, oxybenzone, an ingredient considered safe by the FDA (Food and Drug Administration), is believed to contribute to the recent rise in melanoma cases by increasing the production of DNA-attacking free radicals upon UV exposure.[3] Additionally, studies have shown oxybenzone to behave similarly to the hormone estrogen, suggesting that it may also contribute to the development of breast cancer.[4]

Parabens are absorbed through the skin via cosmetic applications and can be found in nearly all adult urine samples, with the highest concentrations observed in adult females and adolescents.[5] Furthermore, parabens are thought to have estrogenic activity, which affects the expression of genes regulated by the natural form of estrogen, leading to early puberty in girls and an increased risk for the development of breast cancer. While some parabens are banned in European countries, in the U.S., the FDA maintains that they are safe in cosmetic products at levels up to 25%.[6]

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Since the usage of cosmetics formulated with parabens and sunscreen-active ingredients comes with the above mentioned health concerns, it is important for the skincare industry to develop robust analytical methods to maintain compliance regarding safety regulations. With this in mind, this application provides the UHPLC method conditions and performance data for the analysis of parabens and sunscreen-active ingredients found in variations of three cosmetic formulations. Performance data includes repeatability, linearity and LOD/LOQ determination.

Experimental

Hardware/Software

A PerkinElmer Altus™ UPLC® system was used, including the A-30 Sampling and Solvent Delivery Module (quaternary pump), Column Module and A-30 PDA (photodiode array) detector (PerkinElmer, Shelton, CT, USA). A PerkinElmer Brownlee™ SPP C18 2.7-µm 50 x 2.1-mm column was used for all determinations (PerkinElmer, Shelton, CT, USA). All instrument control, analysis, and data processing was performed via Waters® Empower® 3 chromatography data software (CDS).

Method Parameters

The HPLC method parameters are shown in Table 1.

Table 1. HPLC Method Parameters.

HPLC Conditions	
Column:	PerkinElmer Brownlee™ SPP C18 2.7 µm, 50 x 2.1 mm column (Part # N9308402)
Mobile Phase Gradient:	Solvent A: 0.2% acetic acid in water Solvent B: 50/50 methanol/ethanol

	Time (min)	Flow Rate (mL/min)	%A	%B	%C	%D	Curve
1	Initial	0.500	70.0	30.0	0.0	0.0	Initial
2	2.00	0.500	20.0	80.0	0.0	0.0	6
3	6.00	0.500	5.0	95.0	0.0	0.0	6
4	8.00	0.500	5.0	95.0	0.0	0.0	6
5	8.10	0.500	70.0	30.0	0.0	0.0	6

Injection delay time between injections: 10 min.

Detector:	Altus A-30 PDA; wavelength: 254 nm
Oven Temp.:	30 °C
Injection Volume:	1 µL
Sampling (Data) Rate:	10 pts/sec
Pressure (max.):	~4500 psi

Solvents, Standards and Samples

All solvents and diluents used were HPLC grade.

All stock paraben and sunscreen ingredient standards were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO). Parabens included methylparaben, ethylparaben and propylparaben. Sunscreen ingredients included octocrylene, homosalate, octinoxate, avobenzone, octisalate, and oxybenzone.

The following cosmetic samples were obtained from a local drug store: 1) a sunscreen lotion; 2) a skin moisturizer formulated with sunscreen; 3) a lip balm formulated with sunscreen; 4) a skin moisturizer without sunscreen; and 5) a lip balm without sunscreen.

All standard and sample dilutions were prepared in methanol as a diluent, which was also used for all blank injections. Paraben and sunscreen standard solutions were prepared at 1 mg/mL (1000 ppm), followed by a dilution to 1 µg/mL and 0.1 mg/mL, respectively. The latter were used as working stock standard solutions. For sample solutions, 200 mg of each sample was first added to a tared 50-mL volumetric flask. The flask was then filled to mark with diluent and sonicated for 15 minutes.

Prior to injection, all standards and samples were filtered through a 0.22-µm Nylon filter to remove any residual particles.

Results and Discussion

Figure 1 shows the peak profile of three paraben and six sunscreen standards at 254 nm. All paraben and sunscreen-active ingredients are well separated, with the exception of octinoxate and avobenzone. However, it is unlikely that these two components are present in the same sunscreen product, as octinoxate is highly susceptible to photolysis when formulated with avobenzone.[7]

Though not further characterized, two isomers, labeled *Isomer 1* and *Isomer 2,* eluted at 2.8 min and 4.5 min. These were associated with avobenzone and homosalate, respectively.[8,9]

As shown in Figure 2, chromatographic repeatability was confirmed via ten injections of the sunscreen lotion. For the paraben and sunscreen-active ingredients present, retention time repeatability ranged between 0.13 and 0.33 % RSD.

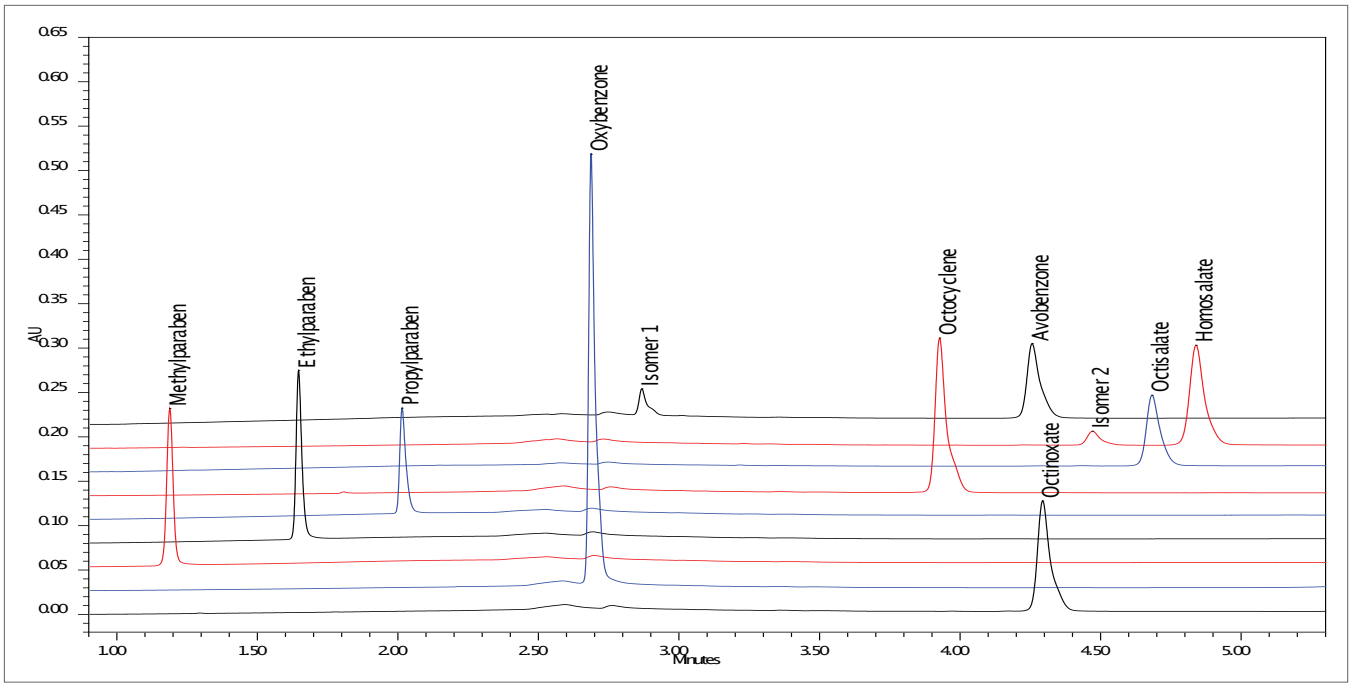

Figure 1. UHPLC chromatogram of a standard mix of three parabens and six sunscreen-active ingredients. Isomer 1 and Isomer 2 are associated with avobenzone and homosalate, respectively.

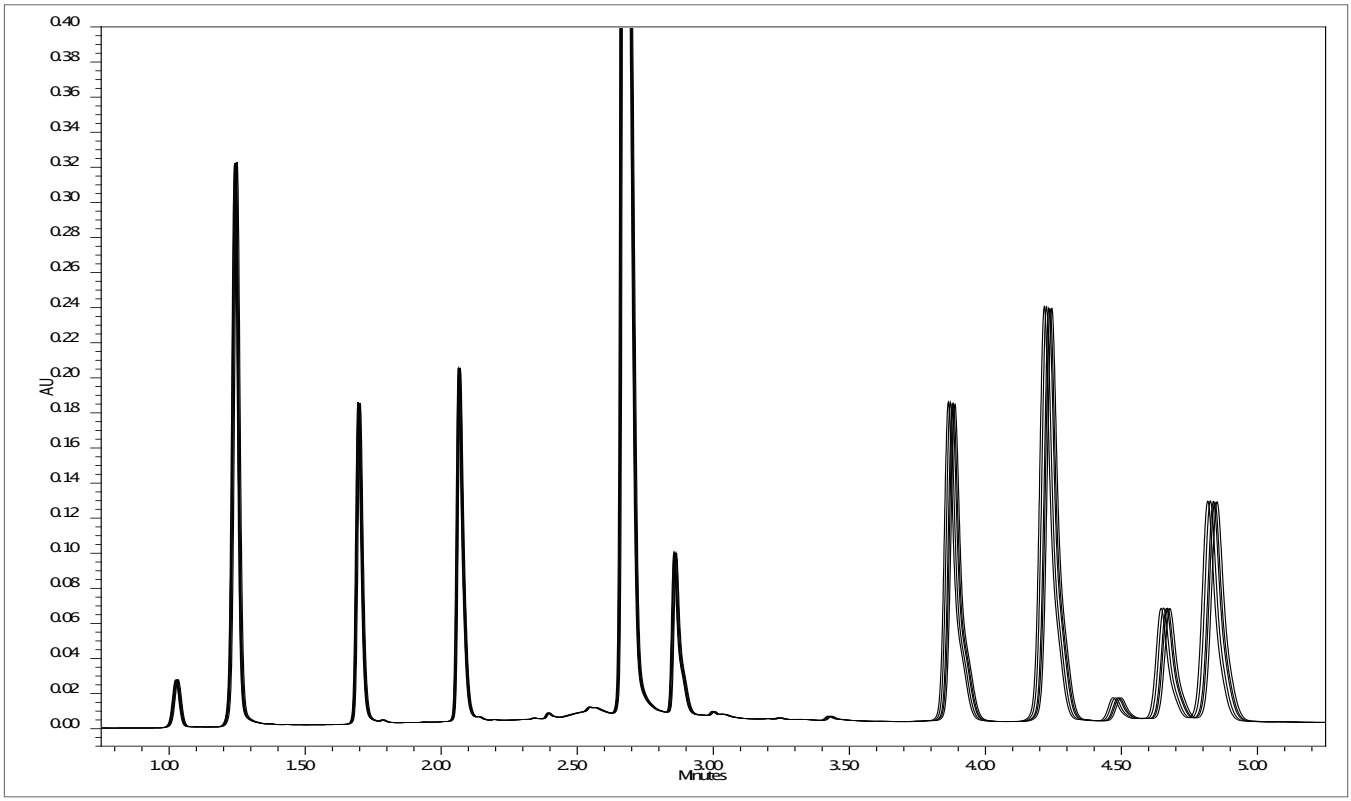

Figure 2. Chromatographic repeatability of sunscreen lotion; wavelength: 254 nm.

Using ethylparaben and oxybenzone as representative examples, Figures 3 and 4 show the respective linearity results. The concentration ranges were 0.5-5 µg/mL and 0.055-0.55 mg/mL, respectively; the concentration range reflecting the levels most commonly found in U.S.-marketed products for each component.[10,11]

Table 2 lists the LOQ and LOD levels that were obtained for four typical paraben and sunscreen components. These levels were based upon the indicated minimum signal-to-noise (s/n) limits.

Table 2. Calculated LOQ and LOD levels for ethylparaben, propylparaben, oxybenzone and octinoxate at 254 nm.

Component	LOQ (µg/mL (ppm)) (s/n ≥ 10/1)	LOD (µg/mL (ppm)) (s/n ≥ 3/1)
Ethylparaben	0.009	0.003
Propylparaben	0.012	0.004
Oxybenzone	0.402	0.121
Octinoxate	0.303	0.091

A comparison of lip balms formulated with and without sunscreen is shown in Figure 5. Methylparaben and propylparaben were present in both formulations, while, as expected, oxybenzone and octinoxate were only present in the lip balm formulated with sunscreen.

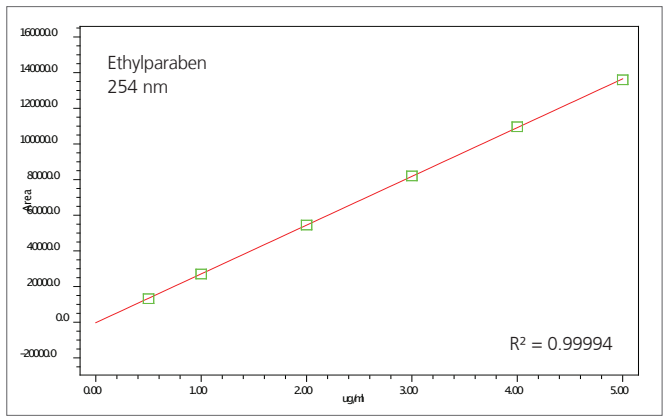

Figure 3. Linearity plot of ethylparaben; concentration range: 0.5-5 µg/mL; wavelength: 254 nm.

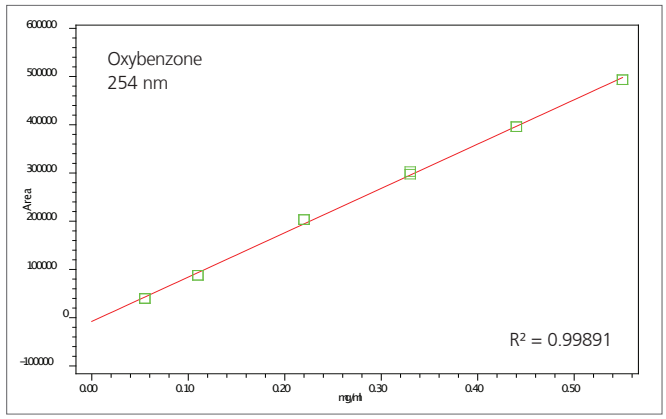

Figure 4. Linearity plot of oxybenzone; range: 0.055-0.55 mg/mL; wavelength: 254 nm.

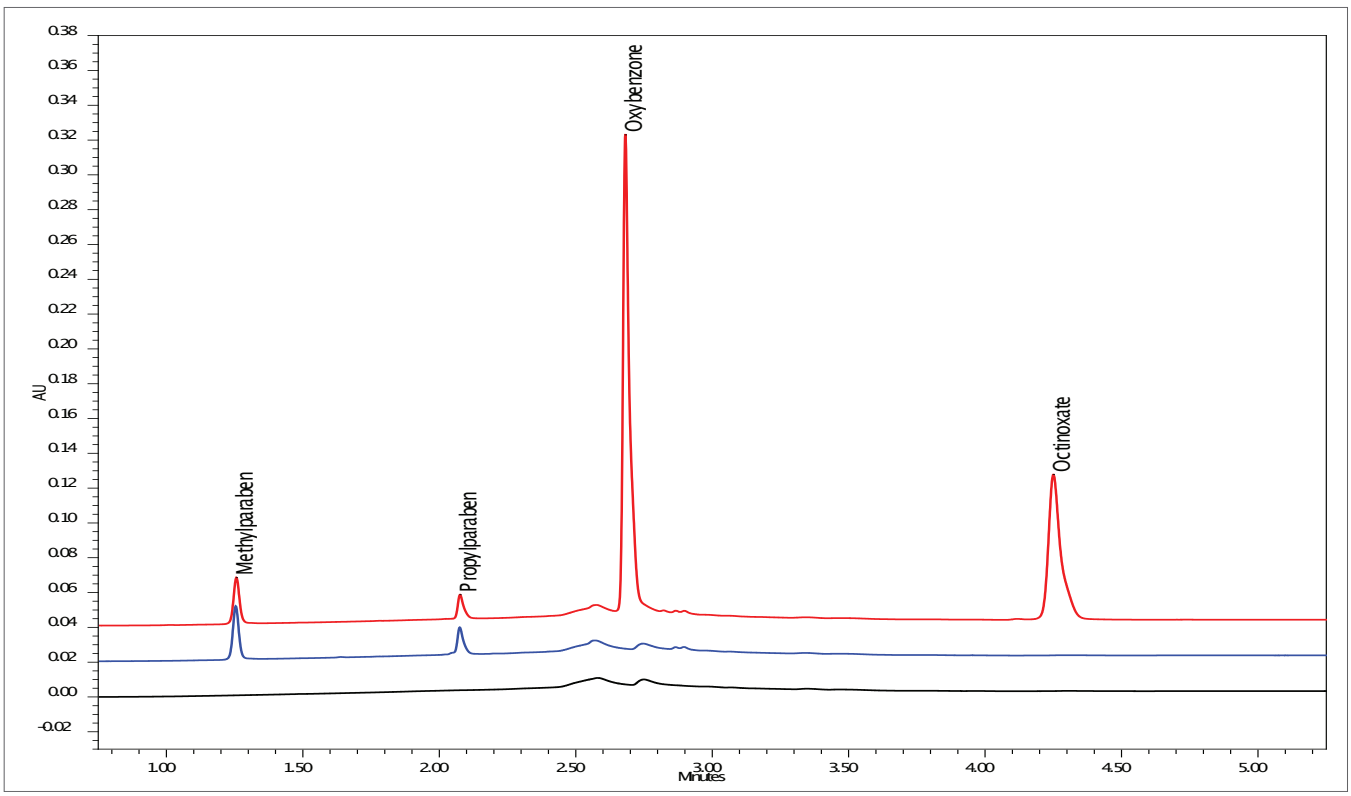

Figure 5. Overlay of a lip balm formulated with sunscreen (red), lip balm formulated without sunscreen (blue), and subsequent blank injection (black).

Figure 6 shows an overlay of moisturizing lotion formulated with and without sunscreen. Methylparaben, ethylparaben, and propylparaben were observed in both lotions, while, as expected, oxybenzone and octinoxate were present only in the skin moisturizer formulated with sunscreen.

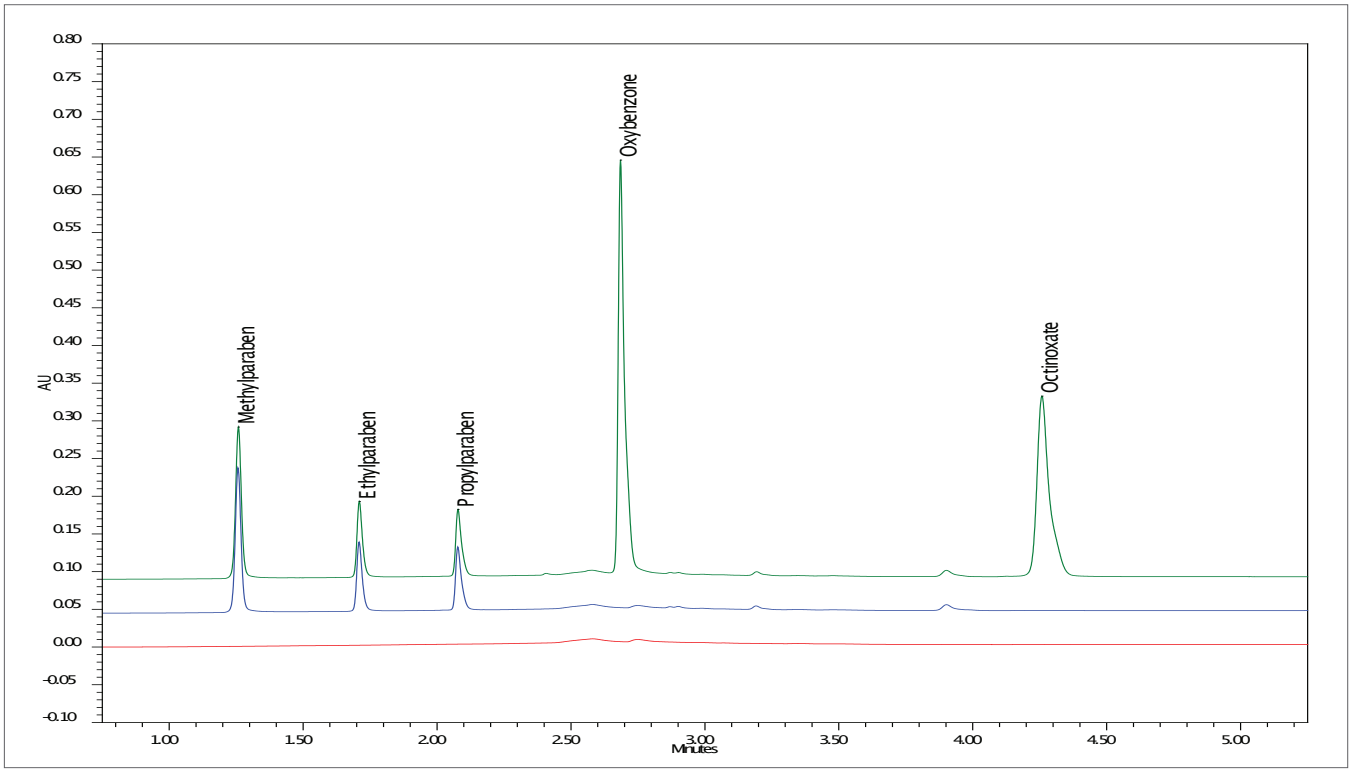

Figure 6. Overlay of skin moisturizer formulated with sunscreen (red), skin moisturizer formulated without sunscreen (blue) and subsequent blank injection (black).

As shown in Figure 7, the sunscreen lotion was found to contain three parabens and five sunscreen-active ingredients.

As shown overlaid in Figures 5-7, a subsequent water blank injection after each sample injection confirmed that there was no noticeable sample carryover in each case.

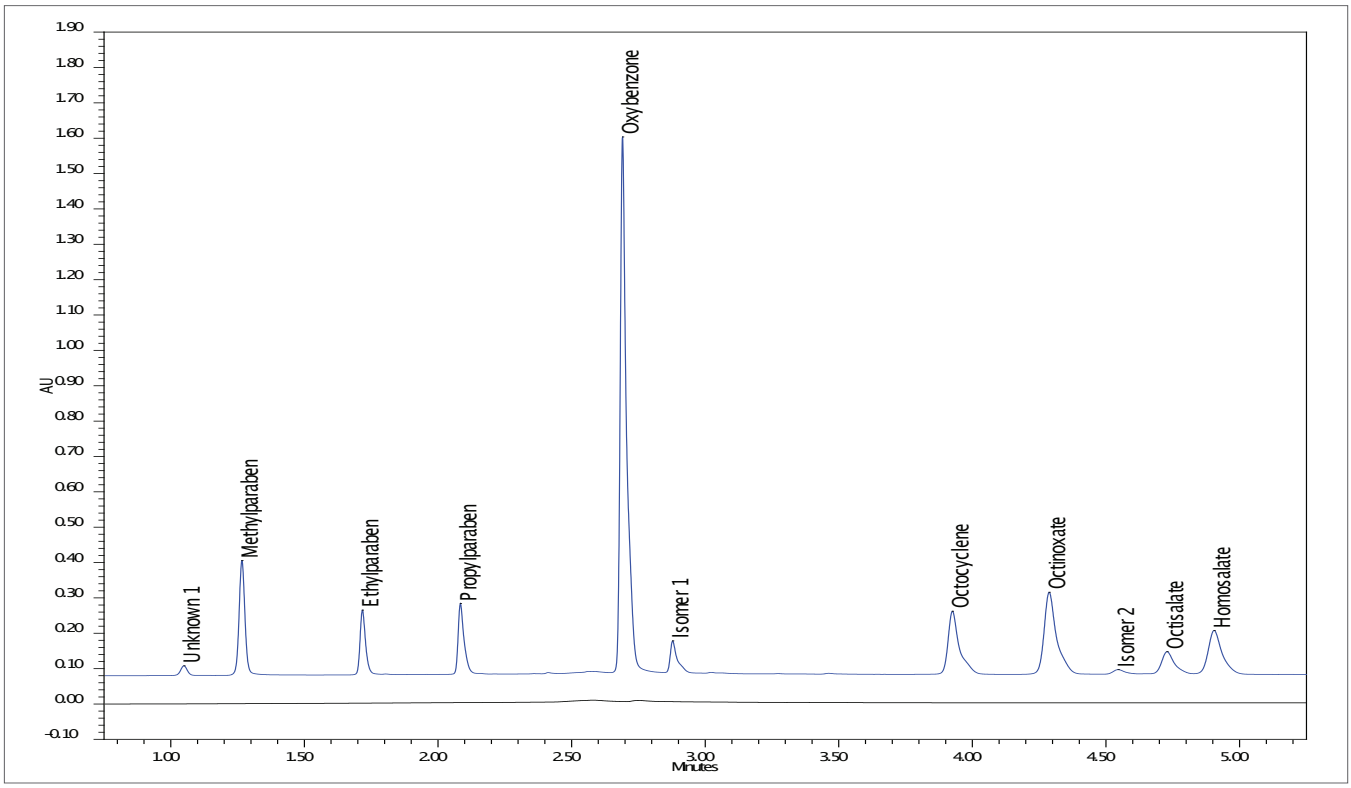

Figure 7. Chromatographic overlay of a sunscreen lotion (blue) and the subsequent blank injection (black).

In Table 3, the calculated amount for each analyzed component and the recovery of sunscreen ingredients in each of the three formulations are listed. It should be noted that octinoxate, oxybenzone, avobenzone and homosalate are unstable and decompose over time. Therefore, depending upon the shelf-life of the product, recoveries of these ingredients may not always be as per the label claim. Also, although the recoveries for octocrylene and oxybenzone were higher than label claim, these two components are primarily added to help preserve the photo and chemical integrity of the more unstable active ingredients.[12,13]

Table 3. Calculated amounts and recoveries of parabens and sunscreen-active ingredients in moisturizer, lip balm and sunscreen lotion, compared to label claim.
Note: for components having a label claim, the amount (%) was entered so as to match the significant figures provided in the label claim.

Moisturizer + Sunscreen	Label Claim (%)	Amount (%); n=2	Recovery (%)
Methylparaben	Not Provided	0.17	-
Ethylparaben	Not Provided	0.09	-
Propylparaben	Not Provided	0.10	-
Oxybenzone	2	2	100
Octinoxate	6	6	100

Lip Balm + Sunscreen	Label Claim (%)	Amount (%); n=2	Recovery (%)
Methylparaben	Not Provided	0.07	-
Propylparaben	Not Provided	0.06	-
Oxybenzone	3.5	3.1	86
Octinoxate	7.5	6.1	81

Sunscreen Lotion	Label Claim (%)	Amount (%); n=2	Recovery (%)
Methylparaben	Not Provided	0.32	-
Ethylparaben	Not Provided	0.19	-
Propylparaben	Not Provided	0.26	-
Oxybenzone	6	8	133
Octocrylene	2.8	5.0	179
Avobenzone	3	3	100
Octisalate	5	5	100
Homosalate	10	10	100

Conclusion

This work demonstrated the fast, effective chromatographic separation of three parabens and six sunscreen ingredients using a PerkinElmer Altus UPLC® system with A-30 PDA. The results exhibited exceptional linearity for the representative paraben and sunscreen-active ingredients over the tested concentration ranges. The results also demonstrated more than ample sensitivity for verifying the label claim of all the analyzed components having published claim values.

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