SPECTRAL ANALYSIS OF BROAD SPECTRUM SUNSCREENS USING HPLC AND A PHOTODIODE ARRAY DETECTOR

Catharine E. Layton, Paul D. Rainville, Amy Woodsmall
Waters Corporation, 34 Maple St., Milford, MA, USA

INTRODUCTION

Ultraviolet (UV) and High-Energy Visible (HEV) electromagnetic radiation is responsible for a variety of chemical reactions including photochemical smog, bleaching of paints, and decay of plastics. Conjugated bonds in organic molecules absorb UV radiation to cause damage and oxidative stress to lipids and proteins resulting in sunburn, hyperpigmentation, photoaging, wrinkles, age spots, broken capillaries, and deadly skin cancer. Regular use of broad-spectrum Sun Protection Factor (SPF) sunscreens has been shown to reduce the risk of skin damage induced by both UV and HEV sources of radiation. In this work, the Alliance™ iS HPLC System with PDA detector is shown to separate and identify compounds, determine spectral purity, and visualize UV absorptive regions for chemical light filters used in sunscreen lotion formulations.

METHODS

LC System:	Alliance™ iS HPLC System with Photodiode Array (PDA) Detector Software, version 1.4.0.
Column:	XBridge™ Premier BEH™ C18 2.5µm, 4.6 x 150mm, p/n: 186009849
Column temperature:	40°C
Sample temperature:	20°C
Injection volume:	35µL
Flow rate:	2.000mL/min
Mobile phase A:	Water / 0.1% formic acid
Mobile phase B:	Acetonitrile / 0.1% formic acid
Needle wash:	50/50 Methanol/Water
2D Wavelength:	254nm
CDS:	Empower™ Software, Version 3.8.0
Diluent:	Ethanol sample dilution, methanol prior to injection
Gradient :	Hold for 1 minute at 70% mobile phase B, linear gradient to 95% mobile phase B over 4 minutes, then re-equilibrated to starting conditions.
Run time(s):	7 minutes for reference standard mixture.15 minutes for lotions.

Fifteen chemical UV filter reference standards were solubilized in methanol to equal a concentration of approximately 4.0mg/mL. Individual PDA spectra were collected from reference standard injections, and a PDA Library created in the Empower Software. A reference standard mixture containing avobenzone (3%), homosalate (13%), octisalate (5%), octocrylene (10%) and oxybenzone (6%) was prepared in methanol. Three OTC sunscreen lotions were weighed and dissolved in ethanol by sonication to equal a final concentration of approximately 5mg/mL (w/v). The supernatant was diluted 1:10 in methanol, and 1mL filtered with a 0.2µm filter prior to injection. Six replicate injections of each sunscreen lotion solution were performed. System repeatability and carryover were investigated. Peak purity and PDA Library identification were determined from the injections.

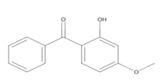
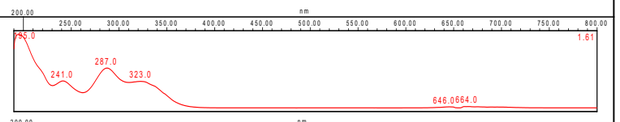
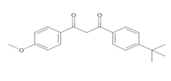
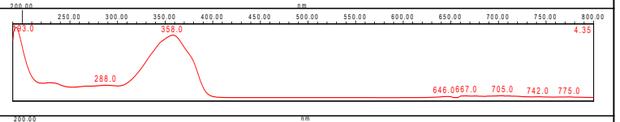
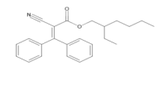
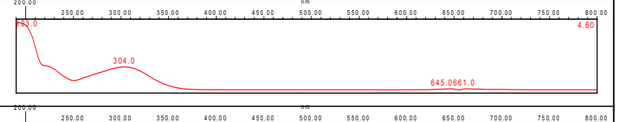
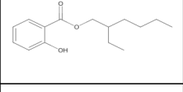
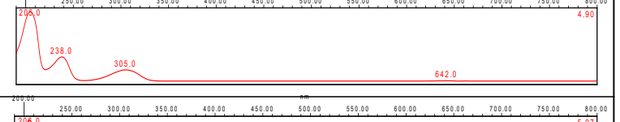
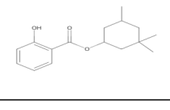
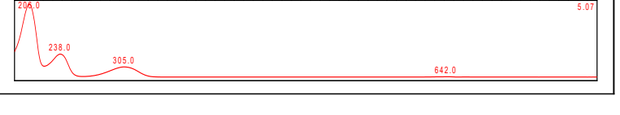
RESULTS AND DISCUSSION

The HPLC method separation baseline resolved the five UV filters in the reference standard mixture (oxybenzone, avobenzone, octocrylene, octisalate, and homosalate) and two related impurities in 5.5 minutes **(Figure 1)**. Retention time repeatability for six replicate injections of the mixture was ≤0.02 RSD, and ≤0.19 RSD for peak area (data not shown). The spectra of 15 individual UV filter reference standards revealed lambda max within the UVC, UVB and/or UVA electromagnetic range. None of the sunscreen compounds showed high absorbance in the HEV blue light spectral range **(Table 1**).

Eluting sunscreen lotion peaks **(Figure 2.A)** were determined to be spectrally homogenous using the Empower 3 Software. This was accomplished automatically by comparing spectra gathered across the eluting peaks with the noise threshold generated by the solvent **(Figure 2.B)**. UV filter peaks were instantly recognized using the PDA Library which utilized both the retention time and spectral profile of the reference standards for identification (**Figure 2.C)**. Sunscreen Lotion A spectra were identified by the PDA Library as; avobenzone (UVA/UVB), homosalate (UVA), octisalate (UVB), octocrylene (UVB), and oxybenzone (UVA/UVB) (**Figure 2.D)**. Sunscreen Lotion B contained four UV filters; avobenzone, homosalate, octisalate and octocrylene, and Sunscreen Lotion C contained only avobenzone, homosalate, and octocrylene.

Contour and 3-Dimensional (**Figure 3.A and B**) plots were used to comprehensively visualize the broad-spectrum UV filter absorbance. Sunscreen lotions, comprised of multiple UV filters, showed absorbance across both the UVB and UVA range. Bemotrizinol, a sunscreen used in formulations internationally, filtered UV radiation in both the UVA and UVB range, but showed less UV coverage around 275nm.

Table 1: Common UV filters in sunscreen lotions formulated in the US.

Compound	Structure	Spectral Range and Absorbance (nm) Wavelength (nm) 200 280 315 400 455 500 800 — UVC UVB UVA Visible
Oxybenzone		
Avobenzone		
Octocrylene		
Octisalate		
Homosalate		

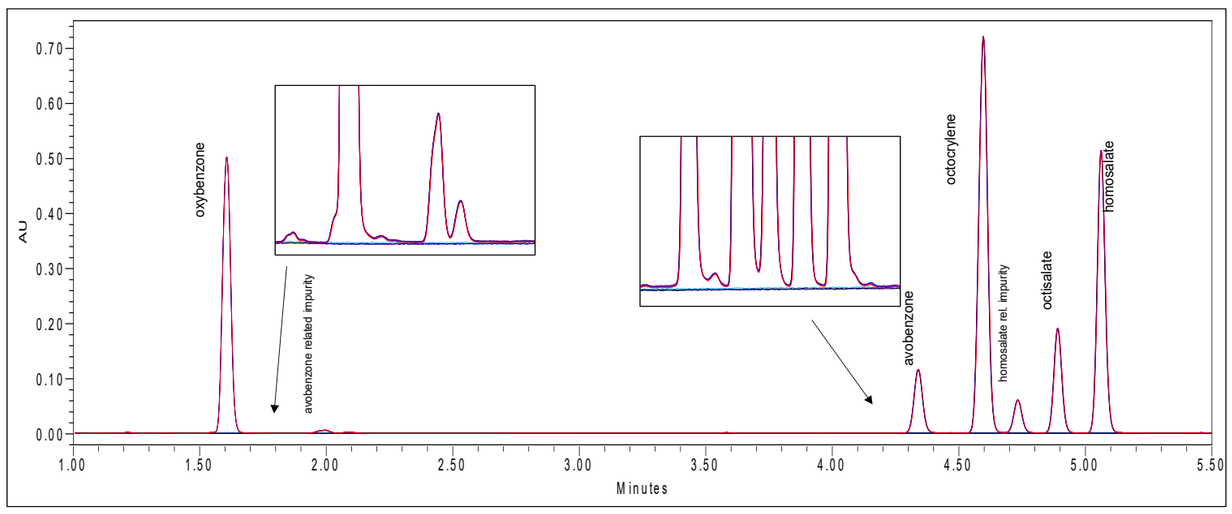
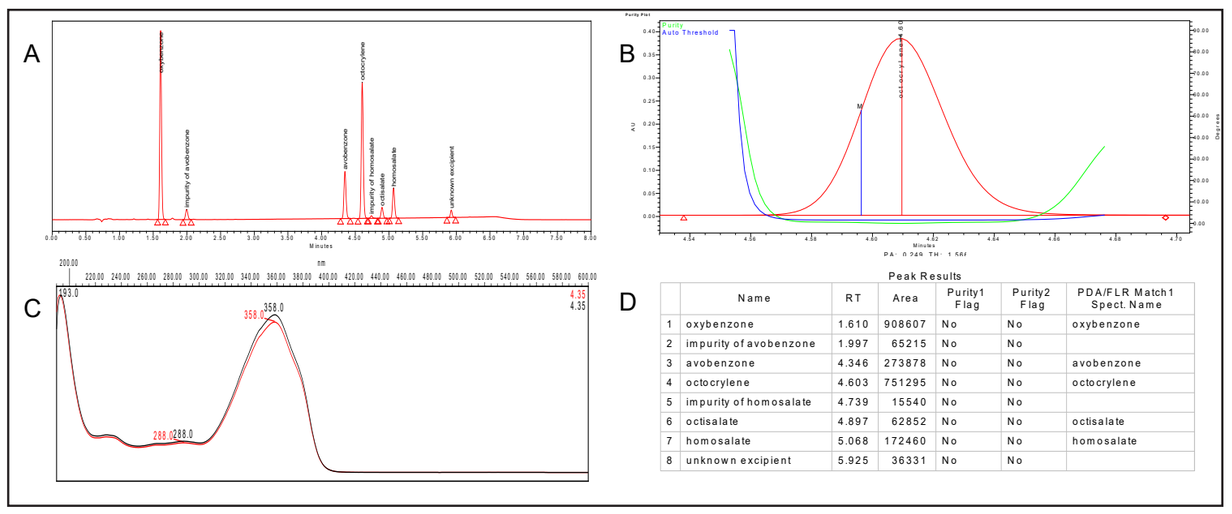


Figure 1: Six replicate reference standard injections overlaid with three consecutive diluent carryover injections (inset– resolution baseline zoom).



	Name	RT	Area	Purity1 Flag	Purity2 Flag	PDA/FLR Match1 Spect. Name
1	oxybenzone	1.610	908607	No	No	oxybenzone
2	impurity of avobenzone	1.997	65215	No	No	
3	avobenzone	4.346	273878	No	No	avobenzone
4	octocrylene	4.603	751295	No	No	octocrylene
5	impurity of homosalate	4.739	15540	No	No	
6	octisalate	4.897	62852	No	No	octisalate
7	homosalate	5.068	172460	No	No	homosalate
8	unknown excipient	5.925	36331	No	No	

Figure 2: A) Chromatogram of Lotion A at 254nm. B) Comparison of octocrylene baseline threshold and purity angle. C) Comparison of avobenzone PDA spectra with the PDA Library spectra for identification. C) Peak Results report summary of two spectral Peak Purity Passes (Purity1 Flag, Purity2 Flag) showing no spectral differences across the peak, and PDA Library identification (PDA/FLR Match1 Spect Name) for all peaks integrated in the Lotion A chromatogram.

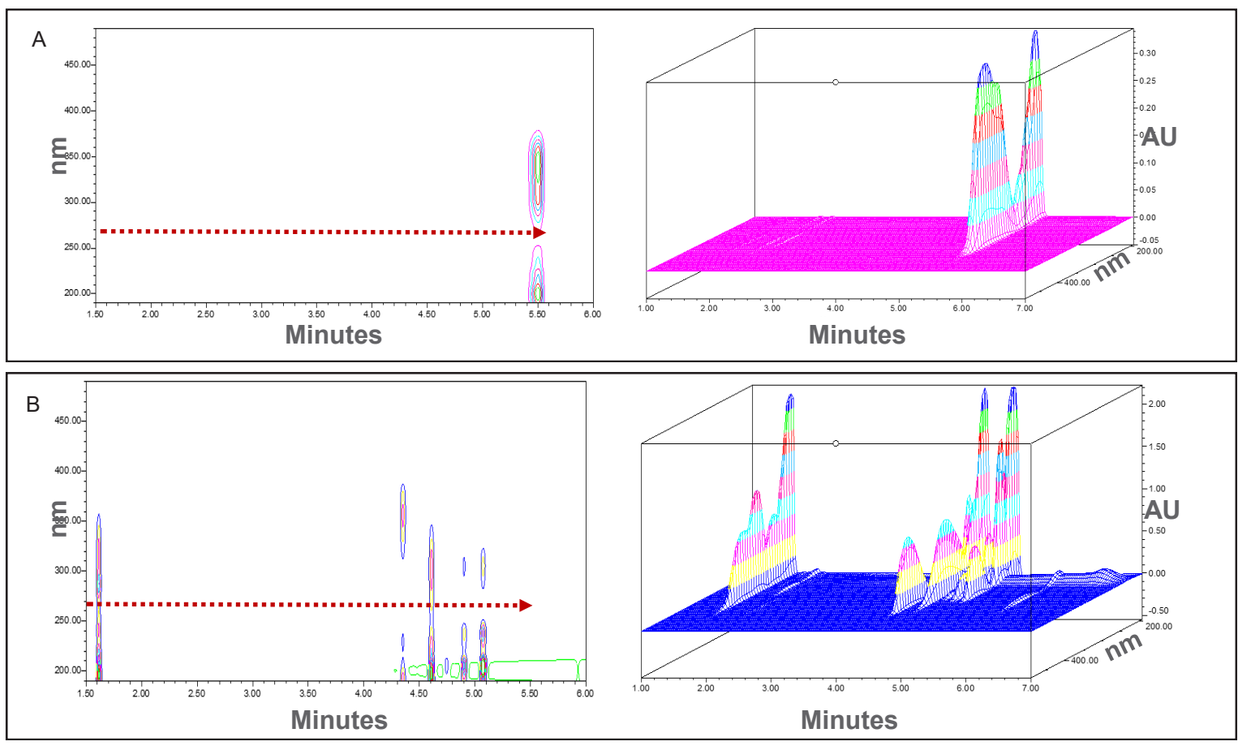


Figure 3: A) Contour plot and 3-Dimensional plot s) for A) bemotrizinol reference standard, under investigation in the US, and B) a broad-spectrum sunscreen lotion formulation containing avobenzone, homosalate, octisalate, octocrylene and oxybenzone.

CONCLUSIONS

The Alliance iS HPLC System PDA detector provided key peak purity and spectral information.

Repeatability of the Alliance iS HPLC System was higher than method validation requirements, and no injection needle carryover was observed. All 15 compounds showed absorbance of UVA and/or UVB radiation, while absorbance within the HEV blue light was minimal.

For the three sunscreen lotions tested, spectral purity was confirmed for all peaks eluting in the chromatographic separation, and UV filters were automatically identified using the Empower 3 Software, PDA library.

Spectral absorbance plots, contour plots, and 3-Dimensional plots provided easy to interpret, full-spectrum visualization of UV and HEV radiation coverage for both individual UV filters and formulated sunscreen lotions.

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