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응용 자료

Spectral Analysis of Broad-Spectrum Sunscreens Using the Alliance® iS HPLC System With Photodiode Array (PDA) Detector

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Abstract

In this application note, the Alliance iS HPLC System with Photodiode Array (PDA) Detector is shown to separate and identify compounds, determine spectral purity, and visualize UV absorptive regions for chemical UV filters used in sunscreen lotion formulations.

Benefits

- The Alliance iS HPLC System with PDA Detector consistently shows low carryover and high repeatability for peak retention time and area
- The Empower 3® Software, when paired with the Alliance iS HPLC System with PDA Detector, can determine peak purity in chromatographic separations
- Chemical UV filters in complex matrices are identified using a Empower 3 Software, PDA Library

- Contour and 3-Dimensional plots provide a visualization of UVC, UVB, UVA, and High-Energy Visible (HEV) blue light broad-spectrum absorbance for sunscreen compounds

Introduction

Ultraviolet (UV) 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. This stress results in sunburn, hyperpigmentation, photoaging of the skin, wrinkles, age spots, broken capillaries, and deadly skin cancer. There are three types of solar UV radiation: UVC (100–280 nm), UVB (280–315 nm), and UVA (315–400 nm). The ozone layer absorbs 100% of UVC, 90% of UVB, and a minimal amount of UVA radiation. The depletion of the ozone layer has resulted in increased concern for overall ground-reaching UV exposure.[1] Additionally, High-Energy Visible (HEV) blue light next to the UVA region (400–450 nm) has also been shown to cause DNA and cellular damage.[2] This radiation is emitted from digital devices, light-emitting diode (LED) light bulbs, and florescent light bulbs. Studies suggest that regular use of broad-spectrum Sun Protection Factor (SPF) sunscreens, ***i.e.*** those that absorb both UVA and UVB radiation, can reduce the risk of skin damage induced by both UV and HEV sources of radiation.[3]

The SPF rating is the minimal dose of a sunscreen formulation that produces perceptible skin erythema using a solar simulated light source.[4,5] UV filters in sunscreens provide a physical or chemical protection barrier. Physical filters work by a similar mechanism as clothing. They include titanium dioxide or zinc oxide and sit on top of the skin to act as a reflective or light scattering barrier. Chemical sunscreens reduce damage from UV radiation by the transfer of energy into a chemical reaction. Compound structure typically exhibits an aromatic functional group, conjugated to a carbonyl group. The compound absorbs high-energy radiation to form an excited state. As the molecule returns to the ground state, it releases the absorbed energy into the skin as heat. Chemical sunscreens can protect against UVB and/or UVA radiation. UVB filters include, aminobenzoates, cinnamates, salicylates, octocrylene, ensulizole, and camphor derivatives. UVA filters include benzophenones, anthranilates, avobenzones, and ecamsules.[6,7]

Both physical and chemical sunscreens, formulated as lotions, oils, sprays creams, gels, paste, and sticks must meet Food and Drug Administration (FDA) over-the-counter (OTC) drug product allowances for concentration and compatibility because of manufacturer package claims to prevent sunburn and skin cancer. Lately,

sunscreens have been placed under increased scrutiny. For example, chemical ingredients used in the United States (US) for many years, trolamine salicylate and aminobenzoic acid (PABA), are now recognized as unsafe or ineffective, and environmental research has shown evidence that oxybenzone and octinoxate cause harm to aquatic environments. Internationally accepted sunscreens, such as bemotrizinol, used for over 20 years in Japan, South Korea, Europe, and Australia, are still under evaluation in the US for both safety, efficacy, and environmental impact. A greater understanding of UV radiation filters formulated as sunscreens across the globe is pertinent.

Chemical sunscreens can be quantified in liquid formulations using liquid chromatography (LC), while physical sunscreens, often inert minerals, require other techniques such Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). In this application note, the Alliance iS HPLC System with Photodiode Array (PDA) Detector was used to determine identity, chromatographic purity, and visualize the spectral absorbance range for fifteen chemical UV filters currently under evaluation for OTC sunscreen formulation approval in the US (Table 1).

Barrier method	Ingredient	Maximum concentration (%) permitted in USA 2025
Chemical	avobenzone	3
	homosalate	15
	octinoxate	7.5
	octisalate	5
	octocrylene	10
	oxybenzone	6
	padimate O	8
	para-aminobenzoic acid (PABA)	Banned 2019
	cinoxate	3
	dioxybenzone	3
	ensulizole	4
	sulisobenzone	10
	meradimate	5
	trolamine salicylate	12
	bemotrizinol	TBD
Physical	titanium dioxide	25
	zinc oxide	25

Table 1. Common UV filters.

Experimental

LC Conditions

LC system:	Alliance™ iS HPLC System with Photodiode Array (PDA) Detector, Software version 1.4.0.
Column:	XBridge™ Premier BEH C_{18} 2.5 µm, 4.6 x 150 mm, p/n: 186009849
Column temperature:	40 °C
Sample temperature:	20 °C
Injection volume:	35 µL
Flow rate:	2.000 mL/min
Mobile phase A:	Water/0.1% formic acid
Mobile phase B:	Acetonitrile/0.1% formic acid
Gradient for formulation analysis:	Hold for 1 minute at 70% mobile phase B, linear gradient to 95% mobile phase B over 4 minutes, then re-equilibrate to starting conditions
Run time(s):	7 minutes for reference standard mixture, 15 minutes for lotion samples
Diluent:	Ethanol sample dilution, methanol prior to injection

Sample filter:	0.2 µm PTFE CE Acrodisk® Minispike Filter, (p/n: WAT200556)
Needle wash:	50/50 Methanol/Water
2D wavelength:	254 nm
3D wavelengths:	190–800 nm
Resolution:	1 nm
Data rate:	10 Hz
Extracted channel:	254 nm
CDS:	Empower® 3, Version 3.8.0

Sample Preparation

Fifteen chemical UV filter reference standards were solubilized in methanol to equal a concentration of approximately 4.0 mg/mL. Individual PDA spectra were collected from reference standard injections, and a PDA Library created in the Empower 3 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 5 mg/mL (w/v). The supernatant was diluted 1:10 in methanol, and 1 mL filtered with a 0.2 µm filter prior to injection. Six replicate injections of each sunscreen lotion solution were performed. System repeatability, carryover, was peak purity and PDA Library identification were determined from the lotion 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 (Figure 2, Table 2) and ≤0.19 RSD for peak area (data not shown). These results were well below the typical method validation acceptance of ≤2.0% RSD. Injection carryover was not observed for the reference standard mixture.

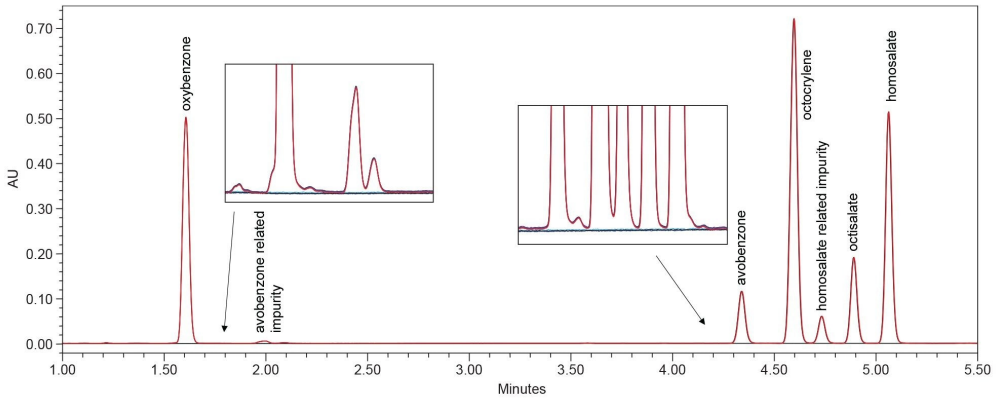


Figure 1. Six replicate reference standard injections overlaid with three consecutive diluent carryover injections (inset).

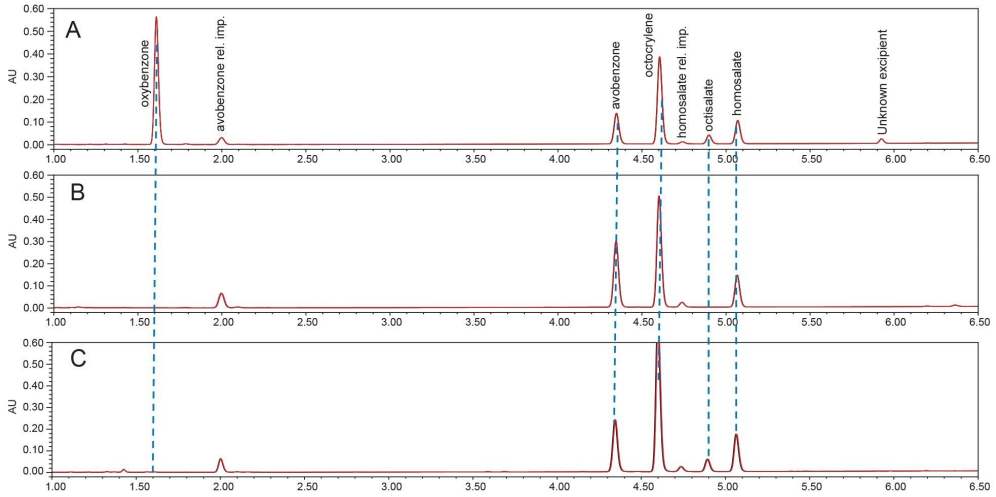
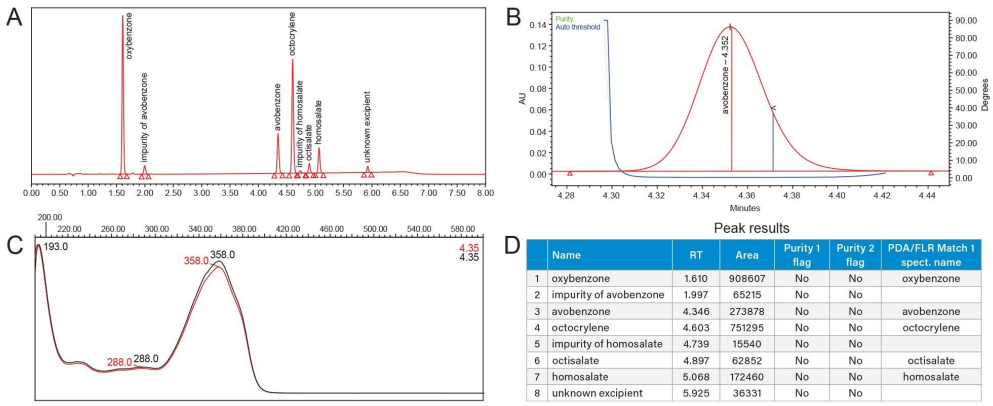


Figure 2. Six replicate injections (repeatability) of three sunscreen lotion formulations (A, B, C).

Sample	Retention time RSD (%)				
	oxybenzone	avobenzone	octocrylene	octisalate	homosalate
Reference standard mixture	0.02	0.02	0.02	0.02	0.02
Lotion A	0.13	0.04	0.04	0.03	0.03
Lotion B	NA	0.08	0.07	0.05	0.07
Lotion C	NA	0.06	0.06	NA	0.05

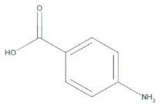
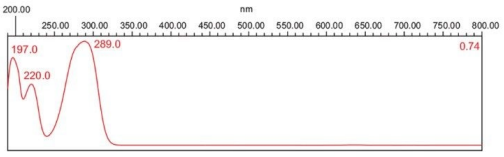
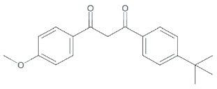
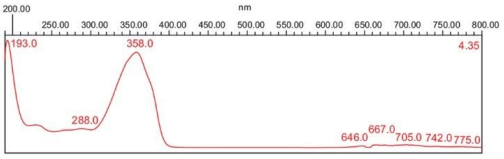
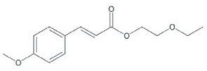
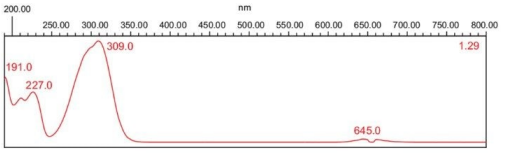
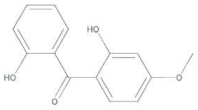
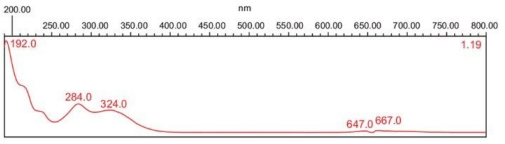
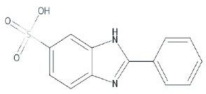
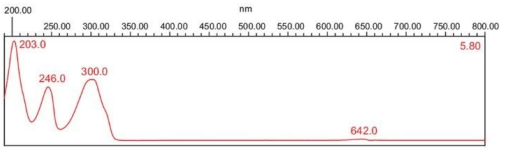
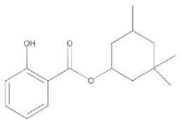
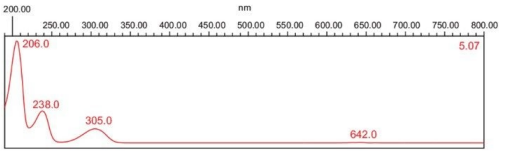
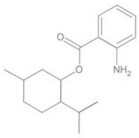
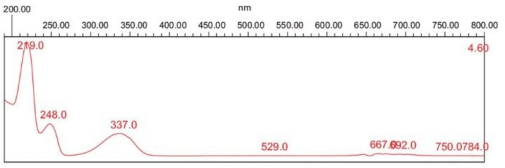
Table 2. Repeatability of retention time for Instrument 1 (n=6) at 254 nm.

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 3). Eluting sunscreen lotion peaks were determined to be spectrally homogenous by 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 3). 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. Sunscreen Lotion A peaks were labeled by the PDA Library as; avobenzone (UVA/UVB), homosalate (UVA), octisalate (UVB), octocrylene (UVB), and oxybenzone (UVA/UVB). Sunscreen Lotion B contained four UV filters; avobenzone, homosalate, octisalate, and octocrylene, and Sunscreen Lotion C contained avobenzone, homosalate, and octocrylene.



	Name	RT	Area	Purity 1 flag	Purity 2 flag	PDA/FLR Match 1 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 3. A) Chromatogram of Lotion A at 254 nm. B) Comparison of avobenzone 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.

Compound	Structure	Spectral range and absorbance (nm)
		Wavelength (nm) 200 280 315 400 455 500 800 — UVC UVB UVA Visible
Para-aminobenzoic acid (PABA)		
Avobenzone		
Cinoxate		
Dioxybenzone		
Ensulizole		
Homosalate		
Meradimate		

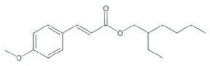
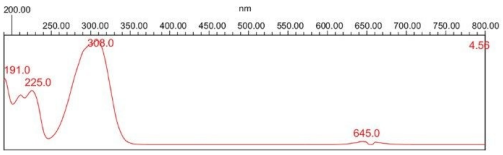
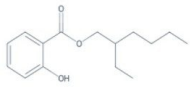
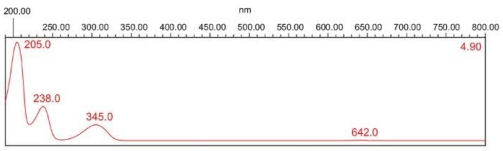
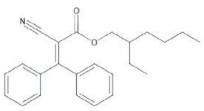
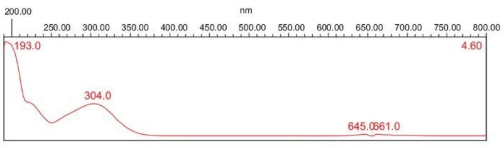
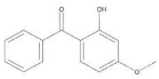
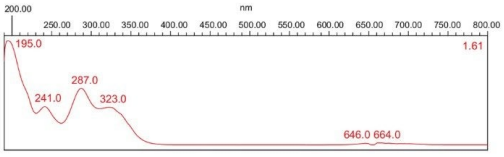
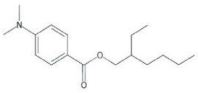
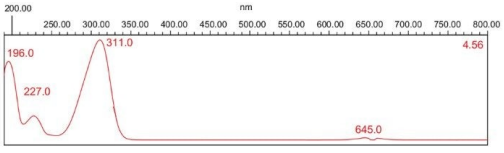
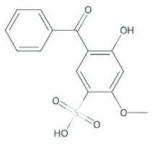
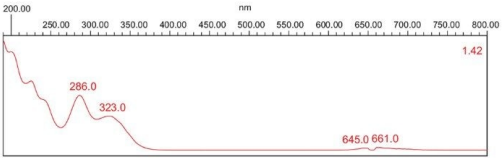
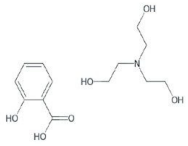
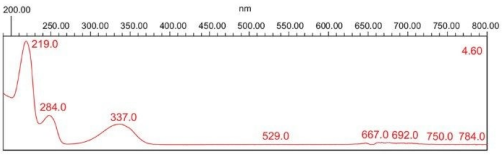
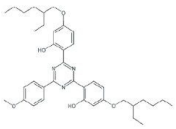
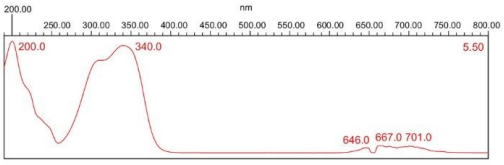
Octinoxate		
Octisalate		
Octocrylene		
Oxybenzone		
Padimate O		
Sulisobenzone		
Trolamine salicylate		
Bemotrizinol		

Table 3. Structure and absorption spectra for UV filters used in sunscreen formulations

between 200–800 nm.[8]

Contour (Figure 4) and 3-Dimensional (Figure 5) 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 275 nm.

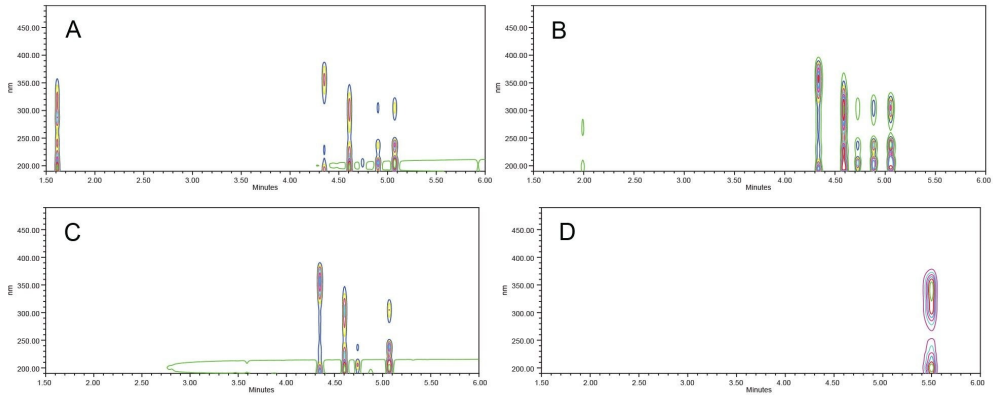


Figure 4. Contour plots showing the chromatographic retention time (x-axis) and radiation wavelengths absorbed (y-axis), for three broad-spectrum sunscreen lotions (A, B, C) and (D) bemotrizinol reference standard, a UV filter under investigation in the US.

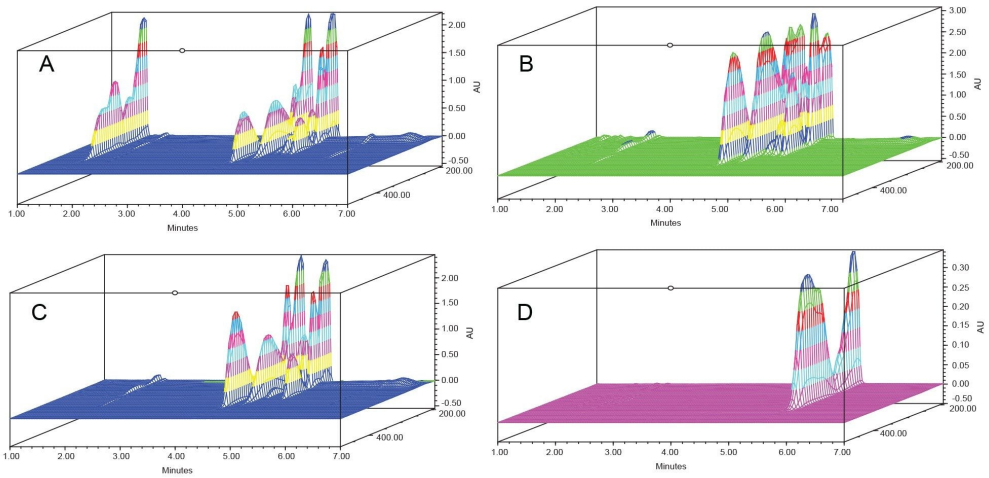


Figure 5. 3-Dimensional plot of the chromatographic retention time (x-axis), UV intensity at the concentration injected (y-axis) and spectral wavelengths absorbed (z-axis) for three broad-spectrum sunscreen lotion formulations (A, B, C) and (D) bemotrizinol reference standard.

Conclusion

The Alliance iS HPLC System with 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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