APPLICATIONS

Analysis of Aromatic Active Ingredients Contained in a Commercially Available Topical Sunscreen

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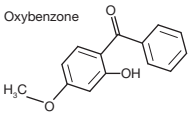
Introduction

The analysis of active ingredients contained in topically administered products can present unique chromatographic challenges. These challenges are associated with the complex cream formulations and unique solubility profiles of the sample matrix. In this application, issues associated with the analysis of cream products were mitigated by leveraging sample preparation and selection of the most relevant LC stationary phase selectivity.

Topical UV protection utilizes organic molecules with active chromophores to absorb harmful UV light from the sun. These organic chromophoric compounds are often closely related aromatic compounds that can be structurally analogous and difficult to resolve. Therefore, in this technical note, we evaluated active organic compounds that are associated with UV protection in a commercially available topical sunscreen. The active ingredients contained in the formulation were identified as oxybenzone, octisalate, homosalate, avobenzone, and octocrylene.

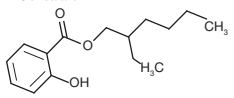
Chemical Structures and LogD

Oxybenzone



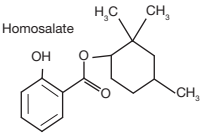
Strongest Acidic pK_a: 7.07
LogD: 3.21 (@ pH 3.0)

Octisalate



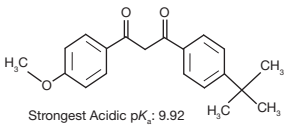
Strongest Acidic pK_a: 9.72
LogD: 5.35 (@ pH 3.0)

Homosalate



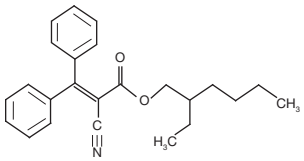
Strongest Acidic pK_a: 9.72
LogD: 5.00 (@ pH 3.0)

Avobenzone



Strongest Acidic pK_a: 9.92
LogD: 4.56 (@ pH 3.0)

Octocrylene

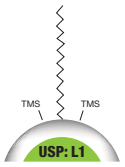


Strongest Acidic pK_a: NA
LogD: 6.78 (@ pH 3.0)

Experimental

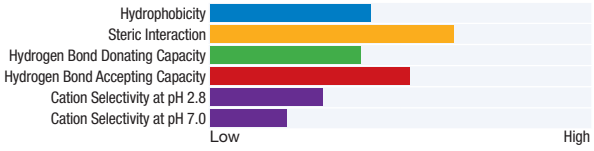
The selectivity implications of 5 active ingredients of a commercially available sunscreen were evaluated with the Kinetex® 2.6 µm C18 and Kinetex 2.6 µm Biphenyl LC columns. The system used for the evaluation was an Agilent® 1100 with a UV-VIS detection at a signal wavelength of 310 nm, a reference wavelength was not utilized. Both the Kinetex C18 and Biphenyl LC columns are based on the highly efficient core-shell solid support particle with unique selectivity characteristics. The core-shell's advantage was leveraged to increase relative peak intensity while operating under nominal HPLC pressures (<400 bar). A mixture of neat active ingredient standards were used to investigate and identify the relative analyte retention and assess selectivity suitability on a commercially available topical cream. A sample of sunscreen was dissolved in 10 mL of reagent grade ethanol, vortexed, centrifuged (@ 4700 rpm), and filtered with a Phenex™ syringe and a 0.45 µm PTFE syringe filter.

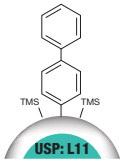
The mobile phase consisted of HPLC grade water, acetonitrile, and trifluoroacetic acid. The use of trifluoroacetic acid was used to avoid potential ketalation between the analytes of interest and accessible stainless steel of the column housing. The column oven was set to 40 °C for this analysis.



Kinetex C18

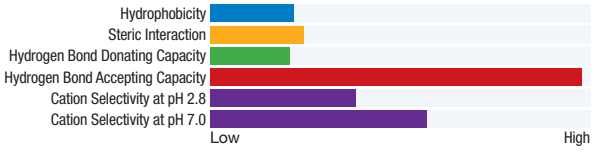
Very well balanced column providing some selectivity through steric, hydrogen, and cationic pathways. This is a great starting point for ultra-high efficiency separations.





Kinetex Biphenyl

100 % aqueous stable reversed phase chemistry with hydrophobic, aromatic, and enhanced polar selectivity.



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Results

Figures 1 and **2** demonstrate the relative selectivity comparison between the Kinetex Biphenyl and C18 in terms of the filtered a standard mixture of the five analytes of interest. Oxybenzone relative retention did not significantly change between both stationary phases. However, for the analytes octisalate, homosalate, avobenzone, and Octocrylene there was an observable difference in terms of retention, elution, resolution, and overall peak shape.

Figure 1.
Standards on Kinetex® C18

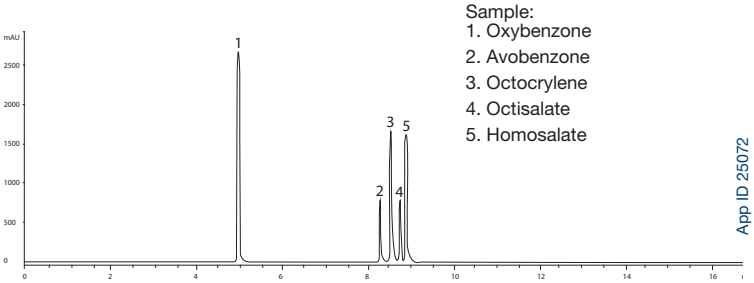
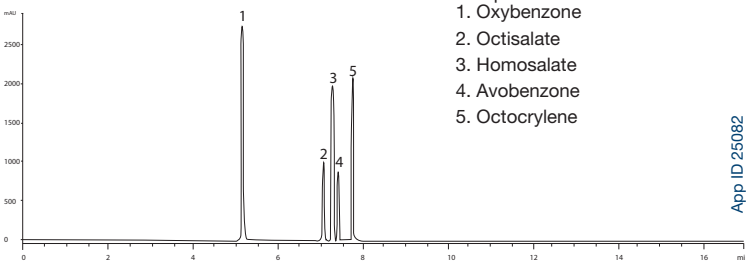


Figure 2.
Standards on Kinetex Biphenyl



Column: As Specified
Dimensions: 100 x 4.6 mm
Part No.: 00D-4622-E0
00D-4462-E0
Mobile Phase: A: Water with 0.1 % TFA
B: Acetonitrile with 0.1 % TFA

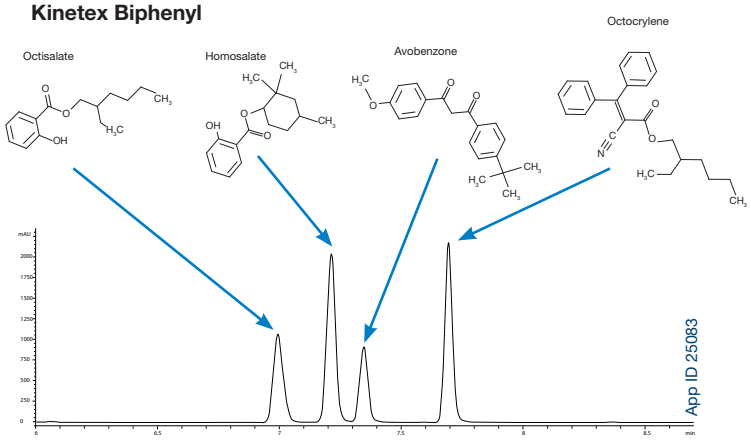
Gradient:	Time (min)	% B
	0	40
	0.5	40
	10	100
	13	100
	14	40
	17	40

Flow Rate: 1.5 mL/min
Injection Volume: 10 µL
Temperature: 40 °C
Detection: UV-VIS (@ 310nm)

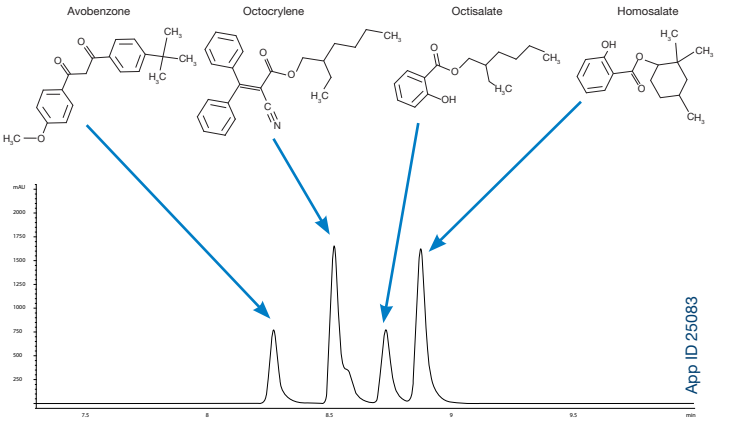
The Kinetex Biphenyl column had an observable increase in relative resolution and overall peak intensity for the given analytes of interest. **Figure 3** focuses on octisalate, homosalate, avobenzone, and Octocrylene in order to better demonstrate the overall chromatographic improvements that can be gainedfrom leveraging the most applicable column selectivity.

Figure 3.
Selectivity Comparison Kinetex C18 vs. Biphenyl

Kinetex Biphenyl



Kinetex C18



Column: As Specified
Dimensions: 100 x 4.6 mm
Part No.: 00D-4622-E0
00D-4462-E0
Mobile Phase: A: Water with 0.1 % TFA
B: Acetonitrile with 0.1 % TFA

Gradient:	Time (min)	% B
	0	40
	0.5	40
	10	100
	13	100
	14	40
	17	40

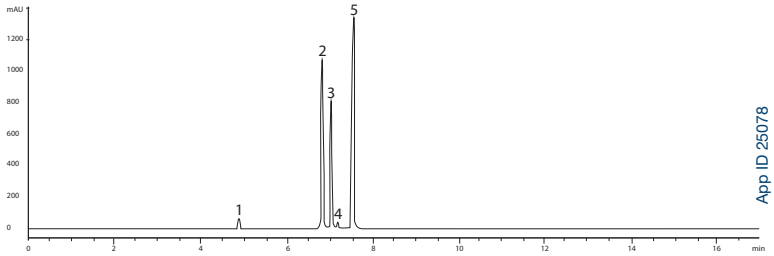
Flow Rate: 1.5 mL/min
Injection Volume: 10 µL
Temperature: 40 °C
Detection: UV-VIS (@ 310nm)

Besides improvements in relative peak shape and height, the overall elution order also changed. This elution change was observed under the identical system and mobile phase conditions and is therefore inherently attributed to the orthogonality of C18 vs phenyl based stationary phase selectivities. For example, the elution of avobenzone and octisalate reverses in respect to one another, dependent upon which stationary phase was used. In respect to analyte hydrophobic nature, avobenzone's LogD value is 4.56 (@ pH 3.0), whereas octisalate's LogD value is (5.35 @ pH 3.0). Therefore, in terms of partitioning coefficient, octisalate has a higher hydrophobicity and is retained longer on a typical reversed phase C18 in comparison to avobenzone.

Conversely, avobenzone has an additional phenyl saturation that has the potential to aromatically interact with the Biphenyl structure of the Kinetex® Biphenyl, leading to increased aromatic selectivity.

Figure 4 displays the chromatographic results of an extracted, centrifuged, and filtered cream sample on the Kinetex 2.6 µm Biphenyl. The respective chromatographic parameters demonstrated good peak symmetry and intensity for all analytes of interest.

Figure 4.
Extracted Sample on Kinetex Biphenyl



Column: Kinetex Biphenyl
Dimension: 100 x 4.6 mm
Part No.: 00D-4622-E0
Mobile Phase: A: Water with 0.1% TFA
B: Acetonitrile with 0.1% TFA

Gradient:	**Time (min)**	**% B**
	0	40
	0.5	40
	10	100
	13	100
	14	40
	17	40

Flow Rate: 1.5 mL/min
Injection Volume: 10 µL
Temperature: 40 °C
Detection: UV-VIS (@ 310 nm)
Sample: 1. Oxybenzone
2. Octisalate
3. Homosalate
4. Avobenzone
5. Octocrylene

	Analyte	Time	Area	Height	Width	Area (%)	Symmetry
1	Oxybenzone	5.1	7309.5	2717.8	0.0419	33.815	0.9
2.	Octisalate	7.1	2873.5	1013.9	0.0426	13.293	0.8
3.	Homosalate	7.3	4835.2	1970.6	0.0386	22.368	1.0
4.	Avobenzone	7.4	1984.5	863.1	0.0358	9.180	0.9
5.	Octocrylene	7.8	4613.6	2076.6	0.0344	21.343	0.9

Upon extraction and centrifugation, the cream sample had considerable turbidity and required filtration. Utilizing a Phenex™ PTFE 0.45 µm syringe filter desk significantly reduced the visual appearance of particulates **(Figure 5)**. In order to confirm the removal of particulates from the respective cream sample, a chromatogram was generated before and after filtration.

Figure 5.
Unfiltered Sample (Left) and Filtered Sample (Right)

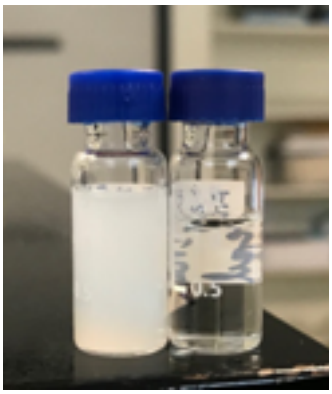


Figure 6.
Chromatographic Overlay

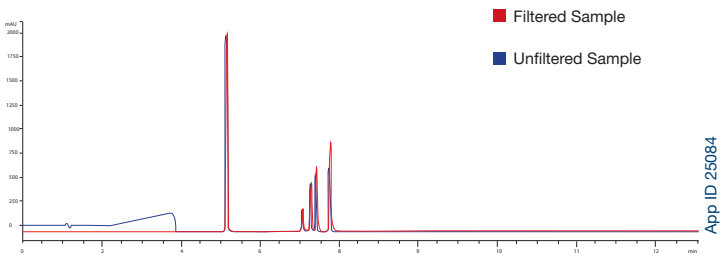


Figure 6 is an overlaid chromatogram, blue represents before filtration and red is post filtration. A significant reduction in chromatographic interference can be observed in the overlay with no loss of analyte intensity.

Column: Kinetex Biphenyl
Dimension: 100 x 4.6 mm
Part No.: 00D-4622-E0
Mobile Phase: A: Water with 0.1% TFA
B: Acetonitrile with 0.1% TFA

Gradient:	**Time (min)**	**% B**
	0	40
	0.5	40
	10	100
	13	100
	14	40
	17	40

Flow Rate: 1.5 mL/min
Injection Volume: 10 µL
Temperature: 40 °C
Detection: UV-VIS (@ 310 nm)
Sample: 1. Oxybenzone
2. Octisalate
3. Homosalate
4. Avobenzone
5. Octocrylene

Conclusion

Analysis of complex formulations presents unique analyte and matrix challenges. In this example, 6 active ingredients in a commercially available topical sunscreen were qualitatively evaluated. The inherent challenges of this analysis were mitigated by the employment of adequate sample preparation and selection of the most relevant LC stationary phase.

APPLICATIONS

Ordering Information

2.6 µm Analytical Columns (mm)						SecurityGuard ULTRA Cartridges‡
Phases	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	3/pk
EVO C18	—	00B-4725-E0	—	00D-4725-E0	00F-4725-E0	AJ0-9296
Polar C18	—	00B-4759-E0	—	00D-4759-E0	00F-4759-E0	AJ0-9532
F5	—	00B-4723-E0	—	00D-4723-E0	00F-4723-E0	AJ0-9320
Biphenyl	—	00B-4622-E0	—	00D-4622-E0	00F-4622-E0	AJ0-9207
XB-C18	—	00B-4496-E0	00C-4496-E0	00D-4496-E0	00F-4496-E0	AJ0-8768
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	AJ0-8768
C8	—	00B-4497-E0	00C-4497-E0	00D-4497-E0	00F-4497-E0	AJ0-8770
HILIC	—	00B-4461-E0	00C-4461-E0	00D-4461-E0	00F-4461-E0	AJ0-8772
Phenyl-Hexyl	—	00B-4495-E0	00C-4495-E0	00D-4495-E0	00F-4495-E0	AJ0-8774

for 4.6 mm ID

	4 mm Diameter for ≤ 2 mL sample volumes		15 mm Diameter for 2 – 10 mL sample volumes		25 – 28 mm Diameter for 10 – 100 mL sample volumes	
Membrane Type/Size	Part No.	Unit	Part No.	Unit	Part No.	Unit
0.20 µm						
Phenex-PTFE [1] (Polytetrafluoroethylene)	AF0-3202-12	100/pk	AF0-2202-12	100/pk	AF0-1202-12	100/pk
	AF0-3202-52	500/pk	AF0-2202-52	500/pk	AF0-1202-52	500/pk
0.45 µm						
Phenex-PTFE [1] (Polytetrafluoroethylene)	AF0-3102-12	100/pk	AF0-2102-12	100/pk	AF0-1102-12	100/pk
	AF0-3102-52	500/pk	AF0-2102-52	500/pk	AF0-1102-52	500/pk

Above syringe filters are non-sterile. Housing is made of medical-grade polypropylene (PP). Luer lock inlet/slip outlet connections unless otherwise indicated.

1. Hydrophobic membrane. Can be made hydrophilic by pre-wetting with IPA.

guarantee

If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

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