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응용 자료

Efficient Method Development for the Analysis of Sunscreen Active Ingredients Using UPLC with Mass Detection and Chromatography Data Software

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Abstract

This application note illustrates how Waters ACQUITY UPLC H-Class PLUS System with 6-port Column Manager coupled with the ACQUITY UPLC PDA and ACQUITY QDa detectors can be used to quickly and efficiently establish a number of method parameters. Empower 3 Software's Custom Reporting, Custom Fields, and Mass Analysis features can be used to streamline the decision making process during method development.

Benefits

- Faster method scouting assays can be performed by combining UPLC with generic gradients, as compared to HPLC
- By using short UPLC columns in method development, many column chemistries can be screened rapidly and automatically using the ACQUITY UPLC Column Manager
- Combining UPLC with mass spectra data facilitates peak tracking during method optimization and complemented UV data for confirming impurities
- Expedite data mining using custom calculation reports in Empower Software

Introduction

Skin cancer is the most common type of cancer diagnosed in the USA, it is estimated one in five Americans will develop skin cancer in their lifetime.[1-4]The most serious class of skin cancers are the melanomas, developing in the melanocyte cells of skin. The World Health Organization (WHO) estimates 65,000 mortalities per year attributed to melanomas.[5] Around 95% of melanomas are caused by exposure to UV radiation.[6]

Due to damaging effects of UV light on skin, increasing numbers of cosmetics and personal care products are formulated with chemicals that actively filter out UV radiation. However long term contact with chemical sunscreens may increase the risk of developing a skin allergy to sunlight.[7] For this reason the type and amount of sunscreen agents in formulations have been strictly regulated around the world and effective methods for simultaneously detecting multiple chemical sunscreen agents in formulations are necessary.[8] HPLC has been applied extensively to this application.[9] However, drawbacks associated with methods

published to date include prohibitive analysis times on HPLC scale columns and/or the use of toxic solvents.

In this application note, we show how Waters ACQUITY UPLC H-Class PLUS System with 6-port Column Manager coupled with the ACQUITY UPLC PDA and ACQUITY QDa detectors can be used to quickly and efficiently establish a number of method parameters. Empower 3 Software's Custom Reporting, Custom Fields, and Mass Analysis features can be used to streamline the decision making process during method development.

Experimental

A panel of seven UV filters from different chemical and regulatory classes (Figure 1) was assembled for method development on the ACQUITY UPLC H-Class PLUS System with PDA/QDa detection equipped with 6-port Column Manager under Empower 3 Software control. These UV filters were deliberately chosen because they have proven difficult to fully resolve by typical HPLC methods: homosalate, octylsalicylate, and avobenzone, and two later generation UV filters that have so far shown to have prohibitive retention times for HPLC analysis: octyltriazone and bemotrizinol.

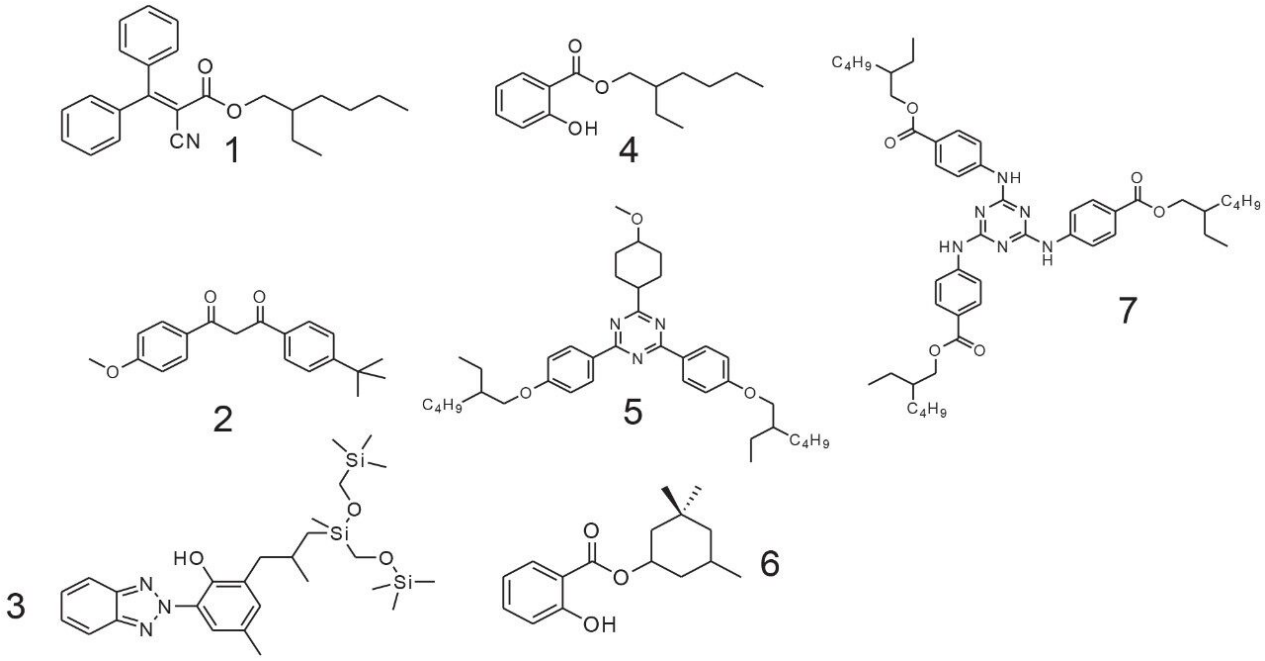


Figure 1. Seven UV filters investigated for method development activities: 1) octocrylene, 2) avobenzone, 3) mexoryl XL, 4) octyl salicylate, 5) bemotrizinol, 6) homosalate, and 7) octyl triazone.

Mixed solvent standards were prepared at 25 ppm in 60:40 methonol: water from individual stock solutions and subjected to a method development screen designed to investigate the column chemistry, organic modifier, column temperature, and mobile phase pH.

UPLC conditions

LC system:	ACQUITY UPLC H-Class PLUS
Detector:	ACQUITY UPLC PDA and ACQUITY QDa
Columns:	ACQUITY UPLC BEH C_{18} Column, 130 Å, 1.7 µm, 2.1 mm × 50 mm, (186002350) ACQUITY UPLC BEH C_8 Column, 130 Å, 1.7 µm, 2.1 mm × 50 mm (186002877) ACQUITY UPLC BEH Phenyl Column, 130 Å, 1.7

	µm, 2.1 mm × 50 mm (186002884) ACQUITY UPLC HSS C_{18} Column, 100 Å, 1.8 µm, 2.1 mm × 50 mm (186003532) ACQUITY UPLC HSS T3 Column, 100 Å, 1.8 µm, 2.1 mm × 50 mm (186003538)
Column temp. 1:	25 °C
Column temp. 2:	60 °C
Sample temp.:	10 °C
Injection volume:	4 µL
Flow rate:	0.5 mL/min
Mobile phase A1:	Water, 0.3% formic acid (pH 2.2)
Mobile phase A2:	20 mM Ammonium formate (pH 4.4)
Mobile phase B1:	Methanol
Mobile phase B2:	Acetonitrile
Gradient:	60 to 95% B over 5 min, 5 min hold at 95% B, return to 60% B and recondition for 1 min

MS conditions

LC system:	ACQUITY UPLC H-Class PLUS
Detector:	ACQUITY UPLC PDA and ACQUITY QDa

MS system:	ACQUITY QDa
Ionization mode:	ESI+
Acquisition range:	100 to 900 Da
Capillary voltage:	0.8 kV
Cone voltage:	8 V

Data management

Empower 3 Chromatography Data Software

Results and Discussion

Peak tracking with Mass Detection

Empower 3 Software's Mass Analysis window can be utilized to link UV and mass data together so that peaks in the UV trace are annotated with a mass-to-charge (*m/z*) value. This enables rapid, selective tracking of peaks and eliminates the requirement to inject individual standards. Consequently as different mobile phases, pHs, and columns are screened, peak migration (i.e., changes in column selectivity) can be immediately assessed and optimum conditions quickly ascertained. Figure 2 shows how changing the column chemistry, organic modifier, and temperature impacts the separation of four of the seven UV filters on moving from (A) a BEH phenyl column run with acetonitrile as the organic modifier at higher pH and temperature, to (B) a BEH C_{18} column in methanol at low pH and low temperature.

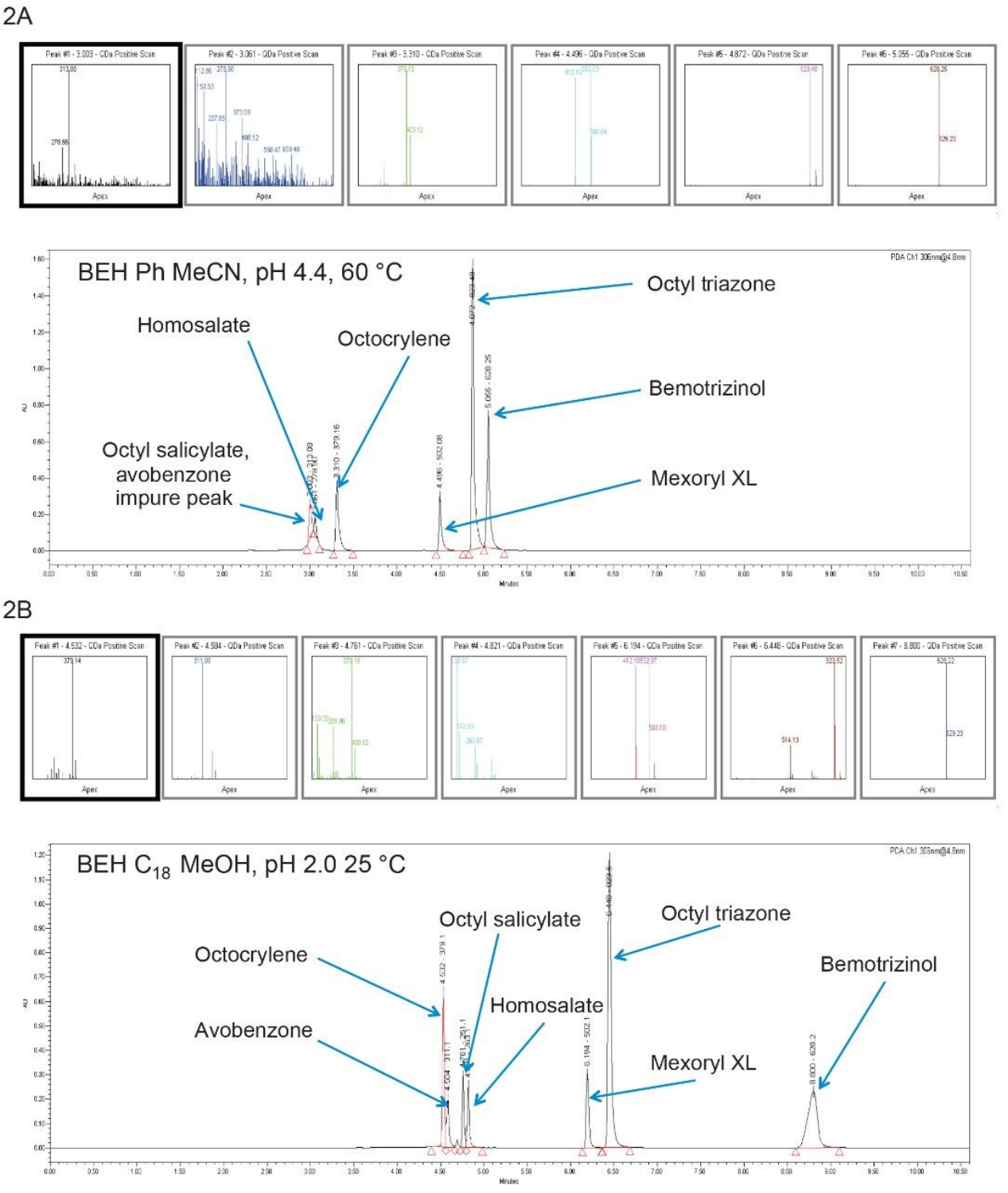


Figure 2. Mass Analysis window showing UV filters annotated with retention times and assigned m/z values.

When evaluating a small number of column chemistries or other method parameters for method development, visual evaluation of scouting runs with the aid of mass detection peak tracking would usually

be sufficient to determine the best parameters for method optimization.

Empower 3 can aid method development by quickly highlighting required information using built-in data analysis tools from large data sets.

Mining the data

Empower 3 Software was utilized to quickly summarize large numbers of injections without the need for manual review of each individual chromatogram within whole data sets. Empower can be used to rapidly summarize the effects of temperature, organic modifier, pH, and column chemistry via simple drop down menus in Empower Report Publisher. Interpretation of the method scouting data of the seven UV filters, in conjunction with these custom reporting features resulted in an easy to read summary report.

Figures 3 and 4 show how the reports summarized each injection in the method development dataset in terms of criteria required for the final method. Figure 3 shows the method development data summarized by total number of peaks in each chromatogram. Figure 4 shows a summary of each injection in terms of its total resolution of all the peaks in the chromatogram. Datasets were separated by mobile phase pH to aid visualization. These values can be automatically combined to determine an injection score for each injection. Injection scores can be configured to account for any chromatographic criteria that the method development group uses to make decisions.

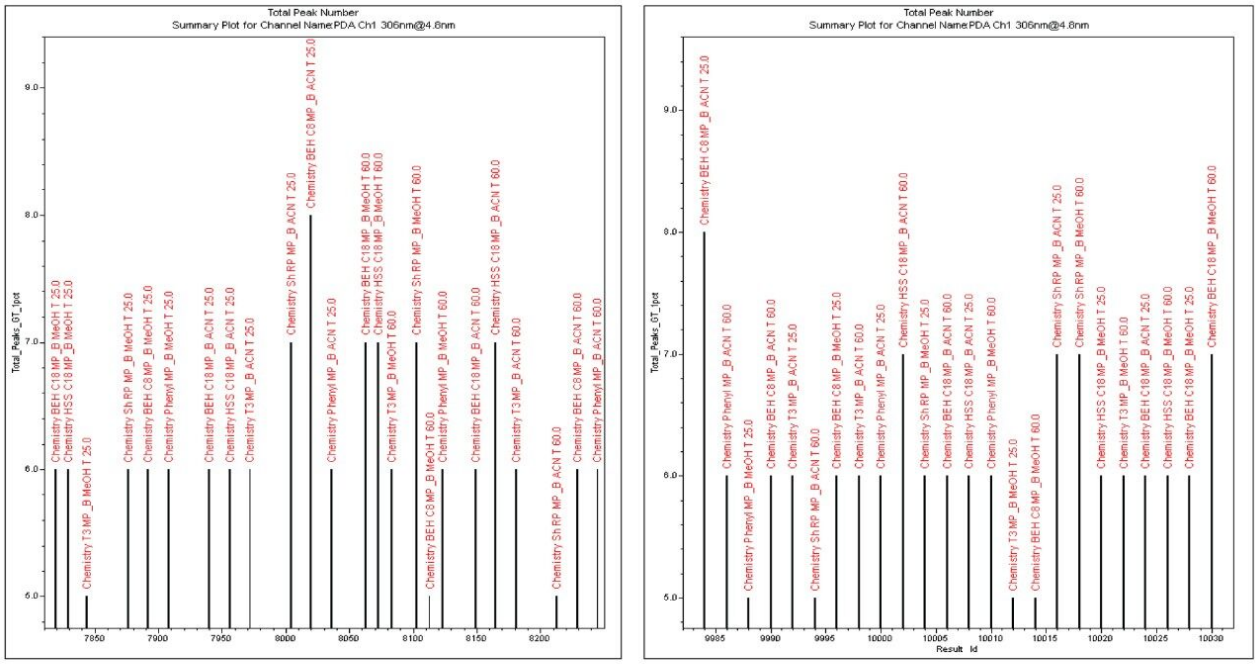


Figure 3. Total peaks summary.

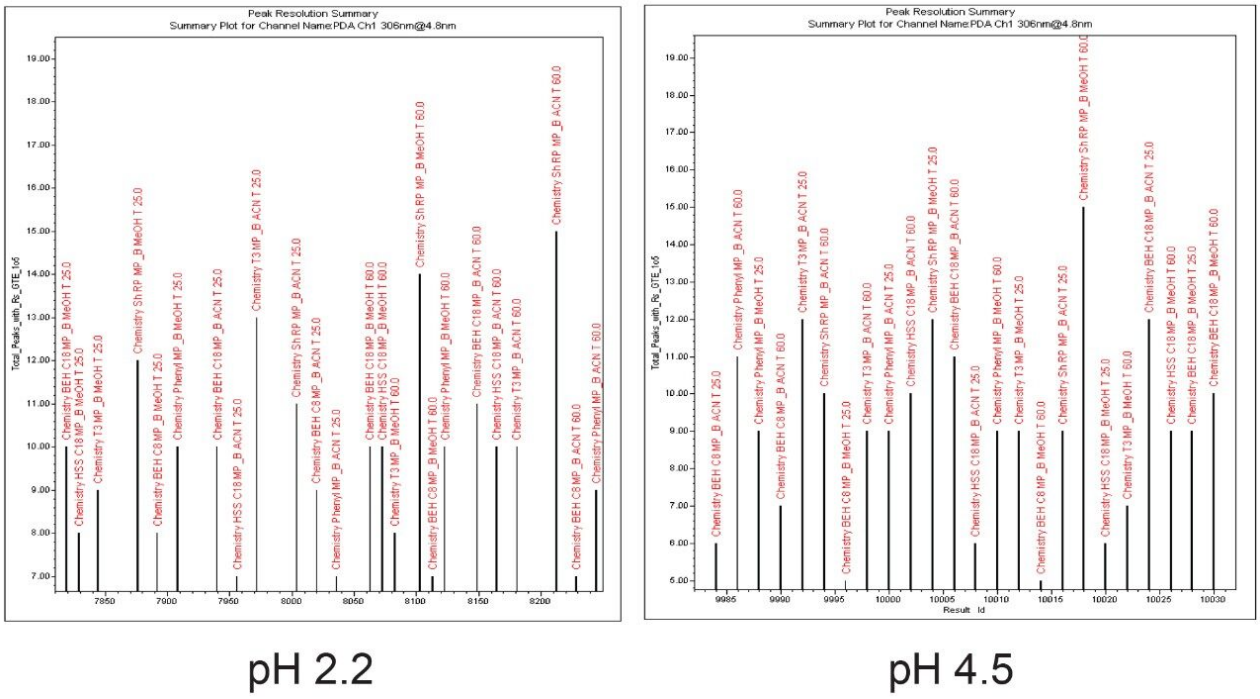


Figure 4. Total resolution score.

Figure 5 shows injection scores for the method development sets combining total peaks, total resolution, and total peak width for each injection. The highest scoring injections are marked by an asterisk. By using Empower 3 custom data mining and custom reporting tools, it was quickly apparent that in the method development exercise employed to optimize UV filter analysis, ACQUITY BEH Shield RP Column offered the most efficient column chemistry and in this case, method optimization activities should be concentrated on a mobile phase at low pH.

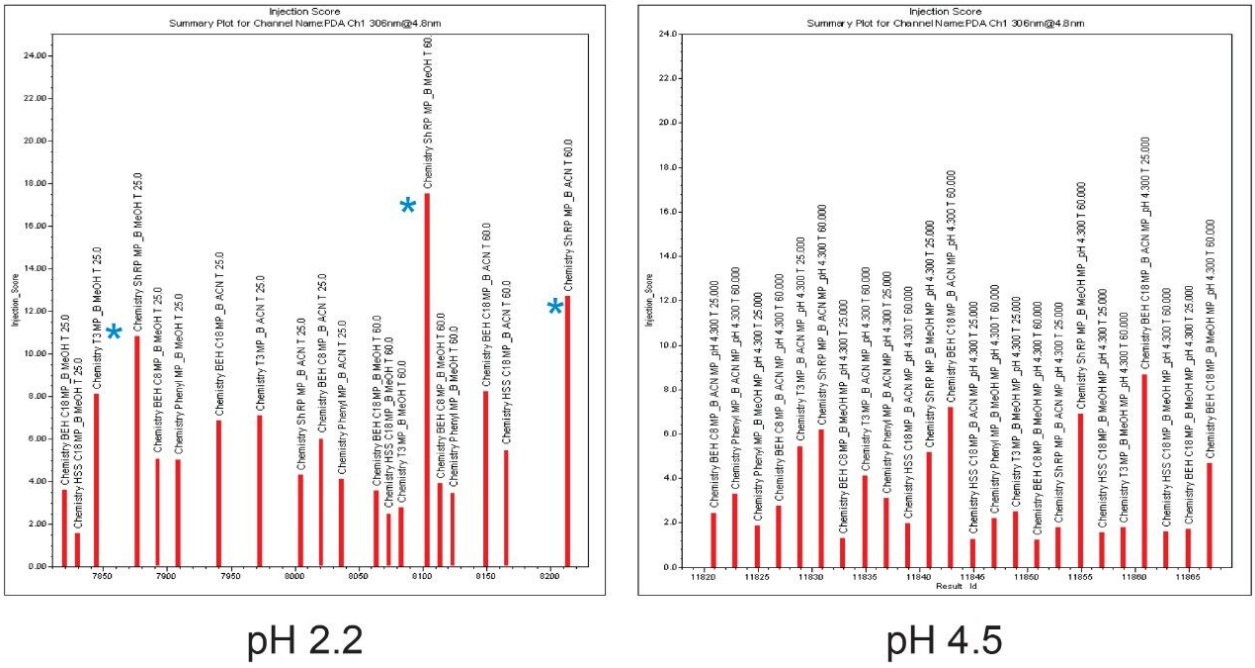


Figure 5. Injection score summary with three highest score highlighted.

The UPLC method was optimized for the ACQUITY UPLC BEH C_{18} Column, (130Å, 1.7 µm, 2.1 mm × 50 mm, 186002350), with formic acid at pH 2.2 and methanol as the mobile phase. Mass confirmation of each UV filter facilitated peak tracking during the method optimization.

Optimal conditions to maximize chromatographic speed and resolution yielded a flow rate of 0.5 mL/min at 35 °C with a gradient from 70% B isocratic for 2 min then to 95% B for 4.5 min. Held at 95% B until 8.5 min then stepped to 100% B for 2 min, returning to 70% B for 1 min re-equilibration. The final method conditions resulted in the chromatogram displayed in Figure 6.

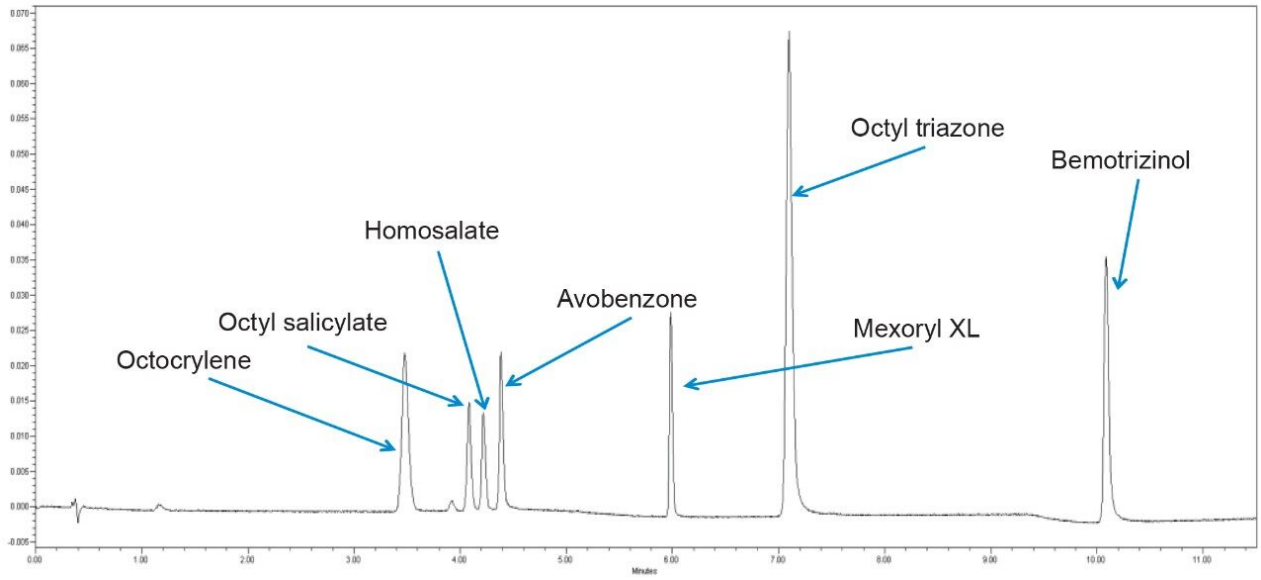
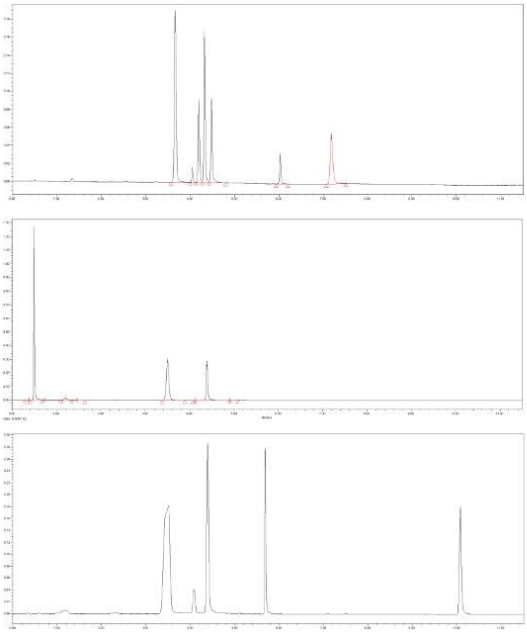


Figure 6. Solvent standard chromatogram, optimized method.

The optimized method was used to analyze three commercially available sunscreen formulations for the presence of UV filter ingredients. Figure 7 shows sample chromatograms and results tables with %weight calculated for each of the UV actives detected. All of the sunscreen formulations tested were found to be in compliance with the regulations for the EU region (Table 1), from which they were sourced, and the UV filters detected were in agreement with the ingredients listed on the formulation labels with no false positives or negatives. Additional optional Empower 3 Software features are available for deployment in a regulatory compliant production or quality control environment. For more information, visit www.waters.com/mvm.



	Name	RT	Area	Height	Amount	Units	Percent_Wt_Wt_Formulation
1	Octocrylene	3.510	2253954	233403	0.107627	mg / mL>	5.381
2	Octyl Salicylate	4.088	810584	207161	0.088972	mg / mL>	4.449
3	Avobenzone	4.388	4476378	1357484	0.089833	mg / mL>	4.492
4	Octyl Triazone	7.096	1132578	283615	0.022965	mg / mL>	1.148
5	Meroxyl XL	5.984	249544	127329	0.019175	mg / mL>	0.959
6	Homosalate	4.225	1434667	420357	0.179234	mg / mL>	8.962

	Name	RT	Area	Height	Amount	Units	Percent_Wt_Wt_Formulation
1	Avobenzone	4.386	3423400	1372719	0.068756	mg / mL>	3.438
2	Octocrylene	3.499	1364819	291855	0.065262	mg / mL>	3.263

	Name	RT	Area	Height	Amount	Units	Percent_Wt_Wt_Formulation
1	Octocrylene	3.506	1805239	181785	0.086247	mg / mL>	4.312
2	Octyl Salicylate	4.084	164003	40432	0.018468	mg / mL>	0.923
3	Bemotrizinol	10.088	547199	172376	0.060804	mg / mL>	3.040
4	Avobenzone	4.386	4868696	1452170	0.097686	mg / mL>	4.884
5	Meroxyl XL	6.063	179	252	0.000201	mg / mL>	0.010

Figure 7. Sample chromatograms and Empower 3 results summarized for three commercially available sunscreen formulations.

		A/B	US	EU	CHN	JP
1	**Octocrylene**	B/A	10%	10%	10%	10%
2	**Avobenzone**	A	3%	5%	5%	10%
3	**Mexoryl XL**	A	**X**	15%	15%	**X**
4	**Octyl salicylate**	B	5%	5%	5%	10%
5	**Bemotrizinol**	A	**X**	10%	10%	3%
6	**Homosalate**	B	15%	10%	10%	10%
7	**Octyl triazone**	Both	**X**	5%	**X**	3%

Table 1. UV filters analyzed, Type of UV radiation filtered and geographical regulatory limits (Formulation % wt/wt). X = Prohibited for use in sunscreen formulations in that geographical area.

Conclusion

- An efficient method development screening process was employed utilizing short UPLC columns and a generic gradient to fast track the method analysis screening time. The process takes advantage of UPLC Technology, delivering rapid method scouting.
- The use of short UPLC columns allowed many column chemistries to be screened quickly in an automated manner using the ACQUITY UPLC Column Manager.
- Further optimization for resolution was achieved using isocratic holds, varying the gradient slope and modulation method temperature.
- Data collected on the ACQUITY UPLC PDA and ACQUITY QDa detectors facilitated peak tracking (mass data), aiding in peak identification and tracking method development.
- The use of specific labeling custom fields in Empower 3 Software allowed for the creation of custom reports to help expedite the mining of the resulting data which would normally take a considerable amount of manual review.
- The utilization of the ACQUITY UPLC H-Class PLUS System and Empower 3 Software provided a rapid solution to the method development challenges associated with cosmetic and personal care formulations.

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