Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Method Equivalence Testing for Sunscreen Actives Using the ACQUITY UPLC H-Class PLUS System and ACQUITY UPLC H-Class System

Michael Jones
Waters Corporation, Wilmslow, UK

GOAL

To demonstrate superior performance of the ACQUITY™ UPLC™ H-Class PLUS System with UV and mass detection for accurate recording of method development activities towards efficient separation, detection, and quantitation of sunscreen actives.

BACKGROUND

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

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

The ACQUITY UPLC H-Class Plus System coupled to Empower™ 3 CDS Software offers powerful tools to streamline method development activities.

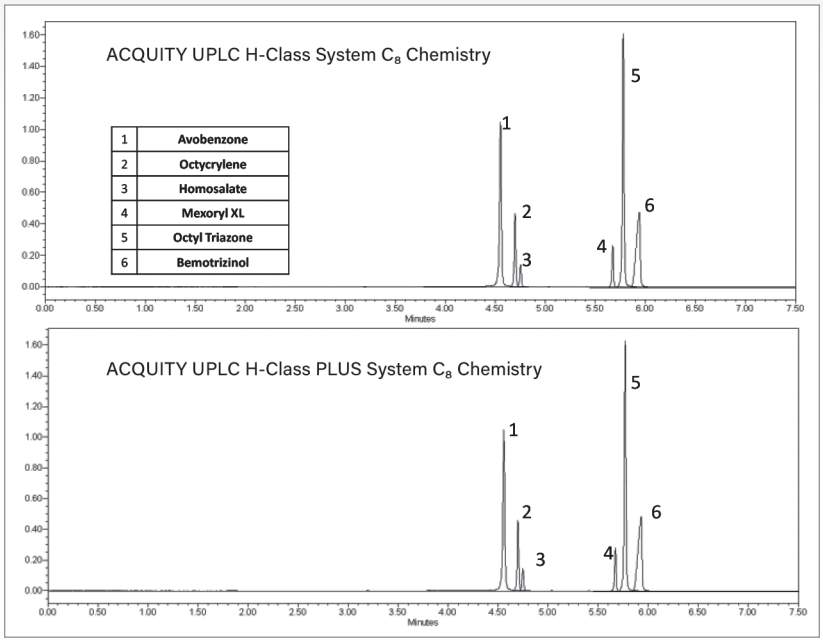

Figure 1. Overlaid chromatograms of individual sunscreen standards, separated by ACQUITY UPLC BEH C_8 Column chemistry on both the ACQUITY UPLC H-Class and ACQUITY UPLC H-Class PLUS systems.

In this technology brief, we demonstrate equivalent performance of Waters™ ACQUITY UPLC H-Class PLUS System for the separation and detection of a mix of six chemical UV filters by measuring and comparing typical system suitability parameters to the data acquired on an ACQUITY UPLC H-Class System on two different column chemistries.

THE SOLUTION

Analytical standards were purchased from Sigma-Aldrich Ltd (Poole, Dorset, UK). The standards were prepared at concentrations of 25 µg/mL in 60:40 water:MeOH for UPLC analysis. UPLC separation was achieved on the ACQUITY UPLC HSS PFP Column (1.8 µm, 2.1 x 50 mm, p/n: 186005965) and the ACQUITY UPLC BEH C_8 Column (1.7 µm, 2.1 x 50 mm, p/n: 186002877) at a flow rate of 0.5 mL/min using a general gradient: 40% B initially to 1 min grading to 95% B at 4.5 min, followed by a 2.5 min hold before returning to 40% B and re-equilibrating for 1 min. The mobile phase consisted of A: water and B: methanol, each with 0.1% formic acid, and the injection volume was 5 µL. The retention times and peak areas were measured by Empower 3 CDS Software and were the average of five replicate injections.

Six sunscreen actives were analyzed on both column chemistries using the same mobile phase, gradient, and injection volume by both the ACQUITY UPLC H-Class and the ACQUITY UPLC H-Class PLUS systems. Figure 1 shows chromatograms for the six compounds on both systems using the ACQUITY UPLC BEH C_8 Columns. Figure 2 shows the same six analytes separated using ACQUITY UPLC HSS PFP Column across both systems. The retention times and peak areas between the two systems, shown in Figure 3, were comparable for all of the compounds tested. Retention time reproducibility as illustrated by %RSD was less than 0.2% and 0.04% for the ACQUITY UPLC H-Class and ACQUITY UPLC H-Class PLUS systems respectively. These data demonstrate the reproducibility across the ACQUITY UPLC H-Class and H-Class PLUS systems and show that methods previously validated on the ACQUITY UPLC H-Class systems should not need revalidating for performing analyses on the ACQUITY UPLC H-Class PLUS System.

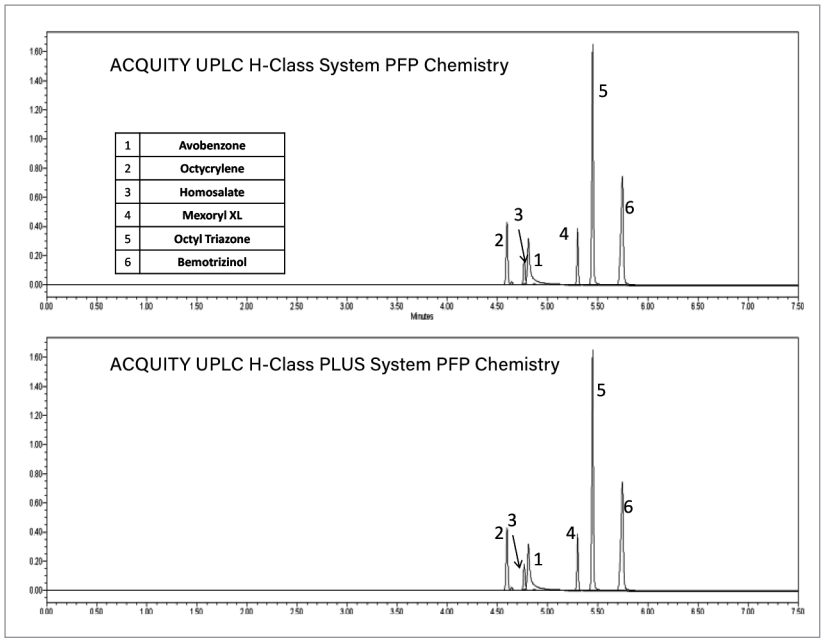

Figure 2. Overlaid chromatograms of individual sunscreen standards, separated using the ACQUITY UPLC HSS PFP Column on both the ACQUITY UPLC H-Class and ACQUITY UPLC H-Class PLUS systems.

ACQUITY UPLC H-Class peak area, retention times

Peak Results

	Name	RT	Area
1	Avobenzone	4.533	1419554.88
2	Avobenzone	4.535	1423840.05
3	Avobenzone	4.536	1419039.00
4	Avobenzone	4.536	1432880.08
5	Avobenzone	4.538	1441333.88
6	Avobenzone	4.550	1451213.06
Mean		4.5	1431306.8
%RSD		0.136	0.9

Peak Results

	Name	RT	Area
1	Octocrylene	4.689	550820.74
2	Octocrylene	4.693	549665.19
3	Octocrylene	4.694	552521.22
4	Octocrylene	4.694	547436.65
5	Octocrylene	4.697	543109.84
6	Octocrylene	4.698	546748.08
Mean		4.7	547933.6
%RSD		0.054	0.6

Peak Results

	Name	RT	Area
1	Bemotrizinol	5.939	1360148.26
2	Bemotrizinol	5.941	1366785.77
3	Bemotrizinol	5.941	1358454.08
4	Bemotrizinol	5.941	1359575.18
5	Bemotrizinol	5.941	1364271.45
6	Bemotrizinol	5.942	1371803.06
Mean		5.9	1363839.6
%RSD		0.018	0.4

Peak Results

	Name	RT	Area
1	Octyl Triazone	5.771	1899755.43
2	Octyl Triazone	5.771	2041283.00
3	Octyl Triazone	5.773	2023904.59
4	Octyl Triazone	5.774	1975133.17
5	Octyl Triazone	5.775	2030027.47
6	Octyl Triazone	5.779	2039803.48
Mean		5.8	1998651.4
%RSD		0.048	2.9

ACQUITY UPLC H-Class PLUS peak area, retention times

Peak Results

	Name	RT	Area
1	Avobenzone	4.554	1316245.78
2	Avobenzone	4.555	1328033.98
3	Avobenzone	4.555	1334896.93
4	Avobenzone	4.555	1311063.00
5	Avobenzone	4.556	1347540.65
6	Avobenzone	4.558	1342976.41
Mean		4.6	1329792.8
%RSD		0.030	1.1

Peak Results

	Name	RT	Area
1	Octocrylene	4.698	540891.27
2	Octocrylene	4.698	540243.36
3	Octocrylene	4.699	545472.97
4	Octocrylene	4.699	550328.23
5	Octocrylene	4.699	544847.22
6	Octocrylene	4.700	538884.93
Mean		4.7	543511.3
%RSD		0.031	0.8

Peak Results

	Name	RT	Area
1	Bemotrizinol	5.925	1371317.42
2	Bemotrizinol	5.926	1353376.16
3	Bemotrizinol	5.928	1351829.18
4	Bemotrizinol	5.929	1373573.84
5	Bemotrizinol	5.929	1356769.05
6	Bemotrizinol	5.929	1375154.03
Mean		5.9	1367503.3
%RSD		0.024	0.6

Peak Results

	Name	RT	Area
1	Octyl Triazone	5.767	2062528.92
2	Octyl Triazone	5.768	2041976.56
3	Octyl Triazone	5.768	2074939.61
4	Octyl Triazone	5.768	2072179.52
5	Octyl Triazone	5.769	2080466.23
6	Octyl Triazone	5.770	2080866.43
Mean		5.8	2068126.2
%RSD		0.018	0.6

Figure 3. Representative retention time and peak area data for the ACQUITY UPLC H-Class and ACQUITY UPLC H-Class PLUS systems.

SUMMARY

The ACQUITY UPLC H-Class PLUS System is a robust and reliable analytical technology for the accurate analysis of sunscreen actives in cosmetic formulations. The enhanced reproducibility of the system will aid in the identification of batch to batch variations in QC monitoring of sunscreen manufacture.

References

1. Guy GP, Thomas CC, Thompson T, Watson M, Massetti GM, Richardson LC. Vital signs: Melanoma incidence and mortality trends and projections–United States, 1982–2030. *MMWR Morb Mortal Wkly Rep*. (2015); 64(21):591–596.
2. Guy GP, Machlin S, Ekwueme DU, Yabroff KR. Prevalence and costs of skin cancer treatment in the US, 2002–2006 and 2007–2011. *Am J Prev Med*. (2015); 48:183–7.
3. Stern RS. Prevalence of a history of skin cancer in 2007: results of an incidence-based model. *Arch Dermatol*. (2010); Mar;146(3):279-82.
4. Robinson JK. Sun Exposure, Sun Protection, and Vitamin D. *JAMA 2005*; 294: 1541-43.
5. World Health Organization, Solar ultraviolet radiation: Global burden of disease from solar ultraviolet radiation. *Environmental Burden of Disease Series*, N.13. (2006).
6. Islami F et al. Proportion and number of cancer cases and deaths attributable to potentially modifiable risk factors in the United States. CA *Cancer J Clin*. Published online Nov. 21, 2017. doi: 10.3322/caac.21440.
7. He QS, Xu, N, Liao SF. Determination of 12 Sunscreen Agents in Cosmetics by High Performance Liquid Chromatography. *Chin. J. Chromatogr*. (2011); 29: 762–767.
8. Pirotta G. *Household and Personal Care Today*. (2015); 10(4): 19–24.
9. Salvador A, Chisvert A. Sunscreen Analysis: A Critical Survey on UV Filters Determination. *Anal Chim Acta*. (2005); 537(1): 1–14.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com