

Note d'application

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

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This is an Application Brief and does not contain a detailed

## Abstract

This application brief demonstrates 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.

## Benefits

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

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## Introduction

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.<sup>1-4</sup> 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.<sup>5</sup> Around 95% of melanomas are caused by exposure to UV radiation.<sup>6</sup>

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.<sup>7</sup> 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.<sup>8</sup> HPLC has been applied extensively to this application.<sup>9</sup> However, drawbacks associated with the methods published to date include prohibitive analysis times on HPLC scale columns and/or the use of toxic solvents.

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.

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## Results and Discussion

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<sub>8</sub> 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<sub>8</sub> 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.

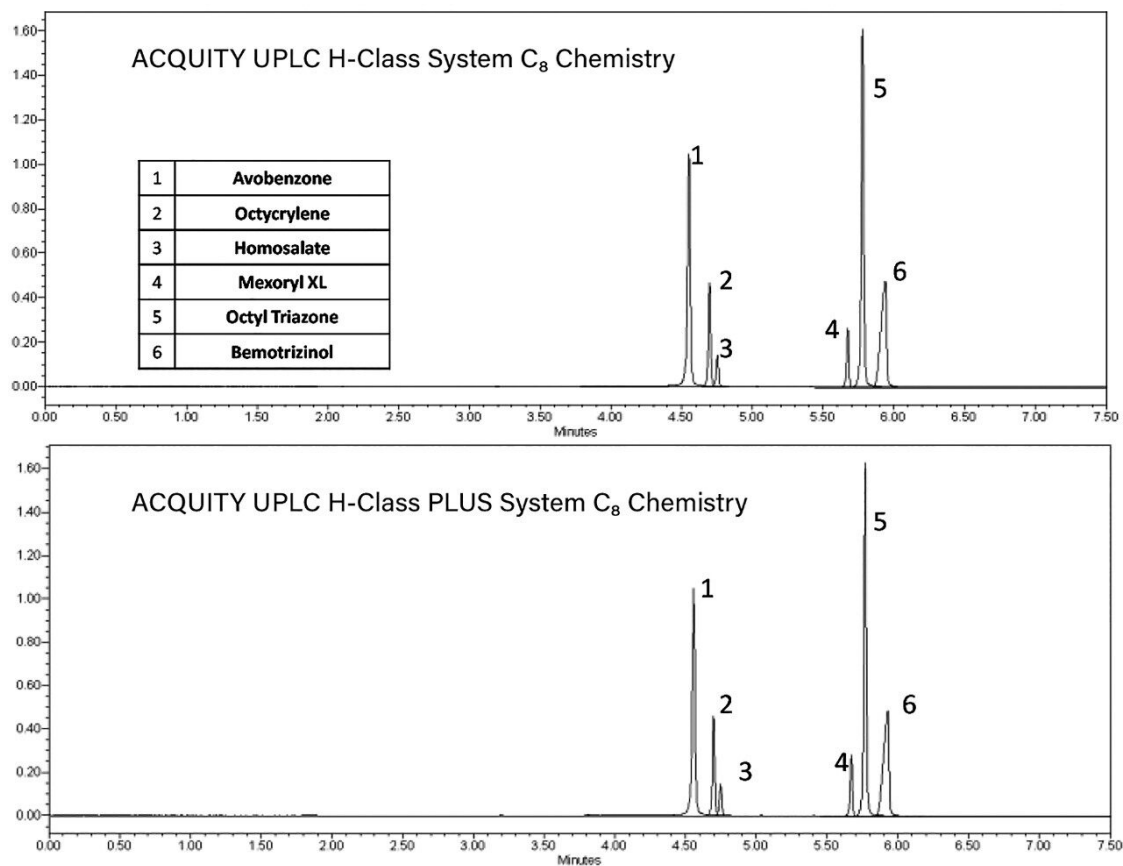


Figure 1. Overlaid chromatograms of individual sunscreen standards, separated by ACQUITY UPLC BEH C<sub>8</sub> Column chemistry on both the ACQUITY UPLC H-Class and ACQUITY UPLC H-Class PLUS systems.

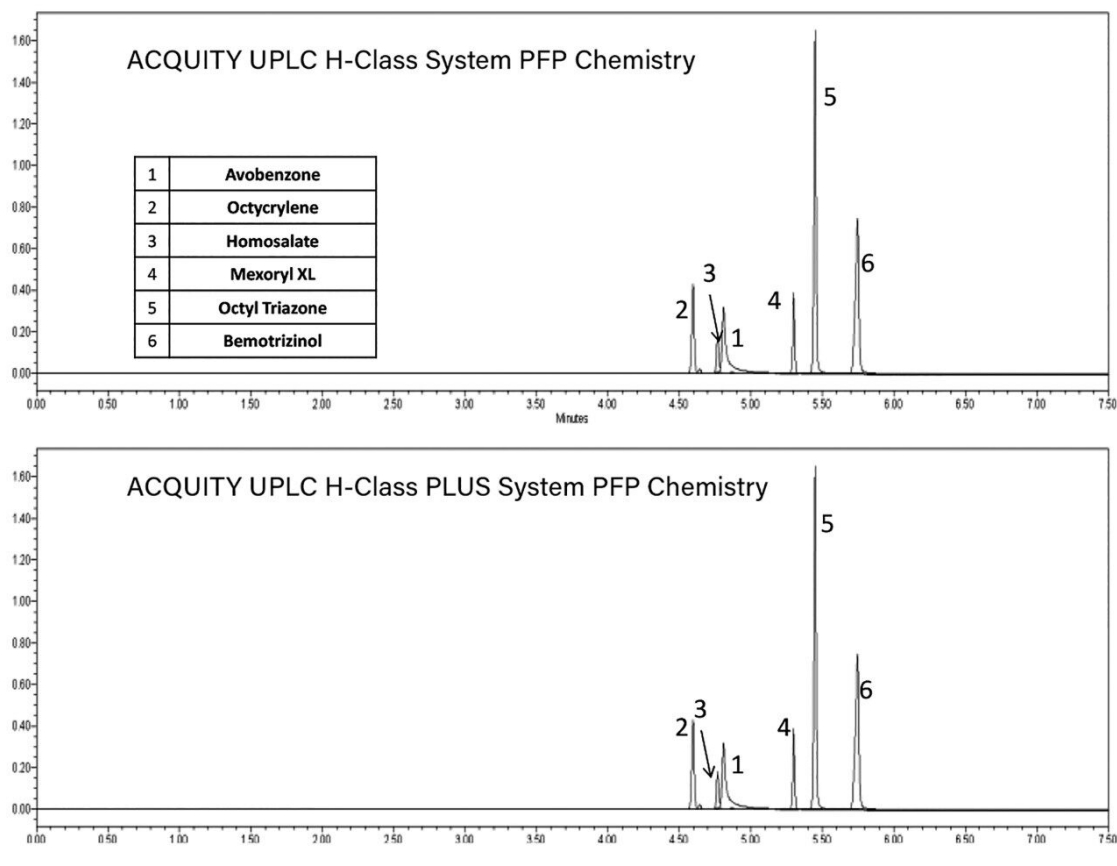


Figure 2. Overlaid chromatograms of individual sunscreen standards, separated using the ACQUITY UPLC HSS PFP Column on both the ACQUITY UPLC H-Class and ACQUITY UPLC H-Class PLUS systems.

## ACQUITY UPLC H-Class peak area, retention times

Peak Results				Peak Results				Peak Results				Peak Results			
	Name	RT	Area		Name	RT	Area		Name	RT	Area		Name	RT	Area
1	Avobenzone	4.533	1419564.88	1	Octocrylene	4.689	560820.74	1	Bemotrizinol	5.939	1360148.26	1	Octyl Triazone	5.771	1889756.43
2	Avobenzone	4.535	1423840.05	2	Octocrylene	4.693	546665.19	2	Bemotrizinol	5.941	1368765.77	2	Octyl Triazone	5.771	2041283.00
3	Avobenzone	4.536	1419039.00	3	Octocrylene	4.694	552521.22	3	Bemotrizinol	5.941	1368454.08	3	Octyl Triazone	5.773	2023804.58
4	Avobenzone	4.536	1432860.08	4	Octocrylene	4.694	547436.65	4	Bemotrizinol	5.941	1369575.18	4	Octyl Triazone	5.774	1979133.17
5	Avobenzone	4.538	1441333.88	5	Octocrylene	4.697	543109.84	5	Bemotrizinol	5.941	1364271.45	5	Octyl Triazone	5.775	200027.47
6	Avobenzone	4.550	1451213.06	6	Octocrylene	4.698	546748.08	6	Bemotrizinol	5.942	1371803.06	6	Octyl Triazone	5.779	2036603.48
Mean		4.5	1431306.8	Mean		4.7	547933.6	Mean		5.9	136369.6	Mean		5.8	1988561.4
%RSD		0.136	0.9	%RSD		0.064	0.6	%RSD		0.018	0.4	%RSD		0.048	2.9

## ACQUITY UPLC H-Class PLUS peak area, retention times

Peak Results				Peak Results				Peak Results				Peak Results			
	Name	RT	Area		Name	RT	Area		Name	RT	Area		Name	RT	Area
1	Avobenzone	4.554	1316245.78	1	Octocrylene	4.696	540891.27	1	Bemotrizinol	5.925	1371317.42	1	Octyl Triazone	5.767	2062528.92
2	Avobenzone	4.555	1326033.96	2	Octocrylene	4.698	540343.36	2	Bemotrizinol	5.926	1363376.16	2	Octyl Triazone	5.768	2041976.56
3	Avobenzone	4.555	1334866.93	3	Octocrylene	4.699	545472.97	3	Bemotrizinol	5.928	1351829.18	3	Octyl Triazone	5.768	2074899.61
4	Avobenzone	4.555	1311063.00	4	Octocrylene	4.699	550328.23	4	Bemotrizinol	5.929	1373573.84	4	Octyl Triazone	5.768	2072179.52
5	Avobenzone	4.556	1347540.65	5	Octocrylene	4.699	544847.22	5	Bemotrizinol	5.929	1369769.05	5	Octyl Triazone	5.769	2060466.23
6	Avobenzone	4.558	1342976.41	6	Octocrylene	4.700	538684.93	6	Bemotrizinol	5.929	1375154.03	6	Octyl Triazone	5.770	2060666.43
Mean		4.6	1329792.8	Mean		4.7	543511.3	Mean		5.9	1367903.3	Mean		5.8	2062126.2
%RSD		0.030	1.1	%RSD		0.031	0.8	%RSD		0.024	0.6	%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.

## Conclusion

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.

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## Featured Products

ACQUITY UPLC H-Class PLUS System <<https://www.waters.com/10138533>>

Empower 3 Chromatography Data Software <<https://www.waters.com/10190669>>

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