

## Application Note

# Monitoring Cell Culture Media for Amino Acids Using an ACQUITY UPLC H-Class PLUS System

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## Abstract

This application brief demonstrates superior performance of an ACQUITY UPLC H-Class PLUS System with UV detection for accurate monitoring of amino acids commonly found in cell culture media.

The ACQUITY UPLC H-Class PLUS System delivered superior results for the analysis of amino acids in cell culture media. Excellent chromatographic resolution and reliable performance ensures accurate and precise qualitative and quantitative results.

Overall, the ACQUITY UPLC H-Class PLUS System integrated with pre-tested derivatization and separation chemistry provides an analytical approach for accurate monitoring media composition during the manufacturing process. It will enable the biopharmaceutical industry to effectively evaluate the robustness and consistency of the cell culture production process, which is essential to manufacturing high quality products.

## Benefits

The ACQUITY UPLC H-Class PLUS System provides a means for the accurate monitoring of amino acids in cell culture media.

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## Introduction

Amino acids are the building blocks of proteins, and thus are essential components of the cell culture media to stimulate cells growth.<sup>1</sup> Cell culture is routinely utilized in production of biopharmaceuticals and cultivated meat, therefore composition of amino acids plays an important key factor in determining yield and quality of the product. The concentration of these acids in the media may often change during the process as the growing cells can either consume or release amino acids. As the cells grow, the consumed amino acids must be supplied to reach the desired level of cell density and product concentrations. Therefore, amino acids must be accurately monitored during the growth of cells in culture using a robust and reliable analytical technique.

In this technology brief, we demonstrate use of an ACQUITY UPLC H-Class PLUS System coupled with UV detection for the analysis of amino acids commonly found in cell culture media. The amino acid analysis was also facilitated by using the Waters UPLC Amino Acid Analysis Application Solution,<sup>2</sup> which includes sample derivatization chemistry, eluents, and chromatographic column to separate derivatives.

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## Results and Discussion

The amino acid analysis in cell culture media was performed following the Waters UPLC Amino Acid Analysis Application Solution.<sup>2</sup> This solution is based on the Waters AccQ•Tag pre-column derivatization and analysis technique.<sup>3</sup> The derivatized amino acids are separated on an AccQ•Tag Ultra, 1.7  $\mu$ m, 2.1 x 100 mm Column (p/n: [186003837](#)) using AccQ•Tag Ultra Eluents A and B. These mobile-phase eluents are provided as concentrates and require dilution with water before use. The pre-configured Empower Software project templates with instrument conditions for separation of amino acids are included in the ACQUITY UPLC H-Class and H-Class Bio Amino Acid Analysis System Guide.

A derivatized standard mixture of cell culture amino acids was run on an ACQUITY UPLC H-Class PLUS System with UV detection at 260 nm. The amino acids commonly found in the cell culture were successfully resolved as displayed in Figure 1. The repeatability of the retentions times and peak areas of 10 replicate injections of the standard solution was measured on the ACQUITY UPLC H-Class PLUS System and compared to the results acquired on an ACQUITY UPLC H-Class System. The %RSD of the peak areas and retention times of the amino

acids are comparable for both systems. The results show that the ACQUITY UPLC H-Class PLUS System delivers reproducible results over a series of injections with excellent separation of amino acids in the culture media.

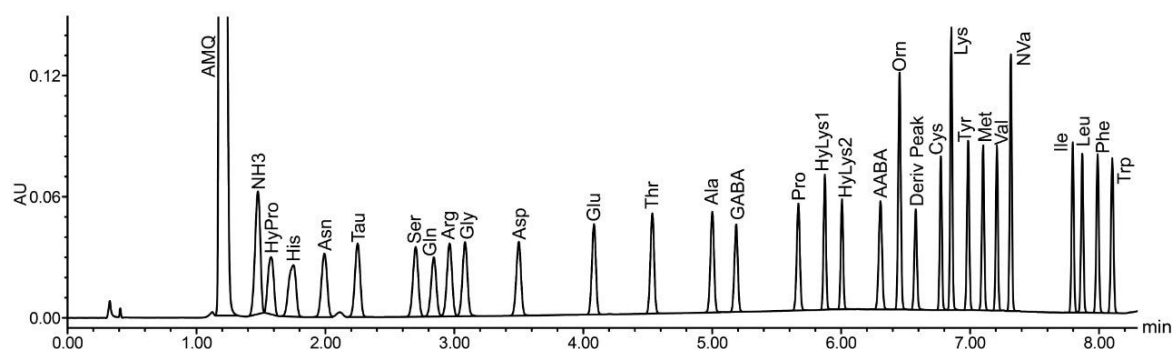


Figure 1. Separation of amino acids commonly found in the cell culture media by an ACQUITY UPLC H-Class PLUS System. UV at 260 nm.

## A. ACQUITY UPLC H-Class

<b>Empower 3</b>	<b>System Suitability</b>
Sample Set ID:	Sample Set Id 2438
Result Set ID:	Result Set Id 3444
Channel Name:	ACQUITY TUV ChA

### System Suitability Separation Results

	Name	# of Inj.	Ave RT	%RSD RT	%RSD Peak Areas
1	HyPro	10	1.651	0.20	0.73
2	His	10	1.855	0.20	0.65
3	Asn	10	2.096	0.15	0.66
4	Tau	10	2.352	0.14	0.73
5	Ser	10	2.805	0.10	0.68
6	Gln	10	2.948	0.09	0.68
7	Arg	10	3.078	0.09	0.79
8	Gly	10	3.179	0.08	0.59
9	Asp	10	3.578	0.07	0.66
10	Glu	10	4.134	0.04	0.67
11	Thr	10	4.568	0.04	0.97
12	Ala	10	5.021	0.03	0.66
13	GABA	10	5.198	0.03	0.68
14	Pro	10	5.666	0.02	0.69
15	HyLys1	10	5.856	0.02	0.74
16	HyLys2	10	5.989	0.02	1.16
17	AABA	10	6.295	0.03	0.67
18	Orn	10	6.430	0.03	0.90
19	Cys	10	6.742	0.02	0.73
20	Lys	10	6.829	0.03	0.77
21	Tyr	10	6.967	0.03	0.69
22	Met	10	7.083	0.03	0.65
23	Val	10	7.190	0.03	0.65
24	NVa	10	7.300	0.03	0.83
25	Ile	10	7.798	0.03	0.60
26	Leu	10	7.875	0.04	0.60
27	Phe	10	8.001	0.04	0.68
28	Trp	10	8.125	0.04	0.71

## B. ACQUITY UPLC H-Class PLUS

<b>Empower 3</b>	<b>System Suitability</b>
Sample Set ID:	Sample Set Id 2261
Result Set ID:	Result Set Id 3527
Channel Name:	PDA Ch1 260nm@4.8nm

### System Suitability Separation Results

	Name	# of Inj.	Ave RT	%RSD RT	%RSD Peak Areas
1	HyPro	10	1.603	0.85	0.99
2	His	10	1.779	0.91	0.51
3	Asn	10	2.025	0.84	0.43
4	Tau	10	2.286	0.82	0.46
5	Ser	10	2.738	0.71	0.48
6	Gln	10	2.881	0.72	0.46
7	Arg	10	3.005	0.70	0.42
8	Gly	10	3.120	0.59	0.47
9	Asp	10	3.534	0.48	0.47
10	Glu	10	4.109	0.33	0.43
11	Thr	10	4.556	0.24	0.43
12	Ala	10	5.018	0.18	0.46
13	GABA	10	5.202	0.15	0.45
14	Pro	10	5.680	0.10	0.47
15	HyLys1	10	5.880	0.06	0.48
16	HyLys2	10	6.012	0.05	0.45
17	AABA	10	6.313	0.06	0.44
18	Orn	10	6.457	0.03	0.46
19	Cys	10	6.776	0.03	0.47
20	Lys	10	6.856	0.03	0.45
21	Tyr	10	6.986	0.03	0.45
22	Met	10	7.102	0.04	0.42
23	Val	10	7.208	0.05	0.44
24	NVa	10	7.316	0.05	0.45
25	Ile	10	7.796	0.06	0.49
26	Leu	10	7.870	0.06	0.50
27	Phe	10	7.990	0.06	0.44
28	Trp	10	8.104	0.06	0.44

Figure 2. Results for 10 replicate injections of the cell culture standard solution performed on ACQUITY UPLC H-Class (A) and ACQUITY UPLC H-Class PLUS (B) Systems. UV at 260 nm.

## Conclusion

The ACQUITY UPLC H-Class PLUS System delivered superior results for the analysis of amino acids in cell culture media. Excellent chromatographic resolution and reliable performance ensures accurate and precise qualitative and quantitative results.

Overall, the ACQUITY UPLC H-Class PLUS System integrated with pre-tested derivatization and separation chemistry provides an analytical approach for accurate monitoring media composition during the manufacturing process. It will enable the biopharmaceutical and cultured meat industries to effectively evaluate the robustness and consistency of the cell culture production process, which is essential to manufacturing high quality products.

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## References

1. <https://www.labome.com/method/Cell-Culture-Media-A-Review.html>
2. UPLC Amino Acid Analysis Application Solution, Waters Brochure, 720001837EN, January 2008.
3. AccQ•Tag Ultra Derivatization Kit, Waters Care and Use Manual, 715001331, July 2014.

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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/513188>>

## Available for Purchase Online

AccQ•Tag Ultra RP Column 130Å 1.7 µm, 2.1 mm 100 mm <  
<https://www.waters.com/waters/partDetail.htm?partNumber=186003837>>

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