SARTORIUS

Application Note

March 22, 2022

Keywords or phrases
LC-MS, BioAccord™, Cell Culture media, amino acid, vitamins, HRMS, Ambr® 15

In-Process Media Monitoring Using the BioAccord™ LC-MS System for the Automated High Throughput Multi-Parallel Ambr® 15 Microbioreactor System

Yun W. Alelyunas[1], Charles Prochaska[2]*, Clint Kukla[2], Mark D. Wrona[1], Ying Qing Yu[1]
1. Waters Corporation 34 Maple St. Milford MA 01757
2. Sartorius Stedim North America, Inc., 545-1 Johnson Avenue, Bohemia, NY 11716

*Correspondence
Email: charles.prochaska@sartorius.com

Abstract

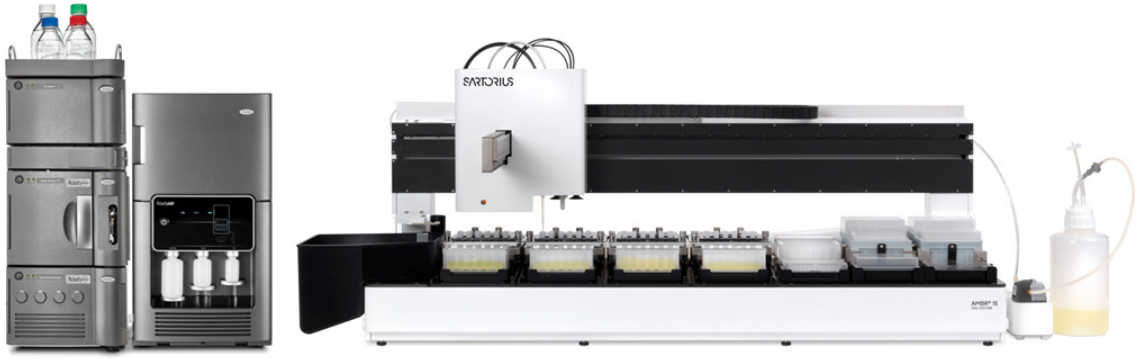
The BioAccord™ LC-MS (liquid chromatography – mass spectrometry) system has been used for the analysis of cell culture media samples from an Ambr® 15 bioreactor. The comprehensive LC-MS methodology, workflow, and batch analysis software allowed bioprocessing engineers to quickly and easily run and process large number of samples. Multivariate data analysis provided an overview of differences in composition and concentration across all vessels. This information, when combined with other process or product related quality attributes, will enable bioprocess groups to quickly gain insight on the impact that critical bioprocess parameters have on products CQAs.

For More Information, Visit
Multi-Parallell Bioreactors | Sartorius

Introduction

Cell culture media provides essential nutrients and components required to sustain cell health and growth and for maximizing biotherapeutic production and quality. There is increasing interest from bioprocess groups to monitor raw media, feed, supplements and spent cell culture media, while providing this information quickly to process engineers. In addition to essential amino acids, vitamins, and other primary feed components, spent media contains hundreds of additional compounds and metabolites.

Routine comprehensive monitoring could provide additional information about cell culture performance during clone selection and process optimization. For this application note, methodology developed for cell culture media monitoring based on the BioAccord™ LC-MS System[1] is applied to the routine screening of in-process samples from an Ambr® 15 high throughput bioreactor system (Scheme 1).



Scheme 1: *A Schematic Illustration of BioAccord™ LC-MS System and Ambr® 15 High Throughput Bioreactor System.*

Methods

LC Conditions

LC-MS System	BioAccord™ LC-MS system with ACQUITY™ Premier BSM
Column(s)	ACQUITY™ Premier HSS T3 2.1 × 150 mm (P | N 186009469)
Column Temp.	40 °C
Sample Temp.	6 °C
Injection Volume	2 µL
Flow Rate	0.25 mL | min
Mobile Phase A	H_2O | 0.1% FA
Mobile Phase B	90% ACN | 10% IPA | 0.1% FA

Gradient Table

Time (min)	Flow (mL/min)	%A	%B	Curve
0	0.25	100	0	6
1.5	0.25	100	0	6
6	0.25	95	5	6
9	0.25	65	35	6
14	0.25	5	95	6
17	0.25	5	95	6
17.1	0.25	100	0	6
20	0.25	100	0	6

Results

MS Conditions

LC-MS System	BioAccord™ LC-MS system with ACQUITY™ Premier BSM	
Ionization Mode	Full scan or Full scan with fragmentation	
Acquisition Range	Low (50 – 2000)	
Polarity	Positive	
	Capillary Voltage	1 kV
	Cone Voltage	20 V
	Fragmentation cone voltage	60 – 80 V
	Negative	
	Capillary Voltage	0.8 kV
	Cone Voltage	15 V
	Fragmentation cone voltage	50 – 70 V
Scan rate	5 Hz	
Desolvation temperature	550 °C	
Intelligent data capture	On	
Dynamic lock mass correction	On	

Data Management

LC-MS software	waters_connect™ Informatics Platform
Informatics	waters_connect™ base kit with UNIFI 1.9 SR13

A mammalian CHO fed batch process was employed for an extended 14-day cultivation on a 24-way Ambr® 15 Cell Culture bioreactor system. Varying media formulations and supplement conditions were used to provide a diverse range of component concentrations. The base cell culture media and feeds used were serum-free Sartorius brand chemically defined media for CHO cell culture. Starting on day 3 samples were taken daily for cell count and spent media measurement using the Ambr® 15 automated liquid handler for vessels: CS1:01 (Media 1-Feed 1), CS1:05 (Media 2-Feed 2), and CS1:09 (Media 1-Feed 3). Media aliquots were centrifuged, filtered, and the filtrate diluted using 0.1% FA at a 1:200 ratio prior to loading onto BioAccord™ System for LC-MS analysis. Detailed LC-MS acquisition parameters using Accurate Mass Screening Workflow have been described previously.[1] All data was collected and processed using the compliance ready waters_connect™ informatics platform.

Compound assignment was based on their retention time and mass/charge (m/z) alignment using the imbedded 200+ compound cell culture media library. The data review panel is shown in Figure 1. On the left side of the screen, the workflow is organized by function, compound class, and | or transformation pathways, thus simplifying and assisting an end user to review the data in a stepwise, systematic fashion. The workflow is customizable if additional information or views are desired. The compound summary table (top right) provides a tabulation of response and other compound related information which can be exported for further data processing. The bottom panel shows the detected peak (extracted ion chromatogram) of the currently selected compound as well as a summary plot, showing its level across all injections. From this trending plot the changes in a compound over the batch process is readily displayed.

Overlaid trend plots of representative compounds across three vessels over the course of the batch are shown in Figure 2. The graph revealed changing responses for some compounds while others remained similar. For example, for alanine, proline, and glutamine, the changes from different vessels followed a similar trend. For asparagine, cystine, and glutamic acid, vessel CS1:05 showed different trends compared to the other two vessels suggesting the starting media composition played a larger role than the feed strategy.

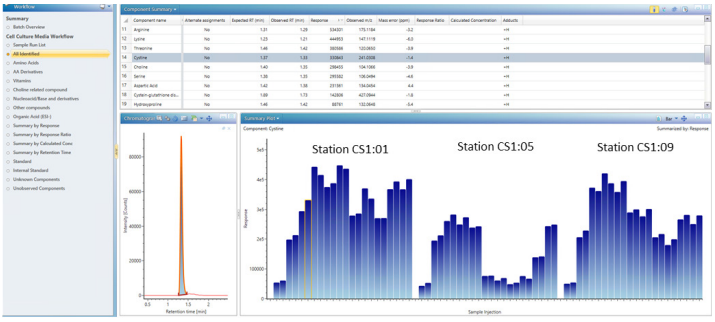


Figure 1: *An Example of waters_connect™ Data Review Panel Showing Custom Workflow, Tabulated Result Data, and Trend Plot of Cystine Response From Different Vessels and Over Sampling Time.*

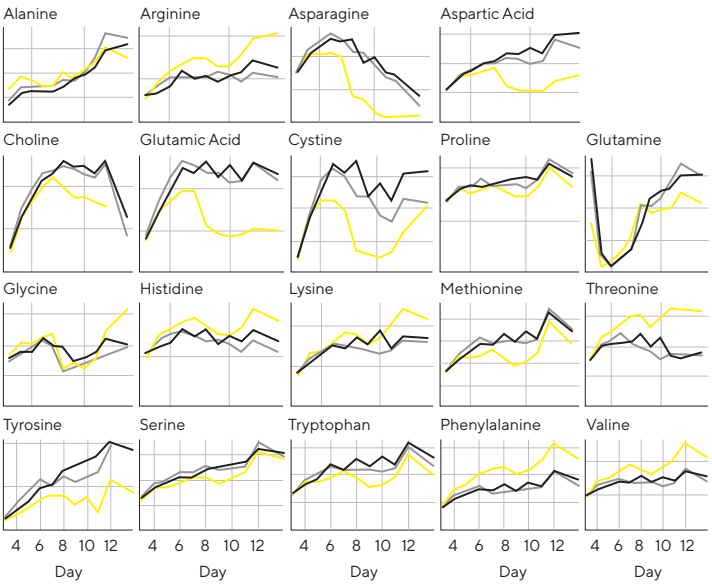


Figure 2: *Overlaid Plot of Sampling Time vs Response of Representative Compound From Three Different Vessels.*
Black Line: Vessel CS1:01 Media 1-Feed 1,
Yellow Line: Vessel CS1:05 Media 2-Feed 2,
Gray Line: Vessel CS1:09 Media 1-Feed 3.

Additional batch processing using multivariate data analysis tools was carried out. A representative PCA (Principal Component Analysis) plot of these bioreactors across sampling time is shown in Figure 3. PCA analysis showed increasing differentiation of media composition over time. Initially, at day 3, media composition of all three vessels are similar. As cultivation process continues, the media changes were similar for vessels CS1:01 and CS1:09, while vessel CS1:05 showed a very different pattern. This information with additional batch comparison analysis, when combined with other process or product related quality attributes, will enable bioprocess groups to quickly gain insight during bioprocess development.

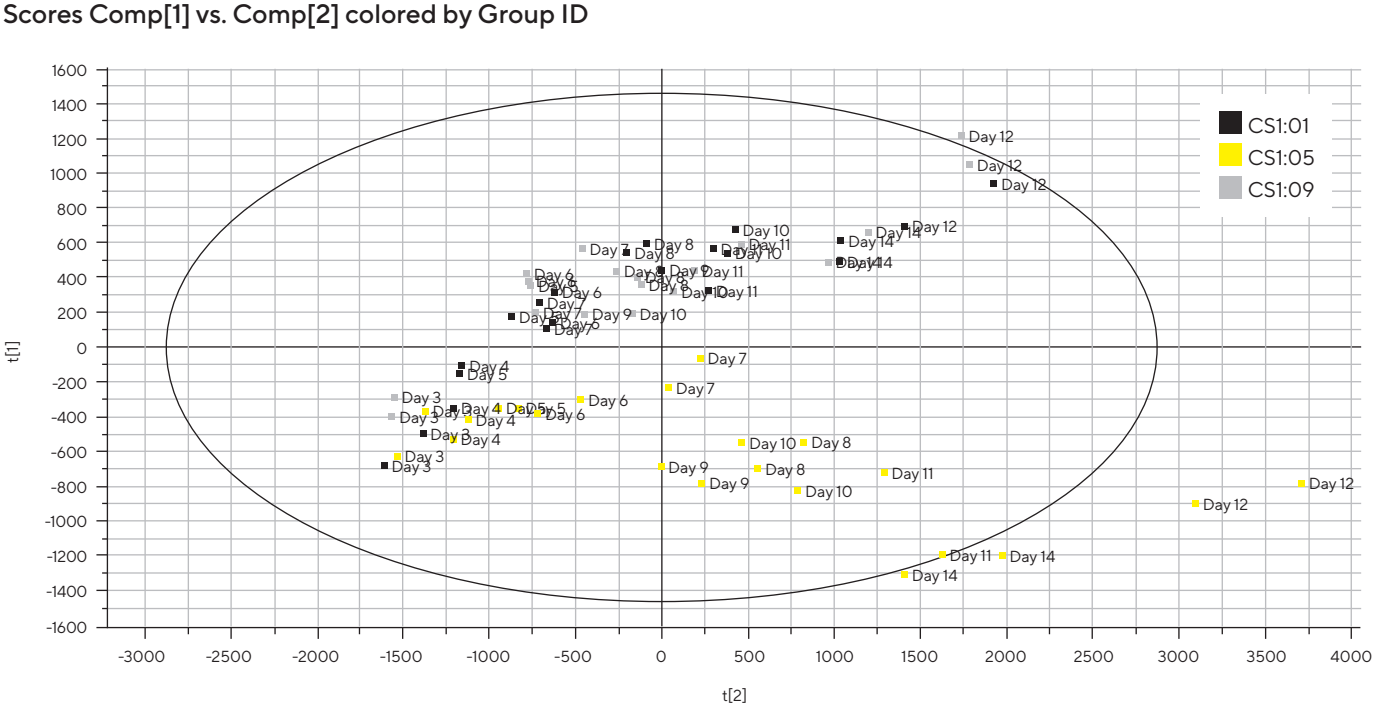


Figure 3: *PCA Plot of Bioreactors Across Sampling Time. The Data Are Coloured by Vessel Name and Labelled by Sampling Time (Day).*

Conclusion

A comprehensive LC-MS methodology and workflow for the analysis of cell culture media samples from the Ambr® 15 bioreactor system is described. Using an Ambr® 15 multi-parallel bioreactor system and BioAccord™ LC-MS System, media collection and LC-MS acquisition could be carried out for multiple bioreactors during the cultivation process on a daily basis. In this way, when the fed-batch process is completed in ~2 weeks, the media data from LC-MS analysis is also complete and readily available for more detailed data mining especially for process related understandings.

The simplicity of BioAccord™ LC-MS system in terms of ease of method setup and long-term performance stability, is an added benefit for bioprocessing engineers with limited LC-MS experience to quickly and easily run and process large numbers of samples. Overall, the routine LC-MS based analysis of Ambr® 15 bioreactor samples enable bioprocess groups to quickly gain insight during bioprocess development.

References

1. Alelyunas YW, Wrona MD, Chen W, Monitoring Nutrients and Metabolites in Spent Cell Culture Media for Bioprocess Development Using the BioAccord™ LC-MS System with ACQUITY Premier, Waters Application Note 720007359, 2021 September

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For More Information, Visit
www.sartorius.com

Status 06 | 17 | 2022