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Application Note

Monitoring Nutrients and Metabolites in Microbial Culture Media using the BioAccord LC-MS System with ACQUITY Premier

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Along with mammalian cell culture systems, microbial based fermentation requires media to support the growth and maintenance of microorganism and high-quality protein production.[1] In this technology brief, we describe the application of the liquid chromatography-mass spectrometry (LC-MS) methodology and workflow developed for cell culture media using the BioAccord LC-MS System for nutrient and metabolite monitoring in growth media for microbial based bioprocessing. The method package includes a comprehensive reversed-phase LC-MS method, a 200+ compound library; a simple, stepwise workflow for data review including trend plots; a suite of tools for unknown screening; multivariate data analysis tools; and reporting template. This media monitoring is a supplement to product quality analysis that BioAccord LC-MS System provides, which includes intact protein analysis, peptide mapping and monitoring (including Multiple Attribute Method), released glycan analysis, and oligonucleotide mass confirmation.

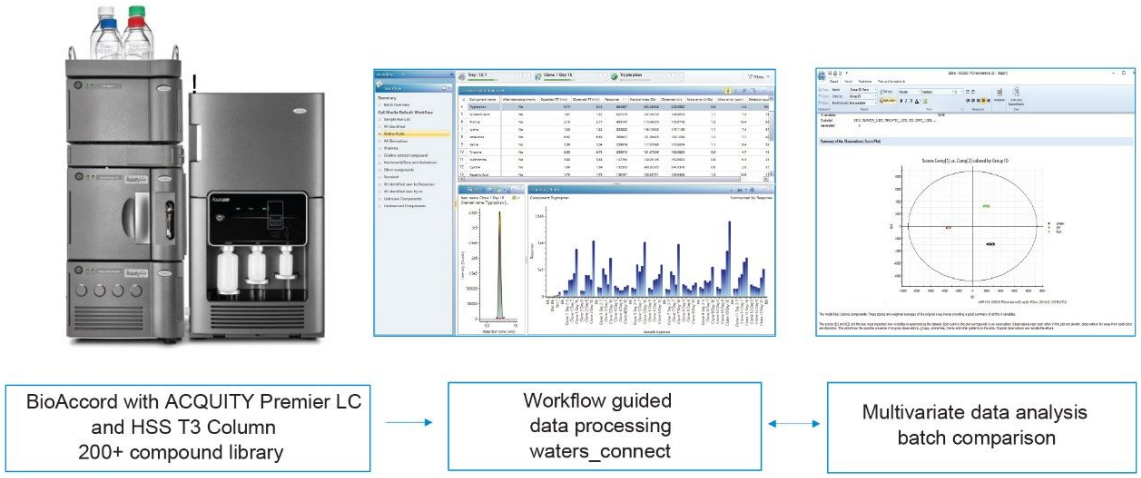
Benefits

- Accelerated media development and monitoring enabled by the direct measurement of microbial growth media attributes with dedicated workflows using the BioAccord LC-MS System, High Performance Surfaces (HPS) Technologies, and waters_connect informatics
- Enable decision making with the acquisition of process inputs and product quality output on a single LC-MS platform supporting media as well as product quality attributes-monitoring, including intact protein analysis, peptide MAM, released glycan, and oligonucleotide mass confirmation

Introduction

In bioprocess development, microorganisms, such as *E. coli*, have been developed as hosts for protein expression and purification, vector construction and gene synthesis.[2] Microbial growth medium is used for the growth and maintenance of microorganism, and protein production. The media, typically formulated from yeast extract, is nutrient-rich containing amino acids, water-soluble vitamins, carbohydrates, peptides, glycerol, and other compounds. It is desirable to have a comprehensive analysis method for their detection.

In this technology brief, the LC-MS methodology and workflow developed using the BioAccord LC-MS System for the analysis of cell culture media[3] is employed for the analysis of microbial growth media. Scheme 1 is a flowchart of the BioAccord LC-MS methodology and workflow. It includes reversed-phase LC separation, HRMS data acquisition, a 200+ compound library, a guided workflow for ease of data review, and multivariate data processing for batch analysis. In this technology brief, two commonly utilized microbial media formulation were obtained and analyzed. Their major components are identified and contrasted.



Scheme 1. A schematic illustration of BioAccord/waters_connect-based workflow for media analysis (adopted from Waters Appnote[3]).

Results and Discussion

Liquid microbial growth media LB broth (Miller) and Terrific broth were purchased from MilliporeSigma (St. Louis, MO). Each broth was diluted 1:100 (V:V) with H_2O. Data acquisition was performed using the BioAccord LC-MS System with ACQUITY Premier Technology as described in a Waters application note.[2] Small molecule mass range of 50–800 *m/z* and dynamic lock mass correction were used. Injection volume was 2 µL.

Samples were analyzed under reversed-phased conditions using the ACQUITY Premier HSS T3 Column (1.8 mm, 2.1 x 150 mm, p/n: 186009469 <https://www.waters.com/nextgen/global/shop/columns/186009469-acquity-premier-hss-t3-column-18--m-21-x-150mm-1-pk.html>). The 200+ compound library was imported for compound analysis and detection. An overlaid chromatogram of detected compounds for Terrific broth media sample using positive or negative ionizations, respectively, is shown in Figure 1. Results showed that 90+ compounds are detected in Terrific broth media sample including all compound classes in the library such as amino acid and its derivatives, vitamins, nucleobase, nucleoside, nucleotide, organic acid, peptide fragments and many other compounds. The most abundant compounds are a panel of amino acids as exhibited in the overlaid chromatogram in Figure 1A.

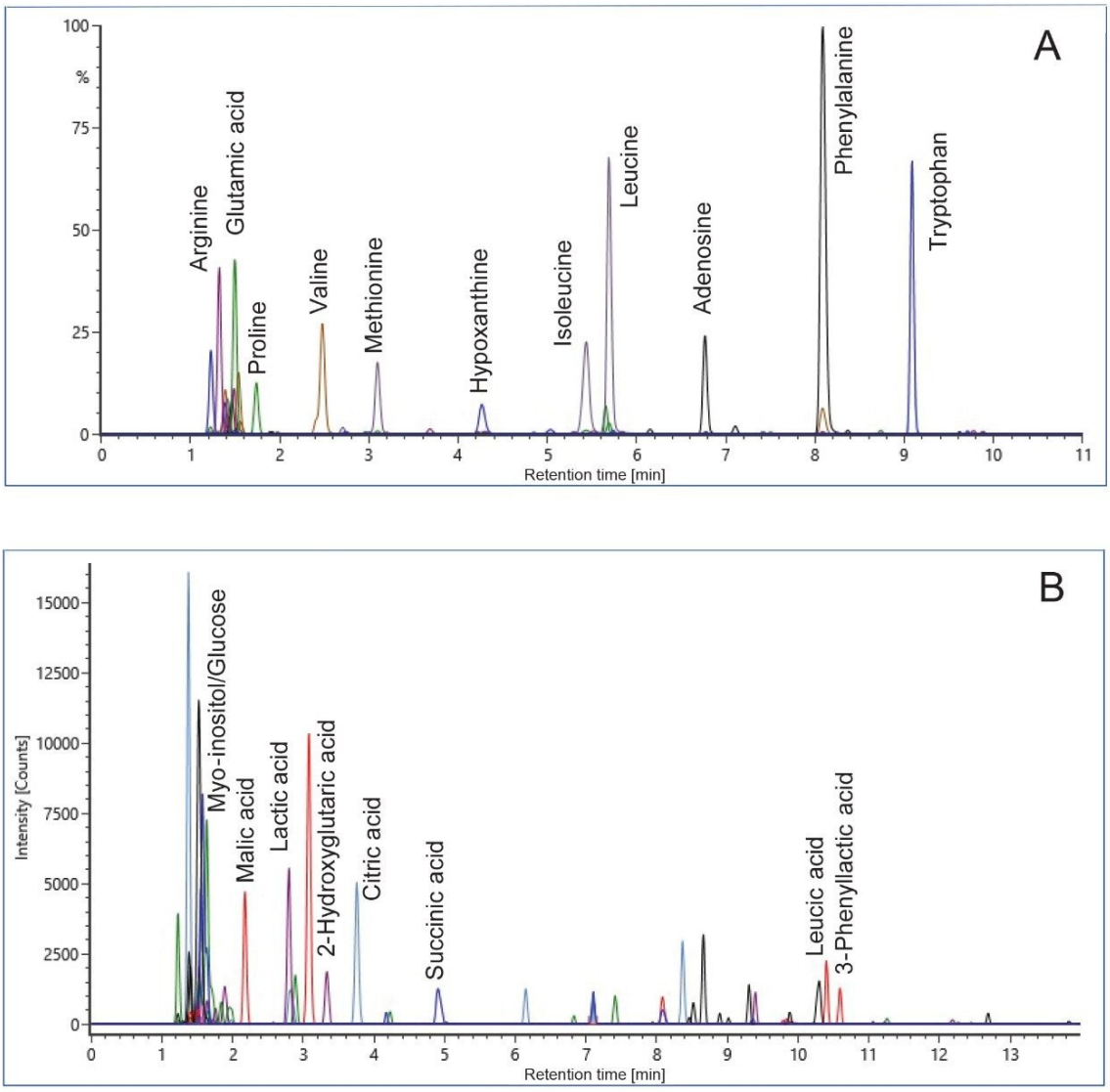


Figure 1. Overlaid chromatogram of Terrific broth media. (A) ESI+ acquisition mode. (B) ESI- acquisition mode. Compounds not labeled under ESI- ionization are generally also detected in ESI+ mode of acquisition.

Similar compounds are also found in LB broth media with amino acids being the most abundant compound detected. A summary display for the response of top 50 compounds detected in both Terrific and LB broth media is shown in Figure 2. Results indicate that Terrific broth in general have higher concentrations compared to LB broth.

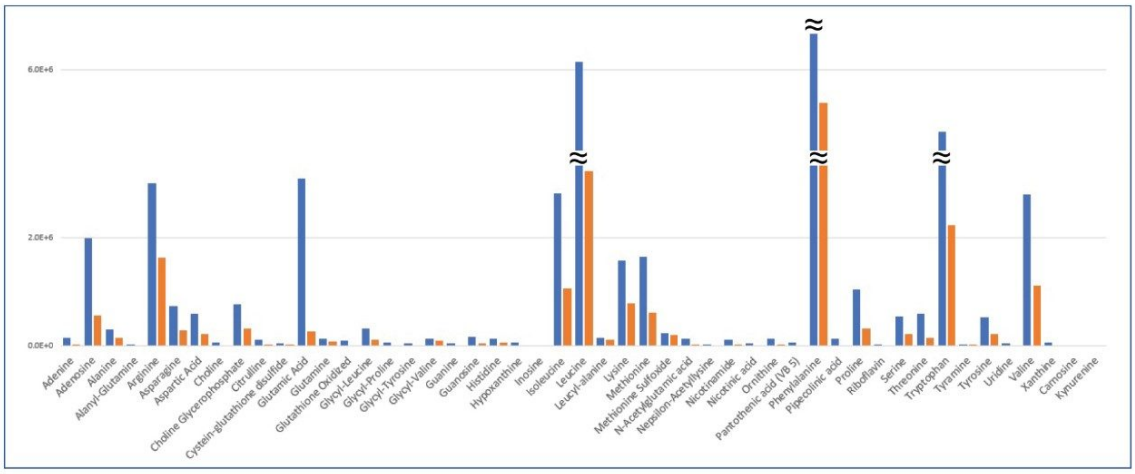


Figure 2. Response comparison of Terrific broth media with LB broth. Blue bars, Terrific broth. Orange bars, LB broth.

LC-MS detection reproducibility data was collected based on six replicate injections of Terrific broth media solution. Data for top 50 compounds in terms of response, retention time, and mass error are summarized in Table 1. Results showed excellent reproducibility with response <5 %RSD, retention time <0.2 %RSD, and mass error <5 ppm. The results also showed that the reproducibility is robust and independent of high or low response range, early or late retention time, and high or low observed mass values. In all, the data demonstrates the methodology previously developed for cell culture media can be readily deployed for the compound monitoring in growth media used in microbial based fermentations.

Component name	Response		rt (min)		Mass error (ppm)		Compound class
	Avg	%RSD	Avg	%RSD	Avg	Stdev	
Alanine	3.0E+05	3.1	1.43	0.03	-3.1	1.9	Amino acid
Arginine	3.0E+06	1.9	1.32	0.01	-2.3	1.4	Amino acid
Asparagine	7.3E+05	4.0	1.39	0.02	-2.9	1.7	Amino acid
Aspartic acid	6.0E+05	3.8	1.42	0.01	-1.2	1.2	Amino acid
Glutamic acid	3.1E+06	2.6	1.50	0.01	-3.4	1.5	Amino acid
Glutamine	1.4E+05	2.7	1.65	0.04	-2.4	1.9	Amino acid
Glycine	2.0E+04	4.2	1.36	0.03	-0.2	3.3	Amino acid
Histidine	1.4E+05	1.0	1.30	0.02	-2.9	1.7	Amino acid
Isoleucine	2.8E+06	2.6	5.43	0.09	-3.2	1.5	Amino acid
Leucine	6.1E+06	4.8	5.69	0.03	-2.9	1.7	Amino acid
Lysine	1.6E+06	1.8	1.23	0.02	-2.6	1.7	Amino acid
Methionine	1.7E+06	2.7	3.09	0.14	-2.7	1.0	Amino acid
Phenylalanine	1.0E+07	1.9	8.09	0.01	-1.8	1.2	Amino acid
Proline	1.1E+06	2.5	1.73	0.09	-3.4	1.9	Amino acid
Serine	5.4E+05	3.2	1.38	0.03	-3.9	2.2	Amino acid
Threonine	5.9E+05	3.2	1.47	0.02	-3.7	1.6	Amino acid
Tryptophan	4.9E+06	1.3	9.09	0.00	-1.3	0.7	Amino acid
Tyrosine	5.3E+05	4.4	5.65	0.03	-2.3	1.0	Amino acid
Valine	2.8E+06	2.9	2.47	0.13	-3.5	1.7	Amino acid
Citrulline	1.1E+05	2.6	1.54	0.03	-1.1	1.4	Amino acid derivatives
Glycyl-leucine	3.2E+05	1.6	8.67	0.00	-1.8	1.5	Amino acid derivatives
Glycyl-valine	1.3E+05	3.8	5.51	0.08	-2.0	1.3	Amino acid derivatives
Leucyl-alanine	1.6E+05	2.7	6.75	0.02	-2.7	0.6	Amino acid derivatives
Methionine sulfoxide	2.4E+05	3.2	1.55	0.04	-2.2	1.2	Amino acid derivatives
N-Acetylglutamic acid	1.3E+05	1.8	5.02	0.14	-1.4	1.3	Amino acid derivatives
Ornithine	1.3E+05	2.1	1.22	0.02	-2.9	1.5	Amino acid derivatives
5-Methylcytidine	1.7E+04	1.8	4.87	0.20	-2.2	1.1	Necleobase, nucleoside, nucleotide
Adenine	1.5E+05	3.2	2.90	0.22	-3.1	2.0	Necleobase, nucleoside, nucleotide
Adenosine	2.0E+06	1.7	6.77	0.02	-2.3	0.6	Necleobase, nucleoside, nucleotide
AMP	2.3E+04	3.5	3.60	0.31	-1.9	1.7	Necleobase, nucleoside, nucleotide
Cytidine	1.7E+04	4.1	2.85	0.44	0.3	1.7	Necleobase, nucleoside, Nucleotide
Guanine	5.0E+04	4.0	2.95	0.21	-4.3	1.0	Necleobase, nucleoside, nucleotide
Guanosine	1.7E+05	2.8	7.10	0.01	-2.3	1.7	Necleobase, nucleoside, nucleotide
Hypoxanthine	5.9E+04	2.8	4.21	0.17	-2.5	1.4	Necleobase, nucleoside, nucleotide
Inosine	2.2E+04	2.4	7.13	0.01	-1.7	2.0	Necleobase, nucleoside, nucleotide
Uridine	4.9E+04	3.1	5.84	0.04	-0.4	1.4	Necleobase, nucleoside, nucleotide
Xanthine	5.8E+04	3.8	5.29	0.13	-2.0	2.1	Necleobase, nucleoside, nucleotide
2-Pyrrolidinone	1.4E+04	4.0	5.87	0.04	-2.8	1.8	Others
4-Guanidinobutanoic acid	1.1E+04	4.4	2.94	0.19	-2.4	1.3	Others
Choline glycerophosphate	7.7E+05	2.2	1.49	0.02	-2.7	1.1	Others
Choline phosphate	1.1E+04	4.2	1.42	0.04	-3.2	1.7	Others
Cystein-glutathione disulfide	5.6E+04	4.3	1.89	0.18	-0.9	0.9	Others
Glutathione oxidized	9.3E+04	1.8	6.15	0.02	0.4	1.3	Others
Pipecolinic acid	1.4E+05	3.8	2.70	0.15	-2.8	1.5	Others
4-Aminobenzoic acid	1.7E+04	3.3	8.75	0.01	-2.5	1.3	Vitamins
Choline	6.3E+04	4.5	1.40	0.01	-2.9	2.5	Vitamins
Nicotinamide	1.2E+05	3.7	3.67	0.21	-4.0	1.3	Vitamins
Nicotinic acid	4.3E+04	3.5	3.19	0.20	-4.0	1.0	Vitamins
Pantothenic acid (VB 5)	6.0E+04	2.9	8.74	0.00	-0.8	1.9	Vitamins
Riboflavin	3.8E+04	3.2	9.62	0.01	-0.6	1.8	Vitamins

Table 1. Summary of reproducibility data for top 50 compounds observed in terrific broth microbial

media based on 6 replicate injections. The data is sorted by compound class followed by component name.

Conclusion

A workflow based on the BioAccord LC-MS System and waters_connect informatics platform has been employed for the analysis of compounds in microbial growth media. Based on compound library included in the workflow, 90+ compounds are detected in the commercially available microbial growth media. The system produced excellent reproducibility across response range, retention time and mass range. BioAccord LC-MS System enables easy deployment, simple operation, and long-term performance stability. This allows added benefit for bioprocessing engineers with no or limited LC-MS experience to quickly and easily run and process large numbers of samples. This workflow built using the BioAccord LC-MS System can be readily deployed to support media monitoring in microorganism based bioprocess development.

References

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2. Baeshen MN, Al-Hejin AM, *et al*. Production of Biopharmaceuticals in *E. coli*: Current Scenario and Future Perspectives. *J Microbiol Biotechnol*. 2015 Jul; 25(7):953–62.
3. 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.

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