

Cell Culture Media LC-MS Application for the BioAccord System

Introduction

Cell culture techniques are routinely used to produce proteins intended for use as biopharmaceuticals. The culture conditions must be optimized to ensure that the protein is produced without structural modification and in the highest possible yield. These preferred conditions will often be different for each clone investigated, so many optimization experiments may be required. This assessment of growth conditions must also consider the changes in the media that occur because of cell growth, that is, the consumption of nutrients and the release of waste products. The monitoring and optimization are complex because of the large number of physical and chemical parameters that have an effect.

When using the BioAccord™ System, this application provides a fast and comprehensive method that allows the screening of 200+ media components in media solution.

- Efficiency:** Monitor multiple compound types within one single analysis
- Specificity:** Compound tracking with high accuracy, reproducibility and sensitivity
- Information rich:** Automated detection of library compound , structural elucidation of unknowns
- Ease of use:** Simple dilute and inject, no derivatization is needed

Required Consumable Materials:

Description	Part Number
Cell Culture Media Starter Kit	176005127
Amino Acid Cell Culture Standard Kit	186009300
ACQUITY™ Premier HSS T3, 1.8 µm, 2.1 x 150 mm Column	186009469
Vion™ Test Mix	186008462



The kit can be used at system level when starting the cell culture media analysis. For detailed methodology of the analysis, please see references below. This guide is intended to supplement the training provided by Waters™ Analytical Professional Services.

Step-by-Step Instructions

STEP 1: Check LC and MS system performance using a system standard.

Check the chromatographic performance and mass accuracy by using the method outlined in the online document:

[How to perform the BioAccord cell culture media system suitability test - WKB231508.](#)

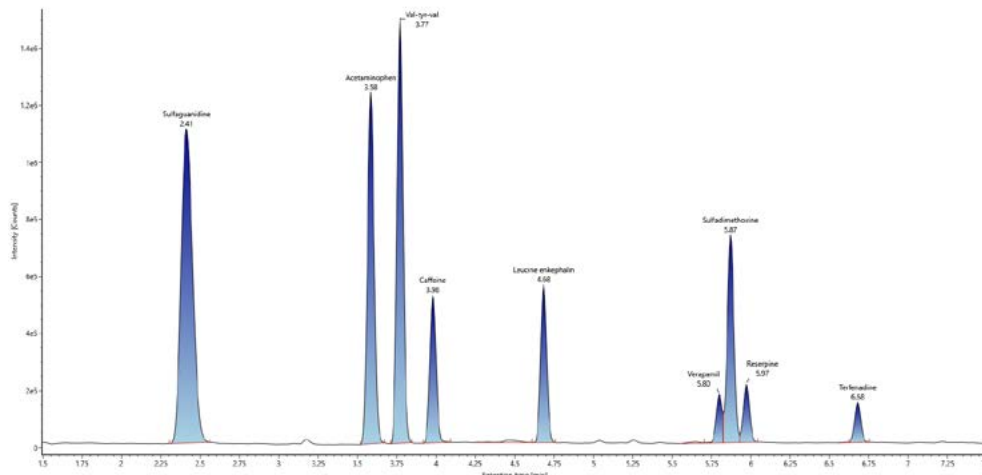





Figure 1. Example chromatogram for system performance.

STEP 2: Prepare the amino acid (AA) cell culture standard and your cell culture media samples for LC-MS analysis.

The amino acid standard is recommended to check the system performance before the analysis of cell culture media samples. It can also be used for amino acid quantification, if required.

RECONSTITUTION RECOMMENDATIONS for underivatized LC-MS-based analysis (Amino Acid Cell Culture Standard Kit, p/n: 186009300)

Amino Acid 17 Hydrolysate Standard (Blue Cap Bottle)	Amino Acid Cell Culture Standard (White Cap Bottle)	HPLC Vial
<p>1 mM 17 AA stock solution</p>  <ol style="list-style-type: none"> 1. Add 1.2 mL 0.1% formic acid 2. Vortex for 10 seconds 3. Solution concentration is 1 mM, except cystine which is at 500 µM 4. Solution is stable up to 60 days in a freezer 	<p>500 µM 26 AA stock solution</p>  <ol style="list-style-type: none"> 1. Add 250 µL 0.1% formic acid 2. Add 250 µL from blue cap bottle 3. Vortex for 10 seconds 4. Solution concentration is 500 µM, except cystine which is at 250 µM 5. Solution is stable up to 60 days in a freezer, except glutamine which is stable for 15 days 	<p>5 µM 26 AA working solution</p>  <ol style="list-style-type: none"> 1. Add 495 µL 0.1% formic acid 2. Add 5 µL from white cap bottle 3. Vortex for 10 seconds 4. Solution concentration is 5 µM, except cystine which is at 2.5 µM
STEP 1	STEP 2	STEP 3

Preparation of cell culture media samples will depend on your sample. Sample recommendations:

- **For commercial media solutions:** Dilute 1/100 (v/v) in water
- **For spent media solutions:** Dilute 1/200 (v/v) in water or water + 0.1 % formic acid

Table 1. List of amino acids for the Amino Acid Cell Culture Standard Kit, p/n: **186009300**

Blue Cap Bottle		White Cap Bottle
Alanine	Lysine	Taurine
Arginine	Methionine	Hydroxyproline
Aspartic acid	Phenylalanine	Asparagine
Cystine	Proline	Glutamine
Glutamic acid	Serine	GABA (γ -Aminobutyric acid)
Glycine	Threonine	Tryptophan
Histidine	Tyrosine	Ornithine
Isoleucine	Valine	AABA (α -Aminobutyric acid)
Leucine		Hydroxylysine

STEP 3: Get your results in 20 min with the BioAccord System, ACQUITY Premier System, and ACQUITY Premier HSS T3 Column.

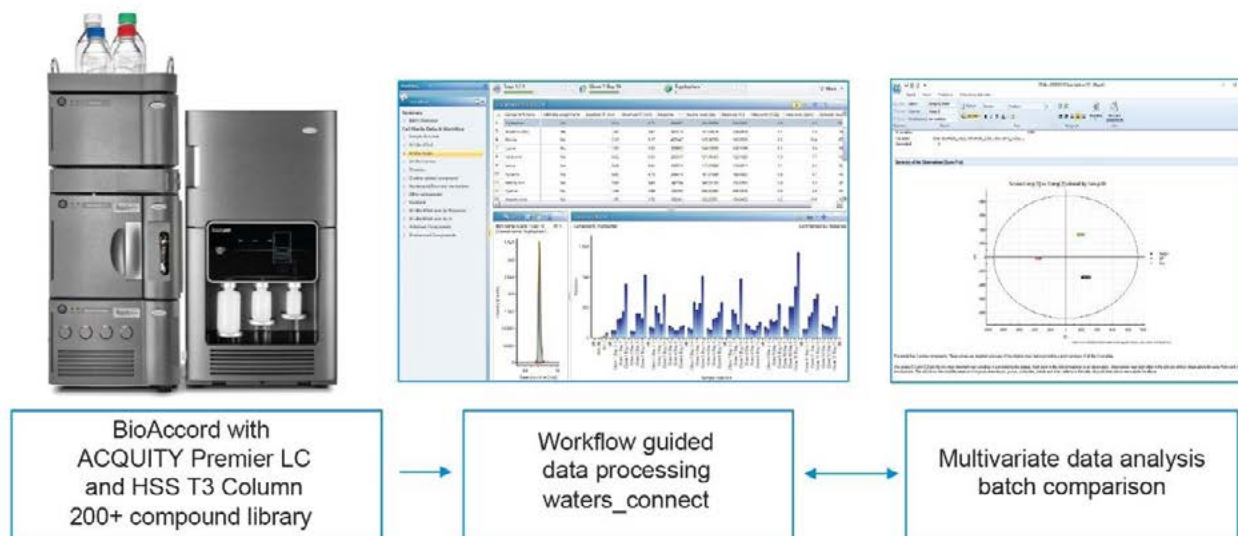


Figure 2. A schematic illustration of BioAccord/waters_connect based workflow for cell culture media analysis.

References

1. Monitoring Nutrients and Metabolites in Spent Cell Culture Media for Bioprocess Development Using the BioAccord LC-MS System with ACQUITY Premier, Waters Application Note, p/n: **720007359**.
2. How to Perform the BioAccord Cell Culture Media System Suitability Test, Waters Article, p/n: **WKB231508**
3. Amino Acid Standard Kits, Waters Care and Use Manual, p/n: **720006663EN**.
4. ACQUITY UPLC™ and ACQUITY Premier HSS Columns, Waters Care and Use Manual, p/n: **715001429**.

Related products

Kairos Amino Acid Internal Standard, p/n: **186009091**

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