

## Quantification of Underivatized Amino Acids in Cell Culture Media Using the BioAccord™ LC-MS System

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### Abstract

Accurate measurement of cell culture media components in bioprocessing is important for raw material testing (batch-to-batch and multiple vendor sourcing), media development, and spent media monitoring of metabolites in flux during the cultivation process. Here, we demonstrate the advantages and capabilities using the BioAccord LC-MS System for routine quantification of amino acids (AA) in cell culture media under underivatized conditions. Results show the method is capable of quantifying AAs with linearity range of three orders of magnitude (0.01  $\mu\text{M}$  to 10  $\mu\text{M}$ ) with high accuracy and excellent precision. The use of an unlabeled compound as the internal standard is effective for response normalization. The data suggest that the method is suitable for the quality control of amino acids in raw media as well as their consumption rates in spent media.

### Benefits

- Quantitative and qualitative monitoring of cell culture media using the simple-to-use BioAccord LC-MS System
  - A single compliant-ready informatics package supporting data acquisition, data review, elucidation of unknowns, report template, and multivariate data analysis
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## Introduction

A reversed phase liquid chromatography and mass spectrometry (LC-MS) method with workflow using ACQUITY™ Premier HSS T3 Column on the BioAccord™ LC-MS System have been developed for the cell culture media analysis (Scheme 1).<sup>1</sup> The Premier system and column incorporate MaxPeak™ High Performance Surfaces Technology in the hardware design. The methodology has been applied to cell culture media monitoring in antibody production,<sup>1</sup> microbial fermentation,<sup>2</sup> and cell and gene therapy.<sup>3</sup> Monitoring changes of the media components in different bioreactors and over time during the cultivation process have shown value for media development and process optimization.<sup>4</sup> Among more than 200 compounds included in the method library, the reversed phase method offers direct, underivatized analysis of amino acids (AAs), with no sample preparation involving derivatization required. In all media analyzed so far, AAs are the most abundant compounds as they are essential building blocks in protein biotherapeutics production. Their monitoring of feed stock and spent media during cultivation process to ensure they are in optimum range is important for therapeutic titer and product quality. This application note focuses on underivatized amino acid quantitation using reversed phase chromatography.



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*Scheme 1. A schematic illustration of BioAccord System/waters\_connect™ based workflow for cell culture media analysis.*

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## Experimental

### Sample and Standard Preparation

An external calibration solution was prepared by serially diluting a 17 amino acids stock solution to concentrations ranging from 0.01 to 10  $\mu\text{M}$ . The diluent used above was 1:1000 diluted Earle's balanced salt (EBS) stock (MilliporeSigma p/n: E2888), using 0.1% formic acid (FA) in  $\text{H}_2\text{O}$  containing 0.1  $\mu\text{M}$  3-chloro-tyrosine as an internal standard. The use of EBS was to mimic general salt conditions in spent media.

Samples used in internal standard studies were commercial media solution IMDM (MilliporeSigma p/n: I3390) and stable isotope labeled (SIL) amino acid standard mixture (Waters p/n: [186009051](https://www.waters.com/nextgen/global/shop/standards--reagents/186009051-kairos-amino-acid-internal-standard-set-100.html) < <https://www.waters.com/nextgen/global/shop/standards--reagents/186009051-kairos-amino-acid-internal-standard-set-100.html> > ). The SIL mixture was used as the internal standard (IS) and spiked into 1:100 diluted IMDM 0.1% FA solution to give a final SIL concentration of 5  $\mu\text{M}$ .

### LC Conditions

LC-MS system	BioAccord LC-MS system with ACQUITY Premier BSM
Column(s)	ACQUITY Premier HSS T3 2.1 x 150 mm (P/N 186009469)
Column temp.	40 °C
Sample temp.	6 °C
Injection volume	2 $\mu\text{L}$
Flow rate	0.25 mL/min
Mobile phase A	0.1% FA in $\text{H}_2\text{O}$
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	60	40	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

## MS Conditions

LC-MS system	BioAccord LC-MS system with ACQUITY Premier BSM		
Ionization mode	Full scan		
Acquisition range	Small molecules (50–800 <i>m/z</i> )		
Polarity	Positive		
	Capillary voltage:	1 kV	
	Cone voltage:	20 V	
Scan rate	5 Hz		
Desolvation temperature	550 °C		
Intelligent data capture	On		
Lock mass correction	Standard		
Informatics	waters_connect 3.1, cell culture media screening workflow		

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## Results and Discussion

### Standard Calibration Curve

Quantitative responses of amino acids using the cell culture media method and BioAccord LC-MS platform were collected using a 17 AA mixture. The calibration standard solutions were prepared using EBS, a balanced salt solution for media preparation, and injected at the beginning and end of a quality control (QC) sample set. The linear response based on log-log linear curve fitting was obtained for each of the amino acids. An example of the

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calibration data is shown in Figure 1, displaying chromatograms, summary response plots, and calibration curves of the two isobaric compounds isoleucine and leucine. For these two compounds, excellent baseline chromatographic separation and reproducible response were observed (Table 1). The observed linear range was 0.01–10  $\mu\text{M}$  or 3-orders of dynamic range with  $R^2 = 0.9996$ . Calibration curves and linear ranges for the rest of AAs are summarized in Figure 2 and Table 1. Data showed that the majority of the compounds displayed linear range of 0.01–10  $\mu\text{M}$  or three orders of dynamic range with  $R^2 > 0.996$ . The exceptions are alanine and glycine with lower sensitivity, and threonine with narrower linear range.

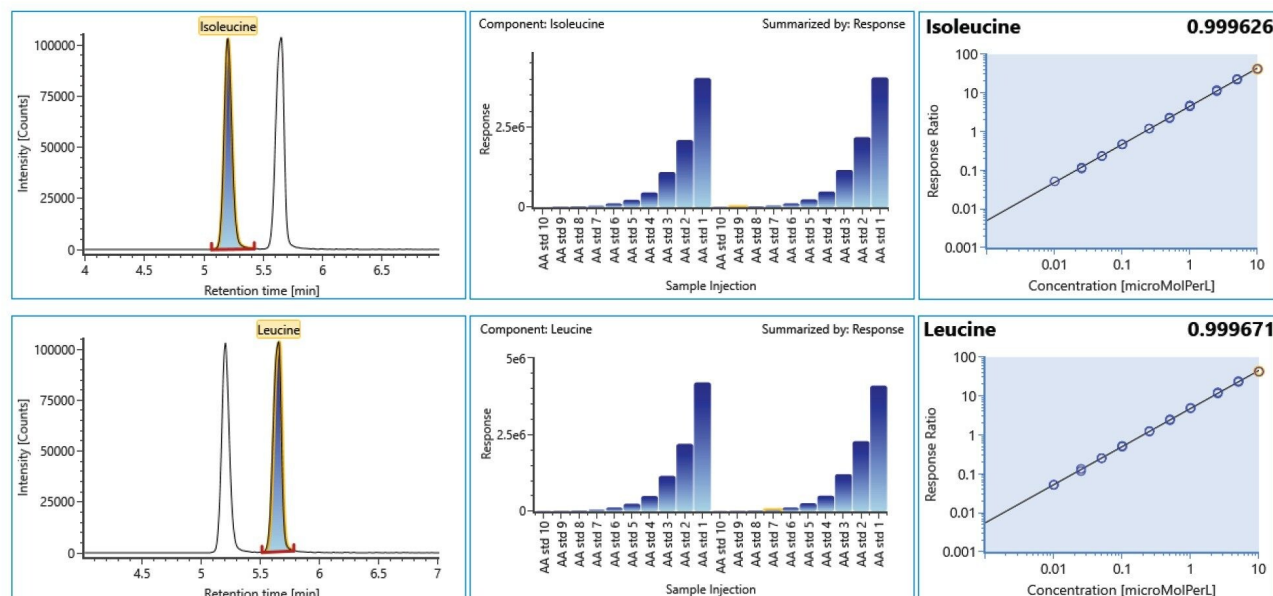


Figure 1. Chromatogram, response bar plots, calibration curve for leucine and isoleucine.

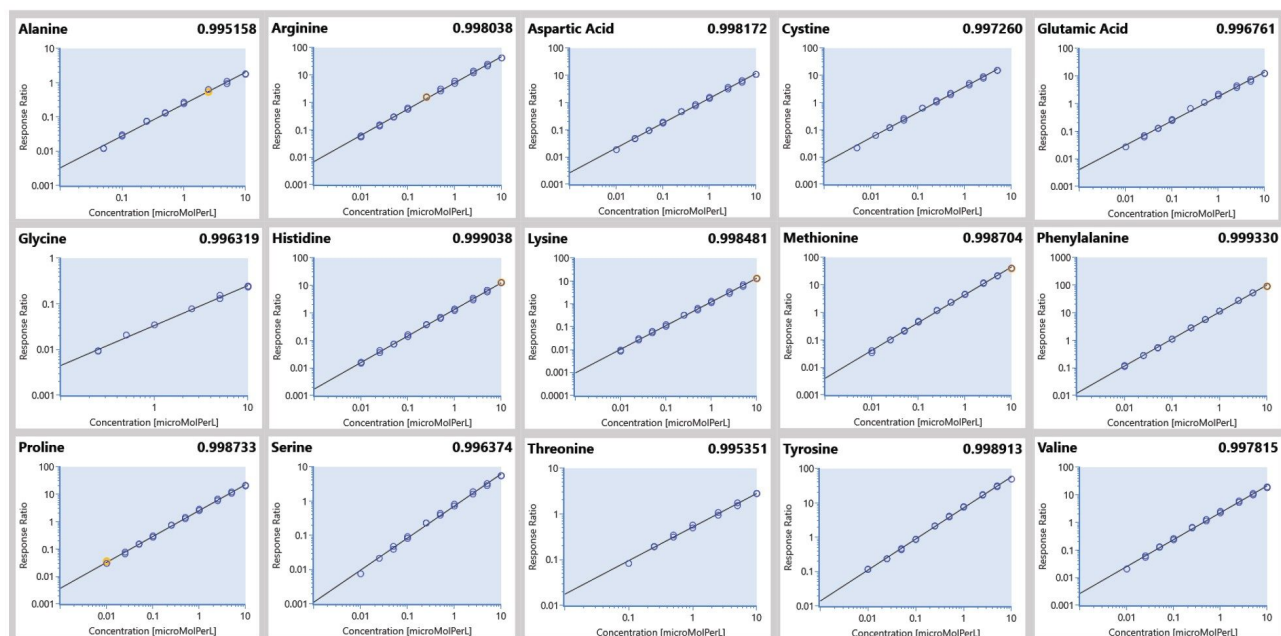


Figure 2. Standard calibration curve of amino acids. Log-log linear calibration was used for curve fitting.

Method accuracy and precision were determined for two QC samples, a low QC at 0.05  $\mu\text{M}$  and a high QC at 5  $\mu\text{M}$ . Data based on triplicate injections are summarized in Table 2. In all, excellent accuracy of 85–115% and reproducibility of <4% at high QC and <15% at low QC are observed. In all, the data suggest that the reversed phase method could be used for quantitative determination of amino acids in cell culture media either for quality control of raw media and/or quantitative monitoring in spent media during cultivation process.

Component name	Neutral mass (Da)	Expected RT (min)	Mass error (ppm)*	Linear range ( $\mu\text{M}$ )	$R^2$	Low QC at 0.05 $\mu\text{M}$ (n=3)		High QC at 5 $\mu\text{M}$ (n=3)	
						%Accuracy	%Precision	%Accuracy	%Precision
Alanine	89.0477	1.42	1.7	0.05–10	0.9951	91	9.2	93	2.2
Arginine	174.1117	1.31	-0.4	0.01–10	0.9980	95	8.5	89	2.7
Aspartic acid	133.0375	1.42	0.1	0.01–10	0.9981	95	8.8	91	1.6
Cystine	240.0239	1.37	-1.7	0.005–5	0.9973	92	11.5	89	1.8
Glutamic acid	147.0532	1.50	0.4	0.01–10	0.9968	92	5.8	88	1.8
Glycine	75.0320	1.36	3.2	0.25–10	0.9963	n/a	n/a	98	3.3
Histidine	155.0695	1.28	1.1	0.01–10	0.9990	96	5.5	95	1.0
Isoleucine	131.0946	5.22	3.4	0.01–10	0.9996	98	7.4	100	1.6
Leucine	131.0946	5.64	3.4	0.01–10	0.9997	97	3.2	101	0.9
Lysine	146.1055	1.23	0.8	0.01–10	0.9985	98	4.1	91	1.1
Methionine	149.0511	3.01	1.2	0.01–10	0.9987	107	4.3	97	3.5
Phenylalanine	165.0790	8.11	1.9	0.01–10	0.9993	95	1.2	102	1.7
Proline	115.0633	1.73	1.5	0.01–10	0.9987	92	7.5	93	0.6
Serine	105.0426	1.38	1.2	0.01–10	0.9964	92	13.6	89	0.5
Threonine	119.0582	1.46	0.6	0.1–10	0.9954	n/a	n/a	87	0.6
Tyrosine	181.0739	5.64	2.2	0.01–10	0.9989	89	1.3	96	1.6
Valine	117.0790	2.39	1.9	0.01–10	0.9978	99	11.7	92	1.5

\*based on sample concentration at 5  $\mu\text{M}$  for all amino acids except cystine at 2.5  $\mu\text{M}$ .

n/a: below detection limit

Table 1. Summary of linearity, precision and accuracy of QC samples from three replicate injections.

## Internal Standard Considerations

In quantitative bioanalysis, internal standard (IS) is added to the sample for signal normalization to ensure the highest reproducibility and accuracy. The use of the SIL version of the same analyte is generally desired. An example using IS correction is shown in Figure 3, displaying trend plots from 100 repeated injections of leucine containing SIL-leucine as the IS. An individual plot of leucine or SIL-leucine showed response drift at the early stage of the injections. By using the SIL correction, variability was significantly reduced, resulting in better accuracy and signal reproducibility.

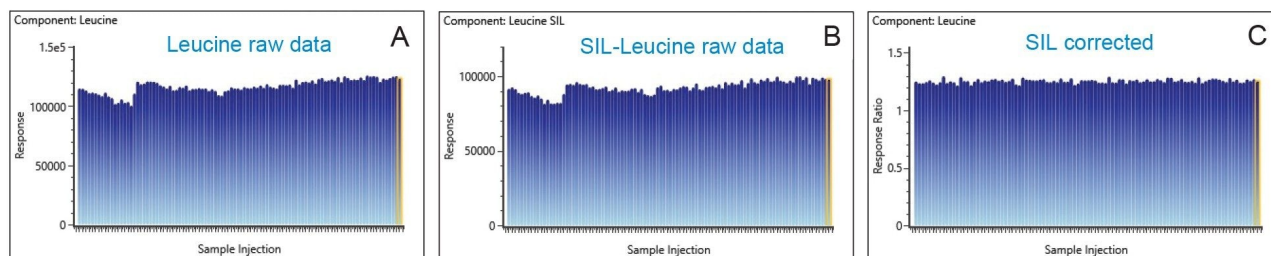


Figure 3. Bar trend plot of raw response from 100 injections. (A) leucine, (b) stable isotope labeled leucine, and (c) response ratio of leucine/SIL-leucine. The plot shows signal variation in raw response is corrected by employing an internal standard.

In this study, response reproducibility based on single IS using different compound is examined. The sample used was basal media mixture IMDM spiked with SIL amino acid mixture and injected 100 times or over 35 hours of data acquisition. Reproducibility of the 100 injections was calculated as follows: (1) no IS, (2) use early eluting compound SIL-proline (Pro) as the IS, (3) use mid eluting SIL-leucine (Leu) as the IS, or (4) use late eluting SIL-phenylalanine (Phe) as the IS.

Figure 4 is a summary plot of %RSD calculated for the 100 injections using above IS corrections. The results showed that when the response was not corrected, the %RSD is ~5%. All method of IS corrections resulted in a reduced %RSD to <3% with the best reproducibility obtained when the corresponding SIL compound was used. These data suggest that while compound specific SIL produced the best reproducibility as expected, using a structurally different compound as the IS can be a practical approach for improved reproducibility. In current raw and spent media analysis, 3-chlorotyrosine has been successfully used as the internal standard.<sup>2-4</sup>



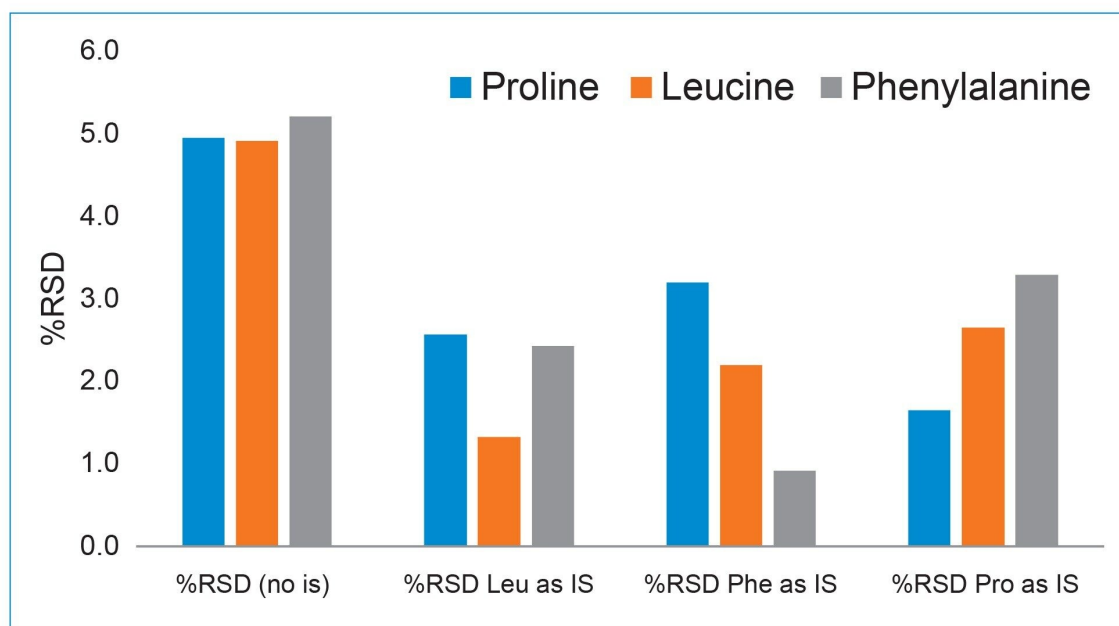


Figure 4. Summary plot of %RSD using different compound as the internal standard. Blue bar: proline (r.t. = 1.73 min), orange bar: leucine (r.t. = 5.64 min), and gray bar phenylalanine (r.t. = 8.11 min).

## Conclusion

- Underivatized amino acids were successfully quantified using the cell culture media method and the BioAccord LC-MS System
- Excellent accuracy and reproducibility are obtained for all amino acids at relevant bioprocessing levels
- Investigation of internal standard suggests the use of a single compound as the internal standard is effective in obtaining good reproducibility
- When standard calibration solutions are available, the method can be potentially deployed in quality control of raw material testing in addition to spent cell culture media monitoring supporting process development

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## References

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## Featured Products

[BioAccord LC-MS System for Biopharmaceuticals <https://www.waters.com/waters/nav.htm?cid=135005818>](https://www.waters.com/waters/nav.htm?cid=135005818)

[ACQUITY Premier System <https://www.waters.com/waters/nav.htm?cid=135077739>](https://www.waters.com/waters/nav.htm?cid=135077739)

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