Comparison of High-Resolution Time-of-Flight Mass Spectrometers for Cell Culture Media Nutrient and Metabolite Analysis

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INTRODUCTION

Quantitative and qualitative analysis of nutrients and metabolites within cell culture media (CCM) is important to monitor in upstream bioprocessing. While the CCM method developed on the BioAccord™ LC-MS system[1] is robust and has been shown to have excellent qualitative and quantitative results for the 200+ compound library, there may be instances where a higher resolution QTof instrument would be preferred for CCM analysis. Such reasons include extended linear range, better sensitivity, or higher resolution for more accurate determination of unknown analytes within media.

Transfer of methods between these two mass spectrometers resulted in a change of ESI source, ion optics, and the addition of the collision cell all which require optimization for the many compounds found in CCM. Without optimization higher in-source fragmentation was observed due to the differences in instrumentation as shown in Figure 1.

Herein is described the optimization of a CCM method on the Xevo™ G3 QTof MS, demonstration of the enhanced capabilities, confirmation of method robustness, and comparison of spent cell media samples on both mass spectrometers.

METHODS

The Waters™ Cell Culture Standard Kit (p/n: 186009300), which contains the standard twenty amino acids plus six amino acid derivatives, was used for optimization experiments. The stock solution was prepared at 2.5 µM using water containing 0.1% formic acid as the diluent for optimization studies.

For comparative studies, a 96-well plate containing media from 8 bioreactor vessels over the course of 13 days was analyzed. Spent media samples were generated by Waters Immerse Delaware center. Samples were diluted 1:400 (V:V) with 0.1% formic acid and an internal standard of 5-methyltryptophan at 0.1 µM.

Analyses were performed on the Xevo G3 QTof MS and BioAccord MS System using waters_connect™ software. The LC method used on both mass spectrometers was the same used in the original 9-minute rapid LC-MS method for CCM.[1] The tested parameters for the Xevo G3 QTof MS are listed in Table 1.

Table 1. Source and transmission parameter ranges used in optimization experiments.

Parameter	Start	End	Increment
Capillary Voltage	0.5 kV	2.5 kV	0.25 kV
Source Temperature	100ºC	150ºC	100,120,150ºC
Cone Gas	10 L/h	90 L/h	20 L/h
Desolvation Gas	400 L/h	1200 L/h	200 L/h
Desolvation Temp.	250ºC	550ºC	50ºC
Cone Voltage	10 V	70 V	10 V
Source offset	0 V	40 V	10 V
StepWave RF	50 V	250 V	50 V
Body Gradient	2 V	20 V	2,5,10,15,20 V
Low Energy transfer	2 V	8 V	2 V
High Energy CE	10-60 V	40-100 V	

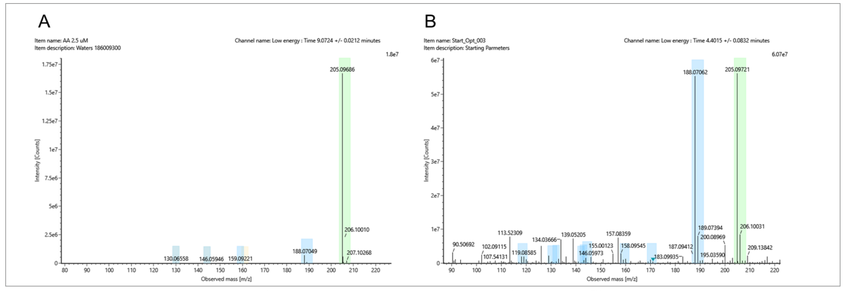

Figure 1. Low Energy XIC for tryptophan from the accurate mass screening workflow in UNIFI™ software for cell culture media on the BioAccord (A) and on the Xevo G3 QTof MS (B) with the molecular ion highlighted in green and in-source fragments highlighted in blue.

RESULTS

Optimization of Source Parameters

The objective of optimizing the source and transfer parameters of the Xevo G3 QTof MS was to improve the ion signal for the smallest, most labile amino acids of glycine (Gly) and alanine (Ala) and to reduce in-source fragmentation for all the analytes.

Figure 2A depicts the change in normalized ion signal for the molecular ion of each amino acid across the range for six ESI source parameters. Figure 2B shows the same conditions for the fragment-to-precursor ion ratio.

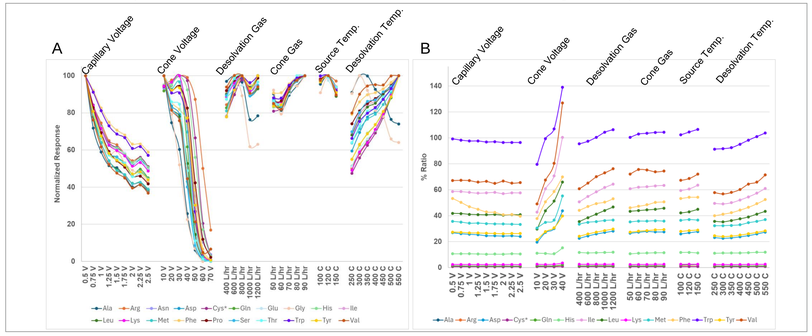

Figure 2. Normalized response for amino acids (A) and fragment-to-precursor ion ratio (% ratio) (B) of fourteen of the amino acids at specified ranges for all ionization source parameters. Each point is an average of triplicate consecutive injections.

Optimization of StepWave™ XS Parameters

Labile species require optimization of ion optics to reduce in-source fragmentation. The StepWave XS has been previously shown to require optimization for labile molecules.[3] Figure 3 depicts the optimization of three parameters of the StepWave ion optics for the molecular ions and the fragment-to-precursor ion ratio for amino acids.

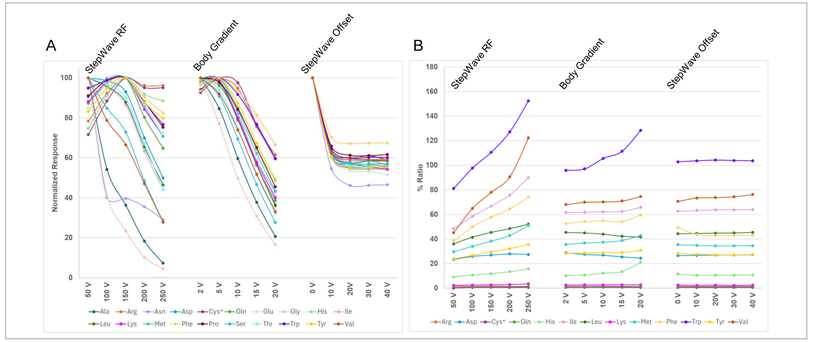

Figure 3. Demonstration of the effect of StepWave XS parameters on normalized response of molecular ion (A) and fragment-to-precursor ion ratio (% ratio) (B). Points represent the average of triplicate consecutive injections.

RESULTS

The smallest, most labile amino acids, Ala and Gly, had the most dramatic signal enhancement, 11x and 7x fold increase respectively, while most small amino acids showed at least a 2-fold increase. The optimized conditions resulted in an overall decrease in the precursor-to-fragment ion ratio, an indication of reduced in source fragmentation. Except for Trp, all monitored fragment-ion-ratios were below 20%. The ratio of Trp decreased from 99% to 25%.

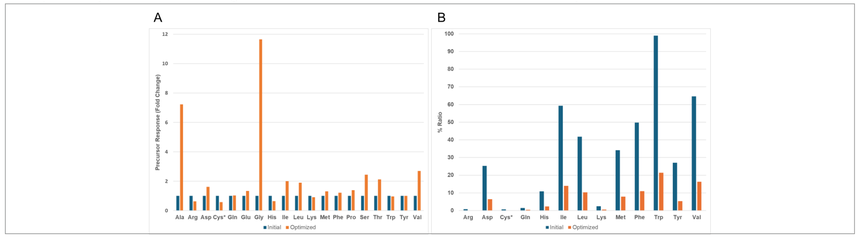

Figure 4. Response of precursor ions at initial and method optimized conditions showing fold changes with optimized parameters (A). Fragment-to-precursor ion ratio at initial and optimized conditions (B).

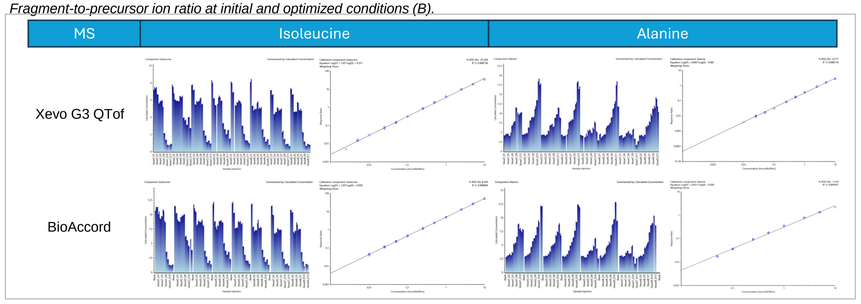

Figure 5. Demonstration of extended linear range for Isoleucine and Alanine on the Xevo G3 compared to the BioAccord. Also showing the calculated concentrations of the spent cell media sample set on both instruments.

CONCLUSION

- LCMS method for CCM was transferred to the Xevo G3 QTof system
- Due to more ion optics and transmission regions, optimization was performed to decrease in-source fragmentation for labile molecules
- Gly and Ala increased in molecular ion response by 11x and 7x after optimization
- In-source fragmentation was reduced shown by the decrease of Trp fragment-to-precursor ion ratio from 99% to 25%
- Analysis of CCM on the Xevo G3 QTof MS provides increased sensitivity as shown by an extended linear dynamic range, compared to the BioAccord system

References

1. Introducing a Rapid Throughput LC-MS Method for Cell Culture Media Nutrient and Metabolite Analysis Supporting Upstream Bioprocessing. Waters Application Note. 720008170,
2. 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.
3. Improved Transmission of Labile Species on the Xevo™ G3 QTof Mass Spectrometer with the StepWave™ XS. Waters Application Note. 720007794.

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