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Analysis of Underivatized Amino Acids and Metabolites in Cell Culture Media by HILIC-LC/MS

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Introduction

Monitoring the wide range of polar compounds found in bioreactors and fermenters is a challenging application for Hydrophilic Interaction Chromatography (HILIC). This is primarily due to the high salt content (e.g., ~110 mM NaCl in DMEM) in the culture media that can cause retention time shifts and signal intensity variations between chromatographic runs. Here, robust HILIC-LC/MS methods were developed for monitoring amino acids, feedstock, and waste products in cell culture media. Moreover, the complete separation of challenging leucine/isoleucine isomers and a wide range of amino acids were achieved with this method.

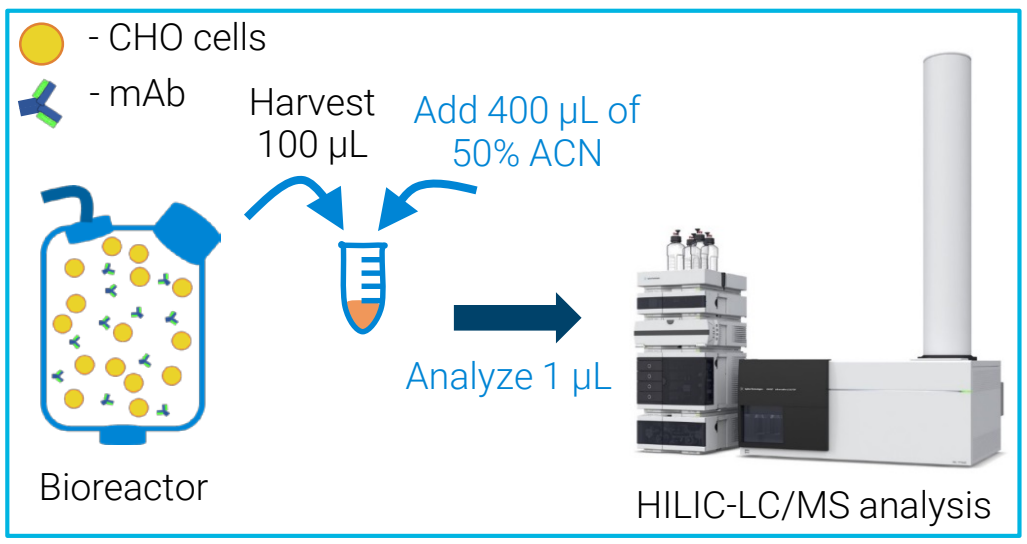
The demonstrated approach provides a new tool to rapidly characterize cell culture media and further evaluate its impact on cell culture performance.

Experimental

Sample Preparation

Spent media samples were harvested from the bioreactor, and diluted either 5-fold with 50% acetonitrile, or 2-fold with Mobile Phase B. Samples were then centrifuged to remove cellular debris.

For mAb titer, samples were analyzed directly.



Mobile Phase Preparation

Mobile phases were prepared by diluting a stock salt solution (as indicated) with either water (for Mobile Phase A) or acetonitrile (for Mobile Phase B).

Instrumentation

Samples were analyzed on one of two systems. Project 1 was performed using an Agilent 1260 Infinity II BioInert LC with an Agilent 6230 TOF. Project 2 was performed using an Agilent 1290 Infinity II LC with an Agilent 6545XT QTOF.

Experimental

Project 1: Optimizing Media Composition

LC Conditions – Spent Media Analysis	
Column	Agilent AdvanceBio MS Spent Media, 2.1 x 100 mm, p/n 675775-901
Column Temp	40 °C
Mobile Phase	Low pH, Positive Ion Mode MS Detection: A = 10% 200 mM ammonium formate in water pH 3, 90% water B = 10% 200 mM ammonium formate in water pH 3, 90% acetonitrile *Final salt concentration is 20 mM.*
Flow Rate	0.5 mL/min
Gradient Program	Time / % B 0 / 100 10 / 75 20 / 20 21 / 20 21.1 / 100 28 / 100

mAb titer was determined by injecting 50 µL of spent media onto an Agilent Bio-Monolith Protein A column (p/n 5069-3639) following the protocol described in Reference 1.

Project 2: Monitoring Nutrient Consumption & Waste Excretion

LC Conditions - High pH, Negative Ion Mode MS Detection	
Column	Agilent AdvanceBio MS Spent Media, 2.1 x 150 mm, p/n 673775-901
Column Temp	30 °C
Mobile Phase	A = 10% 100 mM ammonium acetate in water pH 9, 90% water B = 10% 100 mM ammonium acetate in water pH 9, 90% acetonitrile *Final salt concentration is 10 mM.*
Flow Rate	0.25 mL/min
Gradient Program	Time / % B 0 / 90 2 / 90 12 / 40 13 / 20 16 / 20 17 / 90 25 / 90

Project 1: Selecting an Optimal Cell Culture Media

The samples for these experiments come from two CHO cell cultures where two different, commercially available media products were used (Media 1 and Media 2). When combined with titer determination, analysis of both fresh and spent media can help optimize cell culture composition for maximum production of a uniform protein product. While amino acids are structurally diverse, they do not represent the full range of polar metabolites that may be present in cell culture media. Figure 1 explores the range of polarities that may be present. If the most extreme polyamines are not of interest, a shorter gradient may be used. Figure 2 shows the total compound chromatograms for an early time point and extracted ion chromatograms for the compounds found in Media 1.

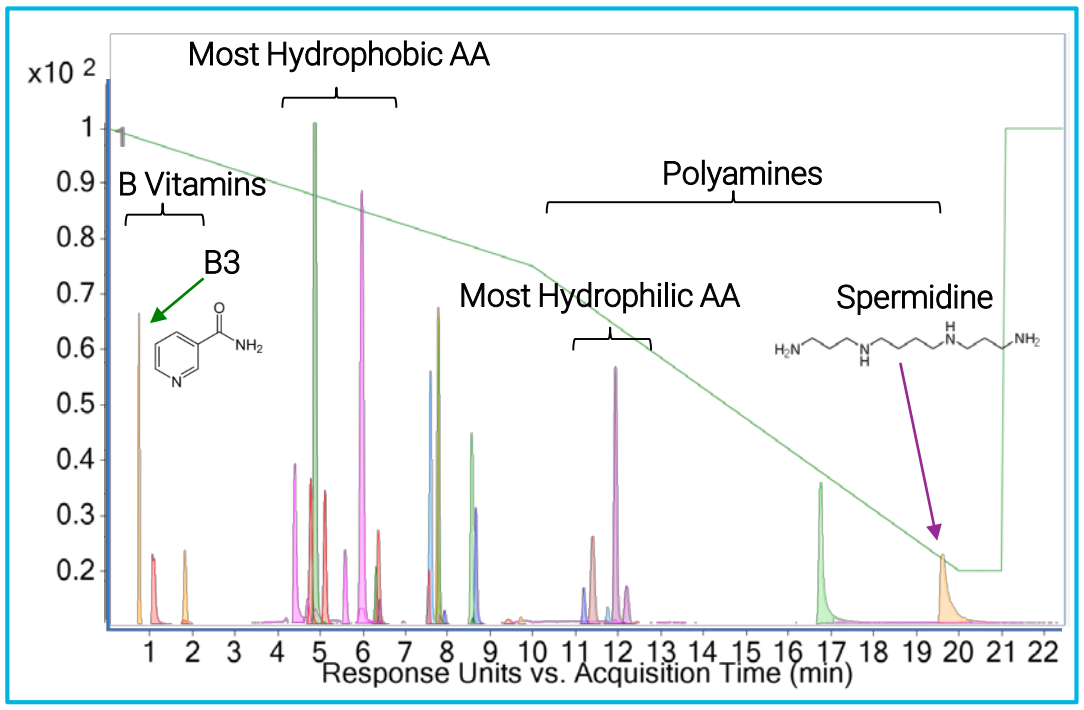


Figure 1. EICs of B vitamin, amino acid, and polyamine standards overlaid with the gradient, illustrating the strength of mobile phase needed to elute various metabolites.

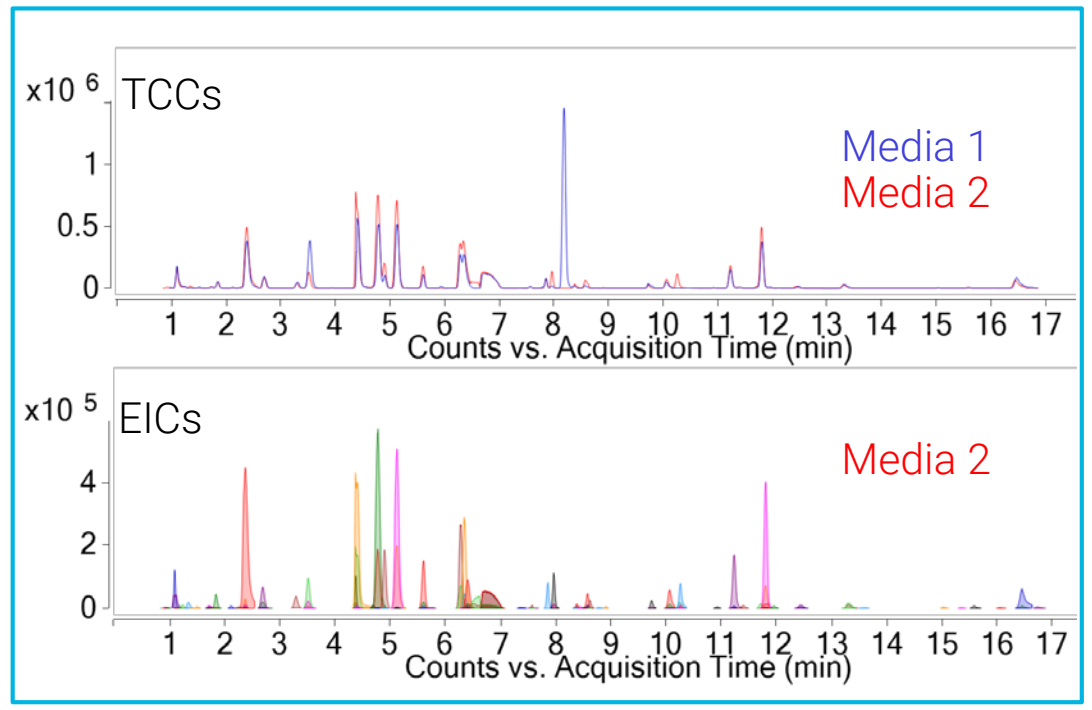


Figure 2. Total compound chromatograms for early timepoint of two media samples (top) and extracted ion chromatograms for all compounds present (bottom).

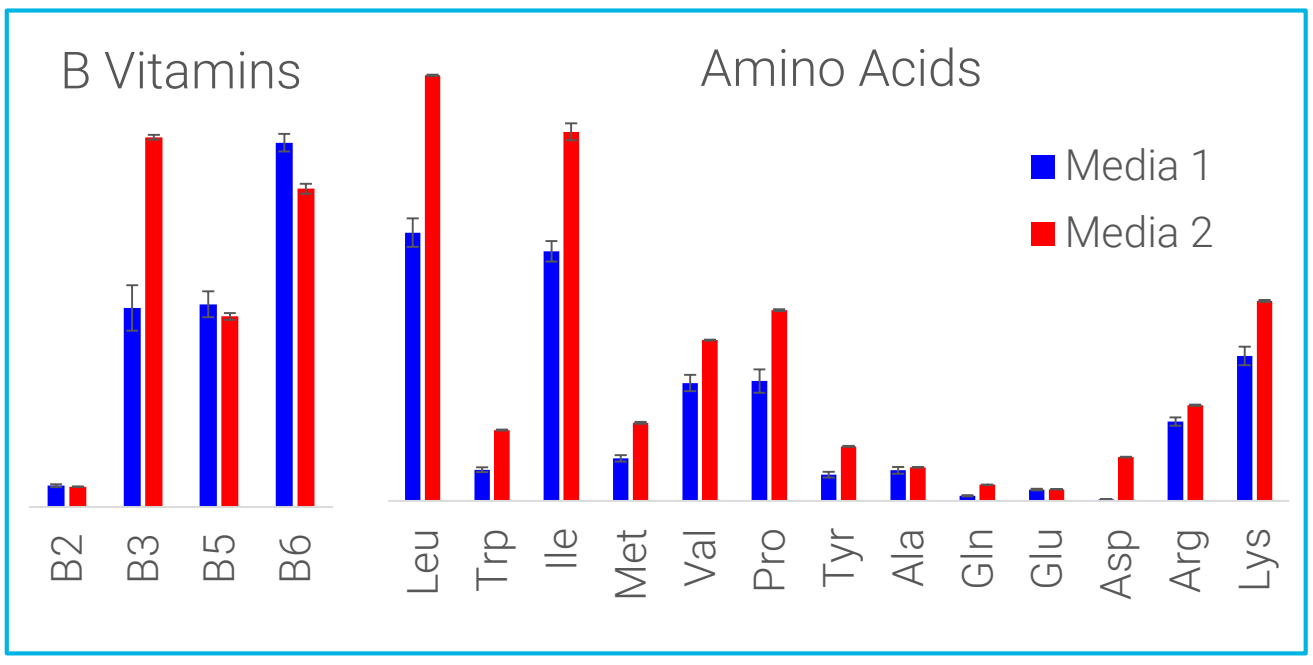


Figure 3. Relative abundances of several B vitamins and amino acids identified from Media 1 and Media 2.

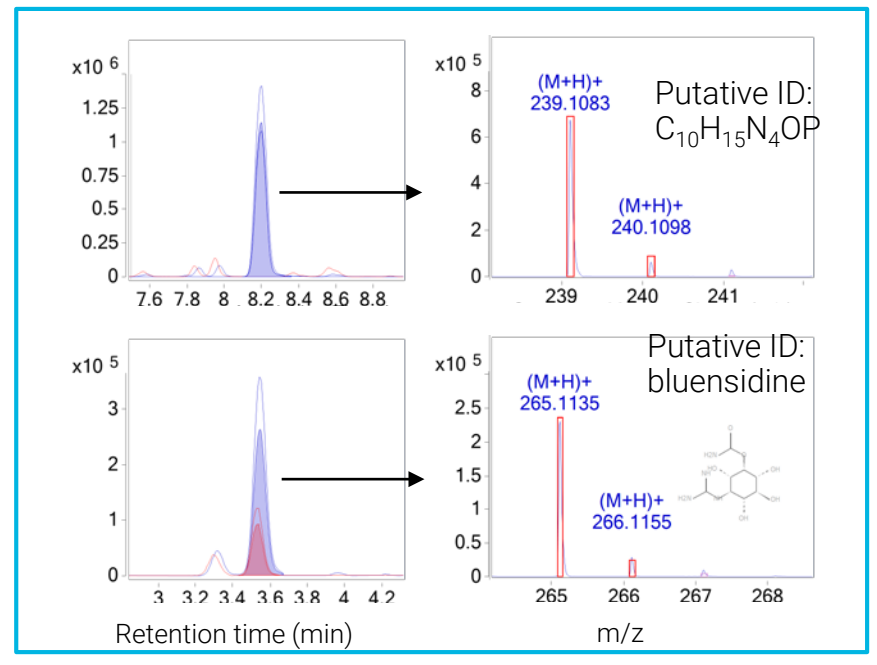


Figure 4. EICs and MS spectra for unidentified differentiating compounds.

At this early time point, Media 2 has higher concentrations of most amino acids, including Gln, a key indicator of cell metabolism. Aside from Vitamin B3, the B vitamins have similar abundances. Because an accurate mass instrument was used, putative IDs can be assigned to unknown compounds using database searching or molecular formula generation. At the two week time point, the cells grown in Media 2 produced nearly three times as much mAb product.

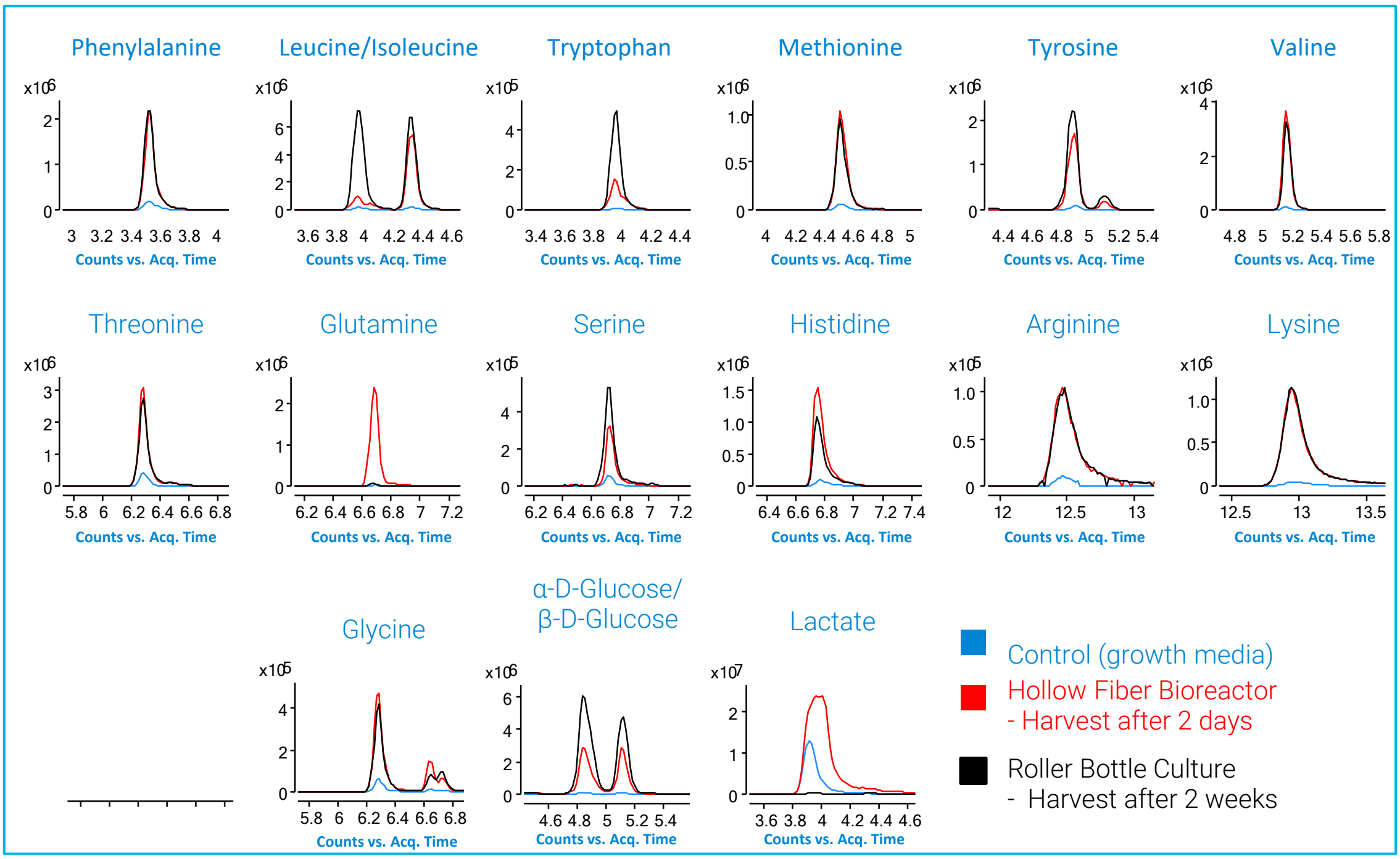
	[mAb]
Media 1	143 mg/L
Media 2	419 mg/L

Table 1. mAb titer from CHO cells grown in different media at 2 weeks growth

Results and Discussion

Project 2: Monitoring Nutrient Consumption & Waste Excretion

The consumption of amino acids and glucose were monitored in negative ion mode so as to simultaneously monitor excretion of lactate, a cellular waste product. The control media is unused commercial media.



Glutamine is higher in abundance in the hollow fiber bioreactor than in the control media because glutamine had to be supplemented. It was not included in the commercial media due to its instability in solution.

Conclusions

- AdvanceBio MS Spent Media column retains a wide range of polar metabolites, easily resolving isomers such as Leu/Ile.
- HILIC-MS workflow provides a flexible and derivatization-free alternative to LC/UV analysis of amino acids.
- Spent media analysis results can be correlated to mAb titer to select or optimize cell culture media
- Nutrient consumption and waste production can be monitored to track cellular metabolism and add nutrients as needed.

References

[1] Dumont, E.; Vandenheede, I.; Sandra, P.; Sandra, K. mAb Titer Analysis with the Agilent Bio-Monolith Protein A Column. Agilent Technologies Application Note. 2014.

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