Identifying and Monitoring Significant Nutrient Factors in Biopharmaceutical Cell Cultures using UPLC and UPLC/MS

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INTRODUCTION

Biopharmaceuticals are most commonly produced in cell culture; the growth conditions in the culture must be optimized for the highest yield of the desired protein at the required purity. Since each clone and each product have different optimum growth conditions, rapid and reliable assays are essential. In addition, it is necessary to monitor the critical nutrients during the growth of the culture so that production may be sustained over time. Many of the important nutrient factors are obvious and well-known, but it is still common to observe differences in growth and productivity between different batches of defined media. We have considered analytical approaches to both kinds of nutrient status. The solutions include defined total solutions, UPLC® methods, and statistical analysis of differences in UPLC/MS data.

The concentration of free amino acids in cell culture media reflects the status of metabolic pathways that affect production of the biopharmaceutical. The UPLC Amino Acid Analysis Solution provides complete monitoring of changes in the amino acids over time. The solution includes the instrument, derivatization and separation chemistry, and analytical software ready to use without development or adjustment. The derivatization steps have now been automated.

The same chromatographic principles are being applied to the water-soluble B-vitamins. The wide ranges of properties and concentrations have placed special constraints on the method, the detection and the sample preparation. The combination of these factors to detect changes in vitamin concentration will be shown.

The recognition of unexpected differences between media batches requires an unbiased analysis that approaches universal detection with relative quantitation. We have used exact mass MS to monitor a UPLC separation of complete media samples. The relative amounts of compounds identified by exact mass and retention time are compared using statistical tests to identify components that are different in good and bad media.

METHODS

UPLC Amino Acid Analysis Solution

Instrument: ACQUITY UPLC® System with TUV
Column: AccQ•Tag™ Ultra 2.1 x 100mm, 1.7µm
Mobile Phases: AccQ•Tag Ultra Eluent A and Eluent B
Flow Rate: 0.7 mL/min
Injection Volume: 1.0 µL
Gradient: Standard Cell CultureGradient
Column Temp: 60 °C
Detection: UV @ 260 nm

UPLC System and Vitamin Analysis

Instrument: ACQUITY UPLC® System with PDA
Column: ACQUITY UPLC® BEH C18 1.7 µ m
Mobile Phase:
A: 25mM KH2PO4, 1 vial Waters Low UV PIC B7, in water
B: 1 vial Waters Low UV PIC B7, in methanol
Flow Rate: 0.4 mL/min
Gradient:

Time	%A	%B
Init	98	2
0.5	98	2
10.0	70	30
10.5	20	80
14.5	20	80
15.0	98	2
22.0	98	2

Injection Volume: 10 µL
Column Temp: 30°C
Detection: UV @ 205nm

UPLC System and Mass Spectrometry

LC Conditions:
UPLC System: Waters ACQUITY™ UPLC
Column: 2.1 x 150 mm BEH, 1.7 µm C_{18}
Mobile Phases:
Mobile Phase A: Water with 0.025, 0.05 or 0.1% (v/v) ion-pairing reagent and 0.1% formic acid (FA)
Mobile Phase B:80% ACN/20% H_2O w/ 0.025, 0.05 or 0.1% (v/v) ion-pairing reagent and 0.1% formic acid (FA)
Flow Rate: 0.5 mL/min
Column Temp: 45 °C
Gradient: 1% -20% B in 10 minutes
Sample Volume: 5 µL

Mass spectrometry:

Instrument:	Waters QTOF Premier™
Capillary Voltage	3.6 KV
Nebulization gas:	800L/min
Desolvation Temperature:	400 °C
Source Temperature:	150 °C
Cone Voltage:	15 V
Collision energy:	2 eV
Lock-spray:	Leucine-enkephalin (0.5 µM)

Samples
Cell culture media samples were diluted 1:20 in Mobile Phase A and injected directly in the LC/MS system.

RESULTS

Amino Acid Analysis

The UPLC Amino Acid Analysis Solution can be used to monitor amino acid concentration during cell culture. This information is used to develop growth conditions for optimum production of the desired biomolecule. The small sample volume needed also ensures that no sample prep is required for analysis using this new methodology.

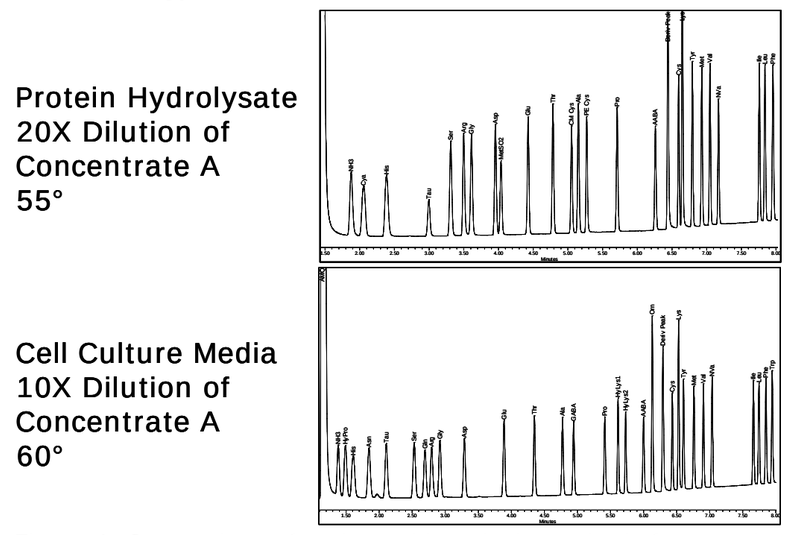
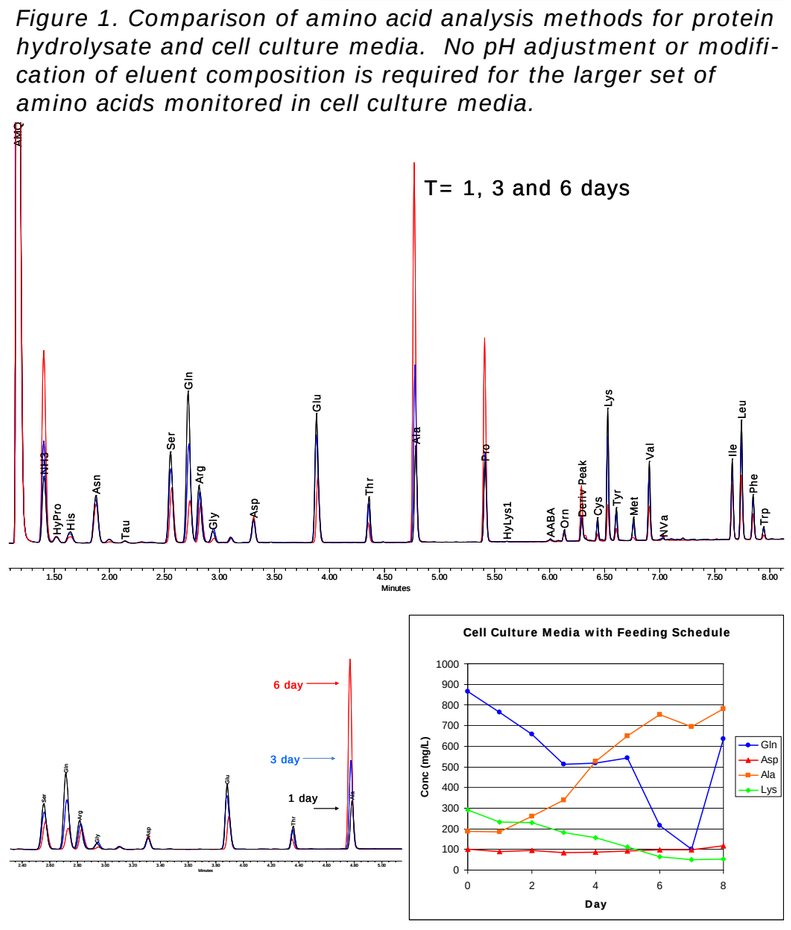


Figure 1. Comparison of amino acid analysis methods for protein hydrolysate and cell culture media. No pH adjustment or modification of eluent composition is required for the larger set of amino acids monitored in cell culture media.



Figures 2A ,2B, & 2C. Changes in amino acid concentration as a function of duration of culture time. Samples were taken from a growing culture on days 1, 3, and 6. The full profile of amino acids is included in the analysis, and the zoomed central region shows the consumption of glutamine and the increase in alanine. Quantitation of all the amino acids shows these changes, as well as indicating that several amino acids do not change with time.

Vitamin Analysis

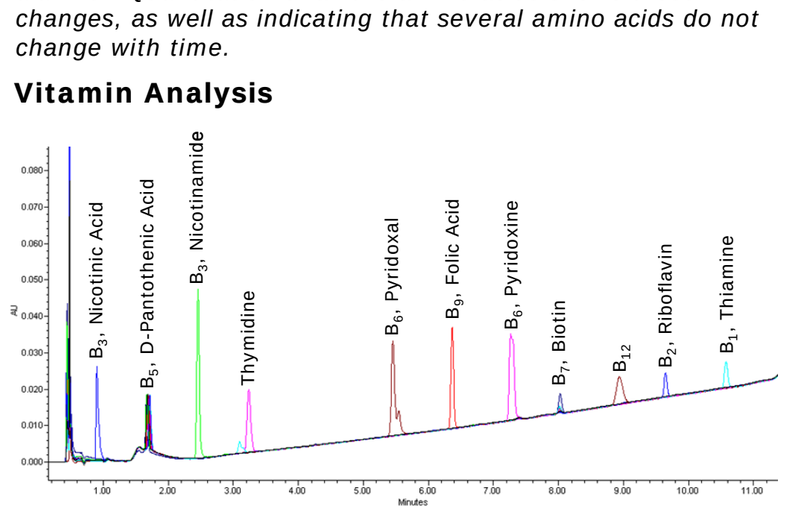


Figure 3. Vitamin Standard Mixture (1ng/µL). The common water-soluble B vitamins can be analyzed at concentrations typical of mammalian cell culture media.

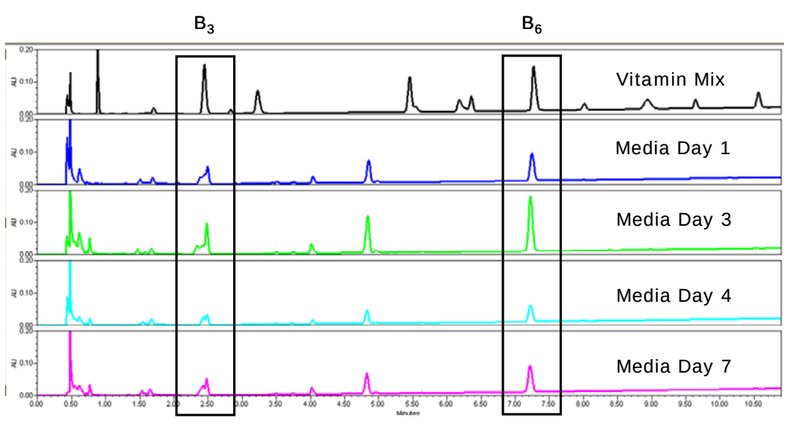


Figure 4. Cell Culture Media over 7 Days of Culture. There are relatively small changes in the relative amounts of vitamins over 7 days of culture.

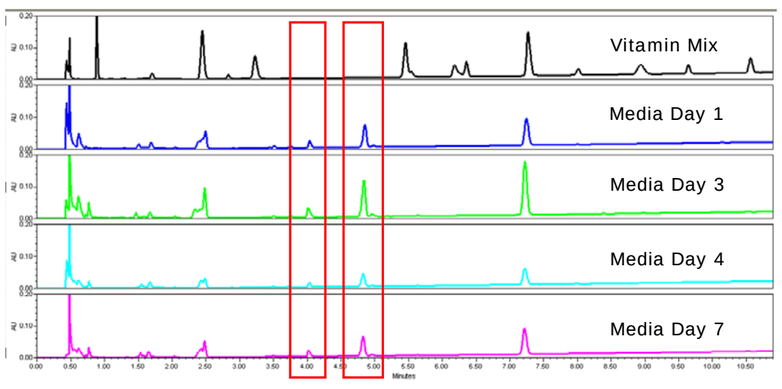


Figure 5. Cell Culture Media over 7 Days of Culture. With the generally broad detection available with low-wavelength UV, it is easy to recognize additional, unidentified media components that elute in the same chromatogram as the water-soluble B vitamins.

LC/MS Analysis

A high-resolution (10,000), high-mass accuracy (<10 ppm) quadrupole time-of-flight mass spectrometer (QTOF Premier) was used for analyte detection. The list of "components" (RT, *m/z* pairs) identified in each LC/MS run were extracted using MarkerLynx data processing software and exported to Easy Info (Waters) for multivariate statistical analysis. Statistically significant compounds, related to the minor differences in sample composition, were identified using PCA (Principal Component Analysis) and OPLS-DA (Orthogonal Partial Least-Squares to Latent Structures Discriminate Analysis).

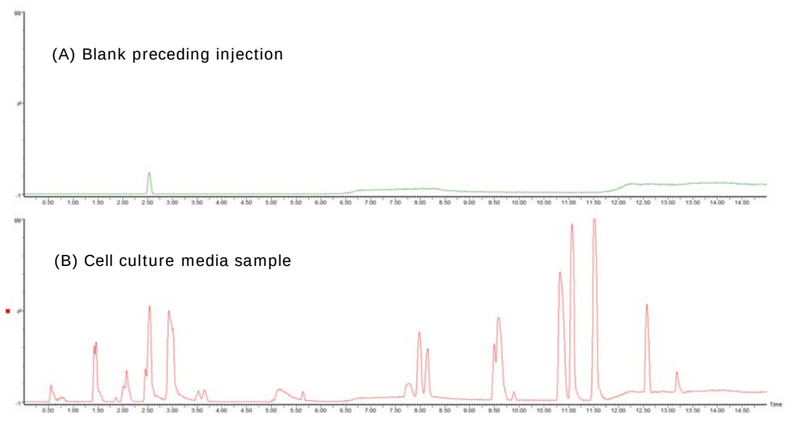


Figure 6. Typical LC/MS base-peak chromatogram of a cell culture media sample. The complexity of the plot reflects the large number of components of growing cell culture media.

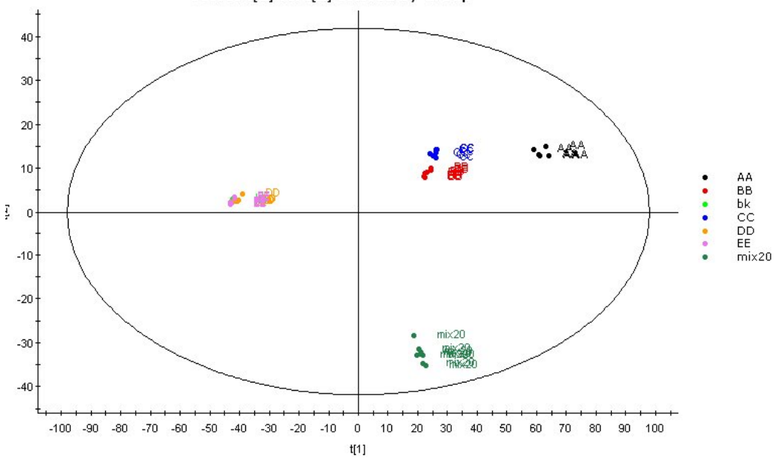


Figure 7. PCA (Principal Component Analysis) reduces the complexity of the LC/MS data to a single plot showing the relationship between different cell culture media. In this example, three media samples (labeled AA, BB and CC), having minor modifications in their chemical composition, can be clearly differentiated.

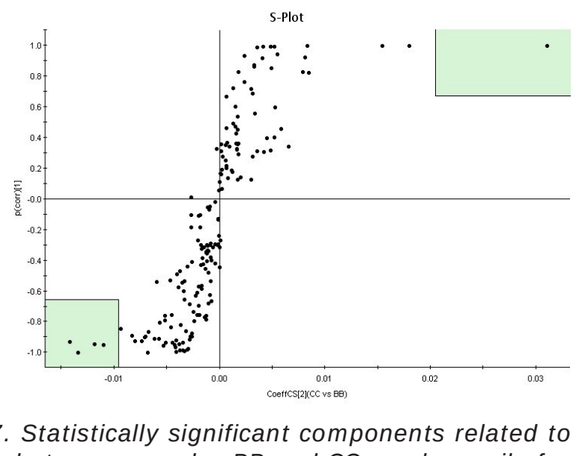


Figure 7. Statistically significant components related to the differences between samples BB and CC can be easily found from the S-plot, the result of the OPLS discriminate analysis. In this case, one marker was found only in sample BB (m/z=239.09), while 4 other markers were present at different concentration levels in both samples.

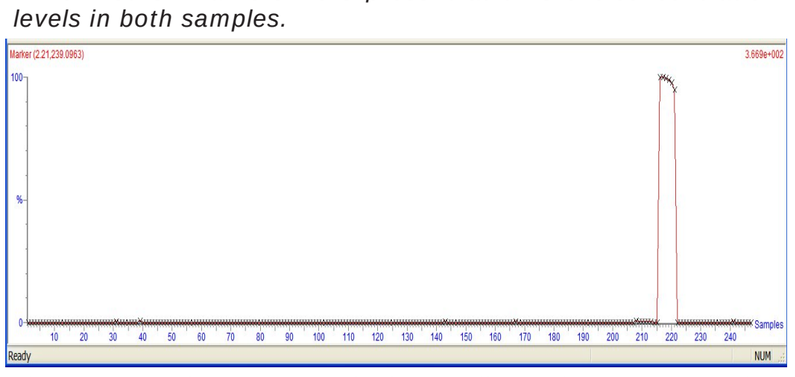


Figure 8. MarkerLynx trend plot confirms the presence of a unique component (m/z = 239.09) in the cell culture media sample labeled BB. In this experiment six replicate injections from 31 different samples were performed.

CONCLUSION

- The UPLC Amino Acid Analysis Solution provides routine monitoring of the changing concentrations of nutrient amino acids in a growing cell culture.
- Water-soluble B vitamins can be analyzed using UPLC with low wavelength UV detection.
- High-resolution/high-mass accuracy mass spectrometer enhances the ability to identify components of cell culture media.
- PCA analysis was successfully used to identify minor changes in the chemical composition of cell culture media.
- Chemometric analyses of LC/MS data can identify changes in media associated with differences in yield.
- LC/MS profiling shows potential for fast analysis of biopharmaceutical grade cell culture media as well as spent media samples obtained during protein production.
- A simple combination of UPLC instrumentation can be used to assess multiple aspects of the nutrient status of growing mammalian cell cultures with appropriate selection of chemistry and methods.

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