MONITORING NUTRIENT STATUS IN CELL CULTURE MEDIA USING UPLC® TECHNOLOGY

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INTRODUCTION

Biopharmaceuticals are most commonly produced in cell culture. The growth conditions 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. Two groups of nutrients, amino acids and vitamins, can provide examples of the analytical approach to this problem. The Waters UPLC® Amino Acid Analysis Solution is a total system solution that combines the well-established AccQ·Tag™ pre-column derivatization and analysis for amino acids with the improved speed, resolution and sensitivity of UPLC® separations. This system has been adapted for tracking both amino acids and water-soluble vitamins in cell culture media. This study illustrates the versatility of UPLC® technology for monitoring the dynamic changes that occur in living and growing cell cultures.

Materials

- Amino Acids
  - UPLC® Amino Acid Analysis Application Solution, as supplied
- Vitamins
  - Waters ACQUITY UPLC® System with the ACQUITY PDA Detector
  - Waters ACQUITY UPLC® HSS T3, 1.8µm, 2.1x50mm Column
  - Heptafluorobutyric Acid, HPLC; Pierce, Rockford, IL
  - Vitamin standards from Sigma-Aldrich, Inc., St. Louis, MO and Acros Organics, Morris Plains, New Jersey
  - Media from Mediatech, Inc., Herndon, VA

AMINO ACID ANALYSIS

Amino acid analysis of complex samples, such as cell culture media often requires sample pre-treatment or preparation. The high sensitivity of the AccQ·Tag™ Ultra method permits direct analysis of media without any sample preparation. Only a small aliquot is required for the analysis. Each sample chromatogram represents 25nL of the culture. In addition, the other components of the media do not interfere with the amino acid analysis. The amino acid analysis method for cell culture media requires the ten-fold dilution of concentrate A and a column temperature of 60˚C.

Protein Hydrolysate 20X Dilution of Concentrate A 55°

Cell Culture Media 10X Dilution of Concentrate A 60°

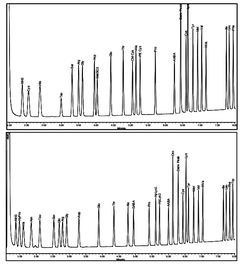


Figure 1. Comparison of amino acid analysis methods for protein hydrolysate and cell culture media.

Amino Acid Analysis of Cell Culture Media

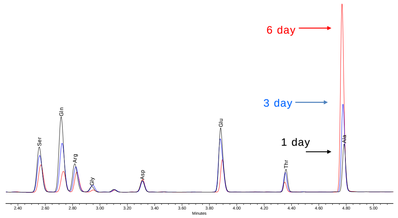


Figure 2. Cell Culture Media at T=1, 3 and 6 days. The accurate quantitation of derivatized amino acids is a useful measure of cell metabolism during the production of a biopharmaceutical. Samples were taken from a bioreactor during the production of a biopharmaceutical at 1, 3, and 6 day intervals. As shown below, some amino acids increase, others decrease, and some remain the same.

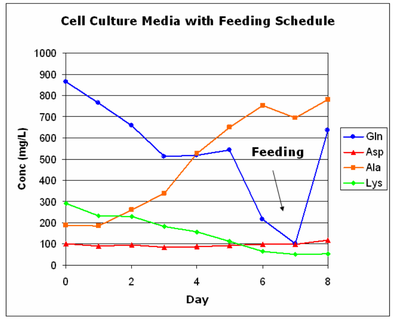


Figure 3. Amino Acid Concentration of Cell Culture Media over Time. The chart above illustrates how several amino acids change over time. This process is summarized for selected amino acids. The compositional impact of feeding the cell culture can be observed at Day 7.

Vitamin Analysis Method Development

Column Selection

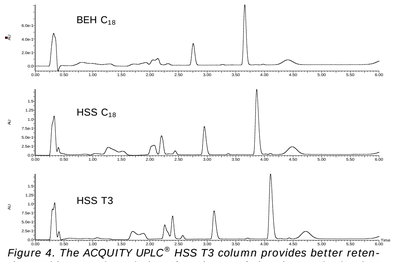


Figure 4. The ACQUITY UPLC® HSS T3 column provides better retention and improved resolution of a mixture of vitamin standards when compared to the ACQUITY UPLC® BEH C_{18} and ACQUITY UPLC® HSS C_{18} columns. All three columns are 2.1x50mm.

Ion-pairing Reagent Selection and Concentration

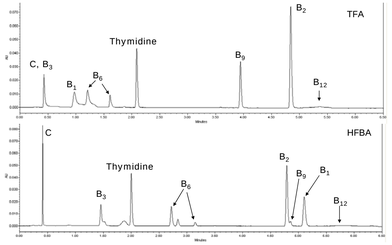


Figure 5. HFBA provides improved resolution, peak shape, and retention when compared with TFA on the ACQUITY UPLC® HSS T3 column.

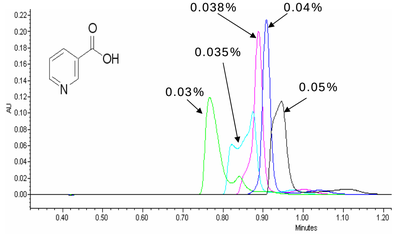


Figure 6. HFBA concentration effect on Nicotinic Acid peak shape and retention.

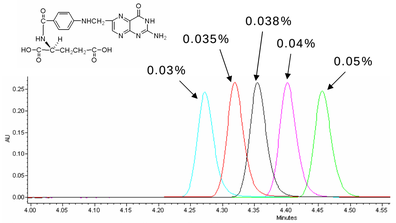


Figure 7. HFBA concentration effect on Folic Acid retention.

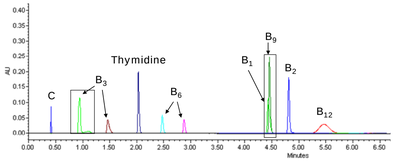


Figure 8. Vitamins B_1 and B_9 coelute with 0.05%HFBA in the mobile phase. Both forms of vitamin B_9 have good peak shape.

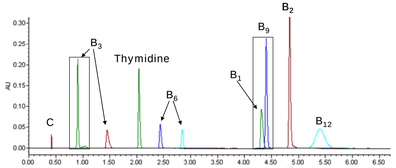


Figure 9. All vitamins have good peak shape with 0.04%HFBA in the mobile phase. Vitamins B_1 and B_9 are close together.

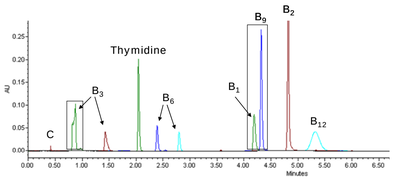


Figure 10. Vitamins B_1 and B_9 are separated with 0.035%HFBA in the mobile phase, but the vitamin B_3 (Nicotinic acid) peak shape deteriorates.

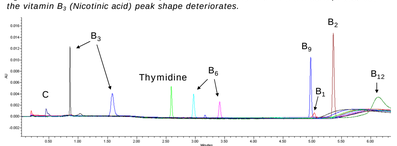


Figure 11. All vitamins are separated and have good peak shape with 0.038%HFBA in the mobile phase. Vitamin B_2 moves to a later elution time.

Figure 12. Vitamin standard at 270nm

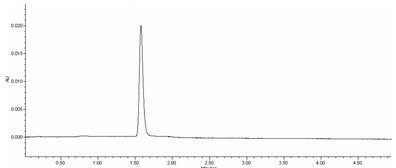
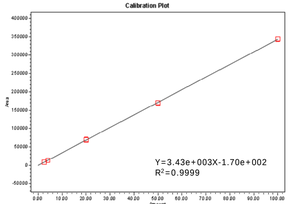


Figure 13. Linearity for vitamin standard at 270nm



- Most of the water-soluble B vitamins absorb at 270nm.
- The limit of quantitation is judged by reproducibility of integration. For most vitamins monitored at 270nm, the assay is linear from 2.5ng on column to 100ng on column.
- The limit of detection is most cases is about 0.5ng on column, but in the most favorable cases, amounts as low as 0.1ng can be detected.

Figure 14. Vitamin standard at 214nm

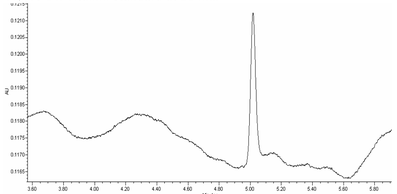
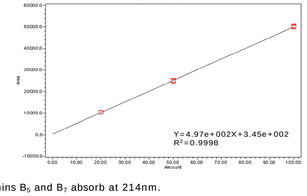
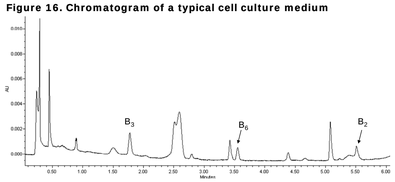


Figure 15. Linearity for vitamin standard at 214nm



- Vitamins B_3 and B_7 absorb at 214nm.
- The limit of quantitation is judged by reproducibility of integration. For vitamins monitored at 214nm, the assay is linear from 20ng on column to 100ng on column.
- The limit of detection is 20ng.

Figure 16. Chromatogram of a typical cell culture medium



Quantitative analysis for vitamins B_3, B_6 and B_2 in cell culture medium

One batch of commercial cell medium was diluted 10-fold with water and assayed. Figure 16 shows a typical chromatogram from the five replicate injections of this single dilution. The tables below show that for those vitamins identified in the cell culture medium, the %RSD for peak area is better than 6%.

Vitamin	Injection	Retention Time	Area
B_3	1	1.799	5257
	2	1.785	5271
	3	1.776	5100
	4	1.798	4910
	5	1.794	4954
Mean		1.791	5079
Std. Dev.		0.009	192
% RSD		0.557	3.78

Vitamin	Injection	Retention Time	Area
B_6	1	3.575	2626
	2	3.565	2616
	3	3.553	2535
	4	3.577	2328
	5	3.578	2448
Mean		3.569	2511
Std. Dev.		0.011	125
% RSD		0.298	4.97

Vitamin	Injection	Retention Time	Area
B_2	1	5.513	2102
	2	5.502	2018
	3	5.511	2024
	4	5.512	2038
	5	5.511	1800
Mean		5.510	1997
Std. Dev.		0.004	115
% RSD		0.081	5.74

CONCLUSIONS

- **The Waters UPLC® Amino Acid Analysis Solution has been adapted using different separation conditions for tracking both amino acids and water-soluble vitamins in cell culture media.**
- **The best retention and peak shape for B vitamins is observed on the ACQUITY UPLC® HSS T3 column.**
- **HFBA provides better retentivity and peak shape than TFA, and selectivity can be adjusted with modifier concentration.**
- **The assay is linear from 2.5ng on column to 100ng on column for most vitamins monitored at 270nm.**
- **For those vitamins identified in cell culture media, the %RSD for peak area is better than 6%.**

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