AMINO ACID ANALYSIS FOR MONITORING CELL CULTURE MEDIA AND PROTEIN STRUCTURE

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INTRODUCTION

A new total application solution for the analysis of amino acids has been recently developed. The analysis provides better resolution and sensitivity than has been possible in existing methods. The enhanced separation ensures that the analysis yields accurate and precise qualitative and quantitative results and that the method is rugged. The method, based on the well understood and widely used AccQ•Tag™ pre-column derivatization chemistry, provides these benefits in a shorter analysis time than previously possible. The derivatives are separated using Waters® ACQUITY UltraPerformance LC® (UPLC®) for optimum resolution and sensitivity. System control, data acquisition, processing, and flexible reporting are provided within Empower™ software. The integrated total application solution ensures successful analyses.

UPLC AMINO ACID ANALYSIS APPLICATION SOLUTION

Design Considerations and Criteria for Success

- Complete resolution of all amino acids
- Adequate resolution for unambiguous identification
- Complete resolution of derivatization by-products from amino acid derivatives
- 3 orders of magnitude linear dynamic range for quantitation
- Adequate electrospray ionization sensitivity and stability

DERIVATIZATION CHEMISTRY

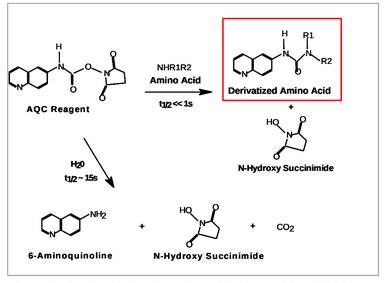

Figure 1. Derivatization Reaction of Amino Acid and AQC

The amino acids are derivatized using AccQ•Tag™ Ultra Reagent (6-aminoquinolyl-N-hydroxy-succinimidyl carbamate). Both primary and secondary amino acids react in a simple batch-wise derivatization.

- Reaction occurs in largely aqueous solution and is therefore tolerant of buffer salts and sample components
- No special sample handling, vacuum drying or extraction is required
- Samples are stable for several days
- Excess reagent naturally hydrolyzes
- Reagent by-product is chromatographically resolved from derivatives

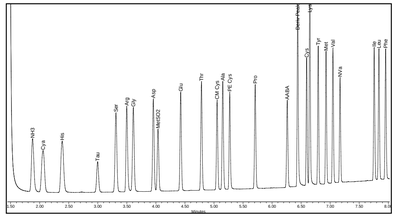

Figure 2A. Amino Acid Standard at 10 pmol on column. Includes common products of stabilizing sulfur amino acids. Both oxidation and alkylation are shown

For monitoring the composition of media during the growth of cells in culture or fermentation media, additional amino acids must be resolved. Method parameters for cell culture media analysis differ only in the dilution of AccQ•Tag Ultra Eluent A Concentrate and a higher column temperature. All other method parameters are identical.

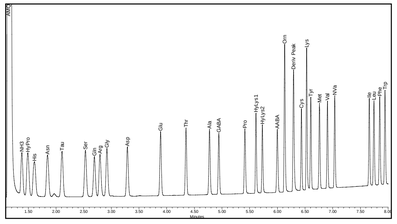

Figure 2B. Cell Culture Standard at 10 pmol on column

PROTEIN STRUCTURE

AMINO ACID COMPOSITION

This methodology can be used for determining amino acid composition of a protein in structure analysis. The common products of stabilizing sulfur-containing amino acids can be measured without modification of the standard method.

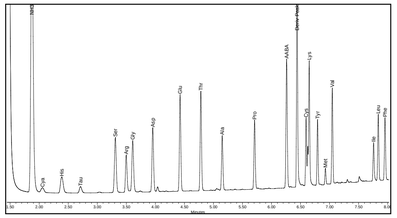

Figure 3A. Hydrolyzed hFSH

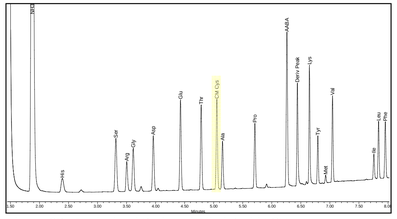

Figure 3B. Analysis of Reduced Alkylated hFSH

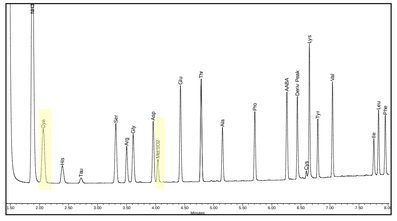

Figure 3C. Analysis of Performic Acid Oxidized hFSH

Determination of Protein Concentration

The results of the analysis can be expressed in any of the usual convenient units, including residue weight of each amino acid. The sum of amino acid weights per injection equals the mass of protein injected. The amount actually submitted for hydrolysis and the concentration of the original sample can then be calculated from the known dilution factors.

Sample	hFSH Hydrolysate	hFSH Reduced Carboxymethylation/Hydrolysate	hFSH Performic Acid Oxidation/ Hydrolysate
Measured amount of protein in tube (mg)	3.43	2.71	5.69

CELL CULTURE MEDIA

AccQ•Tag Ultra™ 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.

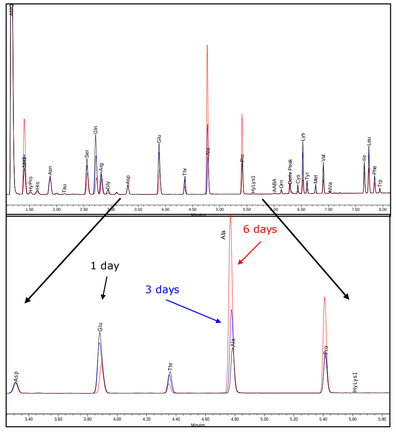

Figure 4A &B. Cell Culture Media at T= 1, 3 and 6 days. Full chromatogram and enlarged view.

Amino acid analysis of complex samples, such as cell culture media often requires sample pre-treatment or preparation. The high sensitivity of this 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 accurate quantitation of derivatized amino acids is a useful measure of cell metabolism during the production of a biopharmaceutical. As shown below, some amino acids increase , others decrease and some remain the same. The table below illustrates how several amino acids change over time. This process is summarized in Chart 1 for selected amino acids. The compositional impact of feeding the cell culture can be observed at Day 7.

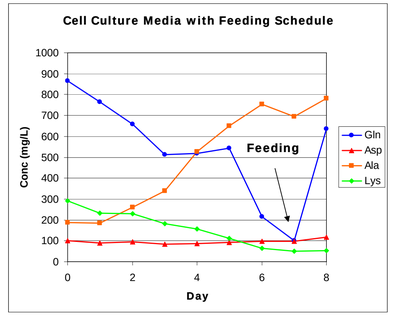

Chart 1. Amino Acid Concentration of Cell Culture Media over Time

ESI-MS COMPATIBILITY

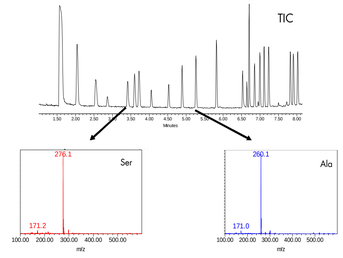

Figure 5. MS TIC and Spectra of Amino Acids

The UPLC® Amino Acid Analysis Application Solution is a UV based method that uses mobile phase eluents that are directly compatible with electrospray mass spectrometry. MS detection is not required for routine peak identification and is not necessary to detect sub picomole levels. However, it is a valuable tool in regards to the following determinations:

- Confirmation of amino acids by molecular weight
- Valuable for initial validation of a method
- Useful in determining unknown or unexpected peaks in a sample

CONCLUSION

- The UPLC® Amino Acid Analysis Solution provides a robust, turnkey AAA method.
- There are standard methods for protein hydrolysates and cell culture media.
- The standard method gives accurate and precise results in a very short run time.
- The results are suitable for use in structural analysis, including the analysis of sulfur-containing amino acids.
- The results also can be used to measure protein concentration.
- Changes in amino acid concentration as a function of growth can be measured in cell cultures used in biopharmaceutical production.
- The method can be used with electrospray ionization mass spectrometry without modification.

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