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A NOVEL METHOD FOR THE ANALYSIS OF AMINO ACIDS

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OVERVIEW

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 Ultra Performance 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

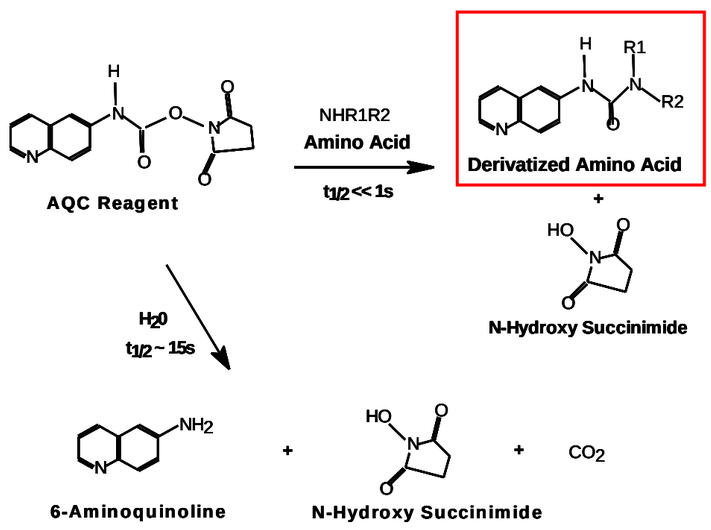
Design Considerations and Criteria for Success

- 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

Complete Turn-key AAA System

- ACQUITY UPLC system provides enhanced resolution for better accuracy, robustness, sensitivity, and speed
- AccQ•Tag™ Ultra Column and packaged reagents/ eluents specifically QC tested with amino acid analysis
- Application-specific Performance Qualification (PQ)
- Same result day-to-day, instrument-to-instrument, lab-to-lab, around the world

DERIVATIZATION CHEMISTRY



The amino acids are derivatized using AccQ•Tag™ Ultra Reagent (6-aminoquinolyl-N-hydroxysuccinimidyl 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

REPRODUCIBILITY

	Mean	Std Dev	%RSD
AMQ	1.459	0.017	1.19
NH3	1.915	0.024	1.26
His	2.401	0.032	1.34
Ser	3.250	0.030	0.93
Arg	3.463	0.027	0.79
Gly	3.566	0.028	0.78
Asp	3.897	0.028	0.72
Glu	4.387	0.024	0.54
Thr	4.766	0.021	0.43
Ala	5.145	0.019	0.37
Pro	5.710	0.016	0.28
Cys	6.569	0.011	0.17
Lys	6.638	0.011	0.16
Tyr	6.768	0.014	0.21
Met	6.917	0.013	0.19
Val	7.040	0.012	0.17
NVa	7.165	0.012	0.17
Ile	7.761	0.011	0.14
Leu	7.844	0.011	0.14
Phe	7.957	0.011	0.14

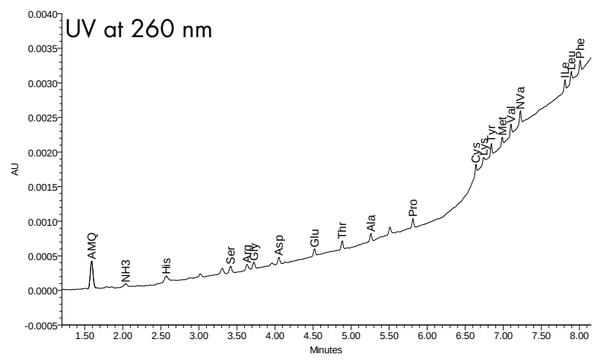
Most Variable

Most Closely Spaced

Retention Time Reproducibility of 25 AccQ•Tag™ Ultra Columns

Peaks in amino acid analysis are identified by retention time. Therefore, qualitative analysis depends upon both resolution and chromatographic reproducibility. The retention time reproducibility of 25 AccQ•Tag™ Ultra columns was tested by 2 operators on 4 systems. Standard deviations range from 0.011 min (0.7 sec) to 0.032 min (2 sec). To determine whether misidentification can occur, we can focus on the most variable peak (Histidine) and the most closely spaced pair (Cys/Lys). A retention time window that is +/- 2 std dev around the mean can be defined for each peak. The window for Histidine does not overlap the windows for the adjacent peaks. Similarly, the windows for the closely spaced Cys and Lys peaks do not overlap. The combination of good resolution and reproducibility ensures that there will be no ambiguity in peak identification.

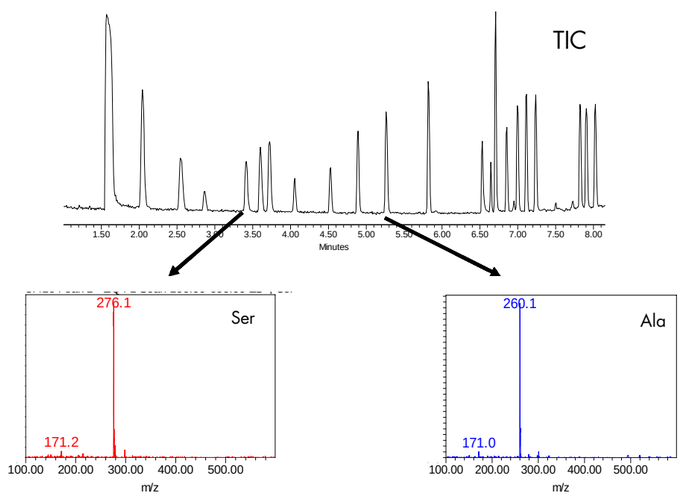
METHOD SENSITIVITY



Protein Hydrolysates at 50 fmol on column

The sensitivity of the method, chemistry and instrument was determined by diluting a known amount of derivatized sample. Levels as low as 50 femtomoles on column were achieved. Sensitivity for samples will be limited by environmental background and contamination.

ESI-MS COMPATIBILITY

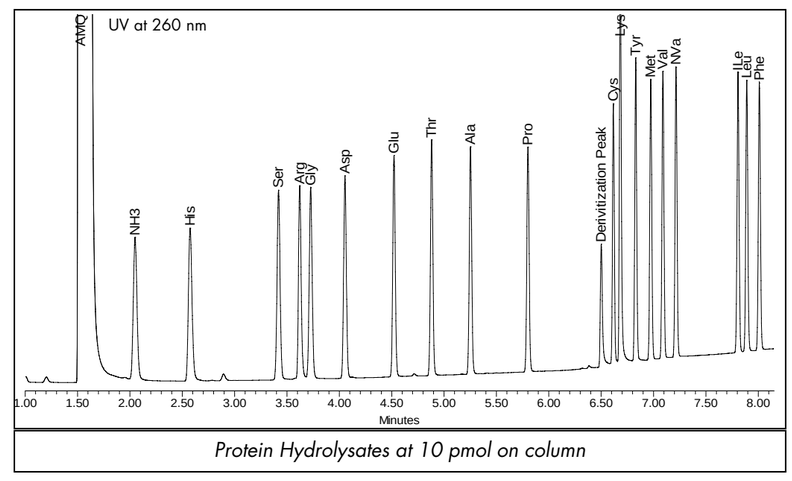


MS TIC and Spectra of Amino Acids

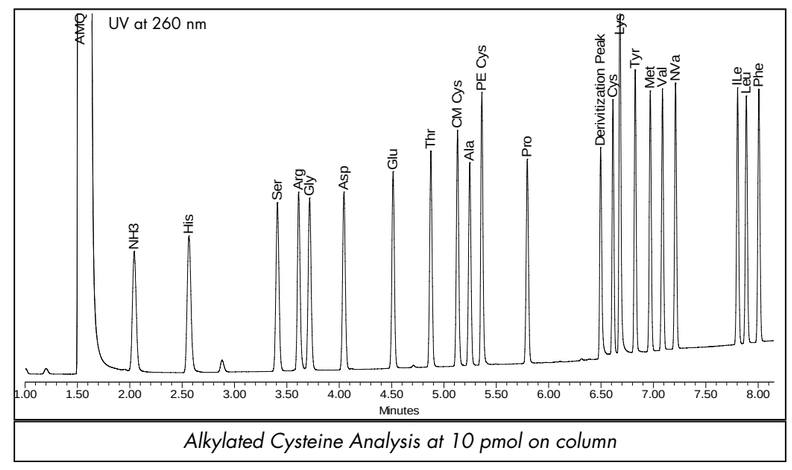
The UPLC™ Amino Acid Analysis Application Solution is a UV based method that uses mobile phase eluents that are directly compatible with electrospray mass spectrometry. MS detection is not required for routine peak identification and is not necessary to detect sub picomole levels. However, it is a valuable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

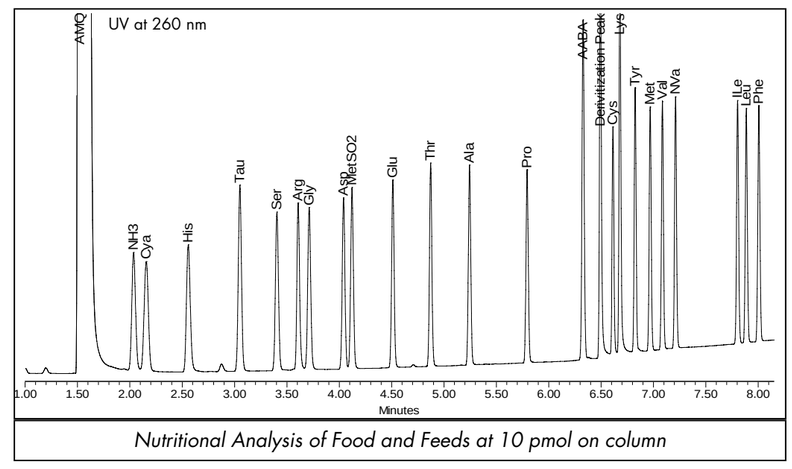
ADDITIONAL APPLICATIONS



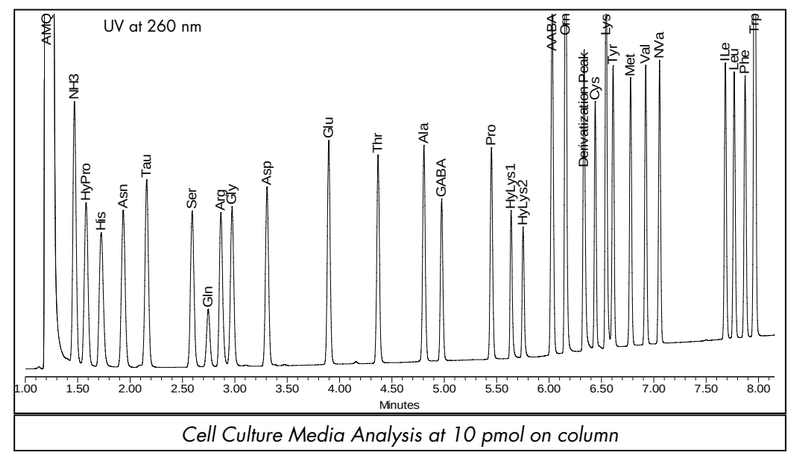
Protein Hydrolysates at 10 pmol on column



Alkylated Cysteine Analysis at 10 pmol on column



Nutritional Analysis of Food and Feeds at 10 pmol on column



Cell Culture Media Analysis at 10 pmol on column

This methodology can be successfully used for a range of applications. The standard method used to separate protein hydrolysates can also resolve the derivatives of cysteine commonly used in protein structure analysis. The products of performic acid oxidation that are part of assessing the nutritional quality of food and feeds are also well separated.

For monitoring the composition of media during the growth of cells in culture, 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.

CONCLUSIONS

- Successful results are assured through the use of pre-tested derivatization and separation chemistry and the high resolution provided with the ACQUITY UPLC™ system.
- This integrated analytical approach provides accurate and precise qualitative and quantitative results for a wide range of applications including protein and peptide hydrolysates, monitoring cell culture media, and measuring the nutritional value of food and feeds.

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