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UPLC AMINO ACID ANALYSIS SOLUTION

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INTRODUCTION

Analysis of amino acids is required in several different areas of research and is also a fundamental tool in various product analysis activities. These applications impose different requirements on the analytical method because the amino acids play different roles.

Amino acids are the basic constituents of proteins. For that reason, qualitative and quantitative analysis of the amino acid composition of hydrolyzed samples of pure proteins or peptides is used to identify the material and to directly measure its concentration.

Amino acids are also intermediates in a myriad of metabolic pathways, often not directly involving proteins. The amino acids are, therefore, measured as elements of physiological and nutritional studies. This has proven particularly important in monitoring the growth of cells in cultures, as used in the production of biopharmaceuticals. Similar considerations lead to the analysis of foods and feeds to ensure that nutritional requirements are met. These diverse sample applications will all benefit from improved amino acid methods.

A comprehensive system-based solution for the analysis of amino acids has been recently developed. This solution provides better resolution and sensitivity, all achieved in a shorter analysis time than with previous methodologies. Its enhanced separation ensures that the analysis yields accurate and precise qualitative and quantitative results and that the method is exceptionally rugged.

This application solution is based on the well-understood and widely-used Waters® AccQ•Tag™ pre-column derivatization chemistry. The derivatives are separated using the Waters ACQUITY UltraPerformance LC® (UPLC®) System 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 Solution.

METHODS AND DISCUSSION

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Ultimately, a new amino acid method must provide the right answer. Increased ruggedness, preferably with reduced labor and run times, are also desired characteristics of a successful laboratory system. These needs are met by combining AccQ•Tag Ultra amino acid analysis chemistries with the proven separation technology of the ACQUITY UPLC System; together they comprise the turnkey application solution called the Waters UPLC Amino Acid Analysis Solution.

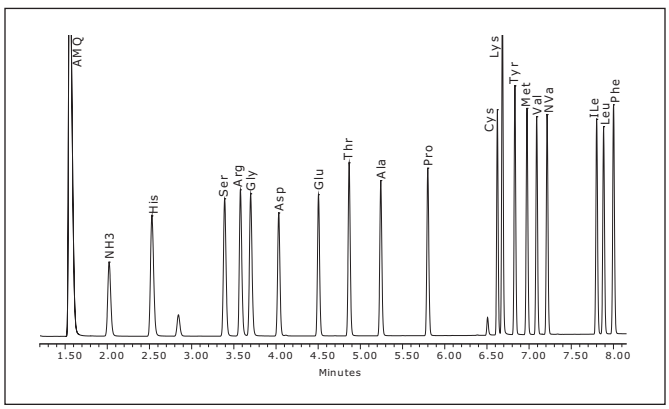

Figure 1. Separation of 50 pmoles of the amino acid hydrolysate standard with the UPLC Amino Acid Analysis Solution.

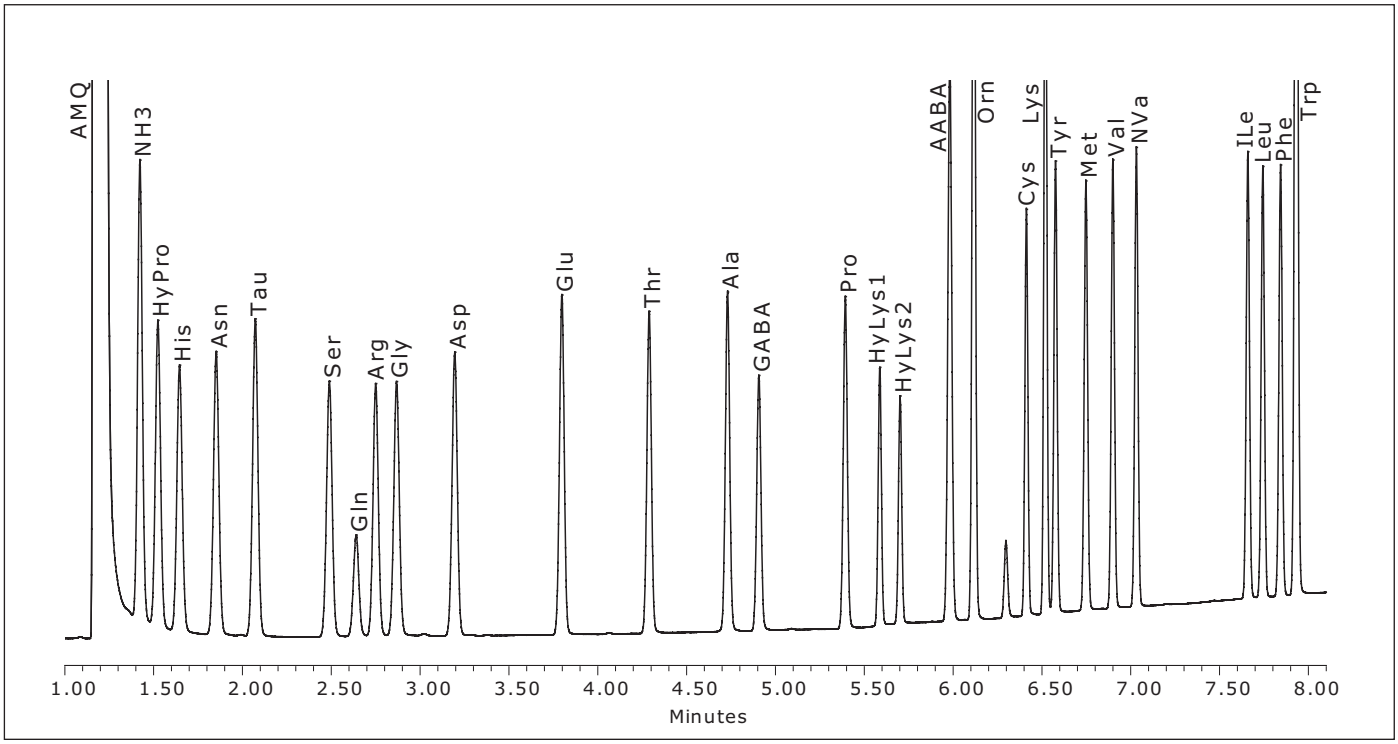

Figure 2. Separation of 10 pmoles of the amino acids commonly found in cell culture media. The UPLC Amino Acid Analysis Solution includes this modified separation method.

Analysis of a hydrolysate standard is shown in Figure 1. The amino acids are derivatized using AccQ•Fluor™ Ultra Reagent (Part Number: 186003836) (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate). Both primary and secondary amino acids react in a simple batch-wise derivatization, and samples are stable for several days. No special sample preparation is required, and the reaction occurs in a largely aqueous solution so it is very tolerant of buffer salts and other sample components. The excess reagent naturally hydrolyzes, and the by-product is chromatographically resolved from the derivatives. No special handling or extraction is required.

The derivatives are separated on an AccQ•Tag Ultra Column, 2.1 x 100 mm (Part Number: 186003837), a bridged ethyl hybrid (BEH) C_{18} 1.7 µm particle specifically tested for separation of the amino acids. Packaged eluents are quality control (QC) tested with amino acid separations. They are provided as concentrates requiring only dilution with water before use. The instrument is the ACQUITY UPLC System with UV detection at 260 nm.

The resolution of the amino acids is 1.6 or greater to ensure accurate quantitation. Retention time reproducibility is on the order of hundredths of minutes, much less than a peak width, to ensure unambiguous identification of the amino acids. The detection is linear, over more than three orders of magnitude, to permit quantitative analysis of samples with disparate ratios of amino acids with an ample margin for samples of different concentration. The sensitivity of the method gives adequate signal-to-noise to quantitate at the level of 50 femtomoles on-column.

The method can be successfully used for a range of applications. The standard method shown in Figure 1 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 foods 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. This requires a different dilution of the AccQ•Tag Ultra Eluent A Concentrate and a higher separation temperature. The chromatogram used for monitoring cell culture media is shown in Figure 2.

CONCLUSION

These results describe the new Waters UPLC Amino Acid Analysis Solution, an Assured Performance Solution (APS), that come complete with application-focused chemistries, innovative UltraPerformance LC and MS technologies, methodology, documentation, and support to deliver the answers you need about amino acids, every time. 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 will give 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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