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A New Amino Acid Analysis Application Solution

Thomas E. Wheat, Eric S. Grumbach, and Jeffrey R. Mazzeo

Waters Corporation

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 exceptionally 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 achieved. 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.

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 methods 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. In addition, amino acids are also intermediates in

myriad many 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, particularly 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.

Ultimately, a new amino acid method must provide the right answer while giving increased ruggedness, preferably with reduced labor and run times. These needs are met by combining the new Waters AccQ•Tag™ Ultra amino acid analysis chemistries with the proven separation technology of ACQUITY UPLC™ resulting in a new turnkey application system called the Waters® UPLC™ Amino Acid Analysis Application Solution. Sample hydrolysates results are shown in Figure 1.

The amino acids are derivatized using AccQ•Tag™ Ultra Reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate). The derivatives are formed for both primary and secondary amino acids in a simple batch-wise derivatization and are stable for several days. No special sample preparation is required, and the reaction occurs in a largely aqueous solution so it is very tol-

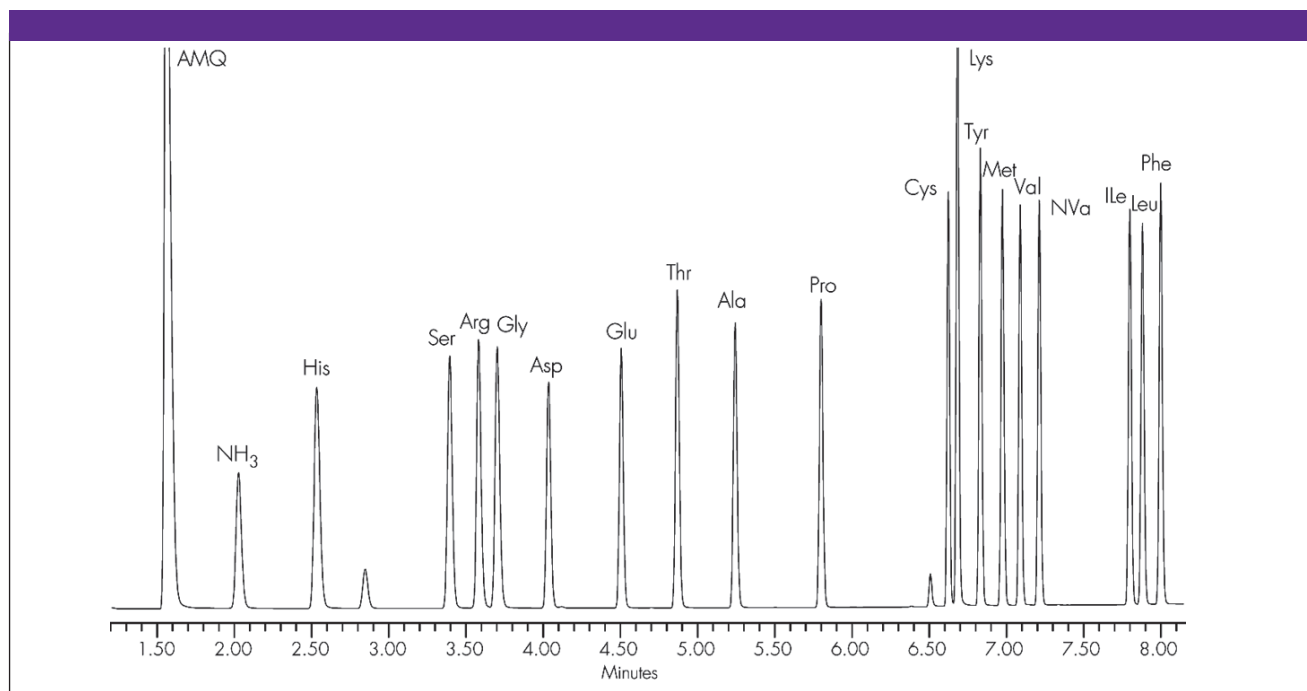


Figure 1: Separation of 50 pmoles of the Amino Acid Hydrolysate Standard with the UPLC™ Amino Acid Analysis Application Solution

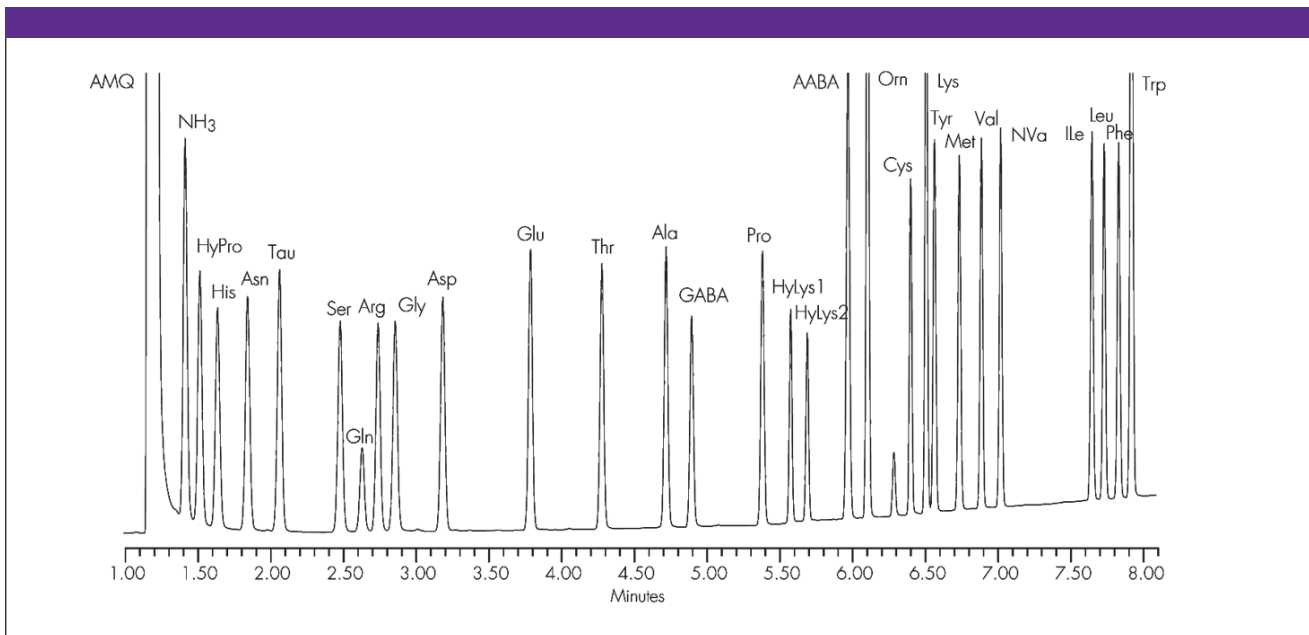


Figure 2: Separation of 10 pmoles of the amino acids commonly found in cell culture media. The ACQUITY UPLC™ Amino Acid Analysis Application Solution includes this modified separation method

erant of buffer salts and other sample components. The excess reagent naturally hydrolyzes, and the by-product is chromatographically resolved from the derivatives so no special handling or extraction is required.

The derivatives are separated on an AccQ•Tag™ Ultra Column, 2.1 × 100 mm, a bridged ethyl hybrid C18 1.7 μm particle specifically tested for separation of the amino acids. Packaged eluents are quality control (QC) tested with amino acid separations and are provided as concentrates requiring only dilution with water before use. The instrument is a Waters® ACQUITY UPLC™ System with UV detection at 260 nm. The resolution of the resolved 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 present used in protein structure determination as well as the products of performic acid oxidation that are part of assessing the nutritional quality of foods and feeds. For monitoring the composition of media during the growth of cells in culture, additional amino acids must be resolved. The only modifications required for such samples are a different specified 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.

These results describe the new Waters UPLC™ Amino Acid Analysis Application Solution. 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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Waters Corporation

34 Maple Street, Milford, MA 01757
tel. (508) 478-2000, fax (508) 478-1990
www.waters.com