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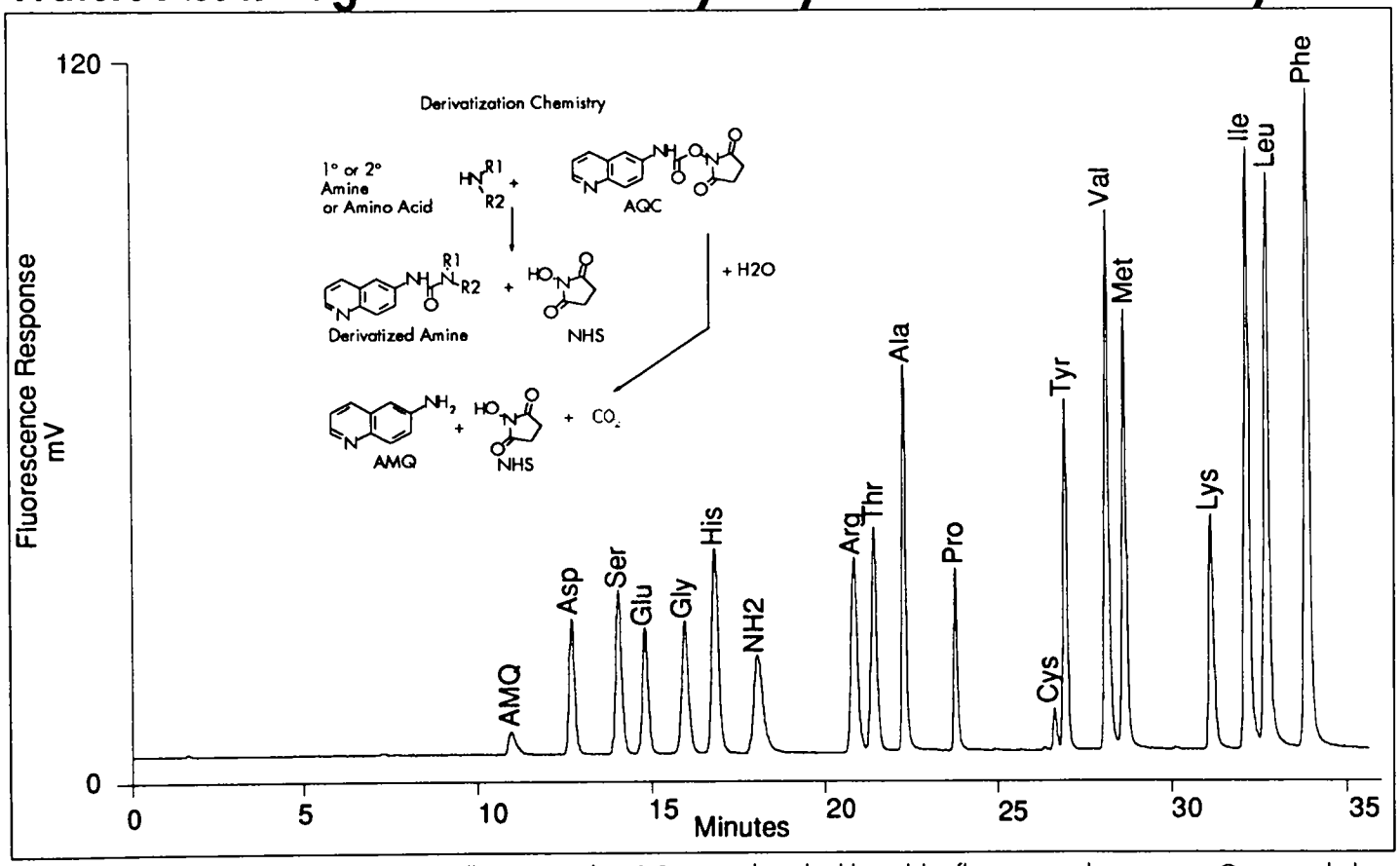
Waters Application Notebook

Waters AccQ•Tag™ Method for Hydrolysate Amino Acid Analysis



Both primary and secondary amines rapidly react with AQC to produce highly stable, fluorescent derivatives. Optimized chromatographic conditions provide baseline or near-baseline separation of the hydrolysate amino acids.

Conditions:

Column: Waters AccQ•Tag Column, 3.9 mm X150 mm

Column Temperature: 37°C

Mobile Phase: A ternary gradient elution profile using
Eluent A: AccQ•Tag Eluent A
Eluent B: Acetonitrile
Eluent C: MilliQ® water

Flow Rate: 1.0 ml/min

Detection: 470 Scanning Fluorescence Detector (5µl flow cell)

EX: 250nm
EM: 395nm
Filter: 0.5
Gain: 100

Injection volume: 5µl

Sample: AQC-Derivatized Amino Acid Standard (50pmoles)

Millipore Corporation Waters Chromatography Division 34 Maple Street Milford Massachusetts 01757 508 478 2000

Objective:

To demonstrate a new amino acid analysis method based on the rapid reaction of primary and secondary amines with AQC (AccQFluor reagent), followed by HPLC analysis.

Details:

Derivatization of amino acids is an important step for detection and quantitation of the amino acids in protein/peptide hydrolysates. Amino acid labelling with phenylisothiocyanate (PITC) is a well-understood reaction chemistry since it is the basis of the Edman degradation procedure for protein sequencing. The Waters Pico•Tag® method based on PITC offers rapid, high-perfomance analysis of all amino acids but the multiple step derivatization protocol can be time consuming. Derivatization with orthophthalaldehyde (OPA), used in Waters Auto•Tag™ OPA method, reacts with only primary amino acids. Another precolumn method based on derivatization with dabsyl chloride has difficulty maintaining optimal chromatography due to the large interfering peak produced by excess reagent. Two other reagents, dansyl chloride and fluorenyl methyl chloroformate (FMOC-Cl), are fluorescent methods which form multiple derivatives with selected amino acids.

Waters AccQ•Tag™ System fulfills the researcher's need for improved protein/peptide compositional analysis by combining a novel precolumn derivatization reagent specifically designed for amino acid analysis[1,2] and optimal HPLC system configuration, including application certified column and eluents, to provide enhanced accuracy and quantitative reproducibility. Waters AccQ•Fluor™reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, AQC) reacts with both primary and secondary amino acids within seconds and excess reagent is hydrolyzed to form N-hydroxysuccinimide, 6-aminoquinoline (AMQ) and carbon dioxide. The derivatized samples can be injected without further workup due to the unique fluorescent properties of AMQ, the only fluorescent by-product of the reaction. This dramatically reduces the magnitude of the AMQ peak so that there is no interference with amino acid quantitation. The reaction yields highly stable, fluorescent quinoline-labelled urea adducts. The AccQ•Tag Chemistry Package, including all the reagents needed for derivatization, is tested and formulated to provide the lowest possible amino acid contamination. A reversed phase separation with near baseline resolution is achieved in a total run time of 45 minutes. The AccQ•Tag System provides researchers a powerful, novel method that may prove useful for many applications in biotechnology.

System:

Waters AccQ•Tag System consisting of a 625LC system equipped with a column heater, 717plus Autosampler with heater/chiller, and 470 Scanning Fluorescence Detector. System control and results management were provided by a Millennium™ 2010 Chromatography Manager using the AccQ•Tag Application Template.

References:

1. Cohen,S. and Michaud, D., Anal. Biochem., (1993) in Press.

2. Cohen, S. A., De Antonis, K, and Michaud, D. P., in Press in Techniques in Protein Chemistry IV (R.H. Angeletti, ed.) Academic Press, (1993) San Diego.

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