

Poster #31 This poster was the initial presentation of the AccQ•Tag method in June 1992. It contains a description of how the reagent works, and examples of hydrolysate analysis of peptides and proteins. Accurate compositional analysis at low levels (100ng hydrolyzed) is the backbone of the method.

HPLC '92

—01

Highly Accurate, High Sensitivity Amino Acid Analysis with Novel Activated Carbamates as Pre-Column Derivatizing Reagents

***S. A. Cohen, D. P. Michaud, Millipore Corp., Waters Chromatography Division 34
Maple Street Milford, MA 01757 USA***

Pre-column derivatization of amino acids has often become the method of choice for amino acid analysis due to several key advantages it offers, including higher sensitivity, faster analysis times, and the use of more flexible chromatographic equipment. Despite these potential improvements, most methods based on such reactions suffer from one or more deficiencies such as poor derivative stability, reaction sensitivity to buffer components, formation of multiple derivatives, low reactivity with selective components, or a complete lack of reactivity with secondary amino acids. We have synthesized a series of new, activated carbamates that exhibit none of these detrimental characteristics and provide a rapid, one-step, quantitative derivatization procedure for all amino acids studied. The synthesis route chosen allows the tagging group to be chosen from a wide variety of commercially available amines and yields high purity product with few components that interfere with analysis. The carbamate analog with aminoquinoline is the basis for an extremely powerful, versatile method for amino acid analysis that can provide quantitative analysis for peptides and proteins that rivals results from post-column derivatization procedures, yet has significant advantages such as detection limits in the 100-200 fmol range. The method requires no removal of the reagent as it is converted to a poorly detected component during the reaction, the derivatives are stable for weeks at room temperature, and low sensitivity to buffer interference has allowed the analysis of a wide variety of samples including foods and feeds, biological fluids and salt-containing protein solutions. This new reagent thus provides the first complete solution for amino acid analysis based on pre-column derivatization.