

Poster #38 This poster has a very good molecular biology slant to it, use it when introducing the method to biotech accounts. Poster # 44 about the Quickblot makes a good pairing for this one. One graphic includes a large phosphate peak running 4mins ahead of the AA peaks posing no problem for the method. All of the work was done on Bovine Cytochrome C.

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The Use of a Precolumn Derivatizing Reagent for obtaining Accurate Amino Acid Compositional Analysis of Chromatographically Separated Proteins

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Biochemists continue to use amino acid analysis as one of their major tools in the characterization process. Since many of their protein samples are obtained from a variety of sources, including HPLC purification and PAGE, the method of choice should be able to tolerate interferences from salts, organic solvents, detergents and other sample contaminants while providing a quantitative method for all the amino acids in a low picomole range. Recently, a novel precolumn derivatizing reagent has been made available to meet these criteria. N-hydroxysuccinimidyl-6-amino quinoline carbamate (AQC)¹ provides the researcher with a simple approach for rapid quantitation of primary and secondary amino acids using HPLC with fluorescence detection. This poster will highlight the principles of a precolumn derivatization method utilizing AQC for the compositional analysis of protein samples that have been separated chromatographically and electrophoretically. Chromatographic methods will include ion exchange and reversed phase HPLC. Data suggests that interferences from commonly used buffers and salts do not compromise the derivatization. In addition, electrophoretic separations will be performed using both polyacrylamide gels and capillary electrophoresis, followed by sample transfer onto PVDF membranes.

[1] Cohen, S.A., and Michaud, D.P. (1993) "Synthesis of a Fluorescent Derivatizing Reagent, 6-Aminoquinolyl-N-Hydroxysuccinimidyl Carbamate, and its Application for the Analysis of Hydrolysate Amino Acids via High Performance Liquid Chromatography", Anal. Biochem., in press.