Poster #39 This poster presents the beginning of the expansion of the use of the AccQ•Fluor reagent past the traditional boundaries of amino acid analysis. This is a key application for pharmaceutical and drug development labs. Position this application just like a Symmetry column, to the methods development chemists. If you can get the chemist to use this reagent as part of their normal quantitation protocol, they'll probably transfer it to QC, where it may be used for years. Poster #48 provides examples of this type of work on amine drugs

American Peptide Symposium '93

-06

Analysis Of Synthetic Peptides Using Derivatization With 6-Aminoquinolyl-N-Hydroxy-Succinimidyl Carbamate

Steven A Cohen*, Kathryn M. De Antonis**, Phyllis R. Brown**, and Charles Van Wandelen*,

*Waters Chromatography Division of Millipore, Milford, MA 01757, ** University of Rhode Island, Kingstown, RI 02881

The reaction of 6-Aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC), a fluorescent amine-labeling compound, with primary and secondary amines provides a facile, rapid, sensitive method for the analysis of both hydrolyzed and intact peptides. AQC has been shown previously to be highly successful for the analysis of amino acids [1]. Derivatization conditions and reversed phase chromatographic parameters have been optimized to provide highly reproducible and accurate analytical results.

A series of synthetic prothrombin leader sequences (ANKGFLEEX) each having a different C terminus have been chosen as model peptides for analysis. Derivatization of the intact peptides occurs at the N terminus and on the amine group of any lysine residues. Under optimal conditions, the reaction is rapid and complete with greater than 98% of the peptide successfully derivatized. Subpicomole amounts of AQC derivatized peptides can be easily detected, and the concentration sensitivity is in the nanomolar range. Good retention of derivatized peptides in the injection mixture allows large volumes of sample to be injected without sacrificing chromatographic peak shape. In addition, a large volume flow cell can be used to enhance the fluorescence signal. Amino acid analysis using the same AQC derivatizing reagent and chromatographic analysis which has been described previously [1] has been done on each peptide to determine its amino acid composition and concentration in solution.

[1] Cohen, S.A., De Antonis, K.M., and Michaud, D.M. (1993) in Techniques in Protein Chemistry iv (R.H. Angeletti, ed.) Academic Press.