Poster#47. Originally presented at PittConn '94. This poster should be used when the customer indicates some doubt in AAA as a truly quantitative method. Low *cvs* and outstanding linearity should be leveraged. Any company dealing with recombinant protein or peptide pharmaceuticals could use this for Q.C.. Indeed 1 New England biotech firm has validated the method for protein quantitation. This poster received over 100 reprint requests at the protein society (a new lab record).

Protein Society '94 -10

Determining The Concentrations of Protein Solutions By Quantitative Amino Acid Analysis

Steven A. Cohen , Charlie Van Wandelen
Waters Chromatography Division of Millipore 34 Maple Street, Milford, MA 01757

Solution concentration of proteins and peptides is often determined using general dye ligand techniques such as the Lowery procedure, bicinchoninic acid procedure, ultraviolet spectroscopy, or total Kjeldahl nitrogen. These methods are adequate for solutions with high protein concentrations (e.g. 0.1 - 10.0 mg/ml), but reduced accuracy is often observed with more dilute samples. Quantitative AAA using ion-exchange with ninhydrin has been reported to provide equivalent results to these procedures, with marked improvement at low concentrations. The recently introduced 6-Aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) method provides a simple alternative to the ninhydrin method for amino acid quantification. Hydrolyzed samples are reconstituted, labeled with a one minute AQC reaction, and then analyzed by reversed phase HPLC. Solutions of highly purified BSA, rcombinantly derived samples, and synthetic peptides have been used to demonstrate excellent linearity and reproducibility for the entire analysis including hydrolysis. Despite the high sensitivity of the methodology, quantification remains linear up to the 5mg/ml level and the subpicomole detection limits enables sample concentrations to be determined at the 5ug/ml level, thus providing three orders of magnitude linear dynamic range. Good recoveries in the presence of many buffer salts often allow derivatization without desalting. In addition to providing solution concentration, the analysis also provides a measure of sample purity by comparing the derived amino acid composition to the expected composition, thus providing confirmation of sample identity.