AccQ•Tag SOLUTIONS

Quantitating Protein Samples

The need for high precision methods for protein quantitation and characterization has led to the development of a diverse group of analytical methods. Dye binding procedures such as Lowery, Bradford, and bicinchoninic acid (BCA) often require refinement before they are precise and accurate. In addition, the assays typically suffer from a limited linear dynamic range. Ultraviolet (UV) absorbance methods, while straightforward to perform, require carefully determined extinction coefficients, and can be easily compromised by the presence of UV active matrix components such as salts, detergents and preservatives.

In light of these limitations, Amino Acid Analysis (AAA), is rapidly gaining acceptance as an alternative quantitation method. Of the methods used today, ion-exchange separation with post-column reaction is most often cited. Precolumn methods of AAA have yet to enjoy full acceptance because of problems with linearity, reproducibility, and/or matrix interferences. Already, the recently developed AccQ•Tag™ AAA method[1] has been shown to be a reliable and reproducible method for determining protein composition (Fig. 1). In this application note we will evaluate the AccQ•Tag method to determine its applicability to the protein quantitation assay[2]. The UV and BCA methods are used as a basis for comparisons. Highly purified bovine serum albumin (BSA), and synthetic Substance P are used as representative analytes.

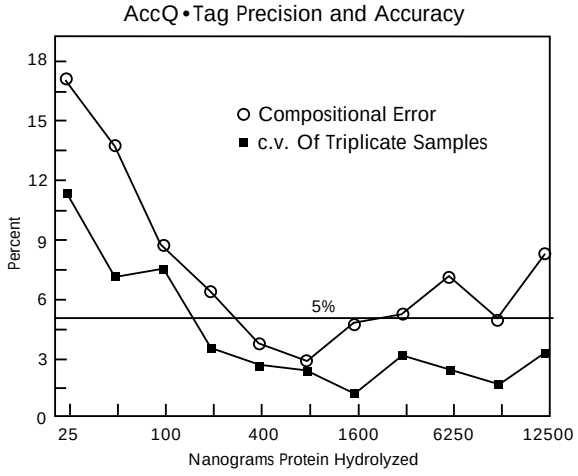

Figure 1 Errors associated with the AccQ•Tag method. The compositional error is an average of the 16 AAs reported. The RSD values reported are for the entire method including hydrolysis. The majority of the compositional error below the 50µg/ml level is due to the loss of Met response; results for the other amino acids still correlate well with the actual composition.

Results of Comparisons with UV and BCA Methods

For the study of response linearity, BSA solutions were prepared at 10 concentration levels. Sample size remained constant within each method for all levels (AccQ•Tag 5µl, UV 500µl and BCA 100µl). Solutions ranging from 5-5000µg/ml were assayed with each method and the results were evaluated using the rigorous measure of linearity described by Dorschel et al.[3] A plot of response/concentration (R/C) vs. concentration (C) theoretically produces a horizontal line for a linear assay, and large deviations from this theoretical result indicate poor linearity. This evaluation is superior to standard R vs. C plots using a linear regression method to calculate a correlation coefficient as this places too much weight on data at the high end of the concentration range. The resulting data (Fig. 2) plotted using the R/C vs. C method illustrates the span over which these methods remain linear. The narrowest range of linearity was for the BCA method, linear between concentrations of ~ 20-450 µg/ml. The responses from the UV method were linear from ~ 80-3000 µg/ml. The linear range of the AccQ•Tag method was widest, from 17-5000 µg/ml. The quantitative results for the UV and AccQ•Tag methods were in close agreement throughout their linear ranges, the average difference being 1.24%.

In order to further evaluate the AccQ•Tag method for quantitative analysis, the peptide content of solid preparations were determined. Substance P was synthesized using Fmoc methodology, and products were isolated from the cleavage solution using parallel methods. One half of the TFA cleavage mixture was precipitated into ethyl ether and filtered, while the other half was diluted with 20 volumes of glacial acetic acid and lyophilized. Quantitative AAA of the samples showed that the preparations contained 77% and 56% peptide content respectively, indicating that minor differences in synthetic protocol can have a significant impact. In contrast, UV quantitation of these samples was not feasible due to absorbance of the synthesis byproducts. Use of the BCA method is not recommended for low molecular weight peptides and was not performed.

Waters

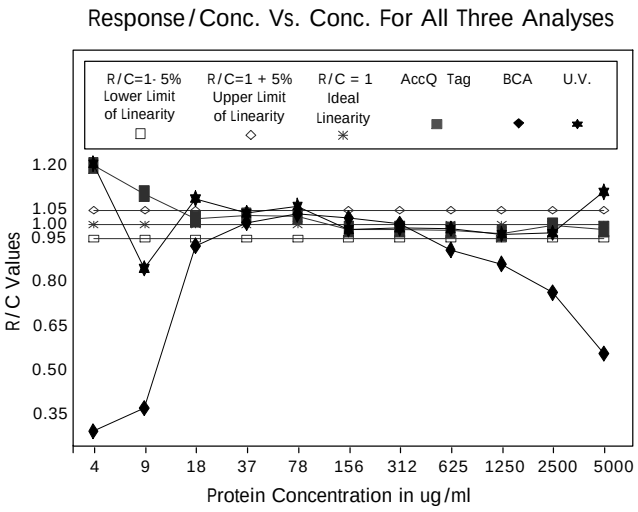

Figure 2 Linearity plots for the three methods tested. Calculated R/C values falling outside ±5% of the average R/C value are considered outside the linear range of the assay.

To assure a high degree of quantitative accuracy on a routine basis, Waters recommends the use of the vapor phase hydrolysis method. Vapor phase hydrolysis is superior to liquid phase because only volatile reagents (HCl/Phenol) contact the sample, which reduces potential contamination to an insignificant level[4]. Uniform results are consistently achieved using the Pico•Tag® Workstation which is capable of hydrolyzing up to 12 samples in the same reaction vial.

Data Analysis and Reporting

Waters Millennium® Chromatography Manager has custom fields which allow protein concentration calculations to be made within the Microsoft Windows™ based software. Variables such as sample size, molecular weight and dilution factors can be entered along with normal sample information prior to HPLC. Calculated results for sample concentration can then be included in the header of the report (Fig. 3) followed by chromatograms and data tables.

Conclusion

As a candidate for protein quantitation, the AccQ•Tag method possesses many desirable qualities including superior dynamic range and broad applicability. In addition, the same analysis provides qualitative information concerning sample composition and purity. These key features, along with superior versatility compared to other pre-column AAA methods, makes the AccQ•Tag method a valuable addition to the modern biotechnology laboratory.

References

1. Cohen, S. A., and Michaud, D. P.(1992) Anal. Biochem. 211, 279-287
2. Cohen S. A., van Wandelen, C. (1994) The Eighth Symposium of the Protein Society, Poster #250
3. Dorschel, C. X., Ekmanis, J.L,, Oberholtzer, J. E, Warren, F.V., Bidlingmeyer, B. A. (1989) Anal. Chem. 61, 951A-958A
4. Cohen, S. A., Tarvin, T. L., Bidlingmeyer, B. A. (1984), American Laboratory, August 48-59

AccQ•Tag Protein Quantitation Report

Sample Name:	PROQNTe21	MW:	66267
Vial:	21	Sample Type:	Unknown
Injection:	1	Run Time:	45.0 min
Volume:	5.00	Date:	12/03/93 02:52 PM
Date Acquired:	11/12/93 12:34		
Samp. Conc.	0.984 µg/ml		
Avg Pmol Hyd	74.246		

Figure 3 An example of a custom report which includes calculated sample concentration (all dilutions accounted for), and other pertinent sample information in the header.

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