Waters
Lab Highlights
An Internal Communication of Applications and Techniques
LAH 0420 12/89
DP/LS/RS/AA/PH

Analysis of Glycoproteins Using the Pico•Tag® Method

Purification and characterization of glycoproteins and glycopeptides are becoming increasingly important procedures in the biochemistry laboratory as the key role of these molecules in biological recognition becomes apparent. With new tools provided by the expanding range of liquid chromatographic applications, studies on the carbohydrate moiety of these molecules are becoming much easier to perform routinely. Despite the focus of much of the research on the oligosaccharides, study of the peptide portion remains an important part of the overall picture. Amino acid analysis (AAA) of the carbohydrate-linked peptide(s) of a glycoprotein can provide valuable information concerning the glycosylation attachment site(s). This Lab Highlight describes modifications to the standard Waters Pico•Tag AAA system operating conditions that provide optimized results for these samples.

Figure 1 below shows the optimized separation for glycoprotein analysis on the Pico•Tag hydrolyzate column (P/N 88131). The need for altered chromatographic

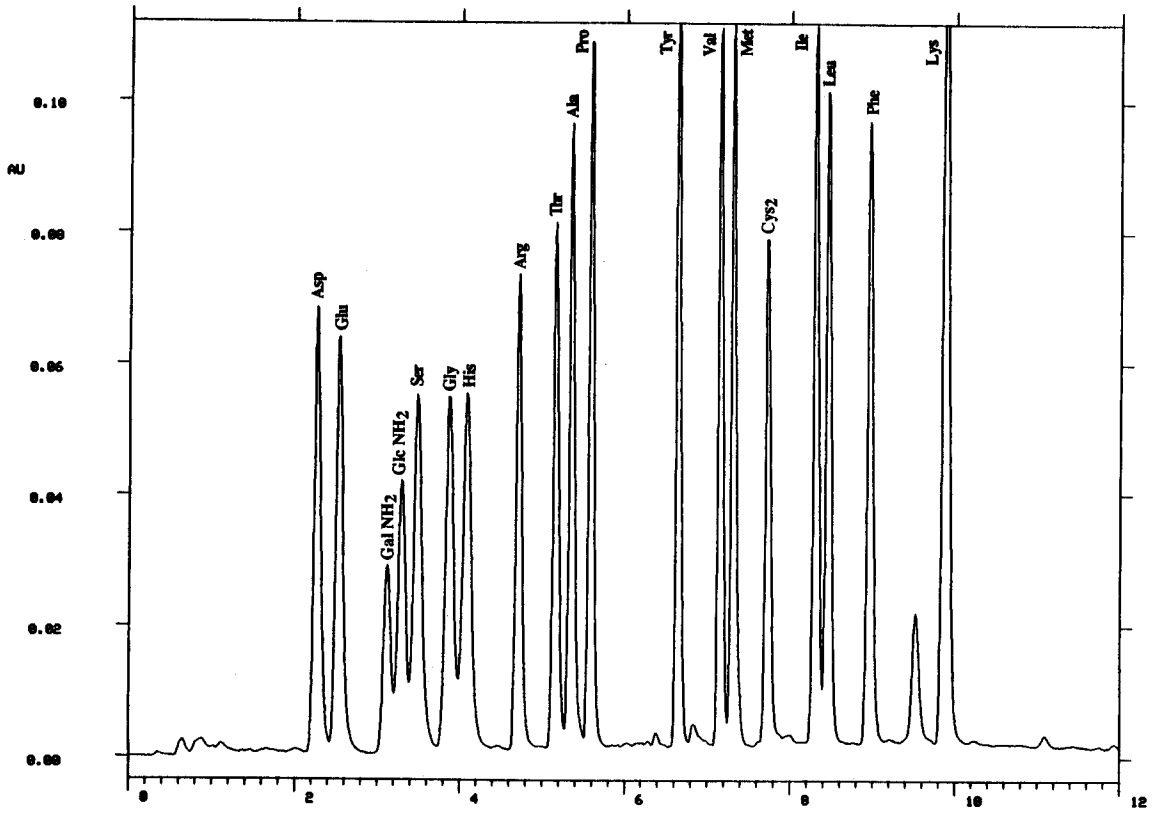

Figure 1. Separation of Hexosamines and Hydrolyzate Standard Mix. For Conditions see Table I.

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conditions stems from the occurrence of N-acetylglucosamine and N-acetylgalactosamine in glycoproteins. Acid hydrolysis of these compounds releases the sugar amines glucosamine and galactosamine, respectively, which react with phenylisothiocyanate to form phenylthiocarbamyl derivatives. Mobile phase compositions, gradients and column temperature have all been adjusted from the standard conditions to obtain resolution of the hexosamines from the hydrolyzate amino acids (Table 1). Without these changes, the hexosamines can interfere with the analysis of Ser and Gly.

Higher resolution can be obtained using the Pico•Tag Free Amino Acid Analysis column (P/N 10950). Conditions for this column and a more in-depth description of the key chromatographic parameters including tips for separation optimization are provided in the recently published: *The Pico•Tag Method: A Manual for Advanced Techniques for Amino Acid Analysis* (Literature #WM02) that is available as an addendum to the Pico•Tag Operators Manual.

Table 1

		Gradient Conditions				
Column:	**Pico•Tag Hydrolyzate Amino Acid Column, 3.9 x 150mm.**	**Time**	**Flow**	**%A**	**%B**	**Curve Number**
Eluent A:	**0.14M Sodium Acetate,**	**Initial**	**1.0**	**100**	**0**	*
		10.0	**1.0**	**54**	**46**	**4**
	0.1% Triethylamine,	**10.2**	**1.0**	**0**	**100**	**6**
	pH 5.30, 6% Acetonitrile	**11.7**	**1.0**	**0**	**100**	**6**
		12.0	**1.5**	**0**	**100**	**6**
Eluent B:	**60% Acetonitrile**	**12.2**	**1.5**	**0**	**100**	**6**
		12.5	**1.5**	**100**	**0**	**6**
Column		**20.0**	**1.5**	**100**	**0**	**6**
Temperature:	**42°C**	**20.5**	**1.0**	**100**	**0**	**6**

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