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應用手冊

UPLC Technology for the Analysis of Antibody Glycopeptides

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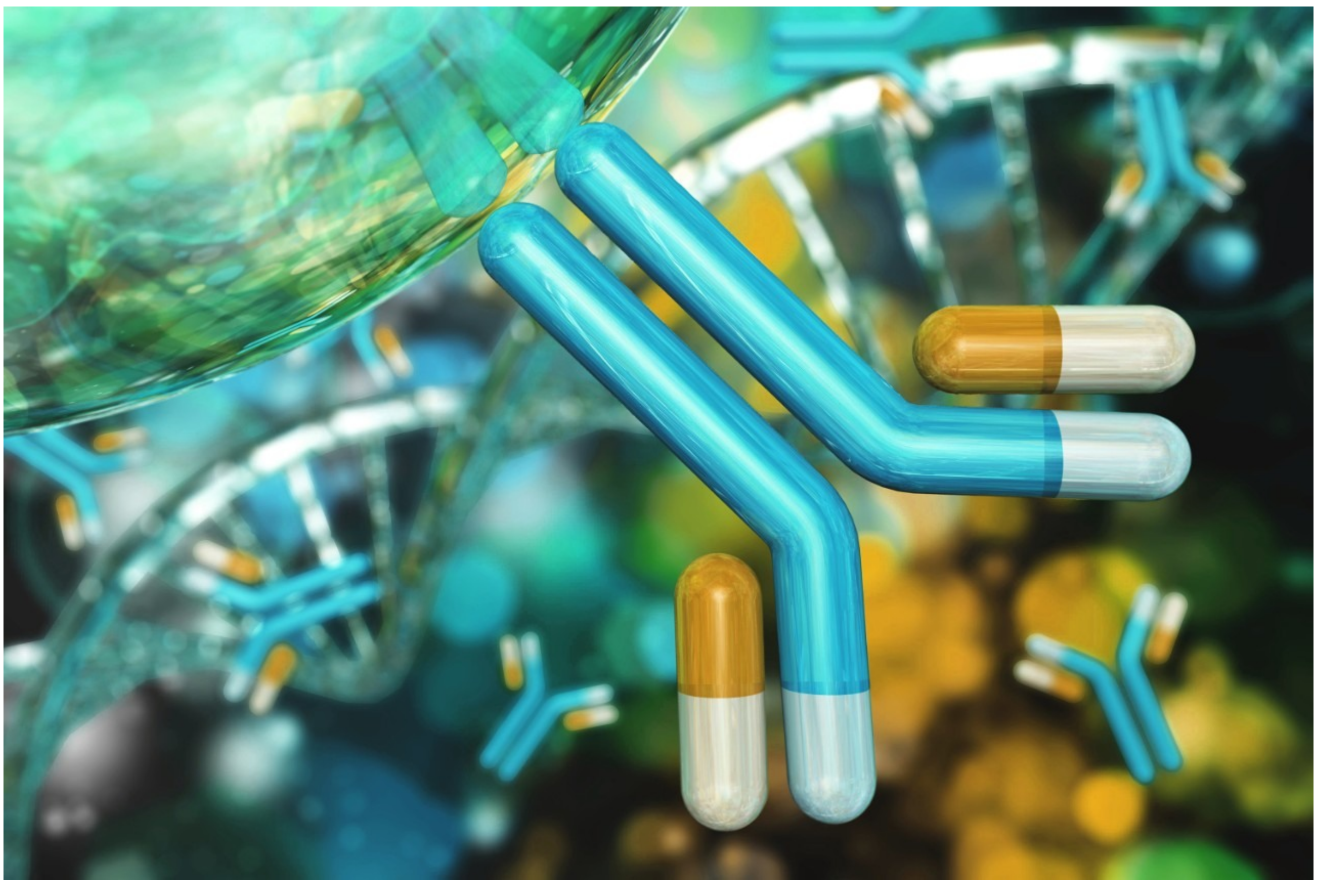

Abstract

The Waters UPLC Peptide Analysis Solution was described as a tool for peptide mapping protein

characterization. The technique has proven useful for improved resolution and speed in peptide mapping. The significantly improved peptide resolution is now shown to provide separation of glycyopeptides with mixed glycan structures, even the separation of glycopeptide positional isomers. Quantitation of the relative amounts of glycan structures can thus be approached within the peptide map without the cumbersome procedures required for released glycan analysis.

Introduction

The development of protein biopharmaceuticals requires complete structural characterization. Definition of a protein structure includes the amino acid sequence, storage-induced modifications, and post-translational modifications of the protein. Glycosylation is one of the most important classes of post-translational modifications. Within the human body many circulating proteins are glycosylated, and therefore the most promising candidate drugs for biopharmaceutical use have glycan chains. The oligosaccharide components can directly affect the efficacy and safety of these drugs by influencing binding, immunogenicity, and turnover. It is, therefore, critical to characterize oligosaccharide structure in biopharmaceutical development.

The analysis of glycoproteins is a challenging enterprise because of the extreme complexity of oligosaccharides and the heterogeneity of the cellular products. The molecular weights and branched structure of glycans that have been released from a protein can be determined by applying MALDI or ESI mass spectrometry with collision-induced dissociation (CID). Proposed structures can also be based on elution times from anion-exchange chromatography or from HILIC on amino phases. These analytical techniques do not, however, yield information about the sites of modifications. To approach the modification site question, glycoproteins are subjected to proteolytic cleavage digestion without removal of the glycans. The reversed-phase HPLC peptide maps usually include glycopeptides as broad, asymmetric, poorly resolved peaks. Better chromatographic separations are required for assessing the safety and efficacy of biopharmaceuticals.

Recently, UltraPerformance LC (UPLC Technology) has been proposed as a tool for improving resolution in peptide mapping. This technique has now been tested for the analysis of glycoforms of a monoclonal IgG.

Figure 1. UPLC Peptide Analysis Solution.

Experimental

Sample Preparation

A monoclonal mouse IgG1 (VICAM division of Waters) was reduced, alkylated, and digested in the presence of 0.1% *Rapi*Gest SF surfactant (Waters Part Number: 186001860 <https://www.waters.com/waters/partDetail.htm?cid=1000941&id=27026>) in a Certified Maximum Recovery vial (Waters, Part Number: 600000670CV <https://www.waters.com/waters/partDetail.htm?cid=10000711&id=28881>). The reducing agent, TCEP (tris[2-carboxyethyl] phosphine, Pierce) was added to a concentration of 10 mM. After 30 minutes of reduction, the alkylation reagent, iodoacetamide, was added to a concentration of 20 mM for 30 minutes. The protein was then digested overnight with Modified Trypsin (Promega) at an enzyme-to-substrate ratio of 1:25.

LC Conditions

Instrument:	ACQUITY UPLC Peptide Mapping System
Detector:	TUV @ 214 nm
Column:	Peptide Separation Technology Column 2.1 x 100 mm, ACQUITY BEH130 C_{18}, 1.7 µm Part Number: 186003555

Column Temp:	40 ˚C
Injection Volume:	10 µL
Mobile Phase A:	0.1% TFA in water
Mobile Phase B:	0.08% TFA in acetonitrile
Flow Rate:	100 µL/min

Gradient

Time (min)	A	B	Curve
2	100%	0%	*
118	50%	50%	6
122	25%	75%	6
123	0%	75%	6
125	100%	0%	6
143	100%	0%	6

MS Conditions

Instrument:	Waters LCT Premier Mass Spectrometer
Ionization Mode:	ESI Positive
Capillary Voltage:	3200 V

Cone Voltage:	30 V
Desolvation Temp:	150 ˚C
Desolvation Gas:	500 L/Hr
Source Temp:	100 ˚C
Acquisition Range:	50-2000 *m/z*

Results and Discussion

The tryptic peptides of the reduced and alkylated monoclonal IgG were separated using UPLC Peptide Mapping System, as shown in Figure 2. The same chromatogram could be observed with either UV or MS detection, and the glycopeptides were recognized by their masses as described in Figures 3 and 4.

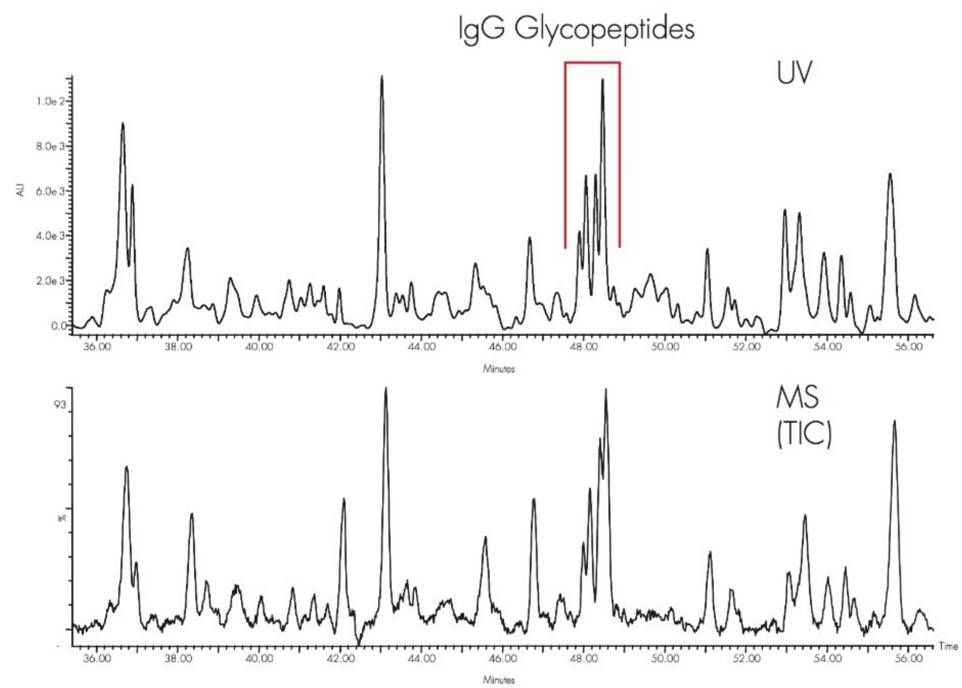

Figure 2. UV and MS UPLC chromatograms of a mouse antibody tryptic digest. The selected time range is focused on the glycopeptides.

The cluster of four marked peaks represent the elution of the major glycoforms of a monoclonal IgG. These antibodies have only one conserved glycosylation site. There are two N-linked glycans tucked between the two heavy chains to which they are attached, one to each heavy chain, at the same conserved sequence. They are heterogeneous with respect to the number of terminal galactose residues, so that a given peptide may exist with zero, one, or two galactose residues. The structures of the “G0F”, “G1F”, and “G2F” glycans are shown in Figure 3.

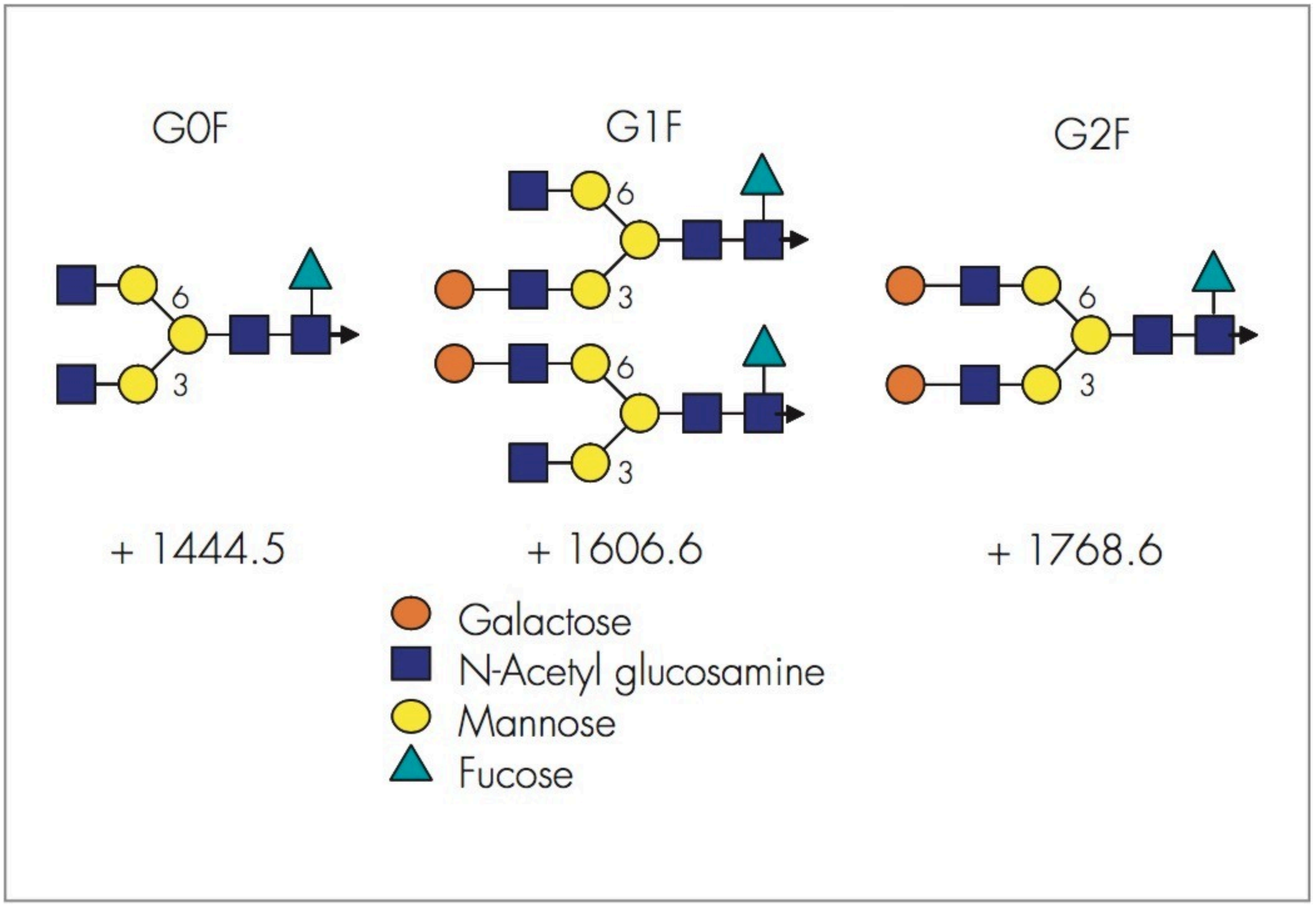

Figure 3. N-linked Glycans of Monoclonal IgG.

Extracted ion chromatograms for the three glycopeptide molecular weights, in each case the triply-charged ion, are shown in Figure 4. The G2F glycoform elutes first in the TFA-containing gradient, followed by baseline-separated glycopeptides displaying the two isomers of G1F. Chromatographic peak widths at half height were 0.11 minutes or 6–7 seconds. The improved resolving power allowed the resolution of individual glycopeptide structures.

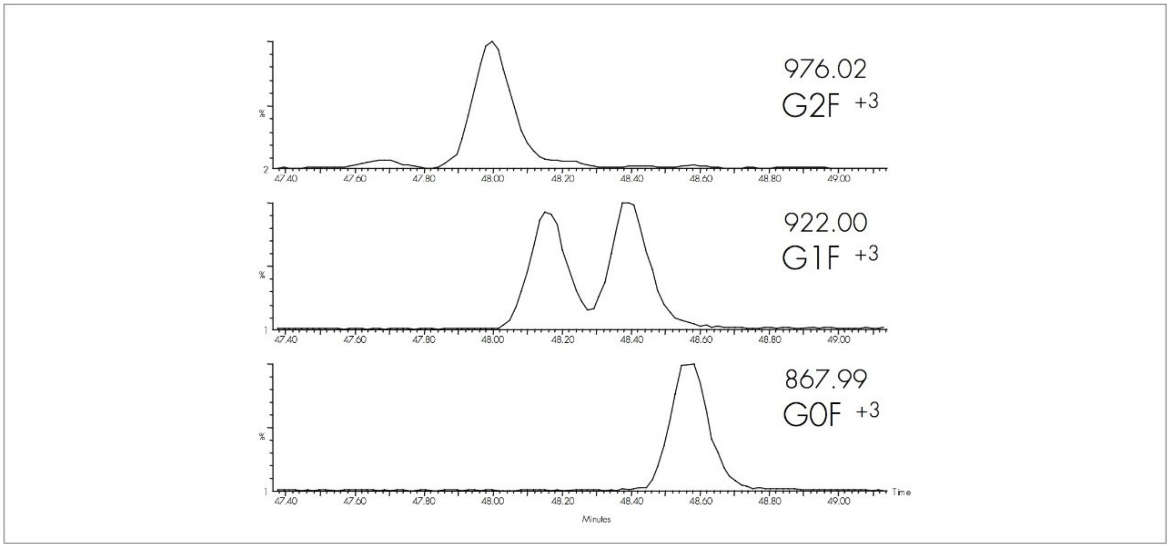

Figure 4. Extracted Ion Chromatograms for the Triply-charged State for Each of the Three Expected Glycopeptides.

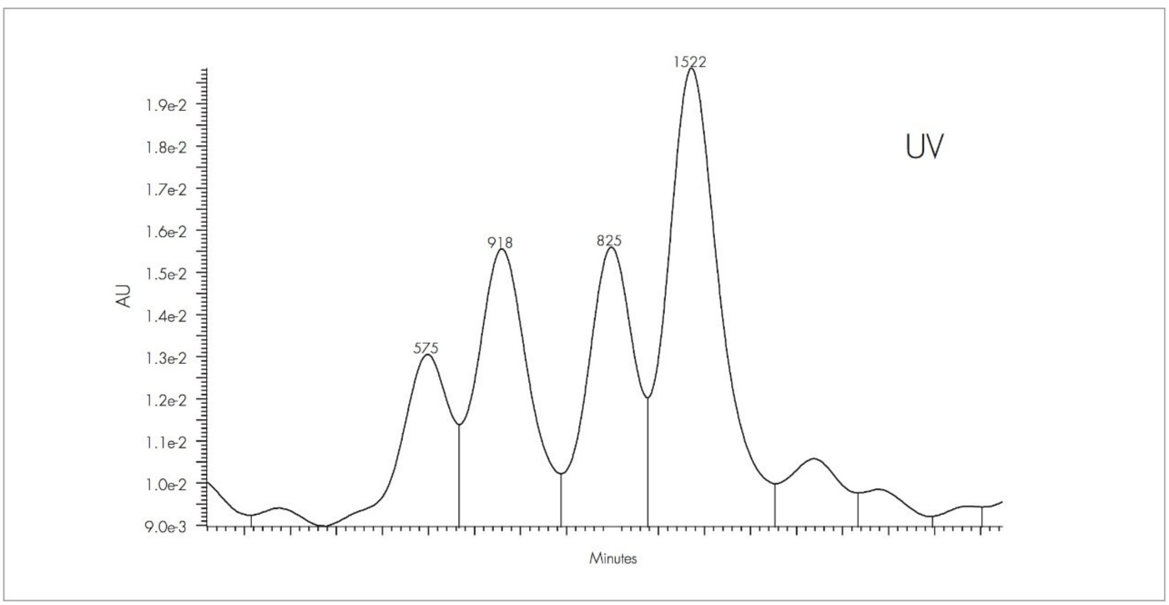

Figure 5. Detection of Four Glycopeptide Species in the UV Trace.

Conclusion

The Waters UPLC Peptide Analysis Solution was described as a tool for peptide mapping protein characterization. The technique has proven useful for improved resolution and speed in peptide mapping. The significantly improved peptide resolution is now shown to provide separation of glycyopeptides with mixed glycan structures, even the separation of glycopeptide positional isomers. Quantitation of the relative amounts of glycan structures can thus be approached within the peptide map without the cumbersome procedures required for released glycan analysis.

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