QUANTITATION OF GLYCOFORMS IN RECOMBINANT IgG1 ANTIBODY BY LC AND LC/MS METHODS

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OVERVIEW

- Recombinant monoclonal antibodies are the fastest growing therapeutics in the biopharmaceutical industry. Recombinant antibodies contain carbohydrate moieties (glycans) as a result of post-translational modifications. The process of adding sugars to the protein at the endoplasmic reticulum and the Golgi apparatus is highly complex, resulting in variation of glycoforms.
- Glycosylation plays a vital role in stability, biodisposition, in-vivo activity, solubility, serum half-life, and immunogenicity of an antibody drug and can affect efficacy, target binding, folding and pharmacokinetic properties. Moreover, glycosylation of recombinant antibodies differs with the cell culturing parameters.
- It is extremely important to accurately quantify the carbohydrate moieties of therapeutic proteins.
- This presentation compares several LC/MS assays with traditional released glycan assay for quantifying glycosylation of a recombinant antibody.

MS Conditions
MS System: Waters Xevo™ QTof MS
Ionization Mode: ESI Positive
Capillary Voltage: 3.0kV
Cone Voltage: 45V/25V
Source Temp: 100°C
Desolvation temp: 350°C
Desolvation Gas: 1000 L/h

LC System: Waters ACQUITY UPLC ®
Detector: ACQUITY UPLC FLR

LC/MS ANALYSIS OF INTACT IgG1 ANTIBODY

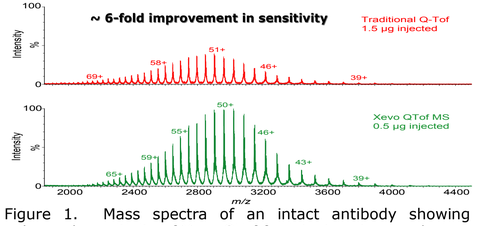

Figure 1. Mass spectra of an intact antibody showing enhanced sensitivity of Xevo QTof for intact protein analysis.

LC/MS ANALYSIS OF INTACT IgG1

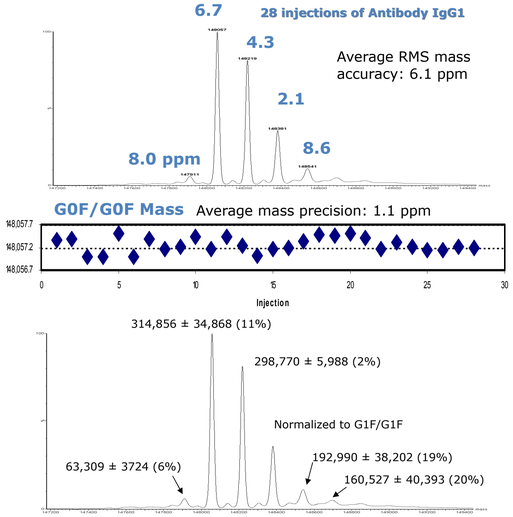

Figure 2. MaxEnt1™ deconvoluted mass spectra of an intact antibody showing mass accuracy, precision and intensity variation for glycoforms on Xevo QTof over 28 injections.

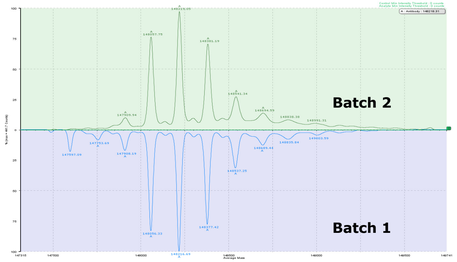

Figure 3. MaxEnt1 deconvoluted mass spectra of an intact IgG1 displayed by BiopharmaLynx™. The glycan profiles and variations from two production batches are clearly illustrated. This approach is fast and provides holistic view of the IgG1 glycoforms and paired masses. Quantitative information on individual glycoforms is unavailable by this approach.

LC/MS ANALYSIS OF HC (REDUCED)

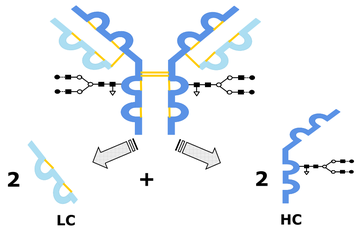

Figure 4. Heavy chain (HC) and light chain (LC) fragments generated by reduction of monoclonal antibody with DTT.

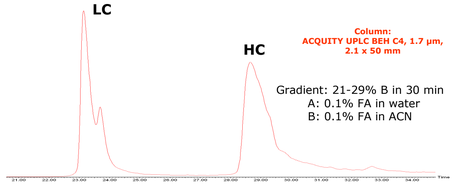

Figure 5. Total ion chromatogram of light chain and heavy chain fragments from a reduced monoclonal antibody. The analysis was done by Acquity UPLC/Xevo QTof MS.

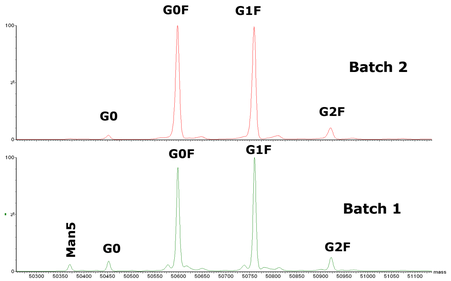

Figure 6. MaxEnt1 deconvoluted mass spectra of IgG HC (~50kDa) generated from two different batches of a recombinant monoclonal antibody. Detected glycoforms were labeled based on the deconvoluted masses. Reduction of the antibody into monomeric HC allowed quantitation of the individual glycoform.

LC/MS ANALYSIS OF IgG1 Fc/2 FRAGMENT

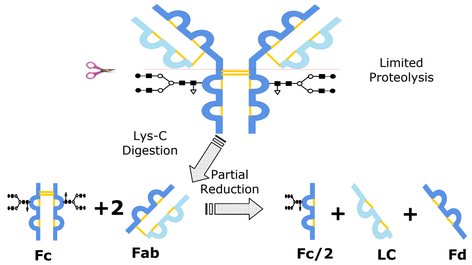

Figure 7. Fragments generated by limited proteolysis of monoclonal antibody with Lys-C followed by partial reduction.

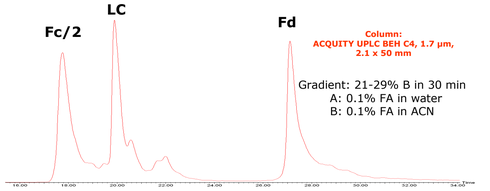

Figure 8. UPLC/MS analysis of Lys-C digested (limited) and partially reduced monoclonal antibody. Total ion chromatogram of Fc/2, LC, and Fd is shown.

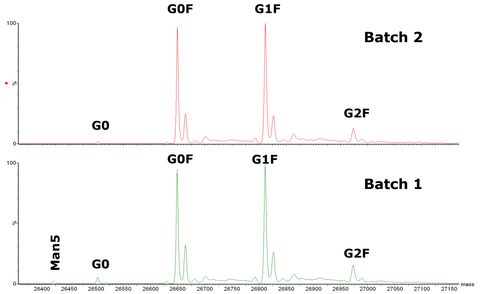

Figure 9. Deconvoluted mass spectra of Fc/2 from the two production batches. Lys-C digestion followed by reduction resulted in monomeric Fc/2 (~25 kD). Detected glycoforms were labeled based on the deconvoluted masses.

Released N-Glycan Assays of the two IgG Batches

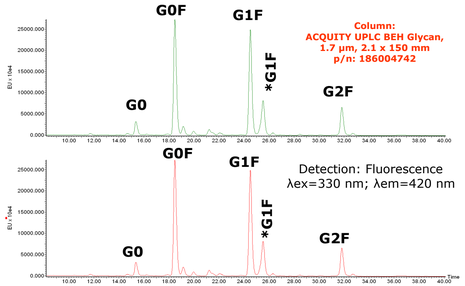

Figure 10. Released N-glycan assay of the recombinant IgG1 from two production batches. The figure shows the chromatograms of 2-AB labeled glycans obtained in HILIC mode. The identification of the 2-AB labeled glycans was achieved by on-line MS analysis (Xevo QTof). The asterisk represents the isobaric form of the G1F separated on the column.

	A	B	C	D	E	F	G
1	Carbohydrates	Release Glycan Batch 1(%)	Release Glycan Batch 2 (%)	Fc/2 Batch 1 (%)	Fc/2 Batch 2 (%)	HC Batch 1 (%)	HC Batch 2 (%)
2	G0F	45.5	45.2	45.1	45.8	45.3	47.0
3	G1F	33.2	33.8	47.6	47.0	47.0	45.6
4	G1F*	12.8	12.6				
5	G2F	8.5	8.4	7.3	7.2	7.7	7.4

Table I. Quantitative comparison of IgG1 glycoforms with different analytical methods. Three glycoforms (G0F, G1F and G2F) were detected by all techniques and were quantitatively compared. Isobaric G1F forms that were separated by free glycan assay are combined for the comparison.

CONCLUSIONS

- Several UPLC/ESI-QTof MS-based methods were compared for the identification and quantitation of various glycoforms in an IgG1 antibody.
- ESI-QTof MS analysis of both Fc/2 (from Lys-C digestion and reduction) and HC (reduced) yielded agreeable quantitation results on common glycoforms in comparison with the N-release assays.
- ACQUITY UPLC BEH Glycan columns offer superior resolution in the separation of released glycans, providing accurate measurement of the individual glycoforms.

References:
1. *Gadgil et.al J. Pharmaceutical Sciences* ***96,*** *2607–2621 (2007)*

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