RECOMBINANT ANTIBODY GLYCOFORMS ASSAY 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.
- 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 glycoforms of a recombinant antibody.

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.

Figure 2. MaxEntTM deconvoluted mass spectra of intact antibody showing mass accuracy, precision and intensity variation for glycans on Xevo QTof over 28 iterations.

Figure 3. MaxEnt deconvoluted mass spectra of Trastuzumab Fc fragment (~50kDa) from three batches displayed by Biopharmasystems. The glycan profiles variations from three production batches are illustrated. This approach provides holistic view of the IgG1 glycans and paired masses. Quantitative information on individual glycans is unavailable by this approach.

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

Figure 5. LC/MS (Acquity UPLC/Xevo QTof) analysis of reduced monoclonal antibody Trastuzumab (Batch 3).

Figure 6. MaxEnt1 deconvoluted mass spectra of IgG1 HC (~50kDa) generated from three different batches of Trastuzumab. Detected glycoforms were labeled based on the deconvoluted masses. Reduction of the antibody into monomeric HC allowed quantification of the individual glycoforms.

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 Trastuzumab, Fc2 fragment from the three production batches. Lys-C digestion followed by reduction resulted in monomeric Fc/2 (~25 kDa). Detected glycoforms were labeled based on the deconvoluted masses.

CONCLUSIONS

- Several UPLC/ESI-QToF MS-based methods were compared for the identification and quantitation of various glycoforms in a IgG1 antibody (Trastuzumab).
- ESI-QToF MS analysis of both Fc2 (from Lys-C digestion and reduction) and HC (reduced) yielded excellent agreement of quantitative results on common glycoforms in comparison with the N-released assays.
- ACQUITY UPLC BEH Glycan columns offer superior resolution in the separation of glycoforms, providing accurate measurement of the individual glycans.

Table 1. Quantitative comparison of Trastuzumab glycoforms with different analytical methods. Glycoforms (G0, G1, G0F, G1F, G2F, and Man5) detected by all techniques were quantitatively compared. Isobestic G0 and G1F forms that were separated by free glycans were combined for the comparison. Percent were calculated from all glycoforms that were detected in a specific batch.

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