BIOTHERAPEUTIC PROTEIN GLYCOSYLATION USING UPLC/QTOF MS

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INTRODUCTION

Glycosylation plays a vital role in the safety and efficacy of many therapeutic proteins such as recombinant monoclonal antibody (*rmAb*). Several studies have shown the correlation between glycosylation variations caused by cell line selection and changes in culture medium parameters. These variations can have profound effect on the biological activities of the biotherapeutics which leads to the changes in drug potency in the final product. In this study, we applied a robust, sensitive and reproducible analytical platform that comprises an Ultra Performance Liquid Chromatography (UPLC) in Hydrophilic Interaction (HILIC) mode, Fluorescence (FLR) detector and a QTof Mass Spectrometer (MS) for Biotherapeutical protein glycosylation analysis.

The scope of the characterization ranged from glycan quantitation and mass profiling for three different **Trastuzumab** batches. In addition to the FLR labeled glycan LC/FLR/MS assay, we also examined glycoform quantitation for the heavy chains, the Fc/2 fragments from limited Lys-C digestion.

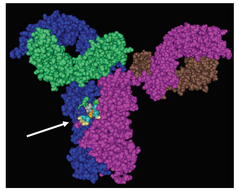
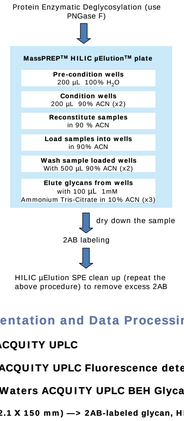


Figure 1: A crystal structure of Trastuzumab. The arrow points to the oligosaccharides that are located in the Fc region of the heavy chain.

METHODS

- **Fluorescent labeled glycan sample preparation**



- **Instrumentation and Data Processing**

LC: Waters ACQUITY UPLC

FLR: Waters ACQUITY UPLC Fluorescence detector

Columns: 1) Waters ACQUITY UPLC BEH Glycan Column

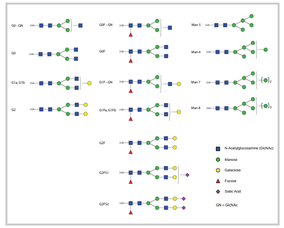
(2.1 X 150 mm) —> 2AB-labeled glycan, HILIC-LC/FLR/MS analysis

2) Waters ACQUITY UPLC BEH C4 column

(2.1 X 50 mm) -> Reversed phase LC/MS analysis for FC/2 and HC

MS: Waters Xevo QTOF MS controlled by MassLynx 4.1

Data Processing: BiopharmaLynx v. 1.2



Proposed glycan structures from Trastuzumab

RESULTS

- **2AB-labeled Glycan Profile (e.g., Batch 2)**

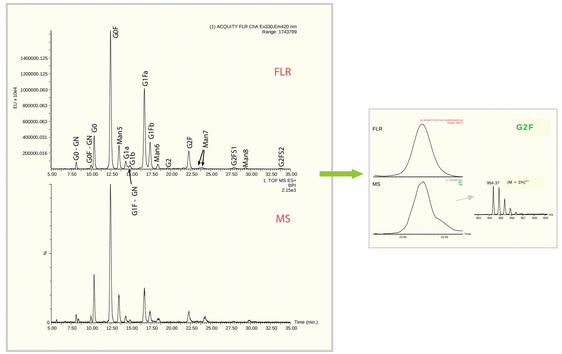


Figure 2. UPLC/FLR/Xevo QTof MS analysis of 2AB labeled glycans from Trastuzumab (Batch 2). The top chromatogram is FLR chromatogram; the bottom is the MS chromatogram. The glycan identify were confirmed by their accurate mass.

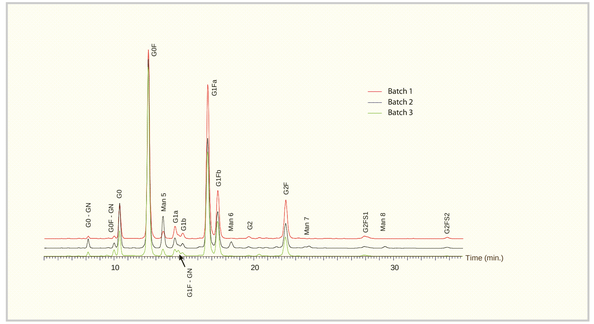


Figure 3. UPLC/FLR chromatograms of 2AB labeled glycans released from three Trastuzumab batches. Man6, Man 7 and Man 8 were observed only in Batch 2.

- **Glycan Profiling Using HC and Fc/2 Portion of the TrastuzumAb**

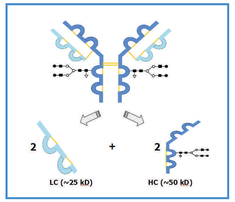


Figure 4a. Heavy chain (HC) and light chain (LC) fragments generated by partial reduction of monoclonal antibody with DTT.

LC/MS

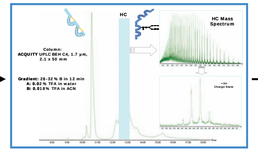
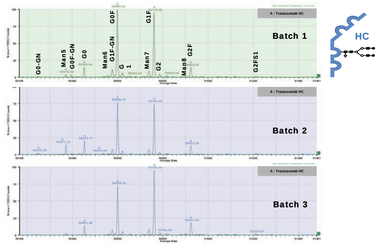


Figure 4b. LC/MS chromatogram of the LC and HC separation. The mass spectrum of the HC and its deconvoluted mass are displayed.

Data processing

BiopharmaLynx result view



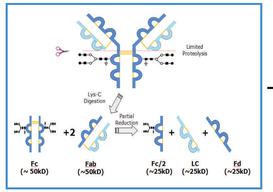


Figure 5a. Fragments generated by limited proteolysis of IgG1 monoclonal antibody, Trastuzumab, with Lys-C protease followed by the partial reduction with DTT.

LC/MS

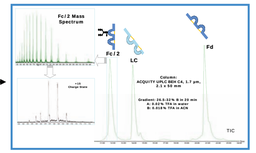
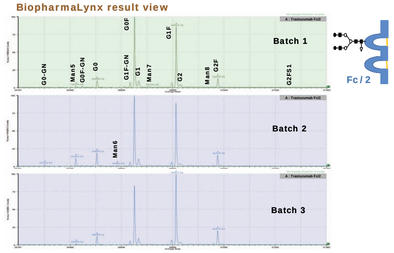


Figure 5b. LC/MS chromatogram of the Fc/2, Fd and LC separation. The mass spectrum of the Fc/2 and its deconvoluted mass are displayed.

Data processing

BiopharmaLynx result view



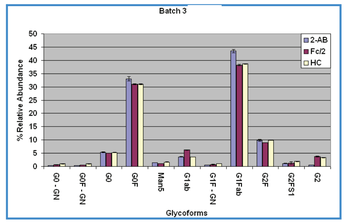


Figure 6. Glycan quantitation comparison of HILIC-LC/FLR vs. directl LC/MS analysis of Fc/2 and HC. Only the common glycans from all three methods were used for the comparison. Fluorescent peak areas from the HILIC-LC/FLR were used to get relative quantitation, while the total ion intensity obtained from BiopharmaLynx data processing were used for the relative quantitation for Fc/2 and HC.

CONCLUSION

- Glycan profiling and relative quantitation can be achieved by released glycan analysis (with Fluorescent dye), or partial cleaved intact protein LC/MS analysis. Results from both methods are agreeable.
- Glycan Characterization Platform, UPLC/FLR/QTof MS, offers a robust, sensitive and versatile analytical solution for batch-to-batch glycan profiling.
- UPLC ACQUITY columns, BEH Glycan and the RP C4 columns offer exceptional separations.
- BiopharmaLynx software enables automated data processing and interpretation for the LC/MS of intact protein and partial cleaved protein. The output data files contains the ion intensity for the identified glycoforms, which can be used for relative glycan quantitation.

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