Reliability of a semi-automated, high throughput (HT) MS based glycomics system for discovery of glycan biomarkers and Quality by Design (QbD) studies of glycoprotein therapeutics

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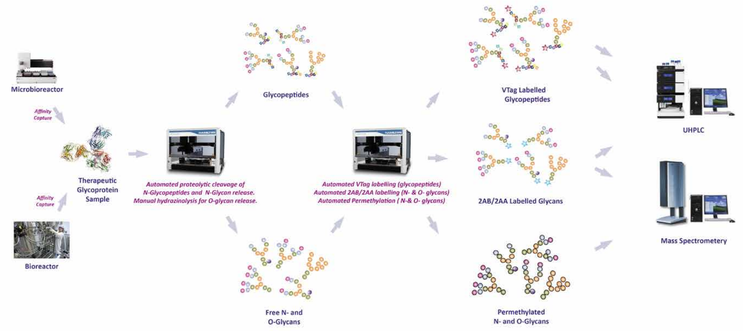
Abstract

For most therapeutic glycoproteins, the glycosylation patterns correlate strongly with the clinical safety and efficacy profiles. In biological tissues these patterns can also correlate with the state of health or disease of the individual. Given this, there is an increasing interest in accurately characterising changes in glycosylation — for example in QbD studies throughout biopharmaceutical development ,as well as in glycan biomarker discovery for medical diagnostics.

To perform these studies, reliable systems for HT glycomics are needed. Despite many advances in glycosylation analysis there are still problems with current technologies, including high cost per sample, low sample throughput and high labour intensity.

This poster presents "LongBow" — a system developed at Ludger for reliable HT glycomics. The "LongBow" system is made up of flexible, modular technologies for semi-automated processing of glycans from a variety of clinical and bio-therapeutic samples as shown in Scheme 1. The emphasis of this poster will be on **Automated and HT Permethylation of N- and O-glycans analysed by MALDI-MS.**



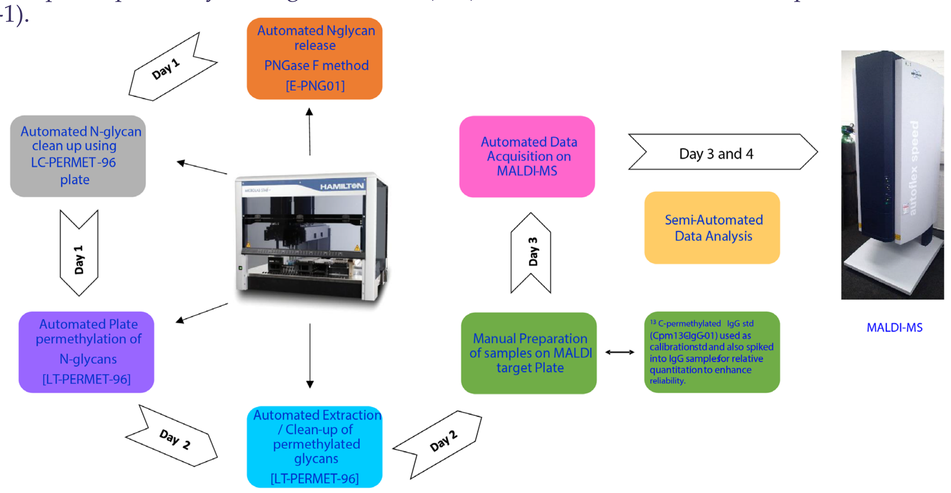
Scheme 1: Semi-automated HT LongBow glycomics workflow for glycoprotein sample preparation for analysis by MS and UHPLC

Introduction

Permethylation is the most popular derivatization of carbohydrates for MS detection, as it enhances ionization efficiency and stabilizes the sialic acids. This modification is the addition of methyl substituent to all of the hydroxyl and *N*-acetyl groups. Permethylation also methyl esterifies the carboxy function on the sialic acid.

The conventional in-solution technique is labor intensive with long turn around times, which prompted us to develop a microplate based permethylation kit which delivers a cost effective, automated and HT method for our QbD and biomarker studies.

However, the reliability of glycan quantitation using this method is challenging and to improve the reliability we :
a) automated sample preparation and clean-up,
b) automated HT permethylation for glycan identification,
c) automated data acquisition on MALDI-MS and perform semi-automated data analysis,
d) use a heavy isotope ^{13}C permethylated IgG standard (Std) for calibration and relative quantitation of IgG samples (Fig-1).



Scheme 2: LongBow system tuned for automated and HT permethylation of glycans and analysis by using MALDI-MS.

Methods

To demonstrate our automated and HT technology, we successfully applied it to various sample subsets and the methods for N- and O-glycan release and derivatization are outlined as follows (see Scheme 2):

Glycan release: N-glycans from Waters IgG standard and human plasma were released using Peptide N Glycosidase F enzyme (PNGase- F, E-PNG01 from Ludger) and cleaned up using a Ludger Clean™ Pre-Permethylation Clean Up Plate (LC-PERMET-96). A manual chemical release method (Hydrazinolysis) was used for release of O-glycans from recombinant human erythropoietin (rhEPO).

Glycan derivatization: The released and purified N-glycans from Waters IgG standard (Fig-2) and human plasma (Fig-3) were permethylated using the prototype LudgerTag™ permethylation microplate kit (LT-PERMET-96). Both the permethylation and post-derivatisation sample clean up steps were performed on the a liquid handling robot and then analysed by flexAnalysis software from Bruker.

The O-glycans released from rhEPO were split into two aliquots to show comparability and orthogonality between two techniques UHPLC and MALDI-MS. Pool 1 samples were reductively aminated with LudgerTag™ 2-AB labelling kit (LT-KAB-VP24), cleaned up using LudgerClean™ T1 cartridges (LC-T1-A6) and analysed by UHPLC (Fig-4a). Pool 2 samples were permethylated using the LT-PERMET-96 kit (Fig-4b).

Results

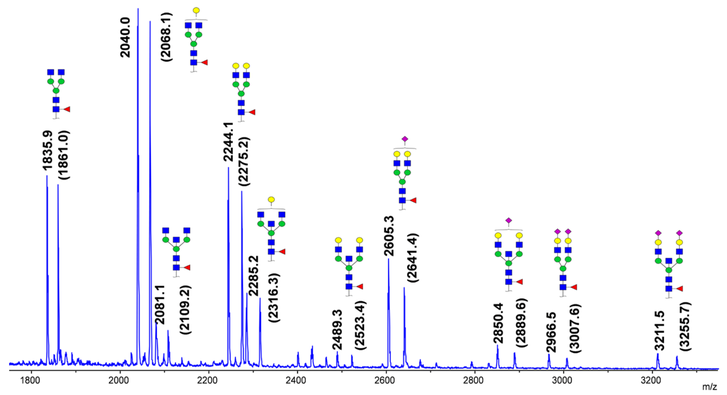


Fig-1: MALDI-TOF-MS spectrum of the ^{12}C and ^{13}C permethylated human-IgG N-glycan standards from Ludger (CPM-^{13}C-IgG-01 and CPM-IgG-01). ^{12}C IgG was spiked with ^{13}C IgG on the same sample spot to show the comparison of relative quantities of the major IgG N-glycans. The mass values shown in the spectra are [M+Na]+ of permethylated glycans, with ^{13}C permethylated masses in parentheses.

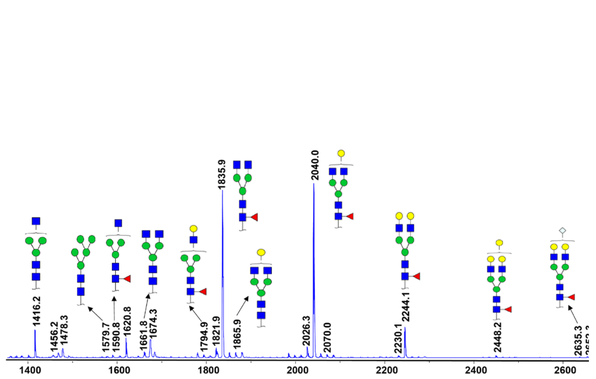


Fig-2: MALDI-TOF-MS spectrum of the permethylated N-glycans from waters IgG standard. *(Typical biopharmaceutical sample used during QbD studies.)*

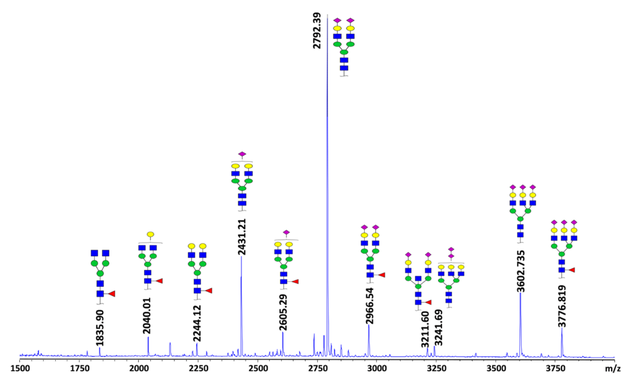


Fig-3: MALDI-TOF-MS spectrum of the permethylated N-glycans from human plasma. *(Typical example of a clinical sample used for biomarker discovery).*

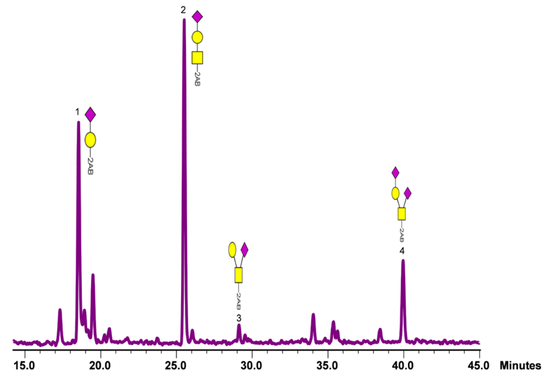


Fig-4a: Typical UHPLC profile of 2-AB labelled biosimilar rhEPO depicting O-glycans. *Note: Measuring 96 samples on UHPLC takes a minimum of 48 hours.*

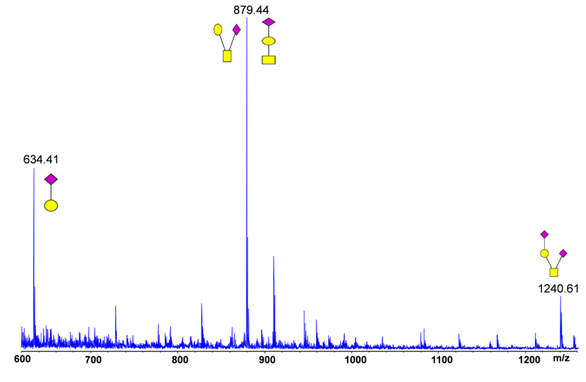


Fig-4b: MALDI-TOF-MS spectrum of the permethylated rhEPO, depicting O-glycans. *Note: Automated data acquisition for 96 samples on MALDI-MS takes under 1 hour.*

Glycan Key Fucose (F) Galactose (H) Mannose (H) N-Acetylglucosamine (N) N-Acetylneuraminic acid (S) N-Glycolylneuraminic acid (G)

The mass values shown in the MALDI-TOF spectra are [M+Na]+ of permethylated glycans.

Conclusions

- We have developed and optimized a new permethylation technique which exploits the use of a liquid handling robot to enable higher throughput and automated glycosylation analysis of N- and O-glycans analysed by using MALDI-MS.
- This HT permethylation technique is comparable to UHPLC results (see Fig 4a and 4b) and it gives a reliable overview of the glycosylation profile in a short timespan.
- The advantages of permethylated glycan analysis using the MALDI-MS are: enhanced signal due to increased ionization efficiency, good sensitivity, and rapid analysis. You can process hundreds of samples per day i.e., on average one sample takes less than a minute to process, so 96 samples can be processed in under 1 hour on MALDI-MS compared to UHPLC which takes more than 48 hours to run 96 samples.
- Therefore, we conclude that our microplate based, automated and HT permethylation technique delivers the most cost effective, fast and reliable method for our QbD and biomarker studies.

References

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