Optimisation of drug glycosylation supported by an automated high throughput permethylation MALDI-MS method

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Introduction

Glycosylation can significantly affect the safety, efficacy and immunogenicity profiles of biologic drugs. Given this, it is important for biopharma companies to optimise Glycosylation Critical Quality Attributes (GCQAs) early on in the product life cycle (particularly during cell line upstream process development). However, current technologies for quantitative measurements of complex and heterogeneous glycan profiles are labor intensive and have poor repeatability. This poster presents a new, high throughput automated method that has very good repeatability.

The gold standard method for N-Glycan analysis involves Ultra High Performance Liquid Chromatography (UHPLC) with fluorescence detection and we compare it with a High Throughput (HTP) and automated N-glycan permethylation (process of converting all the functional groups of free glycans into their methyl derivatives which significantly enhances Mass Spectrometry (MS) analysis) in microplate format for analysis by matrix-assisted laser desorption/ionization (MALDI)-MS. We present an overview of these methods and discuss the advantages and comparability for HTP applications such as clone selection and cell-culture optimisation.

We analysed a Chinese hamster ovary (CHO) cell line (GS-CY01 expressing an IgG4 Monoclonal Antibody (mAb) grown in stirred tank bio-reactors that were cultured at (1) different temperatures (a. mild hypothermic condition at 32°C and b. standard temperature at 37°C) and (2) different aeration conditions (a. direct gas sparging-to give bubbles and b. silicone membrane sparging-with no bubbles).

The effects of varying bioreactor conditions have shown to impact the glycosylation profile (levels of fucosylation, galactosylation and sialylation etc) and here we are focusing only on the alterations of galactosylation levels by measuring the dominant glycan species G0F, G1F and G2F in the IgG4 mAb. We further highlighted our advanced Ludger prototype technology, a 96 well microplate based permethylation kit for N-glycan analysis by MALDI-MS. Permethylation is the process of converting all the functional groups of free glycans into their methyl derivatives and significantly enhances mass spectrometry analysis. The manual permethylation method has numerous extraction steps which are labor intensive which prompted us to develop a microplate based permethylation method which is fast, reliable and comparable to the golden standard UHPLC method.

Methods

Samples of mAbs from bioreactor supernatants were first purified using Protein A then the N-glycans released by automated processing on a Hamilton MICROLAB® STAR Liquid Handling Workstation with PNGase F (Peptide N Glycosidase F (Ludger # E-PNG01) and the LudgerClean™ Post-Exoglycosidase Clean-up Plate (Ludger # LC-PBM-96). The released N-glycans from were aliquoted equally to assure comparability of each sample pool and then they were split into two for further analysis by UHPLC and MALDI-MS as follows:

1. **UHPLC:** Automated reductive amination with LudgerTag™ 2-AB labelling kit (LT-KAB-VP24) and automated clean up for removing contaminants such as excess dye and buffer salts we used LudgerClean T1 cartridges (LC-T1-A6). To obtain high resolution profile these samples were run on a standard Ludger glycoprofiling UHPLC HILIC system.
2. **MALDI-MS:** Automated permethylation of samples was performed in a microplate format using the prototype LudgerTag permethylation kit (LT-PERMET-96) (see Figure 1). The purified permethylated glycans were analysed on a Bruker UltrafleXtreme™ mass spectrometer with control and analysis using the FlexControl 3.4 software.

Fig 1: HT Glycan Permethylation Microplate for mAb glycan analysis by MS
This photo shows the LC-PBM-96 permethylation plate being loaded into the Hamilton robot. Automated permethylation and post-derivatisation sample cleanup are both performed on the plate.

Results

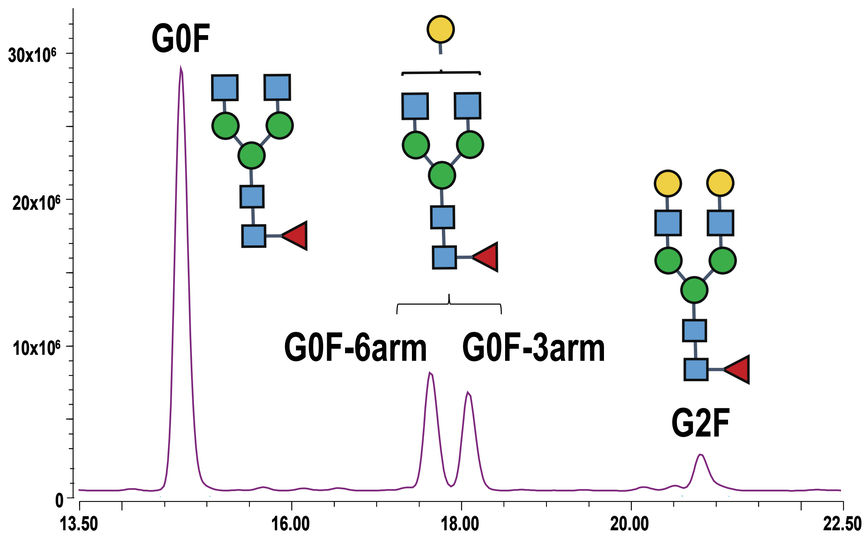

Fig 2: Typical mAb glycan UHPLC profile

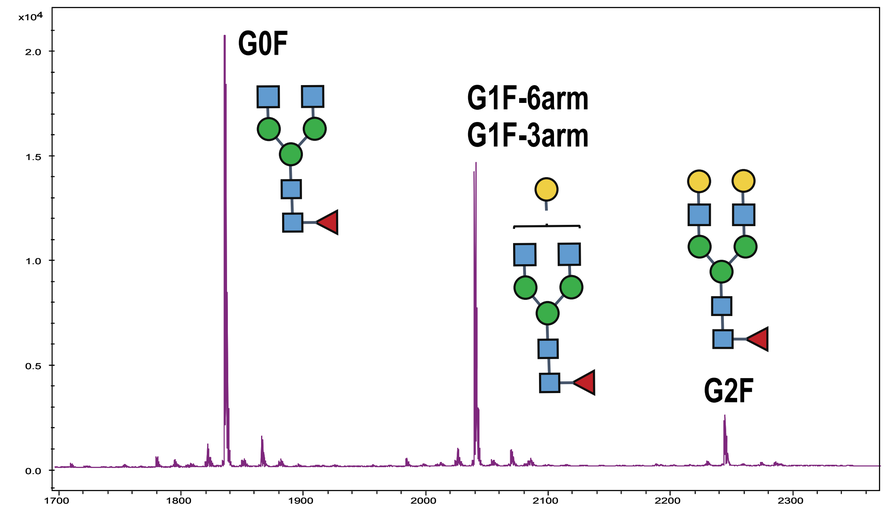

Fig 3: Typical MALDI-MS profile of LT-PERMET-96 permethylated mAb glycans
Note: The two G1F structures are isobaric and are not distinguished in the simple MALDI-MS profile.

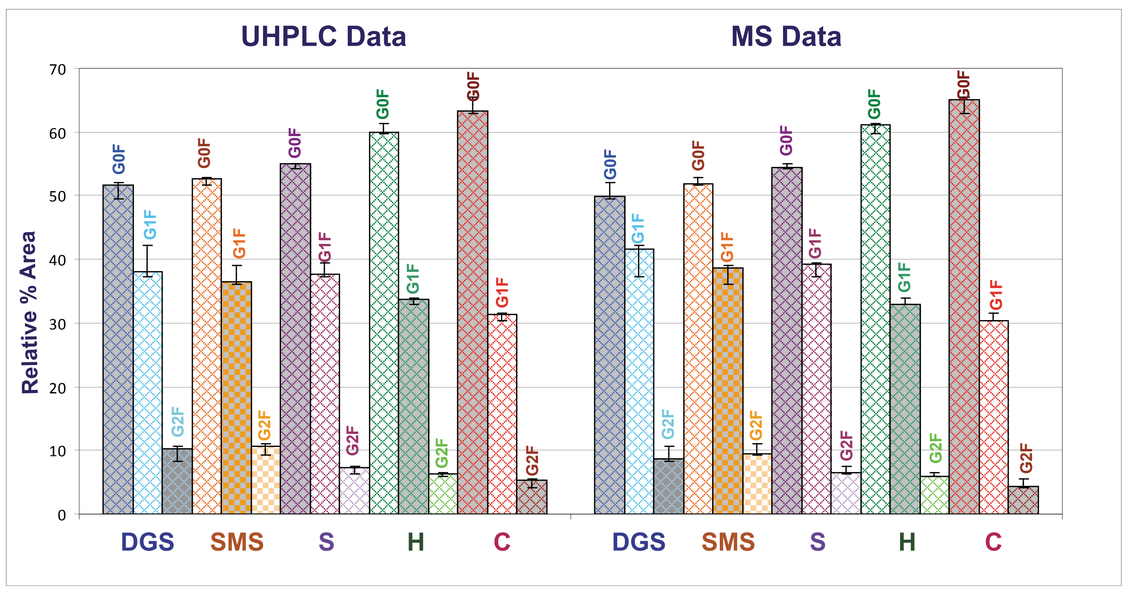

Fig 4: Relative quantitation of glycan species from different bioreactor conditions
Key to bioreactor conditions: DGS- Direct Gas Sparging; SMS-Silicone Membrane Sparging; S-Standard Culture Condition; H-Hypothermic Culture Condition-32°C; C-Control Temperature Condition-37°C.
The relative abundance of G0F increased from 52 %(direct gas sparging culture condition samples) to 63% (Standard temperature-37°C samples) as analysed by UHPLC and similarly the G0F increased from 50% (direct gas sparging culture condition samples) to 65% (Standard temperature-37°C samples) as analysed by permethylated MALDI-MS method.
The data indicates that the relative quantitation data from the two methods (traditional UHPLC and new MALDI-MS) are highly comparable.

The times for preparation and analysis of samples using the permethylation/MS system were significantly shorter than those for the UHPLC method. Typical times for processing 96 released mAb N-glycan samples were 230 min for sample prep and 60 min for analysis (total 290 min = 4.8 hr) by the MS method compared to 250 min for sample prep and 2880 min (2 days) (total 3130 min = 52 hr) for analysis by the UHPLC method.

Conclusions

UHPLC and MALDI-MS analysis have shown to be complimentary platforms for analysis of N-glycans in our study and are truly orthogonal as they are sensitive, reliable and robust methods. We combined sample preparation with the manual Protein A purification, automated enzymatic N-Glycan release with PNGase F and automated clean up which enabled equal sample distribution for comparability between UHPLC Versus MALDI-MS techniques.

- The advantages of fluorescent tagging-UHPLC method for glycan analysis are good sensitivity, accurate sample quantitation, reproducibility and can support an in-depth characterisation of glycan species supported by exoglycosidase sequencing.
- Although a drawback of UHPLC is that sample processing and data analysis is time consuming and therefore supports low throughput samples /day.(I.e. : on an average one sample can take 30 minutes to process so in a day 48 samples can be processed)
- The advantages of automated permethylation MALDI-MS method for glycan analysis are excellent signal enhancement due increased ionization efficiency, sensitivity for measurement of small sample amounts and repeatability.
- Automated micro-plate permethylation is a rapid technique for processing hundreds of samples / day and data acquisition through MALDI-MS is very rapid and hence supports high throughput analysis.(I.e. : on an average one sample can take 1 minute to process, so 96 samples can be processed in 96 minutes)

We conclude that permethylated MALDI-MS is a rapid method for glycan analysis during development and optimization of bio-therapeutics as it gives an accurate and reliable overview of the glycosylation profile in a short span of time.

References

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