WP 023

Automated Glycan Assignment Using Mass Measurement with a Calibrated Retention Time in Glucose Unit

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INTRODUCTION

- This study will focus on determine glycan profile and variation of the innovator, and establishing the degree of comparability with batches of the candidate biosimilar.
- The analytics used for this study was the Waters Biopharmaceutical Platform Solution with UNIFI, comprising an ACQUITY UPLC H-class Bio with fluorescence and Xevo G2-S QTof MS detections.
- The major workflow utilized for this analysis employed collection of dextran ladder calibrated UPLC HILIC retention (Glucose Units or GU) for 2-AB labeled N-glycans. Assignments were produced using an experimentally derived Glycan GU library search, based on GU value and accurate mass search criteria. [1, 2]
- Our results clearly indicate glycan profile differences, likely arising from the differing cell lines (murine vs. CHO cell) used for innovator and biosimilar production.

The Biopharmaceutical Platform Solution with UNIFI supports integrated LC and MS workflows in a single platform that automates acquisition, data processing, bioinformatics, visualization, and reporting within non-regulated and regulated laboratories.

METHODS

Analytical System:

UNIFI Biopharmaceutical Platform solution (1.7 Beta)
ACQUITY UPLC H-class Bio
ACQUITY UPLC BEH Glycan Column: 2.1 x 150 mm
ACQUITY UPLC FLR detector
Xevo G2-S QTof MS

HILIC-LC Method:

Mobile Phases:
A: 50 mM Ammonium Formate (pH 4.4)
B: 100% Acetonitrile

Column Temperature: 40 °C

Gradient Table

Time	Flow Rate	Composition A	Composition B	Composition C	Composition D	Curve
0.00	0.400	30.0	70.0	0.0	0.0	Initial
2.06	0.400	30.0	70.0	0.0	0.0	6
34.80	0.400	47.0	53.0	0.0	0.0	6
36.00	0.250	80.0	20.0	0.0	0.0	6
39.00	0.250	80.0	20.0	0.0	0.0	6
40.00	0.400	30.0	70.0	0.0	0.0	6
45.00	0.400	30.0	70.0	0.0	0.0	6

FLR settings:
λex = 330 nm, λem = 420 nm
10 point/second
Gain = 10

Xevo G2-S QTof:
ESI + mode

Capillary voltage:	2.75 KV
Sample cone:	80 v
Source temperature:	100 °C
Desolvation temperature:	300 °C

Sample Preparation:

3 batches of Innovator Infliximab and 3 batches of a biosimilar candidate were analyzed.

Waters GlycoWorks™ Glycan Sample Preparation Kit:
- Overnight deglycosylation using PNGase F
- Released N-glycans were purified using a HILIC 96-well micro-elution plate operated via a vacuum manifold
- Purified free glycans were lyophilized
- Glycans were labeled with 2-AB (Sigma)
- Excess 2-AB tag was removed using the same HILIC micro-elution plate

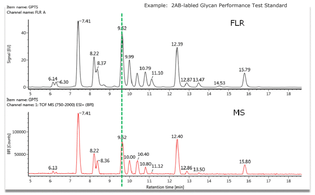
Analytical Standards used:

- 2AB-labeled Dextran ladder (Waters)
- Glycan Performance Test Standard (human IgG glycans spiked with M5 and M6) (Waters)

Glycan Assay (FLR with MS Confirmation) Workflow

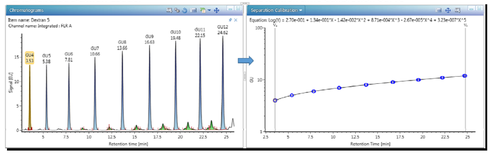
Data Acquisition

Both FLR and MS data channels are time aligned automatically.



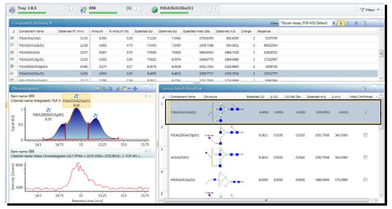
Retention Time Calibration using Dextran Ladder

Retention Time Calibration Using 2AB-Dextran Ladder: calibration curve is automatically applied to analyte of interest to generate GU values

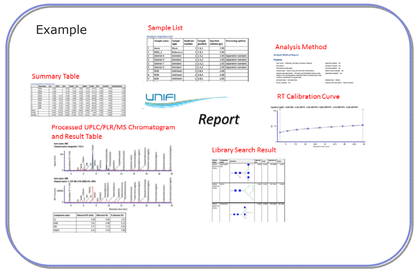


Bioinformatics
Waters Glycan GU Library Search

GU values are searched in the Waters Glycan GU library and the assignment is based on the best matched retention time in GU value and accurate m/z

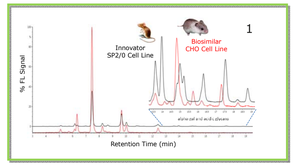


Reporting

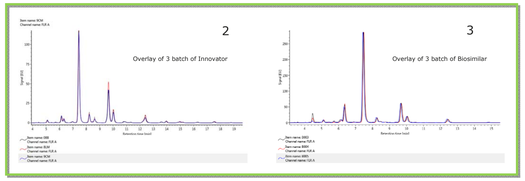


RESULTS

- **N-Glycan profiling for the innovator Infliximab and a biosimilar candidate**

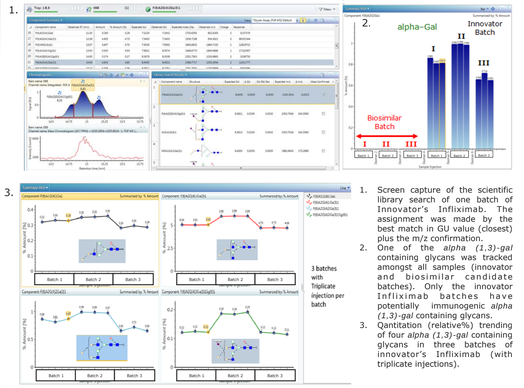


1. Overlay of FLR chromatograms of the innovator and a biosimilar candidate Infliximab. The N-Glycan profiles show significant differences due to the cell line selections.



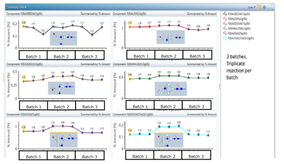
2. Overlay of the FLR chromatograms from three batches of Innovators.
3. Overlay of the FLR chromatograms from three batches of candidate Biosimilar's. Batch to batch differences are observed to be relative% change for some glycans.

- ***Alpha (1,3)-Gal containing glycans in the innovator***

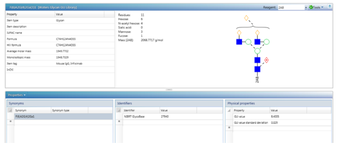


1. Screen capture of the scientific library search of one batch of Innovator's Infliximab. The assignment was made by the best match in GU value (closest) plus the m/z confirmation.
2. One of the *alpha (1,3)-gal* containing glycans was tracked amongst all samples (innovator and biosimilar candidate batches). Only the innovator Infliximab batches have potentially immunogenic *alpha (1,3)-gal* containing glycans.
3. Qantitation (relative%) trending of four *alpha (1,3)-gal* containing glycans in three batches of innovator's Infliximab (with triplicate injections).

- ***NeuGc containing glycans in the innovator***



Line chart trending plots show the amount% of the NeuGc containing glycans from the innovator's batch.
Note: Both alpha-gal and NeuGc were not observed in the Biosimilar candidate batches.



Example of a Glycan database entry: there are a total of 308 common N-linked glycans in the scientific library. Each glycan entry has been characterized structurally using exoglycosidase array and mass confirmed using MALDI MS or ESI MS. "Unknown" glycan assignment is based on best matched experimental retention time that was converted to Glucose Unit (GU) value, and accurate mass measurement. Assignment can be changed to an alternative assignment based on user's interpretation of the data.

CONCLUSION

Highlights for Glycan Analysis in UNIFI System

- FLR and MS data channels are acquired and time aligned automatically.
- Waters Glycan GU Library allows confirmatory analysis of the glycans based on the retention time (in Glucose Unit) and accurate mass measurement.
- Streamlined workflow for quantitative (relative%) and qualitative analysis of N-glycan profiling.
- Using the Glycan application in UNIFI, we demonstrated the N-glycan profile differences between the innovator/biosimilar Infliximab; and the batch to batch variations.
- We envision the UNIFI glycan workflow to be an enabling tool for glycan profiling and comparability assessment for biosimilar drug development.

References

1. Waters Application note (720004202en): http://www.waters.com/waterslibrary/
2. Waters Application note (720004203en): http://www.waters.com/waterslibrary/

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