COMPLETE AUTOMATED WORKFLOW FOR COMPARING THE N-GLYCAN PROFILE OF INNOVATOR AND BIO-SIMILAR INFLIXIMAB SAMPLES

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OVERVIEW

- **Improved N-glycan preparation on the Andrew Alliance pipetting robot with automated vacuum controls**
- **HILIC separation and ToF mass detection for efficient bio-therapeutic characterization using a LC/MS system specifically designed for bio-molecules (Figure 1)**

Figure 1: The BioAccord system consisting of an ACQUITY UPLC I-Class Plus paired with an ACQUITY RDa Time-of-flight mass detector. The LC/MS system is controlled by the UNIFI Scientific Information System. Laid out in front of the system are the materials required to run the GlycoWorks protocol.

- **Automated data processing and reporting of results**
- **Successfully determined similarities and differences between innovator and biosimilar infliximab samples**

METHODS

GlycoWorks *Rapi*Fluor-MS Automated Protocol:

This method, as well as validation, has been previously described in a publication in SLAS Technology. For more information

POSTER **Publication**

LC Conditions:

- Column = ACQUITY Glycan BEH Amide, 1.7 µm, 130 Å, 2.1 X 150 mm
- Sample Vial = 12x32 mm Total Recovery glass vial
- A = 50 mM Ammonium Formate in water, pH 4.4
- B = Acetonitrile

Gradient Table

Time (min)	Flow Rate (mL/min	%A	%B
Initial	0.4	25	75
35	0.4	46	54
36.5	0.2	80	0
39.5	0.2	80	0
43.1	0.2	25	75
47.6	0.4	25	75
55	0.4	25	75

ACQUITY RDa Detector Settings:

- Mass Range = 0-2,000 m/z
- Mode = ESI Positive
- Collection mode = continuum
- Sample rate = 2 points/sec
- Cone voltage = 45V full scan, 80-100 V fragmentation
- Desolvation temperature = 300°C
- Capillary voltage = 1.5 kV
- Lock mass = leu-enkephalin @ 50 fmol/µL in 50/50 water/acetonitrile w/ 0.1% formic Acid
- Informatics = Glycan Application Solution within UNIFI

ACQUITY FLR Detector Settings:

- Excitation λ = 265 nm, Emission λ = 425 nm

RESULTS

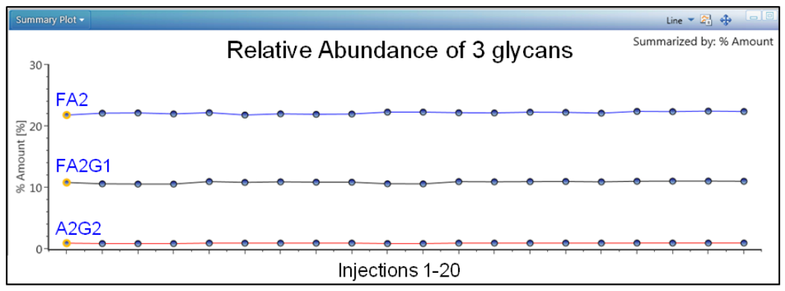

Figure 2: Reproducibility of the LC system demonstrated by monitoring 3 glycoforms of varying relative abundances over 20 consecutive injections. Sample was Waters' RFMS glycan standard. Relative abundance was calculated based on the ratio of area for selected peaks vs the total calculated peak area for all glycoforms.

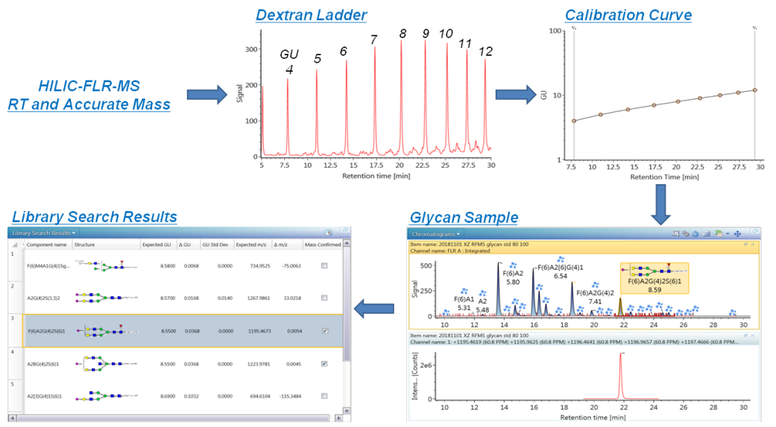

Figure 3: The "Glycan FLR with MS confirmation" workflow within UNIFI. Retention times of glycans are calibrated against a dextran ladder standard, converted to Glucose Unit (GU) values, paired with accurate mass information to conduct a Glycan Scientific Library search within UNIFI for peak identification.

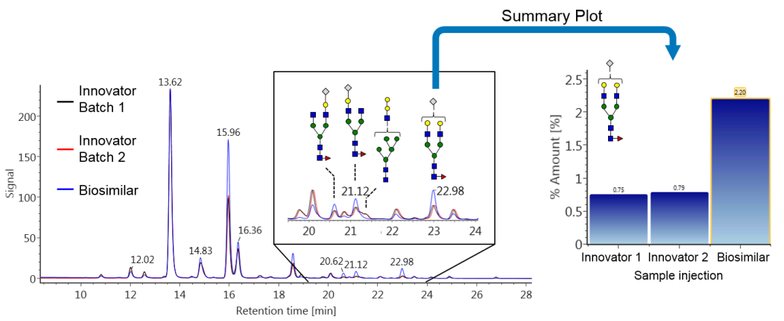

Figure 4: Direct comparison made by overlaying innovator and biosimilar chromatograms. Differences in relative abundance are confirmed by direct comparison in a Summary Plot, which quantitatively compares relative FLR responses.

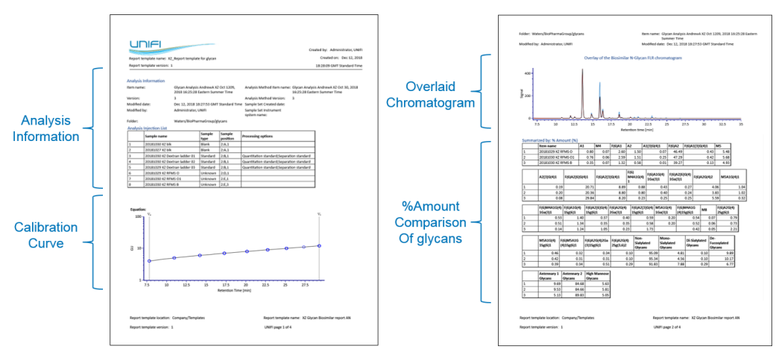

Figure 5: Automatically generated UNIFI report for simplified review, sharing, and filing of acquired data. The customizable template was formatted to include analysis information, a calibration curve, overlaid chromatograms, and a results table.

DISCUSSION

Benefits of automated vacuum control:

- Reduced user interactions during preparation
- Reduced protocol run time by adding pipette measurement and µElution plate well preparation steps during incubations
- Reduced risk of drying out µElution plate wells by allowing the wells to be loaded while the vacuum is off, turned on for a set amount of time that allows for complete flow through, and then turned off for the loading of the next liquid step.

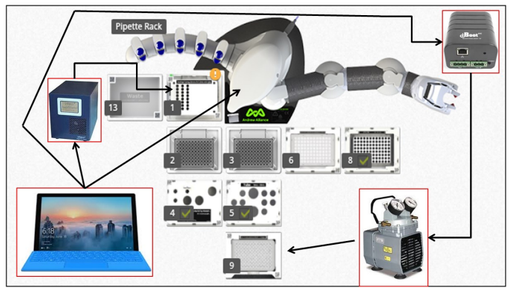

Figure 6: Diagram showing the deck layout of the Andrew system along with the automated control pathways. Features of the control system are outlined in red and include: A) the Surface Pro laptop from Microsoft which runs the Andrew Lab control software to orchestrate B) the INHECO TEC controller for the heating/cooling Domino and C) the iBoot G2+ Advanced Network Power Switch which can switch on/off the vacuum unit which controls suction to the µElution plate Domino.

Infliximab Innovator and Biosimilar Comparison

- Using the Glycan workflow within UNIFI, 25 glycoforms were automatically identified with a mass tolerance of <10 ppm.
- Two innovator samples of infliximab were shown to have a high degree of similarity in their N-glycan profiles while a biosimilar sample was shown to have a different distribution of sialylated glycoforms. (Figure 4)
- Simultaneous acquisition of MS fragmentation data allows improved confidence in structural assignment of closely eluting isobaric glycans with highly similar GU values.

CONCLUSION

- **The GlycoWorks RFMS protocol on the Andrew Alliance robotics platform has been updated with automated vacuum controls and offers a more user-friendly experience**
- **The recently launched BioAccord system, specifically designed for LC-MS analysis of biomolecules, offers robust and reproducible detection of complex N-glycan profiles through the Glycan FLR with MS Confirmation workflow in UNIFI**
- **All-together, the automated sample preparation provided by Andrew along with the automated data processing and reporting afforded by the UNIFI Scientific Information System encapsulates a complete automated workflow for the routine monitoring of N-glycan profiles of biomolecules.**

References

1. Zhang, X.; Reed, C. E.; Shion, H.; et al. Released N-linked Glycan Analysis Using the BioAccord System. Waters Corporation: Waters Corporation, 2019.
2. Reed, C. E.; Fournier, J.; Vamvoukas, N.; et al. Automated Preparation of MS-Sensitive Fluorescently Labeled N-Glycans with a Commercial Pipetting Robot. *SLAS TECHNOLOGY: Translating Life Sciences Innovation* **2018**, 2472630318762384.

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