

## Released N-linked Glycan Analysis Using the BioAccord System

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### Abstract

This technology brief demonstrates the BioAccord System's capability to simplify the analytical workflow to acquire and transform high quality LC/Optical/MS data into meaningful results for glycan analysis.

### Benefits

- The BioAccord System with SmartMS brings comprehensive N-linked glycan analysis to a new level
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### Introduction

As it is well understood the impact glycosylation has on the safety and efficacy of protein drugs, it is critical to characterize and monitor glycoprofiles of biotherapeutics throughout the product lifecycle to ensure the consistency of drug quality. Glycoprofiles are commonly assessed through separation of released N-linked glycans that are labeled with a fluorescence tag. However, conventional LC-fluorescence (FLR) workflows often lack specificity and require complementary techniques for in-depth analysis. Mass Spectrometry (MS) provides

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key structural information and has been extensively used as an orthogonal analytical technique for glycan characterization in biopharmaceuticals development.



*Figure 1. Glycan Application solution using automated GlycoWorks RapiFluor-MS N-Glycan Kit and BioAccord System with UNIFI Software.*

However, analyzing complex glycan samples using high resolution MS generally requires expert users for instrument operation, method optimization, and data interpretation. Therefore, workflows with orthogonal detection capability that can increase confidence and productivity in the lab, and be easily deployed, are highly desirable in the biopharmaceutical industry.

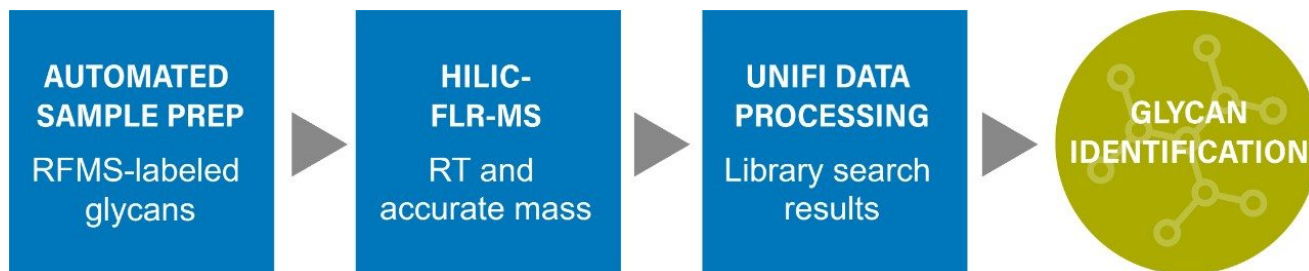


Figure 2. Glycan analysis workflow using the BioAccord System with UNIFI Scientific Information System.

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## Results and Discussion

The BioAccord System is specifically designed to improve the user experience and accessibility in process development and manufacturing environments when deploying LC-MS-based workflows. It consists of an ACQUITY UPLC I-Class PLUS System configured with an optical detector (TUV or FLR) and paired with an ACQUITY RDa Detector which provides robust separations and accurate mass information for efficient characterization and monitoring of biotherapeutics. The LC-MS system is controlled by the compliance-ready UNIFI Scientific Information System software platform, offering streamlined data acquisition, processing, and reporting in an automated fashion (Figure 1).

As one of the purposefully designed workflows supported by the BioAccord System, released glycan analysis was demonstrated using ORENCIA (abatacept), a fusion protein that contains a complex mixture of glycans with a high level of sialylation. As described in Figure 2, N-glycans were released and labeled with the GlycoWorks *Rapi*Fluor-MS (RFMS) N-Glycan Kit reagent via an automated sample preparation platform (Andrew Alliance), and then separated using a 150 mm ACQUITY BEH Glycan Amide Column (p/n: 186004742) followed by fluorescence (FLR) and MS detection in-tandem using the BioAccord System. Within the “Glycan FLR with MS confirmation” analytical workflow in UNIFI, a Glycan GU library search was undertaken in an automated fashion for peak identification based on GU (Glucose Unit) values converted from calibrated retention times (cubic spline curve fitting) and confirmed by the accurate mass information. Figure 3A shows the processed FLR trace with each peak automatically assigned and annotated with its glycan name, detected GU value, and associated structure information. With the ACQUITY RDa Detector, high quality MS full scan and extracted ion chromatogram (XIC) data were obtained for critical glycan species such as Mannose 5 (Figure 3B) at a relative

abundance of 0.2%. The high sensitivity afforded by the ACQUITY RDa Detector resulted in 31 N-linked glycans being identified with less than 10 ppm mass tolerance. Supporting analysis information including GU values and mass are readily accessible via the Component Summary Table (Figure 3C). Collectively, these results demonstrate that the BioAccord System offers an efficient solution to improve specificity and confidence of released glycan analyses in biotherapeutics development.

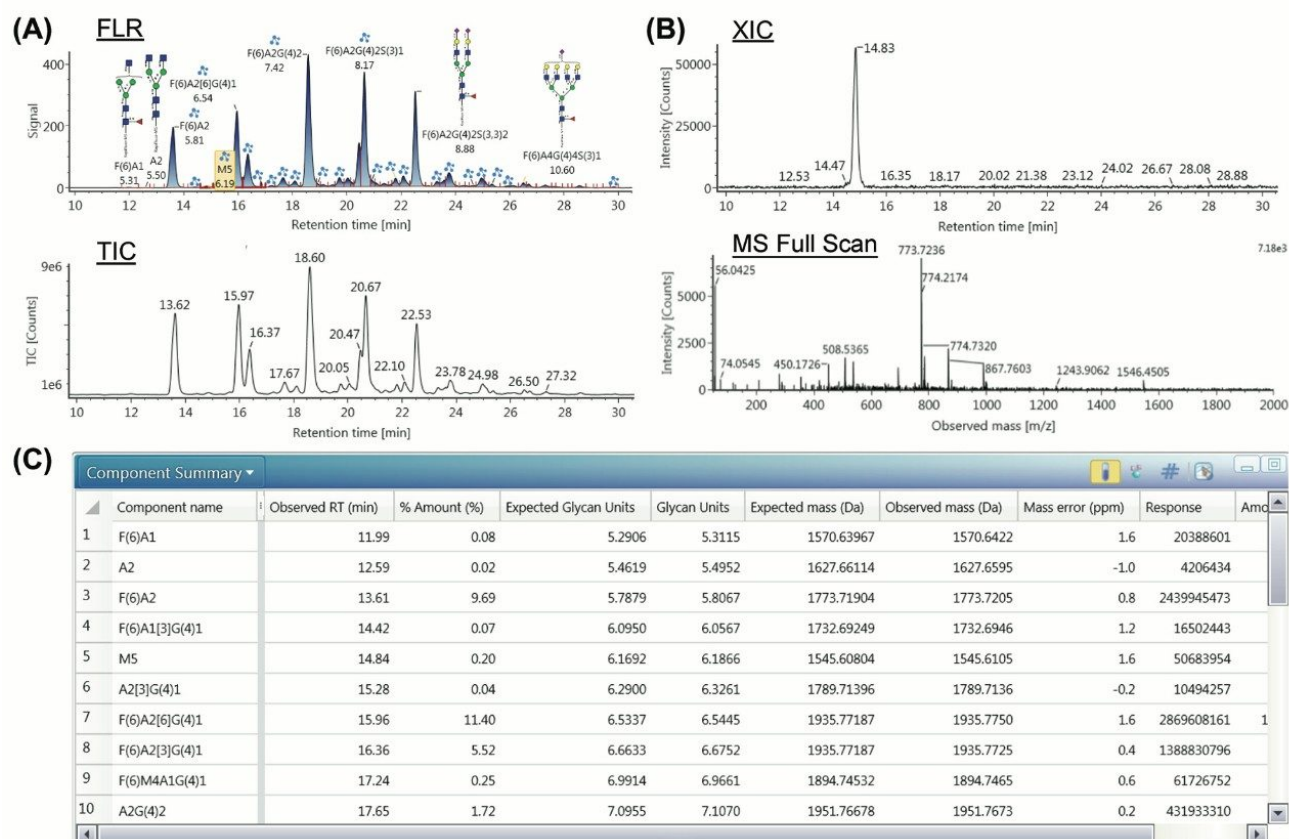


Figure 3. Analysis of N-linked glycans released from a fusion protein, abatacept. Mass load: 4 pmol. (A) Processed results and Total Ion Chromatogram (TIC) of released N-glycan profiling. (B) Extracted ion chromatogram (XIC) and MS full scan spectra of Mannose 5. (C) Component Summary table showing identified glycans within 10 ppm range of mass error.

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## Conclusion

The BioAccord System is purposefully designed to improve the productivity through simple and automated setup for the late stage development and regulated biopharmaceutical labs. Using the released glycan analysis workflow, highly efficient separation and accurate mass information were obtained for quantitative glycan analysis and structure assignment. The workflow was seamlessly integrated by the compliance-ready software UNIFI and can be used to improve the confidence of glycan analyses without compromising productivity in regulated environments.

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