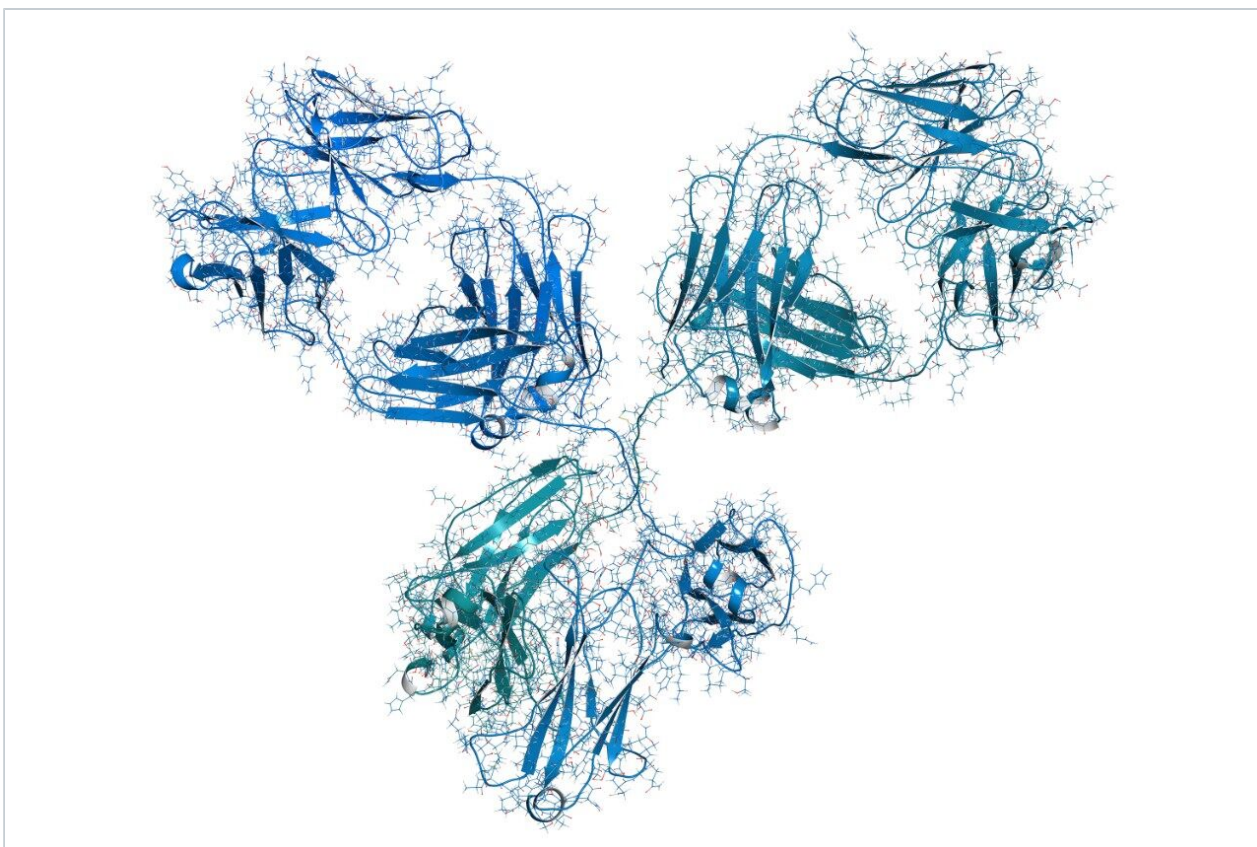


Application Note

Increasing Productivity and Confidence for N-linked Glycan Analysis of Biosimilars Using the BioAccord System

Ximo Zhang, Corey Reed, Henry Shion, William R. Alley, Robert E. Birdsall, Ying Qing Yu

Waters Corporation



Abstract

The objective of this work is to demonstrate that the BioAccord System can increase confidence and productivity in released glycan analysis for the development of biosimilars.

The SmartMS-enabled BioAccord System is an easy-to-use LC-MS platform solution that was purposefully designed for comprehensive analysis of biotherapeutic drug products with built-in analytical workflows for specific analysis such as N-Glycan identification and profiling. Within the BioAccord System, highly robust chromatographic separation and accurate mass information can be obtained using the ACQUITY UPLC I-Class PLUS System and the ACQUITY RDa Detector controlled by UNIFI Scientific Information Software. The ACQUITY RDa Detector is a compact bench top time-of-flight mass detector with built-in self-calibration function and straightforward instrument method setup (Figure 1B and 1C), which substantially reduces the complexity of operating MS instruments. Together, high quality LC-FLR and LC-MS data can be acquired and transformed into meaningful results for released glycan analysis using an all-encompassing workflow, reducing the cost and time for biosimilar development without compromising product quality.

Benefits

- A compliance ready all-encompassing LC-FLR-MS solution for comprehensive N-linked glycan analysis
- Automated workflow from sample preparation to data reporting for improved productivity in biosimilar development
- Robust and specific platform solution that improves the confidence of released glycan analysis

Introduction

Monoclonal antibody (mAb) based therapeutics have been well established in the effective treatment of various diseases due to their high efficacy and specificity. With the up coming patent expiration of several commercially available mAb-based drugs in the next few years, growing efforts are being devoted to developing biosimilars as less expensive alternatives of innovator mAbs. To reduce the need for and size of expensive clinical trials and need to expedite the drug commercialization process, manufacturers must demonstrate high similarity in analytical properties of the biosimilar and its reference product through comprehensive analyses.¹ Due to the impact on drug efficacy and safety, glycosylation is one of the critical

quality attributes of mAb based therapeutics that needs to be well characterized for similarity assessment and quality assurance of biosimilars.² With the high complexity of glycosylation profile of mAbs, orthogonal technologies such as mass spectrometry (MS) are commonly used to complement conventional LC-fluorescence (FLR) based methods for increased specificity. However, high resolution MS often requires experienced scientists for instrument operation, data processing and interpretation, which can be time and resource consuming. To this end, scalable technologies and methods that can add confidence and are easy to deploy for comprehensive glycan analysis in biosimilar development are highly desirable.

The SmartMS-enabled BioAccord System is an easy-to-use LC-MS platform solution that was purposefully designed for comprehensive analysis of biotherapeutic drug products with built-in analytical workflows for specific analysis such as N-Glycan identification and profiling. Within the BioAccord System, highly robust chromatographic separation and accurate mass information can be obtained using the ACQUITY UPLC I-Class PLUS System and the ACQUITY RDa Detector controlled by UNIFI Scientific Information Software (Figure 1A). The ACQUITY RDa Detector is a compact bench top time-of-flight mass detector with built-in self-calibration function and straightforward instrument method setup (Figure 1B and 1C), which substantially reduces the complexity of operating MS instruments. Together, high quality LC-FLR and LC-MS data can be acquired and transformed into meaningful results for released glycan analysis using an all-encompassing workflow, reducing the cost and time for biosimilar development without compromising product quality.

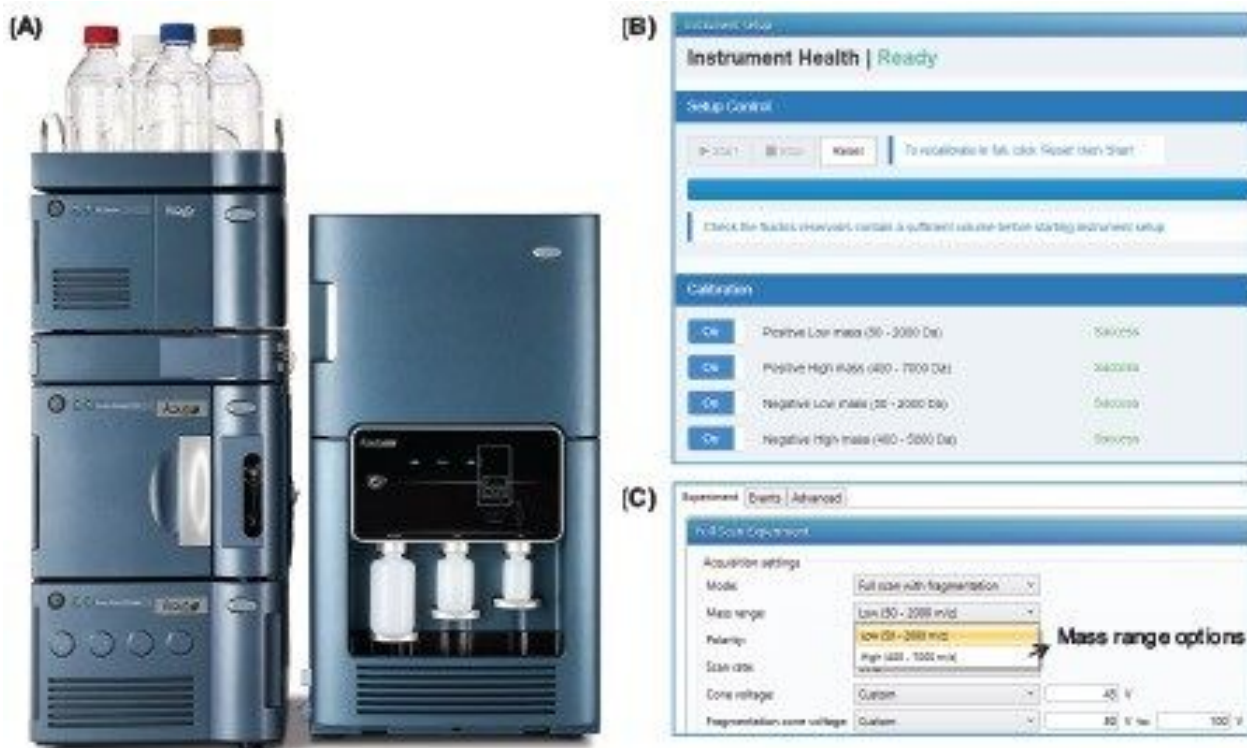


Figure 1. The BioAccord System. (A) Integrated instrument configuration for streamlined released glycan analysis. (B) ACQUITY RDa Detector setup page showing SmartMS™-enabled auto-calibration function for efficient instrument operation. (C) Instrument method page for MS data acquisition. For released glycan analysis, the low mass range option (50–2000 m/z) was selected.

The objective of this work is to demonstrate that the BioAccord System can increase confidence and productivity in released glycan analysis for the development of biosimilars. As an example of assessing the similarity of glycosylation, released glycans from innovator and biosimilar infliximab were analyzed using the BioAccord System.

Experimental

Chemical and reagents

Innovator and biosimilar infliximab samples were donated by external collaborators. LC-MS grade water and acetonitrile were purchased from Honeywell and used as received. Concentrated ammonium formate (p/n

186007081) was used as the additive to prepare mobile phase.

Sample preparation

Two infliximab samples from innovator (Remicade) and one sample from Biosimilar (Inflectra) were diluted with water to a final concentration of 1.5 µg/µL. N-glycans from infliximab were released from 15 µg of diluted mAb samples and labeled using the GlycoWorks *Rapi*Fluor-MS N-Glycan Kit (p/n 176004082)³ via an Andrew Alliance automated sample preparation platform.⁴ An amount of 2.5 pmol released glycan sample was injected for each analysis.

LC Conditions

| | |
|-----------------|---|
| LC system: | ACQUITY UPLC I-Class PLUS |
| Detectors: | ACQUITY FLR Detector, λ_{ex} =265 nm, λ_{em} =425 nm, ACQUITY RDa MS Detector |
| LC column: | ACQUITY Glycan BEH Amide, 1.7 µm, 130 Å, 2.1 × 150 mm, (p/n 186004742) |
| Column temp.: | 60 °C |
| Sample vial: | 12 × 32 mm glass vial Total Recovery, (p/n 600000750cv) |
| Mobile phase A: | Water with 50 mM Ammonium formate, pH = 4.4 |
| Mobile phase B: | Acetonitrile |

Gradient table:

| Time (min) | Flow rate (mL/min) | %A | %B |
|------------|--------------------|----|----|
| Initial | 0.4 | 25 | 75 |
| 35 | 0.4 | 46 | 54 |

| Time (min) | Flow rate (mL/min) | %A | %B |
|------------|--------------------|----|----|
| 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

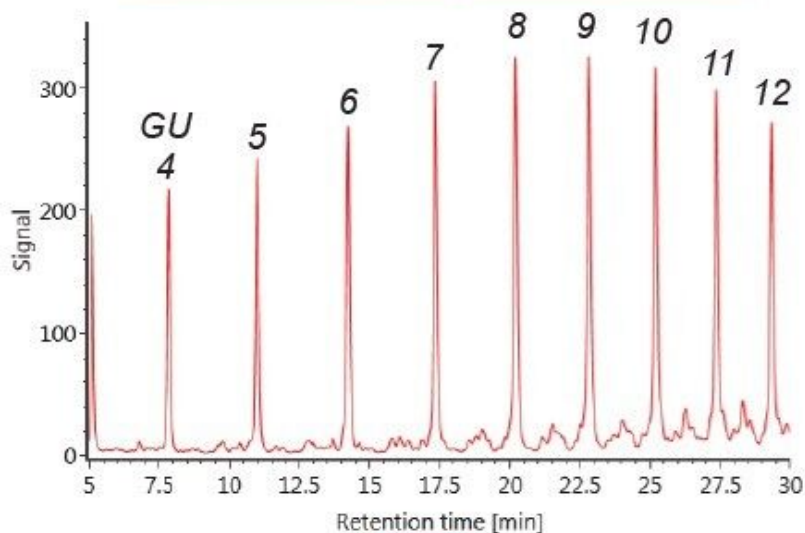
| | |
|--------------------|--|
| Mass range: | 50–2000 <i>m/z</i> |
| Mode: | ESI+ |
| Collection mode: | Continuum |
| Sample rate: | 2 Hz |
| Cone voltage: | 45 V for full scan 80–100 V for fragmentation |
| Desolvation temp.: | 300 °C |
| Capillary voltage: | 1.5 kV |
| Lock mass: | Leu-enkephalin at 50 fmol/μL in 50/50 water/acetonitrile with 0.1% formic acid |
| Informatics: | Glycan Application Solution within UNIFI 1.9.4 |

Results and Discussion

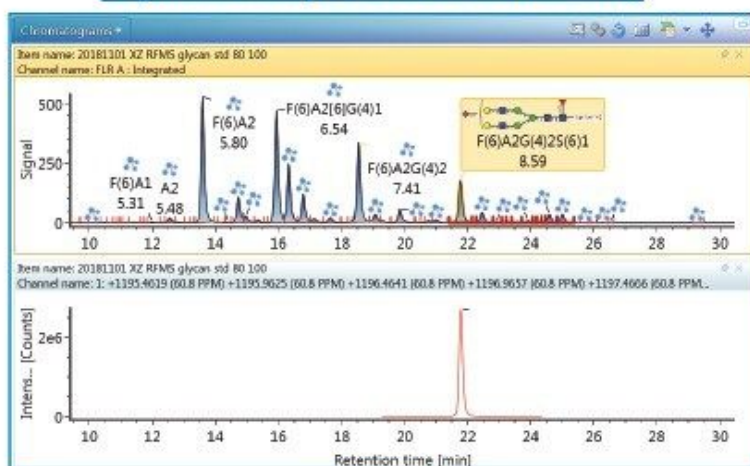
As an integrated solution, the established glycan analysis workflow supported by the BioAccord System can be used to streamline the identification and comparison of glycosylation on biosimilars.⁵ To demonstrate this workflow, *Rapi*Fluor-MS labeled N-linked glycans released from innovator and biosimilar infliximab were analyzed via HILIC separation followed by inline FLR and MS detection using the BioAccord System. With the self-calibration and self-tuning function of the RDa mass detector, high quality MS data were obtained in an efficient manner. The "Glycan FLR with MS confirmation" workflow within UNIFI was used for data interpretation, allowing automated data processing using the retention time and accurate mass information of separated glycans.⁶ As shown in Figure 2, retention times of glycans were calibrated against a dextran ladder standard and converted to Glucose Units (GU) values, and then used along with accurate mass information to conduct a Glycan Scientific Library search within the UNIFI software for peak identification. With the use of GU values for library search, high confidence of peak assignment is ensured by minimizing the potential variation from retention times across analyses.

HILIC-FLR-MS RT and Accurate Mass

RT Calibration Using Dextran Ladder



Glycan Sample Data Processing



Library Search Results

| Component name | Structure | Expected GU | Δ GU | GU Std Dev | Expected m/z | Δ m/z | Mass Confirmed |
|----------------|------------------|-------------|-------------|------------|--------------|--------------|-------------------------------------|
| 1 | F(6)A1G(4)1Sq... | 8.5800 | 0.0068 | 0.0000 | 734.9525 | -75.0063 | <input type="checkbox"/> |
| 2 | A2G(4)2S(3)2 | 8.5700 | 0.0168 | 0.0140 | 1267.9861 | 33.0218 | <input type="checkbox"/> |
| 3 | F(6)A2G(4)2S(6)1 | 8.5500 | 0.0368 | 0.0000 | 1196.4673 | 0.0054 | <input checked="" type="checkbox"/> |
| 4 | A2B(4)2S(6)1 | 8.5500 | 0.0368 | 0.0000 | 1223.9761 | 0.0045 | <input checked="" type="checkbox"/> |
| 5 | A2(3)G(4)2S(6)1 | 8.6000 | 0.1032 | 0.0000 | 694.6104 | -115.3484 | <input type="checkbox"/> |

Figure 2. The integrated "Glycan FLR with MS Confirmation" workflow

2. Shields, R. L. et al. Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcγ₃ and antibody-dependent cellular toxicity. *J Biol Chem*. 2002. 277:26733–26740.
3. Lauber, M. A., et al. Rapid preparation of released N glycans for HILIC analysis using a labeling reagent that facilitates sensitive fluorescence and ESI-MS detection. *Anal. Chem*. 2015, 87, 5401–5409.
4. Reed, C. E., et al. Automated preparation of ms-sensitive fluorescently labeled N-glycans with a commercial pipetting robot. *SLAS TECHNOLOGY*. 2018. 23(6): 550–559.
5. Alley, W. R. J. and Yu, Y. Q. Combining RapiFluor-MS and UNIFI Scientific Information System for a total N-linked glycan solution for innovator vs. biosimilar infliximab comparisons. Waters Application Note. 720005753EN. 2016.
6. Yu, Y. Q. A holistic workflow for acquiring, processing, and reporting fluorescent-labeled glycans. Waters Application Note. 720004619EN. 2016.
7. Food and Drug Administration. Statistical Approaches to Evaluate Analytical Similarity Guidance for Industry. 2017.

Featured Products

[BioAccord System <https://www.waters.com/waters/135005818>](https://www.waters.com/waters/135005818)

720006545, April 2019