

Evaluating BioAccord LC-MS System Performance for Deployment of a Compact, SmartMS-Enabled Biopharma Solution in Late Stage Development and QC Environment - Intact Mass Analysis

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GOAL

To demonstrate the Waters BioAccord™ system-to-system reproducibility for intact mAb glycoprofiling.

BACKGROUND

In 2017, the FDA published a paper entitled "A Retrospective Evaluation of the Use of Mass Spectrometry in FDA Biologics License Applications"¹ that highlights the increased use of mass spectrometry for the characterization of protein-based biopharmaceuticals since 2000. Most recently, there has been a concerted effort from the pharmaceutical industry to expand the use of liquid chromatography and high-resolution mass spectrometry (LC-MS) in late stage development and QC environments. However, the complexity of LC-MS operation requires highly skilled scientists to generate and collect meaningful data. In addition, LC-MS data variability from instrument to instrument poses challenges in developing MS-based assays (such as the Multi-Attribute-Method) in process development and QC laboratories. There is a pressing need for ease-of-use and robust analytical systems for routine analysis of biopharmaceutical drug molecules.

Inter- and intra-system comparison for routine mAb mass confirmation using the BioAccord System platform.

The launch of the BioAccord System is the first step to address the industry's needs. As illustrated in previously published application notes and technology briefs,²⁻⁹ the BioAccord System meets many of these requirements. In this study, we focused on the reproducibility of the BioAccord System using well-designed experiments. This two-part study includes intact mass analysis and peptide mapping/monitoring. This technology brief focuses on the first part: intact mass analysis.

THE SOLUTION

The BioAccord System is physically comprised of an ACQUITY™ UPLC™ I-Class PLUS System configured with an optical detector (TUV or FLR) coupled in-line with an ACQUITY RDa™ Detector (a compact time-of-flight mass detector). The system operates on the UNIFI™ Scientific Information System platform, providing streamlined workflow solutions with automated data acquisition, processing, and reporting (including automating relative abundance calculations). This system is purposely designed for routine biopharmaceutical analysis in both regulated and non-regulated laboratories.

Intact mass analysis by RPLC-MS is a simple and fast way to obtain molecular weight and relative abundance of major glycoforms for mAb products. All the intact mass analyses in this study were carried out using the Waters Humanized mAb Mass Check Standard, originated from NIST mAb reference material (p/n: 186009152). Milli-Q Water (400 µl) was added to 80 µg of intact NIST mAb to produce a solution of 0.2 µg/µL. 0.8 µg was injected for analysis. An ACQUITY UPLC BEH C₄, 1.7 µm, 2.1 mm x 50 mm (p/n: [186004495](#)) was used at 80 °C. Mobile phase A was Milli-Q water with 0.1% formic acid and mobile phase B was acetonitrile with 0.1% formic acid. A linear gradient of 95% A to 15% A from 1 min to 3.7 min was run at 0.4 mL/min, with a total run time of 7 min. The RDa detector was operated in positive

full-scan mode (at 2 Hz) with a mass range of 400 to 7,000 *m/z*. The cone voltage was set at 70 V, capillary voltage at 1.5 KV and the desolvation temperature was kept at 550 °C. A threshold of ≤ 25 ppm mass error was used to confirm the glycosylation forms of the intact monoclonal antibody for all the experiments (Table 1 summarizes mass accuracy).

RESULTS

1. INTER-SYSTEM COMPARISON

Figure 1 displays inter-system reproducibility for intact mass, focusing on %RSD of relative percent of major glycoforms. The experiment was run continuously for 5.5 days (1098 injections) on the BioAccord System. Relative abundances of the five major glycosylation forms (G0F/G0F, G0F/G1F, G1F/G1F, G1F/G2F, and G2F/G2F) were calculated via automated data processing in UNIFI using MaxEnt 1 charge deconvolution. The charge deconvoluted spectrum is displayed along with the %RSD trending plot for the relative abundance of the five glycoforms shown in Figure 1. The result summary table shows that the %RSD of the five major components is about 2%, demonstrating that the BioAccord System can operate continuously with stable and consistent ion signal for a week without the need of recalibration or tuning.

A second experiment was performed to show the reproducibility of the intact mass analysis on one of the systems over a three-month time period. In this work, instead of conducting the experiment continuously on the system, 10 injections were run each day on day 1, 2, 3, 5, 8, 14, and 90. Then the relative abundance of the same five glycosylation forms was plotted in the summary trend plot. The experiment results (data not displayed) show that the %RSD of the relative abundance of the five major glycoforms is less than 8.0%, demonstrating that the BioAccord System is highly stable and robust over a long period of time (three months in this case).

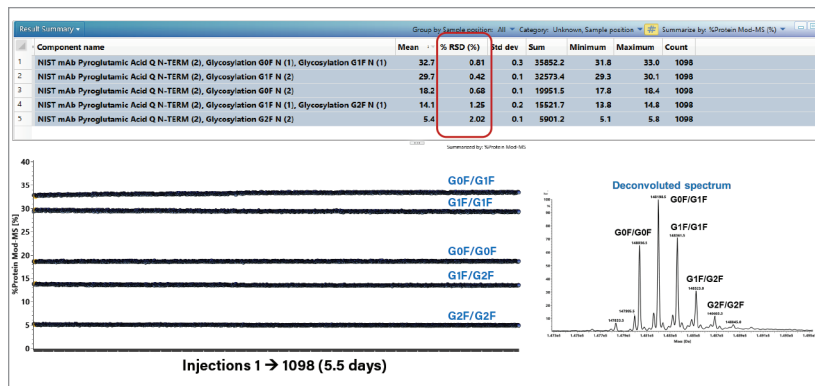


Figure 1. Spectrum quality and data consistency are key contributors for successful intact mass analysis. Relative quantitation of major glycoforms of the Waters Humanized mAb Mass Check Standard (NIST mAb) is displayed here. We were able to obtain consistent relative percentage of the top five glycoforms across 1098 injections. The %RSD is ≤2.0%, highlighted in red in the figure.

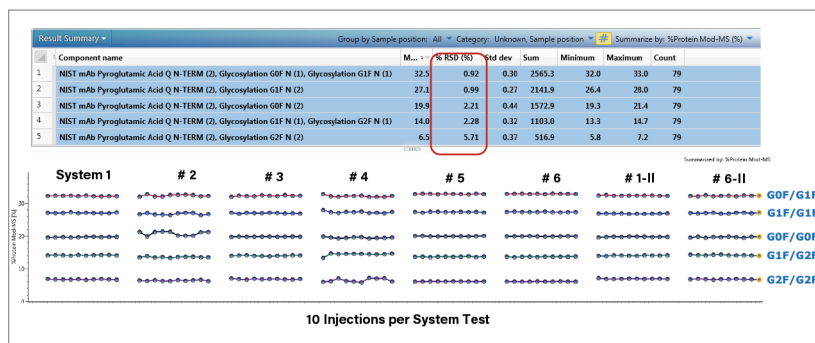


Figure 2. Relative quantitation of the five major glycoforms of the NIST mAb reference standard is shown across six different BioAccord Systems. Consistent relative percentage of glycoforms for the intact NIST mAb sample was obtained from six BioAccord Systems. The %RSD was found to be less than 6.0%, highlighted in red in the result summary table.

Study type	Mass accuracy	% RSD
Inter system study (one week)	Less than 20 ppm	Less than 3.0 %
Inter system study (three months)	Less than 25 ppm	Less than 8.0 %
Intra system study (six systems)	Less than 25 ppm	Less than 6.0%

Table 1. Intact mass analysis reproducibility evaluation.

2. INTRA-SYSTEM INTACT MASS ANALYSIS STUDY

After evaluating the inter-system reproducibility for intact mass analysis, we conducted an intra-system reproducibility study. In this work, we ran the same experiment on six different BioAccord Systems for 10 injections on each system (eight separate experiments in total). Results are shown in Figure 2 (systems one and six had duplicated data sets). The experimental results show that the %RSD of the relative abundance of the five major glycoforms was found to be less than 6.0%, highlighted in red in the result summary table, demonstrating that these six BioAccord Systems have excellent intra-system reproducibility for intact mass analysis.

Table 1 summarizes the experiment results of the major glycoform mass accuracies and the %RSD of the relative abundance, as described in this technology brief.

SUMMARY

The inter-system studies showed that the BioAccord System had excellent reproducibility in glycoform profiling, not only for five and half days continuously run, but also for a 90-day time course study. For the intra-system study, the experiment results showed that the BioAccord System had a high degree of consistency across six different systems. The relative abundance of the top five major glycoforms were found to be less than 6.0% RSD.

Both the inter- and intra-system reproducibility data generated from BioAccord Systems adds confidence in the deployment of MS-based assay in late stage development and QC labs.

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