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應用手冊

A Direct, Conversion-Free Workflow for Processing waters_connect Platform Data with Protein Metrics Software

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Abstract

This application note demonstrates direct processing of data acquired and managed in the waters_connect™ Platform using Protein Metrics Software, including Byos® and Byosphere®, without intermediate file conversion.

Direct data access reduces manual file handling and supports a standardized, vendor-neutral approach to biopharmaceutical mass spectrometry data processing.

Two Protein Metrics workflows were used to evaluate monoclonal antibody characterization datasets: the 1) Intact Mass workflow and 2) the PTM-DIA workflow for Remicade® (infliximab), the reference product, and the Renflexis® (infliximab-abda), an FDA licensed biosimilar to Remicade. These case studies demonstrate how direct connectivity between waters_connect and Protein Metrics software can streamline the transition from data acquisition to interpretation.

Benefits

- Conversion free data access: Process waters_connect Software LC–MS data directly in Protein Metrics workflows without intermediate conversion steps, simplifying project creation and reducing manual data handling
- Traceable acquisition-to-analysis workflow: Application Program Interface (API) enabled connectivity supports consistent transfer of data between waters_connect Platform and Protein Metrics Software, helping reduce file selection errors and improving workflow traceability
- Flexible, vendor-neutral biopharmaceutical characterization: Apply Protein Metrics Intact Mass workflow, and PTM-DIA workflows to data acquired on Waters platforms while retaining waters_connect Software for acquisition and data management.

Introduction

Biopharmaceutical laboratories increasingly rely on complex mass spectrometry workflows to characterize monoclonal antibodies (mAb) reference material and biosimilar candidates and other advanced therapeutic modalities.[1,2] Although vendor-specific data systems support instrument control, data acquisition, and initial data processing, vendor-neutral software can provide complementary capabilities for orthogonal characterization and workflow integration. In this work, the direct access and processing of waters_connect data files are demonstrated using Protein Metrics software workflows.

The workflow was evaluated using mAb characterization datasets for the reference product Remicade® (infliximab) and its biosimilar Renflexis® (infliximab-abda). Intact Mass and Peptide Mapping LC–MS datasets

were acquired using Waters LC-MS system and managed in waters_connect software. Intact Mass data were acquired using a BioAccord™ LC-MS System, whereas Peptide Mapping data were acquired using a Xevo™ G3 QTof Mass Spectrometer. Peptide Mapping data were acquired using LC–MS^E, a Data Independent Acquisition (DIA) approach. Note that Protein Metrics Software can process compatible data generated by a range of Waters high-resolution mass spectrometry (HRMS) platforms.

The datasets were then made directly available for processing in Protein Metrics Software workflows through an application programming interface (API). Protein Metrics Byos was used to assess intact molecular mass and glycoform, as well as peptide-level attributes including sequence coverage and selected post-translational modifications (PTMs).

These case studies demonstrate the applicability of this integrated workflow to biosimilar mAb characterization using complementary Intact Mass and Peptide Mapping analyzes. Such integration can be valuable in biopharmaceutical laboratories, where processing efficiency, reproducibility, data integrity, and scalability are important for analytical characterization and product comparison. Overall, API enabled integration between waters_connect Software and Protein Metrics Software provides a flexible, scalable data-processing environment for biopharmaceutical mass-spectrometry workflows.

Results and Discussion

Data Processing Workflows

LC–MS data were acquired and managed using waters_connect Software v4.0. Data processing projects were created in Protein Metrics Software (v6.0.33) directly the waters_connect data files through an API operating in the background. Figure 1 illustrates the process for uploading waters_connect data files during Protein Metrics Byos project creation. Intact Mass data were analyzed using the Byos Intact Mass workflow, while Peptide Mapping data were processed using the Byos DIA workflow. Theoretical protein sequences, mass tolerances, digestion parameters, modification settings, and other search criteria were defined within the respective Byos project methods.

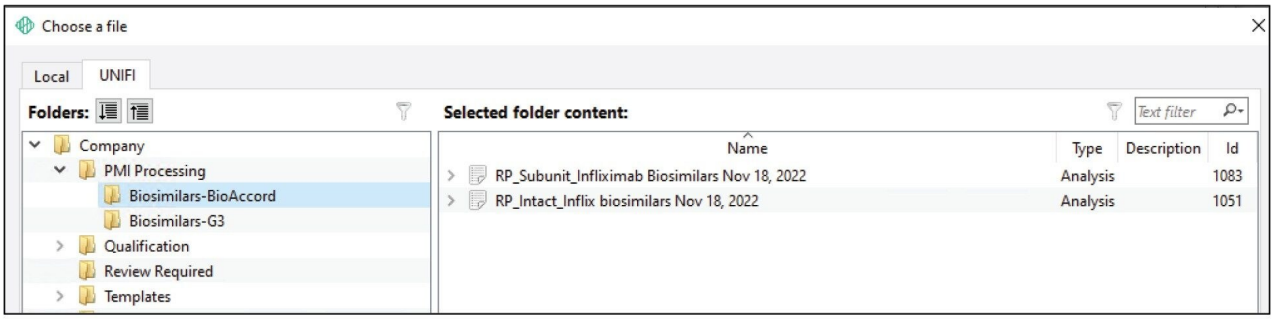

Figure 1. During Protein Metrics Software project creation, scientists can select LC–MS data directly from a waters_connect data repository without an intermediate file-conversion step.

Intact Mass Workflow

Figure 2 shows charge deconvoluted neutral mass spectra for the reference product Remicade® (infliximab) and the biosimilar Renflexis® (infliximab-abda). Both samples were treated with carboxypeptidase B (CpB) before reversed-phase LC–MS analysis. The predominant glycoforms were similar between the two mAbs, although differences were observed in their relative abundances.

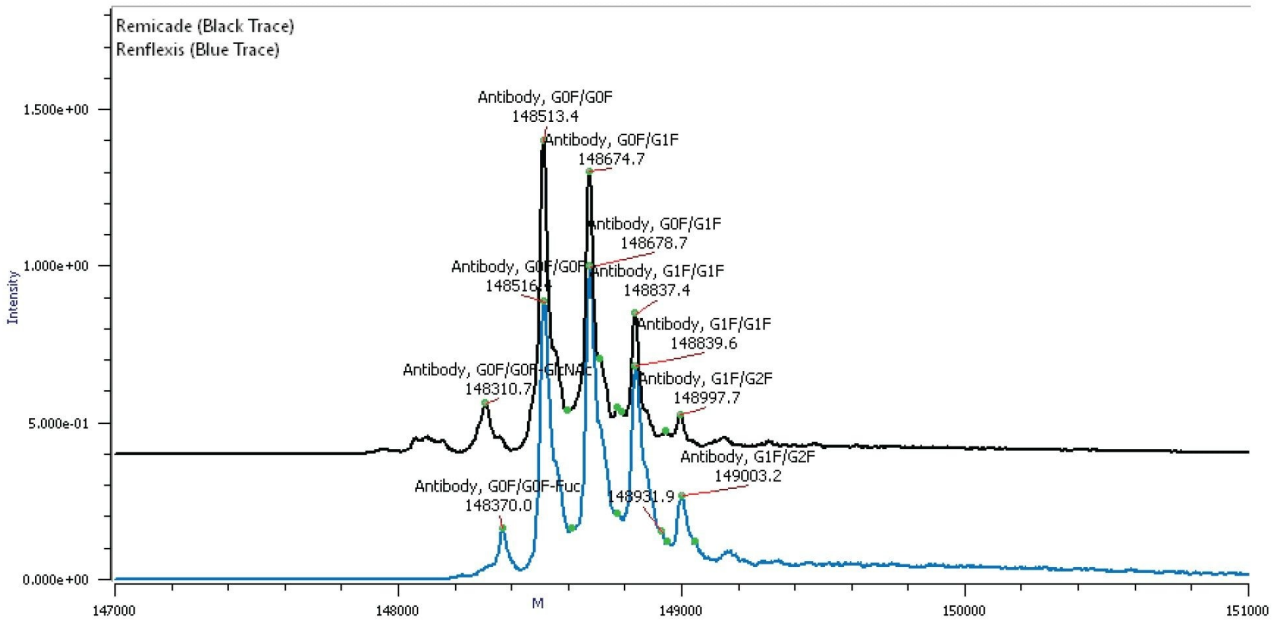

Figure 2. Charge deconvoluted intact mass results for Remicade® (infliximab), and the reference product, Renflexis® (infliximab-abda), a biosimilar. Both samples were treated with carboxypeptidase B before reversed-phase LC–MS analysis.

PTM-DIA Workflow

The PTM-DIA workflow in Byos was used to assess peptide-level sequence coverage and post-translational modifications in Remicade® (infliximab) and Renflexis® (infliximab-abda). Comparable sequence coverage was obtained for the heavy and light chains of both mAbs. The analysis identified glycosylation, deamidation, and oxidation at methionine and tryptophan residues. Figure 3 shows sequence coverage for both mAbs and representative high-energy fragmentation spectra for PTM containing peptides, including glycopeptides and oxidized peptides.

Relative PTM abundances were calculated using the PTM-DIA workflow. For example, the oxidation level of the DTLMISR peptide was 1.9% for Remicade and 2.8% for Renflexis. Although oxidation of the DTLMISR peptide was suppressed by a highly abundant co-eluting peptide, its fragment ions were still identified and matched the historical data.

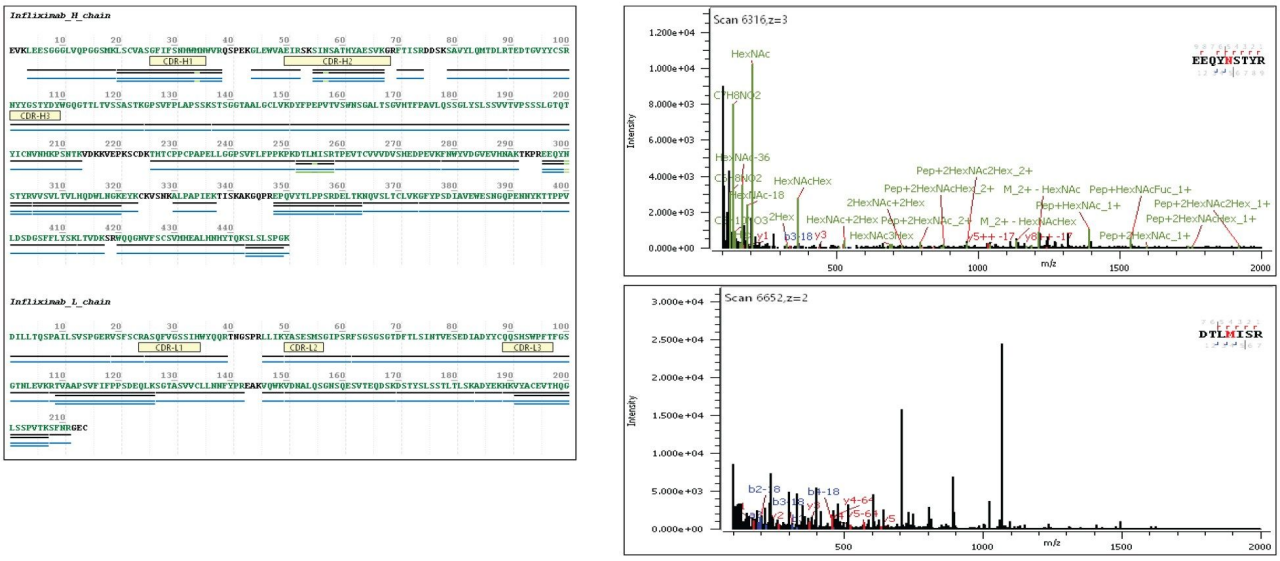

Figure 3. Peptide mapping of Remicade® (infliximab) and Renflexis® (infliximab-abda) using the Byos PTM-DIA workflow. Batch processing enabled determination and comparison of sequence coverage, exceeding 90% for the analyzed chains. Representative high-energy fragmentation spectra for glycopeptides and oxidized peptides are shown. The workflow enabled identification and quantification of low-abundance PTM-containing peptides from DIA data.

Conclusion

Direct access to waters_connect data files from Protein Metrics Software reduces manual file handling and supports consistent, traceable data transfer between acquisition, data management, and data-processing environments. Compared with manual data exchange, API enabled integration can improve processing efficiency, reduce the potential for file selection and transcription errors, and facilitate incorporation of mass spectrometry data analysis into standardized laboratory workflows.

Protein Metrics Software provides a vendor-neutral environment for applying common data processing strategies across multiple instrument platforms and analytical methods. In combination with waters_connect Software for acquisition and data management, this integration can support method harmonization and cross-platform interpretation for biopharmaceutical mass spectrometry workflows.

References

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