

A Robust Workflow for Biosimilar Comparability Assessment via Intact and Subunit RPLC-MS and Native IEX-MS with a Prototype Benchtop QTOF

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Introduction

- Regulatory agencies require comprehensive analyses to prove comparability and quality of biosimilar monoclonal antibodies (mAbs). These are commonly characterized and monitored by LC-MS techniques.
- QToF-based LC-MS analyses have consistently offered the required sensitivity and high m/z mass resolution for streamlined characterization of intact mAb samples in denatured and native forms, revealing key and critical product attributes.
- Routine intact and subunit level LC-MS assays (RP-MS & IEX-MS) were developed on the Xevo™ G3 QToF platform for glycoform and charge variant attribute comparison and applied to a study of infliximab biosimilarity.

Experimental

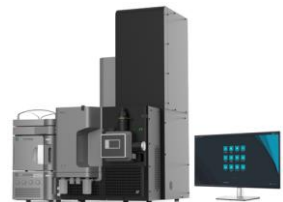


Figure 1. ACQUITY™ Premier I-Class system with ACQUITY Premier BEH™ C4 (RP) column or BioResolve™ SCX mAb (IEX) connected to a Xevo G3 QToF platform controlled by UNIFI™ software under waters_connect platform

Infliximab innovator (Remicade®) & three biosimilars (Inflectra®, Avsola®, and Renflexis®) were analyzed in triplicate at intact and IdeS-digested subunit levels via reversed-phase chromatography (RPLC) and ion exchange chromatography (IEX) coupled to a Xevo G3 QToF mass spectrometer. Data were acquired, processed, and reviewed on the waters_connect™ informatics platform using UNIFI™ and INTACT Mass applications software.

Results

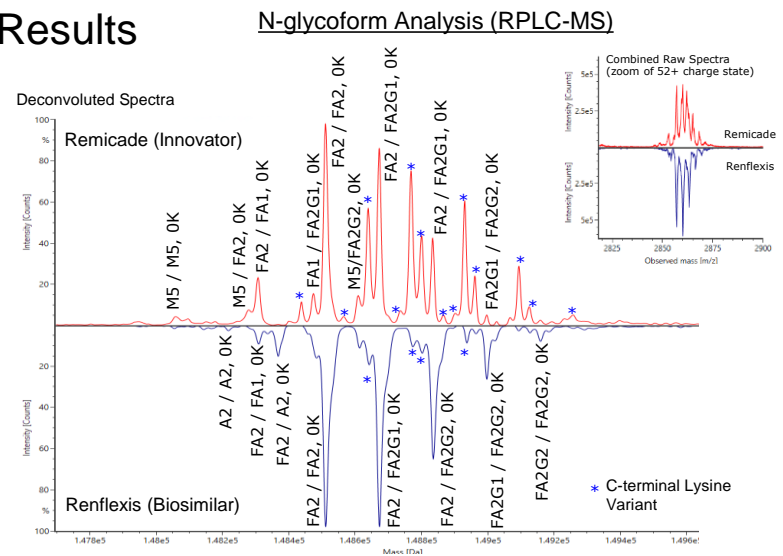


Figure 2. RP Intact: Mirror plot comparing MaxEnt1 deconvoluted mass spectra for Intact Remicade & Renflexis, and data insert (top right) with zoom of mirror plot of combined raw spectra (52+ charge state). Deconvoluted data highlights differences in levels of C-terminal lysine variants and N-glycoforms, with confident assignments down to < 5% relative abundance.

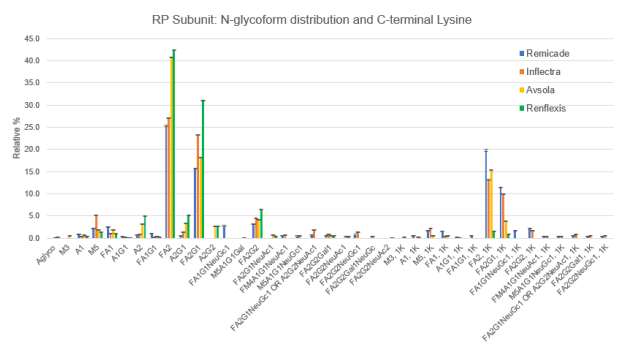


Figure 3. RP Subunit: Confident assignment and relative quantitation of Fc N-glycoforms down to 1%—trends are comparable with intact RP analysis. In addition, immunogenic glycans containing NeuGc and alpha-Gal structures detected for Remicade & Inflectra.

Charge Variant Analysis (IEX-MS)

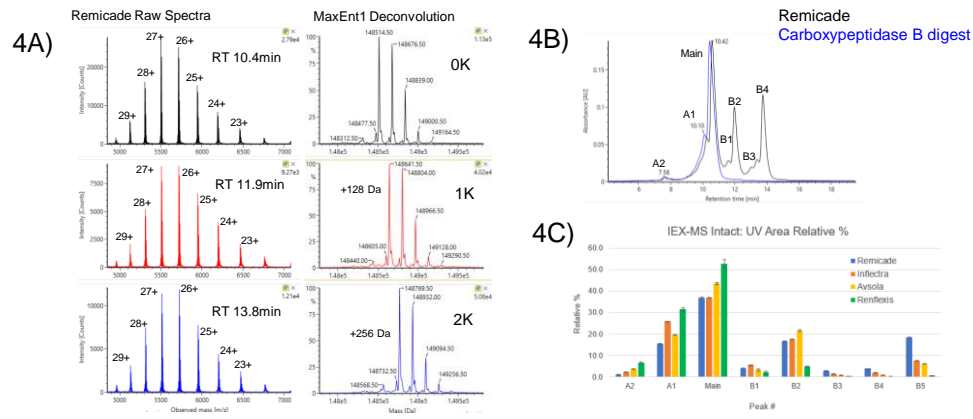


Figure 4. IEX-MS Intact: 4A) Example combined raw spectra for the three major charge variant peaks of Remicade (left) and their corresponding MaxEnt1 deconvoluted spectra, confirming the three C-terminal lysine variants. 4B) UV 280nm overlay of untreated Remicade and carboxypeptidase B-treated Remicade to confirm main species are attributed to C-terminal lysine variants. 4C) Comparison of integrated UV areas for Remicade and three biosimilars.

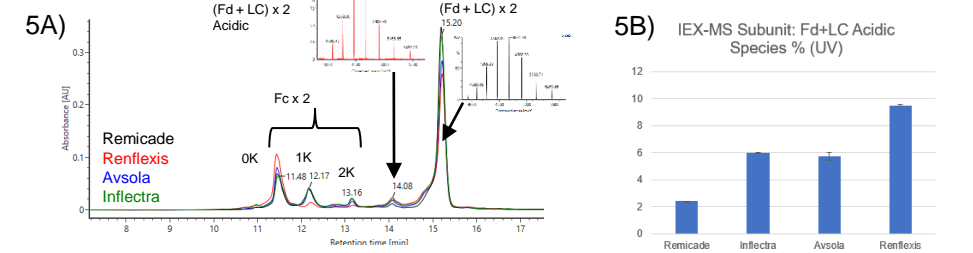


Figure 5. IEX-MS Subunit: 4A) UV 280nm overlay of IdeS-digested infliximab biosimilars; 4B) (Fd + LC) x 2 acidic species relative % comparison (based on UV integration). Native MS was used to confirm the labelled Fc C-terminal lysine variants and (Fd + LC) x 2 species. Deconvoluted mass for the acidic peak of (Fd + LC) x 2 is near-isobaric to the main species, which is consistent with deamidation or conformational variants.

Conclusions

- The Xevo G3 QToF was utilized along with inline UV detection for denatured RP and native IEX-MS, resulting in confident attribute profiling of N-glycoforms (even at low levels, < 5% relative abundance) and charge related variants.
- The resulting RP-MS & IEX-MS intact and subunit workflows were successful in establishing the extent of infliximab biosimilarity, with the most notable differences in levels of C-terminal lysine and variety of N-glycan species.