Biosimilar Peptide Mapping Characterization and MAM Workflow Using Prototype Benchtop QTOF with an App-Based Acquisition and Data Processing Platform

Kellen DeLaney[1], Samantha Ippoliti[1]; Lisa Reid[2]; Owen Cornwell[2]; Ying Qing Yu[1]; Emma Harry[2]; Scott Berger[1]

[1]Waters Corporation, Milford, Massachusetts; [2]Waters Corporation, Wilmslow, United Kingdom

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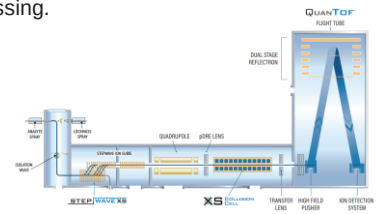
Introduction

Peptide mapping is one of the gold standard techniques for post-translational modification (PTM) assessment not only for characterization, but also for development and comparability, of biologics and biosimilar drugs. This rigorous characterization is crucial to ensure safety and efficacy of biopharmaceuticals. A successful workflow must be robust, easily implemented, and GMP-compliant. This study demonstrates the implementation of a peptide characterization and Multi-Attribute Method (MAM) for infliximab biosimilars on the Xevo™ G3 QTof platform with compliance-ready app-based data acquisition and processing. The Xevo G3 QTof's improved ion optics increases system robustness, and the ability to employ intelligent data capture allows for real-time noise reduction and file size reduction, enabling high-throughput data acquisition and processing.



Experimental

Samples of reduced/alkylated infliximab innovator (Remicade®) and biosimilars (Inflectra®, Avsola®, & Renflexis®) were trypsin-digested prior to analysis via RPLC-MS on an ACQUITY™ Premier UPLC™ System. Data independent acquisition (DIA) MS detection (MS^E) was performed using a Xevo G3 QTof mass spectrometer. Data acquisition, processing, and review were carried out using the UNIFI™ App Peptide Mapping workflow for attribute characterization, and the Peptide MAM App for targeted attribute monitoring.

Results

Peptide Map and PTM Comparison of Biosimilars to Innovator

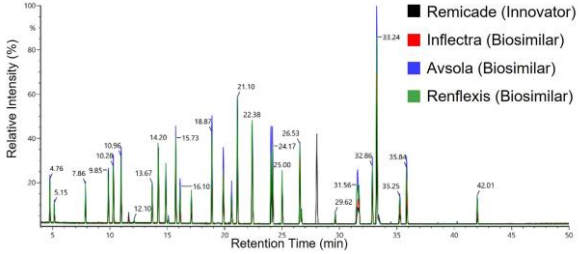


Figure 1. Overlay of chromatograms from innovator and three biosimilars, demonstrating similarity. Peak retention times are annotated. Greater than 95% sequence coverage was achieved for all four products. There are notable differences in N-glycoforms among the four mAbs (see Fig 4 for details).

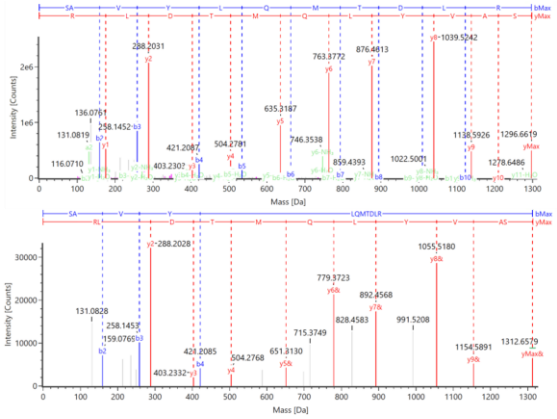


Figure 2. CID high energy spectra of heavy chain tryptic peptide (HT) HT11 in both (top) native and (bottom) oxidized form.

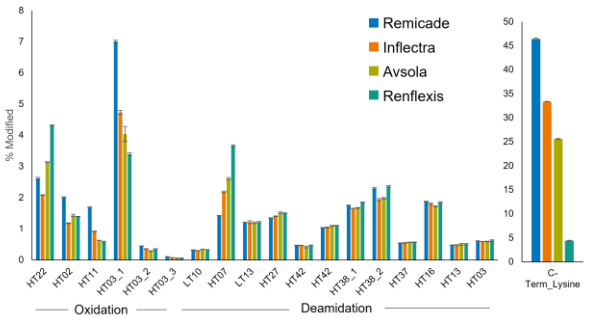


Figure 3. Comparison of attributes (oxidation, deamidation, and C-terminal Lysine conjugation) between innovator and biosimilars. MAM app was used for the study. H heavy chain; L light chain; T tryptic peptide.

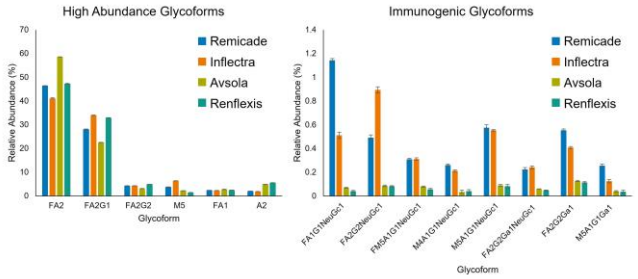


Figure 4. Comparison of relative abundance of detected N-glycoforms between the innovator and biosimilars, showing 13 out of the 28 total glycoforms monitored.

Stress Study

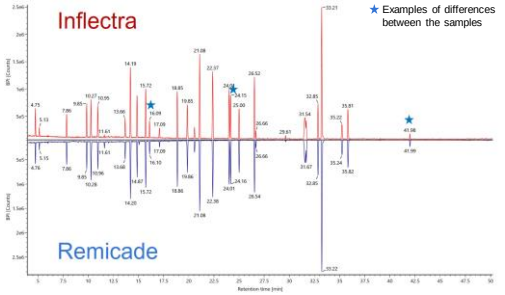


Figure 5. Example of a mirror plot showing comparison of two-week stressed Remicade and Inflectra samples. Only minor differences are observed from the mirror plot. Changes in peptide attributes were quantified using MAM App data processing (see Figure 6).

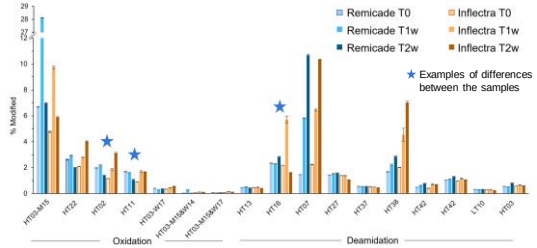


Figure 6. Results from stress study comparing critical quality attributes (CQAs) of Remicade with Inflectra. The average run-to-run relative standard deviation was ~2%, demonstrating excellent method repeatability.

Conclusions

- Peptide maps were generated for innovator and three biosimilar infliximab products with the Xevo G3 QTof MS using a DIA based methodology that produced greater than 95% sequence coverage. Level of major PTMs are compared for the innovator and biosimilar mAbs, including notable differences in relative abundance of glycoforms.
- Attributes of interest were monitored between the innovator and biosimilars, including a thermal stress study, generating results with high reproducibility that were capable of demonstrating trends and differences between molecules.
- The results demonstrate the effective use of the Xevo G3 QTof operated under the waters_connect™ informatics platform for integrated data acquisition, processing, review, and reporting for establishing attribute based biosimilarity of mAbs based biopharmaceuticals.