PEPTIDE CHARACTERIZATION AND MONITORING WORKFLOW FOR BIOSIMILAR mAb DRUG PRODUCTS USING A COMPLIANCE READY LC-MS AND INFORMATICS PLATFORM

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INTRODUCTION

With the increasing commercial opportunities for biosimilar monoclonal antibodies (mAbs), streamlining processes to characterize and monitor mAb attributes is critical. Characterization typically implements peptide mapping to analyze post-translational modifications (PTMs). Additionally, forced-degradation studies are important to establish consistent degradation pathways between biosimilar and innovator drug products. Changes in the abundance of critical quality attributes (CQAs) provide insight into factors related to proteins' efficacy and immunogenicity. Monitoring for these CQAs has been expedited through the use of multi-attribute method (MAM), in which these targeted attributes are relatively-quantified over numerous samples on an LC-MS platform, resulting in greater throughput and greater dynamic range of analysis than non-MS based methods. Here we demonstrate a workflow for characterizing mAbs on a Xevo™ G3 QTof mass spectrometer and migrating to a MAM analysis on a BioAccord™ LC-MS System for monitoring attributes across samples, all within the waters_connect™ Informatics Platform

Key Benefits:

- **High-confidence peptide mapping of innovator and biosimilar mAbs with localization of modifications using the Xevo G3 QTof**
- **Seamless transition of methods across instruments within the network capable waters_connect Informatics Platform**
- **Efficient, compliance-ready workflow for MAM analysis for targeted attribute monitoring and "new peak" detection-based purity analysis**

METHODS

Sample Preparation

Infliximab samples, including innovator Remicade™ and three biosimilars, Inflectra™, Avsola™, and Renflexis™, were incubated at 37 °C for 0 weeks (no stress), 1 week, and 2 weeks. Samples were then reduced, alkylated, desalted, trypsin-digested, and acidified to 0.1% formic acid. The samples were then diluted to a final concentration of 0.2 µg/µL.

Peptide Characterization and Identification of Attributes

The resulting peptide samples were separated with reverse-phase liquid chromatography (RPLC) on an ACQUITY™ Premier UPLC™ System using an ACQUITY Premier Peptide CSH™ Column (1.7 µm, 2.1 × 100 mm) maintained at 60 °C for a 50-minute linear gradient of 1 - 35 %B (80-minute total cycle time). Peptide map characterization was performed on a Xevo G3 QTof mass spectrometer using data independent acquisition (MS^E). Data was acquired within the waters_connect Platform and processed with the UNIFI App peptide mapping workflow. Attributes selected for monitoring were sent to a custom Scientific Library within the waters_connect Platform.

Attribute Monitoring

Attributes added to the scientific library were imported into the Peptide MAM App within the waters_connect Platform for monitoring. Data was acquired on a BioAccord LC-MS System using the same LC conditions as described above for characterization. Biosimilar samples and stressed samples were acquired and processed for relative quantification of targeted attributes. New peak detection was also performed within the Peptide MAM App to detect any new peaks appearing across samples that could represent novel attributes and potential product or process related impurities.

Figure 1. *Workflow within the waters_connect Informatics Platform for protein characterization (attribute definition) and attribute monitoring. Peptide map characterization and attribute definition were carried out using the Xevo G3 QTof with the UNIFI App. Selected attributes were then added to a custom Scientific Library. The library of attributes were then imported into the Peptide MAM App for monitoring across samples using the BioAccord System.*

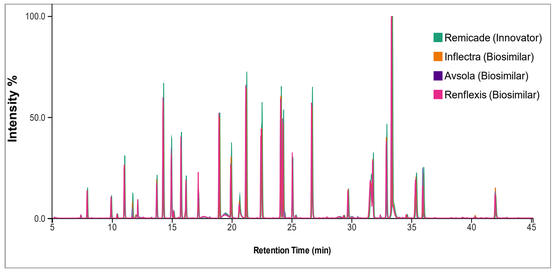

Figure 2. *Overlay of chromatograms acquired on the Xevo G3 QTof from innovator infliximab and three biosimilars, demonstrating similarity. Greater than 95% sequence coverage was achieved for all four products based on accurate mass (within 5 ppm) and fragment ions in the high energy data channel (b/y ions ≥ 3). While the chromatograms appear similar, there are notable differences in lysine clipping and N-glycoforms among the four mAbs (see Fig 5 for details).*

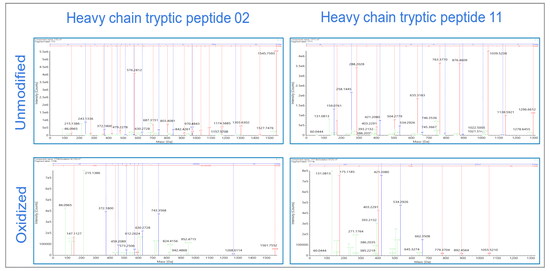

Figure 3. *Xevo G3 QTof LC-MS^E high energy fragmentation spectra of (left) heavy chain tryptic peptide (HT) 02 and (right) HT11, in both their (top) unmodified form and (bottom) oxidized form. Relative oxidation levels were 1.6% and 1.4% for HT02 and HT11, respectively. A total of six oxidation, twelve deamidation, and 28 N-glycosylation PTMs were identified. The high coverage of matching fragment ions across each peptide's amino acid backbone enables both confident identification of peptide assignments and localization of modifications to discrete amino acids within the peptide sequence.*

RESULTS AND DISCUSSION

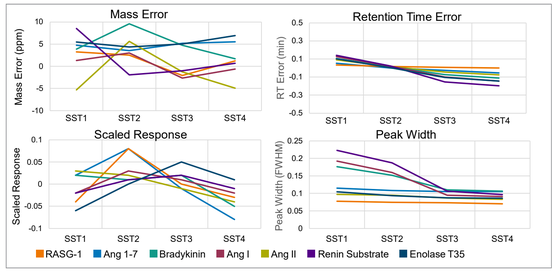

Figure 4. *System suitability injections of a peptide standard mixture on a BioAccord System were used at the beginning and end of the MAM analysis sample sequence to ensure the system was producing results within established performance specifications. These injections are flagged in the Peptide MAM App's sample list and evaluated on specified criteria, including mass error, retention time error, scaled response, and peak width. Shown here are the trends summarized in the Peptide MAM App as part of the BioAccord LC-MS MAM workflow.*

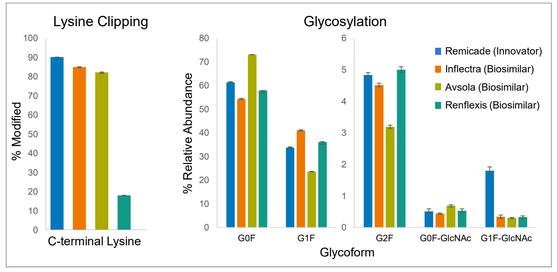

Figure 5. *Comparison of lysine clipping and N-glycosylation across the innovator infliximab (Remicade) and three biosimilar infliximab products (Inflectra, Avsola, and Renflexis) acquired on a BioAccord System. The abundance of C-terminal lysine was similar for Remicade, Inflectra, and Avsola, at 80-90%, while this value was much lower for Renflexis, at less than 20%. The abundances of various N-glycoforms of the heavy chain tryptic peptide 26 were also different between samples. These differences are expected, as the products originate from different cell lines, and such differences are tolerated for biosimilars as long as there is no meaningful risk of differences in clinical outcomes with those changes..*

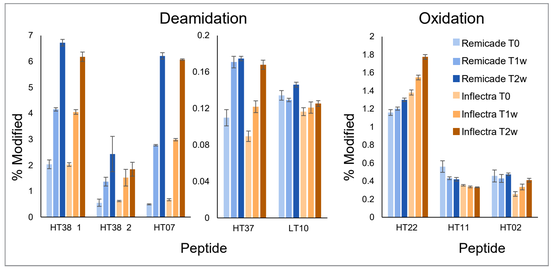

Figure 6. *Forced degradation study (elevated temperature) comparing innovator infliximab (Remicade) and a biosimilar infliximab (Inflectra) at three time points, T0 (no stress), T1w (one week), and T2w (two weeks) acquired on a BioAccord System. Generally, the % deamidation for each peptide was in agreement between the samples and increased with duration of induced stress. Peptide oxidation did not have a clear trend in either sample, though differences were observed.*

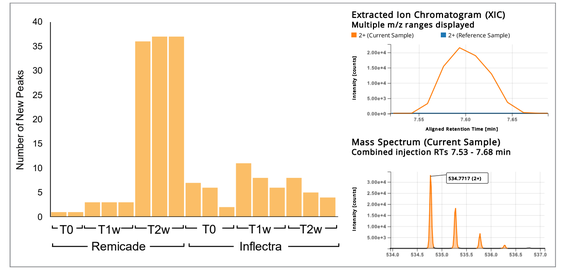

Figure 7. *New Peak Detection results comparing innovator infliximab (Remicade) as the reference sample to a biosimilar sample (Inflectra) in the Peptide MAM App for data acquired on a BioAccord System. A summary of new peaks detected across samples (triplicate injections of each sample) is shown on the left. On the right is a review of the (top) extracted ion chromatogram and (bottom) mass spectrum of a specific new peak, enabling the user to inspect and confirm or reject the "new" peak. The peak shown was identified to be a missed cleavage of heavy chain tryptic peptide 9-10.*

CONCLUSIONS

- **Characterization and monitoring of product attributes is important in evaluating biosimilarity, as any differences in attribute profiles must be evaluated.**
- **The waters_connect Informatics Platform facilitates this peptide map characterization to compliance-ready MAM workflow, even across different instrument systems on a common network.**
- **The Xevo G3 QTof mass spectrometer and UNIFI App peptide mapping workflow enable high sequence coverage and localization of PTMs, which can then be selected for targeted attribute monitoring.**
- **The BioAccord System and Peptide MAM App enable relative targeted quantification of attributes and new peak detection across large sample sets.**
- **This workflow was used to measure differences in infliximab biosimilars, including differences in % modified peptides and changes due to temperature stress.**

References

1. Rogers RS, Nightlinger NS, Livingston B, Campbell P, Bailey R, Balland A. Development of a Quantitative Mass Spectrometry Multi -Attribute Method for Characterization, Quality Control Testing, and Disposition of Biologics. mAbs. 2015 Aug, 7(5), 881-890.
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4. Ranbadige N, Yu YQ. Extending the Capabilities of the BioAccord LC-MS System with a Streamlined Workflow for Compliant-Ready Multi-Attribute Method (MAM). Waters Application Note. 2020 July; 720006963.

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