Routine workflow for comparability assessment of protein biopharmaceuticals

Trastuzumab Intact Analysis using Benchtop X500B QTOF Mass Spectrometer

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Introduction

The development of biopharmaceuticals is complex and requires extensive characterization to ensure safety and efficacy as products progress towards commercialization. While there are many approaches for assessing comparability, intact mass analysis using LC-MS provides a rapid assessment for the mass of the molecule as well as high level heterogeneity information. The ability to accomplish this assay rapidly often makes it a key assay prior to more extensive investigation.

Here we demonstrate a reproducible and robust method for analyzing intact biotherapeutic proteins on the X500B QTOF System with simple and rapid batch processing using BioPharmaView™ Software.

Materials and methods

Biosimilar Trastuzumab therapeutic was obtained from two different manufacturing sources (labeled, Trast-1 and Trast-2). Samples were either diluted in 0.2% formic acid or deglycosylated using PNGase F (New England BioLabs (Ipswich, MA, USA)) using vendors standard protocol.

Chromatography

A total of 0.5 µg of protein was injected onto the ExionLC™ and separated using a Waters Acquity UPLC® Protein BEH C4 column, 300A 1.7 um, 2.1mm x 50mm column 80°C. Standard mobile phases were used (Mobile Phase A: 0.1% formic acid in water, Mobile Phase B: 0.1% formic acid in acetonitrile) with a total run time of 5 min using moving flow rate of 0.2 – 0.5 mL/min. An integrated divert valve was used to flush to waste for the first 0.5 mins of each injection.

Mass spectrometry

Acquisition was performed on X500B QTOF with a Turbo V™ ion source using large protein mode acquisition and decreased detector voltage selected over a range from 900-4000 m/z. Electrospray parameters were as follows:

Curtain gas:	35
Ion source gas 1 (psi):	50
Ion source gas 2 (psi):	50
Temperature (°C):	400

Data processing

Data was processed in BioPharmaView Software using a standardized sample of trastuzumab as reference.

Results and Discussion

Glycosylated Trastuzumab

For this study we used two different lots of trastuzumab. We began with a rapid and simple chromatographic method to deliver a desalted sample for MS analysis. The initial portion of the separation was diverted to waste using the onboard divert valve on the X500B, after desalting the valve was actuated to place the flow in-line with the MS source. As showing in Figure 1, the chromatographic separation is highly reproducible.

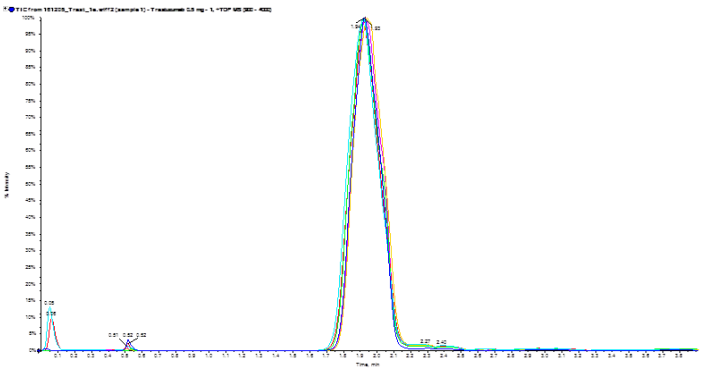

Figure 1: Chromatographic separation of trastuzumab from 2 manufacturers gives reproducible separation.

The data was processed in BioPharmaView using the intact workflow. Once the sequence and expected post translational modification of the protein were defined, the chromatographic window was determined over which to select data. Shown in

Figure 2 are the raw spectra for three replicate injections of one lot of trastuzumb. The replicate spectra overlay very well.

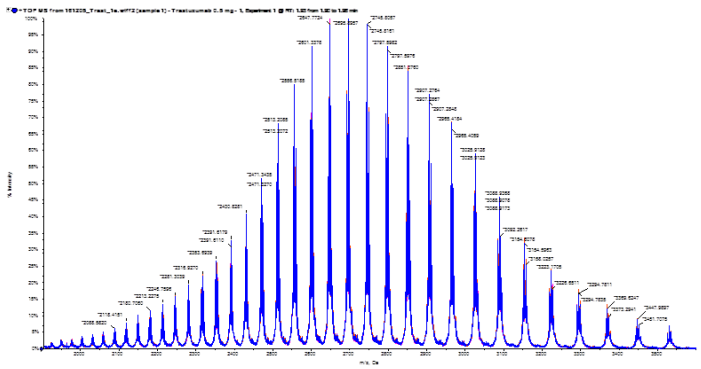

Figure 2: Raw spectrum from three replicate injections of trastuzumab. Each injection is in a different colour (blue, pink, red) and reflects the Gaussian distribution in m/z.

The raw spectra of this sample was compared to a second lot of trastuzumab using BioPharmaView (Figure 3). As shown there are some differences in the intensities of the glycoforms, however the masses of each charge state are very similar.

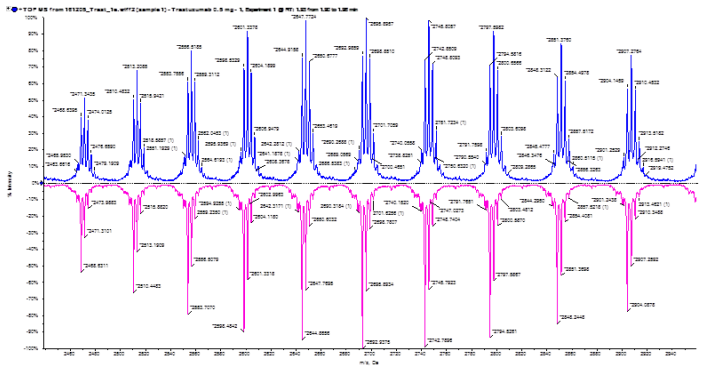

Figure 3: Mirror plot image of one lot of trastuzumab (blue) vs a second lot of trastuzumab (pink) showing a distinct shift in the glycoform pattern.

While evaluation of raw spectra is important to ensure that each charge state represents highly similar profiles, reconstruction of intact mass data is the most common means of comparing data. A range of masses was selected which spanned the expected reconstructed mass for trastuzumab in BioPharmaView. The first lot of the antibody was characterized, verified the identification of each of the reconstructed peaks in the resulting spectrum against previous reports (Figure 4).

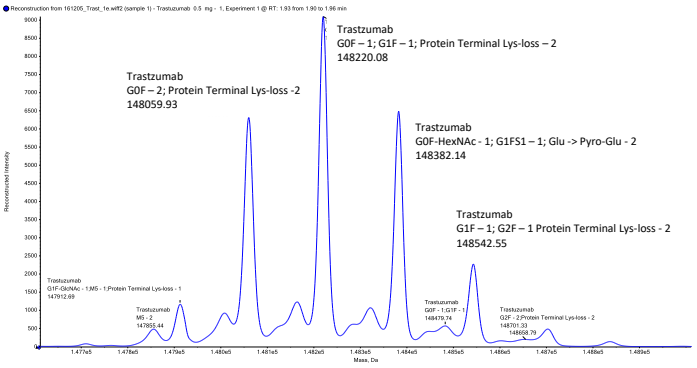

Figure 4: Annotated reconstruction of trastuzumab and all the modifications present including N-terminal lysine loss and glycosylation.

A batch analysis was submitted to compare the second lot of trastuzumab against our initial characterized sample. Consistent with the raw data, our reconstructed spectra showed excellent agreement in the masses of each glycoform, however the intensities were different between the samples (Figure 5).

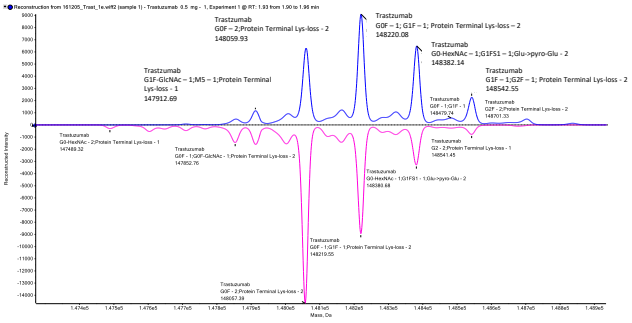

Figure 5: Comparison of glycoforms and intensities of the reconstructed spectra of the two lots of trastuzumab. Lot 1 in blue and Lot 2 in pink.

The replicate injections for each of the lots were plotted in a bar chart to display the relative abundances of each major glycoform as shown in Figure 6. The plot shown was generated automatically in BPV and allows for rapid assessment of the intensity of post translational modifications rapidly.

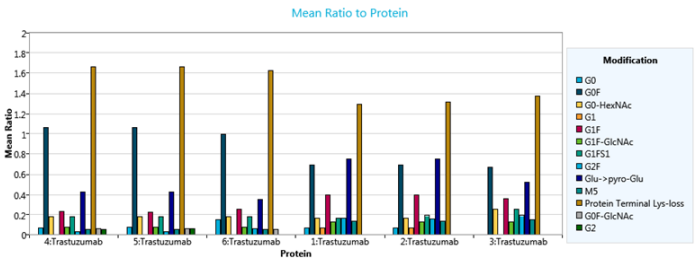

Figure 6: Relative abundances of major glycoforms and other modifications on the two lots. Lot 1 (1:Trastuzumab – 3:Trastuzumab) and lot 2 (4:Trastuzumab – 6:Trastuzumab).

Reviewing the results from Figure 3 highlights the changes in the intensities of the major glycoforms, as well as evidence for the presence of mannose-5 (MAN5) species. To investigate the level of MAN5 species, the plot was customized to display this species in relation to the G1F peak. As shown the relative intensity of the MAN5 peak is greater in the first sample compared to the second and is consistent across replicate analyses.

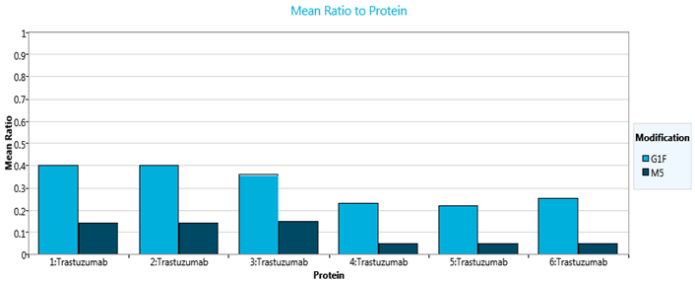

Figure 7: Man5 vs G1F abundances across lot 1 (1:Trastuzumab – 3:Trastuzumab) and lot 2 (4:Trastuzumab – 6:Trastuzumab).

Conclusion

Batch comparisons of biologics is important for the manufacturing process, and enabling rapid comparisons of batches or inter-batch studies allows the quality of product to be monitored and maintained. The benchtop X500B QTOF mass spectrometer was developed, for routine analysis of biologics and rapid batch comparison with the BioPharmaView software. BioPharmaView software was able to rapidly and easily identify the differences between two trastuzumab manufacturing lots based on their distinct glycoform profiles. The visualization tools enable the user to identify, quantify, and track these differences between production lots.

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