Mass Accuracy and Robustness of Intact mAb on the X500B Benchtop Quadrupole-Time-of-Flight Instrument

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ABSTRACT

Biotherapeutics and their increasing take of the pharmaceutical market means companies are having to rethink how they monitor their production and manufacturing. Using mass spectrometry is a viable solution for monitoring production from start to end, looking at the specific critical quality attributes previously targeted with separate methods; now in one complete solution. In this environment, an instrument needs to be compact, easy to use, robust, reliable and sensitive to increase confidence in the data obtained. Here we evaluate the X500B benchtop QTOF which was designed specifically for biologics manufacturing, for its robustness and mass accuracy. A total of 300 injections of antibody were performed and the signal intensity and mass accuracy were monitored. Signal intensity did not change, showing the robustness of the instrument to give the same quality data over time. A single calibration was made prior to analysis of the 300 injections and the mass accuracy calculated based on two glycoforms of the antibody was kept below 25 ppm and showed no drift across time in mass accuracy. Robustness and mass accuracy confirms that the X500B system is an instrument which gives reliable data and increases confidence in the results.

INTRODUCTION

Antibodies and other biologics have been gaining in popularity as biotherapeutics. While much is still being done to learn how to develop such biotherapeutics, more and more are making it through clinical trials and into manufacturing. These compounds, unlike their small molecule predecessors, are far more complex in structure and are of a heterogeneous nature, requiring extensive characterization to ensure safety and efficacy. During production, specific analyses are performed to monitor the product however each analysis is separate and monitors a separate attribute. As more of these biotherapeutics move forward, there is a need to be able to monitor multiple attributes at the same time, reducing analysis time and increasing production time.

What is needed is a compact, user-friendly analytical solution, which is reliable and robust so it can be put onto the production floor and be used to monitor and identify such discrepancies from batch to batch or from time point to time point.

Presented here are the results of a robustness study on the new SCIEX X500B benchtop QTOF which was developed with manufacturing and processing in mind. Using 300 injections of monoclonal antibody (mAb) over the course of 25 hours we monitor signal intensity and mass accuracy with a single calibration prior to injection.

MATERIALS AND METHODS

Sample Preparation:
NIST reference standard was purchased from NIST (Gaithersburg, MD, USA) while trastuzumab and adalimumab were purchased from Myoderm (Norristown, PA, USA). Premixed mobile phases were purchased from VWR (Radnor, PA, USA).

All samples were diluted with 0.1% formic acid in water to a final concentration of 0.1 µg/µl.

HPLC Conditions:
An ExionLC™ system with a Waters MassPREP™ micro desalting (2.1 mm x 5 mm, 1000 Å, 20 µm, Waters, Milford, MA, USA) column at 80˚ C was used for desalting with a gradient consisting of mobile phase A: water + 0.1 % formic acid and mobile phase B: acetonitrile + 0.1 % formic acid. Total flow rate was 0.5 – 0.2 ml/min at for a total run time of 5 min. Gradient went from 5% B to 90% B over 2 min. with a sawtooth gradient to wash the column afterwards. Injection volume was set to 5 µl.

MS/MS Conditions:
An AB Sciex X500B QTOF system with IonDrive™ source and Electrospray Ionization (ESI) probe was used. Data was acquired using intact protein mode turned on and the detector voltage decreased. Data was acquired using a TOF-MS cycle time of 0.5 sec with settings of GS1 50, GS2 50, CUR 35 and TEM of 400˚ C. Acquisition mass range was set from 900 – 4000 m/z.

Data Processing:
Data was processed using BioPharmaView™ software. A reference injection of each mAb was used to compare the data against. Processing parameters were set to use 2000 – 4000 m/z from the data acquisition to reconstruct the protein. Protein reconstruction mass range was set according to the mAb processed. Resolution was set to 5000 and smoothing was set to 5. G0F/G0F and G0F/G1F were used to calculate mass error in ppm.

RESULTS

A single calibration was run after which 3 monoclonal antibodies were run alternating injections across 25 hours, corresponding to 300 injections. Injections were in the running order of NIST standard, trastuzumab and rituximab. Raw data, reconstructed dated and mass error across the injections was interrogated.

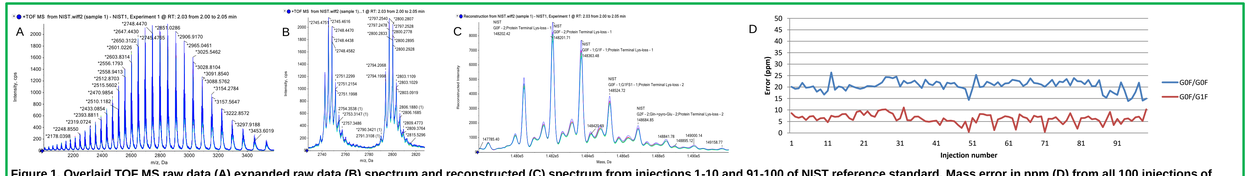

Figure 1. Overlaid TOF MS raw data (A) expanded raw data (B) spectrum and reconstructed (C) spectrum from injections 1-10 and 91-100 of NIST reference standard. Mass error in ppm (D) from all 100 injections of NIST reference standard.

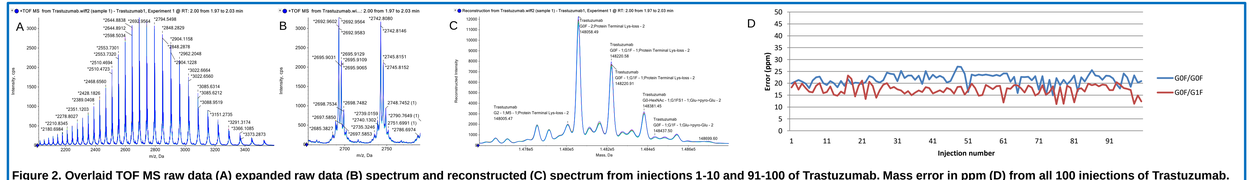

Figure 2. Overlaid TOF MS raw data (A) expanded raw data (B) spectrum and reconstructed (C) spectrum from injections 1-10 and 91-100 of Trastuzumab. Mass error in ppm (D) from all 100 injections of Trastuzumab.

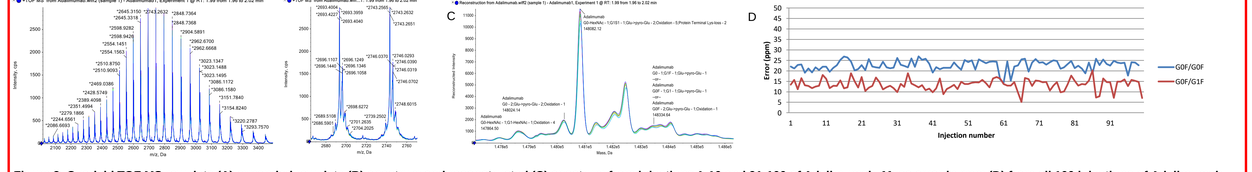

Figure 3. Overlaid TOF MS raw data (A) expanded raw data (B) spectrum and reconstructed (C) spectrum from injections 1-10 and 91-100 of Adalimumab. Mass error in ppm (D) from all 100 injections of Adalimumab.

The first ten and last ten injections from NIST reference standard, trastuzumab and adalimumab were overlaid as raw and reconstructed masses and the signal intensities were compared as shown in Figures 1 (A-C), 2(A-C) and 3(A-C) respectively. It is clearly shown that no significant difference in spectral quality or intensity is detectable. In fact, the groups of spectra are almost perfectly superimposable in all cases investigated. The time difference between the groups of overlaid spectra is calculated to be approximately 25 hours, demonstrating the robustness of the X500B system over a significant period of analytical time.

Another key feature for a routine workflow instrument is the ability to maintain mass accuracy. To show that the X500B not only maintains sensitivity, but also maintains mass accuracy over a similar time period, a single calibration alone was made prior to all 300 injections. Mass accuracy was then monitored over the subsequent batch. Mass error in ppm was extracted from BioPharmaView™ software and the data for the G0F/G0F and G0F/G1F isoforms was plotted against injection number.

Figures 1D, 2D and 3D show the mass error in ppm of G0F/G0F and G0F/G1F glycoforms of NIST reference standard, Trastuzumab and Adalimumab respectively. G0F/G0F and G0F/G1F were chosen since they are common to all three mAb samples analyzed and due to such, comparisons can easily be made. This data shows clearly that following a single calibration, mass accuracy is maintained to less than 25 ppm (some data points are higher than 25ppm) over the course of this 25 hour analytical time window.

CONCLUSIONS

In total, 300 injections were performed over a period of 25 hours, using 3 different mAb, NIST reference standard, Trastuzumab and Adalimumab alternating injections of the three. Comparisons of the first injections to the last injections shows no difference in intensity, confirming that over time there is no loss in sensitivity. Mass accuracy over the 25 hours is maintained under 25 ppm and does not show a mass drift which can be common with TOF instruments.

Biopharmaceutical production requires the ability to carry out batch-to-batch analyses with a high degree of reliability and robustness in a routine environment, to determine whether or not a batch has been manufactured within specified parameters to ensure safety and efficacy. The X500B system has been shown to have these qualities and is capable of generating complex data from these analyses, giving manufacturers high quality information about their biopharmaceutical they can trust, previously unattainable from a single routine analytical workflow.

TRADEMARKS/LICENSING

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