IMPROVEMENTS IN SENSITIVITY FOR BIOTHERAPEUTICS USING A XEVO TQ-XS TANDEM QUADRUPOLE MASS SPECTROMETER

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INTRODUCTION

Biotherapeutics are increasingly becoming a significant part of the pharmaceutical arsenal as more and more companies work towards using them individually or in combination with other large or small molecules as drugs of choice. This trend manifests itself in the growing number of bioanalytical laboratories across the globe incorporating technological and scientific expertise required to deal with the complexities such analyses bring. The diversity within biotherapeutics ranges from small linear or cyclic peptides, all the way to complex monoclonal antibodies and antibody drug conjugates. The instruments of choice for performing these types of analysis are largely tandem quadrupole mass spectrometers, like the Waters Xevo TQ-S.

Because of the nature of biotherapeutics, one of the biggest analytical challenges for scientists is to be able to distinguish and separate out analyte of interest from a very complex matrix background. The resultant increase in signal to noise allows for lower LLOQ's. Sample preparation tools significantly help with this problem. Tandem quadrupole instruments, by principle, further help in attaining this goal by better focusing the ions of interest and removing the neutrals and other matrix ions.

Biotherapeutic peptides and monoclonal antibodies were tested using the Xevo TQ-XS systems. Vancomycin and Infliximab were chosen as candidate molecules to represent some of the common classes of biotherapeutics currently in clinic.

Figure 1. Xevo TQ-XS Tandem Quadrupole Mass Spectrometer

METHODS

Vancomycin

Vancomycin in a branched, tricyclic non-ribosomal peptide antibiotic which was discovered in 1953 and has been used for over 60 years in clinical practice to treat multiple types of infections and inflammation. Vancomycin purchased from Sigma Aldrich was spiked in buffer to obtain a calibration curve from 100 pg/mL to 1 µg/mL. A ACQUITY BEH C18 column was used with generic LC conditions as shown below. The MS was optimized for the Vancomycin transition.

MS Conditions:
Capillary Voltage—3 kV
Cone Voltage—50 V
Source Temperature—150°C
Desolvation Temperature—400°C
Cone Gas Flow—150 L/Hr
Desolvation Gas Flow—800 L/Hr
Transition—724.8—>144.13

LC Conditions:

Time	Flow Rate (µL)	%A	%B	Curve
Initial	0.600	95	5	6
0.50	0.600	90	10	6
3.50	0.600	20	80	6
3.60	0.600	5	95	6
4.00	0.600	5	95	6
4.10	0.600	95	5	6
4.50	0.600	95	5	6

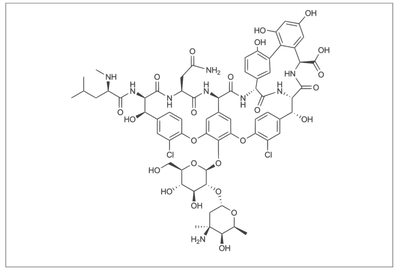

Figure 2. Molecular structure of Vancomycin

Infliximab

Infliximab is a chimeric humanized mouse monoclonal antibody therapeutic developed against TNF-α and is indicated for Crohn's disease, ulcerative colitis, psoriasis, psoriatic arthritis, ankylosing spondylitis and rheumatoid arthritis.

Intact Infliximab was spiked into rat serum to generate a calibration curve and QC samples. All samples and QC's were then digested using the Waters ProteinWorks digest kits. Briefly, the samples were denatured, reduced, alkylated and digested as per the protocol provided in the kit. The protocol has been optimized for use across biological therapeutics or biomarkers in a biological matrix. The simplified protocol reduces method development time significantly and provides scientists with an uncomplicated, kitted approach to digestion which can be very easily standardized and transferred across laboratories.

Generic LC and MS conditions were used and the same set of samples were injected into the Waters Xevo TQ-S and the Xevo TQ-XS. SINS peptide was chosen as the primary quantitation peptide. Waters mAb was added as the internal standard and a signature peptide from the Waters mAb was chosen as the internal standard peptide.

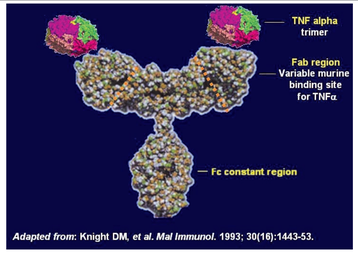

Figure 3. Schematic structure of Infliximab

RESULTS

Significant improvements in analyte area counts were observed for the molecules tested across the entire concentration range.

For Vancomycin, a >10 fold increase in analyte area counts was observed across the range of 100pg/mL to 1 µg/mL. The signal to noise also increased by >5 across the entire concentration range. A chromatographic comparison between the Xevo TQS and Xevo TQ-XS is shown in the figure below.

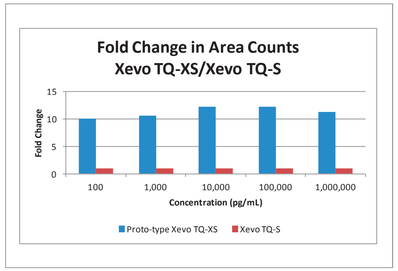

Figure 4. Fold change in area counts for Vancomycin across different concentrations

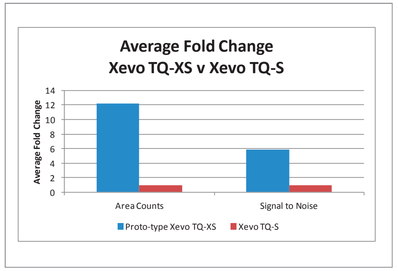

Figure 5. Average fold change in Area counts and Signal to Noise for Vancomycin

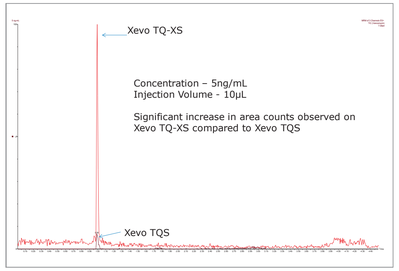

Figure 6. Chromatogram overlay for the same sample injected on Xevo TQ-S and Xevo TQ-XS

For digested Infliximab, data from the most sensitive (SINS) peptide is shown below along with the digested internal standard peptide. On average, there was an increase in the analyte area counts for these peptides between 2-3 fold form a complex digested biological matrix.

The ProteinWorks kit used for digestion yielded a simple protocol with a shortened sample preparation time of around 5 hours including reduction, alkylation and digestion

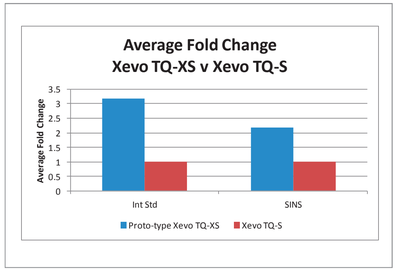

Figure 7. Average fold change in Area Counts for Infliximab and internal standard peptides

DISCUSSION

Quantitation of proteins using the surrogate peptide approach is rapidly becoming a key part of most bioanalytical laboratories have traditionally focused on performing small molecule quantification. Protein/peptide quantification brings with it unique challenges which are different from those typically experienced with a small molecule assay. Scientists are having to learn to navigate these challenges quickly. Advancements in instrumentation technologies continue to enhance the quality and speed of data generated.

The Xevo TQ-XS instrument has a newly designed Stepwave ionguide. The Stepwave has a second stage featuring a segmented quadrupole design for a focused ion beam and increased ion transmission.

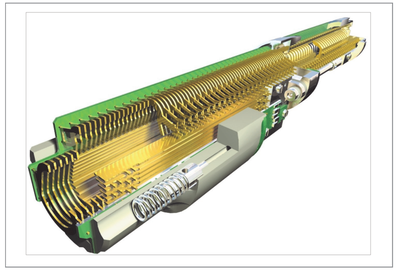

Figure 8. Stepwave device with a second stage segmented quadrupole

CONCLUSION

- **The new Xevo TQ-XS tandem quadrupole Mass Spectrometer has shown significant improvements in analyte area counts as well as signal to noise compared to the current best in class Xevo TQ-S.**
- **These improvements are seen across a wide variety of small and large biomolecules in buffer as well as in matrix.**

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