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

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 mass spectrometry systems, like the Waters Xevo TQ-S.

Because of the emergence 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 result is an increase in signal to noise allows for lower LOD/QC. Sample preparation tools specifically 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 matrix ions and neutrals and other matrix ions.

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

METHODS

Vancomycin

Vancomycin in a branched, tricyclic non–biological 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 UPLC C18 column was used with general LC conditions as shown below. The MS was optimized for the Vancomycin transition.

LC Conditions:
- Capillary Voltage: –3 kV
- Source Temperature: 150°C
- Desolvation Temperature: 400°C
- Cone Gas Flow: 150 L/Hr
- De-Solvation Gas Flow: 600 L/Hr
- Transition: TQ-S

MS Conditions:
- MS/MS Voltage: 50 V

Infliximab

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

Impact Infliximab was spiked into rat serum to generate a calibration curve and QC samples. All LC/MS/MS transitions were generated 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 biotherapeutics 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 should be very easy to standardize and transferred across laboratories.

General 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 ProteinWorks kit was used for digestion which added an internal standard peptide from the Waters MSO kit as the internal standard peptide.

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 100 pg/mL to 1 µg/mL. The signal to noise also increased by >5 compared to the current best in class Xevo TQS.

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 TQS.
• These improvements are seen across a wide variety of large and small biomolecules in buffer as well as in matrix.