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LC-MS/MS ANALYSIS OF MABS USING A MONOCLONAL ANTIBODIES QUANTIFICATION KIT – SPOTLIGHT ON INFLIXIMAB

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INTRODUCTION

Therapeutic monoclonal antibodies (t-mAbs) have been a revolution in the therapeutic tools available to clinicians for treating a variety of conditions. In the era of personalized medicine, there is increasing awareness of the need to measure mAbs for the purposes of dose optimization and cost management. The use of Ligand Binding Assay (LBA) based techniques for measuring mAbs is well established but has some limitations, including poor performance, lack of standardization, a high cost when processing a limited number of samples, limited dynamic range, and the potential for cross-reactivity. Moreover, commercial kits are only available for a limited number of mAbs. Mass spectrometry, a technology widely used in clinical laboratories for monitoring small molecules, is an interesting alternative to overcome these limitations. Here we demonstrate Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS) associated with the ready-to-use commercial mAbXmise™ kit is a simple way to implement mAbs measurement for the anti-TNFα mAb, infliximab, providing high analytical performance, ease of use, and high flexibility for laboratory personnel.

METHODS

Materials

- The mAbXmise Inflammation Kit contains mAb based calibrators, QCs and Internal standards for seven mAbs, including infliximab. In addition, the Kit contains the sample preparation reagents and consumables to extract the mAbs.
- The infliximab calibrators range from 2—100µg/mL, with QCs at 4 and 25µg/mL.

Sample Preparation using the mAbXmise Kit

- The mAbXmise Instructions For Use (IFU) were followed to prepare the samples for LC-MS/MS analysis, with the sample preparation workflow highlighted in Figure 1.
- 20µL plasma samples, including calibrator and quality controls, are dispensed into the mAbXmise plates containing lyophilized stable labelled infliximab.
- Samples are transferred to the PuriXmise plate to perform the immunocapture of infliximab and allows washing of the sample to reduce matrix interferences. Samples are then eluted into a collection plate and evaporated to dryness.
- Samples are resuspended and the protease (CutXmise) is added to the sample to digest infliximab overnight. The digestion is quenched, with the samples immediately ready for analysis.

Figure 1. The LC-MS/MS workflow for the analysis of infliximab using the Promise Proteomics mAbXmise Kit (https://www.mabxmise.com/)

LC-MS/MS Parameters

- Injection was performed in partial loop mode using an ACQUITY™ UPLC™ I-Class FL System. Infliximab tryptic peptides were separated on the XSelect™ Premier HSS T3, 2.1mm x 50mm, 2.5µm Column using an acetonitrile/water/formic acid gradient with a 4.5 minute run time
- Detection was performed using a Xevo™ TQ Absolute Mass Spectrometer, with MRM transitions targeting the infliximab tryptic peptides (Figure 2) and their SIL internal standards.
- Samples were re-analyzed on the Xevo TQ-S micro Mass Spectrometer using the same ACQUITY UPLC System, mobile phase and column.

RESULTS

Analytical Sensitivity

- Multiple signature tryptic peptides were measured for IFX and it was found that SIN peptide followed by ASQ peptide provided the greatest level of analytical sensitivity at the 2µg/mL calibrator standard for IFX with both the highest peak response and Signal:Noise (S/N) using the Xevo TQ-XS MS and Xevo TQ-S micro MS (Figure 2).

INFLIXIMAB CAN BE QUANTIFIED BY LC-MS/MS USING AN EASY TO IMPLEMENT SAMPLE PREPARATION KIT

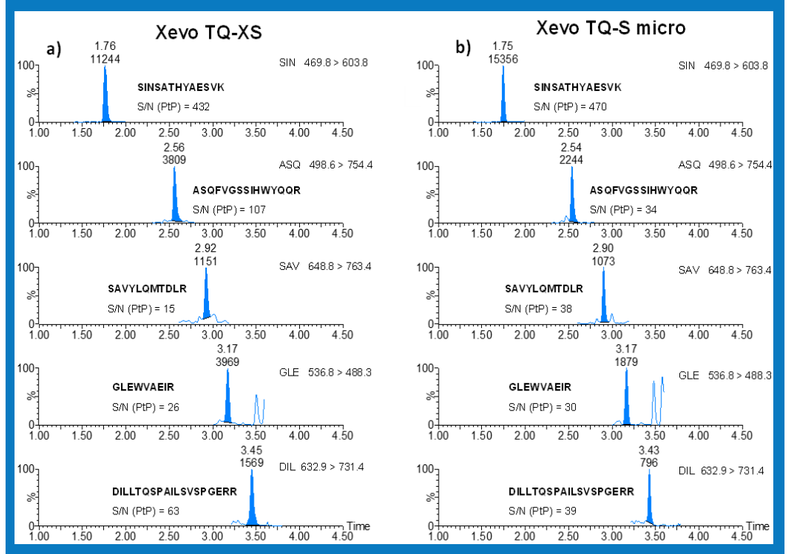

Figure 2. Detection of infliximab signature peptides in the lowest calibrator at 2µg/mL using both the Xevo TQ-XS MS (a) and Xevo TQ-S micro MS (b)

Link to Application Brief

Linearity and QC Precision

- The calibration lines were found to be linear with $r^2 > 0.998$ for each of the peptides on both systems.
- Precision using the Xevo TQ-XS and Xevo TQ-S micro Mass Spectrometers was evaluated using the provided QC material over five replicates at 4 and 25µg/mL.
- Total precision across the QC concentrations were ≤9.5% CV for the signature peptides of IFX (Figure 3) with accuracies ranging from 95 -108% compared to the nominal IFX concentrations.

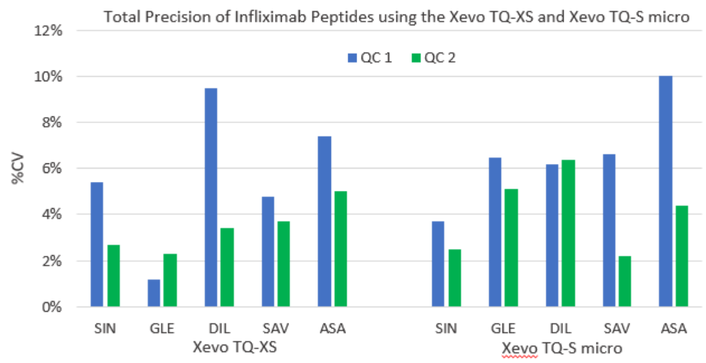

Figure 3. Calculated matrix effects based on both Tg FSP peak area and FSP:FSP SIL response ratio across six individual human serum samples

Peptide Comparison

- 29 anonymized plasma samples from Synnovis, UK were evaluated using the Kit method.
- Box and whisker plots were used to illustrate the differences in infliximab tryptic peptide concentrations for infliximab (Figure 4).

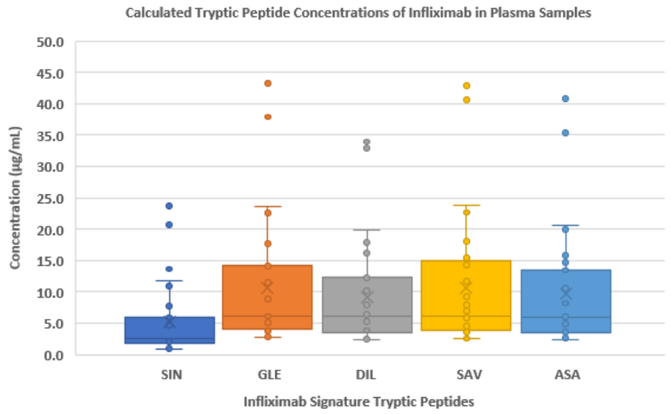

Figure 4. Comparison of the LC-MS/MS signature peptide concentrations for 29 IFX plasma samples, analyzed using the Xevo TQ-XS Mass Spectrometer

- The mean reproducibility for the five peptides across the 29 samples was much higher (CV 29%) compared to the mean reproducibility of the four peptides with SIN removed (CV 10%).
- The reduction in the IFX SIN peptide concentration could be explained by its susceptibility to deamidation at the asparagine-serine (N-S) motif, resulting in a 0.98 Da shift, which is missed during targeted MRM detection.

CONCLUSION

- **A commercially available kit for quantifying Infliximab in plasma was demonstrated on two types of MS/MS system, with analytical sensitivity down to 2µg/mL.**
- **The use of the Promise Proteomics mAbXmise Kit makes the analysis accessible and easy to implement, while being also amenable to automation.**
- **The sample preparation protocol with the Kit reduces matrix interference, improves analytical sensitivity and enables the use of lower sample volumes.**

Disclaimer *The data presented in this poster combine the use of a kit dedicated to the preparation of samples and the use of liquid chro matography and mass spectrometry instrumentation to perform the quantitative analyses. The mAbXmise Kit described has not been cleared by any regulatory entity for diagnostic purposes outside of Europe. The end user is responsible for completion of the method development and validation. Promise Prot eomics mAbXmise Kits are not available for sale in all countries. For information on availability, please contact your local sales representative.*