LC-MS/MS Analysis of mAbs Using a Monoclonal Antibodies Quantification Kit – Spotlight on Infliximab

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Background: The use of immunoassays 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. Using LC-MS/MS associated with the ready-to-use commercial mAbXmise Kit is a simple way to implement mAbs measurement for infliximab, providing high analytical performance, ease of use, and flexibility.

Methods: Briefly, 20µL samples 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 allow washing of the samples. Samples are eluted into a collection plate, evaporated to dryness, 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. Using an ACQUITY™ UPLC™ I-Class PLUS FL System, samples were injected onto a Waters XSelect™ Premier HSS T3 Column, eluted using a water/acetonitrile/formic acid gradient profile and quantified with both a Waters Xevo™ TQ-XS and Xevo TQ-S micro Mass Spectrometer.

Results: The method was linear from 2-100µg/mL, with analytical sensitivity at lowest calibrator of 2µg/mL provided S/N (PtP) ≥15:1 for all tryptic peptides. Coefficients of variation (CV) at 4 and 25µg/mL QC concentrations were all ≤ 10.0% (n=5). The relative concentrations of the tryptic peptides were compared across 29 anonymized samples and good agreement was observed for GLE, SAV, DIL and ASA peptides. The SIN peptide demonstrated significant negative method bias, which could be attributed to its susceptibility to deamidation.

Conclusions: We have demonstrated a clinical research method for quantifying Infliximab in plasma using a commercially available kit for sample preparation followed by LC-MS/MS analysis.

The data presented combines the use of a kit dedicated to the preparation of samples and the use of liquid chromatography 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. Proteomics mAbXmise Kits are not available for sale in all countries.