



A Sensitive and Robust Immunocapture LC-MS/MS Workflow for Quantitation of Infliximab in Human Plasma

Increasing sensitivity for better accuracy, robustness, and LLOQ when quantitating Infliximab in complex biological samples

SCIEX iMethod™ Applications for Pharma and BioPharma

Key Challenges of Infliximab Quantitation Using ELISA Assay

- **Substandard data quality** – Precision and accuracy are compromised at low levels due to interferences.
- **Limited linear dynamic range and hook effect** – Hook effect is known limitation for ELISA assay which causes false negative or artificial lower results. Only up to three orders of dynamic range for most ELISA assay.
- **Limitations on multiplexing assay (MPX):** –MPX assay involves potential interactions between multiple different antibodies and antigens in the sample/assay solution.

Key Benefits of the BioBA Solution for Quantifying Infliximab

- **Sample preparation** – Increased efficiency with included reagent kit, sample preparation SOP, and LC-MS/MS detail method
- **Mass spec selectivity:** – Quantitation infliximab antibody using unique peptide sequence with highly reproducibile and accurate quality data even at low end.
- **Easy to multiplexing on Mass spec:** – By simply adding other biological compound unique peptide MRM transitions, the method can monitor large number of biological analytes in one injection without concerning interferences and compromise data quality.
- **Maximized sensitivity** – QTRAP® 6500 LC-MS/MS Increased ionization efficiency and heat transfer with the new IonDrive™ Turbo V source and Increased ion sampling efficiency and ruggedness with the new IonDrive QJet™ ion guide results in LOQ of 5 ng/ML
- **Large linear dynamic range** – Measurements tested from 5–50,000 ng/mL are linear with 5-orders of magnitude ($r = 0.99943$).
- **Wide mass range** – range of m/z 5 – 2000 provides versatility for large peptide quantitation

Results and Discussion

Sensitivity and linearity of quantitation

A calibration curve of infliximab standards in human whole plasma matrix (5 – 50,000 ng/mL) was generated using MultiQuant™ Software (Figure 1). The tested limit of quantification (LOQ) was 5 ng/mL in plasma. Linearity was achieved from 5-50,000 ng/mL with regression coefficient (r) of 0.99943.

Calibration for YASEMSGIPSR.+2y10 LC: $y = 0.06095 x + 0.10695$ ($r = 0.99943$) (weighting: 1 / x)

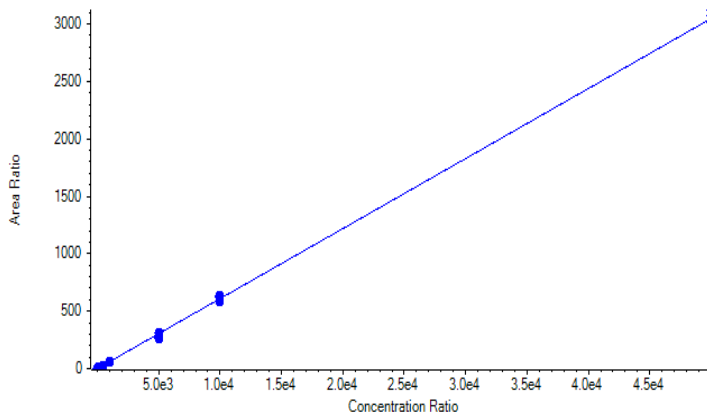


Figure 1: Linear response for Infliximab peptide in human plasma. Calibration range: 5 ng/mL – 50 µg/mL

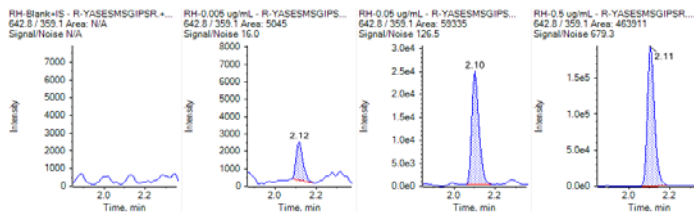


Figure 2: Chromatograms spiked Infliximab in human plasma: blank plasma, 5, 50, and 500 ng/mL

Table 1: Quantitation Statistics of Infliximab in Human Plasma Using Conventional HPLC System

Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy	Value #1	Value #2	Value #3
YASEMSGIPSR +2y10 LC	5.00	3 of 3	5.61	0.49	8.43	116.27	5.95	6.22	5.27
YASEMSGIPSR +2y10 LC	10.00	3 of 3	10.50	0.15	1.44	104.95	10.37	10.66	10.46
YASEMSGIPSR +2y10 LC	50.00	3 of 3	46.80	3.30	7.06	93.60	50.32	46.32	43.76
YASEMSGIPSR +2y10 LC	100.00	3 of 3	99.91	7.43	7.43	99.91	107.46	99.64	92.62
YASEMSGIPSR +2y10 LC	500.00	3 of 3	445.13	7.58	1.70	89.03	451.34	447.37	436.68
YASEMSGIPSR +2y10 LC	1000.00	3 of 3	1016.93	41.30	4.06	101.69	1063.02	983.32	1004.44
YASEMSGIPSR +2y10 LC	5000.00	3 of 3	4856.39	385.98	8.29	93.13	5080.43	4329.52	4563.22
YASEMSGIPSR +2y10 LC	10000.00	3 of 3	10081.91	382.72	3.90	100.82	10332.51	10283.92	9629.31
YASEMSGIPSR +2y10 LC	50000.00	3 of 3	50301.62	891.58	1.77	100.60	50322.63	51162.51	49399.72

Conclusion

- The SCIEX Triple Quad™ LC-MS/MS and QTRAP 6500 systems with IonDrive technology provide high sensitivity to perform high throughput peptide quantitation
- The peptide properties, stability, and non-specific adsorption for insulin infliximab were considered as part of the method development process, resulting in a robust quantitative assay
- Infliximab levels were robustly quantified at 5ng/mL with less than 10% CV in human plasma using a conventional high flow LC methodology. The linear dynamic range was 5– 50,000ng/mL. The quantitation limit and calibration range can be adjusted based on specific assay requirements.

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RUO-MKT-02-2251-A