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 reproducible 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.

**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
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.

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

<table>
<thead>
<tr>
<th>Component/Conc</th>
<th>Actual Concentration (ng/mL)</th>
<th>R.S.D. %</th>
<th>Mean (ng/mL)</th>
<th>R.S.D. %</th>
<th>Mean (ng/mL)</th>
<th>R.S.D. %</th>
<th>Mean (ng/mL)</th>
<th>R.S.D. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>50,000</td>
<td>3.33</td>
<td></td>
<td>100.00</td>
<td>3.33</td>
<td>100.00</td>
<td>3.33</td>
<td>100.00</td>
<td>3.33</td>
</tr>
<tr>
<td>10,000</td>
<td>3.00</td>
<td></td>
<td>100.00</td>
<td>3.00</td>
<td>100.00</td>
<td>3.00</td>
<td>100.00</td>
<td>3.00</td>
</tr>
<tr>
<td>1,000</td>
<td>3.00</td>
<td></td>
<td>100.00</td>
<td>3.00</td>
<td>100.00</td>
<td>3.00</td>
<td>100.00</td>
<td>3.00</td>
</tr>
<tr>
<td>100</td>
<td>3.00</td>
<td></td>
<td>100.00</td>
<td>3.00</td>
<td>100.00</td>
<td>3.00</td>
<td>100.00</td>
<td>3.00</td>
</tr>
<tr>
<td>10</td>
<td>3.00</td>
<td></td>
<td>100.00</td>
<td>3.00</td>
<td>100.00</td>
<td>3.00</td>
<td>100.00</td>
<td>3.00</td>
</tr>
</tbody>
</table>

For Research Use Only. Not for use in diagnostic procedures.
© 2015 AB Sciex. The trademarks mentioned herein are the property of AB Sciex Pte. Ltd. or their respective owners. AB SCIEX™ is being used under license.

RUO-MKT-02-2251-A