A Universal Immunocapture-LC-MS/MS Workflow for Biological Compound Quantitation in Preclinical Studies – Adalimumab

Increasing sensitivity and selectivity for better accuracy, robustness, and LLOQ when quantitating biological compounds in complex pre-clinical biological samples

SCIEX iMethods for Pharma and BioPharma

Key Challenges of Biological Compound Pre-Clinical Quantitation Using ELISA Assay

- **Lack of selectivity** – In discover, generic antibody was typically used in ELISA assay for new biological compound candidate screening which caused lacking of selectivity.
- **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 BioBA Kit integrate with QTRAP® 6500 for quantifying pre-clinical samples

- **Completed solution for sample preparation** – Include BioBA reagent kit, step by step sample preparation SOP, and LC-MSMS detail method
- **Mass spec selectivity:** – Quantitation antibody using unique peptide sequence with highly reproducible and accurate quality data even at low end.
- **Easy to MPX 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 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 5 ng/mL.
  - **Large linear dynamic range** – Measurements tested from 5–100,000 ng/mL are linear with over 5-orders of magnitude (r = 0.99854).
  - **Wide mass range** – range of m/z 5 – 2000 provides versatility for large peptide quantitation

Results and Discussion

**Sensitivity of Quantitation**

A calibration curve of adalimumab standards in rat plasma matrix (5 – 100,000 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 5-100,000 ng/mL with regression coefficient (r) of 0.99854.
Figure 2: Example calibration curve for adalimumab on conventional flow LC

Figure 3: XICs of adalimumab transitions from standard spike-in rat plasma samples (blank, 5 ng/mL, and 50 ng/mL).

Table 1: Statistic of adalimumab quantitation statistics using conventional flow LC

<table>
<thead>
<tr>
<th>Component No.</th>
<th>Actual Conc.</th>
<th>N. of Val</th>
<th>Mean</th>
<th>Standard Dev.</th>
<th>Percent CV</th>
<th>Accuracy 1</th>
<th>Value 1</th>
<th>Error 1</th>
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<tr>
<td>H-APYTFQGGQ</td>
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<td>0.050</td>
<td>0.005</td>
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<td>95.26</td>
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<td>0.044</td>
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<td>7.67</td>
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<td>2.16</td>
<td>100.35</td>
<td>0.029</td>
<td>0.425</td>
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<td>1.59</td>
<td>96.02</td>
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<td>102.54</td>
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<td>100.222</td>
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</tbody>
</table>

Conclusion

- The BioBA solution provided a generic ease of use complete method solution for discovery pre-clinical quantitation analysis with selective and accurate results.
- The mass spectrometer method overcomes the major challenges that ELISA assay encountered. The SCIEX Triple Quad™ and QTRAP® 6500 systems with IonDrive™ technology provide high sensitivity with board linearity range to perform high throughput peptide quantitation.
- Adalimumab peptide properties, stability, and non-specific adsorption were considered as part of the method development process, resulting in a robust quantitative assay.
- Adalimumab levels were robustly quantified using a conventional high flow LC methodology. In tested low end of quantitation 5ng/mL was found to be accurate and reproducible with over 5 orders of linearity dynamic range.