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

Increasing sensitivity for better accuracy, robustness, and LLOQ when quantitating complex biological compounds

SCIEX iMethods™ for Pharma and BioPharma

Key Challenges faced in pre-clinical quantitation of biological compounds 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 BiaoBA 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 below LOQ 5 ng/mL based on sample volume and assay requirement.

Results and Discussion

**Sensitivity and linearity of quantitation**

A calibration curve of trastuzumab standards in rat plasma matrix (10 – 50,000 ng/mL) was generated using MultiQuant™ Software (Figure 1). The tested limit of quantification (LOQ) was 10 ng/mL using 25 uL of rat plasma. Linearity was achieved from 10-50,000 ng/mL with regression coefficient (r) of 0.996.
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.

Trastuzumab peptide properties, stability, and non-specific adsorption were considered as part of the method development process, resulting in a robust quantitative assay.

Trastuzumab levels were robustly quantified using a conventional high flow LC methodology. In tested low end of quantitation 10ng/mL was found to be accurate and reproducible with over 4 orders of linearity dynamic range.

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Actual Conc.</th>
<th>Nom. Value</th>
<th>Mean</th>
<th>Standard Dev.</th>
<th>Percent CV</th>
<th>Accuracy Value</th>
<th>Value 1</th>
<th>Value 2</th>
<th>Value 3</th>
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<tbody>
<tr>
<td>1</td>
<td>Anti-HER2</td>
<td>1.00</td>
<td>3.0</td>
<td>9.0</td>
<td>1.2</td>
<td>11.09</td>
<td>88.00</td>
<td>9.4</td>
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<td>50.0</td>
<td>2.0</td>
<td>101.33</td>
<td>50.00</td>
<td>15.3</td>
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<tr>
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<td>3.0</td>
<td>82.0</td>
<td>4.0</td>
<td>97.50</td>
<td>82.00</td>
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<tr>
<td>4</td>
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<td>3.0</td>
<td>200.0</td>
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<td>10000.00</td>
<td>3500.0</td>
<td>10000.0</td>
</tr>
</tbody>
</table>

Figure 2: Example calibration curve for trastuzumab on conventional flow LC

Figure 3: XICs of trastuzumab transitions from standard spike-in rat plasma samples (blank, 5 ng/mL, 10 ng/mL and 100 ng/mL).

Conclusion

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