



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*TM 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 reproducibile 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.

- **Large linear dynamic range** – Measurements tested from 10–50,000 ng/mL are linear with over 4-orders of magnitude ($r = 0.996$) using 25 μ L of plasma volume.
- **Wide mass range** – range of m/z 5 – 2000 provides versatility for large peptide quantitation

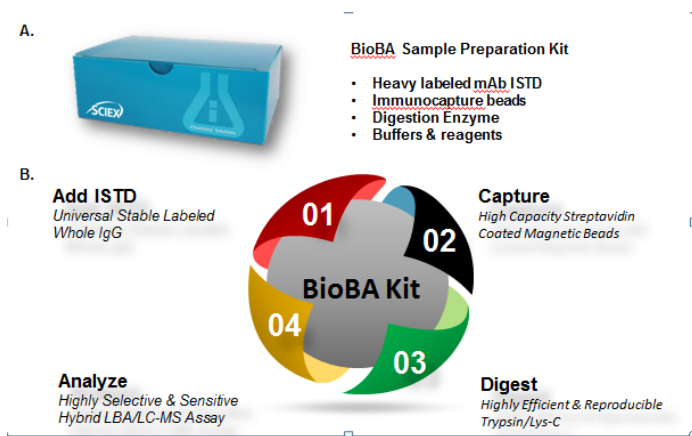


Figure 1. A SCIEX BioBA sample preparation kit. B. Universal immunocapture procedure for human IgG enrichment for preclinical samples

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 μ L of rat plasma. Linearity was achieved from 10-50,000 ng/mL with regression coefficient (r) of 0.996.

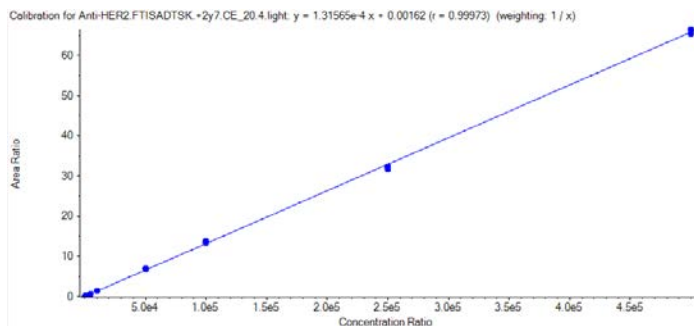


Figure 2: Example calibration curve for trastuzumab on conventional flow LC

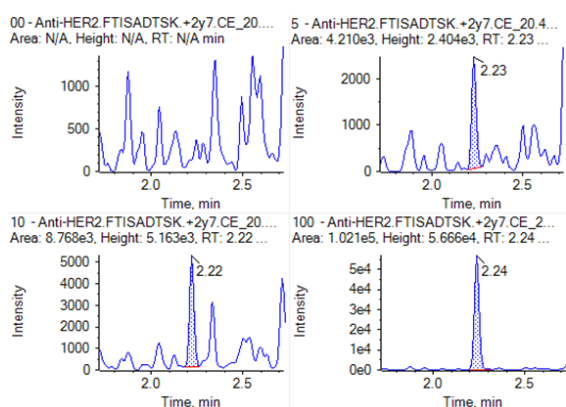


Figure 3: XICs of trastuzumab transitions from standard spike-in rat plasma samples (blank, 5 ng/mL, 10 ng/mL and 100 ng/mL).

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

Row	Component No.	Actual Conc.	Num. Values	Mean	Standard D.	Percent CV	Accuracy	Value #1	Value #2	Value #3
1	Anti-HER2.FTI...	10.00	3 of 3	9.9	1.2	11.99	98.06	9.4	9.0	11.2
2	Anti-HER2.FTI...	50.00	3 of 3	53.7	4.9	9.03	107.43	56.2	48.1	56.9
3	Anti-HER2.FTI...	100.00	3 of 3	97.5	6.3	6.48	97.53	102.3	90.4	99.9
4	Anti-HER2.FTI...	500.00	3 of 3	468.5	17.7	3.78	93.70	454.9	462.0	488.5
5	Anti-HER2.FTI...	1000.00	3 of 3	997.3	46.5	4.66	99.73	1050.9	968.6	972.4
6	Anti-HER2.FTI...	5000.00	3 of 3	5665.4	180.5	3.19	113.31	5738.4	5459.8	5798.1
7	Anti-HER2.FTI...	10000.00	3 of 3	10404.2	217.0	2.09	104.04	10427.3	10176.6	10608.7
8	Anti-HER2.FTI...	50000.00	3 of 3	42701.0	994.5	2.33	85.40	41850.2	42458.3	43794.4

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Conclusion



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