



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

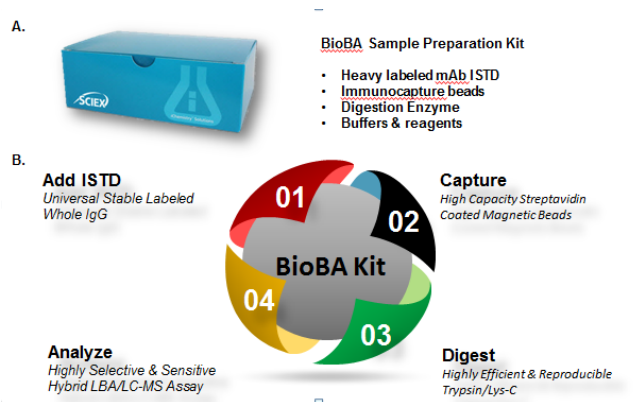


Figure 1. A. SCIEX BioBA sample preparation kit introduction. B. Universal immunocapture procedure for human IgG enrichment for pre-clinical samples

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.

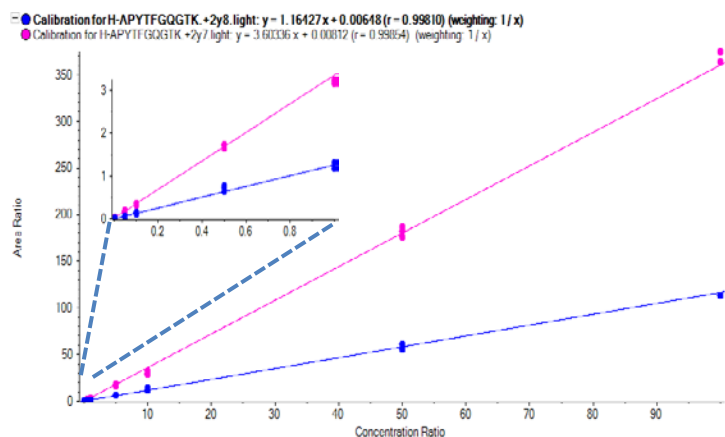


Figure 2: Example calibration curve for adalimumab on conventional flow LC

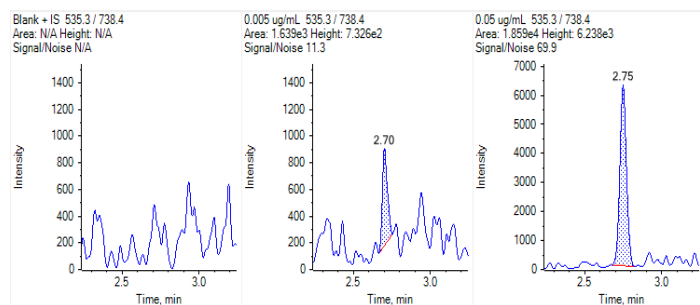


Figure 3: XICs of adalimumab transitions from standard spike-in rat plasma samples (blank, 5 ng/mL, and 50 ng/mL).

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

Component No.	Actual Conc.	Num. Values	Mean	Standard D.	Percent CV	Accuracy	Value #1	Value #2	Value #3
H-APYTFGQG...	0.005	3 of 3	0.005	0.001	14.13	100.63	0.005	0.006	0.004
H-APYTFGQG...	0.050	3 of 3	0.048	0.005	10.84	95.26	0.046	0.044	0.053
H-APYTFGQG...	0.100	3 of 3	0.097	0.007	7.65	97.30	0.089	0.101	0.102
H-APYTFGQG...	0.500	3 of 3	0.502	0.015	2.89	100.35	0.509	0.485	0.511
H-APYTFGQG...	1.000	3 of 3	0.950	0.015	1.55	95.02	0.952	0.964	0.934
H-APYTFGQG...	5.000	3 of 3	5.127	0.321	6.27	102.54	5.162	4.789	5.429
H-APYTFGQG...	10.000	3 of 3	9.029	0.459	5.08	90.29	8.595	8.983	9.508
H-APYTFGQG...	50.000	3 of 3	54.082	1.633	3.02	108.16	52.307	54.419	55.520
H-APYTFGQG...	100.000	3 of 3	110.461	1.939	1.76	110.46	108.222	111.554	111.608

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.

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