A Sensitive and Robust Immunocapture -LC-MS/MS Workflow for Quantitation of Insulin Glargine in Human Plasma

Increasing sensitivity for better accuracy, robustness, and LLOQ when quantitating insulin glargine in complex biological samples

SCIEX iMethods for Pharma and BioPharma

Key challenges of Glargine Quantitation

- **Lack of sensitivity** – Quantification is poorly reproducible at low picogram levels in complex biological matrices.
- **Substandard data quality** – Precision and accuracy are compromised at very low levels, giving results below accepted bioanalytical standards.
- **Background Interferences** – Sample complexity interference even following solid phase extraction cleanup procedures can yield high detectable interferences.

Key benefits of BioBA Solution for Quantifying Insulin Glargine

- **Sample preparation** – Increased efficiency with the included reagent kit, sample preparation SOP, and LC-MS/MS detail method
- **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 LOD of 25 pg/mL and LOQ of 50 pg/ML
- **Large linear dynamic range** – Measurements tested from 50–100,000 pg/mL are linear with close to 5-orders of magnitude (r = 0.99961)
- **Wide mass range** – Range of m/z 5 – 2000 provides versatility for large peptide quantitation

Flexibility of utilizing a Conventional Flow

- **Conventional Flow Robustness** – Be able to utilize a higher injection volume at a higher flow rate maximizes robustness for routine analysis at low picogram levels

Results and Discussion

Sensitivity of Quantitation

A calibration curve of glargine standards in human whole plasma matrix (50 – 100,000 pg/mL) was generated using MultiQuant™ Software (Figure 1). The tested limit of quantification (LOQ) was 50 pg/mL in plasma, and the limit of detection (LOD) was 25 pg/mL in plasma. Linearity was achieved from 50-100,000 pg/mL with regression coefficient (r) of 0.99961.

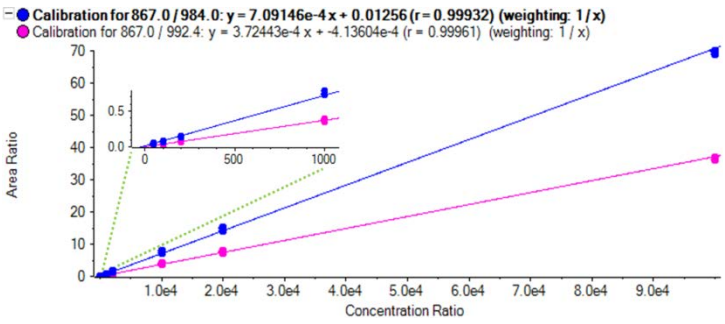

Figure 1: Calibration Curve for Glargine on Conventional Flow HPLC System

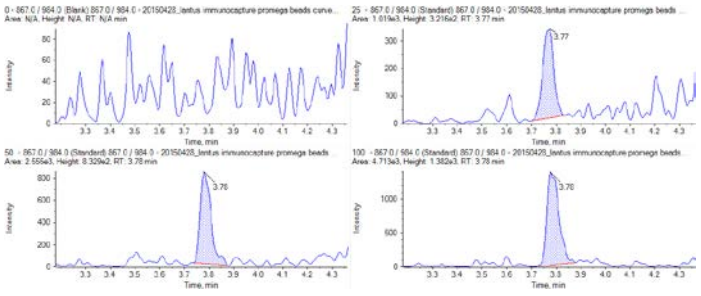

Figure 2: Chromatogram of Glargine for Blank, 25, 50, and 100 pg/mL in Human Plasma using BioBA Sample Preparation Kit on a conventional flow LC.

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

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
1	867.0 / 984.0	50.0	3 of 3	4.9e1	2.1e0	4.2	98.6
2	867.0 / 984.0	100.0	3 of 3	8.9e1	5.7e0	6.4	89.0
3	867.0 / 984.0	200.0	3 of 3	1.8e2	5.8e0	3.3	89.7
4	867.0 / 984.0	1000.0	3 of 3	1.0e3	3.8e1	3.7	104.6
5	867.0 / 984.0	2000.0	3 of 3	2.2e3	3.4e1	1.6	107.7
6	867.0 / 984.0	10000.0	3 of 3	1.1e4	3.5e2	3.2	108.5
7	867.0 / 984.0	20000.0	3 of 3	2.1e4	7.2e2	3.5	103.6
8	867.0 / 984.0	100000.0	3 of 3	9.8e4	8.8e2	0.9	98.3
9	867.0 / 992.4	50.0	3 of 3	5.1e1	6.5e0	12.7	101.6
10	867.0 / 992.4	100.0	3 of 3	9.2e1	8.2e0	8.8	92.3
11	867.0 / 992.4	200.0	3 of 3	1.9e2	1.2e1	6.5	95.4
12	867.0 / 992.4	1000.0	3 of 3	1.0e3	5.4e1	5.4	100.5
13	867.0 / 992.4	2000.0	3 of 3	2.1e3	3.0e1	1.5	102.9
14	867.0 / 992.4	10000.0	3 of 3	1.1e4	2.7e2	2.6	105.9
15	867.0 / 992.4	20000.0	3 of 3	2.0e4	8.8e2	4.3	102.5
16	867.0 / 992.4	100000.0	3 of 3	9.9e4	9.2e2	0.9	98.9

Conclusion

- The SCIEX Triple Quad™ 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 glargine were considered as part of the method development process, resulting in a robust quantitative assay
- Glargine levels were robustly quantified at 50pg/mL in human plasma using a conventional high flow LC methodology. The linear dynamic range was 50 pg/mL – 100,000 pg/mL. The quantitation limit and calibration range can be adjusted based on specific assay requirements.

Publication number: : RUO-MKT-02-2241

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