



# 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.

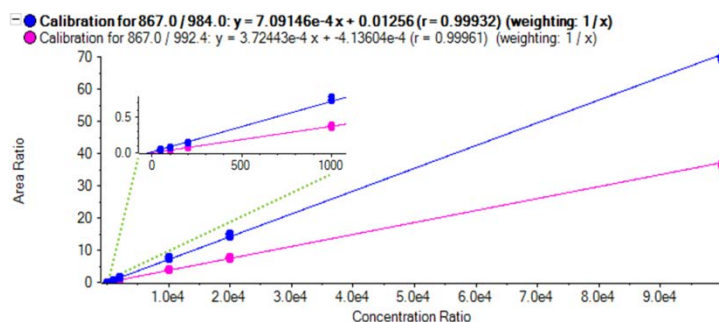


Figure 1: Calibration Curve for Glargine on Conventional Flow HPLC System

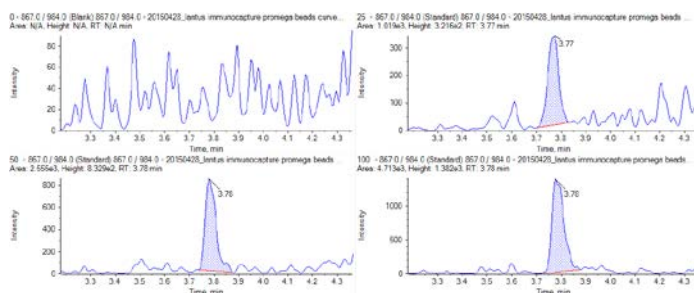


Figure 2: Chromatogram of Glargine for Blank, 25, 50, and 100 pg/mL in Human Plasma using BioBA Sample Preparation Kit on a conventional flow LC.

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.

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