



Highly Sensitive and Accurate Quantification of Glucagon Peptide Hormone in Human Plasma

Increasing sensitivity for better accuracy, robustness, and LLOQ when quantitating Glucagon peptide in complex biological samples

SCIEX iMethods™ for Pharma and BioPharma

Key challenges of Glucagon 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 even following solid phase extraction cleanup procedures can yield detectable interferences.
- **Large linear dynamic range** – Measurements (10–10,000 pg/mL) are linear over 4-orders of magnitude ($r^2 = 0.99964$).
- **Wide mass range** – range of m/z 5 – 2000 provides versatility for large peptide quantitation

Flexibility of utilizing a Conventional Flow or Microflow LC System

- **Conventional Flow Robustness** – Utilizing a higher injection volume at a higher flow rate provides maximal robustness for routine analysis at low picogram levels
- **Microflow Sensitivity** – Minimal injection volumes are needed to achieve low picogram detection for limited samples

Key benefits of QTRAP® 6500 for quantifying Glucagon

- **Maximized sensitivity** – 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 LLOQ of 10 pg/mL

Results and Discussion

Sensitivity of Quantitation

A calibration curve of glucagon standards within human plasma matrix (10 – 10,000 pg/mL) was generated using MultiQuant™ Software (Figure 1). For the conventional and micro flow LC method the limit of quantification (LOQ) was the lowest measured concentration of 10 pg/mL. Linearity was achieved from 10-10,000 pg/mL with regression coefficient (r^2) of 0.99934 and 0.99964 correspondingly .

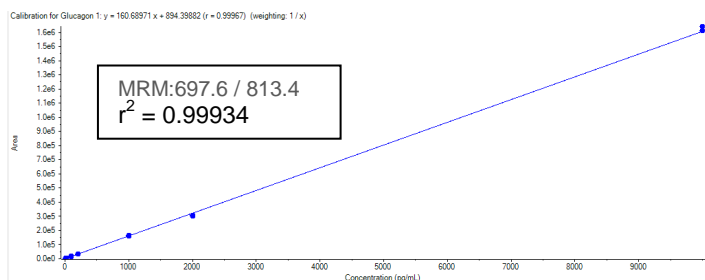


Figure 1: Example Calibration Curve for Glucagon on Conventional flow LC

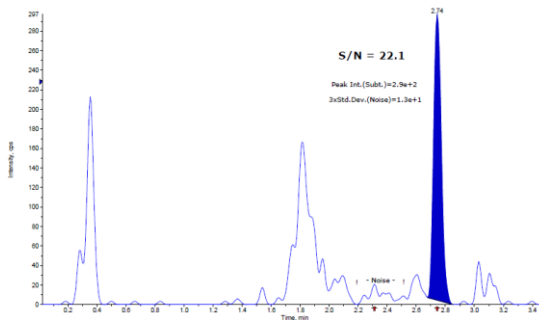


Figure 2: Example Chromatograms of Glucagon in Plasma at 10 pg/mL (697.6 / 813.4) - Conventional flow LC

Table 2: Statistic of glucagon (697.6 / 813.4) quantitation statistics using Conventional flow LC

Actual Concentration	Mean	Standard Deviation	Num. Values	% CV	Accuracy %	Value #1	Value #2	Value #3			
pg/mL									pg/mL		
10	10.3	0.719	3 of 3	6.96	103	9.51	10.8	10.7			
20	19.6	0.974	3 of 3	4.96	98.2	19.4	20.7	18.8			
100	99.5	2.50	3 of 3	2.51	99.5	102	99.2	97.2			
200	204	9.43	3 of 3	4.63	102	199	198	215			
1000	1016	19.7	3 of 3	1.94	102	995	1035	1017			
2000	1893	22.8	3 of 3	1.21	94.6	1909	1867	1903			
10000	10088	109	3 of 3	1.08	101	10033	10214	10018			

Table 3: Glucagon (697.6 / 813.4) quantitation statistics using Microflow LC

Actual Concentration	Mean	Standard Deviation	Num. Values	% CV	Accuracy %	Value #1	Value #2	Value #3			
pg/mL									pg/mL		
10	9.82	0.139	3 of 3	1.41	98.2	9.89	9.91	9.66			
20	18.9	0.605	3 of 3	3.20	94.6	18.6	19.6	18.5			
100	98.9	4.12	3 of 3	4.17	98.9	96.5	103.6	96.5			
200	222	2.15	3 of 3	0.97	111	223	219	222			
1000	974	21.5	3 of 3	2.21	97.4	997	970	954			
2000	2004	53.6	3 of 3	2.67	100	2048	2019	1944			
10000	10003	49.8	3 of 3	0.500	100	10060	9982	9968			

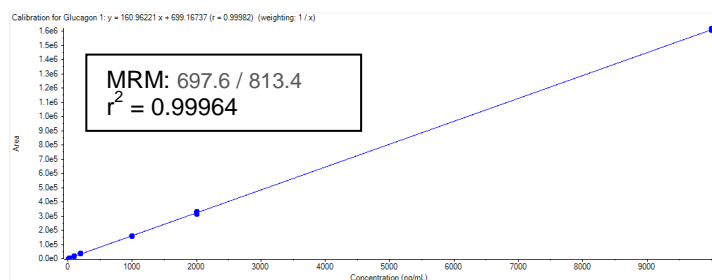


Figure 5: Example Calibration Curve for Glucagon using Microflow LC

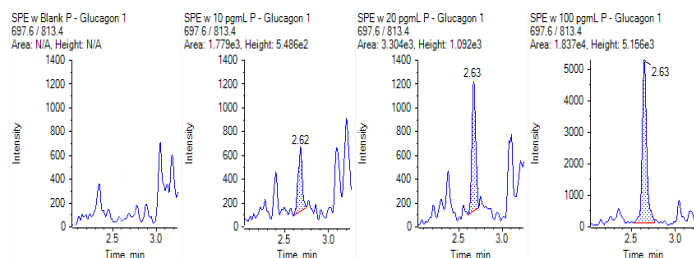


Figure 6: Example Chromatograms of Glucagon (697.6 / 813.4) in Plasma at 0, 10, 20, and 100 pg/mL (Microflow LC)

Conclusion

- The SCIEX Triple Quad™ and QTRAP® 6500 systems with IonDrive™ technology provide high sensitivity to perform high throughput peptide quantitation
- Glucagon peptide properties, stability, and non-specific adsorption were considered as part of the method development process, resulting in a robust quantitative assay
- Glucagon levels could be robustly quantified using a conventional high flow LC methodology using a higher sample injection volume, or utilizing a microflow LC method with lower injection amounts for sample limited assays. In each case 10 pg/mL LOQ was found to be accurate and reproducible.

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