Achieve low-pg/mL quantification of insulin lispro in rat plasma using a high-end triple quadrupole mass spectrometer

Increasing sensitivity for quantification of insulin analogs using the SCIEX 7500 system, powered by SCIEX OS software

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This technical note demonstrates a highly sensitive quantification workflow for insulin analogs on a high-end triple quadrupole mass spectrometer. Intact insulin lispro in rat plasma was used as a model to demonstrate the quantitative performance of the SCIEX 7500 system. A lower limit of quantification (LLOQ) of 50 pg/mL was achieved using a simple sample preparation method paired with an 8-minute LC-MS/MS analysis.

Short-acting insulin analogs, such as insulin lispro, aspart and glulisine, are more readily absorbed and exhibit faster biological actions compared to human insulin.[1] As a result of their key role in insulin therapeutics, it is important to study the pharmacokinetic and pharmacodynamic profiles of insulin analogs. While LC-MS based methods remain the most sensitive and selective platforms for the analysis of insulin therapeutics, analytical challenges prevail. Insulin analogs are often difficult to ionize and fragment, given their high molecular weight and their structural complexity, conferred by disulfide bridging (Figure 1). In addition, insulin analogs are difficult to quantify at low concentrations in biological matrices, given interference from endogenous insulin.

Here, a simple sample preparation paired with a LC-MS/MS method was developed for the sensitive quantification of intact insulin lispro in rat plasma. Enhanced sensitivity for quantification was achieved with front-end improvements on the SCIEX 7500 system for better ion generation, capture and transmission.

Key features of the insulin lispro MRM workflow on the SCIEX 7500 system

- Achieve low-pg/mL level quantification of insulin analogs in rat plasma with outstanding reproducibility, accuracy and linearity using a simple sample preparation and the SCIEX 7500 system
- Reach outstanding sensitivity for insulin analog quantification with improved front-end technology that enables greater ion generation, capture and transmission
- Increase productivity with the user-friendly interface and integrated platform for data acquisition, processing and management in SCIEX OS software

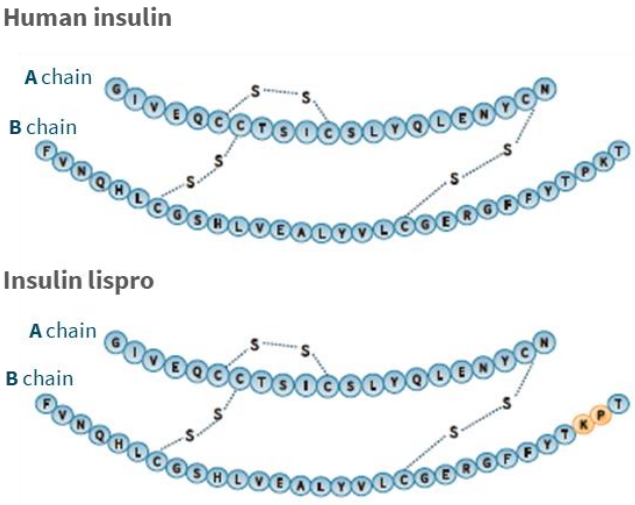


Figure 1. Amino acid sequences of human insulin and insulin lispro. The difference in sequence is highlighted in the B chain of insulin lispro (orange).

Methods

Sample preparation: Insulin lispro standard (50 to 50000 pg/mL) and 10 µL of the internal standard, proinsulin (2000 pg/mL), were spiked into 200 µL of rat plasma. The mixture was precipitated by adding 200 µL of 1:1, methanol/acetonitrile with 1% acetic acid, then centrifuging the mixture at 13,000 rpm for 10 mins. A 400 µL aliquot of the supernatant was mixed with 900 µL of 5% aqueous ammonia. The sample was subjected to solid phase extraction using a Phenomenex StrataX-A microelution 96-well plate following the manufacturer's protocol.

Chromatography: Separation was performed on an Exion LC system, using a Phenomenex Kinetex C18 (3 × 100 mm, 2.6 µm) column. Mobile phase A was 0.2% acetic acid in water and mobile phase B was 0.2% acetic acid in acetonitrile. The operating flow rate was 0.4 mL/min. The column temperature was set to 50°C. The gradient conditions used are summarized in Table 1. A 20 µL aliquot of the sample was injected for LC-MS/MS analysis.

Table 1. Chromatography for intact insulin lispro.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	*90*	*10*
2.0	*50*	*50*
4.0	*10*	*90*
5.0	*10*	*90*
5.1	*10*	*90*
8.0	*90*	*10*

Mass spectrometry: Analysis was performed using a SCIEX 7500 system with the OptiFlow Pro ion source operated in positive MRM mode. The source conditions and MRM transitions used are summarized in Tables 2 and 3, respectively.

Table 2. Source conditions for the SCIEX 7500 system.

Parameter	Settings
Polarity	Positive
Curtain gas	37 psi
Ion source gas 1	40 psi
Ion source gas 2	50 psi
CAD gas	10
Ion spray voltage	2500 V
Source temperature	450°C

Data processing: The MRM data were processed using the Analytics module in the SCIEX OS software, version 2.0 using the MQ4 integration algorithm. A weighting of $1/x^2$ was used for quantification.

Table 3. MRM transitions for insulin lispro and internal standard.

Parameter	Insulin lispro	Internal standard
Precursor ion (m/z)	*968.9*	*994*
Product ion (m/z)	*217.1*	*219.1*
CE (V)	*50*	*45*
CXP (V)	*11*	*14*

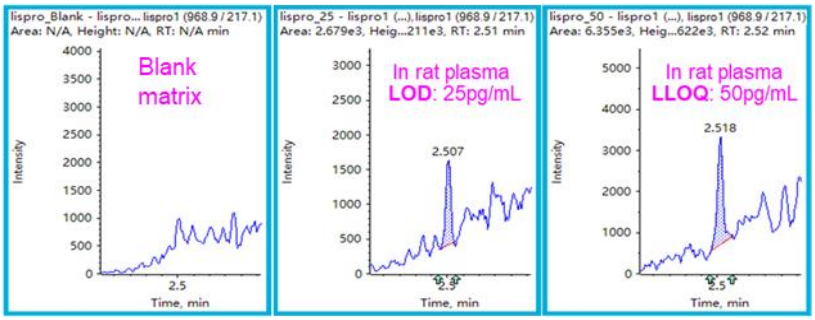
Insulin lispro quantification results

Low abundance, high molecular weight and interference from endogenous components pose significant challenges to the reliable quantification of insulin therapeutics. The SCIEX 7500 system offers enhanced sensitivity for the quantification of insulin analogs in complex matrices with improved front-end features that increase ion generation, capture and transmission.[2]

Quantification of intact insulin lispro in rat plasma was performed using the positive MRM mode on the SCIEX 7500 system (Figure 2). The limit of detection (LOD) was 25 pg/mL and the lower limit of quantification (LLOQ) was 50 pg/mL (Figure 2A). Strong linearity was achieved using a 6-point calibration curve that ranged from 50 to 50000 pg/mL with a correlation coefficient (r^2) of 0.99430 (Figure 2B). The linear dynamic range (LDR) covered 3 orders of magnitude. Figure 2C summarizes the quantitative performance, highlighting the excellent %CV (<12%) and accuracy (95-105%) across the range of 50 to 50000 pg/mL.

Quality control (QC) samples were also analyzed at the low, medium and high concentrations of 100 pg/mL, 500 pg/mL and 20000 pg/mL, respectively. Table 3 summarizes the %CV (<10%) and accuracy (88-100%) for low, medium and high concentrations, demonstrating the excellent assay reproducibility and accuracy.

A



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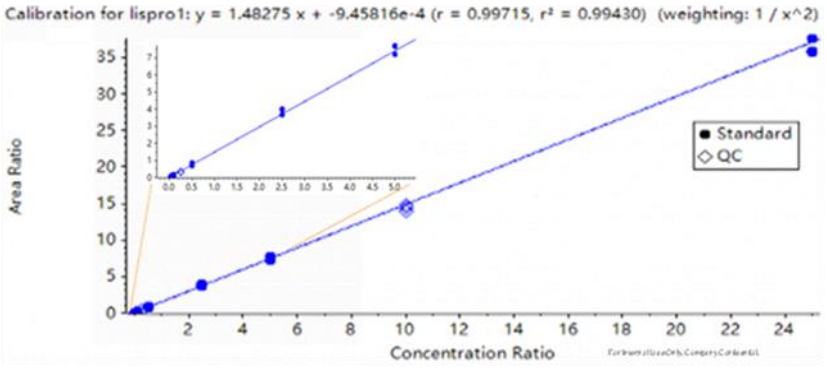

C

Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
50.00	3 of 3	5.054e1	4.329e0	8.57	101.09
200.00	3 of 3	1.913e2	1.206e1	6.31	95.64
1000.00	3 of 3	9.900e2	1.187e2	11.99	99.00
5000.00	3 of 3	5.233e3	2.689e2	5.14	104.66
10000.00	3 of 3	1.014e4	3.881e2	3.83	101.43
50000.00	3 of 3	4.909e4	1.352e3	2.75	98.18

Figure 2. Extracted ion chromatograms (XICs) were used for the quantification of insulin lispro and the generation of calibration curves. XICs of insulin lispro MRM transition in matrix blank and at the LOD and LLOQ are shown (A). Strong linearity was achieved with a correlation coefficient (r^2) of 0.99430 using $1/x^2$ weighting (B). Accuracy and %CV were calculated using triplicate analyses at each concentration (C).

Table 3. Accuracy and %CV of quality control (QC) samples of insulin lispro.

QC concentration (pg/mL)	Calculated concentration (pg/mL)	CV (%)	Accuracy (%)
100	91.0	8.15	91.0
500	443.3	3.78	88.7
20000	19396	2.68	97.0

Conclusions

- Excellent sensitivity, accuracy and %CV were achieved in the quantification of insulin analog using the SCIEX 7500 system with improved front-end technology
- An LLOQ of 50 pg/mL with an LOD of 25 pg/mL was reached for quantification of insulin lispro in rat plasma
- A streamlined data reduction platform was demonstrated on SCIEX OS software for data acquisition, analysis and management

References

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2. Enabling new levels of quantification. SCIEX technical note, RUO-MKT-02-11886-A

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