Ultra-sensitive quantification of intact insulin lispro in rat plasma

Featuring SCIEX Triple Quad™ 7500 LC-MS/MS System – QTRAP® Ready, powered by SCIEX OS Software

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Insulin analogs are altered forms of natural insulin that offer options for insulin replacement strategies with similar or improved actions for glycemic control. Among them are prandial insulin analogs, such as insulin lispro, aspart and glulisine, which tend to be more readily absorbed and act faster than human insulin (Figure 1). It is vital to study the pharmacokinetic and pharmacodynamic profiles of insulin analogs. However, developing sensitive LC-MS methods to quantify them in matrices remains challenging for multiple reasons: 1) the low ionization and CID fragmentation efficiency related to the high molecular weights, 2) low concentrations in matrices, 3) endogenous inferences from natural insulin.

In this technical note, a sensitive LC-MRM method was developed for the quantification of intact insulin lispro in rat plasma using the SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready. The OptiFlow® Pro Ion Source, together with the D Jet™ Ion Guide, offers significantly improved sensitivity for intact insulin analogues. It improves desolvation and focusing to improve instrument sensitivity and thus MRM performance. Insulin lispro was quantified at 0.1 ng/mL in rat plasma without analyte enrichment, with outstanding reproducibility, accuracy and linearity.

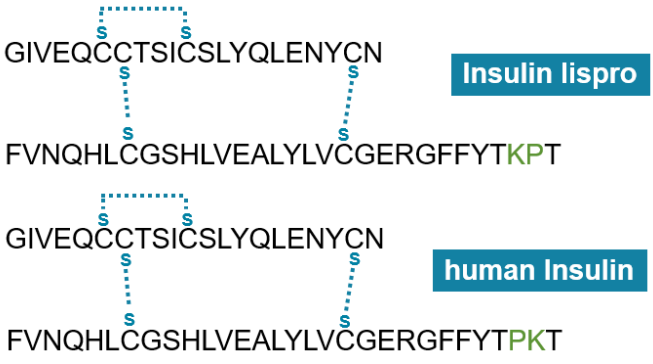

Figure 1. The amino acid sequences of insulin lispro and human insulin. The sequence difference is labeled in green.

Key features of cyclic peptide quantification workflows

- Enhanced sensitivity of the SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready through the introduction of key hardware features that provide significant gains in the generation, capture and transmission of ions[1]: the OptiFlow Pro Ion Source with E Lens™ Technology combined with the D Jet Ion Guide
- Simple sample preparation strategy, requiring no enrichment step
- Robust quantification of insulin lispro at 0.1 ng/mL in rat plasma
- High reproducibility, precision and accuracy across the calibration curve, with excellent linearity
- A 4-fold improvement in S/N was observed when compared to the performance of the SCIEX Triple Quad™ 6500+ LC-MS/MS System
- SCIEX OS Software—a single, compliant-ready platform for acquisition, processing and data management, that is customizable and easy to use

Methods

Sample preparation: Insulin lispro and bovine insulin (internal standard) were spiked into rat plasma. Serial dilution was performed to prepare calibration curve standard samples. The samples were protein precipitated, and the supernatants were collected and processed by mixed-mode SPE. The eluents from the SPE plate were diluted with water and injected directly into the LC-MS/MS for analysis.

LC-MS conditions: Samples were analyzed in triplicate by a SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready, coupled with an ExionLC™ system. The method details are summarized in Table 1 and 2. The same sample set was also injected into a SCIEX Triple Quad 6500+ LC-MS/MS System, coupled with the same HPLC system, to measure the sensitivity difference between the two mass spectrometers. All MRM parameters were optimized on both systems for accurate performance comparison.

Data processing: Calibration curves were processed using Analytics in SCIEX OS Software 2.0.

Table 1. Chromatographic conditions.

Parameter	Value
Column	*Phenomenex bioZen Peptide XB-C18 50×2.1 mm; 2.6 µm*
Mobile Phase A	*Water with 0.1 % formic acid*
Mobile Phase B	*Acetonitrile with 0.1 % formic acid*
Flow Rate	*500 µL/min*
Column Temperature	*40 °C*
Injection Volume	*10 µL*

Time [min]	Mobile Phase A [%]	Mobile Phase B [%]
0	*95*	*5*
0.1	*95*	*5*
5	*60*	*40*
5.1	*10*	*90*
6.0	*10*	*90*
6.1	*95*	*5*
7.0	*95*	*5*

Table 2. MS parameters on SCIEX 7500 System.

Parameter	Value	Parameter	Value
Curtain gas	*40 psi*	*Source temperature*	450 °C
Ion source gas 1	*70 psi*	*Ion source gas 2*	70 *psi*
CAD gas	*12 psi*	*Ion spray voltage*	2000 V

Name	Q1	Q3	CE	CXP
Insulin lispro	*1162*	*217.1*	*46*	*18*
Bovine insulin	*956.4*	*1114.8*	*31*	*18*

Insulin lispro quantification results

The SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready has multiple novel hardware features to improve instrument sensitivity. First is the OptiFlow Pro Ion Source with E Lens Technology.[1] The E Lens Technology creates a stronger field at the ESI probe that the droplets must traverse, leading to more efficient release of ions from the droplet, and deflection of more ions towards the orifice, improving sensitivity.

Next is the addition of the D Jet Ion Guide, which more efficiently captures and transmits the ions in the higher vacuum region behind the orifice plate. Its tapered dodecapole geometry, efficiently focuses the ions into the second stage QJet® Ion Guide.

Together these technologies result in a significant sensitivity improvement in the SCIEX 7500 System over previous generations. To characterize the sensitivity improvement, the insulin calibration curves were analyzed on both the SCIEX 7500 System and the SCIEX Triple Quad 6500+ System. A 4-fold increase in S/N was observed (Figure 2).

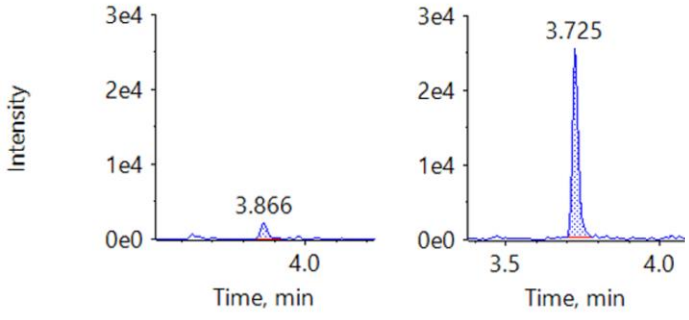

Figure 2. Sensitivity gains with the SCIEX 7500 System. MRM XIC comparison between the SCIEX 7500 System (right) and the SCIEX Triple Quad 6500+ System (left) for insulin lispro at 1 ng/mL.

Insulin lispro shares the same molecular weight, and almost the identical amino acid sequence, with human insulin, with only the two C terminal amino acids in B chain switched in position (Figure 1). This makes the differentiation of insulin lispro from human insulin particularly difficult. The only signature fragment ion that differentiates insulin lispro from human insulin is the y2 ion of B chain. All other y ions have the same m/z as human insulin. Therefore, the y2 ion was used as the quantifier to make the acquisition method applicable to human matrices as well. As shown in Figures 3, 4 and 5, the intact insulin lispro was robustly quantified at 0.1 ng/mL in rat plasma, with tight %CV (<13%) and high accuracies (87-111%) across the entire linear dynamic range (0.1-100 ng/mL). 100 ng/mL was the highest concentration prepared in the sample set, therefore the assay ULOQ was not fully characterized.

Conclusions

- An ultra-sensitive MRM based workflow using the SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready was established for intact insulin lispro quantification in rat plasma
- Insulin lispro was quantified at 0.1 ng/mL in rat plasma with outstanding reproducibility, accuracy and linearity
- Combining the OptiFlow Pro Ion Source with E Lens Technology and D Jet Ion Guide on the SCIEX 7500 System provided a 4-fold improvement in S/N over the SCIEX Triple Quad 6500+ System.

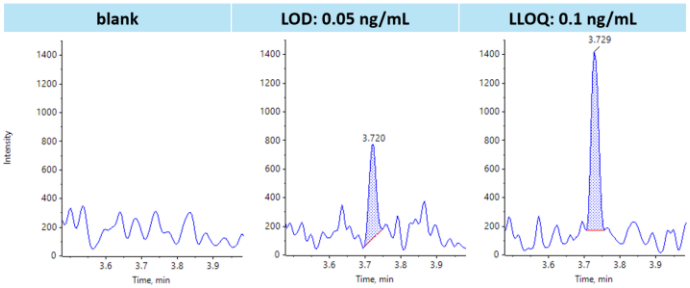

Figure 3. Representative MRM XICs of insulin lispro in rat plasma. From left to right: in matrix blank, at 0.05 ng/mL and 0.1 ng/mL.

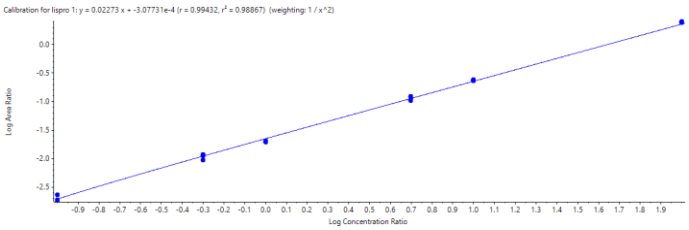

Figure 4. The calibration curve (log-log) of insulin lispro from 0.1 to 100 ng/mL in rat plasma.

Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
0.10	3 of 3	1.018e-1	1.254e-2	12.32	101.76
0.50	3 of 3	4.875e-1	6.202e-2	12.72	97.50
1.00	3 of 3	8.711e-1	4.447e-2	5.11	87.11
5.00	3 of 3	4.940e0	4.156e-1	8.41	98.80
10.00	3 of 3	1.045e1	3.062e-1	2.93	104.51
100.00	3 of 3	1.103e2	4.482e0	4.06	110.32

Figure 5. The quantification summary of insulin lispro in rat plasma.

References

1. Enabling new levels of quantification. SCIEX technical note RUO-MKT-02-11886-A.
2. Improved LC-MRM quantification sensitivity for cyclic peptides from the natriuretic peptide family. SCIEX technical note RUO-MKT-02-11883-A.

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