Quantitation of a Modified Insulin in Rat Plasma Using Triple Quad 6500 LC-MS/MS

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Overview
Using protein precipitation procedure, a sensitive and specific liquid chromatograph-tandem mass spectrometric (LC/MS/MS) method capable of quantifying a modified insulin in rat plasma is described.

In this method, the drug was extracted from a 0.05 mL of rat plasma using simple extraction method. Separation was performed on a reverse phase C18 column. Detection was achieved using a SCIEX 6500 Triple Quad system in the positive ion mode along with multiple reaction monitoring (MRM). The lower limit of quantitation was 2 ng/mL.

This method has been successfully applied to preclinical pharmacokinetic studies.

Introduction

Development of LC-MS/MS assay for intact large biomolecules present many advantages compared to traditional ELISA assay. In recent years, some analytical methods have been developed for determination of intact insulin using LC-MS/MS methods. However, there has been no report on quantitation of any modified insulin that has more than twice of the molecular weight as insulin. In this study, we have developed a rugged, fast method for quantification of a modified insulin (MW >12,000Da, for example, 18,000 Da) in rat plasma using Sciex Triple Quad 6500 mass spectrometer in high-mass positive ESI mode. The lower limit of quantitation is 2 ng/mL (~0.17 pmol/mL).

Sample Preparation:
Plasma samples were extracted by using the 50-µL aliquot of rat plasma. After extraction, the extracts must be centrifuged, and the supernatant were dried and reconstituted. The extract was then transferred to LC vials for LC-MS/MS or stay in the 96-well plate for the analysis.

Liquid Chromatography:
Pump: Shimadzu UFLC LC-30AD
AutoSampler: Shimadzu UFLC SIL-30AC
System Controller: Shimadzu CBM-20A
Analytical Column: C18 column, 50 x 2.1 mm, 1 µm
Gradient: The analyte was eluted using a gradient of mobile phase A (0.1% acetic acid in water) and mobile phase B (0.1% acetic acid in acetonitrile) from 20% to 60% mobile phase B in 3.0 min.
Injection Volume: 5-10 µL

Mass Spectrometry:
MS System: Sciex 6500 Triple Quad
Condition: LC/nanoESI-MS/MS, (High Mass MRM)
MRM transitions: Modified Insulin: 1208.4--→3175.3 Bovine Insulin (ES): 956.7--→3152.2

Results and Discussion

Figure 1. Intact Modified Insulin. Q1 scan (Top), Daughter ion scan for m/z 1208.3 (Bottom).

Figure 2. Representative calibration curve of modified insulin from 2 ng/mL to 200 ng/mL.

Figure 3. Representative chromatograms for double blank (Top) and LLOQ (Bottom) (Note: modified insulin (Left) and bovine insulin (Right)).

Table 1: Validation Data Summary

Table 2: Calibration Data Summary

Table 3: Analytical Precision Summary

Table 4: Repeatability Summary

Table 5: Low Medium High Accuracy Summary

Table 6: Drug/Therapy Summary

Figure 4. Low Medium High Accuracy Summary

Figure 5. Drug/Therapy Summary

Conclusions
A rapid, simple and specific LC/MS/MS method has been developed and validated for quantifying a modified insulin with a lower limit of quantitation of 2 ng/mL from a 0.05 mL plasma sample. For large intact biomolecule quantitation, high sensitivity and high mass MRM with fast scan rate on 6500 Triple Quad will significantly reduce the time to struggle such bioanalytical methods with high quality data.

- Distinct charge states (Figure 1) and selective MRM transitions for the intact modified insulin (Figure 2) facilitates development of a simple, selective and sensitive quantitation method for the modified insulin.
- Excellent linearity was obtained with a correlation coefficient of ≥ 0.9941 for the modified insulin (Figure 2).
- Reproducibility: including LLOQ, the inter-day CV ranged from 4.3% to 13.6% and the biases of the means ranged from 0.2% to 3.7% (Table I).