Detemir Plasma Quantification: Advanced LC-MS/MS Methodology with Shimadzu LC-MS/MS-8060RX

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1. Introduction

Peptide quantification using LC-MS/MS presents significant analytical challenges, particularly for insulin, its synthetic variants, and C-peptide. These biomolecules are critically important for diagnosing and managing diabetes and insulin resistance. Detemir, a long-acting insulin analog, is structurally modified from human insulin by removing threonine at position B30 and attaching a C14 fatty acid chain to amino acid B29 (refer fig.1)[1].

Shimadzu has developed an innovative LC-MS/MS method utilizing the Shimadzu LCMS-8060RX triple quadrupole mass spectrometer coupled with Nexera X3 UHPLC. The method achieves exceptional chromatographic separation and sensitivity, enabling precise Detemir quantification in human plasma with a remarkably lower limit of quantification (LLOQ) at 0.05 ng/mL.

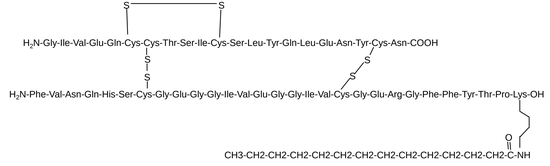

Fig. 1 Structure of Detemir

2. Methods

Sample Preparation: Calibration standards (0.5–100.0 ng/mL) and QC samples (0.5–50.0 ng/mL) were prepared in K_2EDTA human plasma. For each sample, 500 µL plasma was mixed with 600 µL methanol, vortexed for 10 minutes, and centrifuged at 13,700 rpm for 10 minutes at 4 °C. The resulting supernatant was transferred to pre-labeled vials and pretreated with 700 µL of 5 % aqueous ammonia, then vortexed for 30 seconds.

Samples were loaded onto conditioned Mixed mode Anion Exchange, 1 mg 30 CC solid-phase extraction (SPE) cartridges, washed sequentially with 0.5 mL of 5 % ammonia in methanol:water (1:1, v/v) and 0.5 mL of 20 % acetonitrile in water containing 0.05 % formic acid. Analytes were eluted with 0.2 mL of 0.3 % formic acid in acetonitrile:water (1:1, v/v). Finally, 50 µL of the eluate was injected into the LCMS-8060RX system for analysis.

3. LC-MS/MS Conditions

LCMS-8060RX coupled with Nexera™ X3 UHPLC system (Shimadzu Corporation), was used to acquire the data in MRM mode. The instrumental conditions used during the analysis were presented below in Table 1

Fig. 2 Nexera X3 with LCMS-8060RX

Table 1 Instrument Parameters for analysis of Detemir

UHPLC condition (Nexera™ X3)	
Column	Shim-pack™ Scepter C18-120 column (1.9 µm particle size, 100 × 3.0 mm, P/N: 227-31013-03)
Mobile phase	A: 0.1 % formic acid in water, B: 0.1 % formic acid in acetonitrile
Flow rate	0.25 mL/min
Elution mode	Gradient
Column temp	50 ℃

MS parameters (LCMS-8060RX)	
MS interface	Electro Spray Ionization (ESI)
Nitrogen gas flow	Nebulizing gas- 3 L/min; Drying gas- 10 L/min
Ion source voltage	4 kV
MS temp	Desolvation line- 250 °C; Heating block- 400 °C; Interface- 300 °C

4. Results

4.1 Linearity:

Calibration curve of detemir was found linear from 0.05-100.00 ng/mL The goodness of fit was consistently greater than 0.99 during validation with a $1/c^{\wedge}2$ weighting factor (refer fig.3). Representative chromatograms of extracted blank and extracted LLOQ are shown in fig.4

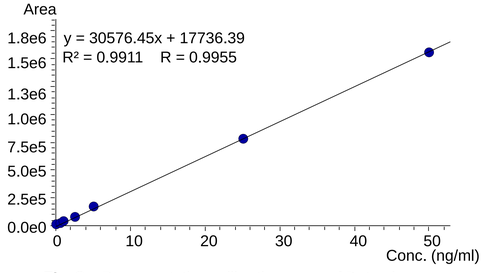

Fig. 3 Representative calibration curve of Detemir

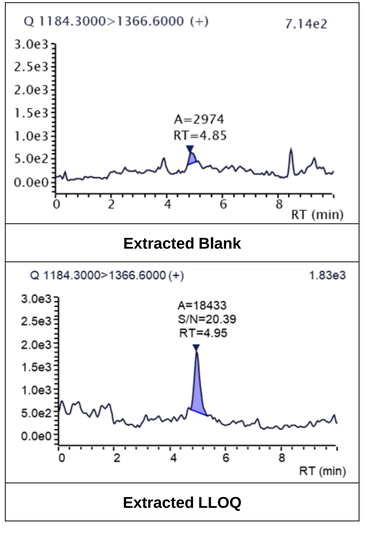

Fig. 4 Chromatograms of extracted blank and extracted LLOQ (0.05 ng/mL)

4.2 Precision and Accuracy:

Intra-day and inter-day precision and accuracy results in plasma quality control samples are summarized in Table 2 and Table 3 and were found within the acceptance criteria[2].

Table 2 Intra-day Precision and Accuracy

QC level	Intra-day (n=6)		
	Mean Conc.	% Accuracy	% CV
LLOQ QC (0.05 ng/mL)	0.05	102.40	10.82
LQC (0.15 ng/mL)	0.16	105.33	6.11
MQC (10.00 ng/mL)	11.03	110.32	3.52
HQC (50.00 ng/mL)	55.11	110.21	9.22

Table 3 Global Precision and Accuracy

QC level	Inter-day (n=12)		
	Mean Conc.	% Accuracy	% CV
LLOQ QC (0.05 ng/mL)	0.05	99.56	10.90
LQC (0.15 ng/mL)	0.16	105.47	7.50
MQC (10.00 ng/mL)	10.94	109.37	3.82
HQC (50.00 ng/mL)	53.10	106.20	11.74

4.3 Carry-over

Carry-over effect was evaluated by injecting extracted samples in the sequence of extracted blank, extracted highest calibrator, extracted blank and extracted lowest calibrator. No carryover was observed at the retention time and MRM transition of detemir in the extracted blank sample following the highest standard calibrator[2].

5. Conclusion

LCMS-8060RX, along with special sample preparation method, optimized chromatography provides a very selective and sensitive method for bioanalysis of Detemir in human plasma. Ultra-high speed and high-separation analysis was achieved on Nexera™ X3 UHPLC by using a simple mobile phase at a minimal gradient flow rate of 0.25 mL/min. By providing these ready to use solutions, we partner with your labs to achieve desired results in your scientific endeavors.

Reference
1) https://go.drugbank.com/drugs/DB01307 (accessed Nov 11, 2024)
2) ICH M10 Bioanalytical Method Validation and Study Sample Analysis. Step 4, May 2022.(accessed 12-01-2025)

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