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MP 383

# Simple, Sensitive and Rapid Quantification of Teriparatide in Human Plasma by LCMS-8060

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## 1. Overview

In this study, we developed a simple, sensitive and rapid LC-MS/MS method for the analysis of teriparatide in human plasma. Teriparatide was extracted from plasma using SPE method and samples were analyzed on LCMS 8060 system.

## 2. Introduction

Teriparatide is a recombinant parathyroid hormone used for the treatment of osteoporosis. Daily injections of teriparatide stimulates new bone formation leading to increased bone mineral density(1). The subcutaneous dose of teriparatide results in very low plasma levels characterised by rapid absorption and elimination, thus requires a highly sensitive method for estimation of analyte in human plasma. In addition, the critical challenges in method development are poor ionization, non-specific adsorption and predominantly carryover issues.

This motivated us to develop a novel and a highly sensitive quantification method for determination of teriparatide in human plasma using Shimadzu LCMS – 8060 triple quadrupole mass spectrometry coupled with Nexera X2 UHPLC.

Shimadzu Application Development Centre (ADC-SAIP), Navi Mumbai has developed and validated a rapid, simple, sensitive and novel method with the lowest limit of quantification (LLOQ) of 5 pg/mL.

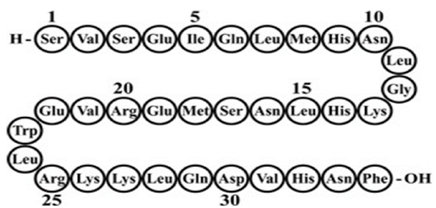


**Figure 1. Structure of Teriparatide(2)**

## 3. Materials and methods

### 3-1. Sample Preparation

**• Preparation of calibration curve standards and quality control (QC) samples**

Calibration standards for teriparatide were prepared in $K_2$ ETDA human plasma at concentration levels ranging from 5.00 to 300.00 pg/mL. Quality control samples were prepared at concentration levels between 5.00 to 250.00 pg/mL for LLOQ QC, LQC, MQC and HQC respectively.

**• Sample extraction**

Teriparatide was extracted from human plasma samples using Solid-Phase extraction technique as per below mentioned protocol:

- Conditioning and equilibration (1mL methanol followed by 1 mL water)
- Sample loading
- Wash 1 (1 mL water x 2 times)
- Wash 2 (1 mL 5% methanol in water x 1 time)
- Elution (1 mL of 100 % acetonitrile x 1 time)
- SPE eluent was blown under nitrogen gas and was reconstituted in 0.2 mL reconstitution solution (80% acetonitrile in water) before analysis on LC-MS/MS system

### 3-2. LC-MS/MS analysis

**Figure 2. Nexera X2 with LCMS-8060**

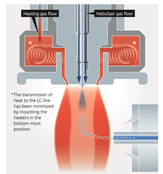


**Figure 3. Heated ESI probe**

LCMS-8060 triple quadrupole mass spectrometer by Shimadzu (Figure 2), sets a new benchmark in triple quadrupole technology with an unsurpassed sensitivity (UFsensitivity), ultra fast scanning speed of 30,000 u/sec (UFscanning) and polarity switching speed of 5 msec (UFswitching). This system ensures highest quality of data, with very high degree of reliability.

In order to improve ionization efficiency, the newly developed heated ESI probe (Figure 3) combines high-temperature gas with the nebulizer spray, assisting in the desolvation of large droplets and enhancing ionization. This development allows high-sensitive analysis of a wide range of target compounds with considerable reduction in background.

The details of analytical conditions are given in Table 1.

**Table 1. Instrument parameters for analysis of Teriparatide**

| UHPLC condition (Nexera X2) | | MS parameters (LCMS-8060) | |
|---|---|---|---|
| Column | Shim-pack GIST C18 column 100 x 2.1 mm, 2.7 µm | MS interface | Electro Spray Ionization (ESI) |
| Mobile phase | A: 0.1% formic acid in water<br>B: Acetonitrile | Nitrogen gas flow | Nebulizing gas- 2 L/min;<br>Drying gas- 10 L/min |
| Flow rate | 0.30 mL/min | Zero air flow | Heating gas- 10 L/min |
| Elution mode | Gradient | MS temp | Desolvation line- 250 °C;<br>Heating block- 300 °C;<br>Interface- 300 °C |
| Column temp | 40 °C | | |

## 4. Results

### 4-1. Selectivity

Selectivity of the method was assessed in six different lots of blank human plasma. Interference from blank plasma was assessed for Teriparatide. No significant interference was observed at the retention time and MRM transition of analyte (refer Figure 4).

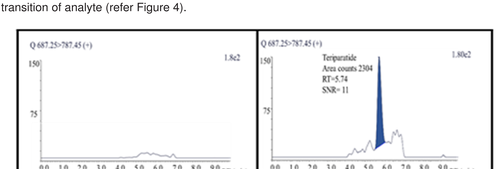


**Figure 4. Chromatograms of extracted blank and extracted LLOQ (5.00 pg/mL)**

### 4-2. Linearity

Calibration curve was found linear from 5.00-300.00 pg/mL (Table 2). The goodness of fit was consistently greater than 0.980 during the course of validation. Signal to noise ratio (s/n) at LLOQ level was found greater than 10:1, across 6 PA batches. Representative calibration curve is shown in figure 5

**Table 2. Representative calibration curve of Teriparatide**

| Calibration curve data for teriparatide (n=6) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Level | CC1 | CC2 | CC3 | CC4 | CC5 | CC6 | CC7 | CC8 |
| Nominal conc | 5.000 | 10.000 | 25.000 | 50.000 | 100.000 | 150.000 | 200.000 | 300.000 |
| Mean (n=6) | 4.991 | 10.340 | 25.189 | 47.218 | 100.265 | 153.552 | 201.328 | 306.717 |
| SD | 0.242 | 1.501 | 2.376 | 3.440 | 8.602 | 9.097 | 11.420 | 34.428 |
| % RSD | 4.86 | 14.52 | 9.43 | 7.29 | 8.58 | 5.92 | 5.67 | 11.22 |
| % Nominal | 99.82 | 103.40 | 100.76 | 94.44 | 100.26 | 102.37 | 100.66 | 102.24 |

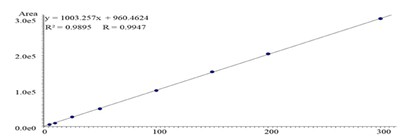


**Figure 5. Representative calibration curve of Teriparatide**

### 4-3. Intra-day Precision and accuracy

Intraday precision and accuracy was evaluated using 6 replicates of LLOQQC, LQC, MQC and HQC in single P&A batch. Precision and accuracy was found well with in acceptance criteria. Statistical data is summarized in table 3.

**Table 3. Intra-day Precision and Accuracy**

| QC (n=6) | LLOQQC | LQC | MQC | HQC |
|---|---|---|---|---|
| Nominal concentration | 5.00 | 10.00 | 150.00 | 250.00 |
| PA batch observed concentration (pg/mL) | 5.308 | 9.073 | 159.932 | 254.29 |
| | 5.364 | 9.204 | 153.143 | 285.953 |
| | 4.614 | 9.832 | 141.414 | 276.757 |
| | 5.773 | 9.521 | 138.772 | 272.599 |
| | 4.944 | 10.629 | 147.793 | 307.919 |
| | 5.645 | 10.739 | 150.36 | 282.333 |
| Mean | **5.275** | **9.833** | **148.569** | **279.975** |
| STDEV | **0.43** | **0.71** | **7.76** | **17.59** |
| % CV | **8.22** | **7.23** | **5.22** | **6.28** |
| % Nominal | **105.49** | **98.33** | **99.05** | **111.99** |

### 4-4. Global Precision and accuracy

Global precision and accuracy was evaluated on 6 PA batches. Precision and accuracy results were found well within the acceptance criteria with % CV < 13.68% and % nominal ranging from 93.13% to 102.17% at LQC, MQC and HQC level. At LLOQQC level, the % CV was found 15.12% and % nominal 102.29% (refer Table 4).

**Table 4. Global Precision and Accuracy**

| QC level (n=36) | Mean Conc. | % Accuracy | % CV |
|---|---|---|---|
| LLOQ (5.0 pg/mL) | 5.11 | 102.29 | 15.12 |
| LQC (10.0 pg/mL) | 9.84 | 98.40 | 13.68 |
| MQC (150.0pg/mL) | 139.69 | 93.13 | 11.37 |
| HQC (250.0 pg/mL) | 255.43 | 102.17 | 12.94 |

### 4-5. Recovery

Recovery experiments was studied at LQC, MQC and HQC level. Recovery was found precise, consistent and reproducible at all levels. Global recovery for teriparatide was found 80.37 % (refer Table 5).

**Table 5. Global Recovery**

| Global recovery | Teriparatide |
|---|---|
| LQC | 85.85 |
| MQC | 73.70 |
| HQC | 81.55 |
| Mean | 80.37 |
| SD | 6.16 |
| % CV | 7.67 |

### 4-6. Matrix effect

Matrix effect was studied at LQC and HQC levels. Mean matrix effect was found 1.03 for teriparatide. Statistical data for matrix effect is shown in Table 6. The results confirm the suitability of the method for quantitative estimation of teriparatide in human plasma.

**Table 6. Matrix effect**

| Teriparatide | Post extracted sample | Aqueous sample | Matrix factor | Teriparatide | Post extracted sample | Aqueous sample | Matrix factor |
|---|---|---|---|---|---|---|---|
| LQC | 7,100 | 6,216 | 1.14 | HQC | 2,16,207 | 1,95,725 | 1.10 |
| | 5,500 | 5,240 | 1.05 | | 1,96,279 | 1,86,088 | 1.05 |
| | 6,500 | 6,676 | 0.97 | | 1,92,251 | 1,96,876 | 0.98 |
| | 6,418 | 6,435 | 1.00 | | 2,14,920 | 1,98,680 | 1.08 |
| | 6,719 | 6,426 | 1.05 | | 2,08,751 | 1,92,847 | 1.08 |
| | 6,700 | 6,830 | 0.98 | | 1,30,259 | 1,50,699 | 0.86 |
| Mean | | | 1.03 | | | | 1.03 |
| SD | | | 0.06 | | | | 0.09 |
| % CV | | | 6.11 | | | | 8.89 |

### 4-7. Carry-over effect

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 teriparatide in the extracted blank sample following the highest standard calibrator.

### 4-8. Other experiments

Based on validation guidelines, method was assessed for following experiments and results were found within acceptance criteria:

- Bench top stability
- Auto sampler stability
- Freeze thaw stability
- Extended batch verification

## 5. Conclusion

LCMS-8060, along with special sample preparation method and optimized chromatography provides a very selective and sensitive method for bioanalysis of teriparatide study samples in human plasma. Ultra-high speed and high-separation analysis was achieved on Nexera X2 UHPLC by using a simple mobile phase at a minimal gradient flow rate of 0.30 mL/min. By providing these ready-to-use solutions, we partner with your labs to achieve desired results in your scientific endeavors.

## 6. References

1. https://go.drugbank.com/drugs/DB06285 (accessed March 08,2022)
2. https://www.rxlist.com/forteo-drug.htm (accessed March 08, 2022)