SHIMADZU
Excellence in Science

Application News

Liquid Chromatograph Mass Spectrometer LCMS-8060

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

Avinash Gaikwad[1], Chaitanya krishna Atmakuri[1], Yogesh Arote[1], Jitendra Kelkar[2], Pratap Rasam[2]
[1] ADC - Shimadzu Analytical (India) Pvt. Ltd., [2] Shimadzu Analytical (India) Pvt. Ltd.,

User Benefits

- Highly sensitive, validated and ready to use teriparatide LC/MS method
- Simple single step SPE method
- Linear dynamic range suitable for pK studies which ranged between 5 pg/mL to 300 pg/mL
- Consistent, reproducible, and precise recovery

1. Introduction

Teriparatide, a mid size peptide shown in Fig. 1, 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 characterized 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, low recovery and predominantly carryover issues.

This motivated us to develop 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), Mumbai has developed and validated a rapid, simple, sensitive and novel method with the lowest limit of quantification (LLOQ) of 5 pg/mL. Precision and accuracy (PA) of the analyte were evaluated at lowest limit of quantification quality control (LLOQ QC), low quality control (LQC), middle quality control (MQC) and high quality control (HQC) samples.

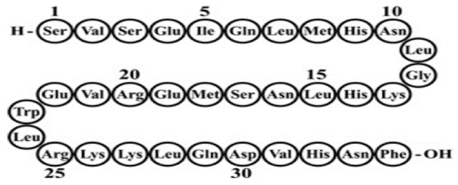

Fig.1 Structure of Teriparatide [(2)]

2. Salient Features

- A rapid, simple and sensitive method was developed for estimation of teriparatide in human plasma.
- Simple extraction procedure enhanced the selectivity of the method.
- Single step SPE method increased sample throughput.
- Heated ESI along with new UF-Qarray ion guide technology contributes by increasing ion production and enhancing transmission respectively. This ensures sensitivity and selectivity of analyte.
- Customized gradient method satisfied the peak shape, retention time and background noise.
- Ready to use validated teriparatide method as per the US major guidelines.

Table 1 Method Validation Summary

Calibration curve range	5.00 to 300.00 pg/mL	
Intraday precision and accuracy (For LLOQ-QC)	Accuracy (%Nominal)	105.49
	Precision (%RSD)	8.22
Intraday precision and accuracy (For LQC, MQC, HQC)	Accuracy (%Nominal)	98.33 to 111.99
	Precision (%RSD)	5.22 to 7.23
Global precision and accuracy (For LLOQ-QC)	Accuracy (%Nominal)	102.29
	Precision (%RSD)	15.12
Global precision and accuracy (For LQC, MQC, HQC)	Accuracy (% Nominal)	93.13 to 102.17
	Precision (%RSD)	11.37 to 13.68
Global % recovery	Recovery (%)	80.37
	Precision (%RSD)	7.67
Matrix effect	Mean matrix Factor	1.03

3. Experimental

3.1. Sample preparation and analytical conditions

Teriparatide was extracted from plasma samples under basic conditions using Solid-Phase extraction technique using the protocol mentioned below:

- Conditioning and equilibration (1 mL methanol followed by 1 mL water)
- Sample loading - Wash 1 (1 mL wash solution 1 x 2 times)
- Wash 2 (1 mL wash solution 2 x 1 time)
- Elution (1 mL of elution solution)
- SPE eluent was blown under nitrogen gas and was reconstituted in 0.1 mL reconstitution solution before analysis on LC-MS/MS system

3.2. Instrument parameters on LCMS-8060

Refer to Table 2 for analytical conditions and instrument parameters and Table 3 for MRM transition.

Table 2 Analytical conditions and instrument parameters

Parameter	HPLC
Column	Shim-pack™ Velox C18 column 100 x 2.1 mm, 2.7 µm (P/N: 227-32015-03)
Mobile Phase	A: 0.1% formic acid in water B: Acetonitrile
Flow Rate	0.3 mL
Oven Temp	40 °C
Injection	20 µL

Table 2 Analytical conditions and instrument parameters (continued)

Parameter	MS
Interface	ESI
Interface temp and Voltage	3 KV and 300 ˚C
MS Mode	MRM, Positive
Heat Block Temp	300 ˚C
DL Temp	250 ˚C
CID Gas	230
Nebulizing Gas	2
Drying Gas	10
Heating Gas	10

Table 3 MRM transition and parameters of Teriparatide on LC/MS

Compound	MRM (m/z)	CE (V)
Teriparatide	687.25-787.45	-20.2

4. Result and Discussion

4.1. Method Development

The initial LC/MS method development for teriparatide showed that the peptide obtained 5+, 6+, and 7+ charge state in MRM mode. However, 6+ charge state gave slightly higher signal in MRM mode compared to other 2 charges. The LC method development for teriparatide showed that analyte eluted off the C18 column under gradient conditions with 22 and 25% organic content. The gradient run was established with total run time of 10 min. The analyte peak eluted from the LC column at the retention time of 5.74 min. A representative chromatogram of extracted blank and LLOQ sample are shown in Fig. 2. Liquid-liquid extraction and solid phase extraction (SPE) were tried initially for extraction of teriparatide from blank plasma. However, SPE extraction method showed consistent, reproducible and precise results without any matrix effect and hence was selected as the final sample extraction method.

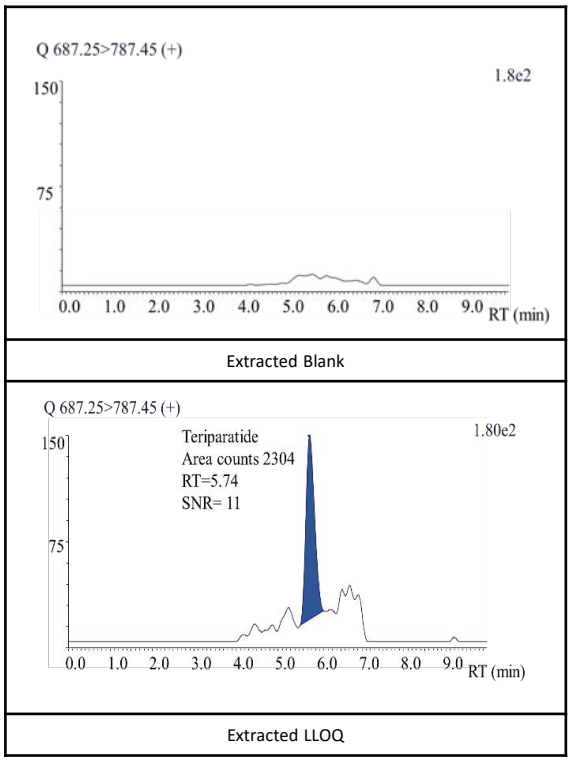

Fig. 2 Chromatograms of Teriparatide

4.2. Method Validation

Selectivity

Six blank human plasma lots were evaluated for their selectivity. No significant interference was observed at the retention time and MRM transition of analyte in any of the human blank plasma lots, refer Table 4 below.

Table 4 Selectivity

Plasma lot no.	Area in blank matrix	LLOQ area	% Interference
V 1403	80	2318	3.45
V 1744	63	3060	2.06
V 2117	79	2204	3.58
V 3509	13	1652	0.79
V 1493	66	2076	3.18
V 8076	93	1601	5.81

Linearity

Calibration curve was found linear from 5.00-300.00 pg/mL. 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 Fig. 3.

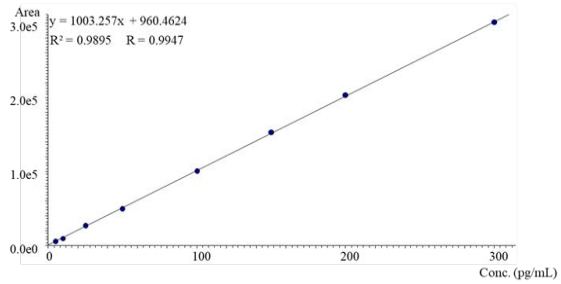

Fig. 3 Calibration curve

Intra-day and Inter-day accuracy and precision

Accuracy and precision of teriparatide was determined for LLOQ QC, LQC, MQC & HQC in biological matrix based on the expected range. Accuracy and precision batches were evaluated on intra-day and inter-day basis. Results of are shown in Table 5. Accuracy and precision results were measured at six determination per concentration at each level with four different concentration levels (LLOQ, LQC, MQC and HQC) and were found within acceptance criteria.

Table 5 Intra-day and Inter-day accuracy and precision

Intra-day (n=6)			
Nominal Conc (pg/mL)	**Observed Conc (pg/mL)**	**Accuracy (%)**	**Precision (%RSD)**
LLOQ (5.0 pg/mL)	5.27	105.49	8.22
LQC (10.0 pg/mL)	9.83	98.33	7.23
MQC (150.0pg/mL)	148.57	99.05	5.22
HQC (250.0 pg/mL)	279.98	111.99	6.28
Inter-day (n=18)			
Nominal Conc (pg/mL)	**Observed Conc (pg/mL)**	**Accuracy (%)**	**Precision (%RSD)**
LLOQ (5.0 pg/mL)	5.11	102.29	15.12
LQC (10.0 pg/mL)	9.84	98.4	13.68
MQC (150.0pg/mL)	139.69	93.13	11.37
HQC (250.0 pg/mL)	255.43	102.17	12.94

Recovery

Recovery experiment was conducted to evaluate precision, reproducibility and consistency of the analyte during extraction at LQC, MQC and HQC level. Recovery of Teriparatide was found precise, consistent, and reproducible at all levels. Global recovery was found 80.37 % refer Table 6.

Table 6 Recovery

QC level	Recovery
LQC (n=6)	85.85
MQC (n=6)	73.70
HQC (n=6)	81.55
Mean	80.37
SD	6.16
%RSD	7.67

Matrix effect

Matrix effect was studied at LQC and HQC levels. Mean matrix factor was found to be 1.03 at both LQC and HQC levels. Representative data of matrix effect is shown in Table 7. The results confirm the suitability of the method for quantitative estimation of Teriparatide in human plasma.

Table 7 Matrix effect

Teriparatide	Aqueous sample	Post extracted sample	Matrix factor
LQC	7,100	6,216	1.14
	5,500	5,240	1.05
	6,500	6,676	0.97
	6,418	6,435	1.00
	6,719	6,426	1.05
	6,700	6,830	0.98
Mean			1.03
SD			0.06
%CV			6.11

Teriparatide	Aqueous sample	Post extracted sample	Matrix factor
HQC	2,16,207	1,95,725	1.10
	1,96,279	1,86,088	1.05
	1,92,251	1,96,876	0.98
	2,14,920	1,98,680	1.08
	2,08,751	1,92,847	1.08
	1,30,259	1,50,699	0.86
Mean			1.03
SD			0.09
%CV			8.91

Carry-over effect

Carryover was evaluated by injecting extracted samples in the sequence of extracted blank, extracted highest calibrator, extracted blank and extracted lowest calibrator. No carryover was present/observed at the retention time and MRM transition of the analyte in the extracted blank sample following the highest standard calibrator.

5. Conclusion

LCMS-8060, along with special sample preparation method, optimized chromatography provides a very selective and sensitive method for bioanalytical assay of Teriparatide. Ultra-high speed and high-separation analysis was achieved on Nexera X2 UHPLC by using a simple mobile phase at a gradient flow rate of 0.3 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)

06-SAIP-LC-056-EN First Edition: May. 2023

Shimadzu Corporation
www.shimadzu.com/an/

Shimadzu Analytical (India) Pvt.Ltd.
www.shimadzu.in