IMPROVING A HIGH SENSITIVITY ASSAY FOR THE QUANTIFICATION OF TERIPARATIDE IN HUMAN PLASMA USING IONKEY/MS

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INTRODUCTION

Teriparatide (FORTEO®), Figure 1, is a recombinant form of a fragment of human parathyroid hormone, used in the treatment of osteoporosis. Osteoporosis is responsible for 1.5 million bone fractures a year and teriparatide is the first treatment that stimulates new bone formation. It is an anabolic drug that acts to build up bones and has the potential to improve skeletal micro architecture and increase bone density. Teriparatide is the first 34 amino acids (the biologically active region) of the 84-amino acid human parathyroid hormone (PTH), and is also referred to as, rhPTH (1-34).[1]

The pharmacokinetics of Teriparatide are characterized by rapid

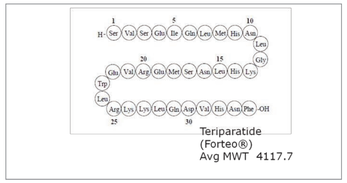

Figure 1: Representative amino acid sequence and molecular weight of Teriparatide.

absorption within 30 minutes and rapid elimination with a half-life of 1hr, resulting in a total duration of exposure (to the peptide) of approximately 4 hours.[1,2] At the practical clinical dose of 20 µg the typical Teriparatide levels are ~50pg/mL, which makes detection by traditional LC-MS/MS even more difficult.

We previously published an analytical scale method for accurate, precise teriparatide quantification, with a detection limit of 15 pg/mL. [3] In this current work we undertook to A.) transfer this method to a the ionKey/MS system (phase 1), and B.) to further improve the method through the inherent characteristics of ionKey/MS (phase 2). This study combines µElution solid-phase extraction (SPE) and the novel and highly efficient ionKey/MS system to improve a quantitative assay for teriparatide in human plasma. In phase 1, we will demonstrate the effective transfer of the previously developed analytical method using a 200 µL sample size to the ionKey/MS system. In phase 2, we show proof of concept for further method improvement, fully capitalizing on the attributes of ionKey/MS: reducing required sample volume, increasing sensitivity, and reducing injection volume.

METHODS

System: ionKey/MS, configured with optional trap and back flush elution
Analytical column: iKey, 150 µm x50 mm BEH PST C18 130Å 1.7 µm
Trap column: Symmetry C18 5 µm, 300 µm x50 mm

Mobile Phases: 0.1% Formic acid in water (A) and in acetonitrile (B)

Gradient: 15 to 45% B over 5 minutes, 2 µL/min

Injection Volume: 15 µL

iKey Temperature: 75°

Sample Preparation: Samples were pretreated using protein precipitation (PPT) and extracted on an Oasis HLB µElution 96-well plate according to a previously published method [3]

MS: Waters Xevo TQ-S

Peptide	MRM Transition	Cone Voltage (V)	Collision Energy (eV)
Teriparatide	687.05 > 787.26	45	18
	824.25 > 983.79	45	25
Human Parathyroid 1-38 (ISTD)	637.58 > 712.61	45	11
	892.22 > 854.80	45	21

Table 1: MRM transitions, collision energies, and cone voltages for teriparatide and internal standard.

RESULTS

Phase 1 results show that we can readily achieve a limit of detection (LOD) of 10 pg/mL with a linear dynamic range of 10-3000 pg/mL in human plasma, using 1/3rd of the injection volume. Representative standard curve statistics are shown in Table 2. Figure 2 contains representative chromatograms for QC samples containing Teriparatide at 25, 50, 80, 200, and 500 pg/mL extracted from 200 µL human plasma as compared to blank extracted plasma. At all levels, QC samples demonstrated very good accuracy and precision, with mean accuracies ranging from 101.2-104.9 and mean %CV's of 2.56-5.09 (Table 3).

Teriparatide Concentration (pg/mL)	Teriparatide/IS Ratio Response	Calculated Teriparatide Concentration (pg/mL)	Mean Accuracy
10.00	0.07	10.56	105.63
20.00	0.14	20.53	102.63
40.00	0.29	38.99	97.58
60.00	0.43	57.58	95.97
100.00	0.73	97.00	97.00
300.00	2.17	286.39	95.50
600.00	4.75	626.81	104.45
1000.00	8.05	1061.49	106.15
3000.00	22.31	2937.14	97.95

Table 2: Representative standard curve statistics for Teriparatide extracted from 200 µL human plasma and analyzed using ionKey/MS.

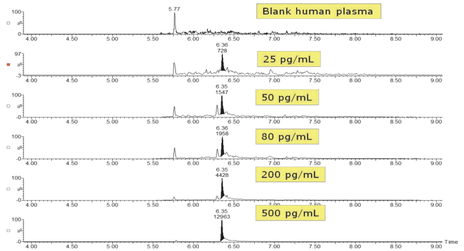

*Figure 2: Representative chromatograms of Teriparatide extracted from **200 µL** human plasma at 25, 50, 80, 200, and 500 pg/mL compared to blank plasma.*

Teriparatide QC Concentration (pg/mL)	Mean (N=5) Calculated Concentration (pg/mL)	SD	%CV	Mean Accuracy
25	25.8887	1.32	5.09	103.6
50	51.4236	1.91	3.72	102.8
80	83.8803	2.15	2.56	104.9
200	202.3569	6.49	3.20	101.2
500	511.1018	15.23	2.98	102.2

Table 3: QC statistics from teriparatide extracted from human plasma.

Phase 2 results show that we were able to reduce the sample and injection volume, and increase signal-to-noise (S:N) by 4X over the 2.1 mm ID scale (Figure 3). While S:N is approximately 11:1 at the 2.1 mm ID scale, it is 45:1 using ionKey/MS with 4X less sample, and half the injection volume. Extracting only 50 µL of human plasma and injecting 15 µL, the method was linear with an R^2 value of >0.99(1/x regression). Representative chromatograms from QC samples are shown in Figure 4.

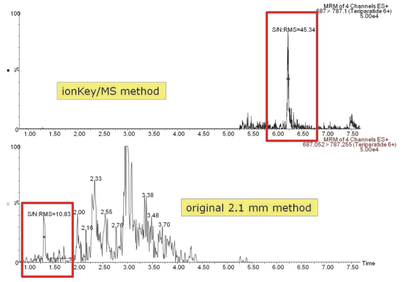

Figure 3: 20 pg/mL Teriparatide extracted from human plasma analyzed using ionKey/MS (top panel, 15 µL injected) and the 2.1mm scale method (bottom panel, 30 µL injection.)

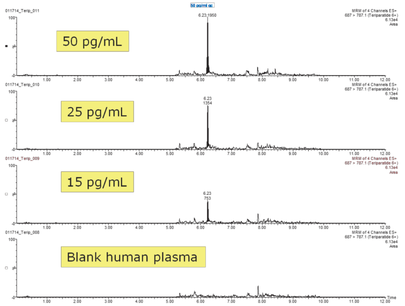

*Figure 4: Representative QC samples extracted from **50 µL** human plasma and analyzed using the ionKey/MS system.*

CONCLUSIONS

- **IonKey/MS provided a 4X improvement in S:N over the 2.1 mm ID scale using 4X less sample and half the injection volume**
- **SPE reduces matrix interferences and enhances selectivity of the extraction for Teriparatide in plasma.**
- **The ionKey/MS method is linear from 10-3000 pg/mL, with no carry-over**
- **Accuracy and precision of QC samples and standard curve points were ±6%**
- **The method can be used for ultra-high sensitivity quantification of Teriparatide in a routine BA lab**

References

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