Therapeutic Teriparatide Peptides Quality Control by Liquid Chromatography Mass Spectrometry

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Introduction

Understanding the impurity profile of peptide drug products is important to ensure product quality and safety, such as potential adverse events associated with impurity-induced immunogenicity. Commonly used Liquid Chromatography-UV peptide impurity methods have limitations, including UV detection is not specific for peptide-related impurities and cannot unequivocally identify these impurities by retention time and UV absorbance, or quantify impurities that co-elute. Here, a sensitive and specific liquid chromatography high resolution mass spectrometry (LC-HRMS) method was developed for characterizing and quantifying peptide-related impurities in teriparatide. Teriparatide is a 34-amino acid peptide drug that is used to treat osteoporosis by acting as a parathyroid hormone analog to stimulate new bone formation. The reference listed drug (RLD) of teriparatide, FORTEO, was manufactured from E. coli using recombinant DNA technology.

Materials and Methods

LC-HRMS analysis was performed using two Instruments. Instrument 1: Agilent 6560A, Accurate-Mass Q-TOF LC-MS mass spectrometer, the Agilent 1290 HPLC system consisted of a 1290 binary pump, thermostat, and auto sampler. Full MS scan mass spectra were acquired from 200-1700 m/z with a scan rate of 4.0 spectra/s in positive ion mode. Instrument 2: Thermo Q-Exactive hybrid quadrupole-orbitrap mass spectrometer with a HESI source. Mass spectra were acquired in positive ion mode in the 350-2000 m/z range with a resolution of 70,000, AGC target of 3e6 and max IT of 100 ms.

A Waters Acquity UPLC BEH C18, 1.7 µm, 2.1 x 100 mm column was used for all separations. Mobile phase A consisted of 0.1% formic acid in water. Mobile phase B was 0.1% formic acid in acetonitrile. Samples were eluted with a gradient (10-26.8% B) at flow rate 0.3 mL/min, total run time including equilibration was 65 min.

Six lots of Forteo samples were tested. All samples were purchased through the US market. Teriparatide injection formulation drugs (250 ug/mL) were diluted to 25 ug/mL with diluent (water: acetonitrile, 9:1).

Results

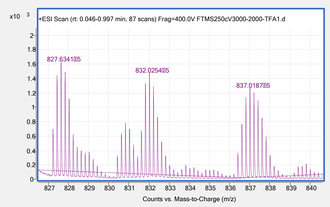

Figure 1. The high-resolution spectrum of peaks zoomed at the +5-charge state of some components in HRMS spectra by direct infusion. The experimental m/z values match theoretical values within 10 ppm mass error. The m/z at 827.6 is teriparatide (1-34) Met +O (8, or 18); The m/z around 831.0 is teriparatide (1-34) methanol ion (+32); The m/z around 832.0 is teriparatide (1-34) potassium ion (+38), and/or teriparatide (1-34) Met +O (8, or 18) sodium ion (+22); The m/z at 835.0 is not observed. The m/z around 837.0 is teriparatide (1-34) potassium and sodium ion (+38+22).

Results

Impurities Identified in Teriparatide Drug Product, FORTEO

Ten peptide-related impurities were investigated and relative area percentages were calculated by integrating peak areas of the three main charge states (+5, 6, 7) using the first three isotope ions at a mass extraction window (MEW) of 50 ppm. Ions in the extracted ion chromatogram (EIC) were adjusted by the observed m/z and RT window. Ions that overlapped/interfered with other species were excluded. Deamidation impurities were difficult to resolve from the API (in the MS, as they are only 1 Da apart, the isotopic distribution peaks overlap with that of teriparatide at +5 charge state) using the current LC-MS EIC method. Thus, the content of deamidation impurities such as Iso-Asp(16), Iso-Asp(30), Asp(33), and Glu(29) were not monitored. Other peptide related impurities can also be monitored once they are separated by LC and/or MS. The LC-MS method can quantify impurities which can't be separated by the LC-UV method, such as LC overlap peaks: rhPTH(1-34) Succinimide(30), Val-Arg rhPTH (1-34) , N-ac rhPTH (1-34) , Teriparatide(4-34), and Teriparatide(1-34).

Compound Name	Monoisotope MW	RT, min	RRT	C470473C	C587623C	C644202D	C650452G	C616383C	C658878C	**Avg**	Std
Teriparatide(1-34)	4115.1305	45.92	1.00	95.94	96.21	96.79	96.26	97.01	97.19	**96.57**	0.50
Teriparatide(1-34) Met +O (8, 18)	4147.1203	38.89	0.85	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	0.00
Teriparatide(1-34) Met +O (8)	4131.1254	41.85	0.91	0.79	0.51	0.65	0.55	0.36	0.32	**0.53**	0.18
Teriparatide(1-34) Met +O (18)	4131.1254	43.47	0.95	1.28	1.15	1.27	1.30	0.92	0.86	**1.13**	0.19
rhPTH(1-30)	3617.8919	42.52	0.93	0.79	0.57	0.40	0.44	0.47	0.42	**0.51**	0.15
rhPTH(1-34) Succinimide(30)	4097.1199	45.71	1.00	0.82	0.66	0.54	0.58	0.59	0.54	**0.62**	0.11
Val-Arg rhPTH (1-34)	4370.3000	45.49	0.99	0.10	0.11	0.09	0.08	0.10	0.11	**0.10**	0.01
N-ac rhPTH (1-34)	4157.1410	45.92	1.00	0.02	0.02	0.02	0.02	0.02	0.02	**0.02**	0.00
rhPTH(1-33)	3968.0621	43.27	0.94	0.20	0.43	0.11	0.23	0.22	0.19	**0.23**	0.11
rhPTH (1-29)	3502.8649	42.19	0.92	0.02	0.31	0.02	0.40	0.29	0.24	**0.21**	0.16
Teriparatide(4-34)	3841.9980	45.13	0.98	0.03	0.02	0.11	0.15	0.03	0.11	**0.07**	0.05

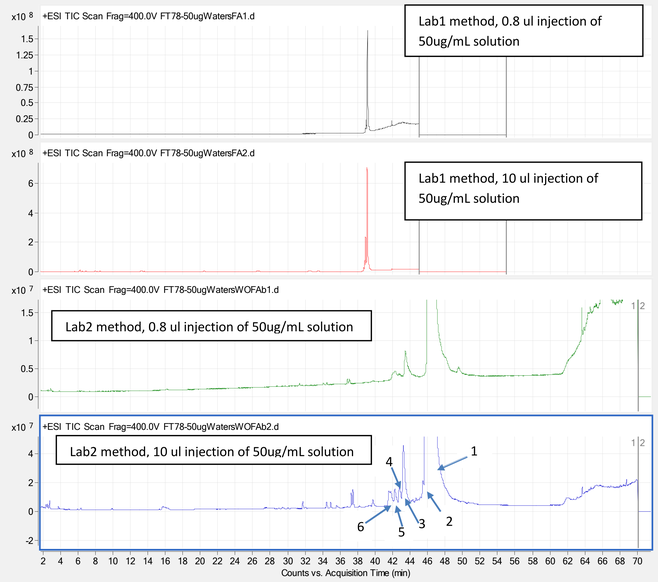

Figure 2. The LC-HRMS TIC chromatograms of teriparatide by both Lab1 and Lab2. The top two chromatograms are from Lab1 with 0.8 and 10 ul injection of 50 ug/mL solution respectively; sharp teriparatide peaks are observed at 39.5 min. The bottom two chromatograms are from Lab2; impurities are better separated, and a sharp teriparatide peak is observed at 46.5 min. Peak 1: teriparatide; 2: Teriparatide(4-34); 3: Teriparatide Met +O (18); 4: rhPTH(1-33); 5: rhPTH(1-30); 6: Teriparatide Met +O (8).

Conclusions

- **All impurity ions monitored were confirmed and correctly assigned accordingly using high resolution mass spectra. These ions were highly resolved and free from overlap/interference with other ions.**
- **Ten peptide related impurities were investigated and relative area percentages were calculated using the EIC method by integrating peaks of the three highest charge states (+5, 6, 7) and three isotope ions at a mass extraction window (MEW) of 50 ppm. The average percentage of impurities in the six RLD lots was found to be about 3 %.**
- **LC-MS can quantify impurities that cannot be separated by the LC-UV method and provides better specificity and sensitivity for peptide impurity profiling.**

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