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Therapeutic Teriparatide Peptides Quality Control by Liquid Chromatography Mass Spectrometry

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PURPOSE

Teriparatide is a 34 amino acid peptide drug that is indicated for the treatment of osteoporosis by acting as a parathyroid hormone analog to stimulate new bone formation. The RLD of teriparatide, “Forteo,” was manufactured from E. coli using recombinant DNA technology. To evaluate the acceptability of peptide-related impurities in such products, the widely used LC-UV approach has limitations. Notably, 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. Understanding the impurity profile is important to ensure drug identity and purity and to assess any potential immunogenicity risks**.** Liquid chromatography coupled with high resolution, high sensitivity mass spectrometry (LC-HRMS) was evaluated in this work for peptide drug quality control purposes.

OBJECTIVE(S)

To develop a sensitive and specific LC-HRMS method for characterizing peptide-related impurities in teriparatide.

METHOD(S)

LC-HRMS analysis was performed using an 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. MS parameters were as follows: the MS was operated in positive ion mode; full scan mass spectra were acquired from 200-1700 m/z with a scan rate of 4.0 spectra/s. 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 (5-47% B) at flow rate 0.3 mL/min.

DS Manufacturer	Name	Lot#
USP	Teriparatide	F015Q0
Bachem	pTH(1-34) TFA	9045679
Bachem	pTH(1-34) Acetate	1065287
PolyPeptide	Teriparatide Acetate	Q# 11702

Drug Name	Manufacturer	Lot#
Forteo	Eli Lilly	C470473C
		C587623C
Forteo	Eli Lilly	C644202D
		C650452G
Forteo	Eli Lilly	C616383C
		C658878C

RESULT(S)

1. The LC-HRMS method for teriparatide had good linearity (R2 =0.9998) and precision (%RSD less than 2.0%) at concentrations between 500 to 10000 ng/mL range. The LOD and LOQ for teriparatide were determined to be 0.02% and 0.05%, respectively, of percent of label claim using the EIC chromatogram area.

2. Impurities identified in teriparatide drug substances and includes the relative percentage area of four lots of drug substances from three different manufactures (Table 1). The relative percentage area of four lots drug substances from three different manufactures. Many impurities were detected by LC-HRMS EIC method. The purity (by HPLC method) of those DS were more than 95% shown in their COAs.

Table1. Impurities identified in teriparatide drug substances.

Compound Name	USP	Bachem	BachemTFA	PolyPeptide
Teriparatide(1-34) MW 4117.8	82.12	82.71	79.36	82.95
Teriparatide(1-34) Met +O (8, 18)	2.48	3.52	3.48	3.39
Teriparatide(1-34) Met +O (8/18)	6.12	4.04	5.20	3.94
rhPTH(1-30)	0.29	0.10	0.14	0.11
rhPTH(1-34) Succinimide(30)	0.25	0.40	0.38	0.55
unkown	8.37	8.19	9.95	8.01
Des-(His32, Asn33, Phe34)-PTH (1-34)	0.03	0.43	0.24	0.22
Des-Ser1-PTH (1-34)	0.02	0.22	0.24	0.04
Endo-Ser1-PTH (1-34)	0.14	0.20	0.77	0.57
Val-Arg rhPTH (1-34)	0.03	0.03	0.06	0.04
N-ac rhPTH (1-34)	0.14	0.16	0.19	0.18
Total	100.00	100.00	100.00	100.00

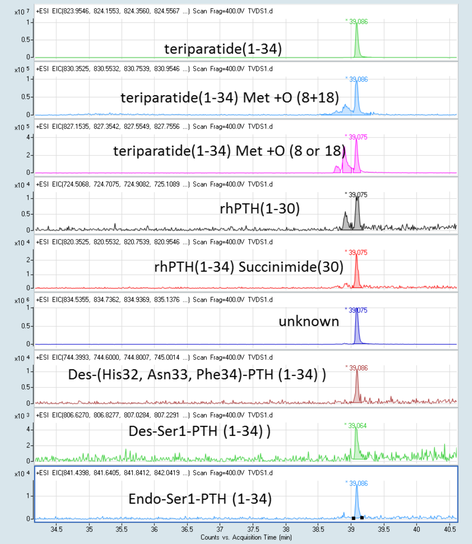

Figure 1. API and impurities LC-HRMS EIC chromatograms of teriparatide.

3. Impurities identified in Teriparatide Drug Product, Forteo (Table 2). The average of normalized relative percentage LC-HRMS EIC area from six lots of teriparatide were as following: teriparatide, 80.38%; teriparatide oxidative degradative impurity Met(O)8+18 , 1.91%; teriparatide oxidative impurities Met(O)8 and Met(O)18, 6.27%; teriparatide1-30 , 0.77%; teriparatide Asp (succinimide)30, 0.53%; unknown (m/z=834.5, z=5), 8.21%.

4. API and impurities LC-HRMS EIC Chromatograms of Teriparatide (Figure 1.) LC-HRMS can identify, separate and quantify impurities co-eluting with the API or other co-eluting impurity peaks with greater sensitivity than LC-UV. For example, teriparatide impurities such as: rhPTH(1-30)(m/z at 724.5856), rhPTH(1-34) Succinimide(30) (m/z at 820.4313), were resolved by LC-HRMS, even when the peaks are not well separated by Chromatography.

Table2. Impurities identified in Teriparatide Drug Product, Forteo.

Compound Name	Monoisotope MW	[M+5H]5+	C470473C	C587623C	C644202D	C650452G	C616383C	C658878C	**Avg**
Teriparatide(1-34) MW 4117.8	4115.130475	824.033365	79.14	79.18	80.06	81.48	81.06	81.35	**80.38**
Teriparatide(1-34) Met +O (8, 18)	4147.120295	830.431329	2.02	1.90	2.16	1.07	2.17	2.14	**1.91**
Teriparatide(1-34) Met +O (8/18)	4131.125385	827.232347	6.57	7.26	6.96	6.66	4.91	5.19	**6.26**
rhPTH(1-30)	3617.89186	724.585642	1.27	0.82	0.42	0.57	1.01	0.52	**0.77**
rhPTH(1-34) Succinimide(30)	4097.119915	820.431253	0.72	0.61	0.46	0.46	0.48	0.42	**0.53**
unkown		834.5321	8.36	8.32	8.02	7.83	8.28	8.42	**8.21**
Val-Arg rhPTH (1-34)	4370.29997	875.067264	0.14	0.12	0.13	0.12	0.18	0.13	**0.14**
N-ac rhPTH (1-34)	4157.141035	832.435477	1.78	1.79	1.79	1.81	1.91	1.83	**1.82**
Total, %			100.00	100.00	100.00	100.00	100.00	100.00	**100.00**

CONCLUSION(S)

The LC-HRMS method detected, resolved and quantified both process- and degradation-related impurities for recombinant or synthetic teriparatide products. The LC-HRMS method was superior to HPLC-UV analysis for teriparatide due to improved specificity and sensitivity.

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