Develop and Validate a LC-MS Method to Establish Impurity Profile in the RLD for Teriparatide

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Introduction

Teriparatide is a 34 amino acid peptide drug that is indicated for treating 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. To evaluate the acceptability of the peptide-related impurities in any product made by recombinant or synthetic processes, the widely used LC-UV approach has limitations. Notably, that 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.[1] Understanding the impurity profile is important to ensure drug quality and safety such as any adverse events associated with immunogenicity. Here, a sensitive and specific LC-MS method was developed for characterizing peptide-related impurities in teriparatide.

Materials and Methods

Synthetic

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

Recombinant

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

DS: Drug Substance

LC-HRMS (liquid chromatography high resolution mass spec) analysis was performed using Agilent 6560A, Accurate-Mass Q-TOF (quadrupole time-of-flight) 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.

Results

The LC-MS method for teriparatide had good linearity (R^2 =0.9998) and precision (%RSD less than 2.0%) at concentrations between 500 to 10000 ng/mL range. The LOD (limit of detection) and LOQ (limit of quantitation) for teriparatide were determined to be 0.02% and 0.05%, respectively, of percent of label claim using the EIC (extracted ion chromatogram) area.

m =	41682.05067	-4571450.16	= b	
	111.0726323	483375.6483	=std	
R^2 =	**0.999765724**	2208692.231		
R =	0.999882855		**% LC**	**LC, ng/mL**
LOD =	38.27	ng/mL	**0.02**	250000
LOQ =	115.97	ng/mL	**0.05**	250000

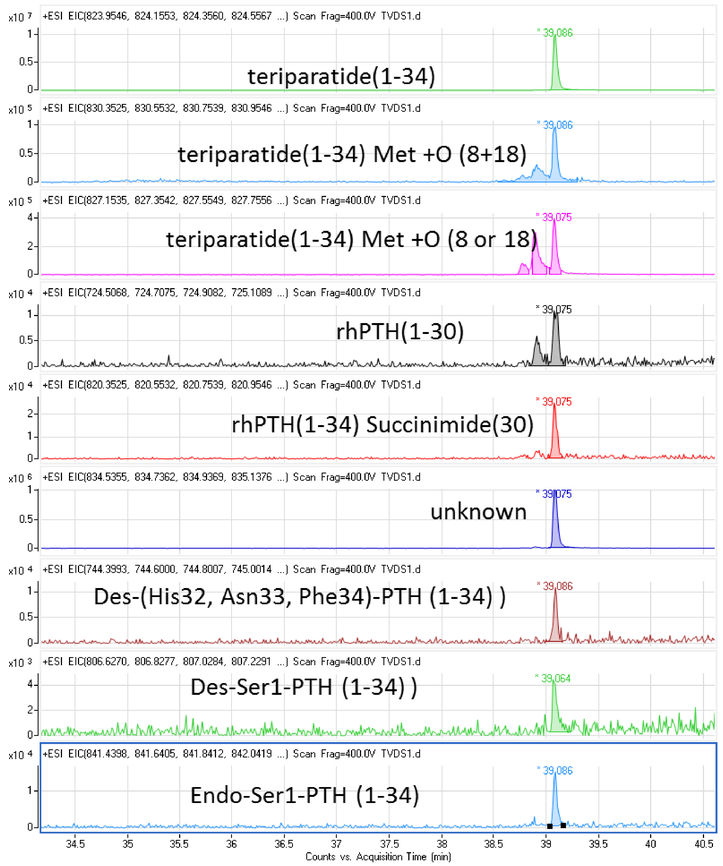
1. Kui et al. AAPS J. 2015 May; 17(3):643-51

Results

Impurities identified in Teriparatide Drug Product, FORTEO

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 , 6.26%; teriparatide1-30 , 0.77%; teriparatide Asp (succinimide)30, 0.53%; unknown (m/z=834.5, z=5), 8.21%.

Compound Name	monoisotope MW	[M+5H]5+	C470473C	C587623C	C644202D	C650452G	C616383C	C658878C	Avg
teriparatide(1-34)	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



API and Impurities LC-HRMS EIC Chromatograms of Teriparatide

LC-MS 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-MS, even when the peaks are not well separated by the chromatography.

Impurities identified in teriparatide Drug Substances (DS)

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 certificates of analysis (COAs).

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)	1.33	0.61	0.00	0.77
teriparatide(1-34) Met +O (18)	4.79	3.43	5.20	3.17
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

Conclusions

The LC-MS method detected, resolved and quantified both process- and degradation-related impurities for recombinant or synthetic teriparatide products. The LC-MS method was superior to HPLC-UV analysis[1] for teriparatide due to the improved specificity and sensitivity of LC-MS.

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Disclaimer

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