Poster T1230-06-42

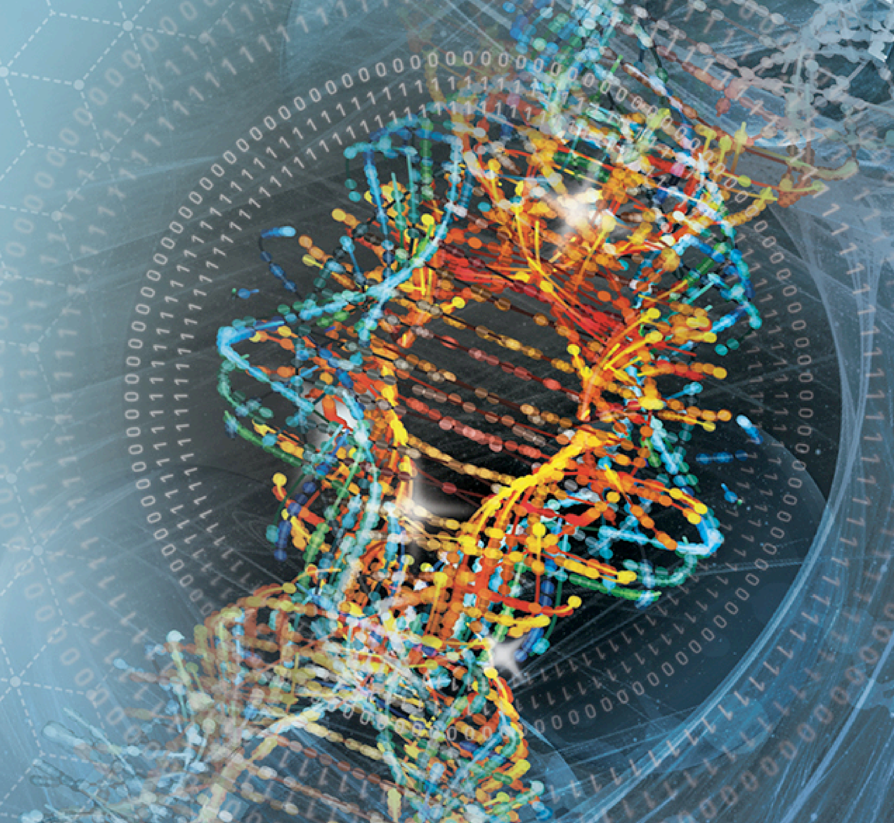
Quantitative comparison of peptide impurities in teriparatide from biological and synthetic origin using LC-MS/MS

Daniel A. Weisz*, Ilan Geerlof-Vidavsky*, Kui Zeng*, Xiaohui Jiang**, Eric Pang**

*Division of Pharmaceutical Analysis, Office of Testing and Research, Office of Pharmaceutical Quality, Center for Drug Evaluation and Research, US Food and Drug Administration, St. Louis, MO USA

**Division of Therapeutic Performance, Office of Research and Standards, Office of Generic Drugs, Center for Drug Evaluation and Research, US Food and Drug Administration, Silver Spring, MD USA

CONTACT INFORMATION: daniel.weisz@fda.hhs.gov



Advancing Pharmaceutical Sciences, Careers, and Community

PURPOSE

The patents for many peptide drugs are expiring within 5-10 years, setting the stage for a wave of generic applications seeking approvals for generic peptide products based on their sameness to the reference products. Assessing sameness requires a quantitative and qualitative comparison of the impurity profiles of the generic and the reference listed drug (RLD) products. Minor differences in the impurity profiles may be justified provided that these differences would not affect the safety and efficacy of the proposed product. In this study, we developed and validated an ultra performance liquid chromatography and mass spectrometry (UPLC-MS) method to identify and quantify differences in the impurity profiles of teriparatide, a 34 amino-acid therapeutic peptide, derived from different origins (recombinant-derived teriparatide drug product and synthetic teriparatide purchased commercially). As recommended in FDA's draft Guidance for Industry, titled "ANDAs for Certain Highly Purified Synthetic Peptide Drug Products that Refer to Listed Drugs of rDNA Origin" (1), the UPLC-MS method should be both sensitive (Limit of Quantitation (LOQ) should be at 0.05%) and robust enough for routine analysis.

OBJECTIVE

To develop and validate a UPLC-MS method to identify and quantify peptide-related impurities in teriparatide products of synthetic and biological origin.

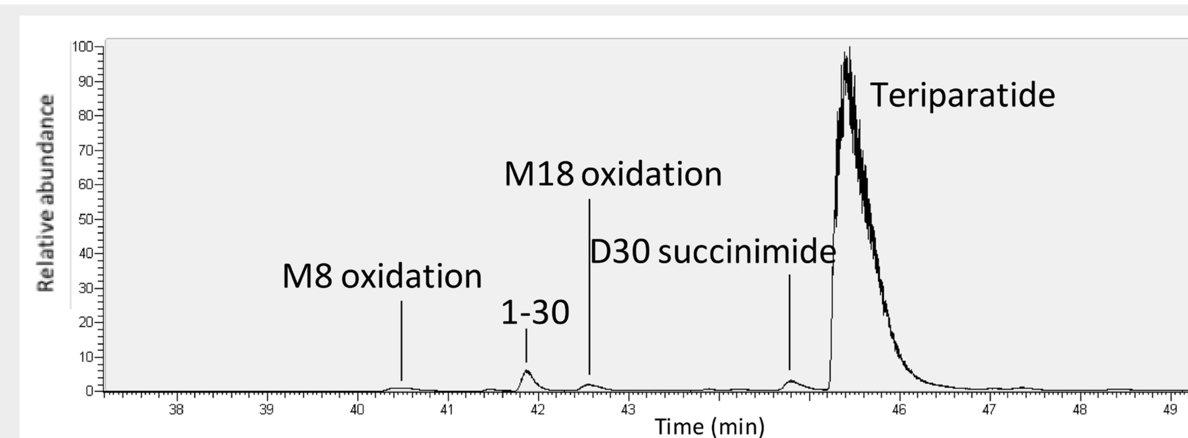
METHODS

Recombinant-derived teriparatide RLD and synthetic teriparatide purchased from Bachem were analyzed by UPLC-MS for relative quantification, and UPLC-MS/MS for impurity identification, on a Thermo Q-Exactive HF-X mass spectrometer. Prior to analysis, synthetic teriparatide was dissolved in 9:1 water : acetonitrile at a final concentration of 0.25 mg/mL, the same concentration as the teriparatide RLD (also referred to as "DP" in "Results"). UPLC separation was performed on a Waters Acquity UPLC BEH C18 1.7µm 2.1 x 150 mm column, with an Agilent 1260/1290 HPLC connected online to the mass spectrometer. Peptide identification and relative quantification was performed using PEAKS X, Thermo Xcalibur integration, and manual verification. The three most intense isotope peaks from each of the three most abundant charge states (+5, +6, and +7) were included in the quantification. Impurity percent values are relative to the total peak area of the main teriparatide peak plus the areas of the sodium and potassium adducts, and the in-source fragment 3-34 (teriparatide with the two N-terminal residues missing).

RESULTS

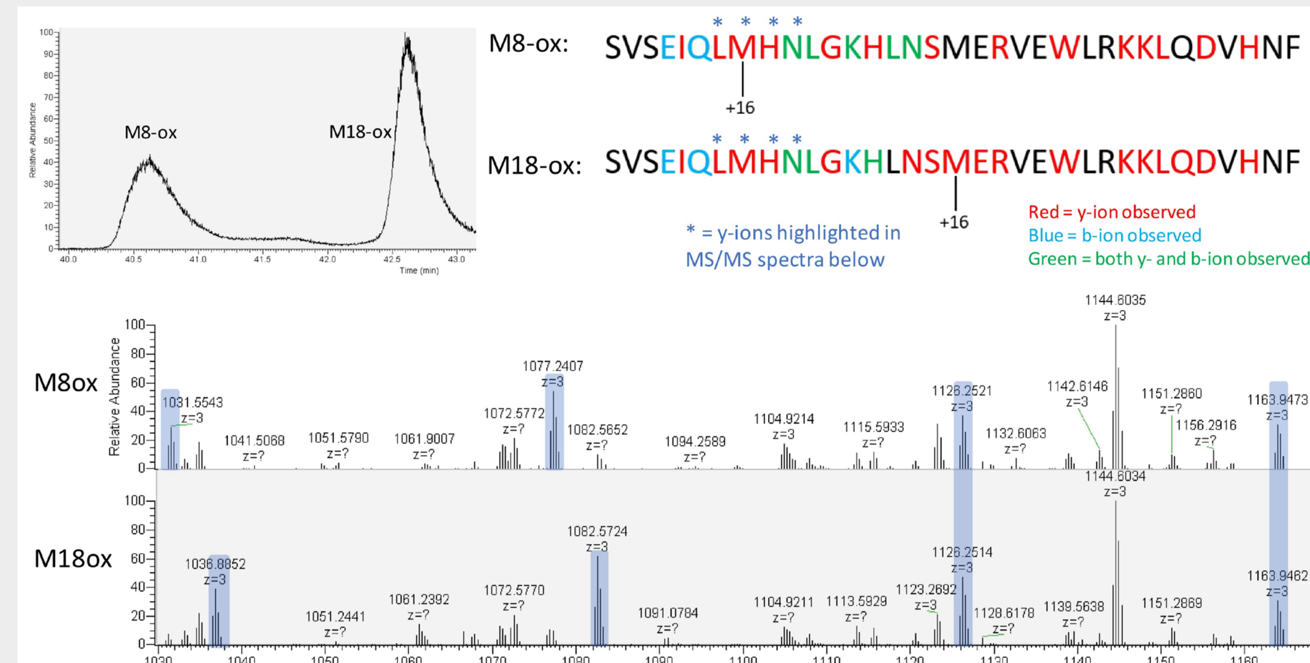
The UPLC method is able to achieved chromatographic separation between teriparatide and other peptide related impurities **(Fig. 1)**.

Fig. 1. Total-ion chromatogram of one drug product lot showing chromatographic resolution of teriparatide and several of the most abundant impurities.



MS/MS analysis enabled residue-level localization of peptide modifications for most impurities. An example is show in **Fig. 2**.

Fig. 2. Example spectra showing the M8 and M18 oxidized peptides. Extracted-ion chromatogram in upper left shows chromatographic resolution of the two peptides, and the MS/MS spectra below show the localization of the modified residues. The blue shaded peaks distinguish between the two modified peptides.

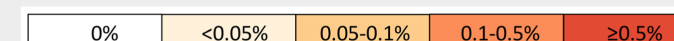


Twenty peptide-related impurities were identified and quantified and are shown in **Table 1**.

The DP lots were manufactured ~3-4 years prior to analysis and were stored at 4ºC in solution during that time, according to manufacturer guidelines. When an impurity's abundance increases with storage time, it would indicate such impurity is most likely formed as a result of drug product degradation under storage conditions. The synthetic teriparatide was considered as time point 0 for this analysis because it had been stored as a solid powder at -80ºC, rendering it less susceptible to various degradation pathways.

Table 1. Impurities identified in teriparatide RLD (DP) and synthetic teriparatide. 1-30: teriparatide truncation product containing residues 1-30 (similar for 1-32, 1-33); VR(1-34): Val-Arg insertion at N-terminus; V(1-30): Val insertion at N-terminus; D: impurity arising from drug product degradation; P: impurity arising from drug production process; DP: drug product.

Modification name	Degradation or prod. process	Synthetic teriparatide	DP lot 1	DP lot 2	DP lot 3
Months since manufacture:			34.5	34.5	50.5
1-30	D	0.03	1.46	1.46	2.26
M18 oxidation	D	0.55	1.19	1.13	1.75
D30 succinimide	D	0.06	1.12	1.11	1.34
M8 oxidation	D	0.25	0.32	0.40	0.79
N33 succinimide	D	0.02	0.27	0.28	0.30
1-30, D30 succinimide	D	0.001	0.12	0.12	0.18
1-30, S17 dehydroalanine	D	0.001	0.10	0.10	0.16
N10 succinimide	D	0.003	0.09	0.09	0.09
Unknown 856.8, RRT 0.9817	D	0.01	0.05	0.05	0.07
1-32	D	0.0004	0.04	0.03	0.05
Unknown 821.02, RRT 1.1360	D	0.003	0.05	0.04	0.01
1-33	D	0.007	0.03	0.02	0.05
VR(1-34)	P	0.002	0.14	0.21	0.17
S1 formaldehyde	P	0.94	--	--	0.02
S1 deletion	P	0.23	--	--	0.002
S18, S19 insertion	P	0.18	--	--	--
V(1-30)	P	0.15	--	--	--
Q29, D30 deletion	P	0.13	--	--	--
Unknown 856.8, RRT 1.0604	P	0.20	--	--	--
K13 deletion	P	0.06	--	--	--



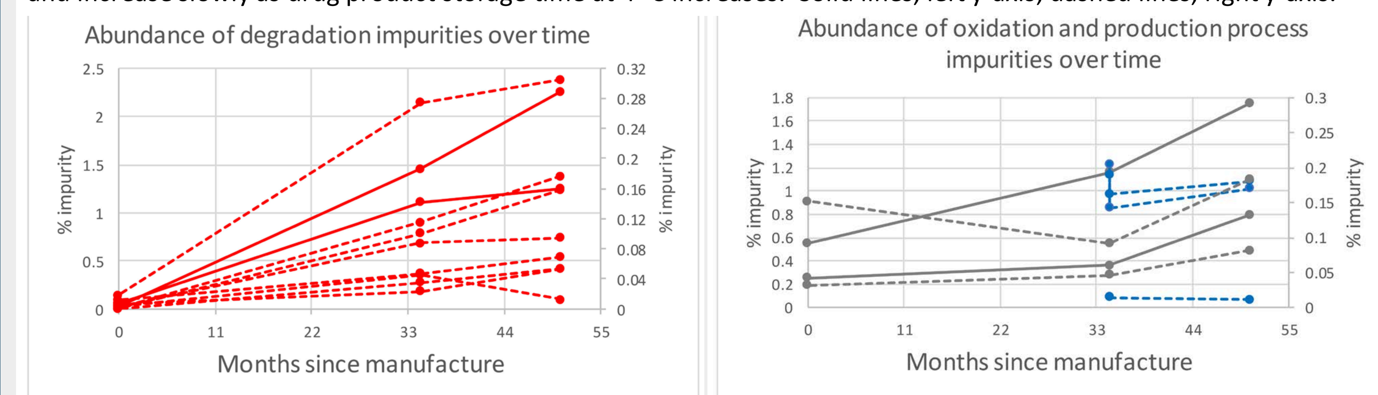
Ten impurities were not found at the initial time points in the synthetic teriparatide, but increased (4-154-fold increase) steadily with storage time (at 4 ºC). **(Fig. 3)**. These impurities were considered to arise from degradation of teriparatide, and all ten were found in all samples tested.

Ten impurities did not increase steadily with time, and displayed instead manufacturing-specific abundance patterns **(Table 1 and Fig. 4)**. These impurities were considered to arise from the particular drug production process employed, either through synthetic or biological process.

The four oxidation impurities were present in relatively high amount in the synthetic teriparatide. They generally rose in abundance with increasing storage time at 4 ºC, but slower than the degradation impurities (only 1-3-fold increase over time).

Figure 3 (left). Degradation impurities are nearly absent in the synthetic teriparatide that had been stored at -80 °C but rise as drug product storage time at 4 °C increases. Solid lines, left y-axis; dashed lines, right y-axis.

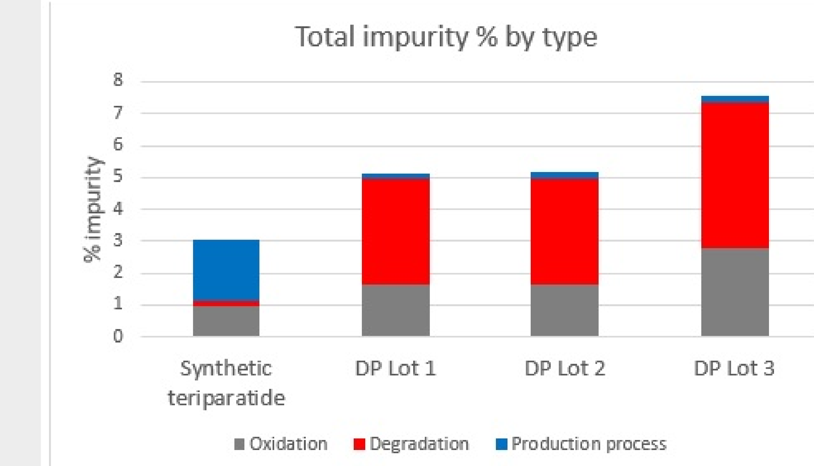
Figure 4 (right). Abundance of each production process impurity in the drug product (blue) is relatively constant over the two time points measured. The oxidation impurities are present at relatively high amount in synthetic teriparatide and increase slowly as drug product storage time at 4 °C increases. Solid lines, left y-axis; dashed lines, right y-axis.



CONCLUSIONS

- A robust UPLC-MS method was implemented that is capable of separating and quantifying peptide-related impurities in teriparatide with an LOQ NMT 0.05%.
- Synthetic teriparatide contained six peptide-related impurities above the recommended identification threshold of 0.10% (1) that were not present in the teriparatide RLD of biological origin, and an additional one just below the threshold (at 0.06%). Five of these impurities (S1 deletion, S18,S19 insertion, V(1-30), Q29,D30 deletion, and K13 deletion) are amino acid variants that likely result from the peptide synthesis process.
- Synthetic teriparatide contained six peptide-related impurities above the recommended identification threshold of 0.10% (1) that were not present in the drug product of biological origin, and an additional one just below the threshold (at 0.06%). Five of these impurities (S1 deletion, S18,S19 insertion, V(1-30), Q29,D30 deletion, and K13 deletion) are amino acid variants that likely result from the peptide synthesis process.
- The teriparatide RLD contained one impurity (VR(1-34)) that arises from the biological production process of this drug.
- No degradation impurities were identified in the recombinant teriparatide RLD that were not already present in the synthetic teriparatide.
- **Fig. 5** summarizes the findings, showing that the peptide synthesis process can introduce new impurities into a drug substance that were not present in the reference product of biological origin. If these impurities were found in the final generic drug product at levels exceeding the recommended levels in FDA's draft guidance (1), justifications and supporting data would need to be provided to demonstrate that these impurities do not impact the safety and efficacy of the proposed generic product.

Figure 5. Total impurity content in each sample is shown, broken down by the three impurity types identified in this study.



REFERENCE

1. FDA Guidance for Industry (draft): ANDAs for certain highly purified synthetic peptide drug products that refer to listed drugs of rDNA origin, October 2017. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/andas-certain-highly-purified-synthetic-peptide-drug-products-refer-listed-drugs-rdna-origin
.

FDA U.S. FOOD & DRUG ADMINISTRATION

DISCLAIMER

The poster reflects the views of the authors and should not be construed to represent FDA's views or policies.