Identification and quantitation of host cell protein impurities in peptide therapeutics using liquid chromatography-mass spectrometry

Xiaoshi Wang[1], Eric Pang[2], Xiaohui Jiang[2], Sarah Rogstad[1]

[1] Division of Pharmaceutical Analysis, OTR, OPQ, CDER, U.S. FDA
[2]Division of Therapeutic Performance, ORS, OGD, CDER, U.S. FDA

Introduction

Over the last decade, peptide therapeutic products have made inroads into the global pharmaceutical market with a wide range of effective applications for numerous disease indications. Concurrently, advanced peptide therapeutic analysis methods are being developed and are improving rapidly with the emergence of modern technologies, such as more efficient solid-phase peptide synthesis and recombinant DNA transgenic cell line peptide production technologies. As the originator products come off patent, the number of peptide therapeutic abbreviated new drug applications (ANDAs) submitted to FDA is also increasing. Since 2003, FDA has approved 38 new drug applications (NDAs) and 40 ANDAs for peptide therapeutics. Figure 1 shows the number of peptide therapeutic approvals from 2003 until March of 2017.

Of the 38 peptide drug NDAs, five were produced recombinantly. One type of impurity for concern in recombinant peptide drugs is host cell proteins (HCPs), which are typically present at levels below 10 ppm in the drug products. Even at very low concentrations, HCPs may have immunogenic effects. Therefore, HCPs need to be monitored and controlled in order to ensure product quality.

Traditionally, many analytical methods have been used for HCP analysis. Among them, ELISA has been used as the 'gold standard' for HCP quantitation as both an in process and a release assay. Table 1 lists the strengths and weaknesses of different analytical methods compared with the LC-MS/MS method. This study used recombinant teriparatide drug product as an example to demonstrate the ability of LC-MS/MS in HCP characterization.

Methods

Teriparatide drug product (FORTEO®, Eli Lilly), lot C365629C (400 µL) was concentrated with a 0.5 mL 10 KDa Amicon filter and buffer exchanged into 100 mM Tris pH 8.0, urea 2M three times. Both the drug product and HCP fractions were reduced in 10 mM DTT at 56 °C for 30 min and alkylated with 20 mM IAM for 30 minutes at RT in the dark, followed by digestion with trypsin at 37°C for 4 hours. Formic acid at added to 1% to quench the digestion for sample loading. The resulting samples were analyzed via LC-MS/MS on a Q-Exactive hybrid quadrupole-orbitrap MS using the Agilent ZORBAX RRHD eclipse plus C18, 2.1x100 mm, 1.8 µm column. Column temperature was maintained at 50 °C. A 100 minute gradient was used with a flow rate of 300 µL/min. Buffer A was 0.1% formic acid in water. Buffer B was 0.1% formic acid in ACN. Data were analyzed with Proteome Discoverer software by searching against a Uniprot *E. Coli* proteome database including the teriparatide sequence.

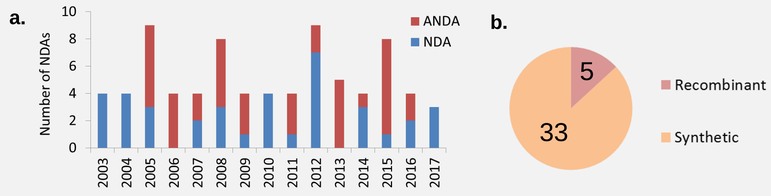

Figure 1: a. Peptide therapeutic approvals over time. b. Distribution of manufacturing types for approved peptide therapeutics in NDAs.

Method	Strength	Weakness
SDS-PAGE /silver stain	➢ Good sensitivity (100pg/band) ➢ Resolves multiple components	➢ Subjective interpretation ➢ Not quantitative ➢ Technique-dependent
HPLC /UV-fluorescent	➢ High resolution ➢ Quantitative	➢ Subjective interpretation ➢ Low sensitivity ➢ Non-specific
Western blot	➢ High sensitivity (0.1-1ng/band) ➢ Semi-quantitative ➢ Immunological identity ➢ Resolves multiple components	➢ Antibody may fail to detect some contaminants ➢ Technique-dependent
ELISA (gold standard)	➢ High sensitivity (1ppm) ➢ Semi-quantitative ➢ Easy to perform	➢ Objective endpoint ➢ Summed value ➢ Bias toward only immunoreactive species ➢ Not transferable (process related)
LC-MS/MS	➢ Identification of individual HCPs ➢ High sensitivity (1ppm) ➢ Quantitative ➢ Process transferable ➢ Useful info for risk assessment	➢ Potential bias towards high abundant species ➢ Technique dependent ➢ Instrument high maintenance

Table 1: Summary of analytical methods for HCP characterization.

Results

Four tryptic peptides and three mis-digested peptides were identified in the digested samples. The sequence coverage of teriparatide was 100%. However, no HCPs were identified after searching the data against an *E. Coli* proteome database. The lack of identifications is likely a result of the extremely low levels of HCPs present in the drug product .

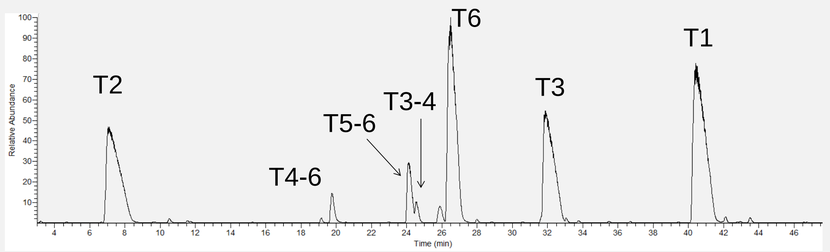

Figure 2: Total ion chromatogram (TIC) of teriparatide drug product after trypsin digestion. (Peptide sequences: T1 is SVSEIQLMHNLSK; T2 is HLNSMER; T3 is VEWLR; T6 is LQDVHNF; T4-6 is KKLQDVHNF; T5-6 is KLQDVHNF; T3-4 is VEWLRK)

Results (continued)

A 10 kDa Amicon filter was applied to fractionate the 4118 Da teriparatide molecule and concentrate the HCPs from 400 µL of RLD teriparatide drug product. After reduction, alkylation and tryptic digestion of the HCP fraction, a LC-MS/MS method was used that detected two *E. Coli* HCPs with at least two unique peptides each. The identified proteins were 50S ribosomal protein L2 and 50S ribosomal protein L31. The peptide sequence, modification, molecular weight, detection accuracy and relative abundance are summarized in Table 2.

Accession	Description	Unique peptide sequence	PTM	MH+ (Da)	ΔM (ppm)	Rel Ratio* ppm
P60422	50S ribosomal protein L2	SANIALVLYK	N/A	1091.6480	1.86	27
		LEYDPNR	N/A	906.4328	1.31	
		NKDGIPAVVER	N/A	1197.6604	1.46	
P0A7M9	50S ribosomal protein L31	STVGHDLNLDVcSK	C12(IAM)	1544.7428	3.50	8
		YEEITAScScGNVMK	C8(IAM) C10(IAM)	1748.7253	-2.00	

Table 2: *E. Coli* proteins and unique peptides identified from concentrated teriparatide HCP fraction.
*Rel Ratio in ppm was calculated as the ratio between averaged area under curves (AUC) of all detectable HCP peptides for the target protein and averaged AUCs of teriparatide tryptic peptides.

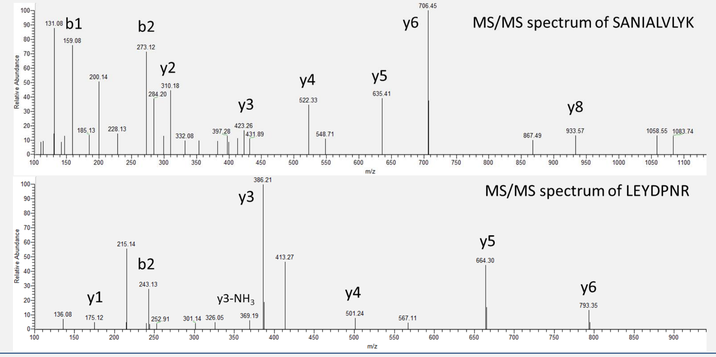

Figure 3: Examples of MS/MS spectra of peptides from 50S ribosomal protein L2.

Conclusions

- HCP fractionation and concentration increased the identification of low abundance HCPs through LC-MS/MS.
- Two *E. Coli* HCPs were identified with at least two unique peptides each in the concentrated HCP fraction of the RLD teriparatide.
- This LC-MS/MS method can be applied to other recombinant peptide drug products for HCP characterization.

Disclaimer

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