Semi-validation of the LC-MS/MS method for identification and comparative quantification of host cell protein (HCP) impurities in teriparatide drug products

Cung Nawl Thawng[1], Cynthia Sommers[1], , Eric Pang[3], David A. Keire[1,2], Hongbin Zhu[1]

1 Division of Pharmaceutical Quality Research II, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, 645 S. Newstead Ave, St. Louis, MO, USA

2 Office of Pharmaceutical Quality Research , Center for Drug Evaluation and Research, U.S. Food and Drug Administration, 645 S. Newstead Ave, St. Louis, MO, USA

3 Division of Therapeutic Performance, Office of Research and Standards, Office of Generic Drugs, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, MD, USA

Introduction

The presence of host cell protein (HCP) impurities is one of the major regulatory concerns for accepting and approving cell-derived peptide drugs. The identification of specific HCPs allows for a better understanding of process development, quality controls, and risk assessments. The goal of this study was to validate a previously developed LC-MS/MS method for identification and comparative quantification of host cell protein (HCP) impurities in teriparatide drug products. The sensitivity and accuracy of the method need to be validated to ensure that it can accurately assess host cell protein (HCP) profiles of products produced through different recombinant process.

Methods

Synthetic standard teriparatide drug (negative control), spiked with Proteomics Dynamic Range Standard Set (UPS-2) which contained 48 proteins ranging from 0.05 to 3000 ppm, was lyophilized and processed with the EasyPep™ Mini MS Sample Prep Kit, without any enrichment or buffer exchange. Peptide Retention Time Calibration (PRTC) was spiked to the sample as internal standard. LC-MS raw data were analyzed using Proteome Discoverer, with Byonic as the searching engine. Protein sequences of the 48 proteins were used for database searching, with a precursor mass tolerance of 5 ppm and a fragment mass tolerance of 0.5 Da applied. Peptide identifications were filtered with a Byonic Score of ≥ 300. Skyline was used for building a spectra library and plotting precursor ion high-resolution MS extracted-ion chromatogram (EIC).

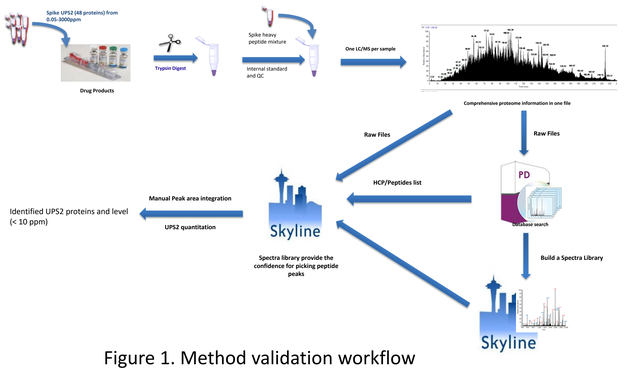

Figure 1. Method validation workflow

Results and discussion

Table 1. List of the protein spiked and the detection and quantification results. In total, 35 of the spiked proteins were detected (33 were identified with ≥ 2 unique peptides).

UniProt #	MW (Da)	fmol spiked	ng spiked	Teriparatide (mg)	ppm	Detected	Quantified
P10145	8386	0.125	0.0010	0.08	0.01	No	No
P05413	14727	0.125	0.0018	0.08	0.02	No	No
P00441	15805	0.125	0.0020	0.08	0.02	No	No
P01375	17353	0.125	0.0022	0.08	0.03	No	No
P02741	23047	0.125	0.0029	0.08	0.04	No	No
P08758	35806	0.125	0.0045	0.08	0.06	No	No
P10636	45719	0.125	0.0057	0.08	0.07	No	No
P02788	76165	0.125	0.0095	0.08	0.12	No	No
P99999	11618	1.25	0.0145	0.08	0.18	No	No
P01579	16879	1.25	0.0211	0.08	0.26	No	No
O00762	20475	1.25	0.0256	0.08	0.32	Yes, 2	No
P01112	21298	1.25	0.0266	0.08	0.33	No	No
P51965	22227	1.25	0.0278	0.08	0.35	No	No
P09211	23225	1.25	0.0290	0.08	0.36	No	No
P01344	7475	12.5	0.0934	0.08	1.17	Yes	Yes
P02787	75181	1.25	0.0940	0.08	1.17	Yes	Yes
P06396	82959	1.25	0.1037	0.08	1.30	Yes	Yes
P61769	11731	12.5	0.1466	0.08	1.83	Yes, 1	No
P01127	12294	12.5	0.1537	0.08	1.92	Yes, 1	No
P10599	12429	12.5	0.1554	0.08	1.94	Yes	Yes
O76070	15363	12.5	0.1920	0.08	2.40	Yes	Yes
P55957	21995	12.5	0.2749	0.08	3.44	Yes	Yes
P08263	25500	12.5	0.3188	0.08	3.98	Yes	Yes
P01008	49039	12.5	0.6130	0.08	7.66	Yes	Yes
Q15843	9072	125	1.1340	0.08	14.18	Yes	Yes
P00709	14078	125	1.7598	0.08	22.00	Yes	Yes
P61626	14701	125	1.8376	0.08	22.97	Yes	Yes
P63279	18007	125	2.2509	0.08	28.14	Yes	Yes
P02753	21071	125	2.6339	0.08	32.92	Yes	Yes
P16083	25821	125	3.2276	0.08	40.35	Yes	Yes
P06732	43101	125	5.3876	0.08	67.35	Yes	Yes
P12081	58233	125	7.2791	0.08	90.99	Yes	Yes
P01133	6353	1250	7.9413	0.08	99.27	Yes	Yes
P00167	16022	1250	20.0275	0.08	250.34	Yes	Yes
P02144	17053	1250	21.3163	0.08	266.45	Yes	Yes
P62937	20176	1250	25.2200	0.08	315.25	Yes	Yes
Q06830	21979	1250	27.4738	0.08	343.42	Yes	Yes
P15559	30736	1250	38.4200	0.08	480.25	Yes	Yes
P63165	38815	1250	48.5188	0.08	606.48	Yes	Yes
P04040	59625	1250	74.5313	0.08	931.64	Yes	Yes
P01031	8536	12500	106.7000	0.08	1333.75	Yes	Yes
P62988	10597	12500	132.4625	0.08	1655.78	Yes	Yes
P69905	15126	12500	189.0750	0.08	2363.44	Yes	Yes
P68871	15867	12500	198.3375	0.08	2479.22	Yes	Yes
P41159	16158	12500	201.9750	0.08	2524.69	Yes	Yes
P00915	28738	12500	359.2250	0.08	4490.31	Yes	Yes
P00918	29115	12500	363.9375	0.08	4549.22	Yes	Yes
P02768	66357	12500	829.4625	0.08	10368.28	Yes	Yes

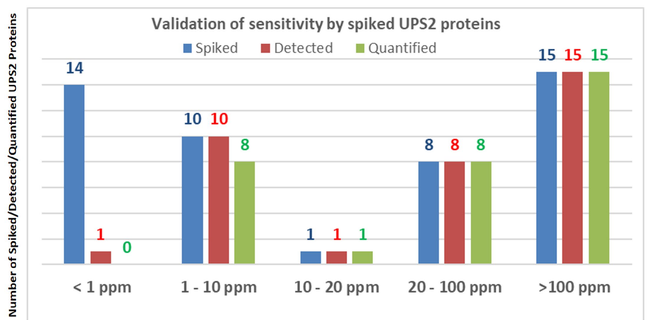

Figure 1. All the spiked protein > 1 ppm are detected (35 out of 48). Eight of the 10 spiked proteins at very low level (1-10 ppm) are quantified. All the spiked protein > 10 ppm quantified.

Results and discussion

Table 2. Quantification results of the protein spiked and the recovery calculated. Recovery (%) were calculated between the relative quantified amount and the amount of the UPS2 proteins (fmol) spiked in the negative control (n=2). More accurate and precision results were observed in higher level protein (≥ 20 ppm)

Protein	MW (fg/fmol)	#PepQuan	#PepID	ppm spiked	ppm quantified	Recovery %
P01344ups-50	7475	2	≥3	1.2	0.7	60
P02787ups-5	75181	2	≥3	1.2	1.9	158
P06396ups-5	82959	1	≥3	1.3	0.3	27
P10599ups-50	12429	2	2	1.9	3.5	179
O76070ups-50	15363	3	≥3	2.4	9.0	373
P55957ups-50	21995	2	2	3.4	1.4	42
P08263ups-50	25500	2	≥3	4.0	4.8	120
P01008ups-50	49039	3	≥3	7.7	8.9	117
Q15843ups-500	9072	2	2	14.2	6.0	42
P00709ups-500	14078	3	≥3	22.0	12.5	57
P61626ups-500	14701	2	≥3	23.0	14.0	61
P63279ups-500	18007	3	≥3	28.1	18.7	66
P02753ups-500	21071	3	≥3	32.9	6.4	19
P16083ups-500	25821	3	≥3	40.3	49.9	124
P06732ups-500	43101	3	≥3	67.3	63.6	94
P12081ups-500	58233	3	≥3	91.0	63.4	70
P01133ups-5000	6353	1	2	99.3	61.1	62
P00167ups-5000	16022	3	≥3	250.3	374.5	150
P02144ups-5000	17053	3	≥3	266.5	255.4	96
P62937ups-5000	20176	3	≥3	315.3	362.6	115
Q06830ups-5000	21979	3	≥3	343.4	511.1	149
P15559ups-5000	30736	3	≥3	480.3	465.8	97
P63165ups-5000	38815	3	≥3	606.5	634.4	105
P04040ups-5000	59625	3	≥3	931.6	1286.0	138
P01031ups-50000	8536	3	≥3	1333.8	147.9	11
P62988ups-50000	10597	3	≥3	1655.8	1642.0	99
P69905ups-50000	15126	3	≥3	2363.4	1469.7	62
P68871ups-50000	15867	3	≥3	2479.2	1656.2	67
P41159ups-50000	16158	3	≥3	2524.7	1675.8	66
P00915ups-50000	28738	3	≥3	4490.3	8933.8	199
P00918ups-50000	29115	3	≥3	4549.2	7990.0	176
P02768ups-50000	66357	3	≥3	10368.3	15923.1	154

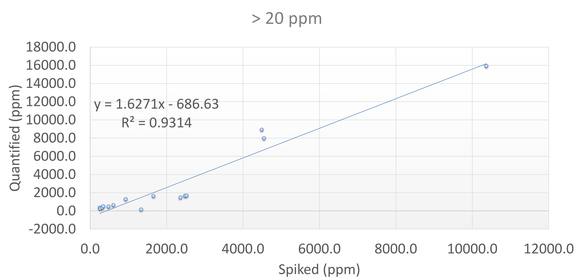

Figure 2. Linear regressions between quantified and spiked proteins 20-10000 ppm).

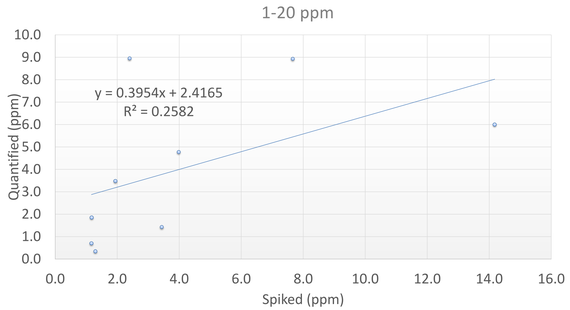

Figure 3. Linear regressions between quantified and spiked proteins 1-20 ppm).

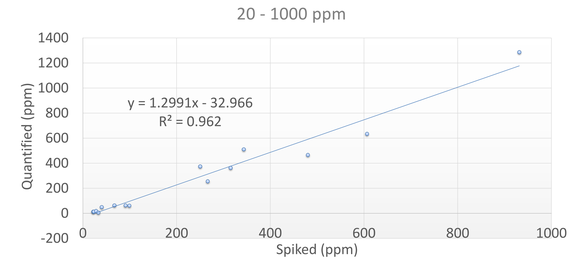

Figure 4. Linear regressions between quantified and spiked proteins 1-20 ppm).

Conclusion

- The method was validated for sensitivity and accuracy by spiking 48 protein standards ranging from 0.01 to 10000 ppm.
- The LC-MS/MS method can be used to detect HCPs in recombinant peptide products at as low as 1 ppm.
- The relative quantitation accuracy is adequate (R^2 = 0.931, average recovery is 97%) for high level proteins (> 20 ppm) and not acceptable (R^2 = 0.258, average recovery is 124%) for lower-level proteins (1-20 ppm).
- The good linearity further proved the acceptable accuracy and the ability of using UPS2 protein standard as external standard for absolute quantification of host cell proteins.