Identification and Quantitation of Host Cell Protein Impurities in Peptide Therapeutics Using Liquid Chromatography-Mass Spectrometry

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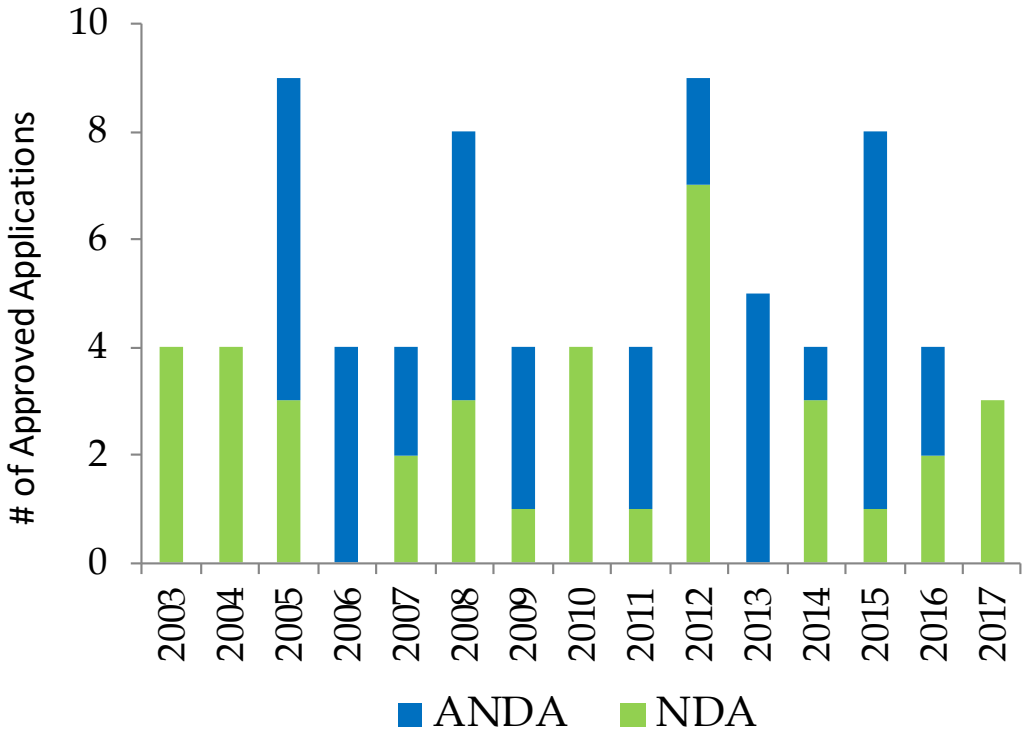
This presentation reflects the views of the author and should not be construed to represent FDA's views or policies

Outline

- ❑ Introduction
  - ➢ Overview of mass spectrometry (MS) usage in FDA approved peptide therapeutic NDAs
  - ➢ Guidelines and methods for host cell protein (HCP) analysis
- ❑ Results
  - ➢ LC-MS/MS workflow for HCP characterization
  - ➢ Identification and quantitation of HCP from teriparatide RLD
- ❑ Conclusions and future work

Peptide Application Approvals

- 38 peptide NDAs and 40 peptide ANDAs have been approved by FDA since 2003.
- 10% were recombinant peptide products.
- 20 Type 1 NDAs (NME) were analyzed for MS usage.
- All NDAs used MS for characterization.



Manufacturing method	Percentage of Type 1 NDAs
SPPS alone	65%
SPPS and LPPS	15%
LPPS alone	10%
Recombinant	10%

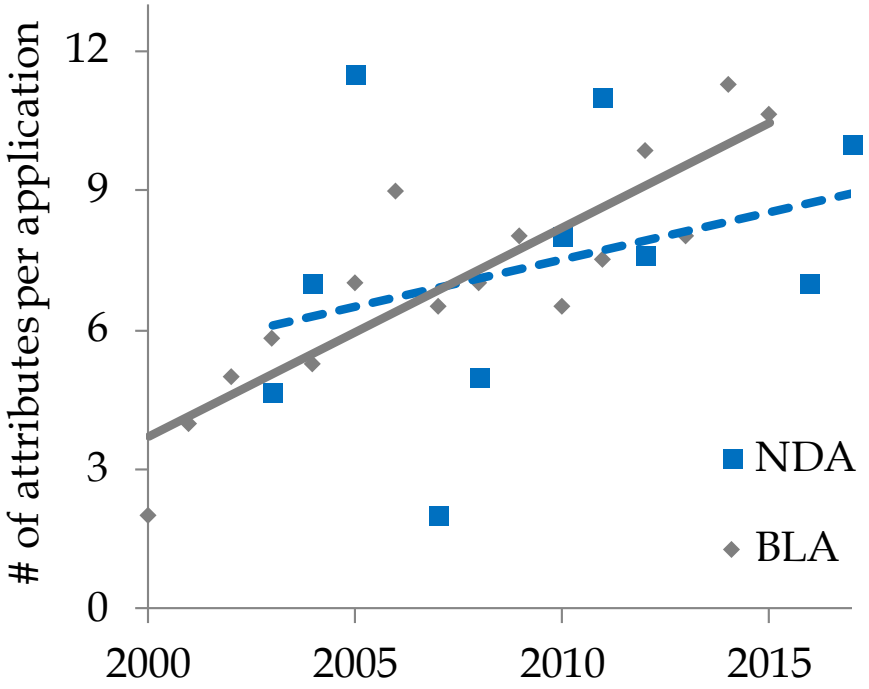
MS in Peptide Characterization

- 28 product quality attributes (PQAs) were monitored by MS.

MS Attribute	% of MS NME NDAs	MS Attribute	% of MS NME NDAs
Molecular weight	100%	Disulfide bonds	20%
Amino acid sequence	85%	Incomplete deprotection	20%
Amino acids deletion/insertion	60%	Dehydration	15%
Terminus modification	55%	Aggregation	10%
Racemisation/diastereomer	50%	Side chain modification caused by solvent	10%
Amino acid enantiomeric purity by GC-MS	45%	Cyclization	10%
Oxidation	45%	Residue solvent by GC-MS	10%
Metals	40%	Thioether	5%
Deamidation	40%	Free thiol	5%
Fragmentation/truncation	35%	Trisulfide	5%
Dimerization/trimerization	30%	PEGylation	5%
Succinimidation/Asu	30%	Palmitoyl	5%
Iso-Asp/beta-Asp	30%	Glycosylation	5%
Acetylation	25%	Mutation	5%

MS PQA Analysis

- A weak correlation was obtained for peptide NDAs (R^2 = 0.10), which is lower than that from protein BLA data (R^2 = 0.77).
- Similar mean numbers of MS attributes per NDA by manufacturing methods and peptide lengths.

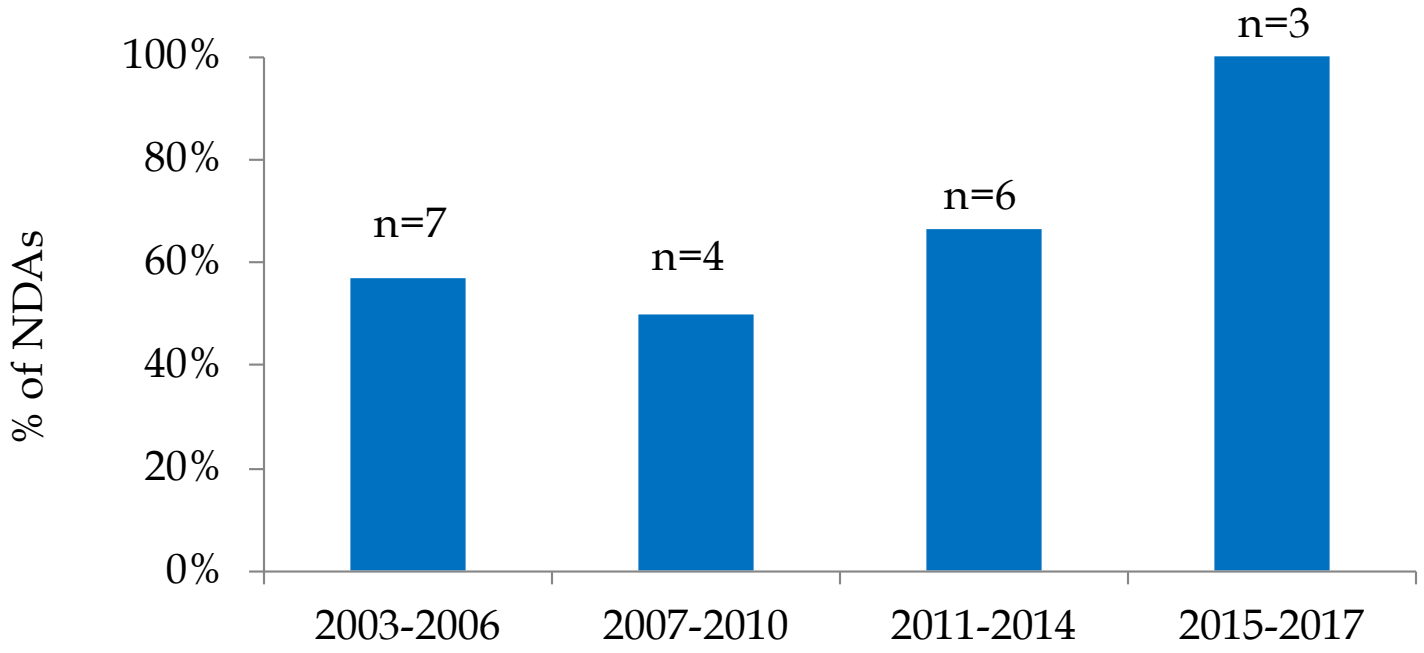


Manufacturing method	# of NDAs	MS attributes per NDA
SPPS alone	13	8.3
SPPS and LPPS	3	6.0
LPPS alone	2	6.0
Recombinant	2	6.5

Peptide length	# of NDAs	MS attributes per NDA
2 aa to 14 aa	8	7.5
15 aa to 29 aa	4	6.8
30 aa to 44 aa	8	8.0

MS in Peptide Quality Control

- MS is commonly used for peptide therapeutics in lot release.
- 65% of the 20 Type 1 peptide NDAs used MS as a release assay for molecular weight measurement.
- MS usage in QC is increasing over time.



HCP in Drug Development

- HCPs are process related impurities produced by the host organisms used to produce recombinant drug products.
- HCP are considered as critical quality attributes (CQAs), HCP associated risks may include:
  - Safety and efficacy of the drug product.
  - Immunogenicity
  - Specific biological activity
  - Product stability issues
- HCP are removed to the lowest feasible extent and HCP levels must be monitored in process and at release.
- Low ppm-level HCPs are typically present in DS/DP following purification processes.

Regulations on HCP

FDA

Regulators classify residual host cell proteins (HCPs) as “process-related impurities.” (US 21 CFR 610.13)

Whenever possible, contaminants introduced by recovery and purification process should be below detectable levels using a highly sensitive analytical methods.

- FDA CBER. Points to consider in the manufacture and Testing of Monoclonal Products for Human Use, Docket No. 94D-0259, Feb 28, 1997

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The ability of the purification process to remove other specific contaminants such as host-cell proteins … should also be demonstrated…

… for HCP, whatever the product or production system, residual HCPs have to be tested for on a routine basis… Results from batch to batch should be consistent and meet specification limits.

-EMEA guideline:CPMP/BWP/382/97

ICH
harmonisation for better health

Regulator guidelines are in place globally (ICH Q6B 2.1.4, 4.1.3, 6.2.1)

For host cell proteins, a sensitive assay – e.g. immunoassay – capable of detecting a wide range of protein impurities is generally utilized.

- ICH Q6B

HCP Characterization Methods

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

LC-MS/MS Workflow for HCP Characterization

Samples
1. Recombinant teriparatide RLD DP from Eli Lilly
2. Synthetic teriparatide from the PolyPeptide Group

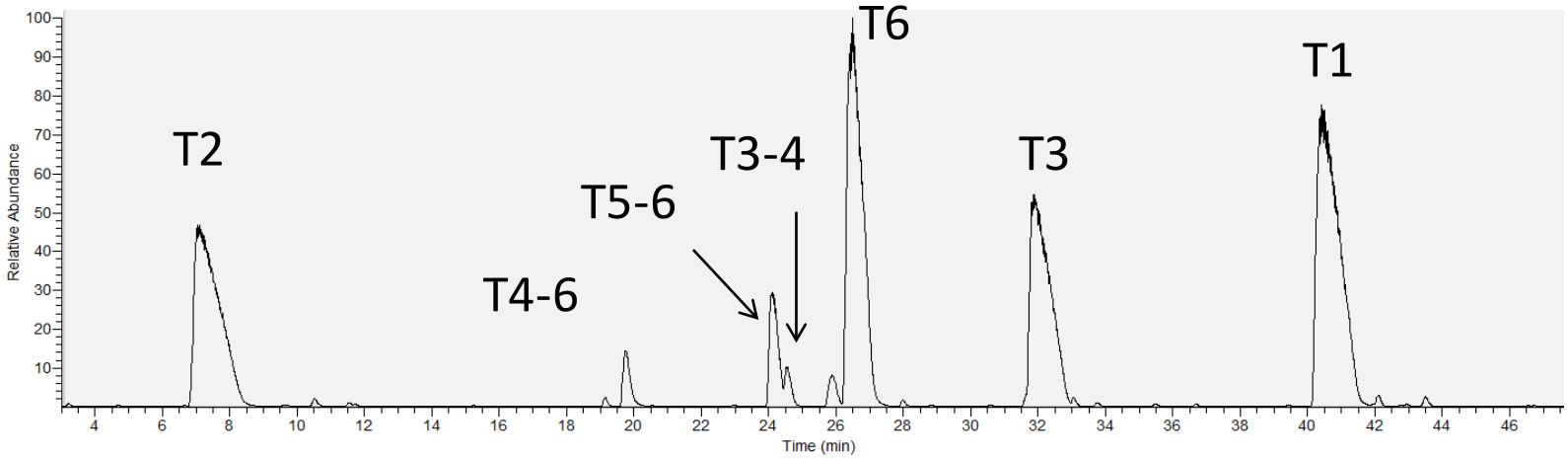
Preparation
1. Fractionation 10KDa filter
2. Denaturation, reduction, alkylation
3. Trypsin digestion

Separation and Detection
LC-MS/MS

Identification
database search

Quantitation
(relative or absolute)

Peptide Map of Teriparatide



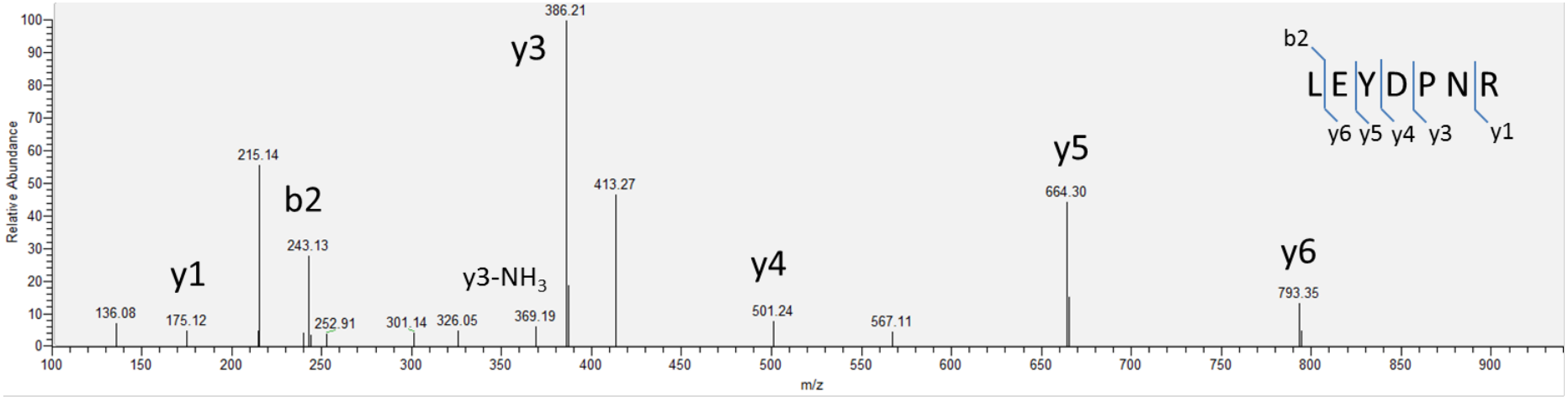
Name	Sequence	Start	End	Theoretical Mass MH+ (Da)	ΔM (ppm)
T1	SVSEQLMHNLGK	1	13	1455.7641	1.10
T2	HLNSMER	14	20	866.4236	4.03
T3	VEWLR	21	25	702.3923	-1.50
T6	LQDVHNF	28	34	872.4275	1.55
T5-6	KLQDVHNF	27	34	1000.5218	0.67
T4-6	KKLQDVHNF	26	34	1128.6181	1.80
T3-4	VEWLRK	21	26	830.4889	0.65

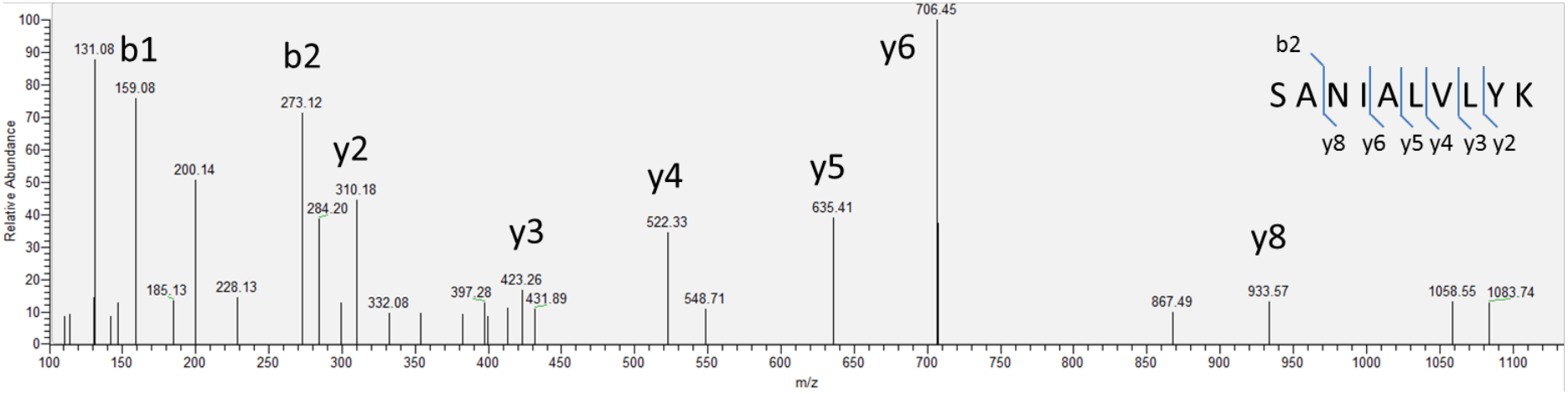
HCPs in Teriparatide RLD

Accession	Description	Unique peptide sequence	PTM	MH+ (Da)	ΔM (ppm)	Rel Ratio (ppm)	
						RLD	Synthetic teriparatide
P60422	50S ribosomal protein L2	SANIALVLYK	N/A	1091.6480	1.86	27	0
		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	0
		YEEITAScScGNVMK	C8(IAM) C10(IAM)	1748.7253	-2.00		

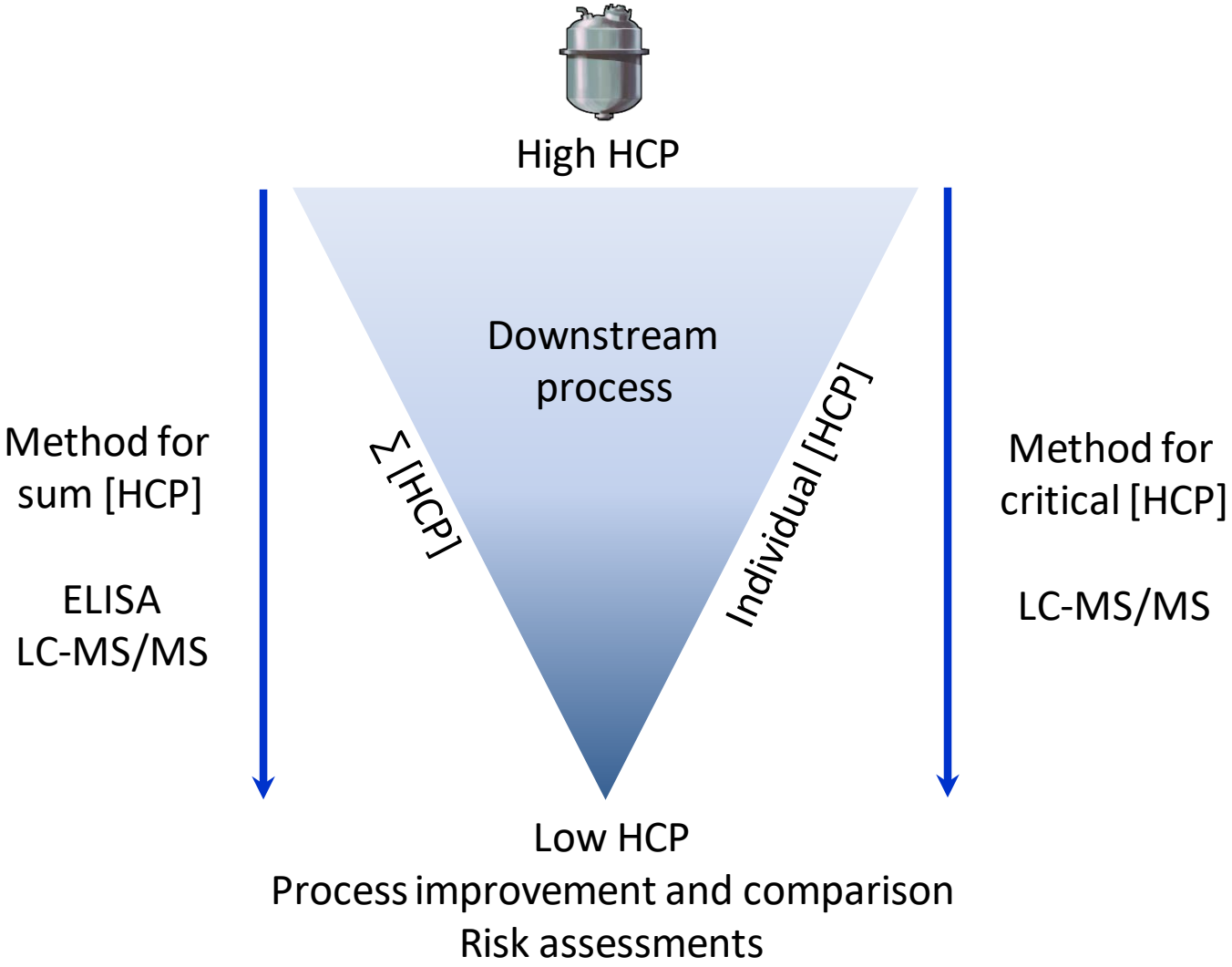
- HCP fractionation (10KDa filter) and concentration increased the chance of low abundant HCP being identified by LC-MS method.
- *Two* E. Coli HCPs were identified with at least two unique peptides each in the concentrated HCP fraction of RLD teriparatide.
- Relative ratios of individual HCPs were quantitated.
- Those peptides were *not* present in commercial available synthetic teriparatide.
- MS/MS b y ion assignments to major peaks according to the sequences.

MS/MS Spectra of Peptides from 50S Ribosomal Protein L2





Future of HCP Characterization



Conclusions and Future Work

- Mass spectrometry is critical for peptide therapeutic characterization and quality control.
- HCP fractionation and concentration increased the identification of low abundant 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.
- Relative ratios of individual HCP were quantitated.

- Absolution quantitation and method validation are ongoing.
- This LC-MS/MS method will be applied to other recombinant peptide drug products for HCP characterization.

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