Research Fueling Approvals: A Case Study of Glucagon

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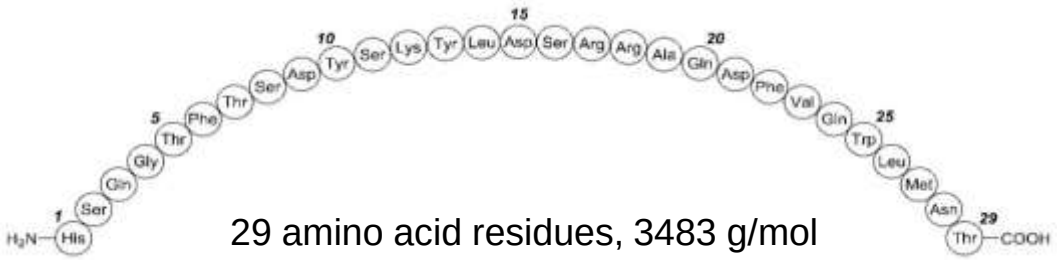
Outline

- Approval of 1st generic Glucagon for Injection
  - Product and lifecycle information
- FDA Guidance for Industry: *ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin* (May 2021)
- Quality considerations when conducting characterization and comparability studies
  - Active ingredient sameness
  - Peptide-related impurities
- Immunogenicity risk mitigation
- FDA supporting research on peptide impurities and higher order structures (HOS)
- Summary

Glucagon for Injection, 1 mg/vial

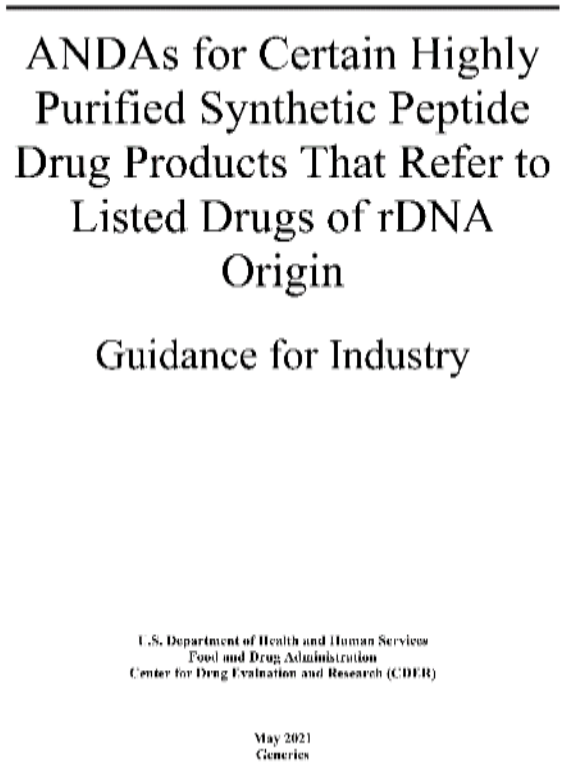


29 amino acid residues, 3483 g/mol

From RCSB Protein Data Bank

- Indicated for the treatment of severe hypoglycemia in pediatric and adult patients with diabetes mellitus, and as a diagnostic aid for use during radiologic examinations to temporarily inhibit movement of the gastrointestinal tract in adult patients
- Lyophilized powder in singe-dose vial containing 1 mg glucagon and 49 mg lactose monohydrate, co-packaged with a 1 ml diluent prefilled syringe containing 12 mg glycerin, 1 mg hydrochloric acid and WFI
- Reference Listed Drug: N020928, Eli Lilly and Co, recombinant API
- **1st Generic ANDA: A208086, Amphastar Pharmaceuticals Inc, approved Dec 28, 2020, synthetic API**

FDA Guidance for Industry


ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

May 2021
Generics

- FDA Guidance for Industry (draft published October 2017, finalized May 2021)
  - Describes a pathway for generic synthetic peptide drug approval under section 505(j) of the FD&C Act for **Glucagon**, Liraglutide, Nesiritide, Teriparatide, and Teduglutide
- FDA Draft Product Specific Guidance (recommended March 2020) for Semaglutide also references the general guidance

Active Ingredient Sameness

- As per the ANDA peptide guidance*, comparative characterization of the following for the proposed product and RLD is recommended using orthogonal analytical methods
  - Primary sequence and physicochemical properties
  - Secondary structure
  - Oligomer/aggregation states
  - Biological activities

* FDA Guidance for Industry: *ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin* (May 2021)

API Characterization and Comparability Studies

- Characterization:
  - Characterization of drug substance primary peptide structure and physicochemical properties
    - Provide evidence to ensure the identity, strength, quality, and purity of the peptide drug substance
    - DMF can be referenced for the API characterization information
- Comparability:
  - Provide evidence to ensure the peptide higher-order structure, aggregation profile and biological activity *in the proposed finished drug product* are comparable to those of the RLD

API Characterization and Comparability Studies

	Attribute	Examples of Characterization/ Comparability Studies	Examples of Methods
DS	Primary sequence and physicochemical properties	Primary peptide structure/sequence Solubility as a function of pH, hygroscopicity, log P, isoelectric point (pI), specific optical rotation	IR, UV, NMR, CD Spectroscopy, Mass Spectrometry, Amino Acid Analysis, L- and D- Amino Acid Content, Amino Acid Sequencing (Edman Degradation and/or HRMS/MS)
DP	Higher Order Structure	Secondary: α-helix, β-sheet, random coil, unordered Tertiary: overall monomer structure	Secondary: Far-UV CD, FTIR, Raman Spectroscopy Tertiary: Near-UV CD, Intrinsic Fluorescence DSC (Differential Scanning Calorimetry), NMR
	Oligomer/ Aggregation States	Reversible non-covalent soluble oligomers, irreversible soluble/insoluble aggregates (may exist as sub-visible or visible particles)	Light Scattering: SEC-UV/MALS, CG-MALS, DLS Analytical Ultracentrifugation: SE-AUC, SV-AUC Gel Electrophoresis: SDS-PAGE, Native Field Flow Fractionation: AF4 MFI (Micro-Flow Imaging)
	Biological Activities	Therapeutically relevant bioassay	Glucagon Bioidentity Tests USP⟨123⟩

Impurity Comparability Studies

- As per the ANDA peptide guidance*, a proposed generic synthetic peptide should not contain impurities at levels greater than those found in the RLD
- Conduct a comparative peptide-related impurity profiling of the RLD and proposed generic product
  - i. Identify each peptide-related impurity present in the proposed drug product at a level of 0.10% of the drug substance or greater
  - ii. For each peptide-related impurity found in both the proposed drug product and the RLD, demonstrate the proposed product level of such an impurity is the same as or lower than that found in the RLD
  - iii. Demonstrate that the proposed drug product does not contain any new peptide related impurities (i.e. not present in the RLD) at levels greater than 0.5% of the drug substance
  - iv. Characterize any peptide related impurity found at levels ≥ 0.10% and ≤ 0.5% of the drug substance that is either not present in the RLD or is present in the proposed drug product at higher levels than that of the RLD, and provide justification as to why such an impurity does not affect the safety, effectiveness or potential for immunogenicity of the proposed product
- Consider the use of UHPLC-HRMS/MS to facilitate peak identification and ensure peak purity (see *Liquid Chromatography-High Resolution Mass Spectrometry for Peptide Drug Quality Control* by Zeng *et al*. *AAPS J.* **2015**, *17*, 643-651)

* FDA Guidance for Industry: *ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin* (May 2021)

Peptide-Related Impurity Limits

- Limits for each peptide-related impurity can be established such that they are either
  1. Qualified by observed RLD levels for each impurity, or
  2. Not more than 0.5% and supported by in vitro and/or in silico studies suggesting the impurity does not add immunogenicity risk to the product, or
  3. Less than 0.10%.

Immunogenicity Risk Mitigation

- For any new or elevated level peptide-related impurities found at levels of ≥ 0.10% and ≤ 0.5%, evaluate the risk of immunogenicity (adaptive immune response)
  - Demonstrate that the impurity does not contain sequences that have an increased affinity for major histocompatibility complex (MHC), known as *T-cell epitopes*
  - Assessment of T-cell activation via binding of peptide-related impurities to MHC
    - *In silico* studies of MHC binding, and
    - *In vitro* binding and functional assays of specific impurities
- Innate immune response comparison between proposed generic and RLD formulated products
  - *In vitro* cell-based assays
  - Animal models

General Considerations for API and Impurity Comparability Studies

- Most peptide drug products are parenteral products (sterile solutions or lyophilized products to be reconstituted for injection)
- Conduct API and impurity comparability studies on the finished drug product where possible (reconstituted as per the label instructions if necessary)
- Provide justification if it is not feasible to conduct a study on the finished drug product; strive to minimize sample manipulation
- Conduct each study on a statistically meaningful number of batches of both the proposed drug product and the RLD
- It is recommended the proposed product be tested on or near release and at the end of the proposed shelf life, and RLD batches of different ages be tested prior to expiry (as available)
- Age proposed product and RLD samples under conditions consistent with the label storage conditions
- Use multiple orthogonal validated methods for each comparability study class and the impurity profiling
- Provide sample ages for the dates of all studies
- Provide drug substance batch # used for each proposed drug product batch

FDA Supporting Research

FDA labs develops analysis methods to support pharmaceutical equivalence claims.

- Active ingredient sameness:
  - Orthogonal methods of chromatography (Rt_1, Rt_2).
  - High Resolving Power Mass Spectrometry (HRMS).
  - Product-Ion Mass Spectrometry (MS/MS) and sequencing.
- Peptide-related impurities:
  - Quantitation by chromatography and HRMS detection (≥0.10%).
  - Characterization of impurities by Product-Ion Mass Spectrometry (MS/MS) and sequencing.
- Secondary Structure and Aggregation:
  - High Order Structure (HOS) comparison by 1D ^{1}H NMR, Mahalanobis Distance (D_M) in PCA space.
  - Comparison of DOSY-NMR and Dynamic Light Scattering for Measuring Diffusion Coefficients and particle size distribution.

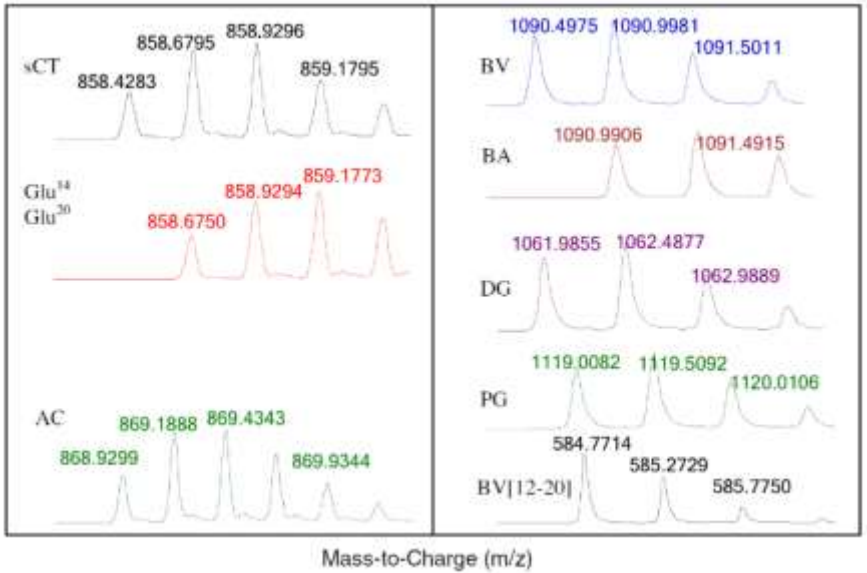
FDA Supporting Research

Peptide Drug Quality control by LC-HRMS

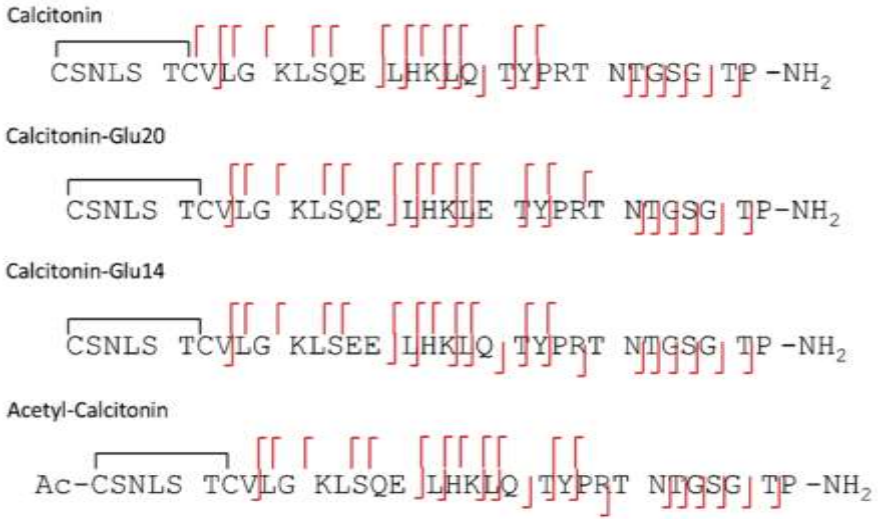
- Three peptide drugs (Calcitonin, Bivalirudin, Exenatide) were analyzed to assess suitability of LC-HRMS approach for monitoring peptide drug product quality.
- Calcitonin and its related impurities displayed linear responses over the range from 0.1 to 10 µM.
- Intra-assay precision in terms of relative standard deviation (%RSD) was less than 10% at all tested concentrations.
- The accuracy of the method was greater than 85% as measured by spiking impurities.
- Limits of detection for were 0.02-0.04 µM, (0.1 µM needed for 0.1% of API).
- Method validation showed the method have high selectivity, sensitivity, precision, and linearity.
- The method is capable of confirming peptide sequence, and quantify impurities, even when they are co-eluting.

"Liquid Chromatography-High Resolution Mass Spectrometry for Peptide Drug Quality Control", K. Zeng, I. Geerlof-Vidavsky, A. Gucinski, X. Jiang, M. T. Boyne, *AAPS J.* 17, 643-651 (2015)

Peptide Drug Quality control by LC-HRMS



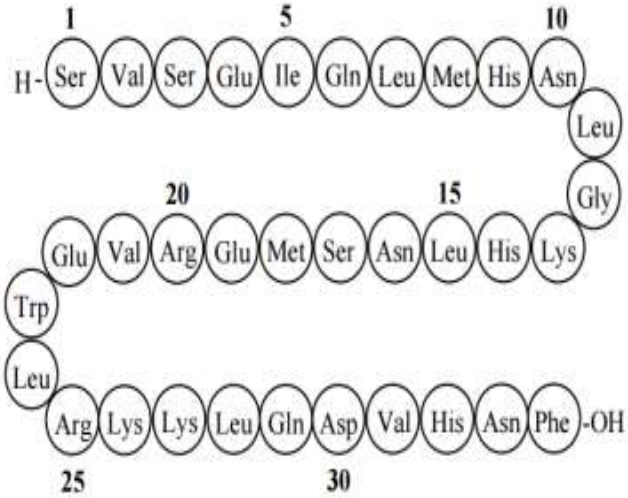
High resolution mass spectra of calcitonin salmon, bivalirudin, and their related impurities. Left: the +4 charge states of sCT and its related impurities are shown. Right: +2 charge states of BV and its related impurities are shown.



Amino acid sequence confirmation for calcitonin salmon and its related impurities by LC-MS/MS.

Teriparatide impurities analysis

34 amino acid peptide used to treat osteoporosis. RLD is from rDNA origin

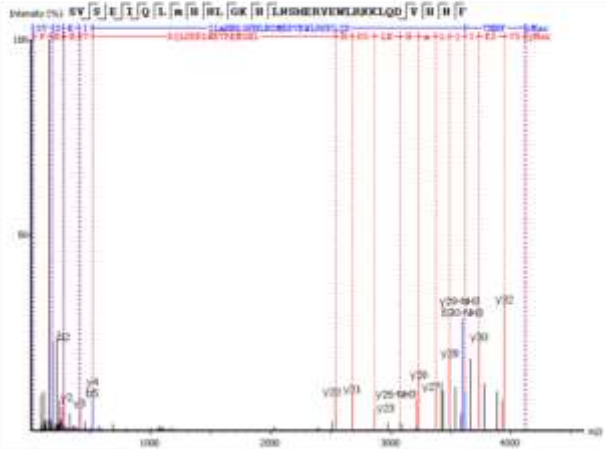


Modification name	Site	Mass shift (Da), theoretical	m/z, observed	RT min.
1-29	-DVHNF 30-34	-612.2656	701.5809	41.46
1-30, dehydroalanine	-VHNF 31-34, S17	-515.2492	720.9842	41.36
1-30, succinimide	-VHNF 31-34, D30	-515.2452	720.9839	43.79
1-30	-VHNF 31-34	-497.2387	724.5862	41.77
V(1-30)	+V1, -VHNF 31-34	-398.1703	744.3995	45.00
1-32	-NF 33-34	-261.1113	771.8114	42.53
Gln, Asp deletion	-Q29, -D30	-243.0855	775.4168	44.53
1-33	-F34	-147.0684	794.6197	42.34
His deletion	-H14	-137.0589	796.6219	46.94
Lys deletion	-K13	-128.0949	798.4147	47.16
Ile deletion	-I5	-113.0841	801.4170	45.11
S1 deletion	-S1	-87.0320	806.6271	45.15
Succinimide	D30	-18.0106	820.4337	44.71
Succinimide	N10	-17.0265	820.6292	43.01
Succinimide	N16	-17.0265	820.6290	42.40
Succinimide	N33	-17.0265	820.6289	48.29
Teriparatide	–	–	824.0330	45.34
Formaldehyde adduct	S1	12.0000	826.4333	47.71
Oxidation	M8	15.9949	827.2328	40.40
Oxidation	M18	15.9949	827.2328	42.47
Formylation	?	27.9949	829.6330	49.24
Formylation	?	27.9949	829.6320	49.75
Acetylation	S1	42.0106	832.4360	50.05
Acetylation	K13	42.0106	832.4357	49.27
Acetylation	K26	42.0106	832.4352	47.96
Acetylation	K27	42.0106	832.4357	51.07
Ser, Ser insertion	S18, S19	174.0641	858.8467	45.06
VR(1-34)	V1, R2	255.1695	875.0675	44.19

"Quantitative comparison of peptide impurities in teriparatide from recombinant and synthetic origin using UHPLC-MS/MS", D. A. Weisz, I. Geerlof-Vidavsky, K. Zeng, E. Pang, S. Rogstad, 2020 CASSS Fall Conference, November 5-6, 2020

Teriparatide impurities analysis

Deconvolved MS² Spectrum



M8-ox: SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNF

+16

M18-ox: SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNF

+16

* = y-ions highlighted in MS/MS spectra below

Red = y-ion observed
Blue = b-ion observed
Green = both y- and b-ion observed

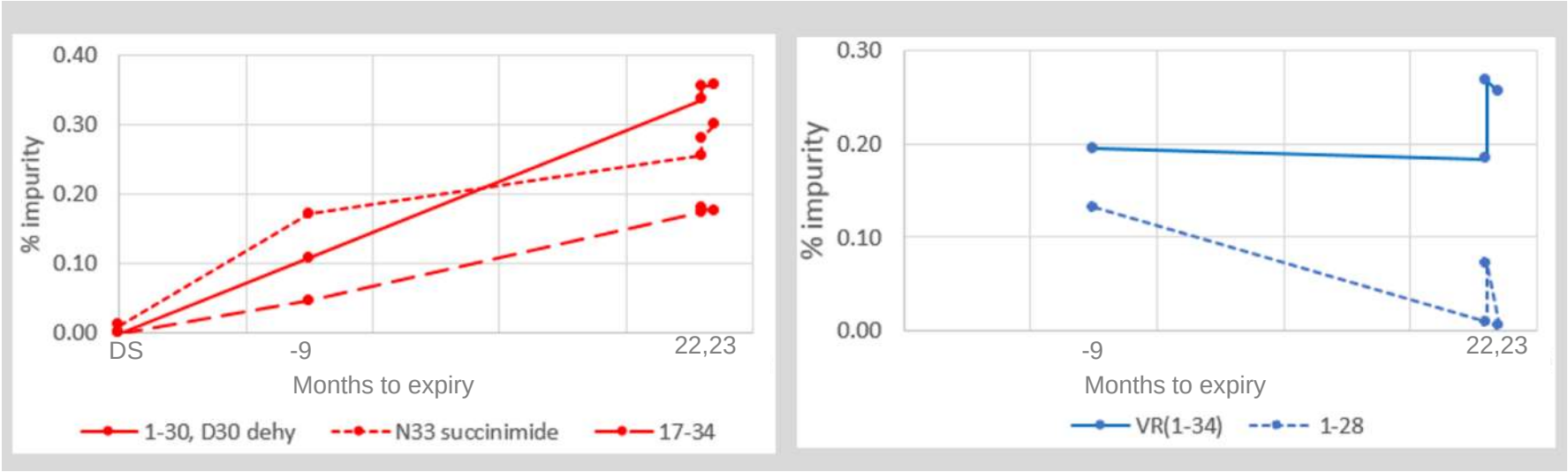
Quantitative comparison of Teriparatide impurities ≥0.10%. DS synthetic peptide (Bachem), DP recombinant teriparatide RLD product.

0%	<0.1%	0.1-0.3%	0.3-0.5%	≥0.5%

Modification name	DS	DP Lot 1	DP Lot 2	DP Lot 3	DP Lot 4
Drug product past expiry (month):	--	-9	22	22	23
Total drug purity	96.07	95.28	92.22	91.21	91.86
1-30	0.02	0.75	2.11	2.14	2.37
M18 oxidation	1.85	1.87	2.26	2.98	2.33
D30 succinimide	0.03	0.82	1.33	1.29	1.50
M8 oxidation	0.32	0.53	0.48	0.99	0.64
1-30, D30 dehydration	--	0.11	0.34	0.36	0.36
N33 succinimide	0.01	0.17	0.25	0.28	0.30
17-34	--	0.05	0.17	0.18	0.17
N33 insertion	0.93	--	--	--	--
S1 deletion	0.24	--	--	--	--
S17 double insertion	0.20	--	--	--	--
1-31	0.13	--	--	--	--
VR(1-34)	--	0.20	0.18	0.27	0.26
1-28	--	0.13	0.01	0.07	0.01

Top group degradation product, bottom process impurities.

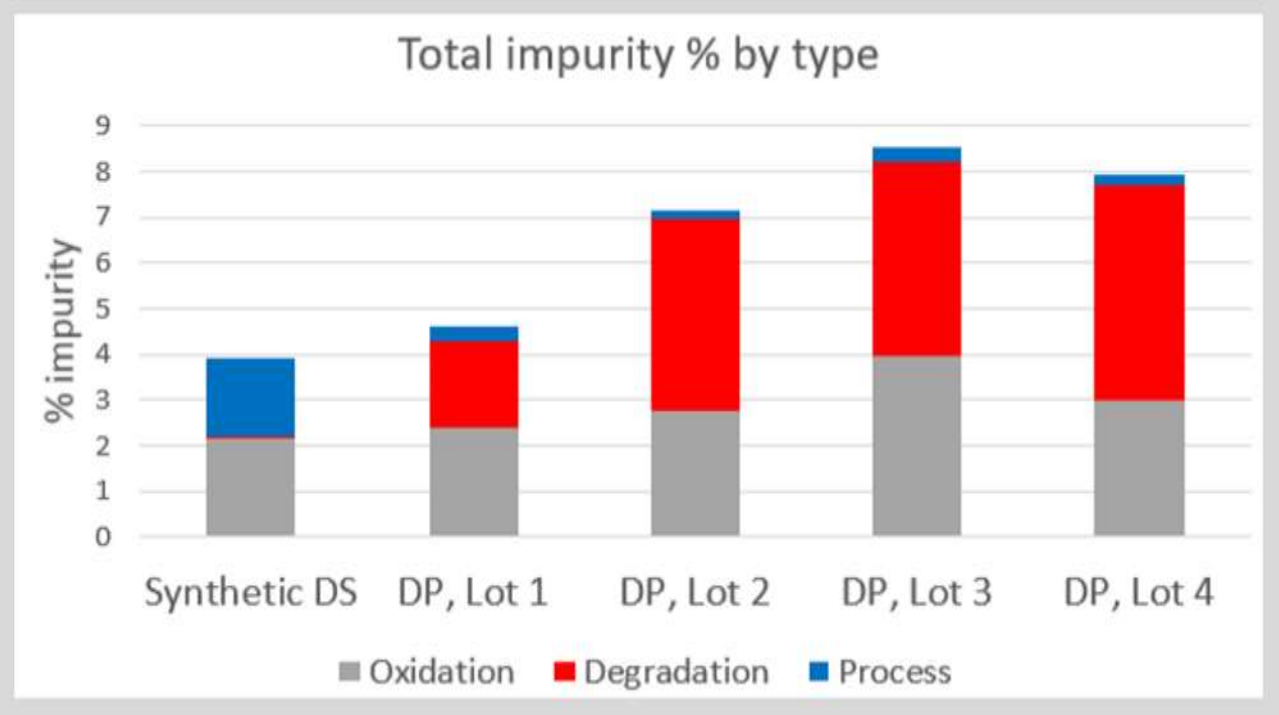
Teriparatide impurities analysis



Degradation impurity levels in DS and DP increase with increasing sample age.

Process impurity levels in DP do not correlate with increasing sample age.

Teriparatide impurities analysis

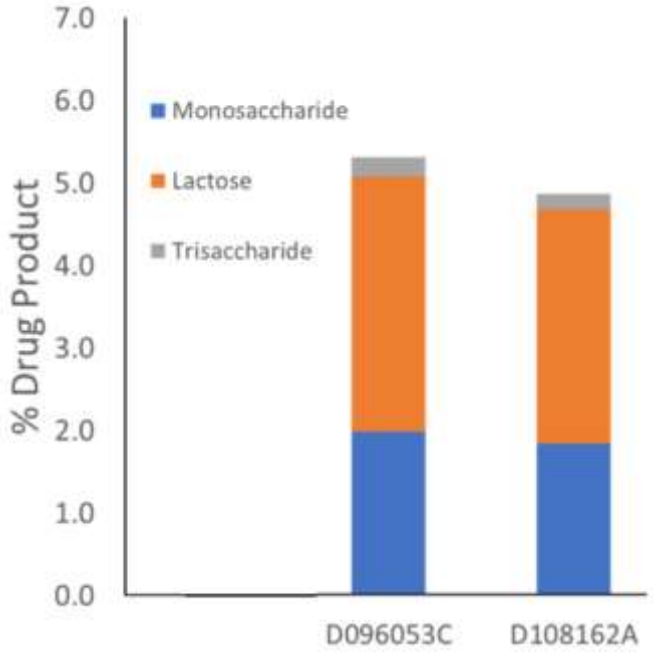


Total impurities content in each lot (≥0.10%), broken by impurity type.

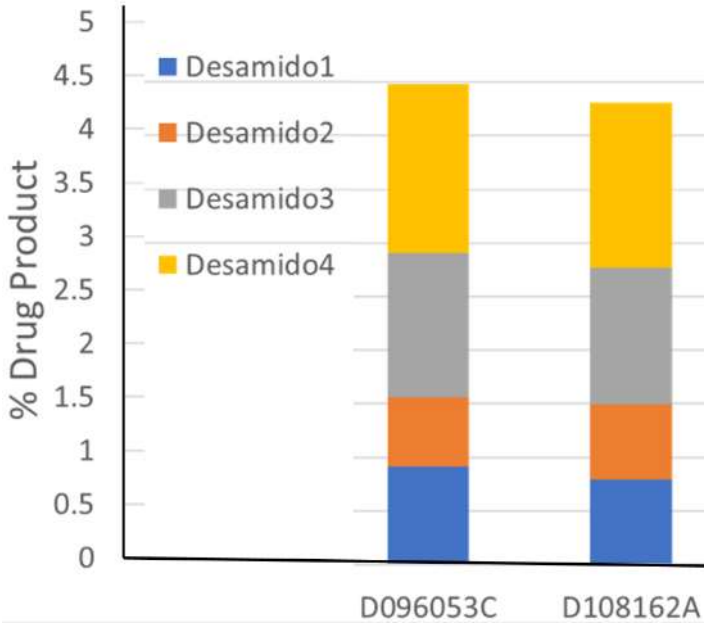
DP lot 1, 9-month to expiry, lot 2, 22-month past expiry, lot 3, 22-month past expiry, lot 4, 23-month past expiry

Glucagon RLD impurities study

29 amino acid peptide used for severe hypoglycemia.
Two lots, 8 and 9 month before expiry, used

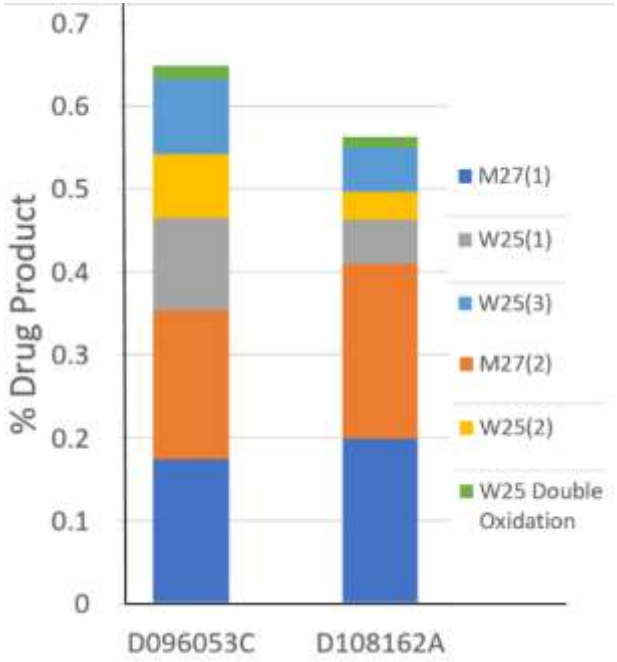


Lactose-related impurities

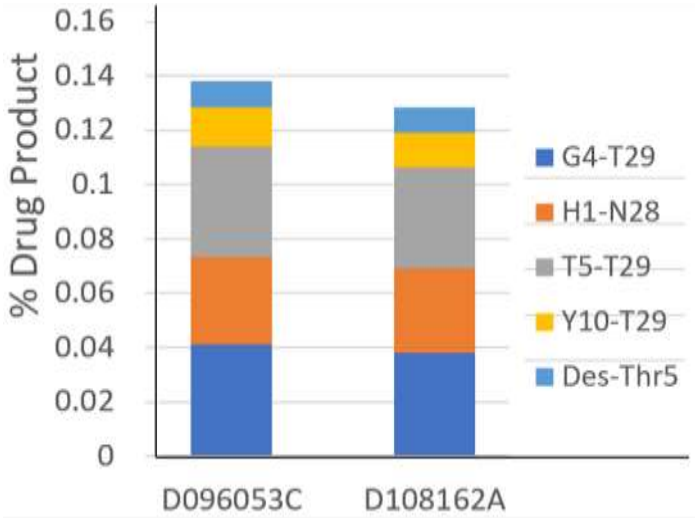


Deamidation

Glucagon RLD impurities study (two lots)



Oxidations



Hydrolysis and Deletion

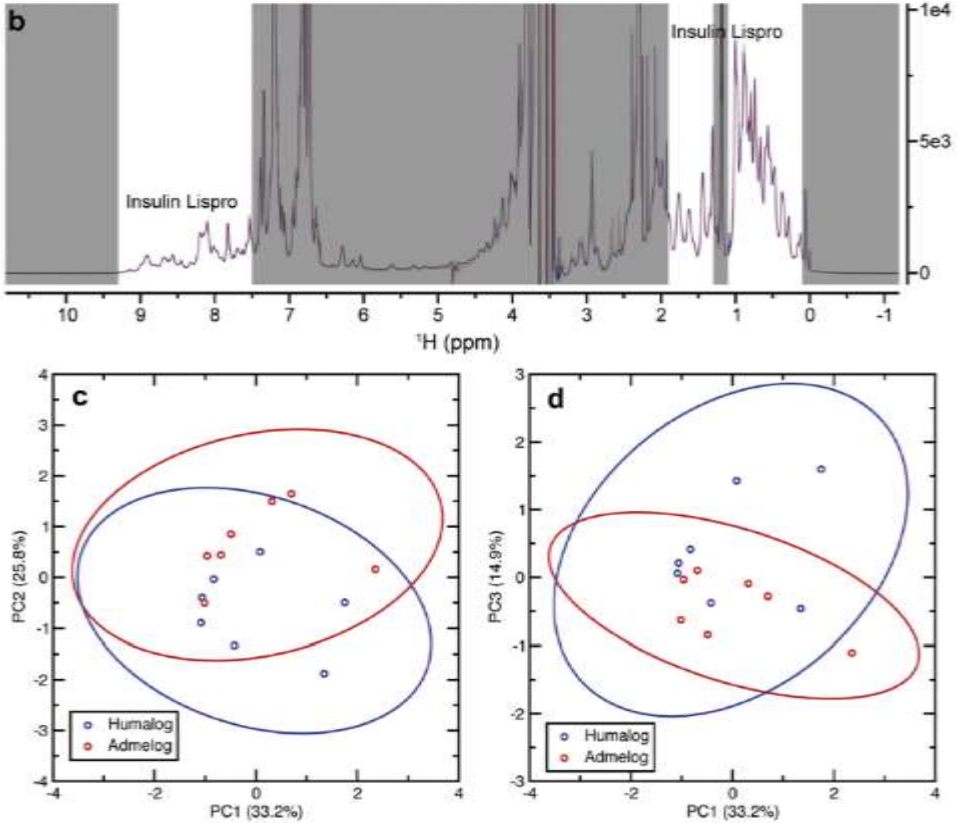
33 impurities were quantified in the RLD.

1D ^{1}H NMR comparisons of HOS

- Protein or peptide higher order structure (HOS) is a quality attribute that could affect therapeutic efficacy and safety.
- HOS similarity between a proposed follow-on product and the reference listed drug should be demonstrated during regulatory assessment.
- HOS differences among U.S. marketed insulin drug products (DPs) were quantified using NMR spectra and principal component analysis (PCA).
- The unitless Mahalanobis Distance (D_M) in PCA space was calculated between insulin analog reference listed drugs and their recently approved follow-on products, and all D_M values were 3.29 or less.
- larger D_M value of 20.5 was obtained between 2 insulin human DPs independently approved. Upon reversible dialysis of the 2 insulin human DPs against the same buffers, the D_M value was reduced to 1.19 or less

"An NMR-Based Similarity Metric for Higher Order Structure Quality Assessment Among U.S. Marketed Insulin Therapeutics", D. Wang, J. Park, S. M. Patil, C. J. Smith, J. L. Leazer Jr., D. A. Keire, K. Chen, J. of Pharm. Sci. 109 (2020) 1519-1528

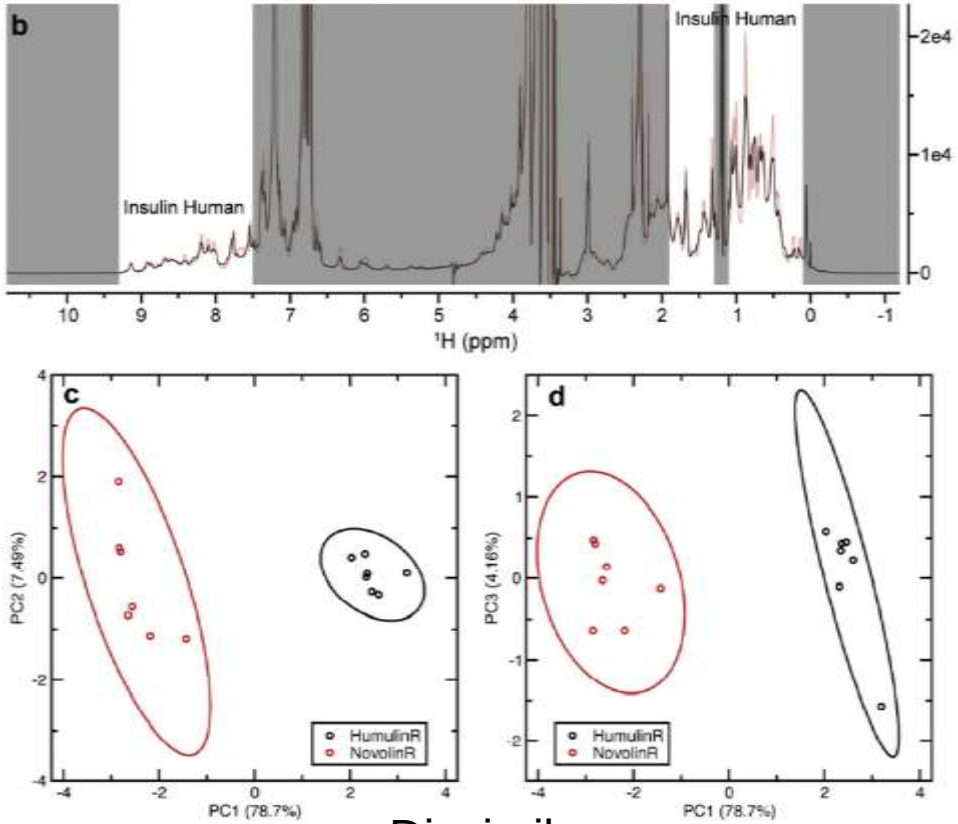
1D ^{1}H NMR comparisons of HOS



Similar

- Quantification of spectral differences for insulin lispro drug products (DP), Humalog® (blue) and Admelog® (red). Vertically zoomed, superimposed 1D ^{1}H NMR spectra of the 2 of DPs.
- Blinded spectral regions shown in gray, were excluded from the principal component analysis (PCA). The PCA scores are plotted between PC1 and PC2 (c) and between PC1 and PC3 (d).
- Mahalanobis Distance (D_M) is **3.29** indicating similar HOS and excipients.

1D ^{1}H NMR comparisons of HOS



Dissimilar

- Quantification of spectral differences for insulin human drug products (DPs), HumulinR® (black) and NovolinR® (red). Vertically zoomed, superimposed 1D ^{1}H NMR spectra of the 2 of DPs.
- Blinded spectral regions shown in gray were excluded from the principal component analysis (PCA). The PCA scores are plotted between PC1 and PC2 (c) and between PC1 and PC3 (d).
- Mahalanobis Distance (D_M) is **20.5** indicating possible dissimilar HOS and/or excipients.
- Upon mass-balanced and reversible dialysis of the 2 insulin human DPs against the same buffers, the D_M value was reduced to **1.19**

Challenge Question #1

Consistent with the ANDA peptide guidance*, when conducting a comparative impurity profiling of the proposed generic drug product and RLD, identify all peptide-related impurities at a level of:

A. > 0.10%

B. > 0.5%

C. ≥ 0.10%

D. ≥ 0.1%

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Summary

- Approval of 1st Generic Glucagon for Injection facilitated by FDA Guidance for Industry *ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin* (May 2021)
- Characterization of the API drug substance
- Comparability studies of API sameness and peptide-related impurities of the proposed finished drug product and RLD
  - Higher order structure
  - Aggregation profile
  - Biological activity
  - Impurity profile
- Immunogenicity Risk Mitigation
- FDA supporting research

U.S. FOOD & DRUG ADMINISTRATION

Questions?

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Office of Pharmaceutical Quality (OPQ) | CDER | US FDA


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