Characterization and Comparative Evaluation Strategies to Demonstrate Complex API Sameness

Complex Generic Drug Product Development Workshop

Session 3: Characterization of Complex Injectable API and Formulations

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Disclaimer

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

Outline

- Introduction: Active Pharmaceutical Ingredient (API) Sameness for an ANDA*
- Characterization Strategies for Complex Injectable API
- Examples

* ANDA: Abbreviated New Drug Application

Generic Drugs and API Sameness

A generic drug must be therapeutically equivalent* to the reference listed drug (RLD)

- **Pharmaceutical equivalent**
- Bioequivalent
- Adequately labeled
- Manufactured in compliance with cGMP regulations

* For definition, see 21 CFR 314.3(b)

Generic Drugs and API Sameness

Pharmaceutical Equivalent (PE):

- **Contains same API**
- Uses same dosage form and route of administration
- Is identical in strength or concentration
- Meets the same compendial standards for strength, quality, purity and identity

API sameness is a requirement for generic drugs

Simple API vs Complex API

Simple API	Complex API
Small molecules with defined structure	Peptides
	Natural and synthetic polymers
Mixture of a few small molecules in a fixed ratio	Heterogenous mixture of small molecules
	Macromolecular complexes

Examples of Complex Injectable API

- Peptides
- Oligonucleotides
- Naturally derived or semi-synthetic mixtures
  - Soybean oil; heparin
  - Enoxaparin
- Synthetic complex mixtures
  - Glatiramer acetate
- Iron complexes
  - Iron Sucrose

Demonstrating Complex API Sameness

Totality of Evidence Approach

- Source of starting material
- Reaction scheme
- Structural signature analysis
- Physicochemical and biological properties/impurities

Characterization Strategies for Complex Injectable API

- Structure confirmation or comparative structural signature analysis
  - Primary structure of peptide/oligonucleotide; structural features and fingerprints; distribution of mixtures, etc.
- Comparative physicochemical property analysis
  - MW distribution, spectroscopic analysis, etc.
- Comparative impurity profile analysis
  - peptide-related impurities in synthetic peptides; (N+1), (N-1) impurities in oligonucleotides
- Comparative biological activity analysis if necessary
  - Confirmatory in vitro and/or in vivo biological activities

Examples

- Peptide: Calcitonin Salmon
- Low Molecular Weight Heparin (LMWH): Enoxaparin Sodium

Peptides

- Any alpha amino acid polymer with a defined sequence that is 40 amino acids or fewer in size;
- No product-specific guidance (PSG) for individual injectable peptide drug;
- Draft guidance for industry: *ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin* (Oct 2017).
  - The general characterization principles and strategies in this guidance may be considered when developing other peptide API products.

Characterization of Peptides

- Primary sequence, amino acid composition
- Optical rotation, other physicochemical properties
- Secondary structure
- Oligomer/Aggregation states
- Biological activities (by in vitro or animal studies)
- Impurities (peptide-related impurities and other impurities)

Use orthogonal analytical methods!

Calcitonin Salmon Impurity Analysis

- Calcitonin Salmon: a 32 amino acid peptide hormone for postmenopausal osteoporosis;
- Several Calcitonin Salmon nasal spray products have been approved (rDNA and synthetic);
- FDA Lab applied a data-dependent acquisition (DDA) LC-MS-MS and data-independent acquisition (DIA) LC-MS^E approach to analyze peptide impurities in Calcitonin Salmon nasal spray products.

Rapid Screening of Peptide Impurities in Calcitonin-Salmon Nasal Spray Using Data-Dependent LC-MS-MS And Data-Independent LC-MS^E, Yang, J., et. al., ASMS Poster, 2017.

Calcitonin-Salmon Impurity Analysis

- Instruments: UHPLC-MS (Thermo Q Exactive Orbitrap and Waters Synapt G2Si mass Spectrometers);
- To identify peptide impurities in all three groups:
  - Impurities observed in total ion chromatogram (TIC);
  - Impurities co-eluting with the API or eluted at its peak tail (challenging for manual screening);
  - Impurities buried under the TIC baseline.

Calcitonin Salmon Impurity Analysis

DIA LC-MS^E approach

Table 1. List of selected peptide impurities observed using DIA approach

Impurity	Rt (min)	M.W. (mono)	Δm/z	% ADC
1	5.8	2195.396	-1234.7	0.01
2		2213.408	-1216.7	0.08
3	21.3	3503.072	73.0	0.02
4		3333.996	-96.1	0.13
5	26.1	3412.084	-18.0	0.01
6	28.7	3412.032	-18.0	0.14
7		3315.960	-114.1	6.45
8		3297.972	-132.1	0.41
9		3477.048	47.0	0.01
10		3572.900	142.8	0.15
11		3214.884	-215.2	0.34
12	30.0	3468.080	38.0	2.18
13		3532.128	102.1	0.02
14		3232.920	-197.2	0.03
15		3501.096	71.0	0.02
16		3451.980	21.9	1.65
17	30.4	3430.072	0.0	0.78
18		2912.788	-517.3	0.01
19		3088.856	-341.2	0.09
20		3316.968	-113.1	0.05
21	31.5	2715.680	-714.4	0.04
22		3431.125	1.1	0.01
23		2930.784	-499.3	0.02
24		3316.968	-113.1	0.05
25		1691.986	-1738.1	0.13
26		3430.072	0.0	0.78
27		3453.000	22.9	0.01
28	32.5	3450.495	20.4	0.73

DDA LC-MS-MS approach

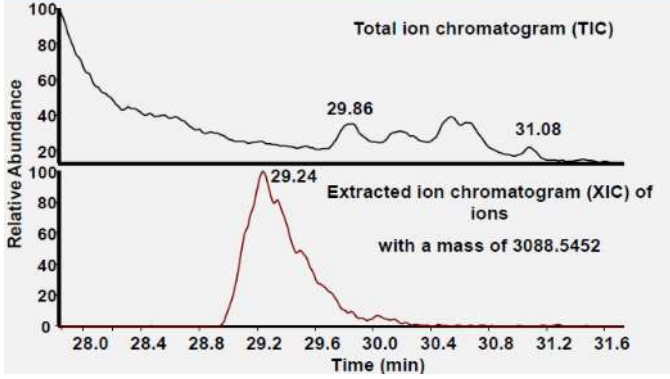
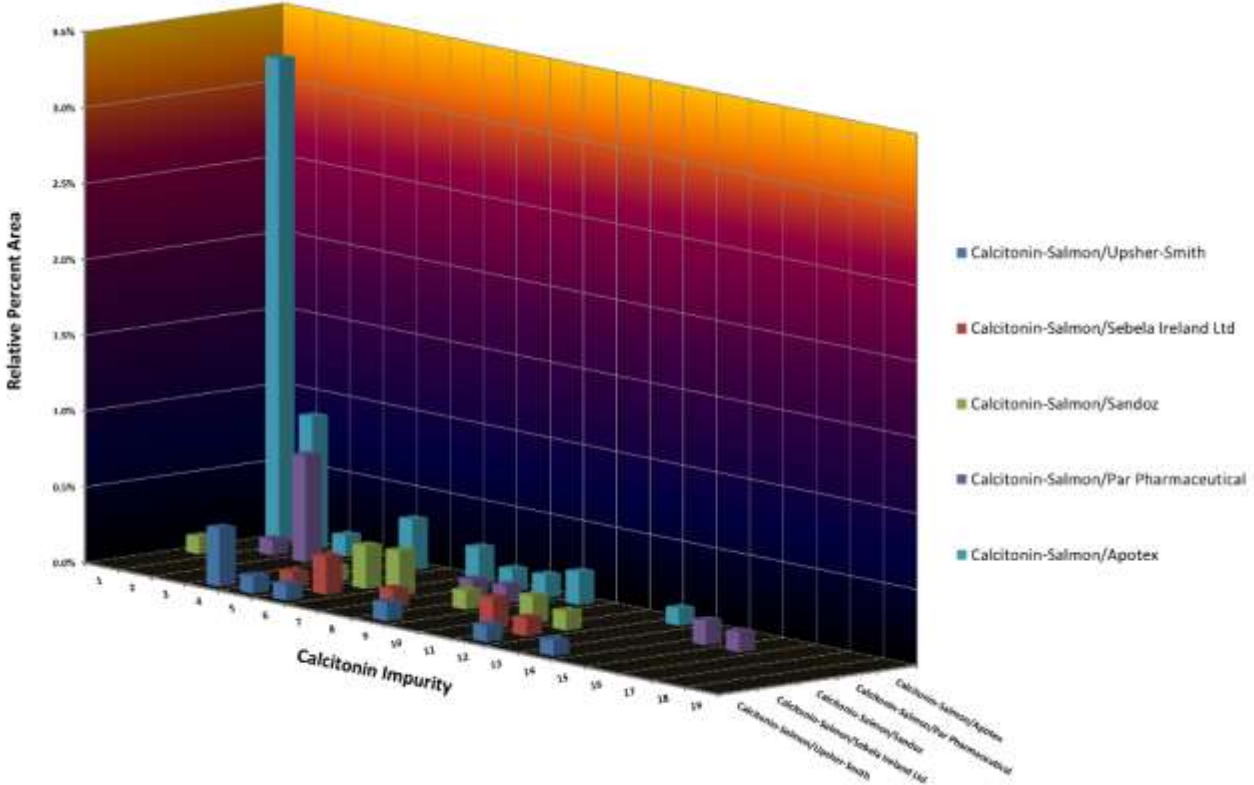


Table 2. List of selected peptide impurities observed in TIC

Index	TIC Peak Retention Time (min)	Monoisotopic Mass	Detected by DDA	Area %
1	5.77	2213.1206	Yes	0.06
2	18.49	1321.6378	No	0.009
3	19.44	1578.7394	No	0.005
4	20.6	3447.7259	Yes	1.17
5		1234.6048	Yes	0.05
6		3333.6461	Yes	0.02
7	24.32	1828.8825	No	0.02
8	24.99	3412.6908	Yes	0.23
9	29.86	2930.4755	Yes	0.04
10	30.4	2715.3867	Yes	0.02
11	30.54	2070.0608	Yes	0.12
12	31.08	1691.8229	Yes	0.06
13	32.71	3471.7261	Yes	0.17
14	35.84	3428.6858	Yes	0.08
15	37.99	3471.7261	Yes	0.02
16	57.07	Only Singly-charged ions observed		
17	58.8	Only Singly-charged ions observed		

API Rt = 28.7 min

Calcitonin Salmon Impurity Analysis



- 13 nasal spray drug products analyzed;
- Over 100 peptide impurities detected by LC/MS;
- 4 were above 0.5%;
- 16 were above 0.1%.

Peptide Impurities: Comparative Evaluation

To ensure impurities will not affect the safety (including the immunogenicity) and efficacy of the generic peptide drug

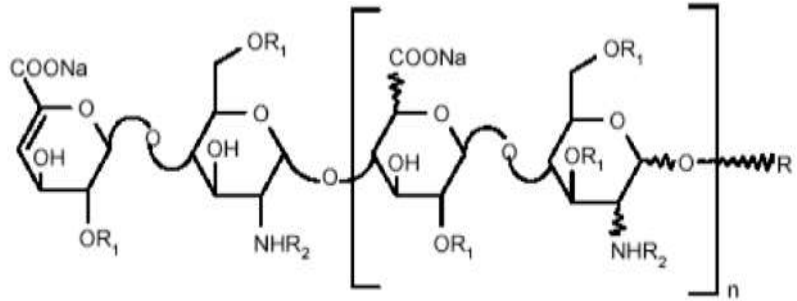
- Existing peptide impurities: generally not higher than those found in the RLD
- New peptide impurities: to identify and characterize above certain threshold
- To assess immunogenicity risk with in silico, in vitro methods

Examples

- Peptide: Calcitonin Salmon
- Low Molecular Weight Heparin (LMWH): Enoxaparin Sodium

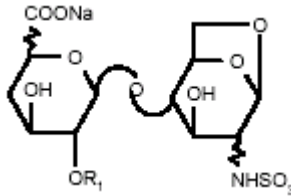
Enoxaparin Sodium

- Derived from porcine heparin through alkaline depolymerization of heparin ester;*
- Average MW 4500 with major components between 2000-8000;
- About 20% (between 15-25%) contains 1,6-anhydro derivative on the reducing end of the polysaccharide chain.



R_1 = H or SO_3Na and R_2 = SO_3Na or $COCH_3$

R = H (n=1-21) or



n=0-20

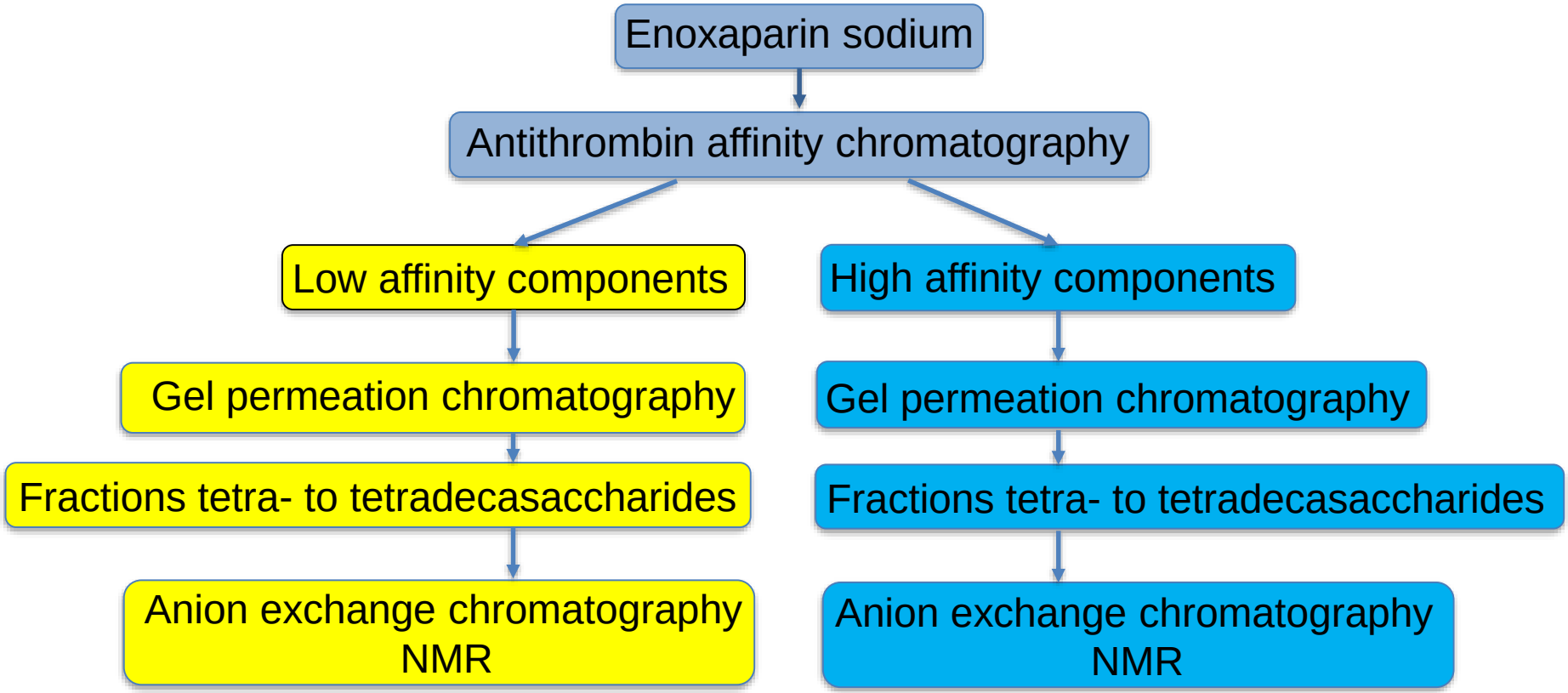
* Enoxaparin Labeling

Comparative Structure Analysis

- PSG recommends structural fingerprinting analysis
  - Oligosaccharide compositions;
  - Sequence of oligosaccharide species;
  - Disaccharide building blocks;
  - Fragment mapping after enzymatic cleavage.

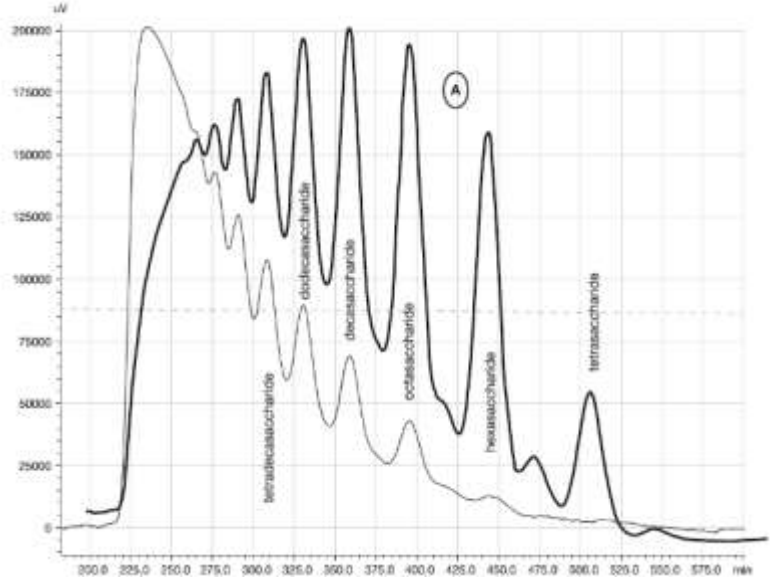
Involves extensive chromatographic separation, Mass spectrometry and 1D, 2D-NMR studies.

Structural Fingerprinting: Composition

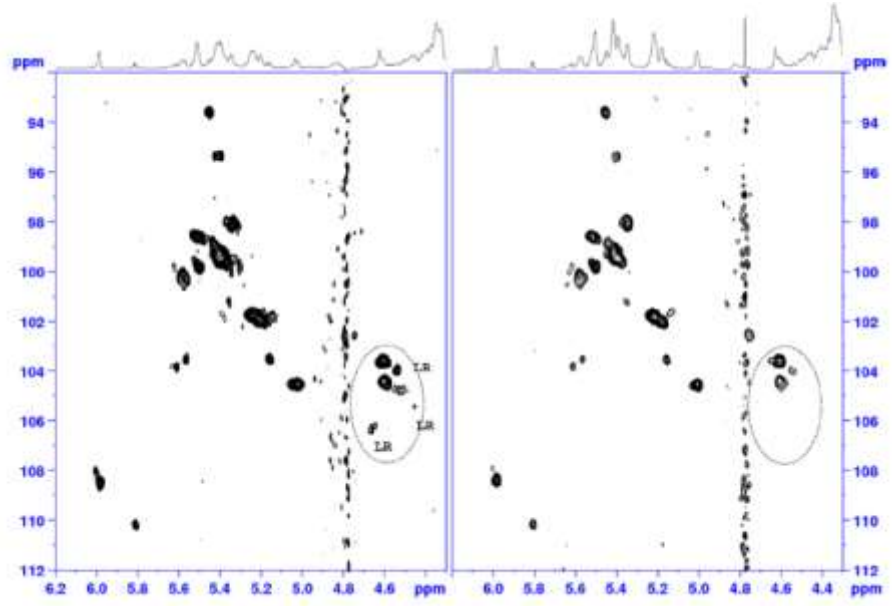


Mourier, P.A., et.al. *JPBA*, 115 (**2015)**, 431-442, with permission

Structural Fingerprinting: Composition



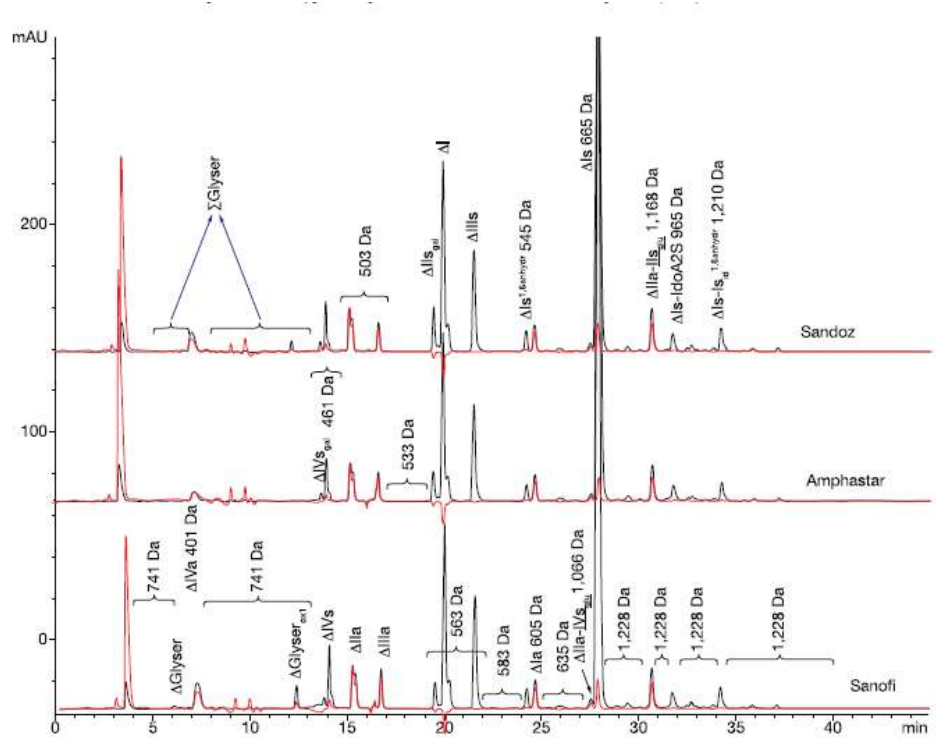
GPC chromatogram of high affinity (thin line) and low-affinity (thick line) enoxaparin by UV (232 nm)



2-D HSQC* ^{1}H-^{13}C NMR spectra of high-affinity tetradecasaccharide fractions on enoxaparin from two manufacturers. Main differences between the spectra are due to a linkage region (LR).

*HSQC = heteronuclear single quantum magnetic resonance

Structural Fingerprinting: Fragment Mapping



Structural symbols

ΔIVa	ΔU-GlcNAc
ΔIVs	ΔU-GlcNS
ΔIIa	ΔU-GlcNAc,6S
ΔIIIa	ΔU 2S-GlcNAc
ΔIIs	ΔU-GlcNS,6S
ΔIIIs	ΔU 2S-GlcNS
ΔIa	ΔU 2S-GlcNAc,6S
ΔIs	ΔU 2S-GlcNS,6S
ΔIVs_{gal}	ΔGalA-GlcNS
ΔIIs_{gal}	ΔGalA-GlcNS,6S

The iduronic (id) or glucuronic (glu) structure of uronic acids is indicated for oligosaccharides, e.g. ΔIs $IIIs_{id}$. Underlined disaccharides have a 3-O-sulfated glucosamine

Δ<u>IIs</u>	ΔU-GlcNS,3S,6S
Δ<u>Is</u>	ΔU2S-GlcNS,3S,6S
IIs_{glu}	GlcA-GlcNS,3S,6S
Δ<u>IIa-IIs_{glu}</u>	ΔU-GlcNAc,6S- GlcA-GlcNS,3S,6S

Enoxaparin batches depolymerized by heparinase enzymes and separated by strong anion exchange chromatography (black line: UV 234 nm; red line: UV 202-242 nm)

Other Characterizations for Sameness

- Physicochemical properties
  - Overall composition, spectroscopic data, certain USP tests
- Biological (in vitro/in vivo) activities
  - In vitro Anti-Factor Xa activity, Anti-Xa/IIa ratio
  - In vivo pharmacodynamic (PD) profile

Totality of Evidence approach for complex APIs

Summary

- Complex API sameness can be demonstrated through a comprehensive, totality of evidence approach
- Each complex injectable API has its own challenges, generic applicants need to evaluate individual situation and apply the principles accordingly
  - Peptide impurity characterization (Calcitonin Salmon)
  - Structure signature characterization of complex mixture (Enoxaparin)

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