# Welcome

# Overview on USP Heparin Standards and Compendial Use

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Scientific Liaison, Global Biologics
19th MAY, 2020


usp
The standard of trust

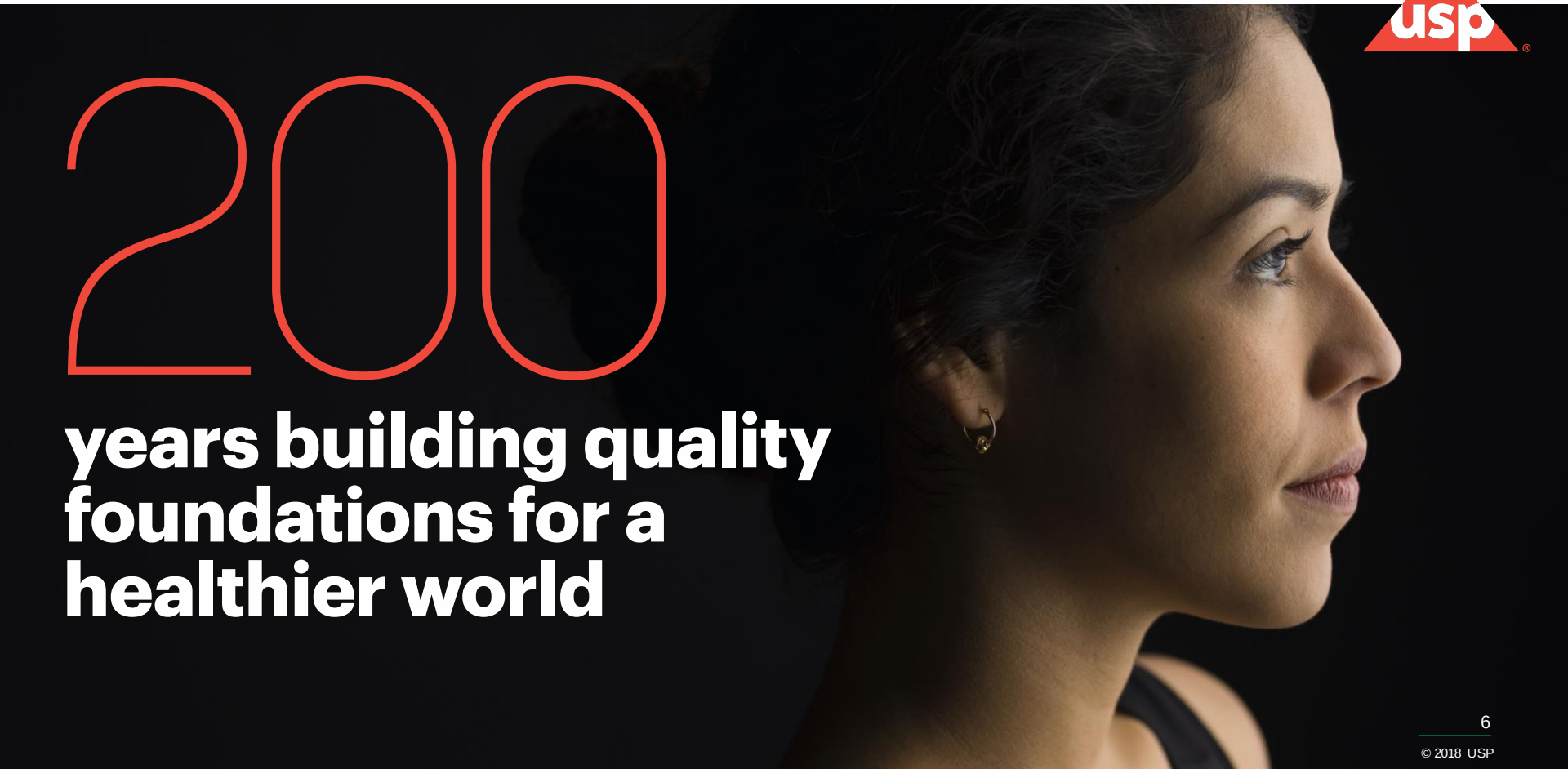
# Agenda

- USP Overview and Standard Setting Process
  - Monograph Development
- Overview on Heparin Standards
  - Product Specific Standards
- Overview of LMWH Standards
  - Product Specific Standards
- Q & A

# USP Overview and Standard Setting Process

# Mission

**To improve global health through public standards and related programs that help ensure the quality, safety and benefit of medicines and foods**


200
years building quality foundations for a healthier world

# Who We Are And Where We Work

- Values-driven organization focused on quality standards for medicine, foods, and dietary supplements

- We work with 900 + scientists, health care practitioners and regulators globally to develop our standards

- Internationally-recognized and globally-focused
- Founded in 1820, non-profit, private and independent
- Headquarter: Rockville, Maryland, USA

# The experts behind our standards

## 2015–2020 Council of Experts

### Healthcare Quality Standards Collaborative Group

- Nomenclature & Labeling
- Compounding
- Healthcare Quality

### Chemical Medicines Monographs Collaborative Group

- Chemical Medicines Monographs 1
- Chemical Medicines Monographs 2
- Chemical Medicines Monographs 3
- Chemical Medicines Monographs 4
- Chemical Medicines Monographs 5
- Chemical Medicines Monographs 6

### Biologics Collaborative Group

- B101 Peptides
- B102 Proteins
- B103 Complex Biologicals
- BIO4 Antibiotics
- GC Biological Analysis

### Excipient Monographs Collaborative Group

- Excipient Monographs 1
- Excipient Monographs 2

### Dietary Supplements/ Herbal Medicines/Foods Collaborative Group

- Non-Botanical Dietary Supplements
- Botanical Dietary Supplements & Herbal Medicines
- Food Ingredients

### General Chapters Collaborative Group

- Chemical Analysis
- Physical Analysis
- Statistics
- Microbiology
- Dosage Forms
- Packaging & Distribution

# Legal Basis for USP Standards

- **Pure Food and Drugs Act of 1906**
- **Federal Food, Drug and Cosmetic (FD&C) Act of 1938**
  - USP standards have been recognized in the Federal Food, Drug and Cosmetic (FD&C) Act since it was first enacted in 1938.
  - The FD&C Act defines term "official compendium" as the official USP, the official NF, the official Homeopathic Pharmacopeia of the United States, or any supplement to them
  - USP–NF standards play a role in the drug adulteration and misbranding provisions of the FD&C Act (which apply as well to biologics, a subset of drugs, under the Public Health Service Act).

**NOTE:** USP has no role in enforcement of these or other provisions that recognize USP–NF standards, which is the responsibility of FDA and other government authorities in the United States and elsewhere.



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# Partnerships at the heart of quality

## Partners in science

With academics, practitioners

## Partners in industry

R&D companies and generic manufacturers

## Partners in government

With regulatory and health authorities

# Regulatory partners around the world

**BRAZIL**
National Health Surveillance Agency (ANVISA) *Signed June 2016*

**CHINA**
Chinese Pharmacopoeia Commission (ChP)
*Renewed October 2016*

**INDIA**
Indian Pharmacopoeia Commission (IPC)
*Renewed March 2017*

**INDIA**
National Institute of Pharmaceutical Education and Research – Hyderabad (NIPER)
*Signed October 2016*

**JAPAN**
Ministry of Health, Labour & Welfare, Pharmaceuticals & Medical Devices Agency (MHLW/PMDA) *Signed September 2016*

**MEXICO**
Permanent Commission of the Pharmacopeia of the United Mexican States, Fed. Commission for the Protection Against Sanitary Risks (FEUM/COFEPRIS)
*Renewing March 2018*

**LATIN AMERICA & CARIBBEAN**
Pan American Health Organization (PAHO)
*Renewed June 2017*

**RUSSIA**
Federal Service on Surveillance in Healthcare (ROSZDRAVNADZOR)
*Renewed June 2015*

**SAUDI ARABIA**
Saudi Food & Drug Authority (SFDA)
*Signed September 2015*

**SOUTH KOREA**
National Institute of Food & Drug Safety Evaluation (NFIDS)
*Renewed April 2015*

**WHO WORLD MEETING OF PHARMACOPEIAS**

# Overview on USP Heparin Standards

# Heparin

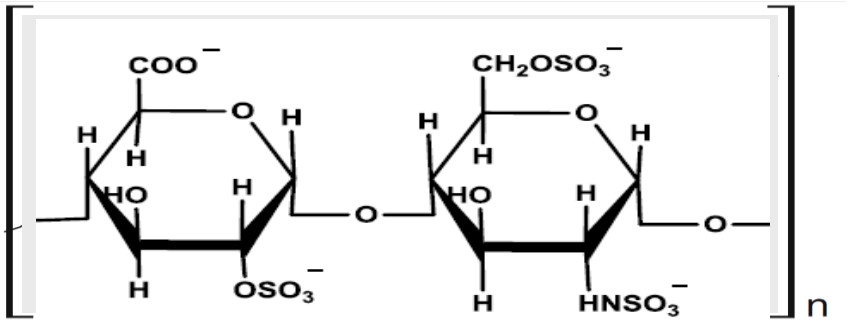
Widely used anti-coagulant

Heparin is a member of the glycosaminoglycan (GAG) family

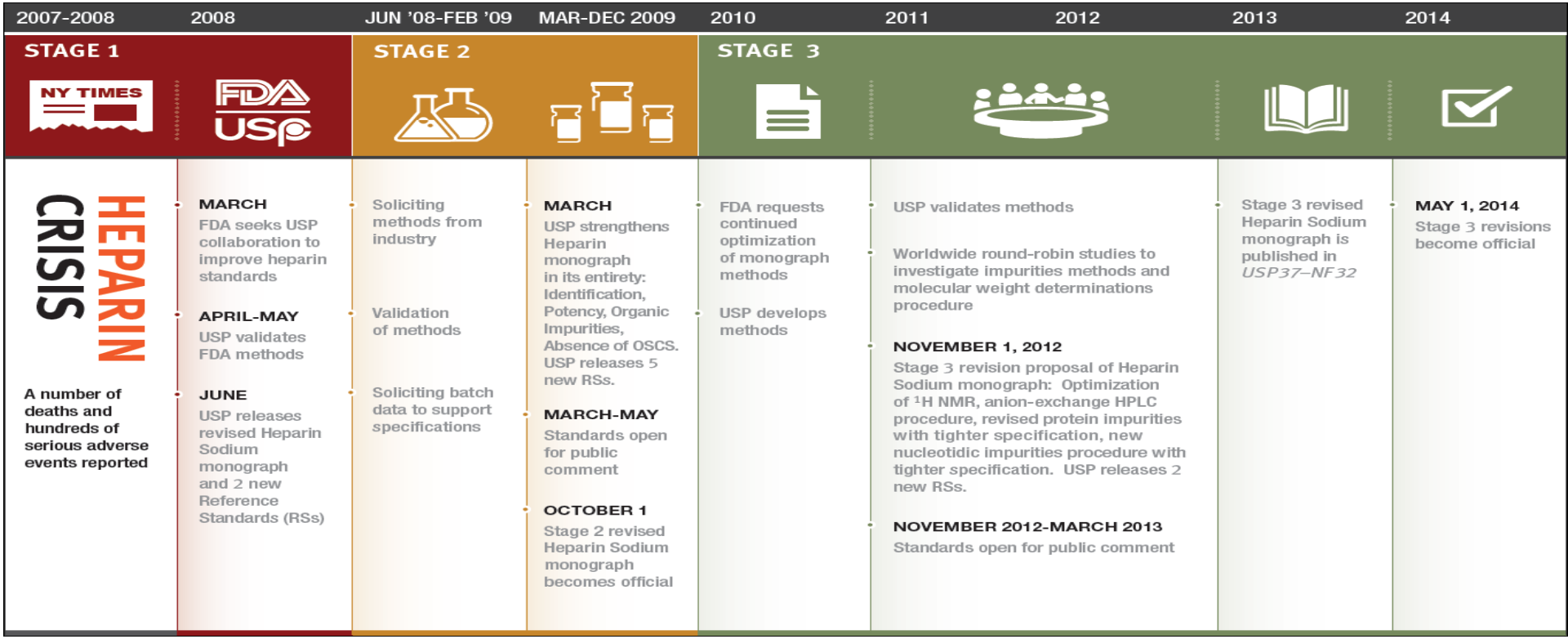
Heparin is a polysaccharide, polysulfated negatively charged heterogeneous mixture with molecular weight range between 2,000 to 50,000 Daltons

Click to add text

The main raw material is pig intestine and majority is sourced from China



# Heparin Timeline



# Current FDA expectations

## Origin of Species

Test and confirm the species origin of crude heparin in each lot of every shipment before use in the manufacture or preparation of a drug.

## Impurity

Test for OSCS in crude heparin in each lot of every shipment before use in the manufacture or preparation of a drug.

## Regulatory

Know the identity and role of the actual manufacturer of crude heparin and any re-packers and distributors who handle crude heparin before receipt and use.

## ICH

Employ the controls described in ICH Q7 to prevent the use of crude heparin containing OSCS or ruminant or unlabeled sources of crude heparin and to fully and promptly investigate and resolve deviations and failures of quality, especially identity and purity.

## Additional Impurity Requirements

Reject for use any crude heparin found to contain any amount of OSCS, or to be derived from, in any amount, ruminant mucosa (unless approved in the drug application).



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# USP Heparin Monographs

Heparin Sodium

Heparin Lock Flush Solution
Heparin Sodium Injection
Anticoagulant Heparin Solution
Antithrombin III Human
Protamine Sulfate
Protamine Sulfate Injection

LMWHs:
Enoxaparin Sodium
Enoxaparin Sodium Injection
Dalteparin Sodium

Fondaparinux Sodium
Fondaparinux sodium Injection

Supported by General Chapters
<207>
<208>
<209>

# Heparin Sodium Monograph after Stage 3

Identification

– **A: 1H NMR spectrum**

– **B: Chromatographic ID**

– **C: Anti-Factor XA and anti-factor IIA Ratio**

– **D: Molecular weight Determinations (revised pore size USP 40)**

– E. Identification tests-General, Sodium **(revised in USP 40)**

**Assay – Anti-Factor IIA Potency**

Other Components: Nitrogen Determination, *Method I* <461>

Impurities

– Residue on Ignition <218>

– ~~Heavy Metals, *Method II* <231>~~ **Removed in USP 40**

– **Limit of Galactosamine in total hexosamine**

– Absence of Oversulfated Chondroitin Sulfate

– **Nucleotidic Impurities (note for nuclease revised > 25 Units/mL from 250)**

– Protein Impurities

# Heparin Sodium Monograph after Stage 3

Specific Tests

– **Bacterial Endotoxins Test <85>**

– Loss on Drying <731>

– pH <791>

– Sterility Tests <71>

# Reference Standards for Current Heparin Sodium

USP Heparin sodium identification

USP Heparin sodium Molecular Weight Calibrant

USP Heparin sodium for Assays

USP Oversulfated Chondrotin sulfate

USP Dermatan sulfate RS

USP Adenosine RS

USP Endotoxin RS

USP Galactosamine hydrochloride RS

USP Glucosamine hydrochloride RS

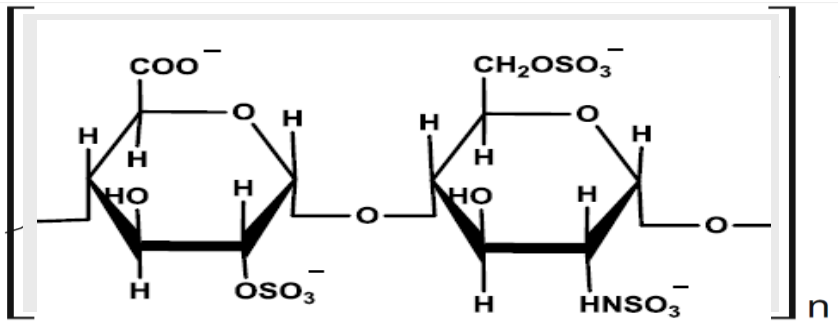
# ID method A. $^{1}$H NMR

**1H NMR:** NLT 500mHz
**Temperature:** 20-30º

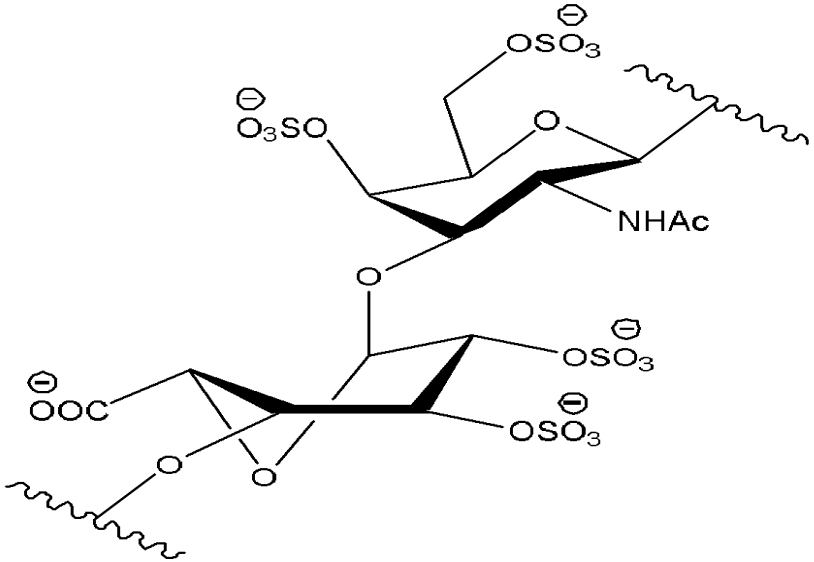
**System suitability solution:** NLT 20 mg/mL USP heparin sodium ID RS with 0.3% OSCS
**Number of transients:** Adjust until the signal-to-noise ratio of the *N*-acetyl heparin signal in the *Standard solution* is at least 1000/1 in the region near 2 ppm.
**Chemical shift:** The TSP methyl signal should be set to 0.00 ppm for all samples.
**Chemical shifts** (for the *N*-acetyl resonance of heparin and oversulfated chondroitin sulfate in the *System suitability solution*): Should be observed at 2.05 ± 0.02 and 2.16 ± 0.03 ppm, respectively.

# ID method A: $^{1}$H NMR Reference Standards

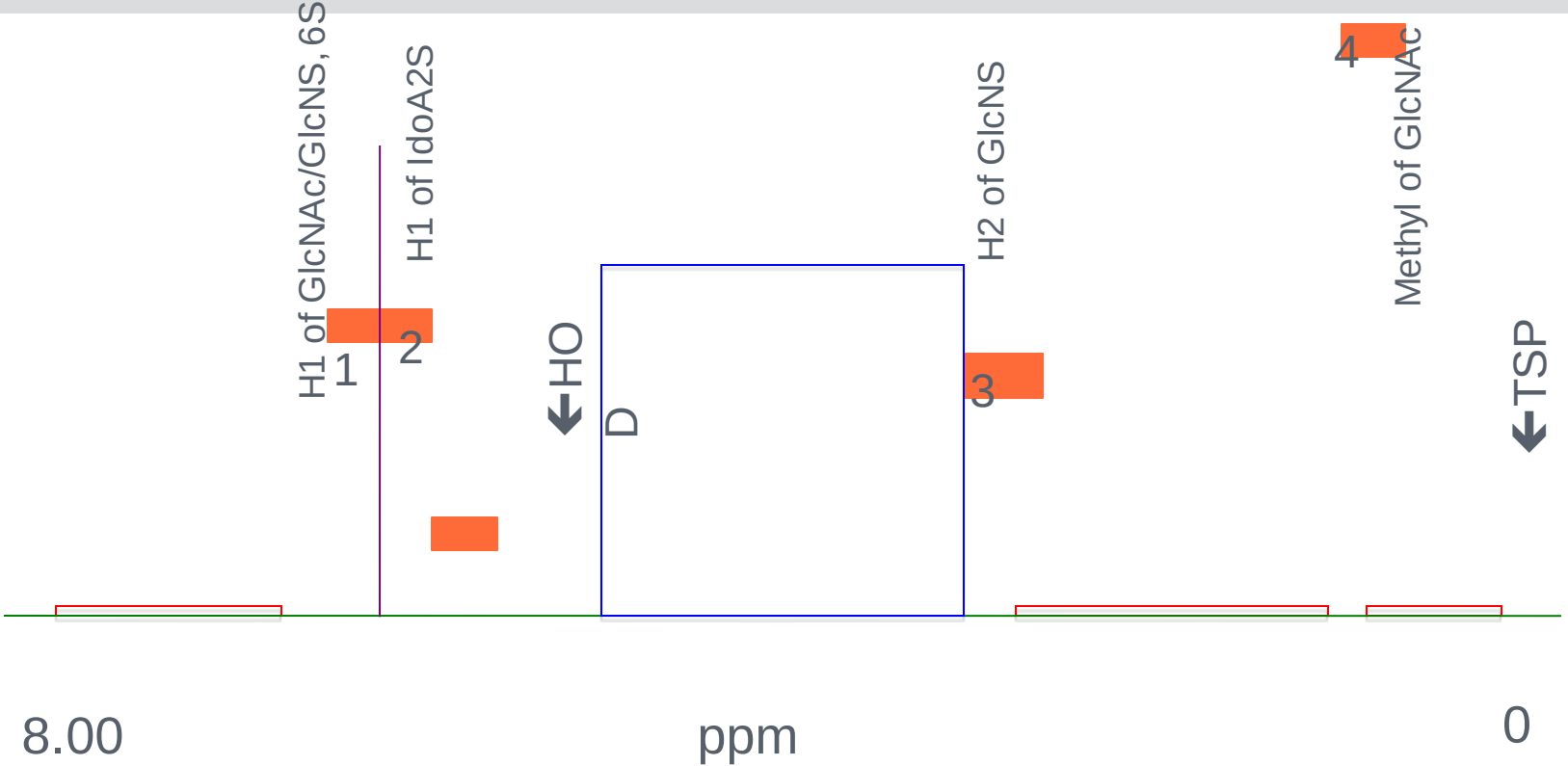
USP Heparin sodium
Identification



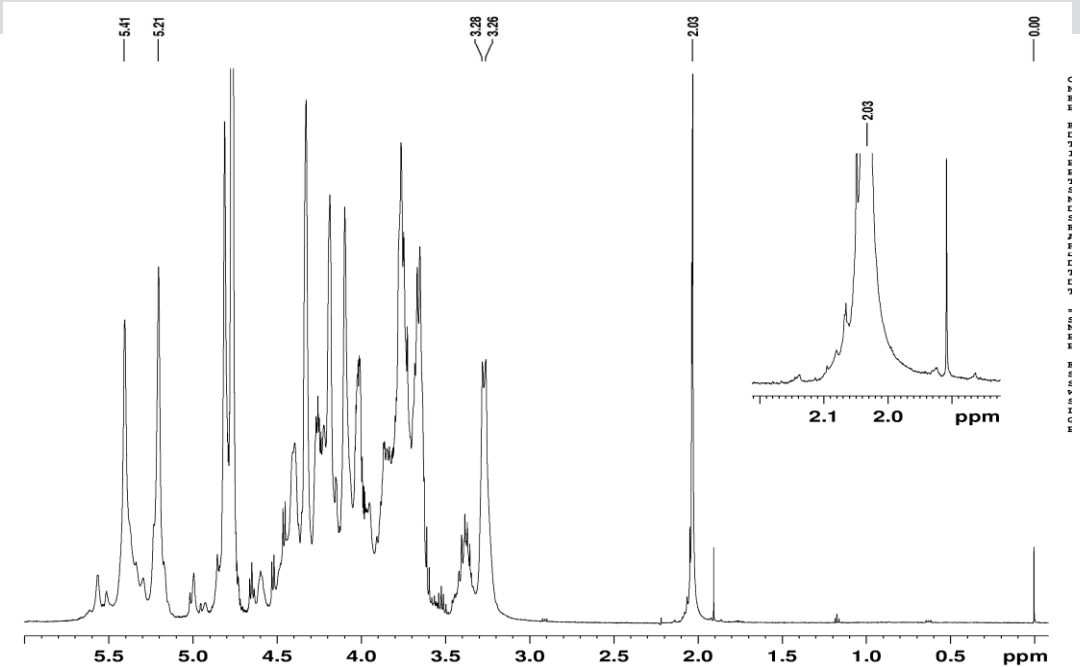

USP Oversulfated
Chondrotin sulfate



# $^{1}$H NMR Specification for Identity of Heparin



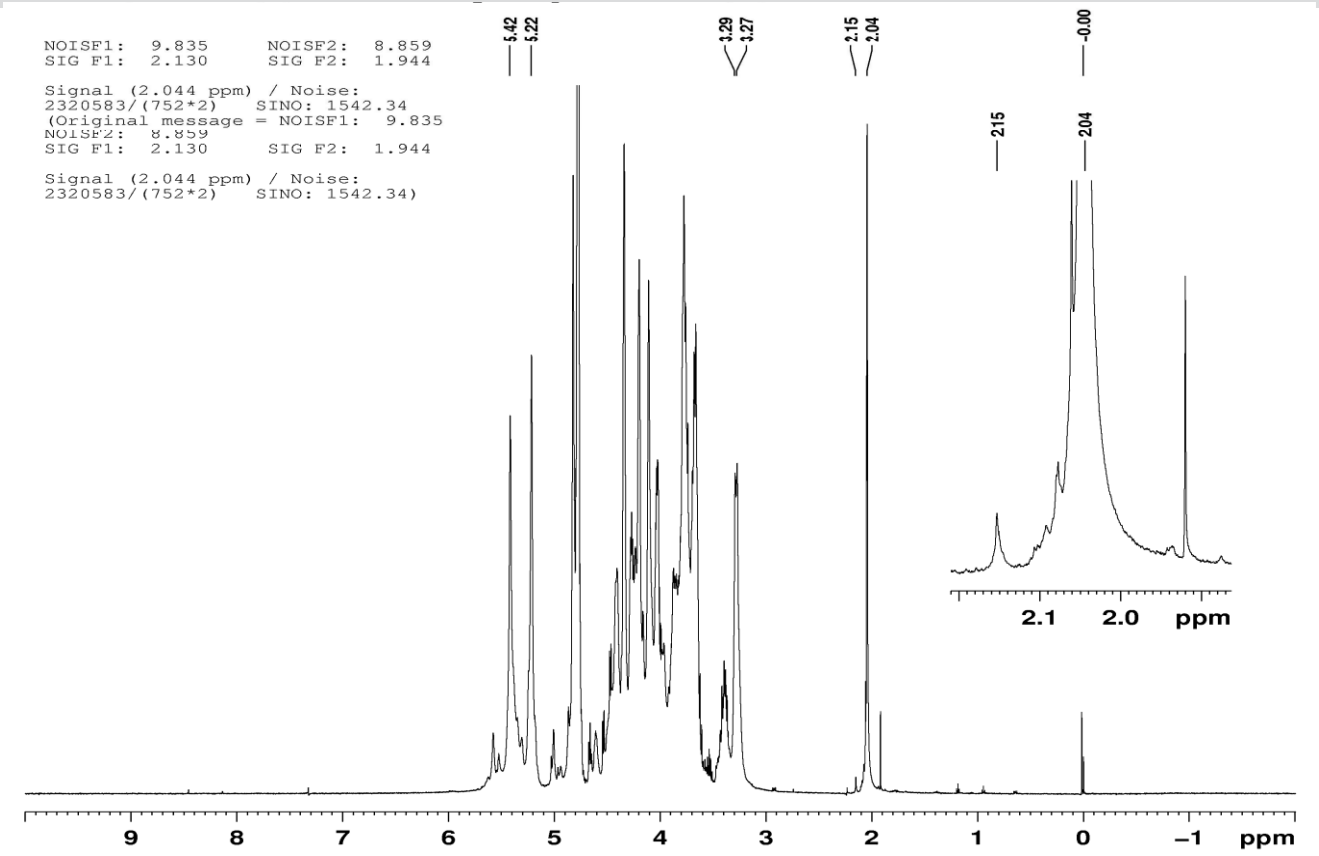
# $^{1}$H NMR Spectrum Heparin Sodium ID RS




## Acceptance Criteria

- No unidentified signals greater than 4% of the mean of signal height of 1 and 2 are present in the following ranges: 0.10-2.00, 2.10-3.20, and 5.70-8.00 ppm.
- No signals greater than 200% signal height of the mean of the signal height of 1 and 2 are present in the 3.35-4.55 ppm for porcine heparin.

# $^{1}$H NMR Spectrum Heparin Sodium System Suitability Solution



# ID method B: Chromatographic ID

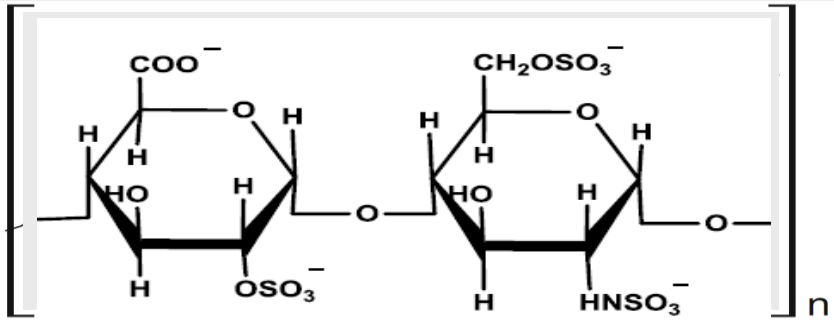
**Mode**: Anion exchange chromatography,
**Column**: 2mm x 25cm (L86)
**Detector**: UV 202nm

**System suitability solution:** NLT 20 mg/mL USP heparin sodium ID RS with 0.1% (w/w) OSCS and 0.5% (w/w) DS
**Resolution:** NLT 1.0 between the dermatan sulfate and heparin peaks, and NLT 1.5 between the heparin and oversulfated chondroitin sulfate peaks
**Relative standard deviation:** NMT 2% for the heparin peak, for 3 replicate injections

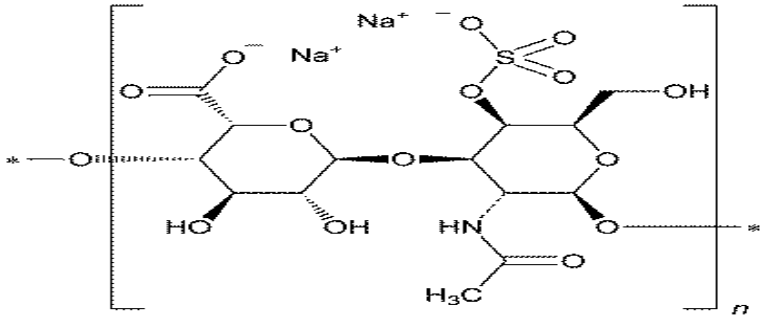
**Acceptance Criteria:** The retention time of the major peak from the *Sample solution* corresponds to that of the *Standard solution*.

# Chromatographic ID Reference Standards

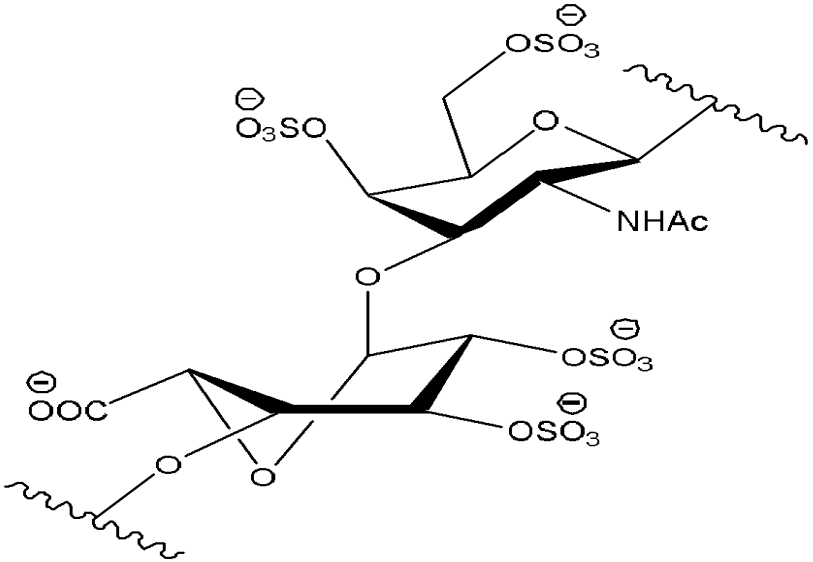
## USP Heparin sodium Identification



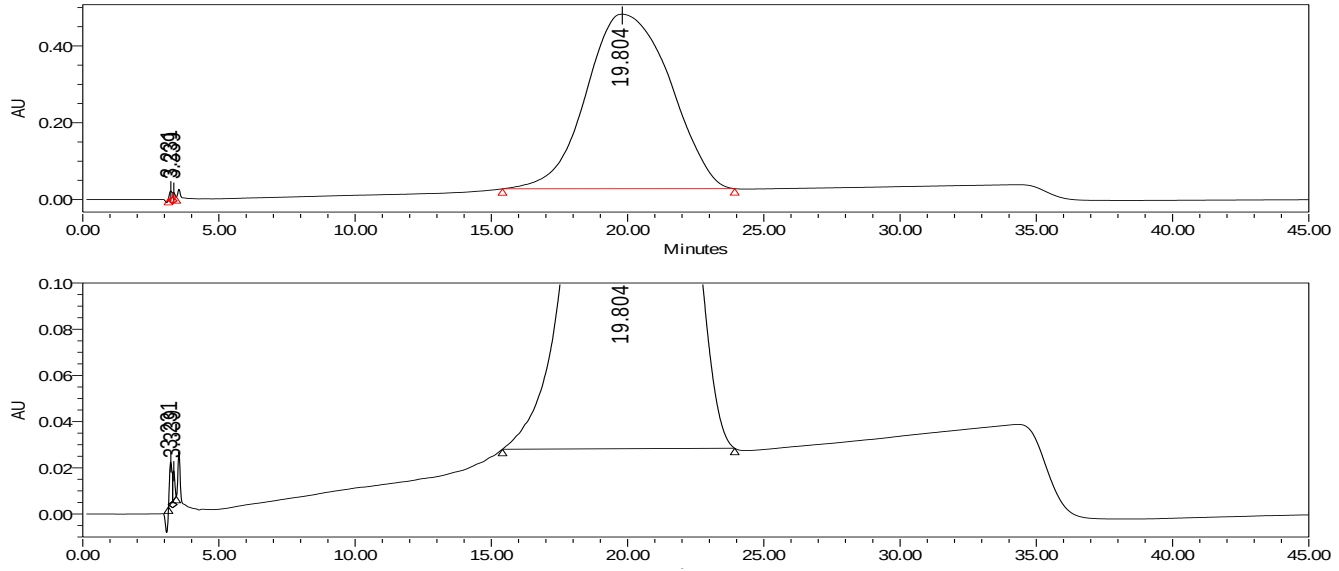

## USP Dermatan Sulfate



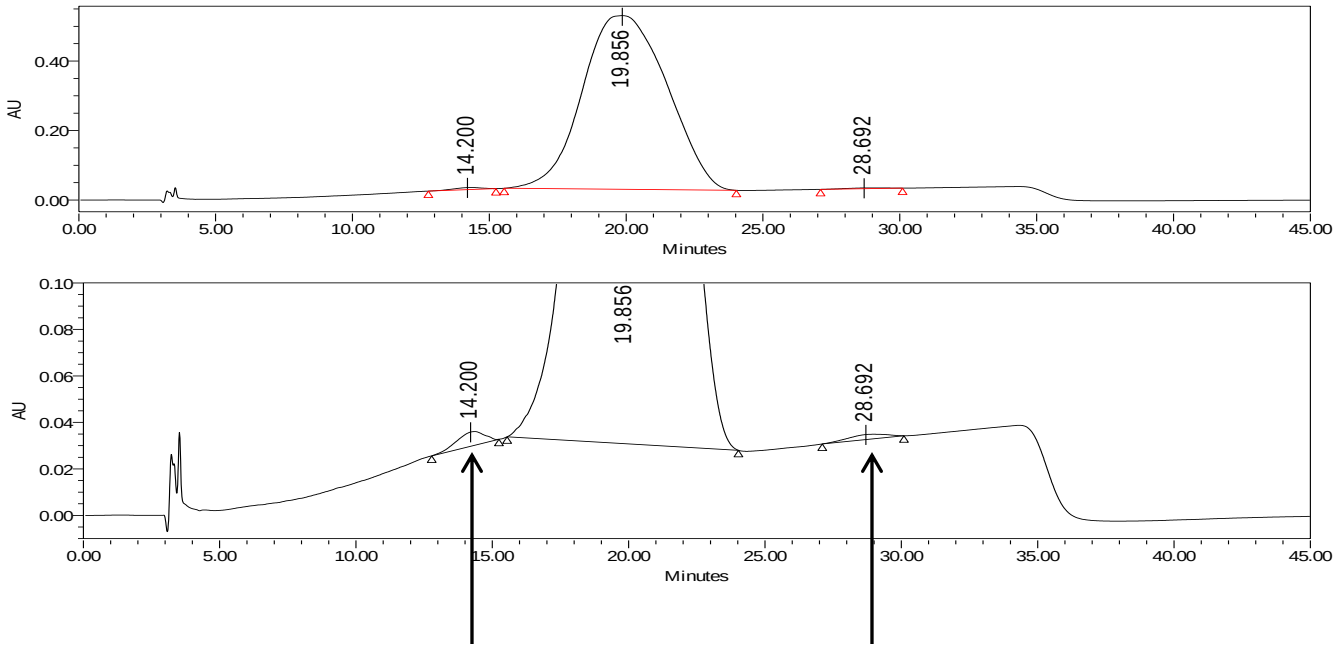

## USP Oversulfated Chondrotin sulfate



# Chromatographic ID Heparin sodium ID RS



# Chromatographic ID System Suitability Solution




Dermatan Sulfate Resolution NLT 1.0

OSCS Resolution NLT 1.5

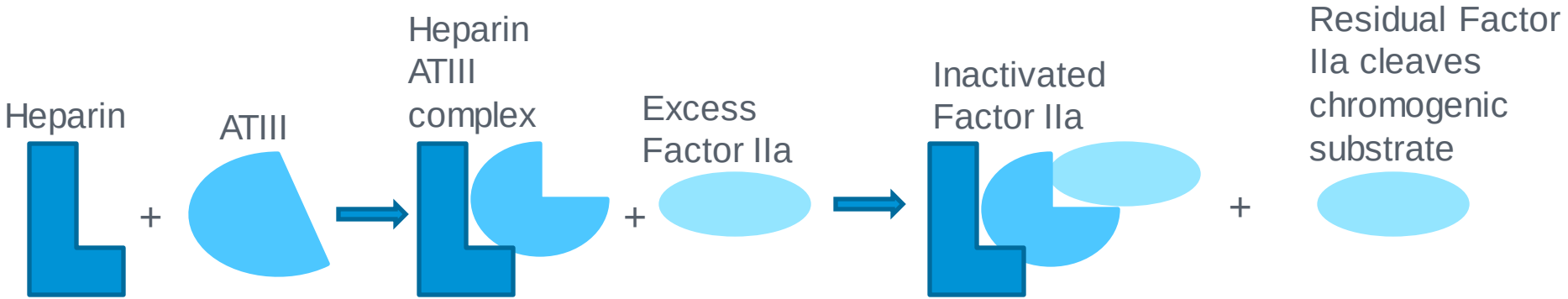
# ID method C. Anti-factor $X_A$ to Anti-factor $II_A$ ratio

Anti-Factor XA and Anti-Factor IIA Assays for Unfractionated and Low molecular Weight Heparins <208>

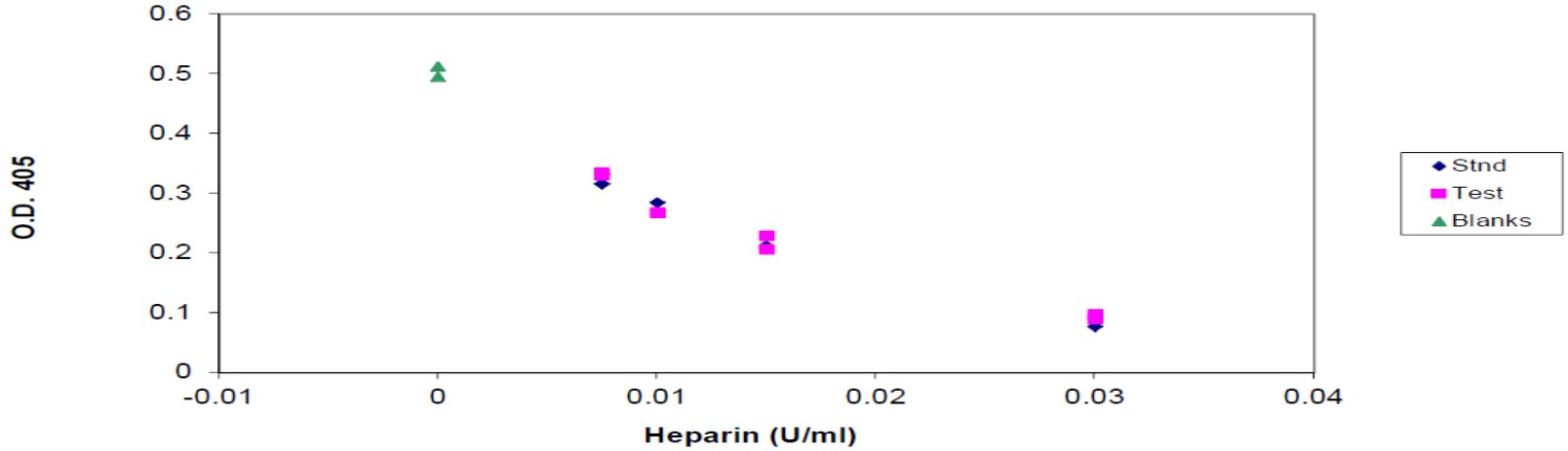
**Acceptance criteria:** 0.9–1.1

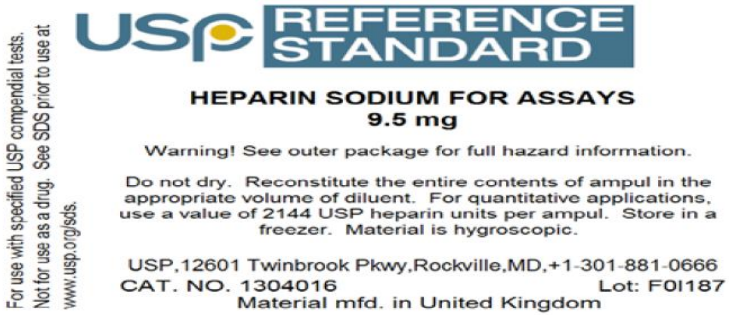
# <208> ANTI-FACTOR Xa AND ANTI-FACTOR IIa ASSAYS FOR UNFRACTIONATED AND LOW MOLECULAR WEIGHT HEPARINS

In the test system, heparin is bound to antithrombin (AT), and factor IIa or factor Xa added to the mixture binds to the heparin–AT complex. The residual factor IIa or factor Xa not inhibited by the heparin–AT complex is quantified by a chromogenic substrate that is specific for either factor IIa or factor Xa and is added in the final step. Analysts note an inverse relationship wherein more color is produced by more residual enzyme, which equates to less heparin activity.



# USP Heparin Sodium for Assay



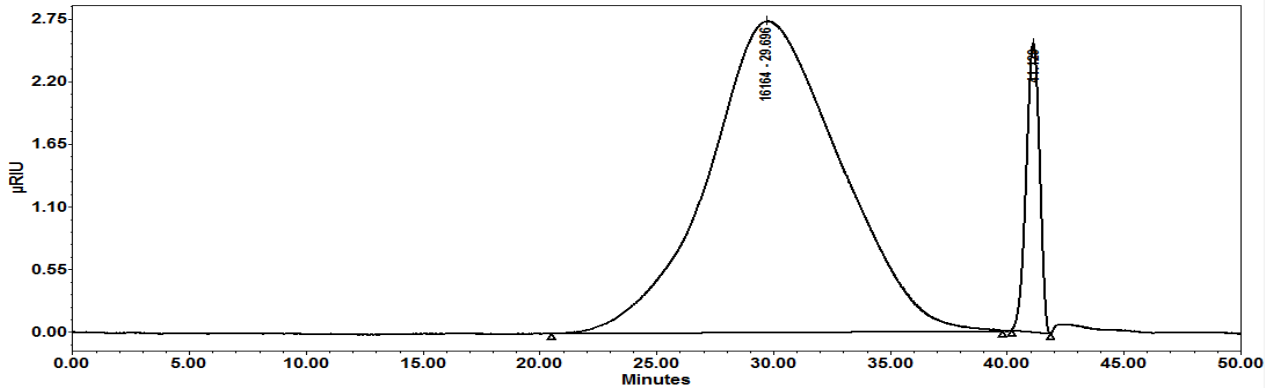



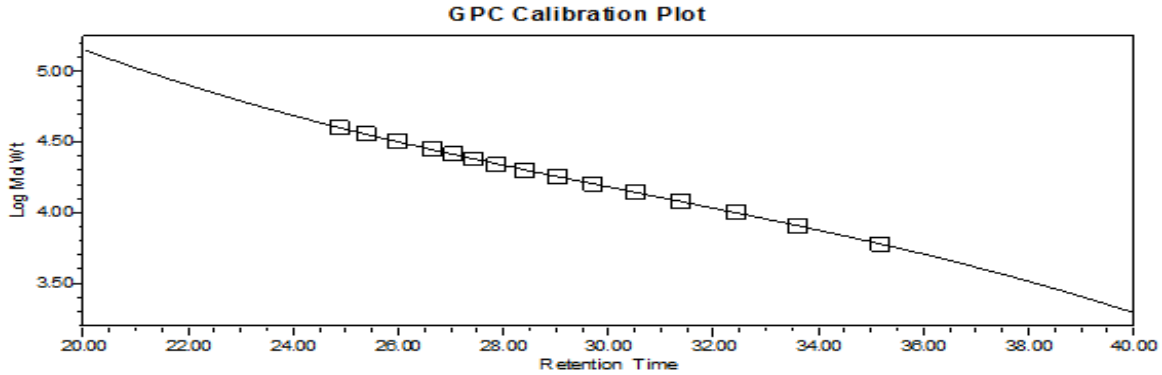
# ID method D. Molecular weight determination

**Mode:** HPLC
**Detector:** Refractive index
**Columns:** One 7.8-mm × 30-cm, **8-µm** packing L59 in series with a 7.8-mm × 30-cm, **5-µm** packing L59 **(revised official in USP 40)**
**Guard column:** 6-mm × 4-cm; 7-µm packing L59
**System suitability Samples:** *Calibration solution* and *System suitability solution* (duplicate injections)
**Suitability requirements Weight-average molecular weight ($M_w$):** The $M_w$ of the *System suitability* sample is within 500 Da of the labeled value as stated in the USP Certificate for USP Heparin Sodium Identification RS.
**Peak molecular weights ($M_p$):** The peak molecular weights ($M_p$) of the duplicate injections of the *System suitability solution* do not differ by more than 5% of the upper value.
**Resolution:** Baseline resolution between the heparin and salt peaks.
**Calibration curve:** $r^2$NLT 0.990, using a third order polynomial equation.

**Acceptance criteria**: *M24000* is NMT 20%, *Mw* is between 15,000 Da and 19,000 Da, and the ratio of *M8000–16000* to *M16000–24000* is NLT 1.0.

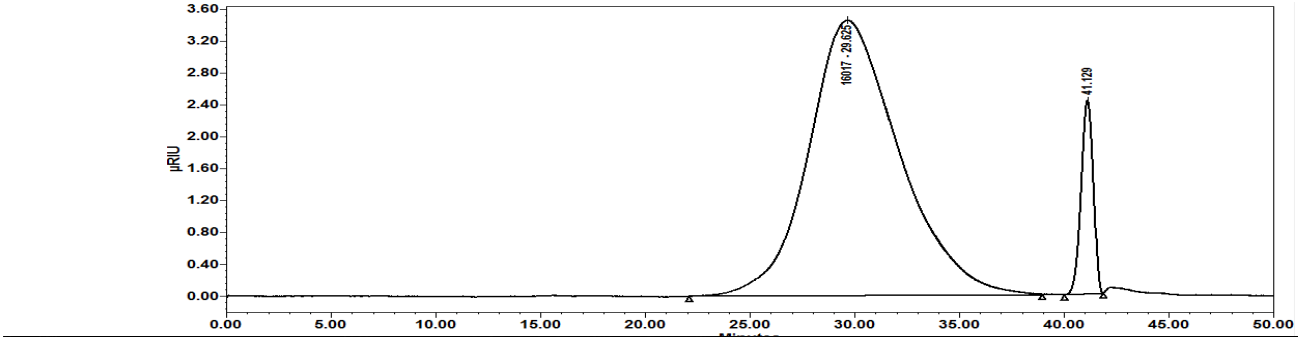
# Molecular weight determination Calibration solution

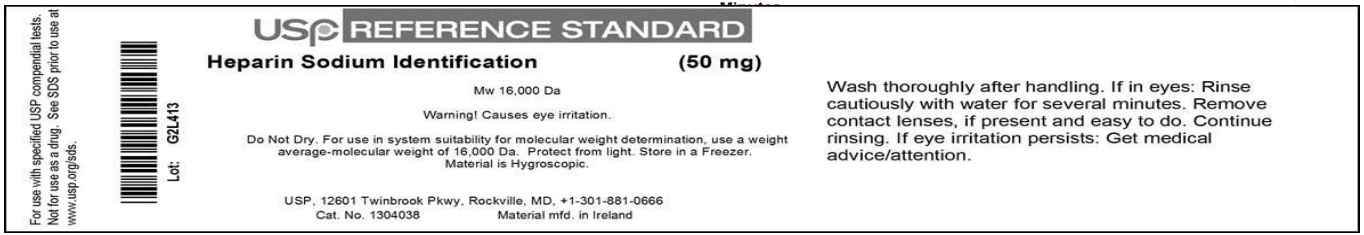



**Calibration curve: $r^2$NLT 0.990, using a third order polynomial equation.**

# Molecular weight determination chromatogram System Suitability solution




**Suitability requirements Weight-average molecular weight ($M_w$):** The $M_w$ of the *System suitability* sample is within 500 Da of the labeled value

**Peak molecular weights ($M_p$):** The peak molecular weights ($M_p$) of the duplicate injections do not differ by more than 5% of the upper value.

**Resolution:** Baseline resolution between the heparin and salt peaks

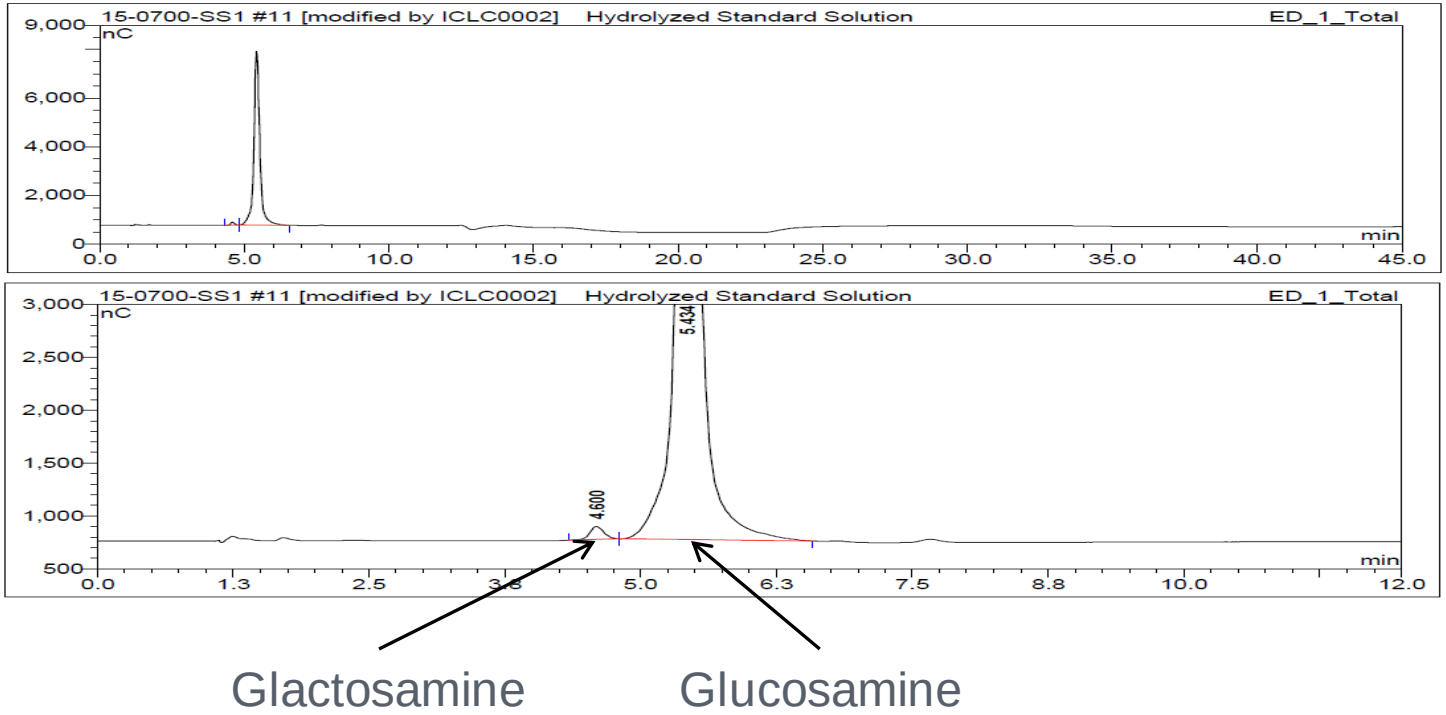
# Impurities: Limit of glactosamine in total Hexosamine

**Mode:** HPIC
**Detector:** Pulsed amperometric detector
**Column:** 3-mm × 3-cm amino acid trap column in series with a 3-mm × 3-cm guard column and a 3-mm × 15-cm column that contains packing L69

**Standard solution:** 0.8 mg/mL USP Glucosamine hydrochloride and 8 ug/mL glactosamine hydrochloride in 5N HCl
**Resolution:** NLT 2 between the galactosamine and glucosamine peaks
**Column efficiency:** NLT 2000 theoretical plates for glucosamine
**Tailing factor:** Between 0.8 and 2.0 for the galactosamine and glucosamine peaks

**Acceptance criteria:** The percent galactosamine peak area of the total hexosamine of the *Hydrolyzed sample solution* must be NMT 1%.

# Impurities: Limit of glactosamine in total hexosamine System Suitability solution




**Resolution:** NLT 2 between the galactosamine and glucosamine peaks

# Impurities: Nucleotidic Impurities

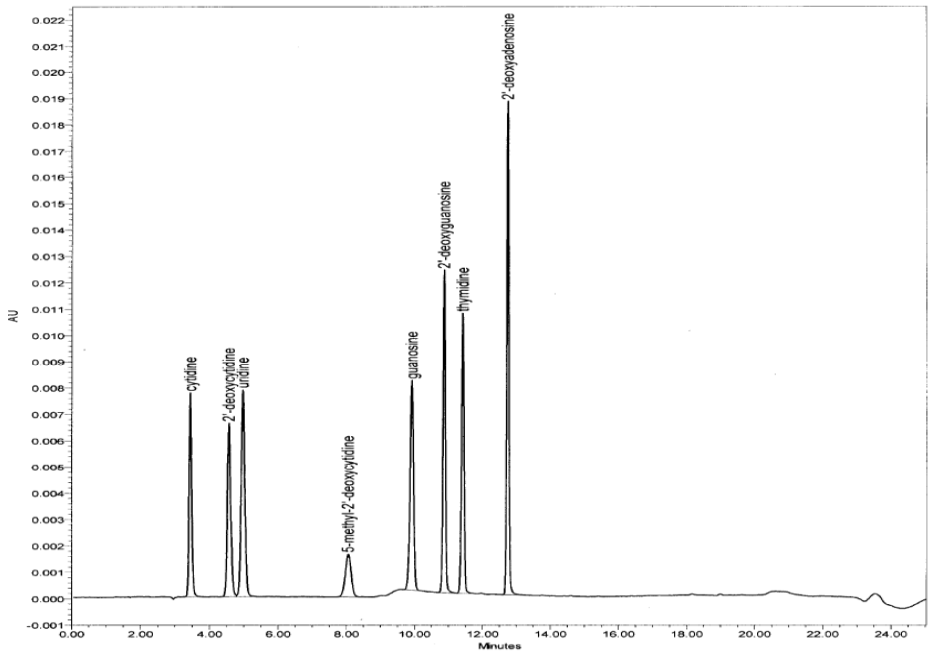
**Mode:** LC
**Detector:** UV 260 nm
**Column:** 4.6-mm × 15-cm; 4-µm packing L1

**System suitability Samples:** *System suitability solution*, *Standard solution*, and *Nucleoside identification solution*
**Suitability requirements Resolution:** The resolution between the 2'-deoxycytidine peak and the uridine peak is NLT 1.3 for the injection of the *Nucleoside identification solution*.
**Relative standard deviation:** Inject six replicates of the *Standard solution* . (%RSD) of the adenosine peak is NMT 10%.
**Signal-to-noise ratio:** NLT 10 for the adenosine peak

**Acceptance criteria**: NMT 0.1% (w/w) is found.

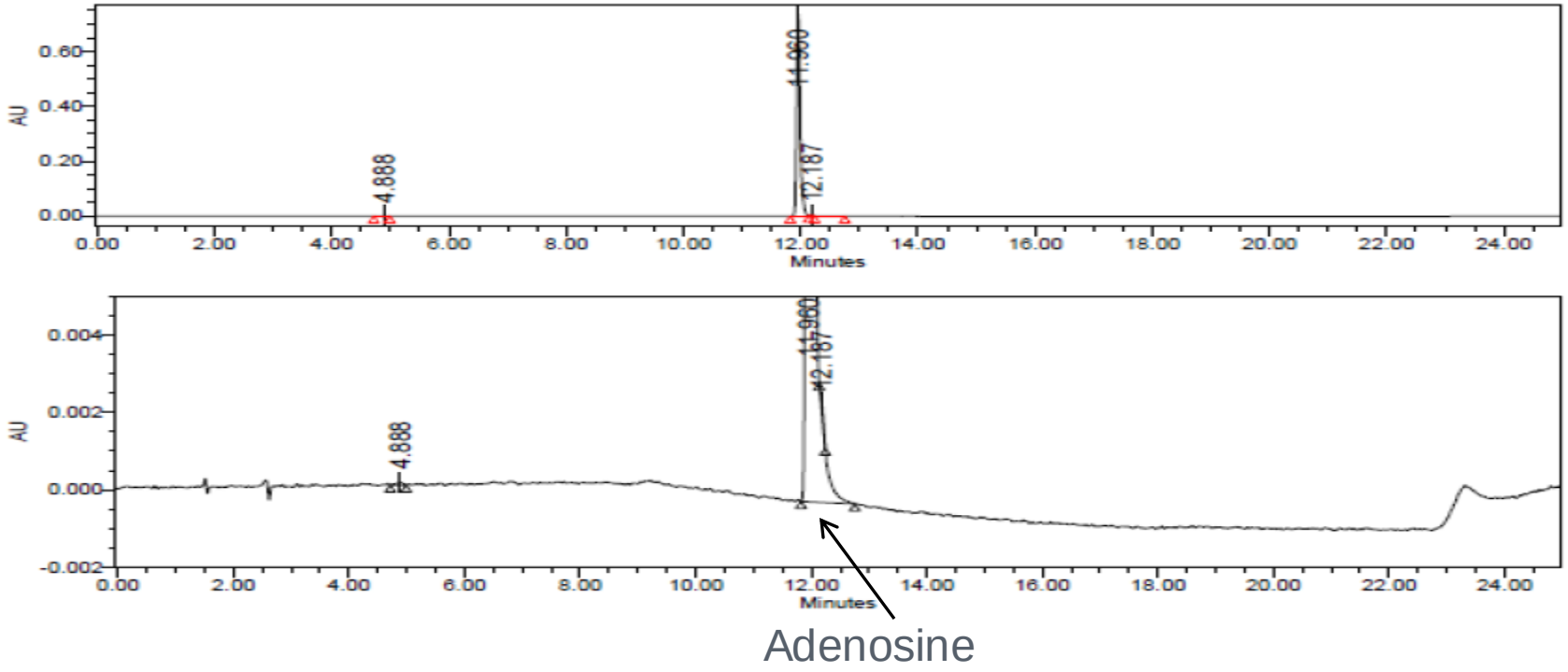
# Nucleoside identification solution

| Name | Relative Retention Time | Relative Response Factor | $MW_{ratio}$ |
|---|---|---|---|
| Cytidine | 0.28 | 0.53 | 1.2548 |
| 2′-Deoxycytidine | 0.38 | 0.56 | 1.2727 |
| Uridine | 0.40 | 0.75 | 1.2537 |
| 5-Methyl-2′-deoxycytidine | 0.66 | 0.25 | 1.2569 |
| Guanosine | 0.81 | 0.74 | 1.2188 |
| 2′-Deoxyguanosine | 0.89 | 0.83 | 1.2319 |
| Thymidine | 0.92 | 0.68 | 1.2558 |
| Adenosine | 1.00 | 1.00 | 1.2319 |
| 2′-Deoxyadenosine | 1.04 | 1.09 | 1.2466 |
| Others | — | 1.00 | 1.0000 |




**Suitability requirements Resolution:** The resolution between the 2'-deoxycytidine peak and the uridine peak is NLT 1.3 for the injection of the *Nucleoside identification solution*

# Nucleotidic impurities, Standard solution




**Relative standard deviation:** Inject six replicates of the *Standard solution* . (%RSD) of the adenosine peak is NMT 10%.

**Signal-to-noise ratio:** NLT 10 for the adenosine peak

# Overview on USP Low Molecular Weight Heparin Standards

# Enoxaparin Sodium Monograph

## Identification

- A: UV Absorption <197K>
- B: **$^{13}$C NMR Spectrum (potential revision)**
- C: **Anti-Factor XA and anti-factor IIA Ratio**
- D: **Molecular weight Determinations**
- E. Identification tests-General, Sodium (191)

## Assay – Anti-Factor $X_A$ Potency

- **Anti-Factor IIa activity**
- Molar Ratio of sulfate to carboxylate

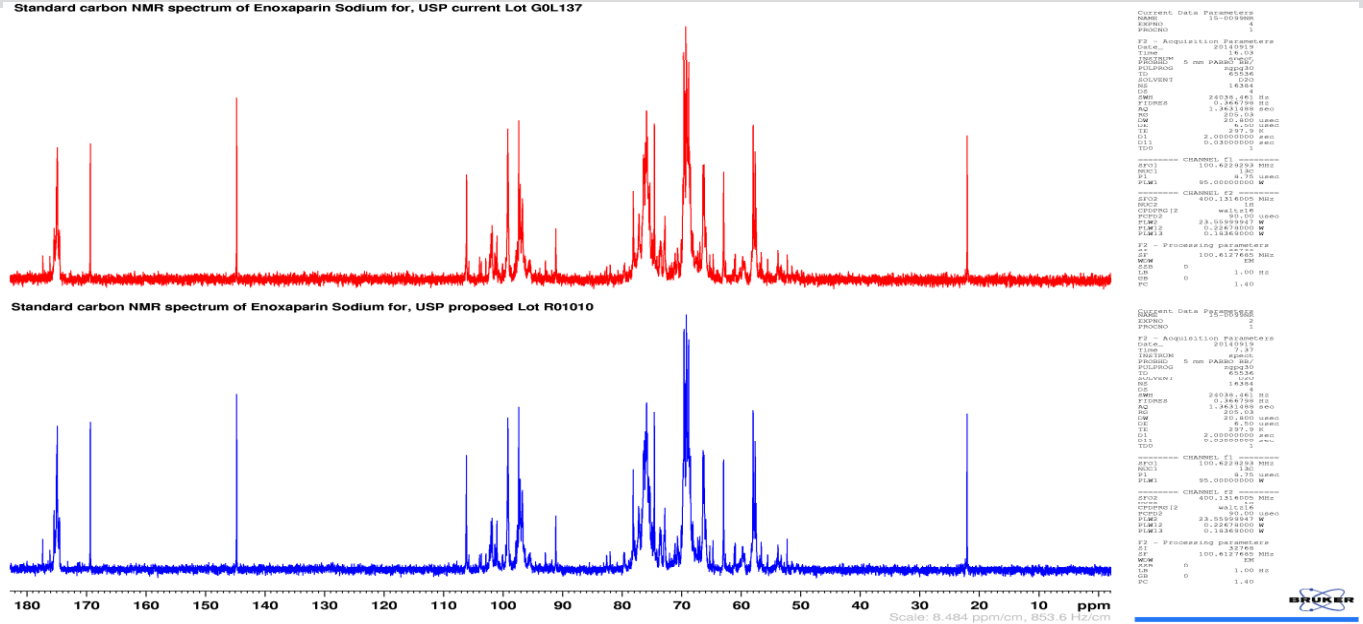
## Other Components:

- Benzyl alcohol determination
- Nitrogen Determination, *Method II*<461>
- Sodium Content

## Specific Tests

- pH <791>
- Loss on Drying <731>
- Specific Absorbance
- Bacterial Endotoxins <85>

# ID B: 13C NMR Spectrum



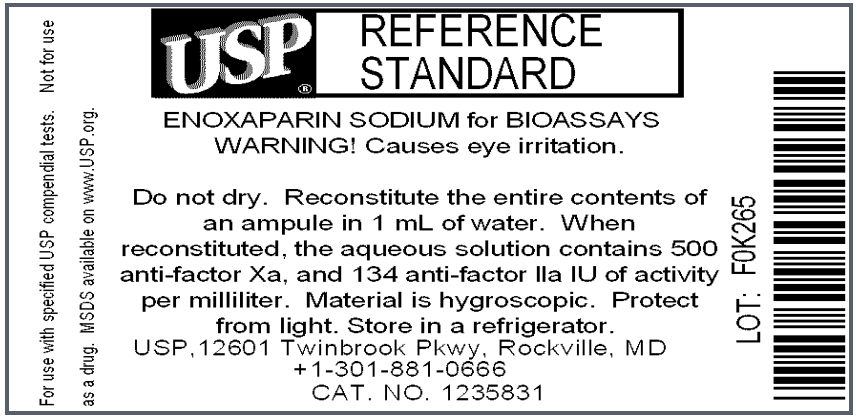

NLT 75Mhz NMR

Acceptance criteria Similar to standard

Potential for Revision to $^{1}H$ NMR

# ID C: Anti-Factor XA and anti-factor IIA Ratio

Use General chapter <208>




System suitability XA:

1. A blank solution gives an increase in absorbance value at 405 nm of NMT 0.20 absorbance units/min (or 0.8 absorbance units in total) when assayed using an appropriate volume (50 mL) of *pH 7.4 buffer* instead of 50 mL of the *Standard solution* or the *Sample solution*.
2. The reading of the blank B2 is not more than ±0.05 absorbance units against blank B1.

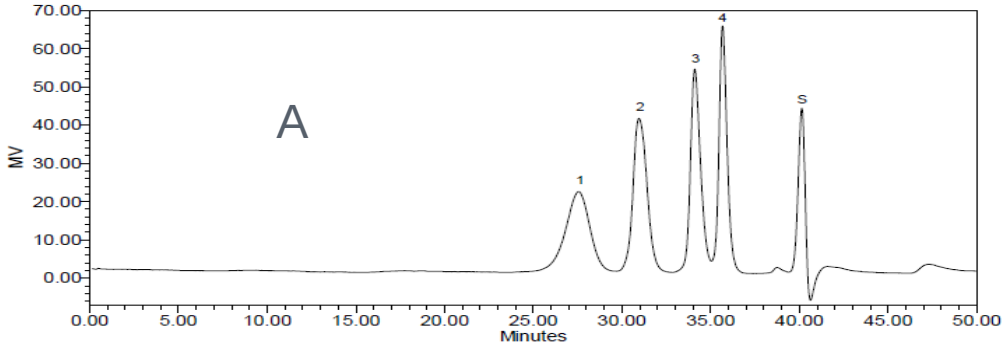
# ID D: Molecular weight determination

**Mode:** HPLC
**Detector:** Refractive index
**Columns:** One 7.8-mm × 30-cm, L59 in series with a 7.8-mm × 30-cm, L59
**Guard column:** 6-mm × 4-cm; 7-µm packing L59

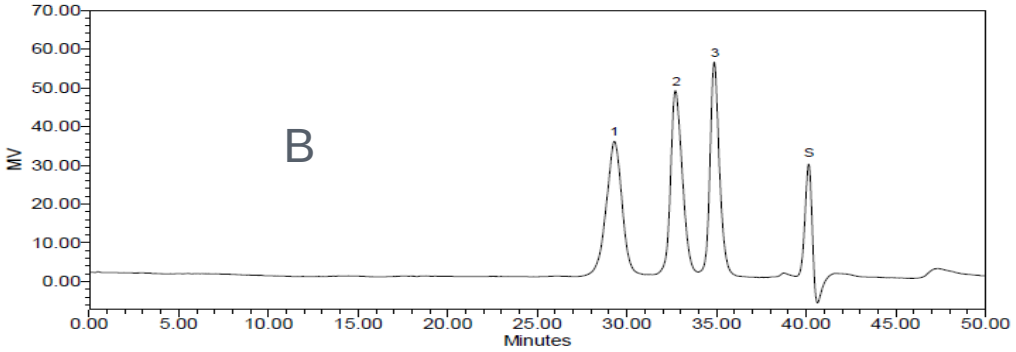
**System suitability Samples:** *Calibration solution* and *System suitability solution* (duplicate injections)
**Suitability requirements Weight-average molecular weight ($M_w$):** The $M_w$ of the *System suitability* sample is within 150 Da of the labeled value as stated in the USP Certificate for USP enoxaparin Sodium RS.
.

**Acceptance criteria**: *M2000* is between 12.0% and 20.0%, *M2000–8000* is between 68.0% and 82.0%, and *M8000* is NMT 18.0%

# Enoxaparin Sodium Molecular weight Calibrants



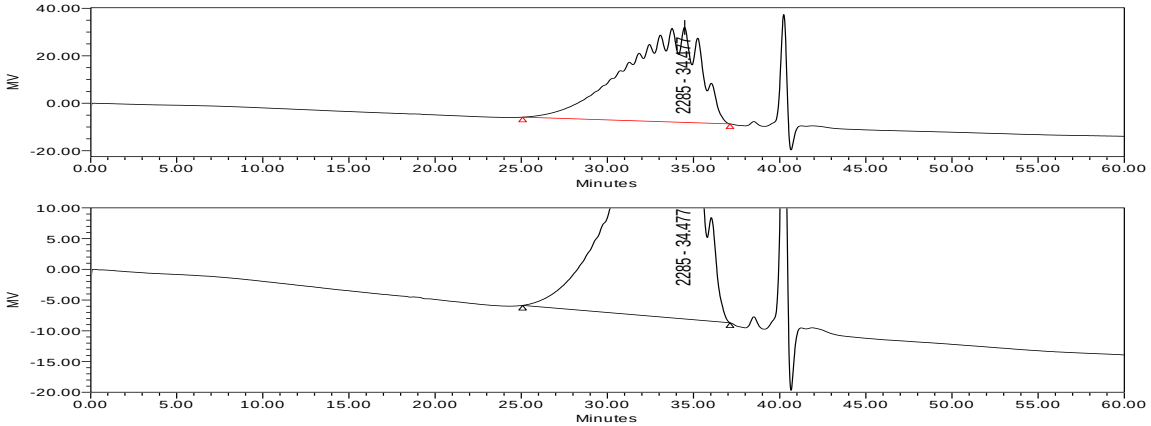

1 – 11000
2 – 5200
3 – 2250
4 – 1400
S – solvent




1 – 7750
2 – 3350
3 – 1800
S – solvent

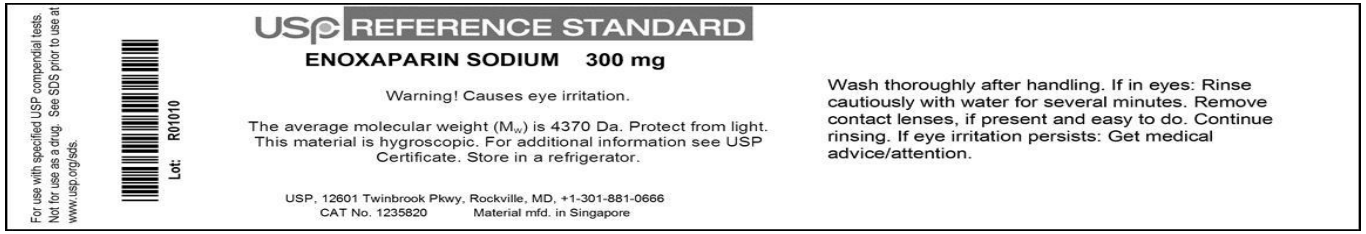
# ID D: Molecular Weight Determinations

## System suitability: 150 Da of USP RS value




**Peak Results**

| | Name | RT | Int Type | %Poly > 8000 | %Poly <2000 | Mn (Daltons) | Mw (Daltons) | MP (Daltons) |
|---|---|---|---|---|---|---|---|---|
| 1 | enoxaparin sodium | 34.477 | bb | 10.519 | 16.542 | 3136 | 4333 | 2285 |



# Dalteparin

Identification

- A: **1H NMR spectrum**
- B: **Molecular weight Determinations <209>**
- C: **Anti-Factor XA and anti-factor IIA Ratio**
- D. Identification tests-General, Sodium (191)

Assay – **Anti-Factor XA Potency**

Other Components:

- Nitrogen Determination, *Method II* <461>
- Sodium Content

Impurities

- Limit of Nitrites
- **Boron** (Revision to Boron test wavelength under consideration)

Specific Tests

- **Anti-Factor IIA Activity**
- Molar Ratio of sulfate to carboxylate

Specific Tests

- **Bacterial Endotoxins Test <85>**
- Loss on Drying <731>
- pH <791>

# ID A: 1H NMR Dalterpain

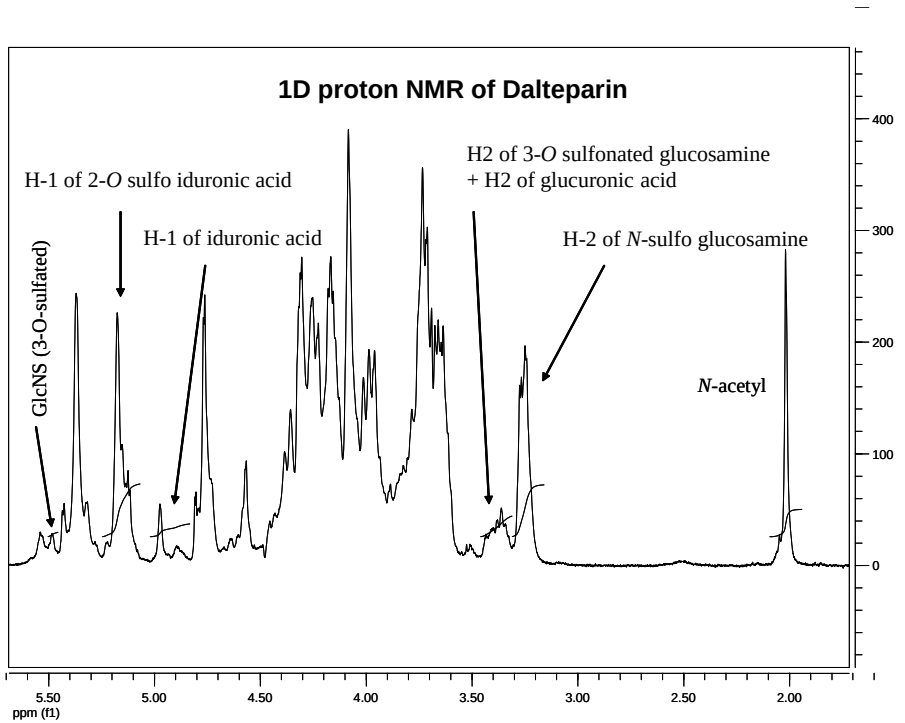
**1H NMR:** NLT 500mHz

**Temperature:** 20-30º

**Chemical shift:** The TSP methyl signal should be set to 0.00 ppm for all samples.

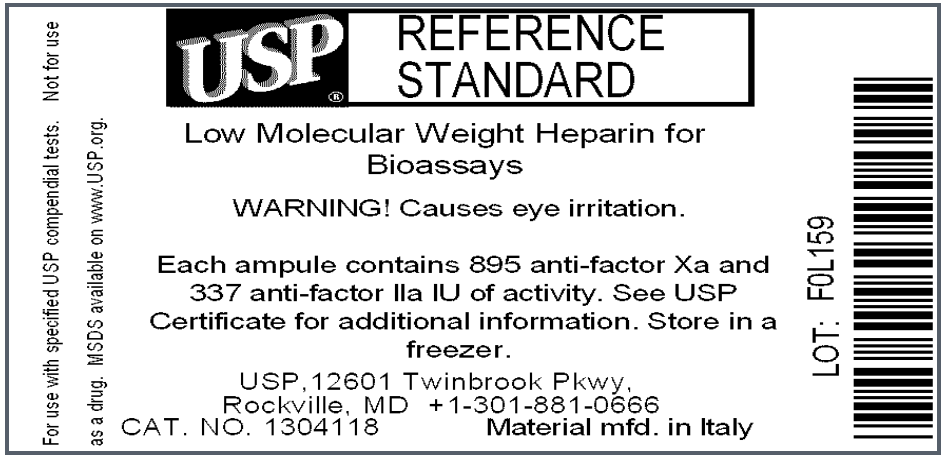
**Chemical shifts** The ppm values for the methyl group of *N*-acetyl, the H-2 of *N*-

# 1H NMR Dalteparin sodium



# Antifactor Xa assay

Use General chapter <208>




System suitability XA:

1. A blank solution gives an increase in absorbance value at 405 nm of NMT 0.20 absorbance units/min (or 0.8 absorbance units in total) when assayed using an appropriate volume (50 mL) of *pH 7.4 buffer* instead of 50 mL of the *Standard solution* or the *Sample solution*.
2. The reading of the blank B2 is not more than ±0.05 absorbance units against blank B1.

# Boron ICP-OES

| | |
|---|---|
| Plasma gas flow | 12 L/min |
| Auxillary gas flow | 0.2 L/min |
| Nebulizer gas flow | 0.75 L/min |
| RF power | 1300 watts |
| Plasma view | Radial |
| Read delay | 20 sec |
| Read parameters (s) | 1.0 min, 5.0 max |
| Peristaltic pump flow rate | 0.44 mL/min |
| Spray chamber | Cyclonic |
| Nebulizer | Meinhard® Type K1 |
| Injector | Alumina, 2.0 mm i.d |
| Quartz torch | Single slot |
| Replicates | 5 |
| Resolution | Normal |
| Wavelength (nm) | 249.772 and 249.677* |

*Wavelength at 249.677 nm was used for reference only, no data reported

Adopted from EP

Boron is determined by measurement of the emission from inductively coupled plasma (ICP) at 249.733 nm or a suitable wavelength.

# <207> TEST FOR 1,6-ANHYDRO DERIVATIVE FOR ENOXAPARIN SODIUM

## DEPOLYMERIZATION OF ENOXAPARIN SODIUM BY HEPARINASES AND RESULTING OLIGOSACCHARIDES

**Mode:** HPLC

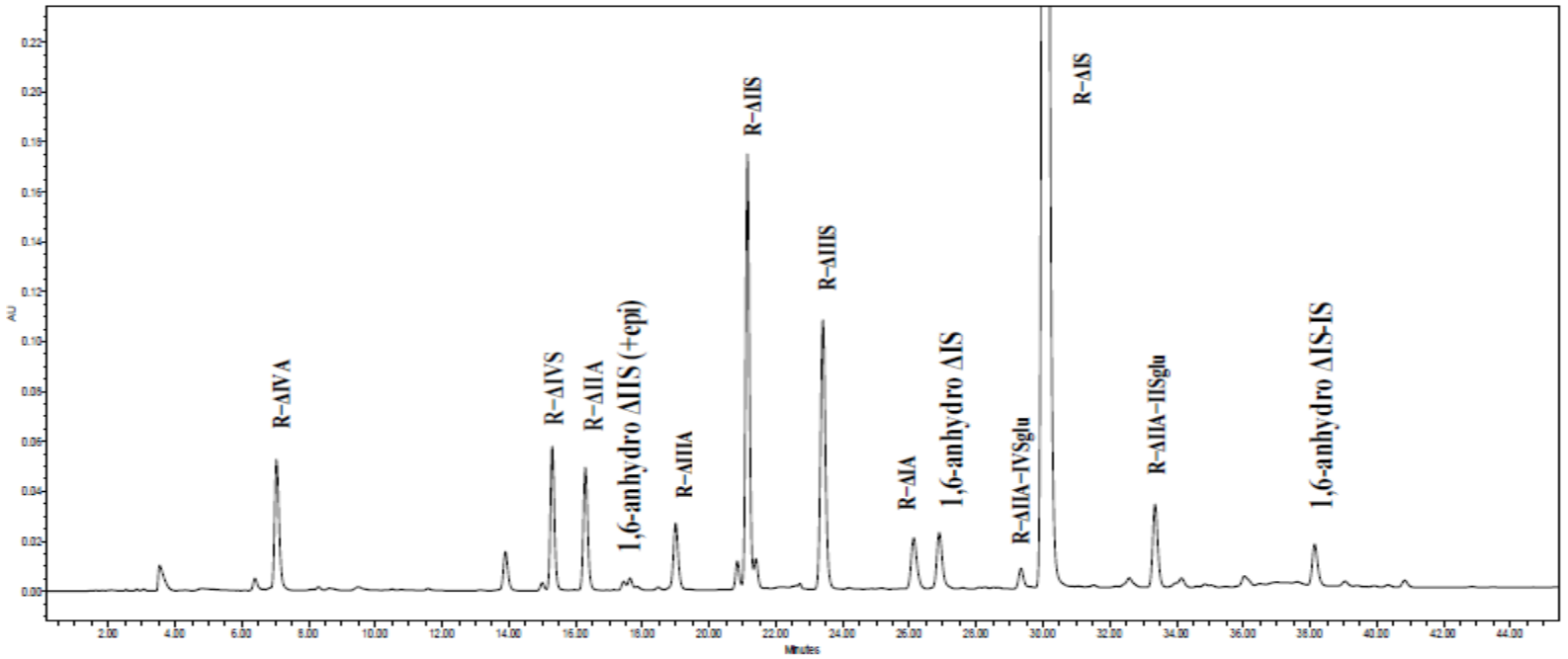
**Detector:** 234nm

**Columns:** 3-mm × 25-cm column that contains 5-mm packing L14

Reduction suitability test requirement of 0.02% to be revised to 2%

Should USP develop Heparinase standards?

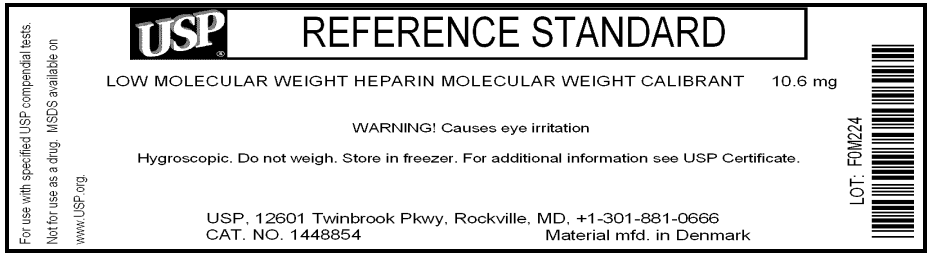
# 1,6-anhydro test USP Enoxaparin sodium

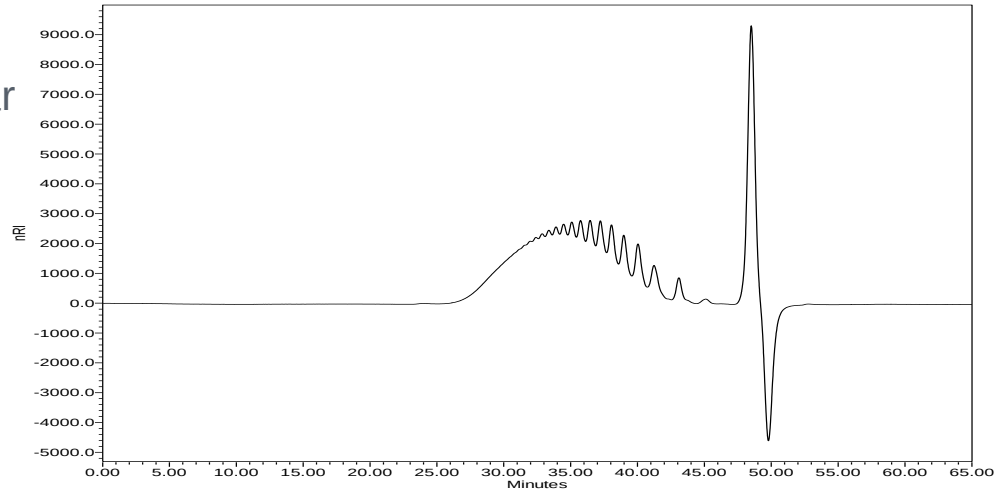


# <209> LOW MOLECULAR WEIGHT HEPARIN MOLECULAR WEIGHT DETERMINATIONS

**Mode:** HPLC
**Detector:** Refractive index
**Columns:**Columns: 7.8-mm × 30-cm; 5-µm packing L59 in series with a 7.8-mm × 30-cm; 5-µm packing L59
**Suitability requirements**
**Resolution:** There is baseline resolution between the last peak of the USP Low Molecular Weight Heparin Molecular Weight Calibrant RS and the salt peak, or negative exchange peaks.
**Calibration curve:** The coefficient of determination of the calibration curve fitted to the Broad Standard Table values must be NLT 0.990, using a third-order polynomial equation.

Cited in Dalteparin sodium



# Fondaparinux Sodium Monograph

Identification

– A: **$^{13}$C NMR Spectrum**

– B: **Chromatographic ID**

– C: Sodium Determination

– E. Identification tests-General, Sodium (191)

Assay – **HPLC**

Other Components: Sodium Determination

Impurities

– Free Sulfate and Residual Chloride Determination

– Organic Impurities

– Pyridine and Ethanol Determination

Specific Tests

– **Bacterial Endotoxins <85>**

– pH <791>

– Microbial Enumeration Tests <61>

– Water Determination <921>

# Fondaparinux Sodium Revisions pf 42 (5)

1. The Assay has been revised to add instructions to filter Solution B, revise the Note in the Assay to include further suggestions on obtaining a stable baseline, a System suitability solution B subsection and related RS have been added, and wording on the resolution requirement has been added for clarity.
2. A hyperlink to the nitric acid reagent has been added in the test for Sodium Determination.
3. The test for Organic Impurities has been revised to specify solutions to be used in the test and include a Note explaining USP's approach to Fondaparinux related impurities as well as process related impurities from the synthesis. The section now includes degradation impurities as well as process related impurities above the unspecified impurity limit.
4. The test for Water Determination has been revised to include a Sample amount and detailed Analysis.

# ID A: $^{13}$C NMR Spectrum

**Mode:** NMR, pulsed (Fourier transform)

**Frequency:** NLT 100 MHz (for $^{13}$C)

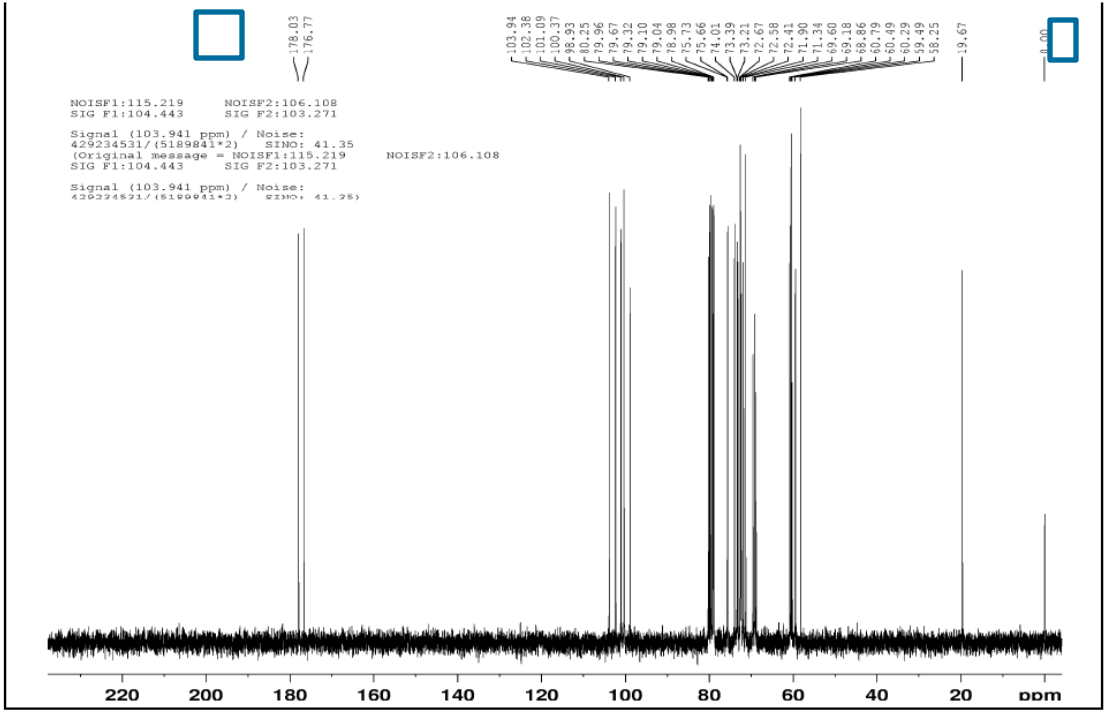
**Temperature:** 40°

**Suitability requirements Number of transients:** The signal-to-noise ratio of the β-D-glucopyranosyluronic acid ring of fondaparinux sodium in the *Standard solution* is at least 20:1 in the region near 103.9 ppm.

**Chemical shift:** The trimethylsilyl resonance for the *NMR reference* should be set to 0.0 ppm, which acts as an external calibration for all samples.

**Chemical shifts for system suitability:** The *O*-methyl and two carbonyl carbons of fondaparinux sodium should be observed at 58.2, 176.7, and 178.0 ppm, all ±0.3 ppm, respectively, in the *Standard solution*.

# ID A: $^{13}$C NMR USP RS Spectrum



# ASSAY

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4-mm × 25-cm; packing L46

**Column temperature:** 25°

Revision added, System suitability solution B: 5.0 mg/mL of USP Fondaparinux Sodium System Suitability Mixture B RS

# Assay system suitability

**Specificity and baseline drift** The chromatogram of the second *Blank* injection shows a baseline drift between 0.00 and 0.02 AU over 30 min. If necessary, adjust the DMSO content of the *Mobile phase* until an acceptable baseline is achieved.

The chromatogram of the second *Blank* injection does not contain peaks between 3.00 and 30.00 min.

**Resolution: Using System suitability solution B, NLT 1.2 between fondaparinux related compound C and fondaparinux related compound D and NLT 1.1 between fondaparinux related compound F and fondaparinux related compound G (see Table 3)2S (USP40)**

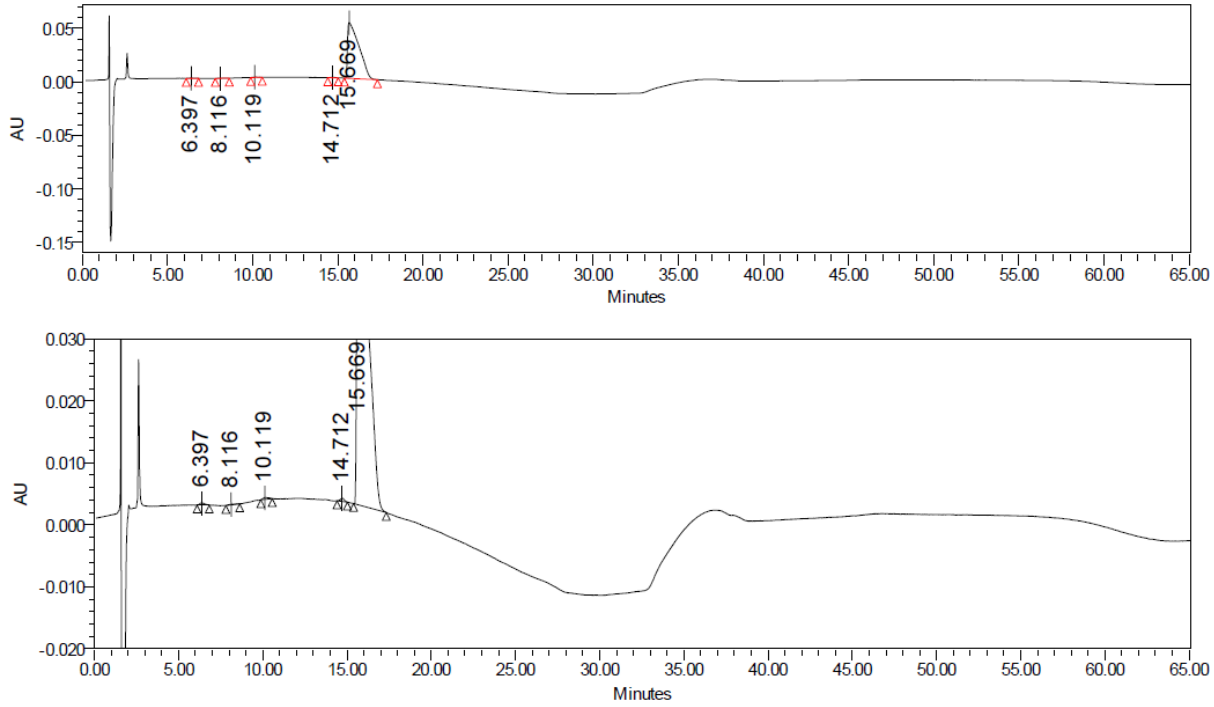
# Assay system suitability

**Relative standard deviation:** For six consecutive injections of the *Standard solution*, the calculated % RSD of the area of the fondaparinux peak is NMT 2.0%. The retention time of the fondaparinux peak should be ±5% of the mean value. The calculated % RSD of the response factors for all replicate injections of the *Standard solution* is NMT 2.0%. The calculated % RSD of the pooled response factors for all injections of the *Standard solution* is NMT 2.0%. The % RSD of the mean response factors for each duplicate *Standard solution* is NMT 2.0%.

**Signal-to-noise ratio:** NLT 10 for the fondaparinux peak in the chromatogram of the *Sensitivity check solution*

**Chromatogram similarity: The chromatograms of *System suitability solution A* and *System suitability solution B*2S (*USP40*) correspond to those of the chromatograms provided with USP Fondaparinux Sodium System Suitability Mixture A RS and USP Fondaparinux Sodium System Suitability Mixture B RS.2S (*USP40*)**

# Fondaparniux sodium for assays



# Organic Impurities

| Name | Relative Retention Time | Response Factor | Acceptance Criteria, NMT (%) |
|---|---|---|---|
| Fondaparinux related compound A[a] | 0.35 | 1.0 | 0.3 (a/a) |
| Fondaparinux related compound B[b] | 0.48 | 70 | 0.15 (w/w) |
| Fondaparinux related compound C[c] | 0.76 | 1.0 | 0.3 (w/w) |
| Fondaparinux related compound D[d] | 0.80 | 1.0 | 0.3 (a/a) |
| Fondaparinux related compound E[e] (impurity peak A) | 0.93 | — | 0.8 (a/a) |
| Impurity peak B[f] | 1.20 | — | 0.6 (a/a) |
| Fondaparinux related compound F[g] | 1.29 | 1.0 | 0.6 (a/a) |
| Fondaparinux related compound G[b] | 1.34 | 100 | 0.1 (w/w) |
| Fondaparinux sodium | — | 1.0 | — |
| Any unspecified impurity | — | — | 0.3 (a/a) |
| Total impurities | — | — | 2.0 |

Comment received in PF that the specifications do not align with all products in US Market

# Revisions to Organic impurities

[NOTE—Manufacturers should determine the suitability of their related substances method for their process related and degradation impurities. For any impurity peak above the limit for unspecified impurity peaks, identification and appropriate qualification is required.]

Individual impurities table added with specifications

Added, System suitability solution B: 5.0 mg/mL of USP Fondaparinux Sodium System Suitability Mixture B RS

# Way forward

What new standards are needed?

How can we Collaborate??

# Questions?

# Stay Connected

+91-88888 76654 | Social Media | skb@usp.org

The standard of trust