

Quality Standards for Medicines, Supplements, and Food Ingredients throughout the World

**Heparin Stage Two Monograph Revisions
Open Microphone Web Meeting
March 3, 2009**

**USP Heparin Monographs-
Stage 1 & 2 Revisions**

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Monograph Revision Stage 1; Official June 18, 2008

- Includes NMR and CE tests for identity
- ID tests reference 2 new USP reference standards (RS):
 - USP Heparin identity RS
 - USP Heparin system suitability RS
- Includes viral inactivation requirement in the definition of the monograph



Stage 2 – Monograph Modernization

- Include robust, sensitive, and quantitative methods to establish heparin quality attributes:
 - Identity
 - Potency
 - Purity
- Development of associated reference standards:
 - Heparin Identity
 - Heparin Potency
 - Oversulfated Chondroitin Sulfate (OSCS)
 - Dermatan Sulfate

Stage 2 Revision Timeline

- **June 19th & 20th:** EDQM-NIBSC-USP Heparin Workshop
- **August 7th & 8th:** USP heparin ad hoc Advisory Panel meets
 - Deliberated sub-team reports
 - Heparin workshop outcomes
 - Narrowed method choices for further consideration
- **October:** Heparin ad hoc Advisory Panel updates USP Blood & Blood Products Expert Committee
- **December 16th & 17th :** Heparin ad hoc Advisory Panel to finalize monograph
- **February 1st 2009:** Publication of USP Proposed Interim Revision Announcement (IRA)
- **May 1st 2009:** web meeting #2
- **May 15th 2009:** deadline for public comment
- **August 2009:** Revised monograph to become official, new reference standards available



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Summary of Stage 2 Revisions & Supporting Analytical Data

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Changes to Heparin Sodium Monograph

Procedure	Stage 1 Revisions	Stage 2 Revisions
Identification	¹ H Nuclear Magnetic Resonance (NMR) Capillary Electrophoresis (CE)	-Expanded ¹ H NMR procedure -Replaced CE method with anion-exchange HPLC procedure -Added ratio of anti-factor Xa activity to anti-factor IIa potency
Potency	No revision	-Replaced sheep plasma clotting assay with chromogenic anti-factor IIa assay
Organic Impurities	No revision	-Added limit of total galactosamine in total hexosamine (a measure of dermatan sulfate and other galactosamines) -Revised protein impurities -Added nucleotidic impurities -Added residual solvents
Absence of OSCS	See Identification	-References Identification A and B
New USP Reference Standards (RS)	-USP Heparin Sodium Identification RS -USP Heparin Sodium System Suitability RS	-USP Oversulfated Chondroitin Sulfate RS -USP Dermatan Sulfate RS -USP Galactosamine Hydrochloride RS -USP Glucosamine Hydrochloride RS

IDENTIFICATION A. ^1H NMR Spectrum

Rationale for retaining and expanding the ^1H NMR method

- The method is sensitive in terms of Limit of Detection (LOD) and extremely sensitive with regard to slight sample variations.
 - Expanded spectral window: at least 10 to -2 ppm
 - Increased S/N ratio requirement to at least 1000/1
- The complexity of spectra is a drawback in the context of purity testing
 - A simplified method of data analysis is proposed
 - The method is supported by USP RS
- Using the full spectrum heparin is easily distinguished from dermatan, chondroitin, and OSCS.
- Any spectral variation alerts users in the event of contamination of unknown origin

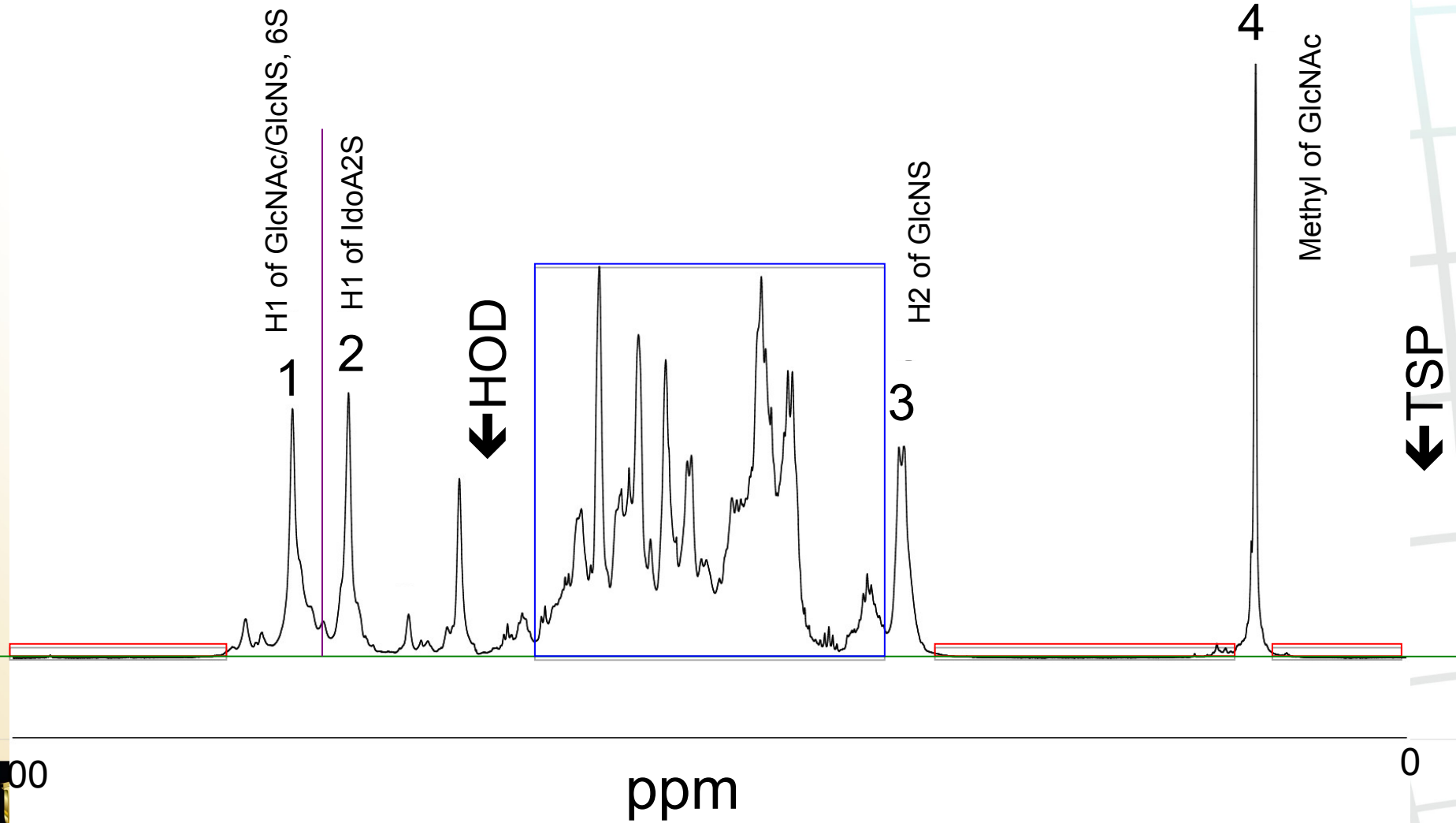


¹H NMR System Suitability and Acceptance Criteria

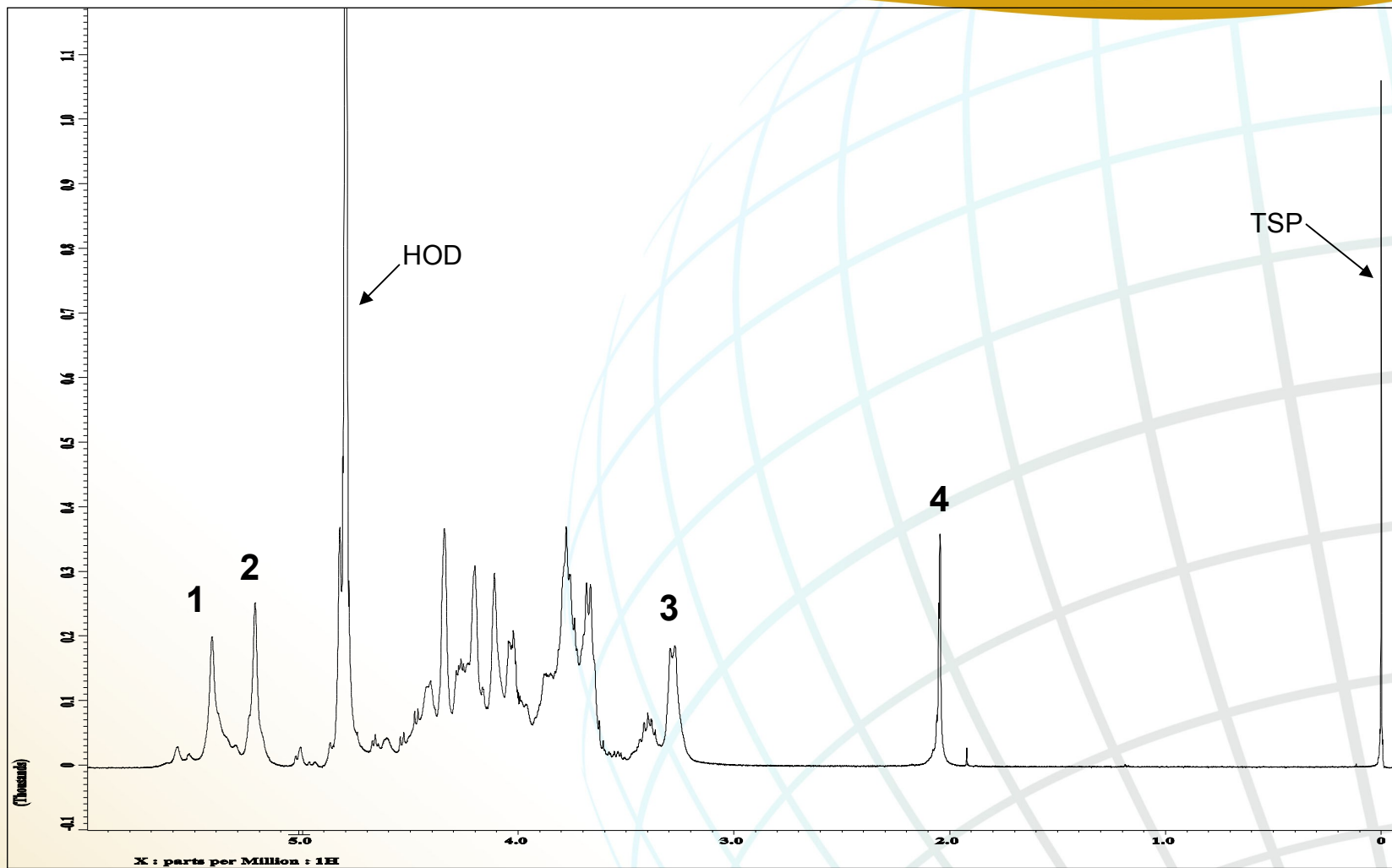
- System Suitability Criteria
 - The S/N of the *N*-acetyl heparin signal in the standard solution is at least 1000/1 in the region near 2 ppm.
 - System suitability solution: Heparin 2.04±0.02 ppm, OSCS 2.16±0.03 ppm
- Acceptance Criteria
 - No unidentified signals greater than 4% of the mean of signal 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.



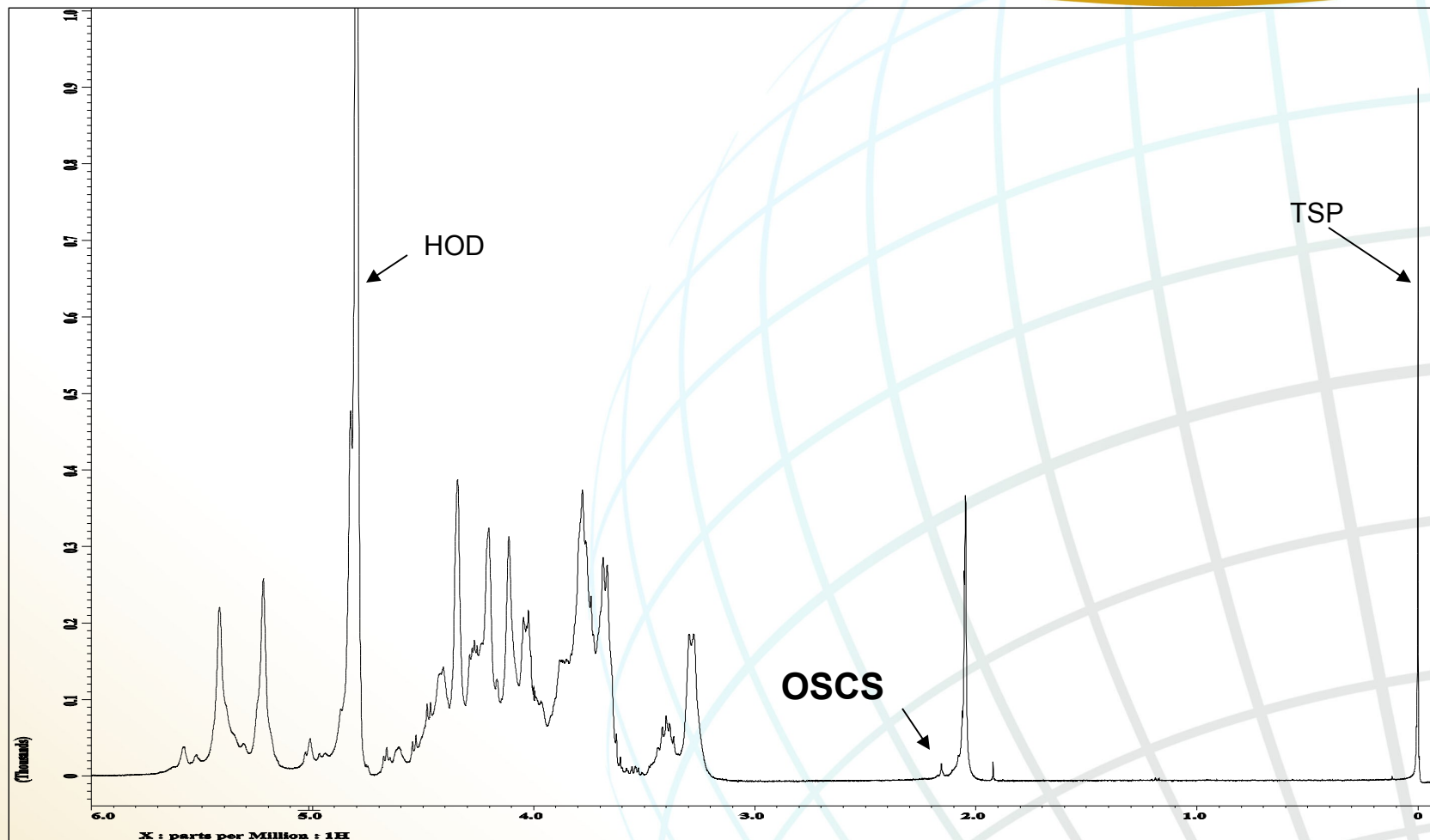
^1H NMR Specification for Identity and Purity of Heparin: Example



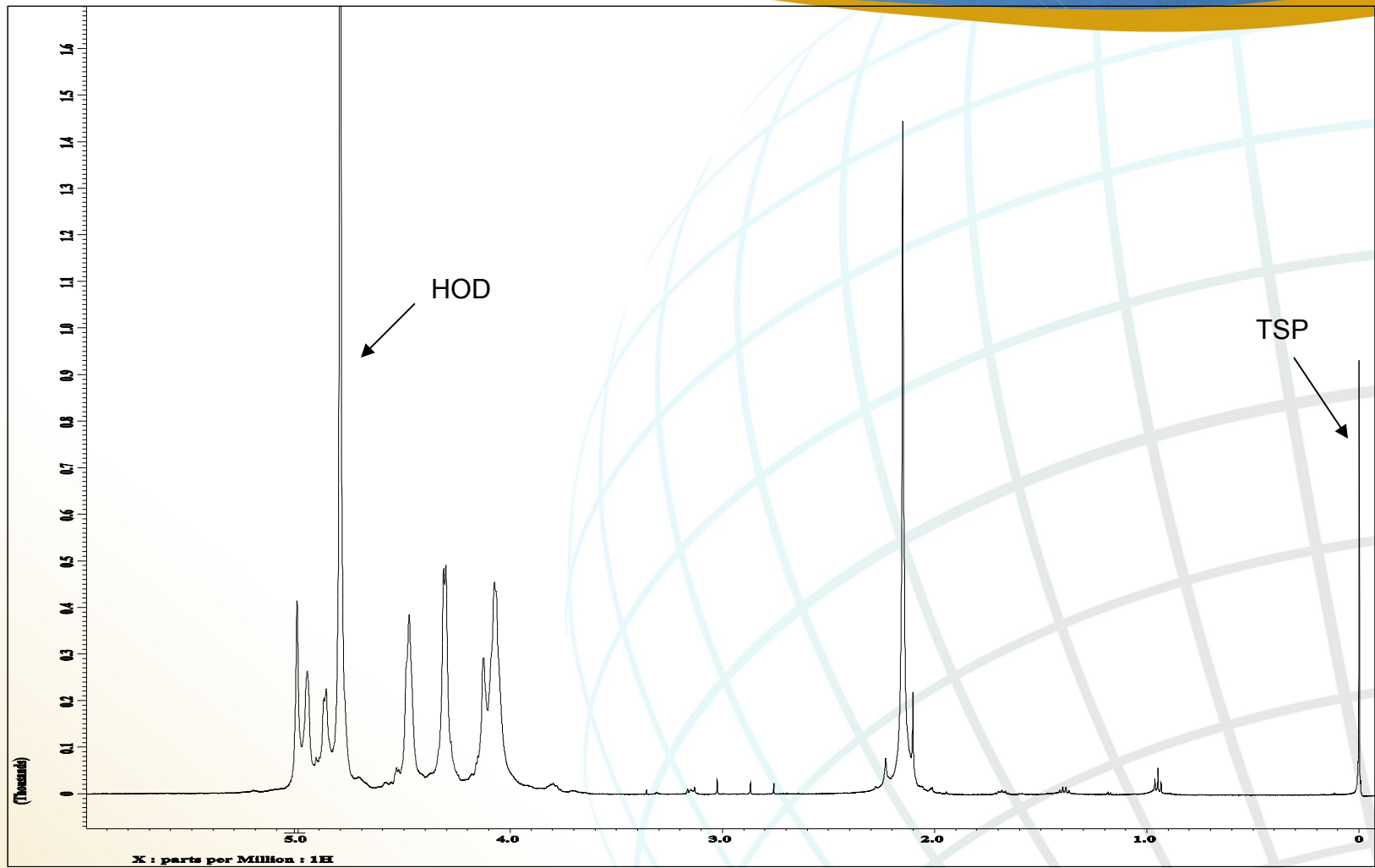
Heparin



1% OSCS in Heparin



Oversulfated Chondroitin Sulfate (OSCS)



Identity Tests: Further Considerations

Why look for CE alternatives?

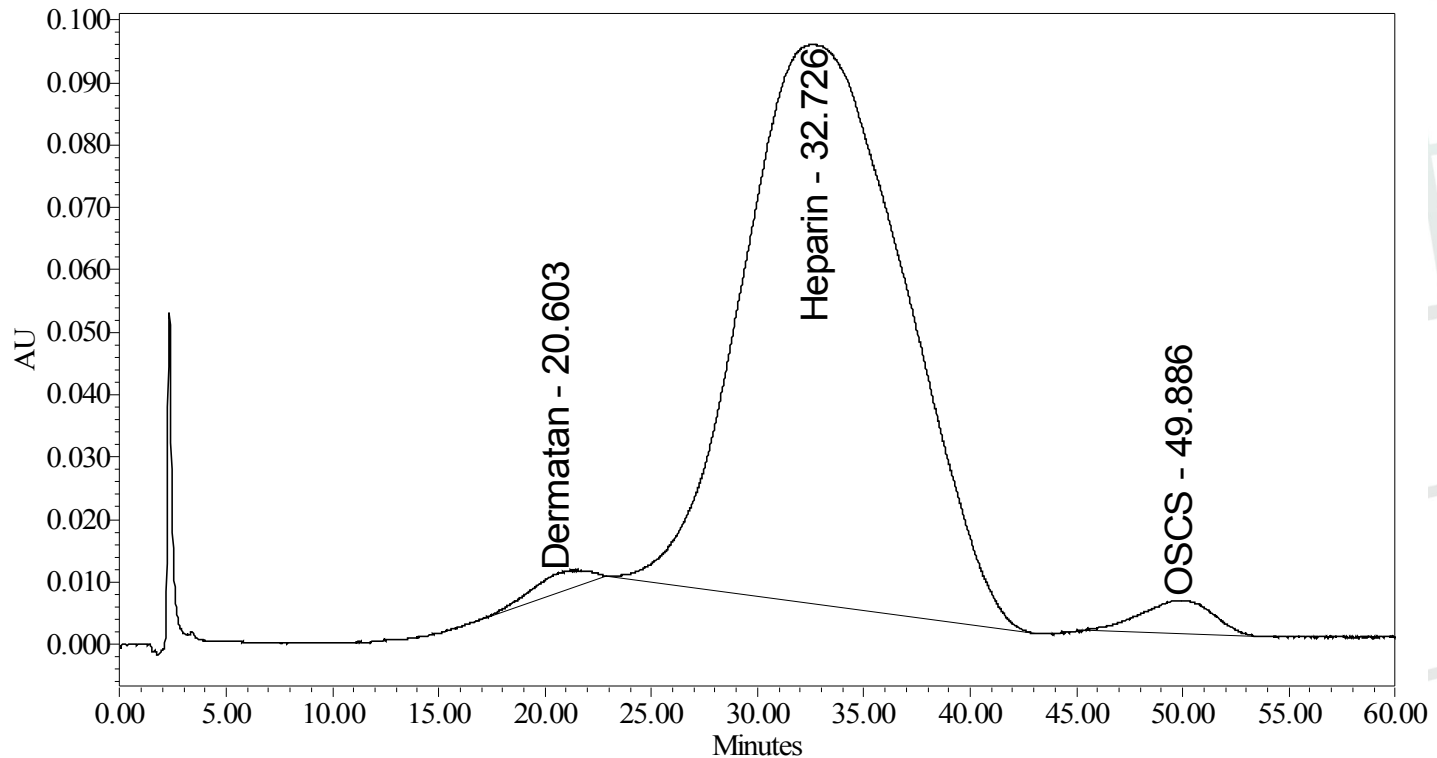
- Use of CE as an identity test presents multiple challenges:
 - Migration time drift and variability between systems and for heparin from different sources
 - Buffer incompatibility for use with heparin calcium
 - Resolution and sensitivity limitations for some impurities

IDENTIFICATION B: Strong Anion Exchange Chromatography Method

- This method is able to detect glycosaminoglycans (GAGs) contaminants
 - Among the liquid chromatography methods evaluated, this method provided the best separation between dermatan and heparin.
 - Heparin, dermatan, and OSCS are completely resolved in the proposed *System suitability solution*.
 - Dermatan sulfate and chondroitin sulfate co-eluted.
- The peaks of 4 heparin sodium samples exhibited similar retention times.
- This method is easy to implement in a QC laboratory.

System Suitability

System suitability solution: Heparin [20 mg/mL], dermatan and OSCS each at 1% (w/w)



Suitability Requirements

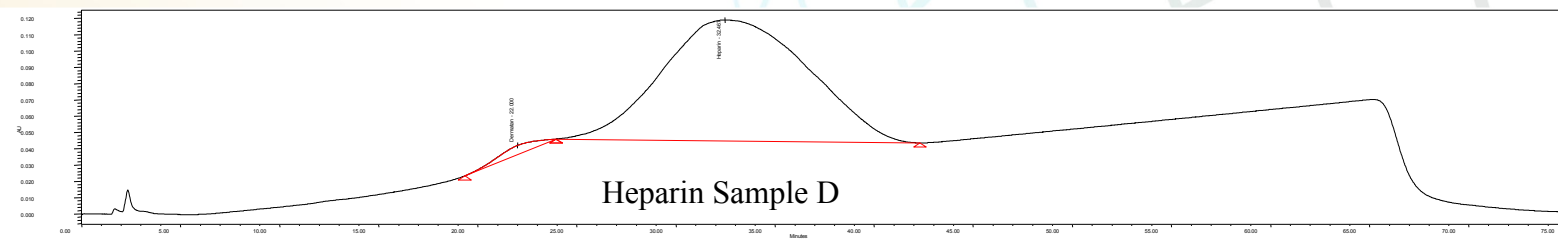
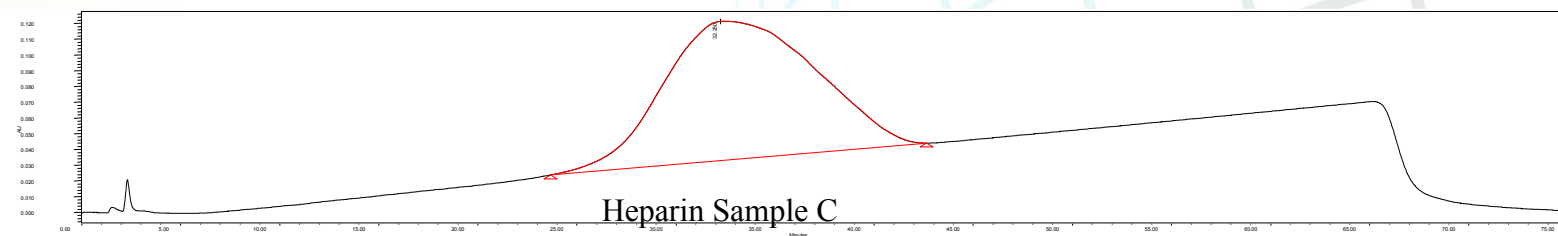
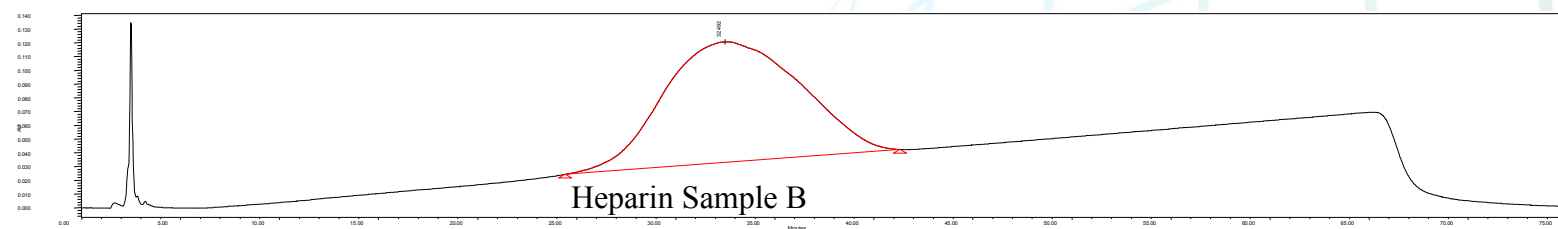
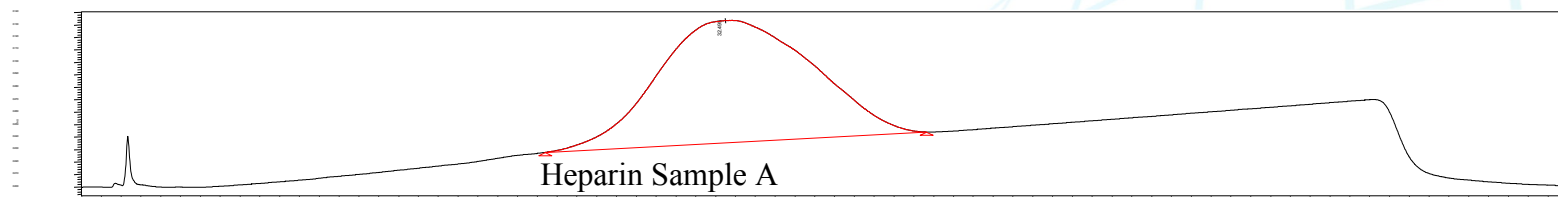
System Suitability solution: Heparin [20 mg/mL], Dermatan sulfate and Oversulfated Chondroitin Sulfate (OSCS) each at 1% (w/w)

	Dermatan	Heparin	OSCS
Retention time (min)	20.8	32.8	50.5
RSD, %	0.5	0.6	0.07
Area	786319	43712417	1286962
RSD, %	1.2	0.6	1.9
Resolution, R		1.2 between Dermatan and Heparin	1.8 between Heparin and OSCS

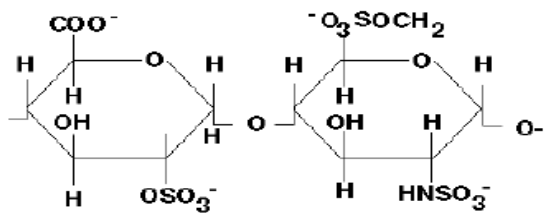
Average of three replicate injections



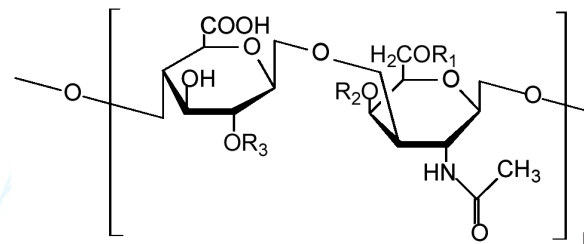
Heparin Sodium Lots



ORGANIC IMPURITIES: Limit of Galactosamine in Total Hexosamine



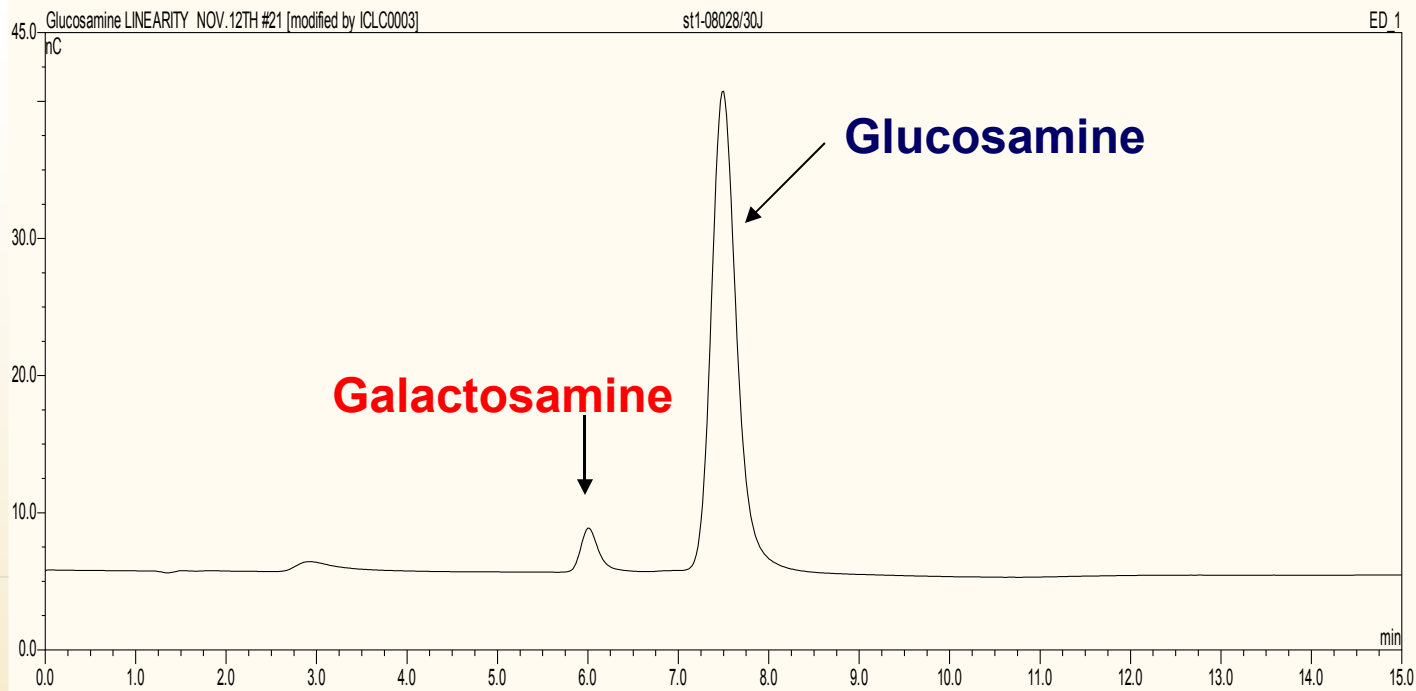
Repeat unit of Heparin



Repeat unit of Chondroitin Sulfate

Glucosamine and Uronic acid

Galactosamine and uronic acid



ORGANIC IMPURITIES: Limit of Galactosamine in Total Hexosamine

A measure of dermatan sulfate and galactosamine containing impurities

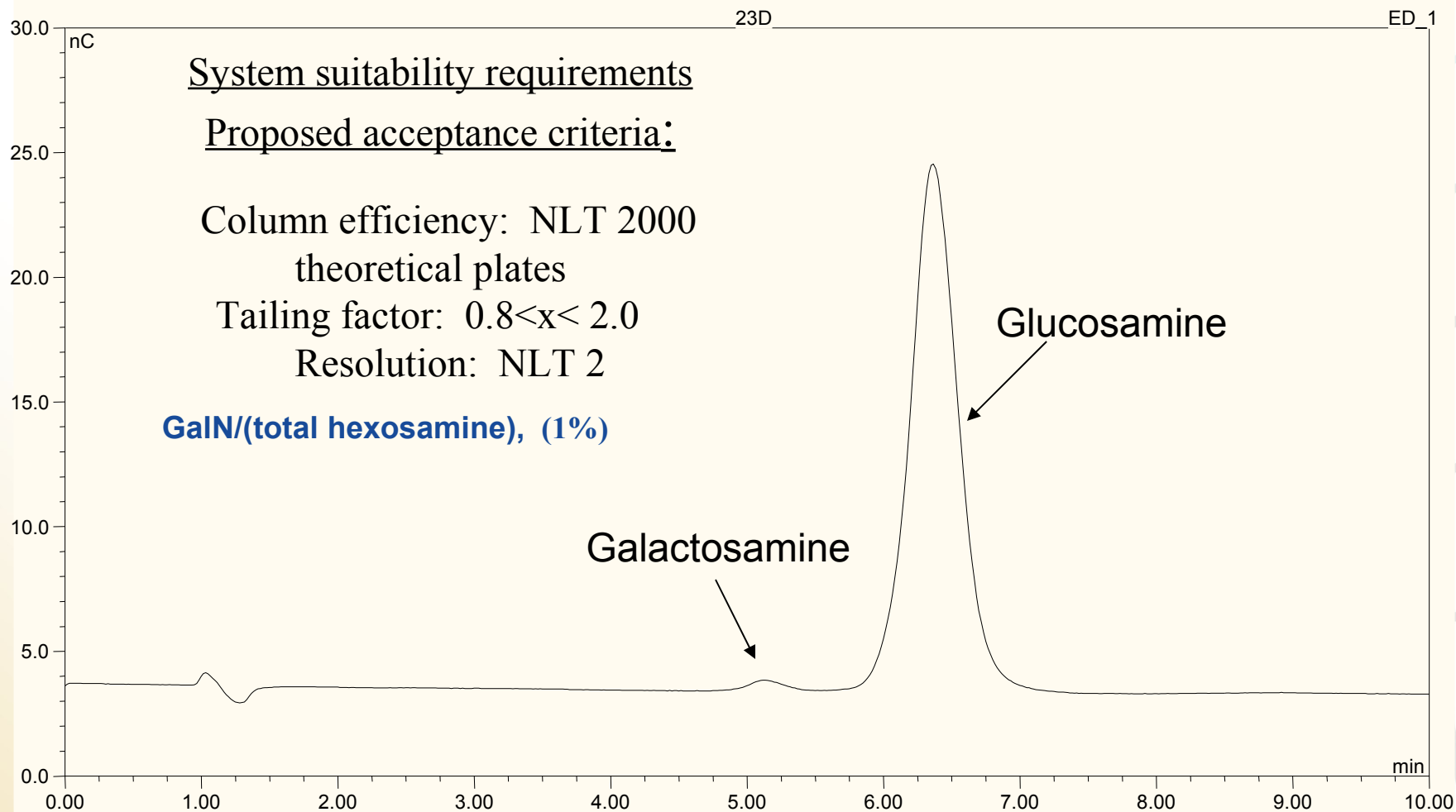
- Oversulfated chondroitins, Dermatan, and Chondroitin Sulfate A all have the same retention time, through digestion converted to galactosamine.

Strengths:

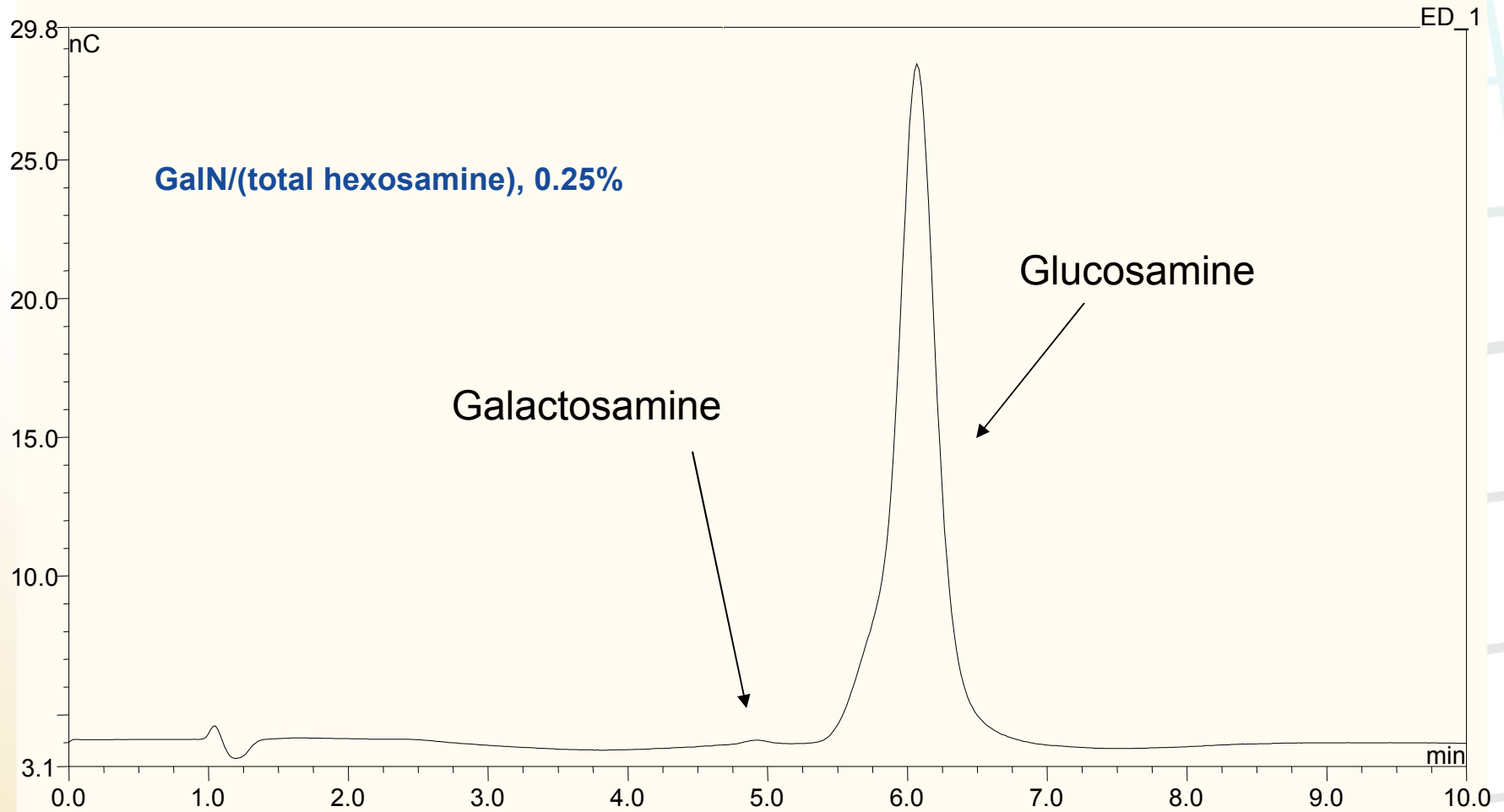
- The method is very sensitive. Able to detect 20 ng of Dermatan in 20 μ g of Heparin
- Heparin has a wide dynamic range.



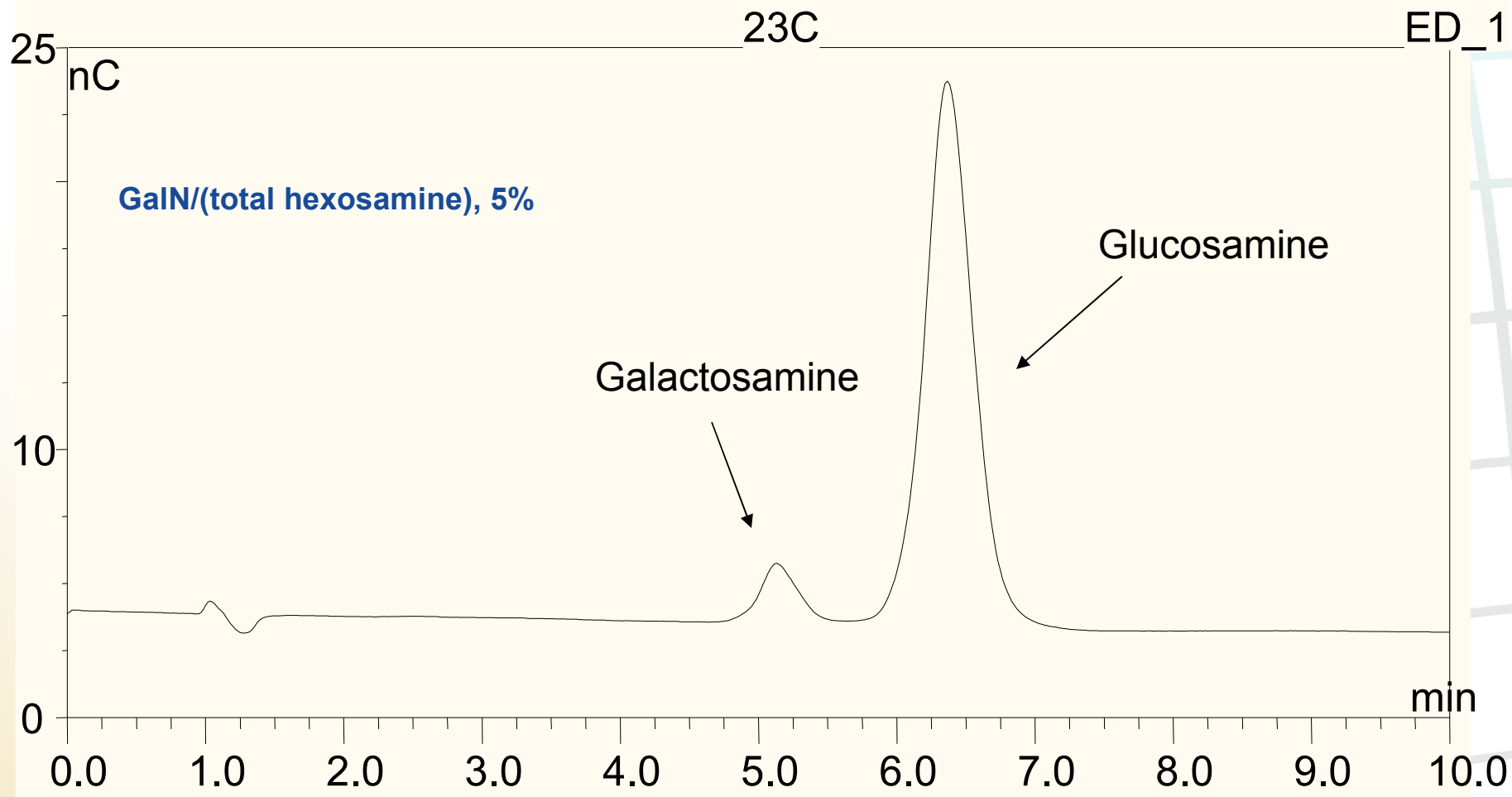
1% Standard Solution



A Passing Lot of Heparin



A Failing Lot of Heparin



Estimated LOD and LOQ

	% GaIN/(total hexosamines)
LOD	0.25
LOQ	0.6

Potency Assay

- Anti-factor IIa chromogenic assay replaces the sheep plasma clotting assay for potency assignment in stage 2 revision of Heparin Sodium monograph
- Minor revision to PF 33 (2) test incorporating recommendations from the Advisory Panel
- Validation study was conducted on revised PF 33 (2) test
- Incorporation of an anti-factor Xa/ anti-factor IIa ratio specification: NLT 0.9 and NMT 1.1
- USP harmonizes units with IU when USP Lot M potency standard is introduced in August 2009



History of Anti-factor IIa Assay Development for UFH

- **1994:** PF 20(6) published a paper by Erwin Coyne *et al.* entitled “Proposal for a New U.S. Pharmacopeial Heparin Assay”, wherein a chromogenic anti-factor IIa assay was proposed as a replacement for the USP sheep-plasma clotting assay.
- **1998-1999:** WHO Working Group on Biological Standardization of Unfractionated Heparin-harmonization and further development of the Anti-factor IIa assay(s).
- **2000-2003:** The Drafting Group developed a harmonized Anti-factor IIa assay and conducted a multi-laboratory validation
- **2004:** The Drafting Group proposed method and study results were published in PF 30(5) “Proposal and Qualification of a Harmonized Anti-factor IIa Assay for Unfractionated Heparin Potency Determination”
- **2006:** USP established an “Heparin Ad hoc Advisory Panel”
- **2007:** PF 33(2) published an “In-Process Revision” wherein the Anti-factor IIa assay was proposed as a replacement for the sheep-plasma Assay of Heparin Sodium (UFH)
- **2008:** USP validation of the previously proposed anti-factor IIa assay
- **2009:** PF35(2) proposes “Interim Revision Announcement” wherein the Anti-factor IIa assay replaces the sheep-plasma Assay in the revised Heparin Sodium monograph.
- **August 1, 2009:** Revised monograph to become official



Rationale for Changing to an Anti-factor IIa Chromogenic Assay

- Impurities such as dermatan can potentially influence the anticoagulant potency of Unfractionated Heparin (UFH) estimated by plasma based assays:
 - Compete for PF4 and other heparin binding proteins
 - Potentiate inactivation of thrombin by heparin co-factor II
- Plasma-based assays are dependent and vary with the quality of plasma used in the assay
- Chromogenic assays can be easily adapted for analysis by automated instruments, therefore less operator dependent
- Anti-Xa to anti-IIa ratios can be used to monitor the consistency and stability of product
- Anti-IIa activity more sensitive to degradation of UFH



Other Changes

- **Revised Protein Impurities**
 - Replaced previous trichloroacetic acid precipitation method with Lowry method
 - NMT 1.0% found
- **Added Nucleotidic Impurities**
 - A_{260} is NMT 0.20.
- **Added Residual Solvents Requirement**
 - It meets the USP <467>*Residual Solvents* requirements

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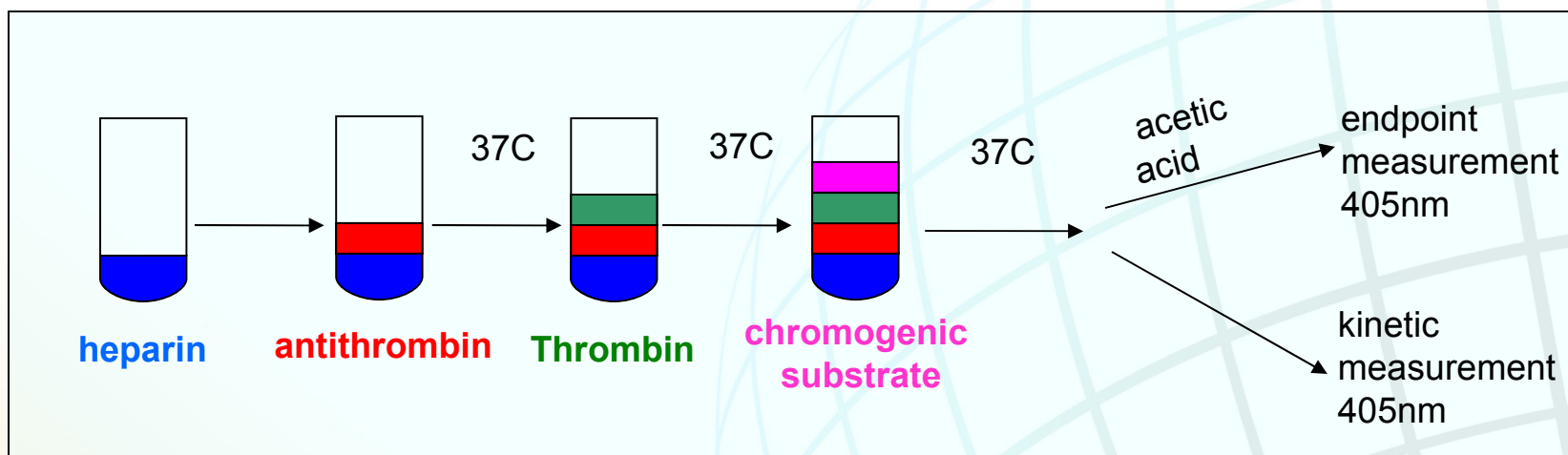
Anti-factor IIa Assay

Michael Ambrose, Ph.D.
Director, B&B Laboratory



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Schematic of Anti-factor IIa



Study Participants

- Three Laboratories participated in the study
 - Laboratory 1 Tube platform
 - Laboratory 2 Tube platform
 - Laboratory 3 Microtiter plate platform
Automated Analyzer
ACT-TOP 500

Linearity

- Linearity was measured at 3 dilutions of the USP Lot L Heparin Sodium (80%, 100 % and 120%).
- Linearity was considered met if the assays fit the model $a=b(\log X)+Y$
 - R^2 for combined tube assays was 0.97
 - R^2 for the MTP platform was 0.99
 - R^2 for the Automated platform was 0.99
- Results showed that the tube platform did show higher assay to assay variability than the MTP or Automated Platform



Accuracy

- Experiment measured the potency of USP Lot L at 3 potencies: 80%, 100% and 120% of the USP Lot L Heparin Sodium (assigned potency of 357 USP units).
- Acceptance criteria was $\pm 10\%$ of the individual potencies.
- Results showed each platform (tube, MTP, Auto) were able to accurately determine the potency of each sample.



Specificity

- Specificity was determined by spiking USP Lot L Heparin Sodium with either Dermatan Sulfate or Oversulfated Chondroitin Sulfate at two concentrations (2% and 10%).
- Heparin Sodium Lot L diluted to 98% and 90% with water was used as un-spiked controls.
- The three platforms were able to determine the expected potencies of spiked Heparin samples with minimal influence when compared to un-spiked controls

Robustness

- Robustness was measured on all three platforms
- Parameters measured included:
 - Incubation time after thrombin addition (± 30 secs)
 - Incubation time after substrate addition (± 30 secs)
 - Incubation temperature of assay ($\pm 1^\circ$)
- Assay designed as 2^k with K being the 3 factors above for a total of 11 variations.
- USP Heparin Lot L was used in the study at 80%, 100 % and 120% of assigned potency.
- ANOVA results showed no effect in determining the expected potency of the samples.



Conclusions

- Anti-factor IIa Chromogenic assay, as written, can be performed in the laboratory on 3 major platforms (tube, MTP, Automated Analyzer) – not platform dependent.
- Linearity – 3 independent dilutions were used for each run
 - Dose response fit $a=b(\log x)+y$
- Range - RS was diluted to 80%, 100% and 120% of the stated potency of the RS
 - Meet criteria for parallelism in the parallel line analysis
- Accuracy – the estimated potency for the 3 runs were calculated.
 - Meet criteria if within $\pm 10\%$ of assigned potency
- Specificity- Using OSCS/DS, showed no significant affect on the determination of estimated potency
- Robustness - ANOVA analysis using 11 variations showed no effects or their interactions are significant for the potency of sample of 80%, 100% and 120% potency

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Reference Standards to Support Stage 2 Revisions for the USP Heparin Sodium Monograph

Fabian A. Jameison, Ph.D.
Reference Standards Evaluation



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Reference Standards

- Six reference standards required to support new applications
 - Dermatan Sulfate – **new RS**
 - Oversulfated Chondroitin Sulfate – **new RS**
 - Heparin Sodium Identification, proposed Lot G (**new Lot**)
 - Heparin Sodium (Assay), proposed Lot M (**new Lot**)
 - USP Galactosamine Hydrochloride RS, Lot F0G137
 - USP Glucosamine Hydrochloride RS, Lot F0C363



Reference Standards–Packaging Configurations

- Heparin Sodium Identification
 - Package at 50 mg/vial
 - Suitable for proposed revised applications
- Glucosamine and Galactosamine Hydrochloride
 - Packaged at 200 and 300 mg/vial respectively
 - Previously evaluated for suitability with HPAEC-PAD method
- Dermatan Sulfate
 - Packaged at 15 mg/vial
 - Need only 20 mg/mL solution



Reference Standards–Packaging Configurations

- Over-sulfated Chondroitin Sulfate
 - Packaged at 25 mg/vial
- Heparin Sodium (Assay)
 - Pre-ampouled, lyophilized powder at 10 mg/ampoule
 - Different formulation from existing USP Heparin RS (liquid formulation)
 - Expected in house late April or early May 2009
 - Collaborative study to begin shortly after receipt of bulk

Timelines

- Suitability of Galactosamine and Glucosamine has been demonstrated in new use studies
 - Will not require new release of RS
- Collaborative study initiated to establish suitability in compendial applications
 - Dermatan sulfate: March 15, 2009
 - Oversulfated chondroitin sulfate: March 30, 2009
 - Heparin Sodium for Identification: March 30, 2009
 - *May have five of six standards available by late April 2009*

All Reference Standards should be available for August 2009 official release of revised monograph



Acknowledgements

- FDA
 - Dr. Ali Al-Hakim
 - Dr. Mohab Nasr
- Baxter
 - Dr. Ed Chess and Dr. Joseph Ray
- USP Heparin Ad hoc Advisory Panel
- USP Heparin Team





Thank You