



Heparin sodium analytics: 2008 and beyond

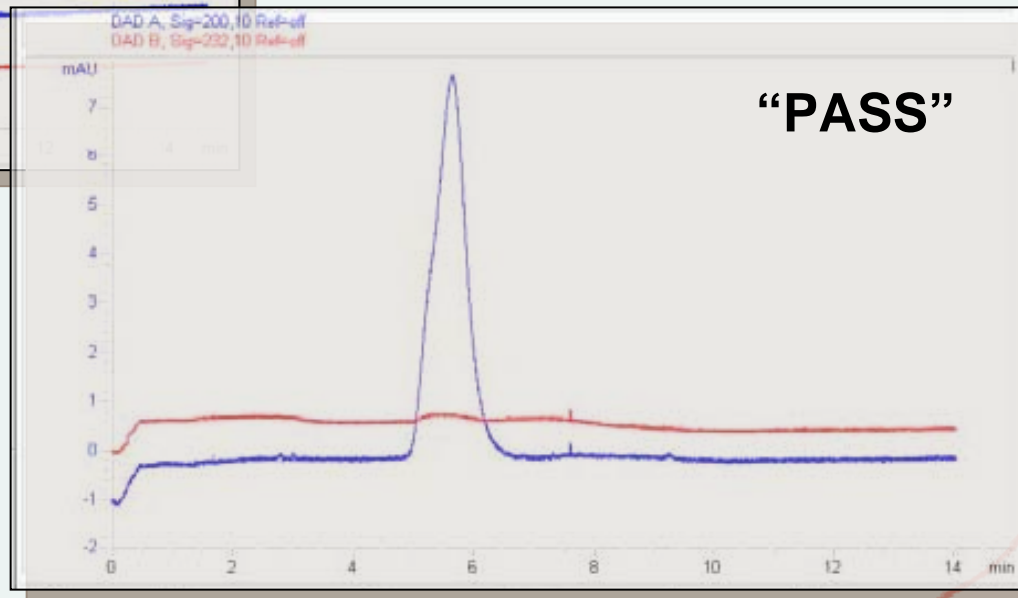
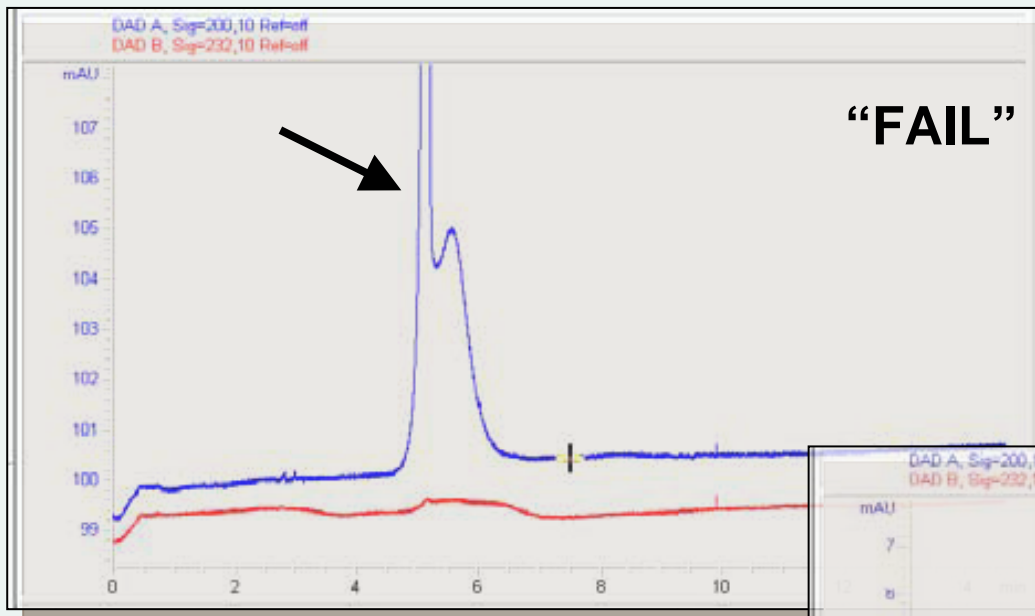
Edwin Kellenbach, Hester Hasper

Objective of this presentation

- Present the sequence of events
 - early 2008: contamination of heparin sodium with OSCS
 - *'the tests in the heparin sodium monograph were not able to detect a contamination with up to 20% OSCS'*
 - manufacturer: ensure safety of the heparin sodium drug substance
 - Immediate action: introduction of CE and NMR (FDA methods)
 - Corrective action: implement stage 1 revision of EP, USP and JP
 - Preventive action: proposed revisions of the USP (stage 2) and EP monographs
 - S-P contribution to monograph revisions
 - Membership of EDQM expert group 6
 - Membership of Heparin ad hoc advisory panel
 - Evaluation of the new methods
- Present the case that the new USP monograph, if implemented in its current form, could negatively impact on heparin supply

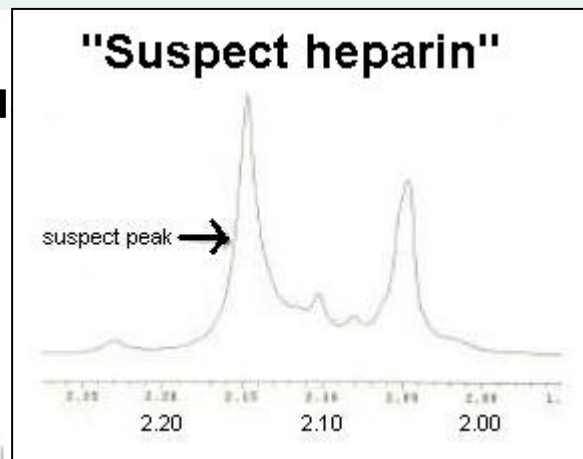
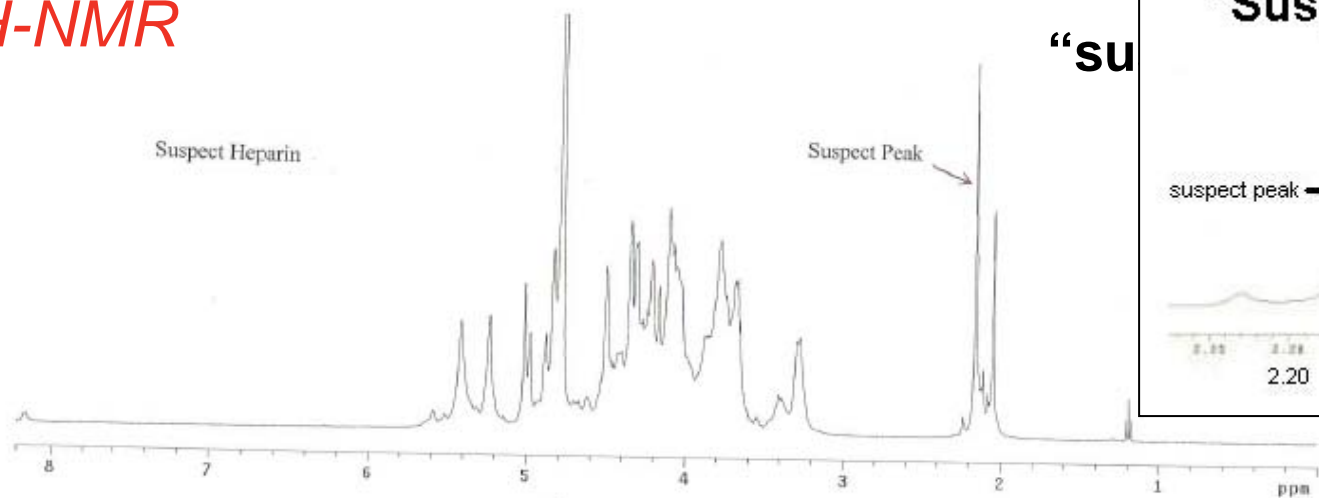
Immediate action: FDA methods

Capillary Electrophoresis



Immediate action: FDA methods

¹H-NMR



Corrective action

1st stage revision of the heparin sodium monograph

- In response to the heparin case, the pharmacopoeias added the CE and ¹H-NMR tests in the following way compared to FDA and each other:

	Capillary Electrophoresis	¹ H-NMR
FDA	Conc: 10 mg/mL, injection 10 s runtime: 14 minutes extra preconditioning step: none No ref. standard and SST	Conc: 25 mg/600 μL D ₂ O + TSP No ref. standard and SST
USP	Conc: 2 mg/mL, injection 30 s runtime: 15 minutes extra preconditioning step: 0.5 min H ₃ PO ₄ Standard: USP reference standard SST: contaminated heparin sodium production lot	Conc: 25 mg/600 μL D ₂ O (TSP not used!) NoF data points: 64 K pulse repetition period: 30 s window factor: 0.3 s SST: contaminated heparin sodium production lot
EP	“method according to competent authorities”	
JP	No CE method	Conc: 20 mg/600 μL D ₂ O+ TSP NoF data points: 32 K pulse repetition period: 20 s window factor: 0.2 s SST: 1% OSCS spiked heparin sodium

Upon the revision of the monographs in USP and JP, equivalence was demonstrated with FDA and/or the other pharmacopoeia

Preventive action: changes

2nd stage revision of the heparin sodium monograph

	USP current	USP draft (e-pub Feb '09)
Identity	Complies with clot assay	Complies with anti-thrombin assay & ratio
	CE, focus on OSCS	SAX HPLC method
	NMR, focus on OSCS	NMR, Total spectrum approach
	Sodium test	<i>Unchanged</i>
	Complies with CE + NMR	Complies with SAX HPLC and NMR
Potency assay	clotassay, ≥ 140 USPU/mg	Chromogenic anti factor IIa assay, NLT 180 USPU/mg
* Anti-Xa		
	$0.80 \leq \text{aXa} / \text{clotassay} \leq 1.20$	$0.9 \leq \text{aXa} / \text{anti-thrombin} \leq 1.1$
* Hexosamines		
	-	Galactosamine in total hexosamine, NMT 1%
* Protein		
	TCA precipitation	Lowry, NMT 1.0%
* DNA		
	-	A260, light scattering corr. <1057>
* Endotoxins		
	<85>; NMT 0.03 EU/USPU	<i>Unchanged</i>
* pH (1% in H2O)		
	5.0 – 7.5	<i>Unchanged</i>
* Heavy metals		
	NMT 30 ppm	<i>Unchanged</i>
* Residue on ignition		
	28 – 41%	<i>Unchanged</i>
* Loss on Drying		
	$\leq 5.0\%$	<i>Unchanged</i>
* Residual solvents		
	<467>; complies	<i>Unchanged</i>
* Sterility		
	complies	<i>Unchanged</i>

Preventive action: evaluation

2nd stage revision of the heparin sodium monograph

	USP draft (e-pub Feb '09)	Evaluation
Identity	Complies with anti-thrombin assay & ratio	Not compatible with existing USP/EP heparin
	SAX HPLC method	Minor problem with SST
	NMR, Total spectrum approach	Potential for other low level component peaks in normal heparin
	Sodium test - <i>Unchanged</i>	-
	Complies with SAX HPLC and NMR	Minor problem with SST
Potency assay	Chromogenic anti factor IIa assay, NLT 180 USPU/mg	Not compatible with existing USP/EP heparin
Compatibility with existing USP/EP heparin		
* Anti-Xa	$0.9 \leq \text{aXa} / \text{anti-thrombin} \leq 1.1$	Not compatible with existing USP/EP heparin
* Hexosamines	Galactosamine in total hexosamine, NMT 1% by HPEAC-PAD	OK
* Protein	Lowry, NMT 1.0%	OK
* DNA	A260, light scattering corr. <1057>	OK
* Endotoxins	<i>Unchanged</i>	-
* pH (1% in H ₂ O)	<i>Unchanged</i>	-
* Heavy metals	<i>Unchanged</i>	-
* Residue on ignition	<i>Unchanged</i>	-
* Loss on Drying	<i>Unchanged</i>	-
* Residual solvents	<i>Unchanged</i>	-
* Sterility	<i>Unchanged</i>	-

Method Evaluations

1. Potency Assay
2. ^1H NMR
3. SAX HPLC
4. A260
5. HPAEC-PAD

Lowry not evaluated; developed and validated in our lab

1-Evaluation of the new potency assay

2nd stage revision of the heparin sodium monograph

Preliminary Results

Heparin Production - Typical Lots	Existing USP Clotting Assay	Proposed Anti-thrombin Assay
	USP Ref Std; Lot L0G091 Acceptance criterion: >140 USPU/mg	USP Ref Std: Lot L0G091 Acceptance criterion: >180 USPU/mg
1	182	155
2	176	157
3	176	125
4	181	156
5	185	150
6	171	153
7	177	160
8	176	167
9	180	137
10	173	132
	(all comply with existing specification)	(all <u>non-compliant</u> with proposed specification using existing Ref Std)

1-Evaluation of the new potency assay

Results:

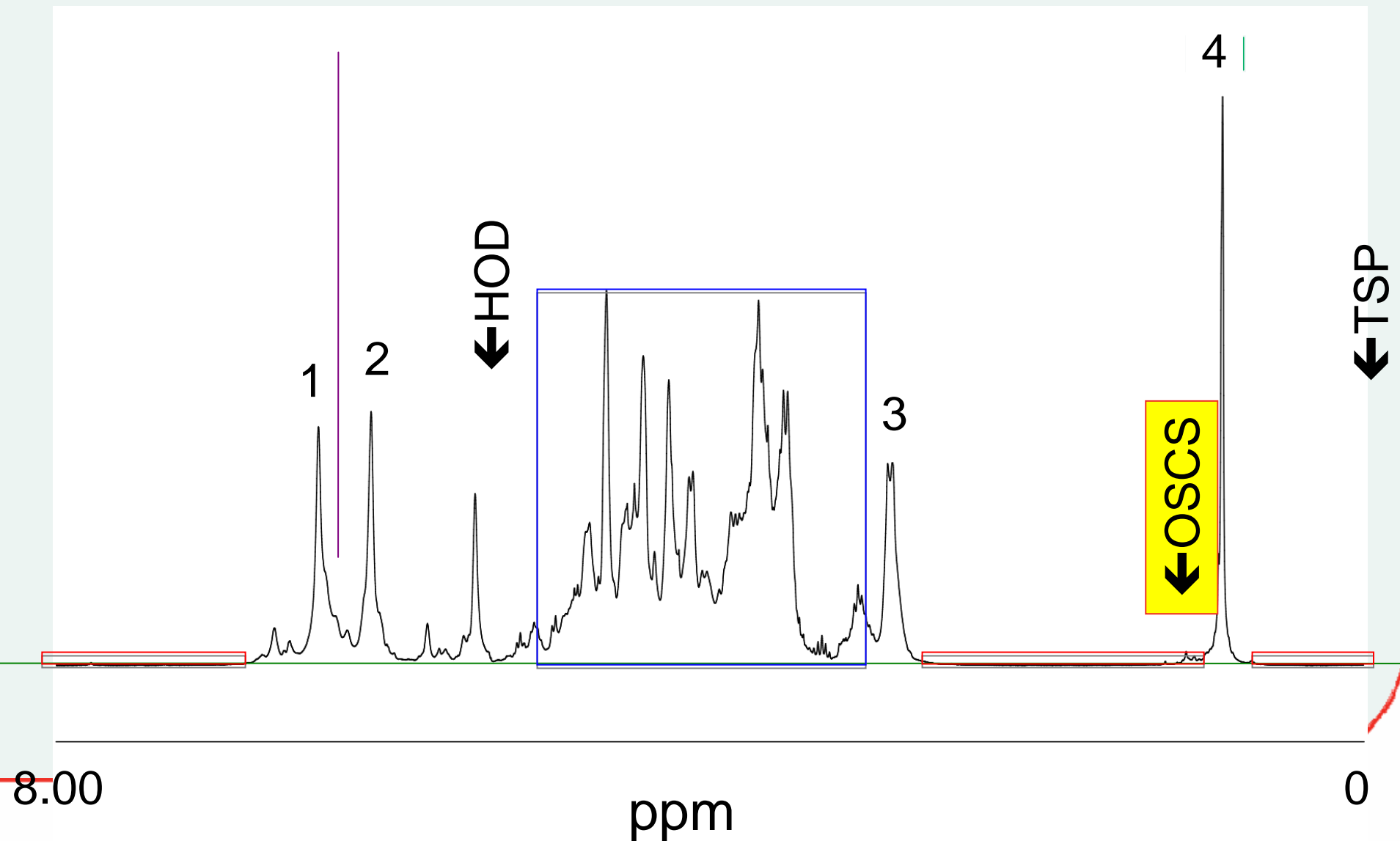
- Method can be run as described
- Anti-thrombin assay results (using current official USP Reference Lot LOG091) are consistently lower than existing USP clotting assay
- Limit of 180 USPU/mg with new assay is significantly higher than current assay limit of 140 USPU/mg
- Consequently, results on typical material using proposed assay do not meet the proposed specification

Additional Points:

- The rationale for the acceptance criterion of 180 USPU/mg specification needs to be clarified
- The monograph cannot be fully evaluated without new official standard for potency
- Changing reference standard, method and specification at one time increases uncertainty and risk
- Similar difficulties expected with narrowed limits for the potency / anti-Xa ratio (0.9 – 1.1 proposed vs. 0.80 – 1.20 current)

2 – Evaluation of the ¹H NMR method

2nd stage revision of the heparin sodium monograph



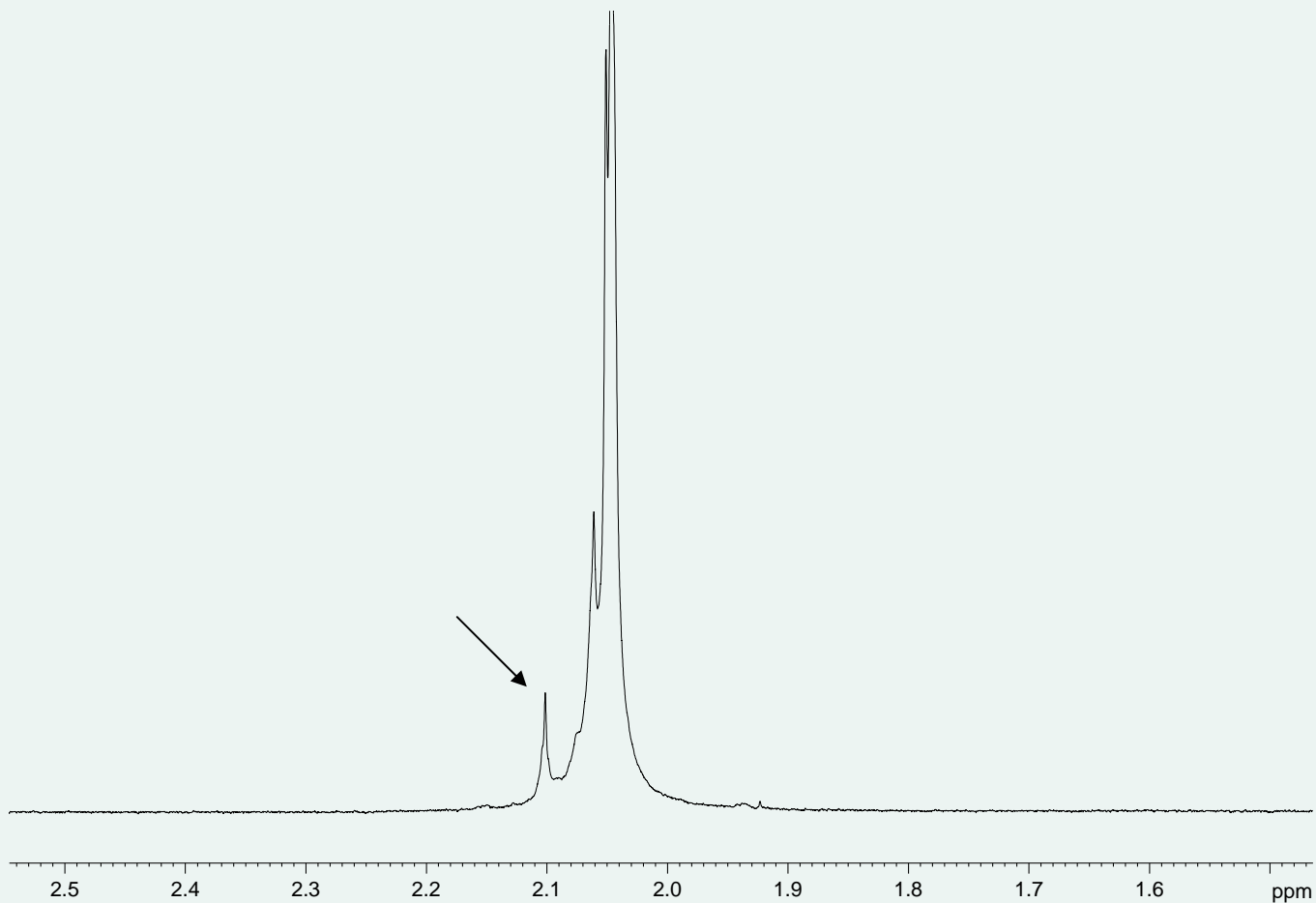
2-Evaluation of the ^1H NMR method

2nd stage revision of the heparin sodium monograph

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.

2-Evaluation of the ^1H -NMR method

2nd stage revision of the heparin sodium monograph



2 – Evaluation of the ^1H NMR method

2nd stage revision of the heparin sodium monograph

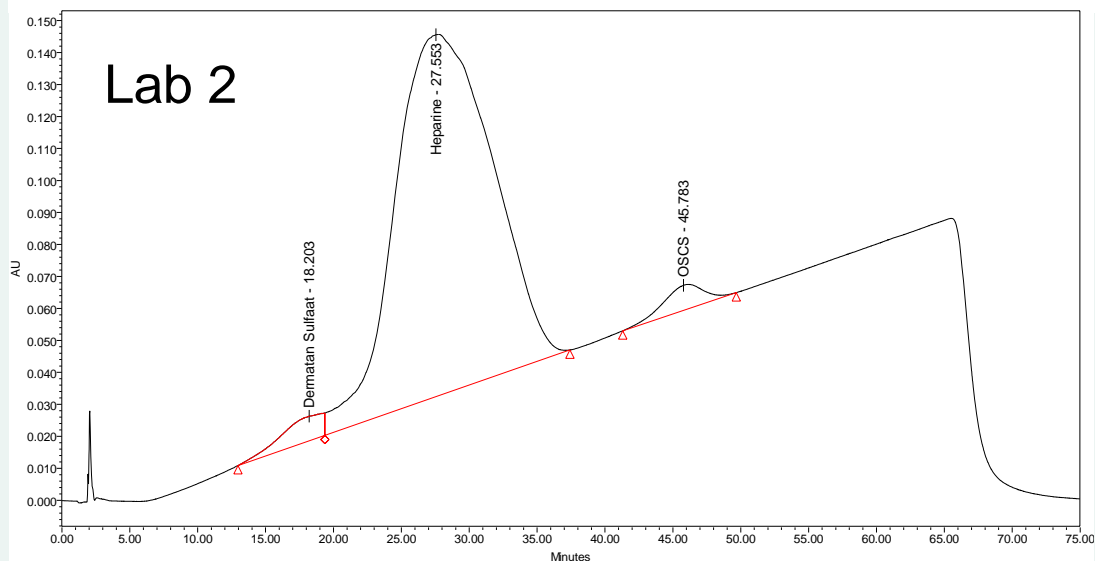
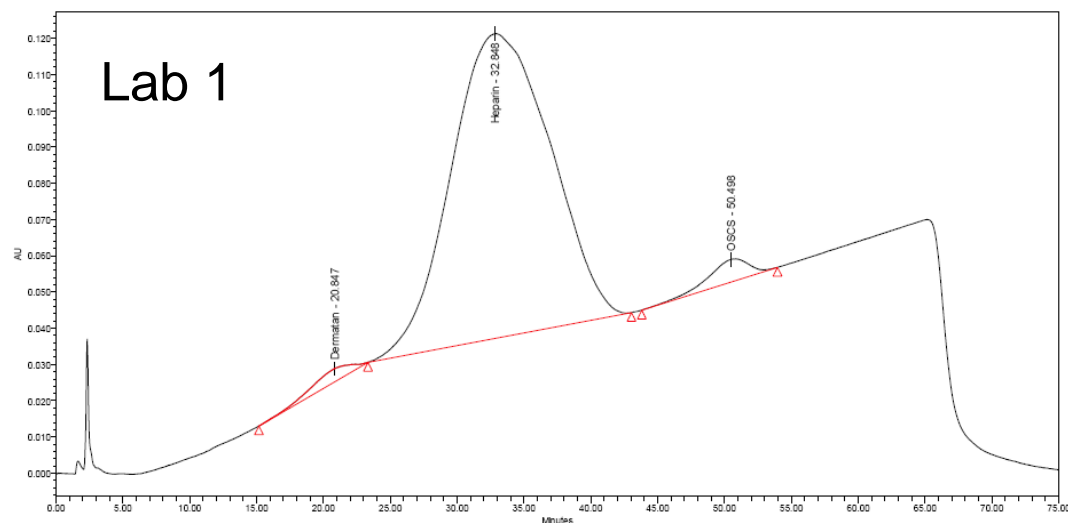
- Results & discussion
 - Method can be run as described
 - Composition of the SST composition: harmonization with JP is highly desirable (\uparrow OSCS)
 - Proposal for S/N to 1000/1: this is unnecessarily high
 - Residual solvents: method does not deal with methanol
 - Detection of OSCS with 600 MHz spectrometer: overlap with ^{13}C satellites, not addressed in monograph

Note:

- Currently acceptable material may contain heparin-related components giving signals in the ‘forbidden’ areas. These components have always been present in these heparins and have never been associated with adverse clinical events

3 – Evaluation of the SAX HPLC

2nd stage revision of the heparin sodium monograph



- Results & discussion
 - Method can be run as described (2 systems tested at 2 labs)
 - Compatible with heparin sodium production lots (criteria are met)
 - Composition of the SST solution: presence of DS poses problems to meet criteria ($R_{\text{DS-heparin}}$) and moreover, it does not have any added value

4 – Evaluation of the A260 method

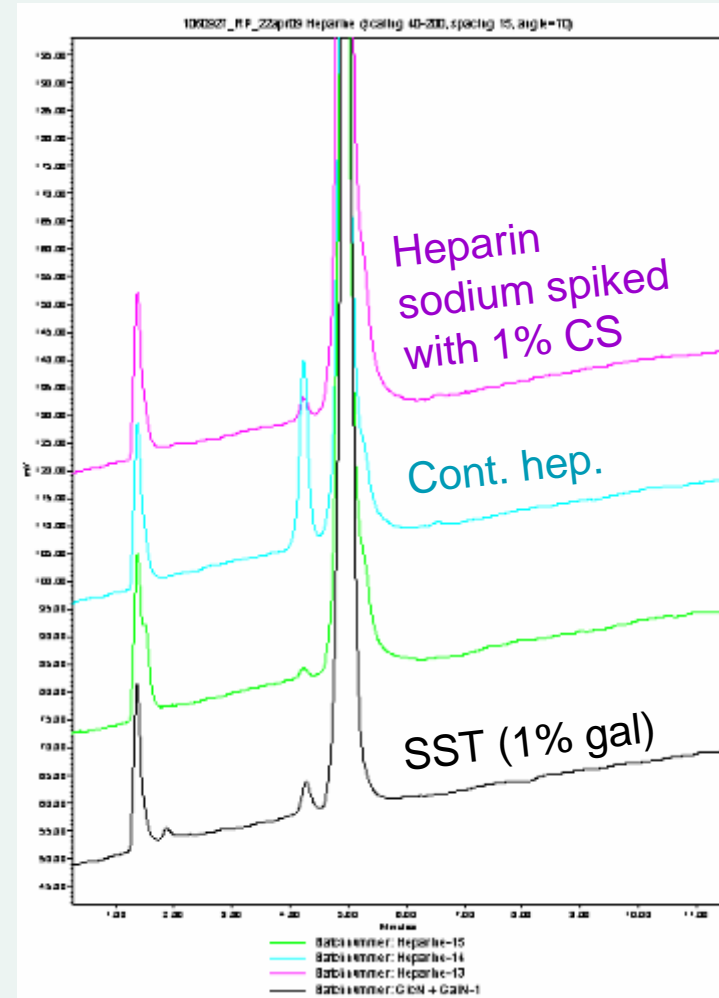
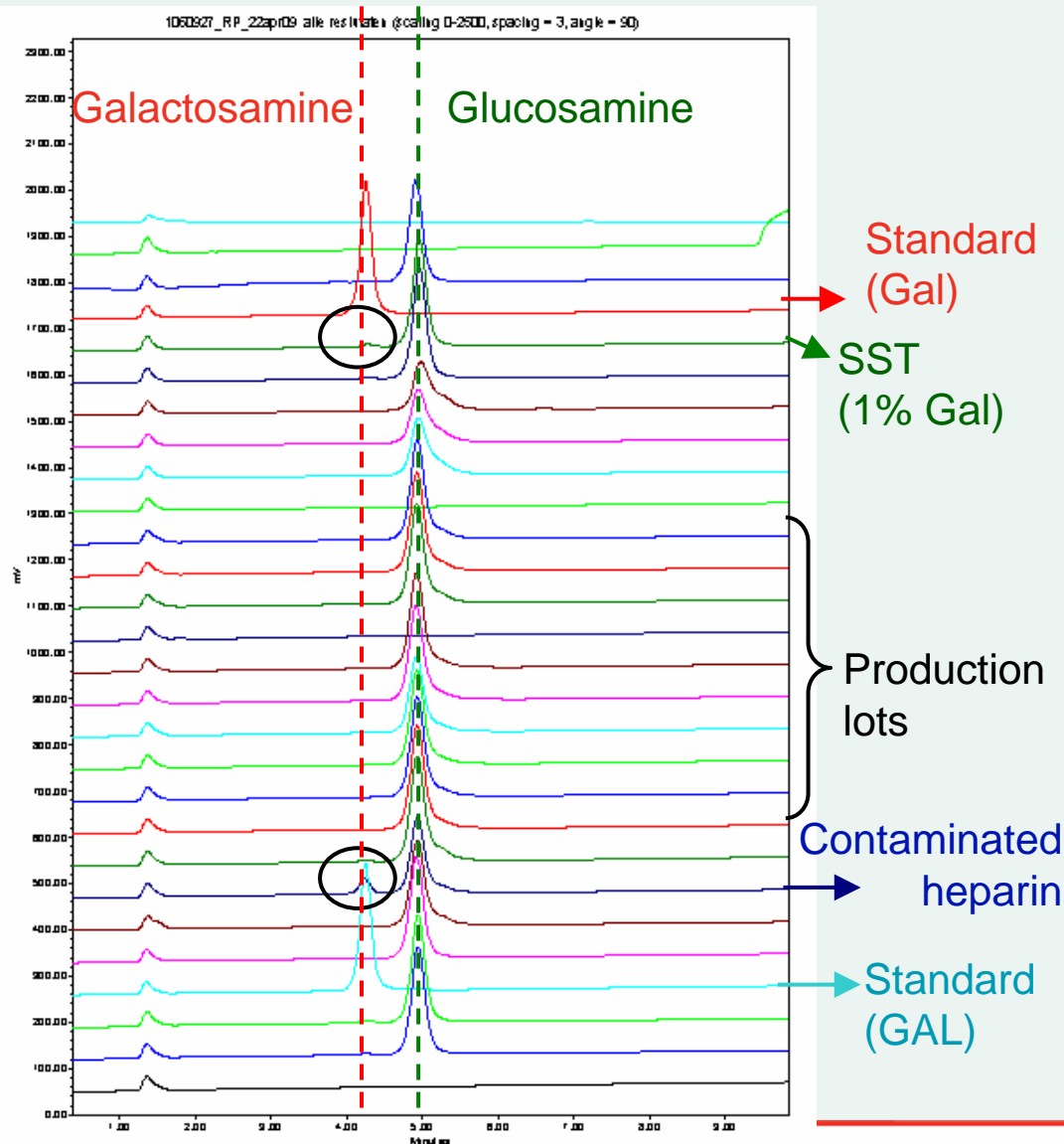
2nd stage revision of the heparin sodium monograph

- Results & discussion
 - Method can be run as described
 - Specifications are met for existing heparin sodium USP or EP
 - Results with correction for light scattering: negative absorbance results

Lot	Observed absorbance at 260 nm	Absorbance at 260 nm due to light scattering	Corrected absorbance at 260 nm
1	0.0498	0.1960	-0.1462
2	0.0513	0.2324	-0.1811
3	0.0437	0.1536	-0.1099
4	0.0468	0.3524	-0.3056
5	0.0565	0.0520	0.0045

5 – Evaluation of the HPAEC-PAD method

2nd stage revision of the heparin sodium monograph



5 – Evaluation of the HPAEC-PAD method

2nd stage revision of the heparin sodium monograph

- Results & discussion
 - Method can be run as described
 - Specifications are met for existing heparin sodium USP or EP grade

Next steps

2nd stage revision of the heparin sodium monograph

- New heparin sodium USP reference standard becomes available August 1st
- Repeat evaluation of typical marketed lots of heparin using the new Anti-thrombin Assay with the new official potency standard
 - Provide feedback to USP
- Industry and USP to engage on rationale for specifications:
 - Potency assay
 - Potency / Anti-Xa ratio
 - ¹H NMR
- Implementation of the new heparin sodium monograph: October 1st

Conclusions

- The majority of the proposed changes to the monograph improve the quality and integrity of the heparin supply chain.
- Proposed tests do not provide equivalent results with existing tests:
 - The proposed NMR and potency specifications are not compatible with existing marketed heparin sodium
 - The reference standard for potency has not been established yet so the monograph cannot be fully evaluated
- The new monograph, if implemented in its current form, may lead to reduced availability of heparin sodium
 - The concerns being brought forward by the industry should be resolved before monograph implementation
- SP is very willing to cooperate with the USP in doing this