



国立医薬品食品衛生研究所

National Institute of Health Sciences

3rd Workshop on the Characterization of Heparin Products

Japanese Pharmacopeia

Revision of heparin sodium and
heparin calcium monographs

National Institute of Health Sciences (MHLW/Japan)

Div. Biol. Chem. & Biologicals

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Outline of the presentation

- 1. Current situation**
- 2. JPXV Supplement 2 in October 2009**
- 3. Proposed monograph for JP partial revision in January 2010**
- 4. Future plans**

1. Current situation

July 30, 2008

JP XV was partly revised

- The OSCS purity test by¹H-NMR was adopted.
in heparin sodium monograph

November 6, 2008

- JP expert committee on biologicals decided to revise
 - Identification tests
 - Purity test for protein
 - nucleotides
 - OSCS
 - galactosamine.

1. Current situation

March 9, 2009

- Draft working group (NIHS and manufacturers) was organized to conduct a draft revision including identification tests as well as OSCS and galactosamine purity tests. Then we started a validation study.

July 10

- The working group proposed a draft revision.

July 17

- JP Expert Committee on Biological released the proposed monograph.

http://www.pmda.go.jp/public/public_kyokuho.html

1. Current situation

August 25, 2009

JP Committee

October 1, 2009

JP XV supplement 2 is enforced.

- A new heparin calcium is listed.
- Definition of heparin sodium is revised.

January, 2010

Partial revision of JPXV

- Identification test, purity test in heparin sodium and heparin calcium monographs will be revised.

2. Biologicals in JPXV supplement 2

Newly listed monograph

Heparin calcium

Hyaluronic acid

Calcitonin (salmon)

Revised monograph

Heparin sodium

Vasopressin injection

Protamine sulfate injection

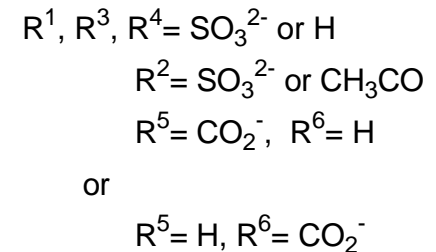
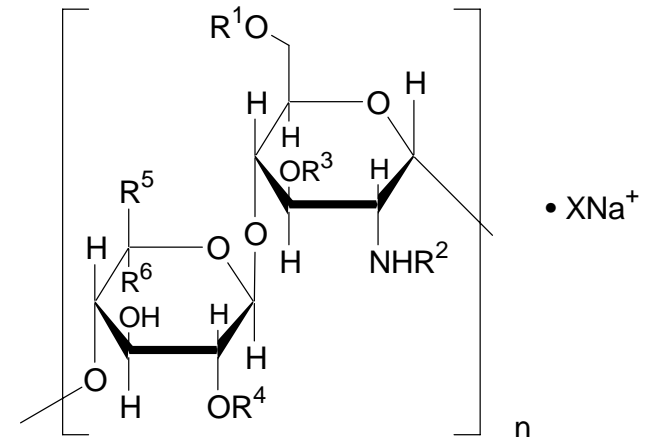
Human menopausal gonadotrophin

2. JPXV supplement 2

Heparin Sodium

Definition

Heparin sodium is a sodium salt of sulfated glycosaminoglycan consisting of D-glucosamine and uronic acid (D-GluA and L-IdoA). It is obtained from the livers, the lungs and the intestinal mucosa of healthy edible animals. Heparin sodium prolongs the clotting time of blood.



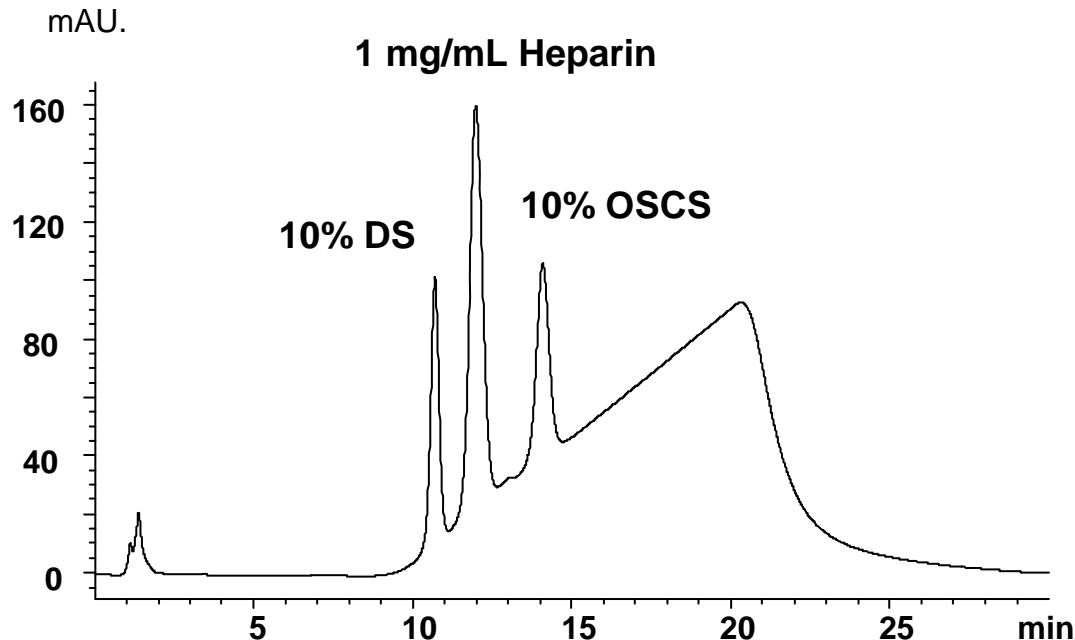
3. Proposed monographs

	Heparin Sodium	Heparin Calcium
Identification	<i>(WAX-HPLC)</i>	<i>(WAX-HPLC)</i>
	<i>(¹H-NMR)</i>	Toluidine blue reaction
		Calcium
Purity Clarity/color	Visual	UV
Cl		Chloride limit test
Metal		Heavy metals limit test
Ba	Turbidity	Turbidity
Residual solvent		Each dossier
Nitrogen	Semimicro-Kjeldahl	Semimicro-Kjeldahl
Protein	TCA	TCA
Nucleotides		
OSCS	¹ H-NMR	¹ H-NMR
GalNH ₂	<i>(ABEE/FL-HPLC)</i>	
Assay	Anti-Xa	Anti-Xa

Proposed identification test by WAX-HPLC

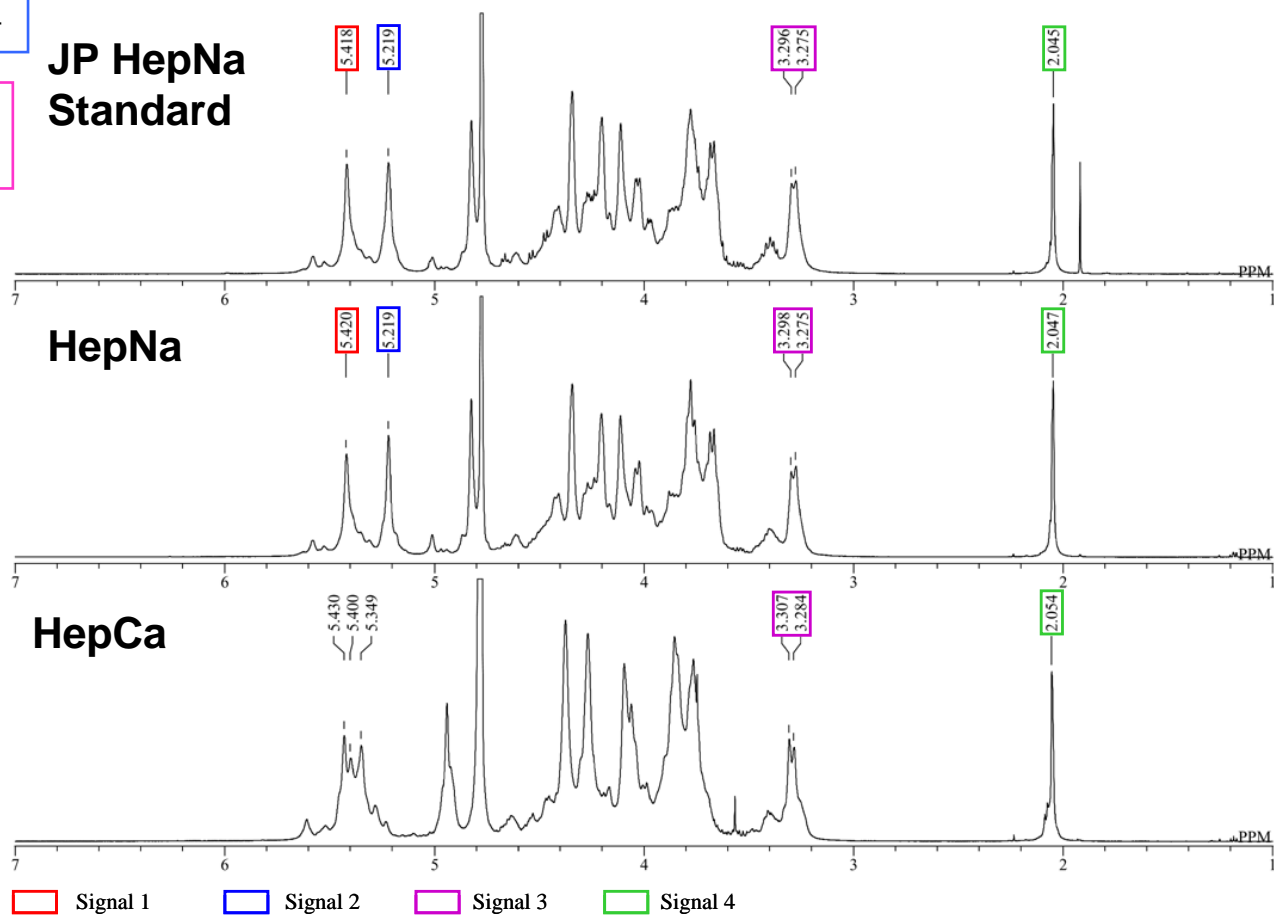
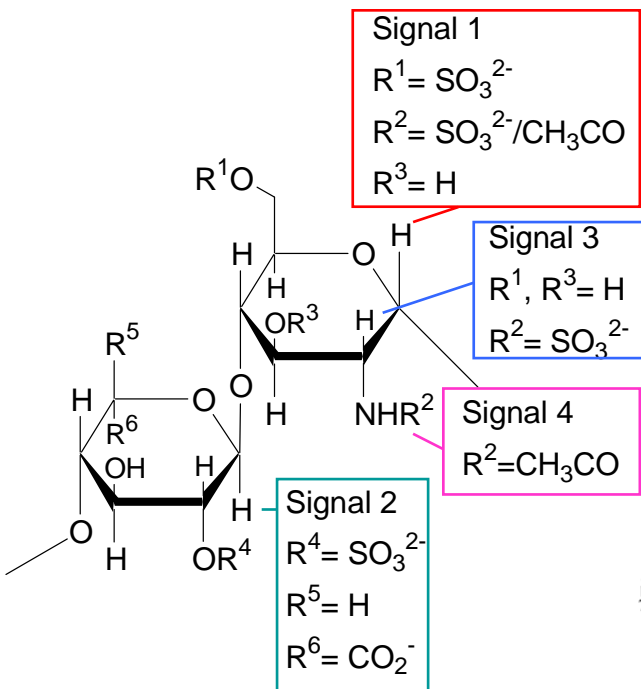
Molecular size, degree of sulfation

- Good separation of DS, heparin and OSCS in 20 minutes
- WAX columns are cheap and available from several manufacturers, including Japanese companies



Proposed identification test by $^1\text{H-NMR}$

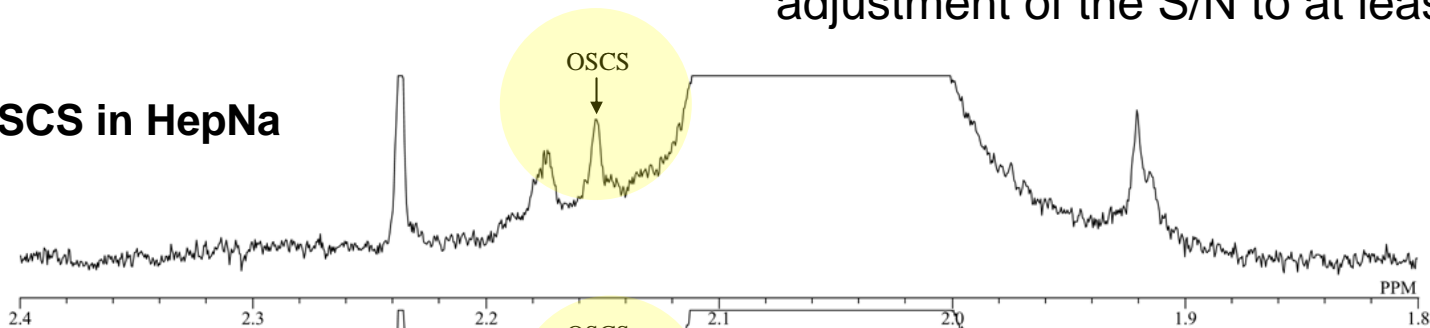
Monosaccharides, linkage, sulfation position



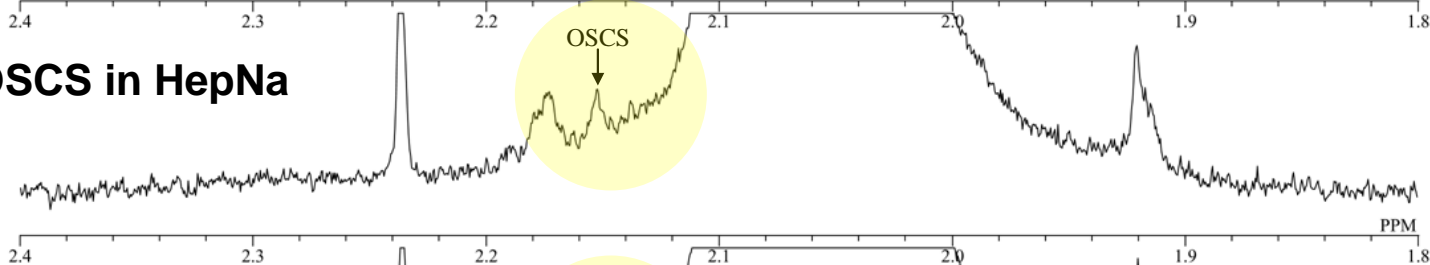
Proposed OSCS purity test by $^1\text{H-NMR}$

adjustment of the S/N to at least 1000

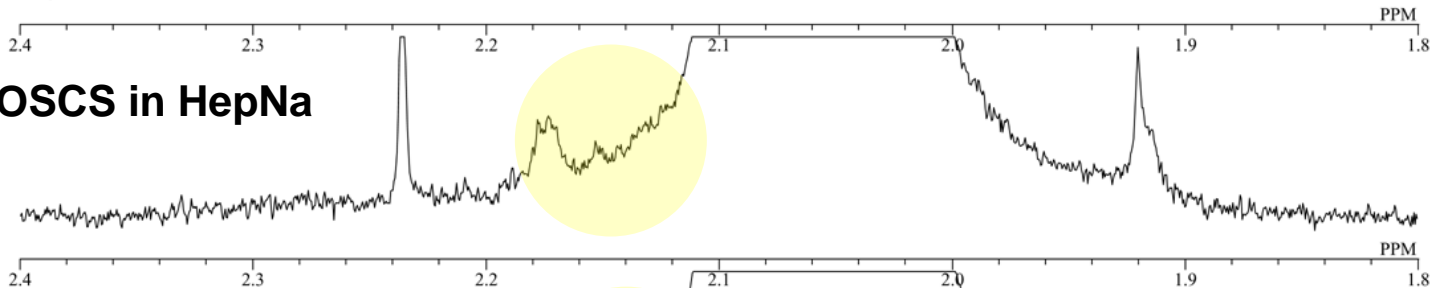
0.1% OSCS in HepNa



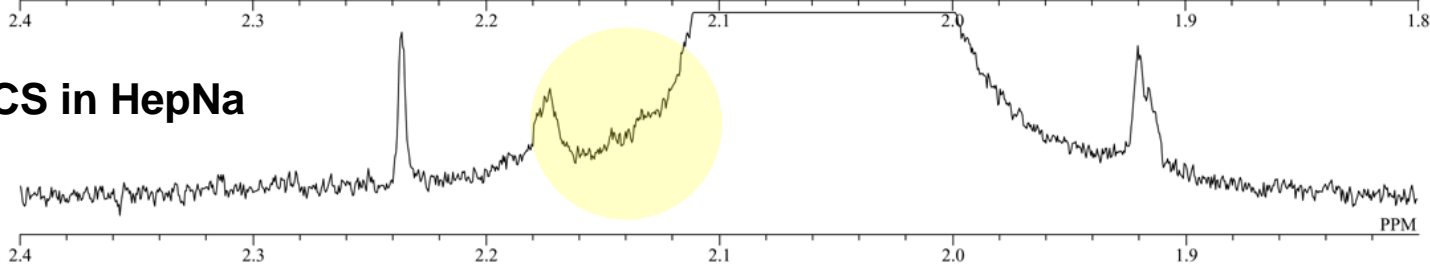
0.05% OSCS in HepNa



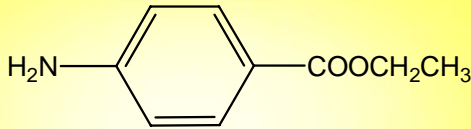
0.025% OSCS in HepNa



0% OSCS in HepNa

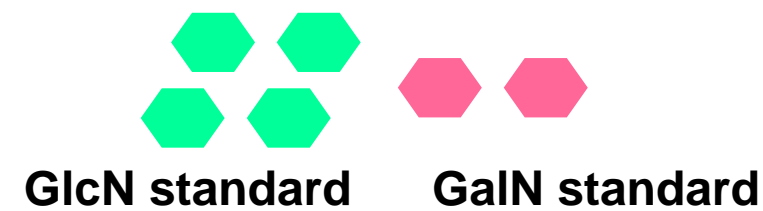


Proposed galactosamine purity test by ABEE-derivatization followed by FL/C18-LC



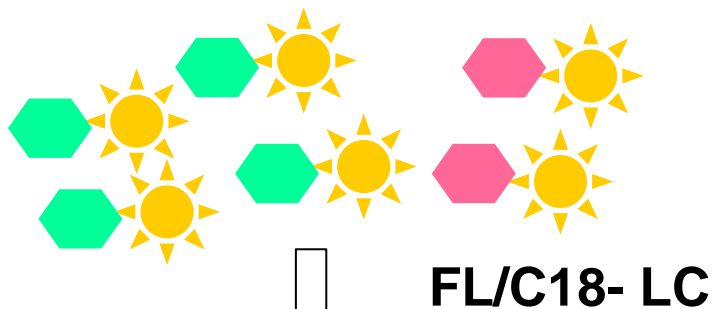
ABEE
Ethyl aminobenzoate

- All GAGs containing GalN can be detected.
- Additional standards for DS, CS, etc. are unnecessary.
- Easy to analyze with conventional instruments.

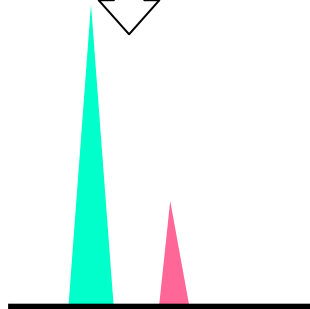


Hydrolysis

Derivatization



Compare the peak ratio
of GalN/GlcN



≡

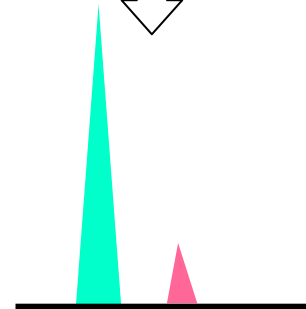
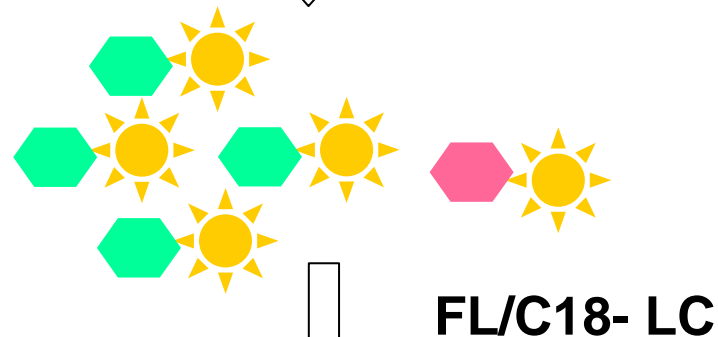
Heparin **DS, CS, OCSC**

Hydrolysis

GlcN

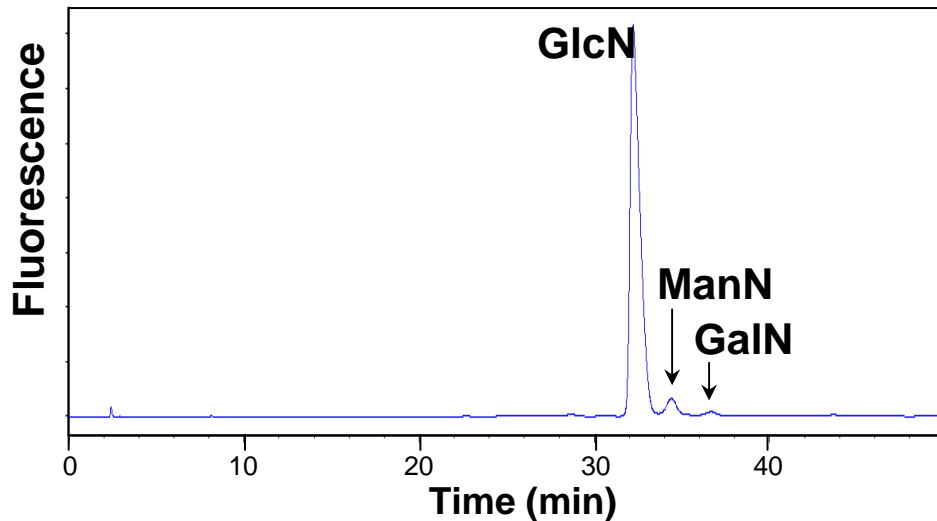
GalN

Derivatization

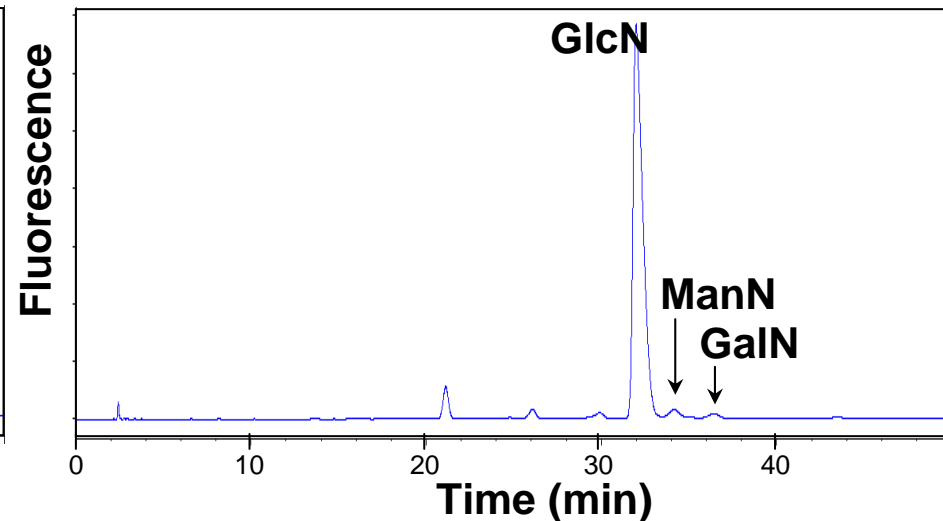


Galactosamine purity test by FL/HPLC

(A) GlcN : GalN (99:1) tentative



(B) Heparin sodium : DS (99:1)



LOD: 0.03 % DS

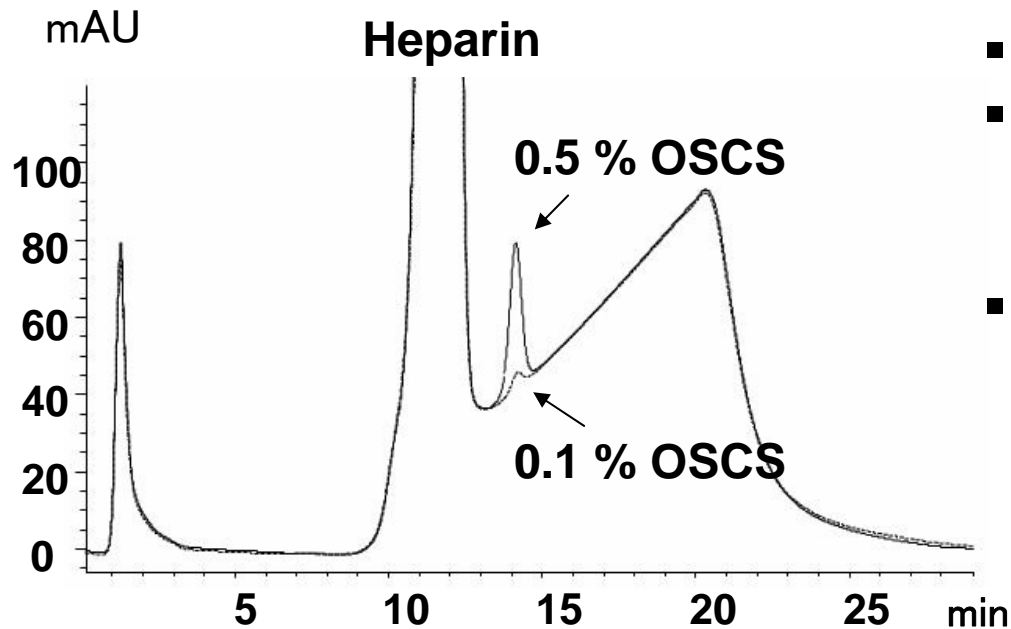
RSD (1 % DS; n=6) : 3.12 %

Linearity (0.1 – 2 %):

$$y=1.0253x + 0.0942; R^2=0.9961$$

Test not adopted in the draft revision

OSCS purity test by WAX-HPLC



LOD: 0.025-0.075 % OSCS

- OSCS (0.1%) can be detected.
- Retention time of OSCS is changeable, depending on the concentration of OSCS.
- Retention time of OSCS in the contaminated heparin is different from the JP OCSC standard which was prepared from bovine CS.

4. Future plan

	Heparin Sodium	Heparin Calcium
Identification	WAX-HPLC	WAX-HPLC
	NMR	¹ H-NMR
	Sodium	Calcium
Purity Clarity/color	Visual test	UV
Cl		Chloride limit test
Metal	Heavy metals limit test	Heavy metals limit test
Ba	Turbidity	Turbidity
Solvent		Each dossier
Nitrogen	Semimicro-Kjeldahl	Semimicro-Kjeldahl
Protein	UV	UV
Nucleotides	UV	UV
OSCS	¹ H-NMR	¹ H-NMR
GalNH2	ABEE/FL-HPLC	ABEE/FL-HPLC
Assay	Anti-Xa	Anti-Xa

Conclusion



- ◆ Heparin calcium monograph will be newly listed in JP on October 1, 2009.
- ◆ Definition of JP heparin sodium will be revised on October 1, 2009.
- ◆ *Identification tests by $^1\text{H-NMR}$ and WAX-HPLC, revised OSCS purity test by $^1\text{H-NMR}$ and galactosamine purity test* have been proposed by the draft working group.
- ◆ JP committee will discuss the revision of heparin monograph including *the tests* on August 25, 2009.
- ◆ *Identification tests and purity tests* in monographs of heparin sodium and heparin calcium will be revised in January 2010.
- ◆ *Other purity tests* will be revised in the future.