

***IMPURITIES IN
BIOLOGICAL DRUG
SUBSTANCES***

PREMESSE

**COME LE IMPUREZZE DEI FARMACI BIOLOGICI
INFLUISCONO NELLO SVOLGIMENTO DELLA
DOCUMENTAZIONE REGOLATORIA**

- Regole per la produzione (GMP)
- Riferimenti per il controllo (Farmacopee)
- Documentazione registrativa (NtA)

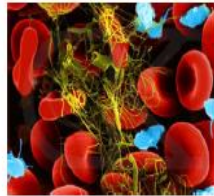
Biological Drug Substances



Blood Derivatives

Whole Blood

Blood Components



Proteins



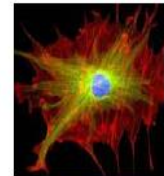
Human Tissues



Vaccines (preventive and therapeutic)



Allergenic Extracts



Cellular & Gene Therapies



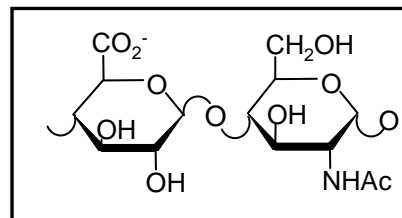
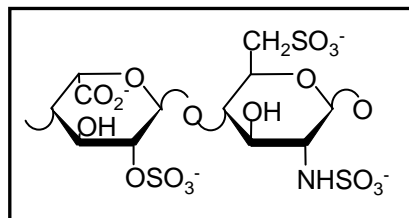
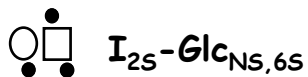
Xenotransplantation Products

EudraLex
The Rules Governing Medicinal Products in the European Union
Volume 4
EU guidelines for
Good Manufacturing Practice for
Medicinal Products for Human and Veterinary Use
Annex 2
Manufacture of Biological active substances and Medicinal Products for Human
Use

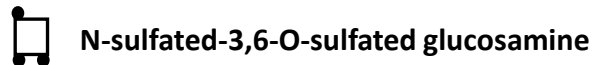
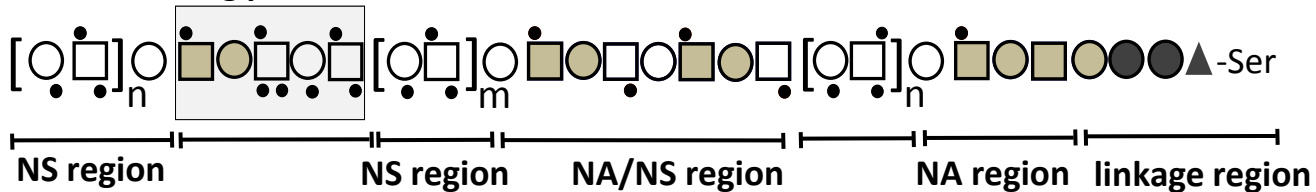
Type and source of material	Example product	Application of this guide to manufacturing steps shown in grey			
1. Animal or plant sources: non-transgenic	Heparins, insulins, enzymes, proteins, allergen extract, ATMPs immunosera	Collection of plant, organ, tissues or fluid	Cutting, mixing, and / or initial processing	Isolation and purification	Formulation, filling

- Che cosa è l'EPARINA
- polisaccaride solfatato
- Unità disaccaridiche
- Acido iduronico 2-solfato e 6-O-solfato
- Glucosamina N-solfato

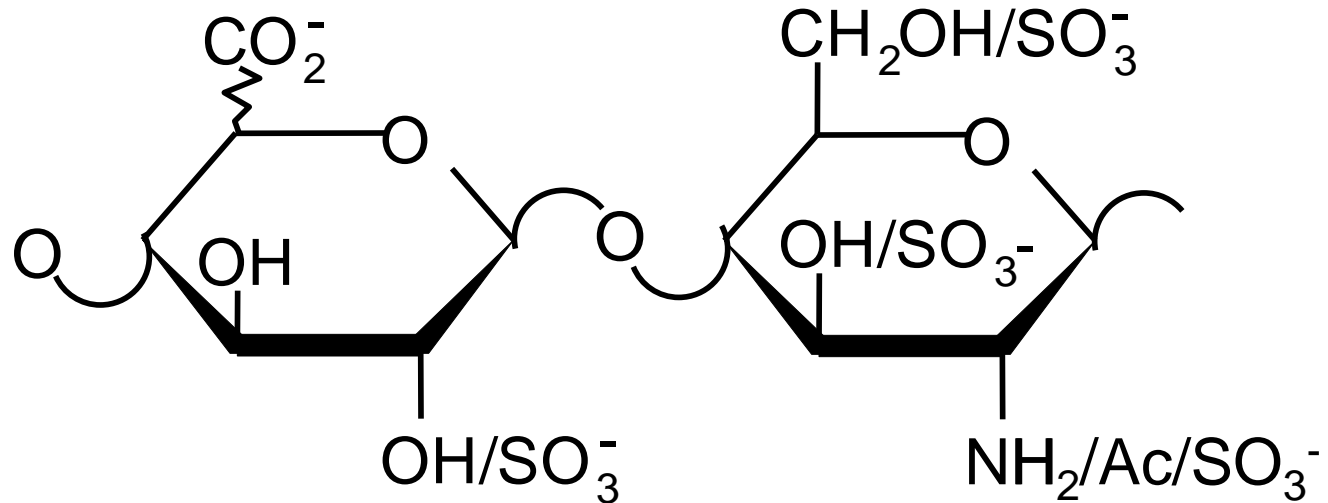
Heparin structure



AT-binding pentasaccharide



Heparin structural complexity



N° building blocks:

DNA

4

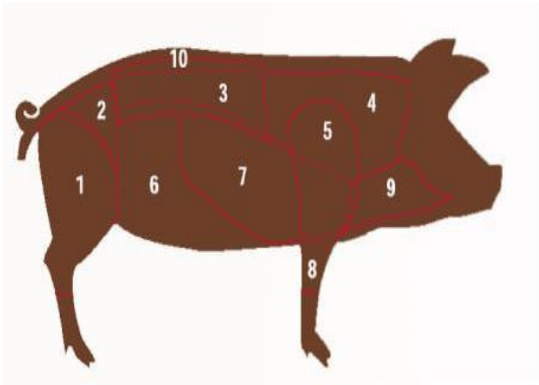
Protein

20

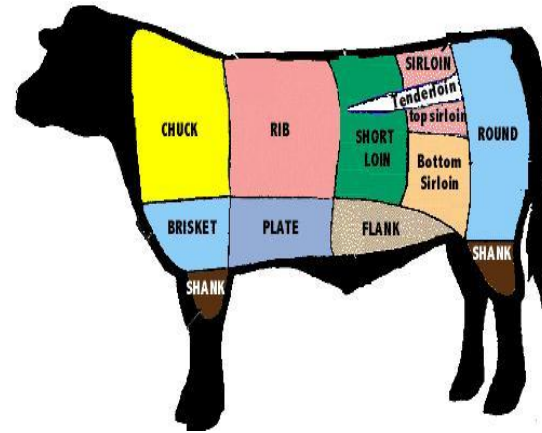
Heparin/HS

48

Heparin production



Pig intestinal mucosa

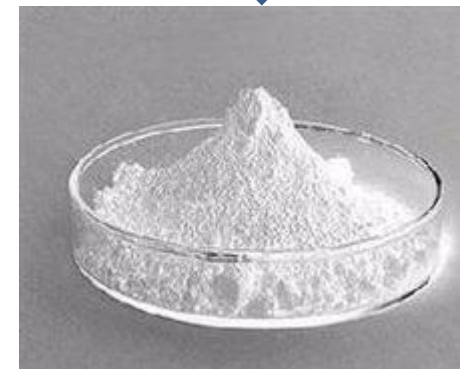


Beef intestinal mucosa and/or lung

Heparin production



Crude heparin



API heparin



Drug

Quali sono le impurezze ?

- **Impurezze o contaminanti naturali**
- **Impurezze legate alle fasi di lavorazione nel processo di produzione**
- **Impurezze dovute a modifiche strutturali**

Heparin structural characterization

Analysis of depolymerized material

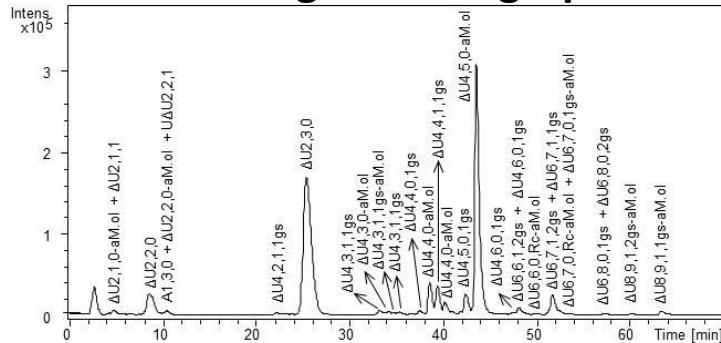


HPLC / CE of disaccharides mixture obtained by enzymatic digestion

HPLC of disaccharides mixture obtained by nitrous acid depolymerization.

UPLC/ESI-IT-MS of partially or totally depolymerized material

Building block fingerprint

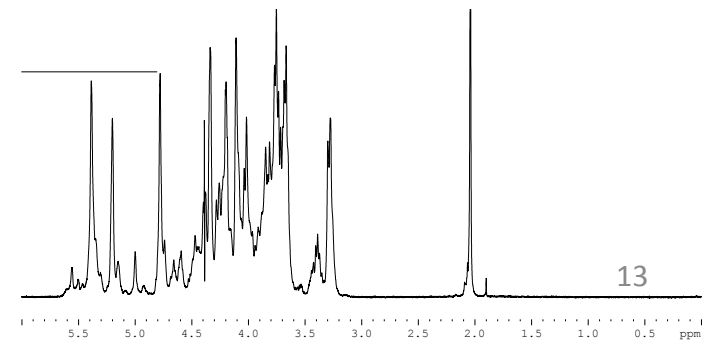


Analysis of intact material

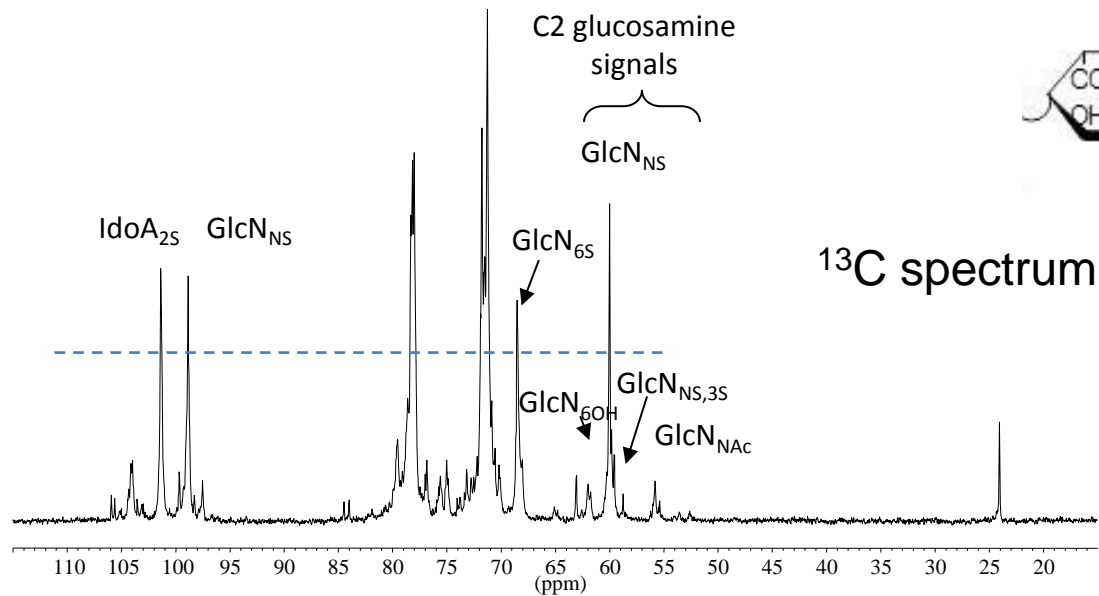
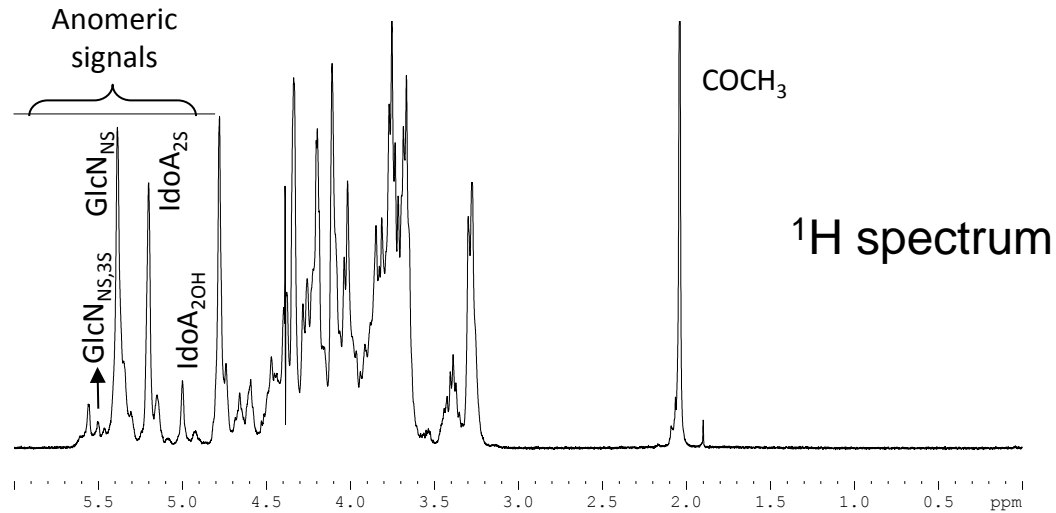


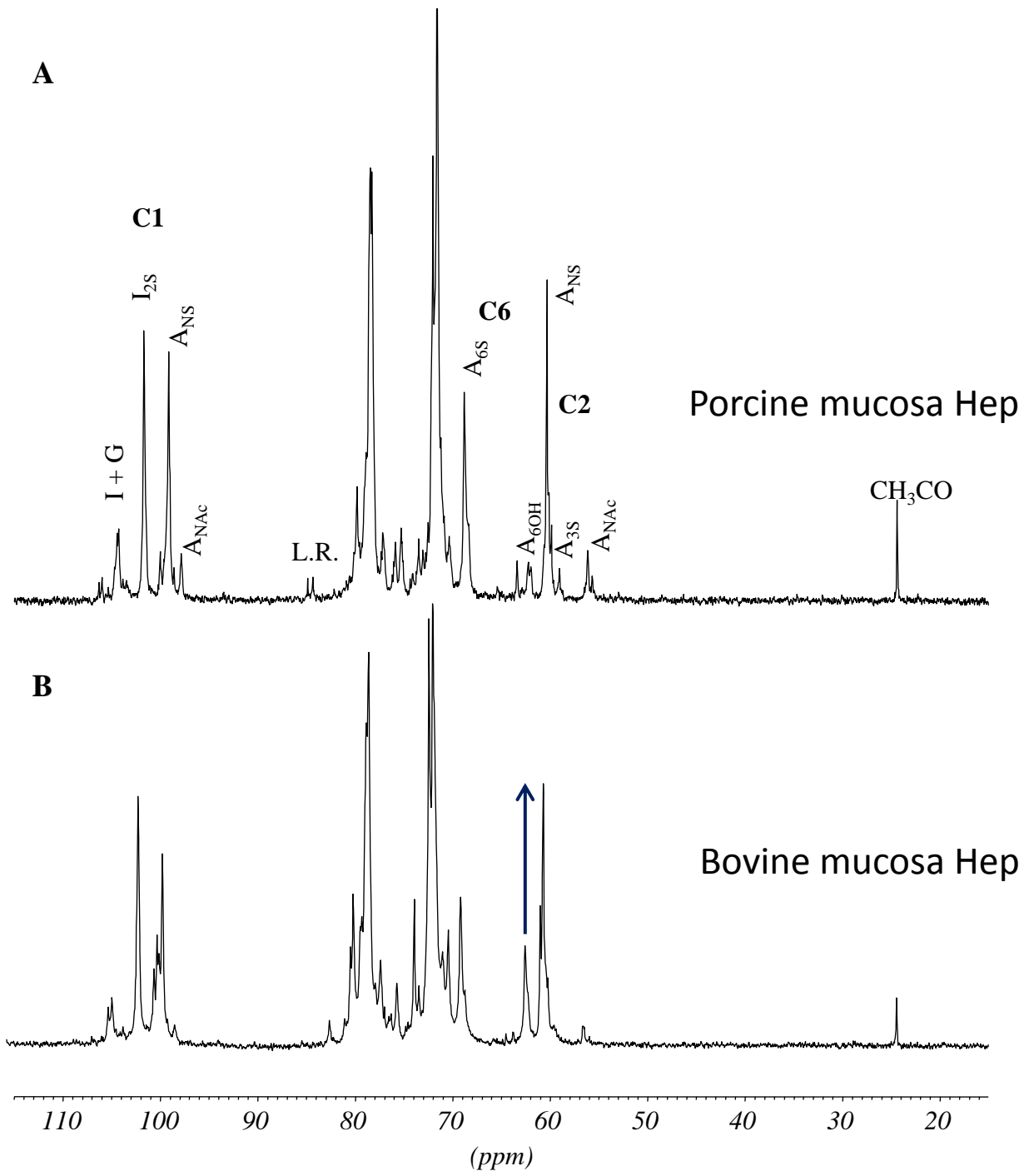
NMR spectroscopy

Product fingerprint



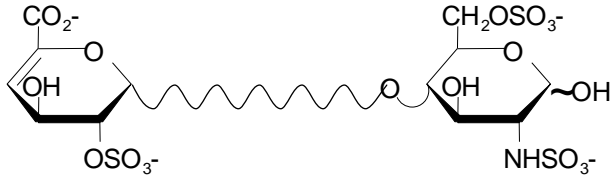
Heparin structure



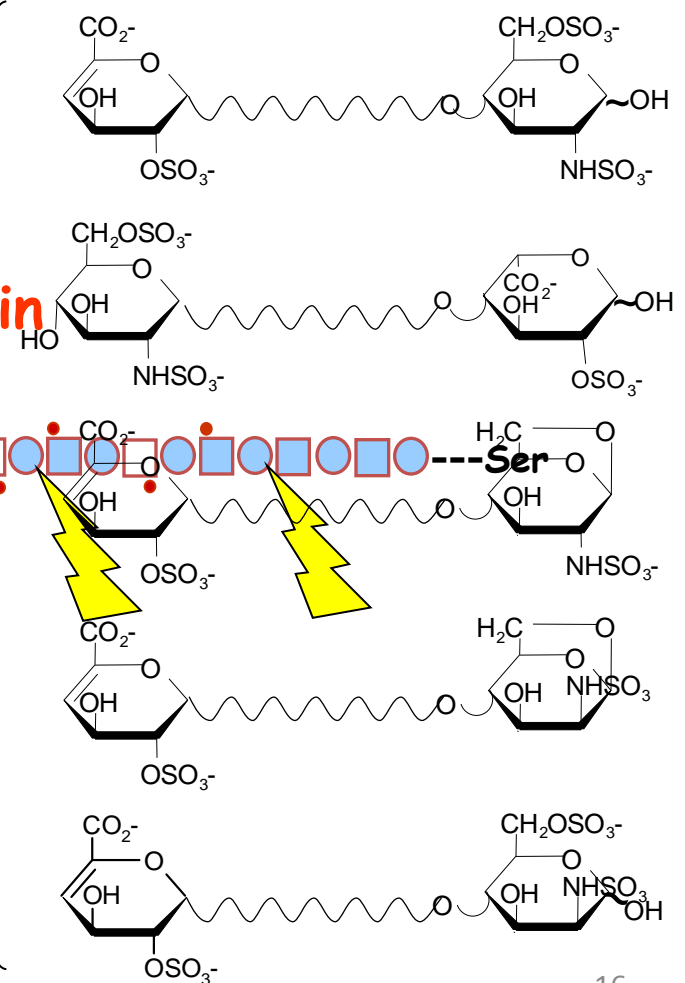


Low Molecular Weight Heparins

**β -eliminative cleavage by heparinase
(Tinzaparin)**



**β -eliminative cleavage of the
benzyl ester by alkaline treatment
(Enoxaparin)**

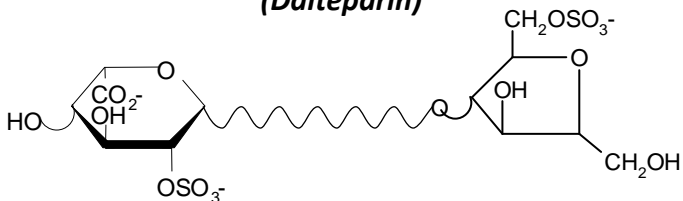


unfractionated heparin
(UFH)



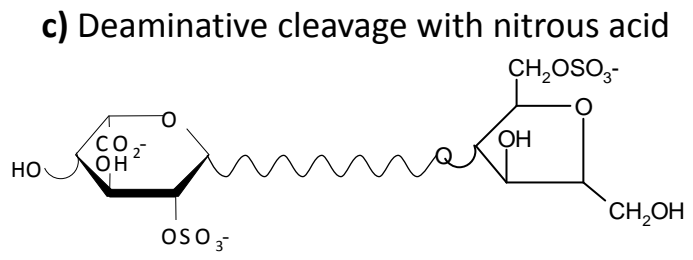
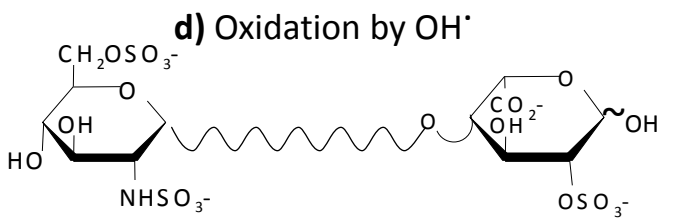
Heparin

**Deaminative cleavage with nitrous acid
(Dalteparin)**

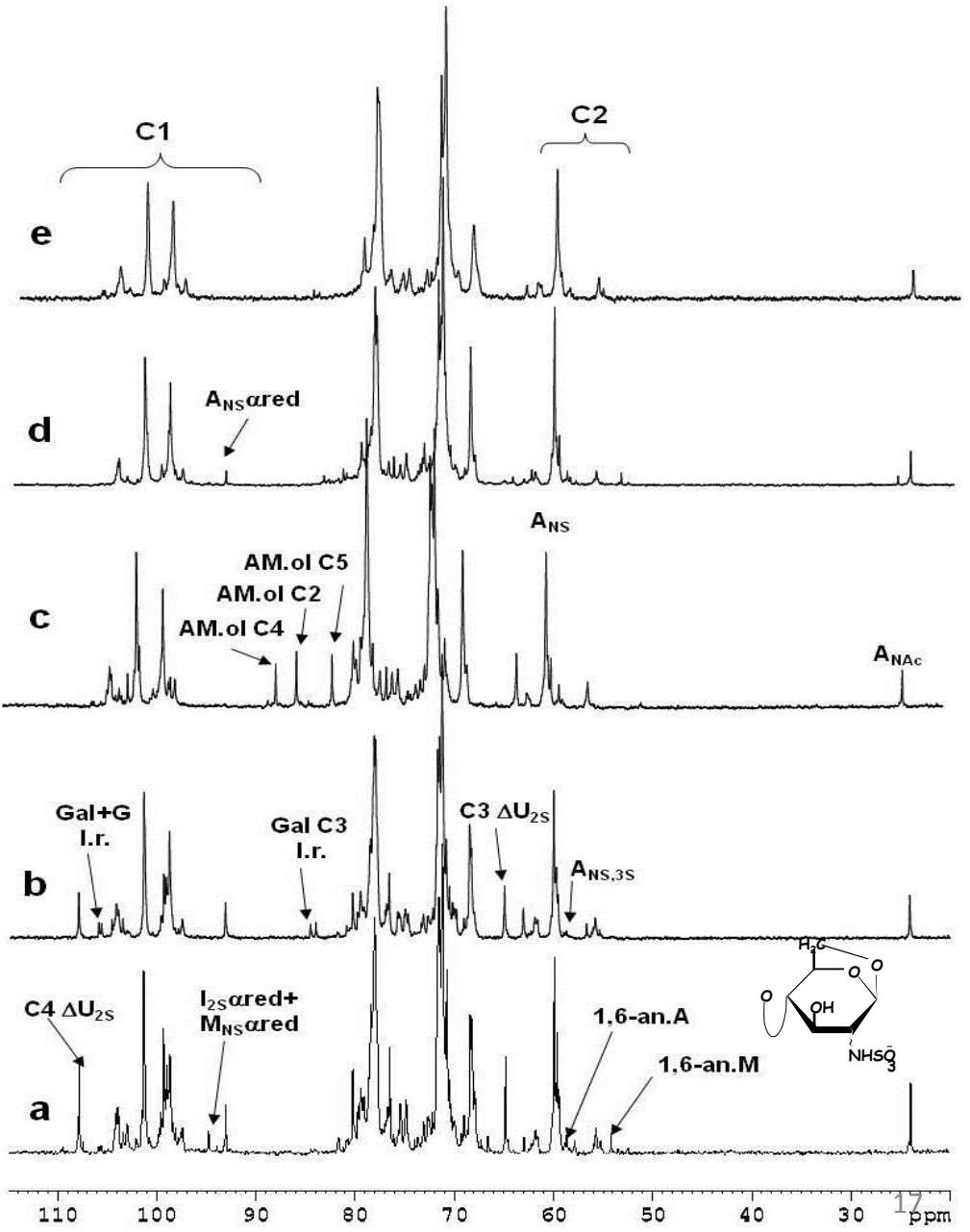
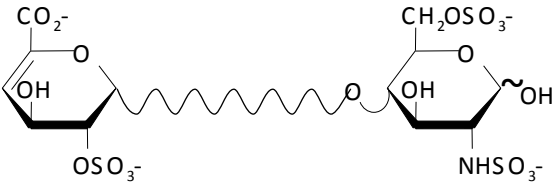


NMR characterization of GAGs

Heparin



β -eliminative cleavage by: **a) chemical;**
b) heparinase



“Impurezze” o Contaminazioni

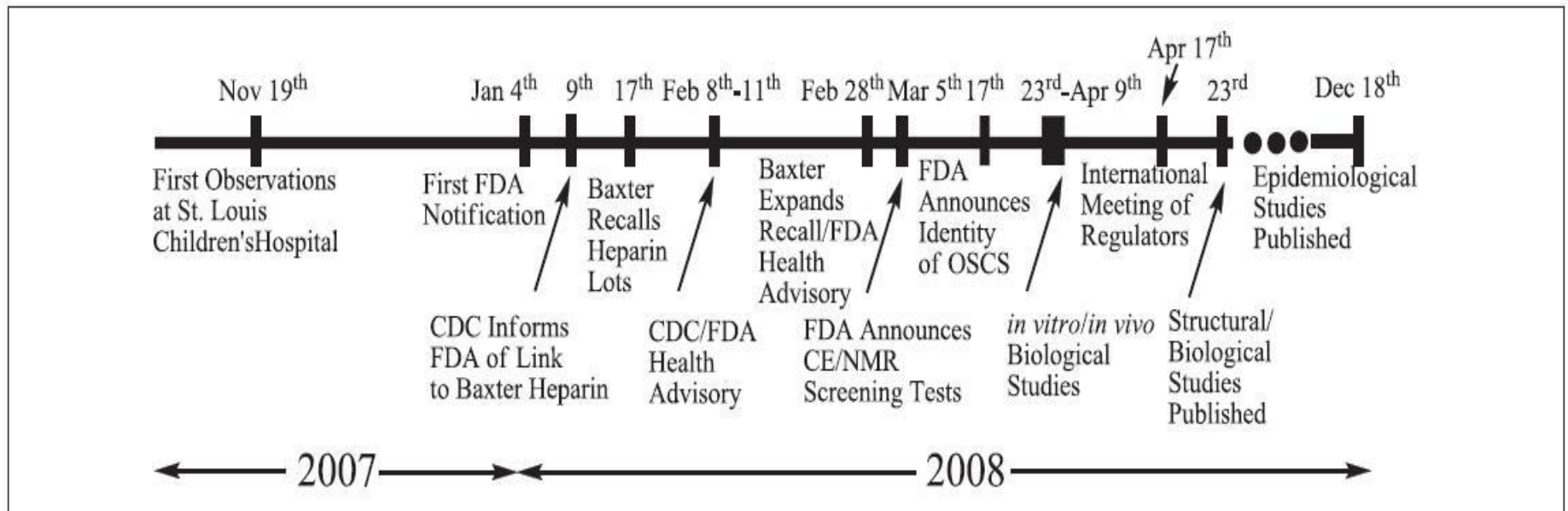
- **BSE (Bovine Spongiform Encephalopathy) e TSE (Trasmitting Spongiform Encephalopathy)**
- **Contaminazione dell'eparina con condroitin supersolfato**

Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3)

**VIRUS VALIDATION STUDIES:
THE DESIGN, CONTRIBUTION AND INTERPRETATION
OF STUDIES
VALIDATING THE INACTIVATION AND REMOVAL OF
VIRUSES
Note for Guidance**

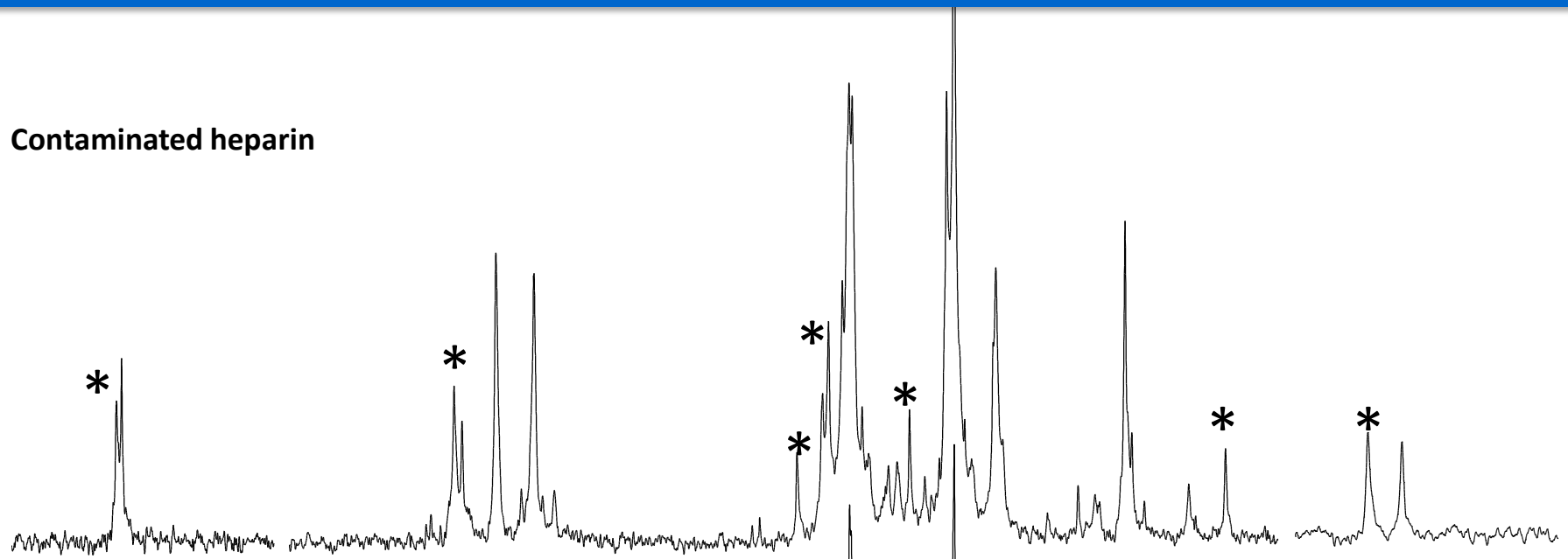
Contaminazione

Guerrini M, Beccati, D., Shriver, Z. et al. *Nat. Biotech.* 2008, 26, 669-675

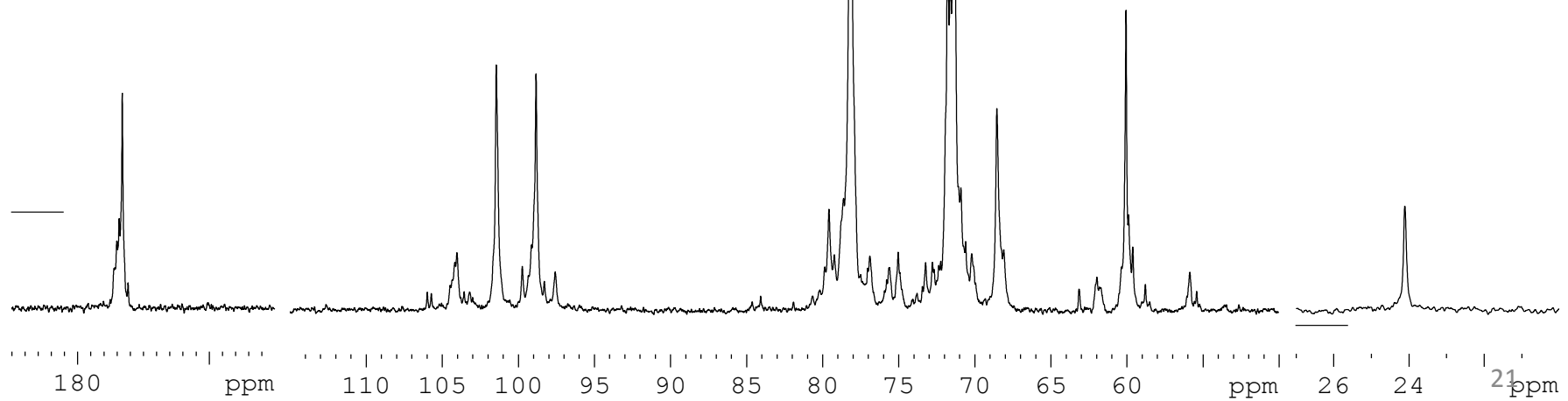


The contaminated Heparin story

Contaminated heparin

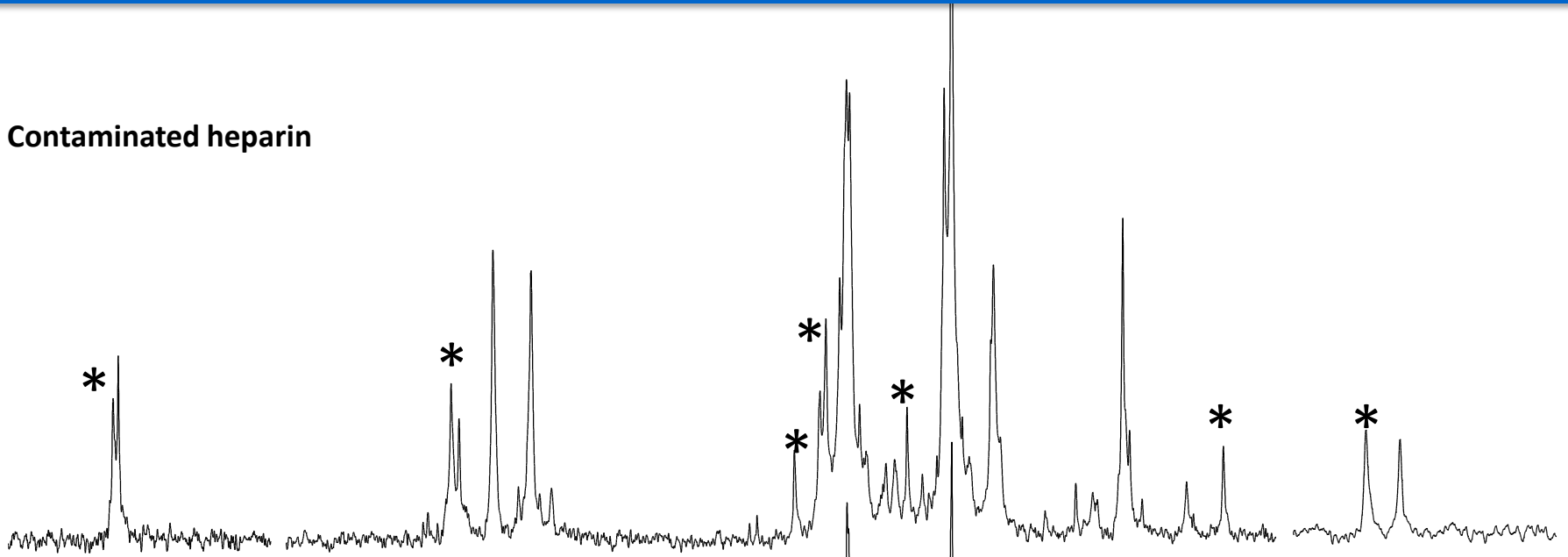


Standard Heparin

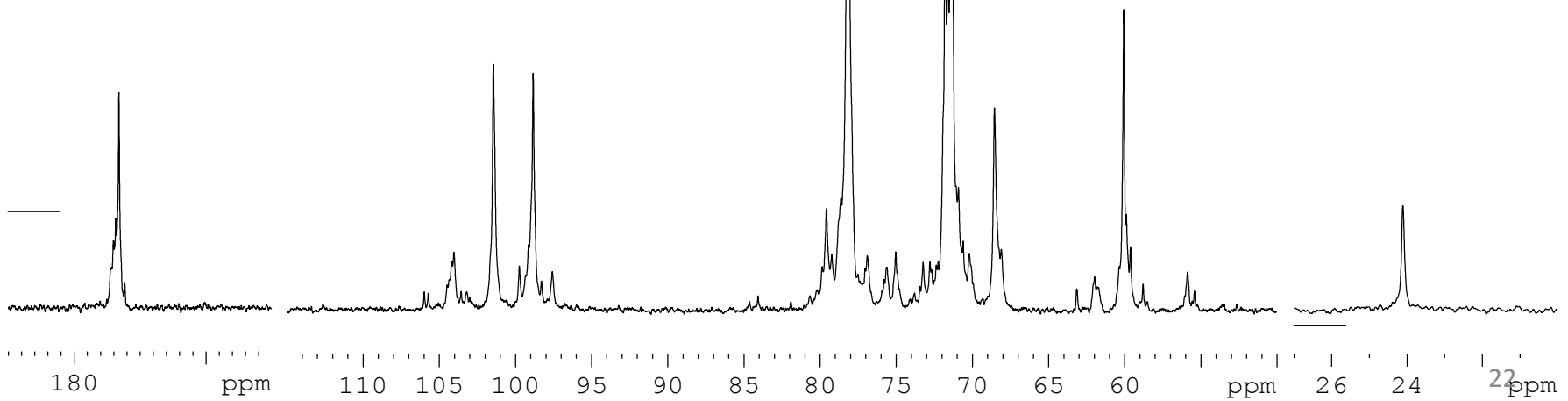


The contaminated Heparin story

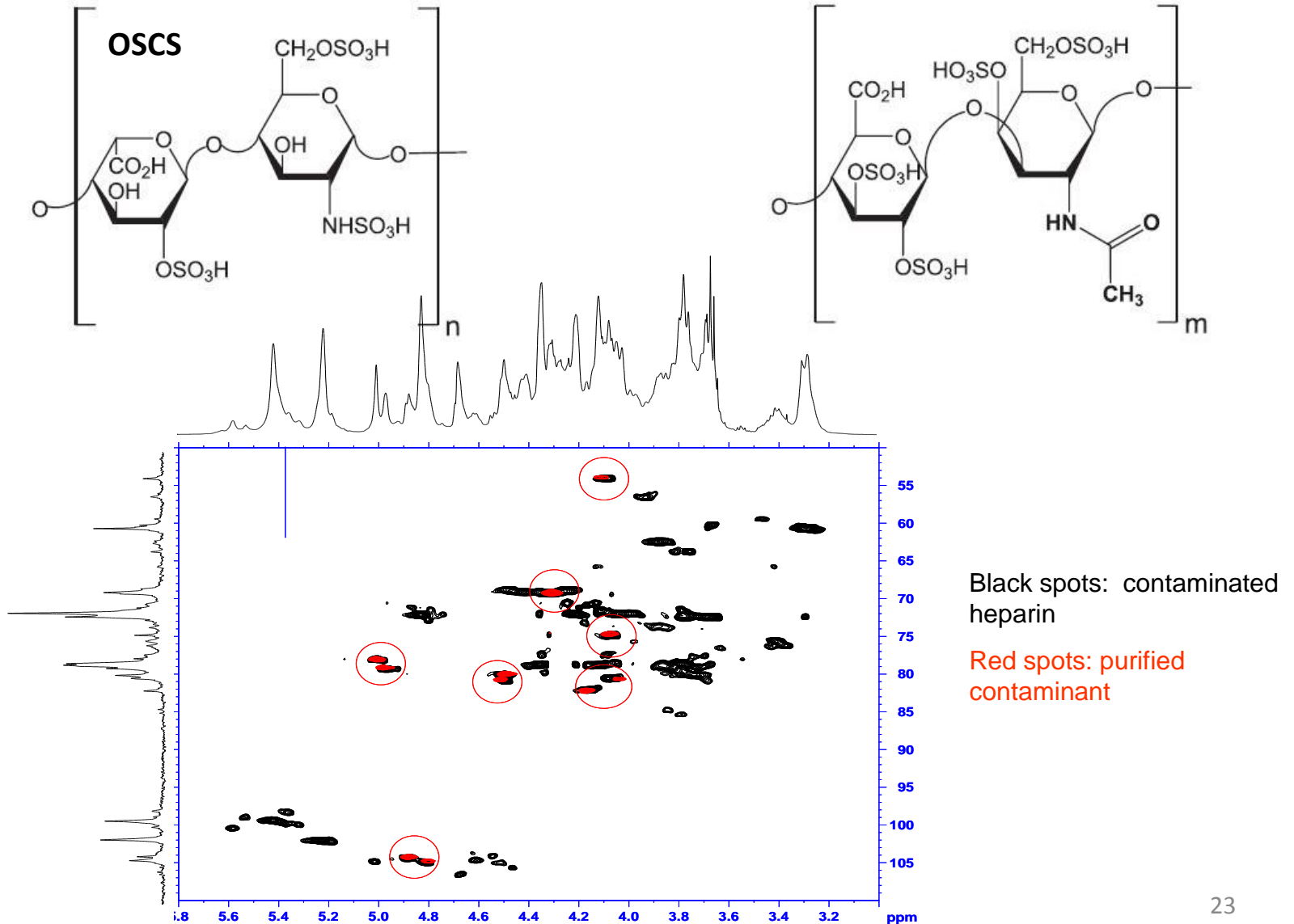
Contaminated heparin



Standard Heparin

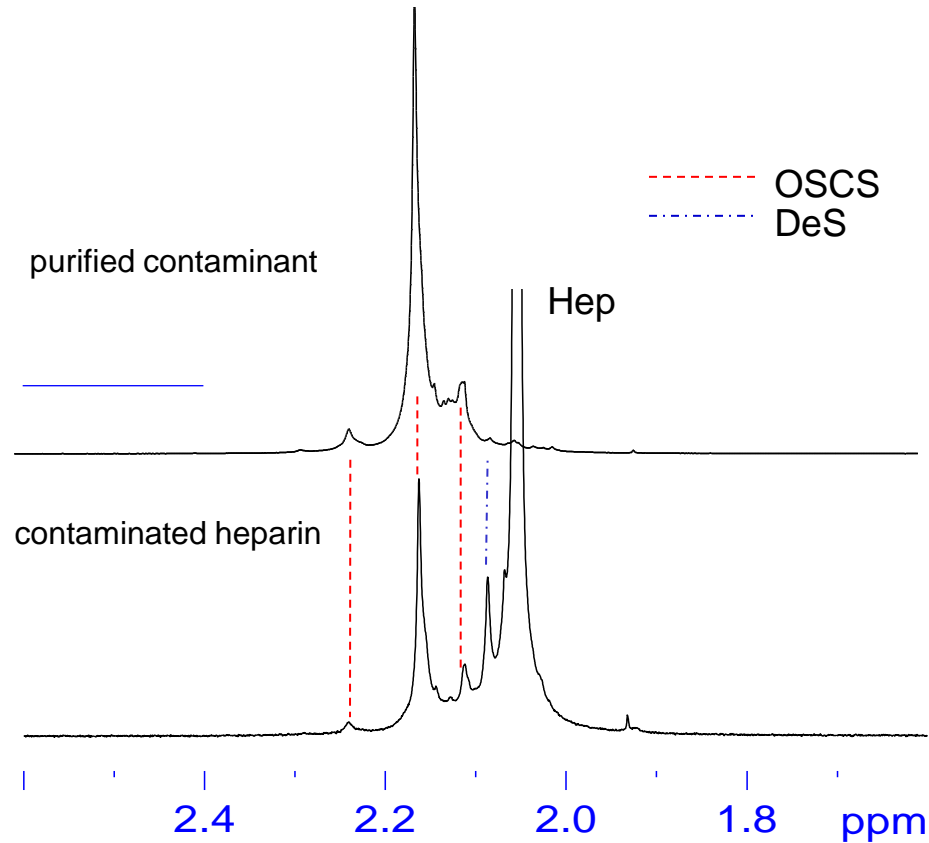


The contaminated Heparin story



The contaminated Heparin story

N-acetyl signals in the ^1H -NMR spectra of a OSCS-contaminated heparin and after heparin removal

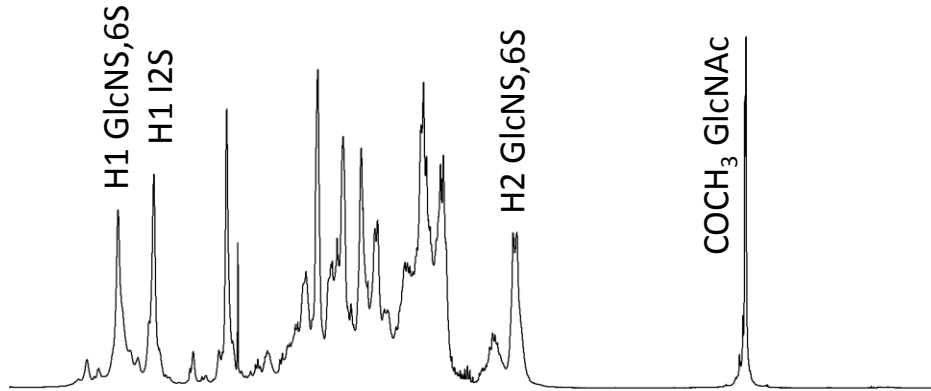


USP Heparin Sodium Specifications: pre and post “Heparin crisis”

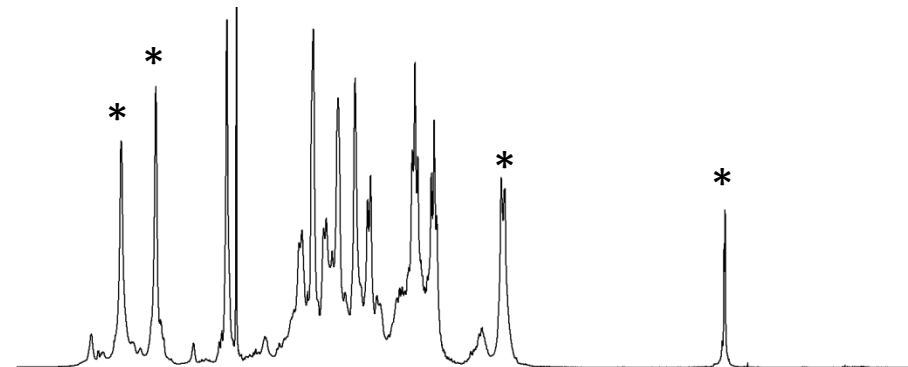
Test	Pre-2008	2008-2014
Identification	Sodium	+ 1H NMR + HPLC + MW
Assay of Heparin Potency (dry basis)	NLT 140 USP U/mg (clotting)	NLT 180 USP U/mg a-IIa assay
Activity	80 to 120% (aXa)	aXa/alla ratio
Absence of OSCS	no	Yes
Organic impurities	no	Galactosamine + nucleotides + residual solvents
Heavy Metals	Conforms	Conforms
Protein (TCA method)	No Turbidity Observed	Revised protein impurities
Bacterial Endotoxins	NMT 0.03 USP EU/USP Unit	NMT 0.01 USP EU/USP Unit
Loss on Drying	Not More than 5%	Not More than 5%
Labeling	species and tissue of origin	species and tissue of origin

Proton spectra of heparin samples extracted from different species/organ sources

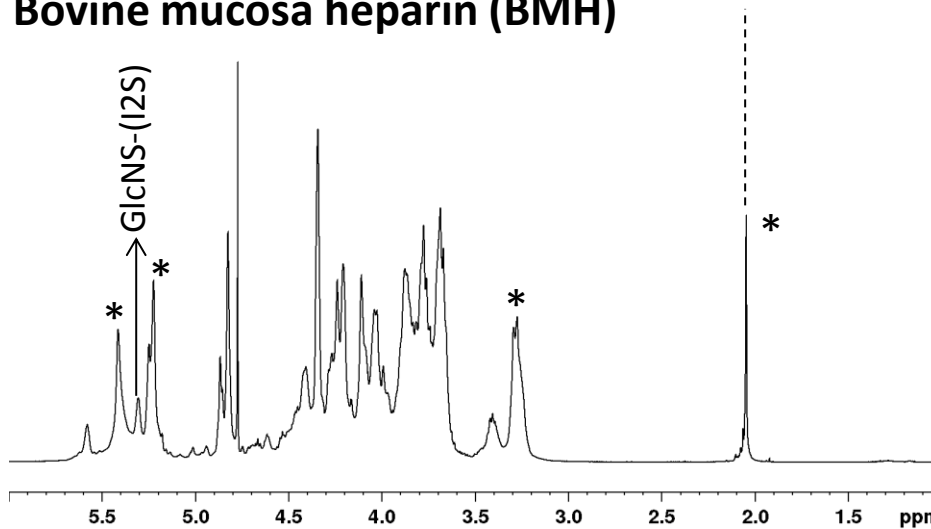
Porcine mucosa Heparin (PMH)



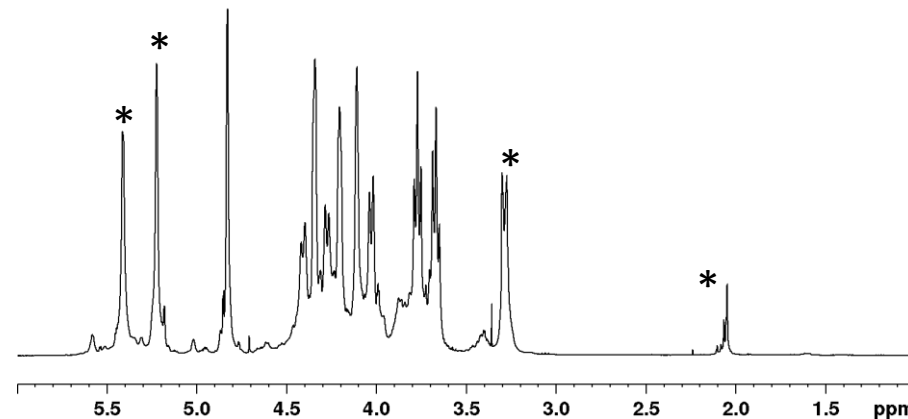
Ovine mucosa heparin (OMH)



Bovine mucosa heparin (BMH)



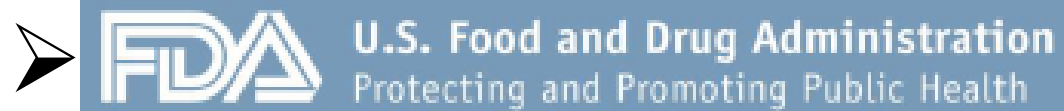
Bovine lung heparin (BLH)



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