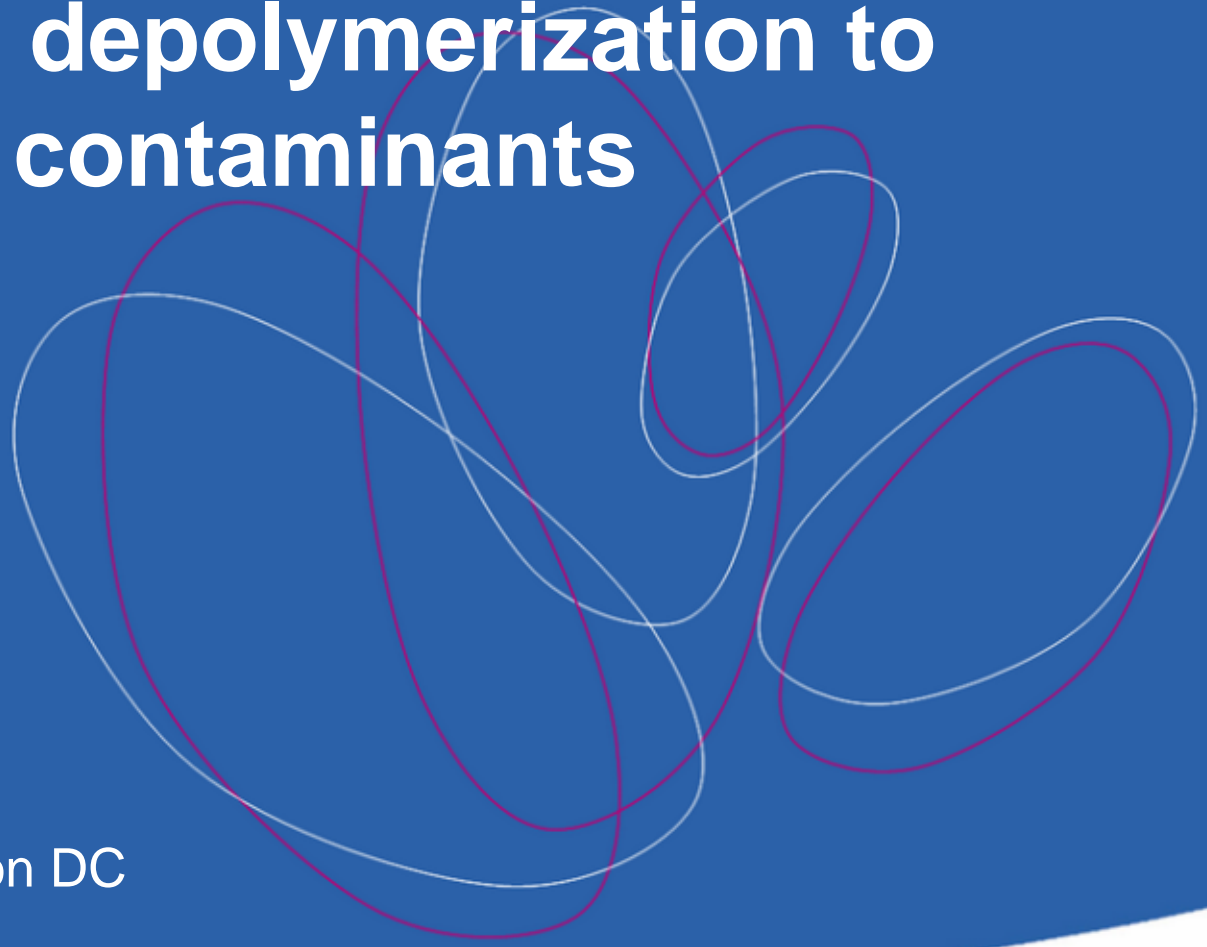


Utility of Anion EXchange HPLC coupled with depolymerization to monitor new contaminants



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Most likely contaminants

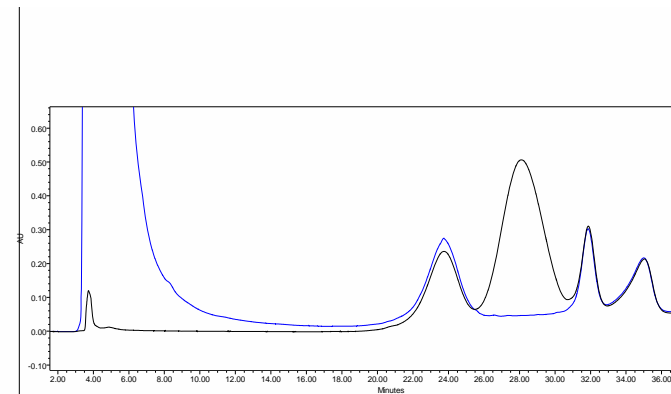
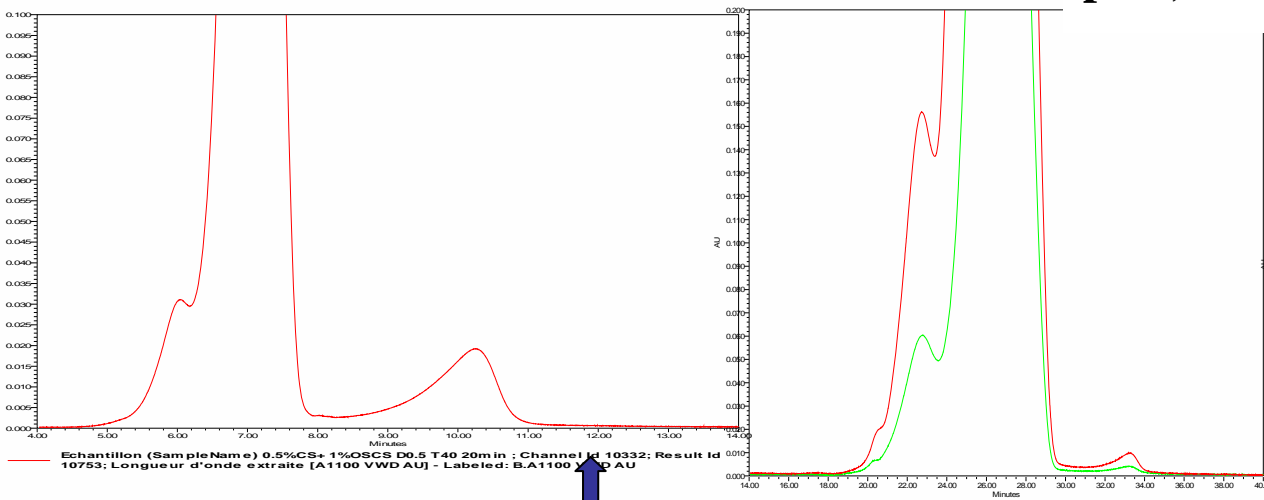
- Need to be cheap, have similar characteristics to that of porcine heparin (some anticoagulant properties, solubility...) to be introduced unnoticed at "some" % in crude heparin
- Oversulfated (OS) polysaccharides and heparin from other animal species are most likely
- Numerous references in the literature (eg chitosans, fucoidans, galactans, cellulose, K5, pullulans, pentosans, partial depolymerization...)
- OSCS is just one of them
- Too numerous possibilities to evaluate all potential products throughout the current tests
- An array of complementary methods is required to lower the LOQ
 - ▶ most useful are $^1\text{H-NMR}$, AEX HPLC, and bioassays
 - ▶ Due to the complexity of heparin, no single method is full-proof



Non heparinoids contaminants by AEX

Heparin spiked with DS 1% and OSCS 1% or 0.1%

Column AS11HC; others are possible
UV 202nm
pH3, NaClO4 gradient



Heparin spiked with DS, OSDS, OSCS 2%
w/o & w HNO2 depolymerization

12min

- ▶ Easy to implement (QC friendly)
- ▶ One of the best LODs for OSCS (<0.01%) on an array of sources
- ▶ Good resolution for OS compounds (eg OSDS)
- ▶ Polydispersity of heparin -> broad peak = blind area (similar issues with other analytical methods like NMR and CE)
- ▶ Remove heparin by depolymerization



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Nitrous depolymerization and limitation

- **Simple** Procedure

- ▶ RT, 30min

- **Moderate** Specificity

- ▶ Cleaves N-sulfated oligosaccharides
- ▶ By products eluted quickly (this is an issue with CE)

- Other limit (general to gradient HPLC or CE)

- ▶ UV absorbing products, even at 202nm

- SAX without and with HNO₂ depolymerization is a routine release test within sanofi-aventis

- ▶ Includes assay of Dermatan Sulfate



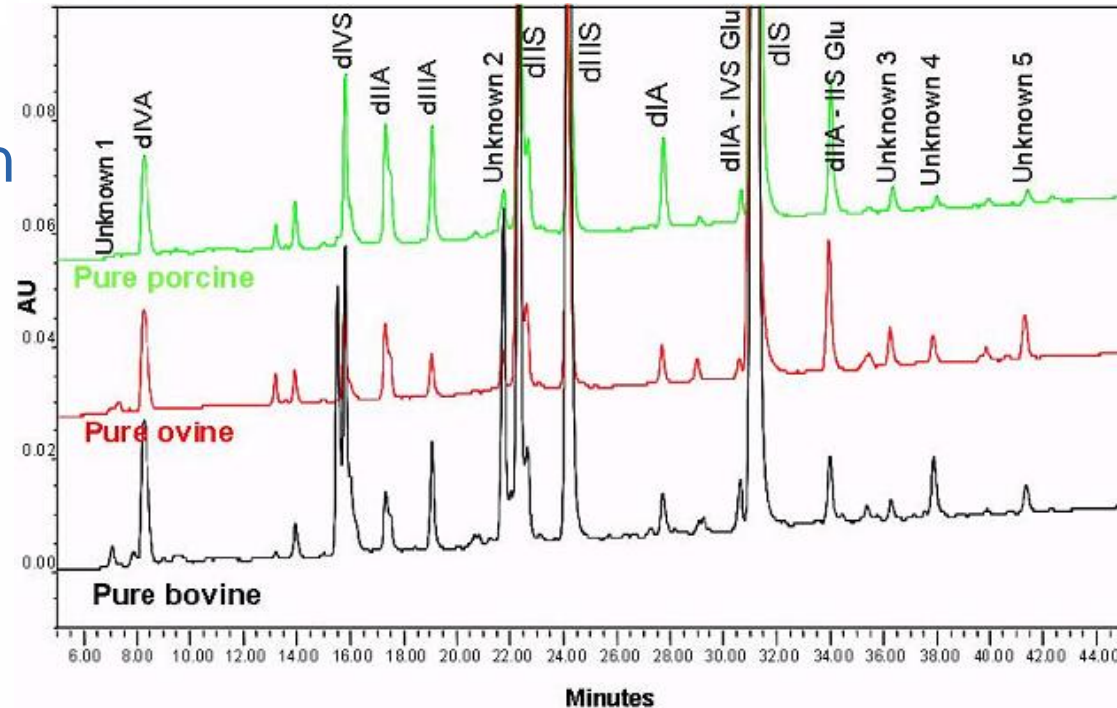
Heparinases depolymerization

- **Less simple** Procedure
 - ▶ Enzymes, 48hours
- **High** Specificity
 - ▶ Very specific to close to natural heparins
 - ▶ Will not cleave most of OS polysaccharides
 - ▶ Cleaves heparin from all animal species
- May be used for non heparinoids contaminants
- Can be used to test for animal species origin in complement to PCR



Non porcine heparin contaminants SAX after heparinases

- ▶ Test for animal species currently not required in monographs
- ▶ Characteristic disaccharide pattern
- ▶ Can be performed on crude heparin



- ▶ Bovine: IIS/Ia
 - ▶ is a good bovine/porcine discriminator
 - ▶ Typically 25-120 for bovine and 4-6 for porcine

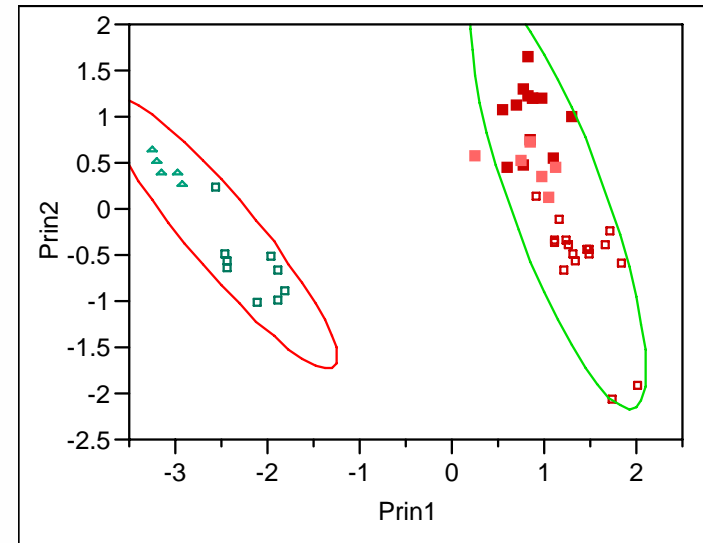
Non porcine heparin contaminants SAX after heparinases

- ▶ Ovine is closer to porcine heparin
- ▶ Use of a set of disaccharides and PCA

Batches of crude (hollow square) and pure Heparin

Red dots: porcine

Green dots: ovine



Comments

- ▶ Other techniques than SAX are possible (eg NMR), but:
 - ┌ SAX is very sensitive and easy to implement (QC friendly)
 - ┌ LOQ mainly relies on the quality of the data base, and the characteristics of the porcine and contaminant heparins
- ▶ Used within s-a to qualify and monitor sources



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CONCLUSION

- SAX HPLC is a very good QC friendly technique to assess various potential contaminants in heparin, in complement to $^1\text{H-NMR}$ and anti-IIa/Xa bioassays
- Focus should not be on OSCS alone with sub 0.1% targets
- HNO_2 depolymerization is very convenient to clear out the black window under heparin
- Heparinases depolymerization is less simple but useful to pick up contamination by other animal species
 - ▶ This requires a comprehensive data base obtained from reliable samples out of many sources of the different animal species



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