

# The limit to common reference standards for the characterization of LMWH

## The case of enoxaparin

Pascal ANGER

July 27/28 2009, Washington DC



**sanofi aventis**

L'essentiel c'est la santé.

# The different LMWH

- Bemiparin, dalteparin, enoxaparin, nadroparin, parnaparin, tinzaparin are all obtained by different processes, and differ very significantly from a structural standpoint
- Question: to which extent is it possible to use generic reference standards and methods?
- The case of enoxaparin



# Identification

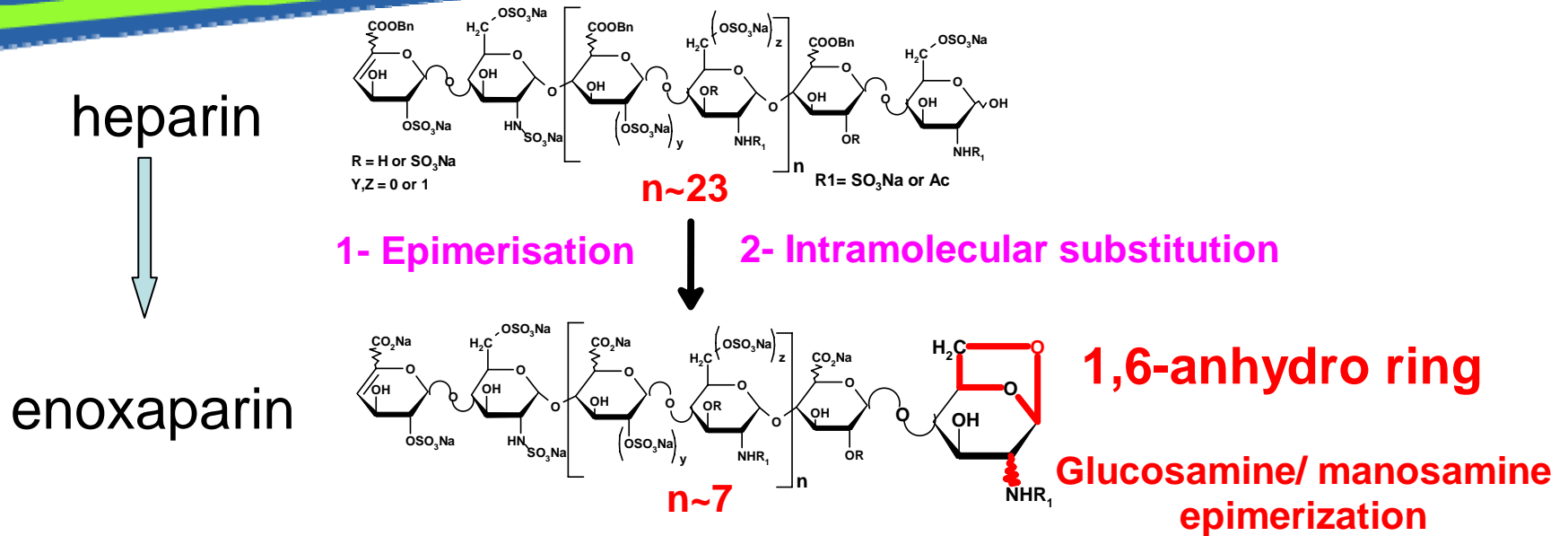
- Whatever the LMWH, obvious need of a specific reference for identification (NMR)
- In case of enoxaparin, this standard is also used in suitability tests by the other characterization techniques



**sanofi aventis**

L'essentiel c'est la santé.

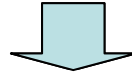
# Specificity of enoxaparin



- Occurs at the reducing end on ~ 20% of the 6-O sulfated glucosamine
- Is a simple (and macroscopic) marker of the enoxaparin process
- Research of other specific oligosaccharides is out of the scope of a monograph

# Assay of 1,6anhydro content

- Unique structural characteristic that distinguishes enoxaparin from all other LMWHs
- Contributes to the overall pharmacologic effects of enoxaparin
- %1.6 anhydro controlled on all enoxaparin batches with specifications 15-25%, as requested by the FDA, Ph.Eur and USP monographs



▶ **Need for a specific method and a specific standard for suitability test with labeled 1,6anhydro  $\pm$  1.5%**

▶ 1,6anhydro % =  $K \times MW(\text{enoxaparin})$

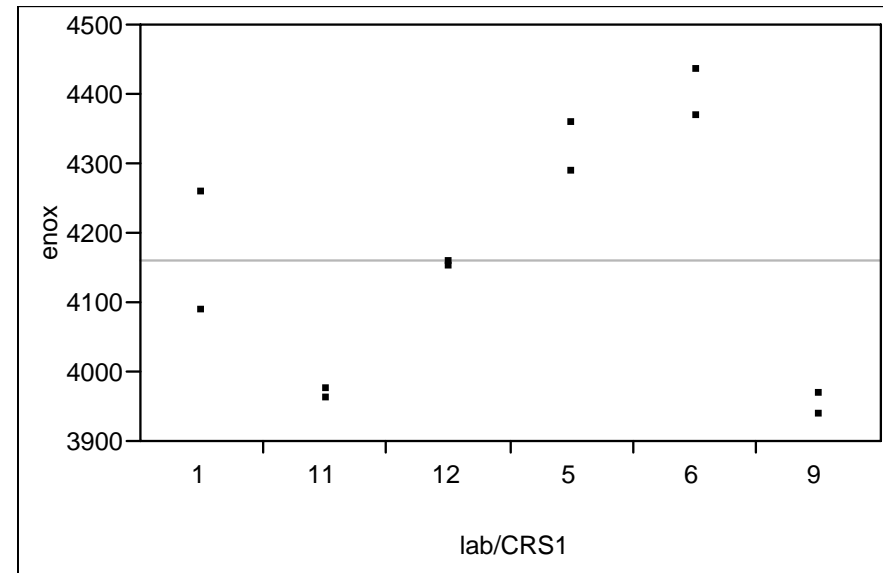


▶ **Additional request for a reproducible MW method**



# Assay of the MW of enoxaparin

- Enoxaparin spec 3800-5000 Da
- Ph.Eur method used in international collaborative study BSP069, 2006
- Due to too high variability, data were centrally reworked whenever possible



## WHY?

Enoxaparin reworked data

- The Ph.Eur method has some interesting features (1 single standard), but also intrinsic issues with most important one being its specific calibration



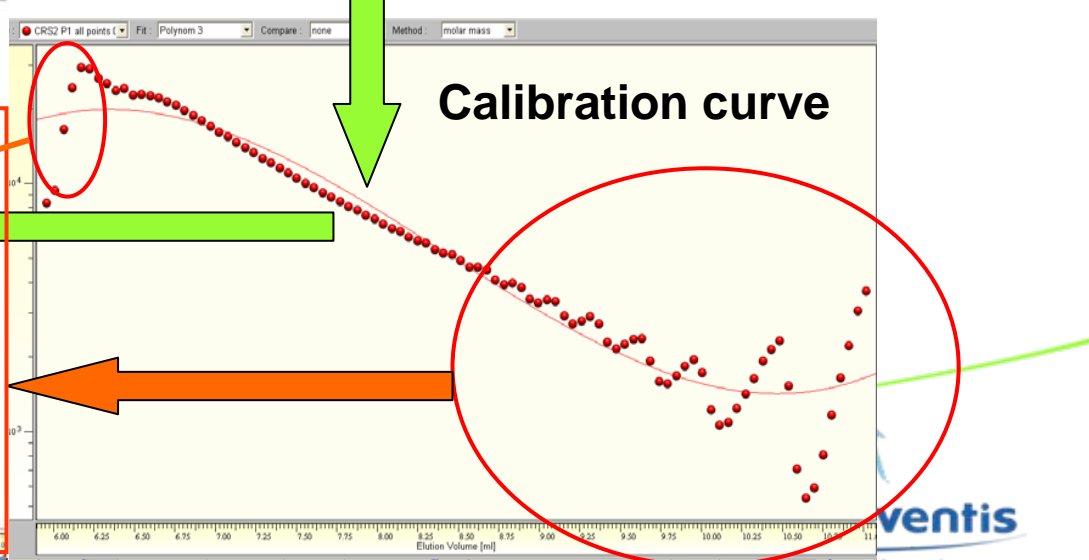
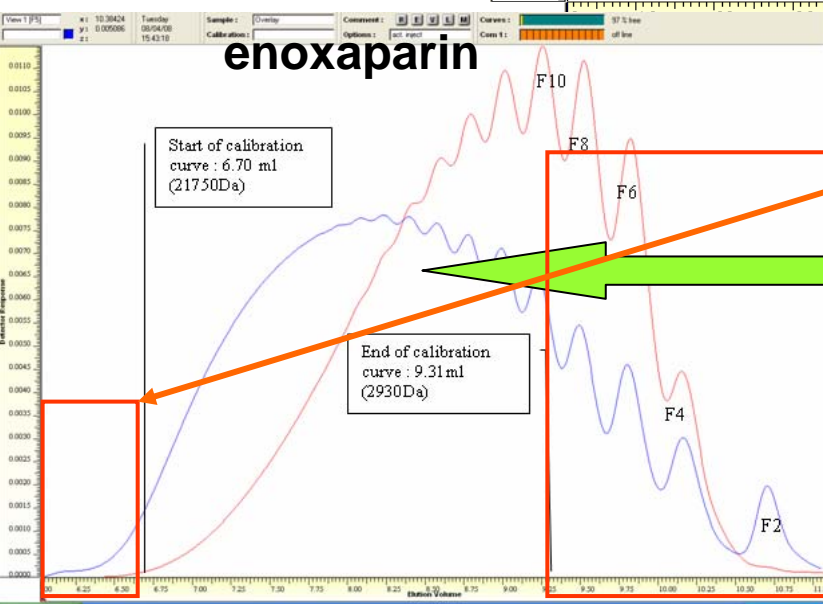
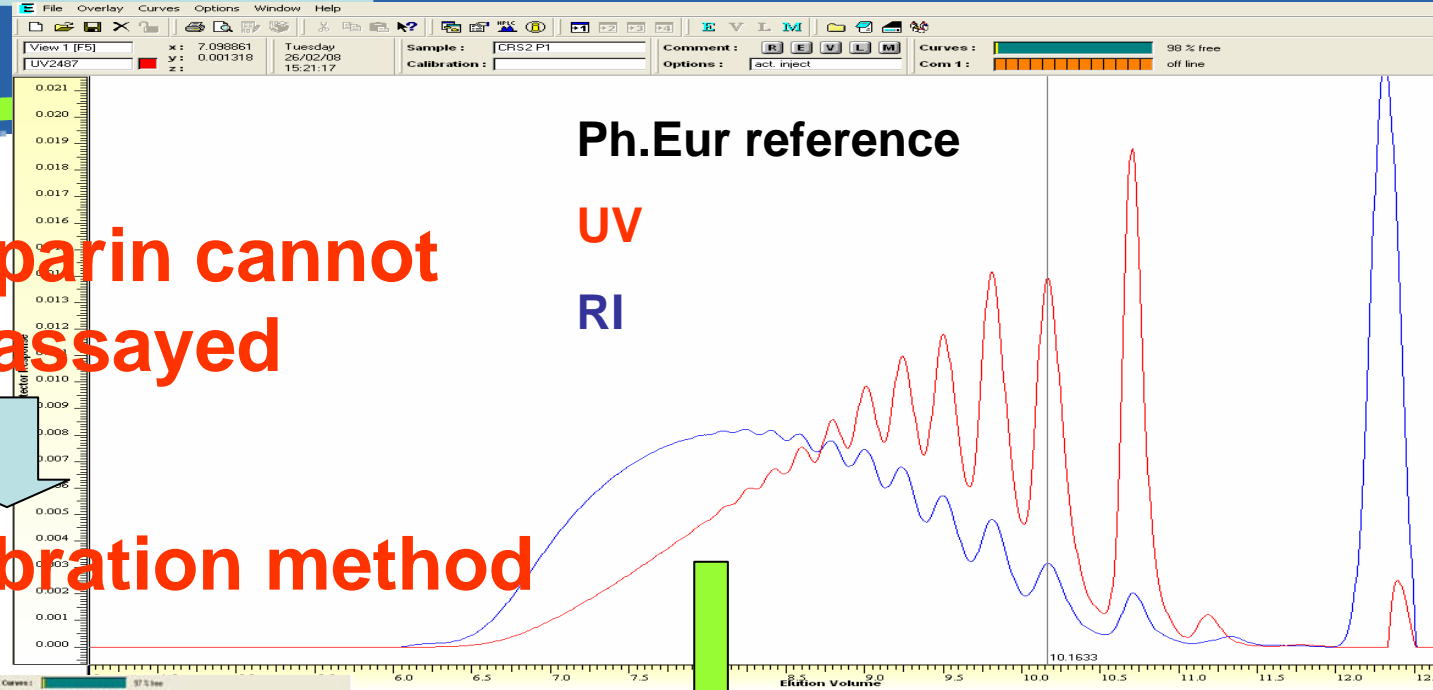
sanofi aventis

L'essentiel c'est la santé.

# Assay of the MW - Ph.Eur calibration

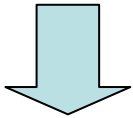
1/3 of enoxaparin cannot be properly assayed

Specific calibration method

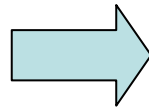


# Assay of the MW - s-a calibration

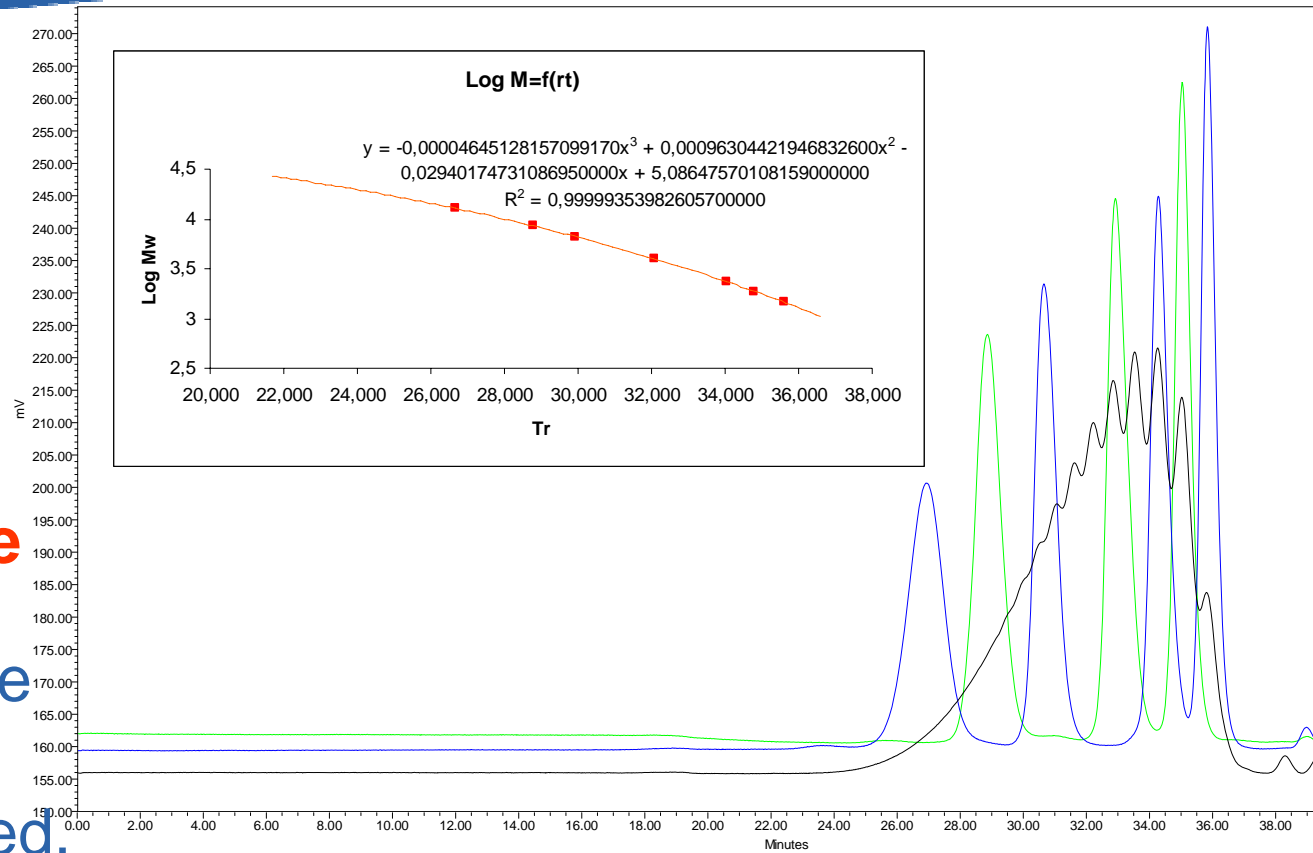
- 2 sets A&B
- 4 & 3 calibrants



- Preparation of **narrow disperse MW enoxaparin calibrants** is time consuming
- But once prepared, consumption is low and the method very reproducible



- Specific standards and suitability test with labeled MW  $\pm 150\text{Da}$**



# Assay of the bioactivity

- Use of a specific standard to guarantee similarity of behavior
  - ▶ General agreement is that best reproducibility is obtained when assaying more similar products
    - Eg. recent NIBSC study for the establishment of the 6th UFH standard (sample V – report CS379 p11)
  - ▶ No international collaborative study has been performed to compare reproducibility obtained with current LMWH IS and with enoxaparin standard
  - ▶ Internal practice is to use an enoxaparin standard to be on the safe side



# Conclusion – Enoxaparin references and methods

	Specific method	Specific reference
Identification	Not necessary	<b>Mandatory</b> (ref#1)
1,6anhydro	<b>Mandatory</b>	<b>Mandatory</b> for suitability test (ref#1)
MW	Not necessary Currently Ph.Eur with improvement	<b>Greatly improves reproducibility</b> Narrow dispersed MW calibrants (ref#2) (ref#1) for suitability test
Bioactivity	Not necessary	<b>Guarantees best reproducibility</b> (ref#3 – solution of #1)

Corresponds to USP draft monograph and s-a practice