

# NMR Spectroscopy

## Identity and purity test of LMWH

## LMWH (Wikipedia entry)

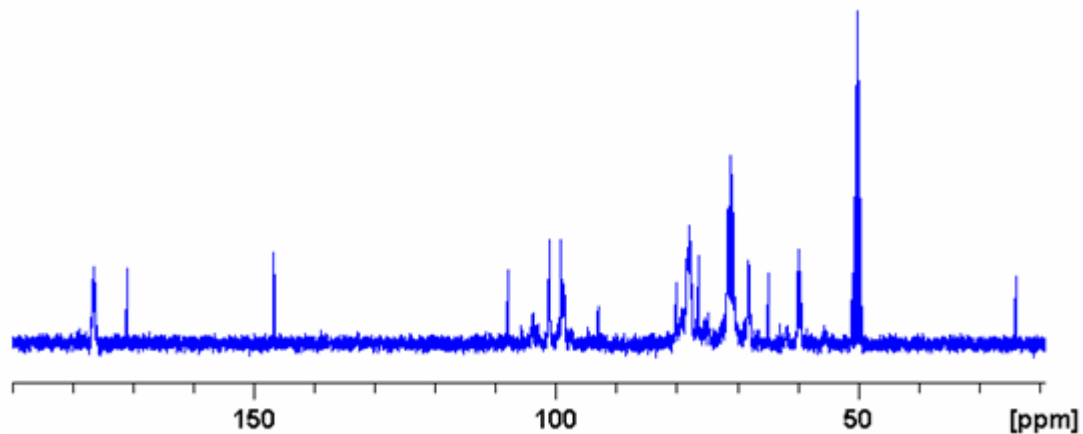
Various methods of heparin depolymerisation are used in the manufacture of LMWH:

1. Oxidative depolymerisation with hydrogen peroxide. Used in the manufacture of [ardeparin](#) (Normiflo)
2. Deaminative cleavage with isoamyl nitrite. Used in the manufacture of [certoparin](#) (Sandoparin)
3. Alkaline beta-eliminative cleavage of the benzyl ester of heparin. Used in the manufacture of [enoxaparin](#) (Lovenox and Clexane)
4. Oxidative depolymerisation with  $\text{Cu}^{2+}$  and hydrogen peroxide. Used in the manufacture of [parnaparin](#) (Fluxum)
5. Beta-eliminative cleavage by the heparinase enzyme. Used in the manufacture of [tinzaparin](#) (Innohep and Logiparin)
6. Deaminative cleavage with nitrous acid. Used in the manufacture of [dalteparin](#) (Fragmin), [reviparin](#) (Clivarin) and [nadroparin](#) (Fraxiparin)



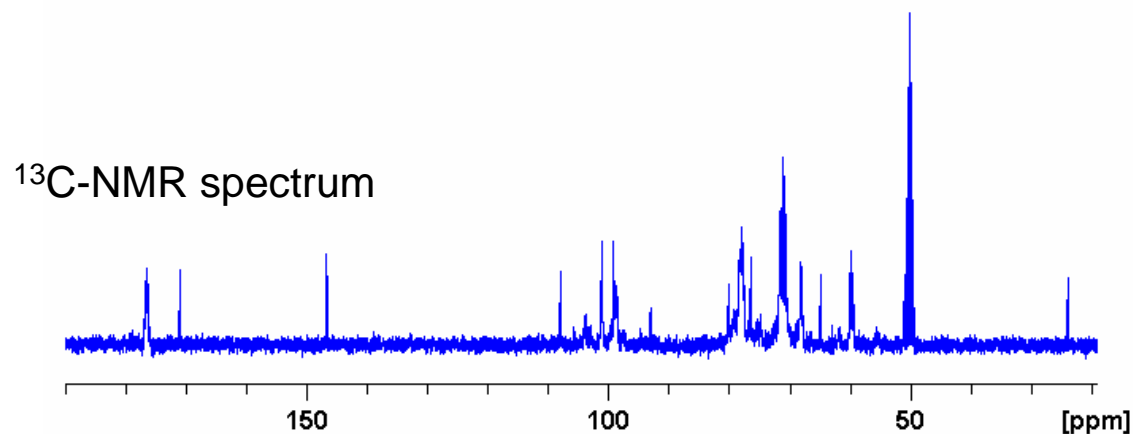
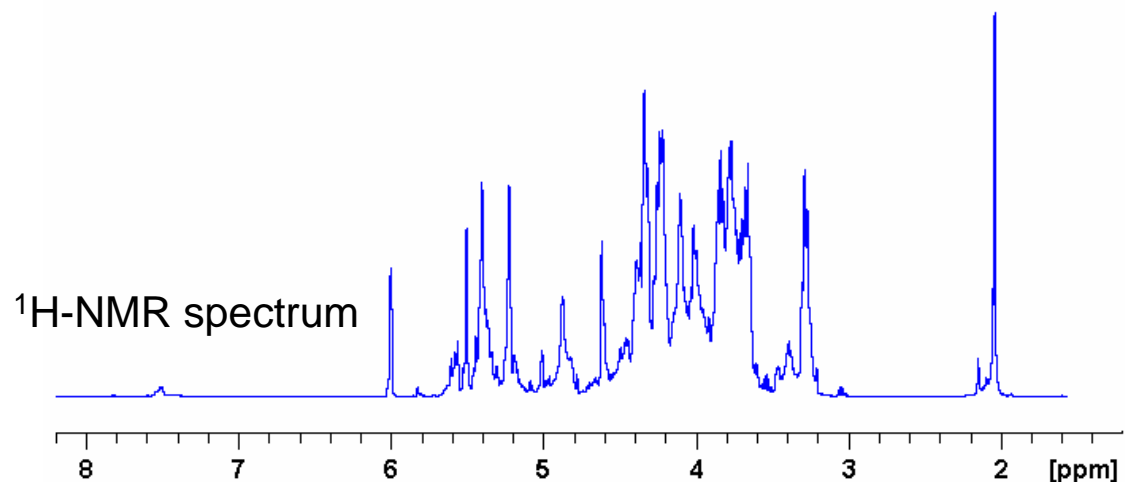
**In different monographs,  $^{13}\text{C}$ -NMR spectra are used for the identification of LMWH**

Could  $^1\text{H}$ -NMR be used instead?



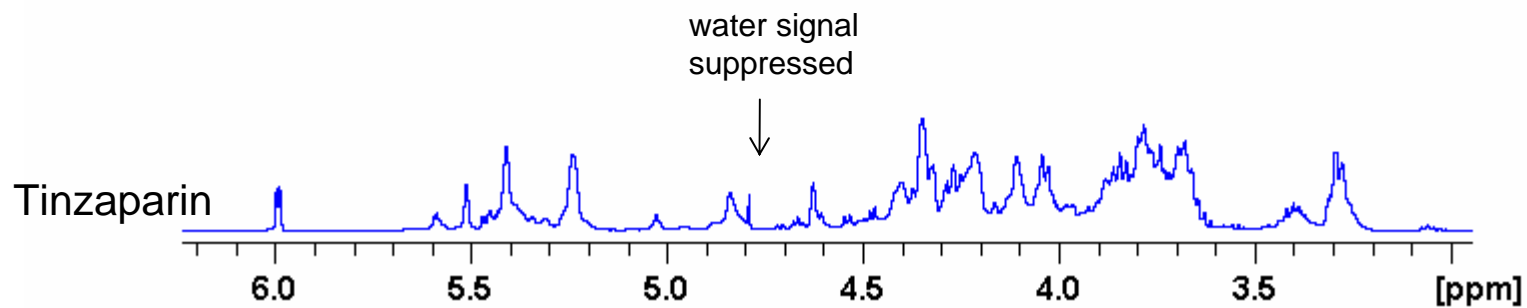
*$^{13}\text{C}$ -NMR spectrum of enoxaparin*

## $^1\text{H}$ -NMR and $^{13}\text{C}$ -NMR spectra of enoxaparin

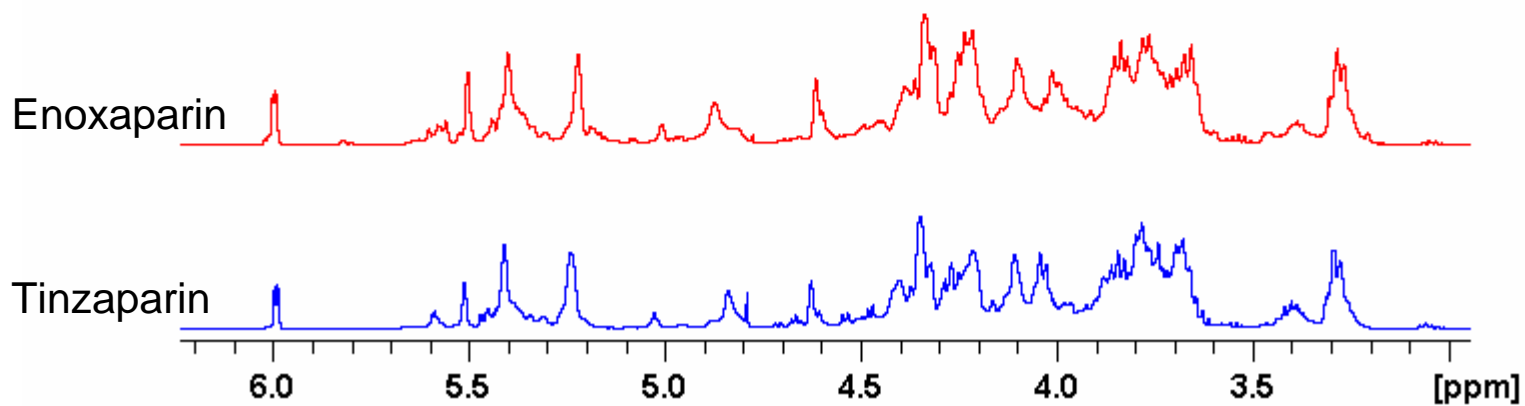


*The  $^1\text{H}$ -NMR spectrum is obtained within 5-15 minutes and the  $^{13}\text{C}$ -NMR spectrum after 8-16 hours; both depending on the strength of the magnetic field of NMR instrument*

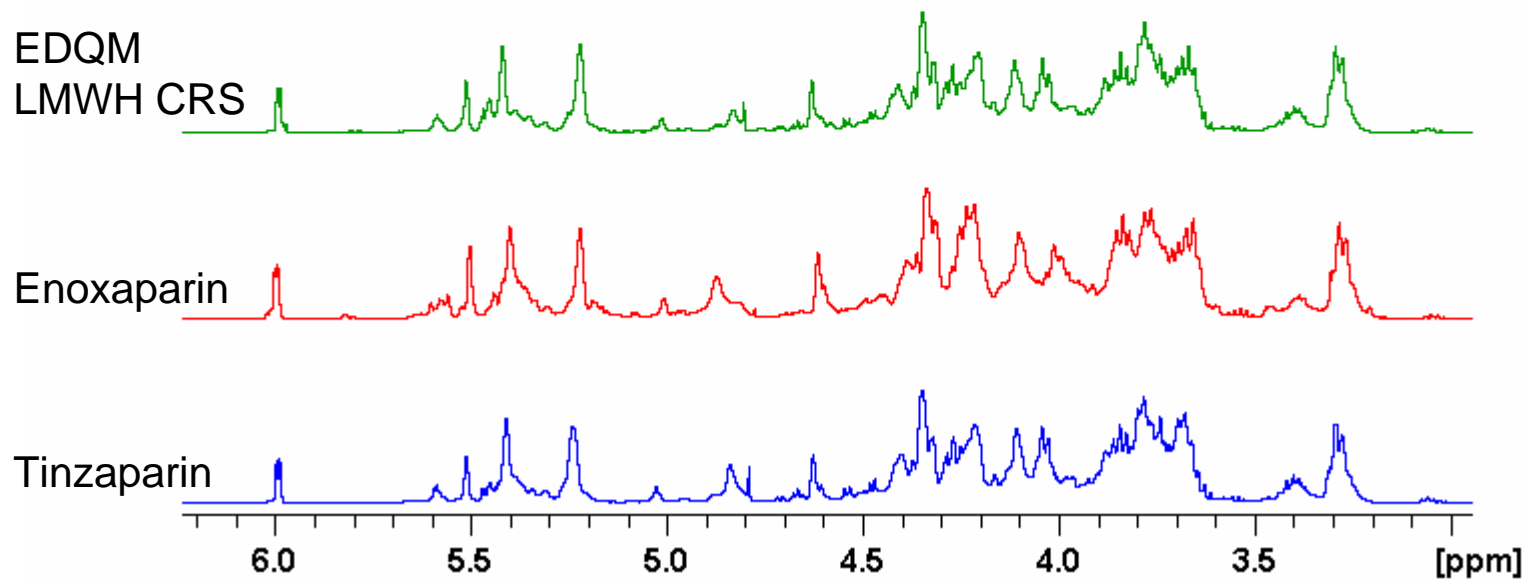
# $^1\text{H}$ -NMR spectra of different LMWH



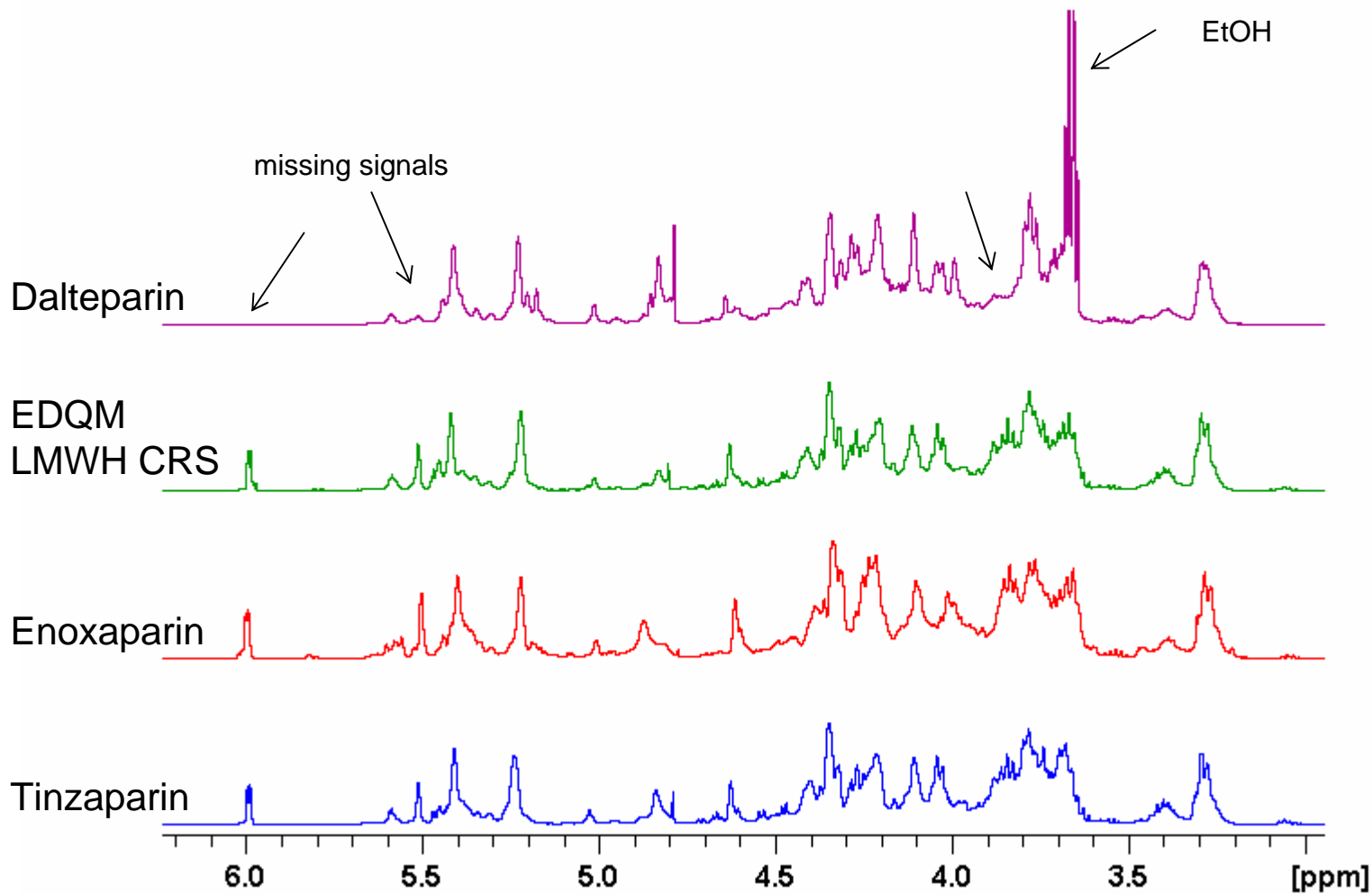
# $^1\text{H-NMR}$ spectra of different LMWH



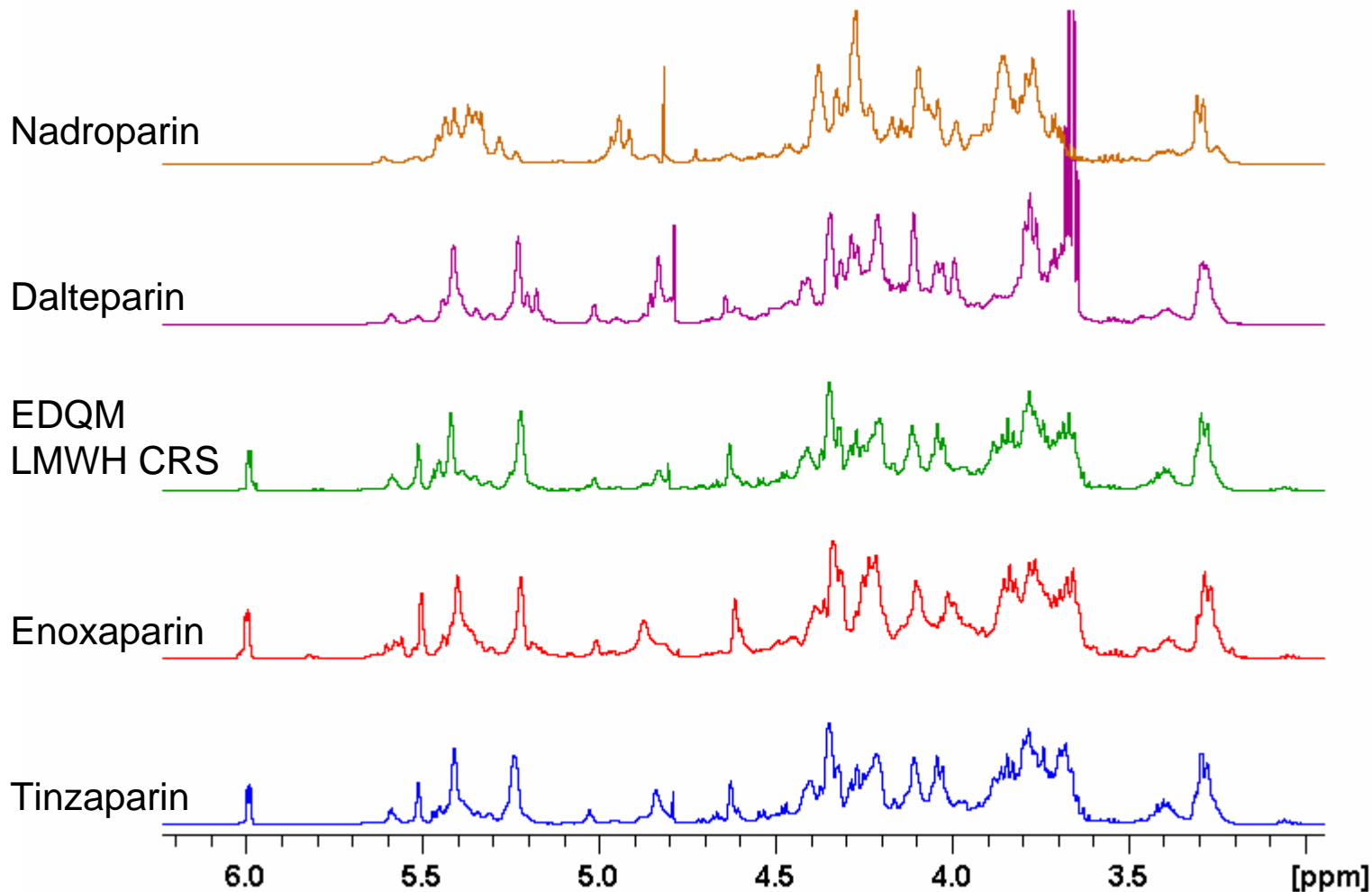
# $^1\text{H-NMR}$ spectra of different LMWH

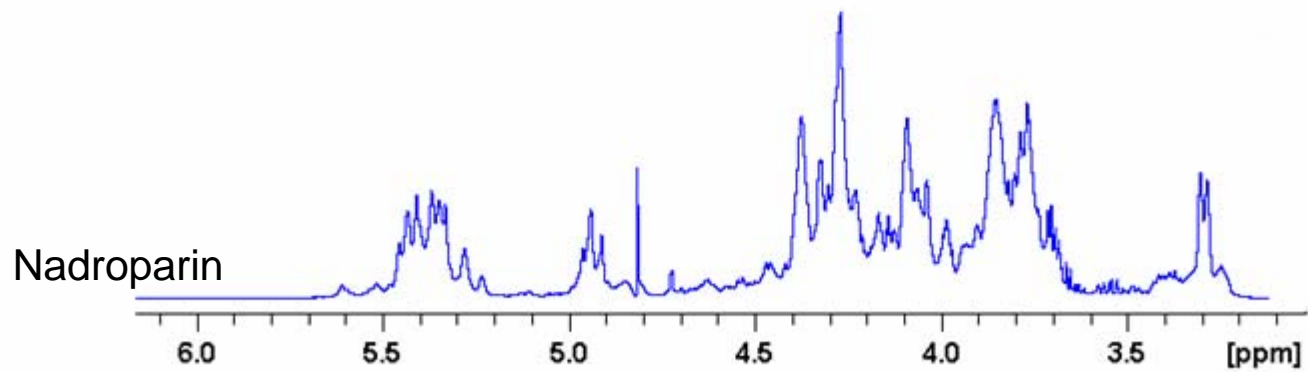


# $^1\text{H-NMR}$ spectra of different LMWH

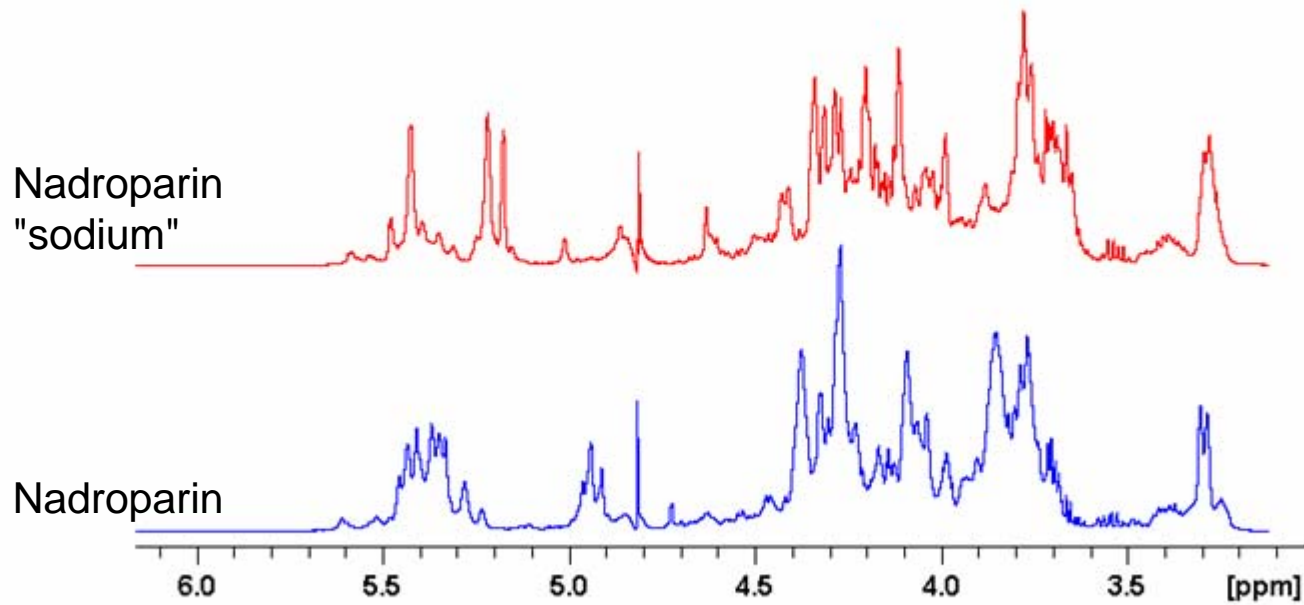


# <sup>1</sup>H-NMR spectra of different LMWH



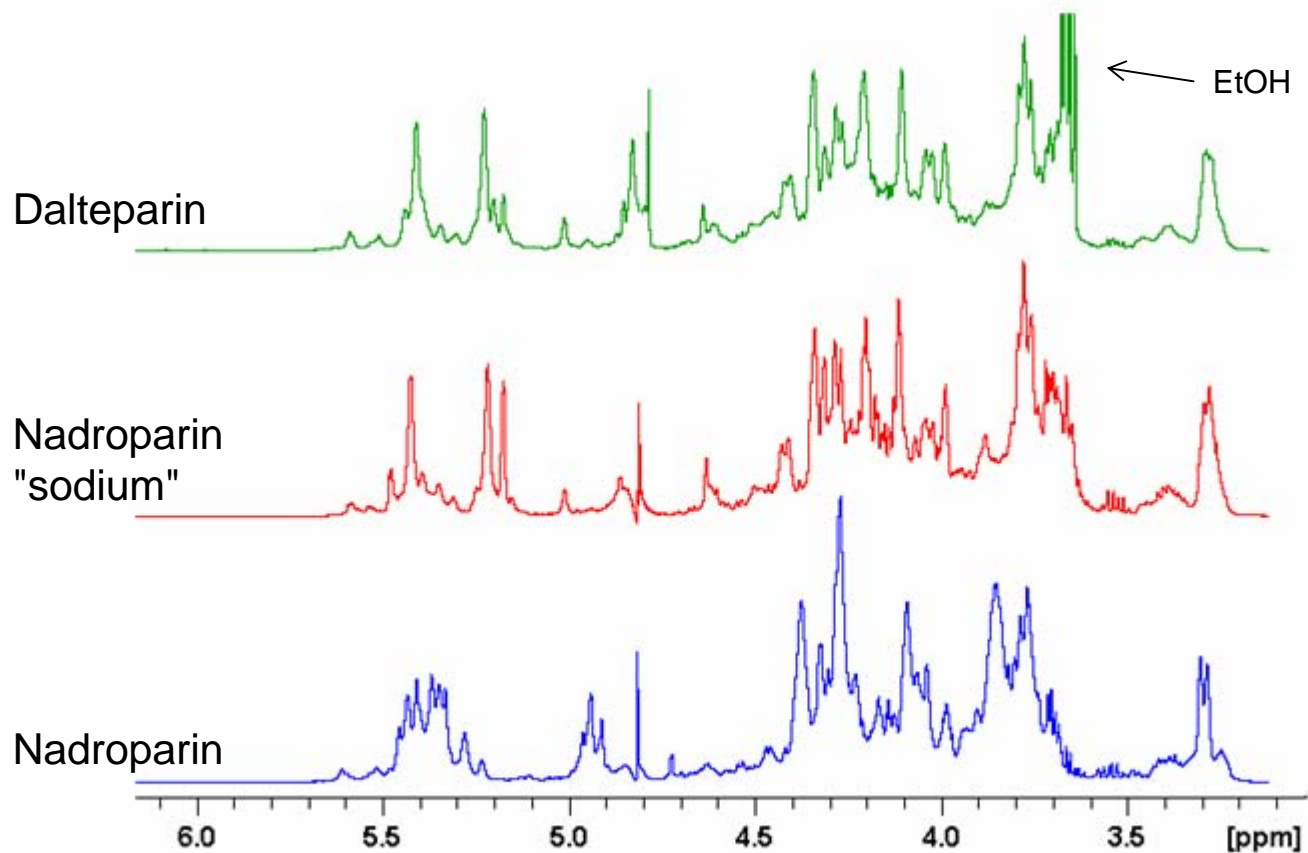


*Nadroparin is a calcium heparin*



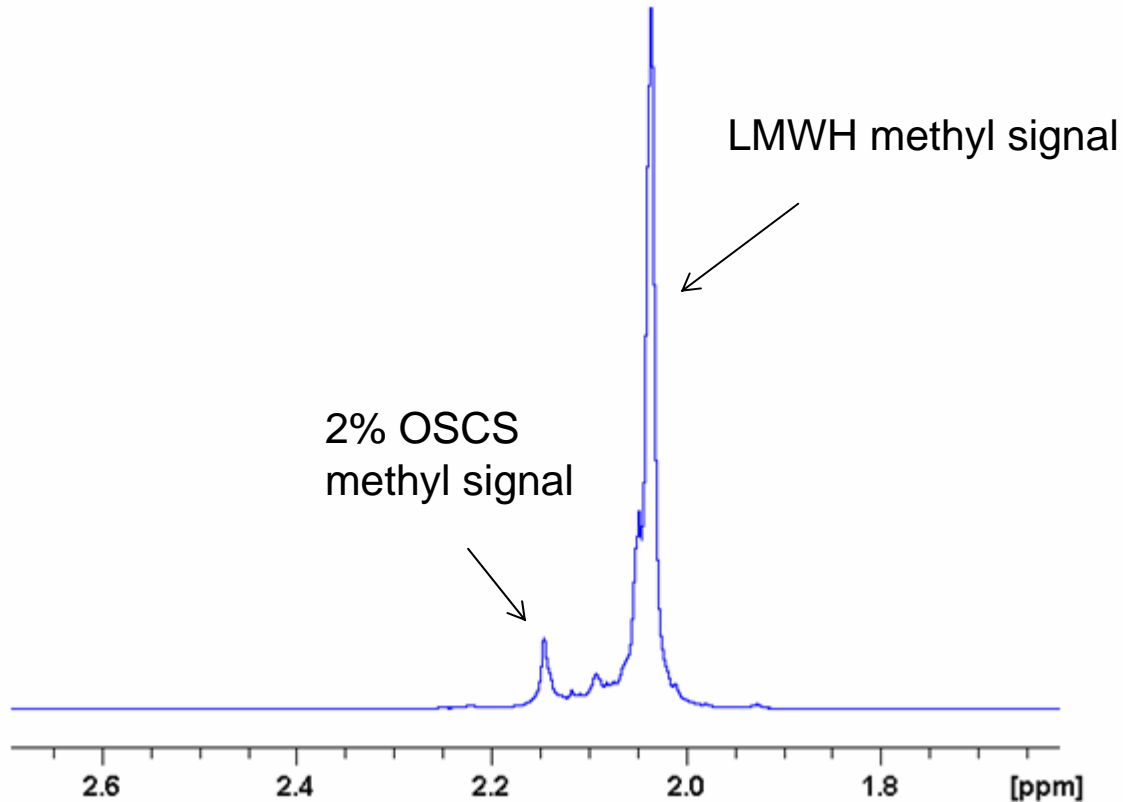
*The calcium ion has been exchanged by sodium ions in nadroparin by passing the solution through Chelex 100*

## $^1\text{H-NMR}$ spectra of nadroparin and dalteparin



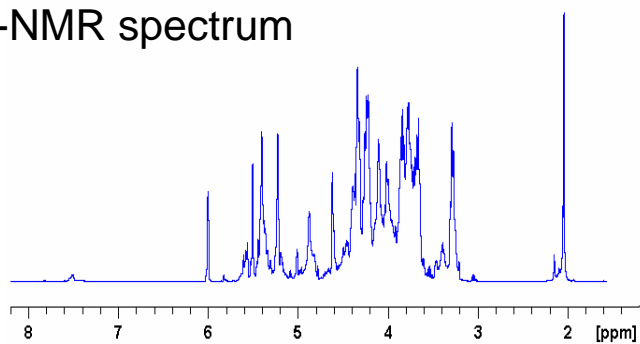
*Nadroparin and dalteparin are manufactured by cleavage with nitrous acid*

## Purity test of LMWH by $^1\text{H-NMR}$

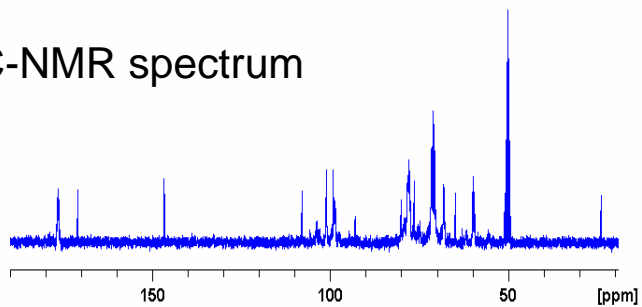


*OSCS is detected by  $^1\text{H-NMR}$  in the same way in LMWH as in UFH, both as APIs and products.*

$^1\text{H}$ -NMR spectrum

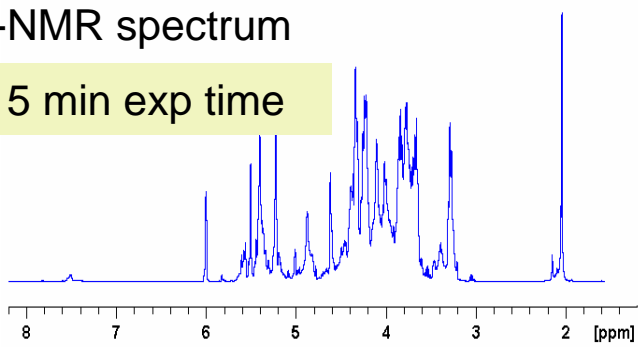


$^{13}\text{C}$ -NMR spectrum



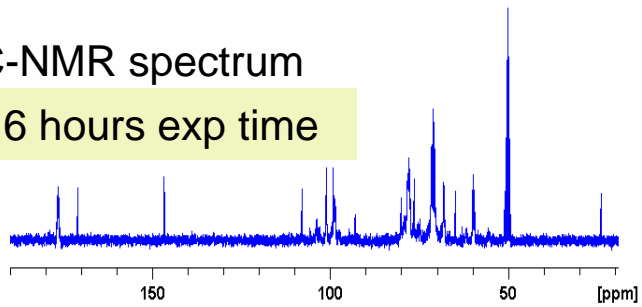
## $^1\text{H}$ -NMR spectrum

5-15 min exp time



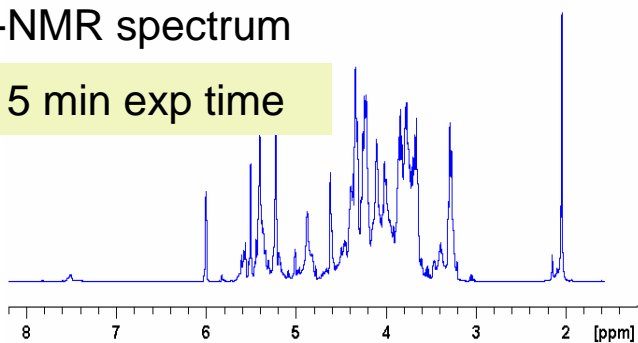
## $^{13}\text{C}$ -NMR spectrum

8-16 hours exp time



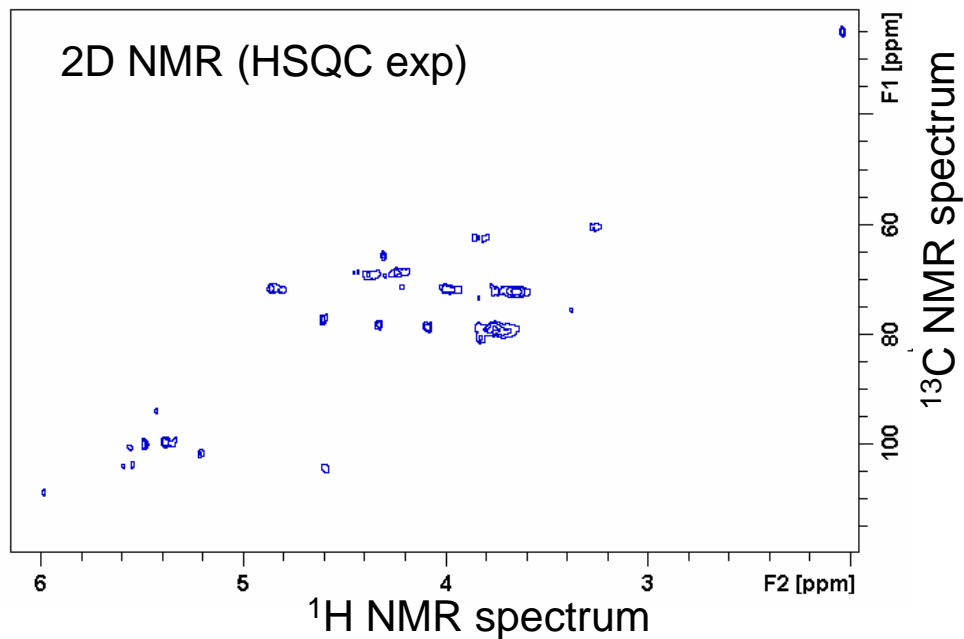
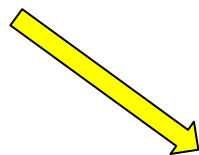
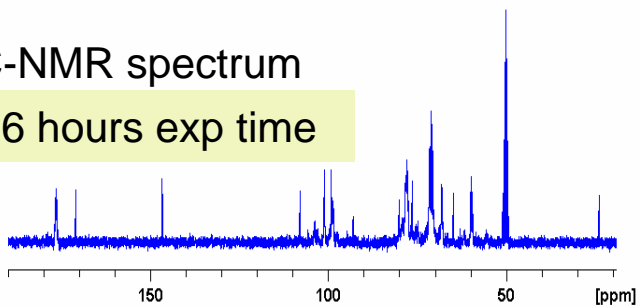
$^1\text{H}$ -NMR spectrum

5-15 min exp time



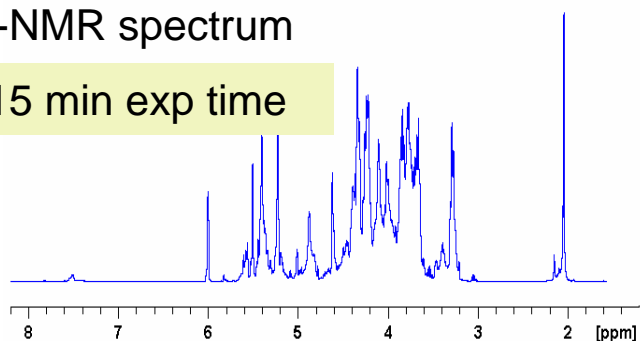
$^{13}\text{C}$ -NMR spectrum

8-16 hours exp time



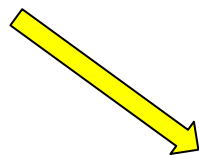
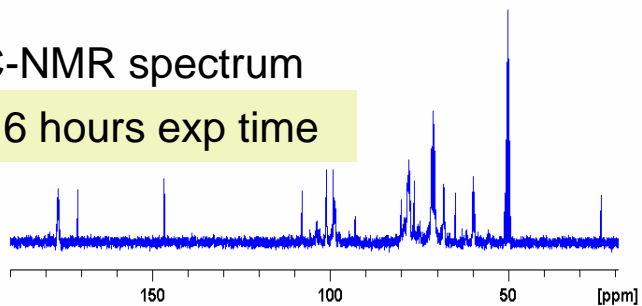
$^1\text{H}$ -NMR spectrum

5-15 min exp time



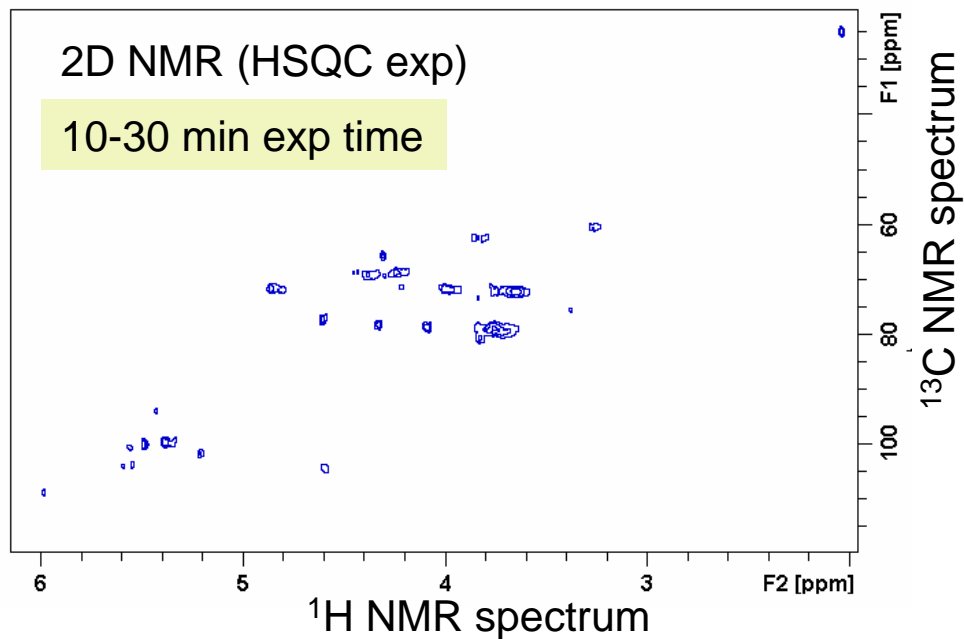
$^{13}\text{C}$ -NMR spectrum

8-16 hours exp time

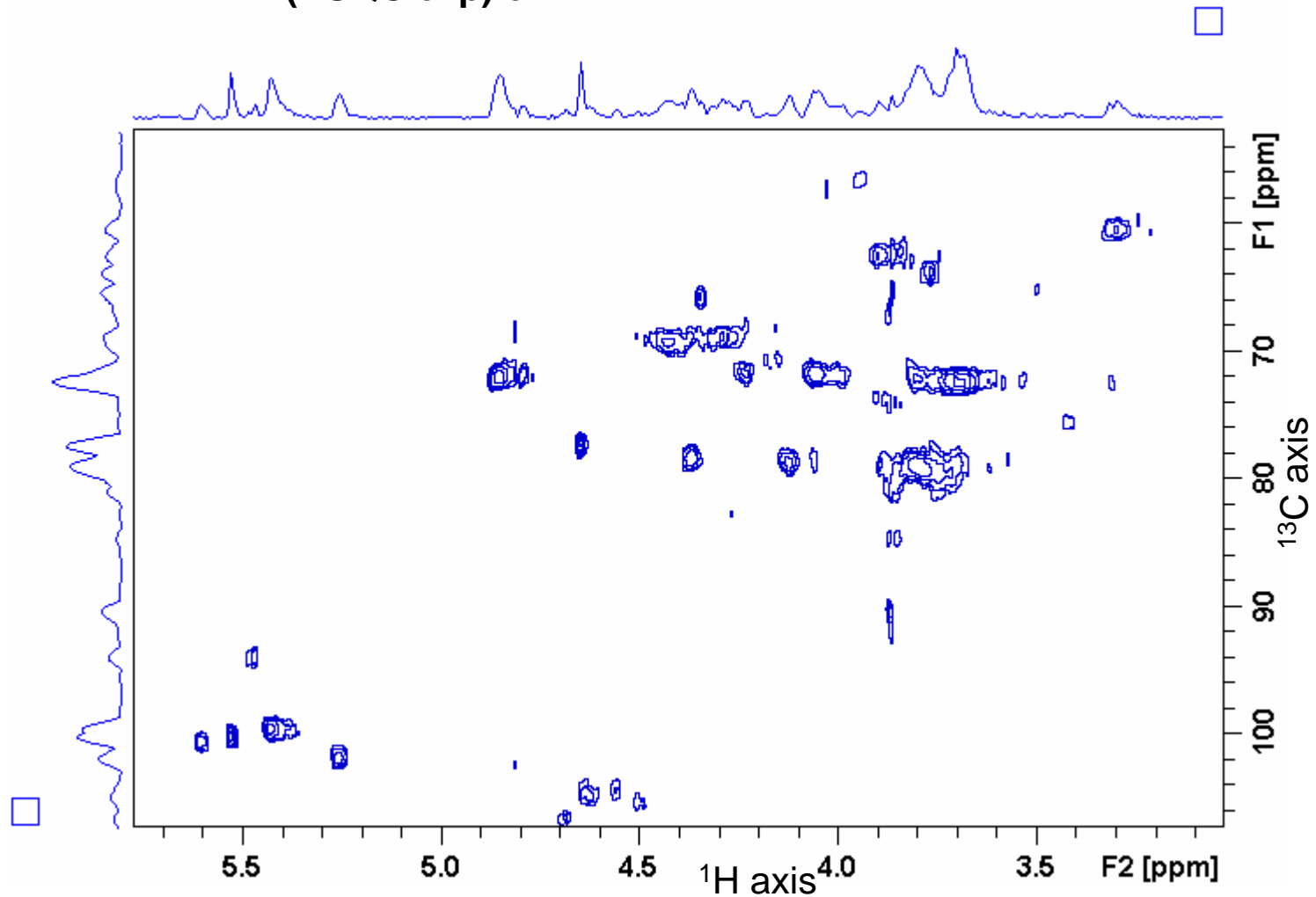


2D NMR (HSQC exp)

10-30 min exp time

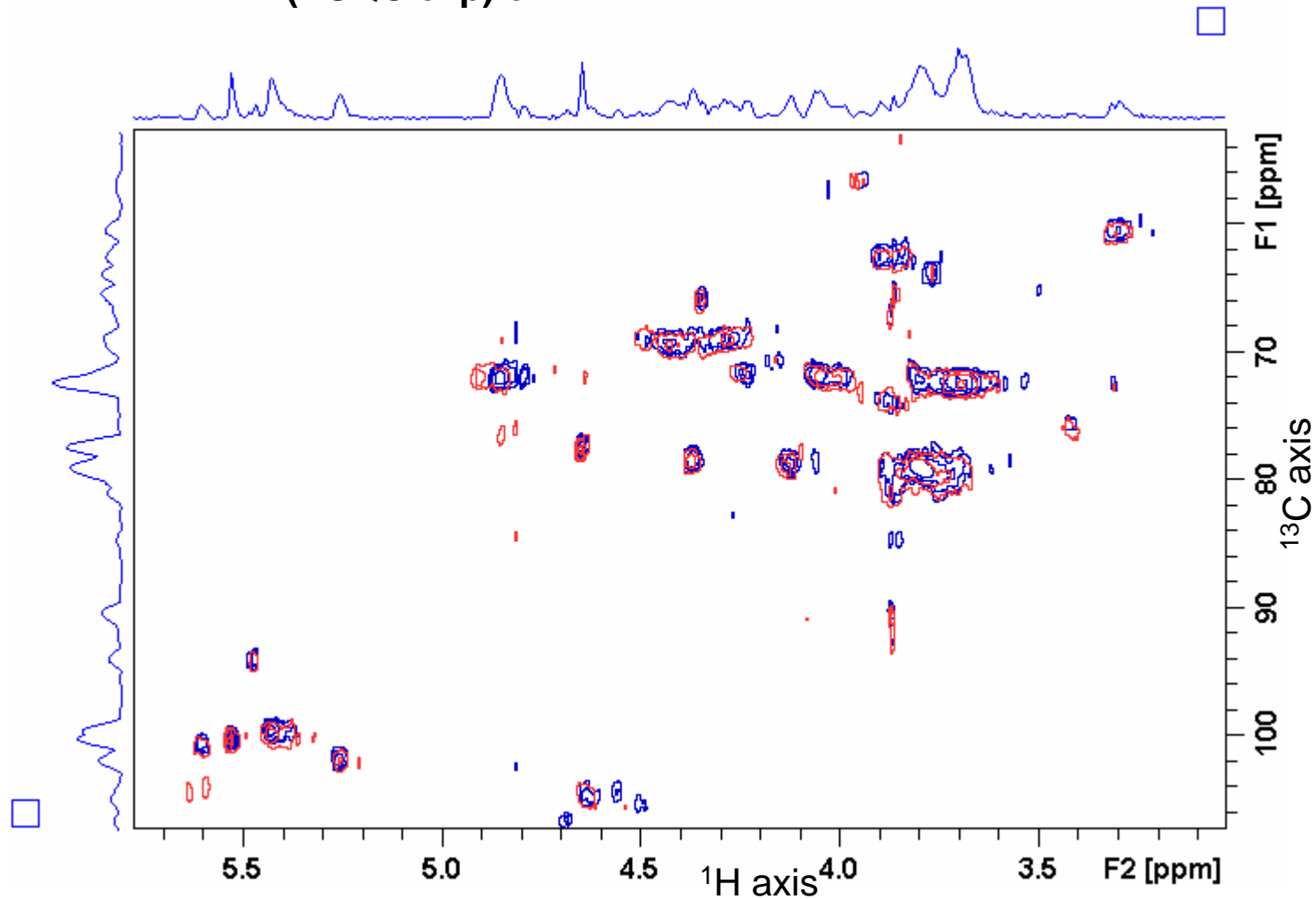


## 2D NMR (HSQC exp) of LMWH



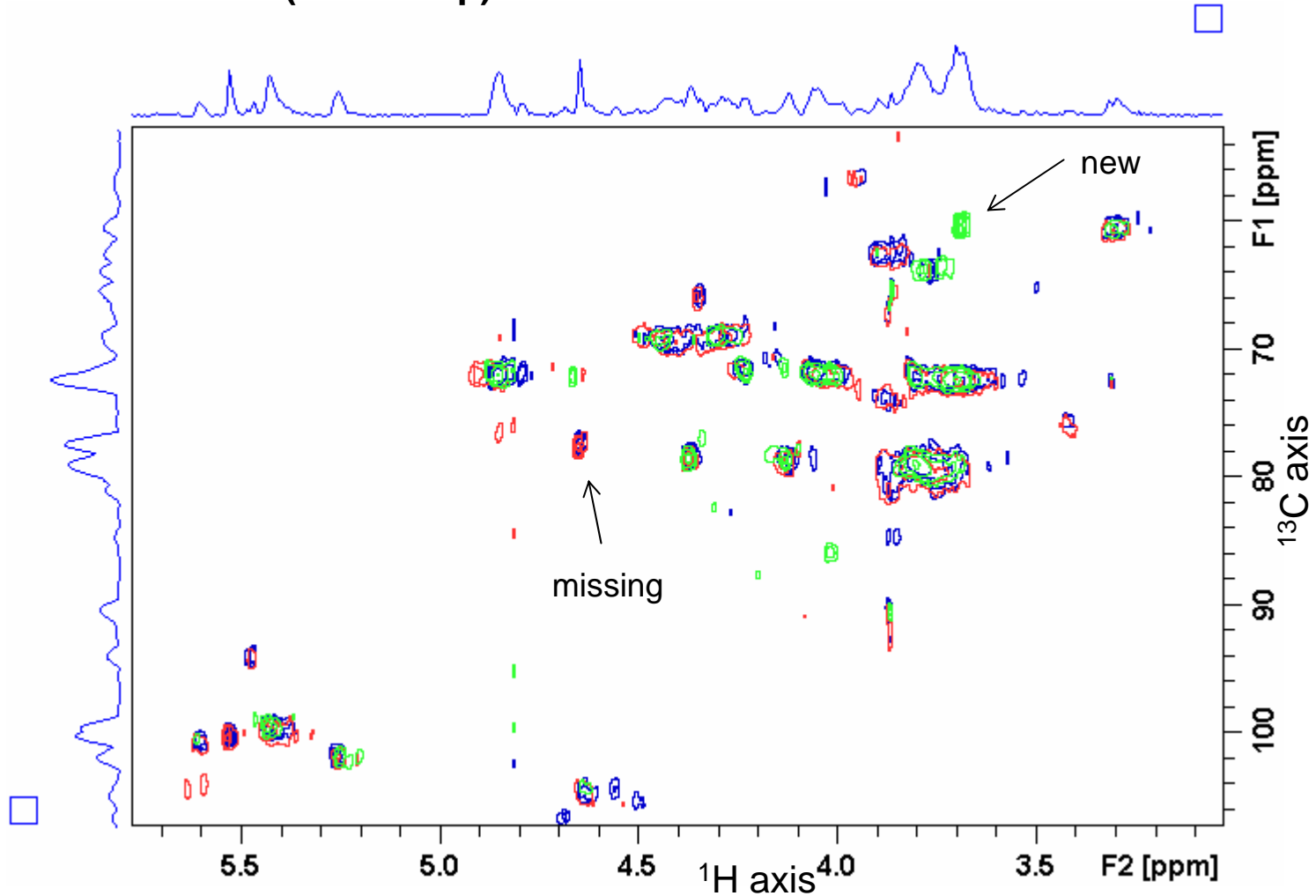
*Blue traces: tinzaparin*

## 2D NMR (HSQC exp) of LMWH



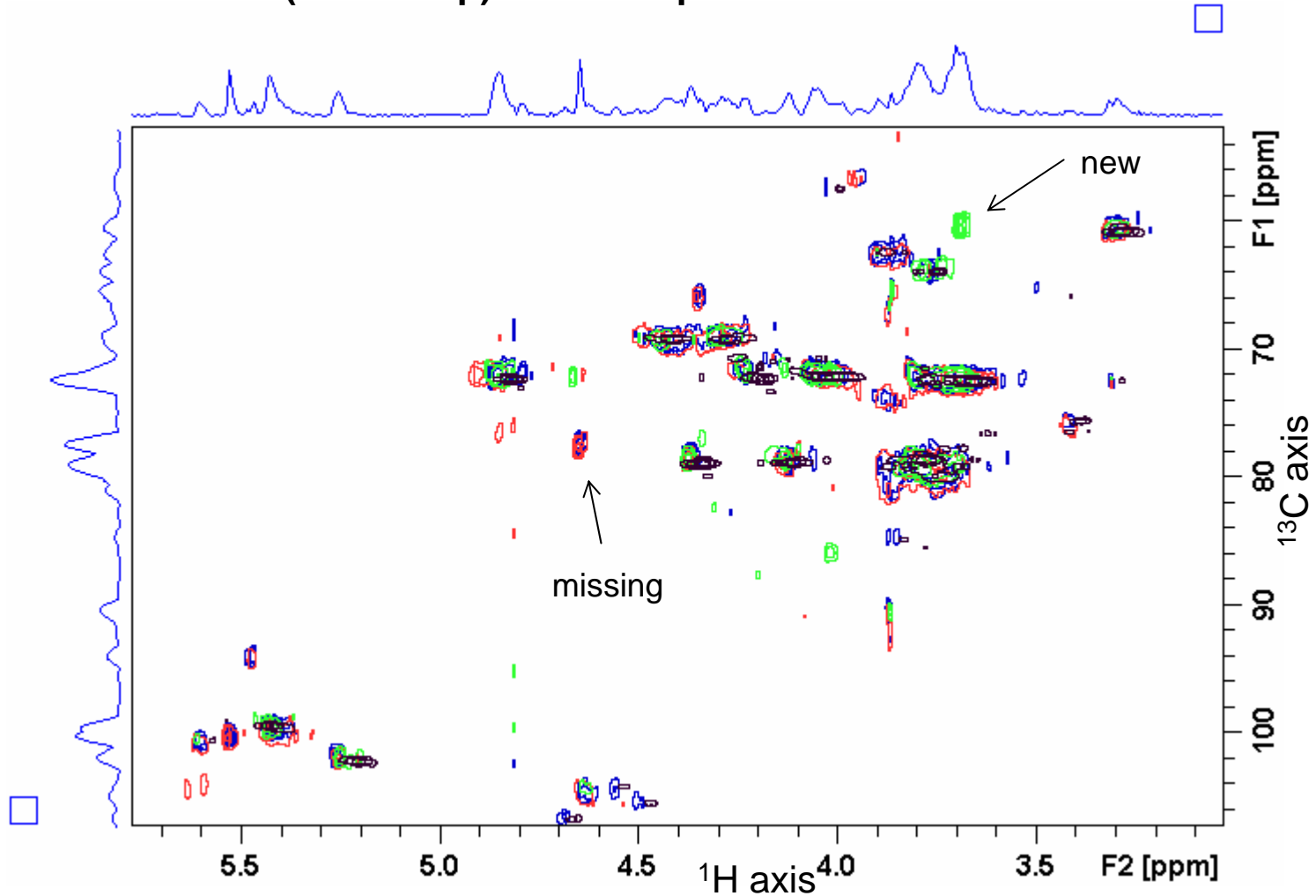
*Blue traces: tinzaparin. Red traces: enoxaparin*

## 2D NMR (HSQC exp) of LMWH



*Blue traces: tinzaparin. Red traces: enoxaparin. Green traces: dalteparin*

## 2D NMR (HSQC exp) of LMWH plus UFH



*Blue traces: tinzaparin. Red traces: enoxaparin. Green traces: dalteparin. Brown traces: UFH*

## Identification by 2D NMR

A proposed list of proton-carbon correlated signals for the identification of LMWH and UFH

Signal	Chemical shifts ( $\delta$ )	
	$^1\text{H} \pm 0.10$ ppm	$^{13}\text{C} \pm 1.5$ ppm
1	5.42	99.3
2	4.34	78.8
3	4.20	72.1
4	3.68	72.4
5	3.28	60.7
6	2.04	24.7

## Conclusions

For the identification of test LMWH samples, a 2D experiment (like HSQC or HMQC) is recommended. It gives an unambiguous answer about the identity of the test sample because the signals in the 2D NMR experiment are well spread. It is like obtaining a  $^1\text{H}$ -NMR and a  $^{13}\text{C}$ -NMR spectrum at the same time

The experiment time is between 10 and 20 minutes depending on the strength of the magnetic field of the NMR instrument and the probe used.

The experiment setup is simple and the processing of data is not complicated