Supplementary Information

Oversulfated Chondroitin Sulfate Control in Low Molecular Weight and Unfractioned Heparins

Diffusion Ordered Nuclear Magnetic Resonance Spectroscopy Method

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Contents:

1. Experimental details
2. Sample preparation
3. Pulsed Field Gradient Spin Echo Experiments

Experimental details

All batches of heparins used were collected from international markets by office of General Pharmaceutical Inspector. The low molecular weight heparin pharmaceutical products based on enoxaparin were taken from market.

The $^1$H NMR spectra were run on VARIAN NMR SYSTEM 500 MHz spectrometer using triple resonance probe equipped with 60 Gauss/cm Z gradient unit, at 25º C. The chemical shifts were referenced vs. internal TSPA standard dissolved in D$_2$O.

S1
Sample preparation

All samples were prepared in 650 µL of H₂O/D₂O (1:5 vol %) with internal TSPA standard, except market product, see below. Samples concentration of solution for each figure are as follows: Fig 1A, OSCS, 10 mg; Fig 1B, LMWH standard, 10 mg; Fig 1C, OSCS –, LMWH standard 1 mg + 16 mg; Fig 2A, Heparin high molecular weight, 25 mg; Fig 2B as in Fig 1B; Fig 2C, heparin high molecular weight + LMWH standard, 10 mg + 10 mg; Fig 3A as in Fig 1A; Fig 3B, LMWH, market product, 110 µL injection + 550 µL D₂O; Fig 3C, contaminated LMWH, market product, 110 mL injection + 550 µL D₂O; Fig 3D, contaminated LMWH, market product + OSCS, 200 mL of injection solution + 450 µL D₂O + 1.3 mg OSCS.

Pulsed Gradient Spin Echo (PGSE) experiments were performed according to the following conditions: 16-20 spectra were acquired using the Dpfdgteste¹, (PFG Double Stimulated Echo with convection compensation) with water presaturation. The z-gradient coil constant was calibrated using known diffusion coefficients of water (Dᵣ = 19.02 x 10⁻¹⁰ m²/s).² The gradient strengths were incremented as a square dependence in the range from 2 to 50 G/cm. The diffusion time (Δ) and the duration of magnetic field gradients (δ) were 400 ms and 2 ms, respectively. Other parameters include the following: a sweep width of 6000 Hz, 32K data points, 64 scans and an acquisition time 2.7 s and relaxation delay of 2 s and 16 dummy scans. The data were processed using Varian DOSY³ package.