

08/2010:0333

HEPARIN SODIUM

Heparinum natricum

DEFINITION

Preparation containing the sodium salt of a sulfated glycosaminoglycan present in mammalian tissues. It is prepared either from the lungs of cattle or from the intestinal mucosae of pigs, cattle or sheep. On complete hydrolysis, it liberates D-glucosamine, D-glucuronic acid, L-iduronic acid, acetic acid and sulfuric acid. It has the property of delaying the clotting of blood.

Potency: minimum 180 IU/mg (dried substance).

PRODUCTION

The animals from which heparin sodium is derived must fulfil the requirements for the health of animals suitable for human consumption. All stages of production and sourcing are subjected to a suitable quality management system. The identity of the source species and the absence of material from the other species is verified by appropriate testing during production.

It is produced by methods of manufacturing designed to minimise or eliminate substances lowering blood pressure.

CHARACTERS

Appearance: white or almost white, hygroscopic powder.

Solubility: freely soluble in water.

IDENTIFICATION

A. It delays the clotting of recalcified citrated sheep plasma (see Assay).

B. Nuclear magnetic resonance spectrometry (2.2.33).

Solution A. A solution in *deuterium oxide R* containing 20 µg/ml of *deuterated sodium trimethylsilylpropionate R* and if the signal at 5.22 ppm is smaller than 80 per cent of the signal at 5.44 ppm, 12 µg/ml of *sodium edetate R*.

Preparation: dissolve 20 mg of the substance to be examined in 0.7 ml of solution A.

Comparison: dissolve 20 mg of *heparin sodium for NMR identification CRS* in 0.7 ml of solution A.

If stored, the sodium edetate and deuterated sodium trimethylsilylpropionate solutions must be kept in high-density, natural polyethylene bottles.

Apparatus: spectrometer operating at minimum 300 MHz.

Acquisition of ¹H-NMR spectra:

- *number of transients*: minimum 16; it is adjusted until the signal-to-noise ratio is at least 1000:1 for the heparin methyl signal at 2.04 ppm;
- *temperature*: about 25 °C; test sample and reference spectra have to be obtained at the same temperature;
- *acquisition time*: minimum 2 s;
- *repetition time* (acquisition time plus delay): minimum 4 s;
- *spectral width*: 10-12 ppm, centred at around 4.5 ppm;
- *pulse width*: to give a flip angle between 30° and 90°.

Processing:

- *exponential line-broadening window function*: 0.3 Hz;
- Fourier transformation;
- trimethylsilylpropionate reference signal set at 0.00 ppm.

Results:

- the large heparin sodium signals must be present: 2.04 ppm, 3.27 ppm (doublet), 4.34 ppm, 5.22 ppm and 5.42 ppm, all within ± 0.03 ppm;
- the ¹H-NMR spectrum obtained with the test sample and that obtained with *heparin sodium for NMR identification CRS* are compared qualitatively after the 2 spectra have been normalised so as to have a similar intensity; dermatan sulfate with a methyl signal at 2.08 ± 0.02 ppm may be observed; no unidentified signals larger than 4 per cent compared to the height of the heparin signal at 5.42 ppm are present in the ranges 0.10-2.00 ppm, 2.10-3.10 ppm and 5.70-8.00 ppm; signals from the solvent or process-related substances may be present and have to be identified to be accepted; variations in the intensity of some signal regions of the spectrum of heparin may occur: the intensity-variable regions are between 3.35 ppm and 4.55 ppm, where the signal pattern is approximately kept but intensity varies.

C. Liquid chromatography (2.2.29) as described in the test for related substances with the following modifications.

Injection: test solution (a) and reference solution (c).

Relative retention with reference to heparin (retention time = about 26 min): dermatan sulfate and chondroitin sulfate = about 0.9; over-sulfated chondroitin sulfate = about 1.3.

System suitability: reference solution (c):

- *peak-to-valley ratio*: minimum 1.3, where H_p = height above the baseline of the peak due to dermatan sulfate + chondroitin sulfate and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to heparin.

Results: the principal peak in the chromatogram obtained with test solution (a) is similar in retention time and shape to the principal peak in the chromatogram obtained with reference solution (c).

D. It complies with the test for sodium (see Tests).

1 TESTS

2 **Appearance of solution.** The solution is clear (2.2.1) and not more intensely coloured
3 than intensity 5 of the range of reference solutions of the most appropriate colour (2.2.2,
4 *Method II*).

5 Dissolve a quantity equivalent to 50 000 IU in *water R* and dilute to 10 ml with the same
6 solvent.

7 **pH (2.2.3):** 5.5 to 8.0.

8 Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

9 **Nucleotidic impurities.** Dissolve 40 mg in 10 ml of *water R*. The absorbance (2.2.25)
10 measured at 260 nm is not greater than 0.15.

11 **Protein:** maximum 0.5 per cent (dried substance).

12 *Solution A.* Mix 2 volumes of a 10 g/l solution of *sodium hydroxide R* and 2 volumes of a
13 50 g/l solution of *sodium carbonate R* and dilute to 5 volumes with *water R*.

14 *Solution B.* Mix 2 volumes of a 12.5 g/l solution of *copper sulfate R* and 2 volumes of a
15 29.8 g/l solution of *sodium tartrate R* and dilute to 5 volumes with *water R*.

16 *Solution C.* Mix 1 volume of solution B and 50 volumes of solution A.

17 *Solution D.* Dilute a phosphomolybdotungstic reagent⁽¹⁾ 2- to 4-fold in *water R*. Suitable
18 dilutions produce solutions of pH 10.25 ± 0.25 after addition of solutions C and D to
19 the test and reference solutions.

20 *Test solution.* Dissolve the substance to be examined in *water R* to obtain a concentration
21 of 5 mg/ml.

22 *Reference solutions.* Dissolve *bovine albumin R* in *water R* to obtain a concentration of
23 100 mg/ml. Prepare dilutions of the solution in *water R* as prescribed in general chapter
24 2.5.33, *method 2*.

25 *Blank: water R.*

26 *Procedure.* To 1 ml of each reference solution, of the test solution and of the blank, add
27 5 ml of solution C. Allow to stand for 10 min. Add 0.5 ml of solution D, mix and allow
28 to stand at room temperature for 30 min. Determine the absorbances (2.2.25) of the
29 solutions at 750 nm, using the solution prepared from the blank as compensation liquid.

30 *Calculations.* As prescribed in general chapter 2.5.33, *method 2*.

31 **Related substances.** Liquid chromatography (2.2.29). *Reference solutions are stable at*
32 *room temperature for 24 h.*

33 *Test solution (a).* Dissolve an accurately weighed quantity of about 50 mg of the substance
34 to be examined in 5.0 ml of *water for chromatography R*. Mix using a vortex mixer until
35 dissolution is complete.

36 *Test solution (b).* Dissolve an accurately weighed quantity of about 0.1 g of the substance
37 to be examined in 1.0 ml of *water for chromatography R*. Mix using a vortex mixer until
38 dissolution is complete. Mix 500 μ l of the solution and 250 μ l of 1 M *hydrochloric acid*,
39 then add 50 μ l of a 250 mg/ml solution of *sodium nitrite R*⁽²⁾. Mix gently and allow to
40 stand at room temperature for 40 min before adding 200 μ l of 1 M *sodium hydroxide*
41 to stop the reaction.

42 (1) Folin-Ciocalteu's phenol reagent from Merck (reference 1.09001.0500) is suitable.

43 (2) Sodium nitrite, analytical reagent grade from Fischer scientific (batch 0886083) is suitable.

1 *Reference solution (a).* Dissolve 250 mg of *heparin for physico-chemical analysis CRS*
 2 in *water for chromatography R* and dilute to 2.0 ml with the same solvent. Mix using a
 3 vortex mixer until dissolution is complete.

4 *Reference solution (b).* Add 1200 µl of reference solution (a) to 300 µl of *dermatan sulfate*
 5 *and over-sulfated chondroitin sulfate CRS*. Mix using a vortex mixer to homogenise.

6 *Reference solution (c).* Add 100 µl of reference solution (b) to 900 µl of *water for*
 7 *chromatography R*. Mix using a vortex mixer to homogenise.

8 *Reference solution (d).* Add 400 µl of reference solution (a) to 100 µl of *water for*
 9 *chromatography R* and mix using a vortex mixer. Add 250 µl of *1 M hydrochloric acid*,
 10 then add 50 µl of a 250 mg/ml solution of *sodium nitrite R*. Mix gently and allow to
 11 stand at room temperature for 40 min before adding 200 µl of *1 M sodium hydroxide*
 12 to stop the reaction.

13 *Reference solution (e).* To 500 µl of reference solution (b), add 250 µl of *1 M hydrochloric*
 14 *acid*, then add 50 µl of a 250 mg/ml solution of *sodium nitrite R*. Mix gently and allow
 15 to stand at room temperature for 40 min before adding 200 µl of *1 M sodium hydroxide*
 16 to stop the reaction.

17 *Precolumn:*

18 – *size:* $l = 0.05$ m, $\varnothing = 2$ mm;

19 – *stationary phase:* *anion exchange resin R* (13 µm)⁽³⁾.

20 *Column:*

21 – *size:* $l = 0.25$ m, $\varnothing = 2$ mm;

22 – *stationary phase:* *anion exchange resin R* (9 µm)⁽⁴⁾;

23 – *temperature:* 40 °C.

24 *Mobile phase:*

25 – *mobile phase A:* dissolve 0.40 g of *sodium dihydrogen phosphate R* in 1 L of *water for*
 26 *chromatography R* and adjust to pH 3.0 with *dilute phosphoric acid R*;

27 – *mobile phase B:* dissolve 0.40 g of *sodium dihydrogen phosphate R* in 1 L of *water for*
 28 *chromatography R*, add 140 g of *sodium perchlorate R*⁽⁵⁾ and adjust to pH 3.0 with
 29 *dilute phosphoric acid R*; filter and degas;

Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 10	75	25
10 - 35	75 → 0	25 → 100
35 - 40	0	100

30 *Flow rate:* 0.22 ml/min.

31 *Detection:* spectrophotometer at 202 nm.

32 *Equilibration:* at least 15 min.

33 *Injection:* 20 µl of test solution (b) and reference solutions (d) and (e).

34 *Relative retention* with reference to heparin (retention time = about 26 min): dermatan
 35 sulfate and chondroitin sulfate = about 0.9; over-sulfated chondroitin sulfate = about 1.3.

36 (3) AG11-HC from Dionex (reference 052963) is suitable.

37 (4) AS11-HC from Dionex (reference 052961) is suitable.

38 (5) Normapur from VWR/Prolabo (reference 27988.232) is suitable.

1 *System suitability:*

- 2
3 – the chromatogram obtained with reference solution (d) shows no peak at the retention
4 time of heparin;
5 – *resolution*: minimum 3.0 between the peaks due to dermatan sulfate + chondroitin
6 sulfate and over-sulfated chondroitin sulfate in the chromatogram obtained with
7 reference solution (e).

8 *Limits:*

- 9
10 – *sum of dermatan sulfate and chondroitin sulfate*: not more than the area of the
11 corresponding peak in the chromatogram obtained with reference solution (e) (2.0 per
12 cent);
13 – *any other impurity*: no peaks other than the peak due to dermatan sulfate
14 + chondroitin sulfate are detected.

15
16 **Nitrogen (2.5.9)**: 1.5 per cent to 2.5 per cent (dried substance), determined on 0.100 g.

17 **Sodium**: 9.5 per cent to 12.5 per cent (dried substance).

18 Atomic absorption spectrometry (2.2.23, *Method I*).

19
20 *Test solution*. Dissolve 50 mg of the substance to be examined in a 1.27 mg/ml solution
21 of *caesium chloride R* in 0.1 M *hydrochloric acid* and dilute to 100.0 ml with the same
22 solvent.

23 *Reference solutions*. Prepare reference solutions containing 25 ppm, 50 ppm and 75 ppm
24 of Na, using *sodium standard solution (200 ppm Na) R* diluted with a 1.27 mg/ml
25 solution of *caesium chloride R* in 0.1 M *hydrochloric acid*.

26
27 *Source*: sodium hollow-cathode lamp.

28 *Wavelength*: 330.3 nm.

29
30 *Atomisation device*: flame of suitable composition (for example 11 L of air and 2 L of
31 acetylene per minute).

32 **Heavy metals (2.4.8)**: maximum 30 ppm.

33
34 1.0 g complies with test F. Prepare the reference solution using 3.0 ml of *lead standard*
35 *solution (10 ppm Pb) R*.

36 **Loss on drying (2.2.32)**: maximum 8.0 per cent, determined on 1.000 g by drying at 60 °C
37 over *diphosphorus pentoxide R* at a pressure not exceeding 670 Pa for 3 h.

38
39 **Bacterial endotoxins (2.6.14)**: less than 0.01 IU per International Unit of heparin,
40 if intended for use in the manufacture of parenteral preparations without a further
41 appropriate procedure for the removal of bacterial endotoxins.

42
43 **ASSAY**

44 Carry out the assay of heparin (2.7.5). The estimated potency is not less than 90 per
45 cent and not more than 111 per cent of the stated potency. The confidence limits of the
46 estimated potency ($P = 0.95$) are not less than 80 per cent and not more than 125 per
47 cent of the stated potency.

1 STORAGE

2 In an airtight container. If the substance is sterile, store in a sterile, airtight, tamper-proof
3 container.
4

5 LABELLING

6 The label states:

- 7 – the number of International Units per milligram;
- 8 – the animal species of origin;
- 9
- 10 – where applicable, that the substance is suitable for use in the manufacture of parenteral
11 preparations.
12

13
14 **Reagents**

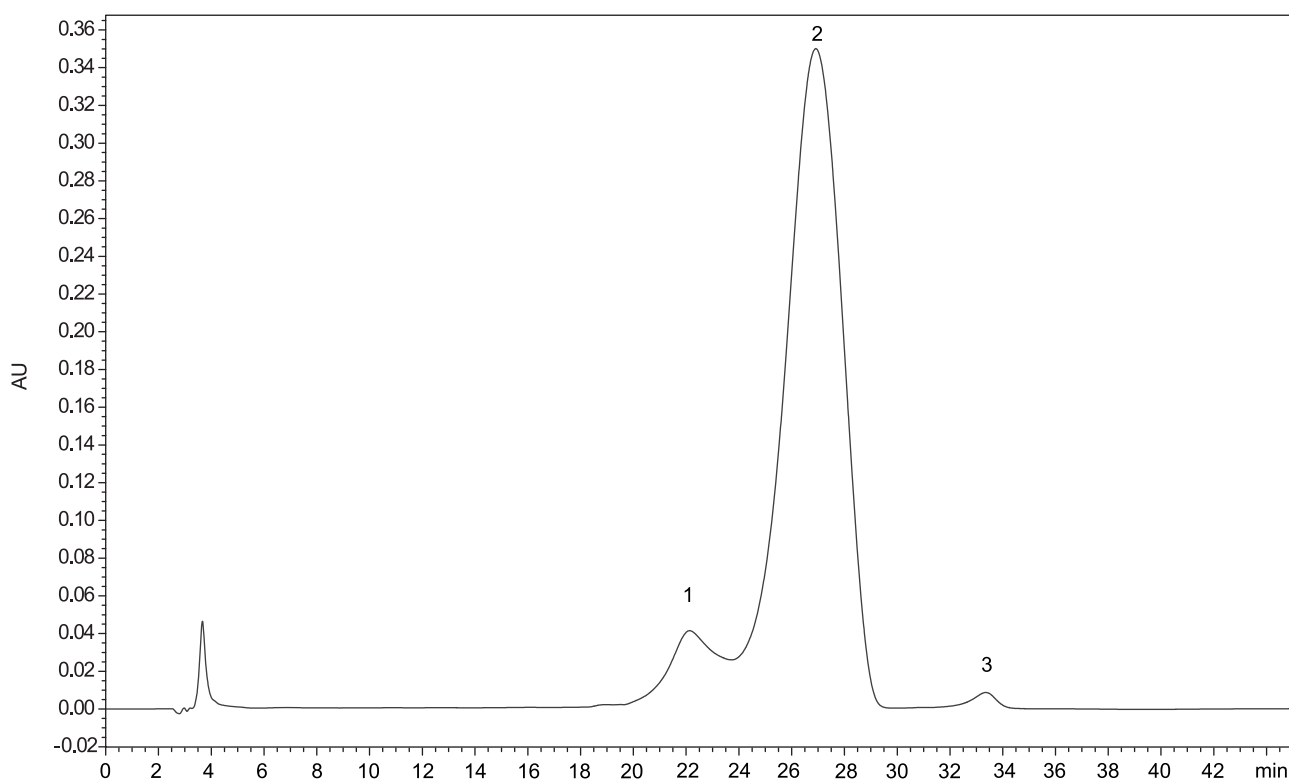
15
16 **Deuterated sodium trimethylsilylpropionate.** $C_6H_9^2H_4NaO_2Si$. (M_r 172.3). XXXXXXXX.
17 [24493-21-8].

18 Sodium 3-(trimethylsilyl)(2,2,3,3- 2H_4)propionate. TSP- d_4 .

19 *Degree of deuteration:* minimum 98 per cent.
20

21 White or almost white powder.

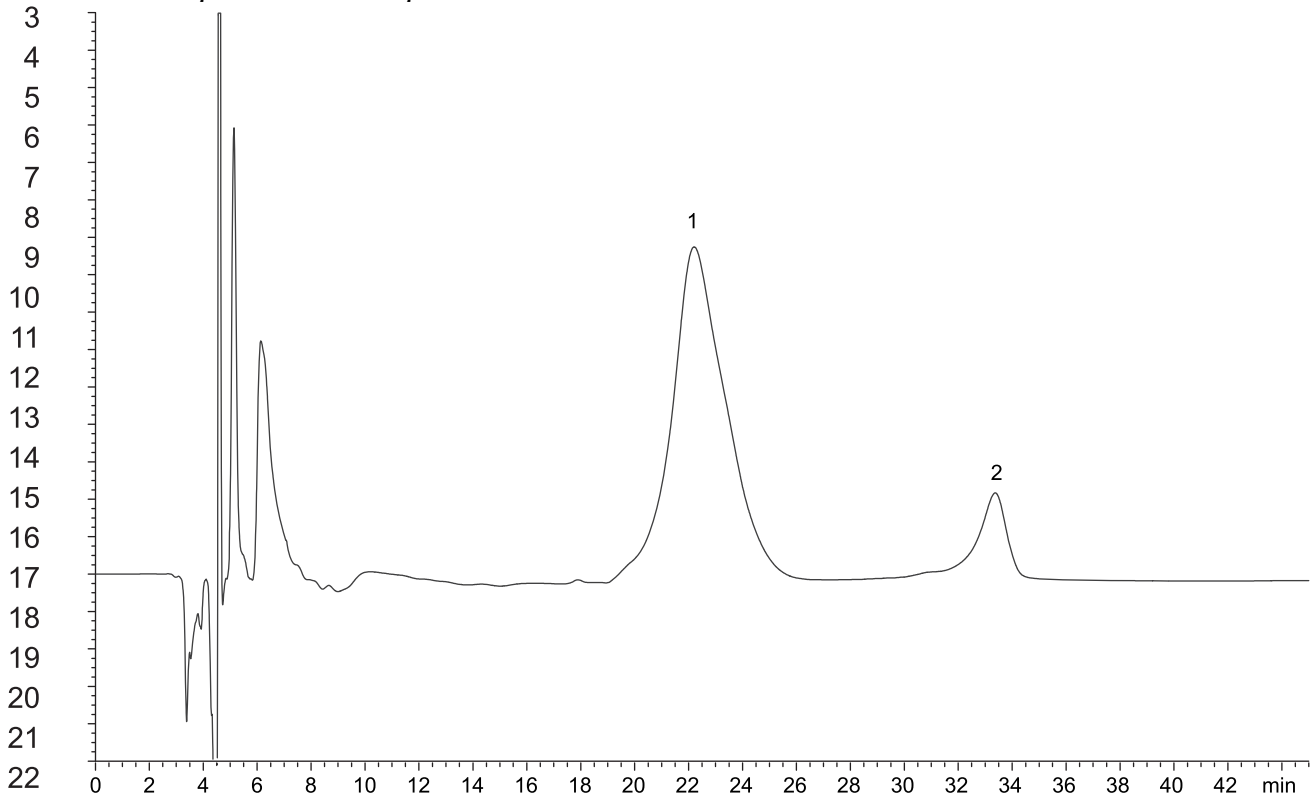
22 *The following chromatogram is shown for information but will not be published in*
23 *the European Pharmacopoeia.*



43
44 1. dermatan sulfate + chondroitin sulfate 2. heparin 3. over-sulfated chondroitin sulfate
45

46 Figure 0333-1. – *Chromatogram for identification test C of heparin sodium:*
47 *reference solution (c) (chromatogram obtained after subtraction of the blank)*

1 The following chromatogram is shown for information but will not be published in
2 the European Pharmacopoeia.



23
24 1. dermatan sulfate + chondroitin sulfate

2. over-sulfated chondroitin sulfate

25 Figure 0333.-2. – Chromatogram for the test for related substances of heparin sodium:
26 reference solution (e) (chromatogram obtained after subtraction of the blank)