



## **Läkemedelsverkets kungörelse om ikraftträdande av reviderade monografier för heparinnatrium och heparinkalcium i Europafarmakopén;**

**LVFS 2010:17**

Utkom från trycket  
den 16 juli 2010

beslutad den 28 juni 2010.

Med stöd av 10 kap. 5 § läkemedelsförordningen (2006:272) beslutar Läkemedelsverket på förslag av Svenska farmakopékommittén att monografi för heparinnatrium (Heparin sodium (no 0333)) och för heparinkalcium (Heparin calcium (no 0332)) i Supplement 6.4 till sjätte utgåvan av Europafarmakopén (European Pharmacopoeia 6.0) ska ersättas med monografier enligt bilaga 1 och 2 till denna kungörelse från den dag denna författning träder i kraft.

Denna författning träder i kraft den 1 augusti 2010.

Läkemedelsverket

MATS LARSSON

Joakim Brandberg

## 1 NOTE ON THE MONOGRAPH

2 This monograph has been thoroughly revised further to the contamination events in  
3 2008 to ensure appropriate quality control for unfractionated heparin. The style and  
4 presentation have also been updated in line with the current version of the Style guide.

5 **Definition:** the minimum potency limit has been raised after an enquiry among  
6 European manufacturers regarding the quality of currently marketed heparin batches;  
7 only 1 grade of heparin has been kept as the present 2-tiered specification no longer  
8 reflects the situation in Europe.

9 **Production:** the tests for nuclear magnetic resonance spectrometry (NMR) and capillary  
10 electrophoresis previously introduced in the 1<sup>st</sup>-step revision applicable from 1 August  
11 2008 have been deleted, as detailed tests are now provided under Identification  
12 and Tests; statements have been added to emphasise the need for a reliable quality  
13 management system throughout production and, based on current practice among  
14 European manufacturers, for confirming the identity of the source species as well as  
15 the absence of any material issued from other species likely to contaminate the drug  
16 substance. This monograph is also revised to harmonise the information related to  
17 the source species for substances of human and animal origin and its presentation  
18 in monographs. The statement relative to the origin of the substance is moved under  
19 Definition accordingly and a paragraph is added regarding the health of the animals  
20 used for the preparation of heparin sodium.

21 **Identification:** the tests for specific optical rotation and zone electrophoresis have  
22 been replaced by the highly specific <sup>1</sup>H-NMR and strong anion-exchange liquid  
23 chromatography (SAX-HPLC) tests; <sup>1</sup>H-NMR has been selected for its ability not only  
24 to allow identification of heparin, but also to alert users to possible contaminations;  
25 identification of the counterion is now based on the test for sodium by atomic absorption  
26 spectrometry described under Tests.

27 **Nucleotidic impurities:** the limit has been tightened, based on current batch data.

28 **Protein:** the Lowry test method has been introduced to replace the absorbance test.

29 **Related substances:** a SAX-HPLC-based test has been introduced, allowing the  
30 differentiation of natural contaminants linked to the production process (such as  
31 dermatan sulfate and chondroitin sulfate) from chemically synthesised contaminants;  
32 a limit for the sum of dermatan sulfate and chondroitin sulfate, which co-elute in this  
33 method, is proposed, further to consideration of current batch data.

34 **Nitrogen:** a lower limit has been added, based on current batch data.

35 **Heavy metals:** method C has been replaced by method F, in line with the general policy  
36 for heavy metals tests.

37 **Sulfated ash:** in view of the highly specific tests introduced into the monograph, this  
38 test has become redundant and has therefore been deleted.

08/2010:0333

## 39 HEPARIN SODIUM

40 Heparinum natricum

## 1 DEFINITION

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

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

## 9 PRODUCTION

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

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

## 18 CHARACTERS

19 *Appearance*: white or almost white, hygroscopic powder.

20 *Solubility*: freely soluble in water.

## 23 IDENTIFICATION

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

25 B. Nuclear magnetic resonance spectrometry (2.2.33).

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

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

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

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

34 *Apparatus*: spectrometer operating at minimum 300 MHz.

35 *Acquisition of <sup>1</sup>H-NMR spectra*:

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

- 1     *Processing:*
- 2     – *exponential line-broadening window function:* 0.3 Hz;
- 3     – Fourier transformation;
- 4     – trimethylsilylpropionate reference signal set at 0.00 ppm.
- 5     *Results:*
- 6
- 7     – the large heparin sodium signals must be present: 2.04 ppm, 3.27 ppm (doublet),
- 8     4.34 ppm, 5.22 ppm and 5.42 ppm, all within  $\pm 0.03$  ppm;
- 9
- 10    – the  $^1\text{H-NMR}$  spectrum obtained with the test sample and that obtained with *heparin*
- 11    *sodium for NMR identification CRS* are compared qualitatively after the 2 spectra
- 12    have been normalised so as to have a similar intensity; dermatan sulfate with a
- 13    methyl signal at  $2.08 \pm 0.02$  ppm may be observed; no unidentified signals larger
- 14    than 4 per cent compared to the height of the heparin signal at 5.42 ppm are
- 15    present in the ranges 0.10-2.00 ppm, 2.10-3.10 ppm and 5.70-8.00 ppm; signals from
- 16    the solvent or process-related substances may be present and have to be identified
- 17    to be accepted; variations in the intensity of some signal regions of the spectrum
- 18    of heparin may occur: the intensity-variable regions are between 3.35 ppm and
- 19    4.55 ppm, where the signal pattern is approximately kept but intensity varies.
- 20
- 21    C. Liquid chromatography (2.2.29) as described in the test for related substances with the
- 22    following modifications.
- 23    *Injection:* test solution (a) and reference solution (c).
- 24    *Relative retention* with reference to heparin (retention time = about 26 min): dermatan
- 25    sulfate and chondroitin sulfate = about 0.9; over-sulfated chondroitin sulfate = about 1.3.
- 26    *System suitability:* reference solution (c):
- 27
- 28    – *peak-to-valley ratio:* minimum 1.3, where  $H_p$  = height above the baseline of the peak
- 29    due to dermatan sulfate + chondroitin sulfate and  $H_v$  = height above the baseline of
- 30    the lowest point of the curve separating this peak from the peak due to heparin.
- 31    *Results:* the principal peak in the chromatogram obtained with test solution (a) is
- 32    similar in retention time and shape to the principal peak in the chromatogram obtained
- 33    with reference solution (c).
- 34
- 35    D. Sodium (see Tests).
- 36
- 37    TESTS
- 38    **Appearance of solution.** The solution is clear (2.2.1) and not more intensely coloured
- 39    than intensity 5 of the range of reference solutions of the most appropriate colour (2.2.2,
- 40    *Method II*).
- 41    Dissolve a quantity equivalent to 50 000 IU in *water R* and dilute to 10 mL with the
- 42    same solvent.
- 43    **pH** (2.2.3): 5.5 to 8.0.
- 44
- 45    Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.
- 46    **Nucleotidic impurities.** Dissolve 40 mg in 10 mL of *water R*. The absorbance (2.2.25)
- 47    measured at 260 nm is not greater than 0.15.

- 1 **Protein:** maximum 0.5 per cent (dried substance).  
2  
3 **Solution A.** Mix 2 volumes of a 10 g/L solution of *sodium hydroxide R* and 2 volumes of a  
4 50 g/L solution of *sodium carbonate R* and dilute to 5 volumes with *water R*.  
5 **Solution B.** Mix 2 volumes of a 12.5 g/L solution of *copper sulfate R* and 2 volumes of a  
6 29.8 g/L solution of *sodium tartrate R* and dilute to 5 volumes with *water R*.  
7  
8 **Solution C.** Mix 1 volume of solution B and 50 volumes of solution A.  
9 **Solution D.** Dilute a phosphomolybdotungstic reagent<sup>(1)</sup> 2- to 4-fold in *water R*. Suitable  
10 dilutions produce solutions of pH 10.25 ± 0.25 after addition of solutions C and D to  
11 the test and reference solutions.  
12 **Test solution.** Dissolve the substance to be examined in *water R* to obtain a concentration  
13 of 5 mg/mL.  
14  
15 **Reference solutions.** Dissolve *bovine albumin R* in *water R* to obtain a concentration of  
16 100 mg/mL. Prepare dilutions of the solution in *water R* as prescribed in general chapter  
17 2.5.33, method 2.  
18 **Blank:** *water R*.  
19  
20 **Procedure.** To 1 mL of each reference solution, of the test solution and of the blank, add  
21 5 mL of solution C. Allow to stand for 10 min. Add 0.5 mL of solution D, mix and allow  
22 to stand at room temperature for 30 min. Determine the absorbances (2.2.25) of the  
23 solutions at 750 nm, using the solution prepared from the blank as compensation liquid.  
24 **Calculations.** As prescribed in general chapter 2.5.33, method 2.  
25  
26 **Related substances.** Liquid chromatography (2.2.29). *Reference solutions are stable at*  
27 *room temperature for 24 h.*  
28 **Test solution (a).** Dissolve an accurately weighed quantity of about 50 mg of the substance  
29 to be examined in 5.0 mL of *water for chromatography R*. Mix using a vortex mixer until  
30 dissolution is complete.  
31  
32 **Test solution (b).** Dissolve an accurately weighed quantity of about 0.1 g of the substance  
33 to be examined in 1.0 mL of *water for chromatography R*. Mix using a vortex mixer until  
34 dissolution is complete. Mix 500 µL of the solution and 250 µL of 1 M *hydrochloric acid*,  
35 then add 50 µL of a 250 mg/mL solution of *sodium nitrite R*<sup>(2)</sup>. Mix gently and allow to  
36 stand at room temperature for 40 min before adding 200 µL of 1 M *sodium hydroxide*  
37 to stop the reaction.  
38 **Reference solution (a).** Dissolve 250 mg of *heparin for physico-chemical analysis CRS*  
39 in *water for chromatography R* and dilute to 2.0 mL with the same solvent. Mix using a  
40 vortex mixer until dissolution is complete.  
41  
42 **Reference solution (b).** Add 1200 µL of reference solution (a) to 300 µL of *dermatan sulfate*  
43 *and over-sulfated chondroitin sulfate CRS*. Mix using a vortex mixer to homogenise.  
44 **Reference solution (c).** Add 100 µL of reference solution (b) to 900 µL of *water for*  
45 *chromatography R*. Mix using a vortex mixer to homogenise.  
46  
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(1) Folin-Ciocalteu's phenol reagent from Merck (reference 1.09001.0500) is suitable.

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

1 Reference solution (d). Add 400 µL of reference solution (a) to 100 µL of water for  
2 chromatography R and mix using a vortex mixer. Add 250 µL of 1 M hydrochloric acid,  
3 then add 50 µL of a 250 mg/mL solution of sodium nitrite R. Mix gently and allow to  
4 stand at room temperature for 40 min before adding 200 µL of 1 M sodium hydroxide  
5 to stop the reaction.

6 Reference solution (e). To 500 µL of reference solution (b), add 250 µL of 1 M  
7 hydrochloric acid, then add 50 µL of a 250 mg/mL solution of sodium nitrite R. Mix  
8 gently and allow to stand at room temperature for 40 min before adding 200 µL of 1 M  
9 sodium hydroxide to stop the reaction.

10 Precolumn:

11 – size:  $l = 0.05$  m,  $\emptyset = 2$  mm;

12 – stationary phase: anion exchange resin R (13 µm)<sup>(3)</sup>.

14 Column:

15 – size:  $l = 0.25$  m,  $\emptyset = 2$  mm;

16 – stationary phase: anion exchange resin R (9 µm)<sup>(4)</sup>;

17 – temperature: 40 °C.

19 Mobile phase:

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

22 – mobile phase B: dissolve 0.40 g of sodium dihydrogen phosphate R in 1 L of water for  
23 chromatography R, add 140 g of sodium perchlorate R<sup>(5)</sup> and adjust to pH 3.0 with  
24 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

31 Flow rate: 0.22 mL/min.

32 Detection: spectrophotometer at 202 nm.

33 Equilibration: at least 15 min.

34 Injection: 20 µL of test solution (b) and reference solutions (d) and (e).

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

38 System suitability:

39 – the chromatogram obtained with reference solution (d) shows no peak at the retention  
40 time of heparin;

41 – resolution: minimum 3.0 between the peaks due to dermatan sulfate + chondroitin  
42 sulfate and over-sulfated chondroitin sulfate in the chromatogram obtained with  
43 reference solution (e).

44 Limits:

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

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

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

- 1 – *sum of dermatan sulfate and chondroitin sulfate*: not more than the area of the  
2 corresponding peak in the chromatogram obtained with reference solution (e) (2.0 per  
3 cent);
- 4 – *any other impurity*: no peaks other than the peak due to dermatan sulfate  
5 + chondroitin sulfate are detected.
- 6
- 7 **Nitrogen (2.5.9)**: 1.5 per cent to 2.5 per cent (dried substance), determined on 0.100 g.
- 8 **Sodium**: 9.5 per cent to 12.5 per cent (dried substance).
- 9 Atomic absorption spectrometry (2.2.23, Method I).
- 10 *Test solution*. Dissolve 50 mg of the substance to be examined in a 1.27 mg/mL solution  
11 of *caesium chloride R* in 0.1 M *hydrochloric acid* and dilute to 100.0 mL with the same  
12 solvent.
- 13 *Reference solutions*. Prepare reference solutions containing 25 ppm, 50 ppm and 75 ppm  
14 of Na, using *sodium standard solution (200 ppm Na) R* diluted with a 1.27 mg/mL  
15 solution of *caesium chloride R* in 0.1 M *hydrochloric acid*.
- 16 *Source*: sodium hollow-cathode lamp.
- 17 *Wavelength*: 330.3 nm.
- 18 *Atomisation device*: flame of suitable composition (for example 11 L of air and 2 L of  
19 acetylene per minute).
- 20
- 21 **Heavy metals (2.4.8)**: maximum 30 ppm.
- 22 1.0 g complies with test F. Prepare the reference solution using 3.0 mL of *lead standard*  
23 *solution (10 ppm Pb) R*.
- 24
- 25 **Loss on drying (2.2.32)**: maximum 8.0 per cent, determined on 1.000 g by drying at 60 °C  
26 over *diphosphorus pentoxide R* at a pressure not exceeding 670 Pa for 3 h.
- 27
- 28 **Bacterial endotoxins (2.6.14)**: less than 0.01 IU per International Unit of heparin,  
29 if intended for use in the manufacture of parenteral preparations without a further  
30 appropriate procedure for the removal of bacterial endotoxins.
- 31
- 32 **ASSAY**
- 33 Carry out the assay of heparin (2.7.5). The estimated potency is not less than 90 per  
34 cent and not more than 111 per cent of the stated potency. The confidence limits of the  
35 estimated potency ( $P = 0.95$ ) are not less than 80 per cent and not more than 125 per  
36 cent of the stated potency.
- 37
- 38 **STORAGE**
- 39 In an airtight container. If the substance is sterile, store in a sterile, airtight, tamper-proof  
40 container.
- 41
- 42 **LABELLING**
- 43 The label states:
- 44 – the number of International Units per milligram;
- 45 – the animal species of origin;
- 46 – where applicable, that the substance is suitable for use in the manufacture of parenteral  
47 preparations.

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**Reagents**

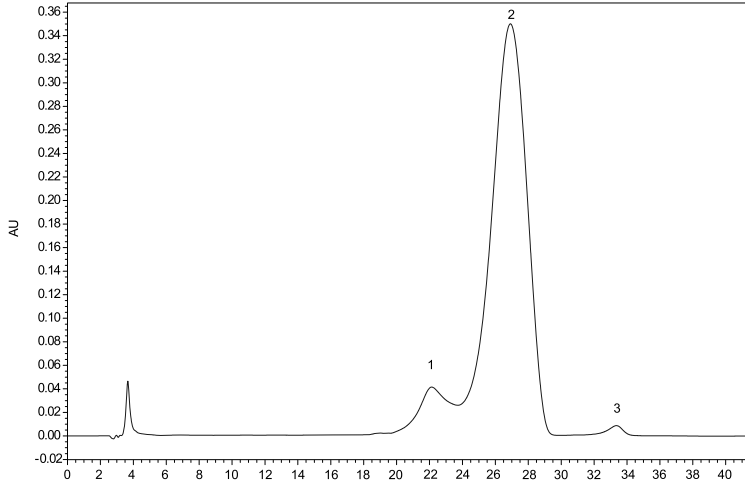
**Deuterated sodium trimethylsilylpropionate.**  $C_6H_9^2H_4NaO_2Si$ . ( $M_r$  172.3). XXXXXXXX.  
[24493-21-8].

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

*Degree of deuteration:* minimum 98 per cent.

White or almost white powder.

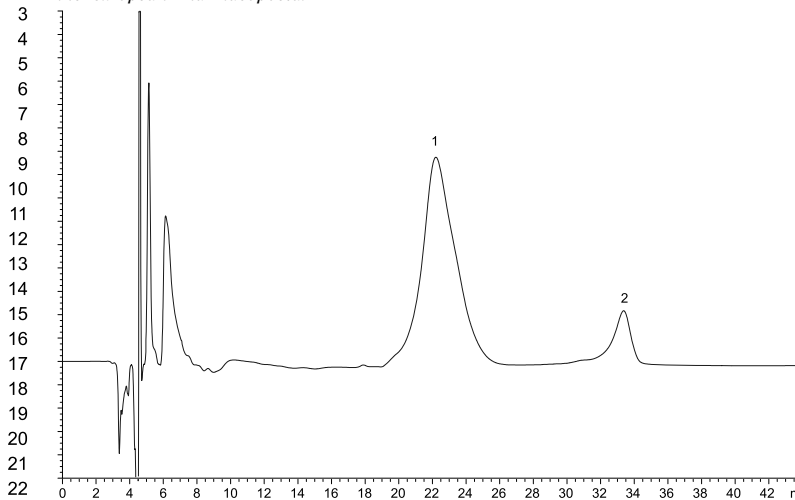
*The following chromatogram is shown for information but will not be published in the European Pharmacopoeia.*



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

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

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2 the European Pharmacopoeia.



23 1. dermatan sulfate + chondroitin sulfate      2. over-sulfated chondroitin sulfate  
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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)  
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5 *presentation have also been updated in line with the current version of the Style guide.*

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7 *European manufacturers regarding the quality of currently marketed heparin batches;*  
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9 *reflects the situation in Europe.*

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08/2010:0332

43  
44  
45 **HEPARIN CALCIUM**46  
47 Heparinum calcicum

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3 mammalian tissues. It is prepared either from the lungs of cattle or from the intestinal  
4 mucosae of pigs, cattle or sheep. On complete hydrolysis, it liberates D-glucosamine,  
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39    same solvent.  
40    **pH** (2.2.3): 5.5 to 8.0.  
41    Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.  
42    **Nucleotidic impurities.** Dissolve 40 mg in 10 mL of *water R*. The absorbance (2.2.25)  
43    measured at 260 nm is not greater than 0.15.  
44  
45    **Protein:** maximum 0.5 per cent (dried substance).  
46    *Solution A.* Mix 2 volumes of a 10 g/L solution of *sodium hydroxide R* and 2 volumes of a  
47    50 g/L solution of *sodium carbonate R* and dilute to 5 volumes with *water R*.

- 1 *Solution B.* Mix 2 volumes of a 12.5 g/L solution of *copper sulfate R* and 2 volumes of a  
2 29.8 g/L solution of *sodium tartrate R* and dilute to 5 volumes with *water R*.  
3  
4 *Solution C.* Mix 1 volume of solution B and 50 volumes of solution A.  
5 *Solution D.* Dilute a phosphomolybdotungstic reagent<sup>(1)</sup> 2- to 4-fold in *water R*. Suitable  
6 dilutions produce solutions of pH 10.25 ± 0.25 after addition of solutions C and D to  
7 the test and reference solutions.
- 8 *Test solution.* Dissolve the substance to be examined in *water R* to obtain a concentration  
9 of 5 mg/mL.
- 10 *Reference solutions.* Dissolve *bovine albumin R* in *water R* to obtain a concentration of  
11 100 mg/mL. Prepare dilutions of the solution in *water R* as prescribed in general chapter  
12 2.5.33, method 2.  
13
- 14 *Blank: water R.*
- 15 *Procedure.* To 1 mL of each reference solution, of the test solution and of the blank, add  
16 5 mL of solution C. Allow to stand for 10 min. Add 0.5 mL of solution D, mix and allow  
17 to stand at room temperature for 30 min. Determine the absorbances (2.2.25) of the  
18 solutions at 750 nm, using the solution prepared from the blank as compensation liquid.  
19
- 20 *Calculations.* As prescribed in general chapter 2.5.33, method 2.
- 21 **Related substances.** Liquid chromatography (2.2.29). *Reference solutions are stable at*  
22 *room temperature for 24 h.*
- 23 *Test solution (a).* Dissolve an accurately weighed quantity of about 50 mg of the substance  
24 to be examined in 5.0 mL of *water for chromatography R*. Mix using a vortex mixer until  
25 dissolution is complete.  
26
- 27 *Test solution (b).* Dissolve an accurately weighed quantity of about 0.1 g of the substance  
28 to be examined in 1.0 mL of *water for chromatography R*. Mix using a vortex mixer until  
29 dissolution is complete. Mix 500 µL of the solution and 250 µL of 1 M *hydrochloric acid*,  
30 then add 50 µL of a 250 mg/mL solution of *sodium nitrite R*<sup>(2)</sup>. Mix gently and allow to  
31 stand at room temperature for 40 min before adding 200 µL of 1 M *sodium hydroxide*  
32 to stop the reaction.
- 33 *Reference solution (a).* Dissolve 250 mg of *heparin for physico-chemical analysis CRS*  
34 in *water for chromatography R* and dilute to 2.0 mL with the same solvent. Mix using a  
35 vortex mixer until dissolution is complete.
- 36 *Reference solution (b).* Add 1200 µL of reference solution (a) to 300 µL of *dermatan sulfate*  
37 *and over-sulfated chondroitin sulfate CRS*. Mix using a vortex mixer to homogenise.  
38
- 39 *Reference solution (c).* Add 100 µL of reference solution (b) to 900 µL of *water for*  
40 *chromatography R*. Mix using a vortex mixer to homogenise.
- 41 *Reference solution (d).* Add 400 µL of reference solution (a) to 100 µL of *water for*  
42 *chromatography R* and mix using a vortex mixer. Add 250 µL of 1 M *hydrochloric acid*,  
43 then add 50 µL of a 250 mg/mL solution of *sodium nitrite R*. Mix gently and allow to  
44 stand at room temperature for 40 min before adding 200 µL of 1 M *sodium hydroxide*  
45 to stop the reaction.  
46

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

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

1 Reference solution (e). To 500 µL of reference solution (b), add 250 µL of 1 M  
2 hydrochloric acid, then add 50 µL of a 250 mg/mL solution of sodium nitrite R. Mix  
3 gently and allow to stand at room temperature for 40 min before adding 200 µL of 1 M  
4 sodium hydroxide to stop the reaction.

5 *Precolumn:*

- 6 – size:  $l = 0.05$  m,  $\emptyset = 2$  mm;
- 7 – stationary phase: anion exchange resin R (13 µm)<sup>(3)</sup>.

9 *Column:*

- 10 – size:  $l = 0.25$  m,  $\emptyset = 2$  mm;
- 11 – stationary phase: anion exchange resin R (9 µm)<sup>(4)</sup>;
- 12 – temperature: 40 °C.

13 *Mobile phase:*

- 14 – mobile phase A: dissolve 0.40 g of sodium dihydrogen phosphate R in 1 L of water for  
15 chromatography R and adjust to pH 3.0 with dilute phosphoric acid R;
- 16 – mobile phase B: dissolve 0.40 g of sodium dihydrogen phosphate R in 1 L of water for  
17 chromatography R, add 140 g of sodium perchlorate R<sup>(5)</sup> and adjust to pH 3.0 with  
18 dilute phosphoric acid R; filter and degas;

20	Time	Mobile phase A	Mobile phase B
21	(min)	(per cent V/V)	(per cent V/V)
22	0 - 10	75	25
23	10 - 35	75 → 0	25 → 100
24	35 - 40	0	100

25 *Flow rate:* 0.22 mL/min.

26 *Detection:* spectrophotometer at 202 nm.

27 *Equilibration:* at least 15 min.

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

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

31 *System suitability:*

- 32 – the chromatogram obtained with reference solution (d) shows no peak at the retention  
33 time of heparin;
- 34 – resolution: minimum 3.0 between the peaks due to dermatan sulfate + chondroitin  
35 sulfate and over-sulfated chondroitin sulfate in the chromatogram obtained with  
36 reference solution (e).

37 *Limits:*

- 38 – sum of dermatan sulfate and chondroitin sulfate: not more than the area of the  
39 corresponding peak in the chromatogram obtained with reference solution (e) (2.0 per  
40 cent);
- 41 – any other impurity: no peaks other than the peak due to dermatan sulfate  
42 + chondroitin sulfate are detected.

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

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

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

- 1 **Nitrogen** (2.5.9): 1.5 per cent to 2.5 per cent (dried substance), determined on 0.100 g.  
2  
3 **Calcium**: 9.5 per cent to 11.5 per cent (dried substance), determined on 0.200 g by  
4 complexometric titration (2.5.11).  
5 **Heavy metals** (2.4.8): maximum 30 ppm.  
6  
7 1.0 g complies with test F. Prepare the reference solution using 3.0 mL of *lead standard*  
8 *solution (10 ppm Pb) R*.  
9 **Loss on drying** (2.2.32): maximum 8.0 per cent, determined on 1.000 g by drying at 60 °C  
10 over *diphosphorus pentoxide R* at a pressure not exceeding 670 Pa for 3 h.  
11  
12 **Bacterial endotoxins** (2.6.14): less than 0.01 IU per International Unit of heparin,  
13 if intended for use in the manufacture of parenteral preparations without a further  
14 appropriate procedure for the removal of bacterial endotoxins. The addition of divalent  
15 cations may be necessary in order to fulfil the validation criteria.  
16

## 17 ASSAY

19 Carry out the assay of heparin (2.7.5). The estimated potency is not less than 90 per  
20 cent and not more than 111 per cent of the stated potency. The confidence limits of the  
21 estimated potency ( $P = 0.95$ ) are not less than 80 per cent and not more than 125 per  
22 cent of the stated potency.  
23

## 24 STORAGE

26 In an airtight container. If the substance is sterile, store in a sterile, airtight, tamper-proof  
27 container.  
28

## 29 LABELLING

31 The label states:

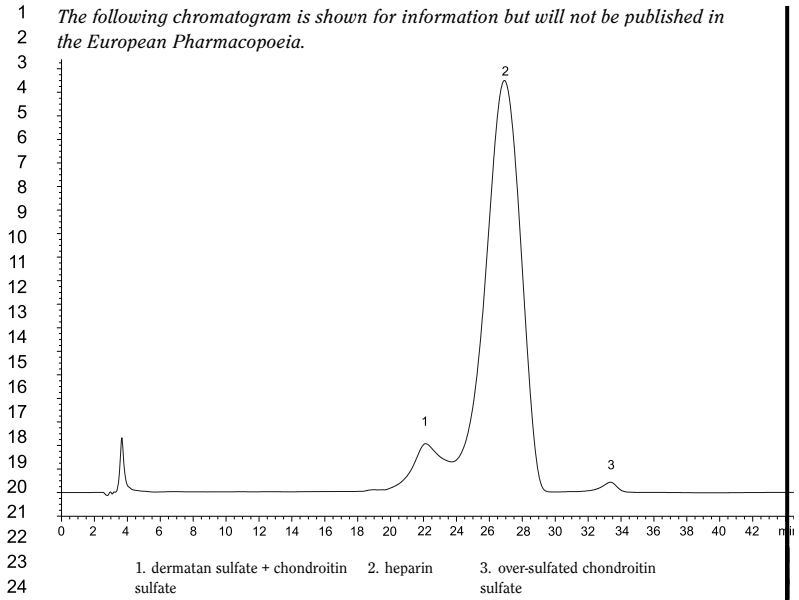
- 33 – the number of International Units per milligram;  
34  
35 – the animal species of origin;  
36  
37 – where applicable, that the substance is suitable for use in the manufacture of parenteral  
38 preparations.  
39

40  
41 **Reagents**

42  
43 **Deuterated sodium trimethylsilylpropionate**.  $C_6H_9^2H_4NaO_2Si$ . ( $M_r$  172.3). XXXXXXX.  
44 [24493-21-8]. Sodium 3-(trimethylsilyl)(2,2,3,3- $^2H_4$ )propionate. TSP- $d_4$ .

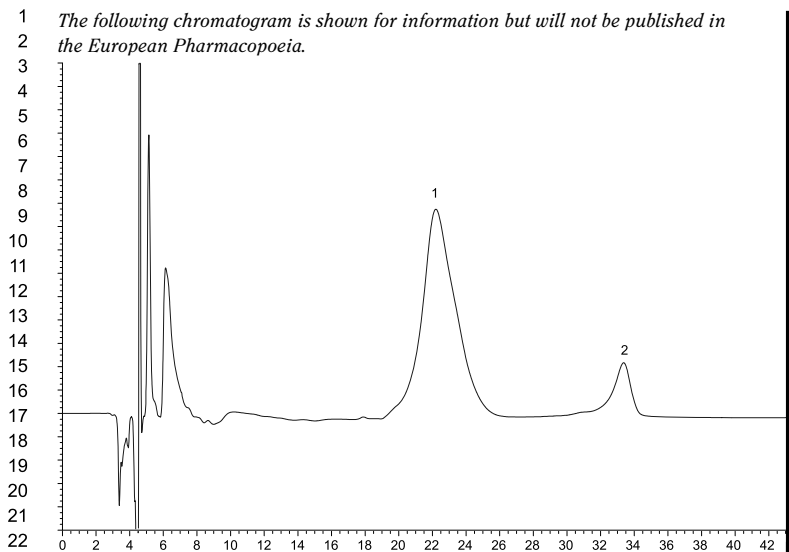
45 *Degree of deuteration*: minimum 98 per cent.  
46

47 White or almost white powder.



25 Figure 0332.-1.- Chromatogram for identification test C of heparin calcium: reference  
26 solution (c) (chromatogram obtained after subtraction of the blank)  
27  
28  
29  
30  
31  
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33  
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46  
47

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



1. dermatan sulfate + chondroitin sulfate

2. over-sulfated chondroitin sulfate

Figure 0332.-2.- Chromatogram for the test for related substances of heparin calcium: reference solution (e) (chromatogram obtained after subtraction of the blank)





