Edition: BP 2025 (Ph. Eur. 11.6 update)

Heparin Injection

General Notices

Action and use

Anticoagulant.

DEFINITION

Heparin Injection is a sterile solution of Heparin Calcium or Heparin Sodium in Water for Injections.

PRODUCTION

The final product is produced from the drug substance where the methods of manufacturing are designed to ensure that substances lowering blood pressure are not introduced and to ensure freedom from contamination by over-sulfated glycosaminoglycans. The production method is validated to demonstrate that the product if tested, would comply with the test for Related substances given below.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Potency

The estimated potency is not less than 90% and not more than 111% of the stated potency.

CHARACTERISTICS

A colourless or straw-coloured liquid, free from turbidity and from matter that deposits on standing.

IDENTIFICATION

A. It complies with the requirements described under Assay.
B. Carry out the assay of anti-factor Xa activity of heparin, Appendix XIV J B2. The ratio of anti-factor Xa activity to anti-factor IIa activity, determined as described under Assay, ranges between 0.9 and 1.1.
C. Carry out the method for *nuclear magnetic resonance spectrometry*, *Appendix II C* using the following solutions.
(1) For injections containing 25 000 IU per mL, dilute 100 µL with 450 µL of *water*. For injections containing 5000 IU, 1000 IU per mL, or lower use 550 µL of each. Add 50 µL of an 0.83% w/v solution of *deuterated sodium trimethylsilylpropionate* in *deuterium oxide*.

For injections containing Heparin Calcium

(2) Dissolve 3.4 mg of *heparin calcium for NMR identification EPCRS* in 700 µL of a solution containing 650 µL of water, 40 µL of *deuterium oxide*, and 10 µL of an 0.83% w/v solution of *deuterated sodium trimethylsilylpropionate* in *deuterium oxide*.

For injections containing Heparin Sodium

(3) Dissolve 3.4 mg of *heparin sodium for NMR identification EPCRS* in 700 µL of a solution containing 650 µL of water, 40 µL of *deuterium oxide*, and 10 µL of an 0.83% w/v solution of *deuterated sodium trimethylsilylpropionate* in *deuterium oxide*.

^{1}H-NMR spectrometer parameters

(a) Operate the spectrometer at a minimum of 400 MHz.
(b) Use a minimum of 256 transients; adjusted until the signal-to-noise ratio is at least 1000:1 for the heparin methyl signal at 2.04 ppm.
(c) Use an ambient temperature; the test sample and reference spectra must be obtained at the same temperature.
(d) Use a minimum of 2 seconds for acquisition time.
(e) Use a minimum of 4 seconds for repetition time.
(f) Use a spectral width of 10 to 16 ppm, centred at around 4.5 ppm.
(g) Use a pulse width to give a flip angle between 30° and 90°.

Processing parameters

(h) Use an exponential line-broadening window function of 0.3 Hz.
(i) Apply Fourier transformation to the collected data.
(j) Use trimethylsilylpropionate as the reference signal, set at 0.00 ppm.

Results for injections containing Heparin Calcium

In the ^{1}H-NMR spectrum obtained with solution (1):

the large heparin calcium signals must be present: 2.05 ppm, 3.29 ppm (broad singlet), 4.37 ppm, 5.35 ppm and 5.43 ppm, all within ± 0.03 ppm.

Compare the ^{1}H-NMR spectrum obtained with solution (2) 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 5.43 ppm are present in the ranges 0.10 to 2.00 ppm, 2.10 to 3.10 ppm and 5.70 to 8.00 ppm; signals from the solvent, excipients or process-related substances may be present and have to be identified to be accepted.

Results for injections containing Heparin Sodium

In the ^{1}H-NMR spectrum obtained with solution (1):

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.

Compare the ^{1}H-NMR spectrum obtained with solution (3) 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 to 2.00 ppm, 2.10 to 3.10 ppm and 5.70 to 8.00 ppm; signals from the solvent, excipients 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.

D. When the injection contains Heparin Calcium, it yields reactions A and B characteristic of *calcium salts*, Appendix VI. When the injection contains Heparin Sodium, it yields reaction A characteristic of *sodium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH, 5.5 to 8.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D using the following solutions. Solutions (3) to (6) are stable at room temperature for 24 hours.

(1) For injections containing 25 000 IU per mL, dilute 100 µL with 400 µL of *water for chromatography*. For injections containing 5000 IU, 1000 IU per mL, or lower use 500 µL of each. Mix using a vortex mixer until dissolution is complete.

(2) Mix 500 µL of solution (1) and 250 µL of 1M *hydrochloric acid*, then add 50 µL of a 25% w/v solution of *sodium nitrite*. Mix gently and allow to stand at room temperature for 40 minutes before adding 200 µL of 1M *sodium hydroxide* to stop the reaction.
(3) Dissolve 0.25 g of *heparin for physico-chemical analysis EPCRS* in *water for chromatography* and dilute to 2.0 mL with the same solvent. Mix using a vortex mixer until dissolution is complete.
(4) Add 1200 µL of solution (3) to 300 µL of *dermatan sulfate and over-sulfated chondroitin sulfate EPCRS*. Mix using a vortex mixer to homogenise.
(5) Add 400 µL of solution (3) to 100 µL of *water for chromatography* and mix using a vortex mixer. Add 250 µL of 1M *hydrochloric acid*, then add 50 µL of a 25% w/v solution of *sodium nitrite*. Mix gently and allow to stand at room temperature for 40 minutes before adding 200 µL of 1M *sodium hydroxide* to stop the reaction.
(6) To 500 µL of solution (4), add 250 µL of 1M *hydrochloric acid*, then add 50 µL of a 25% w/v solution of *sodium nitrite*. Mix gently and allow to stand at room temperature for 40 minutes before adding 200 µL of 1M *sodium hydroxide* to stop the reaction.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 2 mm) packed with *anion-exchange resin* (9 µm) (Dionex Ionpac AS11-HC is suitable) and a stainless steel pre-column (5 cm × 2 mm) packed with *anion-exchange resin* (13 µm) (Dionex Ionpac AG11-HC is suitable).
(b) Use gradient elution and the mobile phase described below.
(c) Use a flow rate of 0.22 mL per minute.
(d) Use a column temperature of 40°.
(e) Use a detection wavelength of 202 nm.
(f) For injections containing 25000 IU per mL or 5000 IU per mL, inject 20 µL of solutions (2), (5) and (6). For injections containing 1000 IU per mL, inject 100 µL of solution (2), and 20 µL of solution (5) and (6).

MOBILE PHASE

Mobile phase A 0.040% w/v of *sodium dihydrogen orthophosphate* in *water for chromatography* adjusted to pH 3.0 with *dilute orthophosphoric acid*.

Mobile phase B 0.040% w/v of *sodium dihydrogen orthophosphate* and 14% w/v of *sodium perchlorate* in *water for chromatography*, adjusted to pH 3.0 with *dilute orthophosphoric acid*, filtered and degassed.

Equilibrate the column with the mobile phase in the initial gradient ratio for at least 15 minutes.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	75	25	isocratic
10-35	75→0	25→100	linear gradient
35-40	0	100	isocratic
40-45	0→75	100→25	linear gradient
45-60	75	25	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to heparin (about 26 minutes) are: dermatan sulfate and chondroitin sulfate, about 0.9; over-sulfated chondroitin sulfate, 1.3.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (5), no peak is present at the retention time of heparin;

in the chromatogram obtained with solution (6), the *resolution* between the peaks due to dermatan sulfate / chondroitin sulfate and over-sulfated chondroitin sulfate is at least 3.0.

LIMITS

In the chromatogram obtained with solution (2):

the area of any peak due to dermatan sulfate and chondroitin sulfate is not greater than 0.25 times the area of the corresponding peak in the chromatogram obtained with solution (6) (2.0%);

The area of any other peak is not greater than 0.01 times the area of the peak due to dermatan sulfate and chondroitin sulfate in the chromatogram obtained with solution (6) (corresponding to a disregard limit of 0.08%). Disregard any peaks that appear during the initial isocratic step.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C.

For a preparation supplied in a container with a nominal content of less than 100 mL, dilute the injection if necessary with *water BET* to give a solution containing 1000 IU of heparin activity per mL (solution A). The endotoxin limit concentration of solution A is 10 IU of endotoxin per mL. Carry out the test using a lysate with a declared sensitivity not less sensitive than 0.0625 IU of endotoxin per mL.

For a preparation supplied in a container with a nominal content of 100 mL or more, the endotoxin limit concentration is 0.25 IU of endotoxin per mL.

For Heparin Injection prepared from Heparin Calcium it may be necessary to add divalent cations in order to achieve the validation criteria.

ASSAY

Carry out the assay of *anti-factor IIa activity of heparin*, Appendix XIV J B2. The fiducial limits of error are not less than 80% and not more than 125% of the stated potency.

STORAGE

Heparin Injection should be kept in a container sealed by fusion of the glass.

LABELLING

The strength is stated as the number of IU (Units) in a suitable dose-volume except that for multi-dose containers the strength is stated as the number of IU (Units) per mL.

The label states (1) whether the material is the calcium or sodium salt; (2) when no antimicrobial preservative is present that the preparation does not contain an antimicrobial preservative and that any portion of the contents not used immediately should be discarded.