Limits:

- *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the peak due to dacarbazine in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total*: not more than 5 times the area of the peak due to dacarbazine in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit*: 0.5 times the area of the peak due to dacarbazine in the chromatogram obtained with reference solution (b) (0.05 per cent).

Impurity D. Head-space gas chromatography (*2.2.28*).

Test solution. Introduce 0.200 g of the substance to be examined into a 20 ml vial and firmly attach the septum and cap. Using a 10 µl syringe, inject 5 µl of *water R* into the vial.

Reference solution (a). Dilute 2.5 ml of *dimethylamine solution R* (impurity D) to 100.0 ml with *water R* (solution A). Firmly attach the septum and cap to a 20 ml vial. Using a 10 µl syringe, inject 10 µl of solution A into the vial.

Reference solution (b). Firmly attach the septum and cap to a 20 ml vial. Using a 10 µl syringe, inject 10 µl of solution A and 10 µl of a 10 g/l solution of *triethylamine R* into the vial.

Column:

- *material*: fused silica;
- *size*: *l* = 30.0 m, Ø = 0.53 mm;
- *stationary phase*: *base-deactivated polyethyleneglycol R* (film thickness 1.0 µm).

Carrier gas: *helium for chromatography R*.

Flow rate: 13 ml/min.

Split ratio: 1:1.

Static head-space conditions that may be used:

- *equilibration temperature*: 60 °C;
- *equilibration time*: 10 min;
- *transfer-line temperature*: 90 °C;
- *pressurisation time*: 30 s.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 3	35
	3 - 11	35 → 165
Injection port		180
Detector		220

Detection: flame ionisation.

Injection: 1 ml.

System suitability: reference solution (b):

- *resolution*: minimum 2.5 between the peaks due to impurity D and triethylamine.

Limit:

- *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*): maximum 0.5 per cent, determined on 1.00 g.

Sulphated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 18.22 mg of $C_6H_{10}N_6O$.

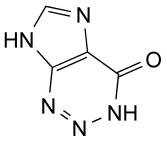
STORAGE

At a temperature of 2 °C to 8 °C, protected from light.

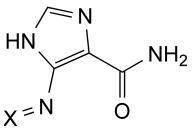
IMPURITIES

Specified impurities: *A, B, D*.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *C*.

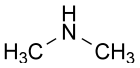


A. 3,7-dihydro-4*H*-imidazo[4,5-*d*]-1,2,3-triazin-4-one (2-azahypoxanthine),



B. X = H_2: 5-amino-1*H*-imidazole-4-carboxamide,

C. X = NH: 5-diazenyl-1*H*-imidazole-4-carboxamide,

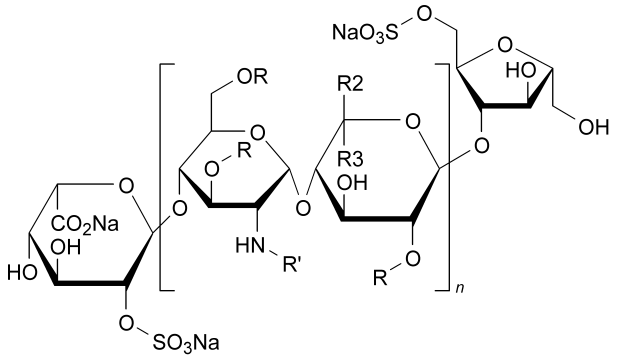


D. *N*-methylmethanamine.

01/2008:1195

DALTEPARIN SODIUM

Dalteparinum natricum



n = 3 to 20 , R = H or SO_3Na , R' = SO_3Na or CO-CH_3
R2 = H and R3 = CO_2Na or R2 = CO_2Na and R3 = H

DEFINITION

Dalteparin sodium is the sodium salt of a low-molecular-mass heparin that is obtained by nitrous acid depolymerisation of heparin from porcine intestinal mucosa. The majority of the components have a 2-*O*-sulpho-α-L-idopyranosuronic acid structure at the non-reducing end and a 6-*O*-sulpho-2,5-anhydro-D-mannitol structure at the reducing end of their chain.

Dalteparin sodium complies with the monograph Low-molecular-mass heparins (0828) with the modifications and additional requirements below.

The mass-average relative molecular mass ranges between 5600 and 6400, with a characteristic value of about 6000.

The degree of sulphatation is 2.0 to 2.5 per disaccharide unit.

The potency is not less than 110 IU and not more than 210 IU of anti-factor Xa activity per milligram, calculated with reference to the dried substance. The anti-factor IIa activity is not less than 35 IU/mg and not more than 100 IU/mg, calculated with reference to the dried substance. The ratio of anti-factor Xa activity to anti-factor IIa activity is between 1.9 and 3.2.

PRODUCTION

Dalteparin sodium is produced by a validated manufacturing and purification procedure under conditions designed to minimise the presence of N-NO groups.

The manufacturing procedure must have been shown to reduce any contamination by N-NO groups to approved limits using an appropriate, validated quantification method.

IDENTIFICATION

Carry out identification test A as described in the monograph *Low-molecular-mass heparins (0828)* using *dalteparin sodium CRS*.

Carry out identification test C as described in the monograph *Low-molecular-mass heparins (0828)*. The following requirements apply.

The mass-average relative molecular mass ranges between 5600 and 6400. The mass percentage of chains lower than 3000 is not more than 13.0 per cent. The mass percentage of chains higher than 8000 ranges between 15.0 per cent and 25.0 per cent.

TESTS

Appearance of solution. Dissolve 1 g in 10 ml of *water R*. The solution is clear (*2.2.1*) and not more intensely coloured than intensity 5 of the range of reference solutions of the most appropriate colour (*2.2.2, Method II*).

Nitrite. Not more than 5 ppm. Examine by liquid chromatography (*2.2.29*). *Rinse all volumetric flasks at least three times with water R before the preparation of the solutions.*

Test solution. Dissolve 80.0 mg of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent. Allow to stand for at least 30 min.

Reference solution (a). Dissolve 60.0 mg of *sodium nitrite R* in *water R* and dilute to 1000.0 ml with the same solvent.

For the preparation of reference solution (b), use a pipette previously rinsed with reference solution (a).

Reference solution (b). Dilute 1.00 ml of reference solution (a) to 50.0 ml with *water R*.

Before preparing reference solutions (c), (d) and (e), rinse all pipettes with reference solution (b).

Reference solution (c). Dilute 1.00 ml of reference solution (b) to 100.0 ml with *water R* (corresponding to 1 ppm of nitrite in the test sample).

Reference solution (d). Dilute 3.00 ml of reference solution (b) to 100.0 ml with *water R* (corresponding to 3 ppm of nitrite in the test sample).

Reference solution (e). Dilute 5.00 ml of reference solution (b) to 100.0 ml with *water R* (corresponding to 5 ppm of nitrite in the test sample).

The chromatographic procedure may be carried out using:

- a column 0.125 m long and 4.3 mm in internal diameter packed with a strong anion-exchange resin;
- as mobile phase at a flow rate of 1.0 ml/min a solution consisting of 13.61 g of *sodium acetate R* dissolved in *water R*, adjusted to pH 4.3 with *phosphoric acid R* and diluted to 1000 ml with *water R*;
- as detector an appropriate electrochemical device with the following characteristics and settings: a suitable working electrode, a detector potential of + 1.00 V versus Ag/AgCl reference electrode and a detector sensitivity of 0.1 μA full scale.

Inject 100 μl of reference solution (d). When the chromatograms are recorded in the prescribed conditions, the retention time for nitrite is 3.3 to 4.0 min. The test is not valid unless:

- the number of theoretical plates calculated for the nitrite peak is at least 7000 per metre per column (dalteparin sodium will block the binding sites of the stationary phase, which will cause shorter retention times and lower separation efficiency for the analyte; the initial performance of the column may be partially restored using a 58 g/l solution of *sodium chloride R* at a flow rate of 1.0 ml/min for 1 h; after regeneration the column is rinsed with 200 ml to 400 ml of *water R*);
- the symmetry factor for the nitrite peak is less than 3;
- the relative standard deviation of the peak area for nitrite obtained from 6 injections is less than 3.0 per cent.

Inject 100 μl each of reference solutions (c) and (e). The test is not valid unless:

- the correlation factor for a linear relationship between concentration and response for reference solutions (c), (d) and (e) is at least 0.995;
- the signal-to-noise ratio for reference solution (c) is not less than 5 (if the noise level is too high, electrode recalibration is recommended);
- a blank injection of *water R* does not give rise to spurious peaks.

Inject 100 μl of the test solution. Calculate the content of nitrite from the peak areas in the chromatogram obtained with reference solutions (c), (d) and (e).

Boron. Not more than 1 ppm, determined by inductively coupled plasma atomic emission spectroscopy.

Boron is determined by measurement of the emission from an inductively coupled plasma (ICP) at a wavelength specific to boron. The emission line at 249.733 nm is used. Use an appropriate apparatus, whose settings have been optimised as directed by the manufacturer.

Test solution. Dissolve 0.2500 g of the substance to be examined in about 2 ml of *water for chromatography R*, add 100 μl of *nitric acid R* and dilute to 10.00 ml with the same solvent.

Reference solution (a). Prepare a 1 per cent *V/V* solution of *nitric acid R* in *water for chromatography R* (blank).

Reference solution (b). Prepare a 11.4 μg/ml solution of *boric acid R* in a 1 per cent *V/V* solution of *nitric acid R* in *water for chromatography R* (STD_{cal}).

Reference solution (c). Dissolve 0.2500 g of a reference dalteparin sodium with no detectable boron in about 2 ml of *water for chromatography R*, add 100 µl of *nitric acid R* and dilute to 10.00 ml with the same solvent (STD_0).

Reference solution (d). Dissolve 0.2500 g of a reference dalteparin sodium with no boron detected in about 2 ml of a 1 per cent *V/V* solution of *nitric acid R* in *water for chromatography R*, add 10 µl of a 5.7 mg/ml solution of *boric acid R* and dilute to 10.00 ml with the same solvent (STD_1). This solution contains 1 µg/ml of boron.

Calculate the content of boron in the substance to be examined, using the following correction factor:

$$f = \frac{(\mathrm{STD_1} - \mathrm{STD_0}) \times 2}{(\mathrm{STD_{cal}} - \mathrm{blank})}$$

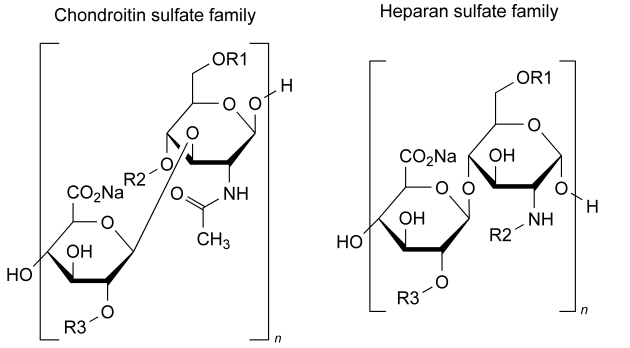
Loss on drying (*2.2.32*). Not more than 5.0 per cent, determined on 1.000 g by drying in an oven at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 670 Pa for 3 h.

Monographs D-E

01/2008:2090

DANAPAROID SODIUM

Danaparoidum natricum



ΔDi	R1	R2	R3
-OS	H	H	H
-6S	SO_3Na	H	H
-4S	H	SO_3Na	H
-US	H	H	SO_3Na
-(U,6)S	SO_3Na	H	SO_3Na
-(U,4)S	H	SO_3Na	SO_3Na
-(4,6)S	SO_3Na	SO_3Na	H
-(U,4,6)S	SO_3Na	SO_3Na	SO_3Na

ΔDiHS	R1	R2	R3
-OS	H	Ac	H
-6S	SO_3Na	Ac	H
-NS	H	SO_3Na	H
-US	H	Ac	SO_3Na
-(U,N)S	H	SO_3Na	SO_3Na
-(6,N)S	SO_3Na	SO_3Na	H
-(U,N,6)S	SO_3Na	SO_3Na	SO_3Na

DEFINITION

Preparation containing the sodium salts of a mixture of sulphated glycosaminoglycans present in porcine tissues. Its major constituents are heparan sulphate and dermatan sulphate. On complete hydrolysis it liberates D-glucosamine, D-galactosamine, D-glucuronic acid, L-iduronic acid, acetic acid and sulphuric acid. It has the characteristic property of enhancing the inactivation of activated factor X (factor Xa) by antithrombin. It has a negligible effect on the inactivation rate of thrombin by antithrombin.

Potency: 11.0 to 17.0 anti-factor Xa units per milligram (dried substance).

PRODUCTION

Danaparoid sodium is prepared from the intestinal mucosa of pigs. It is prepared using a process that ensures that the relative proportion of active sulphated glycosaminoglycans is consistent. It is produced by methods of manufacturing designed to minimise or eliminate endotoxins and hypotensive substances.

CHARACTERS

Appearance: white or almost white, hygroscopic powder.

Solubility: freely soluble in water.

IDENTIFICATION

A. The ratio of anti-factor Xa activity to anti-factor IIa activity, determined as described under Assay and Tests respectively, is not less than 22.

B. It complies with the test for molecular mass distribution (see Tests): the mass-average relative molecular mass ranges between 4000 and 7000.

TESTS

pH (*2.2.3*): 5.5 to 7.0.

Dissolve 0.5 g of the dried substance to be examined in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Anti-factor IIa activity: maximum 0.5 units per milligram (dried substance).

Test solutions. Prepare 2 independent series of dilutions in geometric progression of the substance to be examined in *phosphate buffer solution pH 6.5 R* and in the concentration range of 0.0005 to 0.005 units of anti-factor IIa activity per millilitre.

Reference solutions. Prepare 2 independent series of dilutions in geometric progression of *danaparoid sodium CRS* in *phosphate buffer solution pH 6.5 R* and in the concentration range of 0.0005 to 0.005 units of anti-factor IIa activity per millilitre.

Transfer 50 µl of each solution into the wells of a 96-well microtitre plate. To each well add 50 µl of *antithrombin III solution R3* and 50 µl of *human thrombin solution R1*. Shake the microtitre plate but do not allow bubbles to form. Incubate for 75 min. To each well add 50 µl of *chromogenic substrate R4*. Shake the microtitre plate. Measure the absorbances at 405 nm (*2.2.25*) using a suitable reading device, exactly 4 min after the addition of the chromogenic substrate. The reaction may be stopped using 75 µl of a 20 per cent *V/V* solution of *glacial acetic acid R*. Determine the blank amidolytic activity in a similar manner, using *phosphate buffer solution pH 6.5 R* as the blank solution (minimum 10 blanks per microtitre plate). Calculate the activity of the substance to be examined in units of anti-factor IIa activity per milligram using a suitable statistical method, for example the parallel-line assay.

Chondroitin sulphate and dermatan sulphate. Chondroitin sulphate: maximum 8.5 per cent (dried substance); dermatan sulphate: 8.0 per cent to 16.0 per cent (dried substance).

Determine by selective enzymatic degradation.

Test solutions. Dry the substance to be examined at 60 °C over *diphosphorus pentoxide R* at a pressure of about 670 Pa for 3 h. Dissolve 0.200 g of the dried substance in 10.0 ml of *water R*. Dilute this solution as necessary to obtain 3 test solutions containing 20 mg/ml, 10 mg/ml and 5 mg/ml of the dried substance to be examined in *water R*.