further appropriate procedure for the removal of bacterial endotoxins. The addition of divalent cations may be necessary to fulfill the validation criteria.

ASSAY

The anticoagulant activity of low-molecular-mass heparins is determined in vitro by 2 assays which determine its ability to accelerate the inhibition of factor Xa (anti-Xa assay) and thrombin, factor IIa (anti-IIa assay), by antithrombin III. The International Units for anti-Xa and anti-IIa activity are the activities contained in a stated amount of the International Standard for low-molecular-mass heparin. Low-molecular-mass heparin for assay BRP, calibrated in International Units by comparison with the International Standard using the 2 assays given below, is used as reference preparation.

ANTI-FACTOR Xa ACTIVITY

Reference and test solutions

Prepare 4 independent series of 4 dilutions each, of the substance to be examined and of the reference preparation of low-molecular-mass heparin in tris(hydroxymethyl)aminomethane sodium chloride buffer solution pH 7.4 R; the concentration range should be within 0.025 IU to 0.2 IU of anti-factor Xa activity per millilitre and the dilutions chosen should give a linear response when results are plotted as absorbance against log concentration.

Procedure

Label 16 tubes in duplicate: T1, T2, T3, T4, S1, S2, S3, S4 for the dilutions of the substance to be examined and S1, S2, S3, S4 for the dilutions of the reference preparation. To each tube add 50 µl of antithrombin III solution R2 and 50 µl of the appropriate dilution of the substance to be examined or the reference preparation. After each addition, mix but do not allow bubbles to form. Treating the tubes in the order S1, S2, S3, S4, allow to equilibrate at 37 °C (water-bath or heating block) for 1 min and add to each tube 100 µl of human thrombin solution R. Incubate for exactly 1 min and add 250 µl of chromophore substrate R2. Stop the reaction after exactly 4 min by adding 375 µl of acetic acid R. Transfer the mixtures to semi-micro cuvettes and measure the absorbance (2.2.25) at 405 nm using a suitable reading device. Determine the blank amidolytic activity at the beginning and at the end of the procedure in a similar manner, using tris(hydroxymethyl)aminomethane sodium chloride buffer solution pH 7.4 R instead of the reference and test solutions; the 2 blank values do not differ significantly. Calculate the regression of the absorbance on log concentrations of the solutions of the substance to be examined and of the reference preparation of low-molecular-mass heparins, and calculate the potency of the substance to be examined in International Units of anti-factor Xa activity per millilitre using the usual statistical methods for parallel-line assays.

LABELLING

The label states:

− the number of International Units of anti-factor Xa activity per milligram,
− the number of International Units of anti-factor IIa activity per milligram,
− the mass-average molecular mass and the percentage of molecules within defined molecular mass ranges,
− where applicable, that the substance is free from bacterial endotoxins,
− where applicable, that the contents are the sodium salt,
− where applicable, that the contents are the calcium salt.

STORAGE

In an airtight tamper-proof container. If the product is sterile and free of bacterial endotoxins, store in a sterile and apyrogenic container.

01/2005:1980 corrected

HEPTAMINOL HYDROCHLORIDE

Heptaminol hydrochloride

C19H20ClNO

M̅, 181.7

DEFINITION

(6RS)-6-Amino-2-methylheptan-2-ol hydrochloride.

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: freely soluble in water, soluble in alcohol, practically insoluble in methylene chloride.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.
Hexamidine diisethionate

HEXAMIDINE DISETIONATE

Hexamidini diisethonatis

Heavy metals (2.4.8): maximum 10 ppm.

12 ml of solution S complies with limit test A. Prepare the standard using lead standard solution (1 ppm Pb) R.

Loss on drying (2.2.32): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 100-105 °C for 4 h.

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

ASSAY

Dissolve 0.140 g in 50 ml of alcohol R and add 5.0 ml of 0.01 M hydrochloric acid. Carry out a potentiometric titration (2.2.20), using 0.1 M sodium hydroxide. Read the volume added between the 2 points of inflexion.

1 ml of 0.1 M sodium hydroxide is equivalent to 18.17 mg of C₁₅H₁₉N₅CINO.

IMPURITIES


01/2005:1436

DEFINITION

Hexamidine diisethonatis contains not less than 98.5 per cent and not more than the equivalent of 101.5 per cent of 4,4’-(hexane-1,6-diylidioxy)dibenzenimidamide bis(2-hydroxyethanesulphonate), calculated with reference to the dried substance.

CHARACTERS

A white or slightly yellow powder, hygroscopic, sparingly soluble in water, slightly soluble in alcohol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Examine by infrared absorption spectrophotometry (2.2.24), comparing with the spectrum obtained with hexamidine diisethonatis CRS.

B. Dissolve about 40 mg in 5 ml of water R and add 2 ml of a 200 g/1 solution of ammonium and cerium nitrate R in 4 M nitric acid. An orange-brown colour develops.

B. Infrared absorption spectrophotometry (2.2.24). Comparison: heptaminol hydrochloride CRS.

C. Examine the chromatograms obtained in the test for related substances.

Detection: examine in daylight.

Results: the principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (b).

D. It gives reaction (a) of chlorides (2.3.1).

TESTS

Solution S. Dissolve 5.0 g in carbon dioxide-free water R and dilute to 50 ml with the same solvent.

Appearance of solution. Solution S is clear (2.2.1) and not more intensely coloured than reference solution BY₆ (2.2.2, Method II).

Acidity or alkalinity. To 10 ml of solution S add 0.1 ml of methyl red solution R and 0.3 ml of 0.01 M hydrochloric acid. The solution is red. Add 0.6 ml of 0.01 M sodium hydroxide. The solution is yellow.

Related substances. Thin-layer chromatography (2.2.27).

Test solution (a). Dissolve 0.50 g of the substance to be examined in methanol R and dilute to 5 ml with the same solvent.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10 ml with methanol R.

Reference solution (a). Dilute 3.0 ml of test solution (a) to 10.0 ml with methanol R. Dilute 1.0 ml of this solution to 50.0 ml with methanol R.

Reference solution (b). Dissolve 0.10 g of heptaminol hydrochloride CRS in methanol R and dilute to 10 ml with the same solvent.

Reference solution (c). Dissolve 10.0 mg of heptaminol impurity A CRS in methanol R and dilute to 5.0 ml with the same solvent.

Reference solution (d). Dilute 1.0 ml of reference solution (c) to 10.0 ml with methanol R.

Reference solution (e). To 2.5 ml of reference solution (c) add 0.5 ml of test solution (b) and dilute to 5 ml with methanol R.

Plate: TLC silica gel G plate R.


Application: 10 µl; apply test solutions (a) and (b) and reference solutions (a), (b), (d) and (e).

Development: over 2/3 of the plate.

Drying: in air.

Detection: expose the plate to iodine vapour for at least 15 h.

System suitability: the chromatogram obtained with reference solution (e) shows 2 clearly separated principal spots and the chromatogram obtained with reference solution (a) shows a single principal spot.

Limits: in the chromatogram obtained with test solution (a):

— impurity A: any spot corresponding to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (d) (0.2 per cent),

— any other impurity: any spot, apart from the principal spot and any spot corresponding to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.6 per cent).

A. To 1 ml of solution S (see Tests) add 4 ml of water R and 2 ml of a 200 g/1 solution of ammonium and cerium nitrate R in 4 M nitric acid. An orange-brown colour develops.

To 2.5 ml of reference solution (c) add 0.5 ml of test solution (b) and dilute to 5 ml with methanol R.

Dissolve 0.140 g in 50 ml of alcohol R and add 5.0 ml of 0.01 M hydrochloric acid. Carry out a potentiometric titration (2.2.20), using 0.1 M sodium hydroxide. Read the volume added between the 2 points of inflexion.

1 ml of 0.1 M sodium hydroxide is equivalent to 18.17 mg of C₁₅H₁₉N₅CINO.