IDENTIFICATION

A solution containing the equivalent of 100 IU of hyaluronidase in 1 ml of a 9 g/l solution of *sodium chloride R* depolymerises an equal volume of a 10 g/l solution of *sodium hyaluronate BRP* in 1 min at 20 °C as shown by a pronounced decrease in viscosity. This action is destroyed by heating the hyaluronidase at 100 °C for 30 min.

TESTS

Appearance of solution. Dissolve 0.10 g of the substance to be examined in *water R* and dilute to 10 ml. The solution is clear (*2.2.1*).

pH (*2.2.3*). Dissolve 30 mg in 10 ml of *carbon dioxide-free water R*. The pH of the solution is 4.5 to 7.5.

Loss on drying (*2.2.32*). Not more than 5.0 per cent, determined on 0.500 g by drying at 60 °C at a pressure not exceeding 670 Pa for 2 h.

Bacterial endotoxins (*2.6.14*): less than 0.2 IU per IU of hyaluronidase.

ASSAY

The activity of hyaluronidase is determined by comparing the rate at which it hydrolyses *sodium hyaluronate BRP* with the rate obtained with the International Standard, or a reference preparation calibrated in International Units, using a slope-ratio assay.

Substrate solution. To 0.10 g of *sodium hyaluronate BRP* in a 25 ml conical flask add slowly 20.0 ml of *water R* at 4 °C. The rate of addition must be slow enough to allow the substrate particles to swell (about 5 min). Maintain at 4 °C and stir for at least 12 h. Store at 4 °C and use within 4 days.

For the test solution and the reference solution, prepare the solution and carry out the dilution at 0 °C to 4 °C.

Test solution. Dissolve a suitable amount of the substance to be examined in *hyaluronidase diluent R* so as to obtain a solution containing 0.6 ± 0.3 IU of hyaluronidase per millilitre.

Reference solution. Dissolve a suitable amount of *hyaluronidase BRP* in *hyaluronidase diluent R* so as to obtain a solution containing 0.6 IU of hyaluronidase per millilitre.

In a reaction vessel, mix 1.50 ml of *phosphate buffer solution pH 6.4 R* and 1.0 ml of the substrate solution and equilibrate at 37 ± 0.1 °C. At time t_1 = 0 (first chronometer) add 0.50 ml of the test solution containing E_t mg of the enzyme to be examined, mix, measure the viscosity of the solution using a suitable viscometer maintained at 37 ± 0.1 °C and record the outflow time t_2 using a second chronometer (graduated in 0.1 second intervals), several times during about 20 min (read on the first chronometer). The following viscometer has been found suitable: Ubbelohde microviscometer (DIN 51 562, Part 2), capillary type MII, viscometer constant about 0.1 mm^2/s^2.

Repeat the procedure using 0.50 ml of the reference solution containing E_r mg of *hyaluronidase BRP*.

Calculate the viscosity ratio from the expression:

$$\eta_r = \frac{k \times t_2}{0.6915}$$

k = the viscometer constant in mm^2/s^2 (indicated on the viscometer);

t_2 = the outflow time (in seconds) of the solution;

0.6915 = the kinematic viscosity in mm^2/s of the buffer solution at 37 °C.

Since the enzymatic reaction continues during the outflow time measurements, the real reaction time equals $t_1 + t_2/2$, half of the outflow time ($t_2/2$) for which a certain measurement is valid being added to the time t_1 at which the measurement is started. Plot $(\ln \eta_r)^{-1}$ as a function of the reaction time ($t_1 + t_2/2$) in seconds. A linear relationship is obtained. Calculate the slope for the substance to be examined (b_t) and the reference preparation (b_r).

Calculate the specific activity in International Units per milligram from the expression:

$$\frac{b_t}{b_r} \times \frac{E_r}{E_t} \times A$$

A = the specific activity of *hyaluronidase BRP* in International Units per milligram.

Carry out the complete procedure at least three times and calculate the average activity of the substance to be examined.

STORAGE

Store in an airtight container at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, tamper-proof container.

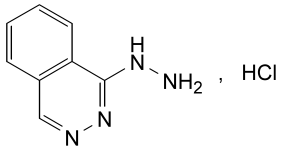
LABELLING

The label states the activity in International Units per milligram.

01/2008:0829

HYDRALAZINE HYDROCHLORIDE

Hydralazini hydrochloridum



$C_8H_9ClN_4$ M_r 196.6

[304-20-1]

DEFINITION

1-Hydrazinophthalazine hydrochloride.

Content: 98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: soluble in water, slightly soluble in ethanol (96 per cent), very slightly soluble in methylene chloride.

mp: about 275 °C, with decomposition.

IDENTIFICATION

First identification: B, E.

Second identification: A, C, D, E.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution. Dissolve 50 mg in *water R* and dilute to 100 ml with the same solvent. Dilute 2 ml of this solution to 100 ml with *water R*.

Spectral range: 220-350 nm.

Absorption maxima: at 240 nm, 260 nm, 303 nm and 315 nm.

Absorbance ratio: A_{240}/A_{303} = 2.0 to 2.2.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: discs.

Comparison: *hydralazine hydrochloride CRS*.

C. Dissolve 0.5 g in a mixture of 8 ml of *dilute hydrochloric acid R* and 100 ml of *water R*. Add 2 ml of *sodium nitrite solution R*, allow to stand for 10 min and filter. The precipitate, washed with *water R* and dried at 100-105 °C, melts (*2.2.14*) at 209 °C to 212 °C.

D. Dissolve about 10 mg in 2 ml of *water R*. Add 2 ml of a 20 g/l solution of *nitrobenzaldehyde R* in *ethanol (96 per cent) R*. An orange precipitate is formed.

E. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S. Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution GY_6 (*2.2.2, Method II*).

Dilute 4 ml of solution S to 20 ml with *water R*.

pH (*2.2.3*): 3.5 to 4.2 for solution S.

Hydrazine. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 0.12 g of the substance to be examined in 4 ml of *water R* and add 4 ml of a 150 g/l solution of *salicylaldehyde R* in *methanol R* and 0.2 ml of *hydrochloric acid R*. Mix and keep at a temperature not exceeding 25 °C for 2-4 h, until the precipitate formed has sedimented. Add 4 ml of *toluene R*, shake vigorously and centrifuge. Transfer the clear supernatant liquid to a 100 ml separating funnel and shake vigorously, each time for 3 min, with 2 quantities, each of 20 ml, of a 200 g/l solution of *sodium metabisulphite R* and with 2 quantities, each of 50 ml, of *water R*. Separate the upper toluene layer which is the test solution.

Reference solution (a). Dissolve 12 mg of *hydrazine sulphate R* in *dilute hydrochloric acid R* and dilute to 100.0 ml with the same acid. Dilute 1.0 ml of this solution to 100.0 ml with *dilute hydrochloric acid R*.

Reference solution (b). Prepare the solution at the same time and in the same manner as for the test solution, using 1.0 ml of reference solution (a) and 3 ml of *water R*.

Plate: *TLC silica gel G plate R*.

Mobile phase: *ethanol (96 per cent) R, toluene R* (10:90 *V/V*).

Application: 20 µl of the test solution and reference solution (b).

Development: over a path of 10 cm.

Drying: in air.

Detection: examine in ultraviolet light at 365 nm.

Limit:

– *hydrazine*: any yellow fluorescent spot due to hydrazine is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b) (10 ppm).

Related substances. Liquid chromatography (*2.2.29*). *The solutions must be injected within one working day.*

Test solution. Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 10.0 ml of reference solution (a) to 50.0 ml with the mobile phase.

Reference solution (c). Dissolve 25.0 mg of *phthalazine R* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 4.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (d). Dilute a mixture of 4.0 ml of the test solution and 10.0 ml of reference solution (c) to 100.0 ml with the mobile phase.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;

– *stationary phase*: *nitrile silica gel for chromatography R1* (10 µm).

Mobile phase: mix 22 volumes of *acetonitrile R* and 78 volumes of a solution containing 1.44 g/l of *sodium laurilsulfate R* and 0.75 g/l of *tetrabutylammonium bromide R*, then adjust to pH 3.0 with *0.05 M sulphuric acid*.

Flow rate: 1 ml/min.

Detection: spectrophotometer at 230 nm.

Injection: 20 µl.

Run time: 3 times the retention time of hydralazine.

Retention time: hydralazine = about 10 min to 12 min; if necessary, adjust the concentration of acetonitrile in the mobile phase.

System suitability:

– the chromatogram obtained with reference solution (d) shows 2 principal peaks;

– *resolution*: minimum 2.5 between the peaks due to hydralazine and phtalazine in the chromatogram obtained with reference solution (d);

– *signal-to-noise ratio*: minimum 3 for the principal peak in the chromatogram obtained with reference solution (b).

Limit:

– *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent).

Heavy metals (*2.4.8*): maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo*.

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

ASSAY

Dissolve 80.0 mg in 25 ml of *water R*. Add 35 ml of *hydrochloric acid R* and titrate with *0.05 M potassium iodate*, determining the end-point potentiometrically (*2.2.20*), using a calomel reference electrode and a platinum indicator electrode.

1 ml of *0.05 M potassium iodate* is equivalent to 9.832 mg of $C_8H_9ClN_4$.

STORAGE

Protected from light.