and 10 mL of *water R* and add 0.1 mL of *sodium nitrite solution R*. Add the second solution to the first and mix. An orange-red colour develops.

TESTS

Solution S. Dissolve 2.5 g in *distilled water R*, heating in a water-bath 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 BY_7 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*): + 11.4 to + 12.4 (dried substance).

Dissolve 2.75 g in 12.0 mL of *hydrochloric acid R1* and dilute to 25.0 mL with *water R*.

Ninhydrin-positive substances. Thin-layer chromatography (*2.2.27*).

Test solution (a). Dissolve 0.10 g of the substance to be examined in *water R* and dilute to 10 mL with the same solvent.

Test solution (b). Dilute 1 mL of test solution (a) to 50 mL with *water R*.

Reference solution (a). Dissolve 10 mg of *histidine CRS* in *water R* and dilute to 50 mL with the same solvent.

Reference solution (b). Dilute 5 mL of test solution (b) to 20 mL with *water R*.

Reference solution (c). Dissolve 10 mg of *histidine CRS* and 10 mg of *proline CRS* in *water R* and dilute to 25 mL with the same solvent.

Plate: *TLC silica gel plate R*.

Mobile phase: *glacial acetic acid R, water R, butanol R* (20:20:60 *V/V/V*).

Application: 5 µL.

Development: over 2/3 of the plate.

Drying: in air.

Detection: spray with *ninhydrin solution R* and heat at 100-105 °C for 15 min.

System suitability: the chromatogram obtained with reference solution (c) shows 2 clearly separated spots.

Limits:

– *any impurity*: any spots in the chromatogram obtained with test solution (a), apart from the principal spot, are not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Chlorides (*2.4.4*): maximum 200 ppm.

Dilute 5 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*): maximum 300 ppm.

Dilute 10 mL of solution S to 15 mL with *distilled water R*.

Ammonium (*2.4.1, Method B*): maximum 200 ppm, determined on 50 mg.

Prepare the standard using 0.1 mL of *ammonium standard solution (100 ppm NH_4) R*.

Iron (*2.4.9*): maximum 10 ppm.

In a separating funnel, dissolve 1.0 g in 10 mL of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 mL, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 mL of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron.

Heavy metals (*2.4.8*): maximum 10 ppm.

Dissolve 2.0 g in a mixture of 3 mL of *dilute hydrochloric acid R* and 15 mL of *water R*, with gentle warming if necessary, and dilute to 20 mL with *water R*. 12 mL of the solution complies with test A. Prepare the reference solution 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 105 °C.

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

ASSAY

Dissolve 0.130 g in 50 mL of *water R*. Titrate with *0.1 M hydrochloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M hydrochloric acid* is equivalent to 15.52 mg of $C_6H_9N_3O_2$.

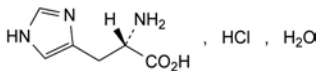
STORAGE

Protected from light.

01/2008:0910
corrected 6.0

HISTIDINE HYDROCHLORIDE MONOHYDRATE

Histidini hydrochloridum monohydricum



$C_6H_{10}ClN_3O_2,H_2O$ M_r 209.6
[5934-29-2]

DEFINITION

Histidine hydrochloride monohydrate contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of the hydrochloride of (*S*)-2-amino-3-(imidazol-4-yl)propanoic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, freely soluble in water, slightly soluble in alcohol.

IDENTIFICATION

First identification: A, B, C, F.

Second identification: A, B, D, E, F.

A. Specific optical rotation (see Tests).

B. pH (see Tests).

C. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *histidine hydrochloride monohydrate CRS*. Examine the substances prepared as discs.

D. Examine the chromatograms obtained in the test for ninhydrin-positive substances. 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 (a).

E. Dissolve 0.1 g in 7 mL of *water R* and add 3 mL of a 200 g/L solution of *sodium hydroxide R*. Dissolve 50 mg of *sulfanilic acid R* in a mixture of 0.1 mL of *hydrochloric acid R* and 10 mL of *water R* and add 0.1 mL of *sodium nitrite solution R*. Add the second solution to the first and mix. An orange-red colour develops.

F. About 20 mg gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S. Dissolve 2.5 g in *carbon dioxide-free water R* prepared from *distilled 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 BY_6 (*2.2.2, Method II*).

pH (*2.2.3*). The pH of solution S is 3.0 to 5.0.

Specific optical rotation (*2.2.7*). Dissolve 2.75 g in 12.0 mL of *hydrochloric acid R1* and dilute to 25.0 mL with *water R*. The specific optical rotation is + 9.2 to + 10.6, calculated with reference to the dried substance.

Ninhydrin-positive substances. Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel plate R*.

Test solution (a). Dissolve 0.10 g of the substance to be examined in *water R* and dilute to 10 mL with the same solvent.

Test solution (b). Dilute 1 mL of test solution (a) to 50 mL with *water R*.

Reference solution (a). Dissolve 10 mg of *histidine hydrochloride monohydrate CRS* in *water R* and dilute to 50 mL with the same solvent.

Reference solution (b). Dilute 5 mL of test solution (b) to 20 mL with *water R*.

Reference solution (c). Dissolve 10 mg of *histidine hydrochloride monohydrate CRS* and 10 mg of *proline CRS* in *water R* and dilute to 25 mL with the same solvent.

Apply separately to the plate 5 µL of each solution. Dry the plate in a current of air. Develop over a path of 15 cm using a mixture of 20 volumes of *glacial acetic acid R*, 20 volumes of *water R* and 60 volumes of *butanol R*. Allow the plate to dry in air. Spray with *ninhydrin solution R* and heat at 100 °C to 105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

Sulfates (*2.4.13*). Dilute 10 mL of solution S to 15 mL with *distilled water R*. The solution complies with the limit test for sulfates (300 ppm).

Ammonium (*2.4.1*). 50 mg complies with limit test B for ammonium (200 ppm). Prepare the standard using 0.1 mL of *ammonium standard solution (100 ppm NH_4) R*.

Iron (*2.4.9*). In a separating funnel, dissolve 1.0 g in 10 mL of *dilute hydrochloric acid R*. Shake with three quantities, each of 10 mL, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 mL of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron (10 ppm).

Heavy metals (*2.4.8*). Dissolve 2.0 g in *water R* and dilute to 20 mL with the same solvent. 12 mL of the solution complies with limit test A for heavy metals (10 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*): 7.0 per cent to 10.0 per cent, determined on 1.000 g by drying in an oven at 145 °C to 150 °C.

Sulfated ash (*2.4.14*). Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.160 g in 50 mL of *carbon dioxide-free water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M sodium hydroxide* is equivalent to 19.16 mg of $C_6H_{10}ClN_3O_2$.

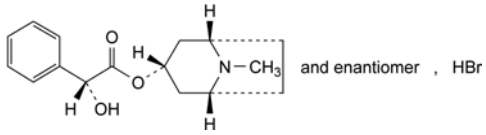
STORAGE

Store protected from light.

01/2008:0500
corrected 6.0

HOMATROPINE HYDROBROMIDE

Homatropini hydrobromidum



$C_{16}H_{22}BrNO_3$ M_r 356.3
[51-56-9]

DEFINITION

(1*R*,3*r*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl (2*RS*)-2-hydroxy-2-phenylacetate hydrobromide.

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

CHARACTERS

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: freely soluble in water, sparingly soluble in alcohol.

mp: about 215 °C, with decomposition.

IDENTIFICATION

First identification: A, C.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *homatropine hydrobromide CRS*.

B. Dissolve 50 mg in 1 mL of *water R* and add 2 mL of *dilute acetic acid R*. Heat and add 4 mL of *picric acid solution R*. Allow to cool, shaking occasionally. Collect the crystals, wash with 2 quantities, each of 3 mL, of iced *water R* and dry at 100-105 °C. The crystals melt (*2.2.14*) at 182 °C to 186 °C.

C. It gives reaction (a) of bromides (*2.3.1*).

TESTS

Solution S. Dissolve 1.25 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*): 5.0 to 6.5 for solution S.

Related substances. Liquid chromatography (*2.2.29*).

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

Reference solution (a). Dilute 5.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (b). Dilute 5.0 mL of reference solution (a) to 25.0 mL with the mobile phase.

Reference solution (c). Dissolve 5.0 mg of *hyoscine hydrobromide CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase. To 10.0 mL of this solution add 0.5 mL of the test solution and dilute to 100.0 mL with the mobile phase.

Column:
- *size*: *l* = 0.1 m, Ø = 4.6 mm,
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm),
- *temperature*: 40 °C.

Mobile phase: mix 33 volumes of *methanol R2* and 67 volumes of a solution prepared as follows: dissolve 6.8 g of *potassium dihydrogen phosphate R* and 7.0 g of *sodium heptanesulfonate monohydrate R* in 1000 mL of *water R* and adjust to pH 2.7 with a 330 g/L solution of *phosphoric acid R*.

Flow rate: 1.5 mL/min.