Histidine hydrochloride monohydrate

**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. It complies with the test for specific optical rotation (see Tests).
B. It complies with the test for the pH (see Tests).
C. Examine by infrared absorption spectrophotometry (2.2.20), 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 sulphanilic 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 BY6 (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.
Homatropine hydrobromide

Reference 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 (c). Dilute 10 mg of histidine hydrochloride monohydrate CRS in water R and dilute to 50 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.

Solubilities. (2.4.13). Dilute 10 ml of solution S to 15 ml with distilled water R. The solution complies with the limit test for sulphates (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 NH₃) 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 R, 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.

Sulphated 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 C₁₆H₂₂BrNO₃.

STORAGE

Store protected from light.