TESTS

Appearance of solution. Dissolve 2.5 g in *methanol R* and dilute to 50 mL with the same solvent. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Related substances. Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel* F_{254} *plate R*.

Test solution. Dissolve 0.5 g of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (a). Dilute 1 mL of the test solution to 10 mL with *methanol R*. Dilute 1 mL of the solution to 20 mL with *methanol R*.

Reference solution (b). Dilute 5 mL of reference solution (a) to 10 mL with *methanol R*.

Reference solution (c). Dissolve 0.5 g of the substance to be examined and 5 mg of *picotamide impurity A CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Apply to the plate 5 μL of each solution. Develop over a path of 15 cm using a mixture 0.8 volumes of *glacial acetic acid R*, 1 volume of *water R*, 2.5 volumes of *methanol R* and 8 volumes of *butanol R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (a) (0.5 per cent) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

Chlorides (*2.4.4*). Dissolve 0.25 g in a mixture of 2.5 mL of *dilute nitric acid R* and 12.5 mL of *water R*. The solution complies with the limit test for chlorides (200 ppm).

Heavy metals (*2.4.8*). Dissolve 1.0 g by gently heating in a mixture of 15 volumes of *water R* and 85 volumes of *methanol R* and dilute to 20 mL with the same mixture of solvents. 12 mL of the solution complies with limit test B for heavy metals (20 ppm). Prepare the standard, using lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 15 volumes of *water R* and 85 volumes of *methanol R*.

Water (*2.5.12*): 4.5 per cent to 5.0 per cent, determined on 0.300 g by the semi-micro determination of water.

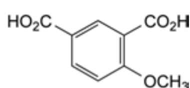
Sulfated ash (*2.4.14*). Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

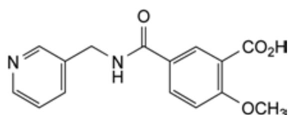
Dissolve 0.150 g in a mixture of 20 mL of *anhydrous acetic acid R* and 20 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 18.82 mg of $C_{21}H_{20}N_4O_3$.

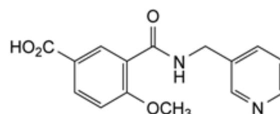
IMPURITIES



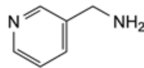
A. 4-methoxybenzene-1,3-dicarboxylic acid,



B. 2-methoxy-5-[[(pyridin-3-ylmethyl)amino]carbonyl]benzoic acid,



C. 4-methoxy-3-[[(pyridin-3-ylmethyl)amino]carbonyl]benzoic acid,



D. (pyridin-3-yl)methanamine.

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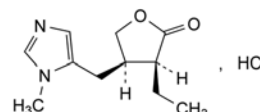
corrected 6.3

PILOCARPINE HYDROCHLORIDE

Pilocarpini hydrochloridum



$C_{11}H_{17}ClN_2O_2$ M_r 244.7
[54-71-7]

DEFINITION

(3*S*,4*R*)-3-Ethyl-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]-dihydrofuran-2(3*H*)-one hydrochloride.

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

CHARACTERS

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

Solubility: very soluble in water and in ethanol (96 per cent).

mp: about 203 °C.

IDENTIFICATION

First identification: A, B, E.

Second identification: A, C, D, E.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *pilocarpine hydrochloride CRS.*

If the substances are examined as discs, prepare them using *potassium chloride R*.

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

Test solution. Dissolve 10 mg in *methanol R* and dilute to 2 mL with the same solvent.

Reference solution. Dissolve 10 mg of *pilocarpine hydrochloride CRS* in *methanol R* and dilute to 2 mL with the same solvent.

Plate: *TLC silica gel G plate R.*

Mobile phase: *concentrated ammonia R, methanol R, methylene chloride R* (1:14:85 *V/V/V*).

Application: 2 μL.

Development: over a path of 15 cm.

Drying: at 100-105 °C for 10 min, then allow to cool.

Detection: spray with *dilute potassium iodobismuthate solution R.*

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

D. Dilute 0.2 mL of solution S (see Tests) to 2 mL with *water R*. Add 0.05 mL of a 50 g/L solution of *potassium dichromate R*, 1 mL of *dilute hydrogen peroxide solution R* and 2 mL of *methylene chloride R* and shake. A violet colour develops in the organic layer.

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

TESTS

Solution S. Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

pH (*2.2.3*): 3.5 to 4.5 for solution S.

Specific optical rotation (*2.2.7*): + 89 to + 93 (dried substance), determined on solution S.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 0.100 g of the substance to be examined in *water R* and dilute to 100.0 mL with the same solvent.

Reference solution (a). Dilute 5.0 mL of the test solution to 100.0 mL with *water R*. Dilute 2.0 mL of this solution to 20.0 mL with *water R*.

Reference solution (b). Dissolve 5.0 mg of *pilocarpine nitrate for system suitability CRS* (containing impurity A) in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (c). To 5 mL of the test solution, add 0.1 mL of *ammonia R* and heat the solution on a water-bath for 30 min, cool and dilute to 25 mL with *water R*. Dilute 3 mL of this solution to 25 mL with *water R*. Mainly pilocarpic acid (impurity B) is formed.

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R1* (5 μm) with a pore size of 10 nm and a carbon loading of 19 per cent.

Mobile phase: mix 55 volumes of *methanol R,* 60 volumes of *acetonitrile R* and 885 volumes of a 0.679 g/L solution of *tetrabutylammonium dihydrogen phosphate R* previously adjusted to pH 7.7 with *dilute ammonia R2.*

Flow rate: 1.2 mL/min.

Detection: spectrophotometer at 220 nm.

Injection: 20 μL.

Run time: twice the retention time of pilocarpine.

Elution order: impurity B, impurity C, impurity A, pilocarpine.

Retention time: pilocarpine = about 20 min.

System suitability: reference solution (b):

- *resolution*: minimum 1.6 between the peaks due to impurity A and pilocarpine.

Limits:

- *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent);
- *sum of impurities A and B*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);
- *sum of impurities other than A and B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent).

Iron (*2.4.9*): maximum 10 ppm, determined on solution S. Prepare the standard using 5 mL of *iron standard solution (1 ppm Fe) R* and 5 mL of *water 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 0.200 g in 50 mL of *ethanol (96 per cent) R* and add 5 mL of *0.01 M hydrochloric acid.* Titrate with *0.1 M sodium hydroxide,* determining the end-point potentiometrically (*2.2.20*). Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 24.47 mg of $C_{11}H_{17}ClN_2O_2$.

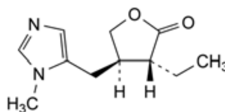
STORAGE

In an airtight container, protected from light.

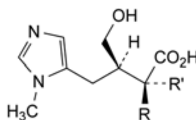
IMPURITIES

Specified impurities: *A, B.*

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*R*,4*R*)-3-ethyl-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]dihydrofuran-2(3*H*)-one (isopilocarpine),



B. R = C_2H_5, R′ = H: (2*S*,3*R*)-2-ethyl-3-(hydroxymethyl)-4-(1-methyl-1*H*-imidazol-5-yl)butanoic acid (pilocarpic acid),

C. R = H, R′ = C_2H_5: (2*R*,3*R*)-2-ethyl-3-(hydroxymethyl)-4-(1-methyl-1*H*-imidazol-5-yl)butanoic acid (isopilocarpic acid).

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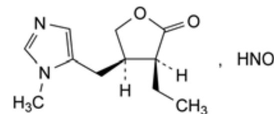
corrected 6.3

PILOCARPINE NITRATE

Pilocarpini nitras



$C_{11}H_{17}N_3O_5$ M_r 271.3
[148-72-1]

DEFINITION

(3*S*,4*R*)-3-Ethyl-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]-dihydrofuran-2(3*H*)-one nitrate.

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

CHARACTERS

Appearance: white or almost white, crystalline powder or colourless crystals, sensitive to light.

Solubility: freely soluble in water, sparingly soluble in ethanol (96 per cent).

mp: about 174 °C, with decomposition.

IDENTIFICATION

First identification: A, B, E.

Second identification: A, C, D, E.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).