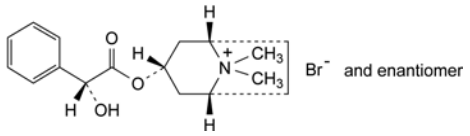
01/2012:0720

HOMATROPINE METHYLBROMIDE

Homatropini methylbromidum



$C_{17}H_{24}BrNO_3$ M_r 370.3
[80-49-9]

DEFINITION

(1*R*,3*r*,5*S*)-3-[[(2*RS*)-2-Hydroxy-2-phenylacetyl]oxy]-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane bromide.

Content: 98.5 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, soluble in ethanol 96 per cent.

mp: about 190 °C.

IDENTIFICATION

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

B. 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*): 4.5 to 6.5 for solution S.

Related substances. Liquid chromatography (*2.2.29*).

Solvent mixture: *acetonitrile R1*, mobile phase A (9:41 *V/V*).

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

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

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

Reference solution (c). Dissolve 5.0 mg of *homatropine hydrobromide CRS* (impurity B) in the solvent mixture and dilute to 50.0 mL with the solvent mixture. To 10.0 mL of the solution add 0.5 mL of the test solution and dilute to 100.0 mL with the solvent mixture.

Reference solution (d). Dissolve 2.0 mg of *homatropine methylbromide for system suitability CRS* (containing impurity A) in 1.0 mL of the solvent mixture.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm);
- *temperature*: 25 °C.

Mobile phase:
- *mobile phase A*: dissolve 3.4 g of *potassium dihydrogen phosphate R* and 5.0 g of *sodium pentanesulfonate monohydrate R* in 980 mL of *water for chromatography R*, adjust to pH 3.0 with a 330 g/L solution of *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*;
- *mobile phase B*: mix 400 mL of mobile phase A and 600 mL of *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	70	30
2 - 15	70 → 30	30 → 70

Flow rate: 1.4 mL/min.

Detection: spectrophotometer at 210 nm.

Injection: 10 µL.

Relative retention with reference to homatropine methylbromide (retention time = about 5 min): impurity A = about 0.9; impurity B = about 1.2.

Identification of impurities: use the chromatogram supplied with *homatropine methylbromide for system suitability CRS* and the chromatogram obtained with reference solution (d) to identify the peak due to impurity A; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity B.

System suitability:
- *resolution*: minimum 2.5 between the peaks due to homatropine methylbromide and impurity B in the chromatogram obtained with reference solution (c);
- *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to homatropine methylbromide in the chromatogram obtained with reference solution (d).

Limits:
- *impurities A, B*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent); disregard the peak due to the bromide ion which appears close to the peak due to the solvent;
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

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 0.300 g in 10 mL of *water R*. Titrate with *0.1 M silver nitrate*. Determine the end-point potentiometrically (*2.2.20*), using a silver indicator electrode and a silver-silver chloride reference electrode.

1 mL of *0.1 M silver nitrate* is equivalent to 37.03 mg of $C_{17}H_{24}BrNO_3$.

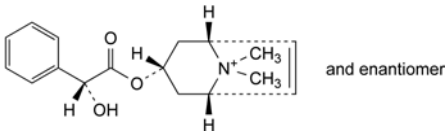
STORAGE

Protected from light.

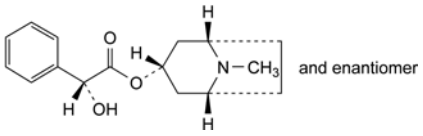
IMPURITIES

Specified impurities: A, B.

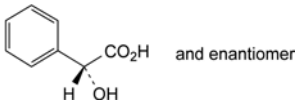
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical 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, D, E, F.*



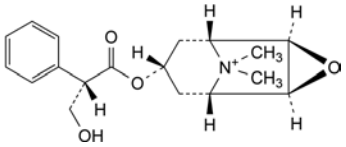
A. (1*R*,3*s*,5*S*)-3-[[(2*RS*)-2-hydroxy-2-phenylacetyl]oxy]-8,8-dimethyl-8-azoniabicyclo[3.2.1]oct-6-ene (methyldehydrohomatropine),



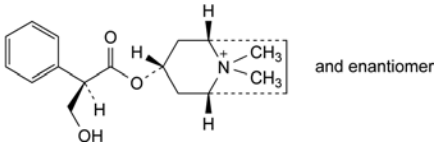
B. (1*R*,3*r*,5*S*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*RS*)-2-hydroxy-2-phenylacetate (homatropine),



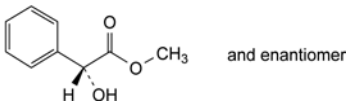
C. (2*RS*)-2-hydroxy-2-phenylacetic acid (mandelic acid),



D. (1*R*,2*R*,4*S*,5*S*,7*s*)-7-[[(2*S*)-3-hydroxy-2-phenylpropanoyl]oxy]-9,9-dimethyl-3-oxa-9-azoniatricyclo[3.3.1.0^{2,4}]nonane (methylhyoscine),



E. (1*R*,3*r*,5*S*)-3-[[(2*RS*)-3-hydroxy-2-phenylpropanoyl]oxy]-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane (methylatropine),



F. methyl (2*RS*)-2-hydroxy-2-phenylacetate (methyl mandelate).

01/2012:0878

HUMAN ANTITHROMBIN III CONCENTRATE

Antithrombinum III humanum densatum

DEFINITION

Human antithrombin III concentrate is a preparation of a glycoprotein fraction obtained from human plasma that inactivates thrombin in the presence of an excess of heparin. It is obtained from plasma that complies with the requirements of the monograph *Human plasma for fractionation (0853)*.

When reconstituted in the volume of solvent stated on the label, the potency is not less than 25 IU of antithrombin III per millilitre.

PRODUCTION

The method of preparation includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for inactivation of viruses during production, the subsequent purification procedure must be validated to demonstrate that the concentration of these substances is reduced to a suitable level and any residues are such as not to compromise the safety of the preparation for patients.

The antithrombin III is purified and concentrated and a suitable stabiliser may be added. The specific activity is not less than 3 IU of antithrombin III per milligram of total protein, excluding albumin. The antithrombin III concentrate is passed through a bacteria-retentive filter, distributed aseptically into its final, sterile containers and immediately frozen. It is then freeze-dried and the containers are closed under vacuum or in an atmosphere of inert gas. No antimicrobial preservative is added at any stage of production.

VALIDATION TEST

It shall be demonstrated that the manufacturing process yields a product that consistently complies with the following test.

Heparin-binding fraction. Examine by agarose gel electrophoresis (*2.2.31*). Prepare a 10 g/L solution of *agarose for electrophoresis R* containing 15 IU of *heparin R* per millilitre in *barbital buffer solution pH 8.4 R*. Pour 5 mL of this solution onto a glass plate 5 cm square. Cool at 4 °C for 30 min. Cut 2 wells 2 mm in diameter 1 cm and 4 cm from the side of the plate and 1 cm from the cathode. Introduce into one well 5 µL of the preparation to be examined, diluted to an activity of about 1 IU of antithrombin III per millilitre. Introduce into the other well 5 µL of a solution of a marker dye such as *bromophenol blue R*. Allow the electrophoresis to proceed at 4 °C, using a constant electric field of 7 V/cm, until the dye reaches the anode.

Cut across the agarose gel 1.5 cm from that side of the plate on which the preparation to be examined was applied and remove the larger portion of the gel leaving a band 1.5 cm wide containing the material to be examined. Replace the removed portion with an even layer consisting of 3.5 mL of a 10 g/L solution of *agarose for electrophoresis R* in *barbital buffer solution pH 8.4 R*, containing a rabbit anti-human antithrombin III antiserum at a suitable concentration, previously determined, to give adequate peak heights of at least 1.5 cm. Place the plate with the original gel at the cathode so that a 2nd electrophoretic migration can occur at right angles to the 1st. Allow this 2nd electrophoresis to proceed using a constant electric field of 2 V/cm for 16 h. Cover the plates with filter paper and several layers of thick lint soaked in a 9 g/L solution of *sodium chloride R* and compress for 2 h, renewing the saline several times. Rinse with *water R*, dry the plates and stain with *acid blue 92 solution R*.

Calculate the fraction of antithrombin III bound to heparin, which is the peak closest to the anode, with respect to the total amount of antithrombin III, by measuring the area defined by the 2 precipitation peaks.

The fraction of antithrombin III able to bind to heparin is not less than 60 per cent.

CHARACTERS

A white or almost white, hygroscopic, friable solid or a powder.

Reconstitute the preparation to be examined as stated on the label immediately before carrying out the identification, tests (except those for solubility, total protein and water) and assay.

IDENTIFICATION

It complies with the limits of the assay.

TESTS

Solubility. It dissolves completely under gentle swirling within 10 min in the volume of the solvent stated on the label, forming a clear or slightly turbid, colourless or almost colourless solution.

pH (*2.2.3*): 6.0 to 7.5.

Osmolality (*2.2.35*): minimum 240 mosmol/kg.

Total protein. If necessary, dilute an accurately measured volume of the preparation to be examined with *water R* to obtain a solution containing about 15 mg of protein in 2 mL. To 2.0 mL of the solution in a round-bottomed centrifuge tube add 2 mL of a 75 g/L solution of *sodium molybdate R* and 2 mL of a mixture of 1 volume of *nitrogen-free sulfuric acid R* and