A. Examine by infrared absorption spectrophotometry (2.2.24), comparing with the spectrum obtained with biotin CRS.

B. Examine the chromatograms obtained in the test for related substances (see Tests). The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve about 10 mg in 20 mL of water R with heating. Allow to cool. Add 0.1 mL of bromine water R. The bromine water is decolourised.

TESTS

Solution S. Dissolve 0.250 g in a 4 g/L solution of sodium hydroxide R and dilute to 25.0 mL with the same alkaline solution.

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

Specific optical rotation (2.2.7). The specific optical rotation is +89 to +93, determined on solution S and calculated with reference to the dried substance.

Related substances. Examine by thin-layer chromatography (2.2.27), using as the coating substance a suitable silica gel (5 μm). Prepare the solutions immediately before use and keep protected from bright light.

Test solution (a). Dissolve 50 mg of the substance to be examined in glacial acetic acid R and dilute to 10 mL with the same solvent.

Test solution (b). Dilute 1 mL of test solution (a) to 10 mL with glacial acetic acid R.

Reference solution (a). Dissolve 5 mg of biotin CRS in glacial acetic acid R and dilute to 10 mL with the same solvent.

Reference solution (b). Dilute 1 mL of test solution (b) to 20 mL with glacial acetic acid R.

Reference solution (c). Dilute 1 mL of test solution (b) to 40 mL with glacial acetic acid R.

Apply to the plate 10 μL of each solution. Develop over a path of 15 cm using a mixture of 5 volumes of methanol R, 25 volumes of glacial acetic acid R and 75 volumes of toluene R. Dry the plate in a current of warm air. Allow to cool and spray with 4-dimethylaminocinnamaldehyde solution R. Examine immediately in daylight. 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) and at most one such spot is more intense than the spot in the chromatogram obtained with reference solution (c) (0.25 per cent).

Heavy metals (2.4.8). 1.0 g complies with limit test C for heavy metals (10 ppm). Prepare the standard using 10 mL of lead standard solution (1 ppm Pb) R.

Loss on drying (2.2.32). Not more than 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

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

ASSAY

Suspend 0.200 g in 5 mL of dimethylformamide R. Heat until the substance has dissolved completely. Add 50 mL of ethanol R and titrate with 0.1 M tetrabutylammonium hydroxide, determining the end-point potentiometrically (2.2.20).

1 mL of 0.1 M tetrabutylammonium hydroxide is equivalent to 24.43 mg of C10H16N2O3S.

STORAGE

Store protected from light.

IMPURITIES

A. di[(3aS,4S,6aR)-2-oxohexahydrothieno[3,4-d]imidazol-4-yl]propanoyl]acetic acid,

B. 4-[(3aS,4S,6aR)-2-oxohexahydrothieno[3,4-d]imidazol-4-yl]butane-1,1-dicarboxylic acid,

C. 5-(3,4-diamino-2-thienyl)pentanoic acid,

D. 2-methyl-5-[(3aS,4S,6aR)-2-oxohexahydrothieno[3,4-d]imidazol-4-yl]pentanoic acid,

E. 5-[(3aS,4S,6aR)-3-benzyl-2-oxohexahydrothieno[3,4-d]imidazol-4-yl]pentanoic acid and 5-[(3aS,4S,6aR)-1-benzyl-2-oxohexahydrothieno[3,4-d]imidazol-4-yl]pentanoic acid.

01/2008:1074

corrected 6.0

BIPERIDEN HYDROCHLORIDE

Biperiden hydrochloridum

C19H30ClNO M_r 347.9
[1235-82-1]

DEFINITION

(1RS)-(1(RS,2SR,4RS)-Bicyclo[2.2.1]hept-5-en-2-yl)-1-phenyl-3-(piperidin-1-yl)propan-1-ol hydrochloride.

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

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: slightly soluble in water and in alcohol, very slightly soluble in methylene chloride.

mp: about 280 °C, with decomposition.
**Identification**

First identification: A, D.
Second identification: B, C, D.

A. Infrared absorption spectrophotometry (2.2.24).

Comparison: biperiden hydrochloride CRS.

B. Thin-layer chromatography (2.2.27).

**Test solution.** Dissolve 25 mg of the substance to be examined in methanol R and dilute to 5 mL with the same solvent.

**Reference solution (a).** Dissolve 25 mg of biperiden hydrochloride CRS in methanol R and dilute to 5 mL with the same solvent.

**Reference solution (b).** Dissolve 5 mg of biperiden impurity A CRS in reference solution (a) and dilute to 2 mL with the same solution.

**Plate:** TLC silica gel F254 plate R.

**Mobile phase:** diethylamine R, methanol R, toluene R (1:1:20 V/V/V).

**Application:** 5 μL.

**Development:** over a path of 15 cm.

**Drying:** in air.

Detection A: examine in ultraviolet light at 254 nm.

Results A: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B: spray with dilute potassium iodobismuthate solution R and then with sodium nitrite solution R and examine in daylight.

Results B: 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 reference solution (a).

**System suitability:** reference solution (b):
- the chromatogram shows 2 clearly separated spots.

C. To about 20 mg add 5 mL of phosphoric acid R. A green colour develops.

D. It gives reaction (a) of chlorides (2.3.1).

**Tests**

**Solution S.** Dissolve 0.10 g in carbon dioxide-free water R, heating gently if necessary, and dilute to 50 mL with the same solvent.

**Appearance of solution.** Solution S is not more opalescent than reference suspension II (2.2.1) and is colourless (2.2.2, Method II).

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

**Related substances.** Gas chromatography (2.2.28).

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

**Reference solution (a).** Dilute 0.5 mL of the test solution to 100 mL with methanol R. Dilute 10 mL of this solution to 50 mL with methanol R.

**Reference solution (b).** Dissolve 5 mg of the substance to be examined and 5 mg of biperiden impurity A CRS in methanol R and dilute to 5 mL with the same solvent. Dilute 1 mL of the solution to 10 mL with methanol R.

**Column:**
- material: fused silica,
- size: l = 50 m, Ø = 0.25 mm,
- stationary phase: poly(dimethyl)(diphenyl)(divinyl)siloxane R (film thickness 0.25 μm).

**Impurities**

**Specified impurities:** A, B, C, F.

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): D, E.
CHARACTERS
Appearance: white or almost white, crystalline powder.
Solubility: practically insoluble in water, soluble in acetone, sparingly soluble in ethanol (96 per cent). It dissolves in dilute mineral acids.

IDENTIFICATION
First identification: C.
Second identification: A, B, D.
A. Melting point (2.2.14): 131 °C to 135 °C.
B. Ultraviolet and visible absorbance spectrophotometry (2.2.25).
Test solution. Dissolve 10.0 mg in a 6 g/L solution of potassium hydroxide R in methanol R and dilute to 100.0 mL with the same solution. Dilute 10.0 mL of this solution to 100.0 mL with a 6 g/L solution of potassium hydroxide R in methanol R.
Spectral range: 220-350 nm.
Absorption maximum: at 248 nm.
Shoulder: at 290 nm.
Specific absorbance at the absorption maximum: 632 to 672.
C. Infrared absorption spectrophotometry (2.2.24).
Comparison: bisacodyl CRS.
If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in chloroform R, evaporate to dryness and record new spectra using the residues.
D. Thin-layer chromatography (2.2.27).
Test solution. Dissolve 20 mg of the substance to be examined in acetone R and dilute to 10 mL with the same solvent.
Reference solution. Dissolve 20 mg of bisacodyl CRS in acetone R and dilute to 10 mL with the same solvent.
Plate: TLC silica gel GF254 plate R.
Application: 10 μL.
Development: over a path of 10 cm.
Drying: in air, if necessary heating at 100-105 °C.
Detection: spray with a mixture of equal volumes of 0.05 M iodine and dilute sulfuric acid R.
Results: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

BISACODYL
Bisacodylum

C_{22}H_{19}NO_4
[603-50-9]

DEFINITION
4,4′-(Pyridin-2-ylmethylene)diphenyl diacetate.
Content: 98.0 per cent to 101.0 per cent (dried substance).

TESTS
01/2008:0595 corrected 6.0

Acidity or alkalinity. To 1.0 g add 20 mL of carbon dioxide-free water R, shake, heat to boiling, cool and filter. Add 0.2 mL of 0.01 M sodium hydroxide and 0.1 mL of methyl red solution R. The solution is yellow. Not more than 0.4 mL of 0.01 M hydrochloric acid is required to change the colour of the indicator to red.

Related substances. Liquid chromatography (2.2.29). Prepare the solutions immediately before use.
Test solution. Dissolve 50 mg of the substance to be examined in 25 mL of acetoniitrile R and dilute to 50.0 mL with the solvent mixture.
Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.
Reference solution (b). Dissolve 2.0 mg of bisacodyl for system suitability CRS (containing impurities A, B, C, D and E) in 1.0 mL of acetoniitrile R and dilute to 20.0 mL with the solvent mixture.