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

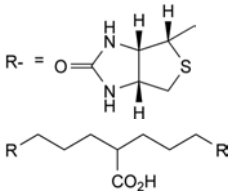
Suspend 0.200 g in 5 mL of *dimethylformamide R*. Heat until the substance has dissolved completely. Add 50 mL of *ethanol R* and titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M tetrabutylammonium hydroxide* is equivalent to 24.43 mg of $C_{10}H_{16}N_2O_3S$.

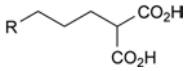
STORAGE

Store protected from light.

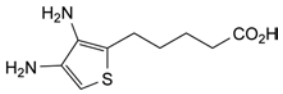
IMPURITIES



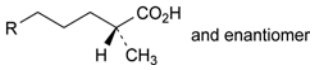
A. di[3-[(3a*S*,4*S*,6a*R*)-2-oxohexahydrothieno[3,4-*d*]imidazol-4-yl]propyl]acetic acid,



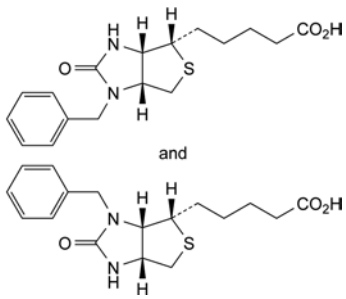
B. 4-[(3a*S*,4*S*,6a*R*)-2-oxohexahydrothieno[3,4-*d*]imidazol-4-yl]butane-1,1-dicarboxylic acid,



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



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

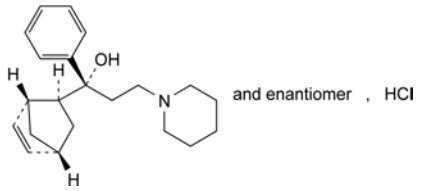


E. 5-[(3a*S*,4*S*,6a*R*)-3-benzyl-2-oxohexahydrothieno[3,4-*d*]imidazol-4-yl]pentanoic acid and 5-[(3a*S*,4*S*,6a*R*)-1-benzyl-2-oxohexahydrothieno[3,4-*d*]imidazol-4-yl]pentanoic acid.

01/2008:1074
corrected 6.0

BIPERIDEN HYDROCHLORIDE

Biperideni hydrochloridum



$C_{21}H_{30}ClNO$ M_r 347.9
[1235-82-1]

DEFINITION

(1*RS*)-1-[(1*RS*,2*SR*,4*RS*)-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 F_{254} 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).

Carrier gas: *nitrogen for chromatography R.*

Flow rate: 0.4 mL/min.

Split ratio: 1:250.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 5	200
	5 - 40	200 → 270
Injection port		250
Detector		300

Detection: flame ionisation.

Injection: 2 µL.

Run time: twice the retention time of biperiden.

Relative retention with reference to biperiden: impurities A, B and C = between 0.95 and 1.05.

System suitability:

– *resolution*: minimum 2.5 between the peak due to biperiden (1st peak) and the peak due to impurity A (2nd peak) in the chromatogram obtained with reference solution (b),

– *signal-to-noise ratio*: minimum 6 for the principal peak in the chromatogram obtained with reference solution (a).

Limits:

– *impurities A, B, C*: for each impurity, maximum 0.50 per cent of the area of the principal peak,

– *any other impurity*: for each impurity, maximum 0.10 per cent of the area of the principal peak,

– *total of impurities A, B and C*: maximum 1.0 per cent of the area of the principal peak,

– *total of impurities other than A, B and C*: maximum 0.50 per cent of the area of the principal peak,

– *disregard limit*: 0.05 per cent of the area of the principal peak.

Impurity F (*2.4.24*): maximum 2 ppm.

Heavy metals (*2.4.8*): maximum 20 ppm.

1.0 g complies with test D. Prepare the reference solution using 2 mL of *lead standard solution (10 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 for 2 h.

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

ASSAY

Dissolve 0.200 g in 60 mL of *alcohol R*. In a closed vessel, titrate with *0.1 M alcoholic potassium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M alcoholic potassium hydroxide* is equivalent to 34.79 mg of $C_{21}H_{30}ClNO$.

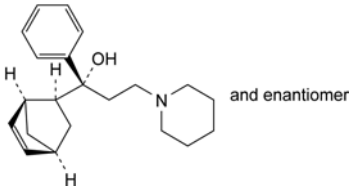
STORAGE

In an airtight container, protected from light.

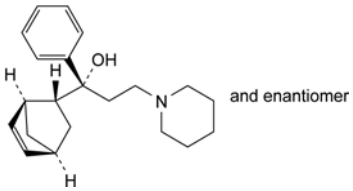
IMPURITIES

Specified impurities: A, B, C, F.

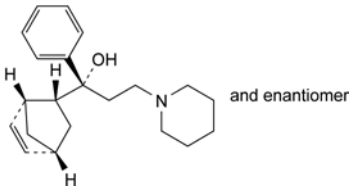
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical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



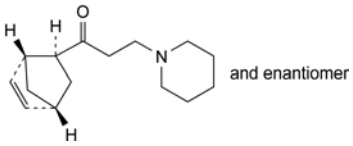
A. (1*RS*)-1-[(1*SR*,2*SR*,4*SR*)-bicyclo[2.2.1]hept-5-en-2-yl]-1-phenyl-3-(piperidin-1-yl)propan-1-ol (*endo* form),



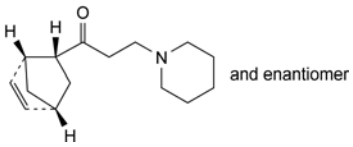
B. (1*RS*)-1-[(1*SR*,2*RS*,4*SR*)-bicyclo[2.2.1]hept-5-en-2-yl]-1-phenyl-3-(piperidin-1-yl)propan-1-ol,



C. (1*RS*)-1-[(1*RS*,2*RS*,4*RS*)-bicyclo[2.2.1]hept-5-en-2-yl]-1-phenyl-3-(piperidin-1-yl)propan-1-ol,



D. 1-[(1*RS*,2*SR*,4*RS*)-bicyclo[2.2.1]hept-5-en-2-yl]-3-(piperidin-1-yl)propan-1-one,



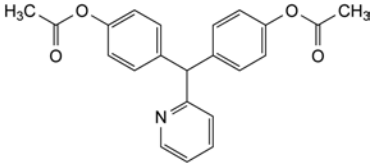
E. 1-[(1*RS*,2*RS*,4*RS*)-bicyclo[2.2.1]hept-5-en-2-yl]-3-(piperidin-1-yl)propan-1-one,

F. benzene.

01/2008:0595
corrected 6.0

BISACODYL

Bisacodylum



$C_{22}H_{19}NO_4$ M_r 361.4
[603-50-9]

DEFINITION

4,4′-(Pyridin-2-ylmethylene)diphenyl diacetate.

Content: 98.0 per cent to 101.0 per cent (dried substance).

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 absorption 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 GF_{254} plate R.*

Mobile phase: *methyl ethyl ketone R, xylene R* (50:50 *V/V*).

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.

TESTS

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.*

Solvent mixture: *glacial acetic acid R, acetonitrile R, water R* (4:30:66 *V/V/V*).

Test solution. Dissolve 50 mg of the substance to be examined in 25 mL of *acetonitrile 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 *acetonitrile R* and dilute to 2.0 mL with the solvent mixture.