– *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

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

0.5 g complies with test D. Prepare the reference solution using 1 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 vacuo* for 16 h.

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

ASSAY

Dissolve 0.200 g in 70 mL of *acetone R*. Titrate with *0.1 M tetrabutylammonium hydroxide in 2-propanol*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M tetrabutylammonium hydroxide in 2-propanol* is equivalent to 30.04 mg of $C_{20}H_{28}O_2$.

STORAGE

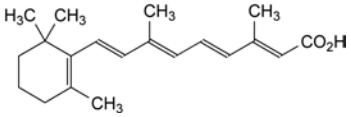
Under an inert gas, in an airtight container, protected from light.

It is recommended that the contents of an opened container be used as soon as possible and any unused part be protected by an atmosphere of inert gas.

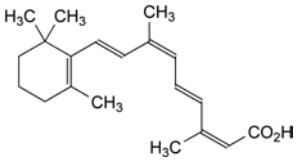
IMPURITIES

Specified impurities: A, H, I.

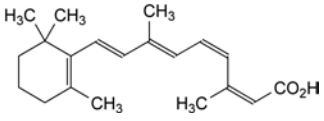
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *B, C, D, F, G.*



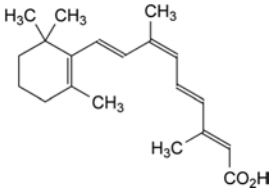
A. (2*E*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenoic acid (tretinoin),



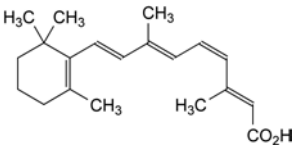
B. (2*Z*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenoic acid (9,13-di*cis*-retinoic acid),



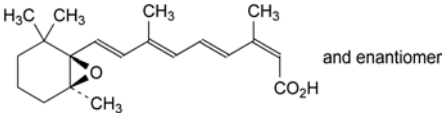
C. (2*Z*,4*Z*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenoic acid (11,13-di*cis*-retinoic acid),



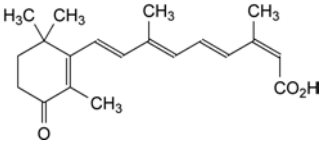
D. (2*E*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenoic acid (9-*cis*-retinoic acid),



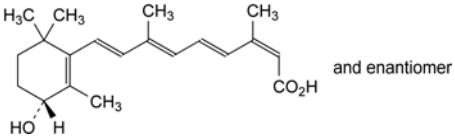
F. (2*E*,4*Z*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraenoic acid (11-*cis*-retinoic acid),



G. (2*Z*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-[(1*RS*,6*SR*)-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl]nona-2,4,6,8-tetraenoic acid (13-*cis*-5,6-dihydro-5,6-epoxyretinoic acid),



H. (2*Z*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethyl-3-oxocyclohex-1-en-1-yl)nona-2,4,6,8-tetraenoic acid (13-*cis*-4-oxoretinoic acid),



I. (2*Z*,4*E*,6*E*,8*E*)-9-[(3*RS*)-3-hydroxy-2,6,6-trimethylcyclohex-1-en-1-yl]-3,7-dimethylnona-2,4,6,8-tetraenoic acid (13-*cis*-4-hydroxyretinoic acid).

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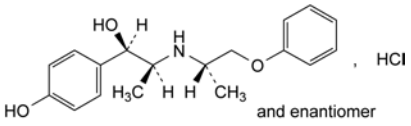
corrected 6.0

ISOXSUPRINE HYDROCHLORIDE

Isoxsuprini hydrochloridum



$C_{18}H_{24}ClNO_3$ M_r 337.8
[579-56-6]

DEFINITION

(1*RS*,2*SR*)-1-(4-Hydroxyphenyl)-2-[[(1*SR*)-1-methyl-2-phenoxyethyl]amino]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: sparingly soluble in water and in ethanol (96 per cent), practically insoluble in methylene chloride.

mp: about 205 °C, with decomposition.

IDENTIFICATION

First identification: B, E.

Second identification: A, C, D, E.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution. Dissolve 50.0 mg in *0.1 M hydrochloric acid* and dilute to 50.0 mL with the same acid. Dilute 10.0 mL of this solution to 100.0 mL with *0.1 M hydrochloric acid*.

Spectral range: 230-350 nm.

Absorption maxima: at 269 nm and 275 nm.

Resolution (*2.2.25*): minimum 1.7 for the absorbance ratio.

Specific absorbance at the absorption maxima:

– at 269 nm: 71 to 74;

– at 275 nm: 70 to 73.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: discs.

Comparison: *isoxsuprine hydrochloride CRS*.

If the spectra obtained show differences, dissolve 50 mg of the substance to be examined and of the reference substance separately in 2 mL of *methanol R*, add 15 mL of *methylene chloride R*, evaporate to dryness and record new spectra using the residues.

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

Test solution. Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution. Dissolve 20 mg of *isoxsuprine hydrochloride CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Plate: *TLC silica gel G plate R*.

Mobile phase: *concentrated ammonia R, methanol R, methylene chloride R* (0.25:15:85 *V/V/V*).

Application: 10 µL.

Development: over a path of 12 cm.

Drying: in a current of warm air.

Detection: spray with a 10 g/L solution of *potassium permanganate 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. To 1 mL of solution S (see Tests) add 0.05 mL of *copper sulfate solution R* and 0.5 mL of *strong sodium hydroxide solution R*. The solution becomes blue. Add 1 mL of *ether R* and shake. Allow to separate. The upper layer remains colourless.

E. 2 mL of solution S gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S. Dissolve 0.50 g, with gentle heating if necessary, in *carbon dioxide-free water R*, cool and dilute to 50.0 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 6.0 for solution S.

Optical rotation (*2.2.7*): – 0.05° to + 0.05°, determined on solution S.

Phenones: maximum 1.0 per cent, calculated as impurity B.

Dissolve 10.0 mg in *water R* and dilute to 100.0 mL with the same solvent. The absorbance (*2.2.25*) of the solution measured at the absorption maximum at 310 nm is not greater than 0.10.

Related substances. Gas chromatography (*2.2.28*). *Prepare the solutions immediately before use.*

Internal standard solution (a). Dissolve 0.1 g of *hexacosane R* in *trimethylpentane R* and dilute to 20 mL with the same solvent.

Internal standard solution (b). Dilute 1 mL of internal standard solution (a) to 50 mL with *trimethylpentane R*.

Test solution. To 10.0 mg of the substance to be examined, add 0.5 mL of *N-trimethylsilylimidazole R*. Heat to 65 °C for 10 min. Allow to cool, then add 2.0 mL of the internal standard solution (b) and 2.0 mL of *water R*. Shake. Use the upper layer.

Reference solution (a). To 10.0 mg of the substance to be examined, add 0.5 mL of *N-trimethylsilylimidazole R*. Heat to 65 °C for 10 min. Allow to cool, then add 2.0 mL of the internal standard solution (a) and 2.0 mL of *water R*. Shake. Dilute 1.0 mL of the upper layer to 50.0 mL with *trimethylpentane R*.

Reference solution (b). To 10.0 mg of the substance to be examined, add 0.5 mL of *N-trimethylsilylimidazole R*. Heat to 65 °C for 10 min. Allow to cool, then add 2.0 mL of *trimethylpentane R* and 2.0 mL of *water R*. Shake. Use the upper layer.

Column:

– *material*: glass;

– *size*: *l* = 1.5 m, Ø = 4 mm;

– *stationary phase*: *silanised diatomaceous earth for gas chromatography R* (125-135 µm) impregnated with 3 per cent *m/m* of *poly(dimethyl)siloxane R*.

Carrier gas: *nitrogen for chromatography R*.

Flow rate: 30 mL/min.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 25	195
	25 - 29	195 → 215
	29 - 39	215
Injection port		225
Detector		225

Detection: flame ionisation.

Injection: 1 µL.

Elution order: isoxsuprine, hexacosane.

System suitability:

– *resolution*: minimum 5.0 between the peaks due to isoxsurpine and hexacosane in the chromatogram obtained with reference solution (a);

– in the chromatogram obtained with reference solution (b), there is no peak with the same retention time as the internal standard.

Limit:

– *total*: calculate the ratio (*R*) of the area of the peak due to the trimethylsilyl derivative of isoxsuprine to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (a); from the chromatogram obtained with the test solution, calculate the ratio of the sum of the areas of any peaks, apart from the principal peak and the peak due to the internal standard, to the area of the peak due to the internal standard: this ratio is not greater than *R* (2.0 per cent).

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

1.0 g complies with test C. 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.

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

ASSAY

Dissolve 0.250 g in 80 mL of *ethanol (96 per cent) R* and add 1.0 mL of *0.1 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 33.78 mg of $C_{18}H_{24}ClNO_3$.

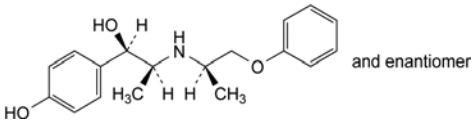
STORAGE

Protected from light.

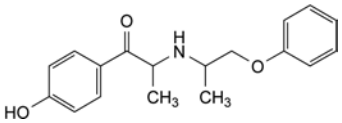
IMPURITIES

Specified impurities: *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*): *A*.



A. (1*RS*,2*SR*)-1-(4-hydroxyphenyl)-2-[[(1*RS*)-1-methyl-2-phenoxyethyl]amino]propan-1-ol,



B. 1-(4-hydroxyphenyl)-2-[(1-methyl-2-phenoxyethyl)amino]-propan-1-one.

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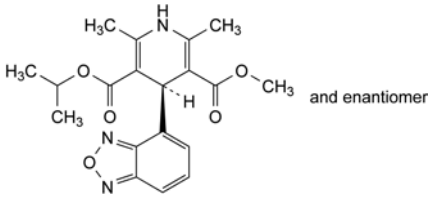
corrected 6.0

ISRADIPINE

Isradipinum



$C_{19}H_{21}N_3O_5$ M_r 371.4
[75695-93-1]

DEFINITION

Methyl 1-methylethyl (4*RS*)-4-(2,1,3-benzoxadiazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate.

Content: 97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: yellow, crystalline powder.

Solubility: practically insoluble in water, freely soluble in acetone, soluble in methanol.

mp: about 168 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: isradipine CRS.

TESTS

Related substances. Liquid chromatography (*2.2.29*).

Test solution (a). Dissolve 50.0 mg of the substance to be examined in 1 mL of *methanol R*, using an ultrasonic bath if necessary, and dilute to 25.0 mL with the mobile phase.

Test solution (b). Dissolve 50.0 mg of the substance to be examined in 2 mL of *methanol R* and dilute to 250.0 mL with the mobile phase.

Reference solution (a). Dilute 1.0 mL of test solution (a) to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b). Dissolve 2 mg of the substance to be examined and 2 mg of *isradipine impurity D CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (c). Dissolve 50.0 mg of *isradipine CRS* in 2 mL of *methanol R* and dilute to 250.0 mL with the mobile phase.

Column:

– *size*: *l* = 0.10 m, Ø = 4.6 mm,
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: *acetonitrile R, tetrahydrofuran R, water R* (125:270:625 *V/V/V*).

Flow rate: 1.2 mL/min.

Detection: spectrophotometer at 230 nm.

Injection: 20 µL of test solution (a) and reference solutions (a) and (b).

Run time: 5 times the retention time of isradipine.

Identification of impurities: use the chromatogram supplied with *isradipine CRS* to identify the peaks due to impurities A and B.

Relative retention with reference to isradipine (retention time = about 7 min): impurity A = about 0.8; impurity D = about 0.9; impurity B = about 1.8.

System suitability: reference solution (b):

– *resolution*: minimum 2.0 between the peaks due to isradipine and impurity D.

Limits:

– *correction factor*: for the calculation of content, multiply the peak area of impurity D by 1.4,
– *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),
– *impurity B*: not more than 8 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.8 per cent),
– *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
– *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
– *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent),
– *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*): maximum 0.2 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

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

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Detection: spectrophotometer at 326 nm.

Injection: test solution (b) and reference solution (c).

Run time: twice the retention time of isradipine.

Calculate the percentage content of isradipine from the areas of the peaks and the declared content of *isradipine CRS*.

STORAGE

Protected from light.

IMPURITIES

Specified impurities: *A, B, D*.