In the chromatogram obtained with the test solution at 220 nm, the area of the peak due to impurity A is not greater than the area of the corresponding peak in the chromatogram obtained with reference solution (b) at the same wavelength (0.5 per cent). In the chromatogram obtained with the test solution at 298 nm, the area of any peak apart from the principal peak is not greater than the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) at the same wavelength (0.5 per cent); the sum of all peak areas at 298 nm, apart from the principal peak and any peak due to impurity B, is not greater than the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.5 per cent). At 298 nm disregard any peak due to the solvent and any peak with an area less than half the area of the peak due to impurity B in the chromatogram obtained with reference solution (c) at the same wavelength.

Heavy metals (2.4.8). 2.0 g complies with limit test C for heavy metals (10 ppm). Prepare the standard using 2 ml of lead standard solution (10 ppm Pb) R.

Loss on drying (2.2.32). Not more than 1.5 per cent, determined on 1.000 g by drying in vacuo at 60 °C over diphosphorus pentoxide R.

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

ASSAY
Dissolve 0.150 g in 20 ml of methanol R. Add 20 ml of water R and titrate with 0.1 M sodium hydroxide, determining the end-point potentiometrically (2.2.20). Carry out a blank titration.

1 ml of 0.1 M sodium hydroxide is equivalent to 20.73 mg of C_{12}H_{17}NO_{2}.

STORAGE
Store protected from light.

IMPURITIES

A. (RS)-2-(3-cyclohexyl-5-methyl-4,5-dihydroisoxazol-5-yl)acetic acid,

B. X = O: 6-cyclohexyl-4-methyl-2H-pyran-2-one,

C. X = NH: 6-cyclohexyl-4-methylpyridin-2(1H)-one.

General Notices (1) apply to all monographs and other texts

CICLOPIROX OLAMINE

Ciclopirox olaminum

C_{14}H_{24}N_{2}O_{3}  \quad M_r 268.4

DEFINITION
6-Cyclohexyl-1-hydroxy-4-methylpyridin-2(1H)-one and 2-aminoethanol.

Content:
- ciclopirox (C_{12}H_{17}NO_{2}; M_r 207.3): 76.0 per cent to 78.5 per cent (dried substance).
- 2-aminoethanol (C_{2}H_{7}NO; M_r 61.1): 22.2 per cent to 23.3 per cent (dried substance).

CHARACTERS
Appearance: white or pale yellow, crystalline powder.
Solubility: sparingly soluble in water, very soluble in alcohol and in methylene chloride, slightly soluble in ethyl acetate, practically insoluble in cyclohexane. It shows polymorphism.

IDENTIFICATION
First identification: A.
Second identification: B.

A. Infrared absorption spectrophotometry (2.2.24).

Comparison: ciclopirox olamine CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of ethyl acetate R, evaporate to dryness on a water-bath and record new spectra using the residues.

B. Thin-layer chromatography (2.2.27).

Test solution. Dissolve 25 mg of ciclopirox olamine CRS in methanol R and dilute to 10 ml with the same solvent.

Reference solution. Dissolve 25 mg of ciclopirox olamine CRS in methanol R and dilute to 10 ml with the same solvent.

Plate: TLC silica gel F_{254} plate R.

Before use wash 2 plates by allowing a mixture of 10 volumes of concentrated ammonia R, 15 volumes of water R and 75 volumes of ethanol R to migrate until the solvent front has reached the top of the plate. Allow the plates to dry in air for 5 min.


Application: 10 µl.

Development: over a path of 15 cm.

Drying: in air for 10 min.

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 the reference solution.
Detection B: spray one plate with ferric chloride solution R3.

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 the reference solution.

Detection C: spray the second plate with ninhydrin solution R. Heat at 110 °C until the spots appear.

Results C: 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.

TESTS

Appearance of solution. The solution is clear (2.2.1) and not more intensely coloured than reference solution BY7 (2.2.2, Method II).

Dissolve 2.0 g in methanol R and dilute to 20 ml with the same solvent.

pH (2.2.3): 8.0 to 9.0.

Dissolve 1.0 g in carbon dioxide-free water R and dilute to 100 ml with the same solvent.

Related substances. Liquid chromatography (2.2.29). Carry out the operations avoiding exposure to actinic light. All materials which are in direct contact with the substance to be examined, such as column materials, reagents, solvents, etc. should contain only small amounts of extractable metal cations.

Test solution. Dissolve 40.0 mg of the substance to be examined (corresponding to about 30 mg of ciclopirox) in a mixture of 20 µl of anhydrous acetic acid R, 2 ml of acetonitrile R, and 15 ml of the mobile phase. If necessary, use an ultrasonic bath. Dilute to 20.0 ml with the mobile phase.

Reference solution (a). Dissolve 15.0 mg of ciclopirox impurity A CRS and 15.0 mg of ciclopirox impurity B CRS in a mixture of 1 ml of acetonitrile R and 7 ml of the mobile phase. Dilute to 10.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 200.0 ml with a mixture of 1 volume of acetonitrile R and 9 volumes of the mobile phase.

Reference solution (c). Dilute 2.0 ml of reference solution (b) to 10.0 ml with a mixture of 1 volume of acetonitrile R and 9 volumes of the mobile phase.

Reference solution (d). Mix 5 ml of reference solution (a) with 5 ml of the test solution.

Column:
- size: l = 80 mm, Ø = 4 mm,
- stationary phase: nitrile silica gel for chromatography R (5 µm),
- rinsing solution: a mixture of 1 volume of anhydrous acetic acid R, 1 volume of acetylacetone R, 500 volumes of acetonitrile R and 500 volumes of water R.

Mobile phase: a mixture of 0.1 volumes of anhydrous acetic acid R, 230 volumes of acetonitrile R and 770 volumes of a 0.96 g/1 solution of sodium edetate R. If the retention time of the principal peak in the chromatogram obtained with the test solution is not between 8 min and 11 min adjust the ratio of the 0.96 g/1 solution of sodium edetate to acetonitrile accordingly.

Flow rate: 0.7 ml/min.

Detection: variable wavelength spectrophotometer capable of operating at 220 nm and 298 nm.

In order to ensure desorption of interfering metal ions, a new column is to be rinsed with the rinsing solution over a period of at least 15 h and then with the mobile phase for at least 5 h at a flow rate of 0.2 ml/min.

Injection: 10 µl; inject the test solution and reference solutions (b), (c) and (d).

Run time: 2.5 times the retention time of ciclopirox.

Relative retention with reference to ciclopirox: impurity A = about 0.5; impurity C = about 0.9; impurity B = about 1.3.

System suitability:
- resolution: minimum of 2.0 between the peaks corresponding to impurity B and ciclopirox in the chromatogram obtained with reference solution (d),
- signal-to-noise ratio: minimum of 10 for the peak corresponding to impurity B in the chromatogram obtained with reference solution (c) at 298 nm,
- symmetry factor: 0.8 to 2.0 for the principal peak in the chromatogram obtained with the test solution.

Limits:
- impurity A at 220 nm: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) at the same wavelength (0.5 per cent),
- any impurity at 298 nm: not more than the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) at the same wavelength (0.5 per cent),
- total at 298 nm apart from impurity B: not more than the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.5 per cent),
- disregard limit at 298 nm: area of the peak due to impurity B in the chromatogram obtained with reference solution (c) at the same wavelength (0.1 per cent).

Heavy metals (2.4.8): maximum 20 ppm.

1.0 g complies with limit test C. Prepare the standard using 2 ml of lead standard solution (10 ppm Pb) R.

Loss on drying (2.2.32): maximum 1.5 per cent, determined on 1.000 g by drying under high vacuum.

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

ASSAY

2-Aminoethanol. Dissolve 0.250 g in 25 ml of anhydrous acetic acid 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 6.108 mg of C6H8NO.

Ciclopirox. Dissolve 0.200 g in 2 ml of methanol R. Add 38 ml of water R, swirl and titrate immediately with 0.1 M sodium hydroxide, determining the end-point potentiometrically (2.2.20). Carry out a blank titration.

Use 0.1 M sodium hydroxide, the titre of which has been determined under the conditions prescribed above using 0.100 g of benzoic acid R.

1 ml of 0.1 M sodium hydroxide is equivalent to 20.73 mg of C12H17NO2.

STORAGE

Protected from light.
Ciclosporin

**DEFINITION**

Ciclosporin contains not less than 98.5 per cent and not more than the equivalent of 101.5 per cent of cyclo[(2S,3R,4R,6S)]-3-hydroxy-4-methyl-2-(methylamino)oct-6-enoyl]-2-aminobutanoyl-N-methylglycyl-N-methylleucyl-N-leucyl-N-methylleucyl-N-leucyl-N-methylleucyl-N-methylleucyl-N,N-diethylmethanamide, calculated with reference to the dried substance. Ciclosporin is a substance produced by *Beauveria nivea* (*Tolypocladium inflatum* Gams) or obtained by any other means.

**CHARACTERS**

A white or almost white powder, practically insoluble in water, freely soluble in ethanol and in methylene chloride.

**IDENTIFICATION**

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

B. Examine the chromatograms obtained in the assay. The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

**TESTS**

**Appearance of solution.** Dissolve 1.5 g in ethanol *R* and dilute to 15 ml with the same solvent. The solution is clear (2.2.1) and not more intensely coloured than reference solution *Y*₉₉, BY₉₉ or R₂ (2.2.2, Method II).

**Specific optical rotation** (2.2.7). Dissolve 0.125 g in methanol *R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is −185 to −193, calculated with reference to the dried substance.

**Related substances.** Examine by liquid chromatography (2.2.29) as prescribed under Assay. Inject separately the test solution and reference solution (b). Continue the chromatography for 1.7 times the retention time of the principal peak. In the chromatogram obtained with the test solution: the area of any peak, apart from the principal peak, is not greater than 0.7 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.7 per cent); the sum of the areas of all the peaks, apart from the principal peak, is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent). Disregard any peak due to the solvent and any peak with an area less than 0.05 times that of the principal peak in the chromatogram with reference solution (b).

**Heavy metals** (2.4.8). The residue obtained in the test for loss on drying complies with limit test *C* for heavy metals (20 ppm). Prepare the standard using 2 ml of lead standard solution (10 ppm Pb) *R*.

**Loss on drying** (2.2.32). Not more than 2.0 per cent, determined on 1.000 g at 60 °C at a pressure not exceeding 15 Pa for 3 h.

**Bacterial endotoxins** (2.6.14): less than 0.84 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins. Dissolve 50 mg of the substance to be examined in a mixture of 280 mg of alcohol *R* and 650 mg of polyoxyethylated castor oil *R* and dilute to the required concentration using water LAL.

**ASSAY**

Examine by liquid chromatography (2.2.29).

**Test solution.** Dissolve 30.0 mg of the substance to be examined in a mixture of equal volumes of acetonitrile *R* and water *R* and dilute to 25.0 ml with the same mixture of solvents.

**Reference solution (a).** Dissolve 30.0 mg of ciclosporin CRS in a mixture of equal volumes of acetonitrile *R* and water *R* and dilute to 25.0 ml with the same mixture of solvents.

**Reference solution (b).** Dilute 2.0 ml of reference solution (a) to 200.0 ml with a mixture of equal volumes of acetonitrile *R* and water *R*.

**Reference solution (c).** Dissolve the contents of a vial of ciclosporin for system suitability CRS in 5.0 ml of the mobile phase.

The chromatographic procedure may be carried out using:

- a stainless steel column 0.25 mm long and 4 mm in internal diameter packed with octadecylsilica silica gel for chromatography *R* (3 µm to 5 µm); the column is connected to the injection port by a steel capillary tube about 1 m long and having an internal diameter of 0.25 mm,

- as mobile phase at a flow rate of about 1.5 ml per minute a mixture of 1 volume of phosphoric acid *R*, 50 volumes of 1,1-dimethylethyl methyl ether *R*, 430 volumes of acetonitrile *R* and 520 volumes of water *R*,