World Health Organization

CLOFAZIMINE

(CLOFAZIMINUM)

Draft proposal for revision in *The International Pharmacopoeia*

(27 August 2024)

DRAFT FOR COMMENTS

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For any technical questions, you may contact **Dr Herbert Schmidt**, Technical Officer, Norms and Standards for Pharmaceuticals, Technical Standards and Specifications (schmidth@who.int), with a copy to Ms Bezawit Kibret (kibretb@who.int)
Comments should be submitted through the online platform by **27 October 2024**. Please note that only comments received by this deadline will be considered for the preparation of this document.
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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/24.954

CLOFAZIMINE

(CLOFAZIMINUM)

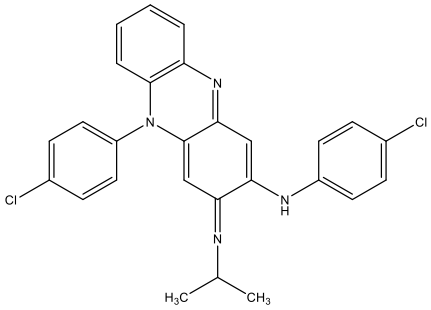
Description	Date
First draft prepared.	April 2024
Discussion at the informal Consultation on Quality Control and Pharmacopoeial Specifications of Medicines	May 2024
Draft revision sent out for public consultation	August to October 2024
Presentation at the 58th Expert Committee on Specifications for Pharmaceutical Preparations	October 2024
Further follow-up action as required.	

CLOFAZIMINE (CLOFAZIMINUM)

Molecular formula. $C_{27}H_{22}Cl_2N_4$

Relative molecular mass. 473.4

Graphic formula.



Chemical name. 3-(*p*-Chloroanilino)-10-(*p*-chlorophenyl)-2,10-dihydro-2-(isopropylimino)phenazine; *N*,5-bis(4-chlorophenyl)-3,5-dihydro-3-[(1-methylethyl)imino]-2-phenazinamine.

CAS Registry No. 2030-63-9.

Description. A reddish brown, fine powder.

Solubility. Practically insoluble in water; very slightly soluble in ethanol (~750 g/L) TS; soluble in dichloromethane R.

Category. Anti-leprosy drug.

Storage. Clofazimine should be kept in a well-closed container.

Additional information. Clofazimine shows polymorphism.

Requirements

Definition. Clofazimine contains not less than 99.0% and not more than 101.0% of $C_{27}H_{22}Cl_2N_4$, calculated with reference to the dried substance.

Identity tests

- Either test A alone, or any two of tests B, C or D, may be applied.

A. Carry out the test as described under *1.7 Spectrophotometry in the infrared region*. The infrared absorption spectrum is concordant with the spectrum obtained from clofazimine RS or with the reference spectrum of clofazimine.

If the spectra thus obtained are not concordant, repeat the test using the residues obtained by separately dissolving the test substance and clofazimine RS in a small amount of dichloromethane R. Evaporate to dryness and record new spectra using the residues. The infrared absorption spectrum is concordant with the spectrum obtained from clofazimine RS.

B. The absorption spectrum of a 5.0 µg/mL solution in hydrochloric acid/methanol (0.01 mol/L) VS, when observed between 230 nm and 600 nm, exhibits 2 maxima at about 283 nm and 487 nm.

C. Carry out the test as described under *1.14.1 Chromatography*, Thin-layer chromatography, using a precoated silica gel R6 plate. Expose the plate immediately before use to ammonia vapour by suspending the plate for 15 minutes in a chromatographic chamber containing a shallow layer of ammonia (~17 g/L) TS. As the mobile phase, use a freshly prepare a mixture of 85 volumes of dichloromethane R and 6 volumes of propanol R as the mobile phase.

Apply separately to the plate 5 µL of each of the following two solutions in dichloromethane R, containing (A) 1 mg of the test substance per mL and (B) 1

mg of clofazimine RS per mL. Develop the plate in a separate chromatographic chamber for 2/3 of its height. After removing the plate from the chromatographic chamber, allow it to dry in air for 5 minutes. Place the same plate back into the chromatographic chamber and develop the plate again for 2/3 of its height. Remove the plate from the chamber and allow it to dry in air for a further 5 minutes. Examine the chromatogram under ultraviolet light (254 nm).

The principal spot in the chromatogram obtained with solution (A) corresponds in position, appearance and intensity to the spot due to clofazimine in the chromatogram obtained with solution (B).

D. Dissolve 2 mg in 3 mL of acetone R and add 0.1 mL of hydrochloric acid (~420 g/L) TS; an intense violet colour is produced. Add 0.5 mL of sodium hydroxide (~200 g/L) TS; the colour changes to orange red.

Heavy metals. Use 1.000 g for the preparation of the test solution as described under *2.2.3 Limit test for heavy metals*, Procedure 3; determine the heavy metals content according to Method A; not more than 10 µg/g.

Sulfated ash *(2.3)*. Not more than 1.0 mg/g, determined on 1.000 g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Related substances. Prepare the solutions immediately before use. Carry out the test as described under *1.14.1 Chromatography*, High-pressure liquid chromatography, using a stainless-steel column (4.6 mm x 25 cm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octylsilyl groups (5 µm).

Prepare an SDS phosphate buffer by dissolving 4.5 g of sodium dodecyl sulfate R, 1.7 g of tetrabutylammonium hydrogen sulfate R, and 1.8 g of disodium hydrogen

phosphate R in 900 mL of water R. Adjust the pH of the solution to 3.0 with phosphoric acid R (~144 g/L) TS and dilute to 1000 mL.

As the mobile phase, use a mixture of 65 volumes of acetonitrile R and 35 volumes of the SDS phosphate buffer.

Operate with a flow rate of 1.0 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 280 nm.

Prepare the following solutions using as a diluent the mobile phase. For solution (1), dissolve 50.0 mg of the test substance and dilute to 100.0 mL. For solution (2), dilute 1.0 mL of solution (1) to 100.0 mL. For solution (3), dilute 5.0 mL of solution (2) to 100.0 mL. For solution (4), dissolve 5 mg of clofazimine for system suitability RS (containing clofazimine and impurity B) in the mobile phase and dilute to 10.0 mL.

Inject 20 µL each of solutions (1), (2), (3) and (4). Record the chromatogram for about 3 times the retention time of clofazimine (retention time about 15 minutes).

Use the chromatogram obtained with solution (4) and the chromatogram supplied with clofazimine for system suitability RS to identify the peak due to impurity B.

The impurities are eluted, if present, at the following relative retentions with reference to clofazimine: impurity A about 0.7 and impurity B about 0.8.

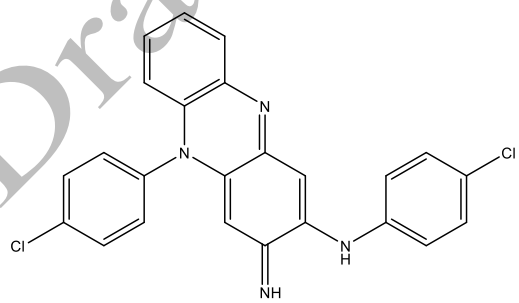
The test is not valid unless, in the chromatogram obtained with solution (3), the resolution between impurity B and clofazimine is at least 2.0. Also, the test is not valid unless, in the chromatogram obtained with solution (3), the signal-to-noise ratio of the peak due to clofazimine is at least 10.

In the chromatogram obtained with solution (1):

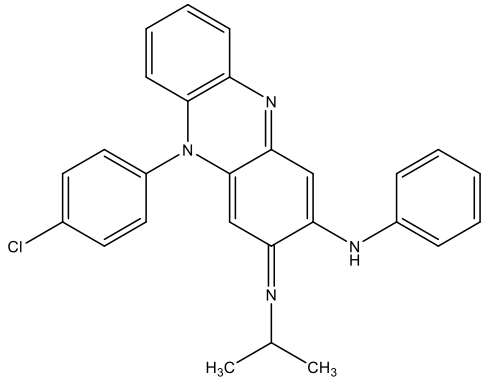
- the area of any peak corresponding to impurity A is not greater than 0.1 times the area of the peak due to clofazimine in the chromatogram obtained with solution (2) (0.1%);
- the area of any peak corresponding to impurity B is not greater than 0.3 times the area of the peak due to clofazimine in the chromatogram obtained with solution (2) (0.3%);
- the area of any other impurity peak is not greater than 0.1 times the peak due to clofazimine in the chromatogram obtained with solution (2) (0.10%).
- The sum of the areas of all impurity peaks is not greater than 0.5 times the area of the peak due to clofazimine in the chromatogram obtained with solution (2) (0.5%). Disregard all peaks with an area of less than 0.05 times the area of the peak due to clofazimine in the chromatogram obtained with solution (2) (0.05%).

Assay. Dissolve 0.400 g in 5 mL of dichloromethane R and add 20 mL of acetone R and 5 mL of anhydrous acetic acid R. Titrate with perchloric acid (0.1 mol/L) VS determining the endpoint potentiometrically, as described under <u>*2.6 Non-aqueous titration*</u>, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 47.34 mg of $C_{27}H_{22}Cl_2N_4$.

Impurities



A. *N*,5-bis(4-chlorophenyl)-3-imino-3,5-dihydrophenazin-2-amine,



B. 5-(4-chlorophenyl)-3-[(1-methylethyl)imino]-*N*-phenyl-3,5-dihydrophenazin-2-amine.

Reference substances to be established.

Clofazimine for system suitability RS

- *It is intended to refer to the corresponding reference substance established for the European Pharmacopoeia.*

Reagent needed to be added into Ph. Int.:

Phosphoric acid R (~144 g/L) TS

Procedure. Dilute 100 mL of phosphoric acid (~1440 g/L) TS with sufficient water to produce 1000 mL.

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