Miltefosine

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Manufacturers, regulatory authorities, health authorities, researchers, and other stakeholders are invited to provide their feedback and comments on this draft proposal. Manufacturers are also invited to submit samples of their products to the IPC to ensure that the proposed monograph adequately controls the quality of the product(s) they manufacture. Comments and samples received after the last date will not be considered by the IPC before finalizing the monograph.

Please send any comments you may have on this draft document to lab.ipc@gov.in, with a copy to Dr. Gaurav Pratap Singh (email: gpsingh.ipc@gov.in) before the last date for comments.

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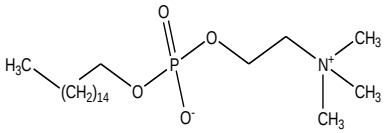
Miltefosine



$C_{21}H_{46}NO_4P$ Mol Wt. 407.6

Miltefosine is hexadecyl 2-(trimethylammonio)ethyl)phosphate.

Miltefosine contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{21}H_{46}NO_4P$, calculated on the anhydrous basis.

Category. Broad spectrum antimicrobial.

Description. A white to off white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *miltefosine IPRS* or with the reference spectrum of miltefosine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 5.5 to 9.0, determined in a 1.0 per cent w/v solution.

Choline chloride. Not more than 0.15 per cent.

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Solvent mixture. 20 volumes of *glacial acetic acid* and 80 volumes of *water.*

Dragendoff reagent. A mixture of 5 volumes of 1.7 per cent w/v solution of *basic bismuth nitrate* (III) in the solvent mixture, 5 volumes of 40 per cent w/v solution of *potassium iodide* in *water*, 20 volumes of *glacial acetic acid* and 70 volumes of *methanol* (*Note – Use the reagent after 24 hours).*

Mobile phase. A mixture of 70 volumes of *chloroform*, 40 volumes of *methanol* and 10 volumes of *1M sodium acetate solution* in *25 per cent ammonia solution*.

Test solution. Dissolve 0.5 g of the substance under examination in the solvent mixture, with the aid of ultrasound and dilute to 5.0 ml with *methanol.*

Reference solution (a). A 0.15 per cent w/v solution of *choline chloride IPRS* in *methanol.*

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with *methanol*.

Reference solution (c). Dissolve 0.5 g of *miltefosine IPRS* in 2 ml of *methanol* with aid of ultrasound, add 0.5 ml of reference solution (a) and dilute to 5.0 ml with *methanol*.

Apply to the plate 50 µl of each solution. After development, dry the plate in air. Wipe the plate immediately with dragendoff reagent with cotton. Put the plate in refrigerator for 45 minutes or till the spots appears. Any spot due to choline chloride in the chromatogram obtained with the test solution is not more intense than the spot due to choline

chloride in the chromatogram obtained with reference solution (b) (0.15 per cent). The test is not valid unless the chromatogram obtained with the reference solution (c) shows two clearly separated spots.

Related substances. A. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.5 g of the substance under examination in the mobile phase with the aid of ultrasound and dilute to 10.0 ml with the mobile phase.

Reference solution (a). A 0.015 per cent w/v solution of *miltefosine IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.15 per cent w/v of *miltefosine impurity G IPRS* and 0.075 per cent w/v, each of, *miltefosine impurity A IPRS, miltefosine impurity B IPRS* and *miltefosine impurity C IPRS* in the mobile phase.

Reference solution (c). Weigh and transfer 3.75 mg of *miltefosine impurity D IPRS* to a 5-ml volumetric flask, add 0.5 ml of *trifluoroacetic acid* and 1 ml of *methanol,* sonicate to dissolve and dilute to volume with *methanol.*

Reference solution (d). A solution containing, 0.075 per cent w/v, each of, *miltefosine impurity E IPRS* and *miltefosine impurity F IPRS* in *methanol*.

Reference solution (e). Weigh and transfer 0.5 g of *miltefosine IPRS* to a 10-ml volumetric flask, add 1.0 ml, each of, reference solution (b), reference solution (c) and reference solution (d) and 4 ml of *methanol,* sonicate to dissolve and dilute to volume with *methanol.*

Reference solution (f). Dilute 3.0 ml of reference solution (a) to 20.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Symmetry shield™ RP 18),
- column temperature: 50˚,
- sample temperature: 20º,
- mobile phase: a mixture of 40 volumes of a buffer solution prepared by dissolving 1.36 g of *sodium acetate trihydrate* in 1000 ml of *water*, adjusted to pH 3.5 with 10 per cent v/v of *trifluoroacetic acid*, 35 volumes of *acetonitrile* and 25 volumes of *methanol*,
- flow rate: 0.7 ml per minute,
- refractometer detector, maintained at 45˚,
- injection volume: 100 µl.

Name	Relative retention time	Correction factor
Miltefosine impurity A[1]	0.25	1.12
Miltefosine impurity B[2]	0.52	1.07
Miltefosine impurity C[3]	0.76	1.36
Miltefosine (Retention time: about 30 minutes)	1.0	---
Miltefosine impurity D[4]	1.34	1.15
Miltefosine impurity E[5]	1.74	1.13
Miltefosine impurity F[6]	2.1	---
Miltefosine impurity G[7]	2.66	1.13

[1]dodecyl(2-(trimethylammonio)ethyl)phosphate.
[2] tetradecyl(2-(trimethylammonio)ethyl)phosphate.
[3]pentadecyl(2-(trimethylammonio)ethyl)phosphate.
[4]2-aminoethyl hexadecyl hydrogen phosphate.
[5]heptadecyl(2-(trimethylammonio)ethyl)phosphate.
[6]octadecyl(2-(trimethylammonio)ethyl)phosphate.
[7]hexadecyl dihydrogen phosphate.

Note – Method is sensitive to decrease and increase of pH and acetonitrile in mobile phase composition.

Inject reference solution (e) to identify the peaks due to miltefosine impurity A, B, C, D, E, F and G.

Inject reference solution (a), (e) and (f). The test is not valid unless the resolution between the peaks due to miltefosine impurity C and miltefosine is not less than 1.2 and between the peaks due to miltefosine and miltefosine impurity D is not less than 1.2 in the chromatogram obtained with reference solution (e), the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 10.0 per cent in the chromatogram obtained with reference solution (a) and the signal-to-noise ratio is not less than 10 in the chromatogram obtained with reference solution (f).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to miltefosine impurity A, B, C, D and E, each of, is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent). the area of any peak corresponding to miltefosine impurity G is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent) and the area of any other secondary peak is not more than 0.33 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). Ignore all the peaks up to 5 minutes and any peak corresponding to miltefosine impurity F.

B. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.15 g of the substance under examination in the mobile phase, with the aid of ultrasound and dilute to 10.0 ml with the mobile phase.

Reference solution (a). A 0.0225 per cent w/v solution of *miltefosine impurity F IPRS* (octadecyl(2-(trimethylammonio) ethyl)phosphate) in *methanol*.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase.

Reference solution (c). A 0.0225 per cent w/v solution of *miltefosine impurity A IPRS* (dodecyl(2-(trimethylammonio) ethyl)phosphate) in the mobile phase.

Reference solution (d). Dilute 1.0 ml, each of, reference solution (a) and reference solution (c) to 10.0 ml with the mobile phase.

Reference solution (e). Dilute 3.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5 µm) (Such as Xterra® MS C18),
- column temperature: 50˚,
- sample temperature: 25º,
- mobile phase: a mixture of 40 volumes of 0.1 per cent v/v solution of *triethylamine* in *water*, adjusted to pH 10.3 with *dilute orthophosphoric acid*, 30 volumes of *acetonitrile* and 30 volumes of *methanol*,
- a flow rate programme using the conditions given below,
- refractometer detector, maintained at 50˚,
- injection volume: 100 µl.

Time (in min.)	Flow rate (ml per min.)
0.0	0.6
20	0.6
23	2.0
57	2.0
60	0.6
80	0.6

Note – This method is sensitive to buffer pH

Inject reference solution (d) to identify the peaks due to miltefosine impurity A and F.

Inject reference solution (b), (d) and (e). The test is not valid unless the resolution between the peaks due to miltefosine impurity A and miltefosine impurity F is not less than 1.2 in the chromatogram obtained with reference solution (d), the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 10.0 per cent in the chromatogram obtained with reference solution (b) and the signal-to-noise ratio is not less than 10 in the chromatogram obtained with reference solution (e).

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to miltefosine impurity f is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent).

The sum of all the impurities from test A and B is not more than 0.75 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Water (2.3.43). Not more than 5.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.25 g of the substance under examination in the mobile phase with the aid of ultrasound and dilute to 50.0 ml with mobile phase.

Reference solution. A 0.5 per cent w/v solution of *miltefosine IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5µm) (Such as Xterra® Shield RP C18),
- column temperature: 45˚,
- sample temperature: 10º,
- mobile phase: a mixture of 40 volumes of a buffer solution prepared by dissolving 1.36 g of *sodium acetate trihydrate* in 1000 ml of *water*, adjusted to pH 3.5 with 10 per cent v/v of *trifluoroacetic acid*, 35 volumes of *acetonitrile* and 25 volumes of *methanol*,
- flow rate: 1.1 ml per minute,
- refractometer detector, maintained at 45˚,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{46}NO_4P$.

Storage. Store protected from moisture under nitrogen atmosphere, at a temperature not exceeding 30°.

Solubility: Freely soluble in *water* and practically insoluble in *acetone.*