Draft Proposal for Comments and Inclusion in The Indian Pharmacopoeia

Tilmicosin Phosphate

Published on: 05 January, 2023 **Last date for comments:** 06 March, 2023

This draft proposal contains monograph text for inclusion in the Indian Pharmacopoeia (IP). The content of this draft document is not final, and the text may be subject to revisions before publication in the IP. This draft does not necessarily represent the decisions or the stated policy of the IP or Indian Pharmacopoeia Commission (IPC).

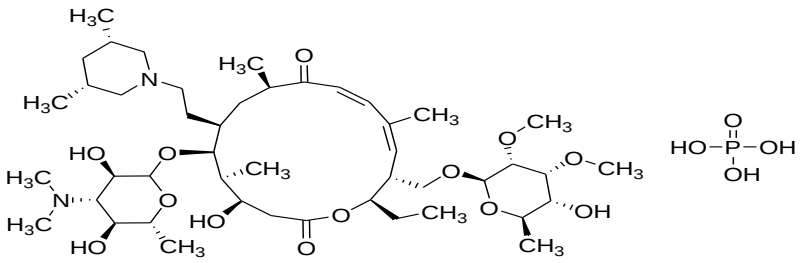
Manufacturers, regulatory authorities, health authorities, researchers, and other stakeholders are invited to provide their feedback and comments on this draft proposal. Manufacturers are also invited to submit samples of their products to the IPC to ensure that the proposed monograph adequately controls 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.

Document History and Schedule for the Adoption Process

Description	Details
Document version	1.0
Monograph proposed for inclusion	IP Addendum 2024
Tentative effective date of monograph	April, 2024
First draft published on IPC website for public comments	05 January, 2023
Draft revision published on IPC website for public comments	-
Further follow-up action as required.	

Tilmicosin Phosphate



$C_{46}H_{83}N_2O_{17}P$ Mol. Wt. 967.1

Tilmicosin phosphate contains not less than 75.0 per cent of tilmicosin, $C_{46}H_{80}N_2O_{13}$, calculated on the anhydrous basis. The content of tilmicosin *cis*-isomers is between 82.0 percent and 88.0 percent, and the content of tilmicosin *trans*-isomers is between 12.0 percent and 18.0 percent of total $C_{46}H_{80}N_2O_{13}$.

CAUTION—Tilmicosin phosphate is irritating to the eyes and may cause allergic reaction. Avoid contact.

Category. In treatment of chronic respiratory diseases in poultry.

Description. A white to almost white powder.

Identification

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

B. In the Assay, the peak due to tilmicosin *trans*-isomer and tilmicosin *cis*-isomer in the chromatogram obtained with the test solution correspond to the peak due to tilmicosin *trans*-isomer and tilmicosin *cis*-isomer in the chromatogram obtained with the reference solution.

C. It gives the reactions of phosphates (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use

Solvent mixture. A 0.57 per cent w/v solution of *orthophosphoric acid* in *water*, adjusted to pH 2.5 with *12.5 M sodium hydroxide.*

Buffer solution. A solution prepared by mixing 70 ml of 10 per cent v/v of *orthophosphoric acid* with stirring to 16.8 ml of *dibutylaminine phosphate*, allow to cool, adjusted to pH 2.5 with *orthophosphoric acid* and dilute to 100 ml with *water* and mix.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of *acetonitrile* with the aid of ultrasound and dilute to 50.0 ml with the solvent mixture.

Reference solution. A 0.025 per cent w/v solution of *tilmicosin IPRS* in the *acetonitrile*. Dilute 5.0 ml of the solution to 25.0 ml with the solvent mixture.

Chromatographic system

– a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),

- mobile phase: A. a mixture of 2.5 volumes of the buffer solution and 97.5 volumes of *water*.
  B. *acetonitrile*,
- flow rate: 1.1 ml per minute,
- a gradient programme using the conditions given below,
- spectrophotometer set at 280 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	82	18
30	82	18
30.1	60	40
35	60	40
35.1	82	18
40	82	18

Name	Relative retention time
Tilmicosin trans-isomers (two incompletely resolved peaks)	0.9
Tilmicosin cis-isomers	1.0
Tilmicosin cis-8-epimer	1.1

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak other then tilmicosin *trans*-isomers, tilmicosin *cis*-isomers and tilmicosin *cis*-8-epimer is not more than 2.4 times the area of the principal peak in the chromatogram obtained with the reference solution (3.0 per cent) and the sum of the areas of all the secondary peaks is not more than 8 times the area of the principal peak in the chromatogram with the reference solution (10.0 per cent), calculated on anhydrous basis.

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

Water (2.3.43). Not more than 7.0 per cent, determined, using 20 ml of a mixture of 75 volumes of *methanol* and 25 volumes of *pyridine* containing 10 per cent w/v of *imidazole* in place of *methanol* in the titration vessel.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use

Solvent mixture. A 0.57 per cent w/v solution of *orthophosphoric acid* in *water*, adjusted to pH 2.5 with *12.5 M sodium hydroxide.*

Buffer solution. A solution prepared by mixing 70 ml of 10 per cent v/v of *orthophosphoric acid* with stirring to 16.8 ml of *dibutylaminine phosphate*, allow to cool, adjusted to pH 2.5 with *orthophosphoric acid* and dilute to 100 ml with *water* and mix.

Test solution. Dissolve 66 mg of the substance under examination in 20 ml of *acetonitrile*, with the aid of ultrasound and dilute to 100.0 ml with the solvent mixture.

Reference solution. Dissolve 25 mg of *tilmicosin IPRS* in 10 ml of *acetonitrile*, with the aid of ultrasound and dilute to 50.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 11.5 volumes of *acetonitrile* and 5.5 volumes of *tetrahydrofuran*, 2.5 volumes of a buffer solution and 80.5 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 10 µl.

NOTE—Mobile phase may be sparged with helium for 2 minutes, before use. Decreasing the portion of acetonitrile or tetrahydrofuran increases resolution.

The relative retention time with reference to tilmicosin *cis*-isomers for tilmicosin *trans* -isomers form is about 0.8.

NOTE—Tilmicosin cis-isomer and tilmicosin cis-8-epimer may co-elute.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to tilmicosin *trans*-isomers and tilmicosin *cis*-isomers is not less than 1.25, the tailing factor is not less than 0.7 and not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution. record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in µg, of tilmicosin *trans*- and *cis*-isomers in the portion of tilmicosin taken by the formula:

$$50(CP/W)(r_i/r_S)$$

Where, C = is the concentration, in mg per ml, of tilmicosin IPRS in the refrence solution*;*

P = is the designated potency, in µg per mg, of the relevant (*trans* or *cis*) tilmicosin isomers in tilmicosin IPRS

W = is the weight, in mg, of Tilmicosin taken to prepare the test solution;

r_i = is the peak area response for the relevant (*trans* or *cis*) tilmicosin isomers obtained from the test solution;

r_S = is the peak area response for the relevant (*trans* or *cis*) tilmicosin isomers obtained from the reference solution.

Calculate the percentage of tilmicosin ($C_{46}H_{80}N_2O_{13}$) in the portion of Tilmicosin taken by the formula:

$$\text{Tilmicosin (\%)} = \frac{0.1(trans + cis)}{100 - water} \times 100$$

Where, *trans* and *cis* are the quantities, in µg per mg, of tilmicosin *trans*-isomers and tilmicosin *cis*-isomers in the Tilmicosin, as determined above.

Calculate the percentages of tilmicosin *trans*-isomers and tilmicosin *cis*-isomers taken by the formula:

$$100\ isomer/(trans + cis)$$

Where, *isomer* is the quantity, in µg per mg, of either the tilmicosin *trans*-isomers or the tilmicosin *cis*-isomers in the Tilmicosin, as determined above.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Solubility: Freely soluble in *water*, insoluble in *acetonitrile.*