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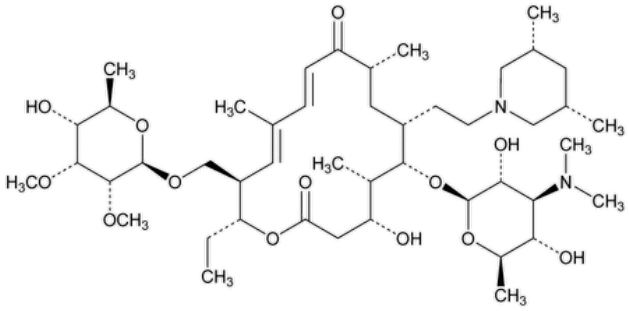
Tilmicosin



$C_{46}H_{80}N_2O_{13}$ 869.13

Tylosin, 4^{A}-*O*-de(2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-20-deoxo-20-(3,5-dimethyl-1-piperidinyl)-, 20(*cis*)-.

4^{A}-*O*-de(2,6-Dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-20-deoxo-20-(*cis*-3,5-dimethylpiperidino)-tylosin CAS RN®: 108050-54-0; UNII: XL4103X2E3.

» Tilmicosin contains not less than 85.0 percent of $C_{46}H_{80}N_2O_{13}$, 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 is irritating to the eyes and may cause allergic reaction. Avoid contact.]

Packaging and storage—Preserve in well-closed, light-resistant containers. Avoid excessive heat.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—

USP Tilmicosin RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩*, Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)

B: The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits peaks for the tilmicosin *trans*-isomer and the tilmicosin *cis*-isomer, the retention times of which correspond to those exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

Water Determination, *Method I* ⟨921⟩**:** not more than 5.0%, 20 mL of a mixture of methanol and pyridine (4:1) containing 10% of imidazole being used in place of methanol in the titration vessel.

Related compounds—

Dibutylammonium phosphate buffer and *Diluent*—Prepare as directed in the *Assay*.

Solution A—To 700 mL of water add 25 mL of *Dibutylammonium phosphate buffer*, dilute quantitatively with water to 1 L, and mix. Degas before use.

Solution B—Use degassed acetonitrile.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Dissolve an accurately weighed quantity of USP Tilmicosin RS in acetonitrile to obtain a solution having a known concentration of about 0.25 mg per mL, sonicating if necessary to dissolve. Transfer 5.0 mL of this solution to a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Test solution—Transfer about 200 mg of Tilmicosin, accurately weighed, to a 50-mL volumetric flask, add 10 mL of acetonitrile, and sonicate briefly to dissolve. Dilute with *Diluent* to volume, and mix. [Note—Use this solution within 24 hours.]

Chromatographic system (see Chromatography ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1 and is programmed for gradient elution by delivering a mixture of *Solution A* and *Solution B* in a ratio of 82:18 initially, and by continuously varying the mixture linearly over a period of 30 minutes until the final ratio is 60:40. The flow rate is about 1.1 mL per minute. Chromatograph the *Standard solution,* and record the responses as directed for *Procedure:* the relative retention times are about 0.9 for the tilmicosin *trans*-isomers (two incompletely resolved peaks), 1.0 for the tilmicosin *cis*-isomer, and 1.1 for the tilmicosin *cis*-8-epimer.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the percentage of each related compound in the portion of Tilmicosin taken by the formula:

$$5(CP/W)(r_c/r_s)$$

in which C is the concentration, in mg per mL, of USP Tilmicosin RS in the *Standard solution; P* is the designated potency, in µg per mg, of tilmicosin in the USP Tilmicosin RS; W is the weight, in mg, of Tilmicosin taken to prepare the *Test solution;* r_c is the area response of the individual related compound peak, other than those obtained for tilmicosin *trans*-isomers, tilmicosin *cis*-isomer, and tilmicosin *cis*-8-epimer; and r_s is the sum of the peak area responses for the tilmicosin *trans*-isomers, the tilmicosin *cis*-isomer, and the tilmicosin *cis*-8-epimer obtained from the *Standard solution*. Not more than 3% of any individual related compound, calculated on the anhydrous basis, is found, and the sum of all the related compounds is not more than 10%, calculated on the anhydrous basis.

Assay—

Dibutylammonium phosphate buffer—Add, with stirring, 70 mL of dilute phosphoric acid (1 in 10) to 16.8 mL of dibutylamine. Allow to cool, and adjust with phosphoric acid to a pH of 2.5 ± 0.1. Dilute with water to 100 mL, and mix.

Mobile phase—To 700 mL of water, add 115 mL of acetonitrile, 55 mL of tetrahydrofuran, and 25 mL of *Dibutylammonium phosphate buffer*. Dilute with water to 1000 mL, and mix. Each component may be degassed before use, or the *Mobile phase* may be sparged with helium for 2 minutes before use. Store the *Mobile phase* in a sealed container when not in use. Make adjustments if necessary (see *System Suitability* under Chromatography ⟨621⟩). [Note—Decreasing the proportion of acetonitrile or tetrahydrofuran increases resolution.]

Diluent—To 900 mL of water, add 5.71 g of phosphoric acid, adjust with 12.5 N sodium hydroxide to a pH of 2.5 ± 0.1, dilute with water to 1000 mL, and mix.

Standard preparation—Transfer about 25 mg of USP Tilmicosin RS, accurately weighed, to a 50-mL volumetric flask, add 10 mL of acetonitrile, and sonicate to dissolve. Dilute with *Diluent* to volume, and mix. [Note—Use this solution on the day prepared.]

Assay preparation—Transfer about 25 mg of Tilmicosin, accurately weighed, to a 50-mL volumetric flask, add 10 mL of acetonitrile, and sonicate to dissolve. Dilute with *Diluent* to volume, and mix. [Note—Use this solution on the day prepared.]

Chromatographic system (see Chromatography ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the responses as directed for *Procedure:* the relative retention times are about 0.8 for the tilmicosin *trans*-isomers and 1.0 for the tilmicosin *cis*-isomers [Note—Tilmicosin *cis*-isomer and tilmicosin *cis*-8-epimer co-elute in this chromatographic system]; the resolution, R, between the tilmicosin *trans*-isomers peak and the tilmicosin *cis*-isomers peak is not less than 1.25; the tailing factors for the peaks are not less than 0.7 and not more than 2; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, 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:

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

in which C is the concentration, in mg per mL, of USP Tilmicosin RS in the *Standard preparation; P* is the designated potency, in µg per mg, of the relevant (*trans* or *cis*) tilmicosin isomers in the USP Tilmicosin RS; W is the weight, in mg, of Tilmicosin taken to prepare the *Assay preparation;* r_i is the peak area response for the relevant (*trans* or *cis*) tilmicosin isomers obtained from the *Assay preparation;* and r_S is the peak area response for the relevant (*trans* or *cis*) tilmicosin isomers obtained from the *Standard preparation*. Calculate the percentage of tilmicosin ($C_{46}H_{80}N_2O_{13}$) in the portion of Tilmicosin taken by the formula:

$$0.1(trans + cis)$$

in which *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:

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

in which *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.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
TILMICOSIN	Documentary Standards Support	SM32020 Small Molecules 3
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

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