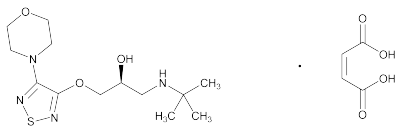
L1. The flow rate is about 1.1 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure:* the relative retention times are about 0.8 for the tilmicosin *trans*-isomers and 1.0 for the tilmicosin *cis*-isomers, the resolution, *R,* between the tilmicosin *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 1.5%.

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 mg, of each of the tilmicosin isomers in each mL of the Injection taken by the formula:

$$0.6(CP / V)(r_I / r_S)$$

in which C is the concentration, in mg per mL, of USP Tilmicosin RS in the *Standard preparation*, P is the potency, in µg per mg, of the relevant (*trans* or *cis*) tilmicosin isomers in the USP Tilmicosin RS, V is the volume of Injection taken to prepare the *Assay preparation*, r_I is the peak response of the relevant 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 quantity, in mg, of $C_{46}H_{80}N_2O_{13}$ in each mL of the Injection taken by adding the quantities, in mg per mL, of *cis*- and *trans*-isomers found.

Timolol Maleate



$C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$ 432.49

2-Propanol, 1-[(1,1-dimethylethyl)amino]-3-[[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy]-, (*S*)-, (*Z*)-2-butenedioate (1:1) (salt);
(–)-1-(*tert*-Butylamino)-3-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxy]-2-propanol maleate (1:1) (salt) [26921-17-5].

DEFINITION

Timolol Maleate contains NLT 98.0% and NMT 102.0% of timolol maleate ($C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** ⟨197M⟩
- **B. Ultraviolet Absorption** ⟨197U⟩
  Analytical wavelength: 294 nm
  Sample solution: 25 µg/mL in 0.12 N hydrochloric acid
  Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**
  Solution A: Dilute 0.5 mL of trifluoroacetic acid with water to 1 L.
  Solution B: Dilute 0.5 mL of trifluoroacetic acid with acetonitrile to 1 L.
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	84	16
2.4	84	16
8	20	80
8.1	84	16
11	84	16

Diluent: Methanol and water (60:40)
System suitability solution: 100 µg/mL of USP Timolol Maleate RS and 10 µg/mL of USP Timolol Related Compound D RS in *Diluent*
Standard solution: 100 µg/mL of USP Timolol Maleate RS in *Diluent*
Sample solution: 100 µg/mL of Timolol Maleate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 295 nm
Column: 2.1-mm × 10-cm; 2.6-µm packing L1
Flow rate: 0.4 mL/min
Injection volume: 2.5 µL
Autosampler temperature: 4°
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0, *Standard solution*
Resolution: NLT 2 between timolol and timolol related compound D, *System suitability solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of timolol maleate ($C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$) in the portion of Timolol Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of timolol from the *Sample solution*
r_S = peak response of timolol from the *Standard solution*
C_S = concentration of USP Timolol Maleate RS in the *Standard solution* (µg/mL)
C_U = concentration of Timolol Maleate in the *Sample solution* (µg/mL)
Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition** ⟨281⟩**:** NMT 0.1%

Delete the following:

- **Heavy Metals,** *Method II* ⟨231⟩**:** 20 ppm (Official 1-Jan-2018)
- **Organic Impurities**
  Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
  System suitability solution: 100 µg/mL each of USP Timolol Maleate RS, USP Timolol Related Compound B RS, USP Timolol Related Compound C RS, USP Timolol Related Compound D RS, USP Timolol Related Compound E RS, and USP Timolol Related Compound F RS in *Diluent*. [NOTE—Prepare fresh and analyze immediately as USP Timolol Related Compound E RS degrades rapidly.]
  Standard solution: 1 µg/mL of USP Timolol Maleate RS and 4 µg/mL each of USP Timolol Related Compound B RS, USP Timolol Related Compound C RS, USP Timolol

USP Monographs

Related Compound D RS, USP Timolol Related Compound E RS, and USP Timolol Related Compound F RS in *Diluent*. Sonicate if needed for 0.5 min.

Sample solution: 1 mg/mL of Timolol Maleate in *Diluent*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between timolol and timolol related compound D, *System suitability solution*

Relative standard deviation: NMT 4.0% for timolol, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each specified impurity in the portion of Timolol Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each specified impurity from the *Sample solution*

r_S = peak response of the corresponding USP Reference Standard from the *Standard solution*

C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (µg/mL)

C_U = concentration of Timolol Maleate in the *Sample solution* (µg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Timolol Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for any individual unspecified impurity from the *Sample solution*

r_S = peak response of timolol from the *Standard solution*

C_S = concentration of USP Timolol Maleate RS in the *Standard solution* (µg/mL)

C_U = concentration of Timolol Maleate in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Timolol related compound B	0.5	0.4
Timolol related compound D	0.8	0.4
Timolol maleate	1.0	—
Timolol related compound E	1.4	0.4
Timolol related compound C	1.8	0.4
Timolol related compound F	2.0	0.4
Unspecified impurity	—	0.10
Total impurities	—	1.0

SPECIFIC TESTS

- **Optical Rotation,** *Specific Rotation* ⟨**781S**⟩
  Sample solution: 50 mg/mL of Timolol Maleate in 1.0 N hydrochloric acid
  Acceptance criteria: –11.7° to –12.5° (λ = 405 nm)
- **pH** ⟨**791**⟩
  Sample solution: 20 mg/mL of Timolol Maleate in water
  Acceptance criteria: 3.8–4.3
- **Loss on Drying** ⟨**731**⟩
  Analysis: Dry under vacuum at 100° to constant weight.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.
- **USP Reference Standards** ⟨**11**⟩
  USP Timolol Maleate RS
  USP Timolol Related Compound B RS
  3-(*tert*-Butylamino)-2-(4-morpholino-1,2,5-thiadiazol-3-yloxy)propan-1-ol.
  $C_{13}H_{24}N_4O_3S$ 316.42
  USP Timolol Related Compound C RS
  N-(*tert*-Butyl)-2,3-bis(4-morpholino-1,2,5-thiadiazol-3-yloxy)propan-1-amine.
  $C_{19}H_{31}N_7O_4S_2$ 485.19
  USP Timolol Related Compound D RS
  4-Morpholino-1,2,5-thiadiazol-3-ol.
  $C_6H_9N_7O_4S$ 187.22
  USP Timolol Related Compound E RS
  (*S*)-3-(*tert*-Butylamino)-1-(4-morpholino-1,2,5-thiadiazol-3-yloxy)propan-2-yl hydrogen maleate.
  $C_{17}H_{26}N_4O_6S$ 414.48
  USP Timolol Related Compound F RS
  3-Chloro-4-morpholino-1,2,5-thiadiazol.
  $C_6H_8ClN_3OS$ 205.67

Timolol Maleate Ophthalmic Solution

» Timolol Maleate Ophthalmic Solution is a sterile, aqueous solution of Timolol Maleate. It contains an amount of $C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$ equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of timolol ($C_{13}H_{24}N_4O_3S$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩**—**
USP Timolol Maleate RS

Identification—Dilute a suitable quantity of Ophthalmic Solution with water to obtain a solution having a concentration of about 20 µg of timolol per mL: the UV absorption spectrum of the solution so obtained exhibits maxima and minima at the same wavelengths as that of a similar preparation of USP Timolol Maleate RS, concomitantly measured.

Sterility Tests ⟨71⟩**:** meets the requirements.

pH ⟨791⟩: between 6.5 and 7.5.

Assay—

pH 2.8 phosphate buffer—Dissolve 11.1 g of monobasic sodium phosphate in 1000 mL of water, adjust with phosphoric acid to a pH of 2.8 ± 0.05, filter, and degas.

Diluent—Prepare a mixture of acetonitrile and *pH 2.8 phosphate buffer* (2:1).

Mobile phase—Prepare a mixture of *pH 2.8 phosphate buffer* and methanol (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩). [NOTE—Minimize the time the Reference Standard, the Ophthalmic Solution, the standard stock solution, the *Standard preparation,* and the *Assay preparation* are exposed to direct light.]

Standard preparation—Transfer about 34 mg of USP Timolol Maleate RS, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Transfer 5.0 mL of this stock solution to a 50-mL volumetric flask, add 15 mL of *Diluent*, dilute with water to volume, and mix.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 5 mg of