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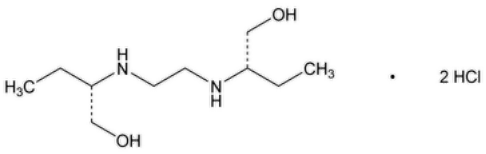
Ethambutol Hydrochloride



$C_{10}H_{24}N_2O_2 \cdot 2HCl$ 277.23

1-Butanol, 2,2'-(1,2-ethanediyldiimino)bis-, dihydrochloride, [*S*-(*R**,*R**)]-;

(+)-2,2'-(Ethylenediimino)-di-1-butanol dihydrochloride;

(2*S*,2'*S*)-2,2'-[Ethane-1,2-diylbis(azanediyl)]dibutan-1-ol CAS RN®: 1070-11-7; UNII: QE4VW5FO07.

DEFINITION

Ethambutol Hydrochloride contains NLT 98.0% and NMT 100.5% of ethambutol hydrochloride ($C_{10}H_{24}N_2O_2 \cdot 2HCl$), calculated on the dried basis.

IDENTIFICATION

- **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197A or 197K
- **B. Identification Tests—General ⟨191⟩, *Chemical Identification Tests, Chloride***

Sample solution: 100 mg/mL in water

Acceptance criteria: Meets the requirements

ASSAY

- **Procedure**

Sample solution: 200 mg of Ethambutol Hydrochloride in a mixture of 100 mL of glacial acetic acid and 5 mL of mercuric acetate TS. Add crystal violet TS.

Analysis: Titrate with 0.1 N perchloric acid VS (the color change at the endpoint is from blue to blue-green). Perform a blank determination, and make any necessary corrections (see *Titrimetry ⟨541⟩*). Each milliliter of 0.1 N perchloric acid is equivalent to 13.86 mg of ethambutol hydrochloride ($C_{10}H_{24}N_2O_2 \cdot 2HCl$).

Acceptance criteria: 98.0%–100.5% on the dried basis

IMPURITIES

- **Organic Impurities**

Protect all solutions containing ethambutol from light.

Solution A: Methanol and water (50:50)

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	82	18
30	82	18
35	0	100
37	0	100
38	82	18
50	82	18

Impurity stock solution A: To 4 mg of USP Ethambutol Related Compound A RS, add 4 mL of acetonitrile and 0.1 mL of triethylamine. Sonicate for 5 min and add 15 µL of (*R*)-(+)-alpha-methylbenzyl isocyanate. Heat the mixture at 70° for 20 min.

Impurity stock solution B: To 4 mg of USP Ethambutol Related Compound B RS, add 4 mL of acetonitrile and 0.1 mL of triethylamine. Sonicate for 5 min and add 15 µL of (*R*)-(+)-alpha-methylbenzyl isocyanate. Heat the mixture at 70° for 20 min.

System suitability solution: To 4 mg of USP Ethambutol Hydrochloride RS, add 4 mL of acetonitrile and 0.1 mL of triethylamine. Sonicate for 5 min and add 15 µL of (*R*)-(+)-alpha-methylbenzyl isocyanate. Heat the mixture at 70° for 20 min. Add 40 µL each of *Impurity stock solution A* and *Impurity stock solution B*.

Standard stock solution: To 4 mg of USP Ethambutol Hydrochloride RS, add 4 mL of acetonitrile and 0.1 mL of triethylamine. Sonicate for 5 min and add 15 µL of (*R*)-(+)-alpha-methylbenzyl isocyanate. Heat the mixture at 70° for 20 min.

Standard solution: Dilute 0.5 mL of *Standard stock solution* with acetonitrile to 100.0 mL.

Sample solution: To 4 mg of Ethambutol Hydrochloride, add 4 mL of acetonitrile and 0.1 mL of triethylamine. Sonicate for 5 min and add 15 µL of (*R*)-(+)-alpha-methylbenzyl isocyanate. Heat the mixture at 70° for 20 min.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Temperatures

Autosampler: 10°

Column: 40°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

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

[Note—The relative retention times for ethambutol related compound B, ethambutol, and ethambutol related compound A are 0.91, 1.0, and 1.3, respectively.]

Suitability requirements

Resolution: NLT 1.2 between ethambutol related compound B and ethambutol; NLT 3.0 between ethambutol and ethambutol related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Ethambutol Hydrochloride taken:

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

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

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

C_S = concentration of USP Ethambutol Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Ethambutol Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ethambutol	1.0	—
Ethambutol related compound A	1.3	1.0
Any individual impurity[a]	—	0.10
Total impurities[a]	—	1.0

[a] Calculate any individual impurity and total impurities from the relative retention times of 0.75 to 1.5 with reference to ethambutol.

Change to read:

• **Limit of Aminobutanol**

Solution A: 12.4 mg/mL of boric acid in water. Adjust with 5 N sodium hydroxide to a pH of 9.0 prior to final dilution.

Fluorescamine solution: 0.1 mg/mL of fluorescamine in acetone

Standard solution: 5 µg/mL of USP Aminobutanol RS

Sample solution: 0.5 mg/mL of Ethambutol Hydrochloride

Instrumental conditions

(See *Fluorescence Spectroscopy ⟨853⟩*.)

Mode: Fluorometry

Analytical wavelength: 485 nm, with the excitation wavelength at about 385 nm

Cell: 1 cm

Analysis

Samples: *Standard solution* and *Sample solution*

Pipet a 10-mL portion of the *Sample solution* into a glass-stoppered, 100-mL conical flask, and add 10 mL of water and 20 mL of *Solution A*. To another 100-mL flask, add 10.0 mL of the *Sample solution,* 10.0 mL of the *Standard solution,* and 20 mL of *Solution A*. Place the flasks on a magnetic stirrer, and while the contents are being stirred rapidly, add 10 mL of *Fluorescamine solution* rapidly. Insert the stoppers in the flasks, invert, and shake briefly. After 1 min, accurately timed, determine the relative fluorescence intensities of both solutions in a suitable fluorometer.

Acceptance criteria: The fluorescence intensity of the solution from the *Sample solution* is NMT ▲▲ (ERR 1-Aug-2020) the difference between the intensities of the two solutions ▲(NMT 1.0%).▲ (ERR 1-Aug-2020)

SPECIFIC TESTS

- **Optical Rotation ⟨781S⟩, *Procedures, Specific Rotation***

Sample solution: 100 mg/mL in water

Acceptance criteria: +6.0° to +6.7°

- **Loss on Drying ⟨731⟩**

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.
- **USP Reference Standards ⟨11⟩**

USP Aminobutanol RS $C_4H_{11}NO$ 89.14

USP Ethambutol Hydrochloride RS

USP Ethambutol Related Compound A RS
((2*R*,2'*S*)-2,2'-[Ethane-1,2-diylbis(azanediyl)]dibutan-1-ol)dihydrochloride.
$C_{10}H_{24}N_2O_2 \cdot 2HCl$ 277.23

USP Ethambutol Related Compound B RS
((2*R*,2'*R*)-2,2'-[Ethane-1,2-diylbis(azanediyl)]dibutan-1-ol)dihydrochloride.
$C_{10}H_{24}N_2O_2 \cdot 2HCl$ 277.23

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

Topic/Question	Contact	Expert Committee
ETHAMBUTOL HYDROCHLORIDE	Documentary Standards Support	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

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