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ETHAMBUTOL DIHYDROCHLORIDE TABLETS

(ETHAMBUTOLI DIHYDROCHLORIDI COMPRESSI)

Draft proposal for revision in The International Pharmacopoeia

(05 November 2024)

DRAFT FOR COMMENTS

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Comments should be submitted through the online platform on or by 05 January 2025. Please note that only comments received by this deadline will be considered for the preparation of this document.

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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/24.960:

ETHAMBUTOL DIHYDROCHLORIDE TABLETS

(ETHAMBUTOLI DIHYDROCHLORIDI COMPRESSI)

Description	Date
Drafting of the text.	August 2024
Presentation at the 58 th meeting of the Expert Committee on Specifications for Pharmaceutical Preparations	October 2024
Draft monograph sent out for public consultation	November 2024 – January 2025
Further follow-up action as required	

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42	ETHAMBUTOL DIHYDROCHLORIDE TABLETS
43	(ETHAMBUTOLI DIHYDROCHLORIDI COMPRESSI)
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45	Category. Antibacterial (antituberculosis).
46	Storage. Ethambutol hydrochloride tablets should be kept in a tightly closed
47	container.
48	Additional information. Strengths in the current WHO Model list of essential
49	medicines: 100 mg (dihydrochloride). Strengths in the current WHO Model list of
50	essential medicines for children: 100 mg (dihydrochloride).
51	Requirements
52	Comply with the monograph for <u>Tablets</u> .
53	Definition. Ethambutol dihydrochloride tablets contain Ethambutol dihydrochloride.
54	They contain not less than 90.0% and not more than 110.0% of the amount of
55	Ethambutol dihydrochloride ($C_{10}H_{24}N_2O_2$, 2HCl) stated on the label.
56	Identity tests
57	• Either tests A, or tests B and C, may be applied.
58	A. Carry out the test as described under <u>1.7 Spectrophotometry in the infrared</u>
59	<u>region</u> . To a quantity of the powdered tablets, nominally containing 0.1 g of
60	Ethambutol dihydrochloride, add 10 mL of methanol R and shake. Filter and
61	evaporate the filtrate to dryness. The infrared absorption spectrum of the
62	residue is concordant with the spectrum obtained from ethambutol
63	dihydrochloride RS or with the reference spectrum of ethambutol
64	dihydrochloride.

65	B.	Carry out the test as described under <u>1.14.1 Chromatography</u> , High-
66		performance liquid chromatography using the conditions given under "Assay".
67		The retention time of the principal peak in the chromatogram obtained with
68		solution (1) corresponds to the retention time of the peak due to ethambutol in
69		the chromatogram obtained with solution (2).
70	C.	Carry out the test as described under <u>1.14.1 Chromatography</u> , Thin layer
71		chromatography, using the conditions given under "Impurity A".
72		Apply separately to the plate 5 μ L of each of the following two solutions in
73		methanol R. For solution (A), transfer a quantity of the powdered tablets,
74		nominally containing 20 mg of Ethambutol dihydrochloride, to a 10 mL
75		volumetric flask, add 8 mL of methanol R, sonicate for 5 minutes, and dilute to
76		volume. Filter the solution and use the filtrate. For solution (B), use a solution
77		containing 2 mg of ethambutol dihydrochloride RS per mL. After removing the
78		plate from the chromatographic chamber, allow it to dry in air or in a current of
79		air.
80		Spray the plate with anisaldehyde/methanol TS and heat it to 105 °C for 10
81		minutes. Allow the plate to cool and examine the chromatogram in daylight.
82		The principal spot in the chromatogram obtained with solution (A) corresponds
83		in position, appearance and intensity with the spot due to ethambutol in the
84		chromatogram obtained with solution (B).
85	Imp	urity A. Carry out the test as described under 1.14.1 Chromatography, Thin-layer
86	chromatography, using silica gel R5 as the coating substance and a mixture of 10	
87	volumes of ammonia (~260 g/L) TS, 15 volumes of water R and 75 volumes of	
88	meth	anol R as the mobile phase.
89	Appl	y separately to the plate 2 μ L of each of the following 3 solutions: For solution
90	(A), transfer a quantity of the powdered tablets, nominally containing 500.0 mg of	

- 91 Ethambutol dihydrochloride, to a 100 mL flask, add 10.0 mL of methanol R and shake
- for 5 minutes. Filter the suspension and use the filtrate. For solution (B), dissolve 50.0
- mg of 2-aminobutanol R (impurity A) in 100.0 mL of methanol. For solution (C),
- prepare a solution containing 5 mg of ethambutol dihydrochloride RS and 0.5 mg of
- 95 2-aminobutanol R per mL.
- Develop the plate for 2/3 of its height. After removing the plate from the
- 97 chromatographic chamber, allow it to dry in air, heat it at 110 °C for 10 minutes, and
- allow it to cool. Spray the plate with ninhydrin/ethanol (1 g/60 mL) TS, heat it at
- 99 110 °C for 5 minutes, and examine the chromatogram in daylight. The test is not valid
- unless the chromatogram obtained with solution (C) shows two clearly separated
- 101 spots.
- Any spot due to impurity A in the chromatogram obtained with solution (A) is not
- more intense than the spot in the chromatogram obtained with solution (B) (1.0%).
- 104 **Impurity B and C.** Prepare the solutions immediately before use. Carry out the test as
- described under <u>1.14.1 Chromatography</u>, High-performance liquid chromatography,
- using a stainless-steel column (10 cm x 4.6 mm) packed with end-capped particles of
- silica gel, the surface of which has been modified with chemically bonded
- 108 octadecylsilyl groups $(3 \mu m)^1$.
- 109 Use the following conditions for gradient elution:
- 110 Mobile phase A: 50 volumes of methanol for chromatography R and 50 volumes of
- 111 water R.
- 112 Mobile phase B: methanol for chromatography R.

Time	Mobile phase A	Mobile phase B	Comments
(minutes)	(% V/V)	(% V/V)	

¹ A Luna C18(2) column has been found suitable.

0–30	71	29	Isocratic
30–35	71 to 0	29 to 100	Linear gradient
35-37	0	100	Isocratic
37–38	0 to 71	100 to 29	Return to initial composition
38-48	71	29	Re-equilibration

Operate with a flow rate of 1.0 mL per minute. Maintain the column temperature at 40 °C. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 215 nm.

Prepare the following solutions: For solution (1), transfer a quantity of the powdered tablets, nominally containing 200.0 mg of Ethambutol dihydrochloride, to a 20 mL volumetric flask, add about 14 mL of water R, sonicate for about 15 minutes, and dilute with water R to volume, mix and filter. Dilute 2.0 mL of the filtrate to 20.0 mL with acetonitrile R. Transfer 4.0 mL of this solution to a test tube, add 100 μ L of triethylamine R and 15 μ L of (R)-(+)- α -methylbenzyl isocyanate R, insert a stopper, mix, and heat at 70 °C for 20 minutes.

For solution (2), dilute 2.0 mL of solution (1) to 200.0 mL with acetonitrile.

For solution (3), transfer 4 mg of ethambutol for system suitability RS (containing ethambutol and impurity B) to a test tube, add 4 mL of mixture containing 9 volumes of acetonitrile R and 1 volume of water and 100 μ L of triethylamine. Sonicate for 5 minutes to dissolve, add 15 μ L of (R)-(+)- α -methylbenzyl isocyanate R, insert a stopper, mix, and heat at 70 °C for 20 minutes.

Inject 10 µL each of solutions (1), (2) and (3) and record the chromatograms.

Use the chromatogram obtained with solution (3) to identify the peaks due to the impurity B. The impurities are eluted, if present, at the following relative retention

with reference to ethambutol (retention time about 14 minutes): impurity C about 0.9 132 and impurity B about 1.3. 133 The test is not valid unless, in the chromatogram obtained with solution (3), the 134 resolution between the peak due to ethambutol and impurity B is at least 4.0. 135 In the chromatogram obtained with solution (1): 136 the area of any peak corresponding to impurity B is not greater than the area of 137 the peak due to ethambutol in the chromatogram obtained with solution (2) 138 (1.0%);139 the area of any impurity peak with a relative retention of 0.75 to 1.5 with 140 reference to ethambutol is not greater than 0.1 times the area of the peak due to 141 ethambutol in the chromatogram obtained with solution (2) (0.10%). 142 The sum of the areas of any peak corresponding to impurity B and of all other 143 impurity peaks with a relative retention of 0.75 to 1.5 with reference to 144 ethambutol is not greater than the area of the peak due to ethambutol in the 145 chromatogram obtained with solution (2) (1.0%). Disregard any peak with an 146 area less than 0.1 times the area of the peak due to ethambutol in the 147 chromatogram obtained with solution (2) (0.1%). 148 **Dissolution.** Carry out the test as described under 5.5 Dissolution test for oral dosage 149 forms, using as the dissolution medium, 900 mL of dissolution buffer, pH 6.8, TS and 150 rotating the paddle at 50 revolutions per minute. At 30 minutes, withdraw a sample of 151 10 mL of the medium through an in-line filter. Allow the filtered sample to cool to 152 room temperature and use it as solution (1). For solution (2), dissolve 56.0 mg of 153 ethambutol dihydrochloride RS in the dissolution buffer. 154 Analyze solutions (1) and (2) as described under 1.14.1 Chromatography, High-155 performance liquid chromatography, using the chromatographic conditions as 156

described under "Assay".

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For each of the six tablets tested, calculate the total amount of ethambutol 158 dihydrochloride (C₁₀H₂₄N₂O₂, 2HCl) in the medium from the results obtained. 159 Evaluate the results as described under 5.5 Dissolution test for oral dosage forms, 160 Acceptance criteria. The amount of ethambutol dihydrochloride released is not less 161 than 75% (Q) of the amount declared on the label. 162 **Assay**. Carry out the test as under 1.14.1 Chromatography, High-performance liquid 163 chromatography, using as the stationary phase a stainless steel column packed with 164 particles of silica gel, the surface of which has been modified with chemically bonded 165 octadecylsilyl groups (5 µm). 166 As the mobile phase, use a solution prepared as follows: transfer 50 g of ammonium 167 acetate R and 0.2 g of copper (II) acetate R to a 1000 mL volumetric flask, add 800 168 mL water R, shake to dissolve, adjust to pH 5.0 with glacial acetic acid R and dilute to 169 volume with water R. Mix 800 mL of this solution with 200 mL of methanol R. 170 As the diluent, use a solution prepared as follows: transfer 7.7 g of ammonium acetate 171 R to a 1000 mL volumetric flask, add 800 mL water R, shake to dissolve, adjust to pH 172 2.0 with phosphoric acid (~1440 g/L) TS and fill up to volume with water R. 173 Prepare the following solutions in diluent. For solution (1), weigh and powder 20 174 tablets. Transfer a quantity of the powder, nominally containing 100.0 mg of 175 Ethambutol dihydrochloride, to a 500 mL volumetric flask. Add 400 mL and shake for 176 about 15 minutes to dissolve. Dilute to volume, mix, and filter. For solution (2), 177 dissolve 50.0 mg of ethambutol dihydrochloride RS in 250.0 mL. 178 Operate with a flow rate of 1.0 mL per minute. As a detector, use an ultraviolet 179 spectrophotometer set at a wavelength of about 270 nm. Maintain the column 180 temperature at 35 °C. 181

Inject 20 µl each of solutions (1) and (2) and record the chromatogram for 15 minutes.

183	Measure the areas of the peaks corresponding to ethambutor obtained in the
184	chromatograms from solutions (1) and (2) and calculate the percentage content of
185	ethambutol dihydrochloride ($C_{10}H_{24}N_2O_2$, 2HCl) in the tablets using the declared
186	content of $C_{10}H_{24}N_2O_2$, 2HCl in ethambutol dihydrochloride RS.
187	Impurities
188	The impurities limited by the requirements of this monograph include those listed in
189	the monograph on Ethambutol dihydrochloride.
190	
191	Reference substance required
192	Ethambutol dihydrochloride ICRS
193	Already established ICRS. Intended uses to be adapted.
194	Ethambutol for system suitability RS (containing ethambutol and the impurity
195	B)
196	It is intended to refer to the corresponding reference substances established for the
197	European Pharmacopeia.
198	Reagent to be added
199	(R) - $(+)$ - α -methylbenzyl isocyanate R
200	C ₉ H ₉ NO.
201	Content. minimum 99.0%.
202	Description. A colourless liquid.
203	Relative density. d_{20}^{20} is about 1.045.

Refractive index. n_D²⁰ is about 1.513.
Boiling point. 55 °C to 56°C at 2.5 mm Hg.
Enantiomeric purity. minimum 99.5.
Storage. At a temperature of 2 °C to 8 °C.
Ninhydrin/ethanol (1 g/ 60 mL) TS
Dissolve 1.0 g of ninhydrin R in 50 mL of dehydrated ethanol R and add 10 mL of glacial acetic acid R.
