



LINEZOLID

(LINEZOLIDUM)

Draft proposal for inclusion for *The International Pharmacopoeia*

(May 2022)

DRAFT FOR COMMENTS

Please send any comments you may have on this draft working document to **Dr Herbert Schmidt**, Technical Officer, Norms and Standards for Pharmaceuticals, Technical Standards and Specifications (email: schmidt@who.int), with a copy to Ms Sinéad Jones (email: jonesi@who.int) by **15 July 2022**.

Our working documents are sent out electronically and they will be placed on the WHO Medicines website (<https://www.who.int/teams/health-product-and-policy-standards/standards-and-specifications/pharmaceuticals/current-projects>) for comments under the “Working documents in public consultation” link. If you wish to receive our draft guidelines, please send your e-mail address to jonesi@who.int and your name will be added to our electronic mailing list.

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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/20.841:

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Description	Date
First draft prepared.	August 2019
Presentation to the 54 th WHO Expert Committee on Specifications for Pharmaceutical Preparations.	October 2019
Discussion at the informal Consultation on Screening Technologies, Laboratory Tools and Pharmacopoeial Specifications for Medicines.	April/May 2020
Discussion at the informal Consultation on Screening Technologies, Laboratory Tools and Pharmacopoeial Specifications for Medicines.	May 2021
First draft sent out for public consultation.	June – July 2021
Revision of the first draft based on the comments received during the public consultation and preparation of Revision 1	August 2021
Presentation at the 56 th Meeting of the Expert Committee on Specifications for Pharmaceutical Preparations	25 April – 2 May 2022
Revision 1 sent out for public consultation.	May – July 2022
Further follow-up action as required.	

[Note from the Secretariat. The draft proposal is based on information submitted by manufacturers and found in other pharmacopoeias and in the scientific literature.

All stakeholders, in particular manufacturers of this product, regulatory authorities, quality control laboratories and procurement agencies, are invited to provide their feedback to the Secretariat of The International Pharmacopoeia. Your support will help ensure that the

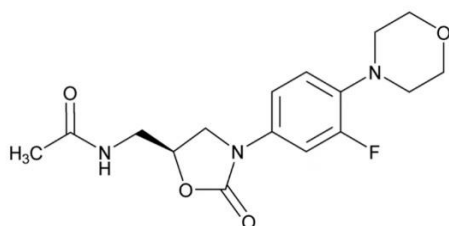
47 *proposed monograph adequately controls the quality of Linezolid active pharmaceutical*
48 *ingredient on the market.]*

49

Draft for comments

LINEZOLID (LINEZOLIDUM)

Graphic formula.



Molecular formula. $C_{16}H_{20}FN_3O_4$

Relative molecular mass. 337.4

Chemical name. *N*-[[*(S)*-3-(3-Fluoro-4-morpholinophenyl)-2-oxo-5-oxazolidinyl]methyl] acetamide; CAS Reg. No. 165800-03-3.

Description. A white to off-white powder.

Solubility. Sparingly soluble in methanol R; soluble in dichloromethane R, slightly soluble in dehydrated ethanol R.

Category. Antituberculosis.

Storage. Linezolid should be kept in an airtight container, protected from light and moisture.

Additional information. Linezolid may exhibit polymorphism.

Requirements

Manufacture. The production method is validated to demonstrate that genotoxic impurities are adequately controlled in the final product.

Definition. Linezolid contains not less than 99.0% and not more than 101.0% of $C_{16}H_{20}FN_3O_4$, calculated with reference to the anhydrous substance.

Identity tests

- Either test A alone, or any two of tests B, C or D may be applied.
- A. Carry out the examination as described under *1.7 Spectrophotometry in the infrared region*. The infrared absorption spectrum is concordant with the spectrum obtained from linezolid RS.

If the spectra thus obtained are not concordant, repeat the test using the residues obtained by separately dissolving the test substance and linezolid RS in a small amount of dehydrated ethanol R at a temperature of about 50 to 60 °C. Evaporate the solvent using a rotary evaporator. The infrared absorption spectrum of the test substance is concordant with the spectrum obtained from linezolid RS.

- B. Carry out the test as described under *1.14.4 High-performance liquid chromatography* using the conditions given under “Related substances”. Prepare the following solutions in a mixture of 20 volumes of acetonitrile R and 80 volumes of 1.36 g/L potassium dihydrogen phosphate in water. For solution (1), dissolve 10 mg of the test substance in 50 mL. For solution (2), dissolve 10 mg of linezolid RS in 50 mL.

Inject 10 µl each of solutions (1) and (2). The retention time of the principal peak obtained with solution (1) corresponds to the retention time of the peak due to linezolid in the chromatogram obtained with solution (2).

C. Carry out the test as described under *1.14.1 Thin-layer chromatography* using silica gel R6 as the coating substance and a freshly prepared mixture of acetone R, toluene R and glacial acetic acid R (45:45:10 V/V/V) as the mobile phase. Apply separately to the plate 2 µL of each of the following 2 solutions in methanol R containing (A) 5 mg of the test substance per mL and (B) 5 mg of linezolid RS per mL. After removing the plate from the chromatographic chamber, allow it to dry in air or in a current of air. Examine the plate under ultraviolet light (254 nm). The principal spot in the chromatogram obtained with solution (A) corresponds in position, appearance and intensity with the spot due to linezolid in the chromatogram obtained with solution (B).

D. Dissolve 20 mg of the test substance in methanol R and dilute to 100 mL with the same solvent. Dilute 1 mL of this solution to 20 mL. Record an absorption spectrum of the solution in the range from 200 nm to 400 nm as described under *1.6 Spectrophotometry in the visible and ultraviolet regions*. The spectrum exhibits a maximum at 258 nm.

Alternatively, in combination with identity test B, where a diode-array detector is available, record the UV spectrum of the principal peak in the chromatograms with a diode array detector in the range of 200 nm to 400 nm. The UV spectrum of the principal peak in the chromatogram obtained with solution (1) corresponds to the UV spectrum of the peak due to linezolid in the chromatogram obtained with solution (2).

Water. Determine as described under *2.8 Determination of water by the Karl Fischer method*, Method A. Use 0.300 g of the test substance. The water content is not more than 5.0 mg/g.

Sulfated ash (2.3). Not more than 2.0 mg/g

Heavy metals. Use 2.0 g of the test substance for the preparation of the test solution as described under 2.2.3 *Limit test for heavy metals*, Procedure 5. Determine the heavy metals content according to Method C; not more than 10 µg/g.

Impurity E (Linezolid R-isomer). Carry out the test as described under 1.14.4 *High-performance liquid chromatography* using a stainless steel column (15 cm x 4.6 mm) packed with silica particles, the surface of which has been modified with chemically-bonded amylose tris-3,5-dimethylphenylcarbamate, (5 µm)¹.

As mobile phase, use a mixture of 960 volumes acetonitrile R, 40 volumes of dehydrated ethanol R, 1 volume of n-butylamine R and 1.6 volumes of trifluoroacetic acid R.

Operate at a flow rate of 0.8 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 254 nm. Maintain the column temperature at 40 °C.

Use a mixture of 960 volumes of acetonitrile R and 40 volumes of dehydrated ethanol R as diluent. For solution (1), dissolve 25.0 mg of the test substance and dilute to 50.0 mL. For solution (2), dilute 1.0 mL of solution (1) to 100.0 mL. Dilute 1.0 mL of this solution to 10.0 mL. For solution (3), dissolve 5.0 mg each of linezolid RS and linezolid impurity E RS and dilute to 200.0 mL.

Inject 5 µL each of solutions (1), (2) and (3). Record the chromatograms for about two times the retention time of linezolid.

In the chromatogram obtained with solution (3), impurity E is eluted with a relative retention of about 0.39 with reference to linezolid. The test is not valid unless, in the chromatogram obtained with solution (3), the resolution between the peak of impurity E and the peak of linezolid is greater than 10. The test is also not valid unless, in the

¹ Phenomenex Lux Amylose-1 or Chiralpak IA column was found suitable.

chromatogram obtained with solution (2), the peak due to linezolid is detected with a signal-to-noise ratio of at least 10.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity E is not greater than 3 times the area of the peak due to linezolid in the chromatogram obtained with solution (2) (0.3%).

Related substances. Carry out the test as described under *1.14.4 High-performance liquid chromatography* using a stainless steel column (15 cm x 4.6 mm), packed with end-capped particles of silica gel, the surface of which has been modified with chemically-bonded octylsilyl groups (3.5 µm)².

Use the following conditions for gradient elution:

- mobile phase A: 90 volumes of phosphate buffer and 10 volumes of methanol R .
- mobile phase B: 30 volumes of phosphate buffer, 50 volumes of acetonitrile R and 20 volumes of methanol R.

Prepare the phosphate buffer by dissolving 1.36 g of potassium dihydrogen phosphate R in 1000 mL of water R.

Time (minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–2	100	0	Isocratic
2–10	100 to 75	0 to 25	Linear gradient
10–15	75	25	Isocratic
15–30	75 to 20	25 to 80	Linear gradient
30–35	20	80	Isocratic

² A Zorbax Eclipse XDB C8 column was found suitable.

35–36	20 to 100	80 to 0	Return to initial composition
36–40	100	0	Re-equilibration

157 Operate with a flow rate of 1.2 mL per minute. As a detector, use an ultraviolet
158 spectrophotometer set at a wavelength of 254 nm. Maintain the column temperature at
159 25 °C.

160 Prepare the following solutions in a mixture of 20 volumes of acetonitrile R and 80
161 volumes of phosphate buffer. For solution (1), dissolve 40.0 mg of the test substance
162 in 50.0 mL. For solution (2), dilute 1.0 mL of solution (1) to 100.0 mL with a mixture
163 of 20 volumes of acetonitrile R and 80 volumes of phosphate buffer. For solution (3),
164 dilute 1.0 mL of solution (2) to 10.0 mL with a mixture of 20 volumes of acetonitrile R
165 and 80 volumes of phosphate buffer. For solution (4), dissolve 5.0 mg each of linezolid
166 RS, linezolid impurity A and linezolid impurity D RS in 100.0 mL of a mixture of 20
167 volumes of acetonitrile R and 80 volumes of phosphate buffer.

168 Inject 10 µL each of solutions (1), (2), (3) and (4).

169 Use the chromatogram obtained with solution (4) to identify the peaks due to linezolid,
170 impurity A and impurity D in the chromatogram obtained with solution (1). The
171 impurity peaks, if present, are eluted at the following relative retention times with
172 reference to linezolid (retention time about 18 minutes): impurity G about 0.49;
173 impurity C about 0.59; impurity H about 0.75; impurity F about 0.80; impurity J about
174 1.28; impurity D about 1.36; impurity B about 1.42; impurity A about 1.50; impurity K
175 about 1.53 and impurity I about 1.66.

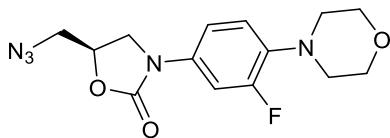
176 The test is not valid unless, in the chromatogram obtained with solution (4), the
177 resolution between the peak due to impurity D and the peak due to impurity A is greater
178 than 15.0. Also, the test is not valid unless in the chromatogram obtained with solution
179 (3), the peak due to linezolid is detected with a signal-to-noise ratio of at least 20.

180 In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity G, when multiplied by a correction factor of 2.2, is not greater than three times the area of the peak due to linezolid in the chromatogram obtained with solution (3) (0.3%);
- the area of any peak corresponding to linezolid related impurity C is not greater than twice the area of the peak due to linezolid in the chromatogram obtained with solution (3) (0.2%);
- the area of any peak corresponding to impurity F is not greater than three times the area of the peak due to linezolid in the chromatogram obtained with solution (3) (0.3%);
- the area of any peaks corresponding to impurity I or K, when multiplied by a correction factor of 1.3, is not greater than 0.8 times the area of the peak due to linezolid in the chromatogram obtained with solution (3) (0.08%);
- the area of any peak corresponding to impurity J, when multiplied by a correction factor of 0.7, is not greater than 0.8 times the area of the peak due to linezolid in the chromatogram obtained with solution (3) (0.08%);
- the area of any other impurity peak is not greater than 0.8 times the area of the peak due to linezolid in the chromatogram obtained with solution (3) (0.08%).
- The sum of the areas of all impurity peaks is not greater than the area of the peak due to linezolid in the chromatogram obtained with solution (2) (0.5%). Disregard any peak with an area less than 0.5 times the area of the peak due to linezolid in the chromatogram obtained with solution (3) (0.05%).

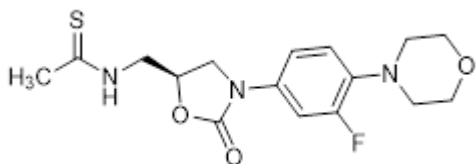
Assay. Dissolve 0.250 mg of the test substance in 30 mL of glacial acetic acid R1, warming slightly if necessary, and titrate with perchloric acid (0.1 mol/L) VS, as described under 2.6 *Non-aqueous titration*, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 33.74 mg of $C_{16}H_{20}FN_3O_4$.

209 **Impurities**

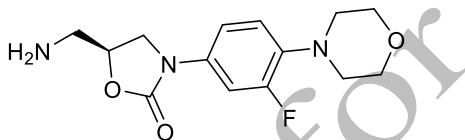


- 211 A. (R)-5-(Azidomethyl)-3-(3-fluoro-4-morpholinophenyl)oxazolidine-2-on
212 (linezolid azide)(synthesis related impurity)

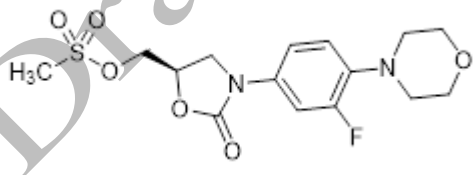
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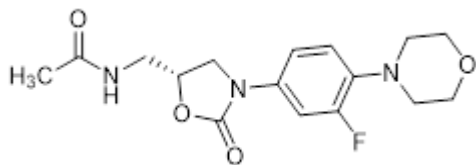
- 215 B. (S)-N-((3-(3-Fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-
216 yl)methyl)ethanethioamide (thiolinezolid)(synthesis related impurity)



- 218 C. (S)-5-(Aminomethyl)-3-(3-fluoro-4-morpholinophenyl) oxazolidin-2-one
219 (linezolid amine) (synthesis related impurity, degradation product).

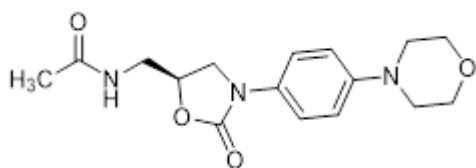


- 221 D. (R)-[3-(3-Fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl] methyl
222 methanesulfonate (synthesis related impurity).



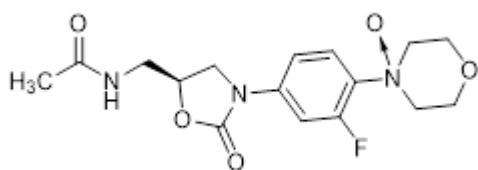
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- 224 E. *N*-[[*(R)*]-3-(3-Fluoro-4-morpholinophenyl)-2-oxo-5-oxazolidinyl] methyl]
225 acetamide (linezolid *R*-isomer) (synthesis related impurity).



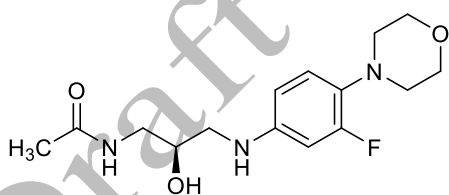
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- 227 F. (*S*)-*N*-{[3-(4-Morpholinophenyl)-2-oxooxazolidin-5-yl] methyl} acetamide
228 (desfluoro linezolid)(synthesis related impurity).



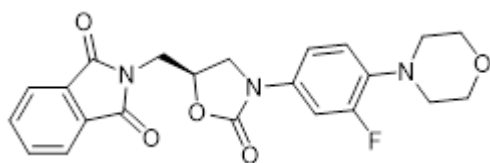
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- 230 G. (*S*)-4-(4-[5-(Acetamidomethyl)-2-oxooxazolidin-3-yl]-2-fluorophenyl)
231 morpholine 4-oxide(linezolid N-oxide) (degradation product).



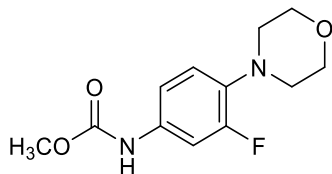
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- 233 H. *N*-[(2*S*)-3-[[3-Fluoro-4-(4-morpholinyl)phenyl]amino]-2-
234 hydroxypropyl]acetamide (degradation product)

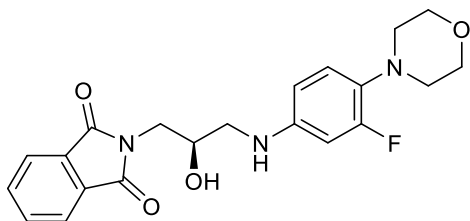


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- 236 I. (S)-2-((3-(3-fluoro-4-morpholino phenyl)-2-oxo-oxazolidin-5-
237 yl)methyl)isoindoline-1,3-dione) (synthesis related impurity).



- 238
239 J. 3-Fluoro-4-morpholine-4-yl-phenyl)carbamic acid methyl ester (synthesis
240 related impurity,).



- 241
242 K. N-[(2R)-3-[[3-Fluoro-4-(4-morpholinyl)phenyl]amino]-2-
243 hydroxypropyl]acetamide (synthetic related impurity)

244 **Reference substances invoked**

245 **Linezolid RS**

246 ICRS to be established.

247 **Linezolid impurity D RS**

248 ICRS to be established.

249 **Linezolid impurity A RS**

250 ICRS to be established.

251 **New reagents**

252 **n-Butylamine**

253 $\text{C}_4\text{H}_{11}\text{N}$; Relative molecular mass 73.1; CAS Reg. No. 109-73-9; Butan-1-amine.

254 Distil and use within one month.

255 *Description.* Colourless liquid, miscible with water, with ethanol (96 per cent).

256 *Relative density.* About 1.401.

257 *Boiling point.* About 78 °C.

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Draft for comments