LINEZOLID TABLETS

(LINEZOLIDI COMPRESSI)

Draft proposal for inclusion in 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: schmidth@who.int), with a copy to Ms Sinéad Jones (email: jonessi@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 jonessi@who.int and your name will be added to our electronic mailing list.

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Please send any request for permission to:

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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/21.888:

LINEZOLID TABLETS

(LINEZOLIDI COMPRESSI)

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

[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 proposed monograph adequately controls the quality of linezolid tablets on the market.]
Following the inclusion of medicines containing linezolid in the WHO Model List of Essential Medicines and, in view of requests from healthcare providers to help ensure that these medicines are of good quality, the World Health Organization (WHO) is also planning to develop monographs on:

- Linezolid dispersible tablets;
- Linezolid intravenous infusion; and
- Linezolid powder for oral suspension.

We would greatly appreciate it if the manufacturers of these products would actively collaborate in the development of these international quality specifications.

For more information, please contact Dr Herbert Schmidt at schmidt@who.int.
LINEZOLID TABLETS

(LINEZOLIDI COMPRESSI)

Category. Antituberculosis, antibiotic.

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

Additional information. Strengths in the current WHO Model List of Essential Medicines (EML): 400 mg, 600 mg. Strengths in the current EML for children: 400 mg, 600 mg.

Requirements

Comply with the monograph for Tablets.

Definition. Linezolid tablets contains not less than 90.0% and not more than 110.0% of the amount of Linezolid (C\textsubscript{16}H\textsubscript{20}FN\textsubscript{3}O\textsubscript{4}) stated on the label.

Identity tests

- Either tests A and C or tests B and C may be applied.

A. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Assay”. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the peak due to linezolid in the chromatogram obtained with solution (2).

B. Carry out 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 two solutions. For solution
(A), transfer a quantity of the powdered tablets, nominally containing 100 mg of
Linezolid, to a flask and add 20 mL of methanol R. Stopper the flask and sonicate
for 5 minutes, filter the suspension and use the clear supernatant. For solution
(B), use a solution contain 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). Spray the plate with basic
potassium permanganate (~5 g/L) TS and examine the plate in daylight. 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).

C. Transfer a quantity of the powdered tablets, nominally containing 25 mg of
Linezolid, to a 50 mL volumetric flask, add 40 mL of methanol R and sonicate
for 5 minutes. Dilute to volume with methanol R and mix. Filter the suspension
and dilute 2 mL of the filtrate to 100 mL with methanol R. Record an adsorption
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 test A, 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).

**Dissolution.** Carry out the test as described under 5.5 *Dissolution test for solid oral
dosage forms*, using as the dissolution medium 900 mL of dissolution buffer, pH 6.8,
TS and rotating the paddle at 50 revolutions per minute. At 30 minutes, withdraw a
sample of about 10 mL of the medium through an in-line filter. Allow the filtered
sample to cool to room temperature. Dilute 1.0 mL of this solution to 50.0 mL with the
dissolution buffer.

Measure the absorbance as described under 1.6 Spectrophotometry in the visible and
ultraviolet regions of the resulting solution in a cuvette with an optical pathlength of 10
mm at the maximum at about 251 nm, using the dissolution buffer as the blank.

For each of the tablets tested, calculate the total amount of linezolid (C₁₆H₂₀F₃N₃O₄) in
the medium using the absorptivity value of 57.1 for linezolid (A₁%cm = 571). Evaluate
the results as described under 5.5 Dissolution test for solid oral dosage forms,
Acceptance criteria. The amount of linezolid released is not less than 80% (Q) of the
amount declared on the label.

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).

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.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–2</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
</tbody>
</table>

1 A Zorbax Eclipse XDB C8 column was found suitable.
Operate with a flow rate of 1.2 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 254 nm. Maintain the column temperature at 25 °C.

For solution (1), transfer a quantity of the powdered tablets, nominally containing 80.0 mg of linezolid, into a 100 mL volumetric flask, add 50 mL of phosphate buffer, sonicate for 5 minutes with intermediate shaking, add 20 mL of acetonitrile R, and sonicate for further 10 minutes with intermediate shaking. Allow the flask to cool to room temperature, dilute to volume with phosphate buffer, mix and filter. For solution (2), dilute 1.0 mL of solution (1) to 100.0 mL with a mixture of phosphate buffer and acetonitrile R (80:20 v/v). For solution (3), dilute 1.0 mL of solution (2) to 10.0 mL with a mixture of phosphate buffer and acetonitrile R (80:20 v/v). For solution (4), dissolve 5.0 mg each of linezolid RS, linezolid impurity A RS and linezolid impurity D RS in 100.0 mL with a mixture of phosphate buffer and acetonitrile R (80:20 v/v).

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

Use the chromatogram obtained with solution (4) to identify the peaks due to linezolid, impurity A and impurity D in the chromatogram obtained with solution (1). The impurity peaks, if present, are eluted at the following relative retention times with reference to linezolid (retention time about 18 minutes): impurity G about 0.49; impurity C about 0.59; impurity H about 0.75; impurity F about 0.80; impurity J about 1.28; impurity D about 1.36; impurity B about 1.42; impurity A about 1.50; impurity K about 1.53 and impurity I about 1.66.
The test is not valid unless in the chromatogram obtained with solution (4) the resolution between the peak of impurity D and the peak of impurity A is greater than 150. Also, the test is not valid unless in the chromatogram obtained with solution (3) the peak due to linezolid is detected with a signal-to-noise ratio of at least 20.

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 3 times the area of the peak due to linezolid in the chromatogram obtained with solution (3) (0.3%);
• the area of any other impurity peak is not greater than twice the area of the peak due to linezolid in the chromatogram obtained with solution (3) (0.17%).
• The sum of the area of all impurity peaks is not greater than the area of the peak due to linezolid in the chromatogram obtained with solution (2) (1.0%). 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. 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

² A Zorbax Eclipse XDB C8 column was found suitable.
Use the following conditions for gradient elution:

- mobile phase A: 70 volumes of phosphate buffer, 15 volumes of acetonitrile R and 15 volumes of methanol R.
- mobile phase B: acetonitrile R.

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

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–7</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
<tr>
<td>7–9</td>
<td>10</td>
<td>90</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>9–15</td>
<td>10</td>
<td>90</td>
<td>Isocratic</td>
</tr>
<tr>
<td>15–16</td>
<td>100</td>
<td>0</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>16–20</td>
<td>100</td>
<td>0</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

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

Use a mixture of 80 volumes of phosphate buffer and 20 volumes of acetonitrile R and as diluent. For solution (1), weigh and powder 20 tablets. Transfer a quantity of the powdered tablets, nominally containing 240.0 mg of linezolid, into a 200 mL volumetric flask, add 100 mL of phosphate buffer and sonicate for 5 minutes. Add 40 mL of acetonitrile R and sonicate for 10 minutes. Allow the solution to cool to room temperature, make up to volume with phosphate buffer, mix and filter. Dilute 5.0 mL of this solution to 50.0 mL with the diluent. For solution (2), dissolve 60.0 mg of linezolid RS in a 50 mL volumetric flask in 30 mL of the diluent and sonicate for 5 minutes. Allow the solution to cool to room temperature and make up to volume with the diluent. Dilute 5.0 mL of this solution to 50.0 mL with the diluent.
Inject 10 μL each of solutions (1) and (2).

Measure the areas of the peaks corresponding to linezolid obtained in the chromatograms of solutions (1) and (2) and calculate the percentage content of linezolid ($\text{C}_{16}\text{H}_{20}\text{FN}_{3}\text{O}_{4}$) using the declared content of $\text{C}_{16}\text{H}_{20}\text{FN}_{3}\text{O}_{4}$ in linezolid RS.

**Impurities.** The impurities limited by the requirements of this monograph include those listed in the monograph on Linezolid, with the exception of impurity E.

**Reference substances evoked**

**Linezolid RS**

ICRS to be established.

**Linezolid related compound D RS**

ICRS to be established.

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