REMDESIVIR

REMDESIVIRUM

Draft proposal for inclusion for The International Pharmacopoeia

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 (schmidt@who.int), with a copy to Ms Claire Vogel
(vogelc@who.int) by 28 February 2021.

Our working documents are sent out electronically and they will also 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.

[Note from the Secretariat. It is proposed to include a monograph on Remdesivir in The
International Pharmacopoeia. The draft monograph is based on information provided by
manufacturers and on laboratory investigations.]
REMDESIVIR

**REMDESVIRUM**

**Molecular formula.** $C_{27}H_{35}N_6O_8P$

**Relative molecular mass.** 602.6

**Graphic formula.**

![Graphic formula](https://example.com/graphic-formula.png)

**Chemical name.** 2-Ethylbutyl (2S)-2-\{[(S)-\{[(2R,3S,4R,5R)-5-(4-aminopyrrolo[2,1-f][1,2,4]triazin-7-yl)-5-cyano-3,4-dihydroxytetrahydrofuran-2-yl]methoxy)(phenoxy)phosphoryl]amino}propanoate or 2-Ethylbutyl $N$-\{[(S)-\{2-C-(4-aminopyrrolo[2,1-f][1,2,4]triazin-7-yl)-2,5-anhydro-d-altrononitril-6-O-yl]phenoxyphosphoryl]-l-alaninate (IUPAC), l-Alanine, $N$-\{(S)-hydroxyphenoxyphosphinyl\}, 2-ethylbutyl ester, 6-ester with $2-C$-(4-aminopyrrolo[2,1-f][1,2,4]triazin-7-yl)-2,5-anhydro-d-altrononitrile (CAS); CAS Reg. No. 1809249-37-3.

**Description.** A white to off-white or yellow crystalline powder.

**Solubility.** It is soluble in ethanol (~750 g/L) TS and freely soluble in methanol R and dimethylsulfoxide R. It is practically insoluble in water R.

**Category.** Antiviral.

**Storage.** Remdesivir should be kept in tightly closed containers, protected from light and moisture.

**Additional information.** Remdesivir exhibit polymorphism.
Requirements

Definition. Remdesivir contains not less than 97.0% and not more than 102.0% (“Assay”. Method A) and not less than 98.0% and not more than 101.0% (“Assay”. Method B) of \( C_{17}H_{35}N_6O_8P \), calculated with reference to the anhydrous substance.

Identity tests

- Either test A alone or any two of tests B, C and D may be applied.

A. Carry out the test as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from remdesivir RS or with the reference spectrum of remdesivir.

If the spectra thus obtained are not concordant repeat the test using the residues obtained by separately dissolving the test substance and remdesivir RS in a small amount of methanol R and evaporating to dryness. The infrared absorption spectrum is concordant with the spectrum obtained from remdesivir RS.

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

C. The absorption spectrum (1.6) of a 0.03 mg per mL solution of the test substance in methanol R, when observed between 220 nm and 400 nm, exhibits a maximum at about 245 nm and at about 275 nm. Where high-performance liquid chromatography with a diode array detector is available, the spectrum may be recorded with this detector and compared with the spectrum of the reference solution.

D. 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 ethyl acetate R, methanol R and glacial acetic acid R (90:9:1 V/V/V) as the mobile phase. Apply separately to the plate 2 µL of each of the following two solutions in methanol R, containing (A) 1 mg of the test substance per mL and (B) 1 mg of remdesivir 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 chromatogram 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 remdesivir in the chromatogram obtained with solution (B).
Specific optical rotation (1.4). Use a 10 mg per mL solution of the test substance in methanol R and sonicate until the substance is dissolved. Calculate with reference to the anhydrous substance; the specific optical rotation $\left[\alpha\right]_{D}^{20}$ is between -18.0 to -25.0.

Sulfated ash (2.3). Not more than 1.0 mg/g, determined on 1.0 g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method. Method A. Use 200 mg of the test substance. The water content is not more than 50 mg/g.

Clarity and colour of the solution. A solution, containing 0.20 g of remdesivir in 20 mL of methanol R, is clear and not more intensely coloured than reference solution Y2, when compared as described under 1.11.2 Degree of coloration of liquids, Method II.

Heavy metals. Use 0.30 g 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.

Related substances. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (4.6 mm x 15 cm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (3 μm).  

Use the following conditions for gradient elution:
- mobile phase A: phosphoric acid solution;
- mobile phase B: use acetonitrile R.

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6 A YMC Hydrosphere C18 column has been found suitable.
Prepare the phosphoric acid solution by diluting 1.2 mL of phosphoric acid (~1440 g/L) TS to 1000 mL with 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>0–3</td>
<td>97</td>
<td>3</td>
<td>Isocratic</td>
</tr>
<tr>
<td>3–45</td>
<td>97 to 30</td>
<td>3 to 70</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>45–53</td>
<td>30</td>
<td>70</td>
<td>Isocratic</td>
</tr>
<tr>
<td>53–54</td>
<td>30 to 97</td>
<td>70 to 3</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>54–65</td>
<td>97</td>
<td>3</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Operate with a flow rate of 1.0 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 237 nm. Maintain the column temperature at 30 °C.

Prepare as a diluent a mixture of 50 volumes of methanol R and 50 volumes of water R. Prepare the following solutions: For solution (1), dissolve 25.0 mg of the test substance in 5 mL of methanol R and dilute to 50.0 mL with the diluent. For solution (2), dilute 1.0 mL of test solution (1) to 100.0 mL with the diluent. For solution (3), dilute 1.0 mL of solution (2) to 10.0 mL with the diluent. For solution (4), dissolve 5 mg of remdesivir for system suitability RS (containing remdesivir and impurity A) in 1 mL of methanol R and dilute to 10 mL with the diluent.

Inject alternately 5 μL each of solutions (1), (2), (3) and (4).

The impurities are eluted, if present, at the following relative retentions with reference to remdesivir (retention time about 28 minutes): impurity E about 0.18; impurity G about 0.46; impurity H about 0.51; impurity C and impurity D about 0.84; impurity A about 0.98; impurity B about 1.03 and impurity F about 1.30.

The test is not valid unless, in the chromatogram obtained with solution (4), the resolution between the peaks due to impurity A and remdesivir is at least 1.5. Also, the test is not valid unless in the chromatogram obtained with solution (3), the peak due to remdesivir is obtained with a signal-to-noise ratio of at least 25.
In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity E, when multiplied with a correction factor of 0.5, is not greater than 1.5 times the area of the peak due to remdesivir in the chromatogram obtained with solution (3) (0.15 %);

- the area of any peak corresponding to impurity F, when multiplied with a correction factor of 1.2, is not greater than 1.5 times the area of the peak due to remdesivir in the chromatogram obtained with solution (3) (0.15 %);

- the area of any peak corresponding to impurity G, when multiplied with a correction factor of 0.8, is not greater than 1.5 times the area of the peak due to remdesivir in the chromatogram obtained with solution (3) (0.15 %);

- the area of any peaks corresponding to impurities A, B or H is not greater than 1.5 times the area of the peak due to remdesivir in the chromatogram obtained with solution (3) (0.15 %);

- the sum of the areas of any peaks corresponding to impurities C and D (impurity C and D co-elute) is not greater than 1.5 times the area of the peak due to remdesivir in the chromatogram obtained with solution (3) (0.15 %);

- the area of any other impurity peak is not greater than the area of the peak due to remdesivir in the chromatogram obtained with solution (3) (0.10 %);

- The sum of the areas of all impurity peaks, including the corrected areas of any peaks corresponding to impurities E, F and G, is not greater than twice the area of the peak due to remdesivir in the chromatogram obtained with solution (2) (2.0%). Disregard all peaks with an area of less than 0.5 times the area of the peak due to remdesivir in the chromatogram obtained with solution (3) (0.05%).

**Assay**

- Either test A or test B may be applied.

A. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (4.6 mm x 25 cm) packed with end-capped particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm).7

Prepare a phosphoric acid solution by diluting 1.2 mL of phosphoric acid (~1440 g/L) TS to 1000 mL with water R. As the mobile phase, use a mixture of 35 volumes of the phosphoric acid solution and 65 volumes of methanol R.

Operate with a flow rate of 1.0 mL per minute.

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7A YMC-Pack ODS-A or an Inertsil ODS-3V column have been found suitable.
As a detector, use an ultraviolet spectrophotometer set at a wavelength of 237 nm. Maintain the column temperature at 25 °C.

Prepare as a diluent a mixture of 50 volumes of methanol R and 50 volumes of water R. Prepare the following solutions: For solution (1), dissolve 40.0 mg of the test substance in 10 mL of methanol R, dilute to 200.0 mL with the diluent. For solution (2), dissolve 40.0 mg of remdesivir RS in 10 mL of methanol R and dilute to 200.0 mL with the diluent.

Inject alternately 5 µL each of solutions (1) and (2) and record the chromatogram for 25 minutes.

Measure the areas of the peaks corresponding to remdesivir obtained in the chromatograms of solutions (1) and (2) and calculate the percentage content of C$_{17}$H$_{35}$N$_6$O$_8$P in the sample using the declared content of C$_{17}$H$_{35}$N$_6$O$_8$P in remdesivir RS.

B. Dissolve 0.400 g in 50 mL of glacial acetic acid R1 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 60.26 mg of C$_{17}$H$_{35}$N$_6$O$_8$P.

**Impurities**

A. 2-Ethylbutyl (2S)-2-\{[(R)-\{[(2R,3S,4R,5R)-5-(4-aminopyrrolo[2,1-f][1,2,4]triazin-7-yl)-5-cyano-3,4-dihydroxytetrahydrofuran-2-yl]methoxy](phenoxy)phosphoryl]amino}propanoate (remdesivir diastereomer) (synthesis related impurity),

B. 2-Ethylbutyl (2S)-2-\{[(S)-\{[(2R,3S,4R,5S)-5-(4-aminopyrrolo[2,1-f][1,2,4]triazin-7-yl)-5-cyano-3,4-dihydroxytetrahydrofuran-2-yl]methoxy](phenoxy)phosphoryl]amino}propanoate (remdesivir diastereomer) (synthesis related impurity),
C. Butyl (2S)-2-\{[(S)-[(2R,3S,4R,5S)-5-(4-aminopyrrolo[2,1-f][1,2,4]triazin-7-yl)-5-cyano-3,4-dihydroxytetrahydrofuran-2-yl]methoxy](phenoxy)phosphoryl]amino\}propanoate (synthesis related impurity),

D. Butyl (2S)-2-\{[(S)-[(2R,3S,4R,5R)-5-(4-aminopyrrolo[2,1-f][1,2,4]triazin-7-yl)-5-cyano-3,4-dihydroxytetrahydrofuran-2-yl]methoxy](phenoxy)phosphoryl]amino\}propanoate (synthesis related impurity),

E. (2R,3R,4S,5R)-2-(4-aminopyrrolo[2,1-f][1,2,4]triazin-7-yl)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-carbonitrile (synthesis related impurity, degradation product),

F. 2-Ethylbutyl (2S)-2-\{[(S)-[(3aR,4R,6R,6aR)-6-(4-aminopyrrolo[2,1-f][1,2,4]triazin-7-yl)-6-cyano-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl]methoxy](phenoxy)phosphoryl]amino\}propanoate (synthesis related impurity),

G. [(2R,3S,4R,5R)-5-(4-aminopyrrolo[2,1-f][1,2,4]triazin-7-yl)-5-cyano-3,4-dihydroxytetrahydrofuran-2-yl]methyl phenyl hydrogenphosphate (synthesis related impurity, degradation product),
H. (3aR,4R,6R,6aR)-4-(4-aminopyrrolo[2,1-f][1,2,4]triazin-7-yl)-6-(hydroxymethyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxole-4-carbonitrile (synthesis related impurity).

**Reference substances to be established**

Remdesivir for system suitability RS (containing remdesivir and impurity A)
- New International Chemical Reference Substance to be established.

Remdesivir RS
- New International Chemical Reference Substance to be established.