MOLNUPIRAVIR
(MOLNUPIRAVIRUM)

Draft proposal for inclusion in The International Pharmacopoeia

(9 September 2022)

DRAFT FOR DISCUSSION

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 4 November 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.

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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/21.906

MOLNUPIRAVIR

(MOLNUPIRAVIRUM)

<table>
<thead>
<tr>
<th>Description</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drafting of the monograph based on information received from manufacturers</td>
<td>December 2021</td>
</tr>
<tr>
<td>Draft revision sent out for public consultation.</td>
<td>January – February 2022</td>
</tr>
<tr>
<td>Presentation to the 56th meeting of the WHO Expert Committee on Specifications for Pharmaceutical Preparations (ECSPP).</td>
<td>April 2022</td>
</tr>
<tr>
<td>Laboratory investigations to verify the analytical provision</td>
<td>May – August 2022</td>
</tr>
<tr>
<td>Preparation of Revision 1 based on the results of the laboratory investigations and the discussion at the 56th meeting of the ECSPP.</td>
<td>August 2022</td>
</tr>
<tr>
<td>Revision 1 sent out for public consultation.</td>
<td>September – November 2022</td>
</tr>
<tr>
<td>Discussion with Experts</td>
<td>TBD</td>
</tr>
<tr>
<td>Further follow-up action as required.</td>
<td></td>
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</tbody>
</table>

[Note from the Secretariat. The monograph on Molnupiravir is proposed for inclusion in The International Pharmacopoeia.

Being the first public standard on Molnupiravir, the monograph is expected to play an important role in ensuring access to safe, effective and quality assured molnupiravir containing medicines. Manufacturers, regulatory authorities, procurement agencies...
and other stakeholders are therefore invited to provide their feedback to the Secretariat of The International Pharmacopoeia.

Manufacturers that have not submitted samples for the elaboration of the monograph are also invited to test their products according the proposed monograph and to submit their results. They will thereby help ensure that the proposed monograph adequately controls the quality of the product(s) they manufacture. For further information, please contact Dr Herbert Schmidt at schmidt@who.int.
MOLNUPIRAVIR (MOLNUPIRAVIRUM)

**Molecular formula.** $C_{13}H_{19}N_3O_7$

**Relative molecular mass.** 329.31

**Graphic formula.**

![Graphic formula]

**Chemical name.** (4-Z)-$N^4$-hydroxycytidine 5’-(2-methylpropanoate) (IUPAC); Uridine, 4-oxime, 5’-(2-methylpropanoate), (4Z)- (CAS); [(2R,3S,4R,5R)-3,4-dihydroxy-5-[(4Z)-(4-(hydroxyimino)-2-oxo-3,4-dihydropyrimidin-1(2H)-yl]oxolan-2-yl]methyl 2-methylpropanoate; CAS Reg. 2492423-29-5.

**Description.** A white to off-white powder.

**Solubility.** It is freely soluble in methanol R and dimethyl sulfoxide R, soluble in water R and , sparingly soluble 2-propanol Rdehydrated ethanol R,ethyl acetate R and acetonitrile R. It is practically insoluble in n-heptane R, dichloromethane R, and n-hexane R.

**Category.** Antiviral.

**Storage.** Molnupiravir should be kept in tightly closed containers, protected from moisture.

**Additional information.** Molnupiravir is slightly hygroscopic and exhibits polymorphism.
Requirements

Manufacture. The production method is validated to demonstrate that the substance hydroxylamine is adequately controlled in the final product. If tested with a suitable method, the substance would comply with a hydroxylamine limit of not more than 14 ppm.

Definition. Molnupiravir contains not less than 97.0% and not more than 102.0% of C₁₃H₁₉N₃O₇, calculated with reference to the dried substance.

Identity tests

- Either tests A or test B or any two of tests C, D, and E 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 molnupiravir RS or with the reference spectrum of molnupiravir.

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

B. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Assay”, but using, as the detector, a diode array detector to record the UV spectrum of the principal peak in each chromatogram in the range of 200 nm to 400 nm. The retention time and the UV spectrum of the principal peak in the chromatogram obtained with solution (1) correspond to the retention time and the UV spectrum of the peak due to molnupiravir in the chromatogram obtained with solution (2).

C. 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 molnupiravir in the chromatogram obtained with solution (2).

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 per mL of the test substance and (B) 1 mg per mL of molnupiravir RS. 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 molnupiravir in the chromatogram obtained with solution (B).

E. The absorption spectrum (1.6) of a 0.03 mg per mL solution of the test substance in methanol R, when observed between 200 nm and 400 nm, exhibits two maxima at about 235 nm and 275 nm.

Specific optical rotation (1.4). Determine the rotation using a 10.0 mg per mL solution of the test substance in methanol R. Calculated with reference to the anhydrous substance. The specific optical rotation \([\alpha]_D^{25}\) is between -8.9 to -7.3.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A. Use 0.500 g of the test substance. The water content is not more than 10 mg/g.

Heavy metals. Use 0.300 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 25 cm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded phenyl groups (3 µm).\(^1\)

Use the following conditions for gradient elution:

- Mobile phase A: pH 2.3 buffer solution;
- Mobile phase B: a mixture of 20 volumes of water R and 80 volumes of the solvent mixture.

Prepare the pH 2.3 buffer solution by dissolving 3.4 g of potassium dihydrogen phosphate R in water R and diluting to 1000 mL with the same solvent. Carefully adjust the pH to 2.30 with phosphoric acid (~105 g/L) TS.

Prepare as the solvent mixture a mixture of 30 volumes of methanol R and 70 volumes of acetonitrile 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>0–5</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
<tr>
<td>5–20</td>
<td>100 to 80</td>
<td>0 to 20</td>
<td>Linear gradient</td>
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<tr>
<td>20–40</td>
<td>80 to 75</td>
<td>20 to 25</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>40–55</td>
<td>75 to 40</td>
<td>25 to 60</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>55–65</td>
<td>40 to 0</td>
<td>60 to 100</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>65–73</td>
<td>0</td>
<td>100</td>
<td>Isocratic</td>
</tr>
<tr>
<td>73–74</td>
<td>0 to 100</td>
<td>100 to 0</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>74–85</td>
<td>100</td>
<td>0</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

\(^1\) A Kromasil 100-5 Phenyl column has been found suitable.
Operate with a flow rate of 0.9 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 230 nm, for impurity F and L, at 260 nm and, for impurity G, at 210 nm. Maintain the column temperature at 25 °C.

Prepare the following solutions freshly and perform the analysis without delay. Use water R as a diluent. For solution (1), transfer 120 mg of the test substance into a 100 mL volumetric flask. Add 60 mL of the diluent, sonicate to dissolve, allow to cool to room temperature and make up to volume. 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), dilute 5.0 mL of solution (2) to 10.0 mL. For solution (4), transfer 1.2 mg of molnupiravir impurity I into a 10 mL volumetric flask. Add 1 ml of acetonitrile R and sonicate to dissolve. Dilute to volume with the diluent. Transfer 1.0 mL of this solution to a 10 mL volumetric flask and make up to volume with solution (1).

Inject 20 µL each of solutions (1), (2), (3) and (4).

The impurities are eluted, if present, at the following relative retentions with reference to molnupiravir (retention time about 23 minutes): impurity D about 0.19; impurity A about 0.23; impurity E about 0.45; impurity K about 0.67; impurity L about 0.82; impurity I about 1.03, impurity F about 1.14; impurity G about 1.70 and 1.72, impurity B about 1.83 and impurity H about 2.04.

The test is not valid unless in this chromatogram obtained with solution (4), the peak-to-valley ratio (Hp/Hv) is at least 3.0, where Hp is the height above the baseline of the peak due to impurity I and Hv is the height above the baseline of the lowest point of the curve separating the peak due to molnupiravir from the peak due to impurity I. Also, the test is not valid unless in the chromatogram obtained with solution (3), the peak due to molnupiravir is obtained 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 A is not greater than 8 times the area of the peak due to molnupiravir in the chromatogram obtained with solution (2) (0.8 %);
- the area of any peak corresponding to impurity B is not greater than 2.2 times the area of the peak due to molnupiravir in the chromatogram obtained with solution (2) (0.22 %);
- the area of any peak corresponding to impurity D, when multiplied with a correction factor of 2.4, is not greater than 0.6 times the area of the peak due to molnupiravir in the chromatogram obtained with solution (2) (0.06 %);
- the area of any peak corresponding to impurity H, when multiplied with a correction factor of 1.6, is not greater than 0.6 times the area of the peak due to molnupiravir in the chromatogram obtained with solution (2) (0.06 %);
- the sum of the areas of any peaks corresponding to impurity G, recorded at 210 nm is not greater than 0.6 times the area of the peak due to molnupiravir in the chromatogram obtained with solution (2), recorded at 210 nm (0.06 %);
- the area of any peak corresponding to impurity F, recorded at 260 nm, when multiplied with a correction factor of 0.7, is not greater than 0.6 times the area of the peak due to molnupiravir in the chromatogram obtained with solution (2), recorded at 260 nm (0.06 %);
- the area of any peak corresponding to impurity L, recorded at 260 nm, is not greater than 0.6 times the area of the peak due to molnupiravir in the chromatogram obtained with solution (2), recorded at 260 nm (0.06 %);
- the area of any other impurity peak is not greater than 0.6 times the area of the peak due to molnupiravir in the chromatogram obtained with solution (2) (0.06 %).
- Determine the areas of all impurity peaks recorded at 230 nm, including the corrected areas of any peak corresponding to impurity D and H. Disregard any
peaks with an area of less than the area of the peak due to molnupiravir in the chromatogram obtained with solution (3), recorded at 230 nm (0.05%). Calculate the percentage concentration of the impurities using the area of the peak due to molnupiravir in the chromatogram obtained with solution (2), recorded at 230 nm, as a reference.

- Determine the corrected areas of any peaks corresponding to impurity F and impurity L, recorded at 260 nm, and calculate their percentage concentration using the area of the peak due to molnupiravir in the chromatogram obtained with solution (2), recorded at 260 nm, as a reference. Disregard any peaks with an area of less than the area of the peak due to molnupiravir in the chromatogram obtained with solution (3), recorded at 260 nm (0.05%).

- Determine the area of any peak corresponding to impurity G, recorded at 210 nm, and calculate its percentage concentration using the area of the peak due to molnupiravir in the chromatogram obtained with solution (2), recorded at 210 nm, as a reference. Disregard any peak with an area of less than the area of the peak due to molnupiravir in the chromatogram obtained with solution (3), recorded at 210 nm (0.05%).

- The sum of the percentage areas of all impurities, recorded at 230 nm, and the percentage areas of the impurities F, L and G, recorded at 260 nm and 210 nm respectively, is not greater than 1.0%.

**Assay.** 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 end-capped particles of silica gel, the surface of which has been modified with chemically-bonded phenyl groups (2.6 µm).²

Use the following conditions for gradient elution:

- mobile phase A: ammonium dihydrogen phosphate solution;

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² A Kinetex Biphenyl column has been found suitable.
• mobile phase B: acetonitrile for chromatography R.

Prepare the ammonium dihydrogen phosphate solution by dissolving 5.75 g of ammonium dihydrogen phosphate R in water R and diluting to 1000 mL with the same solvent.

<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>0–15</td>
<td>90</td>
<td>10</td>
<td>Isocratic</td>
</tr>
<tr>
<td>15–16</td>
<td>90 to 35</td>
<td>10 to 65</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>16–22</td>
<td>35</td>
<td>65</td>
<td>Isocratic</td>
</tr>
<tr>
<td>22–23</td>
<td>35 to 90</td>
<td>65 to 10</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>23–30</td>
<td>90</td>
<td>10</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 260 nm. Maintain the column temperature at 40 °C.

Prepare as the diluent a mixture of 20 volumes of acetonitrile R and 80 volumes of water R.

Prepare the following solutions. For solution (1), transfer 60.0 mg of the test substance into a 50 mL volumetric flask. Add about 30 mL of the diluent, sonicate to dissolve, allow to cool to room temperature, make up to volume, mix and filter. Dilute 5.0 mL of this solution to 50.0 mL. For solution (2), weigh 60.0 mg of molnupiravir RS into a 50 mL volumetric flask. Add 30 mL of the diluent, sonicate to dissolve and make up to volume. Dilute 5.0 mL of this solution to 50.0 mL.

Inject 10 µL each of solutions (1) and (2) and record the chromatograms.
Measure the areas of the peaks corresponding to molnupiravir obtained in the chromatograms of solutions (1) and (2) and calculate the percentage content of Molnupiravir (C_{13}H_{19}N_{3}O_{7}) in the sample using the declared content of C_{13}H_{19}N_{3}O_{7} in molnupiravir RS.

**Impurities**

A. N^4-Hydroxycytidine (synthesis related impurity, degradation product),

B. N^4-Hydroxy-O'2',O'3'-propan-2-yldenecytidin-5'-yl 2-methylpropanoate (dimethyl dioxol impurity, molnupiravir acetonide) (synthesis related impurity),

C. N^4-Hydroxycytidin-5'-yl acetate (molnupiravir acetyl analog (synthesis related impurity))
D. Cytidine

E. $N^4$-Acetylcytosine

F. [(2R,3S,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1-(2H)-yl)3,4-dihydroxytetrahydrofuran-2-yl]methyl isobutyrate (uridine isobutyl ester) (synthesis related impurity, degradation product)

G. ((3aR,4R,6R,6aR)-6-(6-bis(hydroxyamino)-4-(hydroxyamino)-2-oxo-5,6-dihydropyrimidin-1(2H)-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-
4-yl)methyl isobutyrate (hydroxylamino molnupiravir acetonide) (synthesis related impurity)

H. \((2R, 3R, 4R, 5R)-2-(4-(hydroxyamino)-2-oxopyrimidine-1(2H)-yl)-5-((isobutyryloxy)methyl)tetrahydrofuran-3,4-diyil bis(2-methylpropanoate) (molnupiravir triester, triacylated molnupiravir) (synthesis related impurity)

I. \([(2R,3S,4R,5R)-3,4-dihydroxy-5-[4-(hydroxyamino)-2-oxoprimidin-1(2H)-yl]tetrahydrofuran-2-yl]methyl butyrate (molnupiravir N-butyl analog) (synthesis related impurity)

J. \([(2R,3S,4R,5R)-3,4-dihydroxy-5-[4-(hydroxyamino)2-oxopyrimidin-1-yl]oxolan-2-yl]methyl 2-methylpropanate (synthesis related impurity)
K. unknown impurity

\[
\begin{array}{c}
\begin{array}{c}
\text{H}_3\text{C} \quad \text{O} \quad \text{O} \\
\text{CH}_3 \\
\text{HO} \\
\text{OH}
\end{array}
\end{array}
\]

L. [(2R, 3S, 4R, 5R)-5-(4-amino-2-oxopyrimidin-1-(2H)-yl) 3, 4-
dihydroxytetrahydrofuran-2-yl] methyl isobutyrate (cytidine isobutyl ester)
(synthesis related impurity)

Reference substances to be established

Molnupiravir RS

- New International Chemical Reference Substance to be established.