World Health Organization

NIRMATRELVIR

(NIRMATRELVIRUM)

Draft proposal for inclusion in *The International Pharmacopoeia*

(June 2023)

DRAFT FOR COMMENTS

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For any technical questions, you may contact **Dr Herbert Schmidt**, Technical Officer, Norms and Standards for Pharmaceuticals, Technical Standards and Specifications (schmidth@who.int), with a copy to Ms Sinéad Jones (jonessi@who.int, nsp@who.int).
Comments should be submitted through the online platform on or by **23 August 2023**. Please note that only comments received by this deadline will be considered for the preparation of this document.
Our working documents are sent out electronically and uploaded into PleaseReview™. The working documents are also placed on the WHO Medicines website (https://www.who.int/teams/health-product-and-policy-standards/standards-and-specifications/pharmaceuticals/working-documents-public-consultation) under the "Working documents in public consultation".
If you wish to receive all our draft guidelines during the course of the year, please send your full name, organization/ affiliation, and email address to jonessi@who.int, nsp@who.intand your name will be added to our electronic mailing list and review platform.

SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/22.909

NIRMATRELVIR

(NIRMATRELVIRUM)

Description	Date
Drafting of the monograph based on information received from manufacturers	November/December 2022
Discussion at the Consultation on Quality Control and Pharmacopoeial Specifications of Medicines	April 2023
Draft monograph sent out for public consultation.	TBD
Presentation to the WHO Expert Committee on Specifications for Pharmaceutical Preparations.	TBD
Further follow-up action as required.	

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

Being the first public standard, the monograph on Nirmatrelvir is expected to play an important role in ensuring access to safe, effective and quality assured COVID-19 therapeutics. Manufacturers, regulatory authorities, procurement agencies and other stakeholders are therefore invited to provide their feedback.

The draft monograph is based on information and samples received from manufacturers and on laboratory investigations.

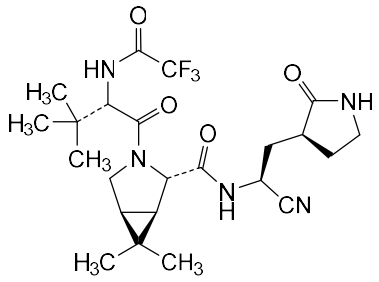
Draft monographs are subject to change.]

NIRMATRELVIR (NIRMATRELVIRUM)

Molecular formula. $C_{23}H_{32}F_3N_5O_4$

Relative molecular mass. 499.54

Graphic formula.



Chemical name. (1R,2S,5S)-N-((1S)-1-Cyano-2-((3S)-2-oxopyrrolidin-3-yl)ethyl)-3-((2S)-3,3-dimethyl-2-(2,2,2-trifluoroacetamido)butanoyl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxamide *(IUPAC);* 3-Azabicyclo[3.1.0]hexane-2-carboxamide, N-[(1S)-1-cyano-2-[(3S)-2-oxo-3-pyrrolidinyl]ethyl]-3-[(2S)-3,3-dimethyl-1-oxo-2-[(2,2,2-trifluoroacetyl)amino]butyl]-6,6-dimethyl-, (1R,2S,5S)- *(CAS)*.

CAS Registry Number. 2628280-40-8

Description. A white to off-white powder.

Solubility. It is freely soluble in dimethyl sulfoxide R and methanol R, soluble in acetonitrile R and dehydrated ethanol R, sparingly soluble in ethyl acetate R, slightly soluble in tert-butyl methyl ether R. It is practically insoluble in water R and heptane R.

Category. Antiviral.

Storage. Nirmatrelvir should be kept in tightly closed containers.

Additional information. Nirmatrelvir exhibits polymorphism.

Requirements

Definition. Nirmatrelvir contains not less than 97.0% and not more than 102.0% of $C_{23}H_{32}F_3N_5O_4$, calculated with reference to the anhydrous substance.

Identity tests

- Either tests A and D or 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 nirmatrelvir RS or with the reference spectrum of nirmatrelvir.

If the spectra thus obtained are not concordant repeat the test using the residues obtained by separately dissolving the test substance and nirmatrelvir RS in a tert-butyl methyl ether R and evaporating to dryness. The infrared absorption spectrum is concordant with the spectrum obtained from nirmatrelvir RS.

B. Carry out the test as described under *1.14.1 Chromatography,* 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 nirmatrelvir in the chromatogram obtained with solution (2).

C. Carry out the test as described under *1.14.1 Chromatography,* Thin-layer chromatography, using silica gel R5 as the coating substance and a freshly prepared mixture of ethyl acetate R and glacial acetic acid R (99:1 *V/V*) as the mobile phase. Apply separately to the plate 5 µL of each of the following two solutions in methanol R, containing (A) 2 mg per mL of the test substance and (B) 2 mg per mL of nirmatrelvir RS. After removing the plate from the chromatographic chamber, allow it to dry in air or in a current of air.

Spray the plate with anisaldehyde/methanol TS and heat it to 105°C for 10 minutes. Allow the plate to cool and examine the chromatogram in daylight.

The principal spot in the chromatogram obtained with solution (A) corresponds in position, appearance and intensity with the spot due to nirmatrelvir in the chromatogram obtained with solution (B).

D. Determine the specific optical rotation using a 10.0 mg per mL solution of the test substance in methanol R. Calculate with reference to the anhydrous substance. The specific optical rotation $[\alpha]_D^{25}$, is between -98.0 to -109.0.

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 5 mg/g.

Sulfated ash (*2.3*)**.** Not more than 1.0 mg/g, determined on 1.000 g.

Heavy metals. Use 1.000 g for the preparation of the test solution as described under *2.2.3 Limit test for heavy metals*, Procedure 3. Determine the heavy metals content according to Method B; not more than 20 µg/g.

Related substances. Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography, using a stainless steel column (2.1 mm x 15 cm) packed with end-capped particles of silica gel, the surface of which has been modified with chemically-bonded phenyl groups (1.8 µm).[1]

Prepare a 0.1% methanesulfonic acid solution by diluting 1.0 mL of methanesulfonic acid R to 1000 mL with water R.

Use the following conditions for gradient elution:

- mobile phase A: 0.1% methanesulfonic acid solution;

[1] An Zorbax RRHD SB-Phenyl column has been found suitable.

- mobile phase B: acetonitrile for chromatography R.

Time (minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–1	95	5	Isocratic
1–5	95 to 78	5 to 22	Linear gradient
5-20	78	22	Isocratic
20–25	78 to 59	22 to 41	Linear gradient
25–35	59 to 30	41 to 70	Linear gradient
35-35.5	30	70	Isocratic
35.5–35.6	30 to 95	70 to 5	Return to initial composition
35.6–40	95	5	Re-equilibration

Operate with a flow rate of 0.475 mL per minute. To avoid excessive system pressure, first equilibrate at a lower flow rate of 0.1 mL/min until the column temperature reaches the set value and then increase the flow rate to 0.475 mL/min. After use, flush the column for at least 1 hour with a mixture of water R and acetonitrile R at room temperature with a flow rate of 0.4 mL/min.

As a detector, use an ultraviolet spectrophotometer set at a wavelength of 205 nm. If configurable, operate with reference wavelength at 400 nm. Maintain the column temperature at 80 °C.

Prepare the following solutions, using as a diluent a mixture of 50 volumes of water R and 50 volumes of acetonitrile R. For solution (1), transfer 55 mg of the test substance into a 50 mL volumetric flask, dissolve in about 30 mL and make up to volume. For solution (2), dilute 1.0 mL of solution (1) to 100.0 mL. For solution (3), dilute 5.0 mL of solution (2) to 100.0 mL. For solution (4), transfer 25 mg of nirmatrelvir RS into a 50 mL volumetric flask, ad 2 mL of sodium hydroxide (~0.4 g/L) TS and mix. After 1 hour, dilute to volume and filter.

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

Use the chromatogram obtained with solution (4) to identify the peaks due to the impurities O, B, I and F.

The impurities are eluted, if present, at the following relative retentions with reference to nirmatrelvir (retention time about 21 minutes): impurity O about 0.29; impurity B about 0.56; impurity I about 0.65; impurity A about 0.83; impurity F about 0.99; impurity J about 1.09; impurity K about 1.18; impurity L about 1.23 and impurity M about 1.31 *[Note from the Secretariat. The relative retention of impurity C will be added at a later stage]*.

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 F and Hv is the height above the baseline of the lowest point of the curve separating this peak from the peak due to nirmatrelvir. Also, the test is not valid unless in the chromatogram obtained with solution (3), the peak due to nirmatrelvir is obtained 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 J is not greater than 0.5 times the area of the peak due to nirmatrelvir in the chromatogram obtained with solution (2) (0.5 %);
- the area of any peak corresponding to impurity M, when multiplied by a correction factor of 1.5, is not greater than 0.3 times the area of the peak due to nirmatrelvir in the chromatogram obtained with solution (2) (0.3 %);
- the area of any peak corresponding to impurity I is not greater than 0.25 times the area of the peak due to nirmatrelvir in the chromatogram obtained with solution (2) (0.25 %);

- the areas of any peak corresponding to impurities A, B, C, F, K, L, N or O are each not greater than 0.15 times the area of the peak due to nirmatrelvir in the chromatogram obtained with solution (2) (0.15 %);
- the area of any other impurity peak is not greater than 0.10 times the area of the peak due to nirmatrelvir in the chromatogram obtained with solution (2) (0.10 %).
- The sum of the areas of all impurity peaks is not greater than twice the area of the peak due to nirmatrelvir in the chromatogram obtained with solution (2) (2.0 %). Disregard all peaks with an area of less than the area of the peak due to nirmatrelvir in the chromatogram obtained with solution (3) (0.05 %).

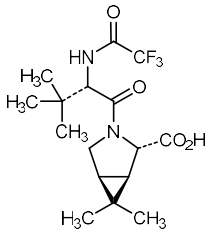
Assay. Carry out the test as described under *1.14.1 Chromatography,* High-performance liquid chromatography, using the conditions given above under "Related substances".

Prepare the following solutions, using as a diluent a mixture of 50 volumes of water R and 50 volumes of acetonitrile R. For solution (1), transfer 55.0 mg of the test substance into a 50 mL volumetric flask, dissolve in about 30 mL and make up to volume. For solution (2), transfer 55.0 mg of Nirmatrelvir RS into a 50 mL volumetric flask, dissolve in about 30 mL and make up to volume.

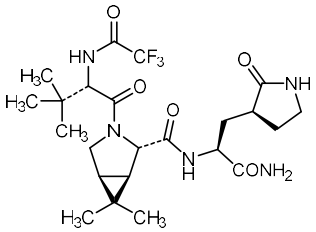
Inject 5 µL each of solutions (1) and (2).

Measure the areas of the peaks corresponding to nirmatrelvir obtained in the chromatograms of solutions (1) and (2) and calculate the percentage content of Nirmatrelvir ($C_{23}H_{32}F_3N_5O_4$) in the sample using the declared content of $C_{23}H_{32}F_3N_5O_4$ in nirmatrelvir RS.

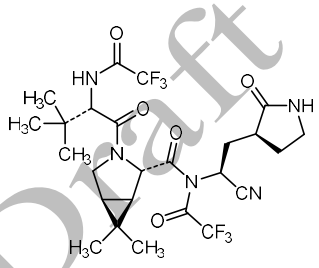
Impurities



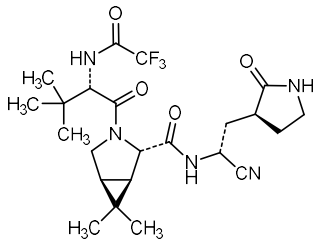
A. (1*R*,2*S*,5*S*)-3-[(2*S*)-3,3-Dimethyl-2-(2,2,2-trifluoroacetamido)butanoyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxylic acid (process related impurity)



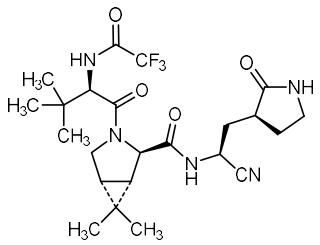
B. (1*R*,2*S*,5*S*)-*N*-{(2*S*)-1-Amino-1-oxo-3-[(3*S*)-2-oxopyrrolidin-3-yl]propan-2-yl}-3-[(2*S*)-3,3-dimethyl-2-(2,2,2-trifluoroacetamido)butanoyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxamide(synthesis related impurity and degradation product),



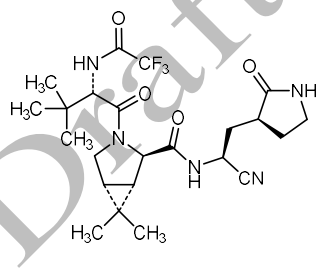
C. (1*R*,2*S*,5*S*)-*N*-{(1*S*)-1-Cyano-2-[(3*S*)-2-oxopyrrolidin-3-yl]ethyl}-3-[(2*S*)-3,3-dimethyl-2-(2,2,2-trifluoroacetamido)butanoyl]-6,6-dimethyl-*N*-(trifluoroacetyl)-3-azabicyclo[3.1.0]hexane-2-carboxamide (synthesis related impurity)



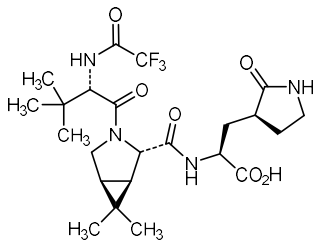
F. (1*R*,2*S*,5*S*)-*N*-{(1*R*)-1-Cyano-2-[(3*S*)-2-oxopyrrolidin-3-yl]ethyl}-3-[(2*S*)-3,3-dimethyl-2-(2,2,2-trifluoroacetamido)butanoyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxamide (epimer of nirmatrelvir)(synthesis related impurity and degradation product),



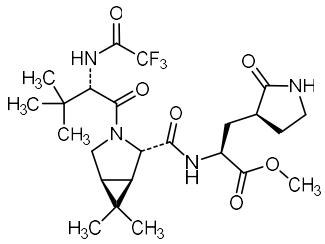
G. (1*S*,2*R*,5*R*)-*N*-{(1*S*)-1-Cyano-2-[(3*S*)-2-oxopyrrolidin-3-yl]ethyl}-3-[(2*R*)-3,3-dimethyl-2-(2,2,2-trifluoroacetamido)butanoyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxamide (diastereomer of nirmatrelvir)(synthesis related impurity)



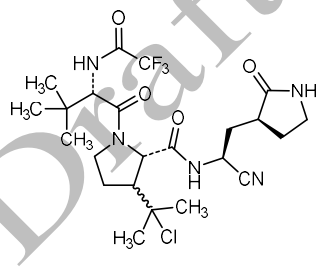
H. (1*S*,2*R*,5*R*)-*N*-{(1*S*)-1-Cyano-2-[(3*S*)-2-oxopyrrolidin-3-yl]ethyl}-3-[(2*S*)-3,3-dimethyl-2-(2,2,2-trifluoroacetamido)butanoyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxamide (diastereomer of nirmatrelvir)(synthesis related impurity)



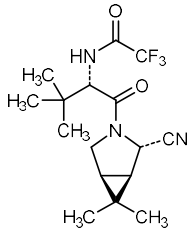
I. (2*S*)-2-({(1*R*,2*S*,5*S*)-3-[(2*S*)-3,3-Dimethyl-2-(2,2,2-trifluoroacetamido)butanoyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carbonyl}amino)-3-[(3*S*)-2-oxopyrrolidin-3-yl]propanoic acid (synthesis related impurity and degradation product),



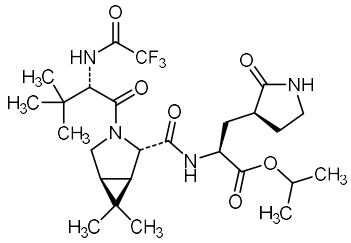
J. Methyl (2*S*)-2-({(1*R*,2*S*,5*S*)-3-[(2*S*)-3,3-dimethyl-2-(2,2,2-trifluoroacetamido)butanoyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carbonyl}amino)-3-[(3*S*)-2-oxopyrrolidin-3-yl]propanoate (synthesis related impurity),



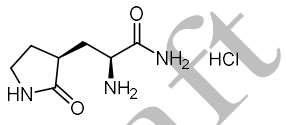
K. (2*S*)-3-(2-Chloropropan-2-yl)-*N*-{(1*S*)-1-cyano-2-[(3*S*)-2-oxopyrrolidin-3-yl]ethyl}-1-[(2*S*)-3,3-dimethyl-2-(2,2,2-trifluoroacetamido)butanoyl]pyrrolidine-2-carboxamide (synthesis related impurity),



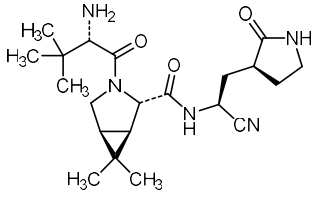
L. *N*-{(2*S*)-1-[(1*R*,2*S*,5*S*)-2-Cyano-6,6-dimethyl-3-azabicyclo[3.1.0]hexan-3-yl]-3,3-dimethyl-1-oxobutan-2-yl}-2,2,2-trifluoroacetamide (synthesis related impurity),



M. Propan-2-yl (2*S*)-2-({(1*R*,2*S*,5*S*)-3-[(2*S*)-3,3-dimethyl-2-(2,2,2-trifluoroacetamido)butanoyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carbonyl}amino)-3-[(3*S*)-2-oxopyrrolidin-3-yl]propanoate (synthesis related impurity),



N. 3-[(3*S*)-2-Oxopyrrolidin-3-yl]-L-alaninamide hydrochloride (1:1) *[**Note from the Secretariat**. In the final version of the monograph, HCl will be removed from structure and the chemical name],*



O. (1*R*,2*S*,5*S*)-*N*-{(1*S*)-1-Cyano-2-[(3*S*)-2-oxopyrrolidin-3-yl]ethyl}-6,6-dimethyl-3-(3-methyl-L-valyl)-3-azabicyclo[3.1.0]hexane-2-carboxamide(degradation product).

Reference substances to be established

Nirmatrelvir RS

- *New International Chemical Reference Substance to be established.*

Reagents to be added

Sodium hydroxide (~0.4 g/L) TS

- A solution of sodium hydroxide R containing about 4 g/L of NaOH (approximately 0.01 mol/L).

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