Nirmatrelvir tablets (Nirmatrelviri compressi)

2025-01

Category. Antiviral.

Storage. Nirmatrelvir tablets should be kept in tightly closed containers protected from light.

Additional information. Nirmatrelvir 150 mg tablets (co-packaged with Ritonavir 100 mg tablets) are listed on the 8th Invitation to Manufacturers of therapeutics against COVID-19 to submit an Expression of Interest (EOI) for Product Evaluation to the WHO Prequalification Unit.

Requirements

Complies with the monograph for *Tablets*.

Definition. Nirmatrelvir tablets contain Nirmatrelvir. They contain not less than 90.0% and not more than 110.0% of the amount of Nirmatrelvir ($C_{23}H_{32}F_3N_5O_4$), stated on the label.

Identity tests

-Test A and test B may be applied.

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).

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. For solution (A), transfer a quantity of the powdered tablets, nominally containing 50 mg of Nirmatrelvir into a 25 mL volumetric flask. Add about 20 mL, sonicate for 10 minutes with intermediate shaking, allow to cool to room temperature and make up to volume, mix and filter. For solution (B), use a solution containing 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).

Dissolution. Carry out the test described under *5.5 Dissolution test for oral dosage forms*, using as the dissolution medium 900 mL of dissolution buffer, pH 6.8, 0.2% SDS and rotating the paddle at 75 revolutions per minute.

For the dissolution buffer, pH 6.8, containing 0.2% SDS, transfer 19 g of trisodium orthophosphate R and 6.2 mL of hydrochloric acid (~420 g/L) TS into a 1000 mL flask, add 900 mL of water R, mix, and adjust to a pH of 6.8 with sodium hydroxide (~80g/L) TS. Add 2.0 g of sodium dodecyl sulfate R, mix, and dilute to 1000 ml with water R.

At 30 minutes, withdraw a sample of 10 mL of the medium through an in-line filter. Allow the filtered sample to cool to room temperature and use it as solution (1). For solution (2), transfer 34.0 mg of nirmatrelvir RS into a 200 mL volumetric flask, dissolve in about 60 mL of dissolution medium and make up to volume with the same solvent.

Determine the amount of Nirmatrelvir released as described under *1.14.1 Chromatography*, High-performance liquid chromatography, using a stainless steel column (10 cm x 4.6 mm) packed with base-deactivated particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (3.5 µm).

Use the following conditions for gradient elution:

-mobile phase A: 0.1 % (*V/V*) solution of perchloric acid (~1170 g/L) TS;

-mobile phase B: acetonitrile for chromatography R.

Time (minutes)	Mobile phase A (% *V/V*)	Mobile phase B (% *V/V*)	Comments
0–5.5	72 to 28	28 to 72	Linear gradient
5.5–5.6	28 to 72	72 to 28	Return to initial composition

5.6–8.0	72	28	Re-equilibration

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 205 nm. Maintain the column temperature at 75 °C.

Inject 15 µL each of solution (1) and (2) and record the chromatograms.

Measure the areas of the peaks corresponding to nirmatrelvir obtained in the chromatograms of solution (1) and (2).

For each of the tablets tested, calculate the total amount of nirmatrelvir ($C_{23}H_{32}F_3N_5O_4$) in the medium, using the declared content of ($C_{23}H_{32}F_3N_5O_4$) in nirmatrelvir RS.

Evaluate the results as described under *5.5 Dissolution test for solid oral dosage forms*, Acceptance criteria. The amount of nirmatrelvir released is not less than 80% (Q) of the amount declared on the label.

Related substances. Carry out the test as described under *1.14.1 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).

Prepare a 0.1% methanesulfonic acid solutionby 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,

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
35.6–42	95	5	Re-equilibration

Operate with a flow rate of 0.48 mL per minute. Maintain the column temperature at 80 °C. 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 0.48 mL/min. After use, flush the column after use for at least 1 hour with a mixture of water R and acetonitrile R (50:50 *V/V*) 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 .

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 a quantity of the powdered tablets, nominally containing 375.0 mg of Nirmatrelvir, into a 250 mL volumetric flask. Add about 150 ml of diluent and stir for 30 minutes. Dilute to volume and mix. Allow the mixture to settle for about 10 minutes, dilute 10.0 mL to 25.0 mL and filter. 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 50.0 mL. For solution (4), transfer 25 mg of nirmatrelvir RS into a 50 mL volumetric flask, add 5 mL of diluent and 2 mL of sodium hydroxide (~0.4 g/L) TS and mix. After 2 hour, dilute to volume with a mixture of phosphate buffer, pH 7.0, TS and acetonitrile R, 50:50 (*V/V*), mix and filter. For solution (5), use the diluent.

Fill the solutions in polypropylene vials because degradation may occur with glass vials.

Inject 3 µL (if a 10 mm flow cell is used) or 5 µL (if a 6 mm flow cell is used)each of solutions (1), (2), (3), (4) and (5).

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

Use the chromatogram obtained with solution (5) to identify interferences from the blank and system peaks.

The impurities are eluted, if present, at the following relative retentions with reference to nirmatrelvir (retention time about 21 minutes): impurity N about 0.04; impurity O about 0.29; impurity B about 0.56; impurity I about 0.65; impurity A about 0.83; impurity G about 0.92; impurity H about 0.92; impurity Q about 0.92 (impurities G, H and Q co-elute); impurity F about 0.98; impurity J about 1.09; impurity K about 1.18; impurity L about 1.23; impurity M about 1.34; impurity C about 1.41. The excipient sodium stearyl fumarate, if present in the sample, is eluted at a relative retention of about 1.65.

The test is not valid unless in this chromatogram obtained with solution (4) the peak-to-valley ration (Hp/Hv) is at least 2.0, 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, 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 0.3 times the area of the peak due to nirmatrelvir in the chromatogram obtained with solution (2) (0.3 %);

the area of any other impurity peak is not greater than 0.2 times the area of the peak due to nirmatrelvir in the chromatogram obtained with solution (2) (0.2 %).

The sum of the areas of all impurity peaks is not greater than three times the area of the peak due to nirmatrelvir in the chromatogram obtained with solution (2) (3.0 %). Disregard all peaks with an area less than the area of the peak due to nirmatrelvir in the chromatogram obtained with solution (3) (0.1 %).

Assay. Determine the content 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), weigh and powder 20 tablets. Transfer a quantity of the powdered tablets, nominally containing 625.0 mg of Nirmatrelvir, into a 500 mL volumetric flask. Add about 250 ml of diluent and stir for 30 minutes. Dilute to volume and mix. Allow the mixture to settle for about 10 minutes, dilute 10.0 mL to 25.0 mL and filter. For solution (2), transfer 60.0 mg of nirmatrelvir RS into a 100 mL volumetric flask, dissolve in about 30 mL of diluent and make up to volume.

Inject 3 µL (if a 10 mm flow cell is used) or 5 µL (if a 6 mm flow cell is used) 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 tablets, using the declared content of $C_{23}H_{32}F_3N_5O_4$ in nirmatrelvir RS.

Impurities

The impurities limited by the requirements of this monograph include those listed in the monograph on Nirmatrelvir.