World Health Organization

ZANAMIVIR

(ZANAMIVIRUM)

Draft proposal for inclusion for *The International Pharmacopoeia*

(July 2020)

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) by **14 September 2020**.

Working documents are sent out electronically and they will also be placed on the WHO Medicines website (http://www.who.int/medicines/areas/quality_safety/quality_assurance/guidelines/en/) for comments under the "Current projects" 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.

SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/20.834:

ZANAMIVIR

(ZANAMIVIRUM)

Description	**Date**
Monograph drafted based on information received from a manufacturer and on laboratory investigations.	February 2020
Discussion at the consultation on Screening Technologies, Laboratory Tools and Pharmacopoeial Specifications for Medicines.	May 2020
Draft monograph sent out for public consultation.	July-September 2020
Presentation to the 55th WHO Expert Committee on Specifications for Pharmaceutical Preparations.	October 2020
Further follow-up action as required.	

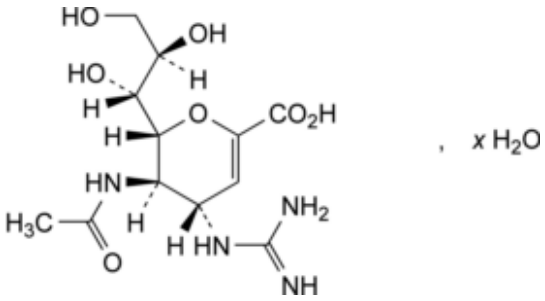
*[**Note from the Secretariat.** It is proposed to include the monograph on Zanamivir in* The International Pharmacopoeia. *The monograph is based on a submission by a manufacturer and on laboratory investigations.]*

ZANAMIVIR (ZANAMIVIRUM)

Molecular formula. $C_{12}H_{20}N_4O_7,xH_2O$

Relative molecular mass. 332.3 (anhydrous substance).

Graphic formula.



Chemical name. (2*R*,3*R*,4*S*)-3-Acetamido-4-carbamimidamido-2-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-3,4-dihydro-2*H*-pyran-6-carboxylic acid hydrate; CAS Reg. No. 551942-41-7.

Description. A white, or almost white, slightly hygroscopic powder.

Solubility. Slightly soluble in water R, practically insoluble in ethanol (~750 g/L) TS and dichloromethane R.

Category. Antiviral.

Storage. Zanamivir should be kept in tightly closed containers, protected from light.

Labelling. The designation on the container should state that the substance is in the form of the hydrate.

Additional information. Zanamivir may exhibit polymorphism.

Requirements

Definition. Zanamivir contains not less than 97.0% and not more than 102.0% of $C_{12}H_{20}N_4O_7$, calculated with reference to the anhydrous and solvent-free substance.

Identity tests

- Either test A or test B 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 zanamivir RS or with the reference spectrum of zanamivir.

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

B. Carry out test B.1 or, where a diode array detector is available, test B.2.

B.1 Carry out the test as described under *1.14.4 High-performance liquid chromatography u*sing 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 zanamivir in the chromatogram obtained with solution (2).

The absorption spectrum (*1.6*) of a 6 µg per mL solution of the test substance in phosphate buffer, pH 7.4, TS, when observed between 200 nm and 400 nm, exhibits a maximum at 260 nm.

B.2 Carry out the test as described under *1.14.4 High-performance liquid chromatography* using the conditions given under "Assay". 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 retention time and the UV

spectrum of the principal peak in the chromatogram obtained with solution (1) correspond to the retention time and UV spectrum of the peak due to zanamivir in the chromatogram obtained with solution (2).

Specific optical rotation (*1.4*). Dissolve 0.250 g in 25.0 mL of water R; sonicate until the substance is dissolved. Calculate with reference to the anhydrous and solvent-free substance; the specific optical rotation is between +36.0 to +38.5.

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. The water content is not less than 40 mg/g and not more than 90 mg/g.

Heavy metals. Use 1.0 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 B; not more than 20 µg/g.

Related substances. Carry out the test as described under *1.14.4 High-performance liquid chromatography*, using a stainless steel column (25 cm x 4.6 mm) packed with particles of cross-linked polyvinyl alcohol polymer with chemically bonded polyamine (5 µm).[1]

As the mobile phase, use a mixture of 60 volumes of acetonitrile R and 40 volumes of a 0.7 g/L solution of sulfuric acid (~1760 g/L) TS previously adjusted to pH 5.5 with ammonia (~1.7 g/L) TS.

Operate with a flow rate of 1.5 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 234 nm and, for impurity I, at 210 nm. For identity test B.2, use a diode array detector in the range of 200 nm to 400 nm. Maintain the column temperature at 30 °C. Prior to first use, rinse the column with

[1]An Asahipak NH2P-50 column has been found suitable.

a 0.7 g/L solution of ammonium sulfate R at 1.5 mL per minute at 30 °C for about 1 hour. Prior to each use, rinse with the mobile phase for at least 8 hours.

Prepare the following solutions. For solution (1), dissolve 23.0 mg of the test substance in 20 mL of water R and dilute to 50.0 mL with acetonitrile R. For solution (2), dilute 1.0 mL of test solution (1) to 100.0 mL with mobile phase. Dilute 1.0 mL or this solution to 10.0 mL with mobile phase. For solution (3), dissolve 5 mg of zanamivir for system suitability RS (containing zanamivir and the impurities A, B, C and E) in 6 mL of water R and dilute to 10 mL with acetonitrile R. For solution (4), dissolve 3.00 mg of zanamivir impurity F RS in mobile phase and dilute to 100.0 mL with mobile phase. Dilute 1.0 mL of this solution to 100.0 mL with mobile phase. Dilute 3.0 mL of this solution to 20.0 mL with mobile phase. For solution (5), dissolve 10 mg of imidazole R in 40 mL of water R and dilute to 100 mL with acetonitrile R. Dilute 1.0 mL of this solution to 100.0 mL with mobile phase.

Inject alternately 20 µL of solutions (1), (2), (3), (4) and (5) and record the chromatogram for 3 times the retention time of zanamivir.

Use the chromatogram obtained with solution (3) to identify the peaks due to the impurities A, B, C and E. Use the chromatogram obtained with solution (4) to identify the peak due to impurity F. Use the chromatogram obtained with solution (5) to identify the peak due to impurity I.

The impurities are eluted, if present, at the following relative retention with reference to zanamivir (retention time about 9 minutes): impurity I about 0.26; impurity F about 0.30; impurity B about 0.60; impurity D about 0.71; impurity C about 0.77; impurity E about 0.83; impurity H 1.14; and impurity A about 2.75.

The test is not valid unless, in the chromatogram obtained with solution (3), the peak-to-valley ratio (Hp/Hv) is at least 2.5, where Hp is the height above the baseline of the peak due to impurity E, and Hv is the height above the baseline of the lowest point of

the curve separating this peak from the peak due to impurity C. Also, the test is not valid unless, in the chromatogram obtained with solution (4), the peak due to impurity F is obtained with a signal-to-noise ratio of at least 10.

Measure the areas of the peaks corresponding to the impurities in the chromatograms obtained with solutions (1) and (4) and the area of zanamivir in the chromatogram obtained with solution (2).

Determine the percentage content of impurity F, considering the concentration of impurity F in solution (4) and the declared content of impurity F in zanamivir impurity F RS.

- The percentage content of impurity F is not greater than 0.01%.

For impurities other than impurity F, compare the peak areas of the impurities with the peak areas of zanamivir obtained with solution (2).

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity A is not greater than 5 times the area of the peak due to zanamivir in the chromatogram obtained with solution (2) (0.5 %);
- the area of any peak corresponding to impurity B is not greater than 3 times the area of the peak due to zanamivir in the chromatogram obtained with solution (2) (0.3 %);
- the area of any peaks corresponding to impurity E, when multiplied with a correction factor of 0.63, is not greater than 2 times the area of the peak due to zanamivir in the chromatogram obtained with solution (2) (0.2 %);
- the areas of any peaks corresponding to impurity C or D is not greater than 2 times the area of the peak due to zanamivir in the chromatogram obtained with solution (2) (0.2 %);

- the area of any peak corresponding to impurity I, recorded at 210 nm, when multiplied by a correction factor of 0.4, is not greater than the area of the peak due to zanamivir in the chromatogram obtained with solution (2), recorded at 210 nm (0.1 %);
- the area of any other impurity peak is not greater than the area of the peak due to zanamivir in the chromatogram obtained with solution (2) (0.10 %).
- Determine the sum of the areas of all impurity peaks recorded at 234 nm, other than any peak corresponding to impurity I and including the corrected area of any peak corresponding to impurity E. Disregard all peaks with an area of less than 0.5 times the area of the peak due to zanamivir in the chromatogram obtained with solution (2) (0.05%). Calculate the percentage concentration of these impurities using the area of any peak corresponding to zanamivir in the chromatogram obtained with solution (2) as a reference. Calculate the percentage concentration of impurity I using the area of any peak corresponding to zanamivir in the chromatogram obtained with solution (2) and recorded at 210 nm as a reference. Add the percentage concentration of impurity I to the percentage concentration of all other impurities. Disregard impurity I if the percentage concentration is less than 0.05%. The percentage concentration of all impurities is not greater than 1.2%.

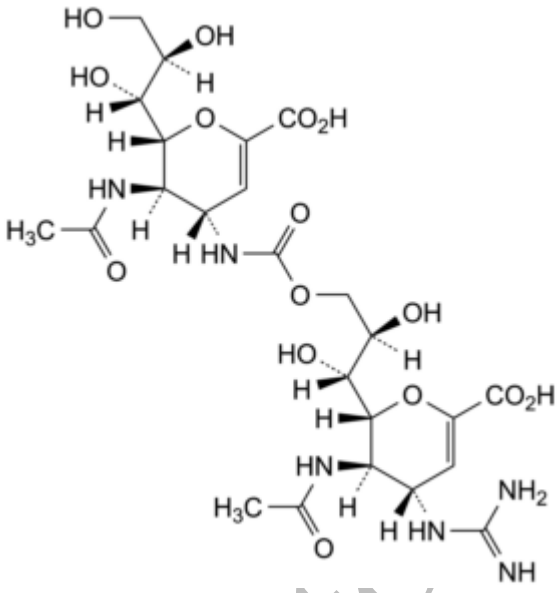
Assay. Carry out the test as described under *1.14.4 High-performance liquid chromatography*, using the conditions given above under "Related substances" with the following modifications.

Prepare the following solutions. For solution (1), dissolve 23.0 mg of the test substance in 20 mL of water R and dilute to 50.0 mL with acetonitrile R. Dilute 5.0 mL of this solution to 50.0 mL with mobile phase. For solution (2), dissolve 23.0 mg of zanamivir RS in 20 mL of water R and dilute to 50.0 mL with acetonitrile R. Dilute 5.0 mL of this solution to 50.0 mL with mobile phase.

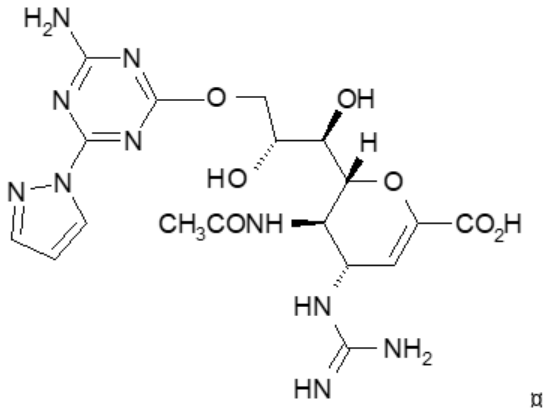
Inject alternately 20 µL of solutions (1) and (2) and record the chromatogram for 3 times the retention time of zanamivir.

Measure the areas of the peaks corresponding to zanamivir obtained in the chromatograms of solutions (1) and (2) and calculate the percentage content of $C_{12}H_{20}N_4O_7$ in the sample using the declared content of $C_{12}H_{20}N_4O_7$ in zanamivir RS.

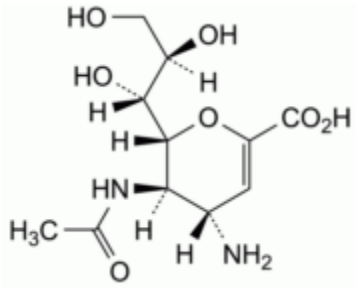
Impurities



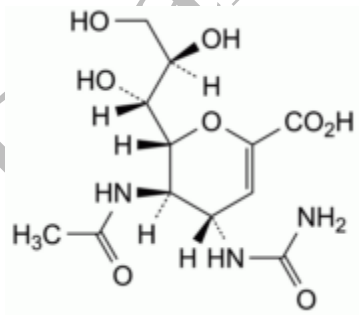
A. (2*R*,3*R*,4*S*)-3-acetamido-2-[(1*R*,2*R*)-3-[[[(2*R*,3*R*,4*S*)-3-acetamido-6-carboxy-2-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-3,4-dihydro-2*H*-pyran-4-yl]carbamoyl]oxy]-1,2-dihydroxypropyl]-4-carbamimidamido-3,4-dihydro-2*H*-pyran-6-carboxylic acid (zanamivir dimer)(synthesis-related impurity).



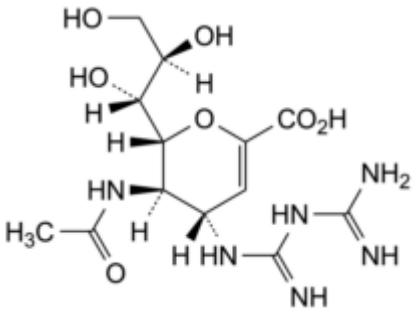
B. 5-Acetamido-9-O-[4-amino-6-(1H-pyrazol-1-yl)-1,3,5-triazin-2-yl]-2,6-anhydro-3,4,5-trideoxy-4-guanidino-d-glycero-d-galacto-non-2-enonic acid (O-triazinyl zanamivir) (synthesis-related impurity).



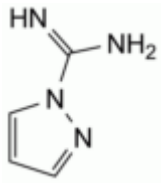
C. (2*R*,3*R*,4*S*)-3-acetamido-4-amino-2-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-3,4-dihydro-2*H*-pyran-6-carboxylic acid (4-amino zanamivir) (synthesis-related impurity).



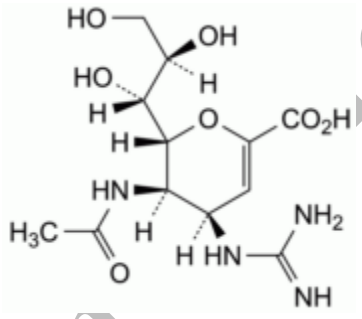
D. (2*R*,3*R*,4*S*)-3-acetamido-4-(carbamoylamino)-2-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-3,4-dihydro-2*H*-pyran-6-carboxylic acid (zanamivir urea analog) (synthesis-related impurity).



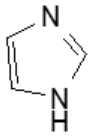
E. (2*R*,3*R*,4*S*)-3-acetamido-4-(*N*′-carbamimidoylcarbamimidamido)-2-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-3,4-dihydro-2*H*-pyran-6-carboxylic acid (4-biguanide zanamivir) (synthesis-related impurity).



F. 1*H*-pyrazole-1-carboximidamide (synthesis-related impurity).



H. (2*R*,3*R*,4*R*)-3-acetamido-4-carbamimidamido-2-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-3,4-dihydro-2*H*-pyran-6-carboxylic acid (*talo*-zanamivir) (synthesis-related impurity).



I. imidazole (synthesis-related impurity).

Reference substances to be established

Zanamivir for system suitability RS (containing zanamivir and the impurities A, B, C and E)

- It is intended to refer to the corresponding reference substance established by the European Pharmacopoeia.

Zanamivir impurity F

- It is intended to refer to the corresponding reference substance established by the European Pharmacopoeia.

Zanamivir RS

- International Chemical Reference Substance to be established.

Reagents to be established

Ammonia (~1.7 g/L) TS

Ammonia (~17 g/L) TS, diluted to contain about 1.7 g of NH_3 per litre (approximately 0.1 mol/L).

***