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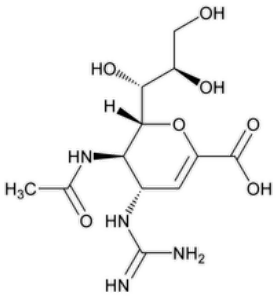
Zanamivir



$C_{12}H_{20}N_4O_7$ 332.31

D-*glycero*-D-*galacto*-Non-2-enonic acid, 5-(acetylamino)-4-[(aminoiminomethyl)amino]-2,6-anhydro-3,4,5-trideoxy-;

5-Acetamido-2,6-anhydro-3,4,5-trideoxy-4-guanidino-D-*glycero*-D-*galacto*-non-2-enonic acid CAS RN®: 139110-80-8; UNII: L6O3XI777I.

DEFINITION

Zanamivir contains NLT 98.0% and NMT 102.0% of zanamivir ($C_{12}H_{20}N_4O_7$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

• **A.** ▲**Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197K or 197M▲ (CN 1-May-2020)

Wavenumber range: 4000 cm^{-1} to 400 cm^{-1}

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Mobile phase: Acetonitrile and 7.5 mM sulfuric acid (60:40). Adjust with ammonia TS to a pH of 6.2.

Resolution solution: Prepare 2.5 µg/mL of *talo*-zanamivir and 0.05 mg/mL of zanamivir from USP Zanamivir Resolution Mixture RS in *Mobile phase*.

Standard solution: 0.045 mg/mL of USP Zanamivir RS in *Mobile phase*

Sample solution: 0.045 mg/mL of Zanamivir in *Mobile phase*

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 234 nm

Column: 4.6-mm × 25-cm; 5-µm packing L82

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *Resolution solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between *talo*-zanamivir and zanamivir, *Resolution solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of zanamivir ($C_{12}H_{20}N_4O_7$) in the portion of Zanamivir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Zanamivir RS in the *Standard solution* (mg/mL)

C_U = concentration of Zanamivir in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• **Organic Impurities**

Mobile phase: Proceed as directed in the *Assay*.

System suitability solution: Prepare 0.45 mg/mL of zanamivir and 9 µg/mL each of imidazole, imidazole carboximidamide, zanamivir urea analog, 4-amino zanamivir, 4-biguanide zanamivir, and *talo*-zanamivir from USP Zanamivir Related Compounds Mixture RS in *Mobile phase*.

Sample solution: 0.45 mg/mL of Zanamivir prepared as follows. Dissolve the sample using 40% of the final volume with water, and dilute with acetonitrile to volume.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 210 nm and 234 nm

Column: 4.6-mm × 25-cm; 5-µm packing L82

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.5 between the peaks of *talo*-zanamivir and zanamivir at 234 nm

Analysis

Sample: *Sample solution*

Calculate the percentage of imidazole in the portion of Zanamivir taken:

$$\text{Result} = [(r_i/F)/(r_i/F + r_z)] \times 100$$

r_i = peak response of imidazole at 210 nm

F = relative response factor for imidazole (see *Table 1*)

r_z = peak response of zanamivir at 210 nm

Calculate the percentage of imidazole carboximidamide and 4-biguanide zanamivir in the portion of Zanamivir taken:

$$\text{Result} = [(r_i/F)/(r_i/F + r_z)] \times 100$$

r_i = peak response of imidazole carboximidamide or 4-biguanide zanamivir at 234 nm

F = relative response factor for imidazole carboximidamide or 4-biguanide zanamivir (see *Table 1*)

r_z = sum of the responses of all the peaks including the zanamivir peak at 234 nm

Calculate the percentage of *O*-triazinyl zanamivir, zanamivir urea analog, 4-amino zanamivir, *talo*-zanamivir, zanamivir dimer, and any other unspecified impurity in the portion of Zanamivir taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of *O*-triazinyl zanamivir, zanamivir urea analog, 4-amino zanamivir, *talo*-zanamivir, zanamivir dimer, or any other unspecified impurity at 234 nm

r_T = sum of the responses of all the peaks including the zanamivir peak at 234 nm

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Imidazole[a]	0.26	2.5	—
Imidazole carboximidamide[b]	0.30	3.3	0.01
O-Triazinyl zanamivir[c]	0.60	—	0.3
Zanamivir urea analog[d]	0.70	—	0.2
4-Amino zanamivir[e]	0.77	—	0.2
4-Biguanide zanamivir[f]	0.83	1.6	0.2
Zanamivir	1.00	—	—
talo-Zanamivir[g]	1.14	—	—
Zanamivir dimer[h]	2.75	—	0.5
Any other unspecified impurity	—	—	0.1
Total impurities	—	—	1.2

[a] 1*H*-Imidazole (No individual limit. Included in the determination of total impurities.)

[b] 1*H*-Imidazole-1-carboximidamide.

[c] 5-Acetamido-9-*O*-[4-amino-6-(1*H*-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.

[d] 5-Acetamido-9-*O*-[4-amino-6-(1*H*-pyrazol-1-yl)-1,3,5-triazin-2-yl]-2,6-anhydro-3,4,5-trideoxy-4-ureido-D-*glycero*-D-*galacto*-non-2-enonic acid.

[e] 5-Acetamido-9-*O*-[4-amino-6-(1*H*-pyrazol-1-yl)-1,3,5-triazin-2-yl]-2,6-anhydro-3,4,5-trideoxy-4-amino-D-*glycero*-D-*galacto*-non-2-enonic acid.

[f] 5-Acetamido-9-*O*-[4-amino-6-(1*H*-pyrazol-1-yl)-1,3,5-triazin-2-yl]-2,6-anhydro-3,4,5-trideoxy-4-(1-biguanidyl)-D-*glycero*-D-*galacto*-non-2-enonic acid.

[g] 5-Acetamido-2,6-anhydro-3,4,5-trideoxy-4-guanidino-D-*glycero*-D-*talo*-non-2-enonic acid (No individual limit. Included in the determination of total impurities.)

[h] 4,4'-(2-Amino-4-oxo-1,3,5-triazapent-2-ene-1,5-diyl) bis(5-acetamido-2,6-anhydro-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid.)

SPECIFIC TESTS

- **Optical Rotation,** *Specific Rotation*⟨781S⟩

  Sample solution: 10 mg/mL in water

  Acceptance criteria: +36.0° to +38.0°, measured at 20°, determined on the anhydrous and solvent-free basis
- **Water Determination,** *Method Ic*⟨921⟩**:** 4.0%–9.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Store in a well-closed container at controlled room temperature.
- **USP Reference Standards** ⟨11⟩

  USP Zanamivir RS

  USP Zanamivir Related Compounds Mixture RS

This Reference Standard is a mixture of zanamivir, imidazole, imidazole carboximidamide, zanamivir urea analog, 4-amino zanamivir, 4-biguanide zanamivir, *talo*-zanamivir, and zanamivir dimer. The chemical names are given below:

Imidazole: 1*H*-Imidazole.

Imidazole carboximidamide: 1*H*-Imidazole-1-carboximidamide.
Zanamivir urea analog: 5-Acetamido-9-*O*-[4-amino-6-(1*H*-pyrazol-1-yl)-1,3,5-triazin-2-yl]-2,6-anhydro-3,4,5-trideoxy-4-ureido-D-*glycero*-D-*galacto*-non-2-enonic acid.
4-Amino zanamivir: 5-Acetamido-9-*O*-[4-amino-6-(1*H*-pyrazol-1-yl)-1,3,5-triazin-2-yl]-2,6-anhydro-3,4,5-trideoxy-4-amino-D-*glycero*-D-*galacto*-non-2-enonic acid.
4-Biguanide zanamivir: 5-Acetamido-9-*O*-[4-amino-6-(1*H*-pyrazol-1-yl)-1,3,5-triazin-2-yl]-2,6-anhydro-3,4,5-trideoxy-4-(1-biguanidyl)-D-*glycero*-D-*galacto*-non-2-enonic acid.
talo-Zanamivir: 5-Acetamido-2,6-anhydro-3,4,5-trideoxy-4-guanidino-D-*glycero*-D-*talo*-non-2-enonic acid.
Zanamivir dimer: 4,4'-(2-Amino-4-oxo-1,3,5-triazapent-2-ene-1,5-diyl) bis(5-acetamido-2,6-anhydro-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid.)

USP Zanamivir Resolution Mixture RS

This Reference Standard is a mixture of zanamivir and *talo*-zanamivir.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
ZANAMIVIR	Documentary Standards Support	SM12020 Small Molecules 1
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

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