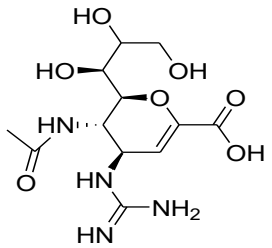
Zanamivir



, x H_2O

$C_{12}H_{20}N_4O_7$, xH_2O Mol. Wt. 332.3 (anhydrous form)

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

Zanamivir contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{12}H_{20}N_4O_7$, calculated on the anhydrous basis.

Category. Neuraminidase inhibitor.

Description. A white crystalline powder, it contains variable quantities of water.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *zanamivir IPRS* or with the reference spectrum of zanamivir.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak chromatogram obtained with reference solution (a).

Tests

Specific optical rotation (2.4.22). + 36.0° to + 38.0°, determined in 1.0 per cent w/v solution at 20º.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 45 mg of the substance under examination in 40 ml of *water* and dilute to 100.0 ml with *acetonitrile*.

Reference solution. A solution containing 0.045 per cent w/v of *zanamivir IPRS* and 0.0009 per cent w/v of *talo-zanamivir IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with polyamine bonded to cross linked polyvinyl alcohol polymer (5 µm) (Such as Shodex Asahipak NH2P-50),
- mobile phase: a mixture of 60 volumes of *acetonitrile* and 40 volumes of *0.0075M sulphuric acid*, adjusted to pH 6.2 with *ammonia*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 210 nm and 234 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Imidazole[1]	0.26	0.4
Imidazole carboximidamide[2]	0.30	0.3
O-Triazinyl zanamivir[3]	0.60	--
Zanamivir urea analog[4]	0.70	--
4-Amino zanamivir[5]	0.77	--
4-Biguanide zanamivir[6]	0.83	0.63
Zanamivir	1.0	--
talo-Zanamivir[7]	1.14	--
Zanamivir dimer[8]	2.75	--

[1] 1*H*-imidazole (No individual limit, included in the determination of total impurities),
[2] 1*H*- imidazole -1-carboximidamide,
[3] 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,
[4] 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,

[5]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,
[6]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-(1-biguanidyl)-D-*glycero*-D-*galacto*-non-2-enonic acid,
[7]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),
[8]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.

Inject the reference solution at 234 nm. The test is not valid unless the resolution between the peaks due to talo-zanamivir and zanamivir is not less than 1.5.

Inject the test solution at 210 nm. Calculate the percentage of imidazole impurity by using following expression.

$$\frac{A_I \times C}{A_I \times C + A_Z} \times 100$$

Where, A_I = peak response of imidazole,
A_Z = peak response of zanamivir,
C = correction factor.

Inject the test solution at 234 nm. The area of any peak corresponding to imidazole carboximidamide is not more than 0.01 per cent and the area of any peak corresponding to 4-biguanide zanamivir is not more than 0.2 per cent, calculated by using the expression.

$$\frac{A_C \times C}{A_C \times C + A_Z} \times 100$$

Where, A_C = peak response of imidazole carboximidamide or 4-biguanide zanamivir,
A_Z = sum of the responses of all the peaks including the zanamivir,
C = correction factor for imidazole carboximidamide or 4-biguanide zanamivir.

In the chromatogram obtained with the test solution at 234 nm, the area of any peak corresponding to *O*-triazinyl zanamivir is not more than 0.3 per cent, the area of any peak corresponding to zanamivir urea analog and 4-amino zanamivir, each of, is not more than 0.2 per cent, the area of any peak corresponding to zanamivir dimer is not more than 0.5 per cent and the area of any other secondary peak is not more than 0.1 per cent, calculated by area normalization. The sum of all the impurities is not more than 1.2 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 4.0 per cent to 9.0 per cent, using method 3.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 45 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (a). A 0.0045 per cent w/v solution of *zanamivir IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.00025 per cent w/v of *talo-zanamivir IPRS,* 0.005 per cent w/v *zanamivir IPRS* in the mobile phase.

Use chromatographic system as described under Related substances with the following modification.
– spectrophotometer set at 234 nm,

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to talo-zanamivir and zanamivir is not less than 1.5 in the chromatogram obtained with reference solution (b) and the relative standard deviation for replicate injections is not more than 1.5 in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the content of $C_{12}H_{20}N_4O_7$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

2.4.26. Solubility.

Zanamivir. Sparingly soluble in *water,* insoluble in *acetone*, in *acetonitrile*, in *ethanol* and in *isopropyl alcohol.*