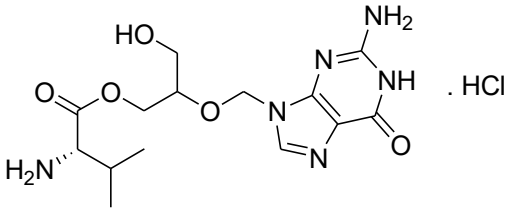
Valganciclovir Hydrochloride



$C_{14}H_{22}N_6O_5$,HCl Mol. Wt. 390.8

Valganciclovir Hydrochloride is L-Valine, ester with 9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]guanine, monohydrochloride.

Valganciclovir Hydrochloride contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{14}H_{22}N_6O_5$,HCl, calculated on the anhydrous and solvent free basis.

Category. Antiviral

Description. A white to off-white powder.

Identification

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

B. When examined in the range of 200 nm to 400 nm (2.4.7), a 0.001 per cent w/v solution in *methanol,* shows absorption maxima at the same wavelength as obtained with the same concentration of the reference solution.

C. A 5.0 per cent w/v solution gives reaction (A) of chlorides (2.3.1).

Tests

Limit of isopropyl alcohol. Not more than 1.0 per cent.

Determine by gas chromatography (2.4.13).

Internal standard solution. A 0.1 per cent v/v solution of *1,4-dioxane* in *dimethylformamide.*

Test solution. Dissolve 100 mg of the substance under examination in 2.0 ml of internal standard solution.

Reference solution (a). Dilute 1.0 ml of *isopropyl alcohol* and 0.1 ml of *toluene* to 100.0 ml with *dimethylformamide.*

Reference solution (b). Dilute 0.1 ml of reference solution (a) to 2.0 ml with the internal standard solution.

Chromatographic system

- a capillary column 30 m x 0.53 mm, coated with 6 per cent cyanopropylphenyl- 94 per cent dimethylpolysiloxane (film thickness 3.0 µm) (Such as DB-624),
- temperature:
  column. 40° for 10 minutes, 40° to 240° @ 25° per minute and hold at 240° for 15 minutes,
- inlet port at 250° and detector at 300°,
- flame ionization detector,
- split ratio :1:15,
- flow rate: 10.5 ml per minute, using nitrogen as the carrier gas.
- injection volume: 0.5 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to 1,4-dioxane and toluene is not less than 8.0, the column efficiency is not less than 6000 theoretical plates for 1,4-dioxane peak and the relative standard deviation of peak area ratios of isopropyl alcohol to the 1,4-dioxane for replicate injections is not more than 15.0 per cent.

Inject reference (b) solution and the test solution.

Calculate the content of isopropyl alcohol using ratio of peak area of isopropyl alcohol to that of peak area of 1,4-dioxane.

Related substances

A. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in 100.0 ml of *0.001 M hydrochloric acid.*

Reference solution. A solution containing 0.02 per cent w/v of *valganciclovir hydrochloride IPRS* and 0.001 per cent w/v of *methoxymethylguanine IPRS* in *0.001 M hydrochloric acid.*

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5 µm) (Such as zorbax SB C18),
- mobile phase: A. a buffer solution prepared by dissolving 11.5 g of *monobasic ammonium phosphate* in 900 ml of *water,* adjusted to pH 2.8 with *orthophosphoric acid* and dilute to 1000 ml with *water,*
  B**.** *methanol,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	92	8
5	92	8
15	80	20
30	30	70
30.1	92	8
45	92	8

Name	Relative retention time	Correction factor
Guanine*	0.28	0.53
Ganciclovir*	0.42	0.71
Methoxymethylguanine*	0.81	---
Valganciclovir	1.00	---
Isovalganciclovir*#	1.26	---
Monoacetoxyganciclovir*	1.36	0.77
Homologue of valganciclovir (H)	1.47	0.77
Homologue of valganciclovir (I)	1.52	0.71
Bis-valine ester of ganciclovir**	1.61	1.41
Homologue of valganciclovir(G)*#	1.66	---
Genciclovir monopropionate**	2.09	0.91
Valganciclovir dimer* (stereoisomer A)	2.49	---
Valganciclovir dimer* (stereisomer B)	2.52	---
Valganciclovir dimer* (stereisomer C)	2.54	---

*Specified impurity
*# Reported as the sum of diasteromers
** Other identified impyrity

Note- The retention time for the second peak of valganciclovir is between 5 and 8.5 minutes.

Inject the reference solution. The test is not valid unless the resolution between the first peak of valganciclovir and methoxymethylguanine peak is not less than 1.0 and the resolution between the two peaks of valganciclovir (R and S esters of L-valine) is not less than 3.0, the column efficiency is not less than 8000 theoretical plates and the tailing factor is not more than 1.4 for the second peak of valganciclovir.

Inject the test solution. The area of any peak corresponding to bis-valine ester of ganciclovir, homologue of valganciclovir (H), homologue of valganciclovir (I), valganciclovir dimer (stereisomer A), valganciclovir dimer (stereisomer B) and valganciclovir dimer (stereisomer C), each of, is not more than 0.1 per cent, the area of any peak corresponding to monoacetoxyganciclovir and genciclovir monopropionate, each of, is not more than 0.15 per cent, the area of any peak corresponding to guanine and homologue of valganciclovir (G), each of, is not more than 0.25

per cent, the area of any peak corresponding to methoxymethylguanine is not more than 0.3 per cent, the area of any peak corresponding to isovalganciclovir is not more than 0.5 per cent, the area of any peak corresponding to ganciclovir is not more than 1.5 per cent. The sum of the areas of peaks corresponding to bis valine ester of ganciclovirs and ganciclovir mono propionate is not more than 0.25 per cent, the area of any other secondary peak is not more than 0.1 per cent and the sum of areas of all other secondary peaks is not more than 0.25 per cent, calculated by area normalization.

Diastereomer ratio. Using the chromatogram obtained with the test solution under Related substances A. Calculate the percentage of valganciclovir (R and S esters of L-valine) by the following expression.

$$100[A_1/ (A_1+A_2)]$$

$$100[A_2/ (A_1+A_2)]$$

where, A_1 and A_2 are the peak areas of the valganciclovir (R and S esters of L-valine). The diastereomer ratio is (45:55) to (55:45).

B. Determine by liquid chromatography (2.4.14), as described under Related substances test A with the following modifications.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with phenyl groups bonded to porous silica (3.5 µm) (Such as zorbax SB phenyl),
- mobile phase: A. a buffer solution prepared by diluting 2.5 ml of *triethylamine* to 1000 ml *water,* adjusted to pH 3.0 with *trifluoroacetic acid,*
  B**.** *methanol,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	93	7
10	93	7
20	70	30
21	93	7
35	93	7

Name	Relative retention time
Valganciclovir	1.00
Ganciclovir mono-N-methyl valinate	1.2

Note- The retention time for the second peak of valganciclovir is between 6 and 9 minutes.

Inject the reference solution. The test is not valid unless the resolution between the two peaks of valganciclovir (R and S esters of L-valine) is not less than 1.3, the column efficiency is not less than 8000 theoretical plates and the tailing factor is not more than 1.2 for the second peak of valganciclovir.

Inject the test solution. The area of any peak corresponding to ganciclovir mono-N-methyl valinate (sum of distereomers) is not more than 0.3 per cent, the area of any other secondary peak is not more than 0.1 per cent and the sum of areas of all other secondary peaks is not more than 0.25 per cent, calculated by area normalization.

The sum of all the impurities calculated under method A and B is not more than 3.0 per cent.

Enantiomeric purity. Not less than 97.0 per cent.

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 10 mg of the substance under examination in *0.001 M hydrochloric acid* and dilute to 50.0 ml with *0.001 M hydrochloric acid.*

Reference solution. A solution containing 0.02 per cent w/v of *valganciclovir hydrochloride IPRS* and 0.002 per cent w/v of *D-valganciclovir IPRS* in *0.001 M hydrochloric acid.*

Chromatographic system

– a stainless steel column 15 cm x 4.0 mm, packed with crown ether coated bonded to porous silica (5 µm) (Such as crownpack CR (+),
– mobile phase: a 1.62 per cent w/v solution of *perchloric acid* in *water,*
– flow rate: 0.8 ml per minute,
– spectrophotometer set at 254 nm,
– injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to second peak of D-valine ester and first peak of valganciclovir is not less than 3.5, the column efficiency is not less than 1800 theoretical plates for the second peak of valganciclovir.

Inject the test solution and calculate the enantiomeric purity by the following expression.

$$100[A/ (A+B)]$$

Where, A is the sum of the peak area of valganciclovir (R and S esters of L-valine) and B is the sum of the peak area of enantiomeric impurities (R and S esters of D-valine).

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). Not more than 8.0 per cent, determined on 0.1 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in *0.001 M hydrochloric acid* and dilute to 100.0 ml with *0.001 M hydrochloric acid.*

Reference solution (a). A 0.02 per cent w/v solution of *valganciclovir hydrochloride IPRS* in *0.001 M hydrochloric acid.*

Reference solution (b). A solution containing 0.02 per cent w/v of *valganciclovir hydrochloride IPRS* and 0.001 per cent w/v of *methoxymethylguanine IPRS* in *0.001 M hydrochloric acid.*

Chromatographic system
– a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5 µm) (Such as Zorbax SB C-18),
– mobile phase: a mixture of 92 volumes of buffer solution prepared by dissolving 11.5 g of *monobasic ammonium phosphate* in 900 ml of *water,* adjusted to pH 2.8 with *orthophosphoric acid* and dilute to 1000 ml with *water* and 8 volumes of *methanol*,
– flow rate: 1 ml per minute,
– spectrophotometer set at 254 nm,
– injection volume: 20 µl.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the first peak of valganciclovir and methoxymethylguanine peak is not less than 1.0 and the resolution between the two peaks of valganciclovir (R and S esters of L-valine) is not less than 3.0, the column efficiency is not less than 8000 theoretical plates for the second peak of valganciclovir and the tailing factor is not more than 1.4 for the second peak of valganciclovir in the chromatogram obtained with reference solution (b). The relative standard deviation of the correction factor (C) for replicate injections is not more than 1.0 per cent in the chromatogram obtained with reference solution (a), the correction factor (C) calculate by the following expression.

$$C = (Ws/Rs)/100$$

where, Ws is the weight (in mg) of *valganciclovir IPRS* in reference solution (a) preparation and Rs is the sum of the areas of the two peaks of valganciclovir diastereomers in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the content of $C_{14}H_{22}N_6O_5$,HCl

Storage. Store protected moisture, at temperature not exceeding 25º.

Solubility. Very slightly soluble in *ethanol*; practically insoluble in *2-propanol*, in *hexane*, in *acetone*, and in *ethyl acetate*.