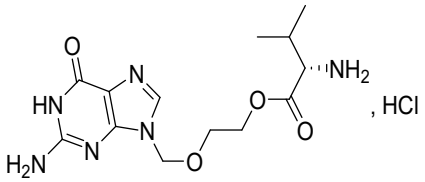
Valacyclovir Hydrochloride



$C_{13}H_{20}N_6O_4$,HCl Mol. Wt. 360.8

Valacyclovir Hydrochloride is L-Valine, 2-[(2-amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methoxy] ethyl ester, monohydrochloride.

Valacyclovir Hydrochloride contains not less than 95.0 per cent and not more than 102.0 per cent of $C_{13}H_{20}N_6O_4$.HCl, calculated on the anhydrous 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 v*alacyclovir hydrochloride IPRS* or with the reference spectrum of valacyclovir hydrochloride.

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

C. A 5 per cent w/v solution gives the reaction (A) of chlorides (2.3.1).

Tests

Related substances

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*. (prewash the plate with *methanol*)

Mobile phase. A mixture of 54 volumes of *methylene chloride*, 34 volumes of *methanol*, 12 volumes of *tetrahydrofuran* and 3 volumes of *ammonia solution.*

Test solution. Dissolve 0.25 g of the substance under examination in 2 ml of *water,* with the aid of ultrasound for 20 minutes and dilute to 5.0 ml with *ethanol,* filter.

Reference solution (a). Dissolve 5 mg, each of, *valacyclovir related compound D IPRS*, and *valacyclovir related compound G IPRS*, 10 mg, each of, *valacyclovir related compound E IPRS* and *valacyclovir related compound F IPRS* in 2 ml of *water* and 6 ml of *ethanol,* with the aid of ultrasound for 20 minutes and dilute to 10.0 ml with *ethanol.*

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with *ethanol*.

Reference solution (c). Dilute 0.5 ml of reference solution (a) to 10.0 ml with *ethanol*.

Apply to the plate 4 µl of the test solution and reference solution (b) and (c). Allow the mobile phase to rise 10 cm. Dry the plate in air. Examine the plate under ultraviolet light at 254 nm and visually estimate the valacyclovir related compounds E and G in the sample using the appropriate reference solution spots. The chromatograms obtained with the reference solution (b) and (c) each show three clearly separated spots due to valacyclovir related compound D, E, and G. Spray the plate with 0.01 per cent w/v of *fluorescamine* in *ethylene dichloride* and examine the plate under ultraviolet light at 365 nm to estimate the level of valacyclovir related compound F in the sample using the appropriate reference solution spot.

Name	Relative Rf value
Valacyclovir hydrochloride	1
Valacyclovir related compound D[1*]	1.1
Valacyclovir related compound E[2]	1.3
Valacyclovir related compound F[3]	1.8
Valacyclovir related compound G[4]	1.9

*This impurity is controlled in Method B of Related substances,
[1]2-[(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)methoxy]ethyl N-ethyl-L-valinate,

[2]2-[(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)methoxy]ethyl N-[(benzyloxy)carbonyl]-L-valinate,
[3]2-hydroxyethyl-L-valinate,
[4]N,N-dimethylpyridin-4-amine.

In the chromatogram obtained with the test solution, any spot corresponding to valacyclovir related compound E is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b) (0.2 per cent), any spot corresponding to valacyclovir related compound F is not more intense than the corresponding spot in the chromatogram obtained with reference solution (c) (0.1 per cent) and any spot corresponding to valacyclovir related compound G is not more intense than the corresponding spot in the chromatogram obtained with reference solution (c) (0.05 per cent).

B. Determine by liquid chromatography (2.4.14).

Solvent mixture. 20 volumes of *ethanol* and 80 volumes of *water*.

Test solution. Dissolve 40 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution. A solution containing 0.04 per cent w/v of *valacyclovir hydrochloride IPRS*, 0.00008 per cent w/v of *valacyclovir related compound C IPRS* and 0.00016 per cent w/v of *acyclovir related compound A IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with phenyl group bonded to porous silica (5µm),
- column temperature: 15º,
- mobile phase: A. a 0.3 per cent w/w solution of *trifluoroacetic acid* in *water*,
  B. a 0.3 per cent w/w solution of *trifluoroacetic acid* in *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
5	90	10
35	60	40
35.1	90	10
45	90	10

Name	Relative retention time
Guanine[1,2]	0.31
Acyclovir[1,3]	0.42
Acyclovir alaninate[4]	0.54
Valacyclovir	1.00
Valacyclovir related compound C[5]	1.06
Acyclovir related compound A[1,6]	1.09
Valacyclovir related compound D[7]	1.17
Acyclovir isoleucinate[8]	1.30
N-formyl valacyclovir[9]	1.61
Guaninyl valacyclovir[10]	1.66
Bis valacyclovir[11]	2.00

[1]This impurity is controlled in Method C of Related substances,
[2]2-amino-1H-purin-6(9H)-one (guanine),
[3]9-[(2-hydroxyethoxy)methyl]guanine (acyclovir),
[4]9-[(2-hydroxyethoxy)methyl]guanine L-alaninate,
[5]2-[(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)methoxy]ethyl N-methyl-L-valinate,
[6]2-[(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)methoxy]ethyl acetate,
[7]2-[(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)methoxy]ethyl N-ethyl-L-valinate,
[8]9-[(2-hydroxyethoxy)methyl]guanine L-isoleucinate,
[9]9-[(2-hydroxyethoxy)methyl]guanine N-formyl-L-valinate,
[10][N^2-(guanine-N^2-yl)methyl]-9-[(2-hydroxyethoxy)methyl]guanine L-valinate,
[11]2,2'[Methylenebis[imino(6-oxo-1,6-dihydro-9H-purine-9,2-diyl)methylene-oxy]]diethyl di(L-valinate).

Inject the reference solution. The test is not valid unless the resolution between the peaks due to valacyclovir and valacyclovir related compound C is not less than 1.5 and valacyclovir related compound C and acyclovir related compound A is not less than 1.5 and the tailing factor is not more than 1.5 for valacyclovir peak.

Inject the test solution. The area of any peak corresponding to acyclovir alaninate, acyclovir isoleucinate and guaninyl valacyclovir, each of, is not more than 0.2 per cent, the area of any peak corresponding to valacyclovir related compound C, bis valacyclovir, each of is not more than 0.3 per cent, the area of any peak corresponding to valacyclovir related compound D is not more than 0.5 per cent, the area of any peak corresponding to N-formyl valacyclovir is not more than 0.8 per cent and the area of any other secondary peak is not more than 0.1 per cent, calculated by area normalization.

C. Determine by liquid chromatography (2.4.14),

Test solution. Dissolve 50 mg of the substance under examination in *0.05 M hydrochloric acid* and dilute to 100.0 ml with the *0.05 M hydrochloric acid*.

Reference solution (a). A 0.05 per cent w/v solution of *valacyclovir hydrochloride IPRS* in *0.05 M hydrochloric acid.*

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with *0.05 M hydrochloric acid*.

NOTE –Valacyclovir hydrochloride IPRS contains a detectable quantity of D-valacyclovir.

Chromatographic system

- a stainless steel column 15 cm x 4.0 mm, a crown ether coated on silica gel substrate. The active site is (S)-=18-crown-6-ether (5 µm) (Such as Crown Pak CR (+)),
- column temperature: 10º,
- mobile phase: a mixture 19 volumes of *water*,1 volume of *methanol* and 0.1 volume of *perchloric acid*,
- flow rate: 0.75 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Guanine and acyclovir[1,2]	0.18	0.66
Acyclovir related compound A[3]	0.42	0.89
D-Valacyclovir[4]	0.55	--
Valacyclovir	1.0	--

[1]2-amino-1H-purin-6(9H)-one (guanine),
[2]9-[(2-hydroxyethoxy)methyl]guanine (acyclovir),
[3]2-[(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)methoxy]ethyl acetate,
[4]D-Valine, 2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy] ethyl ester, monohydrochloride.

Inject reference solution (a) and (b).The test is not valid unless the resolution between the peaks due to valacyclovir hydrochloride and D-valacyclovir is not less than 2.0 in the chromatogram obtained with reference solution (a) and the relative standard deviation for replicate injections is not more than 5.0 per cent, in the chromatogram obtained with reference solution (b).

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to guanine and acyclovir is not more than twice the area of principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent), the area of any peak corresponding to acyclovir related compound A is not more than 0.2 times the area of principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any peak due to D-valacyclovir is not more than 3 times the area of the principal peak in the chromatogram obtained with the reference solution (b) (3.0 per cent).

The sum of all the impurities from related substance test A, B and C is not more than 5.0 per cent.

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

Water (2.3.43). Not more than 2.0 per cent (for the anhydrous form) and 5.0 to 11.0 per cent (for the hydrous form).

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances C.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to valacyclovir hydrochloride and D-valacyclovir is not less than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{13}H_{20}N_6O_4$,HCl.

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

Solubility. Soluble in *water*; and insoluble in *dichloromethane*.