Vaccinia Immune Globulin

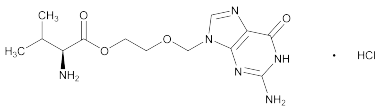
» Vaccinia Immune Globulin conforms to the regulations of the FDA concerning biologics (see *Biologics* ⟨1041⟩). It is a sterile, nonpyrogenic solution of globulins derived from the blood plasma of adult human donors who have been immunized with vaccinia virus (Smallpox Vaccine). It is standardized for viral neutralizing activity in eggs or tissue culture with the U.S. Reference Vaccinia Immune Globulin and a specified vaccinia virus. It contains not less than 15 g and not more than 18 g of protein per 100 mL, not less than 90.0 percent of which is gamma globulin. It contains 0.3 M glycine as a stabilizing agent, and contains a suitable antimicrobial agent.

Packaging and storage—Preserve at a temperature between 2° and 8°.

Expiration date—The expiration date is not later than 3 years after date of issue.

Labeling—Label it to state that it is not intended for intravenous injection.

Valacyclovir Hydrochloride



$C_{13}H_{20}N_6O_4 \cdot HCl$ 360.80

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

L-Valine, ester with 9-[(2-hydroxyethoxy)methyl]guanine, monohydrochloride [124832-27-5].

DEFINITION

Valacyclovir Hydrochloride contains NLT 95.0% and NMT 102.0% of $C_{13}H_{20}N_6O_4 \cdot HCl$, calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

- **A. Infrared Absorption** ⟨**197K**⟩
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. Identification Tests—General,** *Chloride* ⟨**191**⟩
  Sample solution: 50 mg/mL in water
  Acceptance criteria: Meets the requirements

ASSAY

- **Procedure**
  Mobile phase: Methanol, water, and perchloric acid (1: 19: 0.1)
  Standard solution: 0.5 mg/mL of USP Valacyclovir Hydrochloride RS in 0.05 M hydrochloric acid. [Note—USP Valacyclovir Hydrochloride RS contains a detectable quantity of D-valacyclovir.]
  Sample solution: 0.5 mg/mL of Valacyclovir Hydrochloride in 0.05 M hydrochloric acid
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4-mm × 15-cm; 5-µm packing L66
  Column temperature: 10°
  Flow rate: 0.75 mL/min
  Injection size: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Resolution: NLT 2.0 between valacyclovir hydrochloride and D-valacyclovir
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of valacyclovir hydrochloride ($C_{13}H_{20}N_6O_4 \cdot HCl$) in the portion of Valacyclovir Hydrochloride taken:

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

r_U = peak response of valacyclovir from the *Sample solution*

r_S = peak response of valacyclovir from the *Standard solution*

C_S = concentration of valacyclovir hydrochloride in the *Standard solution* (mg/mL)

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

Acceptance criteria: 95.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **Residue on Ignition** ⟨**281**⟩**:** NMT 0.1% on a 2-g sample
- **Heavy Metals,** *Method II* ⟨**231**⟩**:** NMT 20 ppm
- **Limit of Palladium** (if present)
  (See *Plasma Spectrochemistry* ⟨730⟩.)
  Diluent: Dimethyl sulfoxide and hydrochloric acid (98:2)
  Blank solution: *Diluent*
  Standard solutions: Dilute with *Diluent* any commercially available standard stock solution of 1 mg/mL of palladium to prepare the following two solutions: 1 µg/mL of palladium and 10 µg/mL of palladium.
  Sample solution: 10 mg/mL of Valacyclovir Hydrochloride in *Diluent*
  Analytical wavelength: 340.458 nm
  Spectrophotometer system: Use a suitable standard inductively coupled plasma–optical emission spectrophotometric system, and construct a calibration curve.
  System suitability
  Samples: *Blank solution* and *Standard solutions*
  Suitability requirements
  Relative standard deviation: NMT 2.0%, *Standard solutions*
  Correlation coefficient: NLT 0.999, *Blank solution* and *Standard solution*
  Analysis
  Samples: *Blank solution* and *Sample solution*
  Calculate the concentration of palladium using the calibration curve corrected for the emission response of the *Blank solution* and sample weight. Calculate the amount of palladium in the Valacyclovir Hydrochloride taken to prepare the *Sample solution*.
  Acceptance criteria: NMT 10 ppm
- **Organic Impurities, Procedure 1** (for related compounds E, F, and G)
  Developing solvent: Methylene chloride, methanol, tetrahydrofuran, and ammonia solution (54:34:12:3)
  Standard stock solution: Transfer 5 mg each of USP Valacyclovir Related Compound D RS, USP Valacyclovir Related Compound E RS, and USP Valacyclovir Related Compound G RS, and 8.4 mg of USP Valacyclovir Related Compound F RS into a 10-mL volumetric flask. Add 2 mL of water with swirling, followed by 6 mL of alcohol, and

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sonicate for 20 min. Allow to cool, and dilute with alcohol to volume.

Standard solutions: Transfer 1.0 and 0.5 mL of *Standard stock solution* into two separate 10-mL volumetric flasks. Dilute the solution in both flasks with alcohol to volume.

Sample solution: Transfer 250 mg of Valacyclovir Hydrochloride into a 5-mL volumetric flask. Add 2 mL of water, and sonicate for 20 min to dissolve. Add alcohol to about 95% volume of the flask. Cool, and dilute with alcohol to volume. Pass through a suitable filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: TLC
Detector: UV, long and short wavelength
Plate: TLC plate coated with 0.25-mm layer of chromatographic silica gel mixture. Prewash the plate with methanol.
Developing distance: NLT 7 cm from the origin
Application size: 4 µL

Analysis
Samples: *Standard solutions* and *Sample solution*
Develop the plate to the specified distance. Remove the plate from the solvent chamber, and allow to dry. Examine the plate under short-wavelength UV light, and visually estimate the valacyclovir related compounds E and G in the sample using the appropriate standard spots. The chromatograms obtained with the *Standard solutions* each show three clearly separated spots due to valacyclovir related compounds D, E, and G. Spray the plate with 0.01% fluorescamine in ethylene dichloride, and examine the sprayed plate under long-wavelength UV to estimate the level of valacyclovir related compound F in the sample using the appropriate standard spot. The relative R_F values and limits for each impurity are provided in *Table 1*.

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative R_F Value	Acceptance Criteria, NMT (%)
Valacyclovir hydrochloride	1	—
Valacyclovir related compound D[a]	1.1	—
Valacyclovir related compound E[b]	1.3	0.2
Valacyclovir related compound F[c]	1.8	0.1
Valacyclovir related compound G[d]	1.9	0.05

[a] This impurity is quantitated using *Procedure 2*.
[b] 2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-[(benzyloxy)carbonyl]-L-valinate.
[c] 2-Hydroxyethyl-L-valinate.
[d] *N*,*N*-Dimethylpyridin-4-amine.

- **ORGANIC IMPURITIES, PROCEDURE 2**

Solution A: 0.3% w/w trifluoroacetic acid solution in water
Solution B: 0.3% w/w trifluoroacetic acid solution in methanol
Diluent: Alcohol and water (1:4)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	90	10
5	90	10
35	60	40
35.01	90	10
45	90	10

System suitability solution: 0.4 mg/mL of USP Valacyclovir Hydrochloride RS, 0.8 µg/mL of USP Valacyclovir Related Compound C RS, and 1.6 µg/mL of USP Acyclovir Related Compound A RS in *Diluent*
Sample solution: 0.4 mg/mL of Valacyclovir Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L11
Column temperature: 15°
Flow rate: 0.8 mL/min
Injection size: 10 µL
System suitability
Sample: *System suitability solution*
Resolution: NLT 1.5 between valacyclovir and valacyclovir related compound C, and NLT 1.5 between valacyclovir related compound C and acyclovir related compound A
Tailing factor: NMT 1.5 for the valacyclovir hydrochloride peak
Analysis
Sample: *Sample solution*
Calculate the percentage of each individual impurity in the portion of Valacyclovir Hydrochloride taken:

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

r_U = peak response of any impurity in the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Guanine (near solvent front)[a,b]	0.31	—
Acyclovir[a,c]	0.42	—
Acyclovir alaninate[d]	0.54	0.2
Valacyclovir	1.00	—
Valacyclovir related compound C[e]	1.06	0.3
Acyclovir related compound A[a,f]	1.09	—
Valacyclovir related compound D[g]	1.17	0.5
Acyclovir isoleucinate[h]	1.30	0.2
N-Formyl valacyclovir[i]	1.61	0.8
Guaninyl valacyclovir[j]	1.66	0.2
Bis valacyclovir[k]	2.0	0.3
Any unspecified impurity	—	0.1

[a] This impurity is quantitated by the *Procedure 3* method.
[b] 2-Amino-1*H*-purin-6(9*H*)-one (guanine).
[c] 9-[(2-Hydroxyethoxy)methyl]guanine (acyclovir).
[d] 9-[(2-Hydroxyethoxy)methyl]guanine L-alaninate.
[e] 2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-methyl-L-valinate.
[f] 2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl acetate.
[g] 2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-ethyl-L-valinate.
[h] 9-[(2-Hydroxyethoxy)methyl]guanine L-isoleucinate.
[i] 9-[(2-Hydroxyethoxy)methyl]guanine *N*-formyl-L-valinate.
[j] [N^2-(guanine-N^2-yl)methyl]-9-[(2-hydroxyethoxy)methyl]guanine L-valinate.
[k] 2,2′-[Methylenebis[imino(6-oxo-1,6-dihydro-9*H*-purine-9,2-diyl)methylene-oxy]]diethyl di(L-valinate).

- **ORGANIC IMPURITIES, PROCEDURE 3**

Mobile phase, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

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Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Valacyclovir Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of guanine plus acyclovir or acyclovir acetate or D-valacyclovir from the *Sample solution*
r_S = peak response of valacyclovir from the *Standard solution*
C_S = concentration of USP Valacyclovir Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Valacyclovir Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 4*)
Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Guanine and acyclovir[a,b]	0.18	1.51	2.0
Acyclovir related compound A[c]	0.42	1.12	0.2
D-Valacyclovir[d]	0.55	1.0	3.0
Valacyclovir	1.0	—	—

[a] 2-Amino-1*H*-purin-6(9*H*)-one (guanine).
[b] 9-[(2-Hydroxyethoxy)methyl]guanine (acyclovir).
[c] 2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl acetate.
[d] D-Valine, 2-[(2-amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methoxy] ethyl ester, monohydrochloride.

Total organic impurities: NMT 5.0% for the sum of all impurities from *Organic Impurities, Procedures 1*, *2*, and *3*

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** For the anhydrous form: NMT 2.0% (200 mg of sample); if labeled as the hydrous form: 5.0%–11.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at a temperature below 30°.
- **LABELING:** Where it is the hydrous form, the label so indicates.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Acyclovir Related Compound A RS
  [NOTE—USP Acyclovir Related Compound A AS is equivalent.]
  2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl acetate.
  $C_{10}H_{13}N_5O_4$ 267.24
  USP Valacyclovir Hydrochloride RS
  USP Valacyclovir Related Compound C RS
  2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-methyl-L-valinate hydrochloride.
  $C_{14}H_{22}N_6O_4 \cdot HCl$ 374.82
  USP Valacyclovir Related Compound D RS
  2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9yl)methoxy]ethyl *N*-ethyl-L-valinate.
  $C_{15}H_{24}N_6O_4$ 352.39
  USP Valacyclovir Related Compound E RS
  2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-benzyloxy)carbonyl]-L-valinate.
  $C_{21}H_{26}N_6O_6$ 458.47
  USP Valacyclovir Related Compound F RS
  2-Hydroxyethyl valinate para-toluenesulfonate salt.
  $C_7H_{15}NO_3 \cdot C_7H_8O_3S$ 333.40
  USP Valacyclovir Related Compound G RS
  N,N-Dimethylpyridin-4-amine.
  $C_7H_{10}N_2$ 122.17

Valacyclovir Tablets

DEFINITION
Valacyclovir Tablets contain an amount of Valacyclovir Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of valacyclovir ($C_{13}H_{20}N_6O_4$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨**191**⟩**:** Meets the requirements

ASSAY
- **PROCEDURE**
  Diluent: 0.1% (v/v) phosphoric acid in water
  Mobile phase: Methanol and *Diluent* (5:95)
  Standard solution: 0.1 mg/mL of USP Valacyclovir Hydrochloride RS in *Diluent*. [NOTE—USP Valacyclovir Hydrochloride RS contains a detectable quantity of D-valacyclovir.]
  Sample solution: Transfer NLT 5 Tablets into a suitable volumetric flask, and add 0.1 M hydrochloric acid (approximately 80% of the volume of volumetric flask). Mechanically shake the sample until the Tablets disintegrate into a fine suspension (60 min), and sonicate for 10 min. Cool to ambient temperature, dilute with 0.1 M hydrochloric acid to volume, and mix to obtain a solution having a concentration of 2.5 mg/mL. Dilute a portion of the sample with *Diluent* to obtain a nominal concentration of 0.1 mg/mL of valacyclovir, and mix. Pass a portion of this solution through a membrane filter of 0.45-µm or finer pore size, and use the filtrate.
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4-mm × 15-cm; 5-µm packing L66
  Column temperature: 10°
  Flow rate: 0.75 mL/min
  Injection size: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Resolution NLT 1.3 between the D-valacyclovir and valacyclovir peaks
  Tailing factor: NMT 2.0 for the valacyclovir peak
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of $C_{13}H_{20}N_6O_4$, based on the label claim, in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Valacyclovir Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of valacyclovir in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of valacyclovir, 324.34
M_{r2} = molecular weight of valacyclovir hydrochloride, 360.80

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