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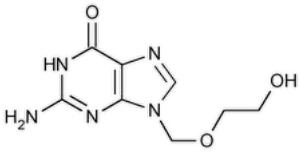
Acyclovir

Change to read:



$C_8H_{11}N_5O_3$ ▲225.21▲ (IRA 1-Jun-2024)

6*H*-Purin-6-one, 2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-;

9-[(2-Hydroxyethoxy)methyl]guanine CAS RN®: 59277-89-3; UNII: X4HES1O11F.

Change to read:

DEFINITION

Acyclovir contains NLT 98.0% and NMT ▲102.0%▲ (IRA 1-Jun-2024) of acyclovir ($C_8H_{11}N_5O_3$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197K or 197A
- **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

Change to read:

- **Procedure**

Mobile phase: Glacial acetic acid in water (1 in 1000)

Standard stock solution: 0.5 mg/mL of USP Acyclovir RS prepared as follows. Dissolve about 25 mg of USP Acyclovir RS in 5 mL of 0.1 N sodium hydroxide in a 50-mL volumetric flask, and dilute with water to volume.

Standard solution: 0.1 mg/mL of USP Acyclovir RS from *Standard stock solution* in 0.01 N sodium hydroxide

Sample stock solution: 0.5 mg/mL of Acyclovir prepared as follows. Dissolve about 100 mg of Acyclovir in 20 mL of 0.1 N sodium hydroxide in a 200-mL volumetric flask, and dilute with water to volume.

Sample solution: 0.1 mg/mL of Acyclovir from *Sample stock solution* in 0.01 N sodium hydroxide

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 10-µm packing L1

Flow rate: 3 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT ▲0.73%▲ (IRA 1-Jun-2024)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of acyclovir ($C_8H_{11}N_5O_3$) in the portion of Acyclovir taken:

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

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

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

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

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

Acceptance criteria: 98.0%–▲102.0%▲ (IRA 1-Jun-2024) on the anhydrous basis

IMPURITIES

Change to read:

- **Organic Impurities**

Buffer A: 3.48 g/L of ▲dibasic potassium phosphate anhydrous▲ (IRA 1-Jun-2024) in water. Adjust with phosphoric acid to a pH of 3.1.

Buffer B: 3.48 g/L of ▲dibasic potassium phosphate anhydrous▲ (IRA 1-Jun-2024) in water. Adjust with phosphoric acid to a pH of 2.5.

Solution A: Acetonitrile and *Buffer A* (1:99)

Solution B: Acetonitrile and *Buffer B* (50:50)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
27	80	20
40	80	20

Diluent: Dimethyl sulfoxide and water (20:80 v/v)

System suitability solution: 0.01 mg/mL of USP Acyclovir RS and 0.5 µg/mL each of USP Acyclovir Related Compound A RS and USP Acyclovir Related Compound F RS prepared as follows. Dissolve a suitable amount of USP Acyclovir RS, USP Acyclovir Related Compound A RS, and USP Acyclovir Related Compound F RS in 20% of the ▲▲ (IRA 1-Jun-2024) flask volume of dimethyl sulfoxide and dilute with water to volume.

Standard stock solution: 0.1 mg/mL of USP Acyclovir RS prepared as follows. Dissolve a suitable amount of USP Acyclovir RS in 20% of the ▲▲ (IRA 1-Jun-2024) flask volume of dimethyl sulfoxide and dilute with water to volume.

Sensitivity solution: 0.3 µg/mL of USP Acyclovir RS from *Standard stock solution* in *Diluent*

Standard solution: ▲0.001 mg/mL▲ (IRA 1-Jun-2024) of USP Acyclovir RS from *Standard stock solution* in *Diluent*

Sample solution: 1 mg/mL of Acyclovir prepared as follows. Dissolve a suitable amount of Acyclovir in 20% of the ▲▲ (IRA 1-Jun-2024) flask volume of dimethyl sulfoxide and dilute with water to volume.

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*

▲[Note—The relative retention times in *Table 2* are provided as information that could aid in peak assignment.]

Table 2▲ (IRA 1-Jun-2024)

Name	Relative Retention Time
Guanine▲[a]▲ (IRA 1-Jun-2024)	0.40
Hydroxyethyl guanine[b]	0.74
N^7-Isomer[c]	0.94

Name	Relative Retention Time
Acyclovir	1.0
Specified impurity 1[d]	1.34
Specified impurity 2[d]	1.39
▲*N*-Acetylguanine[e]	1.48▲ (IRA 1-Jun-2024)
7,9'-Diguanyl analog[f]	1.53
9,9'-Diguanyl analog[g]	1.59
Acyclovir related compound F	1.79
Acyclovir related compound A	1.83
Diacetyl guanine[h]	▲2.36▲ (IRA 1-Jun-2024)
Bis-acyclovir[i]	2.44
▲Acyclovir related compound G▲ (IRA 1-Jun-2024) [j]	2.67

▲[a] 2-Amino-1,7-dihydro-6*H*-purin-6-one.▲ (IRA 1-Jun-2024)

[b] ▲2-Amino-9-(2-hydroxyethyl)-1,9-dihydro-6*H*-purin-6-one.▲ (IRA 1-Jun-2024)

[c] 2-Amino-7-[(2-hydroxyethoxy)methyl]-1,7-dihydro-6*H*-purin-6-one.

[d] Unknown structure.

▲[e] *N*-(6-Oxo-6,9-dihydro-1*H*-purin-2-yl)acetamide.▲ (IRA 1-Jun-2024)

[f] ▲2-Amino-9-({2-[(2-amino-6-oxo-1,6-dihydro-7*H*-purin-7-yl)methoxy]ethoxy}methyl)- 1,9-dihydro-6*H*-purin-6-one.▲ (IRA 1-Jun-2024)

[g] 1,2-Bis[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethane.

[h] ▲*N*-(9-Acetyl-6-oxo-6,9-dihydro-1*H*-purin-2-yl)acetamide.▲ (IRA 1-Jun-2024)

[i] Bis({9-[(2-hydroxyethoxy)methyl]guanine}-N^2-yl)methane.

[j] 2-{[2-(Acetylamino)-6-oxo-1,6-dihydro-9*H*-purin-9-yl]methoxy}ethyl acetate.

Suitability requirements

Resolution: NLT 1.0 between acyclovir related compound F and acyclovir related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any ▲▲ (IRA 1-Jun-2024) impurity in the portion of Acyclovir taken:

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

r_U = peak response of ▲each▲ (IRA 1-Jun-2024) impurity from the *Sample solution*

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

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

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

F = relative response factor (See ▲*Table 3*▲ (IRA 1-Jun-2024))

Acceptance criteria: See ▲*Table 3*.▲ (IRA 1-Jun-2024) Reporting threshold is 0.03%.

▲**Table 3**▲ (IRA 1-Jun-2024)

Name	Relative Response Factor	Acceptance Criteria, NMT (%)
Guanine	1.0	0.7
Hydroxyethyl guanine	1.0	0.2
N^7-Isomer	0.43	0.1
Specified impurity 1	1.0	0.1
Specified impurity 2	1.0	0.3
7,9'-Diguanyl analog	0.66	0.1
9,9'-Diguanyl analog	1.0	0.2
Acyclovir related compound F	1.0	0.1
Acyclovir related compound A	1.0	0.1
Diacetyl guanine	1.0	0.1
Bis-acyclovir	1.0	0.2
▲Acyclovir related compound G▲ (IRA 1-Jun-2024)	1.0	0.1
Any ▲▲ (IRA 1-Jun-2024) unspecified impurity	1.0	0.05
Total impurities	—	1.0

SPECIFIC TESTS

• **Water Determination** ⟨921⟩, *Method I*: NMT 6.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers. Store at room temperature. Protect from light and moisture.

Change to read:

• **USP Reference Standards** ⟨11⟩

USP Acyclovir RS

USP Acyclovir Related Compound A RS

2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl acetate.

$C_{10}H_{13}N_5O_4$ ▲267.25▲ (IRA 1-Jun-2024)

USP Acyclovir Related Compound F RS

N-[9-[(2-Hydroxyethoxy)methyl]-6-oxo-6,9-dihydro-1*H*-purin-2-yl]acetamide.

$C_{10}H_{13}N_5O_4$ ▲267.25▲ (IRA 1-Jun-2024)

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

Topic/Question	Contact	Expert Committee
ACYCLOVIR	Documentary Standards Support	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

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