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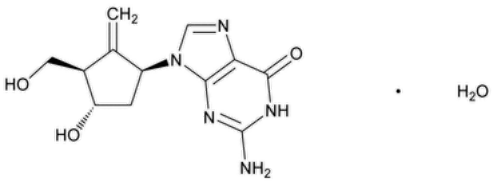
Entecavir



$C_{12}H_{15}N_5O_3 \cdot H_2O$ 295.29

6*H*-Purin-6-one, 2-amino-1,9-dihydro-9-[(1*S*,3*R*,4*S*)-4-hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]-, monohydrate;

9-[(1*S*,3*R*,4*S*)-4-Hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]guanine monohydrate CAS RN®: 209216-23-9; UNII: 5968Y6H45M.

Anhydrous 277.28

DEFINITION

Entecavir is a monohydrate and contains NLT 98% and NMT 102% of entecavir ($C_{12}H_{15}N_5O_3$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A.** ▲**Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197A or 197K▲ (CN 1-May-2020)

• **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

• **Procedure**

Solution A: Acetonitrile and water (3:97)

Solution B: Acetonitrile

Mobile phase: See *Table 1*. [Note—The gradient elution times are established on an HPLC system with a dwell volume of approximately 1.0 mL.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
8	100	0
50	77	23
75	17	83
90	100	0
100	100	0

System suitability stock solution: 1.0 mg/mL of USP Entecavir System Suitability Mixture RS in methanol

System suitability solution: 0.2 mg/mL of USP Entecavir System Suitability Mixture RS in *Solution A* from *System suitability stock solution*

Standard stock solution: 1.0 mg/mL of USP Entecavir Monohydrate RS in methanol. Sonicate as needed.

Standard solution: 0.2 mg/mL of USP Entecavir Monohydrate RS in *Solution A* from the *Standard stock solution*

Sample stock solution: 1.0 mg/mL of Entecavir in methanol. Sonicate as needed.

Sample solution: 0.2 mg/mL of Entecavir in *Solution A* from *Sample stock solution*

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* and *Standard solution*

[NOTE—See *Table 2* for the relative retention times of the components in the *System suitability solution*.]

Suitability requirements

Resolution: NLT 3.5 between entecavir 1-epimer and entecavir; NLT 2.0 between entecavir and 8-hydroxy entecavir, *System suitability solution*

Tailing factor: 0.8–1.5 for entecavir, *System suitability solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of entecavir ($C_{12}H_{15}N_5O_3$) in the portion of Entecavir taken:

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

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

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

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

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

Acceptance criteria: 98%–102% on the anhydrous basis

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, System suitability stock solution, System suitability solution, Sample stock solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution: Use the *Standard solution* from the *Assay*.

Standard solution: 0.2 µg/mL of USP Entecavir Monohydrate RS in *Solution A* from the *Standard stock solution*

System suitability

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

[NOTE—See *Table 2* for relative retention times of the components in the *System suitability solution*.]

Suitability requirements

Resolution: NLT 3.5 between entecavir 1-epimer and entecavir; NLT 2.0 between entecavir and 8-hydroxy entecavir, *System suitability solution*

Tailing factor: 0.8–1.5 for entecavir, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of each impurity in the portion of Entecavir taken:

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

r_U = peak response of each impurity from the *Sample solution*

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

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

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

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Furoentecavir[a]	0.73	1.0	0.1
Entecavir 1-epimer[b]	0.93	1.0	0.1
Entecavir 3-epimer[c]	0.96	1.0	0.1
Entecavir	1.0	—	—
8-Hydroxy entecavir[d]	1.03	0.67	0.1
Entecavir 4-epimer[e]	1.08	1.0	0.1
8-Methoxy entecavir[f]	1.27	0.67	0.1
4-Dimethylsilyl entecavir[g]	1.84	1.0	0.1
Entecavir related compound A	3.41	—	—[h]
Any unspecified impurity	—	1.0	0.1
Total impurities[i]	—	—	0.3

[a] 9-[(3a*S*,4*S*,6*S*,6a*R*)-3a,6-Dihydroxyhexahydro-1*H*-cyclopenta[c]furan-4-yl]guanine.

[b] 9-[(1*R*,3*R*,4*S*)-4-Hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]guanine.

[c] 9-[(1*S*,3*S*,4*S*)-4-Hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]guanine.

[d] 8-Hydroxy-9-[(1*S*,3*R*,4*S*)-4-hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]guanine.

[e] 9-[(1*S*,3*R*,4*R*)-4-Hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]guanine.

[f] 8-Methoxy-9-[(1*S*,3*R*,4*S*)-4-hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]guanine.

[g] 9-[(1*S*,3*R*,4*S*)-4-Hydroxydimethylsilyl-3-(hydroxymethyl)-2-methylenecyclopentyl]guanine.

[h] For information only; quantitated in the test for *Limit of Entecavir Related Compound A*.

[i] Includes the sum of all the impurities found in the tests for *Limit of Entecavir Related Compound A* and *Organic Impurities*.

- **Limit of Entecavir Related Compound A**

Solution A: 0.1% (v/v) trifluoroacetic acid in water

Solution B: 0.1% (v/v) trifluoroacetic acid in acetonitrile

Mobile phase: See *Table 3*. [Note—The gradient elution times are established on an HPLC system with a dwell volume of approximately 1.0 mL.]

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	65	35
8	53	47
8.1	65	35
11	65	35

Standard solution: 2 µg/mL of USP Entecavir Related Compound A RS in methanol

Sample solution: 1.0 mg/mL of Entecavir in methanol. Sonicate as needed.

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

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

Temperatures

Autosampler: 4°

Column: 30°

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.5

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of entecavir related compound A in the portion of Entecavir taken:

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

r_U = peak response of entecavir related compound A from the *Sample solution*

r_S = peak response of entecavir related compound A from the *Standard solution*

C_S = concentration of USP Entecavir Related Compound A RS in the *Standard solution* (mg/mL)

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

Acceptance criteria: NMT 0.1%

SPECIFIC TESTS

- **Water Determination ⟨921⟩, *Method I, Method Ic*:** 5.5%–7.0%
- **Optical Rotation ⟨781S⟩, *Procedures, Specific Rotation***

Sample solution: 10 mg/mL of Entecavir in a mixture of dimethylformamide and methanol (50:50)

Acceptance criteria: +24° to +30°

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light. Store at room temperature.
- **USP Reference Standards ⟨11⟩**

USP Entecavir Monohydrate RS

USP Entecavir Related Compound A RS

3-Benzyl-4-silyl entecavir;
9-[(1*S*,3*R*,4*S*)-4-Dimethylphenylsilyl-3-(benzyloxymethyl)-2-methylenecyclopentyl]guanine.
$C_{27}H_{31}N_5O_2Si$ 485.65

USP Entecavir System Suitability Mixture RS

The mixture contains entecavir monohydrate and the following impurities (other impurities may also be present):
Entecavir 1-epimer.
8-Hydroxy entecavir.
8-Methoxy entecavir.

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

Topic/Question	Contact	Expert Committee
ENTECAVIR	Documentary Standards Support	SM12020 Small Molecules 1
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

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