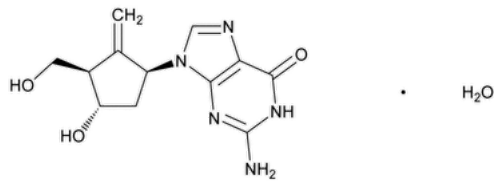


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Entecavir



$C_{12}H_{15}N_5O_3 \cdot H_2O$ 295.29
6H-Purin-6-one, 2-amino-1,9-dihydro-9-[(1S,3R,4S)-4-hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]-, monohydrate;
9-[(1S,3R,4S)-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