Status: Currently Official on 14-Feb-2025
Official Date: Official as of 01-Dec-2017
Document Type: USP Monographs
DocId: GUID-2E5A2907-819F-41CE-9336-B05A133E2A37_1_en-US
DOI: https://doi.org/10.31003/USPNF_M1987_01_01
DOI Ref: u3hu2

Entecavir Tablets

DEFINITION
Entecavir Tablets contain NLT 90.0% and NMT 105.0% of the labeled amount of entecavir ($C_{12}H_{15}N_5O_3$).

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*.

ASSAY
• **Procedure**

Solution A: Acetonitrile, trifluoroacetic acid, and water (1:0.1:99)

Solution B: Acetonitrile, trifluoroacetic acid, and water (30:0.1:70)

Mobile phase: See *Table 1*. [Note—The gradient elution times are established on an HPLC system with a dwell volume of approximately 1.1 mL.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3.5	100	0
21	69	31
24	51	49
27	0	100
28	100	0
35	100	0

Diluent: 0.01 N hydrochloric acid

Standard stock solution: 0.2 mg/mL of USP Entecavir Monohydrate RS prepared as follows. Transfer a suitable quantity of USP Entecavir Monohydrate RS into an appropriate volumetric flask. Dissolve in NMT 20% of the flask volume of methanol, and sonicate if necessary. Dilute with *Diluent* to volume.

Standard solution: 10 µg/mL of USP Entecavir Monohydrate RS in *Diluent* from *Standard stock solution*

Sample solution: Nominally 10 µg/mL of entecavir prepared as follows. Transfer NLT 5 Tablets to an appropriate volumetric flask. Add 80% of the flask volume of *Diluent*, and sonicate for 30 min. Cool to room temperature. Dilute with *Diluent* to volume, and centrifuge for 10 min. Pass the supernatant through a suitable filter, and use the filtrate for analysis.

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Temperatures

Column: 30°

Autosampler: 4°

Flow rate: 1 mL/min

Injection volume: 75 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

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

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

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

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

Acceptance criteria: 90.0%–105.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩

Test 1

Medium: Simulated intestinal fluid TS without enzyme; 1000 mL

Apparatus 2: 50 rpm

Time: 30 min

Mobile phase: Acetonitrile and water (8:92)

Standard stock solution: 0.1 mg/mL of USP Entecavir Monohydrate RS in *Medium* prepared as follows. Transfer a suitable amount of USP Entecavir Monohydrate RS to a suitable flask and add *Medium* to about 66% of the flask volume. Sonicate until dissolved. Dilute with *Medium* to volume.

Standard solution: Dilute an appropriate volume from the *Standard stock solution* in *Medium* to obtain a similar concentration as the *Sample solution*. Prepare fresh on the day of use.

Sample solution: Pass the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 3-µm packing L1

Flow rate: 1 mL/min

Injection volume: 100 µL

Run time: NLT 2 times the retention time of entecavir

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of entecavir ($C_{12}H_{15}N_5O_3$) dissolved:

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

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)

V = volume of *Medium*, 1000 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of entecavir ($C_{12}H_{15}N_5O_3$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium: pH 6.8 phosphate buffer; 1000 mL

Apparatus 2: 50 rpm

Time: 15 min

Buffer: 2 g/L of ammonium acetate in water. Adjust with glacial acetic acid to a pH of 5.0. Pass through a suitable filter.

Mobile phase: Acetonitrile and *Buffer* (7:93)

Standard stock solution: 0.6 mg/mL of USP Entecavir Monohydrate RS in methanol prepared as follows. Transfer a suitable amount of USP Entecavir Monohydrate RS to a suitable volumetric flask and add methanol to about 20% of the flask volume. Sonicate until dissolved. Dilute with methanol to volume.

Standard solution: Dilute an appropriate volume from the *Standard stock solution* in *Medium* to obtain a similar concentration as the *Sample solution*.

Sample solution: Pass the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

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

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 100 µL

Run time: NLT 2 times the retention time of entecavir

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of entecavir ($C_{12}H_{15}N_5O_3$) dissolved:

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

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)

V = volume of *Medium*, 1000 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of entecavir ($C_{12}H_{15}N_5O_3$) is dissolved.

• **Uniformity of Dosage Units ⟨905⟩:** Meet the requirements

IMPURITIES

• **Organic Impurities**

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

Analysis

Sample: *Sample solution*

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

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

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

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: Disregard any peak less than 0.10%.

Individual impurities: NMT 0.5%

Total impurities: NMT 2.0%

SPECIFIC TESTS

• **Microbial Enumeration Tests ⟨61⟩ and Tests for Specified Microorganisms ⟨62⟩:** The total aerobic microbial count is NMT 10^3 cfu/g. The total yeasts and molds count is NMT 10^2 cfu/g. It meets the requirements of the tests for absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers. Store at controlled room temperature.

• **Labeling:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

• **USP Reference Standards ⟨11⟩**

USP Entecavir Monohydrate RS

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

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

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. PF 40(5)

Current DocID: GUID-2E5A2907-819F-41CE-9336-B05A133E2A37_1_en-US

DOI: https://doi.org/10.31003/USPNF_M1987_01_01

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