Entecavir Tablets

Type of Posting	Revision Bulletin
Posting Date	27–Jan–2017
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Expert Committee	Chemical Medicines Monographs 1
Reason for Revision	Compliance

In accordance with the Rules and Procedures of the 2015-2020 Council of Experts, the Chemical Medicines Monographs 1 Expert Committee has revised the Entecavir Tablets monograph. The purpose of the revision is to add *Dissolution Test* 2 for a drug product approved by the FDA. This analytical procedure is validated using Waters Symmetry C18 brand of L1 column. The typical retention time for entecavir is about 5.5 minutes.

Additionally, the calculations in the *Assay* and *Dissolution Test* 1 have been updated to delete the molecular weight correction, because the USP Certificate for the USP Entecavir Monohydrate RS includes the assay value for anhydrous entecavir.

The Entecavir Tablets Revision Bulletin supersedes the currently official monograph. The Revision Bulletin will be incorporated in the *Second Supplement to USP 40–NF 35*.

Should you have any questions, please contact Shankari Shivaprasad, Ph.D. Senior Scientific Liaison (301-230-7426 or sns@usp.org).

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

Change to read:

- **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:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \bullet_{\text{(RB 1-Feb-2017)}} \times 100$$

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)

•(RB 1-Feb-2017)•
Acceptance criteria: 90.0%–105.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩

•**Test 1**• (RB 1-Feb-2017)
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:

$$\text{Result} = (r_U/r_S) \times C_S \bullet_{\text{(RB 1-Feb-2017)}} \times V \times (1/L) \times 100$$

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)
•• (RB 1-Feb-2017)
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.• (RB 1-Feb-2017)

• **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.

Add the following:

•• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.• (RB 1-Feb-2017)

• **USP REFERENCE STANDARDS ⟨11⟩**
USP Entecavir Monohydrate RS