Entecavir Tablets

Type of Posting                Revision Bulletin
Posting Date                  27–Jan–2017
Official Date                 01–Feb–2017
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 (C12H15N5O3).

**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>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>3.5</td>
<td>100</td>
<td>0</td>
</tr>
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<td>28</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>35</td>
<td>15</td>
<td>0</td>
</tr>
</tbody>
</table>

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.)

**Acceptance criteria:** 90.0%–105.0%

**PERFORMANCE TESTS**

**Change to read:**

- **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:** 75 µL
**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 (C12H15N5O3) dissolved:

\[
\text{Result} = \left( \frac{t_1}{t_2} \right) \times \frac{(C_d/C_s)} {\text{· (RB 1-Feb-2017)} \times 100}
\]

\[
r_1 = \text{peak response from the Standard solution}
\]
\[
r_2 = \text{peak response from the Sample solution}
\]
\[
C_s = \text{concentration of USP Entecavir Monohydrate RS in the Standard solution (mg/mL)}
\]
\[
C_d = \text{nominal concentration of entecavir in the Sample solution (mg/mL)}
\]

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Entecavir

\[ r_U = \text{peak response of entecavir from the Sample solution} \]
\[ r_S = \text{peak response of entecavir from the Standard solution} \]
\[ C_S = \text{concentration of USP Entecavir Monohydrate RS in the Standard solution (mg/mL)} \]
\[ V = \text{volume of Medium, 1000 mL} \]
\[ L = \text{label claim (mg/Tablet)} \]

**Tolerances:** NLT 80% (Q) of the labeled amount of entecavir (C\(\text{H}_\text{N}_\text{O}_\text{S}\)) 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

**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.)

**Detector:** UV 254 nm

**Column:** 4.6-mm \(\times\) 25-cm; 5-µm packing L1

**Column temperature:** 40°C

**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\(\text{H}_\text{N}_\text{O}_\text{S}\)) dissolved:

\[ \text{Result} = \left( \frac{r_U}{r_S} \right) \times C_S \times V \times \left( \frac{1}{L} \right) \times 100 \]

\[ r_U = \text{peak response of entecavir from the Sample solution} \]

**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:

\[ \text{Result} = \left( \frac{r_U}{r_T} \right) \times 100 \]

\[ r_U = \text{peak response of each individual impurity from the Sample solution} \]

\[ r_T = \text{sum of all the peak responses from the Sample solution} \]

**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.

**USP Reference Standards (11)**

USP Entecavir Monohydrate RS