A VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF EMTRICITABINE IN BULK AND CAPSULES

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Abstract

A rapid, precise, accurate, specific and simple RP-HPLC (reversed phase – high performance liquid chromatography) method was developed for the assay of Emtricitabine from tablets. A High performance liquid chromatograph 10AT SHIMADZU- SPD10A, using Phenomenex - Luna RP-18(2),250X4.6mm, 5 µm column, with a mobile phase composition of buffer : acetonitrile [85:15 %(v/v)] was used. The flow rate of 1.0 mL min⁻¹ and the effluent was detected at 280 nm. The retention time of Emtricitabine was 9.341 minutes. Linearity was observed over the concentration range of 20-600 µg mL⁻¹. The limit of detection was found to be 5.539µg mL⁻¹ while the quantification limit was 16.786µg mL⁻¹. The accuracy of the proposed method was determined by recovery studies and was found to be 99.468% to 101.110 %. The commercial tablet formulation was successfully analyzed using the developed method and the proposed method is applicable to stability studies and routine analysis of Emtricitabine in bulk and pharmaceutical formulations.

The proposed method was validated for various ICH (International Conference on Harmonization) parameters like linearity, limit of detection, limits of quantification, accuracy, precision, range and specificity.

Keywords: Emtricitabine, RP-HPLC, Stability studies, ICH guidelines

Introduction

Emtricitabine is chemically 4-amino-5-fluoro-1-[(2R, 5S)-2-(hydroxymethyl)-1, 3-oxathiolan-5-yl] pyrimidin-2-one (Fig. 1). It is a white
crystalline powder used as an antiretroviral agent, for the treatment of HIV infection. It has an empirical formula of C₈H₁₀FN₃O₃S and the molecular weight of 247.2470. Emtricitabine belongs to a class of antiretroviral drugs known as nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1) [1]. A literature survey reveals that very few analytical methods have been established for the estimation of Emtricitabine: simultaneous ultraviolet spectrophotometric estimation of Tenofovir and Emtricitabine in Bulk and in Tablet dosage form [2], a validated RP - HPLC Method for Simultaneous Estimation of Emtricitabine and Tenofovir in a Tablet Dosage Form [3], a simple HPLC method for quantitation of Emtricitabine in capsule dosage form [4], Simultaneous determination of Emtricitabine and Tenofovir by area under curve and dual wavelength spectrophotometric method [5], emtricitabine: Inhibitor and substrate of multidrug resistance associated protein [6].

Figure 1
Chemical structure of Emtricitabine

There was no reported stability-indicating analytical method for the analysis of Emtricitabine in the presence of its degradation products in bulk and pharmaceutical dosage forms. The objective of this work was to develop a new, simple, economic, rapid, precise, and accurate stability-indicating HPLC method for the quantitative analysis of Emtricitabine, and to validate the method in accordance with ICH guidelines [7] with shorter retention time, runtime, and economic mobile phase.

Materials and Methods
Pure standard of Emtricitabine (assigned purity 99.98%) was obtained as a gift sample from Ranbaxy labs Pvt. Ltd, Gurgaon, India. The gift samples were used as standard without further purification. HPLC grade water, acetonitrile and methanol (Qualigens), hydrochloric acid, sodium hydroxide, potassium dihydrogen phosphate (S.D. fine chemicals, Mumbai, India), were used throughout the experiment. The commercial pharmaceutical preparation (Emtriva®) which claimed to contain 200mg of Emtricitabine was used in analysis. The chemical structure and purity of the
sample obtained was confirmed by TLC (thin layer chromatography), IR (Infrared spectroscopy), melting point studies.

**Instrumentation and chromatographic conditions**

A high performance liquid chromatography system, Shimadzu pump LC-10AT VP equipped with universal injector (Hamilton 25 µL) SPD10A, UV-VIS detector SPD10A-10A VP (Shimadzu) was used. Isocratic elution of mobile phase comprising buffer 85% and acetonitrile 15% [(Solvent A) - Buffer: 100 g of potassium dihydrogen phosphate dissolved in 800 mL of water, the pH was adjusted to 2.5 with hydrochloric acid and the volume was brought to 1000 mL with water (solvent B); Acetonitrile] with a flow rate of 1.0 mL min⁻¹ was performed on a C18 column (250x 4.6 mm, 5µm). The effluent was detected at 280 nm. The retention time of Emtricitabine was 9.341 minutes. The column temperature was maintained at room temperature level and the volume of injection was 20 µL. Prior to injection of analyte, the column was equilibrated for 30-40 min with the mobile phase.

**Preparation of mobile phase**

The HPLC grade solvents were used for the preparation of mobile phase, isocratic elution of mobile phase comprising buffer 85%, and acetonitrile 15%. The contents of the mobile phase were filtered before use through a 0.45µm membrane filter, sonicated and pumped from the solvent reservoir to the column at a flow rate of 1 mL/min.

**Preparation of standard solution**

A stock solution of drug was prepared by dissolving 100 mg of pure Emtricitabine in a 100 mL volumetric flask containing a sufficient amount of methanol (HPLC grade) to dissolve the drug, it was sonicated for about 15 min and then brought to volume with mobile phase. Daily working standard solutions of Emtricitabine were prepared by suitable dilution of the stock solution with the mobile phase. Six sets of drug solution were prepared in the mobile phase containing Emtricitabine at a concentration of 250-500µg/mL. Each of these drug solutions (20µL) was injected six times into the column, the peak area and retention times were recorded.

**Procedure for sample solution**

Twenty capsules were accurately weighed and the outer shells of these capsules were removed, the powder of these twenty capsules being collected. An amount of the powder, equivalent to 200 mg of Emtricitabine (the content of one capsule) was dissolved in 50 mL of mobile phase. The solution was stirred for 10 min using a magnetic stirrer and filtered into a 100 mL volumetric flask through a 0.45 µm membrane filter. The residue was washed 3 times with 10 mL of mobile phase, and then the volume was
completed to 100 mL with the same solvent. Further it was added mobile phase in order to obtain a stock solution of 1000µg/mL. An aliquot of this solution (1 mL) was transferred to a 10 mL volumetric flask and brought to volume with the mobile phase to give an expected concentration of 100µg/mL. All determinations were conducted in triplicate.

**Stability studies**

**Thermal degradation at different temperature levels and different time interval**

About 2 to 3 g of sample were exposed at different time intervals: 0, 90 and 180 days and at different temperatures: -20°C, 25°C and 40°C.

**Photochemical degradation**

The photochemical stability of the Emtricitabine was studied by exposing the methanolic stock solution to direct sunlight for 8 h (from 9 AM to 5 PM, at 20°C).

**Thermal stress (test sample exposed to sunlight)**

About 2 to 3 g of sample were transferred into a clean dry watch glass and spread evenly. It was exposed to sunlight for 10 hours. After the sample got exposed to the prescribed time, 25 mg of sample were accurately weighed into a clean dry 50 mL volumetric flask, dissolved and diluted to the mark with mobile phase, finally making a concentration of 100µg/mL with mobile phase and injecting 20µL of this sample into HPLC system, observing the degradation.

**Forced degradation of Emtricitabine and capsules of Emtricitabine**

In order to establish whether the analytical method and the assay were stability indicating, the tablets and pure active pharmaceutical ingredient of Emtricitabine were stressed under various conditions to promote degradation. As this drug was freely soluble and stable in methanol this solvent was used in all forced degradation studies. All solutions used in forced degradation studies were prepared by dissolving Emtricitabine or the drug product in a small volume methanol and later diluted with 3% hydrogen peroxide, 0.1N hydrochloric acid and 0.1N sodium hydroxide to achieve the concentration of 100µg/mL and inject 20µL of this sample into HPLC system, observing the degradation.

**Results and Discussion**

**Stability study**

The stability study was carried out by employing the following tests: hydrolysis (neutral, acidic and basic), photolysis and thermolysis. No decomposition was observed when Emtricitabine was exposed to sunlight, temperature, UV light whereas significant changes (about 20 to 25 %) were
observed when the sample was treated with 0.1N NaOH and 0.1N HCl. The sample treated with 3 % H₂O₂ was almost completely degraded.

**Validation of analytical method**

**Linearity**

Acceptance criteria: Coefficient of correlation (r²) should be greater than 0.998

Procedure: A stock solution of drug was prepared by dissolving 100 mg of pure Emtricitabine in a 100 mL volumetric flask containing a sufficient amount of methanol (HPLC grade) to dissolve the drug, was sonicated for about 15 min and then made up to volume with mobile phase. Daily working standard solutions of Emtricitabine were prepared by suitable dilution of the stock solution with the mobile phase. Six sets of the drug solution were prepared in the mobile phase containing Emtricitabine at a concentration of 250-500µg/mL. Each of these drug solutions (20µL) was injected in six concentrations in three replicates times into the column, the peak area and retention times were recorded. (Table I and Figure 2)

<table>
<thead>
<tr>
<th>Table I</th>
<th>Peak Area of Emtricitabine*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicate</td>
<td>Dilution I</td>
</tr>
<tr>
<td>1</td>
<td>33271318</td>
</tr>
<tr>
<td>2</td>
<td>33263258</td>
</tr>
<tr>
<td>3</td>
<td>33123584</td>
</tr>
<tr>
<td>Average</td>
<td>33219387</td>
</tr>
<tr>
<td>SD</td>
<td>83065.360</td>
</tr>
<tr>
<td>RSD%</td>
<td>0.25</td>
</tr>
</tbody>
</table>

*Average of five determinations

![Figure 2](overlay spectra for Linearity Chromatogram)
The correlation coefficient ($r^2$) for Emtricitabine was found to be 0.9992, indicating the linearity and that the method is linear between the concentrations of 250-500µg mL$^{-1}$.

Accuracy

The accuracy is the closeness of the measured value to the true value for the sample. Accuracy was found out by recovery study from prepared solution (three replicates) with standard solution, of the label claim. Aliquots of 1 mL, 2 mL and 3 mL of sample drug (Emtricitabine) solution of 1000µg/mL were pipetted into each of three volumetric flasks. To this 2 mL of standard drug (Emtricitabine) solution of 1000µg/mL was added to each volumetric flask respectively. The volume was made up to 10 mL with mobile phase. 20 µL of each solution was injected and chromatograms were recorded. The range was found between 99.468 to 101.110 % respectively. The % recovery values were obtained within the standard limit which confirms that the method is accurate and free from any positive or negative interference of the excipients.

The results of recovery studies of the drug are presented in table II.

<table>
<thead>
<tr>
<th>Conc. taken in µg/ml (A)</th>
<th>Std addition in µg/ml (B)</th>
<th>Total drug conc. in µg/ml (A+B)</th>
<th>Peak Area*</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>200</td>
<td>300</td>
<td>35856287</td>
<td>99.468</td>
</tr>
<tr>
<td>200</td>
<td>200</td>
<td>400</td>
<td>42323609</td>
<td>100.473</td>
</tr>
<tr>
<td>300</td>
<td>200</td>
<td>500</td>
<td>48838595</td>
<td>101.110</td>
</tr>
</tbody>
</table>

*Average of three determinations

The percentage recovery by the proposed method was ranging from 99.468 to 101.110 % indicating no interference of the tablet excipients with drug under analysis.

Precision

Precision is the measure of repeatability or reproducibility and it was determined by injecting 5 times the expected operating range concentration. The chromatograms were recorded to determine the mean standard deviation and the relative standard deviation. (Table III)  

Acceptance criteria: RSD<2.0% for peak area and retention time.
From the above analytical data it is observed that RSD for the assay is 0.19 which indicates that the method is precise and reproducible.

**Specificity**

Specificity is the ability to assess the analyte in the presence of components that may be expected to be present in the sample matrix (USP 2004). For demonstrating the specificity of the method for drug formulation the drug was spiked and the representative chromatogram was recorded (Figure 3).

![Chromatogram registered for the Specificity studies](image-url)
The excipients used in different formulation products did not interfere with the drug peak and thus, the method is specific for emtricitabine.

**Limits of detection and quantification**

The detection limit (LOD) is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. It may be expressed as a concentration that gives a signal-to-noise ratio of 2:1 or 3:1. The lower limit of detection for emtricitabine is 5.539 µg/mL in the reference material and formulation. The limit of Quantification (LOQ) is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. A signal-to-noise ratio of 10:1 can be taken as LOQ of the method. The LOQ values were found to be 16.786 µg/mL for raw material and formulations.

**System suitability**

A solution of 350 µg mL\(^{-1}\) (approx.) of Emtricitabine (in five replicates) was prepared and was injected, then the system suitability parameters were calculated from the following chromatogram (Table IV and Figure 4).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Data obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretical plates per column</td>
<td>4921</td>
</tr>
<tr>
<td>Symmetry factor/Tailing factor</td>
<td>1.21</td>
</tr>
</tbody>
</table>

![Figure 4](image_url)

*Chromatogram registered in order to show the system suitability*
Conclusion
The proposed RP-HPLC method is found to be accurate, precise, linear, stable, specific, and simple, for the quantitative estimation of Emtricitabine in raw material and pharmaceutical formulations. Hence, the present RP-HPLC method is suitable for the routine assay of Emtricitabine in raw materials and in pharmaceutical formulations in the quality control laboratories.

Acknowledgements
The authors thank Ranbaxy labs Pvt. Ltd, Gurgaon, India, for providing a sample of Emtricitabine as a gift.

References
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