

## VALIDATED HPTLC METHOD FOR THE DETERMINATION OF EMTRICITABINE AS BULK DRUG AND IN CAPSULE DOSAGE FORM

Pradeep Kumar<sup>\*1</sup>, S.C.Dwivedi<sup>1</sup>, Ashok Kushnoor<sup>2</sup>

<sup>1</sup>School of Pharmacy, Suresh Gyan Vihar University, Jaipur, Rajasthan, (INDIA)

<sup>2</sup>Shri Gopichand College of Pharmacy, Baghpat, Uttar Pradesh, (INDIA)

E-mail of Corresponding Author: [pradeep\\_alpine@yahoo.co.in](mailto:pradeep_alpine@yahoo.co.in)

### Abstract

A simple, accurate, precise, and rapid high performance thin layer chromatographic method has been developed and validated for the estimation of Emtricitabine in capsule dosage forms. The method employed TLC aluminium plates precoated with silica gel 60 F 254 as the stationary phase. The mobile phase used was a mixture of (Chloroform: Methanol 8.5: 1.5v/v). The detection of spot was carried out at 275nm. The calibration curve was found to be linear between 200 to 2200 ng mL<sup>-1</sup> with regression coefficient of 0.9992. The proposed method can be successfully used to determine the drug content of marketed formulation. The accuracy of the proposed method was determined by recovery studies and found to be 98.79 to 99.61 %. The proposed method is applicable to routine analysis of Emtricitabine in bulk and pharmaceutical formulations. The proposed method was validated according to various ICH parameters like linearity, accuracy, precision, specificity, limits of detection, limits of quantification, range and solution stability.

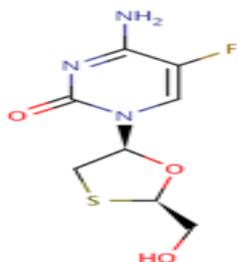
**Keywords:** Emtricitabine, Validation, ICH guidelines, HPTLC

### 1. 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 antiretroviral agents, for the treatment of HIV infection. It has an empirical formula of C<sub>8</sub>H<sub>10</sub>FN<sub>3</sub>O<sub>3</sub>S and 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)<sup>1</sup>. Literature survey reveals that very few analytical methods has been established for estimation of Emtricitabine viz. Simultaneous ultraviolet spectrophotometric estimation of Tenofovir and Emtricitabine in Bulk and in Tablet dosage form<sup>2</sup>, A Validated RP - HPLC Method for Simulataneous

Estimation of Emtricitabine and Tenofovir in a Tablet Dosage Form<sup>3</sup>, A simple HPLC method for quantitation of Emtricitabine in capsule dosage form<sup>4</sup>, Simultaneous determination of Emtricitabine and Tenofovir by area under curve and dual wavelength spectrophotometric method<sup>5</sup>, Emtricitabine: Inhibitor and substrate of multidrug resistance associated protein<sup>6</sup>, Extractive spectrophotometric methods for the determination of emtricitabine in dosage form using safranin<sup>7</sup>, Development and validation of Emtricitabine and Tenofovir in Pure and in fixed dose combination by UV Spectrophotometry<sup>8</sup>, Low Level Determinations of Methyl Methanesulfonate and Ethyl Methanesulfonate Impurities in Emtricitabine Active Pharmaceutical Ingredient by LC/MS/MS Using Electropray Ionization<sup>9</sup>.

Fig. 1: Chemical structure of Emtricitabine



The objective of this work was to develop a new, simple, economic, rapid, precise, and accurate HPTLC method for quantitative analysis of Emtricitabine, and to validate the method in accordance with ICH guidelines<sup>10</sup>.

## 2. Materials and Methods

### 2.1. Materials

Pure standard of Emtricitabine (Assigned purity 99.98%) was obtained as a gift sample from Ranbaxy labs Pvt. Ltd, Gurgaon, India. The gift sample was used as standard without further purification. Silica gel 60 F 254 TLC plates (20x10cm) were used as stationary phase. All chemicals and reagents used were of analytical grade and obtained from Qualigens. Commercial pharmaceutical preparation (Emtriva) which was claimed to contain 200mg of Emtricitabine is used in analysis. The chemical structure and purity of the sample obtained was confirmed by TLC, IR, Melting point studies.

### 2.2. Equipment

The instrument used in the present study was Camag Linnomat V- automatic sample applicator, Hamilton syringe (100 $\mu$ l), Camag TLC scanner 3, Camag Twin trough chamber of appropriate size, Analytical weighing balance (Shimadzu AX 200), Sonicator (model SONICA 2200MH) were used throughout the experiment. Camag Wincats software was used for acquisition, evaluation and storage of chromatographic data.

### 2.3. Preparation of Standard Solution

A stock solution of drug was prepared by dissolving 100 mg of Pure Emtricitabine in a 100 ml volumetric flasks containing sufficient amount of methanol to dissolve the drug, sonicated for about 15 min and then made up to volume with methanol (1 mg/ml). A standard solution was prepared by dilution of the stock solution with methanol to give in concentration of 100 $\mu$ g/ml. Further dilutions were made with methanol to give a solution in concentration range of 200-2200ng/ml.

### 2.4. Procedure for Sample Solution (From Formulation)

To determine the content of the drug in a solid dosage form, 20 capsules of emtricitabine (200mg) were accurately weighed, their average weight was calculated. Powder equivalent to 200 mg of the drug (content of one capsule) was dissolved in sufficient amount of methanol to dissolve the drug, sonicated for about 15 min. and then filtered into a 100 ml volumetric flask through 0.45  $\mu$ m membrane filter. The residue was washed 3 times with 10 ml of methanol, and then the volume was completed to 100 ml with the same solvent. Make further dilutions with methanol to obtain a stock solution of 10 $\mu$ g/ml. An aliquot of this solution (1 ml) was transferred to a 10 ml volumetric flask and made up sufficient volume with the methanol to give an expected concentration of 1  $\mu$ g/ml.

### 2.5. Prewashing of TLC plates

HPTLC was performed on 20 cm  $\times$  10 cm precoated silica gel 60 F 254 TLC plates. The adsorbent has a very large surface area; it may absorb air and other impurities from atmosphere, particularly volatile impurities, after the pack has been opened. The non-volatile impurities adsorbed by layer can lead to irregular baseline in scanning densitometry. To avoid possible interference from such impurities in quantitative analysis, plates were

prewashed with methanol, dried, and activated for 30 min. at 110 C, with the plates being placed between two sheets of glass to prevent deformation of the aluminum during heating.

## 2.6. Procedure

A methanolic solution of Emtricitabine (1 mg/ml) was prepared. This solution was further diluted with methanol to yield a solution containing 1 $\mu$ g/ml. Different concentrations of Emtricitabine in a concentration range of 200- 2200ng/ml were applied on plates as 8 mm bands, 8 mm apart and 1 cm from edge of the plate, by means of Camag Linomat V automatic sample applicator fitted with 100  $\mu$ l Hamilton syringe. A methanol blank was applied to parallel track. The mobile phase, Chloroform: Methanol 8.5: 1.5v/v was poured into the twin trough glass chamber and the glass chamber left to equilibrate for 10 min at 25  $\pm$  2<sup>0</sup> C. After that the plate was placed in Camag twin trough glass chamber. After development, the plate was removed from the chamber, dried in current of hot air, and scanned at 275 nm, using a deuterium lamp, by means of Camag TLC scanner III densitometer. Densitograms were obtained by HPTLC of Emtricitabine at various concentrations. This method was followed for all quantitative analysis. The Wincats software was used for data acquisition and processing of the plate. Peak height and peak area were integrated for the entire track. The calibration curve was established by plotting the obtained peak area on ordinate against corresponding concentration on abscissa.

### 2.6.1 Validation of Analytical Method

Validation of an analytical method is process to establish by laboratory studies that the performance characteristics of the method meet the requirements for the intended analytical application. Performance characteristics are expressed in terms of analytical parameters.

Typical analytical parameters used in validation area:

1. Linearity
2. Accuracy
3. Precision
4. Specificity
5. Limit of detection
6. Limit of quantification
7. Range
8. Solution stability

### 2.6.2. Linearity

**Acceptance criteria:** Coefficient of correlation (r<sup>2</sup>) 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 flasks containing sufficient amount of methanol to dissolve the drug, sonicated for about 15 min and then made up to volume with methanol (1 mg/ml). A standard solution was prepared by dilution of the stock solution with methanol to give in concentration of 100 $\mu$ g/ml. Further dilutions were made with methanol to give a solution in concentration range of 200-2200ng/ml. (Graph No. 1 and chromatogram No. 1)

### 2.6.3. Accuracy

The accuracy is the closeness of the measured value to the true value of the sample. To evaluate the accuracy of the method, known amount of pure drug was added to the previously analyzed solution containing pharmaceutical formulation and the mixture was analyzed by the proposed method and the recoveries were calculated. Accuracy was found out by recovery study from prepared solution (three replicates) with standard solution, of the label claim. Aliquots of 0.1 ml, 0.5ml and 0.9 ml of sample drug (Emtricitabine) solution of 10 $\mu$ g/ml were pipetted into each of three volumetric flasks. To this 0.5 ml of standard drug (Emtricitabine) solution of 10 $\mu$ g/ml was added to each volumetric flask respectively. The volume was made

up to 10 ml with methanol. The range of recovery studies were found between 98.79 to 99.61 %. The values of recovery justify the accuracy of the method. 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. (Table No. 1)

#### 2.6.4. Precision

Precision is measure of repeatability or reproducibility and it was determined by injecting 5 times the expected operating range concentration. The chromatograms were recorded to determine mean standard deviation and relative standard deviation. (Table No. 2)

**Acceptance criteria:** RSD<2.0% for peak area.

#### 2.6.5. 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 drugs was spiked and observe the chromatogram (chromatogram No.2).

#### 2.6.6. 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 4.320ng/ml in reference material and formulation. Limit of Quantification (LOQ) is the lowest amount 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 13.092ng/ml for raw material and formulations.

#### Range

The specific range derived from the linearity studies. The range was calculated from the linearity graph. From the lower to higher concentration between which the response is linear, accurate and precise.

**Acceptance criteria:** RSD < 2.0

#### 2.6.7. Solution Stability

The solution stability of the standard and sample prepared in methanol was studied for 5 days at bench top. The solution under study was compared with freshly prepared standard solution

### 3. Results and Discussion

**3.1. Linearity:** Correlation coefficient ( $r^2$ ) for Emtricitabine was found to be 0.9992, indicating the linearity and the method is linear between the concentrations of 200-2200ng/ml with Rf value 0.56±0.02.

**3.2. Accuracy:** The percentage recovery by the proposed method was ranging from 98.79 to 99.61 % indicating no interference of the capsule excipients with drug under analysis.

**3.3. Precision:** From the analytical data it is observed that RSD for the assay is 0.346 which indicates that the method is precise and reproducible.

#### 3.4. Specificity:

The excipients used in different formulation products did not interfere with the drug peak and thus, the method is specific for Emtricitabine.

**3.5. Limits of detection and Quantification:** The LOQ values were found to be 13.092ng/ml for raw material and formulations.

**3.6. Range:** The range for Emtricitabine was found to be 200-2200 ng/ml.

**3.7. Solution Stability:** When The solution under study was compared with freshly prepared standard solution, the samples were found to be stable for period of more than 48 hours.

### Conclusion

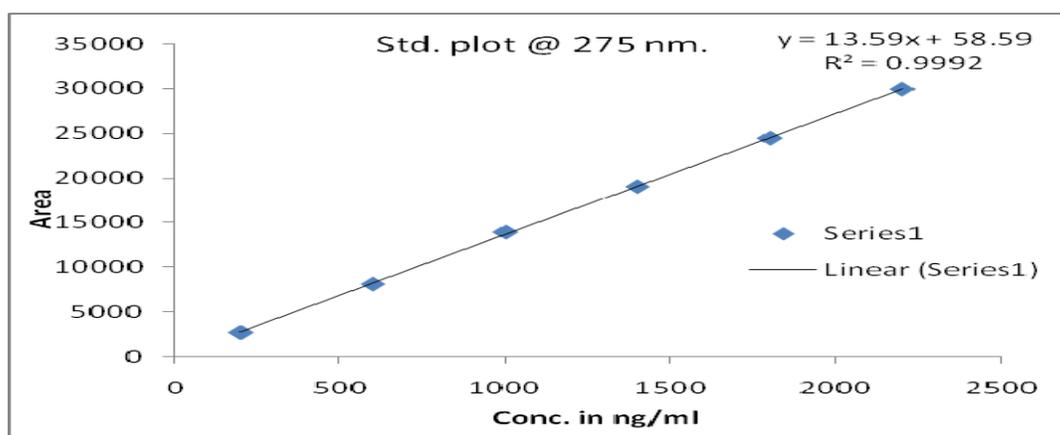
The proposed HPTLC method is found to be accurate, precise, linear, stable, specific, and simple, for quantitative estimation of Emtricitabine in raw material and pharmaceutical formulations. Hence the present HPTLC method is suitable for routine assay of Emtricitabine in raw materials and in pharmaceutical formulations in the quality control laboratories.

### Acknowledgement

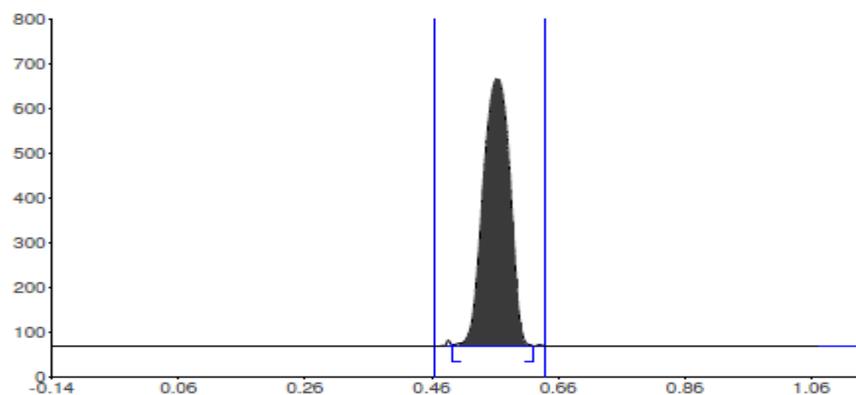
The authors thank Ranbaxy labs Pvt. Ltd, Gurgaon, India, for providing a sample of Emtricitabine as a gift.

### References

- 1 <http://www.drugbank.ca/drugs/DB00879>
- 2 T. Sudha, J. Saminathan, P.V. Hemalatha, V.R. Ravikumar, International Journal of Biopharmaceutics, 2010:1, 26-30
- 3 Rajesh Sharma, Pooja Gupta, Eurasian J. Anal. Chem. 2009, 4(3): 276-284
- 4 Arjun Goje, Sathis Kumar. D, Yogeswaran. P, Sathyabrata Jena, David Banji, KNV Rao, Der Pharma Chemica. 2010, 2(2): 281-285
- 5 Swapnil A Ghorpade, Monali S. Sali, Atul H. Kategaonkar, Dhaval M. Patel, Vishnu P Choudhari, Bhanudas S Kuchekar, J. Chil. Chem. Soc. 2010, 55(1):115-117
- 6 Laurence Bousquet, Alain Pruvost, Nathalie Didier, Robert Farinotti, Aloïse Mabondzo, European Journal of Pharmaceutical Sciences.2008, 35(4):247-256
- 7 S. Sharma, M.C. Sharma, American-Eurasian journal of toxicological sciences 2011, 3(3): 138-142
- 8 K. Anandakumar, K. Kannana, T. Vetrichelvan, Digest Journal of Nanomaterials and Biostructures 2011, 6(3): 1085-1090
- 9 P.R. Kakadiya, T.G. Chandrashekhar, S. Ganguly, D.K. Singh, V. Singh, Analytical Chemistry Insights 2011,6: 21-28
- 10 International Conference on Harmonization (ICH), Validation of Analytical Procedures: Text and Methodology Q2 (R1), November 2005



Graph No. 1



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.49	3.0	0.56	597.6	100.00	0.62	1.2	19060.7	100.00

Chromatogram No. 1

Table No. 1

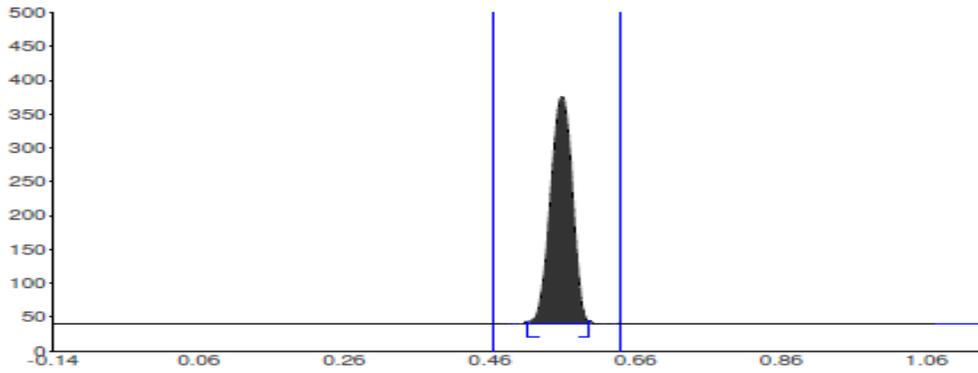
Conc. taken in ng/ml (A)	Std addition in ng/ml (B)	Total drug conc. in ng/ml (A+B)	Peak Area*	% Recovery	Average	% RSD
100	500	600	8009.8	98.79	99.26333	0.427571
500	500	1000	13842.9	99.39		
900	500	1400	19008.2	99.61		

\*Average of three readings

Table No.2

Area Response*	
S.No.	
1	9140.7
2	9167.2
3	9097.5
4	9178.6
5	9158.8
Average	9148.56
S.D	31.71597
R.S.D	0.346677

\*Average of three readings



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.51	3.2	0.56	336.1	100.00	0.60	4.2	8113.4	100.00

**Chromatogram No. 2**