# Development and validation of RP-HPLC methods for estimation of Emtricitabine

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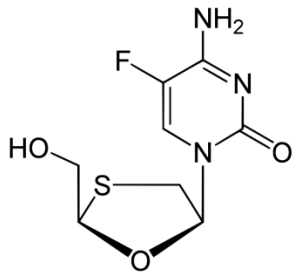
***Abstract :*** High-performance liquid chromatographic (HPLC) was developed and validated for the quantitative determination of Emtricitabine. The RP-HPLC method was developed by the isocratic technique on a reversed-phase, Phenomenex column. The retention time for EC was 5.9 min, this method can be a cheap, reliable and less time consuming chromatographic analysis. The proposed method is highly sensitive, precise and accurate and hence successfully applied for determining the assay of a marketed formulation.

***Key Words:*** *Emtricitabin, Correlation equation, retention time, RP-HPLC.*

## INTRODUCTION

Emtricitabine is a nucleoside analogue and reverse transcriptase inhibitor used for the treatment and prevention of human immunodeficiency virus (HIV) infection and the acquired immunodeficiency syndrome (AIDS). Its chemical name is 4-amino-5-fluoro-1-[(2R,5S)-2(hydroxymethyl)-1,3-oxathiolan-55-yl]-1,2-dihydropyrimidine-2-one.A fixed-dose triple combination of Emtricitabine, Tanofavir and efavirenz (Sustiva, marketed by Bristol-Myers Squibb) was approved by the U.S. Food and Drug Administration (FDA) on July 12, 2006 under the brand name Atripla. Emtricitabine/Tanofavir was approved for medical use in the United States in 2004 (WHO, 2019).




Literature survey reveals stability indicating UV spectrophotometric, RP-HPLC (Gerber, et.al., 2014, Morgan,et.al.,2015, Karger, et.al.,1997, Henry, et.al., 2009). HPLC in human serum, HPTLC (Geiss. et,al.,1987, Jork.et.al.,1990, Hahn.et.al.,2000) and LC–MS (Chaimbault,et.al.,2014, Dass.et.al.,2007, Pitt.et.al.,2017 Niessen.et.al.,2006) method for the estimation of EC. Several analytical methods have been reported for the quantitative determination of EC such as high performance liquid chromatography, HPTLC- densitometry and LC–MS . A new analytical method needs to be established. In the present work we have developed a simple UV method for estimation of EC correlation equation; obtained from absorbance maxima of EC. The values obtained from the developed equation were also verified using HPLC. The existing HPLC method was further modified to reduce the retention time. Both these methods are used to calculate drug content in marketed formulation and in dissolution media.

## RESULTS AND DISCUSSION

## MATERIALS AND METHODS :

### EMTRICITABINE :

The Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) was carried out on Waters Alliance Series with 2695 Separations Module and 2998 Photodiode Array Detector using Empower 2 software.

### ANALYTICAL CONDITIONS

The active ingredient content of Emtricitabine was determined by HPLC analysis. The following HPLC conditions were used in the analysis.

| Mobile phase | Column | Column Specifications | Column Temperature | Flow Rate | Injection Volume | Detector | Detector Wave length | Retention Time |
|---|---|---|---|---|---|---|---|---|
| Acetonitrile : HPLC Water adjusted pH 4.0 with Orthophosphoric acid (75 : 25% v/v) | Phenomenex | 250 mm x 4.6 mm, 5µm | 30 °C | 1.0 mL/min | 10µL | PDA | **230nm** | 5.9min (approximately) |

**Table 1**

### RECOMMENDED PROCEDURES:

After systematic and detailed study of the various parameters involved, as described under results and discussion in this paper, the following procedure was recommended for the determination of ECB in bulk samples and pharmaceutical formulations.

**PREPARATION OF SOLUTIONS:**

**PREPARATION OF STANDARD SOLUTION:**

0.01840 g [18.40 mg] of Emtricitabin standard was weighed accurately into a clean and dry 25 ml volumetric flasks and dissolved with 5 ml of Acetonitrile (HPLC grade) and made upto the mark with the same solvent. These solutions were marked as R1A respectively.

**PREPARATION OF SAMPLE SOLUTION:**

0.01820 g [18.20mg], 0.02030 g [20.30mg], 0.01860 g [18.60mg], 0.01850 g [18.50 mg] and 0.01880 g [18.80mg] of Emtricitabine was weighed accurately into a clean and dry five separate 25 ml volumetric flasks and dissolved with 10 ml of Acetonitrile (HPLC grade) respectively and made upto the mark with same solvent.

**PREPARATION OF LINEARITY SOLUTIONS:**

0.03320 g [33.20 mg] of Emtricitabine standard (99.5%) was weighed accurately into a clean and dry 10 ml volumetric flask and dissolved with 5 ml acetonitrile (HPLC grade) and made up to the mark with the same solvent. This prepared solution is equivalent to 3303 mg/L. Lower concentrations were made by appropriate dilutions as mentioned in the table 2.22.

| Concentration of solution taken for dilution (µg/ml) | Dilution details | | Concentration of diluted solution (µg/ml) |
|---|---|---|---|
| | Volume taken (ml) | Volume made up (ml) | |
| 3303 | 7.569 | 10 | 2500 |
| 2500 | 8.000 | 10 | 2000 |
| 2000 | 7.500 | 10 | 1500 |
| 1500 | 6.667 | 10 | 1000 |
| 1000 | 5.000 | 10 | 500 |

**Table 2.22.: Dilution details linearity solutions**

**PREPARATION OF FORTIFICATION STANDARD SOLUTION**0.01850 g [18.50 mg] and 0.01840 g [18.40 mg] of Emtricitabine standard was weighed accurately into two separate clean and dry 25 ml volumetric flasks and dissolved with 10 mL of acetonitrile (HPLC grade) and made upto the mark with the same solvent. These solutions were marked as R1A and R1B respectively

**PREPARATION OF 5 G/KG FORTIFICATION LEVEL SOLUTION**

0.0186 g [18.6 mg] of Emtricitabine technical (test item) was weighed accurately into a clean and dry 25 ml volumetric flask, 0.093 ml (93 µL) of linearity prepared solution of (1000 µg/ml) was added to it and dissolved with acetonitrile (HPLC grade) and made upto the mark with the same solvent. This solution marked as T1 was equivalent to dose concentration (5 g/kg).

**PREPARATION OF 10 G/KG FORTIFICATION LEVEL SOLUTION**

0.0186 g [18.6 mg] of Emtricitabine technical (test item) was weighed accurately into a clean and dry 25 ml volumetric flask, 0.186 ml (186 µL) of linearity prepared solution of (1000 µg/ml) was added to it and dissolved with acetonitrile (HPLC grade) and made upto the mark with the same solvent. This solution marked as T2 was equivalent to dose concentration (10 g/kg).

**DETERMINATION OF ACTIVE INGREDIENT CONTENT**

**PREPARATION OF STANDARD SOLUTION OF EMTRICITABIN**

0.01850 g [18.50 mg] and 0.01840 g [18.40 mg] of Emtricitabine standard was weighed accurately into two separate clean and dry 25 ml volumetric flasks and dissolved with 10 ml of acetonitrile (HPLC grade) and made upto the mark with the same solvent. These solutions were marked as R1A and R1B respectively.

**PREPARATION OF SAMPLE SOLUTION FOR ACTIVE INGREDIENT**

0.01820 g [18.20 mg] and 0.01850 g [18.50 mg] of Emtricitabine TC was weighed accurately into two different clean and dry 25 ml volumetric flasks and dissolved with 10 ml of acetonitrile (HPLC grade) and made up to the mark with same solvent. These solutions were marked as R2A and R2B respectively.

**LINEARITY**

| Sample code | Standard CS1 | Standard CS2 | Standard CS3 | Standard CS4 | Standard CS5 | Slope | Correlation Co-efficient | Intercept |
|---|---|---|---|---|---|---|---|---|
| Conc. (µg/ml) | 500 | 1000 | 1500 | 2000 | 2500 | | | |
| Observed Area | 6971035 | 12174534 | 17394224 | 22615938 | 27636329 | 10354 | 0.999 | 1826814 |

**Table No.2.23: Results of linearity.**

Each of these solutions (2500, 2000, 1500 ,1000 and 500 mg/L) were injected into HPLC under the given conditions and the peak area was recorded and a graph of detector response (peak area) versus concentration in mg/L was plotted. The values for slope (m), intercept (b) and the linear regression coefficient (R2) were calculated.

The detector response to varying concentrations of Emtricitabin was found to be linear (R2 = 0.9998) in the range of 500 to 2500 mg/L. The plot of concentrations versus detector response along with the regression parameters was attached and the results are presented in Table. No. 2.23

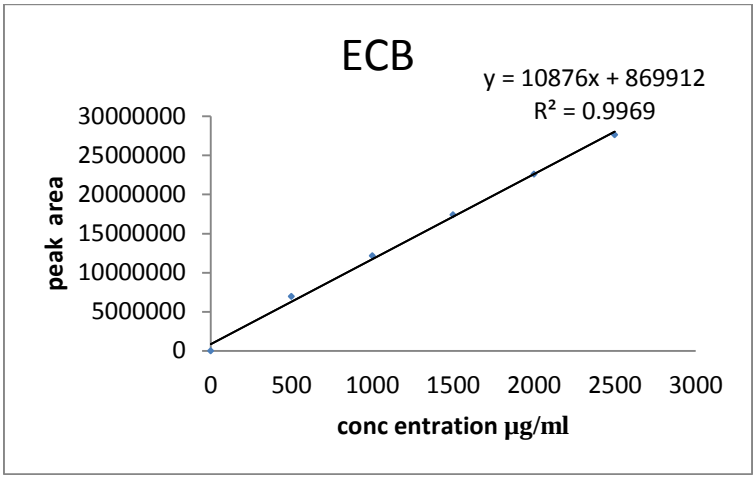



**Fig. Linearity curve of Emtricitabin reference item**

**ACTIVE INGREDIENT CONTENT**

The active ingredient is the drug substance and expedients which are ingredient of the tablet. it has the property of long term stabilization, and bulking of up solid formulation which contain small amount to a therapeutic enhancement. The above prepared Emtricitabine standard solutions and sample solutions were injected into a High performance liquid chromatography (HPLC) by using the analytical conditions. Emtricitabine active ingredient content, present in the sample was quantified by injecting reference standard and comparing the peak area of standard with the peak area of sample. The results are presented, in table no.2.26.

| Sample Code | Standard Wt (mg) | Standard Purity (g/kg) | Standard Area | Mean Standard Area | Sample Area | Sample Wt (grams) | A.I. Content (g/kg) | A.I. Content (% w/w) | Mean A.I. Content (% w/w) |
|---|---|---|---|---|---|---|---|---|---|
| R1A1 | 18.50 | 995.00 | 8686035 | | | | | | 97.70 |
| R2A1 | | | | 8686715 | 8384660 | 18.20 | 976.23 | 97.62 | |
| R2A2 | | | | | 8397127 | | 977.68 | 97.77 | |
| R1B1 | 18.40 | | 8678334 | | | | | | |
| R1A2 | 18.50 | | 8687395 | | | | | | 97.66 |
| R2B1 | | | | 8677025 | 8559508 | 18.50 | 976.22 | 97.62 | |
| R2B2 | | | | | 8566189 | | 976.98 | 97.70 | |
| R1B2 | 18.40 | | 8675715 | | | | | | |
| Mean | | | | | | | | | 97.68 |
| Standard deviation | | | | | | | | | 0.03 |
| Relative standard deviation (%RSD) | | | | | | | | | 0.03 |

**Table 2.26 Results of Active Ingredient:**

**CONCLUSIONS**

Emtricitabine was evaluated as per the guidelines of ICH. The method was validated for the determination of Active Ingredient Content in EC for the test item EC meets the acceptance criteria. The active ingredient content of the test item EC was found to be 97.68(%w/w) by using HPLC method.

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