



**STABILITY-INDICATING RP-HPLC METHOD FOR ANALYSIS OF  
EMTRICITABINE IN THE BULK DRUG AND IN A PHARMACEUTICAL DOSAGE  
FORM**

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**ABSTRACT**

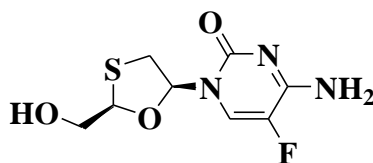
A novel stability-indicating RP-HPLC method has been developed and validated for quantitative analysis of Emtricitabine (EMT) in the bulk drug and in a pharmaceutical dosage form. An isocratic separation of EMT was achieved on Thermo C<sub>18</sub> column (4.6 x 250mm, 5 μ particle size) as the stationary phase with a flow rate of 1 ml/min and using a UV detector to monitor the eluate at 254 nm. The mobile phase consisted of 10mM KH<sub>2</sub>PO<sub>4</sub>: methanol (20:80v/v) and pH adjusted 3.0 by orthophosphoric acid enabled separation of the drug from its degradation products. The method was validated for linearity, accuracy (recovery), precision, specificity, and robustness. The linearity of the method was satisfactory over the range 5-25 μg/ml (correlation coefficient 0.999). The limits of detection and quantification were 0.35 and 1.05 μg/ml respectively. Recovery of EMT from the pharmaceutical dosage form ranged from 98.50 to 99.00%. EMT was subjected to stress conditions (hydrolysis (acid, base), oxidation, photolysis, and thermal degradation) and the samples were analyzed by this method. Extensive degradation was found under acid, photolytic and oxidative stress. The degradation products were well resolved from main peak. The forced degradation study proves the stability indicating power of the method and therefore, the validated method may be useful for routine analysis of EMT as bulk drug, in respective dosage forms, for dissolution studies and as stability indicating assay method in pharmaceutical laboratories and industries.

**Keywords:** RP-HPLC, Emtricitabine, forced degradation, Method validated

**INTRODUCTION**

Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example temperature, humidity, light and enables storage conditions, retest periods and shelf life to be Recommended<sup>1,2</sup>. The two main aspects of study

of the stability of a drug product that play an important role in shelf life determinations are assay of the active drug and the degradation products generated during stability studies <sup>3</sup>. Assay of a drug product in a stability test sample must be performed with stability indicating method, as recommended by the International Conference on Harmonization (ICH) <sup>4</sup>. Emtricitabine (EMT) is a nucleoside reverse transcriptase inhibitor (NRTI). Chemically, it is 5-fluoro-1-(2R, 5S)-[2-(hydroxymethyl)- 1,3-oxathiolan-5-yl] cytosine (Figure 1). EMT is the (–) enantiomer of the thio analog of cytidine, which differs from other cytidine analogs in that it has fluorine in the fifth position. EMT is an antiviral agent used for the prevention of perinatal HIV-1 reverse transcriptase <sup>5</sup>. It is also active against the hepatitis B virus <sup>6,7</sup>. EMT is phosphorylated by cellular enzymes to form EMT 5-triphosphate, which competes with deoxycytidine 5- triphosphate and terminates the amino acid chain of newly forming viral DNA. A literature survey reveals several analytical methods available for the determination of EMT individually and in combination with other drugs by UV <sup>8</sup>, HPLC in pharmaceutical formulations <sup>8</sup>, drug substance, and biological matrices <sup>9-11</sup>, LC/MS/MS <sup>12</sup>, and stability-indicating liquid chromatographic methods <sup>13</sup> were reported. This paper mainly deals with the forced degradation of EMT under the stress conditions such as acidic & basic hydrolysis, oxidation, heat, light and validation of the method for accurate quantification of EMT in the bulk drug & pharmaceutical dosage form.



**Figure 1 Chemical structure of emtricitabine**

## MATERIALS AND METHODS

### Instrument

Liquid chromatographic system from Waters model no 784 comprising of manual injector, water 515 binary pump for constant flow and constant pressure delivery and UV-Visible detector connected to software Data Ace for controlling the instrumentation as well as processing the generated data.

### Reagents and chemicals

Emtricitabine was obtained as pure sample from Hetero Pharma Limited, Hyderabad, India, as gift samples along with their analytical reports. HPLC grade methanol and acetonitrile was obtained from Merck (India) limited. Potassium Dihydrogen orthophosphate and Ortho-phosphoric acid (GR grade) was obtained from S.D. Fine Chemicals Ltd, Mumbai, India. All other chemical used were of analytical grade. Triple distilled water was used for whole experiment was generated in house. Capsules Emtriva, 200 mg, Gilend Sciences Inc. UK, was purchased from local market.

### **Chromatographic conditions**

The isocratic mobile phase consisted of 10mM KH<sub>2</sub>PO<sub>4</sub> (pH-3.0 with OPA): methanol (20:80 v/v), flowing through the column at a constant flow rate of 1.0 ml/ min. The mobile phase was filtered through nylon 0.22 µm membrane filters and was degassed before use (30 min). A Thermo (C-18) Column (5 µm, 250mm x 4.60mm) was used as the stationary phase. By considering the chromatographic parameter, sensitivity and selectivity of method for drugs, 254 nm was selected as the detection wavelength for UV-Visible detector.

### **Standard preparation**

#### *Standard stock solution*

Accurately weighed 10 mg of EMT was transferred into 10 ml volumetric flask, dissolved in 5ml of methanol and volume was made up to 10ml with methanol to get concentration of solution 1000µg/ml (Stock-A), 5ml of stock-A was taken and diluted up to 50ml with methanol to get concentration of 100µg/ml (Stock-B).

#### *Working standard solution*

Working standard solutions were prepared by taking dilutions ranging from 5-25 µg/ml for EMT.

### **Analysis of dosage forms**

For analysis of the capsule formulation (Emtriva 200mg), weight equivalent to weight 10 mg of EMT was transferred to 10 ml volumetric flask and dissolved in methanol. The solution was shaking vigorously for 20 mins and filtered through Whatman filter paper no.41, then volume was made up to mark with methanol. From the above solution 1 ml of solution was taken and diluted to 10 ml with methanol to get a solution containing 100µg/ml. From the above solution 1 ml of solution was taken and diluted to 10 ml with methanol to get a solution containing 10 µg/ml of EMT. The amounts of EMT in capsule formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with capsule formulation.

### **Forced degradation study**

In order to determine whether the method is stability indicating, forced degradation studies were conducted on emtricitabine powder and the analysis was carried out by HPLC with a UV detector. 20µl of each of forced degradation samples were injected.

#### *Acid degradation*

50 mg of EMT sample was taken into a 50 ml round bottom flask, 50 ml of 0.1 M HCl solution was added and contents were mixed well and kept for constant stirring for 8 hr at 80°C. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of EMT.

### ***Alkaline hydrolysis***

50 mg of EMT sample was taken into a 50 ml round bottom flask, 50 ml of 0.1 M NaOH solution was added and contents were mixed well and kept for constant stirring for 8 hr at 80°C. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of EMT.

### ***Oxidative degradation***

50 mg of EMT sample was taken into a 50 ml round bottom flask, 50 ml of 3% hydrogen peroxide solution was added and contents were mixed well and kept for constant stirring for 24 hr at room temperature. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of EMT.

### ***Thermal degradation***

50 mg of EMT sample was taken in to a petri dish and kept in oven at 50°C for 4 weeks. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of EMT.

### **Method validation**

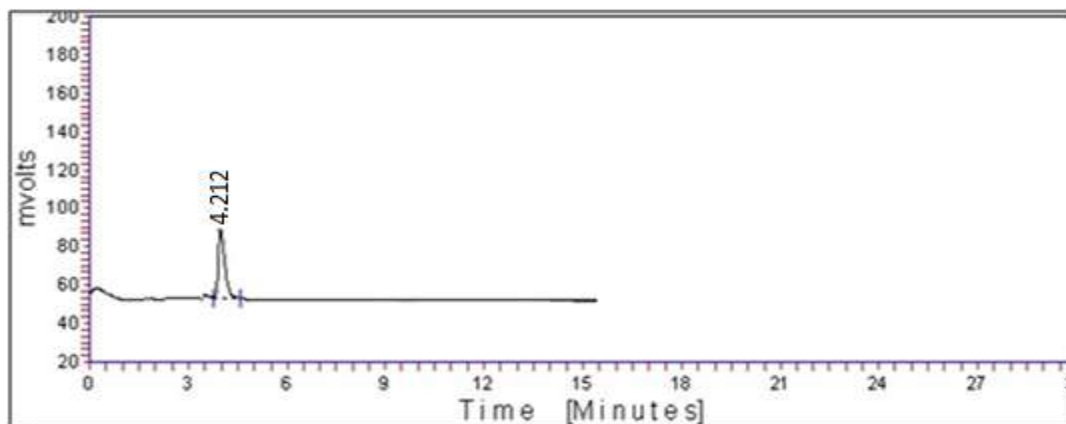
The method was validated for linearity, specificity, limits of detection (LOD) and quantification (LOQ), system suitability, accuracy, precision, robustness and stability in accordance with ICH guidelines. To assess specificity, peak purity was determined by use of the photodiode-array detector. To check linearity, test solutions of EMT were prepared at six concentrations 5-25µg/ml. Each solution was injected in triplicate and calibration graphs were obtained by plotting peak area against concentration. Linearity was checked over the same concentration range on three consecutive days. RSD (%) of the slope and Y-intercept of the calibration plot were also calculated. The limits of detection (LOD) and quantification (LOQ) for EMT were determined, as the amounts for which signal-to-noise ratios were 3:1 and 10:1, respectively, by injecting a series of dilute solutions of known concentration. Precision, as RSD (%) was determined by measuring the concentration of drug in the injection six times. Intermediate (inter-day) precision was evaluated by two analysts on different days in the same laboratory. The accuracy of the method was studied by measurement of recovery after adding known amounts of the drug (80, 100 and 120% of the label claim of known amount of EMT per injection) to the placebo. Three samples were prepared for each recovery level and results were calculated by use of the calibration plot. The robustness of the method was assessed by deliberate alteration of the experimental conditions and determining the effect on resolution of EMT from the main product obtained by degradation under basic conditions. The change was made by altering the pH and / or concentration of the mobile phase to check the method capacity to remain unaffected. The effect of change in pH of

mobile phase, flow rate, mobile phase ratio on the retention time, theoretical plates, area under curve and percentage content of EMT was studied. During these tests all other conditions were held constant at the optimum values. The stability of EMT and sample solutions (at ambient temperature) were tested by analysis after 24, 48 and 72 hrs, comparison of the results with those obtained from freshly prepared standard solutions and calculation of RSD.

## Results and discussion

### *Optimization of chromatographic condition*

The primary objective in developing this stability indicating HPLC method was to achieve resolution between EMT and its degradation products. To achieve this, Waters model no 784 comprising of manual injector, water 515 binary pumps for constant flow and constant pressure delivery and UV-Visible detector connected to software Data Ace for controlling the instrumentation as well as processing the generated data and Thermo C<sub>18</sub> column was employed for envisaged work. Combination of 10mM KH<sub>2</sub>PO<sub>4</sub> (pH-3.0 with OPA): methanol (20:80 v/v), as mobile phase was attempted for quantitation of EMT with acceptable system suitability parameters (RT, tailing factor, number of theoretical plates and HETP) at 254 nm as detection wavelength. Linearity was found 5-25µg/ml with correlation coefficient  $r^2 = 0.999$  having equation as: 24.29 conc. + 4.366. The column temperature was 25°C. The tailing factor for EMT was <2 and retention times were approximately 4.212 ± 0.3 min for main peak and less than 10 min for the degradation products (Figure 2). This low total runs time resulted in high productivity and low cost of analysis as per sample.



**Figure 2 Standard chromatogram of emtricitabine**

### *Forced degradation study*

Bakshi *et al.*<sup>14</sup> suggested target degradation of 20-80% when establishing the stability-indicating properties of analytical methods, because even intermediate degradation products should not interfere with any stage of drug analysis. Although conditions used for forced degradation were adjusted to achieve degradation in this range, this could not be achieved for conditions other than exposure to acid, base and oxidising agent, even after long exposure. Peak purity test results confirmed that the EMT peak

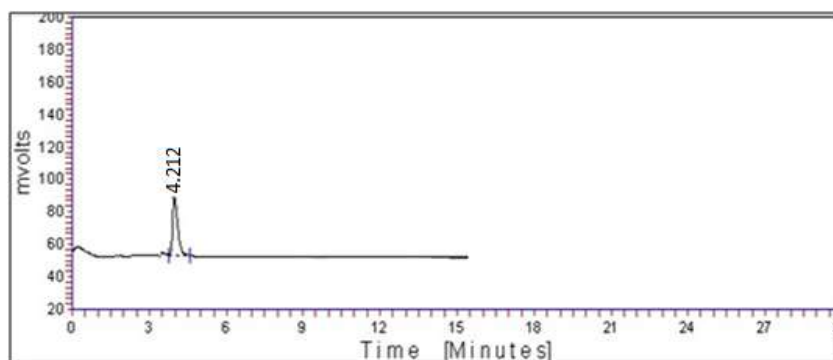
was homogeneous under all the stress conditions tested. The mass balance of EMT in stress samples was close to 100% and moreover, assay of unaffected EMT in the injection confirmed the stability-indicating nature of the method. The results from forced degradation studies are summarized in Table 1. Chromatographic peak-purity data were obtained from the spectral analysis report; peak purity greater than 99 is indicative of a homogeneous peak. The peak purity for EMT from degradation studies was in the range 99.9-100.0, indicating homogeneous peaks and thus establishing the specificity of the method. No degradation peaks co-eluted with the EMT peak, suggesting the method enabled specific analysis of EMT the presence of its degradation products.

**Table 1 Results of forced degradation studies of emtricitabine**

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.15	0
Acidic hydrolysis	85.98	14.02
Alkaline hydrolysis	92.32	7.68
Oxidative degradation	89.65	10.35
Photolytic degradation	87.12	12.88

### **Method validation**

Peak purity was >99.9% for drug substance and drug degradation products at 254 nm, which showed that the analyte peaks were pure and that formulation excipients and degradation products were not interfering with analyte peaks Figure 3. LOD and LOQ for EMT were 0.35 and 1.05 $\mu$ g/ml, respectively for 20 $\mu$ l injection volumes. Results from regression analysis are listed in Table 2 with system suitability data. When precision was determined by six fold analysis of drug injection, the RSD of ERP peak area was less than 2%, indicating that the method is reliable. Results from assessment of precision are listed in Table 3. Results obtained from determination of recovery are listed in Table 4 and capsules analysis was shown in Table 5.



**Figure 3 Chromatograms of emtricitabine (15 $\mu$ g/ml) in a capsule formulation**

**Table 2 Results from regression analysis and system suitability data**

Parameters	Emtricitabine
Retention time*	4.212 ± 0.3 min
Tailing factor*	1.13
Theoretical plate*	2950
Linear range (µg/ml)	5-25
LOD (µg/ml)	0.35
LOQ (µg/ml)	1.05
Linear equation	AUC=24.29conc.+ 4.366
Slope	24.29
Intercept	4.366
Correlation coefficient (r <sup>2</sup> )	0.999

\*Mean of six readings

**Table 3 Result of precision of test method of emtricitabine**

PARAMETER	% RSD
Repeatability	0.767
Precision (%R.S.D.)*	Day to Day 1.224
	Analyst to Analyst 1.870

\*Average of five determination

**Table 4 Recovery of emtricitabine**

Recovery Level %	% Recovery (Mean±SD)*	% RSD
80	99.00±0.030	0.379
100	98.50±0.176	1.785
120	98.67±0.406	0.470

\*Average of five determination

**Table 5 Analysis of capsules sample**

S. No	Drug
	Emtricitabine
1	Mean 99.15
2	S.D. 0.120
3	% RSD 0.121

## Conclusion

The method developed for quantitative analysis of emtricitabine is rapid, precise, accurate and selective. Peak purity studies under all the stress conditions showed the drug peak to be pure and hence the method is stability indicating. In other words, it can be mentioned that the method developed can be utilized for the successful quantification of the drug in presence of its degradation product and excipients. The

method was completely validated and satisfactory results were obtained for all the characteristics tested. The method is stability indicating and can be used to assess the stability of emtricitabine in the bulk drug. The method can be conveniently used for routine analysis of emtricitabine as bulk drug, in respective dosage forms, for dissolution studies and as stability indicating assay method in pharmaceutical laboratories and industries.

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