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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_{18} 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 254nm. The mobile phase consisted of 10mM KH_2PO_4: 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 range5-25µg/ml (correlation coefficient 0.9998). The limits of detection and quantification were 0.23 and 0.96µg/ml respectively. Recovery of EMT from the pharmaceutical dosage form ranged from 98.00 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 proved 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 [1, 2].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 [3]. 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) [4]. 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-1reverse transcriptase [5]. It is also active against the hepatitis Bvirus [6, 7]. 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 [8], HPLC in pharmaceutical formulations, drug substance, and biological matrices [9-11], LC/MS/MS [12], and stability-indicating liquid chromatographic methods [13] 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.

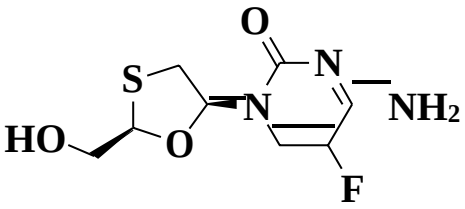

Figure 1 Chemical structure of emtricitabine

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. Potassuim 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_2PO_4 (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

Twenty capsules were weighed and their mean weight was determined. Weight equivalent to 10mg of EMT was dissolved in 10 ml of methanol and then sonicated for 30 min and filtered through whatman paper no. 41. The filtrate was appropriately diluted to get concentration of 10 µg/ml and analyzed. The amounts of EMT in capsules formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with 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 80ºC. 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 80ºC. 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 50ºC 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 byuse 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 (atambient temperature) were tested by analysis after24, 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 conditions

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_{18} column was employed for envisaged work. Combination of 10mM KH_2PO_4 (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 r2 = 0.999 having equation as: 41.28 conc.+12.47. 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.

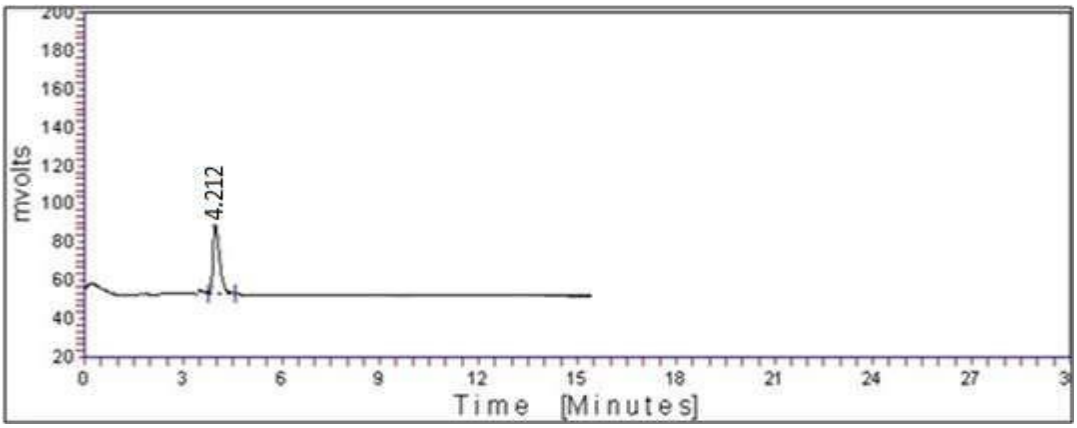

Figure 2 Standard chromatogram of emtricitabine

Forced degradation study

Bakshi*et al.*[14]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 range99.9-100.0, 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	99.85	0
Acidic hydrolysis	85.23	14.77
Alkaline hydrolysis	91.25	8.75
Oxidative degradation	80.23	19.77
Photolytic degradation	85.41	14.59

Method validation

Peak purity was >99.9% for drug substance and drug degradation products at 254nm, 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 0.23 and 0.96µg/ml, respectively for 20 µl 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 results from robustness and capsules analysis was shown in Table 5& 6.

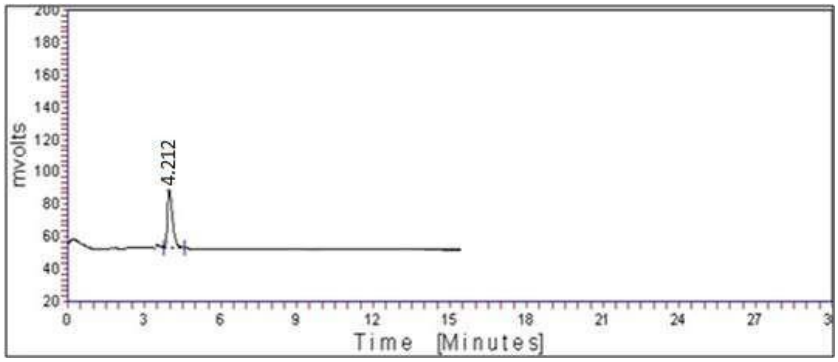

Figure 3 Chromatograms of emtricitabine (15µg/ml) in a capsule formulation

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*	14127
Linear range (µg/ml)	5-25
LOD (µg/ml)	0.23
LOQ (µg/ml)	0.96
Linear equation	AUC=41.28 conc.+12.47
Slope	41.28
Intercept	12.47
Correlation coefficient (r^2)	0.999
S.D. of slope	0.0351
% RSD of slope	0.08
S.D. of intercept	0.0642
% RSD of intercept	0.511

*Mean of six readings

Table 3 Result of precision of test method of emtricitabine

Std. conc. (µg/ml)	**Repeatability**	**Intermediate precision**	
		Day to day	**Analyst to analyst**
5	100.2	99.80	99.26
10	102.7	103.9	100.19
15	99.60	102.3	99.80
20	101.00	100.5	99.02
25	100.19	100.9	100.12
Mean	100.738	101.48	99.678
S.D.	1.205	1.632	0.519
% R.S.D.	1.196	1.608	0.520

*Mean of fifteen determinations (3 replicates at 5 concentration level)

Table 4 Recovery of emtricitabine

Level of addition	Std. drug sol. Added (µg/ml)	% mean* recovered
80	8	98.000
100	10	99.000
120	12	98.646

*Average of five determination

Table 5 Results from robustness testing

Parameter	Percentage (Mean ± S.D*)	Percentage RSD*
Mobile phase	97.24 ±0.17	0.179
Flow rate	98.40 ±0.09	0.091

*Mean obtained at three concentrations and three replicate

Table 6 Analysis of capsules sample

S. No		Drug Emtricitabine
1	Mean	99.25
2	S.D.	0.110
3	% RSD	0.115

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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