WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 6, 499-505. Research Article ISSN 2277 – 7105

RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF EMTRICITABINE IN SYNTHETIC MIXTURE

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Article Received on
01 June 2014,

Revised on 26 June 2014,
Accepted on 21 July 2014

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ABSTRACT

A facile, sensitive, accurate and validated HPLC method has been developed to determine Emtricitabine in bulk drug and synthetic mixture. Chromatographic separation is conducted on Phenomenox C18 (250mm x 4.6mm,5µm) column at ambient temperature using sodium dihydrogen orthophosphate (0.02M) and methanol (50:50v/v) as a mobile phase and at a flow rate 1ml/minute at a wavelength of 280 nm. The retention time for Emtricitabine was found to be 3.5minute. The Calibration graph was plotted over the range of 80-240µg/ml with correlation coefficient 0.9990. The limit of detection (LOD) and limit of quantification (LOQ) for Emtricitabine were 0.0112 and 0.0375µg/ml. Accuracy was between 99.53% and 100.01%. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The newly developed method was validated according to the ICH guidelines with respect to linearity, accuracy, precision and specificity.

KEY WORDS: Emtricitabine, Validation, Synthetic mixture, ICH Guidelines.

INTRODUCTION

Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) and chemically it is 5-fluoro-1-(2R, 5S)-[2-(hydroxymethyl)-1, 3–oxathiolan–5-yl] cytosine[1]. It is analogue of cytidine. The drug works by inhibiting reverse transcriptase, the enzyme that copies HIV RNA into new viral DNA. By interfering with this process, which is central to the replication of HIV, It helps to lower the amount of HIV or viral load in patient's body and can indirectly increase the number of immune system (called T cells / Cd4+ cells).[2] Emtricitabine is commercially available and is approved by FDA for treatment of HIV infection.

Emtricitabine exhibits chemical activity against the hepatitis B virus (HBV) among individuals with chronic HBV infection, treatment results in significant histologic, virologic and biochemical improvement. It is not approved by FDA for the treatment of HBV infection. The most common adverse effects are diarrhea, headache, nausea and rash. Among the other side effects patients may experience to hepatotoxicity or lactic acidosis.[3] Emticitabine has determined by UV-spectroscopic[4], HPTLC[5] and RP-HPLC[6-11] methods in single and combined dosage form. Literature reveals that few chromatographic methods in biological fluids were reported along with other antiretroviral drugs.[12-14] Even though various methods has been reported to carry out emtricitabine in individual / bulk drug or combination with other drugs, no method has been so far for estimation of emtricitabine in synthetic mixture. The present study was aimed to carry out the estimation of emtricitabine in synthetic mixture by RP-HPLC method using isocratic mode.

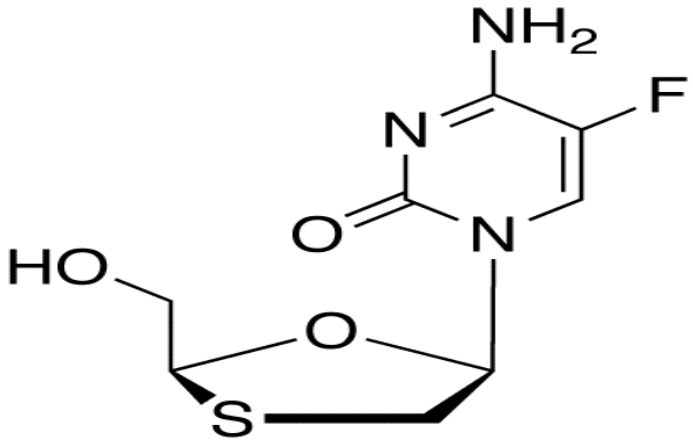

Figure 1. Structure of Emtricitabine

MATERIALS AND METHOD

Experimental Section

Emticitabine was procured as a gift sample. Methanol (HPLC grade) and all other analytical grade reagents were obtained from Fischer. Water (HPLC grade) was obtained by distillation in glass and passage through Milli-Q water purification system.

Instrumentation

HPLC analysis was carried out using Shimazdu system (LC 2010 AHT) with variable wavelength programmable UV detector and Rheodyne injector with 20 µl fixed loop were used for chromatographic separation. Class VP software were used for data analysis. Chromatographic separation was carried out on Phenomenox C18 column (250mm X 4.5mm). For Spectroscopic detection perkin elmer UV visible spectrophotometer was used.

For weighing purpose electronic balance was used. Degasser, ultrasonic bath sonicator was used.

Chromatographic condition

Mobile phase consists of 0.02 M Buffer and methanol in the ratio of 50: 50 was prepared. The prepared mobile was filtered through nylon disc filter of 0.45µm (Millipore) and sonicated for 15 min before use. The flow rate was fixed to 1ml /min and the injection volume was 20 µl. The detection wavelength was fixed at 280nm. Chromatographic condition was achieved at ambient temperature.

Preparation of mobile phase

0.02M sodium dihydrogen orthophosphate (1.56 gm was dissolved in 500 ml HPLC grade water) and methanol (500 ml) was prepared and mixed in the ratio of 50: 50 V/V.

Preparation of standard stock solution

Weigh accurately a quantity of 50mg of emtricitabine and dissolved in 50 ml of mobile phase to get a concentration of 1000µg / ml. Further dilution was made to obtain 80 µg/ml using mobile phase.

Preparation of synthetic mixture

Emtricitabine was mixed with starch, Magnesium stereate, Talc and Carboxy methyl cellulose. Total 300mg of synthetic mixture was prepared. Weigh accurately a quantity of the powder equivalent to 50 mg of emticitabine was transferred to 50 ml volumetric flask, added 30 ml of mobile phase and sonicated for 20 min with occasional shaking and make the required quantity up to 50ml with mobile phase. Filter the solution using 0.45µm whattmann filter paper. Further it was diluted to get the concentration of 80 µg/ml using mobile phase. The chromotagram was shown in Fig.no.2

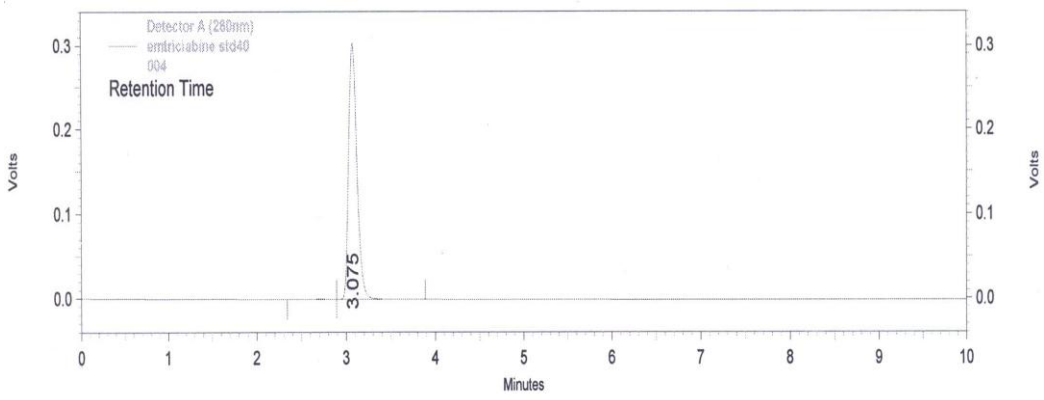

Fig.no.2: Chromotagram of Emtricitabine

Method Validation

Chromatographic condition was optimized and validated by Linearity, Accuracy, Precision, Specificity, Limit of detection and limit of quantification in accordance with ICH guidelines.

Linearity

Standard stock solution was diluted to prepare linearity standard solution in the concentration range of 80 -240 µg/ml. Three sets of standard solution were prepared. Each set was analyzed to plot a calibration graph. Slope, intercept and coefficient of determination (r^2) of calibration curves were calculated to ascertain linearity.

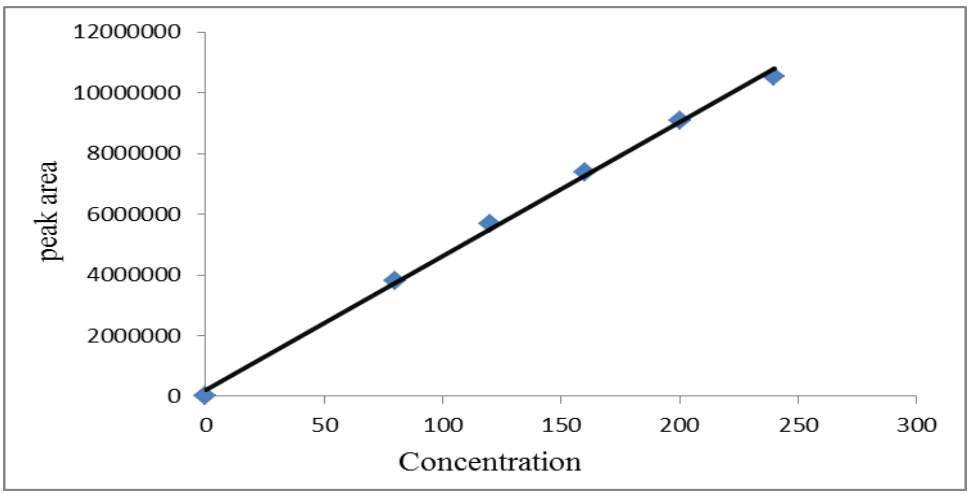

Figure no. 2. Linearity of Emtricitabine

Accuracy

The sample was spiked using synthetic mixture with 80%, 100% and 120% standard solution. The proposed method was used to analyze the synthetic mixture. Triplicate experiments were carried out and recovery (%), RSD (%) of the spiked synthetic mixture was calculated.

Precision

Precision was calculated by carrying out six independent assays of the text sample by the proposed method. RSD (%) of six assays obtained was calculated.

Limit of detection and Limit of Quantification

From the calibration graph the detection and quantification limits were evaluated in the concentration range between 80 -240 µg/ml of emtricitabine. For this replicate injection the acceptance criterion was RSD not more than 30% for LOD concentration and not more than 10 % for LOQ concentration. LOD and LOQ concentration for the standard drug solution were injected six times.

Specificity

By assessing the chromatograms obtained from the sample solution the method was found to be specific. The excipients used in the synthetic mixture have no interference with the analytes of interest.

RESULT AND DISCUSSION

Mobile phase of different composition were tried to resolve the peaks of Emtricitabine the mobile phase which was found to ideal was 0.02M sodium dihydrogen orthophosphate: methanol in the ratio of 50:50 V/V and it was selected for the proposed method. Quantification was achieved with UV detection at 280 nm. A representative of chromatogram was shown in figure 3. The validation parameters were represented in table 1. Linear regression data showed a good linear relationship over a concentration range of 80 -240 µg/ml for emtricitabine. The correlation coefficient was found to be 0.9990. Limit of detection and limit of quantification was found to 0.0112 and 0.0375 µg/ml respectively. In this method the values obtained indicate that it is more sensitive. Precision (%RSD) was calculated and it was found to be 0.0020%. It indicates that the method is more précised. Accuracy was calculated in three different concentrations like 80%, 100% and 120% standard solution. The average percentage recovery was found to be 99.53%, 99.05% and 100.01% respectively. Assay was carried out using synthetic mixture and the value was found to be 99.16%. The proposed RP-HPLC method is accurate, précise, specific and rapid for the estimation of emtricitabine in synthetic mixture.

Table 1. Summary of validation parameters

Parameters	Results
Accuracy(Percentage of recovery) at 80% 100% 120%	 99.53% 99.05% 100.01%
Precision % of RSD	 0.0020%
Linearity concentration	80 -240 µg/ml
Equation for regression line	Y= 42127.34+ 555161.2x
Correlation coefficient	0.9990
Specificity	Specific
LOD	0.0112 µg/ml
LOQ	0.0375 µg/ml
Assay	99.16%

CONCLUSION

The developed RP HPLC method was used to determine Emtricitabine quantitatively in bulk and synthetic mixture by isocratic mode. The HPLC method was validated and showed good linearity, precision, accuracy and specificity. The method was validated as per international conference on harmonization (ICH) guidelines.

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