



METHOD DEVELOPMENT AND VALIDATION OF RP- HPLC IN THE APPLICATION OF IN-VITRO DISSOLUTION STUDY OF EMTRICITABINE IN BULK DRUG AND TABLET FORMULATION

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ABSTRACT

A new RP-HPLC method was developed for the determination of Emtricitabine in the bulk drug and tablet dosage forms and it was applied for the *in vitro* drug dissolution studies. Isocratic elution mode with a mixture of methanol and water in the ratio of (89:11) was selected as the mobile phase with a C₁₈ column (250 x 4.6mm, 5μ) for separation. This mixture was found to be appropriate allowing good elution for the Emtricitabine at retention time 2.72 minute at flow rate of 1 ml/min and detection wavelength at 272nm. The linearity was found in the concentration range of Emtricitabine 5-100 μ g/ml. The liquid chromatography method was extensively validated for linearity, range, accuracy, precision (intermediate precision, repeatability) and specificity. All these analytical validation parameters were observed and the %RSD was determined which indicates the usefulness of RP-HPLC method for determination of Emtricitabine in the bulk drug & tablets dosage form.

Key words: Emtricitabine; Validation; *In-Vitro* studies; RP-HPLC

INTRODUCTION

Nucleoside reverse transcriptase inhibitor (NRTIs) was the first class of drug those were introduced as antiretroviral agents for the treatment of infection with human immunodeficiency virus (HIV). Emtricitabine (2'-deoxy-3'-thiacytidine: 3TC) is a pyrimidine analog reverse transcriptase inhibitor that is active against HIV-1, HIV-2, and HBV. The molecule has two chiral centers and is manufactured as the pure 2 R, cis (-)-enantiomer. The racemic mixture from which Emtricitabine originates has antiretroviral activity but it has less potency and is substantially more toxic than the pure (-)-enantiomer. Compared with the (+)-enantiomer, the phosphorylated (-)-enantiomer is more resistant to cleavage from nascent RNA/DNA duplexes by cellular 3'-5' exonucleases, which may contribute to its greater potency.¹⁻³ Fig.1

There have been several publications describing analytical methods for the determination of Emtricitabine. Few methods have been described for the simultaneous determination of other antiretroviral drugs in biological samples and pharmaceutical dosage forms⁴⁻¹⁴. These are time consuming and costly methods. No method has been developed and applied to *in-vitro* pharmacokinetic studies for different classification of antiretroviral drug of Emtricitabine by using single method.

Drug dissolution testing is an integral part of pharmaceutical development and routine quality control monitoring of drug release characteristics. The profiles, obtained from dissolution rate studies, have also been used in an attempt to characterize the *in-vitro* behavior of drug with success. They have to be performed under precisely specified conditions (i.e; temperature, volume, and stirring rate) that mimic process in the gastrointestinal tract.

The present work reports the development and validation of RP-HPLC for the determination of Emtricitabine in bulk drug and tablet formulation and application of this method to *in-vitro* dissolution studies.

MATERIAL & METHODS

Chemicals

Mobile phase consisted of methanol (SD Fine Chem. Ltd, HPLC grade) and water (Millipore HPLC grade) was used during analysis. For *in-vitro* dissolution studies was used. Emtricitabine reference standard was generous gift from Ranbaxy Laboratories Limited, Gurgaon, India. Emtricitabine tablets were procured from the local market Emtriva 200 mg, Truvada 200mg.

Instrument

Shimadzu LC-20AT liquid pump, SPD-20A UV-Visible detector, a Luna 5μ C-18 RP-HPLC column (250 x 4.6mm, 5 μ m, ID), 25ml Hamilton injecting syringe and spinchrom software were used. Sartorius (CP225D) electronic balance was used for weighing the materials. The dissolution rate studies of Emtricitabine from tablets were performed on a Veego, VDA-8DR USP dissolution apparatus.

Method development

Various solvent systems were tried for the development of suitable HPLC method for the analysis of Emtricitabine in the bulk and tablet formulation. The suitability of the solvent system was decided as the basis of the sensitivity of the assay, suitability for stability studies and availability of cost effective solvents.

Preparation of stock solution

Accurately weighed 100 mg of Emtricitabine standard was taken in separate 100 ml volumetric flasks and dissolved with 70 ml of mobile phase in each volumetric flask and sonicated for 5 min and then mark with mobile phase to get final concentration of 1mg/ml which was considered as stock solutions.

Preparation of standards solution

Standard stock solution was further diluted with mobile phase to obtain the concentration of solution in the range of 5-100 μ g/ml was performed with UV detector for Emtricitabine 272nm in 20μ l of each prepared solutions were injected with a flow rate of 1ml/min and the peak area were

recorded. Table 1

The operation were carried out in triplicate group was plotted between concentration vs. peak area.

Selection of analytical wavelength

By appropriate dilution of standard stock solution with mobile phase, various concentration of Emtricitabine was prepared accurately. The solutions were scanned between the wavelengths range 400-200 nm using the Shimadzu double beam UV visible spectrophotometer in the spectrum mode.

Preparation of sample solutions

For the estimation of Emtricitabine from twenty tablets were weighed separately and powder equivalent to 100 mg of the drugs were accurately weighed and transferred to separate 100 ml volumetric flasks and sonicated for 5 minutes. Volumes were made up to the mark with mobile phase and filtered through 0.45 μ m filter. The concentrations of sample solution was prepared and analyzed with UV detection for Emtricitabine at 272 nm, with a flow rate of 1ml/min.

In Vitro Dissolution Studies from Tablet Dosage Form

Drug dissolution studies were carried out in 900 ml of 0.1N HCl (pH 1.2, simulated gastric medium) according to the USP 2000 dissolution procedures the single entity products with the use of a USP paddle type of apparatus at a stirring rate of 75 rpm. The temperature of the cell was maintained at 37 \pm 0.5°C. Aliquots of Emtricitabine were withdrawn at predetermined time intervals at 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 min replaced with equal volume of pre-warmed 0.1N HCL solution to maintain the sink condition. The sample solutions were diluted with methanol (HPLC grade) and it was analyzed by using methanol and water (89:11%, v/v) ratio and concentrations were determined at 272nm for Emtricitabine peaks are given in Figure-2. The retention time of Emtricitabine is 2.86 min. The cumulative percentage of drug released in the media was plotted against time in order to determine the release profile from the tablet formulations. The dissolution test data were obtained by averaging three parallel studies. Table.2

RESULTS AND DISCUSSION

Method development

Effect of incorporating methanol

Methanol is a polar solvent being widely used in RP-HPLC. The primary effects it was found that the retention factor (k) was decreases with increases in the concentration of sample. The peak obtained was broad and showed severe tailing. However the final concentration of methanol was adjusted to achieve a good capacity factor. The chromatogram revealed that with increased in methanol concentration the retention time was reduced. The peak obtained was best acceptable at the methanol concentration of 89%. The peak was found sharp and reproducible.

Effect of changing the flow rate

To determine the effect of flow rate, the programmed controller was set at different flow rates 0.5 mL/min, 0.7 mL/min, 0.9 mL/min 1.0 mL/min, 1.1 mL/min, 1.2 mL/min and 1.5 mL/min. operate were performed at each flow rate. The optimum flow rate was also chosen keeping in mind the recommended flow rate for a column with a given internal diameter.

Method validation

Linearity and range

Under the optimized conditions, a calibration curve was used for Emtricitabine. Six standard mixtures of different concentrations were prepared. The corrected peaks were used for constructing the calibration graph. Linearity range, regression equation, correlation coefficient, slope, standard errors of slope, standard errors of intercept, are shown in Table 2 The plot of peak area of each sample against respective concentration of Emtricitabine was found to be linear in the range of 5-100 μ g/ml, using regression analysis of the linear equation for Emtricitabine $Y = 24.60x + 7.205$ with correlation coefficient of $r^2 = 0.9999$. Linearity data obtained from the measurements are given in Table 3.

Accuracy

In order to examine the accuracy of the method and to check the interference from excipients used in tablet dosage formulation, the recovery studies were carried out by standard addition method. In this method, three different amounts of Emtricitabine were added to a constant known concentration of the composite tablet solution. Each solution was injected five times and the amounts determined were compared to theoretical amounts. The results were summarized in Table 4. The recovery range was found from 99.01 to 99.69 for Emtricitabine. The relative standard deviation (% RSD) of Emtricitabine 1.42 was observed which indicates that the method gives sufficient accuracy.

Precision

Precision of the method was tested by performing intra-day and inter-day studies. For intra-day studies, triplicate of prepared samples were analyzed within same day. For inter-day validation, concentrations were determined on three separate days. The % RSD values obtained from peak area for LAM was observed. It can be seen from Table 5, a good precision was obtained for drug.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) for Emtricitabine was found to be 1.705, 5.16 μ g/ml. The typical chromatogram of Emtricitabine was shown in Figure 3. A mixture of methanol and water in the ratio of (89:11, v/v) was found to be most suitable to obtain a peak well defined and free from tailing.

Specificity

The effect of inactive ingredients (placebo) on the determination of the drug in tablet formulation was studied. Each tablet contains 200mg of Emtricitabine along with some inactive ingredients, such as titanium dioxide, red oxide, yellow oxide, talc and methyl cellulose. In the chromatogram, no interfering peaks were observed in the region of analytes.

Ruggedness

A different analyst, using a different batch of chemicals, column, tried the method. There was not much variation in results calculated by % RSD. These studies showed that the method is rugged.

System suitability parameter

The system suitability test was carried out on freshly prepared standard stock solution of Emtricitabine. Parameters such as efficiency, resolution, peak tailing HETP,

LOD, and LOQ that were studied to evaluate the suitability of the system are given in Table 6.

Applied to the dissolution studies

The method was also applied to determine the release rate pattern of the drug from the tablet dosage form of Emtricitabine in dissolution rate studies. The release data were evaluated according to different models namely Zero order, First order, Higuchi and Korsmeyer & Peppas function. The regression coefficient values obtained after fitting the dissolution data to various kinetic models is shown in Table 7. The regression value of Emtricitabine was found to be following the Korsmeyer-Peppas release pattern following diffusion controlled release of the contained drug.

The assay of Emtricitabine tablet was found to be 191.18, & 199.66. The results are given in Table 8. The absence of additional peaks in the chromatogram indicates non interference of the common excipients used in the tablets. Emtricitabine was found to give individual identical results when compared to their peak area and peak height when samples were collected and analyzed by developed RP-HPLC method using the same solvent system. Drug release kinetics was determined by analyzing the regression values. Fig.4

The present study indicates the possibility of using the same solvent system for quality control samples and for analyzing the drug release pattern from the tablet dosage forms.

CONCLUSION

Developed reverse phase high performance liquid chromatographic method is simple, reliable, linear, accurate, sensitive and reproducible as well as economical for the effective qualitative and quantitative analysis of Emtricitabine in bulk drug and tablet formulations. The method does not involve any costly sample extraction procedure in the method which highlights its usefulness in routine quality control testing. In addition, the reported method can also be used for pharmacokinetic studies.

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Table 1: Calibration values of Emtricitabine in standard drug

Concentration µg/ml	Peak area (mV.s)
5	123.881
10	269.736
15	371.494
20	503.710
30	748.583
40	983.960
50	1226.043
80	2006.804
100	2448.166

Table 2: Percentage cumulative release of Emtricitabine

Time (min.)	Peak area	Concentration (x)	Amount in 5 ml	Amount in 900 ml	CR	%CR
10	374.946	14.924	0.746	134.316	134.316	89.544
20	403.805	16.097	0.804	144.873	145.619	97.079
30	398.445	15.879	0.793	142.911	143.715	95.81
40	396.665	15.807	0.790	142.263	143.056	95.370
50	403.521	16.086	0.804	144.774	145.564	97.042
60	400.115	15.947	0.797	143.523	144.327	96.218
70	398.066	15.864	0.793	142.776	143.573	95.715
80	405.272	16.157	0.807	145.413	146.206	97.470
90	405.269	16.157	0.807	145.413	146.22	97.48
100	400.260	15.953	0.790	143.577	144.384	96.256
110	391.241	15.586	0.779	140.274	141.071	94.047
120	395.392	15.755	0.787	141.795	142.574	95.049

Table 3: Data for the calibration graphs of Emtricitabine

Parameters	Emtricitabine
Linearity & range	5-100 µg/ml
Regression equation	$y = 24.60x + 7.205$
Correlation coefficient	0.9999
Slope	24.60
Intercept	7.205
Standard error of slope	0.0600
Standard error of intercept	3.525

Table 4: result of accuracy

Analyte	Label claim (mg)	Fortified amount (mg)	Amount found (mg)	% Recovery	% RSD
Emtricitabine	200	10	9.93	99.01	1.05
		20	19.16	99.22	1.89
		30	29.04	99.69	1.42

Table 5: Intra & inter-day Precision

Analyte	Concentration (µg/ml)	Intra-day	%RSD	Inter-day	%RSD
Emtricitabine	20	493.104	0.907	504.424	1.052
	40	970.377	0.513	986.628	0.545
	80	1973.850	0.879	1960.273	0.815

Table 6: System suitability test for Emtricitabine

Parameters	Emtricitabine
Retention Time	2.7
HETP(mm)	0.022
Tailing Factor	1.12
Theoretical plates/m	32247.057
LOD (µg/ml)	1.705
LOQ (µg/ml)	5.16

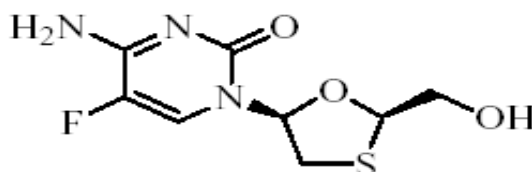
Table 7: Kinetic assessments of release data

Kinetics	Parameters	Emtricitabine
Zero Order	Kr^0	0.328
	r^2	0.231
First Order	Kr^1	0.001
	r^2	0.044
Highuchi	Kr^2	0.122
	r^2	0.021
Korsmeyer & Peppas	Kr^3	0.350
	r^2	0.705

Kr^0 Kr^1 Kr^2 Kr^3 = slope values, r^2 = regression values

Table 8: Results of Emtricitabine analysis in tablet dosage forms

Sample	Label claim (mg)	Amount found	%Recovery	%RSD
Tablet A	200	191.18	99.59	0.730
Tablet B	200	199.66	99.83	0.669

**Fig.1: Chemical Structure of Emtricitabine**

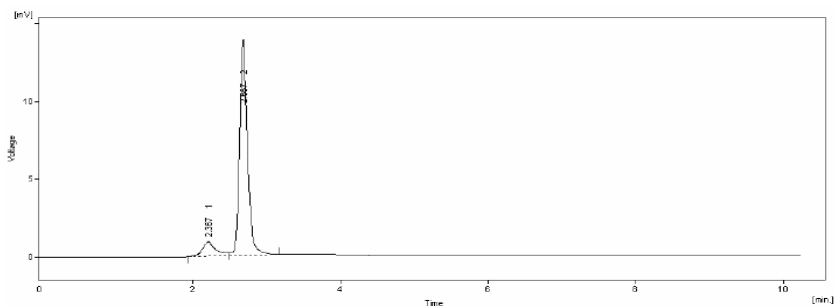


Fig. 2: RP-HPLC Chromatograms of Emtricitabine by dissolution in -vitro kinetics studies

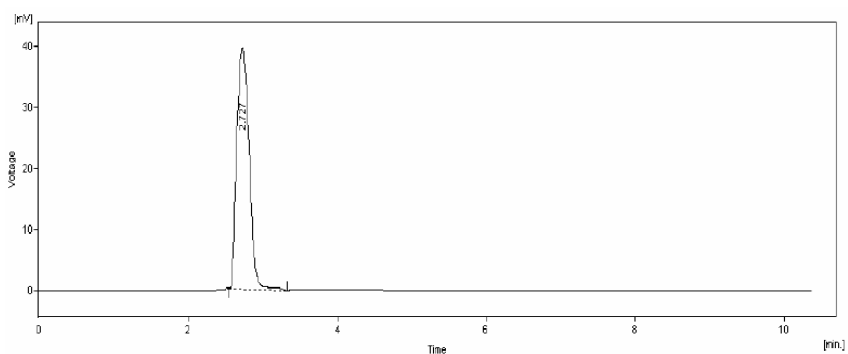


Fig.3 : RP-HPLC Chromatograms of Emtricitabine

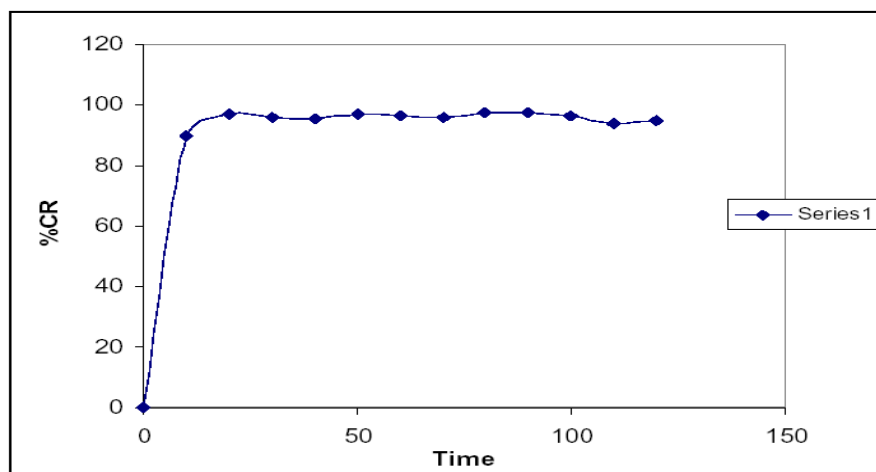


Fig.4: in-vitro dissolution profile of Emtricitabine

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