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MULTIVARIATE UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF EMTRICITABINE IN BULK FORM

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ABSTRACT

To develop a simple, precise and accurate UV-method with multivariate calibration technique for estimation of emtricitabine in bulk form. This technique is based on the use of the linear regression equations by using relationship between concentration and absorbance at five different wavelengths like 287, 289, 291, 293 and 295. The results were treated statistically and were found highly accurate, precise and reproducible. The emtricitabine shows absorption maxima at 291 nm and obeyed Beer's law in the range of 1-10 µg/mL. The % recovery of capsules was found to be in the range of 99.16-100.41. The low % RSD values are indicates the accuracy and precise of the method. The proposed methods can be successfully applied for method development, validation and multivariate analysis of emtricitabine.

Keywords: Emtricitabine, UV spectroscopy, Development, Validation and Multivariate technique.

INTRODUCTION

Emtricitabine is a human immunodeficiency virus type 1 (HIV-1) specific nucleoside reverse transcriptase inhibitor (NRTI). Emtricitabine works by inhibiting reverse transcriptase, the enzyme that copies HIV RNA into new viral DNA. Emtricitabine is phosphorylated by cellular enzymes to form emtricitabine 5'-triphosphate, which is responsible for the inhibition of HIV-1 reverse transcriptase. It competes with the natural substrate deoxycytidine 5'-triphosphate and incorporates into nascent viral DNA, resulting in early chain termination [1]. Emtricitabine efficiently reduces the viral load and is well tolerated. Emtricitabine is used individually or in combination with other antiretroviral agents in the treatment of HIV infection in adults [2,3]. It is chemically known as(–)-4-amino-5-fluoro-1-[(*2R*,*5S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1*H*)-one and structure is shown in Figure 1.

Literature survey reveals that there are few analytical methods such as UV spectrophotometric methods [4-6], extractive spectroscopy [7], RP-HPLC method in bulk, formulations and biological fluids [[8-12], Stability indicating LC method [13] and HPTLC method [14], were developed for the analysis of emtricitabine in different solvents.

There are no reported method on the calibration of multivariate spectrophotometric analysis for the estimation of emtricitabine in bulk form. Multivariate calibration refers to the process of constructing a mathematical model that relates a property such as content or identity to the absorbance of a set of known reference samples at more than one wavelength [15]. If the absorbance of an analyte (Z) is measured at five wavelengths set, straight line equation can be written as; $A_\lambda = aX(Cz+k)$ where A_λ represent the absorbance of the analyte, A is the slope and k is the intercept of the linear regression function of the analyte. C_Z represents the concentration of analyte. At five selected wavelengths, the equation system can also be summed as; $A_T = aX\ (C_Z + b)\ X\ (C_Z + c)\ X\ (C_Z + d)\ X\ (C_Z + e)\ X\ (C_Z + K_T)$, which can be simplified to $A_T = C_Z\ (a+b+c+d+e) + K_T$ where a, b, c, d, e are the slopes, A_T and $K_T$represents the sum of absorbance obtained and sum of intercepts of regression equations at five-wavelength set respectively. The concentration of the Z analyte in a mixture can be calculated by using the Eqn. $C_Z = A_T - K_T / (a+b+c+d+e)$ [16]. This paper describes the application of UV spectral multivariate calibration technique having simple mathematical content for the quantitative determination of emtricitabine in pharmaceutical formulation. Spectrophotometric methods of analysis are more economic and simpler, compared to methods such as chromatography and electrophoresis.

MATERIALS AND METHODS

Materials

Emtricitabine was gift sample from Hetero

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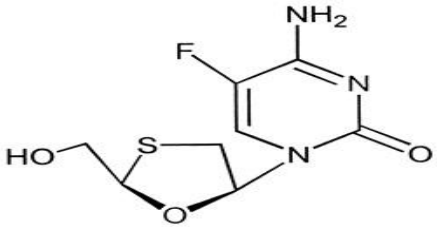
Laboratories Ltd. (Hyderabad, A.P, INDIA). Methanol was purchased from Merck Chemical Company (India). What man no 5 filter paper was obtained from Modern Science lab, (Nasik, India).All chemicals used were analytical grade and glass wares used were Class A grade.

Figure 1. Chemical Structure of Emtricitabine



Instruments

The multivariate technique was performed in double beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe was employed with spectral bandwidth of 1nm and wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells and sonicator.

Preparation of Standard stock solution of emtricitabine

Standard stock solution of emtricitabine (1mg/mL) was prepared by transferring 10 mg of emtricitabine into a 10 ml volumetric flask containing 4mL of (7:3) methanol and 0.1N HCl. It was then sonicated for 15 minutes and solution was diluted up to the volume by methanol and water. From these, further dilutions were made using (7:3) methanol and 0.1 N HCl to produce solution of emtricitabine (100µg/ml).

Selection of wavelength for analysis of emtricitabine

1 ml of standard stock solution of emtricitabinewas transferred into a 10 ml volumetric flask and diluted to a mark with methanol: 0.1N HCl (7:3) to give concentration of 10 µg/ml. The resulting solution was scanned in the UV range (200–400 nm).

Preparation of sample solution

The outer shells of the twenty capsules were removed, the powder of these twenty capsules being collected, weighed accurately. A powder equivalent to 20mg of Emtricitabine was transferred carefully to 100mL volumetric flask and about 70ml methanol: 0.1N HCl (7:3) was added. The mixture was sonicated for 10 minutes. The volume was made up to 100ml with diluent, filtered through what man no. 5 filter paper. From the filtrate 0.1ml was pipetted out and diluted to 10mL with. The final solution was scanned in the UV range (200–400 nm).

VALIDATION OF PROPOSED METHOD

The method was validated according to ICH guidelines in order to determine the linearity, precision, accuracy and ruggedness of the method [17].

Linearity

Linearity was evaluated by seven point standard curve in concentration range of 1-10 µg/ml (1, 2, 4, 6, 8 and 10 µg/ml) of emtricitabine. The calibration curve was obtained by plotting absorbance against concentration (µg/ml) for five different wavelengths. Each set was analyzed to plot a calibration curve. Standard deviation (SD), slope, intercept, and correlation coefficient of determinations (r^2) of the calibration curves were calculated to ascertain the linearity of the method.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solution (n=6) for emtricitabine (2µg/ml) without changing the parameter of the proposed UV method. The %RSD was calculated.

Intermediate Precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses on the same day and next day for three different concentration of standard solution of emtricitabine (2, 6 and 10µg/ml). The result was reported in terms of relative standard deviation (%RSD).

Accuracy

Accuracy of the proposed method was determined using recovery studies by standard addition method. The recovery studies were carried out by adding different amounts (50, 100 and 150%) of the pure drug to the pre-analysed formulation. The solutions were prepared in triplicates and the % recovery was calculated.

Limit of Detection and Limit of Quantitation

The limit of quantification (LOQ) and limit of detection (LOD) were based on the residual standard deviation of the response and the slope of the constructed calibration curve (n=3), as described in International Conference on Harmonization guidelines Q2 (R1).

$LOD = 3.3 \times \sigma/S$, $LOQ = 10 \times \sigma/S$

Where σ = the standard deviation of the response and S = slope of the calibration curve

Ruggedness Studies

Ruggedness studies were performed by preparing three replicates of 4 µg/ml, analysing by two different analysts and on two different instruments and the results are reported as % RSD.

RESULTS AND DISCUSSION

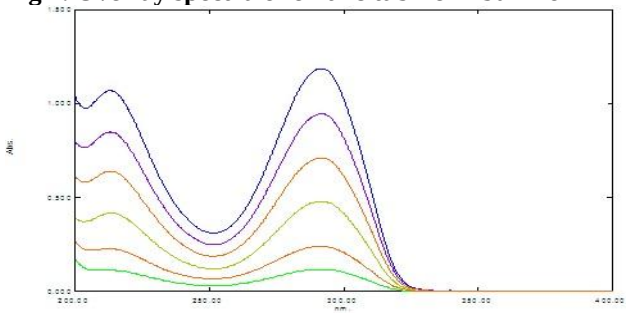
Method validation

The proposed method was validated as per ICH guidelines in order to determine the linearity, precision, accuracy and ruggedness of the method. The optical parameters are shown in Table 1.

Linearity

Standard solutions of emtricitabine in the concentration range of 1 to 10µg/ml were observed in UV spectroscopy at 291nm. A graph of absorbance (on Y-axis) versus concentration (on X-axis) was plotted (overlay of absorbance) was shown in Figure 2.

Fig 2. Overlay spectra of emtricitabine in bulk form



In order to improve this correlation and minimize instrumental fluctuations, absorbance's of these solutions were measured over a range surrounding 291 nm i.e., 287, 289, 291, 293, 295 nm are shown in Table 2. The calibration curves of emtricitabine at different wavelengths are shown in Figure 3.

Method precision (repeatability)

Repeatability was determined by analyzing 2 µg/ml concentration of emtricitabine for six times and % RSD was found to be < 2 which shown in Table 3.

Intermediate Precision (reproducibility)

The precision of the developed method was expressed in terms of percent relative standard deviation (% RSD). These results show reproducibility of the assay. The % RSD values were found to be less than 2 that indicate this method precise for the determination of the pure form. The interday and intraday precision results were mentioned in Table 4.

Accuracy

Accuracy is determined by performing recovery studies at 3 levels in which known amount of analyte shall be added and recovery shall be carried out in three replicates of each concentration level and the % recovery was calculated. The mean recovery was found between 99.89-100.05 and %RSD between 0.176-0.293. The accuracy results are shown in Table 5.

Limit of Detection and Limit of Quantitation

The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. LOD and LOQ values are 0.079 and 0.231.

Ruggedness Studies

This study was performed by analysing 4µg/ml of by two different analysts and on two instruments, results of the study were given in Table 6 and % RSD obtained was less than 2 which is within the acceptance limits.

Application of the Proposed Method for Pharmaceutical Formulation

The proposed method was able to remove the interferences of the other excipients present in the pharmaceutical formulations (tablets) are assessed with a high percent of recovery. The percentage recovery for tablet formulation was found to be 99.16-100.41 enlisted in Table 7. The results for assay are within acceptable limits.

Fig 3. Calibration curves of emtricitabine at different wavelengths (a).287, (b).289, (c).291, (d). 293 and (e).295 nm

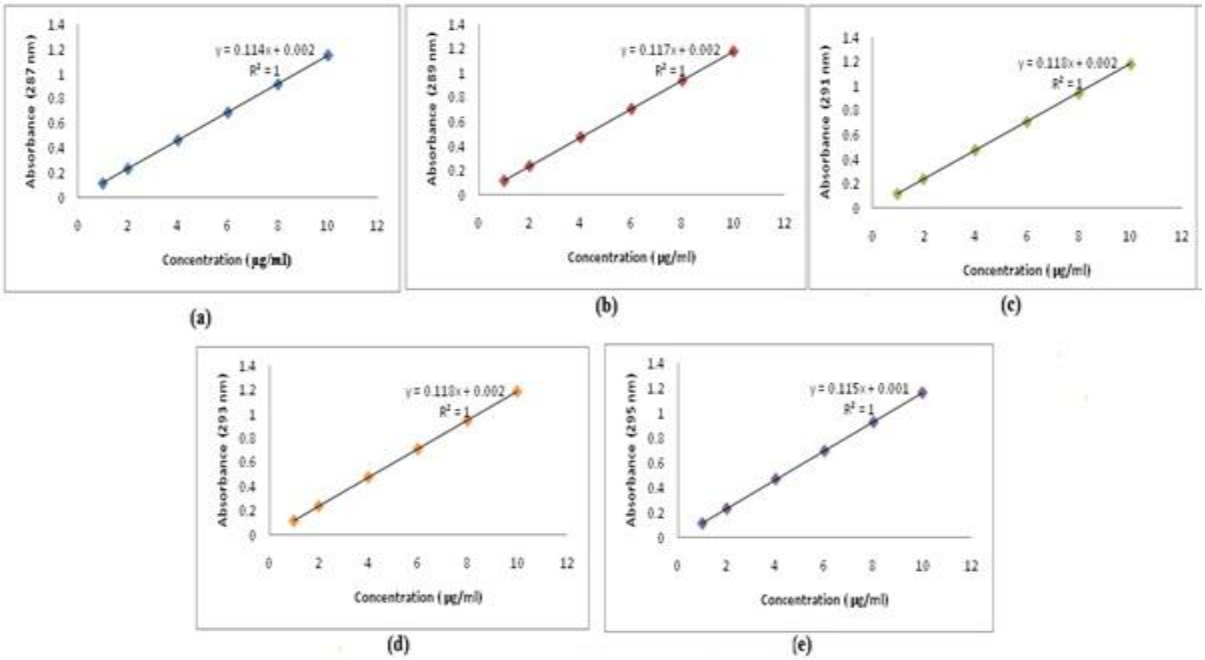


Table 1. Summary of optical characteristics

Parameters	Method
λmax (nm)	291 nm
Beers law limit (µg/ml)	1-10
Molar absorptivity (L mol^{-1} cm^{-2})	0.12×10^3
Correlation coefficient (r^2)	1
Regression equation (y=mx+c)	Y=0.1181x+0.0026
Slope(m)	0.1181
Intercept(c)	0.0026
Accuracy (%)	99.89-100.05
Precision (%RSD)	0.613
LOD(µg/ml)	0.079
LOQ(µg/ml)	0.239
Sandell's sensitivity (µ cm^{-2}/0.001 absorbance units)	0.0083
90 % Confidence limits 95% Confidence limits	0.9401(+), 0.2859(-) 1.0027(+), 0.2233(-)

Table 2. Calibration data of proposed method by using multivariate technique

Concentration [µg/ml]	Absorbance at 287nm	Absorbance at 289nm	Absorbance at 291nm	Absorbance at 293nm	Absorbance at 295nm
1	0.115	0.117	0.118	0.118	0.115
2	0.233	0.238	0.24	0.239	0.234
4	0.464	0.474	0.479	0.478	0.47
6	0.689	0.704	0.711	0.709	0.696
8	0.917	0.936	0.945	0.944	0.926
10	1.152	1.175	1.185	1.184	1.162

Table 3. Repeatability studies of Emtricitabine

Concentration [µg/ml]	Absorbance at 291nm	Absorbance Mean	SD	%RSD
2	0.240	0.240	0.001	0.613
2	0.241			
2	0.239			
2	0.242			
2	0.238			
2	0.241			

Table 4. Intraday and Interday precision of Emtricitabine

Concentration (µg/ml)	Absorbance mean± S.D. (n=3)	%RSD	Absorbance mean± S.D. (n=3)	%RSD
2	0.240±0.0015	0.636	0.240±0.0010	0.417
6	0.711±0.0032	0.452	0.711±0.0020	0.281
10	1.185±0.0030	0.253	1.185±0.0021	0.176

Table 5. Recovery studies of Emtricitabine

Spiked level (%)	Formulation Conc (µg/ml)	Pure Drug Conc (µg/ml)	Amount Conc recovered (µg/ml)	% Recovery	% Mean recovery	%RSD
50	4	2	6.01	100.14	100.05	0.293
	4	2	5.98	99.72		
	4	2	6.02	100.28		
100	4	4	7.97	99.68	99.89	0.212
	4	4	7.99	99.89		
	4	4	8.01	100.11		
150	4	6	9.97	99.75	99.94	0.176
	4	6	10.01	100.08		
	4	6	10	100		

Table 6. Ruggedness of Emtricitabine

Parameter	Concentration(µg/ml)	Absorbance mean± S.D. (n=3)	%RSD
Analyst 1	4	0.479±0.0015	0.319
Analyst 2	4	0.478±0.0021	0.435
Instrument 1	4	0.479±0.0015	0.319
Instrument 2	4	0.480±0.0017	0.361

Table 7. Results of Assay

Labeled claim (mg)	Amount found(µg/ml)	%recovery	%RSD
200	200.83	100.41	0.638
200	198.33	99.16	
200	199.16	99.58	

CONCLUSION

The above proposed UV method is very simple, precise, accurate, rapid and cost effective for the quantification ofemtricitabine from its pharmaceutical dosage forms by the multivariate spectrophotometric method. Hence it can be utilized for routine analysis in bulk and pharmaceutical dosage forms.

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