Research Article

UV VISIBLE SPECTROPHOTOMETRIC ESTIMATION OF EMTRICITABINE

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ABSTRACT

A low cost precise spectrophotometric method has been developed for the estimation of Emtricitabine in bulk and tablet dosage form. Emtricitabine shows maximum absorbance at 298nm in presence of solvent chloroform and phosphate buffer of pH 7.4. The Beer's law is obeyed in the concentration range of 2-20 μg/ mL and the graph of the drug shows a straight line with correlation coefficient of 0 .9910. The assay method of the drug was validated by accuracy and precision of the proposed method. The results are validated as per the directions of International conference on Harmonization.

Keywords: Emtricitabine, UV spectrophotometry, Beer's law, validation

INTRODUCTION

Emtricitabine, is a specific synthetic nucleoside analogue with activity against human immune deficiency virus type 1 reverse transcriptase inhibitor. The molecular formula of Emtricitabine is $C_8H_{10}FN_3O_3S$[1], molecular weight is 247.24g/mol . The colour of this drug is a white to off-white powder with a solubility of approximately 112 mg/mL in water at 25°C. The chemical name of Emtricitabine is 5-fluoro-1-(2R,5S)-[2-(hydroxymethyl)-1,3 oxathiolan-5-yl]cytosine. It is soluble in water but freely soluble in chloroform, methanol, ethanol, and sodium hydroxide. The molecular structure of the drug is shown in the Figure 1. Literature survey of Emtricitabine reveals that some analytical methods were reported namely CLC [2], liquid chromatography[3] and Different spectrophotometric methods have been reported for the determination of Emtricitabine which includes using methanol and acetonitrile (70:30) by Syeda Kulsum *et al*[4], distilled water by Heba K. Ashour *et al* [5], high purity distilled water by Mohammad H. Abdel Hay *et al*[6], methanol by Nagaraju P.T *et al*, Vishnu P. *et al*, and Ingale K. D *et al* [7-9], Double distilled water by P. Ananda kumar, *et al*[10]. The comparisons of the proposed method with other existing methods for the assay of Emtricitabine in pharmaceutical formulations have shown in table: 4 . Reported methods are highly expensive and time consuming .Therefore an attempt was made to develop a low cost precise, accurate spectrophotometric method for the estimation of Emtricitabine in bulk and tablet dosage form.

MATERIALS AND METHOD
Instruments and Apparatus

The absorbance of the drug were carried out by using shimadzu company model 1700 UV-visible double beam spectrophotometer with 1 cm matched quartz cell, spectral band width is 1 nm, supported by UV win 5.0 software .

Reagents and Chemicals

All chemicals are AR grade. Chloroform, double distilled water and phosphate buffer of pH 7.4 is used throughout the analysis. Pharmaceutical formulation of Emtricitabine was supplied by Emcure pharmaceuticals, Pune, (Maharastra). Chloroform, double distilled water and phosphate buffer 7.4 purchased from Merck India Ltd, Mumbai. Commercially available tablets namely Emtriva (100mg), Atripla (100mg), procured from Apollo pharmacy Chennai (Tamilnadu).

Selection of Solvent
Chloroform, double distilled water and phosphate buffer of pH 7.4[12] are used throughout the analysis.

Selection of Method and Wave Length

UV scan range of 200 nm to 400 nm was selected for the proposed method of Emtricitabine. The wavelength corresponding to maximum absorbance was found at 298 nm and calibration curve was taken at 298 nm. The intercept of calibration line of the drug was determined by linear regression Analysis.

Preparation of Standard Solutions of Emtricitabine

The 100 mg of standard (pure) drug of Emtricitabine is weighed accurately and dissolved in 100 ml chloroform solvent then transferred into 100 ml volumetric flasks to prepare 1000 μg/ mL[11] stock solution of the drug. The different aliquots of 2, 4,6,8,10,12,14,16,18, and 20 μg/mL were taken in ten 10 ml volumetric flasks and make up volume with double distilled water .To each flask 2mL of phosphate buffer of pH 7.4 solution is added, then all stock solutions of the drug were scanned in the UV scan range of lambda max (λmax) 200 nm to 400 nm to determine maximum absorbance for this method .The calibration curve was plotted in the concentration range of 2-20 μg/ mL. The wavelength corresponding to maximum

absorbance of Emtricitabine measured at 298 nm against chloroform as blank.

Preparation of Sample Solutions of Emtricitabine

For the analysis of Emtricitabine two commercial brands namely Emtriva (100mg) and Atripla (100mg) tablets were purchased from Apollo pharmacy , Chennai (TN).Twenty tablets of the drug was weighed accurately and powdered, then 100 mg of the drug in powdered form dissolved in 40 ml of chloroform and sonicated for few minutes and filtered by using Whatman filter paper No.42. The filtrate formed is again diluted with double distilled water to get 10µg/mL concentration, taken in a ten 10 ml volumetric flasks. To each 10 ml flask 2mL of phosphate buffer of pH 7.4 solution is added. Then absorbance of Emtricitabine measured at298 nm against chloroform as blank.

Determination of λ Max

UV scan range of 200 nm to 400 nm was selected to determine maximum absorbance by using 10 µg/ml solution of the drug, the wave length corresponding to maximum absorbance was found at 298 nm. The spectrophotometric spectrum is shown in Figure 2

Preparation of Calibration Curve

On the basis of experimental results, calibration curve was plotted and shown in fig: 3 in the concentration range of 2-20 µg/ mL of eight standard solutions of Emtricitabine in chloroform as blank . UV scan range of 200 nm to 400 nm was selected to determine maximum absorbance of the drug. In this method the wavelength corresponding to maximum absorbance was found at 298 nm.

Validation of Method[13]

The spectrophotometric estimation of Emtricitabine is validated as per the directions of International conference on Harmonization to determine linearity, precision, accuracy, LOD and LOQ of the proposed method.

Linearity and Range

Standard stock solution of Emtricitabine in appropriate dilution were assayed as per the proposed method According to Beer's – Lambert's law the concentration range of Emtricitabine was found to be 2-20 µg/ mL, So that the calibration curve in the figure : 3 is linear in the given concentration range.

Precision

The precision of the proposed method of Emtricitabine was estimated by using concentrations of the drug were analyzed six times in a day (intra-day precision) and six continuous days (inter-day precision). Data is given in the Table 2.

Accuracy

The Accuracy of the proposed method of Emtricitabine was estimated by using standard addition method .This process is carried out by adding different amounts namely 80% ,100% and 120% of the pure sample of the drug to the pre-analysed formulation. Accuracy data of the drug is shown in the table-2

LOD and LOQ

LOD is Limit of Detection and LOQ is Limit of Quantitation . The LOD and LOQ of Emtricitabine were determined (Table : 1) by using standard deviation of the response and slope approach as per the directions of International Conference on Harmonization (ICH) guidelines. The limits of detection (LOD) is calculated by using the equation $LOD = \frac{3s}{k}$ Where, S = intercept of the standard deviation K = The slope of the calibration curve (mean) The limits of quantitation (LOQ), is calculated by using the equation $LOQ = \frac{10 S}{K}$ Where, S = intercept of the standard deviation K = The slope of the calibration curve (mean).

Recovery Studies of Emtricitabine

Recovery studies of Emtricitabine were performed to know the accuracy of the proposed method. This process is done by adding a known quantity of pure drug to a pre-analysed sample. The result of analysis of the drug is notified in the Table 3.

Table 1: Optical Parameters of Emtricitabine

S.No	Parameter	Emtricitabine
1	λMax (nm)	298nm
2	Beer's Law Limit (µg/ mL)	2-20
3	Correlation Coeffcient(r^2)	0.9910
4	Regression Equation (Y= a+bc)	Y=0.013X+0.049
5	Intercept (a)	0.0490
6	Slope (c)	0.0130
7	SD	5.627
8	Mean	11
9	Variance	31.6666
10	LOD (%)	0.129
11	LOQ (%)	0.432

Table 2: Determination of Accuracy and Precision of Emtricitabine

| S.No	Name of the sample	Labeled Amount (mg/capsule)	Amount Found* (mg)	Precision	
				Interday	Intraday
1	EMTRIVA	100	99.90	0.0082	0.0069
2	ATRIPLA	100	99.80	0.0094	0.0073

(*average of 6 determinations)

Table 3: Recovery Studies of Marketed Formulations of Emtricitabine

S.No	Name of the sample	Labeled Amount (mg/capsule)	% Level	Amount Found* (mg)	% Recovery
1	EMTRIVA	100	80	99.90	99.9
2	ATRIPLA	100	100	99.80	99.8

Table 4: Comparisons of the Proposed Method with other existing Methods for the Assay of Emtricitabine

S.NO	Reagent	λ Max	Beer's law limits µg mL^{-1}	Reference
1	Methanol-Acetonitrile (70:30)	296 nm	2-20	4
2	Distilled water	228 nm 260 nm	2-40	5
3	High purity distilled water	298.5 nm	0.5–40	6
5	MethanoL	241.1 nm	5-30	7
6	MethanoL	302.17 nm 306.88 nm	2-14 4-20	8
7	MethanoL	281 nm	6-48	9
8	Double distilled water	281 nm	4-24	10
9	Chloroform, Double distilled water, Phosphate Buffer 7.4	298nm	2-20	Proposed method
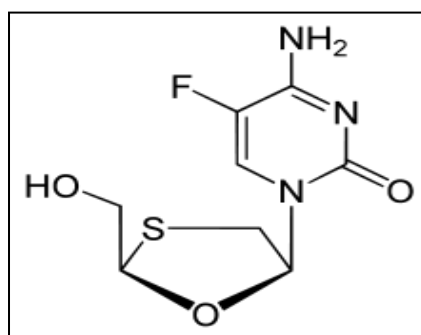
Figure 1: Structure of Emtricitabine
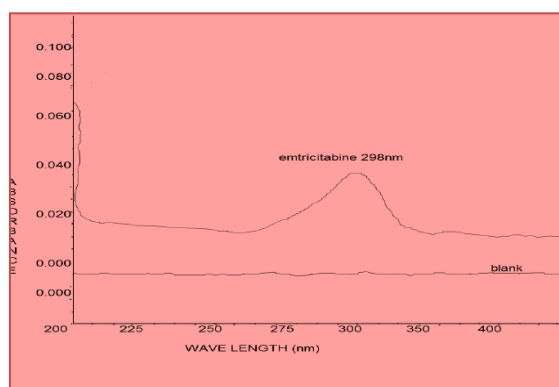
Figure 2: **U V Visible Spectrum of Emtricitabine**
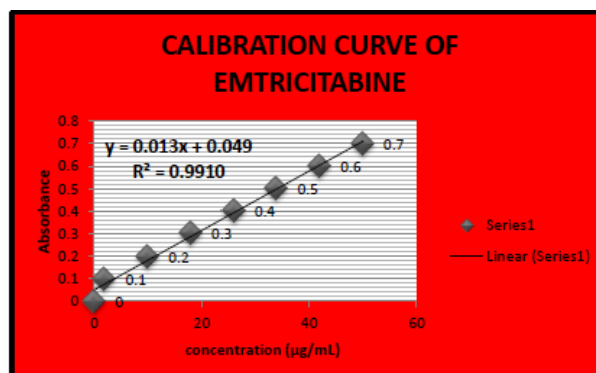
Figure 3: Calibration Curve of Emtricitabine

RESULTS AND DISCUSSION

The UV Spectrum of standard stock solutions of Emtricitabine shows absorption maximum at 298 nm, then the calibration curve is obtained by plotting a graph of absorbance verses concentration, the Beer –lamberts' law was verified from the data of calibration curve of the drug under investigation. The calibration curve of the drug is shown in the Figures 3. The linearity was observed between 2-20 µg/ mL and the graph of this drug shows a straight line with correlation coefficient of 0 .9910. The assay method of the drug was validated by the accuracy and precision of the proposed method shown in Table 2. The % recovery of 99.9-99.8 shows accuracy of the proposed method. The validated optical, statistical parameters, LOD and LOQ data of the drug was shown in Table 1.

CONCLUSION

In this paper a low cost simple, precise and more economical UV visible spectrophotometric method for the determination of Emtricitabine in bulk and pharmaceutical formulation has been developed and validated as per the directions of International conference on Harmonisation.

ACKNOWLEDGEMENT

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