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UV Visible Spectrophotometric Estimation of AntiRetroviral Drugs

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ABSTRACT

A new spectrophotometric method has been developed for the estimation of antiretroviral drugs namely Efavirenz and Emtricitabine in bulk and tablet dosage form. Efavirenz shows maximum absorbance at 256nm and Emtricitabine 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 4-28 µg/ mL for Efavirenz and 2-20 µg/ mL for Emtricitabine. The graph of both drugs shows a straight line with correlation coefficient of 0.9970 for Efavirenz and 0 .9910 for Emtricitabine. The assay method of both drugs was validated by of accuracy and precision of the proposed method. The results are validated as per the directions of International conference on Harmonization.

Keywords: Efavirez, Emtricitabine, UV spectrophotometry, Beer's law, validation, Tablet Analysis.

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INTRODUCTION

Efavirenz is a non –nucleoside reverse transcriptase inhibitor of HIV-1. It is an antiretroviral agent, because it blocks the activity of the reverse transcriptase of a retrovirus, so that it is also called Reverse transcriptase inhibitor. The chemical name of efavirenz is (4S)-6-chloro-4-(cyclopropylethynyl)-1, 4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one, the chemical formula is $C_{14}H_9ClF_3NO_2$, It has a calculated molecular mass of 315.7 g/mol. It is insoluble in water but freely soluble in chloroform, methanol, ethanol, and sodium hydroxide. The colour of this drug is white to pink. The structure of Efavirenz is shown in Figure 1

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 $C_8H_{10}FN_3O_3S$, molecular weight is 247.24g/mol . The colour of this drug is a white to off-white powder. 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 structure of Emtricitabine is shown in Figure 2

Literature survey of efavirenz reveals that an RP-HPLC method[1] and HP-TLC[2], for Emtricitabine some analytical methods reflected in literature namely CLC[3], liquid chromatography [4] and spectrophotometric analysis [5] . The comparisons of the proposed method with different existing methods for the assay of Efavirenz and Emtricitabine in pharmaceutical formulations have shown in Table: 4 & 5. Therefore, an attempt was made to develop a low cost precise and accurate spectrophotometric method for the estimation of Efavirenz and Emtricitabine in bulk and tablet dosage form.

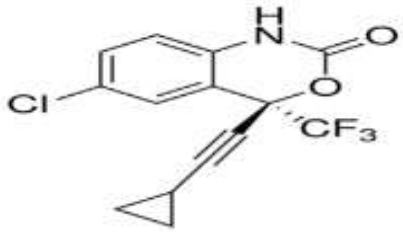

Figure 1: Structure of Efavirenz

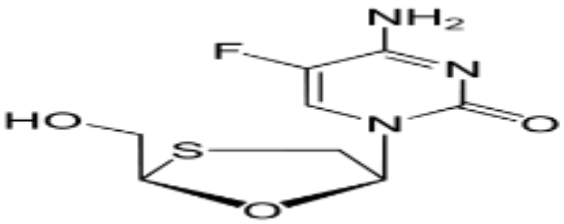

Figure 2: Structure of Emtricitabine

MATERIALS AND METHOD

Instruments and Apparatus

The absorbance of both drugs 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 used in this analysis are AR grade. Chloroform and phosphate buffer 7.4 is used throughout the analysis. Pharmaceutical formulation of Efavirenz and Emtricitabine was supplied by Emcure pharmaceuticals, Pune, (MH). Chloroform and phosphate buffer 7.4 procured from Merck india Ltd, Mumbai. Commercially available tablets namely Estiva (100mg),Stocrin (100mg), Emtriva (100mg), Atripla(100mg), procured from Apollo pharmacy Chennai (TN).

Selection of Solvent:

Chloroform and phosphate buffer 7.4 [21] are used throughout the analysis.

Selection of Method and Wave Length:

UV scan range of 200 nm to 400 nm was selected for both drugs of the proposed method and the wavelength corresponding to maximum absorbance was found at 256 nm for Efavirenz and calibration curve was taken at 256 nm. For 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 both drugs was determined by Linear regression Analysis.

Preparation of Standard Solutions of Efavirenz and Emtricitabine :

The 100 mg of standard (pure) drug of Efavirenz and Emtricitabine were weighed accurately and then transferred into 100 ml volumetric flasks to prepare 1000 µg/ mL[20] stock solution of both drugs and dissolved in chloroform solvent, to get the desired concentration of stock solutions . Both drugs were diluted with double distilled water to get different aliquots of 2, 4,6,8,10,12,14,16,18,20,22,24,26 and 28 µg/mL were taken in a ten 10 ml volumetric flasks separately and make up volume with double distilled water .To each flask 2mL of phosphate buffer 7.4 solution is added, then all dilutions of both drugs 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 4-28 µg/ mL and 2-20 µg/ mL for determination of Efavirenz and Emtricitabine in chloroform as blank. .The wavelength corresponding to maximum absorbance was found at 256 nm and 298 nm for Efavirenz and Emtricitabine respectively.

Table 1: Optical Parameters of Efavirenz and Emtricitabine

S.no	Parameter	Efavirenz	Emtricitabine
1	λMax (nm)	256nm	298nm
2	Beer's Law Limit (µg/ mL)	4-28	2-20
3	Correlation Coefficient(r^2)	0.9970	0.9910
4	Regression Equation (Y= a+bc)	Y=0.018X+0.012	Y=0.013X+0.049
5	Intercept (a)	0.0120	0.0490
6	Slope (c)	0.0180	0.0130

7	SD	8.1547	5.627
8	Mean	16.4	11
9	Variance	66.5	31.6666
10	LOD	0.135	0.129
11	LOQ	0.453	0.432

Preparation of Sample Solutions of Efavirenz and Emtricitabine

For the analysis of Efavirenz two commercial brands namely Estiva (100mg),Stocrin (100mg), tablets and Emtriva (100mg), Atripla(100mg) tablets for Emtricitabine were procured from Apollo pharmacy , Chennai (TN).Ten tablets of each brand of both drugs weighed accurately and powdered . 100 mg of both drugs in powdered form dissolved in 40 ml of chloroform separately and sonicated for few minutes and filtered by using whatmann filter paper No.42. The filtrate formed is again diluted with double distilled water to make 10µg/mL concentration of sample solution. To each flask 2mL of phosphate buffer of pH 7.4 solution is added. Then all dilutions of both drugs were scanned in the UV scan range of lambda max (λmax) 200 nm to 400 nm to determine maximum absorbance for this method. The absorbances of Efavirenz and Emtricitabine measured at256 nm and 298 nm respectively 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 both drugs separately the wave length corresponding to maximum absorbance was found at 256 nm and 298 nm for Efavirenz (Figure :3) and Emtricitabine (Figure :4) respectively.

Preparation of Calibration Curve:

The calibration curve was plotted in the concentration range of 4-28 µg/ mL and 2-20 µg/ mL of ten standard solutions of Efavirenz and Emtricitabine respectively in chloroform as blank. UV scan range of 200 nm to 400 nm was selected to determine maximum absorbance for both drugs in this method and the wavelength corresponding to maximum absorbance was found at 256 nm and 298 nm for Efavirenz and Emtricitabine (Figure : 5&6) respectively.

Validation of Method

The spectrophotometric studies of drugs namely Efavirenz and Emtricitabine are validated as per the directions of International conference on Harmonization to determine linearity, precision, accuracy, LOD and LOQ of the proposed method[2].

Linearity and Range

Standard stock solutions of both drugs namely Efavirenz and Emtricitabine in appropriate dilution were assayed as per the proposed method, According to Beer's–Lambert's law the

concentration range was found to be 4-28 µg/ mL for Efavirenz and 2-20 µg/ mL for Emtricitabine. So that the calibration curves of both drugs (Figure: 5&6) are linear in the concentration range.

Precision

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

Accuracy

The Accuracy of the proposed method of Efavirenz and 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 both drugs to the pre-analyzed formulation. Accuracy data of both drugs shown in the Table-2.

LOD and LOQ

LOD is Limit of Detection and LOQ is Limit of Quantitation. The LOD and LOQ of Efavirenz and 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 $\text{LOD} = \frac{3s}{k}$ 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 $\text{LOQ} = \frac{10\ S}{K}$ Where, S = intercept of the standard deviation K = The slope of the calibration curve (mean) [22].

Recovery Studies of Efavirenz and Emtricitabine

Recovery of Efavirenz and 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-analyzed sample. The result of analysis of both drugs and recovery studies are notified in the table: 3

RESULTS AND DISCUSSION

The U.V Spectrum of standard stock solutions of Efavirenz shows absorption maximum at 256 nm and Emtricitabine shows absorption maximum at 298 nm , then 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 both drugs. The calibration curve of both drugs shown from Figure 5 -6. The linearity was observed between 4-28 µg/ mL for Efavirenz and 2-20 µg/ mL for Emtricitabine. The graph of both drugs shows a straight line with correlation coefficient of 0.9970 for Efavirenz and 0 .9910 for Emtricitabine. The assay method of both drugs was

validated by the accuracy and precision of the proposed method shown in Table 2. The % recovery of 98.2-99.8 shows accuracy of the proposed method.

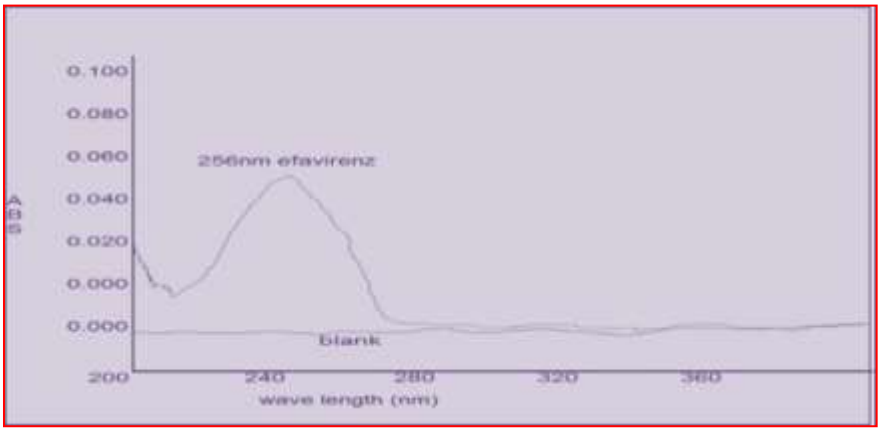

Figure 3: UV Visible Spectrum of Efavirenz

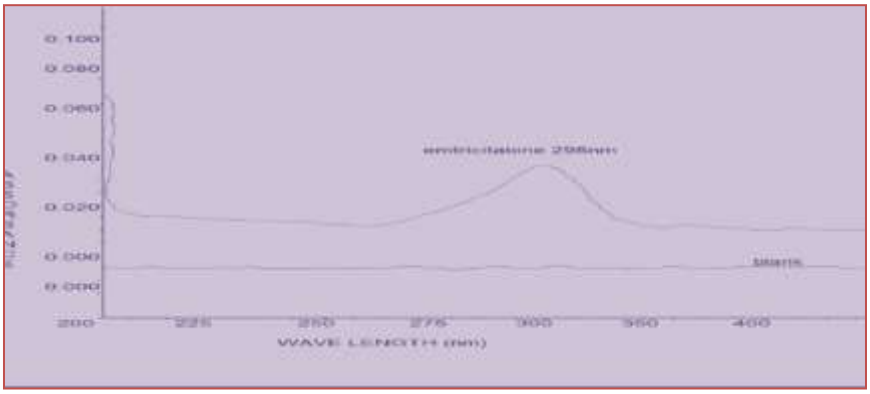

Figure 4: UV Visible Spectrum of Emtricitabine

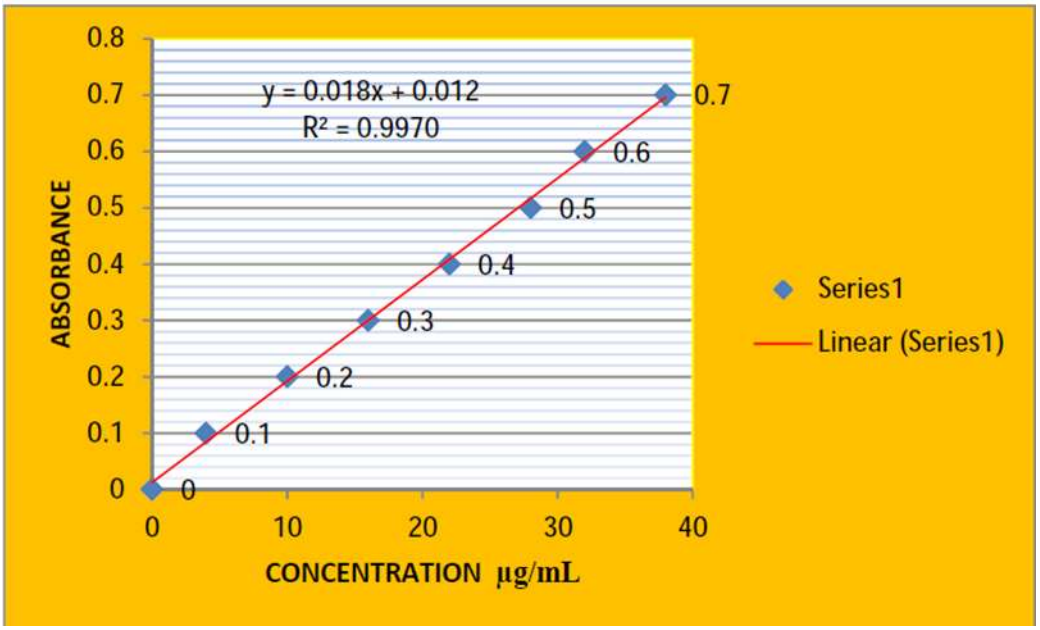

Figure 5: Calibration Curve of Efavirenz

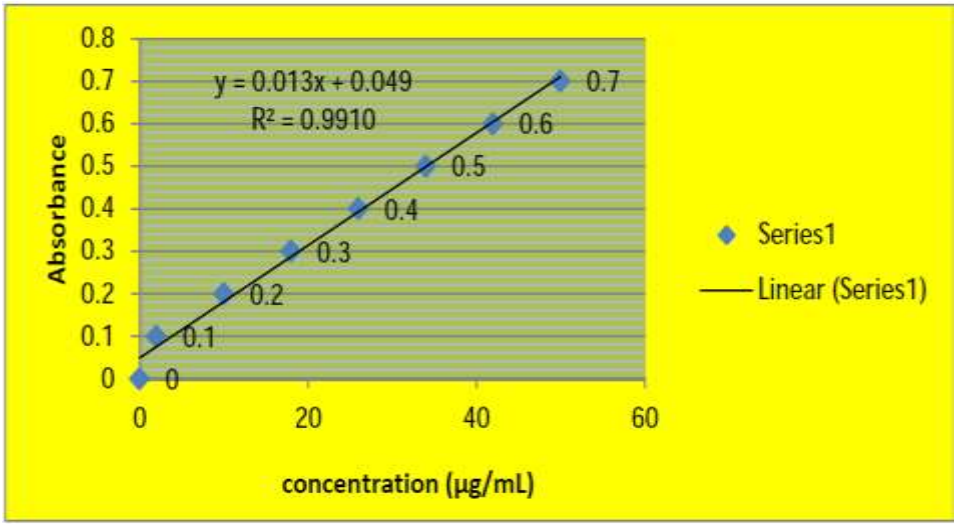

Figure 6: Calibration Curve of Emtricitabine

Table 2: Determination of Accuracy and Precision of Efavirenz and Emtricitabine

S.NO	Name of the Sample	Labeled Amount (mg/capsule)	Amount Found* (mg)	Precision	
				Inter day	Intraday
1	ESTIVA	100	98.20	0.0062	0.0064
2	STOCRIN	100	98.14	0.0096	0.0078
3	EMTRIVA	**100**	**99.90**	**0.0082**	**0.0069**
4	ATRIPLA	**100**	**99.80**	**0.0094**	**0.0073**

Table 3: Recovery Analysis Marketed Formulations of Efavirenz and Emtricitabine

S.NO	Name of The Sample	Label Claim In (mg)	% Level	Amount Found (mg)	% Recovery
1	ESTIVA	100	80	98.20	98.2
2	STOCRIN	100	100	98.10	98.1
3	EMTRIVA	100	80	99.90	99.9
4	ATRIPLA	100	100	99.80	99.8

Table 4: Comparisons of the proposed method with other existing methods for the assay of Emtricitabine in pharmaceutical formulations.

S.no	Reagent	λMax	Beer's law limits µg mL^{-1}	Reference
1	Methanol-acetonitrile (70:30)	296 nm	2-20 µg mL^{-1}	6
2	Distilled water	228 nm 260nm	2-40µg mL^{-1}	7
3	High purity distilled water	298.5 nm	0.5–40 µg mL^{-1}	8
5	Methanol	241.1 nm	5-30µg mL^{-1}	9
6	Methanol	302.17 nm 306.88 nm	2-14 µg mL^{-1} 4-20 µg mL^{-1}	10
7	Methanol	281 nm	6-48µg mL^{-1}	11
8	Double distilled water	281 nm	4-24µg mL^{-1}	12
9	Chloroform, phosphate buffer 7.4	298nm	2-20µg mL^{-1}	Proposed method

Table 5: Comparisons of the proposed method with other existing methods for the assay of Efavirenz in pharmaceutical formulations

S.no	Reagent	λ Max	Beer's law limit µg mL^{-1}	Reference
1	Beta naphthol, sodium nitrite, methanol	561 nm	10-20µg mL^{-1}	13
2	Methanol-phosphate buffer(70:30)	254nm	2-12µg mL^{-1}	14
3	Methanol-sodium lauryl sulphate	247 nm	1-20 µg mL^{-1}	15
4	DMSO	264nm	2-18µg mL^{-1}	16
5	Mixture of water, acetonitrile, methanol, sodium hydroxide and hydrochloric acid	247 nm	10-40µg mL^{-1}	17
6	Methanol	248 nm	5-40µg mL^{-1}	18
7	Methanol	239 nm	5-40µg mL^{-1}	19
8	Chloroform, phosphate buffer of pH 7.4,	256 nm	4-28 µg mL^{-1}	Proposed method

CONCLUSION

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

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