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Development and Validation of Spectrophotometric Method for Estimation of Emtricitabine in Tablet Dosage Form

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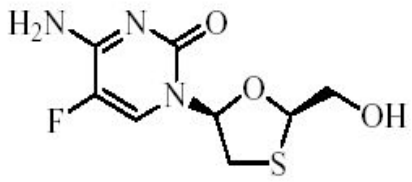
Abstract: Two simple, precise and economical UV methods have been developed for the estimation of Emtricitabine in bulk and pharmaceutical formulations. Emtricitabine has the absorbance maxima at 241.1nm (Method A), and in the first order derivative spectra, Showed zero crossing at 241.1nm, with a sharp peak at 232.7nm when n=1 (Method B).Drug followed the Beer's Lamberts range of 5–30 µg/ml for the Method A&B.The limits of detection were found to be 0.0684 µg/ml and 0.185 µg/ml for Method Aand Method B respectively. The limit of quantification for Method A and Method B were found to be 0.207µg/ml and 0.555 µg/ml respectively. Results of analysis were validated statistically and by recovery studies and were found to be satisfactory.
Key words: Emtricitabine, UV Spectrophotometry, Derivative Spectroscopy.

1. INTRODUCTION:

Emtricitabine is an analogue of cytidine[1]. 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, emtricitabine can help to lower the amount of HIV, or "viral load", in a patient's body and can indirectly increase the number of immune system cells (called T cells or CD4+ T-cells). Both of these changes are associated with healthier immune systems and decreased likelihood of serious illness.Chemical name of Emtricitabine is 5-fluoro-1-(2R, 5S)-[2-(hydroxymethyl)- 1, 3- oxathiolan-5-yl]cytosine. It has a molecular formula of $C_8H_{10}FN_3O_3S$ and a molecular weight of 247.24[2].



Literature survey reveals thatHPLC [3] and LC [4]methods as well asHPLC [5, 6, 7, 8] in plasma, HPTLC [9] and AUC [10] methods for simultaneous estimation wereexisted for estimation of Emtricitabine. No single UV method for Emtricitabine is reported till date using derivative spectroscopy. Hence an attempt has been made to develop new UV method for its estimation in bulk and pharmaceutical formulations with good accuracy, simplicity, precision and economy.

2. EXPERIMENTAL

2.1 Instruments and reagents

A Shimadzu UV - 1800 UV/VIS spectrophotometer was used with 1 cm matched quartz cell.
All the chemicals used were of analytical grade. Methanol A.R. grade was procured from Loba Chem. Ltd., Mumbai. An analytically pure sample of Emtricitabine was obtained from Hetero Pharmaceutical Limited, Hyderabad as a gift sample. A Shimadzu was used with 1 cm mathes quartz cell. Tablet of 200mg were procured from local pharmacy.

2.2 Preparation of standard stock solution

Standard stock solution was prepared by dissolving accurately weighed 100 mg of Emtricitabine in Methanol and the volume was made up to 100 ml with Methanol in 100 ml volumetric flask (Stock solution-I, 1000 µg/ml). 10 ml of stock solution-I was diluted to 100 ml with Methanol (Stock solution-II, 100 µg/ml). 1 ml of stock solution-II was taken in 10 ml standard flask diluted to 10 ml with Methanol to get the concentration 10 µg/ml. The absorbance of resulting solution was measured against respective blank solution in the UV region of 200-400 nm, which shows maximum absorbance at 241.1 nm.

2.3 Zero order spectroscopic method

Use above stock solution-II to prepare rang of standard solution from 5, 10, 15, 20, 25 and 30. The solutions were scanned in the range from 400-200nm (method A), and the peaks were observed at 241.1nm and 282.6nm. The wavelength selected for the analysis of the drug was 241.1nm **(figure 1)**. The drug followed the Beer's-Lamberts law in the range of 5-30 µ g/ml. Using calibration curve the concentration of the sample solution can be determined.

2.4 First order derivative spectroscopic method

The first order derivative spectra at n=1 (method B)[11], showed a sharp peak at 232.7 **(figure 2)**. The absorbance difference at n=1 (dA/d) is calculated by the inbuilt software of the instrument which was directly proportional to the concentration of the standard solution. The standard drug solution was diluted so as to get the final concentration in the range of 5-30 µg/ml and scanned in the first order derivative spectra. The calibration curve of dA/d against concentration of the drug showed linearity.

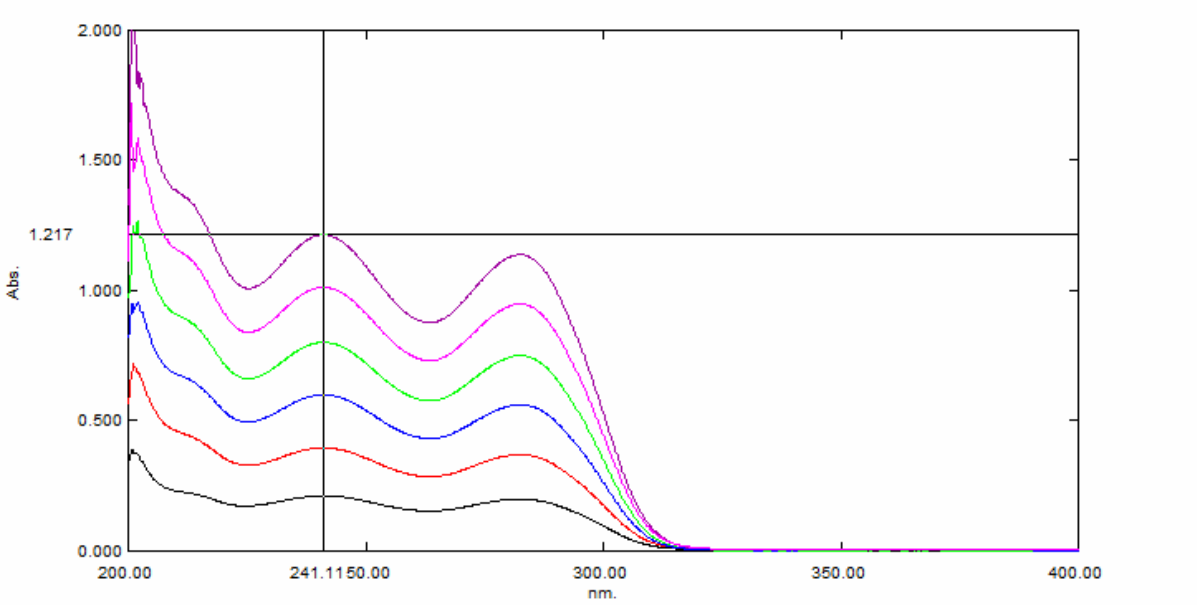
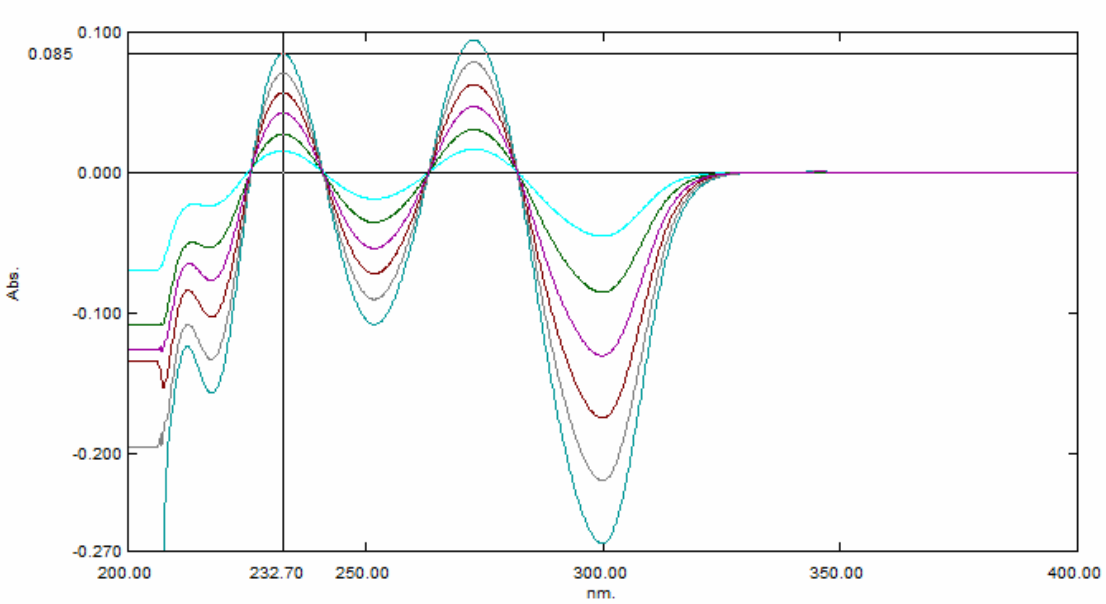


Figure 1: Zero orderderivative spectra of Emtricitabine



.**Figure 2: First order derivative spectra of Emtricitabine with n=1**

2.5 Analysis of the Tablet formulation

20 tablets of Emtricitabine were weighed, powdered in glass mortar and the powder equivalent to 10 mg of Emtricitabine was weighed accurately and transferred into a 100 ml standard volumetric flask. The contents were dissolved in Methanol and sonicated for thirty minutes. This solution was filtered through 0.45 micron Whatmann filter paper. 1 ml of the filtrate was diluted to 10 ml with Methanol to get the solution of 100 µg/ml. An aliquot of 1 ml of test solution was diluted to 10 ml with Methanol in 10 ml standard volumetric flask to produce the concentration 10 µg/ml.

2.6 Validation of the method

All these methods were validated according to ICH guidelines[12, 13] by carrying out analysis of six replicate sample of tablet. Recovery studies were carried out at three different levels i.e. 50%, 100%, and 150% by adding the pure drug to previously analysed tablet powder sample. From the amount of drug found, percentage recovery wascalculated.Precision method was studied as intra-day and inter-day variations. The Ruggedness of marketed formulation was carried out.

Table: 1Results of calibration curve

Sr. No.	Conc. (µg/ml)	Method A	Method B
		Absorbance at 241.1 nm	Absorbance at 232.7 nm
1	5	0.212	0.014
2	10	0.397	0.027
3	15	0.600	0.043
4	20	0.802	0.057
5	25	1.013	0.071
6	30	1.214	0.085

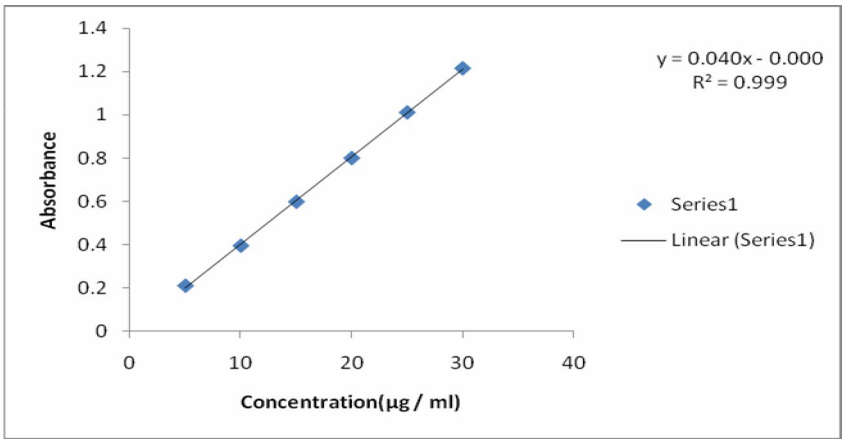


Fig: 3Linearity curve forEmitricitabine at 241.1 nm by Zero order derivative spectroscopy

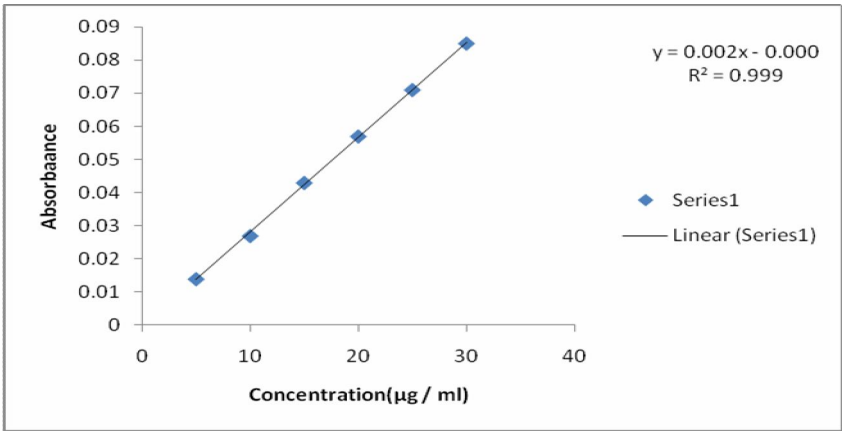


Fig: 4Calibration curve for Emtricitabine at 232.7 nm by first order derivative spectroscopy

Table: 2Optimum conditions, Optical characteristics and Statistical data of the Regression equation in UV method

Parameters	Method A	Method B
λ_{max} (nm)	241.1	232.7
Beer's law limits (µg/ml)	5-30	5-30
Molar extinction coefficient (L mol^{-1} cm^{-1})	$0.0465X10^4$	$0.017 X 10^4$
Sandell's sensitivity (µg/cm^2-0.001 absorbance units)	0.0215	0.588
Regression equation (Y*)	Y= 0.0404 C - 0.0007	Y=0.0029C-0.0006
Slope (b)	0.0404	0.0029
Intercept (a)	0.0007	0.0006
Correlation coefficient(r^2)	0.9996	0.9995
% RSD**	0.668	0.341
Limit of detection (µg/ml)	0.0684	0.185
Limit of quantitation (µg/ml)	0.2073	0.555

*Y= bC + a where C is the concentration of Emtricitabine in mcg/ml and Y is the absorbance at the respective $\lambda_{max.}$

**Average of six determinations.

Table: 3 Determination of Accuracy results for Emtricitabine by zero order derivative spectroscopy

Brand name	Concentration of sample (µg/ml)	Amount of pure drug added(µg/ml)	Amount Recovered (µg/ml)	% Recovery± SD**
Emtriva	10	5	4.98	99.74 ± 0.591
	10	10	9.98	99.85 ± 0.832
	10	15	15.03	100.24 ± 0.613

**Average of six determinations.

Table: 4 Determination of Accuracy results for Emtricitabine by First order derivative spectroscopy

Brand name	Concentration of sample (µg/ml)	Amount of pure drug added(µg/ml)	Amount Recovered (µg/ml)	% Recovery± SD**
Emtriva	10	5	4.98	99.68 ± 0.547
	10	10	9.96	99.67 ± 0.796
	10	15	14.95.	99.71 ± 0.643

**Average of six determinations.

Table: 5 Determination of Precision results for Emitricitabine at 241.1 nm by Zero order derivative spectroscopy

Conc. (µg/ml)	Inter-day Absorbance Mean ± SD**	% RSD	Intra-day Absorbance Mean ± SD**	% RSD
5	0.213 ± 0.0016	0.751	0.211 ± 0.0016	0.758
10	0.375 ±0.0015	0.400	0.377 ±0.0011	0.291
15	0.601 ±0.0012	0.199	0.599 ±0.0018	0.300
20	0..802 ±0.0017	0.211	0.806 ±0.0017	0.210
25	1.016± 0.0011	0.108	1.012 ±0.0012\	0.118
30	1.212 ± 0.0018	0.148	1.208 ± 0.0014	0.115

**Average of six determinations.

Table: 6 Determination of Precision for Emtricitabine at 232.7 nm by firstorder derivative spectroscopy

Conc. (µg/ml)	Inter-day Absorbance Mean ± SD**	%RSD	Intra-day Absorbance Mean ± SD**	% RSD
5	0.015 ± 0.0006	4	0.017 ± 0.0004	2.352
10	0.028 ±0.0009	3.214	0.029 ±0.0007	2.413
15	0.042 ±0.0011	2.619	0.040 ±0.0010	2.5
20	0.056 ±0.0014	2.5	0.055 ±0.0019	3.454
25	0.071 ± 0.0015	2.112	0.073 ± 0.0012	1.643
30	0.085 ± 0.0018	2.117	0.084 ± 0.0017	2.023

**Average of six determinations.

Table: 7 Ruggedness results for Emtricitabine at 241.1 nm by Method A and at 232.7 nm by Method B

Brand name		Label claim (mg)	Analyst I		Analyst II	
			Amount found (mg)	Recovery ± SD** (%)	Amount found (mg)	Recovery ± SD** (%)
Emtriva	**Method A**	200	198.4	99.2 ± 0.16	198.1	99.05 ± 0.19
	Method B	200	197.5	98.7 ±0.21	198.2	99.1 ± 0.18

**Average of six determinations.

3. RESULTS AND DISCUSSION

All the methods A and B for the estimation of Emtricitabine in tablet dosage were found to be simple, accurate and reproducible. Beer-lambert's law wasobeyed in the concentration range of 5-30 µg/ml in all these methods. The accuracy of the method was assessed by recovery studies at three different levels i.e. 50%, 100%, 150%. The values of standard deviation were satisfactory and the recovery studies were close to 100%.The results for percentage recovery obtained from the amount of drug are given in table 3&4. The %RSD value was less than 2 indicative of accuracy of the method. Results for precision study are reported in Table 5&6. The results of analysis of marketed formulation are shown in Table 7. The values obtained were found to be within the limit.Hence these methods can be useful in routine analysis of Emtricitabine in bulk drug and formulations.

4. CONCLUSION

The developed method was found to be simple, sensitive, accurate and reproducible and can be used for routine quality control analysis of Emtricitabine in bulk and in pharmaceutical formulations.

5. ACKNOWLEDGEMENT

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