Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2014, 5(4):10-15

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Spectrophotometric methods for the determination of emtricitabine in bulk and in its pharmaceutical formulations using aromatic aldehydes as chromogenic reagents

P. Saifulla Khan*, P. Raveendra Reddy and V. Krishna Reddy

Department of Chemistry, Sri Krishnadevaraya University, Anantapuramu, India

---

ABSTRACT

Two simple, accurate, sensitive and extraction-free spectrophotometric methods (A and B) have been developed for the determination of emtricitabine in bulk and in its pharmaceutical dosage forms. Both A and B methods are based on the reaction of emtricitabine with p-Dimethylaminobenzaldehyde (PDAB) and 4-Hydroxy-3-methoxy benzaldehyde (HMBA) in the presence of dilute sulphuric acid gives red colored chromogens, which show maximum absorbance at 495nm and 480nm respectively. Beer's law is obeyed in the concentration range 1.0-40.0 µg mL^{-1} for method A, and 2.5-35.0µg mL^{-1} for method B respectively. The molar absorptivity and sandell's sensitivity values are 6.1 $x10^3$ L $mo1e^{-1}cm^{-1}$ and 0.04 µg cm^{-2} for method A, and 6.9 $x10^3$ L $mo1e^{-1}cm^{-1}$ and 0.035 µg cm^{-2} for method B respectively. The results of these methods are validated statistically and by recovery studies. The proposed methods are economical, sensitive and can be used for the determination of emtricitabine in bulk and in its pharmaceutical formulations.

Key words: UV-Visiblespectrophotometric; *p*-Dimethylaminobenzaldehyde (PDAB); 4-Hydroxy-3-methoxy benzaldehyde (HMBA); Sulphuric acid; Methanol.

INTRODUCTION

Emtricitabine (EMT) has the chemical name 4-amino-5-fluoro-1-[(2R, 5S)-2-(hydroxymethyl)-1, 3-oxathiolan-5-yl]-1, 2-dihydropyrimidin-2-one [1]. It is white to off-white crystalline powder with a molecular formula of $C_8H_{10}FN_3O_3S$ and a molecular weight of 247.24 g $mole^{-1}$. The structural formula is shown in Fig.1.

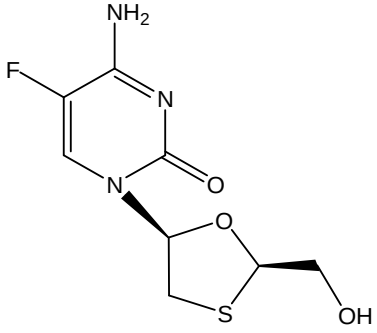


Fig.1. Structure formula of Emtricitabine

Emtricitabine is a nucleoside reverse transcriptase inhibitor related to cytosine with antiretroviral activity against HIV. Emtricitabine works by inhibiting reverse transcriptase enzyme that copies HIV RNA into new viral DNA. It

can help lower the level of HIV in the patient's body and can indirectly increase the number of immune system cells (called T cells or CD_{4+} T-cells). It is used for the prevention of perinatal HIV-1 reverse transcriptase [2]. It is also active against Hepatitis-B-virus [3, 4]. Literature survey indicated that few analytical methods are reported for the analysis of emtricitabine by HPLC [5], LC [6], HPLC-in plasma[7-10] , HPTLC [11] and few UV-spectrophotometric methods [12-17] either alone or combined with other drugs. To the best of our knowledge, only one visible spectrophotometric method was reported by Janakipathi et al [18]. In the present study we are reporting two simple, sensitive, accurate and economical spectrophotometric methods for the determination of emtricitabine using *p*-Dimethylaminobenzaldehyde (PDAB), and 4-Hydroxy-3-methoxybenzaldehyde (HMBA).

MATERIALS AND METHODS

Instruments used:
Shimadzu UV-1700 Pharma spec with 1cm matched quartz cell was used for spectral measurements.

Preparation of reagents:
All the chemicals and reagents used were of analytical grade and the solutions were prepared in double distilled water

P-Dimethylamino benzaldehyde (PDAB) and 4-Hydroxy-3-methoxybenzaldehyde (HMBA) (5.0 % w/v):
Accurately weighed 5.0 g of respective p-dimethylaminobenzaldehyde or 4-Hydroxy-3-methoxybenzaldehyde and dissolved in methanol and made up to the mark with methanol in 100 mL volumetric flask.

Sulphuric acid (0.1N):
0.27 mL of concentrated sulphuric acid was diluted to 100mL with distilled water in a 100mL beaker and standardize volumetrically.

Preparation of standard drug solution:
A standard stock solution containing 1.0 mg mL^{-1} was prepared by dissolving 10 mg of emtricitabine in 10 mL of distilled water. From this, a working standard solution containing 100µg mL^{-1} was prepared by appropriately diluting the stock solution with distilled water.

Recommended Procedures:
The following procedures were recommended for the assay of emtricitabine in bulk and pharmaceutical formulation samples.

For Bulk samples
Aliquots of standard drug solution of emtricitabine 0.1 – 4.0 mL (100µg mL^{-1}) were taken and transferred into a series of 10 mL volumetric flasks. To each flask 2mL of methanolic *p*-Dimethylaminobenzaldehyde (PDAB) (5%w/v) or 4-Hydroxy-3-methoxybenzaldehyde (HMBA) (5%w/v) and 0.5mL of H_2SO_4 (0.1N) were added. After thoroughly shaking, the volumetric flasks were set aside for 10 minutes, for the reaction to complete. The volumes in each flask were diluted to 10 mL with methanol. The absorbances of the orange red colored solutions were measured at 495 nm (PDAB) and 480 nm (HMBA) against the reagent blank and the calibration curves were plotted.

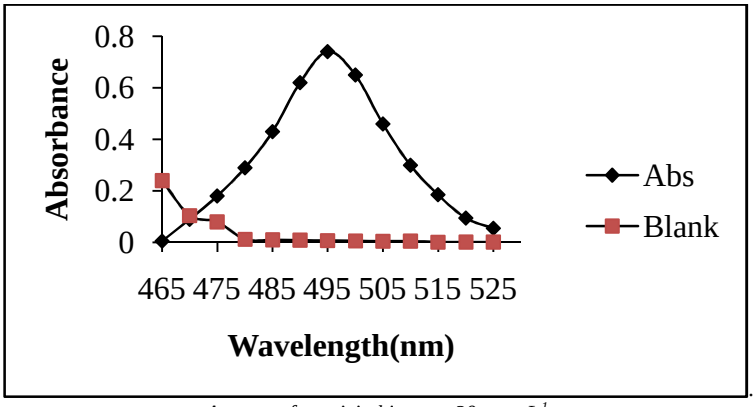
For pharmaceutical formulations
The methods proposed for the bulk samples were extended for the determination of emtricitabine in tablet formulations, 20 tablets of Emtriva® 200 mg (Gilead Sciences Inc) were weighed, powdered and the powder equivalent to 10 mg of emtricitabine was dissolved in distilled water in a 10 mL volumetric flask. 10mL of the above solution was further diluted to 100mL and analyzed as described, in the above mentioned methods. The analysis procedure was repeated three times to evaluate the precision.

RESULTS AND DISCUSSION

Absorption spectra:
In a 10 ml volumetric flask, 2mL of methanolic *p*-Dimethylaminobenzaldehyde (PDAB) (5%w/v) or 4-Hydroxy-3-methoxybenzaldehyde (HMBA) (5%w/v) and 0.5mL of H_2S0_4 (0.1N) were mixed and made up to the mark with methanol. In another 10 ml volumetric flask, 2mL of methanolic *p*-Dimethylaminobenzaldehyde (PDAB) (5%w/v) or 4-Hydroxy-3-methoxybenzaldehyde (HMBA) (5%w/v) , 0.5mL of H_2S0_4 (0.1N) and specified amounts of EMT in final solutions (30 µg $mL^{-1}$for EMT-PDAB and 20 µg $mL^{-1}$for EMT-HMBA) were taken and the resultant orange

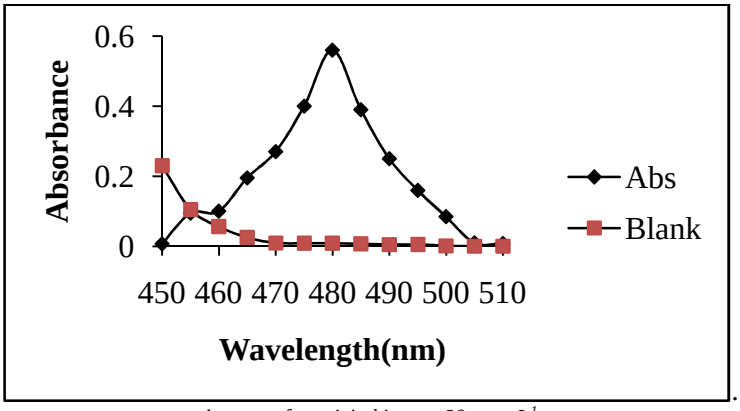
red colored solution was diluted up to the mark with methanol. The absorbances of both the solutions were measured at different wavelengths in the region 350-600 nm against the corresponding blanks.

The plots drawn between the wavelength and absorbance values are shown in Fig.2 and Fig.3 for EMT-PDAB, and EMT-HMBA respectively which indicate that the colored solution possesses maximum absorbances at 495 nm, and 480 nm where the reagent blank is showing relatively very low absorbance respectively. Therefore, further analytical studies were carried out at 495 nm, and 480 nm using the reagent blank.



Amount of emtricitabine = 30 µg mL^{-1}
Concentration of PDAB = 1.0% w/v

Fig .2: Absorption spectrum of emtricitabine with PDAB in sulphuric acid



Amount of emtricitabine = 20 µg mL^{-1}
Concentration of HMBA = 1.0% w/v

Fig .3: Absorption spectrum of emtricitabine with HMBA in sulphuric acid

Optical Characteristics:

In order to verify the applicability of Beer's law for the formed colored species in these proposed methods, the absorbances at appropriate wavelengths of a set of solutions containing varying amounts of EMT and specified amounts of reagent(as given in the recommended procedure) were recorded against the corresponding reagent blank. The Beer's law plots of these systems were recorded. Beer's law limits, molar absorptivity, Sandell's sensitivity, LOD and LOQ for emtricitabine were calculated and recorded in Table 1.

Interference studies:

To assess the applicability of the method developed for the analysis of the emtricitabine, the effect of a different range of additives usually present in the formulations under optimum conditions were investigated separately. In all the methods proposed, the commonly used additives in the preparation of formulations did not interfere with the assay of emtricitabine by proposed methods.

Table 1: Optical characteristic, precision and accuracy data of the proposed methods for emtricitabine

Parameters	EMT-PDAB	EMT-HMBA
λ_{max} (nm)	495	480
Beer's law limits(µgmL^{-1})	1-40	2.5-35
Molar Absorptivity(L mole^{-1}cm^{-1})	6.099 x10^{3}	6.922x10^{3}
Sandell's Sensitivity (µg cm^{-2} /0.001 absorbance unit)	0.0405	0.035
Regression Equation (Y= mx +c) Slope(m) Intercept(c)	 0.024 + 0.007	 0.028 + 0.005
Limit of detection(LOD) (µgmL^{-1})	0.3025	0.2357
Limit of quantification(LOQ) (µgmL^{-1})	0.9166	0.7142
Correlation Coefficient (r)	0.999	0.997
Precision(% RSD)	0.524	0.450
Standard error of estimate	0.0502	0.0328

Analysis of Emtriva tablets

To evaluate the applicability of the proposed methods for the analysis of synthetic drug samples, Emtriva tablets (200mg) (Gilead Science, Inc) were powdered thoroughly. Known quantities of this powder were analyzed for their emtricitabine content by the proposed methods and the results were compared with the reported labeled amount by calculating the % error. The results are presented in Table 2.

To study the accuracy and reproducibility of the developed methods, known quantities of emtricitabine were spiked into the drug sample and the total emtricitabine contents were determined by the present methods (standard addition method). From the obtained values the recovery percentage of the drug were calculated and given in Table 3.

Table 2: Assay of emtricitabine in Emtriva® tablet formulations

Sample No.	Labeled amount (mg)	*Amount obtained by proposed method (mg)		Error (%)	
		PDAB	HMBA	PDAB	HMBA
1	200	199.5	198.8	0.25	0.60
2	200	199.7	199.6	0.15	0.20
3	200	198.9	199.2	0.55	0.40

** Average of three determinations*

Table 3: Results of recovery study by standard addition method

EMT in tablet (µg mL^{-1})	Amount of EMT spiked (µg mL^{-1})	Drug added (%)	*Amount found (µg mL^{-1})		% Recovery by the proposed method	
			PDAB	HMBA	PDAB	HMBA
12.0	3.0	25	14.92	15.05	99.26	100.13
12.0	6.0	50	18.12	17.97	100.46	99.83
12.0	12.0	100	24.08	24.13	100.35	100.15

** Average of three determinations.*

Chemistry of the formed colored species

Emtricitabine possesses primary amine group which may be undergoing condensation reaction with aromatic aldehydes i.e. PDAB or HMBA. In the present study, the drug involves in quantitative reaction with PDAB or HMBA reagents. The reaction is based on the condensation of emtricitabine with methanolic p-Dimethylamino benzaldehyde or 4-Hydroxy-3-methoxybenzaldehyde in acidic medium there by producing orange red colored Schiff's base complexes with maximum absorbance at 495 nm and 480 nm respectively. Stability study of the developed chromogen was carried out by measuring the absorbance values at a time intervals of 15 mins for 5 hrs, and it was found to be stable for more than 3 hrs at room temperature. The linearity was found to be in the concentration range of 1-40 µg mL^{-1} and 2.5-35 µg mL^{-1}with PDAB and HMBA respectively. Based on the analogy, the proposed structures of formed colored complexes are given in Fig. 4 and Fig. 5.

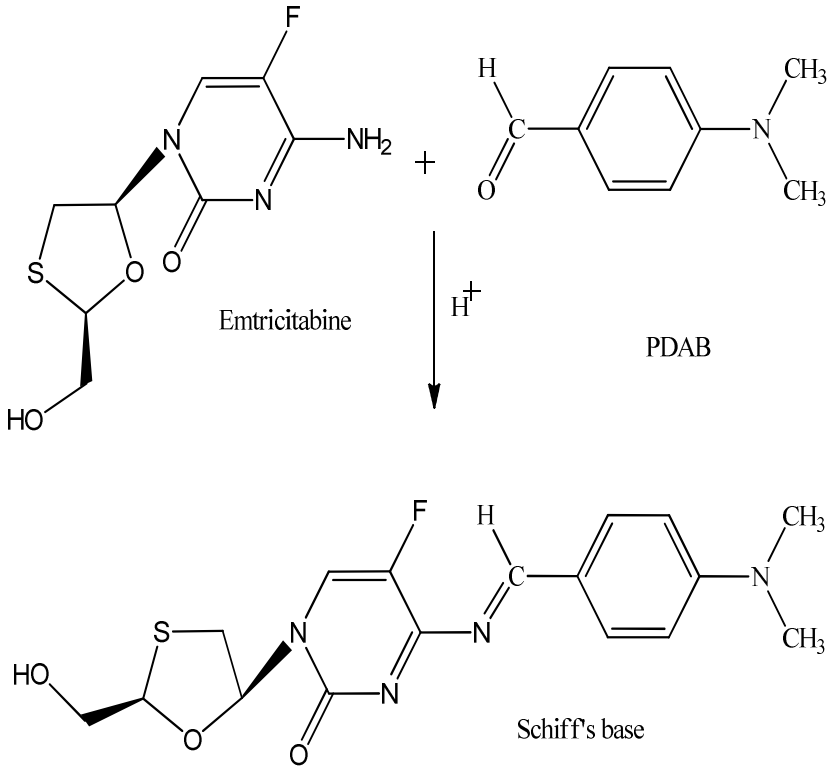


Fig 4.7: Probable reaction mechanism of emtricitabine with PDAB in H_2SO_4

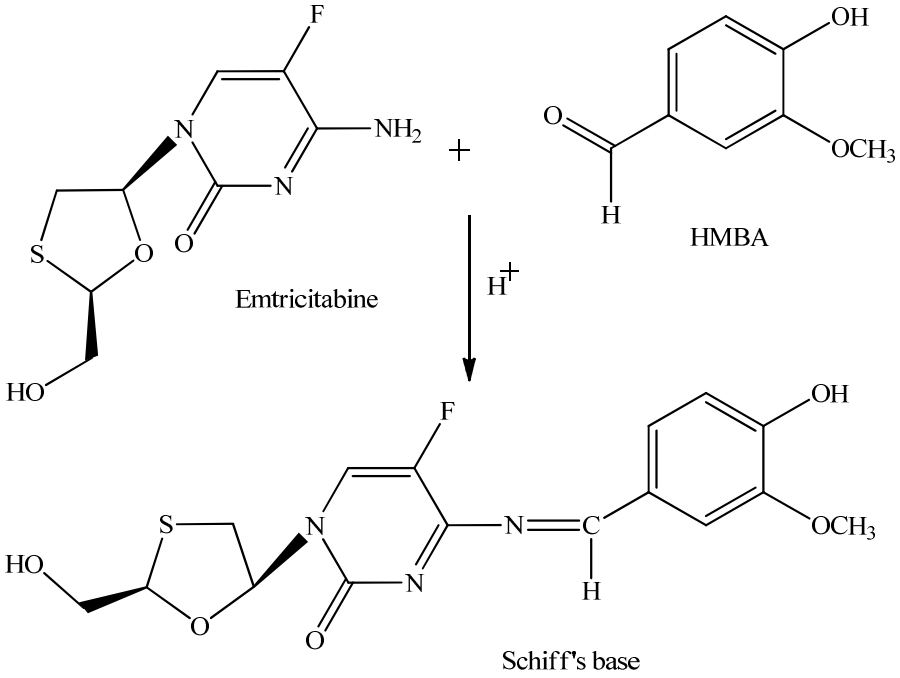


Fig 4.8: Probable reaction mechanism of emtricitabine with HMBA in H_2SO_4

CONCLUSION

In the present study the author has been developed two simple, accurate and sensitive extraction-free spectrophotometric methods for the determination of emtricitabine in bulk and pharmaceutical dosage forms. These methods are developed based on Schiff's base complexes formed between emtricitabine and PDAB or HMBA in acidic medium. Based on molarabsorptivity data and Beer's law range, it may be concluded that method EMT-HMBA is more sensitive than method EMT-PDAB. The proposed methods are simple, sensitive, accurate and economical for routine analysis of emtricitabine in bulk and its parenteral formulations.

Acknowledgement
The author acknowledges M/s Vishnu Chemicals Limited, Hyderabad for the supply of emtricitabine as gift sample.

REFERENCES

[1] S.C.Sweetman,(Ed)., Martindale-The complete drug reference, vol.1, (The pharmaceutical press, London, U K, 36th edition, **2009).**
[2] S. Budawari, The Merck Index, (Merck and Co. Inc., New Jersey, **2001**) 3568,9151.
[3] C.S. Sean., Martindale: The complete drug reference, (pharmaceutical press, London, **2002**) 620,642.
[4] R.G.Gish, H. Trinh et.al, *J. Hepatol.*, **2005**, 43(1), 60-66.
[5] G. Arjun, D. Satish Kumar, P. Yogeswaran, J. Sathyabrata , B. David , et al, *Der Pharma Chemica.*, **2010,** 2(2), 1-2.
[6] P. Murali, A.M. Dunge , H. Jos ,V.S. Ann , A. Erwin , *Talanta.*, **2010**, 82(1), 125-128.
[7] J.A.H. Droste, R.E. Aarnoutse, D.M. Burger, *J Liq Chromatogr Relat Technol.*, **2007**, 30(18),2769-2778.
[8] L.R. Naser , D. Rustin, Crutchley, D.M.K. Angela, *J Chromatogr B.*, **2005**, 822 (1-2), 201-208.
[9] N. Stefania, B. Alessio, I. Giuseppe, N. Pasquale , P.P. Leopoldo, et al, *J Chromatogr B.*, **2006**,831(1-2), 258-266.
[10] R. Herve, M. Bernard, C. Corinne, B. Pierre-Antoine, *J Chromatogr B.*, **2007**, 850(1-2), 376-383.
[11] Y.B Chavan , Z. Rafiq, B. Rauza, *Indian J Pharma Sci .*, **2009**,71(1),95-97.
[12] A.G Swapnil, S. Monali, Sali, H.K. Atul, M.P. Dhaval, et al, *J Chil Chem Soc.*, **2010**, 55(1),115-117.
[13] P.T. Nagaraju , K.P. Channabasavaraj, P.T. Shanthakumar, *International Journal of Chem Tech Research.*, **2011**, 3(1), 23-28.
[14] C. M. Bhaskar Reddy and G.V. Subba Reddy, *International research journal of pharmacy*. **2012**, 3(8), 268-270.
[15] H. Mohammad, Abdel hay et al, *Journal of spectroscopy*., **2013,** article ID 937409, 7.
[16] Anindita. Behera et.al, International *journal of Pharm Tech research*., **2011**, 3(3), 1874-1882.
[17] A. Nagendra, D. Deepika and M.M. Annapurna, *Chem. Sci.Trans.*, **2013**, 2(3), 981-982.
[18] P. Janaki pathi, P. Raveendra Reddy and N. Appalaraju, *Research journal of pharmacy and Technology.*, **2011,** 4(3), 437-440.