Spectrophotometric Determination of Emtricitabin through Shiff's base System Using Vanillin and Pdab in Pharmaceutical Preparations

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Abstract - Two simple, sensitive and reproducible Spectrophotometric methods have been developed for the determination of emtricitabin either in pure from or in the table form. The proposed methods are based on the condensation reaction of amine group present in emtricitabin with aromatic aldehyde namely vanillin and p-dimethyl amino benzaldehyde (PDAB) to yield rose red coloured shiff's bases. The formed shiff's base is quantified spectrophotometrically at (λ_{max}) 512nm for vanillin and 512nm for PDAB. Beer's law is obeyed in the concentration ranges 60-100µgmL^{-1} and 5 to 40µgmL^{-1} with a limit of detection (LOD) of 1.8056 µgmL^{-1} for vanillin and 1.33 µgmL-1 for PDAB. The mean percentage recovery was found to be 100.17±1.336 and 97.86±1.303 for the two methods respectively. The proposed methods was successfully applied determine. The emtricitabin resent in tablet and the results were compared to that of reference methods. The proposed methods are recommended for quality control and routine analysis.

Keywords: P-dimethyl amino benzaldehyde (PDAB), limit of detection (LOD)

I. INTRODUCTION

Emtricitabin is a nucleoside reverse transcriptase inhibitor (6) (NRTI) administrator for the treatment of HIV infection. Its chemical designation is 4-amino-5-fluoro-1-[(2R, 5S)-2(hydroxymethyl)-1, 3-oxathiolan-55-yl]-1, 2-dihydropyrimidine-2-one.emtricitabin is used in combination with other antiretroviral agents for the treatment of HIV infection in adults and children. It is available and approved by FDA for treatment of HIV infection.ECB helps to lower the amount of HIV or' viral load', in a patient's body by converting HIV RNA to anew viral DNA thus increasing the number of immune system cells. A few physico-chemical methods like HPLC and UV-Visible spectrophotometry are used in the determination of ECB in pharmaceutical formulation. The drug has been determined by variety of analytical techniques such as development and validation of spectrophotometric method for estimation of ECB in tablet and dosage form. This paper describes two simple and sensitive methods (A and B) using reagents vanillin and PDAB resp for assay of ECB is bulk and pharmaceutical formulation. The drug was condensed with vanillin and PDAB in acidic medium. The effect of various parameters such as concentration and volume of vanillin and PDAB, nature and strength of acid, order of addition of reagents, solvent for final dilution were studied by means of control experiments by varying one parameter at a time.

II. MATERIALS AND METHODS

I. *Preparation of vanillin:*

(BDH, 0.4%2.63×10^{-3}M) prepared by using 400mg of vanillin in 100 mL of methanol.

II. *H2SO4: Concentrated (merck)*

III. *PDAB solution:* (E-merck, 0.5%, 6.31×10^{-3}M) prepared by dissolving 500mg of p-dimethyl amino benzaldehyde in 100mL of methanol.

IV. *Preparation of standard drug solution:*

About 100mg of emtricitabin was accurately weighed a dissolved completely in methanol and make up to the mark in 100mL volumetric flask. This can b brought to 100µg/mL for method A, and 40µg /mL for method B.

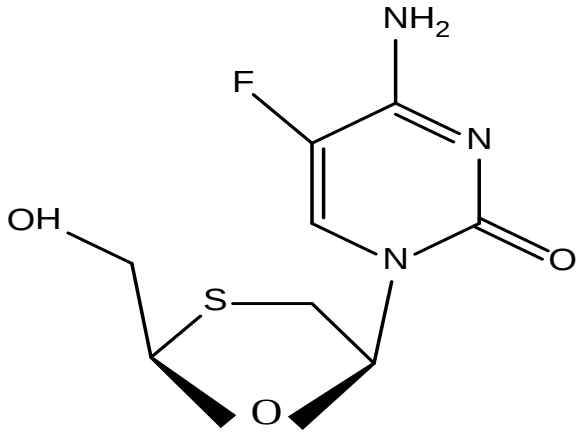


Fig. 1.1 Structure of EMTRICITABIN

III. PROPOSED METHODOLOGY

3.1 Method A:

Aliquots of standard solution (0.5-3.0ml,100µg/mL) were delivered in to a series of 10mL calibrated l tubes ,2.0mL of vanillin and 3.0mL of concentrated sulphuric

acid were added successively and the total volume in each flask was brought to 9mL by the addition of methanol and placed in a heating water bath for 15min.Then the flasks were cooled and made upto the mark with methanol and absorbance was measured after 5min.At 512nm against a reagent blank prepared in a similar way. The amount of ECB in sample solution was obtained from the beer's Lambert's plot.

3.2 Method B:

Aliquots of standard solution (0.5-3.0Ml,100µg/mL) were delivered in to a series of 10mL calibrated l tubes ,2.0mL of p-dimethyl amino benzaldehyde and 3.0mL of concentrated sulphuric acid were added successively and the total volume in each flask was brought to 9mL by the addition of methanol and placed in a heating water bath for 15min.Then the flasks were cooled and made upto the mark with methanol and absorbance was measured after 5min.At 512nm against a reagent blank prepared in a similar way. The amount of ECB in sample solution was obtained from the beer's lambert's plot.

IV. CHEMISTRY COLOURED SPECIES:

Method A&B:

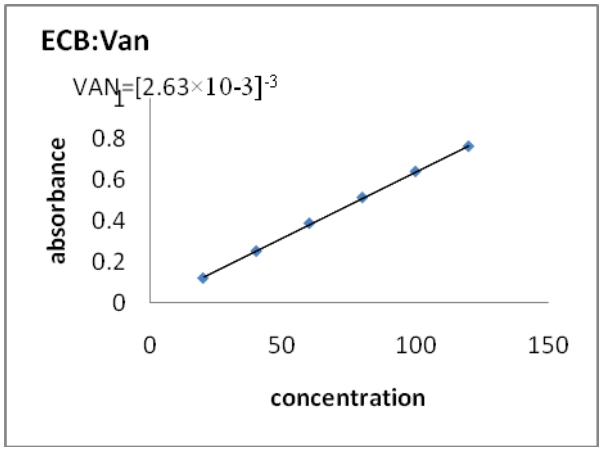
In the present investigation, it is observed that under acidic conditions the amino group in substituted pyrimidine of ECB is stabilized and behaves as amino compound (primary amine) forming coloured condensation product with aromatic aldehyde [vanillin (p-hydroxy-m-methoxy benzaldehyde), PDAB (p-dimethyl amino benzaldehyde)] in the presence of H_2SO_4 in nonaquous medium. The nature of cloured species obtained with PDAB and vanillin are presents in the scheme2.

V. RESULTS AND DISCUSSION:

Beer's law limits, molar absorptivity ,san dell's sensitivity ,% range ,standard deviation are summarized in the table.1.The regression analysis using the method of least squares was made slope (b),Intercept(a) and correlation co-efficient (r) obtained from different concentrations are given in table 5.1.The results showed that these methods have reasonable precision .The optimum conditions colour development for methods A and B have been established by varying parameters one at a time and keeping the other parameters fixed and observing the effects of product on the absorbance of decoloured species.

To evaluate the validity and reproducibility of the methods known amounts of pure drug were added to the previously analyzed pharmaceutical dosage forms and the mixtures were analyzed by the proposed methods .The percent recoveries are given in table 5.2.The interference studies revealed that the common excipients and other additives that are usually present in the tablet dosage forms did not interfere at their regularly added levels.

Beer's law plot of ECB-VAN (A) Method



Beer's law plot of ECB-PDAB (B) Method

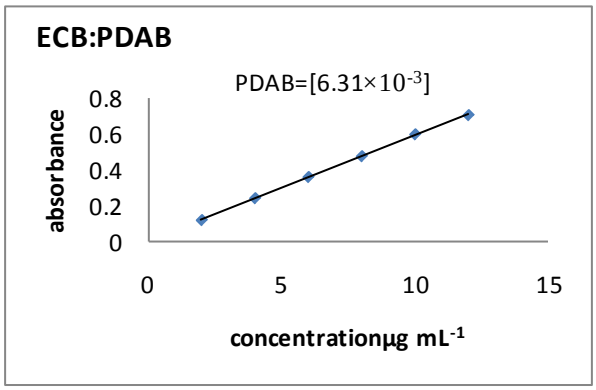


Table: 5.1 Optical and Regression characteristics, precision and accuracy of the proposed methods for ECB

formulation	Labeled amount in mg	Amount found by proposed methods MethodA MethodB	%Recovery by proposed methods MethodA MethodB
Tablet-I	200mg	200.34±2.67 195.73±2.606	100.17±1.336 97.86±1.303
Tablet-II	100mg	99.01±1.35 98.76±1.202	99.01±1.35 98.76±1.202

*: Average of six determinations considered **: Average of three determinations

Table 5.2: ASSAY OF ECB IN PHARMACEUTICAL FORMULATIONS

Parameter	MethodA	MethodB
λ_{max} (nm)	512	512
Beer's law limits(µgml^{-1})	60-100	May-40
Detection limits (µg ml^{-1})	1.8056	1.33

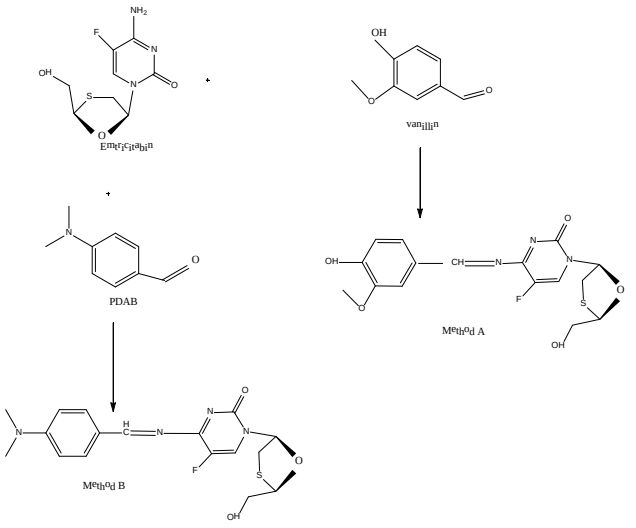
Molar absorptivity (1 mole cm^{-1})	6.143×10^{3}	1.4573×10^{4}
Sandell's sensitivity ($\mu g\ cm^{-2}$ / 0.001 absorbance unit)	0.0205	0.0344
Regression equation (Y = a + bC) Slope (b)	0.006	0.058
Standard deviation of slope (S_b)	0.0585	3.321×10^{-3}
Intercept (a)	0.003	0.001
Standard deviation of intercept (S_a)	4.56	2.584×10^{-4}
Standard error of estimation (S_e)	4.923	0.02779
Correlation coefficient (r^2)	0.999	0.999
Relative standard deviation (%)*	0.596	2.831
% Range of error (Confidence limits)*0.05 level	0.625	2.971
0.01 level	0.981	4.659
% Error in bulk samples **	1.23	2.65

*: Average ± standard deviation of six determinations; the t- and F-values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit t=2.57, F=5.05.

**: After adding2different amounts of the pure labeled to the pharmaceutical formulations, each value is an average of 3 determinations

$: UV Reference method.

Chemistry of proposed method A &B:



VI. CONCLUSION

The proposed methods are found to be simple ,sensitive, accurate and economic for routine analysis of ECB in bulk and pharmaceutical formulation .Based on molar absorptivity data and Beer's law range ,it may be concluded that among the proposed methods ,method is more sensitive than method A.

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