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A SIMPLE SPECTROPHOTOMETRIC ASSAY OF LAMIVUDINE IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple, sensitive and accurate spectrophotometric method has been developed for the estimation of lamivudine in bulk and pharmaceutical dosage forms. The amino group in lamivudine is diazotised with sodium nitrite and hydrochloric acid at $0^{o}C$ temperature. After diazotisation, the diazonium salt is coupled with resorcinol. The orange red coloured chromogen formed in the method is stable for more than 24 hours. The orange red coloured chromogen is measured at the wavelength of maximum absorbance 460 nm against the reagent blank. The results obtained with the proposed method are in good agreement with labeled amounts, when marketed pharmaceutical preparations are analyzed.

KEYWORDS: Spectrophotometry, resorcinol, lamivudine, Pharmaceutical and Formulation.

INTRODUCTION

Lamivudine, (2R-cis)-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone[31], is a synthetic nucleoside analogue with activity against the human immunodeficiency virus (HIV) and hepatitis B virus (HBV). The molecule has two chiral centers and is manufactured as the pure 2R, cis*(−)*-enantiomer. The racemic mixture from which lamivudine originates has antiretroviral activity but is less potent and substantially more toxic than the pure (−)-enantiomer. Compared with the (+)-enantiomer, the phosphorylated (−)-enantiomer is more resistant to cleavage from nascent RNA/DNA duplexes by cellular 3'-5' exonucleases, which may contribute to its greater potency. Lamivudine is either formulated alone as a tablet/oral formulation or in combination with zidovudine.

Various spectrophotometric[1-6] methods, titrimetric and spectrophotometric methods[7,8], Simultaneous Spectrophotometric estimation of Lamivudine[9-12] and other combination drugs and high performance liquid chromatography[13-19], RP-HPLC method[20] has been reported in the literature for its pharmaceutical formulations.

Lamivudine could be readily diazotized in acid medium and the resultant diazonium cation would then react with coupling reagent resorcinol by electrophilic substitution at the position ortho to the phenolic hydroxyl group resorcinol and results in the formation of the coloured product. The main purpose of the present study was to establish relatively simple, sensitive and validated visible spectrophotometric methods for the determination of lamivudine in pure form and in pharmaceutical dosage forms

Assay Procedure

To study the effect of drug concentration on the absorbance of the coupling reaction under optimal conditions now arrived is studied by the following method to know the suitability of the method for the assay of Lamivudine.

Various aliquots of the standard Lamivudine solution ranging from 0.5-2.5 ml are transferred into a series of 10ml volumetric flasks. To each flask, 2.0ml of 0.1N hydrochloric acid solution and 1.5ml of cold 0.1N sodium nitrite solution are added. The resultant solution in each flask is well shaken and allowed to stand for five minutes at 0-5^{0}C temperature for diazotization to complete. 1.0ml of 1% urea solution is added to each flask and the solution is shaken frequently to allow nitrogen gas to escape. Then 1.0ml of 0.1N sodium hydroxide solution and 1.0ml of 1% resorcinol solution are added and the volume in each flask is made upto 10ml with methanol. A orange red colour is formed. The maximum absorbance of the orange red colour solution is measured at 460nm against the reagent blank. Calibration graph is obtained by plotting absorbance values against the concentration of lamivudine solution. The calibration curve is found to be linear over a concentration range of 50 to 250µg/ml of lamivudine. The amount of lamivudine present in the sample is estimated from the calibration graph. The results are presented in fig.1.

Assay of lamivudine in pharmaceutical formulations

The proposed procedure for the assay of lamivudine is applied for its determination in commercial tablets.

Preparation of the sample solution

Powdered tablet equivalent to 50mg of the drug is weighed accurately and transferred into a 50 ml beaker and mixed well with 30ml of methanol. The solution is filtered and transferred into a 50ml volumetric flask and the volume is made up to 50ml with methanol. The concentration of the drug solutions is now 1mg/ml. This stock solution is further diluted to obtain the working concentration of 100µg/ml.

RESULTS AND DISCUSSIONS

Lamivudine undergoes diazotisation when treated with sodium nitrite and hydrochloric acid. The excess nitrous acid during the diazotisation is removed by the addition of urea solution. The solution was shaken frequently to allow the nitrogen gas to escape. The diazonium cation reacts with the coupling reagent, resorcinol by electrophilic substitution at the o-position of the coupling agent to produce an orange red azo product. This orange red colour product shows maximum absorbance at 460nm. The colour of the product is stable for more than 24 hours. The calibration curve (concentration vs. absorbance) is linear over the range of 50-250 µg/ml of lamivudine. The optical characteristics of the proposed method such as absorption maxima, Beer´s law limits, molar absorptivity and Sandell´s sensitivity are presented in Table.1. The molar absorptivity and Sandell´s sensitivity values shows sensitivity of the method. The regression analysis using method of least squares was made for the slope (b), intercept (a) and correlation (r) obtained from different concentrations and results are summarized in the Table1. The value of correlation coefficient was 0.999, which indicated the good linearity of calibration lines. The percent relative standard deviation calculated from the five measurements of lamivudine shown in Table.2. The % RSD is less than 2, which indicates that the method has good reproducibility. The values of standard deviation, coefficient of variation values are low, indicates high accuracy and reproducibility of the method. The‘t’ calculated values are compares well with the theoretical value of 2.78 there by indicating that the precision of the method is good. There no effect of additives and excipients such starch, calcium lactose and glucose in the concentrations those present in general pharmaceutical preparations.

The proposed method is found to be simple, precise, accurate and time saving, reproducible and can be conveniently adopted for routine analysis of estimation of lamivudine in bulk drugs samples and pharmaceutical formulations.

Table: 1. Optical characteristics of proposed method

parameters	Proposed method
λmax (nm)	460
Beer's law limit (µg/ml)	50-250
Molar absorptivity (l mole-^{1} cm-^{1})	1.634x10^{3}
Sandell's sensitivity (µg cm-^{2} / 0.001 absorbance unit)	0.0611
Regression equation (Y = a + bx)	Y=0.0063x+0.0062
Slope (b)	0.0063
Intercept (a)	0.0062
Correlation coefficient (r)	0.9997

*Y = a+bx, where Y is the absorbance and X concentration in µg / ml

Table: 2. Assay of lamivudine in tablets

S.No	Sample (mg)	*Amount Found(mg) ±S.D*	% label claim	%RSD*	*t_{cal}
1	150	150.09±0.45	100.06	0.3052	0.4392
2	150	149.8±0.34	99.86	0.2299	0.1298

***Average of five determination based on the label claim**

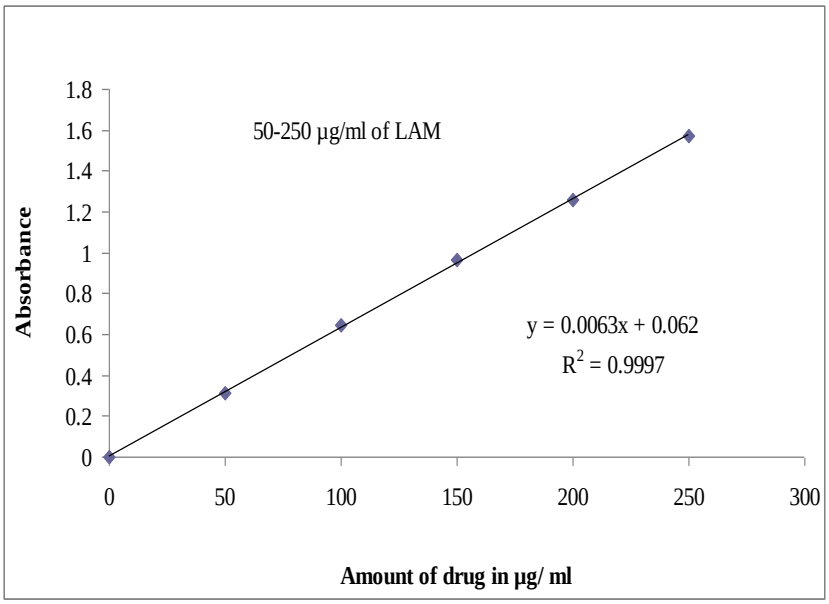

Fig: 1 Calibration curve of lamivudine

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