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Method development and validation of RP-HPLC method for simultaneous determination of Lamivudine and Zidovudine

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ABSTRACT

A rapid, sensitive and specific RP-HPLC method involving UV detection was developed and validated for determination and quantification of Lamivudine and Zidovudine. Chromatography was carried out on a Phenomenex – Luna, C18 (250 x 4.6 mm i.d., 5 μ) column. using filtered and degassed mixture of Buffer: Methanol (0.1M Ammonium acetate)(50:50) Ph-3.8 as mobile phase at a flow rate of 1.5ml/min and effluent was monitored at 270nm. The method was validated in terms of linearity, precision, accuracy, and specificity, limit of quantification and limit of detection. The assay was linear over the concentration range of Lamivudine and Zidovudine was 3.75 to 22.5 mcg/ml and 7.5 to 45mcg/ml respectively. Accuracy of the method was determined through recovery studies by adding known quantities of standard drug to the pre analyzed test solution and was found to be 99.06-99.96% and 99.99%-100.25% within precision RSD of 0.42 and 0.53 for Lamivudine and Zidovudine respectively. The system suitability parameters such as theoretical plates and tailing factor were found to be 3115,1.75 and 4398,1.33 respectively for Lamivudine and Zidovudine. The method does require only 7 minutes as run time for analysis which prove the adoptability of the method for the routine quality control of the drug.

Key words: Lamivudine, Zidovudine, Method development, Validation.

INTRODUCTION

Lamivudine is chemically 1[(2R,5S)-2-(Hydroxy methyl)-1-3 oxathiolan-5yl] cytosine and used as an antiretroviral activity [6,7]. Lamivudine is an analogue of cytidine. It can inhibit both types (1 and 2) of HIV reverse transcriptase and also the reverse transcriptase of hepatitis B. It needs to be phosphorylated to its triphosphate form before it is active. 3TC-triphosphate also inhibits cellular DNA polymerase. Zidovudine is chemically 1-[(2R,4S,5S)-4azido-5-

(hydroxymethyl)tetrahydrofuran-2-yl]-5-methylprimidine-2,4(1H,3H)-dione and used as an antiretroviral activity [6,7]. There is a plethora of analysis of such formulations without prior separation. For the estimation of multi-component formulation, the instrumental techniques, which are commonly employed, are spectrophotometry, GLC, high performance thin layer chromatography (HPTLC), HPLC etc. These methods are based upon the measurement of specific and nonspecific physical properties of the substances. The literature survey [8-14] reveals that there is some HPLC methods have been reported. But the present study is to develop an accurate and reliable HPLC [1-5] method for simultaneous estimation of Lamivudine and Zidovudine in solid dosage form.

In this paper we describe a simple, inexpensive, sensitive and validated HPLC method for the simultaneous determination of Lamivudine and Zidovudine in pharmaceutical formulation.

EXPERIMENTAL SECTION

Working standards of Lamivudine and Zidovudine were obtained from well reputed research laboratories. HPLC grade Methanol, Merck grade Ammonium acetate and Milli-Q water were procured from the market. The separation was carried out on isocratic HPLC system Shimadzu 2010A HT(Class vp6.13v) with pre-packed Phenomenex – Luna, C18 (250 x 4.6 mm i.d.,5 μ) column using filtered and degassed mixture of 0.1M Ammonium acetate buffer:Methanol (50:50) as mobile phase.

Standard preparation: Weight accurately about 30mg of Lamivudine and 60mg of Zidovudine and transferred in to a clean 100 ml volumetric flask dissolved in few ml of mobile phase and make up to the volume with mobile phase. Sonicate for 10 minutes and filtered through membrane filter and marked as standard stock solution Pipette out 5ml from the standard stock solution into a clean 100ml standard flask and make up the volume with mobile phase and marked as standard stock solution A.

Chromatographic conditions: Flow rate 1.5ml/min; detection wavelength 270nm; injection volume 10 μ l; column used Phenomenex – Luna, C18 (250 x 4.6 mm i.d.,5 μ); column temperature: 25°C; mobile phase: Buffer: Methanol (50:50).

Method development: Working standard of various concentrations was prepared by taking aliquots of standard solution and diluted to get required concentration for calibration plot and which was injected.

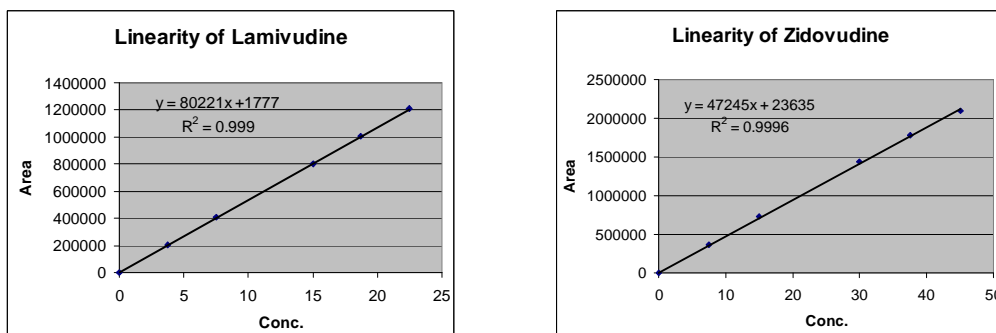
Assay preparation for commercial formulation: Weigh and powder 20 tablets, Transfer an accurately weighed quantity of powder equivalent to the average weight of one tablet into a clean 200 ml standard flask. Add 100 ml of mobile phase and dissolved, make up the volume with mobile phase. The solution is sonicated for 15 minutes and filtered through membrane filter, and marked as sample stock solution.

Pipette out 5 ml from the sample stock solution in to a clean 25 ml standard flask and make up the volume 25 ml with mobile phase (sample stock solution A). Then further dilute 5 ml from the sample stock solution A in to a clean 50 ml standard flask and make up the volume 50 ml with mobile phase (sample stock solution B) and mix.

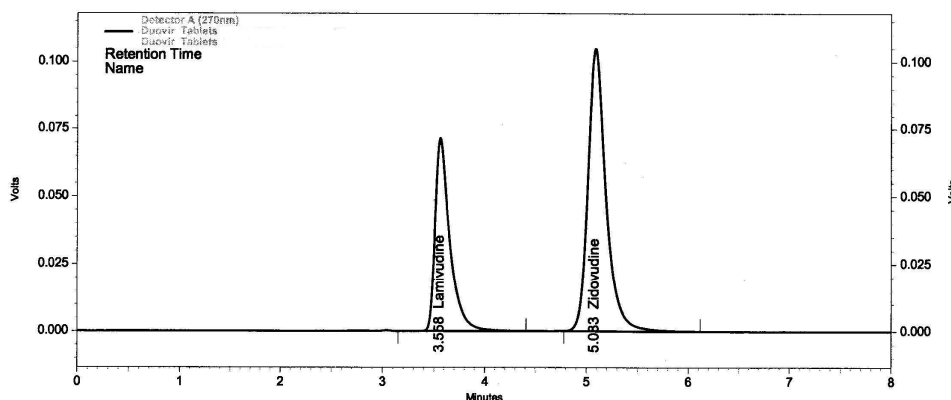
10 μ l of the standard preparation and assay preparation were separately injected and chromatographed.

Method validation[15-24]

Linearity: Linearity was demonstrated by analysing six different concentrations of active compound. Peak areas were recorded for all the peaks and calibration plot was constructed by plotting peak area vs concentrations of Lamivudine and Zidovudine which were found to be linear in the range of 3.75 to 22.5 mcg/ml and 7.5 to 45mcg/ml respectively. Coefficient of correlation was 0.9999 and 0.9999(Fig-1 and 2).

Fig-1 & 2: Linearity of Lamivudine and Zidovudine

Accuracy: Accuracy was done by recovery study using standard addition method, known amount of standard Lamivudine and Zidovudine in to pre-analysed samples and subjected to proposed HPLC method. The results of recovery studies are shown in Table-1.

Fig-3: RP-HPLC estimation of Lamivudine and Zidovudine**Table-1: Analysis of tablet containing Lamivudine and Zidovudine**

Formulation	Drug	Injected sample (mcg/ml)	Amount found (mcg/ml)	Found (%)	Amount std. added	Amount recovered (mcg/ml)	Recovery (%)
Tablet	Lamivudine	75	74.10	98.80	75	73.875	98.50
	Zidovudine	150	148.95	99.30	150	148.35	98.90

Precision: To demonstrate agreement among results, a series of measurements are done with Lamivudine and Zidovudine six replicate injections of the specific standard at various time intervals on the same day were injected into the chromatograph and the value of %RSD was found to be 0.42 and 0.53 for Lamivudine and Zidovudine respectively. In inter-day precision same standard was injected on different system and the found %RSD were 0.26 and 0.023 for Lamivudine and Zidovudine respectively.

Table-2: Precision

Amount found on	Method Precision		System Precision	
	Mean %	RSD (%)	Mean %	RSD (%)
Lamivudine	100.2	0.42	99.2	0.26
Zidovudine	100.2	0.53	98.7	0.02

RESULTS AND DISCUSSION

The regression value was found to be 0.9999 and 0.9999 for Lamivudine and Zidovudine respectively, which shows the response, is linear from 3.75 to 22.5 mcg/ml and 7.5 to 45mcg/ml respectively. Coefficient of correlation was 0.9999 and 0.9999. Selectivity experiment showed that there is no interference or overlapping of the peaks either due to excipients or diluents with the main peak of Lamivudine and Zidovudine. The percentage RSD for precision is <2 which confirms that method is sufficiently precise.

CONCLUSION

The total run time required for the method is only 7 mins for eluting both Lamivudine and Zidovudine. The proposed method is simple, fast, accurate, and precise and can be used for routine analysis in quality control of Lamivudine and Zidovudine.

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