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Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2010, 2(1): 478-481

ISSN No: 0975-7384

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 [1-5] method involving UV detection was developed and validated for determination and quantification of Lamivudine and Zidovudine. Chromatography was carried out on a pre-packed AltimaC18 5µ (150*4.6mm) column using filtered and degassed mixture of Ammonium acetate buffer:Methanol (80:20) as mobile phase at a flow rate of 1.0ml/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 37.5mcg-112.5mcg/ml and 75mcg to 225mcg/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 98.50%-99.9% and 98.30%-100.10% within precision RSD of 0.71 and 0.82 for Lamivudine and Zidovudine respectively. The system suitability parameters such as theoretical plates and tailing factor were found to be 3189.33, 1.12 and 7852.83, 1.05 respectively for Lamivudine and Zidovudine. The method does require only 20 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,3H0-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 method for simultaneous estimation of Lamivudine and Zidovudine in solid dosage form.

Objective

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

Materials and Methods

Experimental work

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 (AGILENT1100) with pre-packed AltimaC18 5µ (150*4.6mm) column using filtered and degassed mixture of Ammonium acetate buffer:Methanol (80:20) as mobile phase.

Standard preparation

About 75mg of Lamivudine and 150mg of Zidovudine were accurately weighed and transferred to a 100ml volumetric flask individually and dissolved in the water by sonication to give standard stock solution. Take 5ml of the aliquot in 50ml standard flask and make up the volume with 50ml with the diluents.

Chromatographic conditions

Flow rate 1.0ml/min; detection wavelength 270nm; injection volume 10µl; column used AltimaC18 5µ (150*4.6mm); column temperature: 25^{o}C; mobile phase: Buffer: Methanol (80:20).

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

Twenty capsules were taken; average weight was determined and mixed well fine powder. Powder equivalent to 75mg of Lamivudine and 150mg of Zidovudine was transferred into 100ml volumetric flask and dissolved in sufficient amount of diluent and sonicated to dissolve. Take 5ml of the aliquot in 50ml standard flask and make up the volume with 50ml with the diluents. Solution was filtered through 0.45µ membrane filter and then the filtrate was further diluted to get the required concentrations.

Procedure

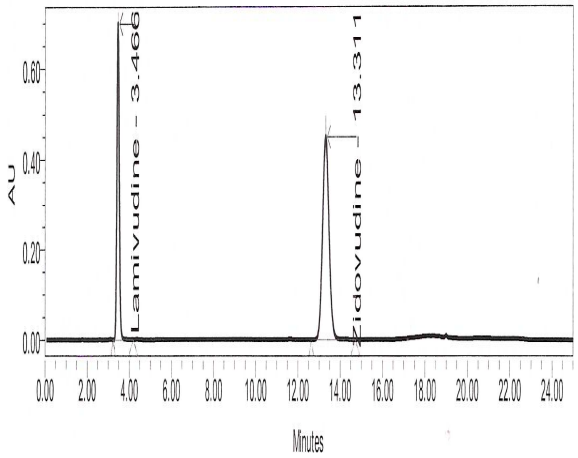
10µl of the standard preparation and assay preparation were separately injected and chromatographed.

Method validation[15-16]

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 37.5mcg-112.5mcg/ml and 75mcg to 225mcg/ml respectively. Coefficient of correlation was 0.9988 and 0.9987(Fig-1).

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.

RP-HPLC estimation of Lamivudine and Zidovudine



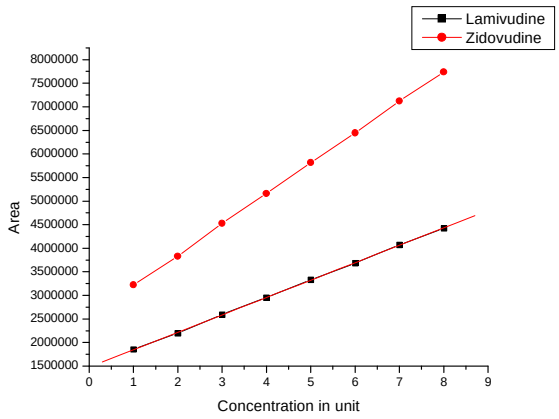


Table-1 Analysis of capsule containing Lamivudine and Zidovudine

Formulation	Drug	Injected sample (mcg/ml)	Amount found (mcg/ml)	Found (%)	Amount std. added	Amount recovered (mcg/ml)	Recovery (%)
Capsule	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 0.23 and 0.37 for Lamivudine and Zidovudine respectively. In inter-day precision same standard was injected on different days and the found %RSD were 0.36 and 0.38 for Lamivudine and Zidovudine respectively.

Amount found on	Intra-day		Inter-day	
	Mean %	RSD (%)	Mean %	RSD (%)
Lamivudine	99.2	0.23	98.8	0.36
Zidovudine	98.3	0.37	98.2	0.38

Results and Discussion

The regression value was found to be 0.9998 and 0.9997 for Lamivudine and Zidovudine respectively, which shows the response, is linear from 37.5mcg-112.5mcg/ml and 75mcg to 225mcg/ml respectively. Coefficient of correlation was 0.9998 and 0.9997. 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 and the total run time required for the method is only 20mins 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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