Research Article

Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Lamivudine, Zidovudine and Nevirapine from Bulk and Tablet Dosage Form

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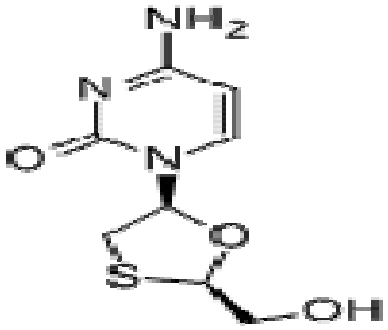
ABSTRACT

A simple, fast and precise reverse phase high performance liquid chromatographic method developed for the simultaneous determination of Lamivudine, Zidovudine & Nevirapine in its tablets form. Nucleodur C18 250 x 4.6 mm(l x d),5µ in isocratic mode with mobile phase Buffer and Methanol in the ratio of 60 : 40 %v/v were used. The flow rate was 0.8ml/min. Linearity for Lamivudine, Zidovudine & Nevirapine were 24mcg/ml to 36mcg/ml,48mcg/ml to 72mcg/ml and 32mcg/ml to 48mcg/ml respectively. The correlation coefficient (r^2) was found to be greater than (0.999). Amount of Lamivudine, Zidovudine & Nevirapine present in each tablet were found to be 150.20mg/tab and 300.07mg/tab and 199.52mg/tab respectively. The %RSD values were less than 2% for method precision. The mean percentage recovery of Lamivudine, Zidovudine & Nevirapine were found to be 100.313, 100.79, & 99.96.respectively.The proposed method is accurate, precise, selective and rapid for the simultaneous estimation of Lamivudine, Zidovudine & Nevirapine.

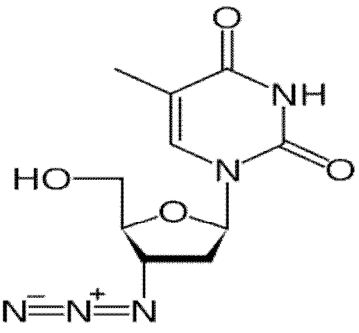
Keywords: HPLC, Validation, Lamivudine, Zidovudine, Nevirapine.

INTRODUCTION

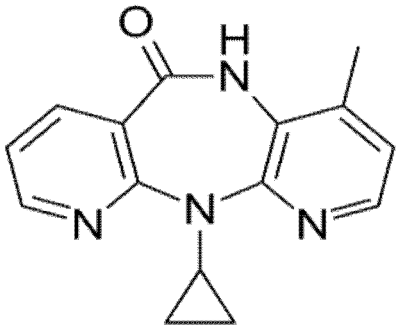
Lamivudine is used to treat Human Immunodeficiency Virus Type 1 (HIV-1) and hepatitis B (HBV). Chemically the drug is 4-amino-1-[(2R, 5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one.It has the following structural formula.



Several methods such as HPLC[1],HPLC-MS/MS[2],UV-VIS SPECTROPHOTOMETRY[3] have been reported in the literature. Zidovudine is a potent inhibitor of HIV replication, acting as a chain-terminator of viral DNA during reverse transcription. It improves immunologic function, partially reverses the HIV-induced neurological dysfunction, and improves certain other clinical abnormalities associated with AIDS. Chemically the drug is 1-[(2R,4S,5S)-4-azido-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidine-2,4-Dione.It has the following structural formula.



Several methods such as HPLC[4],HPTLC[5] have been reported in the literature. Nevirapine is used for treatment of HIV-1 infection in combination with other antiretroviral agents.Chemically the drug is 11-cyclopropyl-4-methyl-5, 11-dihydro-6H-dipyrido[3,2-b:2′,3′-e][1,4]diazepin-6-one.It has the following structural formula.



Several methods such as HPLC[6], GC-MS[7], UV-VIS SPECTROSCOPY[8], HPTLC[9] have been reported in the literature.

MATERIALS AND METHODS

Reference standard of Lamivudine, Zidovudine, Nevirapine. Trovir film coated tablet contain label claim of Lamivudine 30mg, Zidovudine 60mg and Nevirapine 40mg was procured from market. Methanol HPLC grade, Water HPLC grade, Ammonium acetate AR grade, Acetic acid AR grade reagents were used. Nucleodur C18 250 x 4.6 mm (L x D) column was used.

Preparation of Diluents

Methanol and water was mixed in the ratio of 1:1.

Preparation of Buffer

0.1M ammonium acetate in 0.1% acetic acid in water and adjust pH 2.5 with ortho phosphoric acid.

Preparation of Mobile Phase

Buffer: Methanol in the ratio of 60:40 is mixed, filtered through membrane filter of micron 0.45mm, degassed and this used as the mobile phase.

Preparation of Standard Stock Solution

Weigh accurately about 30 mg of Lamivudine, 60mg of Zidovudine and 40mg of Nevirapine were transferred into 100 ml volumetric flask and dissolve in 20ml of methanol and dilute with diluents to volume and mix.

Preparation of Working Standard Solution

Dilute 5ml of the above solution to 50ml with the diluents to obtain the concentration of 30 µg/ml of Lamivudine, 60µg/ml of Zidovudine and 40µg/ml of Nevirapine.

Sample preparation

Weigh 20 tablets and grind to fine powder in a dry mortar. Transfer 185 mg of the powder into a 100 ml volumetric flask. Add 20 ml of methanol and dissolve and add 25 ml of diluents and sonicate for 30 minutes and shake for 30 minutes. Dilute to volume with diluent and mix. Filter through 0.45µ membrane filter by discarding the first 5 ml. Dilute 5 ml of the above solution to 50 ml with the diluents.

RESULTS AND DISCUSSION

Assay: 20µl working standard solution and sample solution (n=4) were injected in to an injector of liquid chromatograph. From the peak area of Lamivudine, Zidovudine & Nevirapine, the amount of drugs in sample (n=4)were computed. A typical HPLC chromatogram of Lamivudine, Zidovudine & Nevirapine as shown in fig 1. In replicate analysis n=4 of the drug by the proposed method the content of Lamivudine, Zidovudine & Nevirapine in the Trovir film coated tablet were 150.20mg/tab, 300.07mg/tab & 199.52mg/tab respectively. The results obtained by the proposed method were close to the label claim of these three drugs indicating that the method is precise and accurate.

Linearity Study

In to a series of five standard measuring flask, varying amount of standard stock solution of combination of Lamivudine, Zidovudine & Nevirapine were taken and made up to various concentrations of 24,27,30,33,36 µg/ml for Lamivudine,48,54,60,66,72 µg/ml for Zidovudine and 32,36,40,44,48 µg/ml for Nevirapine, 20µl was injected from each flask. The peak area response of the solutions were recorded at 270nm.The plot of peak area versus the respective concentrations of Lamivudine, Zidovudine & Nevirapine were found to be linear in the range of 24-36 µg/ml,48-72 µg/ml & 32-48 µg/ml respectively with coefficient of correlation (r=0.9994), (r=0.9982) & (r=0.9978) respectively as shown in fig.2.

Accuracy

Accuracy studies were performed at 80%, 100%, 120% spiked sample. Three replicate of each concentration were performed. The mean % recovery of Lamivudine, Zidovudine & Nevirapine in the drug Trovir were 100.313, 100.79, & 99.96 respectively. Since there is no significant difference between the theoretical and actual, the method is shown to be accurate and selective.

Robustness

Robustness of the method was evaluated by deliberately altering the method conditions from the original method parameters (changing

the mobile phase's composition) and verifying compliance of the system suitability requirements. Insignificant differences in pea areas and less variability in retention time were observed and results (system suitability results, assay values) were found to be satisfactory.

System Suitability

System suitability tests were carried out on freshly prepared working standard solution of Lamivudine, Zidovudine & Nevirapine and the parameters obtained with 20µl injection volume and standard solution n=6 are shown in Table 1.

Method Precision

The precision of the method was demonstrated by intraday variation studies. The assay on six test preparations were performed be injected into the chromatographic system as per the test method. The % assay of drug and % RSD were calculated. The % assay for the Lamivudine, Zidovudine & Nevirapine were 100.6,100.1 & 99.9 respectively. %RSD for the Lamivudine, Zidovudine & Nevirapine were 0.40, 0.38 & 0.33 respectively.

Specificity

A placebo was prepared which contained all the ingredients except Lamivudine, Zidovudine and Nevirapine in the same proportion as present in the formulation. A sample solution from this preparation was injected into the system. No peaks were observed at the retention times of Lamivudine, Zidovudine and Nevirapine. Hence concluded that the method is specific to the active ingredients only.

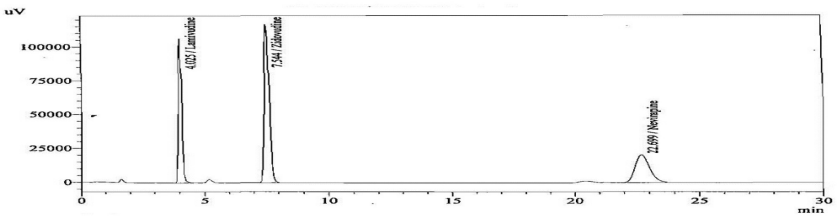
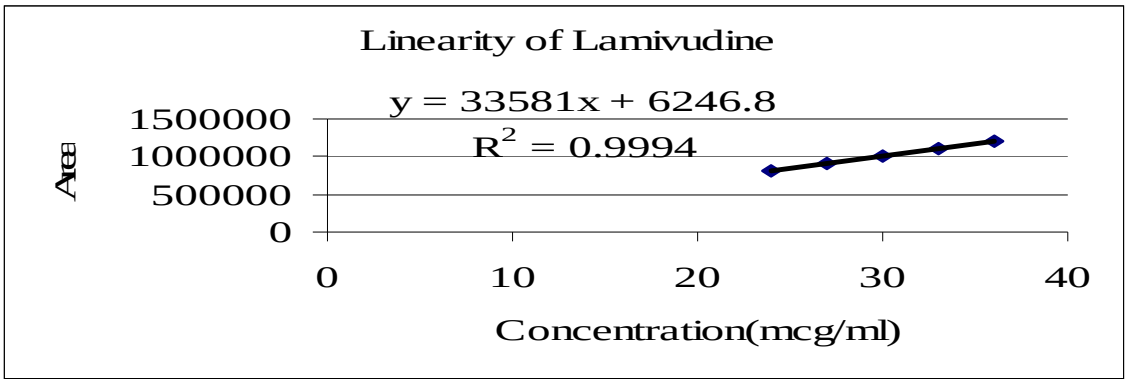
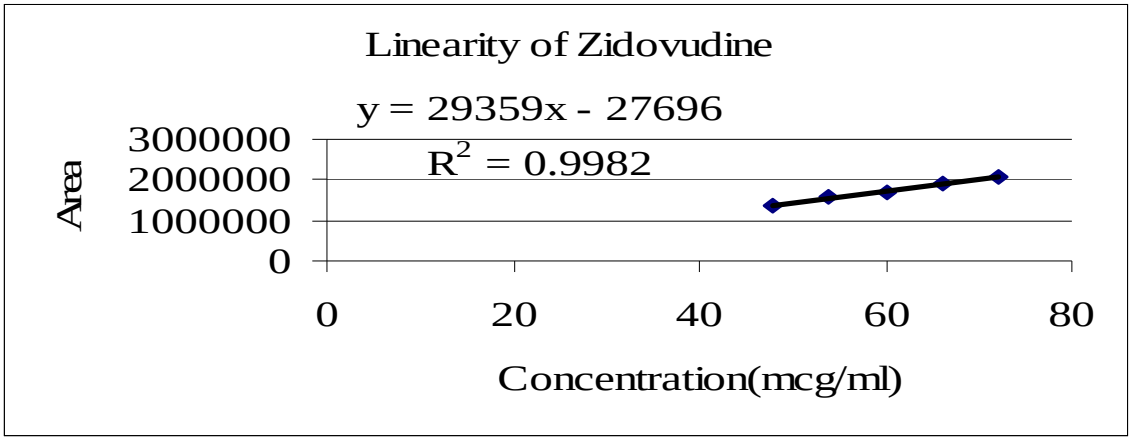


Fig.1: Typical HPLC Chromatogram of Lamivudine, Zidovudine & Nevirapine





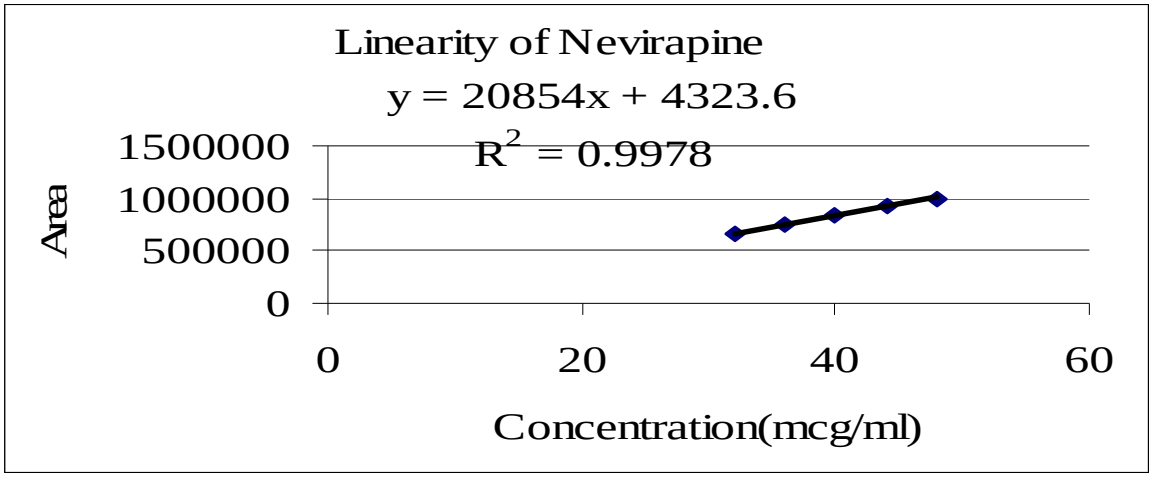


Fig.2: Typical Linearity curve of Lamivudine, Zidovudine & Nevirapine

Table 1: System suitability parameters

Parameters	Lamivudine	Zidovudine	Nevirapine
Retention time	4.01	7.50	22.57
Tailing factor	1.236	1.196	1.256
Theoretical plate	4151.745	6259.386	7391.881
Peak area	984973.5	1661873	819193.3
%RSD of peak area	0.08	0.08	0.59

CONCLUSION

The proposed method is simple, precise and accurate for the simultaneous determination of Lamivudine, Zidovudine & Nevirapine in tablet dosage. In routine quality control or in test laboratories, when we have this formulation to be analysed, the method is best suited.

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