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Development and Validation of a New RP-HPLC Method for the Simultaneous Estimation of Lamivudine, Zidovudine and Nevirapine in Tablet Dosage Forms.

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ABSTRACT

An accurate, precise and reproducible high performance liquid chromatographic method was developed for the simultaneous estimation of lamivudine, zidovudine and nevirapine in pharmaceutical dosage forms. Phenomenex C18 column (250 x 4.6 mm; 5µ) was employed for the separation of drugs. A mixture of 0.02 M trichloroacetic acid (6.8 pH) and methanol in the ratio of 40:60 v/v was used as the mobile phase and pumped at a flow rate of 1ml/min. The detection wavelength was set at 265 nm. The linearity of quantification was observed in the range of 7.5-112.5, 10-150 and 15-225 µg/ml for lamivudine, zidovudine and nevirapine respectively. The proposed method was validated according to ICH guidelines. The method was found to be suitable for simultaneous and accurate determination of these drugs in tablet dosage forms without any interference from the excipients.

Keywords: Lamivudine, Zidovudine, Nevirapine, Tablets, Method Development, HPLC

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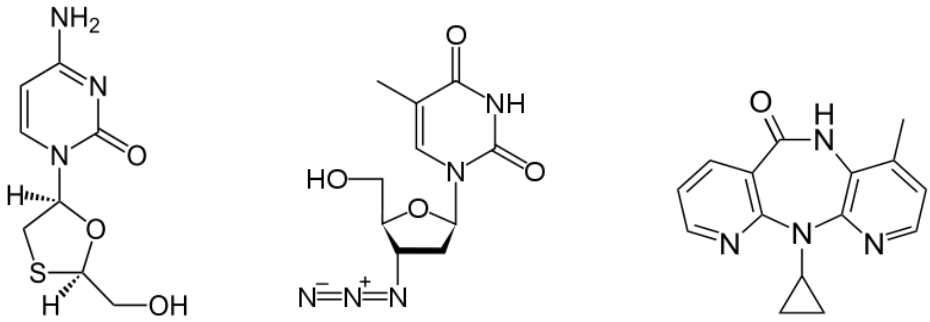
INTRODUCTION

Chemically, lamivudine (Figure. 1(a)) is (-)-1-[(2*R*, 5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine, zidovudine (Figure. 1(b)) is 3'-azido-3'-deoxythymidine, and nevirapine (Fig. 1(c)) is 11- cyclopropyl-4-methyl-5,11-dihydro-6*H*- dipyrido [3,2-*b*:2',3'-e][1,4] diazepin-6-one. The active triphosphate metabolites of lamivudine and zidovudine act against HIV by inhibition of reverse transcriptase via DNA chain termination after incorporation of the nucleotide analogue. Nevirapine, a non- nucleoside reverse transcriptase inhibitor, binds directly to reverse transcriptase and blocks the RNA-dependent and DNA-dependent DNA polymerase activities by causing a disruption of the enzyme's catalytic site. The activity of nevirapine does not compete with template or nucleoside triphosphates. All the three drugs are official in Indian Pharmacopoeia. [1]



(a) **(b)** **(c)**

Figure 1. Chemical structures of lamivudine (a), zidovudine (b) and nevirapine (c).

A literature survey reveals the report of analytical methods for the determination of these drugs individually and in combination with one another in biological samples and in pharmaceutical dosage forms based on spectroscopy, HPLC and LC-MS/MS.[2-11] Very few HPLC methods has been reported for the simultaneous estimation of lamivudine, zidovudine and nevirapine. Hence, the authors have attempted to develop a simple, rapid, precise and accurate method for the simultaneous estimation of these drugs in tablet dosage forms. The method was duly validated as per ICH guidelines.[12]

MATERIALS AND METHODS

Drugs, Chemicals and Solvents

The reference samples of lamivudine, zidovudine and nevirapine were obtained from Hetero Labs Ltd. (Hyderabad, India) as gift samples. Trichloroacetic acid and Ammonia liquor were purchased from Qualigens. HPLC grade methanol was procured from Merck Co. HPLC grade water was prepared by using Millipore Milli-Q system.

Equipment and Chromatographic Conditions

A Shimadzu Prominence chromatograph equipped with a Phenomenex C18 column (250 x 4.6 mm; 5µ), LC-20AD pumps and an SPD-20A Prominence UV/VIS detector was employed for the study. Samples were injected into the system through a Rheodyne 7725 injection valve via a 20 µl loop. The output signal was monitored and integrated by LC Solutions software. A mixture of 0.01 M trichloro acetic acid (pH 6.8) and methanol in the ratio of 40:60 v/v was used as the mobile phase and pumped at a flow rate of 1ml/min. The detector wavelength was set at 265 nm. The injection volume was 20µL. The chromatography was carried out at 26°C.

Preparation of Buffer solution

1.63 g of trichloroacetic acid was transferred into a beaker containing 1000 ml of water and mixed. The pH of the solution was adjusted to 6.8 with liquid ammonia. The solution was then filtered through a 0.45µ membrane filter and sonicated.

Preparation of Mobile Phase

The above buffer (pH 6.8) was mixed thoroughly with methanol in the ratio of 40:60 v/v. This solution was used as the mobile phase.

Preparation of Diluent

A mixture of the buffer and methanol in the ratio of 50:50 v/v was used as the diluent.

Preparation of Stock and Working Standard Solutions of Lamivudine, Zidovudine and Nevirapine

75 mg of lamivudine, 150 mg of zidovudine and 100 mg of nevirapine were separately weighed and transferred into three separate 50 mL volumetric flasks. About 20 mL of methanol was added in to each flask and sonicated. The volumes were made up to 50 mL with methanol and mixed well. A quantity of 5 mL of each of the above drug solutions was transferred into a 50 mL volumetric flask and the volume was made up with the diluent to get concentrations of 150, 300 and 200 µg/mL of lamivudine, zidovudine and nevirapine respectively. This solution was used as the stock solution.

5 mL of the above stock solution were transferred into a 10 mL volumetric flask and diluted to volume to get concentrations of 75, 150 and 100 µg/mL of lamivudine, zidovudine and nevirapine respectively. This was used as the mixed working standard solution.

Calibration Curve

Various dilutions of the mixed standard stock solution of lamivudine, nevirapine and zidovudine were prepared at different concentration levels including the working concentration. Twenty microlitres of each concentration were injected into the HPLC system. The response was read at

265 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were calculated and linearity plots of concentration over the mean peak area were constructed for the individual drugs.

Estimation of the Drugs from Tablet Dosage Forms

Ten tablets of the commercial formulation Duovir-N were weighed and ground to a fine powder. An amount equivalent to about 150 mg of lamivudine was transferred into a 100 mL volumetric flask and to it 60 mL of methanol was added and sonicated for 20 min. The volume was made up with methanol. A portion of this solution was filtered through a 0.22 µm membrane filter (discarding the first few mL of the filtrate). 5 mL of this filtrate was transferred into a 100 mL volumetric flask containing 50 mL of the diluent. The volume was made up with the diluent and the contents mixed well. The above solution was then chromatographed six times. The mean peak areas of the drugs were calculated and the drug content in the formulation was calculated by using the regression equation of the method.

RESULTS AND DISCUSSION

During method optimization studies, trials were conducted by using a C_{18} column for the effective separation of all the three drugs within short runtimes. Different flow rates and compositions of the mobile phase were tested. After evaluating the column efficiency parameters like theoretical plates, resolution and tailing the chromatographic conditions as shown in Table 1 were used for the analysis. Under the optimized conditions, the retention times of lamivudine, zidovudine and nevirapine were found to be 3.065 min, 3.800 min, and 5.043 min respectively. The proposed method was also applicable to tablet formulations.

Table 1. Optimized chrmatographic conditions

Stationary Phase	Phenomenex C18 column (250 x 4.6 mm; 5µ)
Mobile Phase	0.02 M trichloroacetic acid buffer - Methanol (40:60 v/v)
Diluent	0.02 M trichloroacetic acid buffer - Methanol (50: 50 v/v)
Flow Rate	1.0 mL/min
Column Temperature	26°C
Injection Volume	20 µL
Detection Wavelength	265 nm
Run Time	10 min

Specificity

A good analytical method should be able to measure the analytes accurately in the presence of suspected interferences such as blank, excipients, and degradation products. Figure. 2 shows chromatographic base-line separation of lamivudine, nevirapine and zidovudine from working standard solution. Figure. 3 demonstrates that no interferences were found at the retention times

of lamivudine, nevirapine and zidovudine in their tablet dosage form due to excipients.

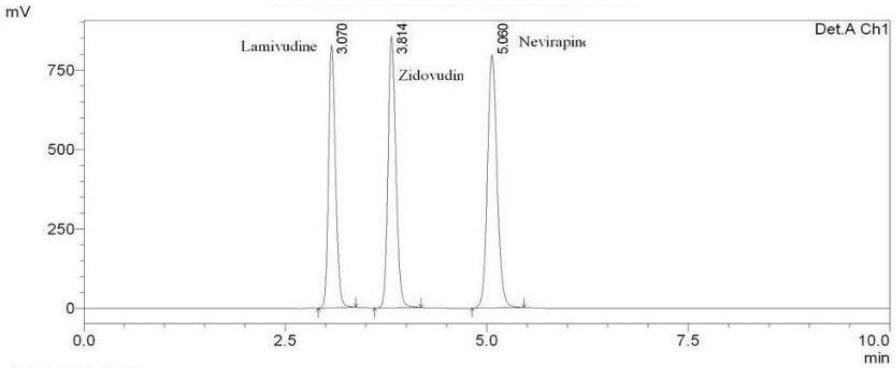


Figure 2. Representative chromatogram showing the separation of lamivudine, zidovudine and nevirapine from the working standard solution.

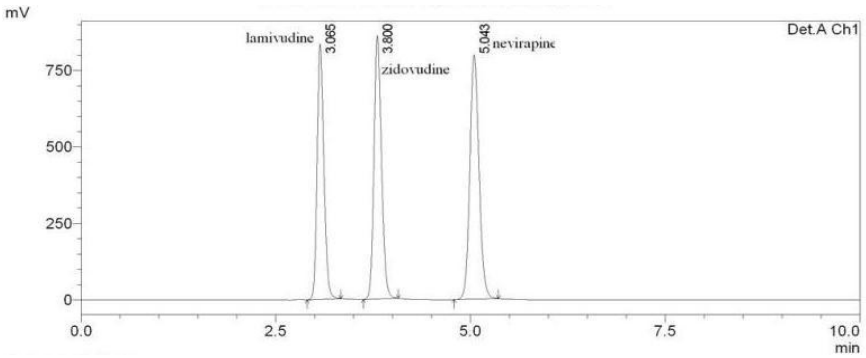


Figure 3. Representative chromatogram obtained from analysis of lamivudine, zidovudine and nevirapine and from formulation sample solution.

Linearity

The regressions of the plots were computed by least square regression method and were shown in the Figures 4, 5 and 6. The calibration curves (n=3) constructed for each drug were linear over the concentration range of 7.5-112.5 µg/mL for lamivudine, 15-225 µg/mL for zidovudine and 10-150 µg/mL for nevirapine. The correlation coefficients were greater than 0.999 and the %RSD for each concentration studied was less than 2%.

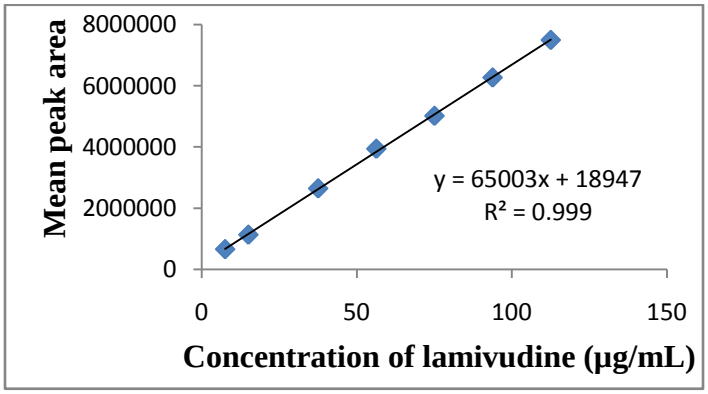


Figure 4. Linearity plot for lamivudine

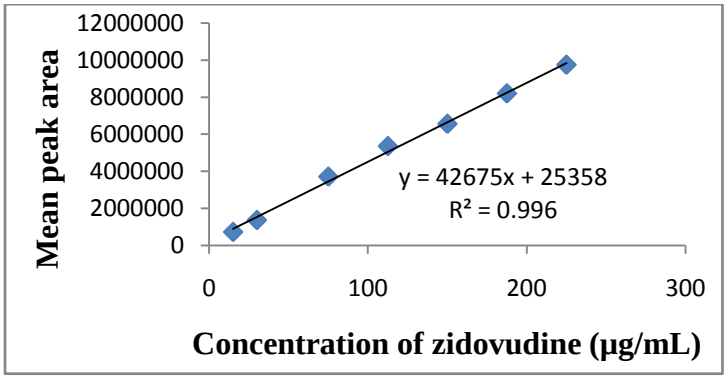


Figure 5. Linearity plot for zidovudine

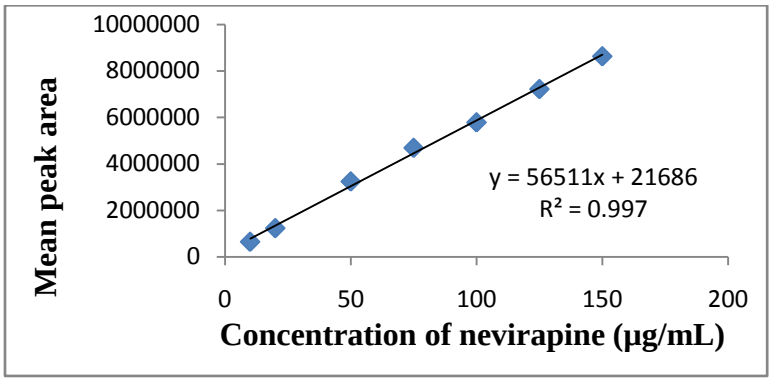


Figure 6. Lineaity plot for nevirapine

Accuracy and Precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery and standard deviation of the percentage recovery were calculated and represented in Table 2. The high percentage of recovery indicates that the proposed method is highly accurate. The precision of the method was demonstrated by inter-day and intra-day variation studies. Six replicate injections of sample solutions were made and the percentage RSD was calculated and represented in Table 3. From the data obtained the developed RP-HPLC method was found to be precise.

Table 2. Accuracy data of the proposed method

Analyte	Amount of the analyte taken(µg/mL)	Mean recovery (µg/mL) ± SD	% Mean recovery ± SD
Lamivudine	60	60.02 ± 0.110	100.03 ± 0.183
	75	75.01 ± 0.028	100.01 ± 0.037
	90	90.11 ± 0.170	100.12 ± 0.189
Nevirapine	80	80.02 ± 0.100	100.02 ± 0.125
	100	100.02 ± 0.181	100.02 ± 0.181
	120	120.07 ± 0.182	100.06 ± 0.152
Zidovudine	120	120.11 ± 0.166	100.09 ± 0.138
	150	150.15 ± 0.180	100.10 ± 0.120
	180	180.10 ± 0.311	100.06 ± 0.172

Table 3. Precision data for the proposed method

Analyte	Intra-day precision		Inter-day precision	
	Measured peak area ± SD	%RSD	Measured peak area ± SD	%RSD
Lamivudine	5016577 ± 14668.84	0.29	5023870 ± 11507.47	0.23
Nevirapine	5778757 ± 14657.35	0.25	5771057 ± 17754.8	0.31
Zidovudine	6552708 ± 17447.52	0.27	6534962 ± 20279.84	0.31

System Suitability Parameters

System suitability parameters were studied with six replicates of standard sample solutions and the parameters are presented in Table 4.

Table 4. System suitability parameters of the proposed method

Parameter	Lamivudine	Zidovudine	Nevirapine
Retention time (min)	3.070	3.814	5.060
Tailing factor	1.1	1.1	1.1
Theoretical plates	5218.9	6587.7	8104.5
HETP	0.0479	0.0379	0.0309
Resolution	-	4.149	6.030

Method Suitability

The commercial tablet formulation, Duovir-N (CIPLA) was analyzed by the proposed method and the results are shown in Table 5. The values were found to be in good agreement with the labeled amounts, which confirms the suitability of the method for the analysis of drugs in pharmaceutical dosage forms.

Table 5. Recovery of lamivudine, nevirapine and zidovudine from Duovir-N tablets

Drug	Labeled amount (mg)	Amount recovered (n=6)	% Recovery
Lamivudine	150	149.35	99.57
Nevirapine	200	200.25	100.12
Zidovudine	300	299.75	99.92

CONCLUSION

The proposed RP -HPLC method is sensitive, precise and accurate and can be used for the routine quality control analysis for the simultaneous determination of the lamivudine, nevirapine and zidovudine in their tablet dosage forms.

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