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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ZIDOVUDINE, LAMIVUDINE AND NEVIRAPINE TABLETS BY RP-HPLC

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ABSTRACT

An accurate, precise and reproducible high performance liquid chromatographic method was developed for the estimation of Zidovudine, Lamivudine, and Nevirapine in pharmaceutical dosage form. In this method Hypersil BDS C18 column (250mmx4.6mm I.D., 5 μ m particle size) with mobile phase consisting of 0.01M ammonium acetate buffer and acetonitrile in the ratio of 75:25 v/v was used. The flow rate was 1 ml/min and the detection wavelength was 270 nm. The linearity was observed in the range of 33-100 μ g/ml, 22-67 μ g/ml, and 16-50 μ g/ml with a correlation coefficient of Zidovudine, Lamivudine and Nevirapine were 0.9996, 0.9996 and 0.9997 respectively. The proposed method was validated for its linearity, accuracy, precision and robustness. The proposed method is simple, rapid, accurate, precise and reproducible hence can be applied for routine quality control analysis of zidovudine, Lamivudine and Nevirapine in pharmaceutical dosage forms.

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Key Words

Zidovudine, Lamivudine and Nevirapine, HPLC, Estimation, Validation.

INTRODUCTION

Zidovudine is 1-[(2*R*, 4*S*, 5*S*)-4-azido-5-(hydroxymethyl)oxolan-2-yl]-5-methyl-1, 2, 3, 4-tetrahydropyrimidine-2, 4-Dione, Lamivudine is 4-amino-1-[(2*R*, 5*S*)-2-(hydroxyl methyl)-1, 3-oxathiolan-5-yl]-1, 2-dihydropyrimidin-2-one, Nevirapine is 11-cyclopropyl-4-methyl-5, 11-dihydro-6*H*-dipyrido [3, 2-*b*: 2', 3'-*e*][1,4]diazepin-6-one. Zidovudine, a structural analog of thymidine, inhibits the activity of HIV-1 reverse transcriptase (RT) both by competing with the natural substrate dGTP and by its incorporation into viral DNA. Lamivudine is a synthetic nucleoside analogue and is phosphorylated intracellularly to its active 5'-triphosphate metabolite, lamivudine triphosphate (L-TP). This nucleoside analogue is incorporated into viral DNA by HIV reverse transcriptase and HBV polymerase, resulting in DNA chain termination. Nevirapine binds directly to reverse transcriptase (RT) 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 tri phosphates. All the three drugs are official in I.P¹ and are indexed in other sources ^{2, 3} also. 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 their dosage forms based on HPLC, HPTLC and LC-MS/MS ⁴⁻¹³. This report presents an HPLC assay for the simultaneous estimation of Zidovudine, Lamivudine and Nevirapine in pharmaceutical dosage form.

MATERIAL AND METHODS

Working standard of Zidovudine, Lamivudine and Nevirapine was obtained from well reputed research laboratories. HPLC grade acetonitrile, ammonium acetate, methanol, and ortho phosphoric acid from Merck chemical Ltd were procured from the market. The separation was carried out on isocratic HPLC system with Hypersil BDS C18 column (250X4.6mm, 5 μ) column using filtered and degassed mixture of 0.01M Ammonium acetate: Acetonitrile (750:250) as mobile phase.

Preparation of Buffer solution

0.7708 gm of Ammonium Acetate Salt was dissolved in 1000ml of milli Q water. Filtered through 0.45 μ m or a finer porosity membrane filter and degassed.

Preparation of Mobile Phase

Mixed Buffer and Acetonitrile in the ratio of 750:250v/v respectively and degassed.

Preparation of Diluent

Mixed Water and Acetonitrile in the ratio of 25:75v/v respectively and degassed.

Preparation of Standard solution

Weighed accurately and transferred about 33mg of Lamivudine working standard, 44mg of Nevirapine working standard and 66mg of zidovudine working standard into a 100ml volumetric flask and dissolved in diluent and was made up to the volume with diluent. Further diluted 5ml of the above solution to 50ml with diluent and mixed. Filtered the solution through 0.45 μ m nylon filter or 0.45 μ m PVDF membrane filter

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

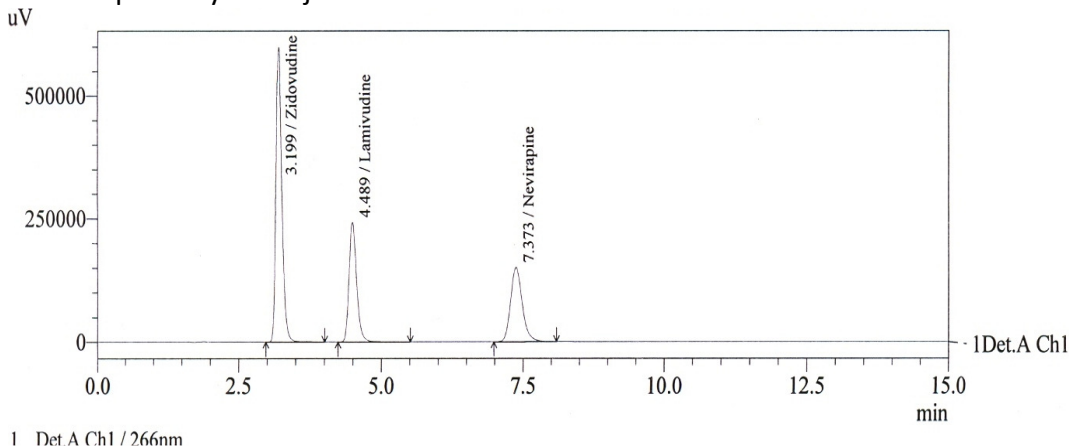
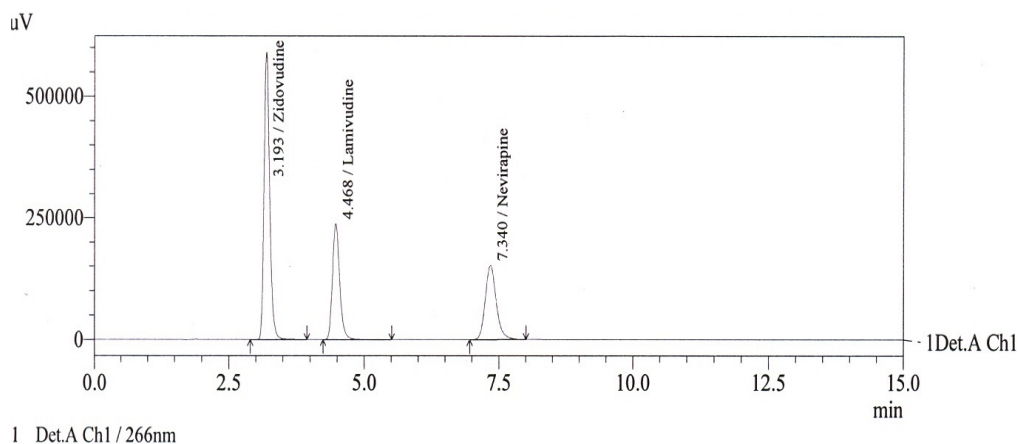
20 tablets were weighed and powdered. Weighed accurately a quantity equivalent to 66mg of Zidovudine, 33mg of Lamivudine and 44mg of Nevirapine were transferred into a 100ml volumetric flask and diluted to the volume with diluent. Further diluted 5ml of the above solution to 50ml with diluent and mixed. The above solution was filtered through 0.45 μ m nylon filter/0.45 μ m PVDF membrane filter. The result were shown in the table no.1.

Table No.1: Analysis of Tablet Formulation

S.No	Parameters	Drug		
		Zidovudine	Lamivudine	Nevirapine
1	Label claim(mg/tab)	300mg	150mg	200mg
2	Drug content (%)	99.1	98.5	99.5
3	% RSD	0.665	0.662	1.2

Procedure 10 µl of the standard preparation and assay preparation were separately injected and

chromatographed. It was shown in the figure no.1 and 2 and the results were tabulated in table no.1.

**Fig.no.1:** Chromatogram of standard**Fig.no.2:** Chromatogram of sample

Validation parameters

Accuracy

Accuracy was confirmed by recovery study as per ICH norms [14] at three different concentration levels 50%, 100%, 150% by replicate analysis (n = 3). Here to a pre analysed sample solution, standard drug solutions were added and then percentage of drug content was

calculated. The result of accuracy study and assay of tablet were tabulated in was Table 2, 3 and 4. From the recovery study it is clear that the method is accurate for quantitative estimation of Zidovudine, Lamivudine and Nevirapine in tablet dosage form as the statistical parameters are within the acceptance range (S.D. < 2.0)

Table no.2: Accuracy result for Zidovudine

Conc.	Sample ID	Amount added (ppm)	Amount found (ppm)	% Recovery	% Mean recovery
50%	Sample-1	34.1	34.13	100.1	100.4
	Sample-2	34.08	34.15	100.2	
	Sample-3	34.05	34.32	100.8	
100%	Sample-1	66.4	66.24	99.8	100.1
	Sample-2	66.25	66.47	100.3	
	Sample-3	66.32	66.44	100.2	
150%	Sample-1	99.8	98.44	98.6	99.1
	Sample-2	99.76	98.54	98.8	
	Sample-3	99.65	99.42	99.8	

Table no.3: Accuracy result for Lamivudine

Conc.	Sample ID	Amount added (ppm)	Amount found (ppm)	% Recovery	% Mean recovery
50%	Sample-1	16.63	16.57	99.6	100.0
	Sample-2	16.55	16.57	100.1	
	Sample-3	16.60	16.65	100.3	
100%	Sample-1	33.70	33.23	98.6	98.8
	Sample-2	33.65	33.31	99.0	
	Sample-3	33.72	33.31	98.8	
150%	Sample-1	49.20	48.54	98.7	98.6
	Sample-2	49.42	48.57	98.3	
	Sample-3	49.61	49.05	98.9	

Table no.4: Accuracy result for Nevirapine

Conc.	Sample ID	Amount added (ppm)	Amount found (ppm)	% Recovery	% Mean recovery
50%	Sample-1	22.50	22.89	101.8	101.6
	Sample-2	22.62	22.93	101.4	
	Sample-3	22.68	23.06	101.7	
100%	Sample-1	44.57	44.81	100.5	100.8
	Sample-2	44.51	44.88	100.8	
	Sample-3	44.46	44.96	101.1	
150%	Sample-1	66.83	66.24	99.1	99.5
	Sample-2	66.75	66.29	99.3	
	Sample-3	66.79	66.83	100.1	

Precision

The precision of the method was demonstrated by interday and intraday precision studies. For the intraday precision, injections of the three mixed standard solutions were repeated thrice in a day and % RSD was calculated. In the interday studies, injection for standard solutions was made on 3 consecutive days and % RSD was calculated. The interday % RSD for Zidovudine, Lamivudine and Nevirapine. From the data obtained, the developed RP-HPLC method was found to be precise.

Linearity and Range

Table No.5: Linear regression data of Zidovudine, Lamivudine and Nevirapine.

S.No	Parameters	Zidovudine	Lamivudine	Nevirapine
1	Linear Range ($\mu\text{g/ml}$)	33-100 $\mu\text{g/ml}$	16-50 $\mu\text{g/ml}$	22-67 $\mu\text{g/ml}$
2	Correlation coefficient (r^2)	0.9996	0.9997	0.9996
3	LOD	0.8641	0.1487	0.0460
4	LOQ	1.4571	0.6530	0.4274
5	Tailing factor	1.19	1.57	1.29

Robustness

The robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. Robustness of the method was determined by carrying out the analysis under conditions during which wavelength was changed. Variation is seen to have impact on the resolution than other parameters and hence should be controlled. No significant change was found in the AUC value.

RESULT AND DISCUSSION

For HPLC analysis, initially various mobile phases were tried to attempt to obtain the best separation and resolution between Zidovudine, Lamivudine and Nevirapine. The mobile phase consisting of Ammonium acetate buffer:Acetonitrile in the ratio of 75:25v/v was found to be appropriate mobile phase allowing adequate separation of all the compounds using Hypersil BDS C18 column(250X4.6mm) at a flow rate of 1ml/min. As Zidovudine, Lamivudine and Nevirapine

According to USP 80% to 120% of test concentration was taken and dilution was done appropriately. The observations are shown in Table no.5. The graph was plotted % test concentration Vs peak area and correlation coefficient was calculated.

Limit of Detection (LOD) and Limit of Quantization (LOQ) The LOD and LOQ of Zidovudine, Lamivudine and Nevirapine by proposed methods were determined using calibration standards. LOD and LOQ were calculated as $3.3s/S$ and $10s/S$ respectively, where S is the slope of the calibration curve and s is the standard deviation of response. The results of the same are shown in Table 5.

exhibit significance absorbance at wavelength 270nm it was selected as detection wavelength for simultaneous estimation of Zidovudine, Lamivudine and Nevirapine in pharmaceutical dosage forms. The retention time was found to be 4.08 min, 6.75min and 3.2min respectively. The calibration curve was linear in concentration range of 33-100 $\mu\text{g/ml}$, 22-67 $\mu\text{g/ml}$, and 16-50 $\mu\text{g/ml}$ for Zidovudine, Lamivudine and Nevirapine respectively. The limit of detection was calculated and it was found to be 0.8641, 0.1487, and 0.0460 for Zidovudine, Lamivudine and Nevirapine respectively. The limit of quantification was calculated and it was found to be 1.4571, 0.6530 and 0.4274 for Zidovudine, Lamivudine and Nevirapine respectively. The developed method were validated in terms of linearity and range, limit of detection and limit of quantification, recovery study, interdays study, intraday study and study by different analysts. Recovery studies were carried out to study of the methods.

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