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Research Article

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 andNevirapine in pharmaceutical dosage forms. In this method, Hypersil BDS (Base Deactivated Silica) C18 column (150x4.6mm) with mobile phase consisting of Potassium Dihydrogen Phosphate buffer and methanol in the ratio of 60:40 v/v was used. The flow rate was 0.9ml/min and the detection wavelength was 270nm. The linearity was observed in the concentration range of 50%, 75%, 100%, 125%, 150% for the 3 drugs of Zidovudine, Lamivudine and Nevirapine and the correlation coefficients were 1.000,1.000.1.000 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 the routine quality control of analysis of Zidovudine, Lamivudine and Nevirapine in pharmaceutical dosage forms.

Key words: Zidovudine, Lamivudine, Nevirapine, RP-HPLC, Validation

INTRODUCTION

Zidovudine is 1- [(2R, 4S, 5S) – 4- azido – 5 – (hydroxymethyl) – 2- yl] - 5 – methyl -1,2,3,4 – tetrahydropyrimidine – 2,4- Dione.Lamivudine is 4 – amino – 1 - [(2R, 5S) -2 – (hydroxylmethyl) -1,3-oxathiolan-5-yl) -1, 2 – dihydropyrimidin – 2 – one. Nevirapine is 11-cyclopropyl-4-methyl-5, 11-dihydro 6H-dipyrido [3,2-b:2',3'-e] [1,4] diazepin - 6-one. Zidovudine, a structural analogue 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 active5'- 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 disruption of the enzyme's catalytic site. The activity of Nevirapine does not compete with template or nucleoside tri phosphates. All the drugs are official in I.P. and are indexed in other sources too.

Literature survey revealed that a number of methods are available for estimation of these drugs in pharmaceutical dosage form as well as biological fluids like HPLC, RP-HPLC, LC/MS etc. [1-9]

Literature survey does not reveal any simple RP-HPLC method for simultaneous estimation these combined dosage forms. The present communication describes simple, sensitive, accurate and precise RP-HPLC method for simultaneous estimation of both drugs in their combined tablet dosage forms.

MATERIALS AND METHODS

Working standards of Zidovudine, Lamivudine and Nevirapine were obtained from Ranbaxy Laboratories Ltd., Dewas, (M.P.) India.

HPLC grade acetonitrile, methanol, Potassium Dihydrogen phosphate, Orthophosphoric acid, triethylamine ,Mili Q Water, were purchased from Ranbaxy Laboratories Ltd., (New Delhi, India). .

The separation was carried out on an isocratic HPLC system with Hypersil BDS (Base Deactivated Silica) C18 column (150x4.6mm) column using filtered and degassed mixture of Potassium Dihydrogen Phosphate: methanol (600:400) as mobile phase.

Chromatographic conditions :

Flow rate	: 0.9 ml/min
injection volume	: 10 µl
Column	: Hypersil BDS C18,150×4.6mm

Detector wavelength : 270 nm
Run time : 14 mins

Preparation of Buffer solution :

1.2g of K2HPO4 (Potassium Dihydrogen Phosphate) was accurately weighed and dissolved in HPLC Milli Q water. The above solution was sonicated for 15mins. Then the above solution was made upto 1000ml with HPLC water, then filtered and the PH was made upto 7.3 by adding Triethylamine, and then degassed.

Preparation of Mobile Phase:

Buffer and Methanol in the ratio of 600:400 v/v were taken respectively and degassed

Preparation of Diluent:

Acetonitrile and Methanol were mixed in the ratio of 50:50 and degassed.

Preparation of Standard solution:

Weighed accurately and transferred about 30mg of Zidovudine working standard, 15mg of Lamivudine working standard and 20mg of Nevirapine working standard are transferred into a 100ml volumetric flask and dissolved in diluent and made upto the required volume with the diluent. 5ml of the above solution was diluted to 50ml with diluent and mixed. The above solution is filtered through 0.45um nylon filter.

Method Development

Stock solutions of Zidovudine, Lamivudine and Nevirapine were prepared by dissolving 30 mg of Zidovudine, 15 mg of Lamivudine, and 20 mg of Nevirapine in three different 100 ml volumetric flasks containing 50 ml of methanol and all the solutions were sonicated for 20 minutes and then made up to the mark with methanol to get a concentration of 1 mg / ml. 1 ml of the above stock solution was transferred to 50 ml volumetric flask and the volume was made up to the mark with mobile phase for the three drug solutions. Subsequent dilutions of these three solutions were made with the mobile phase to get a concentration of 50 – 150 µg/ml. The solutions were injected into the 10 µl loop and the chromatograms obtained were recorded as shown in the Fig.1. The retention time of Zidovudine, Lamivudine and Nevirapine were found to be 2.2, 4.06 and 11.62 minutes respectively. The calibration curve was constructed by plotting concentration vs peak ratios. The amount of Zidovudine, Lamivudine and Nevirapine present in the sample was caliculated through the standard calibration curve. The liniearity experiment was carried out in triplicate to ascertain accuracy and precision of the method. The peak area ratios of the drug vs concentration were found to be linear and the results are shown in Table-5.

Preparation of sample:

Twenty tablets were accurately weighed and powdered. Quantity equivalent to 30mg of Zidovudine, 15mg of Lamivudine and 20mg of Nevirapine were transferred into a 150ml volumetric flask and diluted to the required volume with diluent. 5ml of the above solution is further diluted to 50ml with the diluent and mixed. The above solution was filtered through 0.45 um nylon filter.

Validation parameters:

Specificity

The specificity was established by preparing a Zidovudine, Lamivudine and Nevirapine standard at 0.5% level of test concentration and injected 6 times into HPLC system as per the test procedure

Accuracy:

Accuracy was confirmed by recovery studies as per ICH (International Conference on Harmonization) norms at three different concentration levels of 50%, 100%, 150% by the replicate analysis (n=3).

The results of accuracy studies and the assay of tablets were tabulated in the below tables. From the recovery studies, it is clear that the method is accurate for the quantitative estimation of Zidovudine, Lamivudine and Nevirapine in tablet dosage forms as the statistical parameters are within the acceptance range.

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 (Relative Standard Deviation) was calculated. The interday % RSD for Zidovudine, Lamivudine and Nevirapine were calculated.

The % RSD values Zidovudine, Lamivudine and Nevirapine were found to be 0.9, 0.9 and 0.8

Linearity and Range:

The test concentrations were taken accurately and the dilutions were done appropriately. The observations are shown in the below Tables-8, 9, & 10. The graphs were plotted as % test concentration Vs peak area. figures-1, 2 & 3 the correlation coefficients were calculated

Limit of Detection (LOD) & Limit of Quantification (LOD):

The LOD and LOQ values of Zidovudine, Lamivudine and Nevirapine by the proposed methods were determined using the calibration standards. LOD and LOQ were calculated as 3.3 s/n and 10 s/n respectively, where s/n refers to

signal to noise ratio. The results of all the above values are shown in the below Table-1.

Robustness:

Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RPHPLC method developed, are rugged and robust.

System suitability studies:

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions. The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 %standard deviation range during routine performance of the method.

RESULTS OF 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 Potassium Dihydrogen phosphate buffer: Methanol in the ratio of 60:40 v/v was found to be appropriate mobile phase allowing adequate separation of all the compounds using Hypersil BDS (Base Deactivated Silica) C18 column (150x4.6mm) at a flow rate of 0.9ml/min. As Zidovudine, Lamivudine and Nevirapine exhibit significant absorbance at wavelength 270nm, it was selected as a detection wavelength for the simultaneous estimation of Zidovudine, Lamivudine and Nevirapine in pharmaceutical dosage forms. The retention times were found to be 2.22min, 4.06 and 11.62min respectively. The calibration curve was linear in the concentration range of 50-150 ug/ml for Zidovudine, Lamivudine and Nevirapine respectively. The limit of detection was calculated and it was found to be 0.0004, 0.0001, 0.002 for Zidovudine, Lamivudine and Nevirapine respectively. The limit of quantification was calculated and it was found to be 0.001, 0.0005 and 0.006 respectively. The developed method was validated in terms of linearity and range, precision limit of detection and limit of quantification.

Table1: Linearity and Range:

S.No.	Parameters	Zidovudine	Lamivudine	Nevirapine
1	Linear Range (ug/ml)	50-150 ug/ml	50-150 ug/ml	50-150ug/ml
2	Correlation Coefficient (r2)	0.9993	0.999	0.9997
3	LOD	0.0004	0.0001	0.002
4	LOQ	0.001	0.0005	0.006
5	Tailing factor	1.34	1.18	1.10

Table-2: Accuracy results for Lamivudine:

Concentration	Sample ID	Amount Added (µg/ml)	Amount found (µg/ml)	% Recovery	%Mean Recovery
	Sample-1	73.6935	75.4482	102.38	
	Sample-2	73.5447	74.7249	101.60	
50%	Sample-3	72.9492	74.4100	102.00	102
	Sample-4	72.8003	74.4686	102.29	
	Sample-5	72.3537	74.6839	103.22	
	Sample-6	72.6514	74.6622	102.77	
	Sample-1	142.9208	146.6442	102.61	
100%	Sample-2	143.2186	145.8023	101.80	103
	Sample-3	142.9208	148.2761	103.75	
	Sample-1	203.9599	211.5372	103.72	
	Sample-2	204.1088	211.3474	103.55	
150%	Sample-3	203.6621	211.5698	103.88	103
	Sample-4	208.4262	211.7819	101.61	
	Sample-5	202.7689	209.9293	103.53	
	Sample-6	203.2155	210.5002	103.58	

Table-3: Accuracy results for Zidovudine

Concentration	Sample ID	Amount Added (µg/ml)	Amount found (µg/ml)	% Recovery	%Mean Recovery
	Sample-1	148.8910	151.6057	101.82	
	Sample-2	148.5902	150.4275	101.24	
50%	Sample-3	147.3871	149.6915	101.56	102
	Sample-4	147.0863	149.6597	101.75	
	Sample-5	146.1839	150.2055	102.75	
	Sample-6	146.7855	150.2916	102.39	
	Sample-1	288.7584	293.9706	101.81	
100%	Sample-2	289.3599	292.3009	101.02	102
	Sample-3	288.7584	297.2213	102.93	
	Sample-1	412.0822	426.2978	103.45	
	Sample-2	412.3830	425.5055	103.18	
150%	Sample-3	411.4807	425.9858	103.53	103
	Sample-4	409.0743	426.1705	104.18	
	Sample-5	409.6759	422.1525	103.05	
	Sample-6	410.5783	423.5490	103.16	

Table-4: Accuracy results for Nevirapine:

Concentration	Sample ID	Amount Added (µg/ml)	Amount found (µg/ml)	% Recovery	%Mean Recovery
	Sample-1	98.2581	99.8838	101.65	
	Sample-2	98.0596	99.0776	101.04	
50%	Sample-3	97.2655	98.3817	101.15	102
	Sample-4	97.0670	98.6139	101.59	
	Sample-5	96.4715	98.4750	102.08	
	Sample-6	96.8685	98.7823	101.98	
	Sample-1	190.5611	196.5348	103.13	
100%	Sample-2	190.9581	194.5885	101.90	103
	Sample-3	190.5611	198.5836	104.21	
	Sample-1	271.9465	265.2218	97.53	
	Sample-2	272.1450	264.6651	97.25	
150%	Sample-3	271.5495	265.4588	97.76	98
	Sample-4	269.9615	264.6313	98.03	
	Sample-5	270.3585	262.6400	97.15	
	Sample-6	270.9540	263.9225	97.40	

Table-5: Robustness for Lamivudine:

S No	Sample name	Change	Name	RT	Area	Tailing	Platecount
1	Flow1	1.2ml/min	Lamivudine	4.528	8386440	1.26	6395
2	Flow2	0.8ml/min	Lamivudine	2.896	5323963	1.20	5519
3	Temp1	50^{o}C	Lamivudine	3.513	6571444	1.20	5767
4	Temp2	40^{o}C	Lamivudine	3.519	6541305	1.19	6280

Table-6: Robustness for Nevirapine:

S No	Sample name	Change	Name	RT	Area	Tailing	Platecount
1	Flow1	1.2ml/min	Nevirapine	11.068	2251174	1.13	8709
2	Flow2	0.8ml/min	Nevirapine	7.112	1409108	1.08	7535
3	Temp1	50^{o}C	Nevirapine	8.691	1743023	1.09	7855
4	Temp2	40^{o}C	Nevirapine	8.239	1743217	1.11	9233

Table-7: Robustness for Zidovudine :

S No	Sample name	Change	Name	RT	Area	Tailing	Platecount
1	Flow1	1.2ml/min	Zidovudine	2.732	4856479	1.43	3810
2	Flow2	0.8ml/min	Zidovudine	1.746	3050410	1.36	3401
3	Temp1	50°C	Zidovudine	2.124	3773955	1.34	3539
4	Temp2	40°C	Zidovudine	2.132	3755377	1.34	3625

Table-8: Linearity for Zidovudine

S NO	Conc	Area
1	0	0
2	50	1892468
3	75	2801562
4	100	3721121
5	125	4518896
6	150	5434286

Table-9: Linearity for Lamivudine:

S No	conc	Area
1	0	0
2	50	3296291
3	75	4886174
4	100	6494139
5	125	7805165
6	150	9434410

Table-10:Linearity for Nevirapine:

S No	conc	Area
1	0	0
2	50	866461
3	75	1288465
4	100	1724135
5	125	2102429
6	150	2533494

Fig:1 Calibration curve for Zidovudine

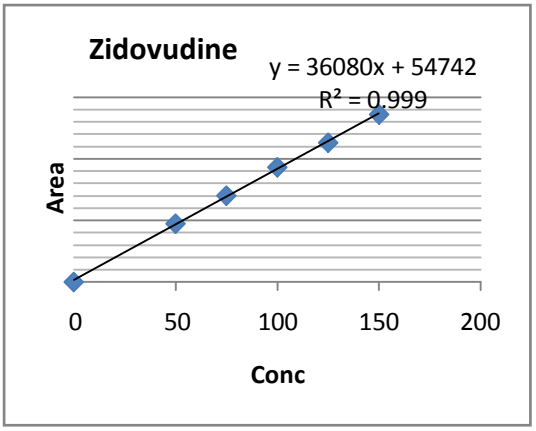


Fig:2 Calibration curve for Lamivudine

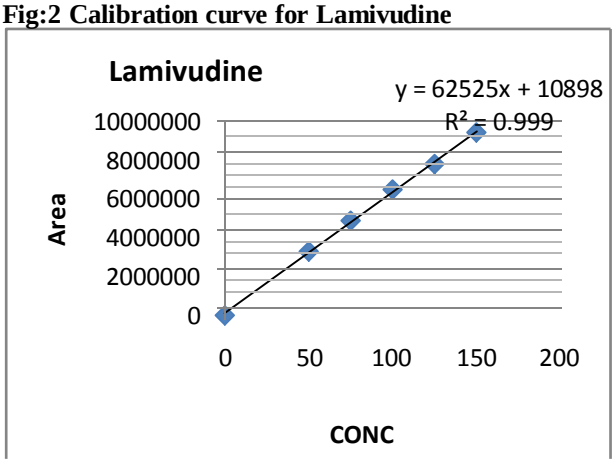


Fig 3: Calibration curve for Nevirapine

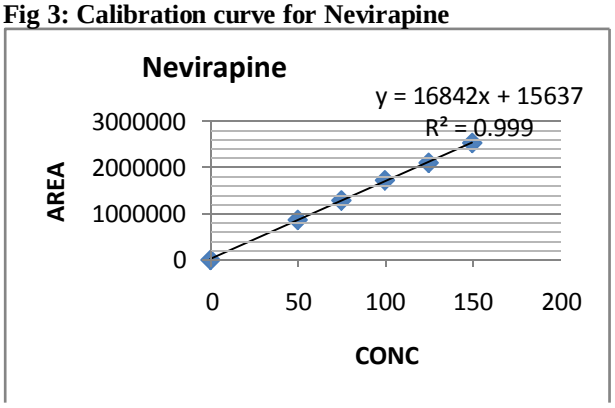


Fig 4: Chromatogram for Standard

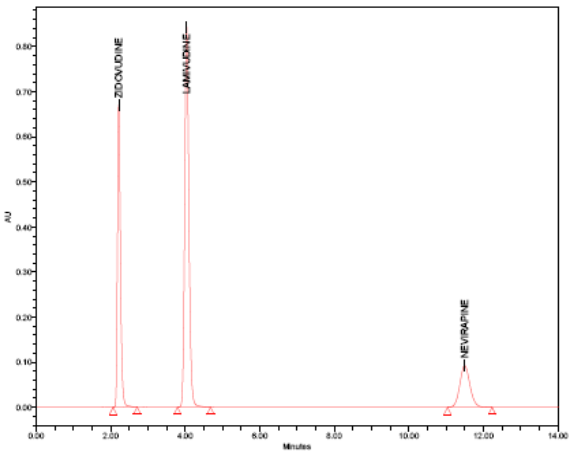
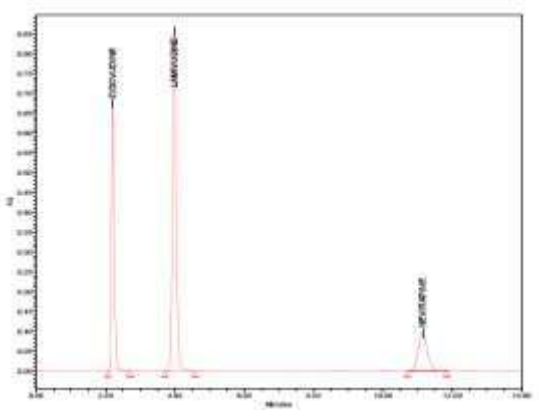


Fig 5: Chromatogram for Sample:



CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the Simultaneous determination of Zidovudine, Lamivudine and Nevirapine in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Zidovudine, Lamivudine and Nevirapine in pure and its pharmaceutical dosage forms

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