International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com

Research Article

Quantitative Estimation and Validation Of Lamivudine, Zidovudine And Nevirapine In Pharmaceutical Formulation

Shinde G.S*,Gayke.A.U, Bagul.T.P, Aglave .S.B, Rawat.S.S, and N.P.Jain

Department of Quality Assurance Technique,S.N.D. College of pharmacy, yeola.
*Corresponding author

Abstract

The RP-HPLC method for Lamivudine, Zidovudine and Nevirapine was developed using Inertsil ODS 3V C18 column (5µm, 150mm × 4.6mm) as stationary phase and 0.01M 1-octane Sulphonic acid : Methanol (60:40v/v, pH2.6) as mobile phase. The mobile phase was maintained at a flow rate of 1ml/min,run time 15 min and detection was carried out at 270nm.Lamivudine, Zidovudine and Nevirapine were found to be linear in the concentration range of 75-225µg/ml, 150-450µg/ml and 100-300µg/ml respectively. The result of % assay of marketed formulation was found as 101.93±0.1527, 100.86±0.2021and 94.2±0.1527 for Lamivudine, Zidovudine and Nevirapine respectively. Accuracy of the method was determined by performing recovery study and the result were found in the range of 100.9-101.4%, 100.1-100.7% and 99.0-99.9% for Lamivudine, Zidovudine and Nevirapine respectively. Percentage RSD of precision study of these drugs were found less than 2 percent which indicated good precision of the developed method. The proposed method was validated for linearity, accuracy, precision, and robustness. The proposed method is simple,rapid.precise and reproducible hence can be applied for routine quality control analysis of Lamivudine, Zidovudine and Nevirapine in pharmaceutical dosage form.

Keywords: Inertsil, Lamivudine, Zidovudine , Nevirapine, Sulphonic acid : Methanol .

Introduction

One of the deadliest and unmanageable chronic health catastrophes is HIV/AIDS. Fixed dose combinations(FDCs) form the main stay in clinical management of HIV-1 infection as they offer several advantages over single products with respect to storage, prescribing, dispensing, patient use, consumption and disease management.[1].Lamivudine is chemically 1[(2R,5S)-2-(Hydroxy methyl)-1-3 oxathiolan-5yl] cytosine and Lamivudine is an analogue of cytosine. Polymerase, Zidovudine is chemically 1-[(2R,4S,5S)-4azido-5-(hydroxymethyl) tetrahydrofuran-2-yl]-5-methylprimidine-2,4(1H,3H0-dione and Nevirapine is chemically 11-cyclopropyl-4-methyl-5, 11-dihydro-6*H*-dipyrido [3, 2-*b*: 2', 3'-*e*][1,4] diazepin-6-one and are reverse transcriptase (RT) inhibitors of human immune deficiency virus types (1 and 2) of HIV reverse transcriptase and also the reverse transcriptase of hepatitis B.[2,3]. Literature revealed that few methods have been reported for the individual estimation of Zidovudine and Lamivudine and in combination with other drugs [4-9]. The method developed is precise, simple and precise RP-HPLC method to determine Zudovudine and Lamivudine in pharmaceutical dosage forms.

Materials and Methods

Materials

Lamivudine Zidovudine and nevirapine pure standards were received as gift samples from micro labs Pharmaceuticals banglore (India). All other reagents used were HPLC grade.

Apparatus:

The HPLC system was waters HPLC consisted with the pump-alliance 2690 seperation module, column Inertsil ODS-3V, C18, (150 × 4.6 mm, 5µ), auto sampler, detector was waters 2489 and UV–Visible Detector, data processor Empower 2 (Water). Other instruments used were Melter balance AY 220, Eutech pH meter, MelterUltrasonicatior and Millipore membrane filter.

Method

Preparation of Buffer

2.16gm of 1-Octane sulphonic acid sodium salt was weighed and dissolved in 1000 ml of Milli Q water and adjusted the pH to 2.6 ± 0.05 with 1% orthophosphoric acid. The solution was filtered through 0.45 µ membrane filter and sonicated for 10min to degas.

Preparation of mobile phase

Buffer and methanol was mixed in the ratio 60:40 v/v and filtered through 0.45 µ membrane filter and degassed by sonication.

Preparation of diluent:

Mixture of methanol and water in the ratio of 50:50 v/v was prepared and degassed.

Preparation of Standard Solution:

Standard Stock Solution of Lamivudine, Zidovudine, Nevirapine

About 37.5 mg of Lamivudine, 75.0 mg of Zidovudine**,** 50.0 mg of Nevirapine standard were weighed accurately into 25ml of volumetric flask. 20ml of methanol was added and sonicated for 5 minutes. After sonication, the volume was made up to the mark with same solvent to obtain final concentration of 1500 µg/ml, 3000 µg/ml, 2000 µg/ml of Lamivudine, Zidovudine, Nevirapine respectively. From the above solution 10 ml was pipetted out in a 100 ml volumetric flask and the volume was made up to the mark with diluent to obtain final concentration of 150 µg/ml, 300 µg/ml, 200 µg/ml of Lamivudine, Zidovudine, Nevirapine respectively.

Assay preparation of Tablet formulation

20 tablets were accurately weighed and average weight was determined. Powdered tablet equivalent to 300 mg of Lamivudine was transferred into a 200 ml volumetric flask. 10 ml of water was added and sonicated for 10 minutes to disperse the tablet powder. Then 130 ml of methanol was added and sonicated for 25 minutes with intermittent shaking. The volume was made upto mark with methanol after cooling and mixed well, filtered through 0.45 µ nylon filter. First 2 ml of the filtrate was discarded and 5 ml of this filtrate was diluted to 50 ml with diluent and mixed well to get concentration of Lamivudine as about 150µg/ml, Zidovudine 300µg/ml, Nevirapine 200µg/ml. The blank, standard and sample solutions were injected and result are given in table no 1

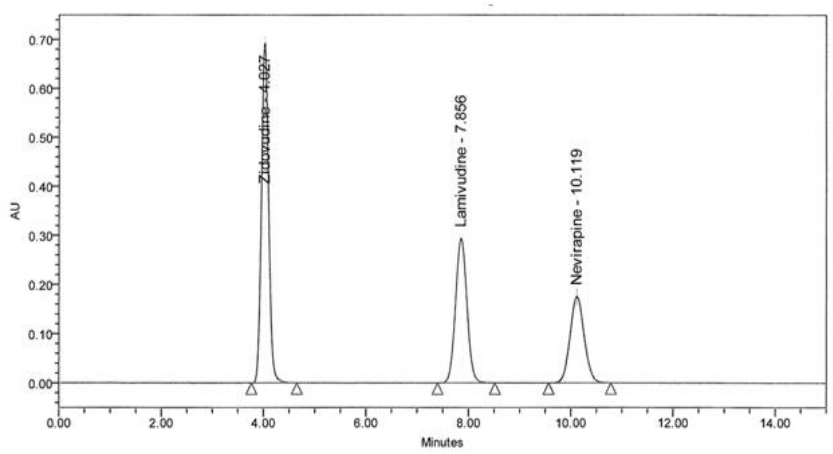

Fig: 1 Chromatogram of Combined Standard Solution of Lamivudine, Zidovudine and Nevirapine

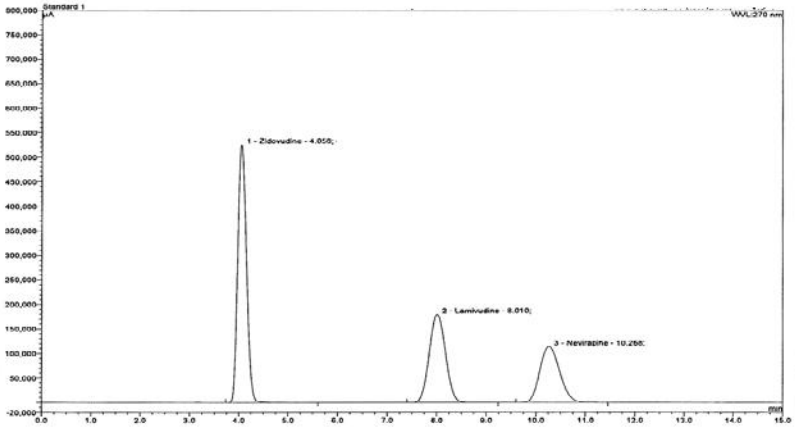

Fig:2 Chromatogram of Lamivudine, Zidovudine and Nevirapine Sample Solution.

Table no 1: Analysis of Tablet formulation

Sr no	parameters	Drug		
		Lamivudine	Zidovudine	Nevirapine
1	Label claim (%/tab)	150 mg	300 mg	200mg
2	Drug content(%)	101.9	100.8	94.3
3	% RSD	0.14	0.20	0.16

Validation parameters

Accuracy

The method was validated in accordance to ICH guidelines Recovery study was performed by standard addition method by adding the known amount of Lamivudine, ZidovudineNevirapine (Reference standard) to the placebo at three different concentration levels i.e 50%, 100% and 150% of assay concentration and % recovery for all these drug were calculated.% Recoveries of Lamivudine, Zidovudine, and Nevirapine were found in the range of 100.9-101.4 %, 100.1-100.9 % and 99.0-99.99% respectively(RSD < 2) as in table no 2.

Table 2 Accuracy (Recovery studies)

Sr no	Level of % Recovery	Weight Taken (mg)	Mg Recovered	% recovery	% mean recovery
Lamivudine	50	150.05	152.74	101.8	101.4
	100	300.40	304.79	101.05	
	150	450.05	454.07	100.9	
Zidovudine	50	300.20	305.18	101.7	100.7
	100	599.50	604.81	100.9	
	150	899.00	899.54	100.1	
Nevirapine	50	199.70	199.91	100.1	99.9
	100	399.90	398.61	99.7	
	150	599.20	593.37	99	

Precision

The system precision was checked by using standard Lamivudine, Zidovudine and Nevirapine to ensure that the analytical system is precise.

Intra-day Precision

Intra-day precision was determined by analyzing the combined standard solutions of Lamivudine, Zidovudine and Nevirapine (150, 300, 200 µg/ml) at three different time intervals on same day. The % RSD of precision study of these drugs was found to be less than 1 %.

Inter-day Precision

Inter-day precision was determined by analyzing the combined standard solutions of Lamivudine, Zidovudine and Nevirapine (150, 300, 200 µg/ml) on three consecutive days. The % RSD of precision study of these drugs was found to be less than 1 %.

Table 3 Result of Intraday and Interday precision of Lamivudine (150µg/ml)

Conc. µg/ml	Time (hr.)	Mean Peak Area n=6	Std. Deviation n=6	%RSD	Time (days)	Mean Peak Area n=6	SD n=6	% RSD
150 (Lamivudine)	0	4152892	3613.939	0.08%	1	4156201	5091.232	0.12%
	2	4139386	3583.033	0.08%	2	4138035	3296.168	0.07%
	4	4142319	4589.268	0.11%	3	4155800	8119.642	0.19%

Table 4 Result of Intraday and Interday precision of Zidovudine (300µg/ml)

Conc. µg/ml	Time (hr.)	Mean Peak Area n=6	Std. Deviation n=6	%RSD	Time (days)	Mean Peak Area n=6	SD n=6	% RSD
300 (zidovudine)	0	6636319	11496.57	0.17%	1	6641437	10733.47	0.16%
	2	6485820	7281.153	0.11%	2	6485858	7267.965	0.11%
	4	6536319	11496.57	0.17%	3	6536291	11534.14	0.17%

Table: 5 Result of Intraday and Interday precision of Nevirapine (200µg/ml)

Conc. µg/ml	Time (hr.)	Mean Peak Area n=6	Std. Deviation n=6	%RSD	Time (days)	Mean Peak Area n=6	SD n=6	% RSD
200 Nevirapine	0	3192686	38231.68	1.19%	1	3184422	31298.39	0.98%
	2	3154635	1728.371	0.054%	2	3153290	7658.574	0.24%
	4	3260535	27990.07	0.85%	3	3245086	29443.96	0.90%

Linearity and Range

The solutions for linearity were prepared in the concentrations as follows and chromatographic peak area was plotted against the concentration of the each drugs. From the data obtained, co-relation coefficient, slope and y-intercept were calculated (fig 3,4,5)

Table 6 Results of linearity for RP-HPLC Method

Parameter	LAMI	ZEDO	NEVI
Linearity and Range	75-225	150-450	100-300
Slope(m)	27974.69	21915.68	15950.85
Intercpt	-0.8280	-0.6870	-0.7489
Correction 3coefficient(R^2)	0.9999	0.9999	0.9999

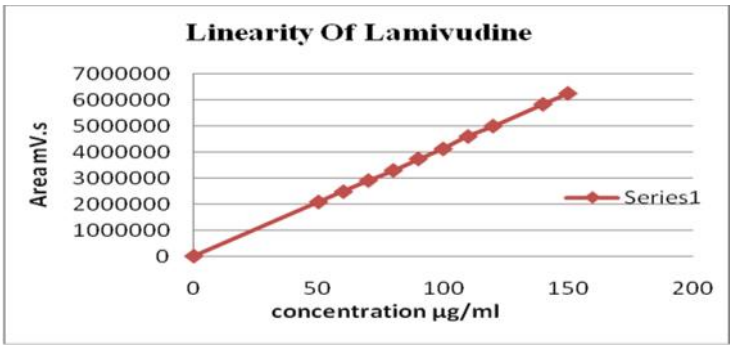

Fig no 3: Linearity of Lamivudine

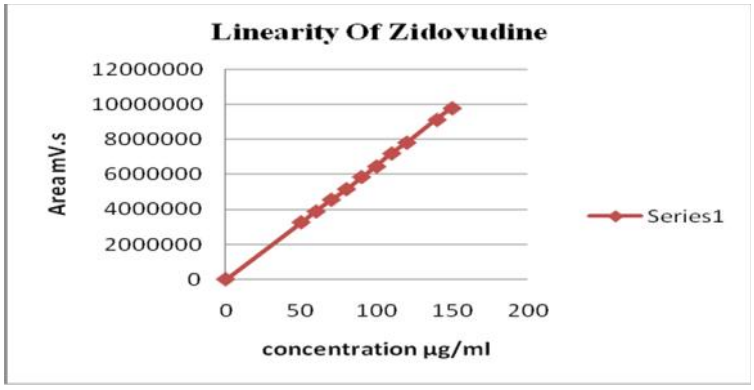

Fig no 4: Linearity of zidovudine

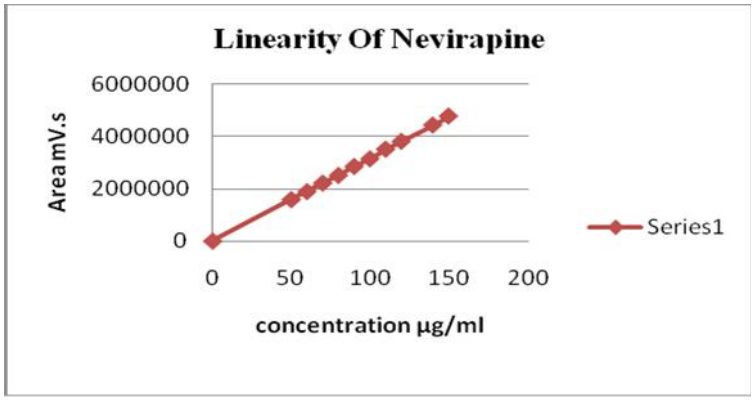

Fig no 5: Linearity of Nevirapine

Robustness:

Robustness was done by changing the column temperature (±5°C), flow rate (±10%), wavelength (±5 nm) .It was observed that % RSD of Peak Area was less than 2% for all the drugs in the conditions.

Table 7 : Variation in column Temperature

Column Oven Temperature	Analyte	Retention Time* (min)	Tailing Factor (T)	Resolution (R)	Theoretical Plates (N)	%RSD Peak Area
25°	LAMI	8.77	1.06	12.23	6038	1.15
	ZIDO	4.25	1.10	NA	3813	1.13
	NEVI	10.87	1.08	4.01	5581	1.17
30°	LAMI	7.856	1.05	11.42	5963	0.10
	ZIDO	4.027	1.10	NA	3990	0.10
	NEVI	10.11	1.07	4.85	6365	0.08
35°	LAMI	7.05	1.05	10.41	5671	0.43
	ZIDO	3.82	1.11	NA	4054	0.42
	NEVI	9.44	1.08	5.64	6860	0.38

Table 8 : Variation in Flow Rate (ml/min)

Flow rate (ml/min)	Analyte	Retention Time* (min)	Tailing Factor (T)	Resolution (R)	Theoretical Plates (N)	%RSD Peak Area
0.9ml/min	LAMI	8.6	1.05	11.56	6007	0.36
	ZIDO	4.450	1.11	NA	4132	0.36
	NEVI	11.14	1.09	4.88	6675	0.43
1.0ml/min	LAMI	7.856	1.05	11.42	5963	0.10
	ZIDO	4.027	1.10	NA	3990	0.10
	NEVI	10.11	1.07	4.85	6365	0.08
1.1ml/min	LAMI	7.159	1.05	11.08	5658	0.17
	ZIDO	3.665	1.09	NA	3736	0.18
	NEVI	9.21	1.07	4.69	5884	0.14

Table 9 : Variation in Wavelength (nm)

Wavelength in nm	Analyte	Retention Time* (min)	Tailing Factor (T)	Resolution (R)	Theoretical Plates (N)	%RSD Peak Area
265nm	LAMI	7.85	1.06	11.32	5983	0.40
	ZIDO	4.02	1.11	NA	3949	0.40
	NEVI	10.12	1.09	4.80	6009	0.41
270nm	LAMI	7.856	1.05	11.42	5963	0.10
	ZIDO	4.027	1.10	NA	3990	0.10
	NEVI	10.11	1.07	4.85	6365	0.08
275nm	LAMI	7.85	1.06	11.27	5745	0.05
	ZIDO	4.03	1.11	NA	3937	0.05
	NEVI	10.12	1.08	4.87	6494	0.06

System Suitability Parameters

The system suitability tests are carried out to evaluate the reproducibility of the system for the analysis to be performed. The results of system suitability tests are given in Table 10, showing that the parameters are within the suitable range.

Table 10 : System Suitability Parameters

Analyte	Retention Time* (min)	Tailing Factor* (T)	Theoretical Plates* (N)	Resolution* (R)
LAMI	8.016	1.05	2795	8.45
ZIDO	4.059	1.18	2428	-
NEVI	10.274	1.16	3087	3.36
Required limits	--	T < 2	N > 2000	R >2

Results and discussion

The scope of the present work is to expand the optimization of the chromatographic conditions, to develop RP-HPLC method for the estimation of drugs in selected multi-component dosage forms. The developed method was also validated. Accuracy of the method was determined by performing recovery study and the result were found in the range of 100.9-101.4%, 100.1-100.7% and 99.0-99.9% for Lamivudine, Zidovudine and Nevirapine respectively. Percentage RSD of precision study of these drugs were found less than 2 percent which indicated good precision of the developed method. The proposed method was validated for linearity, accuracy, precision, and robustness. The proposed method is simple,rapid.precise and reproducible hence can be applied for routine quality control analysis of Lamivudine, Zidovudine and Nevirapine in pharmaceutical dosage form.

Conclusion

The method gives good resolution for both the drugs with a short analysis time. Percentage recovery shows that the method is free from interference of the excipient used in the formulation. Proposed study describes an HPLC method for the estimation of Lamivudine, Zidovudine and Nevirapine in pharmaceutical dosage form. The validated isocratic reversed phase method employed here proved to be simple, sensitive, fast, accurate, precise and robust. Therefore, the proposed method can be used for routine analysis of in pharmaceutical dosage form in combined dosage form.

References

1. MahuaSarkar, ‘et al’,2006 Development and validation of RPHPLC and ultraviolet spectrophotometric methods of analysis for the quantitative estimation of antiretroviral drugs in pharmaceutical dosage forms. Journal of Chromatography B 830, 349–354 .
2. USP-NF, The Official Compendia of Standards, published by The United States Pharmacopoeia Convention, City Press, Baltimore, US, 2009, Vol III, pp 3072-3.
3. Indian pharmacopoeia 2007,ministry of health and family welfare, controller of publications, Delhi
4. VibhutiKabra,‘et al’2009. Simultaneous quantitative determination of zidovudine and nevirapine in human Plasma Using Isocratic, Reverse Phase High Performance Liquid Chromatography, TJPR,8(1): 79-86.
5. PrasadaRao C H, ‘et al’ 2009.Channabasavaraj K P, Lakshmi Aswini G, Development and validation of RP-HPLC method for the estimation of Nevirapine in bulk drug and tablets, J PharmaSci and Res, 1(2): 78-82.
6. AnandBabu K,‘et al’2011., Analytical method development and validation for simultaneous estimation of Zidovudine, Lamivudine and Nevirapine tablets by RP-HPLC, International Journal of Pharmaceutical Research and Development,3(7): 9-14
7. Venkatesh P‘et al’,2011. Sepectrophotometric determination of nevirapine in pharmaceuticals after derivatization with 2,4-dinitro phenlyhydrazine. Asian J. Pharm. Health Sci.,1 (1): 33-4.

8. RohiniP ,‘et al’,2011. Method development and validation for estimation of Nevirapine from tablets by RP-HPLC, Int. J Pharma, 1(1): 29-33.
9. Purnima D Hamrapurkar,‘et al’,2009 .Quantitative estimation of Nevirapine by high performance thin layer chromatography, JPRHC,1(2):197-216.