RP- HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF LAMIVUDINE, ZIDOVUDINE AND NEVIRAPINE FROM THEIR COMBINED TABLET DOSAGE FORM

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ABSTRACT

A novel, sensitive and selective reverse phase high performance liquid chromatographic method was developed and validated for the simultaneous determination of lamivudine, zidovudine, and nevirapine in tablet formulation. The chromatographic separation was achieved on Inertsil, ODs C18 4.6 x 150mm, 3.5mm column by means of a mobile phase containing a mixture of Ammonium acetate buffer pH 5.5, acetonitrile and methanol in the ratio of 50:30:20 v/v at a flow rate of 1.2 ml/min at an ambient temperature and detection was carried out at 260nm.Clear chromatography peaks were identified with retention times of 2.457min for lamivudine, 3.327 min for zidovudine and 4.813 min for nevirapine. The urbanized technique was validated as per ICH guidelines regarding specificity, linearity, accuracy, precision, LOD, LOQ and robustness and the method shows excellent linearity and correlation coefficients of lamivudine, zidovudine and nevirapine were 0.9997, 0.9998 and 0.9998 correspondingly. The % means recoveries of drugs were in between 98.5-100.03 and %RSD for repeatability and intermediate precision was less than 2%. Therefore, the proposed method can be in use for the determination of Lamivudine, Zidovudine, and Nevirapine in various pharmaceutical dosage forms during regular and quality-control analysis.

Keywords: lamivudine, zidovudine and nevirapine, Simultaneous determination, RP-HPLC, tablets

INTRODUCTION

Human Immunodeficiency Virus (HIV) has been one of the most devastating blood-borne viruses affecting a large pediatric and adult population across the earth. Due to the development thereafter of various antiretroviral (ARV) agents, it has led to the major decrease in the morbidity and mortality rates of the HIV infected population. The standard ARV therapy in the treatment of these HIV-infections entail the use of combination drug therapy, usually a triple combination such as two-nucleoside or nucleotide reverse transcriptase inhibitors (NRTIs) as a backbone and a non nucleoside reverse transcriptase inhibitor (NNRTI) [1,2,3].Out of various existing ARV drugs currently recommended by World Health Organization for HIV infection in the pediatric population in resource-limited countries, the combination of zidovudine/lamivudine (NRTI backbone) and nevirapine (NNRTI) is considered as one of the preferred ARV treatment Lamivudine and Zidovudine are synthetic nucleoside analogs and are phosphorylated intracellularly by kinases to its active 5'-triphosphate metabolites, lamivudine triphosphate (LTP) and zidovudine triphosphate (ZDV-TP). These active forms are incorporated into HIV DNA by HIV reverse transcriptase resulting in chain termination. Chemically lamivudine is 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one having a molecular formula of $C8H11N3O3S$, molecular weight is 229.3 gm/ mol. and melting point is 160-162°C. Soluble in water, sparingly soluble in methanol and the PKA value is 14.29. Chemically zidovudine is 1-[(2R,4S,5S)-4-azido-5-(hydroxymethyl)oxolan-2-yl]-5-methyl-1,2,3,4-tetrahydropyrimidine-2,4-dione having a molecular formula of $C10H13N5O4$, molecular weight is 267.2413 gm/ mol. and melting point is 159-160°C.Soluble in water and the PKA value is 9.95. Nevirapine is a potent, non-nucleoside reverse transcriptase inhibitor (NNRTI) used in combination with nucleoside analogs. Nevirapine directly binds to reverse transcriptase (RT) and blocks the RNA and DNA-dependent polymerase actions by producing a disturbance of the enzyme's dynamic site HIV-1 RNA to induce a conformational change at the active site and interrupt catalytic activity of the virus to transcribe viral RNA into DNA[4]. Chemically nevirapine is 2-cyclopropyl-7-methyl-2,4,9,15 tetraazatricyclo [9.4.0.0^{3,8}] pentadeca-1(15),3,5,7,11,13-hexane-10-one having a molecular formula of $C15H14N4O$, molecular weight is 266.2 gm/ mol. and melting point is 196.06 °C. Soluble in DMS, sparingly soluble in DCM

and the PKA value is 10.37. These drugs, when given as a fixed dose combination product rather than individual entities, has shown to get better therapy in situation of continued virological suppression and a significant reduction in the mortality rates of the HIV/AIDS infected people [5,6].Progress of fixed-dose combination is frequently related with many propose challenges such as drug-drug or drug-excipient interactions that could compromise the stability and efficacy of the therapeutic agents.

More number of drugs are introduced into the market every year.Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopeias. Hence, standards and analytical procedures for these drugs may not be available in the pharmacopeias. Therefore, it becomes necessary to develop newer analytical methods for such drugs. Analytical methods for the drugs in formulations are not available owing to the interference caused by the excipients. Analytical methods for combination of drugs may not be official in pharmacopeias. LC represents an increasing growth that makes it the most popular method used in a pharmaceutical analysis.

 Literature survey reveals a number of analytical methods have been reported for simultaneous determination of lamivudine, zidovudine, and nevirapine in bulk, pharmaceutical dosage forms and biological fluids and also in combination with other antiretroviral drugs. These methods are spectrophotometric techniques, such as; UV Spectrophotometric methods (Vaishali P. Nagulwar* et al., 2009; *Ramesh Jayaprakash et al., 2014) [7,8], as well as in HPLC methods (J.V.L.N. Seshagiri Rao* et al., 2010; Patel, Vandana B et al., 2010 P. V. Vamshi Krishna* et al., 2012; .*Anjali Joshi1 et al., 2012,;Sindhura D* et al., 2013; S. Dharmaraj et al., 2013; T T. Sunitha* et al., 2013; Kokkula Sandhya1 * et al., 2014; Shinde G.S* et al., 2014; P.Satyanarayana1* et al., 2015; Vanitha Prakash1 et al., 2015) [9-19] and also LC-MS (Seshagiri Rao JVLNc * et al., 2012; Utpal Nandi et al., 2013; Arindam Mukhopadhyay* et al., 2016) [20-22].

The objectives of our study is that, the existing analytical methods require more expensive reagents and solvents and also involve cumbersome extraction and separation processes and also some analytical procedures are not available in the literature due to patent regulations. So, a need was felt to develop an eco-friendly analytical method for simultaneous analysis of Lamivudine, Zidovudine, and Nevirapine by reducing the solvent consumption, by minimizing the buffer strength and pH of mobile phase than

reported methods. The proposed method was validated with respect to specificity, linearity, precision, accuracy, and robustness as required by the International Conference on Harmonization to support the suitability of the method[23].
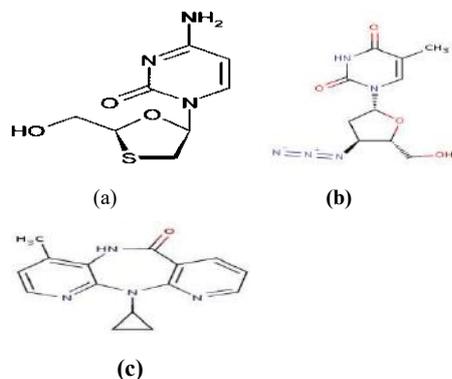

Figure 1: Chemical structures of (a) Lamivudine, (b) Zidovudine and (c) Nevirapine drugs

MATERIALS AND METHODS

Lamivudine, Zidovudine and Nevirapine standard drugs were obtained as a contribution sample from Chandra labs, Hyderabad, India. Methanol, water, acetonitrile and glacial acetic acid of HPLC grade and other chemicals of A.R grade were purchased from E. Merk (India) Ltd. Worli, Mumbai, India. The 0.45μ nylon filters were purchased from Millipore. Lamivudine (150mg), Zidovudine (300mg) & Nevirapine (200mg) tablets as DUOVIR-N were obtained from Mukesh pharmacy, Hyd.

Instruments and chromatographic conditions

The HPLC system (waters) Autosampler separation module 2695 consisted of high-pressure pump, PDA detector 996, and 20 μL capacity injector loops. The system was well equipped with empower 2 software for monitoring and processing of data. Other types of equipment like Sartorius digital weighing balance, Poloman pH meter and Fastclean sonicator was used for sample and standard preparations. The analytical column used was Inertsil, ODs C18 4.6 x 150mm, 3.5mm packed with a particle size of 5.0μ. Ammonium acetate buffer pH 5.5, acetonitrile and methanol in the ratio of 50:30:20 was used as mobile phase at a flow rate of 1.2 ml/min and the injection volume of 20 ml, column oven temperature of ambient and UV detection at 260 nm

Method development

Various mobile phases with different ratios were tested during the development of HPLC method suitable for the estimation of lamivudine, zidovudine, and nevirapine in tablet formulation. These involve Phosphate buffer pH 4.0 and ACN in the ratio of 40:60, Phosphate buffer: pH 3.5 ACN and MeOH in the Ratio of 20:60:20, Phosphate buffer pH 4.0, ACN and MeOH in the Ratio of 15:65:20, Ammonium acetate buffer pH 5.5, ACN and MeOH in the Ratio of 50:10:40 Ammonium acetate buffer pH 5.5, ACN and MeOH in the Ratio of 50:20:30 and Ammonium acetate buffer pH 5.5, ACN and MeOH in the Ratio of 50:30:20. The mobile phase was selected based on the sensitivity of the process, the time necessary for the

analysis, easily available solvents and simplicity of preparation. The mobile phase was premixed and filtered through a 0.45μm filter and sonicated for 10min to remove gases. Optimization of mobile phase was taken based on various parameters such as retention time, number of theoretical plates and resolution. Methanol was used as a diluent.

Preparation of standard stock solution of lamivudine

Lamivudine working standard of about 10 mg was weighed accurately and transferred into a 100ml volumetric flask and dissolved then diluted to 100 ml with diluent. 10 µg /ml of solution was prepared by diluting 1 ml of this solution to 10 ml with the diluent.

Preparation of standard stock solution of zidovudine

Zidovudine working standard of about 10 mg was weighed accurately and transferred into a 100ml volumetric flask and dissolved then diluted to 100 ml with diluent. 10 µg /ml of solution was prepared by diluting 1 ml of this solution to 10 ml with the diluent.

Preparation of standard stock solution of nevirapine

Nevirapine working standard of about 10 mg was weighed accurately and transferred into a 100ml volumetric flask and dissolved then diluted to 100 ml with diluent. 10 µg /ml of solution was prepared

by diluting 1 ml of this solution to 10 ml with the diluent.

Preparation of standard solutions of lamivudine, zidovudine, and nevirapine

Lamivudine, Zidovudine and Nevirapine working standards of about 50 mg, 100mg and 66.6mg have been weighed precisely and transferred into a 100ml volumetric flask and dissolved then diluted to 100 ml with the mobile phase. 50µg/ml of lamivudine, 100µg/ml of zidovudine and 66.6µg/ml of nevirapine were prepared by diluting 1ml of the above solutions to 10ml with the mobile phase.

Preparation of sample solutions

Twenty tablets, containing 150mg of lamivudine, 300mg of zidovudine and 200 mg of nevirapine, were weighed accurately and grounded to fine powder, the powder which is equivalent to 50 mg of lamivudine, 100mg of zidovudine and 66.6 mg of nevirapine were precisely weighed and transferred to100 ml of volumetric flask and dissolved in sufficient mobile phase, filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and then diluted to 100ml with mobile phase.50µg/ml of lamivudine, 100µg/ml of zidovudine and 66.6µg/ml of nevirapine were prepared by diluting 1ml of above solutions to 10ml with mobile phase.

Preparation of acetate buffer PH 5.5

1.540 gm of Acetate buffer was weighed and dissolved in water and diluted to 1000

ml with water and the pH was adjusted using glacial acetic acid. The buffer was filtered through 0.45µ filters to remove all fine particles.

Preparation of mobile phase

50 volumes of Acetate buffer pH 5.5, 30 volumes of Acetonitrile and 20 volumes of methanol were mixed together. The solution was degassed in an ultrasonic water bath for 10 minutes and filtered through a 0.45µm filter under vacuum.

Determination of working wavelength (λmax)

10 µg/ml solutions of all three drugs in methanol were scanned using UV-Visible spectrophotometer in the wavelength region of 200–400 nm against methanol as blank. In the absorption curve, the characteristic absorption maxima (λmax) for Lamivudine, zidovudine and nevirapine drugs in the mixture were at 270 nm 265 nm and 230 nm. The resulting spectra were shown in the fig: 2
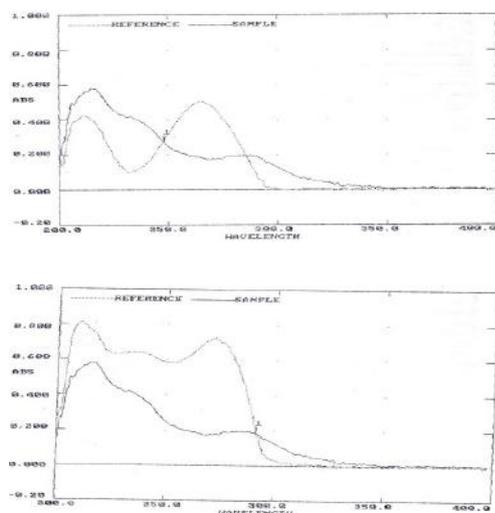

Fig: 2 Overlay UV spectrum of of Lamivudine, zidovudine and nevirapine drugs

Procedure

A solvent system of Ammonium acetate buffer pH 5.5, ACN and MeOH in the Ratio of 50:30:20%v/v was found to be the most suitable mobile phase for ideal separation of Lamivudine, zidovudine, and nevirapine drugs. The mobile phase was pumped through the column at a flow rate of 1.2 ml/min. The column was at an ambient temperature and equilibrated with mobile phase for 30 minutes prior to the injection of solutions. 20µl of five repeat injections of both standard and sample solutions were injected into the chromatographic system and the areas of Lamivudine, zidovudine and nevirapine peak were measured. The detection of drugs was monitored at 260 nm. The runtime was set at 8 min. Under these optimized chromatographic conditions, the retention time for the Lamivudine was 2.457min, zidovudine was 3.327 min and nevirapine was 4.813 min, respectively. The typical chromatogram of all three drugs showing the separation of drugs is given in Figures 3and 4. From the peaks of drugs, the % assay was calculated using the following formula.

$$\%Assay = ATAS \times WSDS \times DTWT \times P100 \times Avg\ WtLC \times 100$$

Method validation

The validation of analytical method verifies that the characteristics of the method if they satisfy the requirements of the method. The developed method was validated according

to ICH guidelines for specificity, linearity, accuracy, and precision, LOD,LOQ and robustness.

RESULTS AND SCUSSION

Method optimization

Different mobile phases were experienced to develop a liquid chromatographic method for the assay of Lamivudine, zidovudine, and nevirapine.The HPLC method was optimized through the evaluation of several solvent mixtures. Mobile phase of Ammonium acetate buffer pH 5.5, ACN and MeOH in the Ratio of 50:30:20%v/v on Inertsil, ODsC18 4.6 x 150mm, 3.5mm, column resulted in sharp, well-defined peaks with good resolution and low retention times were about 2.457 min for Lamivudine, 3.326 min zidovudine and 4.810 min for nevirapine at the flow rate of 1.2 ml/min. The % assay of Lamivudine, Zidovudine, and Nevirapine present in the tablet dosage form was found to be 98.59 %, 98.69 %, and 100.27% respectively. Results are shown in table 1

Table: 1 Assay Results of Lamivudine, Zidovudine and Nevirapine

Standard Area			
Injection No	**Lamivudine**	**Zidovudine**	**Nevirapine**
1	1529.340	3107.866	2016.568
2	1529.798	3117.090	2004.559
3	1516.743	3100.112	1991.608
4	1526.475	3118.032	2005.731
5	1524.718	3119.845	2012.314
Average	1525.415	3112.589	2006.156
Sample area			
1	1528.533	3119.690	2008.710
2	1522.402	3104.454	2082.342
3	1516.743	3100.112	2091.608
4	1526.475	3118.032	2005.731
5	1524.718	3119.845	2012.314
Average	1523.774	3112.427	2040.141
Tablet average weight	750.3 mg	750.3 mg	750.3 mg
Standard	50 mg	100 mg	66.6 mg
weight			
Sample weight	250.1 mg	250.1 mg	250.1 mg
Label amount	150 mg	300 mg	200 mg
Std.purity	98.7	98.7	98.7
Cal.:	147.89 mg	296.08 mg	200.54 mg
%Assay	98.59%	98.69%	100.27%

Study of retention time

A standard dilution of pure drugs having 50µg/ml of lamivudine, 100µg/ml of zidovudine and 66.6µg/ml of nevirapine were prepared in a diluent and loaded injection port of instrument fitted with a 20µl fixed loop. The solution was injected and chromatogram was recorded. The retention time of Lamivudine was 2.457min, zidovudine was 3.327 min and nevirapine was 4.813 min. The respective chromatograms of drugs are shown in figures 3 and 4.

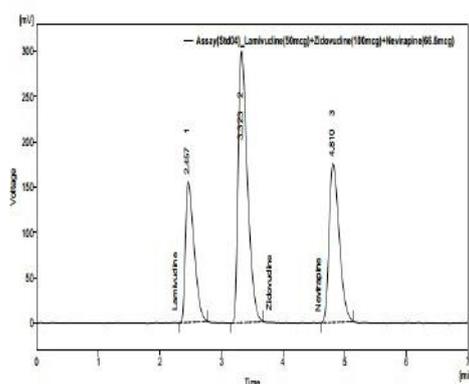

Fig. 3: Typical chromatogram of Lamivudine, zidovudine and nevirapine from standard solution
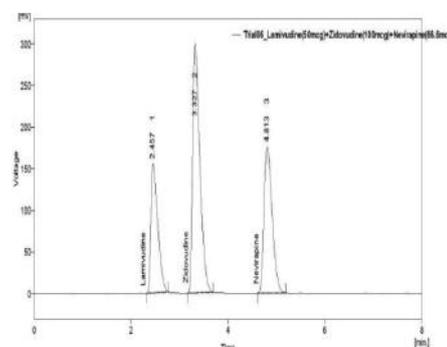

Fig. 4: Typical chromatogram of Lamivudine, zidovudine and nevirapine from sample solution

Method validation

System suitability

After equilibration of the column with mobile phase, five replicates injections of a 20µl standard solution was injected. The System suitability of the method was evaluated using parameters from the recorded chromatograms by complete separation of lamivudine, zidovudine and nevirapine peaks from standard solution and %RSD of replicating injections was less than 1%. The system suitability results are publicized in Table 2.

Table 2: System suitability results

Name of the peak	Rt min	Peak Area	Tailing	Plate count	Re Solution	% RSD
LMV	2.46	1524.71	1.68	3166	-	0.75
ZDV	3.32	3119.84	1.56	2297	3.154	0.56
NVR	4.81	2012.31	1.51	3967	5.058	0.38

Specificity

The specificity of the process was evaluated by injecting blank, sample, and standard preparations into the chromatographic system and chromatograms were compared. It was observed that there was no interference due to excipients from tablet dosage form and also from solvent at retention time of analyte peaks and further more showed good resolution.
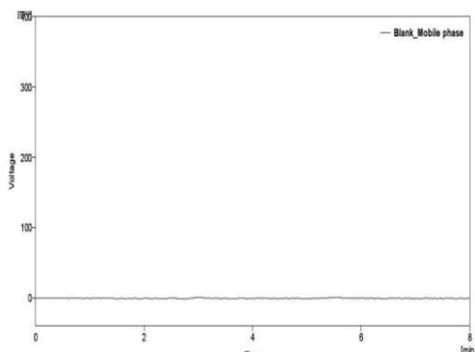

Fig: 5 Chromatogram of blank preparation

Linearity

The linearity of response (peak area) for Lamivudine, Zidovudine, and Nevirapine in relation to concentration was determined in a concentration ranging from 30-70µg/ml for Lamivudine, 60-140µg/ml for Zidovudine and 39.9-93.2µg/ml for Nevirapine. Each concentration level was injected in replicate in to the HPLC system. The linearity was evaluated by the value of the correlation coefficient. These values for Lamivudine, Zidovudine, and Nevirapine were 0.9997, 0.9998 and 0.9985 and good correlation was obtained between the peak areas and concentrations as shown in figures 6,7and8 and the results are shown in Table 3, 4 and 5

Table 3: Linearity results for Lamivudine

Lamivudine(µg/ml)	Peak area
30	1052.087
40	1330.588
50	1508.091
60	1986.835
70	2107.992
Slope	40.05
Y- intercept	243
Correlation coefficient (R2)	0.9997
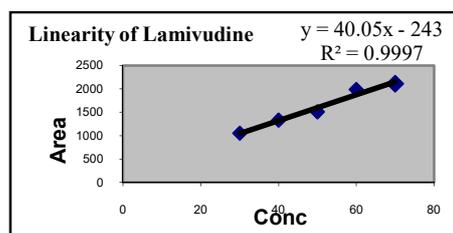
Fig: 6 calibration curve of Lamivudine

Table 4: Linearity results for Zidovudine

Zidovudine (µg/ml)	Peak area
60	2147.573
80	2713.131
100	3084.988
120	3805.769
140	4230.274
Slope	50.45
Y- intercept	50.72
Correlation coefficient (R2)	0.9998
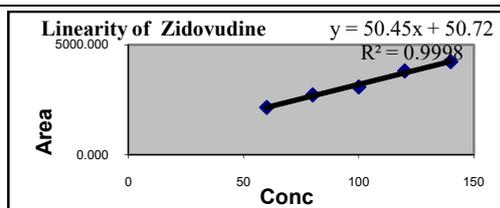
Fig: 7 calibration curve of Zidovudine

Table 5: Linearity results for Nevirapine

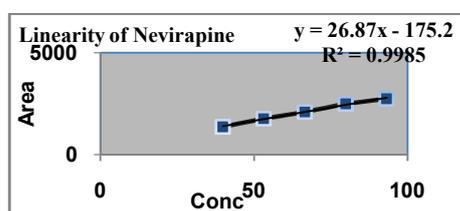| Nevirapine (µg/ml)	Peak area
39.9	1363.369
53.2	1759.538
66.6	2092.019
79.9	2486.364
93.2	2742.958
Slope	26.87
Y- intercept	175.2
Correlation coefficient (R2)	0.9985

Fig: 8 calibration curve of Nevirapine

Accuracy

The accuracy of the method was determined by recovery experiments and was performed by the standard addition method at three concentration levels of 80%, 100% and 120%. Each level was injected thrice into the chromatographic system and the percentage and mean % recoveries for all three drugs were calculated and the results are given away in Table 6, 7 and 8.

Table 6: Recovery results of Lamivudine

Accu racy level (%)	Amount added (in µg)	Amount recovered (in µg)	% Recove ry	Mean % recovery
80	40+10	49.82	99.64	
80	40+10	49.80	99.60	99.60
80	40+10	49.78	99.56	
100	50+10	59.46	99.10	
100	50+10	59.52	99.20	99.15
100	50+10	59.50	99.16	
120	60+10	69.78	99.68	
120	60+10	69.81	99.72	99.72
120	60+10	69.85	99.78	

Table 7: Recovery results of Zidovudine

Accu racy level (%)	Amount added (in µg)	Amount recovered (in µg)	% Recove ry	Mean % recovery
80	80+20	98.34	98.34	
80	80+20	98.55	98.55	98.54
80	80+20	98.75	98.75	
100	100+20	120.05	100.04	
100	100+20	120.01	100.00	100.03
100	100+20	120.04	100.05	
120	120+20	137.55	98.25	
120	120+20	138.01	98.57	98.58
120	120+20	138.50	98.92	

Tab le :8 Recovery results of Nevirapine

Accu racy Level (%)	Amount added (in µg)	Amount recovered (in µg)	% Recove ry	Mean % recov ery
80	53.2+13.34	66.52	99.96	
80	53.2+13.34	66.49	99.92	99.91
80	53.2+13.34	66.45	99.86	
100	66.6+13.34	79.90	99.94	
100	66.6+13.34	79.86	99.89	99.89
100	66.6+13.34	79.82	99.84	
120	79.9+13.34	99.92	99.65	
120	79.9+13.34	99.88	99.61	99.60
120	79.9+13.34	92.82	99.54	

Precision

The precision of the method was demonstrated by repeatability and intermediate precision studies. Intermediate precision (inter-day precision) was evaluated by assaying six injections of sample solution according to the description of the analytical method by different analysts on different days using different HPLC and columns of the same make but different lot number. The % RSD for response factor of all three drugs was found to be less than 2% and results are revealed in tables 9,10 and 11.

Table: 9 Repeatability Results for Lamivudine

S.No	Peak name	Rt (min)	Peak Area	Plate count	Asym metry
1	LMV	2.463	1532.16	4370	1.346
2	LMV	2.457	1533.75	4362	1.346
3	LMV	2.457	1533.46	4362	1.480
4	LMV	2.460	1527.64	4366	1.440
5	LMV	2.463	1533.01	4370	1.346
6	LMV	2.457	1528.52	4362	1.346
	Mean	2.4595	1531.42		
	SD	0.003	2.338		
	%RSD	0.13	0.15		

Table: 10 Repeatability Results for Zidovudine

S.No.	Peak name	Rt (min)	Peak Area	Plate count	Asym metry
1	ZDV	3.330	3114.84	3303	1.172
2	ZDV	3.323	3125.29	3294	1.100
3	ZDV	3.323	3117.27	3294	1.067
4	ZDV	3.327	3118.77	3298	1.172
5	ZDV	3.330	3119.67	1.346	1.100
6	ZDV	3.323	3112.33	3203	1.100
	Mean	3.326	3118.03		
	SD	0.003	4.451		
	%RSD	0.10	0.14		

Table: 11 Repeatability Results for Nevirapine

S.N	Peak name	Rt (min)	Peak Area	Plate count	Asym metry
1	NVR	4.817	2016.05	3967	1.757
2	NVR	4.810	1998.32	3813	1.707
3	NVR	4.807	2013.29	3951	1.730
4	NVR	4.813	2008.86	3961	1.730
5	NVR	4.817	2014.13	3967	1.180
6	NVR	4.807	2009.16	3951	1.730
	Mean	4.812	2009.97		
	SD	0.005	6.368		
	%RSD	0.10	0.32		

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection and quantification were assessed based on the signal to noise ratio. This ratio for LOD is 3:1 and LOQ is 10:1.The limit of detection and quantification were evaluated from the calibration curves by applying statistical calculations and results are shown in table 12.

The limit of detection and the limit of quantification were expressed as :

LOD=3.3 σ/S
LOQ=10.0 σ/S
Where;

σ = Standard deviation of the response

S = Slope of the regression line

Table:12 LOD and LOQ results for Lamivudine, Zidovudine and Nevirapine

Name of the analyte	LOD µg/ml	LOQ µg/ml
Lamivudine	1.30	3.95
Zidovudine	3.97	12.03
Nevirapine	2.66	8.06

Robustness

Robustness of the method were demonstrated by analyzing the system suitability parameters under deliberately modified chromatographic conditions such as flow rate, and wavelength on lower and higher side of the original values. There was no significant change in the retention time between the original method and changes made to the method. The results are illustrated in Table 13,14 and 15

Table: 13 system suitability results for robustness study of Lamivudine

Robust condition	Rt (min)	Peak Area	Tailing	Plate count	% RSD
Normal	2.460	1524.71	1.68	3166	0.75
FR 1.0 ml	2.937	1839.01	1.50	4323	0.79
FR 1.4 ml	2.140	1313.54	1.12	4427	0.82
WL 258nm	2.457	1505.60	1.34	4362	0.86
WL 262 nm	2.460	1611.07	1.34	3198	0.73

Table: 14 system suitability results for robustness study of zidovudine

Robust condition	Rt (min)	Peak Area	Tailing	Plate count	Resolution	% RSD
Normal	3.327	3119.84	1.56	2297	3.15	0.56
FR 1.0 ml	3.967	3764.63	1.29	3254	3.13	0.65
FR 1.4 ml	2.890	2648.87	1.96	3365	3.22	0.76
WL 258 nm	3.320	3004.85	1.06	4366	3.14	0.72
WL 262 nm	3.327	3312.39	1.06	3207	3.15	0.58

Table: 15 system suitability results for robustness study of Nevirapine

Robust condition	Rt (min)	Peak Area	Tailing	Plate count	Resolution	% RSD
Normal	4.817	2012.31	1.51	3967	5.05	0.38
FR 1.0 ml	5.743	2435.68	1.86	3893	5.05	0.45
FR 1.4 ml	4.180	1714.57	0.15	3944	5.11	0.62
WL 258 nm	4.807	2084.01	1.73	3951	5.04	0.52
WL 262 nm	4.817	2022.59	1.68	3824	5.01	0.36

CONCLUSION

From the above experimental results, the projected method for the simultaneous estimation of Lamivudine, Zidovudine and Nevirapine in their combined dosage form was found to be simple, precise, accurate and high resolution. Also, the lower solvent consumption and shorter retention time lead to more acceptable, cost effective and Eco friendly chromatographic procedure. Hence it can be conveniently adopted for routine analysis in quality control department

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