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Simultaneous Determination of Lamivudine, Zidovudine and Nevirapine in Tablet Dosage Forms by RP-HPLC

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ABSTRACT

An accurate, precise and economic reversed phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the estimation of lamivudine, zidovudine and nevirapine in pharmaceutical dosage forms. In this method Qualisil BDS C8 column (250mmx4.6mm i.d., 5µm particle size) with mobile phase containing water and acetonitrile in the ratio of 70: 30 v/v with pH adjusted to 5 with ortho phosphoric acid (OPA). The flow rate was 1mL/min and the detection wavelength was 250nm. The linearity was observed in the range of 1-15µg/mL for lamivudine, 3-24 µg/mL for zidovudine and 2.5-20 µg/mL for nevirapine. Retention times were 3.1min, 4.4min, and 7.0min for lamivudine, zidovudine and nevirapine respectively. The proposed method was validated as per ICH guidelines for linearity, accuracy, precision and robustness and can be applied for routine quality control analysis of pharmaceutical dosage forms used for multidrug therapy containing lamivudine, zidovudine and nevirapine.

Keywords: RP-HPLC, OPA, Multidrug therapy, ICH, Validation.

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INTRODUCTION

Lamivudine chemically (2R, cis)-4- amino-l-(2-hydroxymethyl-l, 3-oxathiolan-5-yl)-(lH)-pyrimidin-2-one and zidovudine chemically 1- (3- azide-2, 3-di deoxy-β-D-ribofuranosyl)-5-methyl Pyrimidin-2, 4 (1H, 3H) – dione are synthetic nucleoside analogs showing a potent and synergistic effect on inhibition of the human immunodeficiency virus (HIV-1), the causative agent of Acquired Immuno-Deficiency Syndrome(AIDS). HIV encodes at least three enzymes protease, reverse transcriptase and endonuclease. Lamivudine and zidovudine belong to the class of nucleoside reverse transcriptase inhibitors (NRTI)[1] Nevirapine chemically 2-cyclopropyl-7-methyl-2,4,9,15-Tetraazatricyclo 4.0{3,8}]pentadeca-1(15),3,5,7,11,13-hexane-10-one is a non-nucleoside reverse transcriptase inhibitor (NNRTI) with activity against human immunodeficiency virus type 1 (HIV-1) that is already marketed for the treatment of HIV-1 infected adults. New therapeutic strategy of AIDS treatment requires the combination of NRTI's with NNRTI's. The introduction of highly effective combination regimens of antiretroviral drugs has led to substantial improvements in morbidity and mortality[2]. Intra cellularly, lamivudine is phosphorylated to its active 5'- triphosphate metabolite, lamivudine triphosphate (3TC-TP), intracellularly, zidovudine is phosphorylated to its active 5'-triphosphate metabolite, zidovudine triphosphate (ZDV-TP)[3.] Nevirapine is metabolized by cytochrome P450 (CYP3A4) and is a relatively potent inductor of the enzyme; consequently, it has the ability to reduce plasma concentrations of other drugs that are also biotransformed by CYP3A4 as Protease Inhibitors [4.] Lamivudine, zidovudine and nevirapine are official in IP, BP, and USP.

A literature survey revealed that few analytical methods were available for estimation of lamivudine, zidovudine and nevirapine in multi-component pharmaceutical formulations [5-8] and in human plasma [9-11.] It was found that all the reported methods demonstrated separation on C_{18} column and there were no methods available on C_8 hence the present RP-HPLC method utilized C_8 and also differs in eluent, runtime and the UV detection wavelength. This paper describes development of reversed phase HPLC on C_8 column and validation for combined assay method for lamivudine, zidovudine and nevirapine in tablet formulations.

MATERIALS AND METHODS

Pure forms of lamivudine, zidovudine and nevirapine were obtained as generous gifts from Hetero Labs, Hyderabad, India. HPLC grade Acetonitrile was procured from Merck, Mumbai, India, and Ortho- phosphoric acid was purchased from S.D. Fine Chem Ltd., Mumbai, India. All other chemicals and materials were of analytical grade. Water used in the HPLC analysis was

prepared in-house. The mobile phase and all the solutions were filtered through a 0.45mm Fisher brand (Mumbai, India) membranes prior to use.

Instrument and Software

HPLC system (Agilent LC 1120) consisting of isocratic pump, manual rheodyne injector and UV- VIS (VWD) detector and EZ Chrom elite software were used for analysis. The quality control samples were processed by using micropipettes (Eppendorf research, USA). The mobile phase and all other solutions were degassed with ultra Sonicator (pci, Mumbai, India).

Preparation of Mobile phase or Eluent

70ml of water was taken into a 100ml measuring cylinder and to that 30ml of Acetonitrile was added and mixed well. The pH of the solution was adjusted to 5.0 with Ortho-Phosphoric acid. The above solution was filtered through 0.45mm filter membrane and then degassed through an ultra sonicator.

Preparation of stock solutions

Accurately weighed 10mg of lamivudine, zidovudine and nevirapine and were transferred into three clean dried 10ml volumetric flasks and dissolved by adding sufficient amount of mobile phase and the final volume was made up to 10mL with mobile phase.

RESULTS AND DISCUSSION

Method development

Preliminary studies involved trying C_{18} and C_8 reverse phased columns and testing several mobile phase compositions were conducted for the separation of lamivudine, zidovudine and nevirapine with good chromatographic parameters like less peak tailing and good symmetry. A C_8 column (250mm x 4.6mm i.d., 5µ) as a stationary phase with a mobile phase of water: acetonitrile (70: 30) at a flow rate of 1.0mL/min and a detection wavelength of 250nm afforded the best separation of lamivudine, zidovudine and nevirapine with retention times of 3.1, 4.4 and 7.0min respectively.

Method validation

After development of method, validation of the method for simultaneous estimation of Lamivudine, Zidovudine and Nevirapine was performed in accordance with ICH guidelines which include System suitability, Linearity and Range, Accuracy, Precision, LOD and LOQ, Specificity and Robustness.

System suitability

System suitability of the method is determined by injecting a mix solution containing three drugs

of 10 µg/mL concentration and analyzing. System suitability parameters that are to be monitored are plate count, asymmetry, peak tailing, resolution, and capacity factor and height equivalent to theoretical plates (HETP)[12]. (Table- 1)

Table- 1 System suitability data for the developed method

	Values obtained			Limit
	Lami	Zido	Nevi	
Retention time(min)	3.100	4.40	7.00	--
Plate count	12366± 116	14803± 127	16773± 137	>2000
Asymmetry (10%)	1.007± 0.01	1.0143±0.01	0.9968± 0.01	0.9-1.2
Tailing factor	1.00± 0.01	1.166± 0.02	1.142± 0.02	≤ 2.0
Resolution	--	10.55± 0.05	15.24± 0.05	>2
HETP	0.002	0.0016	0.0014	--
Capacity factor	0.5044	0.7618	1.8003	0.5- 20

Linearity and range

The linearity of a method is a measure of how well a calibration plot of response Vs concentration approximates a straight line. To evaluate linearity of the method eight different concentrations for lamivudine (1, 3, 5, 7, 9, 11, 13 and 15µg/mL), zidovudine (3, 6, 9, 12, 15, 18, 21 and 24 µg/mL) and nevirapine (2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5 and 20 µg/mL) were prepared and analyzed. Plot of peak area vs. drug concentration was plotted and linearity was observed in the specified range with correlation coefficients of 0.9992, 0.9992 and 0.9991 for lamivudine, zidovudine and nevirapine respectively. This result demonstrates linearity of the method over the specified range. (Table- 2)

Table- 2 Linearity, LOD and LOQ data

S.No	Parameter	Lamivudine	Zidovudine	Nevirapine
1.	Linearity and correlation coefficient	1-15 µg/mL, 0.9992	3-24 µg/mL, 0.9992	2.5-20 µg/mL, 0.9991
2.	LOD[a]	45ng/mL	75ng/mL	500ng/mL
3.	LOQ[a]	150ng/mL	224ng/mL	1550ng/mL

[a] *LOD and LOQ are not required for assay method validation.*

Accuracy and percentage recovery

Accuracy of the method was tested on dispersible tablets obtained from Ranbaxy Laboratories Ltd. as gift samples. Twenty tablets were powdered and an amount equivalent to 9mg of tablet powder was weighed accurately and dissolved in 10ml of solvent (70: 30 water: acetonitrile) and filtered through 0.45mm filter membrane and then degassed. From this solution 0.1mL was taken with a micro pipette and transferred into a 10mL volumetric flask, dissolved and final volume was made upto the mark with mobile phase. Then the solution was injected into the HPLC system and analyzed. Peak areas were noted and percentage purity was determined. Percentage

recovery was determined by spiking the drug concentrations to 80%, 100% and 120% of pre-analyzed samples. The solutions were injected and analyzed. Peak areas were noted and percent recovery was determined. (Table- 3 and 4)

Table- 3 Assay report on tablet formulation

S.No.	Parameters	Drug content		
		Lamivudine	**Zidovudine**	**Nevirapine**
1.	Label claim (mg)	30	60	50
2	Drug content (%)	100.3411	98.7211	101.2377
3	% RSD	0.8491	0.5975	0.5155

Table- 4 Percentage recovery report

S.No.	Drug	% Recovery	% RSD
		80%	0.6475
1.	Lamivudine	100%	0.8301
		120%	0.7923
		80%	0.5462
2.	Zidovudine	100%	0.6687
		120%	0.6278
		80%	0.6371
3.	Nevirapine	100%	0.6733
		120%	0.6651

Precision

System (repeatability) and Method precision (Reproducibility)

To determine system and method precision was evaluated by calculating the RSD of the peak areas of six replicate injections for standard concentration (100%) of lamivudine, zidovudine and nevirapine. The %RSD values were 0.4105, 0.8036 and 0.9372 for lamivudine, zidovudine and nevirapine respectively. (Table- 5)

Intraday and Interday precision (Intermediate)

To determine intraday precision three replicates of three concentrations of lamivudine, zidovudine and nevirapine were injected at three time intervals of a single day and analyzed. To determine Interday precision three replicates of single concentration of drugs were injected for three days at regular time intervals. The % RSD was calculated for each injection. Intraday precision values were 0.5347, 0. 7447 and 0.5039 and Interday precision were 0.7758, 0.4819 and 0.4458 for lamivudine, zidovudine and nevirapine respectively. (Table- 5)

LOD and LOQ

LOD and LOQ were determined using mathematical method and S/N ratio method. The results were displayed in (Table -2).

Table- 5 Precision data

S.No.	Precision	%RSD		
		Lami	Zido	Nevi
1	System precision	0.5714	0.7968	0.7624
2	Method precision	0.5843	0.6934	0.7138
3	Intraday precision	0.5327	0.7447	0.5039
4	Interday precision	0.7758	0.4819	0.5458

Specificity

Specificity can be defined as the ability to measure accurately the concentration of an analyte in the presence of all other sample materials. In this method other sample materials include excipients involved in preparation of tablet dosage forms. No other peaks except drug peaks were observed in the chromatogram. This demonstrates that the method is specific.

Robustness

Robustness of the current method was investigated by analyzing samples of lamivudine, zidovudine and nevirapine tablet formulation using the same chromatographic conditions set forth in method development but (a) using flow rate of 0.9mL/min and 1.1mL/min instead of 1.0mL/min, (b) volume fraction of acetonitrile 28% and 32% instead of 30% and (c) pH of 4.9 and 5.1 instead of 5.0. %RSD was calculated and was observed to be less than 2.0%.

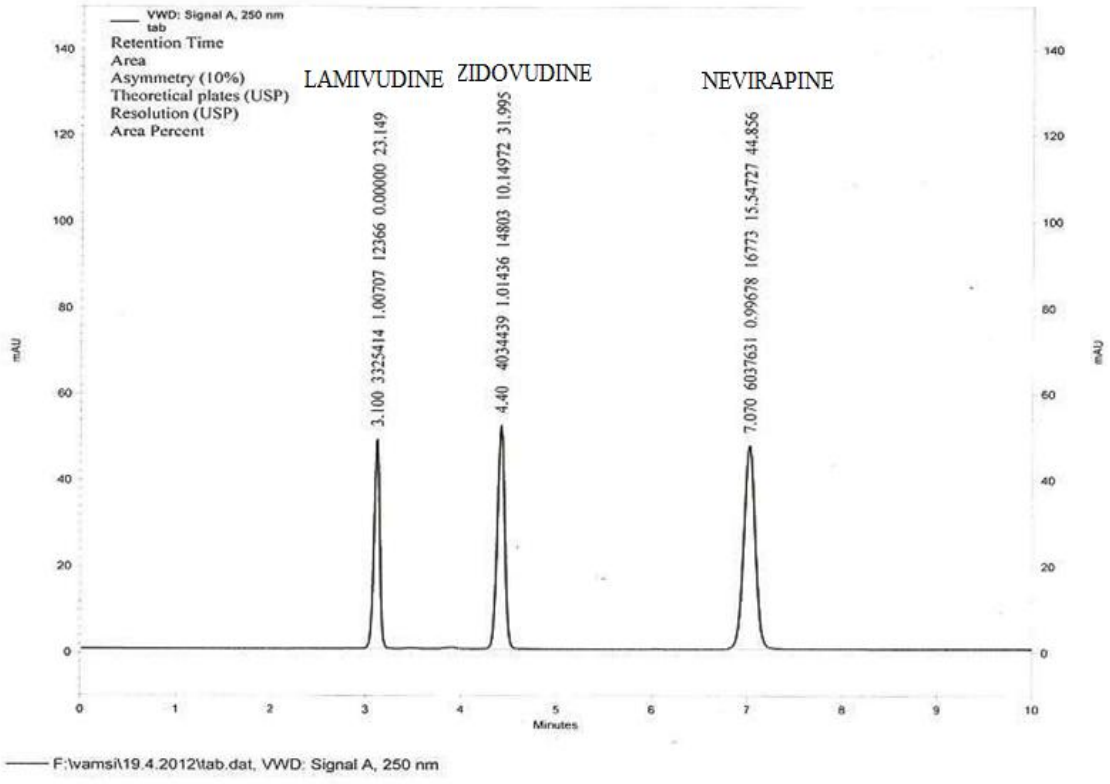

Figure 1 Chromatogram of Lamivudine, Zidovudine and Nevirapine in tablet dosage form.

Solution state stability

To generate reproducible and reliable results, the sample, standards and reagents used for the HPLC method must be stable for reasonable time (e.g. one day, one week, one month, etc. depending on need). Solution state stability was tested by preparing a 10 µg/mL solution of all three drugs in mobile phase and injected for three days at regular time interval of 24Hrs. and observed for extra peaks. No peaks were observed other than the drug peaks at specified retention times. This infers the solution state stability of the drugs.

Mobile phase: Water: Acetonitrile (70: 30 v/v), pH adjusted to 5.0 with OPA. Flow rate- 1.0mL/min, injection volume- 20µL, column- C8 (250x 4.6mm, 5µ), UV detection at 250nm. Retention times were 3.10, 4.40 and 7.00, Peak asymmetry was 1.0070, 1.0143 and 0.9967 and Plate count was 12366, 14803 and 16773 for Lamivudine, Zidovudine and Nevirapine respectively.

CONCLUSION

The Proposed RP-HPLC method is a suitable technique for simultaneous determination of lamivudine, zidovudine and nevirapine in multi drug therapy without any interferences form each other and the method demonstrated the elution of all analytes before 8min. thus the present method is suitable for quick analysis when compared to other methods. All the parameters for three drugs met the criteria of ICH guidelines for method validation. LOD and LOQ of the method were found to be very low and may be considered as more sensitive. The developed method may be recommended for routine and QC analysis of the investigated drugs to provide simple, accurate and reproducible quantitative analysis for the determination of lamivudine, zidovudine and nevirapine in the antiretroviral formulations.

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