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STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS OF ZIDOVUDINE AND LAMIVUDINE FROM THEIR COMBINATION DRUG PRODUCT

Firoj A .Tamboli*[1], P.R.Dhangar[1], H.N. More[1], S.U. Jadhav[1], A.A. Gosavi[1], T.S. Ghatge[2]

*[1] Department of Pharmacognosy, Bharati Vidyapeeth College of Pharmacy, Near Chitranagari, Kolhapur-416013, Maharashtra, India

[2]Ph.D Scholar, Department of Pharmacy, Birla Institute of Technology & Science, Pilani, Hyderabad, Secunderabad,-500078, Telangana, India

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ABSTRACT

A simple and precise chromatographic stability indicating reverse phased HPLC method for estimation zidovudine (ZDV) and lamivudine (LMV) from their combination product has been developed and validated using different validation parameters. In the developed RP HPLC method, YMC, Pack ODS A, (250 mm X 4.6 mm), 5 µm column is used. The mobile phase consisting of acetate buffer pH 4.0 and methanol in the proportion of 80:20 (v/v). The detection was done using photodiode array detector at 270 nm. The combination drug product of ZDV and LMV was exposed to thermal, photolytic, hydrolytic, and oxidative stress conditions, and the stressed samples were analysed by the designed method. The data of peak homogeneity in stressed sample chromatograms of ZDV and LMV peaks, illustrated the specificity of the method for their estimation in presence of degradants. The described method was linear over a range of 0.025 ppm to 0.75 ppm for zidovudine and 0.05 ppmto 1.50 ppm for lamivudine. The mean recoveries were 101.24and 97.63% for zidovudine and lamivudine, respectively.

Keywords: Zidovudine, Lamivudine, HIV, RP-HPLC method

INTRODUCTION

Infections caused by retroviruses such as HIV are treated by a specific category of drugs called antiretroviral drugs. Lamivudine and zidovudine are categorized as antiretroviral drugs, primarily used for the treatment of HIV. Lamivudine (LMV) is a nucleoside reverse transcriptase inhibitor (NTRI). It is analogue of nucleoside that is phosphorylated to lamivudine triphosphate by cellular kinases.[1] Lamivudine acts by inhibiting the enzyme reverse transcriptase of human immuno deficiency viruses (HIV) and hepatitis B(HBV)[2] and it is indicated for the treatment of the same. Its chemical name is expressed as 2R (cis)-4-amino-l-(2-hydroxymethyl-l,3 oxathiolan-5-yl)-(lH)-pyrimidin-2-one.[3]

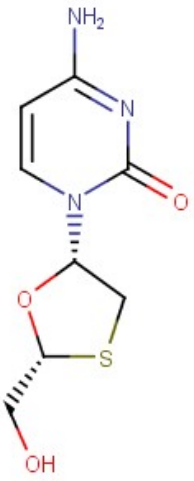

Figure 1: Structure of lamivudine

Zidovudine (ZDV) a nucleoside reverse transcriptase inhibitor, is chemically expressed as thymidine,3'-azido-3'deoxy-3'azido-3'deoxythymidine.[4] It is dideoxynucleoside reverse transcriptase inhibitors. It acts by inhibiting HIV-1 reverse transcriptase and is used for treatment as well as prevention of HIV.[5,6]

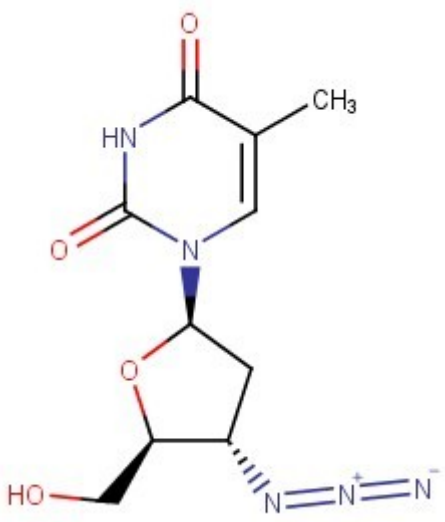

Figure 2: Structure of zidovudine

Combinations of two or more drugs in the pharmaceutical dosage forms are very much useful in multiple therapies. Lamivudine and zidovudine when combined have synergistic effect in the treatment of HIV and it also prevents mutation in the HIV virus in the combination therapy.[7] Lamivudine and zidovudine acts by competitively inhibiting and minimizing the activity of reverse transcriptase (RT).[8] Stability testing is critical part in the process of development of a drug product. High performance liquid chromatography is one of the most accurate chromatographic methods widely used for the quantitative as well as qualitative analysis of drug product and is used for determining drug product stability.[9,10] Stability testing is a technique for determining the change in the quality of drug under the effect of a variation in environmental factors such as temperature, light, and humidity. The various drug related impurities that are formed during the synthesis or manufacture of drug product are separated by using stability indicating HPLC methods.[10] It also provides information to establish the recommended period for storage conditions, shelf lives, and retest periods. The degradants generated and the assay of drug is important in determination of the shelf life of the drug product during the stability study. The stability testing of the drug product needs to be performed as per the International Conference on Harmonization (ICH) guidelines.[11,12]

There are several reported analytical methods for determination of lamivudine and zidovudine individually. However, there are only a few methods[13] reported for estimation zidovudine (ZDV) and lamivudine (LMV) from their combination product by reverse phase HPLC.

The main objectives of this work are to develop a simple and precise chromatographic stability indicating method of multicomponent antiretroviral tablet dosage form and to validate developed method by using different validation parameters.

EXPERIMENTAL

Chemicals and Reagents

Lamivudine and zidovudine were procured from Asrix Lab. Ltd. of AR grade. Water of HPLC grade was obtained from S.D. Fine Chemicals Ltd., India, and acetonitrile of HPLC grade was obtained from Qualigens Fine Chemicals Ltd., Mumbai, India. Dipotassium hydrogen orthophosphate and Potassium dihydrogen phosphate of AR grade were obtained from Ranbaxy Chemicals Ltd., New Delhi, India.

HPLC Instrumentation and Conditions

The HPLC system consisted LC-2010AHT auto injector and SPD-M 10-AVP- PDA Detector of Shimadzu, Japan. The chromatographic separations were performed using YMC, Pack ODS A, (250 mm X 4.6 mm), 5 µm column, the temperature of which was maintained at 28 °C using column oven and with the flow rate of 0.8 ml/min. The mobile phase consisting of acetate buffer pH 4.0 and methanol in the proportion of 80:20 (v/v). It was filtered through 0.45 µm membrane filter and degassed by sonication for 20 min prior to use. measurements were made with injection volume 20 µl and ultraviolet (UV) detection at 270 nm. The forced degradation samples were analysed by scanning the PDA detector in the range of 220-400 nm and desired peak coverage of 100%. Peak homogeneity obtained from the spectral analysis report was expressed in terms of peak purity values.

Standard and Sample Preparation

12.5 mg of lamivudine working standard and 25 mg of zidovudine working standard was weighed accurately and transferred to 250 ml volumetric flask; about 170 ml of diluent was added and sonicated to dissolve with intermittent swirling. It was further diluted to make volume with diluent.

Analysis of Dosage Form

Twenty tablets were accurately weighed and subsequently crushed to form a fine powder. The tablet powder equivalent to about 50 mg of lamivudine was accurately weighed and transferred to a volumetric flask. Then, 170 ml of mobile phase was added to the flask and sonicated for about 15 min with intermittent swirling, allowed equilibration to room temperature. It was further diluted to make up the volume with mobile phase. This solution was filtered through 0.45 µ nylon filter paper discarding first 3 ml of the filtrate. The subsequent filtrate was collected and used for injection on HPLC.

Procedure for Forced Degradation Study

For determination of stability, forced degradation carried out under photolytic, thermal and humidity, oxidative, and acid/base hydrolytic stress conditions. For photolytic degradation, tablet powder was exposed in the photo stability chamber, as per ICH guideline (An overall illumination of not less than 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200-watt hours/square meter). For thermal and humidity degradation, sample was exposed at 40°C/75% RH for 24 hours and analyzed by HPLC. For thermal degradation, tablet powder was heated at 80°C for 24 hours in oven and allowed to cool. For oxidative degradation, 3.0 % H_2O_2 solution was added to the solution of drug product and kept at room temperature for 90 minutes. For acid degradation, solution of drug product was exposed to 5 M HCL solution at 80°C for 30 minutes. The solution was cooled and neutralized before analysis. For base degradation, solution of drug product was exposed to 5 M NaOH solution at 80°C for 30 minutes. The solution was cooled and neutralized before analysis.

RESULT AND DISCUSSION

Stability study was carried out with the aim to reveal the effect of some factor like acid, base, heat, oxidation, sunlight and photolytic on zidovudine and lamivudine in tablet dosage form. To separate the drug and degradation product selectively the HPLC method was developed and validated. For HPLC method development, the retention behavior of lamivudine and zidovudine and it degradation product were initially studied using C- 18 column. Mobile phase containing methanol and water was found to be not suitable, as the compound showed very close retention times and splitting of the chromatogram. Various ratio of methanol: water, buffer, reagent were tried and it was found that acetate buffer pH 4: methanol adjust pH to 7 (80:20 v/v) of mobile phase gave good resolution shown in Figure 3. A flow rate of 0.8 ml/ min resulted in drug retention time within 10 minutes; hence, this flow rate was selected for degradation studies. The samples were scanned in range of 200 -400 wavelength by preparing sample solution of ppm and 270 nm was selected as suitable wavelength to the determination of lamivudine and zidovudine in combination and it degradation study.

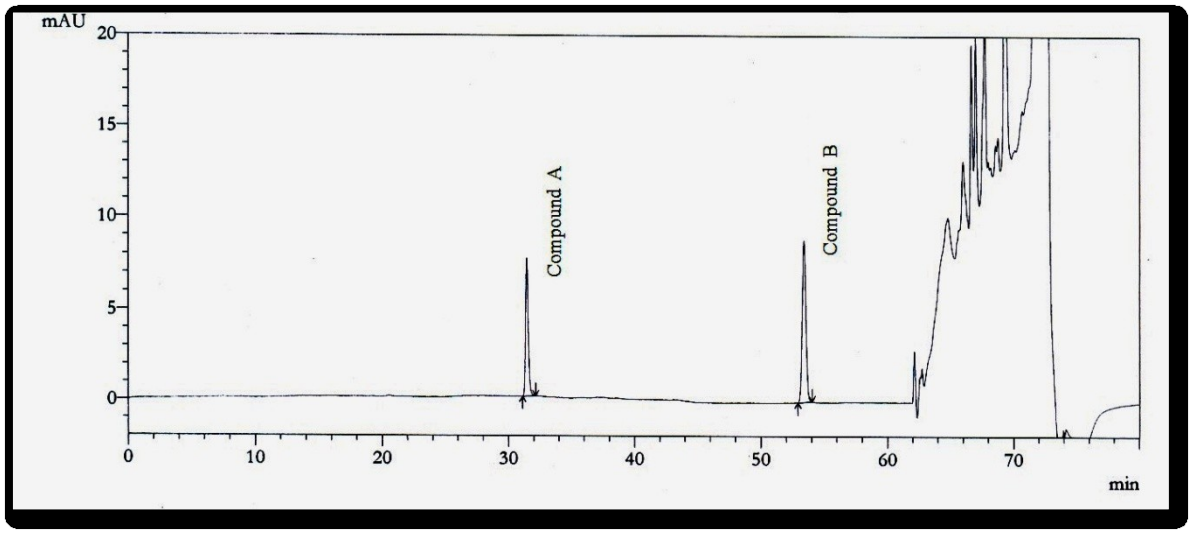

Figure 3: Chromatogram of Sample solution

The method has been validated as per ICH Q2A guidelines by applying parameter like specificity, for response function, accuracy, and intermediate precision. The nominal concentrations of standard and test solutions for zidovudine and lamivudine were 0.50 and 1 ppm, respectively. All parameter was determined by preparing standard solution at five different concentration levels ranging from 0.025 to 0.75 for zidovudine and 0.05to 1.50 for lamivudine. The standard solutions for linearity were prepared and inter-run precision for slope of regressed line was found to be 51.76 and 42.26 of zidovudine and lamivudine respectively. (Table 1 and Table 2) The correlation coefficients were found to be more than 0.997 for both the drugs (Figure 2 and Figure 3). Accuracy and precision of the method was determined by performing the recovery experiment. Recovery study was performed at three levels, in which sample stock solutions were spiked with standard drug solution containing 50, 100, and 150% of labeled amount of both the drugs in tablets. Three replicate samples of each concentration level were prepared and the % recovery at each level (n = 3), and mean% recovery (n=9)were determined (Table 3). The mean recovery was 101.24 and 97.63% for zidovudine and lamivudine, respectively. A batch of tablets was analyzed by two different analysts on different days using different columns to establish the intermediate precision of the method (Table 4).

Table 1: Result of linearity study

Concentration of Zidovudine (ppm)	AUC	Concentration of Lamivudine (ppm)	AUC
0.25	1.18563	0.50	2.00623
0.40	12.29843	0.80	20.35904
0.45	19.93425	0.90	32.69705
0.50	22.40187	1.00	36.57738
0.55	25.01051	1.10	41.10375

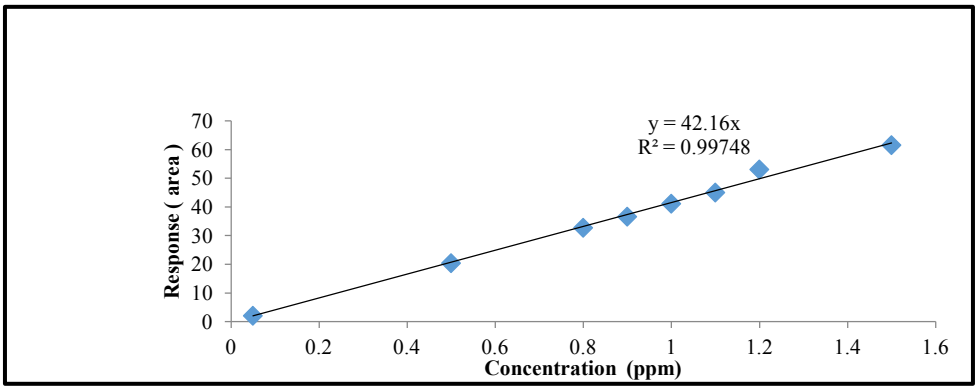

Figure 4: Calibration curve of zidovudine

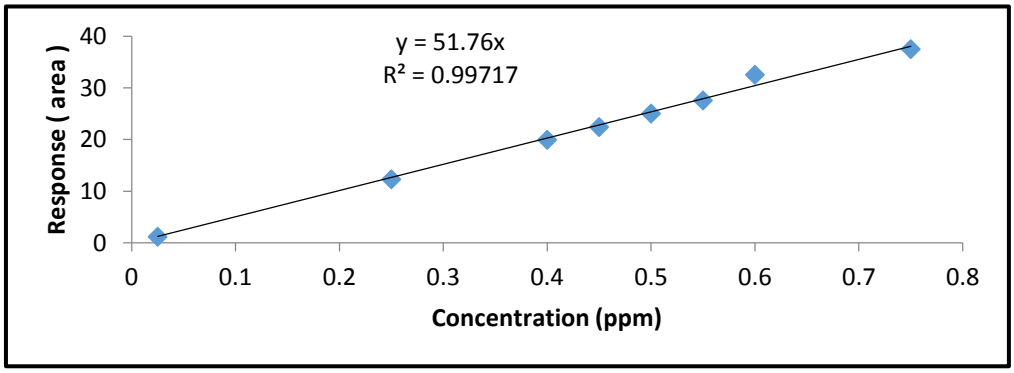

Figure 5: Calibration curve of lamivudine

Table 2: Regression equation data for drugs

Regression equation data for drugs, Y = A + B*C		
Drug Name	Zidovudine	Lamivudine
Slope (B)	51.76595	42.16187
Intercept (A)	-0.32888	-0.43324
Correlation Coefficient	0.99717	0.99748
Range (µg/ml)	0.25 - 0.55	0.50 - 1.10

Table 3: Accuracy results for zidovudine and lamivudine

Level of recovery %	Zidovudine			Lamivudine		
	Concentration taken (ppm)	Concentration recovered (ppm)	% recovery mean	Concentration taken (ppm)	Concentration recovered (ppm)	% recovery mean
50	0.250	0.250	99.98	0.504	0.463	96.23
100	0.501	0.504	101.2	1.008	0.972	97.56
150	0.750	0.754	101.3	1.512	1.468	98.95

Table 4: Precision results for zidovudine and lamivudine

Concentration (µgm/ mL)	% Concentration Recovery (% RSD)			
	Zidovudine		Lamivudine	
	Intra- day Precision		Inter- day Precision	
	Morning Precision	Evening Precision	Day 1	Day 2
10	100.85(0.017)	95.07(0.013)	99.18(0.016)	99.23(0.016)
30	98.35(0.018)	95.01(0.014)	98.17(0.015)	98.56(0.016)
50	95.13(0.017)	100.26(0.016)	97.56(0.014)	99.58(0.017)

After validating the HPLC method, the forced degradation of zidovudine and lamivudine in combination tablet form was studied by checking effect at various condition such as acid, base, heat, sunlight photolytic and oxidative condition and various time interval. From the degradation it was confirmed zidovudine and lamivudine that does not degraded at thermal, acidic and basic condition Figure 5,7,8 however lamivudine show degradation at photolytic condition Figure 4 and zidovudine show degradation at oxidative condition Figure 6. Results presented in table indicate that effect of extent of degradation at various concentration Table 5.

Table5: Results of analysis of forced degradation study samples using proposed method, indicating percentage degradation of AM and BH, and purity of zidovudine and lamivudine peaks in chromatograms

Stress condition/duration/state	Zidovudine		Lamivudine	
	(%) Degradation	Peak purity	(%) Degradation	Peak purity
Thermal/80ºC/48 h/solid	0.0	999	0.0	999
Photo/UV366nm/48 h/solid	0.0	999	4.58	999
Acidic/5M HCl/80ºC / 48 h	0.0	999	0.0	999
Alkaline/5 M NaOH/90ºC /48h	0.0	999	0.0	999
Oxidative/3.0%/48 h	8.14	999	0.0	999

*Peak purity values in the range 990–1000 indicate a homogenous peak.

Forced Degradation Study of Related Substances

Photolytic degradation

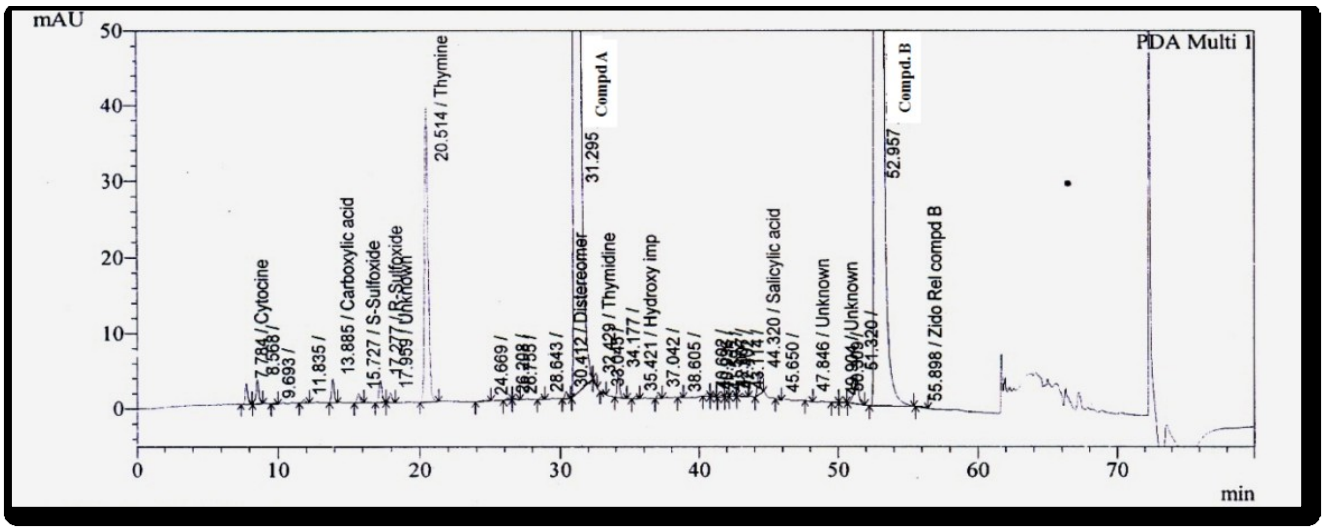

Figure 6: Chromatogram of photolytic sample

Thermal degradation

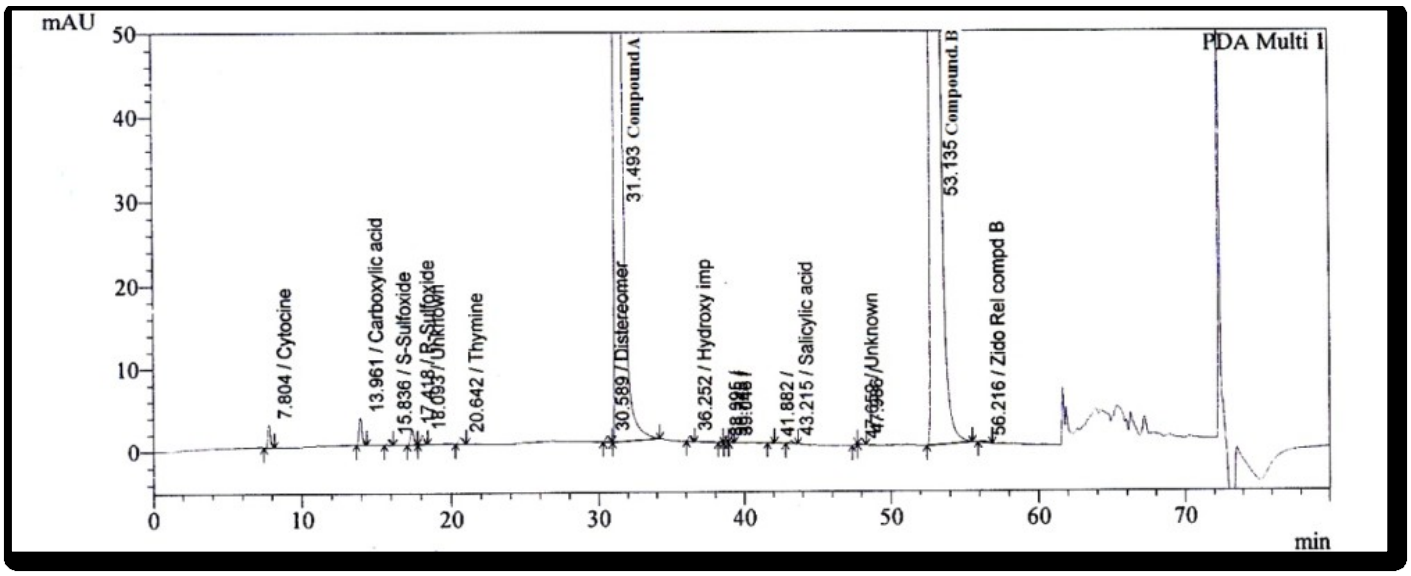

Figure 7: Chromatogram of thermal sample

Oxidative Degradation

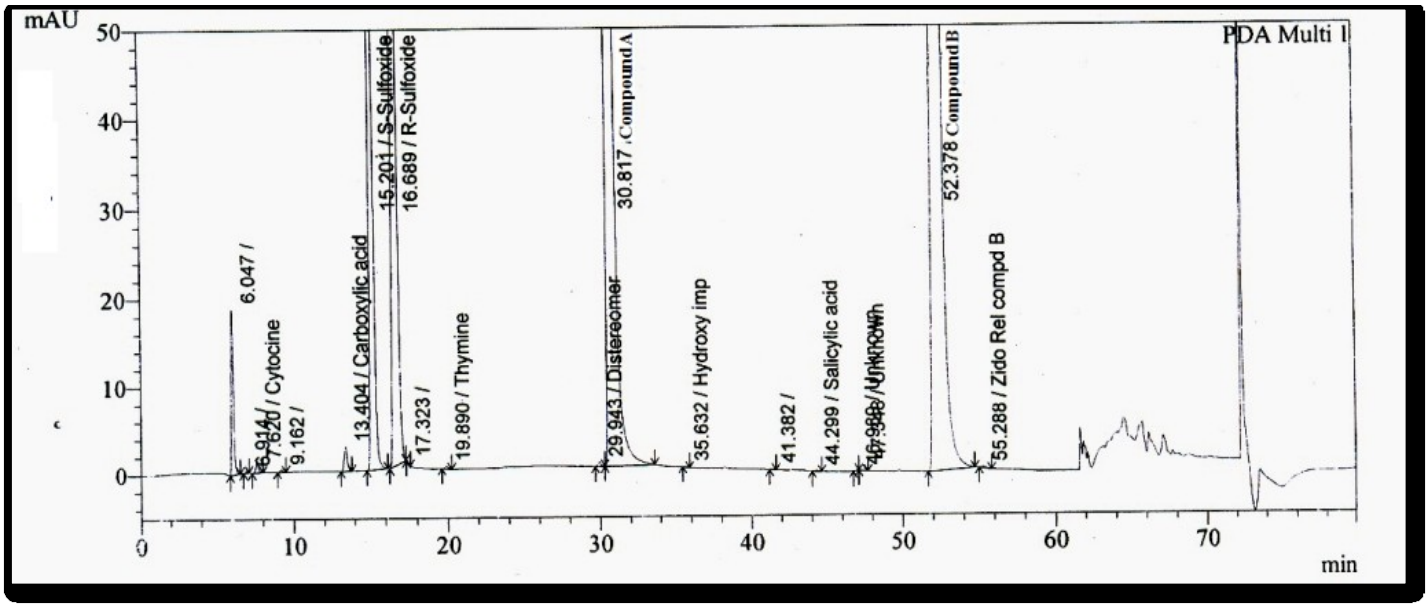

Figure 8: Chromatogram of oxidative sample

Acid Degradation

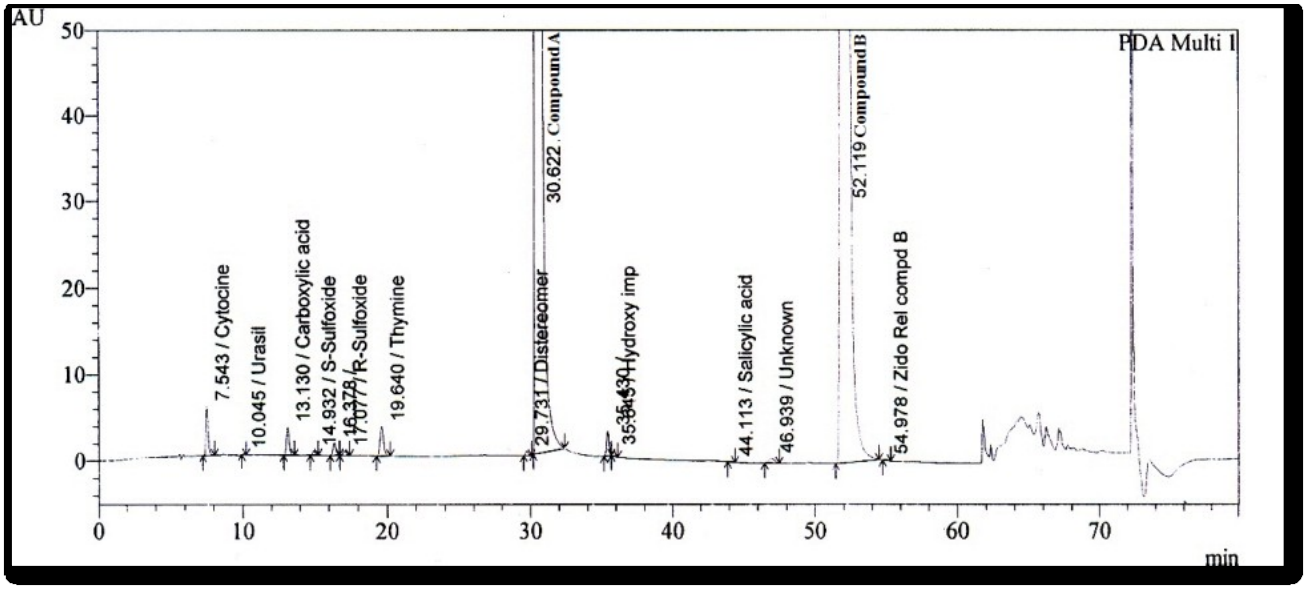

Figure 9: Chromatogram of acid sample

Base Degradation

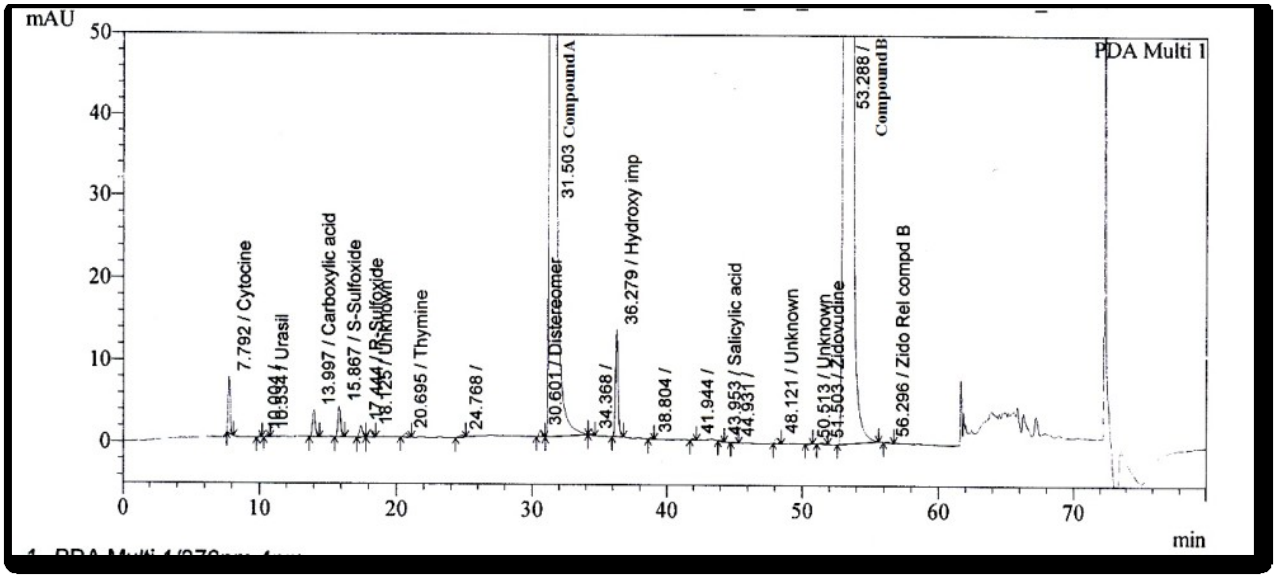

Figure 10: Chromatogram of base sample

CONCLUSION

A simple and precise chromatographic stability indicating reverse phased HPLC method for estimation zidovudine (ZDV) and lamivudine (LMV) from their combination product has been developed and validated. The developed method was validated as per ICH guideline Q_2 (R_1) for specificity, relative retention factor, and linearity, precision. From the degradation studies, it can be concluded that combination product of zidovudine and lamivudine are relatively stable in acid, base, humidity, thermal and UV. Hence, it can be concluded that the method is suitable for its intended use, i.e. for estimation and determination of % degradation in zidovudine and lamivudine tablets.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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*** Correspondence Author:**
Dr. Firoj A.Tamboli
HOD- Pharmacognosy,
Bharati Vidyapeeth college of Pharmacy,
Near chitranagari,
Kolhapur -416013
Maharashtra, India

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