DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR ESTIMATION OF ZIDOVUDINE

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ABSTRACT:

A simple, precise and sensitive stability indicating high performance thin layer chromatographic (HPTLC) method has been developed and validated for the analysis of Zidovudine in bulk and in tablet dosage form. The separation was performed on pre-coated silica gel 60 GF_{254} plates using Ethyl acetate : Methanol (6:4 v/v)as mobile phase. The retention factor (R_f) was found to be 0.43 ± 0.07. The detection of band was carried out at 265nm.The drug was subjected to different stress conditions like acid, base hydrolysis, oxidation, thermal degradation and photolysis. The method was successfully validated according to ICH Q_2(R1) guidelines. The linear regression analysis data for the calibration plot showed good linear relationship with R^2= 0.9928 in the range of 500-3000ng band^{-1}. The method found to be accurate as results of the recovery studies are close to the 100 %. The developed method can be adopted for routine analysis of Zidovudine in bulk and pharmaceutical dosage form.

***KEYWORDS*:**High performance thin layer chromatography (HPTLC), Zidovudine, method validation, stability indicating method.

INTRODUCTION:

Zidovudineischemically 1-[(2R,4S,5S)-4-azido-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidine-2,4-dione.Zidovudine, also known as azidothymidine.is an antiretroviral medication used to prevent and treat HIV/AIDS. It is generally recommended for use with other antiretrovirals. It may be used to prevent mother-to-child spread during birth or after a needlestick injury or other potential exposure. Literature survey reveals that few analytical methods have been reported for the estimation of Zidovudine in pharmaceutical dosage form including UV-Vis spectroscopy[7], high performance liquid chromatography (HPLC)[1,3,4], LC-MS/MS[2] , high performance thin layer chromatography(HPTLC), FT-IR[5], and reverse phase high performance liquid chromatography (RP HPLC)[6].

MATERIALS AND METHODS

Reagents and chemicals

Chromatographic conditions:

Chromatographic separation of drug was performed on aluminum plates precoated with silica gel 60 F_{254} (10 cm × 10 cm with 250 µm layer thickness). Sample was applied on the plate as a band of 6 mm width using Camag 100 µl sample syringe (Hamilton, Switzerland) with a linomat 5 applicator (Camag, Switzerland). The mobile phase was composed of Ethyl acetate : Methanol (6:4 v/v)10 cm × 10 cmCamagtwin trough glass chamber was used for linear ascending development of TLC plate under 15 min saturation conditions and 10 ml of mobile phase was used per run. Migration distance was 80 mm.Densitometric scanning was carried out using Camag TLC scanner at 295 nm, operated by win CATS software (version 1.4.3), slit dimensions were 4.00 × 0.45 mm and Deuterium lamp was used as a radiation source.

Selection of detection wavelength

From the standard stock solution (1000µg ml^{-1}) further dilutions were made using methanol and scanned over the range of 200-400 nm and the spectra was obtained. It was observed that the drug showed considerable absorbance at 265 nm. Representative UV spectrum of Zidovudine is shown in Fig 1.

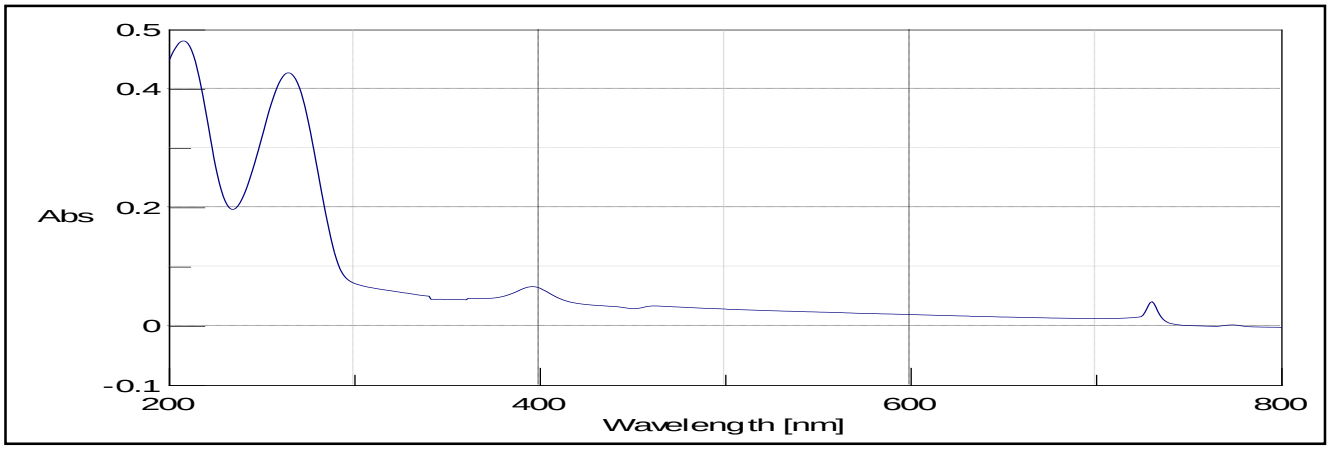

Fig. 1: The UV spectrum of Zidovudine (10 µg ml^{-1})

Preparation of Standard stock solution

Standard stock solution of drug was prepared by dissolving 10 mg of the drug in 10 ml of methanol to get concentration of 1000 µg ml^{-1}. From the standard stock solution, working standard solution was prepared containing 100 µg ml^{-1} of Zidovudine.

Preparation of sample solution:

For determination of the content of Zidovudine in Zidovudine tablets (label claim: 300 mg Zidovudine per tablet), twenty tablets were weighed; average weight was determined and were finely powdered. A quantity of powder equivalent to 10 mg of Zidovudine was transferred to a 10 ml volumetric flask containing 5 ml of methanol. The mixture was ultra sonicated for 10 min and the resulting sample stock solution was filtered with Whatman filter paper 41 and the volume was made up with the methanol. 5.0 ml of this solution was diluted to 10 ml with the methanol to prepare a final sample stock solution of 500 µg ml^{-1}.

Densitogram

Solution of Zidovudine (100µg ml^{-1}) was prepared. 10 µl (1000 ngband^{-1}) of solution was applied on pre-activated TLC plate with the help of Hamilton syringe (100 µl), using Linomat 5 sample applicator. The development chamber was saturated with mobile phase for 15 min. The spotted plate was placed in the saturated chamber and developed up to 80 mm distance. The plate was dried and was scanned over 90 mm distance at 265 nm. The retention factor was found to be 0.43 ± 0.07.Representative densitogram of Zidovudine (1000 ngband^{-1}) is shown in Fig 2.

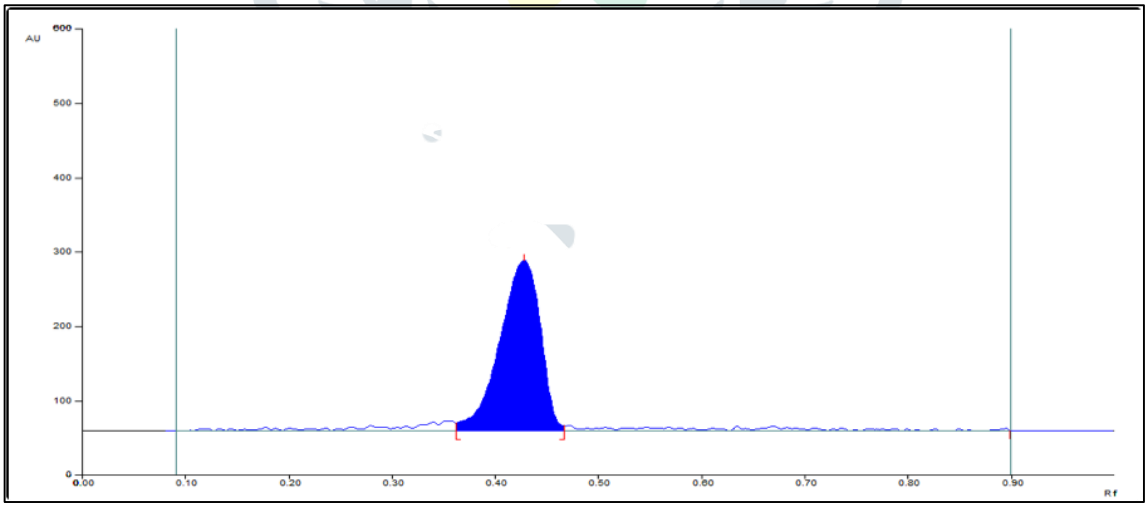

Fig 2: Densitogram of standard solution of Zidovudine (1000 ngband^{-1})

Chromatographic parameters

Sr. No.	Parameter	Conditions used for Analysis

1	Stationary phase	TLC aluminium plate precoated with silica gel 60 F_{254}
2.	Mobile phase	Ethyl acetate: MeOH (6:4 v/v).
3.	Detection Wavelength	265 nm
4.	Saturation time	15 min.
5.	Band width	6 mm

Stress degradation studies of bulk drug:

Stress degradation studies were carried under condition of acid as well base hydrolysis, oxidation and dry heat. Dry heat and photolytic degradation were carried out in solid state.

Acid Degradation Studies

From the Stock-1, 250 µl of the solution were transferred to the 5 ml pre-calibrated volumetric flask and volume was made up to 5 ml with 0.1 N HCl (Theoretical concentration: 500 µg/ml). The volumetric flask was allowed to stand for 2 hrs in water bath at 80^0C. After 2 hrs, solution was neutralized with equivalent volume of 0.1N NaOH. The solution was vortexed and filtered through 0.22 µm syringe filter. Filtered solution was analyzed for Zidovudine content by using prevalidated HPTLC method. Chromatograms were recorded to assess the stability of Zidovudine.

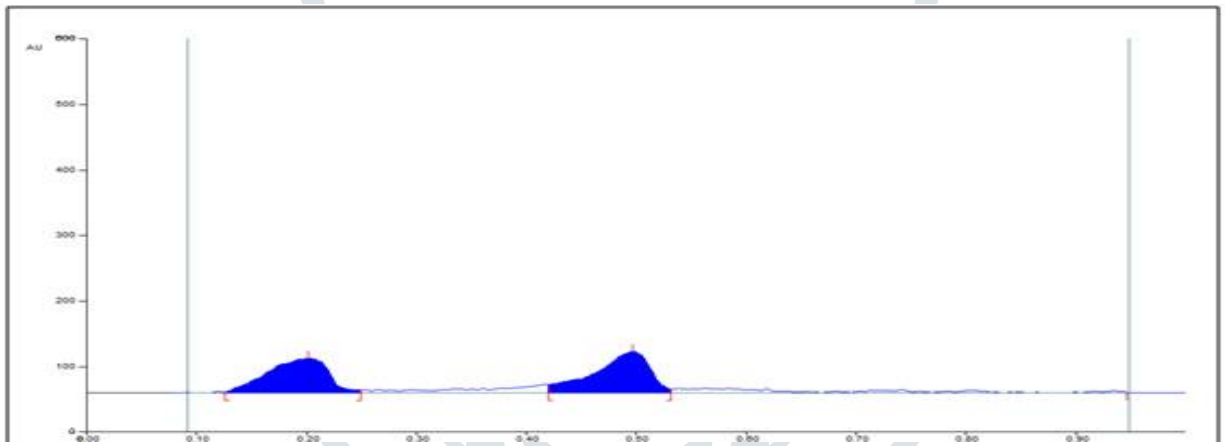

Fig 3: Chromatograph of Zidovudine stressed under 0.1 N HCl

Alkali Degradation Studies

From the Stock-1, 250 µl of the solution were transferred to the 5 ml pre-calibrated volumetric flask and volume was made up to 5 ml with 0.1 N NaOH (Theoretical concentration: 500 µg/ml). The volumetric flask was allowed to stand for 2 hrs in water bath at 80^0C. After 2 hrs, solution was neutralized with equivalent volume of 0.1N HCl. The solution was vortexed and filtered through 0.22 µm syringe filter. Filtered solution was analyzed for Zidovudine content by using prevalidated HPTLC method. Chromatograms were recorded to assess the stability of Zidovudine.

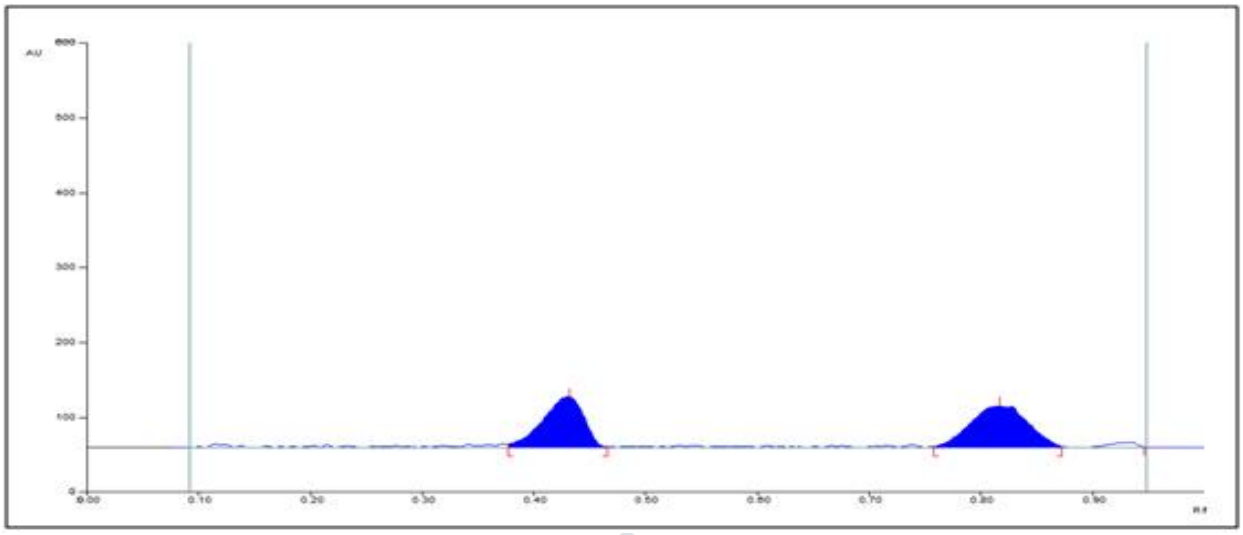

Figure 4: Chromatograph of Zidovudine stressed under 0.1 N NaOH

Oxidation Studies

From the Stock-1, 250 µl of the solution were transferred to the 5 ml pre-calibrated volumetric flask and volume was made up to 5 ml with 10 % hydrogen peroxide (H_2O_2) solution (Theoretical concentration: 500 µg/ml). The volumetric flask was allowed to stand for 2 hrs in water bath at 80^0C. After 2 hrs, solution was diluted with equivalent volume of HPLC grade water. The solution was vortexed and filtered through 0.22 µm syringe filter. Filtered solution was appropriately diluted with methanol and was analyzed for Zidovudine content by using prevalidated HPTLC method. Chromatograms were recorded to assess the stability of Zidovudine.

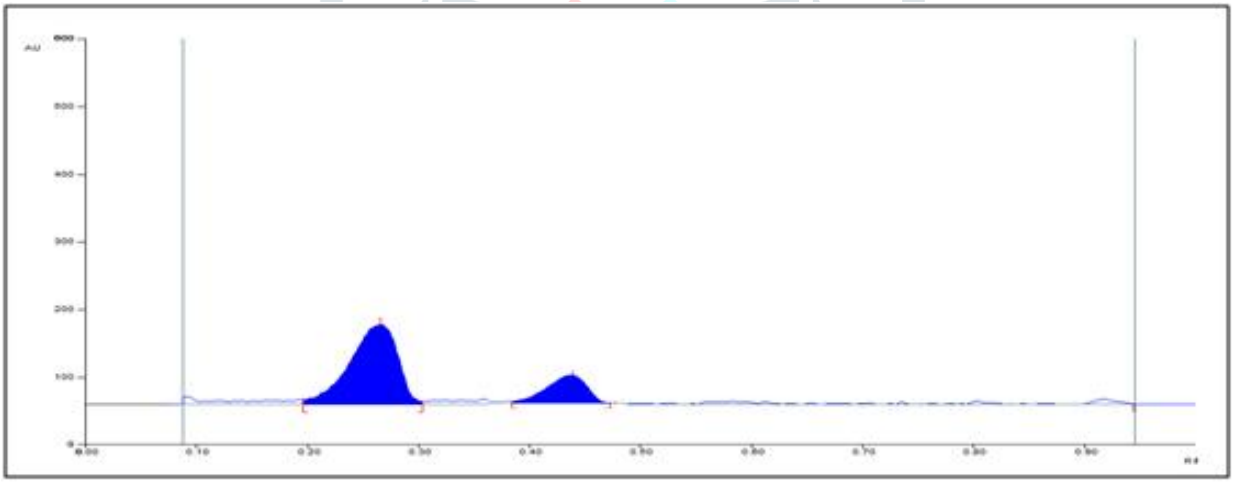

Fig 5: Chromatograph of Zidovudine stressed under 10% Hydrogen peroxide

Degradation under dry heat

Dry heat studies were performed by keeping drug sample in oven (80^0 C) for a period of 4 hours. A sample was withdrawn after 4 hour, and stock 1 solution was prepared. From the Stock-1, 250 µl of the solution were transferred to the 5 ml pre-calibrated volumetric flask and volume was made up to 5 ml with HPLC grade water (Theoretical concentration: 500 µg/ml). Solution was diluted with equivalent volume of HPLC grade water. The solution was vortexed and filtered through 0.22 µm syringe filter. Filtered solution was analyzed for Zidovudine content by using prevalidated HPTLC method. Chromatograms were recorded to assess the stability of Zidovudine.

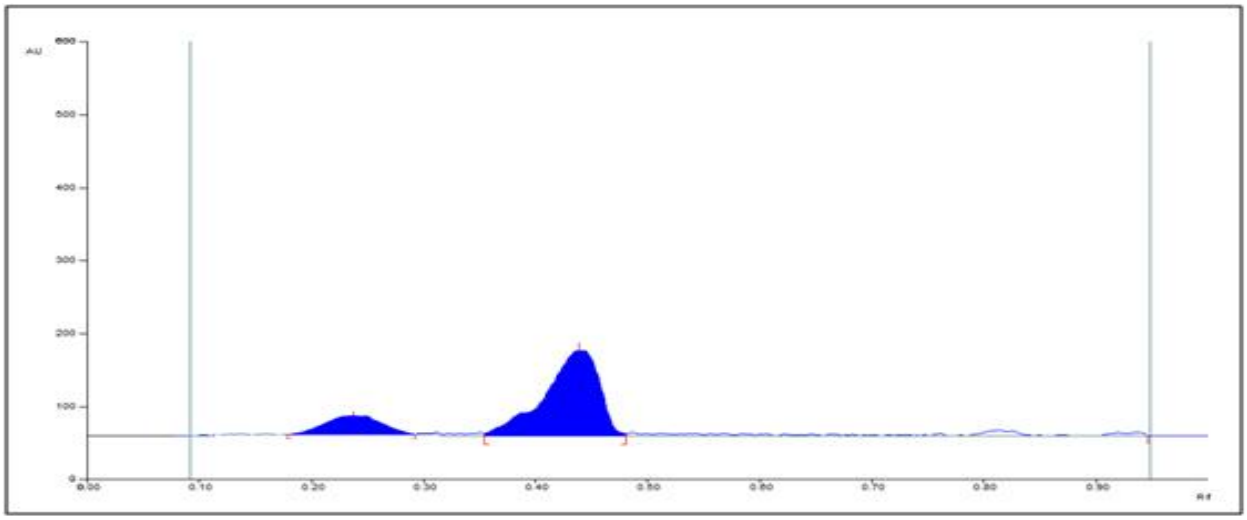

Fig 6: Chromatograph of Zidovudine stressed under dry heat

Photo Stability Studies:

The photochemical stability of the drug was also studied by exposing the 1 mg drug to UV light in UV chamber for 2 Hrs. After 2 hrs, sample was dissolved to achieve the theoretical concentration of 500 µg/ml and diluted suitably with equivalent volume of HPLC grade water. The solution was vortexed and filtered through 0.22 µm syringe filter. Filtered solution was analyzed for Zidovudine content by using prevalidated HPTLC method. Chromatograms were recorded to assess the stability of Zidovudine.

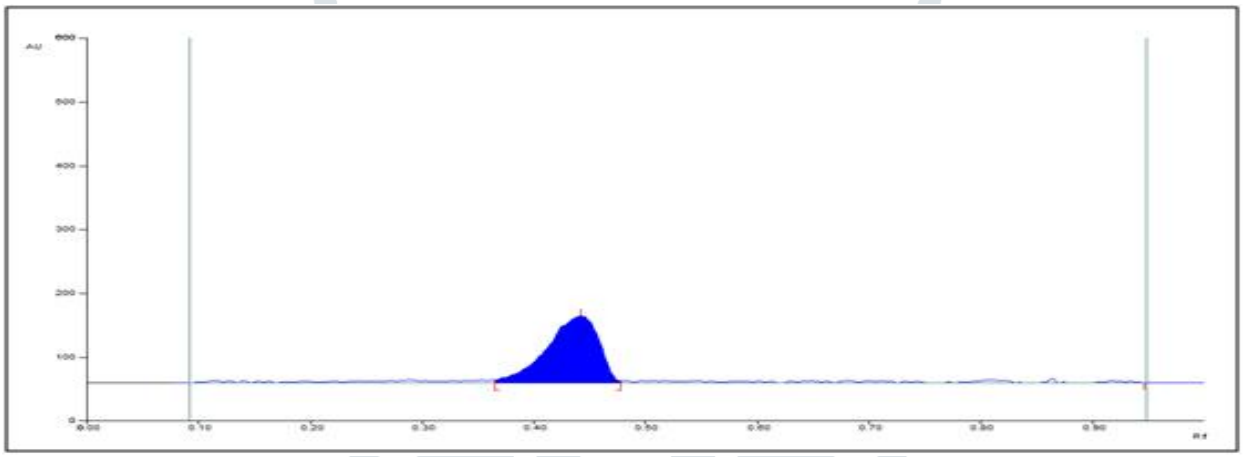

Fig 7: Chromatograph of Zidovudine stressed under UV light

Summary of degradation parameters:

Sr.	Stress Condition	% Assay of Zidovudine
1.	Acid (0.1 N HCl)	76.014
2.	Alkali (0.1 N NaOH)	88.084
3.	Oxidation (10% H_2O_2)	9.192
4.	Heat (80ºC)	70.690
5.	UV Exposure	98.244

VALIDATION OF ANALYTICAL METHOD

Specificity

The specificity of the method was ascertained by peak purity profile studies. The peak purity values were found to be more than 0.998, indicating the no interference of any other peak of degradation product, impurity or matrix.

Linearity

From the standard stock solution (1000 µgml^{-1}) of Zidovudine, solution was prepared containing 500 µg/ml of Zidovudine. This solution was further used for spotting. Six replicates per concentration were spotted. The linearity (relationship between peak area and concentration) was determined by analysing six concentrations over the concentration range 500-3000 ngband^{-1} to obtain

calibration curve. The results found to be linear with regression equation of y = 5.5173x + 2453.8 and R^2= 0.9928. The results obtained are shown in Table 7.4. The calibration curve is shown in Fig. 3

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Table of Linearity study of Zidovudine

Replicate	Concentrations of Zidovudine (ngband^{-1})					
	500	1000	1500	2000	2500	3000
	Peak area					
1	4688.2	8447.7	10625.5	13595.5	16565.1	18059.5
2	4611.6	8570.7	10503.3	13980.1	16551.3	18719.9
3	4677.7	8596.6	10856.6	13513.9	16301.8	18865
4	4681.5	8543.5	10575	13977.3	16231.4	18581.1
5	4610.5	8572.8	10578.4	13469.7	16670.5	18564.5
6	4694.4	8531.1	10547.3	13528.8	16552.6	18594.5
Avg	4660.650	8543.733	10614.350	13677.550	16478.783	18564.077
SD	38.844	52.439	125.282	236.740	171.718	272.268
% RSD	0.833	0.614	1.180	1.731	1.042	1.467

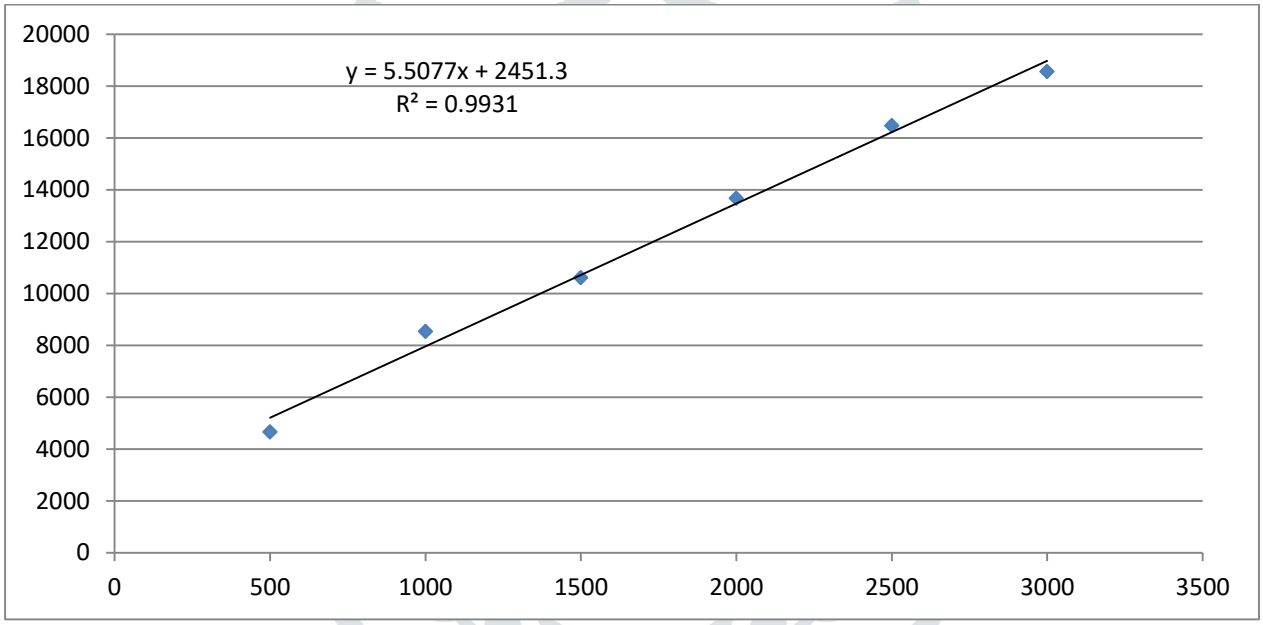

Fig8: Calibration curve of Zidovudine (500-3000ng band^{-1}) reference standard

Range:

Zidovudine = 500-3000 ngband^{-1}

Precision:

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the inta-day studies 3 replicates of 3 concentrations were analysed on the same day, and % RSD was calculated. For the interday variation studies, 3 concentraions were analysed on 3 consecutive days and % RSD was calculated. For intraday precision and interday precision results obtained are shown .

Table of Intraday variation studies data for Zidovudine

Conc. (ng/band)	Area	% recovery	Average	SD	%RSD
1000	8072.8	102.079	101.422	0.614	0.605
	8031.1	101.322			
	8005.9	100.864			
1500	10578.4	98.385	98.734	0.513	0.520
	10587.3	98.493			
	10655.9	99.323			
2000	13528.8	100.576	101.343	0.669	0.660
	13646.8	101.648			
	13664.2	101.806			

Table of Interday variation studies data for Zidovudine

Conc. (ng/band)	Area	% recovery	Average	SD	%RSD
1000	1010.785	101.078	101.114	0.170	0.168
	1012.982	101.298			
	1009.641	100.964			
1500	1484.327	98.955	99.375	1.254	1.261
	1511.765	100.784			
	1475.775	98.385			
2000	2023.641	101.182	100.733	0.395	0.392
	2008.823	100.441			
	2011.529	100.576			

Limit of Detection (LOD) and Limit of quantitation (LOQ):

LOD and LOQ are calculated from the formula: -

$$LOD = \frac{3.3\ \sigma}{S} \qquad LOQ = \frac{10\ \sigma}{S}$$

Where,
σ = Standard deviation of Y intercept;
S = Average of slope of the calibration curve

Table of LOD and LOQ of OND HCl

Method	Avg slope	S.D	LOQ (ng/band)	LOD (ng/band)
Using S.D of y-intercept	5.523	94.028	170.24	56.18

Assay:

Zidovir 300 tablet formulation analysis was carried out as mentioned under section preparation of sample solution. Procedure was repeated for six times. 2 µl volume of sample solution was applied and area was recorded. Basic concentration of sample chosen was 1000 ngband^{-1} from tablet solution. Concentration and % recovery was determined from linear equation. Assay results obtained are shown in table.

Table of Assay of marketed formulation

Sr. No.	Peak area	Amount recovered (ng/band)	% recovery
1	7999.8	1007.534	100.753
2	7947.6	998.055	99.806
3	8003.2	1008.152	100.815
4	7993.8	1006.445	100.644
5	7983.4	1004.556	100.456
6	8000.7	1007.698	100.770
Mean	7988.083	1005.407	100.541
SD	21.064	3.825	0.382
%RSD	0.264	0.380	0.380

Accuracy:

To check accuracy of the method, recovery studies were carried out by spiking the standard drug to the tablet solution, at three different levels 50, 100 and 150%. Basic concentration of sample chosen was 1000 ng/band. % recovery was determined from linear equation. Accuracy results obtained are shown in Table .

Table of Accuracy studies of Zidovudine

Level	Amount of sample taken (ng/band)	Amount of standard spiked (ng/band)	Area	Amount recovered (ng/band)	% recovery (Mean ±%RSD)

50%	1000	500	10784.6	1513.218	100.390 ± 0.446
			10735.2	1504.248	
			10712.2	1500.071	
100%	1000	1000	13648.2	2033.211	100.992 ± 1.227
			13658.5	2035.081	
			13417.1	1991.246	
150%	1000	1500	16199.5	2496.494	100.564 ± 0.882
			16433.6	2539.003	
			16256.4	2506.826	

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which chamber saturation time, mobile phase composition, time from spotting to development, time from development to scanning was changed and the effect on the area was noted. It was found that method is robust. The results obtained are shown in table .

Table of Robustness study

Sr. No.	Parameters	Robust condition	%RSD
1	Saturation time (15 min) ± 2 min	13 min	0.94
		15 min	1.47
		17 min	0.85
2	Time from spotting to development	Immediate	1.47
		After 30 min	1.11
		After 1 hr	0.75
3	Time from development to scanning	Immediate	1.47
		After 30 min	1.46
		After 1 hr	0.87
4	Mobile phase ratio variation (Ethyl acetate: Methanol v/v)	5.8:4.2	1.43
		6:4	1.47
		6.2:3.8	0.88

Summary of validation study

Table of Summary of Validation Parameters

Sr. No.	Validation parameters	Zidovudine
1.	Linearity equation R^2 Range	y = 5.507 x + 2451.309 R^2= 0.993 500-3000 ng/band
2.	Precision	(%RSD)
	Intraday	1.417
	Interday	1.028
3.	Assay	100.541 ± 0.380
4.	Accuracy	
	50	100.390 ± 0.446
	100	100.992 ± 1.227
	150	100.564 ± 0.882
5.	Limit of detection	56.18 $ngband^{-1}$
6.	Limit of quantitation	170.24 $ngband^{-1}$
7.	Specificity	Specific
8.	Robustness	Robust

CONCLUSION:

A simple, precise, accurate, reproducible and stability indicating HPTLC method without interference from the excipients or from degradation products has been developed and validated for the determination of Zidovudine as bulk drug and in tablet dosage form. The developed method can be used for quantitative analysis of Zidovudine in pharmaceutical dosage form. The method was developed by using easily available and cheap solvents for analysis of drug hence can be considered as economic.

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