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Validated Stability Indicating High-Performance Thin-Layer Chromatographic Determination of Didanosine in Tablet Dosage Form

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ABSTRACT

A simple, accurate, precise and selective stability-indicating high-performance thin-layer chromatographic (HPTLC) method for determination of Didanosine has been developed and validated as bulk drug and in the tablet dosage form. As stability testing is a major step in the development of the new drug as well as formulation, stress degradation studies were carried out according to ICH guidelines. Didanosine was found susceptible to all the analyzed stress conditions except photolysis. Chromatographic resolution of Didanosine and its degradation products was achieved by using precoated silica gel 60 F_{254} aluminum plates as stationary phase and Ethyl acetate :Ethanol: Glacial acetic acid (8.5: 1: 0.5, v/v/v) as mobile phase. Densitometric detection was carried out at 250 nm. The retention factor was found to be 0.25 ± 0.02. The developed method was validated concerning linearity, accuracy, precision, the limit of detection, the limit of quantitation and robustness as per ICH guidelines. Results found to be linear in the concentration range of 200-1200 ng band$^{-1.}$ The developed method has been successfully applied for the estimation of the drug in the capsule dosage form. The proposed method can be used for routine analysis of drugs in quality control laboratories and can also help monitor the potency during shelf life.

1.0 INTRODUCTION

Didanosine, chemically, 9-[(2*R*,5*S*)-5-(hydroxymethyl)oxolan-2-yl]-1*H*-purin-6-one is reverse-transcriptase inhibitor used to treat Human immunodeficiency virus infection and acquired immune deficiency syndrome and used in combination with other medications as part of highly active antiretroviral therapy (HAART) [1].

An extensive literature review concerning analytical methods revealed that methods such as Spectrophotometry [2, 3] and High-Performance Liquid Chromatography (HPLC) [4-9] have been reported for the determination of didanosine as bulk drug and in tablet dosage form either as single drug or in combination with other drugs. Analytical reports on the quantization of didanosine using high-performance liquid chromatography-tandem mass spectrometry [LC-MS] [10, 11] were also available in the literature. Micellar electrokinetic capillary chromatography method for analysis of didanosine has also been reported [12].

To best of our information, no reports were found in the literature for the determination of didanosine in tablet dosage form by stability-indicating high-performance thin-layer chromatographic (HPTLC) method. This paper describes the development and validation of simple, precise, accurate and selective stability-indicating HPTLC method for the determination of didanosine by International Conference on Harmonisation Guidelines [13, 14].

2.0 MATERIALS AND METHODS

2.1 Chemicals and reagents

Analytically pure didanosine working standard was obtained as a gift sample from Hetero Drugs Ltd., (Hyderabad, India). The pharmaceutical dosage form used in this study was Videx tablets labeled to contain 100 mg of didanosine was procured from the local pharmacy. Ethyl acetate, Ethanol, Glacial acetic acid (all AR grade) was purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

2.2 Instrumentation and chromatographic conditions

Chromatographic separation of the drug was performed on precoated silica gel 60 F_{254} (10 cm ×10 cm with 250 µm layer thickness) Merck TLC plates as stationary phase using a Camag

Linomat V sample applicator (Switzerland). Samples were applied on the plate as a band with a 6 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland).

Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) with the use of a solvent mixture comprising of ethyl acetate: methanol: glacial acetic acid (8.5: 1: 0.5, v/v/v) as mobile phase. The mobile phase was saturated in the chamber for 20 min. After development, TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on Camag thin layer chromatography scanner at 250 nm for all developments operated by winCATS software version 1.4.2. The source of radiation utilized was a deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

2.3 Preparation of standard stock solution

The standard stock solution was prepared by dissolving 10 mg of drug in 10 mL of methanol to get a solution of concentration 1000 ng μL^{-1} which was further diluted to 10 mL to furnish the final concentration 100 ng μL^{-1}.

2.4 Selection of detection wavelength

After chromatographic development bands were scanned over the range of 200-400 nm. It was observed that the drug showed considerable absorbance at 250 nm. So, 250 nm was selected as the wavelength for detection.

2.5 Estimation of the drug in capsule dosage form

Commercial brand of tablets namely Videx containing 100 mg of drug was used to estimate the amount of didanosine in available tablet formulation. For this, 20 tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 100 mg was transferred to 100 mL volumetric flask containing 50 mL of methanol and the contents were sonicated for 15 min. The solution was filtered using Whatman paper No. 41 and the volume was made up to the mark with methanol to obtain the final concentration of 1000 ng μL^{-1}. The above solution was diluted further with methanol to get the final concentration of 100 ng μL^{-1}. Four µL volume of this solution was applied on a TLC plate to obtain a final sample concentration of 400 ng $band^{-1}$. After chromatographic development peak areas of the bands were measured

at 250 nm and the amount of drug present in the sample was estimated from the calibration curve. The procedure was repeated six times for the analysis of the homogenous sample.

2.6 Forced degradation study

The stability studies were performed by subjecting the bulk drug to the physical stress (hydrolysis, peroxide, heat, and light) and stability was accessed. The study was carried out at a concentration of 1000 µg μL^{-1}. The hydrolytic studies were performed by treatment of a stock solution of a drug separately with 0.1N HCl, 0.1 N NaOH and water at room temperature for a period of 1 h. The acid and alkali stressed samples were neutralized with NaOH and HCl, respectively to furnish the final concentration of 800 ng $band^{-1}$. The oxidative degradation was carried out in 15 % H_2O_2 at room temperature for 30 min and the sample was diluted with methanol to obtain an 800 ng $band^{-1}$ solution. Thermal stress degradation was performed by keeping the drug in the oven at 50ºC for a period of 48 h. Photolytic degradation studies were carried out by exposure of the drug to UV light up to 200-watt h square $meter^{-1}$ for 2 d. Thermal and photolytic samples were diluted with methanol to get a concentration of 800 ng $band^{-1}$.

3.0 RESULTS AND DISCUSSION

3.1 Optimization of chromatographic conditions

The current research work was undertaken to develop a stability-indicating HPLTC method which would be capable to give the satisfactory resolution between didanosine and its degradation products. Diverse solvent systems containing various ratios of benzene, ethyl acetate, glacial acetic acid, chloroform, toluene, ethanol, and methanol were examined (data not shown) to separate and resolve spot of didanosine from its impurities and other excipients present in the formulation. Finally, the mobile phase comprising ethyl acetate: ethanol: glacial acetic acid (8.5: 1: 0.5, v/v/v) gave the best separation of drug with a sharp symmetric peak. The densitometric evaluation was carried out at 250 nm. After the developed method had been fully optimized, compact, sharp, and the symmetric peak was obtained for didanosine with retention factor value 0.25 ± 0.02 as presented in the densitogram in Figure 1.

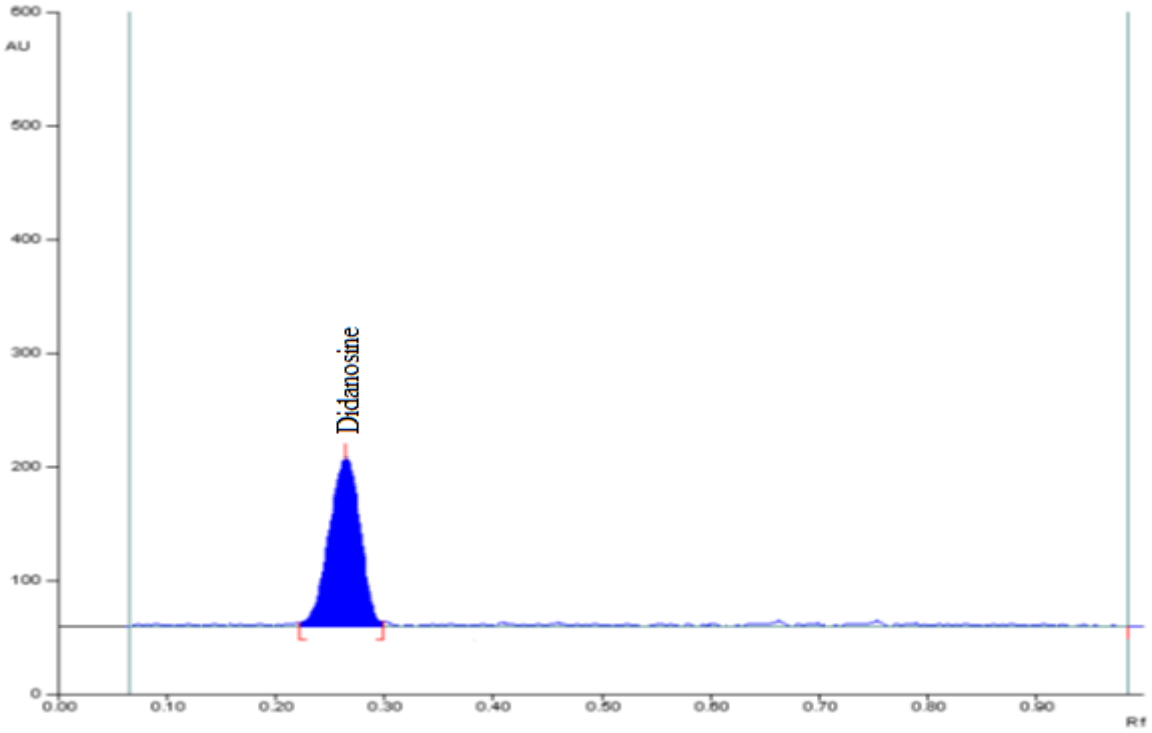

Figure No. 1: Representative densitogram of a standard solution of Didanosine (600 ng band^{-1}, Rf= 0.25 ± 0.02)

The forced degradation results indicated susceptibility of the drug to hydrolytic, oxidative, thermal and photolytic stress conditions. Significant degradation product peaks were observed in acidic, basic, oxidative and photolytic conditions. The degradation products observed after stress degradation was not interfering with the active drug indicating the specificity of the developed procedure. Figures 2-4 represent the densitograms of acid, alkali and oxidative degradation, while Figure 5 depicts the densitogram of photolytic degradation. The findings of degradation studies are represented in Table 1.

Table No. 1: Summary of forced degradation studies

Stress conditions/ duration	**% Recovered**	**% of Degradation**
Acidic / 0.1 N HCl/ Kept at RT for 1 h	81.21	18.78
Basic/0.1 N NaOH/ Kept at RT for 1 h	74.79	25.20
Neutral/H_2O/ Kept at RT for 1 h	88.69	11.30
Oxidative /15 % H_2O_2 / Kept at RT for 30 min	86.49	13.50
Dry heat/ 50ºC/ 48 h	90.43	9.56
Photolysis: UV light 200-watt h square meter^{-1} 2 d	85.30	14.69

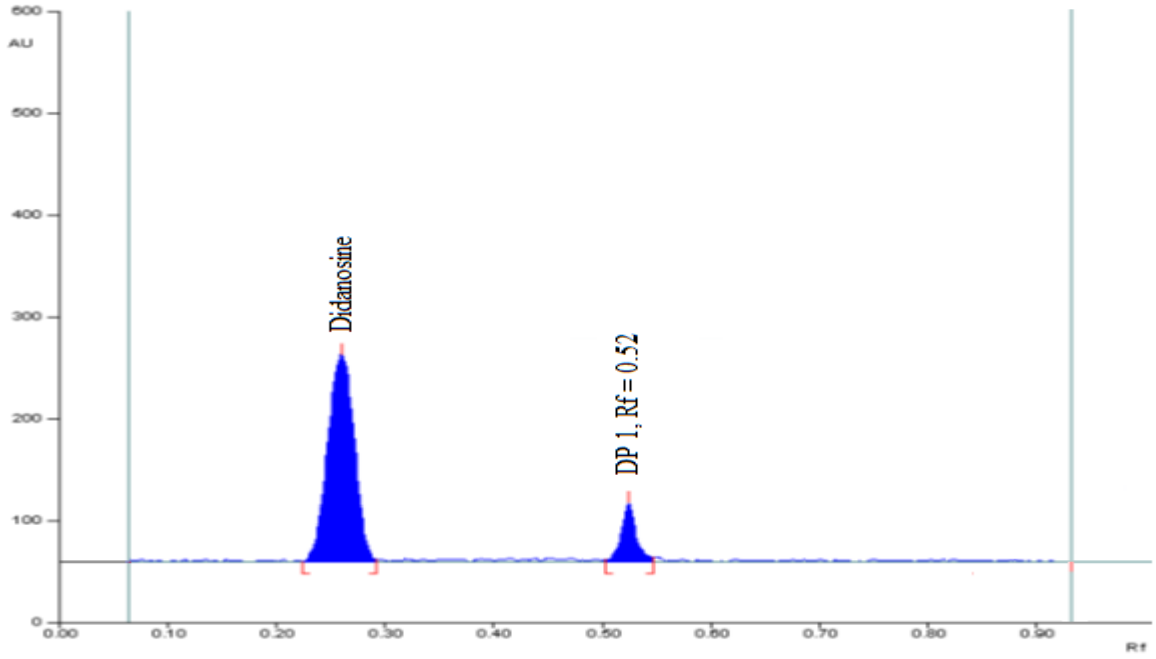

Figure No. 2: Densitogram after acid hydrolysis with degradation product (DP1, Rf = 0.52)

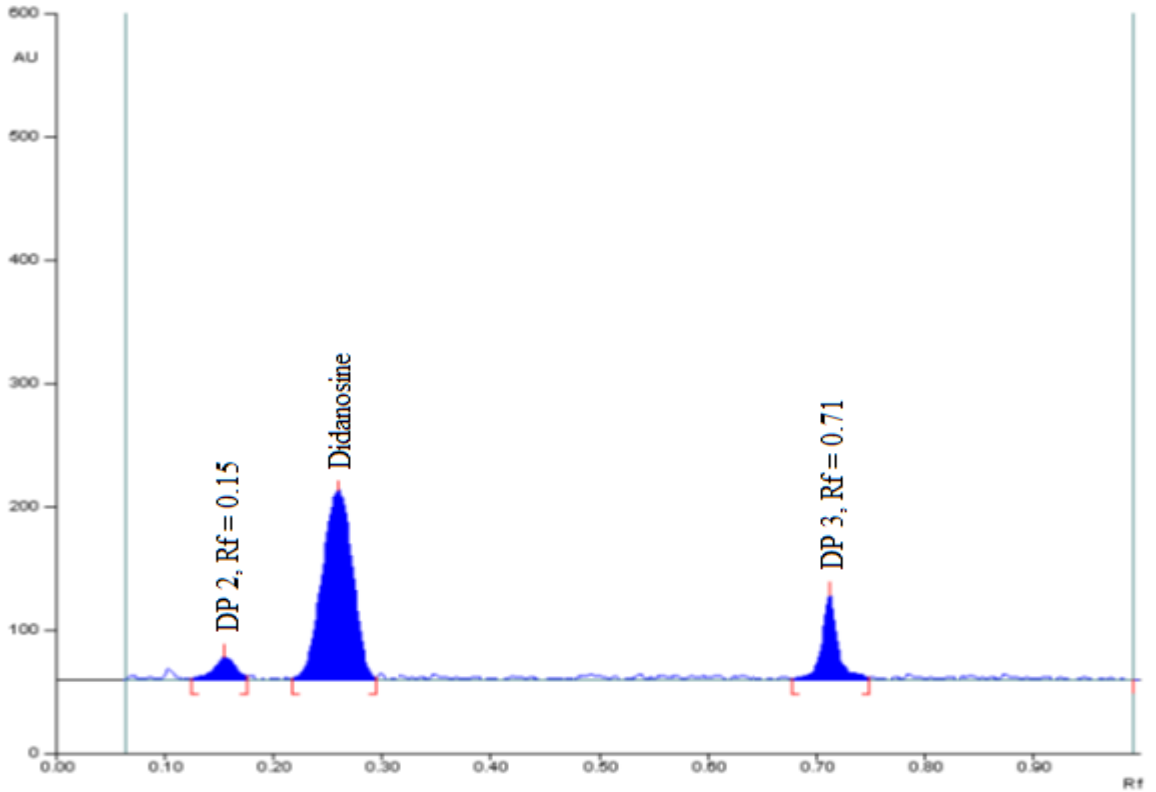

Figure No. 3: Densitogram after alkali hydrolysis with degradation peaks (DP2, Rf = 0.15) and (DP3, Rf = 0.71)

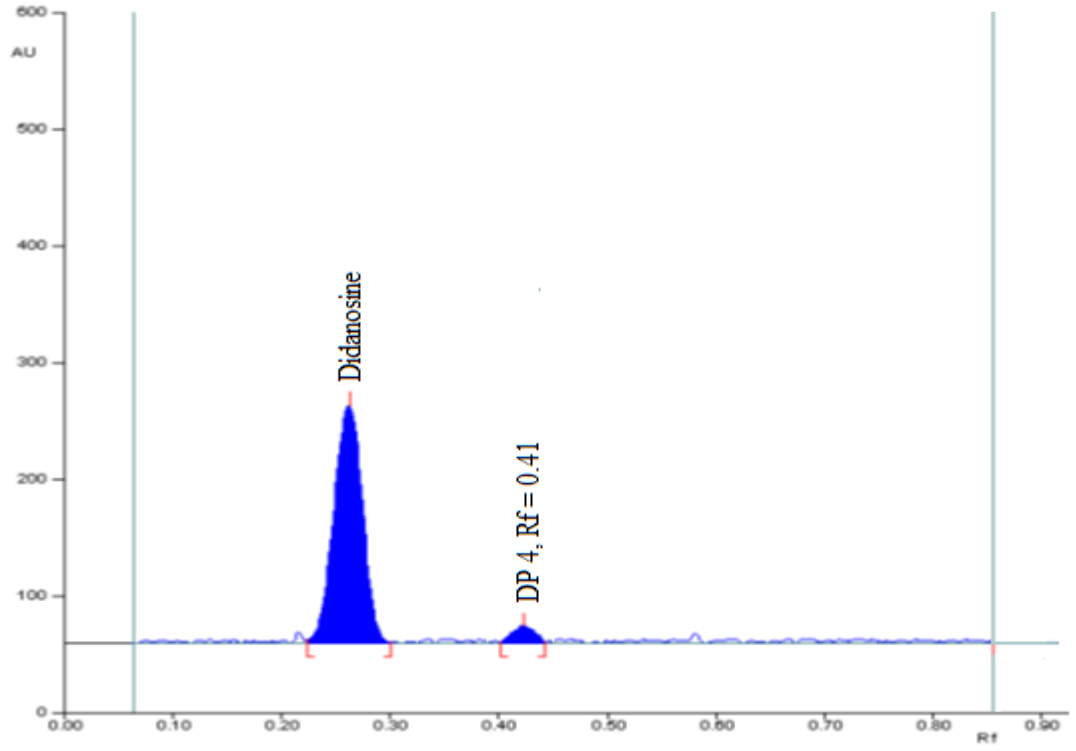

Figure No. 4: Densitogram after oxidative degradation with degradation product (DP4, Rf = 0.41)

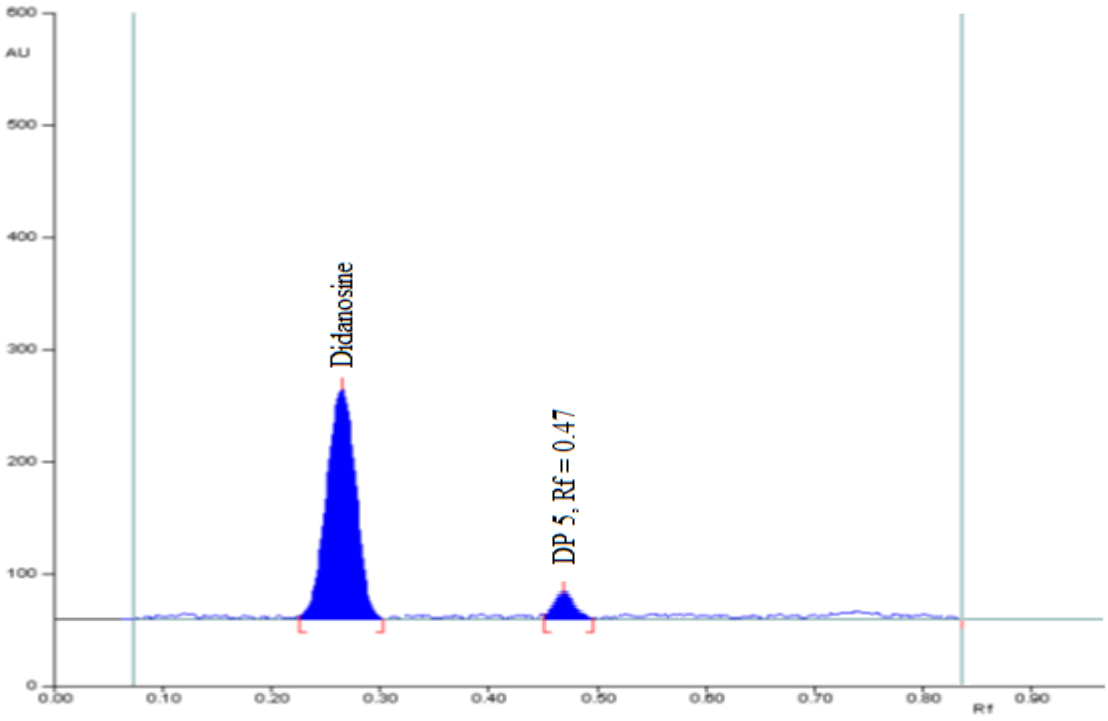

Figure No. 5: Densitogram obtained after photolysis ((DP4, Rf = 0.47)

3.2 Method Validation

The developed method was validated in terms of linearity, accuracy, intra-day and inter-day precision, the limit of detection, the limit of quantitation and robustness, by ICH guidelines [13, 14].

3.2.1 Preparation of calibration curve

For the preparation of a calibration plot, volumes 2, 4, 6, 8, 10 and 12 µL of a standard solution of Didanosine (100 ng μL^{-1}) were spotted onto the TLC plates, developed and scanned under optimized chromatographic conditions. The developed method was found to be linear in the concentration range of 200-1200 ng $band^{-1}$ with the high correlation coefficient. The linear regression equation was found to be y = 9.1123x + 567.56 having a correlation coefficient of 0.996. The calibration curve obtained by plotting concentration vs peak area is represented in Figure 6.

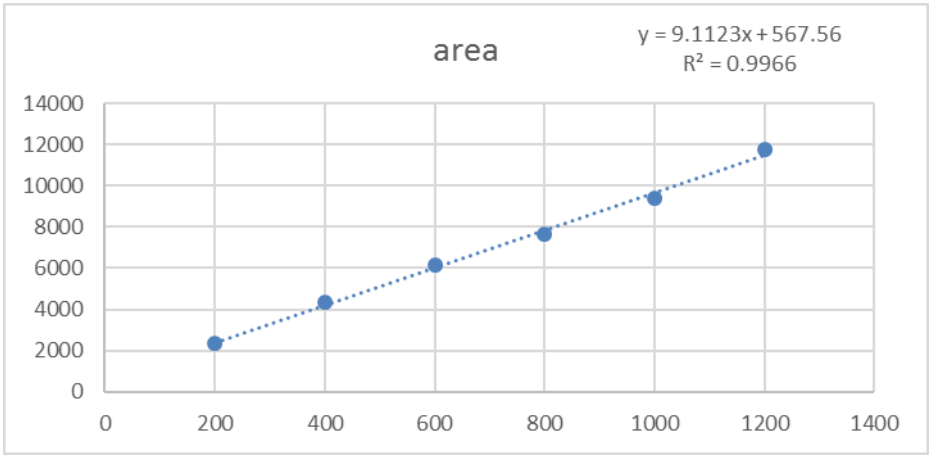

Figure No. 6: Calibration curve for Didanosine (200-1200 ng $band^{-1}$)

3.2.2 Precision

Set of three different concentrations (400, 800, 1200 ng $band^{-1}$) in three replicates of standard solutions of Didanosine were prepared. All the solutions were analyzed on the same day to record any intraday variations in the results. Intra-day variation, as RSD (%), was found to be in the range of 0.53 to 1.16. For the Inter day variation study, three different concentrations of the standard solutions in the linearity range were analyzed on three consecutive days. Interday variation, as RSD (%), was found to be in the range of 0.76 to 1.03. The lower values of % R.S.D. (< 2) indicated that the method was found to be precise.

3.2.3 Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ were found to be 20.02 ng band^{-1} and 60.69 ng band^{-1}, respectively.

3.2.4 Recovery studies

Accuracy of the developed method was carried out by recovery studies by standard addition method which involved the addition of a known quantity of the standard drug to pre-analyzed sample solution at three different levels 80, 100 and 120 %. The basic concentration of the sample chosen was 400 ng band^{-1} from the tablet solution. The drug concentrations were calculated from the linearity equation. The results of the recovery studies indicated the accuracy of the method for the estimation of the drug in the tablet dosage form. The results obtained are represented in Table 2.

Table No. 2: Recovery studies

Drug	Amount took (ng band^{-1})	Amount added (ng band^{-1})	Amount recovered (ng band^{-1})	% Recovery±R.S.D.*
Didanosine	400	320	716.27	99.48±1.02
	400	400	806.97	100.87±0.91
	400	480	878.60	99.84±0.86

*Average of three determinations

3.2.5 Robustness

Robustness of the method was determined by making intentional variations in method parameters during which mobile phase composition (± 2 % ethanol), wavelength (± 1 nm) was altered and the effect on the area of the drug was noted. The areas of peaks of interest remained unaffected by small changes of the operational parameters indicating that the method is robust.

4.0 CONCLUSION

Stability-indicating HPTLC-densitometric method without interference from the excipients or degradation products has been developed and validated for the determination of Didanosine

as bulk drug and in the tablet dosage form. The developed method is simple, precise, accurate, reproducible and specific. The developed method can be used for the quantitative analysis of drugs in the pharmaceutical dosage form. As the method is stability-indicating one it may be extended to study the degradation kinetics of drugs.

5.0 ACKNOWLEDGEMENT

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