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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF DIDANOSINE IN TABLET DOSAGE FORM

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ABSTRACT

The present work describes development and validation of a new simple, accurate, precise and selective stability-indicating reverse phase high performance liquid chromatography (RP-HPLC) method for determination of Didanosine as bulk drug and in tablet dosage form. As stability testing is key step in new drug as well as formulation development, stress degradation studies were performed according to ICH guidelines. Chromatographic resolution of Didanosine and its degradation products was accomplished by use of Jasco HPLC system equipped with Grace C_{18} column (150 x 4.6 mm i.d.) as stationary phase and mixture comprising of Acetonitrile: Methanol (85: 15, v/v) the pH was adjusted to 8 with triethylamine as optimum mobile phase. Densitometric detection was carried out at 250 nm. The retention time was found to be 2.58 ± 0.04 min. The developed method was validated with respect to linearity, accuracy, precision, limit of detection, limit of quantitation and robustness as per ICH guidelines. Results were linear in the range of 5-30 µg mL^{-1}. The developed method has been successfully applied for the estimation of drug in tablet dosage form.

KEYWORDS: Didanosine, RP-HPLC, Forced degradation, Tablet dosage form.

INTRODUCTION

Didanosine, chemically, 9-[(2R,5S)-5-(hydroxymethyl)oxolan-2-yl]-1H-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 RP-HPLC method. This paper describes the development and validation of simple, precise, accurate and selective stability-indicating RP-HPLC method for the determination of didanosine by International Conference on Harmonisation Guidelines.[13,14]

MATERIALS AND METHODS

Chemicals and reagents

Analytically pure didanosine working standard was obtained as 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. Acetonitrile (HPLC grade), Methanol (HPLC grade), Triethylamine (HPLC grade) were purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions

JASCO HPLC system equipped with Model PU 2080 Plus pump, Rheodyne sample injection port (20 µl), MD 2010 PDA detector and Borwin- PDA software (version 1.5). A chromatographic column Grace C_{18} (150 x 4.6 mm i.d. 3µm) was used. Separation was carried out at flow rate of 1 mL min^{-1} using Acetonitrile: Methanol (85: 15 v/v) the pH was adjusted to 8 with triethylamine as mobile phase and detection was carried out at 250 nm.

Preparation of standard stock solution

Standard stock solution of Didanosine was prepared by dissolving 10 mg drug in 10 mL methanol to get concentration of 1000 µg mL^{-1} which was further diluted with mobile phase to get final concentration 100 µg mL^{-1}.

Tablet formulation analysis

Twenty tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 10 mg of Didanosine was weighed and transferred to 100 mL volumetric flask containing about 60 mL of methanol and ultrasonicated for 15 min and filtered through Whatman paper No. 41 and volume was made upto the mark with the mobile phase. One mL of this solution was transferred to 10 mL calibrated volumetric flask and volume was made up to the mark with the methanol to get solution of concentration 10 µg mL^{-1} for Didanosine. After setting the chromatographic conditions, the tablet sample solution was injected, chromatogram was obtained and the peak areas were recorded. The injections were repeated six times and the amount of drug present per tablet was estimated from the calibration curve.

System suitability

The system suitability was assessed by six replicate injections of the standard Didanosine having concentration 15 µg mL^{-1}. The resolution, peak asymmetry, number of theoretical plates and height equivalent to theoretical plate (HETP) were calculated. The values obtained demonstrated the suitability of the system for the analysis of drug. The results obtained are represented in Table 1.

Table 1: System suitability parameters for proposed RP-HPLC method.

Sr. No.	Parameters	Didanosine
1	Theoretical plates	4123.41
2	HETP (cm)	0.0046
3	Resolution	3.50
4	Asymmetry factor	1.19

Stress degradation study

The stability studies were accomplished by subjecting the bulk drug to the physical stress and stability was accessed. The study was carried out at concentration of 20 µg $µL^{-1}$. The hydrolytic studies were performed by treating the stock solution of drug with 0.1N HCl and 0.1N NaOH at room temperature for 1 h. The stressed samples of acid and alkali were neutralized with NaOH and HCl, respectively to furnish the final concentration of 20 µg $µL^{-1}$ The oxidative degradation was carried out in 15 % H_2O_2 at room temperature for 30 min and sample was diluted with mobile phase to obtain 20 µg $µL^{-1}$ solution. Thermal stress degradation was performed by keeping drug in oven at 50ºC for period of 48 h. Photolytic degradation studies were carried out by exposure of drug to UV light up to 200 watt h square

meter^{-1} for 2 d. Thermal and photolytic samples were diluted with mobile phase to obtain 20 µg µL^{-1} concentration.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The primary target in developing this stability indicating RP-HPLC method is to achieve the resolution between Didanosine and its degradation products. To achieve the separation, we used Grace C_{18} column as stationary phase and mixture of acetonitrile: methanol (85: 15, v/v) the pH was adjusted to 8 with triethylamine as mobile phase. The tailing factor obtained was less than two and retention time was 2.58±0.04. The representative chromatogram of the standard drug solution is shown in Fig. 1.

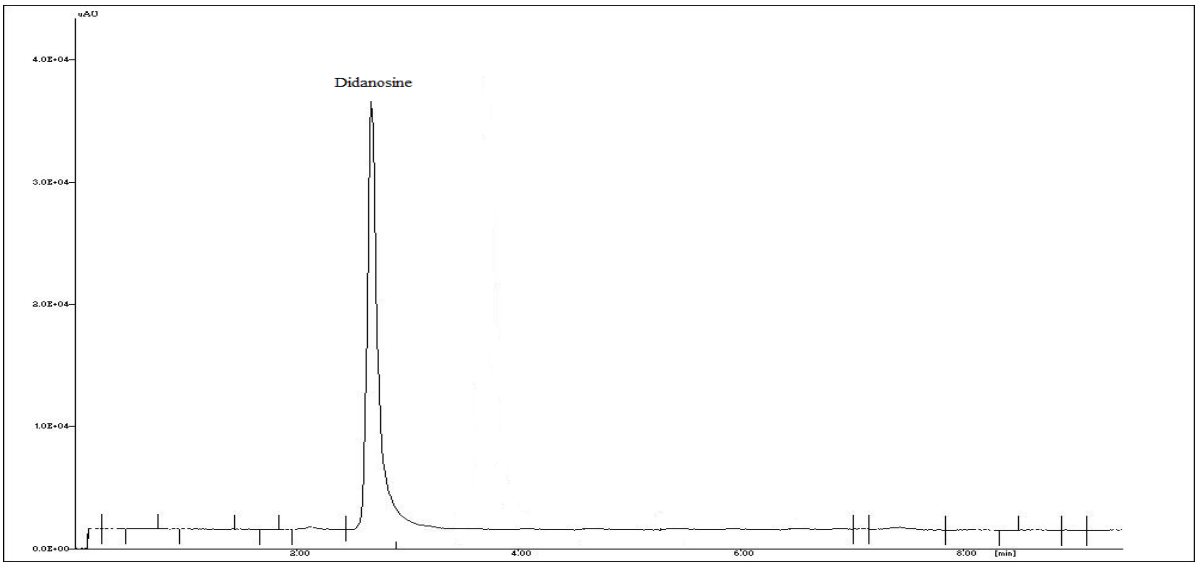

Fig. 1: Representative chromatogram of standard drug solution (15 µg mL^{-1}, 2.58 min).

The stress 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. Stress degradation study demonstrated that the method is highly specific as no degradation products were eluted at retention time of drug. Fig. 2 and 3 symbolizes the chromatograms of acid and alkali degradation while Fig. 4-6 show the chromatograms of oxidative, thermal and photolytic degradation. The stress degradation studies data is summarized in Table 2.

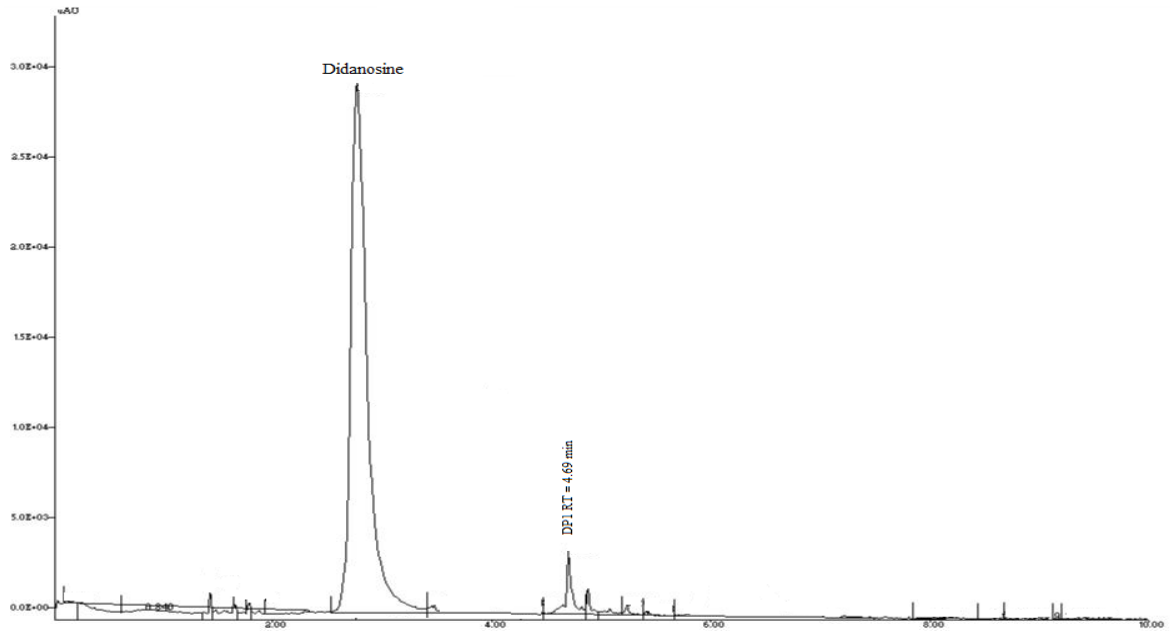

Fig. 2: Chromatogram obtained after acid treatment with degradation product. (DP1, RT = 4.69 min)

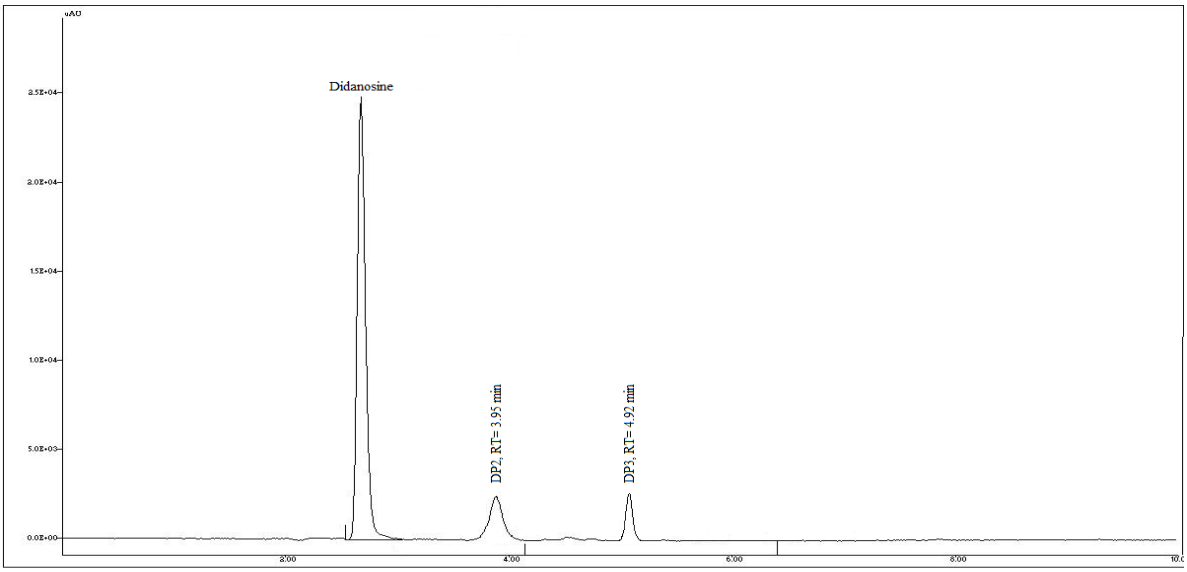

Fig. 3: Chromatogram obtained after alkali treatment with degradation products. (DP2, RT = 3.95 min) and (DP3, RT= 4.92 min)

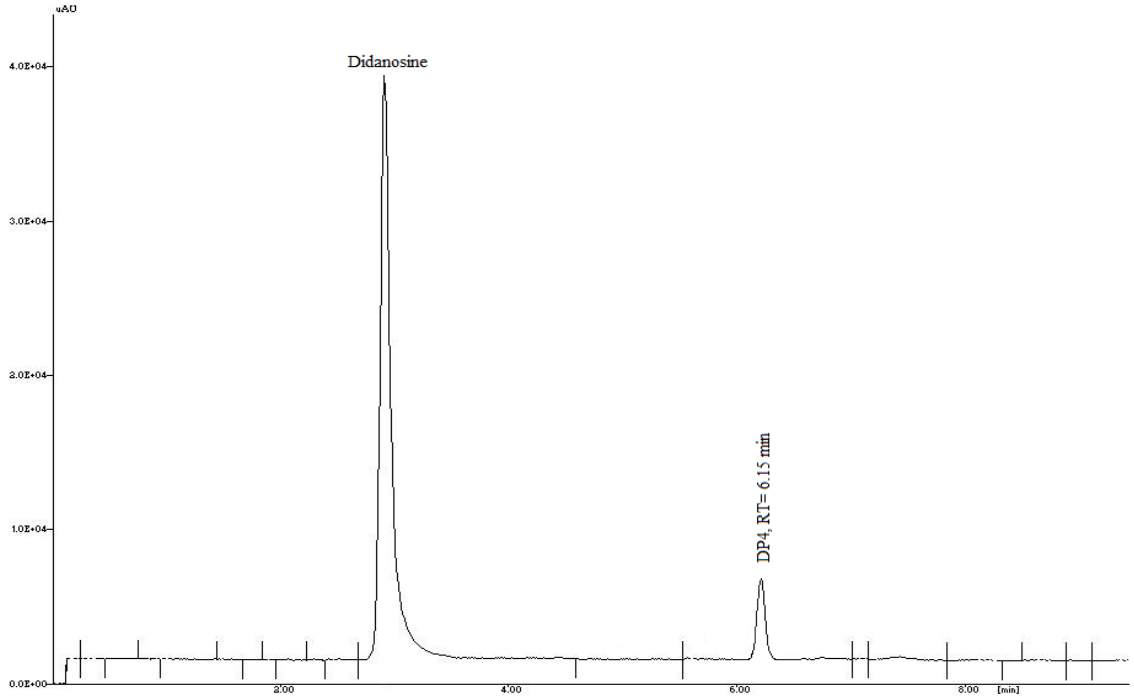

Fig. 4: Oxidative degradation chromatogram with degradation product.

(DP4, RT = 6.15 min)

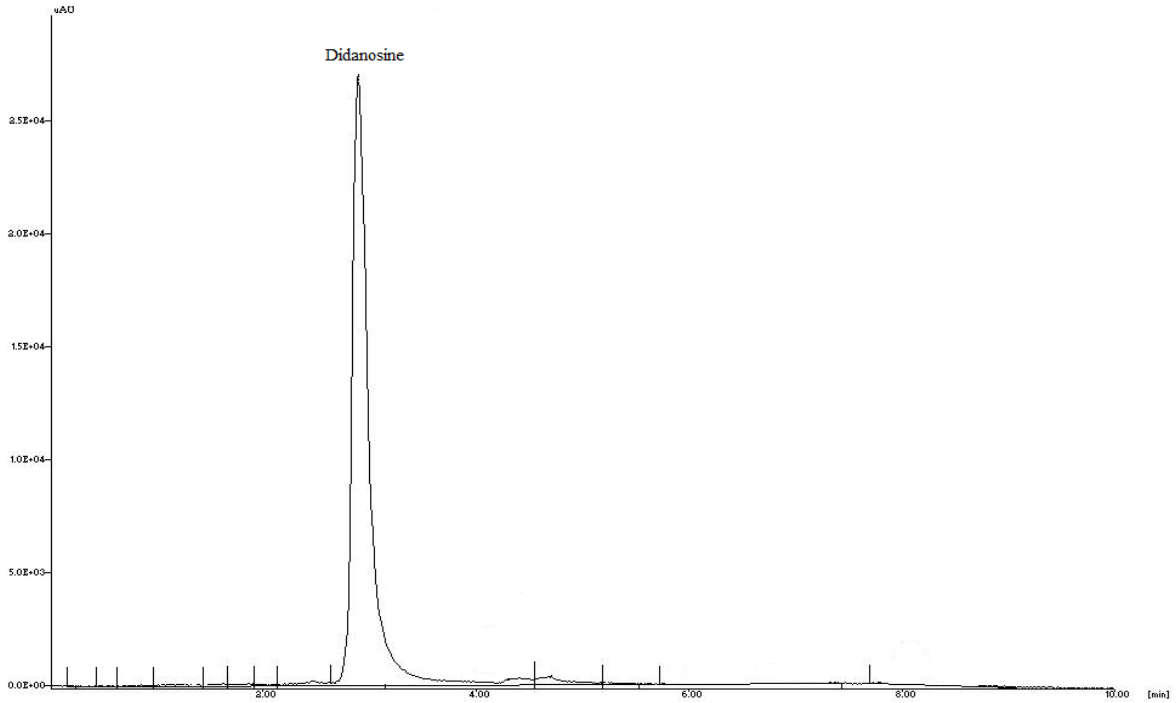

Fig. 5: Chromatogram obtained after thermal stress.

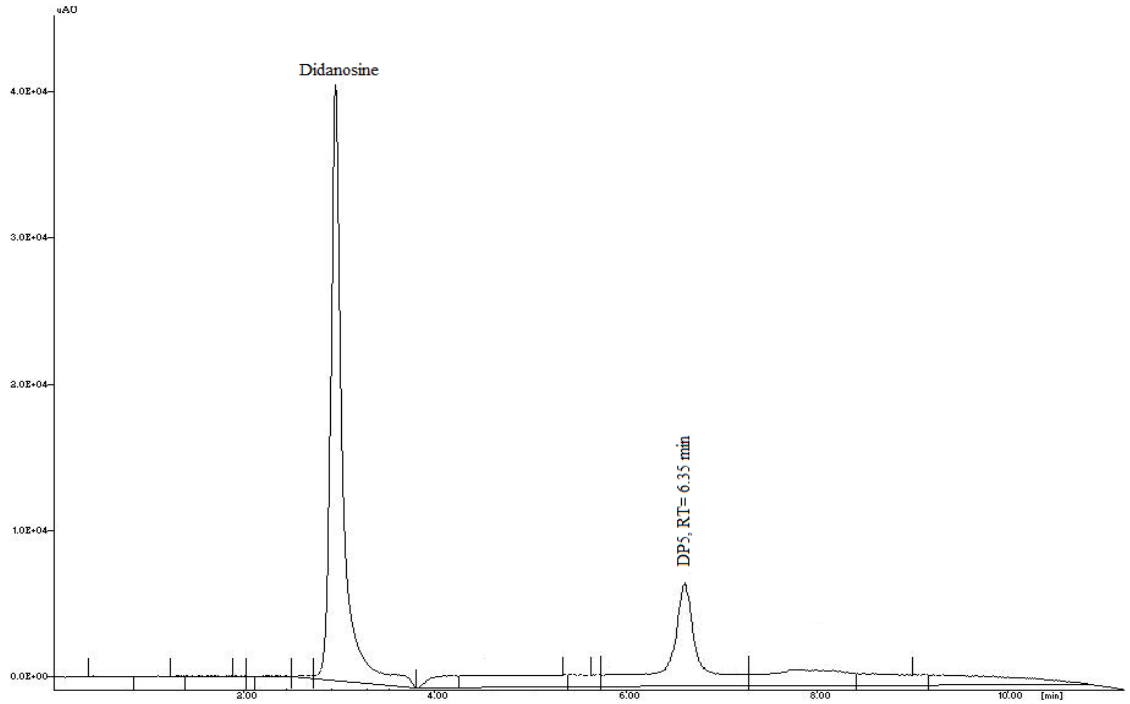

Fig. 6: Photolytic UV degradation chromatogram with degradation product. (DP5, RT = 6.35 min)

Table 2: Summary of stress degradation studies.

Stress conditions/ duration	% Recovered	% Degradation
Acidic / 0.1 N HCl/ Kept at RT for 1 h	85.35	14.61
Alkaline /0.1 N NaOH/ Kept at RT for 1 h	80.51	19.48
Oxidative /15 % H_2O_2 / Kept at RT for 30 min	85.95	14.04
Dry heat/ 50ºC/ 48 h	90.62	9.37
Photolysis: UV light 200 watt h square meter^{-1} 2 d	82.81	17.18

Method Validation

The method was validated for linearity, accuracy and intra-day and inter-day precision, specificity and robustness, in accordance with ICH guidelines. [13, 14]

Linearity

The linearity of the response of the drug was verified at six concentration levels, ranging from 5-30 µg mL^{-1}. The calibration graph was obtained by plotting peak area versus the concentration and data was treated by least-squares linear regression analysis. The equation of the calibration curve was y = 48703x + 52036. The calibration graph was found to be linear in the plotted concentrations with coefficient of correlation 0.992. The calibration curve obtained is represented in Fig. 7.

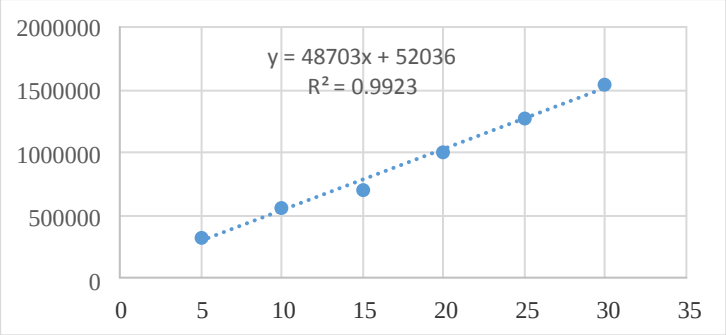

Fig. 7: Calibration curve for Didanosine.

Precision

One set of three different concentrations of standard solution of Didanosine (10 µg mL^{-1}, 20 µg mL^{-1}, 30 µg mL^{-1}) were prepared. All the solutions were analyzed thrice, in order to record any intraday variations in the results. Intra-day variation, as RSD (%), was found to be in the range of 0.16 to 0.86. For Inter day variations study three different concentrations of the standard solution in linearity range were analyzed on three consecutive days. Interday variation, as RSD (%) was found to be in the range of 0.55 to 1.33.

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 values were found to be 0.51 µg mL^{-1} and 1.55 µg mL^{-1}, respectively.

Accuracy

Recovery studies were carried out by standard addition method to check the accuracy of the method. It involved addition of standard drug solution to pre-analyzed sample solution at three different levels 80 %, 100 % and 120 %. Basic concentration of sample chosen was 10 µg mL^{-1} from tablet solution. The drug concentrations were calculated from linearity equation. The results of recovery studies indicated the accurateness of the proposed method for estimation of drug in tablet dosage form. The results obtained are shown in Table 3.

Table 3: Recovery studies.

Drug	Amount taken (µg mL^{-1})	Amount added (µg mL^{-1})	Total amount found (µg mL^{-1})	% Recovery*	% R.S.D.[a]
Didanosine	10	08	18.02	100.10	0.69
	10	10	19.88	99.36	1.53
	10	12	22.07	100.31	1.19

*Average of three determinations, R.S.D. is relative standard deviation

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 992, indicating the no interference of any other peak of degradation product, impurity or matrix.

Robustness

The deliberate variations in method parameters were made to check the robustness of the method. The parameters varied were flow rate of the mobile phase (± 0.1 mL min^{-1}), mobile phase composition (± 2 % acetonitrile) and the effect on the area of drug was noted. It was observed that there were no marked changes in the chromatograms and peak areas of drug, which demonstrated that the developed RP-HPLC method is robust.

CONCLUSION

Stability indicating RP-HPLC method without interference from excipients or degradation products has been developed and validated for determination of Didanosine in tablet dosage form. The developed method is specific, accurate, precise, and robust. As the developed method is stability indicating, it can be used for assessing the stability of Didanosine in bulk drug and in pharmaceutical tablet dosage form.

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