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DEVELOPMENT AND VALIDATION OF NEW UV-SPECTROPHOTOMETRIC ASSAY METHOD FOR DIDANOSINE IN PURE AND IN FORMULATIONS

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ABSTRACT

A simple, accurate, economical and convenient method for the analysis of didanosine using UV-spectrophotometry. λmax selected for quantitation was 246nm. In the developed method, the linearity was observed in the concentration of 25-150mcg/mL. Accuracy of the proposed method was ascertained by recovery studies were found in the range of 98.17 - 99.39 %. The values of standard deviation and %RSD were satisfactorily low indicating the good precision of the proposed method. The results of analysis for assay and recovery studies for formulation was 99.95% respectively concluding that this proposed method being accurate, precise, robust, economical, simple and time saving can be used in laboratories and also for the routine analysis of didanosine in bulk preparation and in tablet dosage without any interference from excipients

KEYWORDS: Didanosine, Validation, ICH guidelines.

INTRODUCTION

Didanosine[Figure.1] (2',3'-dideoxyinosine)[1-7] is an antiretroviral medication used to treat HIV/AIDS in combination with other medications as part of highly active antiretroviral therapy (HAART). An extensive review of the literature revealed a few analytical methods [8-16] were reported for the estimation of didanosine in dosage forms. Therefore, attempts were made to develop and validate simple and precise new UV-spectrophotometric method for the determination of didanosine in pure and in pharmaceutical formulations. This papers presents a new UV-spectrophotometric method for the determination of didanosine in pure and formulations. The developed method had sufficiently good accuracy, precision and permitted a simple, time and money-saving assay of didanosine in formulations. This method was also validated for various parameters according to ICH guidelines.

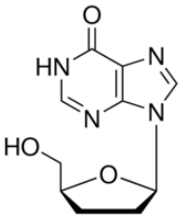

FIG.1: MOLECULAR STRUCTURE OF DIDANOSINE

A.EXPERIMENTAL

i. APPARATUS & CHEMICALS: All the Spectral and absorbance measurements in the present assay were carried out by using Shimadzu UV/Vis spectrophotometer model UV-2450 equipped with 1.0cm thickness matched quartz cells. A Sartorius TE2145 analytical balance (USA) was used in this assay. Didanosine (99% pure) was procured as a gift sample from Torrent Pharmaceutical Limited, Ahmedabad and pharmaceutical formulation in the brand name of videx claimed to contain 100mg of didanosine (oral tablets from Lifestar Pharma PVT.Ltd.) were purchased from commercial sources in the local market. Acetonitrile of analytical grade was obtained from E Merck Ltd, Mumbai, India. Potassium dihydrogen phosphate and Orthophosphoric acid of AR grade was procured from Qualigens Fine Chemicals, Mumbai, India.

ii.PREPARATION OF DILUENT: The diluent for didanosine was prepared by using 0.025M potassium dihydrogen ortho phosphate buffer pH-3.0 combined with acetonitrile in the ratio of 65:35 v/v. Filtered through a membrane filter unit and degassed by sonication under vacuum for prior to the assay.

PREPARATION OF 0.025M POTASSIUM DIHYDROGEN ORTHO PHOSPHATE PH 3.0 BUFFER:1.7 gms of potassium dihydrogen ortho phosphate was dissolved in 400mL of HPLC-grade water and adjusted to pH 3.0 with diluted ortho phosphoric acid; the final volume was made up to 500mL using triple distilled water.

iii.PREPARATION OF STANDARD STOCK SOLUTION: Weigh and transfer 50mg of didanosine (pure) into 50mL volumetric flask, add 40mL of diluent[Methanol] and sonicate to dissolve and dilute to volume with diluent. The volume was adjusted with the same diluent up to the mark to give stock standard solution of final strength i.e. 100µg/mL. From this stock solution different aliquots were transferred into a series of 100 mL volumetric flasks and diluted upto the mark with the same diluent to get concentration range of 25-150µg/mL respectively.

iv.SELECTION OF WAVELENGTH FOR ANALYSIS OF DIDANOSINE: Appropriate volume 2.0mL of standard stock solution of didanosine was transferred into 100 mL volumetric flask, diluted to mark with distilled water to give concentration of 20µg/mL.The resulting solution was scanned in UV range (200nm- 400nm). In spectrum Didanosine showed absorbance maximum at 246nm (Fig.2).

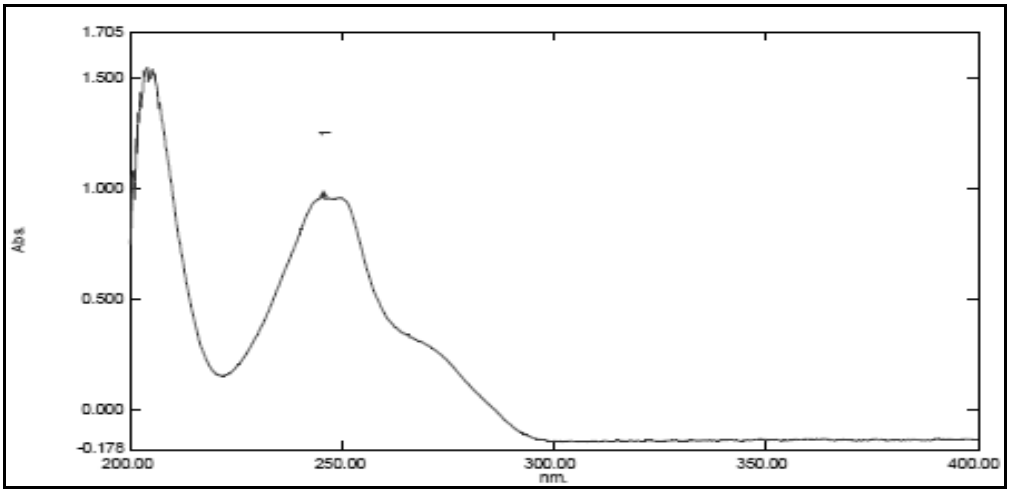

FIG:2 - A TYPICAL UV SPECTRUM OF DIDANOSINE

v.VALIDATION OF THE METHOD: The developed UV-Spectrophotometric method was validated in accordance to ICH norms in terms of linearity, accuracy, precision, and ruggedness.

i.LINEARITY STUDY: Different aliquots of didanosine (Stock solution)in range 0.5-3.0mL were transferred into series of 100mL volumetric flasks and the volume was made up to the mark with distilled water to get working standard concentrations 25, 50, 75, 100, 125 and 150µg/mL, respectively. These solutions were scanned on UV-Spectrophotometer in the UV range at an absorption maxima of 246nm and the absorbance were recorded. A calibration plot was constructed with respect to the absorbance recorded vs concentration.

ii. SENSITIVITY: The sensitivity of the developed method was estimated in terms of the Limit of Quantification (LOQ) and Limit of Detection (LOD). The LOQ and LOD were calculated using equation LOD = 3.3 x N/B and LOQ = 10 x N/B, where, 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve.

iii.ACCURACY: To the pre analyzed sample solutions, a known amount of standard stock solution was added at different levels i.e. 50%, 100% and 150 %. The solutions were analyzed by proposed method.

iv.PRECISION: The precision of present UV-Spectrophotometric method was ascertained from the absorbance values obtained by the determination of six replicates of a fixed amount of didanosine (100µg/mL) in total solution.

v.RUGGEDNESS: Ruggedness of the proposed method was determined for a fixed concentration (100µg/mL) of didanosine by analyzing two analysts using same operational and environmental conditions.

vi.STABILITY OF SOLUTIONS: In order to obtain reliable experimental results, it is essential to evaluate the stability of standard solution. The stability of the solution was validated as per ICH guidelines. Standard and sample solution of a fixed concentration was stored at room temperature for one day (intra-day precision) and subsequent injections of the above solutions were subjected to spectrophotometer in triple were made at 0,12 and 24hrs interval and the corresponding spectra's were recorded and the %recovery was calculated.

vii.APPLICATION OF PROPOSED METHOD FOR PHARMACEUTICAL FORMULATION: For the analysis of formulation twenty tablets of didanosine (VIDEX-100mg) were purchased, weighed and their average weight was determined and grinded fine to powder. This tablet powder equivalent to 100mg of didanosine was accurately weighed and transferred into 50mL volumetric flask containing 30mL of diluent, sonicate the flask to dissolve for 10mins and dilute to volume with diluent. This solution was then filtered through whatmann filter paper no.45. From this solution different were pipetted and were transferred to a series of 100mL volumetric flask and the volume in each flask was made up to the mark with same diluent to get concentrations that obey within the linearity range. These solutions were scanned on spectrophotometer in the UV range at 246nm and the concentrations of the drug were calculated from linear regression equation.

B.RESULTS AND DISCUSSION

i. LINEARITY STUDIES: The linearity was performed by preparing standard solutions of didanosine at different concentration levels as mentioned in experimental condition i.e. 25.0-150µg/mL. Twenty micro liters of each concentration was subjected in duplicate into the spectrophotometer and the absorbance was read at 246nm. From these spectras, a linearity plot was constructed with the absorbance recorded over concentration and the regression of the plot was computed by least square regression method, and the results were presented in Table.1. The linear regression data for the deduced calibration curve showed a good linear relationship over the concentration range 25.0-150.0µg/ml for didanosine and the regression equation was found to be Y = 0.0101X - 0.0475 (r^2=0.9991).

TABLE: 1: LINEARITY RESULTS OF DIDANOSINE WITH THE PROPOSED METHOD

% LEVEL	CONCENTRATION (µg/mL)	AVERAGE ABS.
25	25.00	0.196
50	50.00	0.464
75	75.00	0.708
100	100.00	0.981
125	125.00	1.234
150	150.00	1.453
	Slope:	0.0101
	Intercept:	- 0.0475
	r2	0.9991
	LOD (µg/mL)	4.29
	LOQ (µg/mL)	14.30

TABLE:2: RESULTS OF METHOD PRECISION STUDIES OF DIDANOSINE WITH THE PROPOSED METHOD

S NO	NAME	ABS
1	SOLUTION-1	0.981
2	SOLUTION-2	0.976
3	SOLUTION-3	0.973
4	SOLUTION-4	0.969
5	SOLUTION-5	0.972
6	SOLUTION-6	0.974
AVG*		0.974
STD DEV*		0.00407
% RSD		0.42

*Average of six determinations

TABLE:3: RESULTS OF RECOVERY STUDIES OF DIDANOSINE WITH THE PROPOSED METHOD

ACCURACY LEVEL	50%	100%	150%
S NO	AREA	AREA	AREA
INJECTION-1	0.484	0.978	1.45
INJECTION-2	0.482	0.979	1.448
INJECTION-3	0.48	0.976	1.446
AVG*	0.482	0.978	1.448
AMT RECOVERED*	49.08	99.39	147.45
%RECOVERY*	98.17	99.39	98.30

*Average of three determinations

ii. SENSITIVITY: The LOD and LOQ of TOLP by the proposed method were found 4.29µg/mL and 14.30µg/mL, respectively.

iii. PRECISION: The, %RSD values of intraday variation was found well within 2% limit, indicating that the current the proposed method is precise for the determination of didanosine in formulations (Table.2).

iv. ACCURACY: Accuracy of the present UV-method was determined by recovery tests. Pre-analysed tablet powder was spiked with pure didanosine at three concentration levels (50.100,150% of that in tablet powder) and the total was found by proposed methods. In all the %levels, the added didanosine recovery percentage values ranged between 98.17 - 99.39 (Table.3), which indicated the accuracy of the proposed.

v. RUGGEDNESS: Ruggedness of the proposed method was determined by analyzing aliquots from homogenous slot by two analyst using same operational and environmental conditionsand the results are reported in Table.4 and are in the acceptable range for didanosine by the developed method indicating that the developed method is rugged.

vi. STABILITY OF SOLUTIONS: The results had shown that standard solution of the analyte was stable for at 24hrs and 35ºC revealing the stability of the proposed method (Table.5).

vi. DETERMINATION OF DIDANOSINE IN MARKETED FORMULATIONS: The proposed method has been applied for the assay of commercial tablets (VIDEX - 100mg) containing didanosine. The drug sample was analyzed for six times after extracting the drug as mentioned in assay sample preparation of the experimental section. The concentration of didanosine was calculated from linear regression equation at 246nm and the results are shown in Table.6. The results of analysis showed that the amount of drug was in good agreement with label claim of didanosine formulation.

TABLE:4. RESULTS OF RUGGEDNESS STUDIES FOR DIDANOSINE WITH THE PROPOSED METHOD

		ANALYST -1	ANALYST -2
S No	Name	ABSORBNACE	ABSORBANCE
1	SCAN-1	0.981	0.988
2	SCAN-2	0.976	0.989
3	SCAN-3	0.973	0.982
4	SCAN-4	0.969	0.985
5	SCAN-5	0.972	0.981
6	SCAN-6	0.974	0.979
AVG*		0.974	0.984
STD DEV*		0.00407	0.004
% RSD*		0.42	0.407

*Average of six determinations

TABLE:5. RESULTS OF STABITY STUDIES OF STANNADARD AND SAMPLE SOLUTIONS OF DIDANOSINE WITH THE PROPOSED METHOD

TIME INTERVAL (Hrs)	%RECOVERY	
	STANDARD(n=3)	SAMPLE(n=3)
0	99.99	99.99
12	99.87	99.98
24	99.99	99.80

TABLE:6.ANALYSIS DIDANOSINE IN FORMULATIONS

S.NO	NAME OF FORMULATION	LABELED AMOUNT mg/tablet	AMOUNT FOUND* mg	PERCENTAGE OF RECOVERY STUDIES
1.	VIDEX	100mg	99.95	99.95

*Average of three determinations

CONCLUSIONS

The UV-spectrophotometric method for the estimation of didanosine in bulk and tablet formulation was found to be accurate, precise and robust. The method was found to be linear over a convenient range, economical and utilized a solvent which can be easily prepared. The above factors make this method suitable for the estimation of didanosine in bulk drug and in pharmaceutical dosage forms. It can therefore be concluded that this method being accurate, precise, robust, economical, simple and time saving, can be used in laboratories and also for the routine analysis of didanosine in bulk preparation and in tablet dosage without any interference from excipients.

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