Research Article

Spectrophotometric Estimation of Didanosine in Bulk Drug and its Formulation

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ABSTRACT

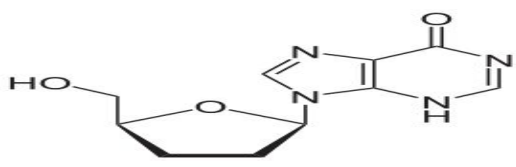
Didanosine is used in the treatment of AIDS. Three simple, sensitive, accurate and economical spectrophotometric methods have been developed for the estimation of Didanosine in bulk drug and pharmaceutical formulation. Method A is based on UV spectrophotometric measurements in which Didanosine was dissolved in double distilled water and exhibited absorption maximum at 249nm and obeyed Beer's law in the concentration range of 2-20µg/ml. Method B is based on measurements of first order, second order and third order derivative spectroscopy adopted to eliminate spectral interference, in which derivative amplitude was measured at 261nm, 248nm, and 222nm respectively. Method C is based on calculation of area under curve (AUC) for analysis of Didanosine in wavelength range of 245 to 255nm. The drug obeys Beer's law in the concentrations range of 2-20µg/ml. The results of analysis were validated for accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ). These results were found to be satisfactory. The proposed methods are simple, sensitive, rapid, economical and suitable for routine quality control applications in pharmaceutical formulation.

Keywords: Didanosine, UV spectrophotometry, Derivative spectroscopy, Area under curve (AUC).

INTRODUCTION

Didanosine is chemically known as 2',3'-dideoxyinosine (DDI) and is sold under the trade names Videx and Videx EC. It is a reverse transcriptase inhibitor, effective against HIV and used in combination with other antiretroviral drug therapy as part of highly active antiretroviral therapy (HAART). Didanosine (DDI) is a nucleoside analogue of guanosine. It differs from other nucleoside analogues, because it does not have any of the regular bases, instead it has hypoxanthine attached to the sugar ring. Within the cell, DDI is phosphorylated to the active metabolite of dideoxyadenosine triphosphate, ddATP, by cellular enzymes. Like other anti-HIV nucleoside analogs, it acts as a chain terminator by incorporation and inhibits viral reverse transcriptase by competing with natural ddATP.

Structure



MATERIALS AND METHODS

INSTRUMENTS

(1) The instrument used for the present study was PC based Jasco V-530 UV-Visible double beam Spectrophotometer with 1 cm matched pair quartz cell and spectral bandwidth of 2 nm.
(2) Shimadzu AUX 220 digital balance
(3) Sonicator Sonica ultrasonic cleaner model 2200 MH

Chemicals and Reagents

Didanosine was obtained as generous gift sample from Emcure Pharmaceuticals, Pune, India, and double distilled water prepared in laboratory was used as solvent throughout the analysis.

Preparation of stock solution

Accurately 10 mg of Didanosine was weighed in to 100 ml of clean and dry volumetric flask and dissolved in 70 ml double distilled water and the volume was made up to 100 ml to get a stock solution of 100µg/ml. This stock solution was used

for making dilutions for calibration curve. All solutions were freshly prepared prior to analysis.

Method A: UV Spectroscopy

Dilutions of stock solution (100µg/ml) were prepared in the range of 2-20µg/ml and scanned in the spectrum mode from 200nm-400nm. From the spectra of drug (Figure A), λ max of Didanosine was found to be 249 nm. The calibration curve (Figure F) was prepared in the range 2-20µg/ml. The amount of drug present in the sample solution was computed from its calibration curve.

Method B: UV Derivative spectroscopy

In this method, 2-20µg/ml solutions of Didanosine were prepared from stock solution (100µg/ml) and scanned in the spectrum mode from 200 nm to 400 nm. First order derivative spectra were selected for analysis of drug. First order derivative spectra of drug showed a sharp peak at 261 nm (Figure B) which was selected for its quantification. Similarly second order and third order derivative spectroscopy adopted to eliminate spectral interference, in which derivative amplitude were measured at 248nm (Figure C) and 222nm (Figure D) respectively. The calibration curves for Didanosine of first order (Figure G), second order (Figure H) and third order (Figure I) were plotted in the concentration range of 2-20µg/ml. The amount of drug present in the sample solution was computed from its calibration curve.

Method C: Area under curve

For the selection of analytical wavelength, 14µg/ml solution of Didanosine was prepared from stock solution (100µg/ml) and scanned in spectrum mode from 200nm-400nm. From the spectra of drug, area under curve in the range of 245-255nm was selected for the analysis (Figure E). The calibration curve was prepared in the concentration range of 2-20µg/ml at their respective AUC range (Figure J). By using the calibration curve, the concentration of the sample solution can be determined.

METHOD OF VALIDATION

Linearity

A calibration curves were constructed at optimum experimental conditions using zero, first, second, third order derivative and AUC absorbance's versus concentration in the range of 2-20µg/ml. From calibration curve data, high value of the correlation coefficient was found and the value of the intercept on ordinate which is Zero shows very good linearity of the calibration graph and adherence of the method to Beer's law.

Accuracy

The accuracy of the method was assessed, based on recovery study. The technique of standard addition method was used to assess accuracy of the method. For this purpose a concentration of 80%, 100%, 120% was selected. In this the absorbance of the sample after standard addition were measured in triplicate. The results are reported in terms of % recovery in table 1.

Precision

For Intraday and Interday precisions of the method, solutions of Didanosine were prepared at three concentration levels (low, mid and high) each in triplicate. These solutions were analyzed respectively three times within one day and three consecutive days.

Limit of Detection and Limit of Quantification

The LOD and LOQ were separately determined based on calibration curve. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines were used to calculate the LOD and LOQ.

Assay Determination

Ten capsules were weighed to obtained granules. An accurately weighed 10 mg granules of Didanosine and transferred to clean and dry 100 ml volumetric flask and dissolved in 70 ml of double distilled water. The volume was made up to the mark using double distilled water to get concentration of 100µg/ml. The resulting solution was filtered through whatman

filter paper no.42. From the above prepared solution (filtrate), further dilution was prepared to get the concentration of 10µg/ml. The absorbance was measured at the selected wavelength and concentrations were determined. The analysis was done in triplicate.

RESULTS AND DISCUSSION

First order, second order and third order derivative spectroscopy was adopted in estimation of Didanosine to eliminate spectral interference, in which derivative amplitude was measured at 261 nm, 248nm and 222nm respectively. UV spectrophotometric measurements in which Didanosine was dissolved in double distilled water and exhibited absorption maximum at 249 nm and area under curve (AUC) for analysis of Didanosine in wavelength range of 245 to 255 nm have been used for drug to obey Beer's law in the concentration range of 2-20µg/ml. The method was validated for various parameters like linearity, accuracy, precision and recovery shown in table 2.

CONCLUSION

The developed U.V. spectroscopic, derivative spectroscopic and area under curve method gives sensitive, accurate, precise and economical results for determination of Didanosine in marketed formulation (capsule) and easily applied for routine analysis. The most striking feature of these methods is its simplicity and rapidity. The developed method was validated for various parameters like linearity, accuracy, precision, LOD and LOQ. The developed methods were successfully applied for determination of the drug in commercial formulation.

ACKNOWLEDGEMENT

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Table 1: Recovery result of Didanosine

Method	% Recovery	S.D.	% RSD
1. UV Spectroscopy	100.48	1.146	0.86
2. Derivative Method			
a. First Order	99.92	0.985	1.19
b. Second Order	99.68	0.536	0.72
c. Third Order	99.22	1.012	0.98
3. AUC Method	101.56	0.857	1.54

Table 2: Optical characteristics of Didanosine by UV, First Order, Second order, Third order and AUC

Parameters	UV spectroscopy	Derivative spectroscopy			AUC
		First order	Second order	Third order	
λ max (nm)	249	261	248	222	245-255
Linearity (µg/ml)	2-20	2-20	2-20	2-20	2-20
Regression equation (Y = mx + c)	y =0.0539x + 0.0266	y = 0.0018x + 0.0002	y = -0.0003x - 0.00005	y = 0.00003x + 0.00002	y = 0.5163x + 0.2615
Intercept (c)	0.0266	0.0002	-0.00005	0.00002	0.2615
Slope (m)	0.0539	0.0018	-0.0003	0.00003	0.5163
Correlation Coefficient (r)	0.9976	0.9992	0.9995	0.9992	0.9975
Accuracy	100.48	99.92	99.68	99.22	101.56
Precision (S.D.)	1.362	0.922	1.125	0.984	1.462
LOD	0.1678	0.1657	0.1660	0.1677	0.0010
LOQ	0.6056	0.6078	0.5078	0.5054	0.0030

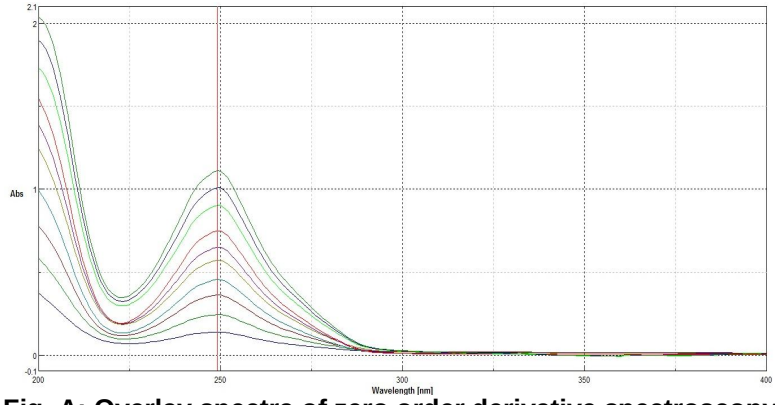

Fig. A: Overlay spectra of zero order derivative spectroscopy

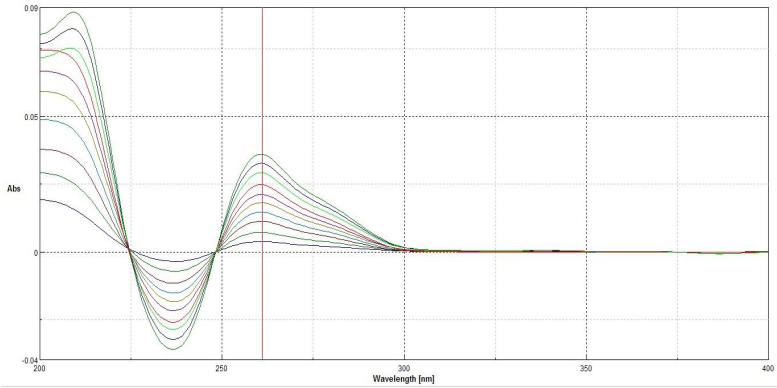

Fig. B: Overlay spectra of first order derivative spectroscopy

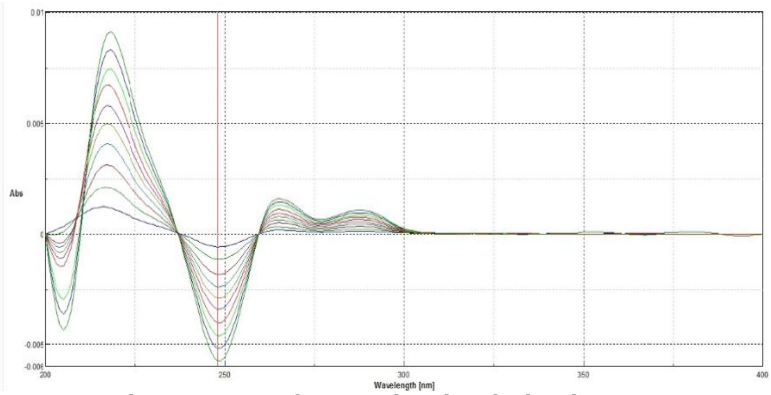

Fig. C: Overlay spectra of second order derivative spectroscopy

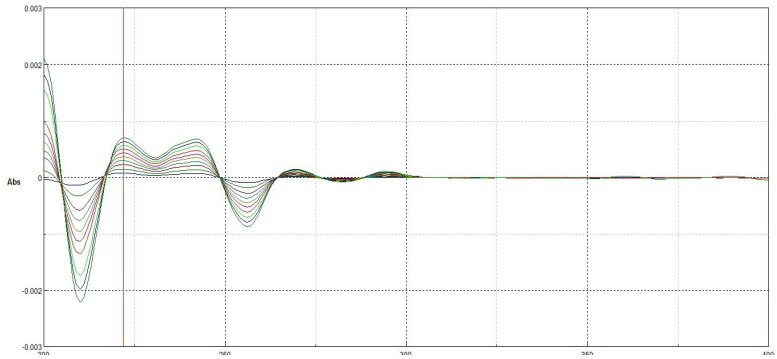

Fig. D: Overlay spectra of third order derivative spectroscopy

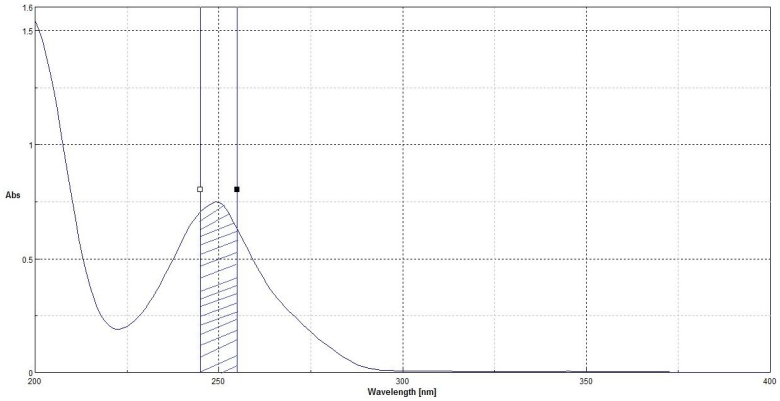

Fig. E: AUC calibration curve of Didanosine

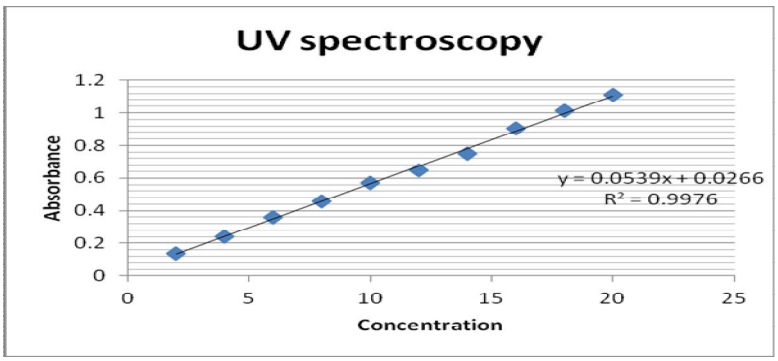

Fig. F: Zero order calibration curve of Didanosine

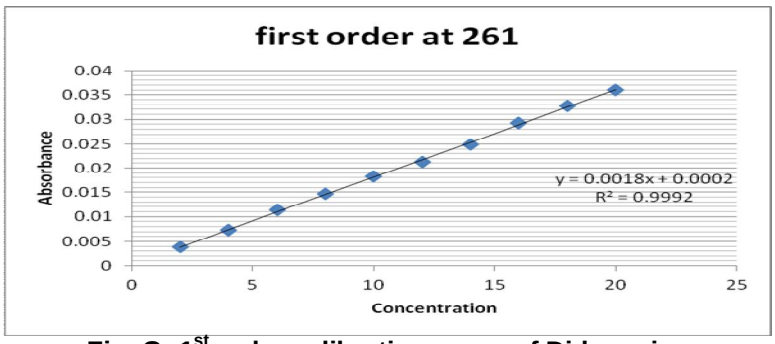

Fig. G: 1st order calibration curve of Didanosine

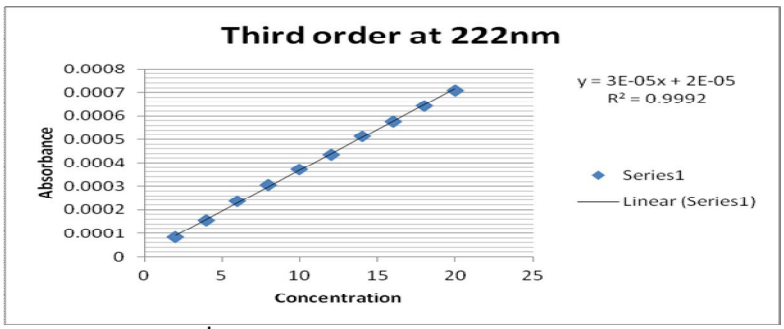

Figure H: 2nd order calibration curve of Didanosine

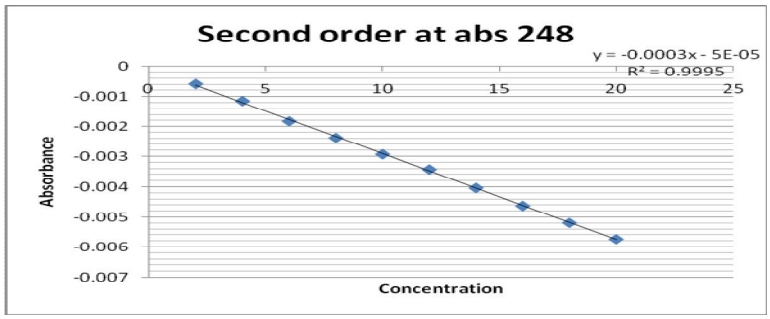

Fig. I: 3rd order calibration curve of Didanosine

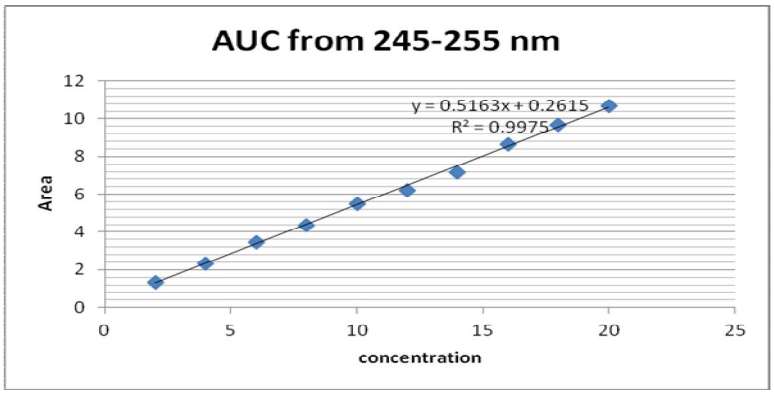

Fig. J: AUC calibration curve of Didanosine

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