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Spectrophotometric Determination of Didanosine in Bulk and Tablet Formulation

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Abstract: A simple, accurate, rapid and sensitive method is developed for the estimation of Didanosine in bulk and tablet formulation. In the proposed method orange red colour was observed due to reaction of keto group of Didanosine with 2, 4-dinitrophenyl hydrazine in dilute sulphuric acid which absorbs maximally at 424 nm. Molar absorptivity of the drug was found to be at 1.38×10^5 . The Beer's law was obeyed in the concentration range of and $20-80 \mu\text{g mL}^{-1}$. The proposed method can be used as a routine quality control test for determination of didanosine in bulk and tablet formulation.

Key words: Didanosine, 2, 4-dinitrophenyl hydrazine, spectrophotometry

INTRODUCTION

Didanosine, (2, 3-dideoxyinosine; ddi) is a dideoxy analogue of the purine nucleoside inosine that potentially inhibits the replication of the human immunodeficiency virus (Fig. 1) (De Clercq, 2004). So far there are some methods available for determination of didanosine in biological fluids (Clarke *et al.*, 2006; Estrelarde *et al.*, 2003). Only one HPLC method is available for its determination in Formulation (De Oliveira *et al.*, 2005). In the present research, we are reporting first spectrophotometric method for determination of didanosine in bulk and tablet formulation.

MATERIALS AND METHODS

Apparatus and Reagents

A Shimadzu model 1601 double beam UV-Visible Spectrophotometer with a pair of 1 mm matched quartz cell was used to measure absorbance of the resulting solutions. A Shimadzu analytical balance, Didanosine standard, sulphuric acid, 2, 4-dinitrophenylhydrazine were also used in the study.

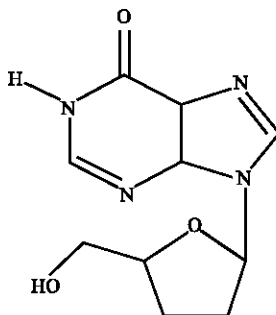


Fig. 1: Chemical structure of didanosine

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Preparation of Standard Solutions

Didanosine standard stock solution (1 mg mL^{-1}) was prepared in distilled water. From this stock solution working standard solutions of $100 \text{ } \mu\text{g mL}^{-1}$ was prepared by appropriate dilution with water. Solution of 2, 4-dinitrophenyl hydrazine (2% w/v) was prepared in 0.1 N sulphuric acid.

General Procedure for Assay

Aliquots of the working standard solution of Didanosine (20 to $80 \text{ } \mu\text{g mL}^{-1}$, i.e., 2 to 8 mL) were transferred in a series of 10 mL volumetric flask. These drug solutions were mixed with 2 mL of 2, 4-dinitrophenyl hydrazine. Final volume was adjusted with distilled water. After thoroughly shaking, the flasks were kept aside for 15 min for colour development. Absorbance of the resulting orange red solution was measured at 424 nm and the calibration curve was plotted.

Procedure for Assay of Didanosine in Tablet Formulation

Twenty tablets were weighed and crushed in powder. An accurately weighed quantity of powder equivalent to 10 mg of drug was dissolved in 100 mL of distilled water. The procedure was continued as described under general procedure.

RESULTS AND DISCUSSION

Determination of Absorption Maximum

Didanosine when treated with 2, 4-dinitrophenyl hydrazine orange red colour is formed. To determine absorption maximum, $40 \text{ } \mu\text{g mL}^{-1}$ solution of drug was reacted with 2, 4-dinitrophenyl hydrazine. After 15 min absorption spectra was recorded against reagent blank (Fig. 2). Absorption maximum wavelength was found to be at 424 nm .

Optimization of Variables

To study the effect of concentration of 2, 4-dinitrophenyl hydrazine on maximum absorbance a number of preliminary experiments were carried out. In a series of 10 mL volumetric flask containing $40 \text{ } \mu\text{g mL}^{-1}$ of the drug solution, the concentration of 2, 4-dinitrophenyl hydrazine was varied and the mixture was diluted up to mark with distilled water. After 10 min absorbance of each solution was measured at 424 nm . It was found that 2% w/v solution of 2, 4-dinitrophenyl hydrazine in a range of $2\text{-}3 \text{ mL}$ was necessary to achieve maximum color intensity. Therefore 2 mL of 2% 2, 4-dinitrophenyl hydrazine was recommended for all measurements. Results obtained for optimization of variables are presented in Fig. 3.

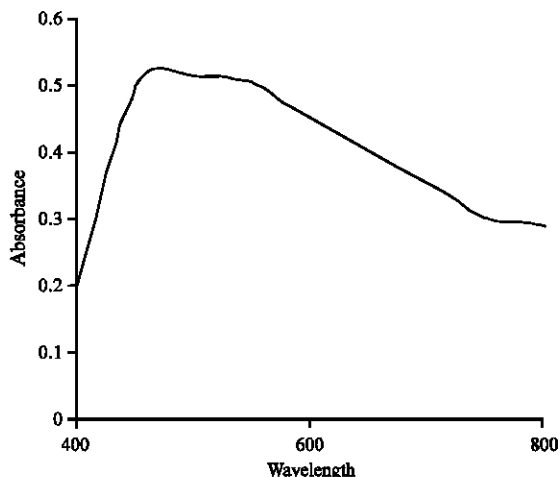


Fig. 2: Absorption spectra of didanosine

The effect of time on maximum absorbance was also tested by measuring the absorbance of solutions at regular interval and it was found that solution showed maximum absorbance after 15 min and was stable for further 3 h.

Optical Characteristics and Validation of the Method

Optical characteristics such as Beer's law limit, molar absorptivity and Sandell's sensitivity for the proposed method is given in Table 1. The accuracy and precision of the method were checked by analyzing 6 replicate samples within Beer's law range containing same amount of drug. Values of RSD were below 0.9%. Lower values of RSD indicate good precision and reproducibility of the method.

Interference Studies

To study the potential interference problems from commonly used excipients and other additives such as glucose, dextrose, lactose, sodium alginate, talc, recovery studies were carried out. Under the experimental condition employed, to a known amount of drug ($40 \mu\text{g mL}^{-1}$), excipients in different concentration were added and analysed. Results of the recovery analysis are presented in Table 2. Excipients up to the concentration shown in table do not interfere with the assay. In addition recoveries in most cases were 100% and the lower values of the RSD indicate the good precision of the method.

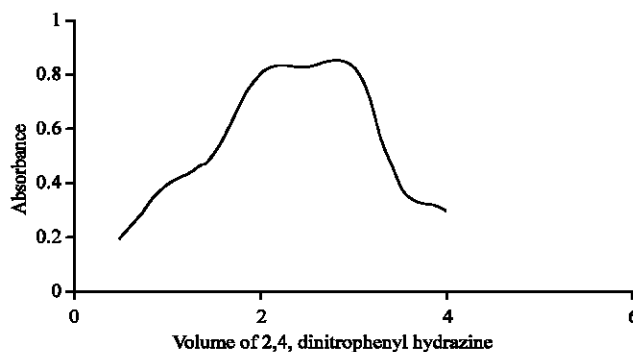


Fig. 3: Effect of Concentration of 2, 4-dinitrophenyl hydrazine on absorption maximum
* $60 \mu\text{g mL}^{-1}$ of didanosine was used

Table 1: Optical characteristics of the proposed methods

Parameter	Values
λ max (nm)	424
Beer's law limit ($\mu\text{g mL}^{-1}$)	20-80
Molar absorptivity ($\text{mol}^{-1} \text{cm}^{-1}$)	1.41×10^5
Sandell's sensitivity ($\mu\text{g cm}^{-2} 0.001 \text{ A}$)	2.05×10^{-2}
Correlation coefficient (r)	0.9993
Regression equation	
Slope	0.0045
Intercept	0.005

Table 2: Determination of didanosine in the presence of excipients^a

Excipients	Amount taken (mg mL^{-1})	(%) Recovery $\pm$ (%) RSD ^b
Glucose	45	98.60 $\pm$ 0.56
Lactose	40	98.95 $\pm$ 0.12
Dextrose	50	99.34 $\pm$ 0.56
Sodium alginate	25	99.34 $\pm$ 0.45
Talc	25	98.40 $\pm$ 0.84

^a $40 \mu\text{g mL}^{-1}$ of didanosine was used, ^bAverage of 6 replicate analyses

Table 3: Analysis of didanosine in tablet formulation

Formulation	Label claim (mg)	(%) of label claim* $\pm$ SD	Amount add (mg)	(%) recovery* $\pm$ SD	t-value
T ₁	100	99.18 $\pm$ 0.176	100	98.79 $\pm$ 0.152	0.544
T ₂	100	98.92 $\pm$ 0.322	100	99.44 $\pm$ 0.749	0.323

Where, T₁ and T₂ are two different brands of Tablet formulation. *Denotes n = 6, average of six readings

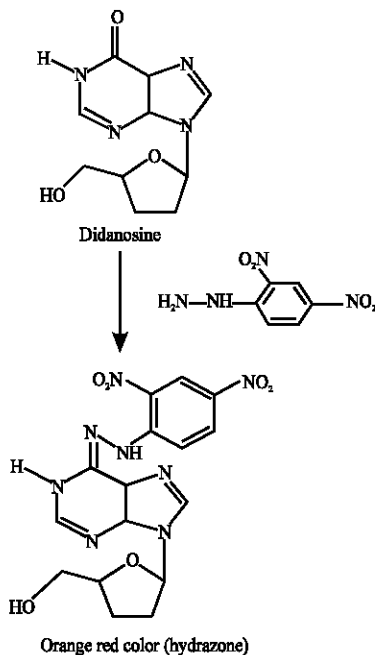


Fig. 4: Colour reaction of didanosine with 2, 4-dinitrophenyl hydrazine

Applicability of the Method

The applicability of the proposed spectrophotometric procedure was tested by analyzing various available commercial formulations. Table 3 result shows that the data are consistent with label claim of the formulations. The calibration curves shows linear response over the range of concentration used in the assay procedure. RSD values are in the range of 0.154-0.649 which shows that the method is precise and accurate. The precision and accuracy of the method was further compared statically using students t test. The calculated t values do not exceed the tabulated values. The low SD shows that the excipients in formulation do not interfere in analysis.

Mechanism of Colour Reaction

There are many methods reported for estimation of drugs using 2, 4-dinitrophenyl hydrazine. In the proposed method condensation of didanosine with 2, 4-dinitrophenyl hydrazine results in formation of orange red colored hydrazones with removal of water molecule. The possible reaction is given in Fig. 4.

CONCLUSIONS

The proposed spectrophotometric method for determination of didanosine is simple, sensitive, accurate, precise and reproducible. Color reaction neither requires any stringent condition nor any specific reagent or buffers. This method can be successfully applied for routine estimation of didanosine in bulk and pharmaceutical dosage forms.

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