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Determination of Didanosine in Pharmaceutical Dosage Forms by RP-HPLC

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A new reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and used for the estimation of didanosine (DDI) in bulk and pharmaceutical dosage forms using RPC-18 column using an isocratic HPLC system. The mobile phase consisted of acetonitrile and 0.05 M potassium dihydrogen phosphate (pH 4.2) in the ratio of 50 : 50 at a flow rate of 1 mL/min. The run time was 15 min. Nelfinavir mesylate (NEM) (50 μg/mL) was used as internal standard. The detection was carried out at 248 nm and the linearity was found to be in the range of 0.1-200 μg/mL. The retention times for didanosine and internal standard (NEM) were 3.183 and 10.675 min, respectively. Recovery studies shown that about 99.96 % of DDI could be recovered from the pre-analyzed samples indicating high accuracy of proposed method. There was no intra-day and inter-day variation found in the method of analysis. The mean drug content in branded DDI tablet dosage forms was quantified and found to be between 98.9 to 101.24 %.

Key Words: RP-HPLC, Didanosine, Nelfinavir mesylate.

INTRODUCTION

Didanosine (DDI) is a purine nucleoside analogue used against HIV-1 and HIV-2 in the treatment of AIDS. It is 2',3'-dideoxyinosine classified under nucleoside reverse transcriptase inhibitors category of antiretroviral drugs[1]. It is more active in quiescent cells and non-dividing human monocyte/macrophages[2]. Some analytical methods for the estimation of didanosine were reported such as amperometry[3] and HPLC[4-7]. The present study is aimed at developing a simple, reproducible and sensitive reverse phase high performance liquid chromatographic method for the estimation of DDI in bulk and pharmaceutical dosage forms using nelfinavir mesylate (NEM) as an internal standard (IS).

EXPERIMENTAL

An isocratic HPLC system (Shimadzu®) consisting of LC-10 AT liquid pump, SPD-10A UV-visible detector, a ODS-18 RP column (4.6 mm I.D. X 25 cm length), 25 μL Hamilton® injecting syringe and MS Windows based

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Single channel software (Class VP®). Afcoset® electronic balance was used for weighing the materials. Pure samples of didanosine and nelfinavir mesylate were obtained from Matrix Laboratories, Hyderabad, India. Acetonitrile of HPLC grade and potassium dihydrogen phosphate of AR grade were purchased from E. Merck (India) Ltd., Mumbai. Water used was triple distilled prepared by all glass distillation apparatus.

Chromatographic conditions: The optimized chromatographic conditions were as follows:

Chromatograph	Schimadzu HPLC system
Mobile phase	Acetonitrile : 0.05 M potassium dihydrogen phosphate (pH 4.2) (50 : 50)
Column	ODS C-18 RP column (4.6 mm I.D. × 25 cm length)
Flow rate	1 mL/min
Detection	UV set at 248 nm
Injection volume	20 µL
Temperature	Ambient
Retention time	
of Drug	3.183 min
of IS	10.675 min
Run time	15 min

Stock solutions of DDI and NEM were prepared by dissolving accurately weighed 25 mg of DDI and NEM in 25 mL of acetonitrile:0.05 M potassium dihydrogen phosphate (50:50) to obtain 1 mg/mL solutions. From these solutions 2.5 mL was pipetted out into 25 mL volumetric flask and diluted with the same solvent system to obtain 100 µg/mL solutions. Working standard solutions of DDI each containing internal standard (NEM) solution in the concentration of 50 µg/mL were prepared by taking required aliquots of DDI solutions and then diluted with the same solvent system. The standard solutions prepared above were injected five times into the column at a flow rate of 1 mL/min. The ratios of AUC of drug to I.S. were calculated for each of the drug concentrations. The regression equation of drug concentration over the ratio of drug peak is to that of internal standard was obtained. The regression equation was used to estimate the amount of DDI in pharmaceutical tablet dosage forms.

The proposed HPLC method was tested for intra-day and inter-day variations. The recovery studies were carried out by adding known amounts of (10 and 20 µg) of the DDI to the pre-analyzed samples and subjecting them to the proposed HPLC method.

Estimation of didanosine in commercial formulations: Contents of ten tablets containing didanosine were pooled and powdered. The powder equivalent to 25 mg of DDI was extracted into acetonitrile and the volume was adjusted to 25 mL, mixed and filtered through a 0.45 µ filter. From the filtrate 0.1 mL was pipetted into a 10 mL graduated test tube and spiked with the

required aliquot of IS solution and then the volume was adjusted to 10 mL with the mobile phase such that the concentration of IS in each sample was 50 μg/mL and was injected five times into HPLC column. The mean concentration of didanosine corresponding to the ratio of AUC of didanosine to that of IS was calculated from the standard graph. The same procedure was followed for remaining tablet brands.

RESULTS AND DISCUSSION

The present study was carried out to develop a specific sensitive, precise and accurate HPLC method for the analysis of didanosine in pharmaceutical tablet dosage forms. The column pressure varied from 160-170 kgf/cm^2. The retention times for DDI and IS (NEM) were 3.183 and 10.675 min, respectively. Each of the samples was injected five times and almost the same retention times were observed in all the cases.

TABLE-1
CALIBRATION OF HPLC METHOD FOR ESTIMATION OF DIDANOSINE

Concentration of Didanosine (μg/mL)	Mean ratio of AUC of drug to IS (n = 5)	CV (%)
0.1	0.0094	2.75
0.5	0.0445	2.28
1	0.0852	2.72
2	0.1651	1.66
5	0.4115	1.47
10	0.9594	2.99
20	1.7030	2.43
40	3.4213	1.98
80	6.8336	2.84
100	8.5336	3.04
160	13.3599	3.18
200	17.0791	2.16

C.V.= coefficient of variation, regression equation (from 0.1 to 200 μg/mL)

The ratio of peak area of DDI to peak area of IS for different concentrations set up as above were calculated and the average values for five such determinations are shown in Table-1. The peak areas of both drug and internal standard were reproducible as indicated by the low coefficient of variation (3.18 %). A good linear relationship (r = 0.9998) was observed between the concentration of drug and the respective ratio of peak areas. The calibration graph was found to be $y = 0.0847x + 0.0177$ (where y = ratio of peak area of drug to that of internal standard and x = concentration of drug in the range 0.1-200 μg/mL). When DDI solutions containing 10 and 30 μg/mL were analyzed by the proposed HPLC method for finding out intra-day and inter-day variation, a low coefficient of variation was observed (< 2.36 %) showing that the method is highly precise (Table-2). About 99.96 % of DDI could be recovered from the preanalyzed samples indicating high accuracy of proposed method as shown in Table-3.

TABLE-2
PRECISION OF THE PROPOSED RP-HPLC METHOD

Didanosine concentration (μg/mL)	Concentration of didanosine (μg/mL) found			
	Intra-day		Inter-day	
	Mean (n = 5)	CV(%)	Mean (n = 5)	CV(%)
10	10.17	1.87	10.14	2.36
30	30.09	1.36	30.12	2.11

TABLE-3
RECOVERY STUDIES OF DIDANOSINE

Amount of drug added (μg)	Mean (± S.D.) amount (μg) found (n = 5)	Mean recovery (%)
10	9.996 (± 0.06)	99.96
20	20.015 (± 0.08)	100.05

The DDI content in branded tablet formulations was quantified using the proposed analytical method and details are shown in Table-4. The absence of additional peaks indicated no interference of the excipients used in the tablets. The tablets were found to contain 98.9-101.24 % of the labeled amount. The low per cent of CV (2.84 %) indicates the reproducibility of the assay of DDI in the tablet dosage forms. The proposed method was found to be simple, precise, accurate, specific and economical.

TABLE-4
ASSAY OF DIFFERENT BRANDS OF DIDANOSINE TABLETS

Brand	Labeled amount of drug (mg)	Mean % of labeled amount (n=5)	CV (%)
1	100	101.24	2.84
2	100	99.84	1.76
3	100	98.90	2.49

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