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Research Article

Analytical method development and validation of different marketed Didanosine tablets by RP-HPLC

R. Sathiya sundar*[1], I. Carolin nimila[1], J. Ashok kumar[1], T.M. Vijaya kumar[2], G. Poovi[2], R. Sankar anand[3]

[1] Faculty of Pharmacy, PRIST University, Thanjavur-614904, Tamilnadu, India
[2] GIET School of Pharmacy, Rajamundary, Andhra Pradesh, India
[3] Jagans college of Pharmacy, Nellore, Andhra Pradesh, India

ABSTRACT

A Simple and suitable analytical method for validation of Didanosine (DDI) by reverse phase high performance liquid chromatographic (RP-HPLC) method. The method was performed on a RP-HPLC (Agilent 1120 LC Germany) model, C-18 Zorabax column, 4.6 mm X 250 mm, 5µm particle size. The mobile phase was mixture of methanol and 0.01M sodium acetate buffer adjusted to the pH 6.5 (75:25) at flow rate of 1.2 ml/min. UV detection was performed at 250 nm and the linearity was found to be 10 to 60 µg/ml with a correlation coefficient of 0.9998. The retention time for DDI was 5.17 min. The method showed good recovery and the relative standard deviation of intra and inter day assay results were 99.96% to 100.05 %. This method used for the analysis of different marketed DDI tablets.

Keywords: Didanosine; RP-HPLC; Analytical method development; validation.

INTRODUCTION

Didanosine (DDI) is a purine nucleoside analogue used against HIV-1 and HIV-2 in the treatment of AIDS. It is chemically 2', 3' dideoxyinosine classified under nucleoside reverse transcriptase inhibitors category of antiretroviral drugs DDI is the second drug approved for the treating of HIV infection (Lippincott's illustrated reviews 4th Edition). Didanosine were reported such as amperometry and HPLC in single.(Helena C. Castro et'al 2005), combination with other drugs and also reported in biological fluids(John Conte Jr.et'al 2004) . It is degraded at low pH the buffer to minimize the degradation. (Goodman & Gilman's 10th Edition) A simple, reverse phase high performance liquid chromatographic method was inconvenient determination and run time were rather long. Thus an attempt was made to develop a simple, precise, accurate and economical RP-HPLC method for estimation of Didanosine in solid dosage form. The chemical structure of Didanosine are shown in Fig.1

EXPERIMENTAL

Chemicals and Reagents

Analytical grade Sodium acetate, Ammonia solution and HPLC-Grade Methanol were procured from Merk (Mumbai, India) and pure standard of Didanosine was obtained as gift sample from Orchid Chemicals & Pharmaceuticals Ltd (Chennai India). HPLC-Grade water was collected from Millipore-Q.

* Corresponding Author
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Contact: +91-
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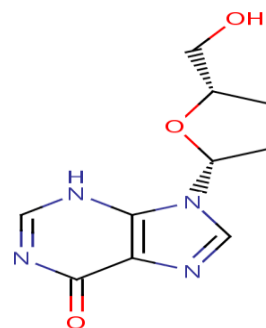

Figure 1: Chemical structure of Didanosine

Instrumentation and Chromatographic conditions

Chromatography was performed with a Agilent Technologies 1120 compact LC (Germany) gradient pump, an variable wavelength detectors and a rheodyne 9013 injector with 20µl loop, C18 Zorbax column (4.6 mm X 250 mm, 5µm particles) was used for Chromatographic separation under suitable condition. Detection was carried out at 250 nm and the software used was EZ chrome elite version 3.3. Chromatographic condition was showed on Table.1

Preparation of Standard Solution

Accurately weighed about 50 mg of Didanosine and transferred into a 50 ml volumetric flask. Then add 30ml of the mixture of methanol : 0.01M Sodium ace-

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tate buffer (75:25) to dissolved and sonicate to makeup the mark with the same mixture of mobile phase(stock solution). Transfer 0.5 ml of this solution was diluted to 10ml with the mobile phase. The final concentration of this solution is 50 µg/ml. The standard solution was filtered through 0.45µ membrane filter and sonicated before use. Then the solution was injected into the column. The Retention time of DDI was 5.17 min; a typical chromatogram is showed on Fig.2

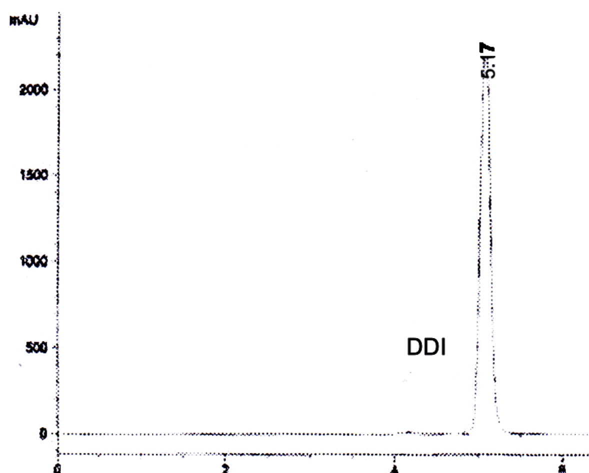

Figure 2: Standard chromatogram

Assay Procedure

Twenty tablets, each containing DDI (100 mg) tablets weighed, finely powdered and weighed accurately about powder equivalent of 50mg of DDI sample and transfer it into a 50ml volumetric flask. The sample was extracted with methanol and volume was adjusted into 50ml. The solution was filtered through 0.45µ membrane filter and sonicated before use. From the filtrate 0.5ml was transferred into volumetric flask and make up the volume with mobile phase. The above indices procedure was followed for all marketed products. The report for different brands DDI were showed on Table.2

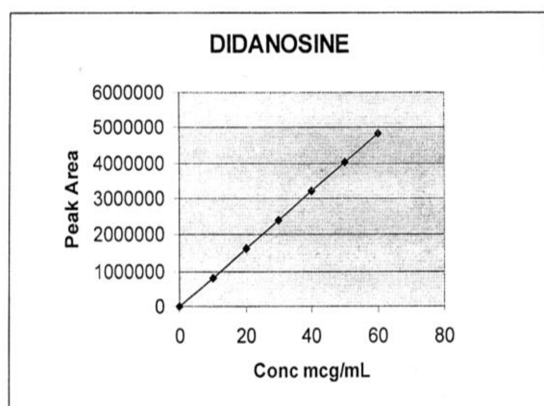

Figure 3: Linearity of Didanosine

Table 1: Results for Chromatographic condition

Chromatograph	Agilent HPLC system
Mobile phase	methanol:0.01M Sodium acetate buffer pH6.5 (75:25)
Column	C-18 Zorabax Column (4.6 mm×250 mm length)
Flow rate	1.2 ml/min
Wavelength Detection	VWD at 250nm
Injection volume	20µL
Temperature	Ambient
Retention time of DDI	5.17 min
Run time	10min

Validation

Linearity and Range

The linearity of the detector response is established by plotting a graph concentration Vs Area. From the standard stock solution 0.1 to 0.6 ml was transferred to 10 ml volumetric flask and get the solution in concentration ranging from about 10 to 60 µg/ml. These standard solutions were injected for construction of calibration plots by plotting drug peak-area ratio (y) for each of the drugs against concentration (x).The analysis was performed in ambient temperature. The linearity graph and peak area were showed on Table.2 and Fig.3.

Table 2: Results from Assay of marketed Didanosine tablets

Name of the Brand	Labeled amount of Drug(mg)	Mean% of labeled amount(n=5)	% RSD
DINEX (Cipla)	100	101.24	0.84
DINOSIN (Genix)	100	99.84	0.76
VIDEX(mexico)	100	98.90	0.49

Recovery

Recovery was determined spiking the formulation with standards of each drug equivalent to 50, 100, and 150% of amount originally present. The sample was filtered in 0.45µ membrane and injected into the HPLC system. Each sample was ran for 5 times and the mean recovery were reported on Table.4

Precision

The precision of method was done by replicate (n=5) analysis of tablet preparations. The precision of inter and intraday changes in peak area of drug solution on the same day and alternative days on a weak. The precision expressed as % RSD in this report showed on Table.5

Table 3: Results from Liniarity

Concentration of Didanosine (μg/ml)	Peak Area
10	805327
20	1610655
30	2415983
40	3221311
50	4026639
60	4831966

Robustness

The Robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. Robustness of an analytical method was analysis as different pH (6.5±0.5), Composition of mobile phase ±2 (75:25), flow rate ±0.2 (1.2). The robustness expressed as % RSD and the result were showed on Table.6

Table 4: Result from Recovery studies

Amount of drug added μg	Amount found in μg (n=5) Mean (±S.D.)	Mean recovery (%)
10	9.996(±0.06)	99.96
20	20.015(±0.08)	100.05
30	29.970(±0.05)	99.94

Results and Discussion

DDI standard having concentration 10 μg/ml was scanned in UV region 200-400 nm. The λ max of DDI was found to be at 250 nm. The DDI peak in this sample was identified to comparing with DDI standard and Retention time was found to be 5.17min. The estimation of different of DDI tablets were carried out by RP-HPLC using mobile phase having the composition of methanol: 0.01M sodium acetate buffer pH 6.5 (75:25). Finally filtered using 0.45μ membrane filters and degassed in sonicated for 10 min. The column was used as a Zorbax C-18 column. Flow rate of mobile phase was 1.2 ml/min. System suitability 6 replicated injections was found to be less than 2%, theoretical plate 4427, Tailing factor 1.47. The validation of developed method shows the drug stability is well within the limits. The linearity of detector response was found to be linear from 10 to 60μg/ml of targeted concentration for DDI standard with a correlation co-efficient value is 0.9998. The assay value of marketed tablet was found to be 98.9-101.2%. The accuracy limit is described by the percentage of recovery in the range of 99.96-100.06%. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy. The robustness of the analytical method was studied by different pH (6.5) ±0.5, ratio of mobile phase ±2 (75:25), flow rate ±0.2 (1.2). Robustness expressed as % RSD three replicate determinations was found with small variation in method parameter. The LOD and LOQ were studied by different concentration. All the parameters are showed in table. 6

Table 5: Result from Precision of the proposed RP-HPLC method

Didanosine Concentration (μg/ml)	Concentration of Didanosine (μg/mL) found			
	Intra-day		Inter-day	
	Mean (n=5)	% RSD	Mean (n=5)	% RSD
10	10.17	1.87	10.14	0.36
30	30.09	1.36	30.12	0.11

Table 6: Results from LOD, LOQ and ruggedness

Parameter	Didanosine
Calibration range (μg/ml)	10 to60
Theoretical plates	4427
Resolution	--
Tailing factor	1.47
LOD	1ppm
LOQ	3ppm
% Recovery of ruggedness Analyst-1	**99.06**
Analyst-2	**101.58**

Conclusion

RP-HPLC is at present one of the most sophisticated tools of analysis. The mobile phase consists of methanol: 0.01M sodium acetate buffer pH6.5 (75:25). The detection is carried out using UV detector set at 250 nm. The values of % RSD are less than 2.0 % indicating the accuracy and precision of the method the percentage recovery 99.96% to 100.05% for Didanosine. The proposed RP-HPLC method simple, precise and accurate, robust and linearity i.e, it follows Lambert-Beer's Law the determination of DDI tablet dosage form so it can be easily and conveniently adopted routine QC analysis for raw materials, formulations and also for dissolution studies.

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