Available online on 15.08.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Open Access Research Article

RP-HPLC Method Development and Validation for Determination of Didanosine in Pharmaceutical Dosage Forms

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ABSTRACT

To develop a simple, cheap, accurate, and rapid Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method and validate as per ICH guidelines for estimation of Didanosine in pharmaceutical dosage forms. The separation was conducted by using mobile phase consisting of methanol: water in the ratio (30:70). The wavelength was found at 246nm. Agilent 1220 Infinity LC with ezchrome software is used for chromatographic determination. The separation was conducted by using Zebra Eclipse XDB-C-18 (4.6×250×5µm) at the flow rate of 1.0 ml/min using variable wavelength detector. The developed method resulted in didanosine eluting at 4.650 min. The method was found to be linear over the concentration range 2-12µg/ml with coefficient regression R2-0.997. Mean recovery was found to be in the range of 99.99%, during accuracy studies. The limit of detection (LOD) and limit of quantitiation (LOQ) was found to be 5 mg/ml and 16 mg/ml respectively. A cheap, accurate, precise, linear and rapid RP-HPLC method was developed and validated for the quantitative estimation of Didanosine as per ICH guidelines.

Keywords:-RP-HPLC, Didanosine, Method Validation

Article Info: Received 18 June 2019; Review Completed 24 July 2019; Accepted 28 July 2019; Available online 15 August 2019

Cite this article as:

Gholve S, Gangapure S, Birajdar M, Mujewar I, Bhusnure OG, RP-HPLC Method Development and Validation for Determination of Didanosine in Pharmaceutical Dosage Forms, Journal of Drug Delivery and Therapeutics. 2019; 9(4-s):343-347 **http://dx.doi.org/10.22270/jddt.v9i4-s.3328**

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INTRODUCTION:

Didanosine chemically 2', 3'- dideoxyinosine (fig1) is an antiretroviral medication used to treat HIV/AIDS in combination with other medications as part of highly active antiretroviral therapy. An extensive review of the literature revealed a few analytical methods (1-12), was reported for the estimation of didanosine in dosage forms. Didanosine is a dideoxy analogue of the purine nucleoside inosine that potentially inhibits the replication of the human immunodeficiency virus. Analogue to other nucleoside inhibits, this compound also requires intracellular metabolism to the active triphosphate, 2', 3'- dideoxyinosine-5-triphosphate (ddATP), which act as a competitive inhibitors of HIV reverse transcriptase or as a DNA chain transmitter**. (13-14)** Validation is the process of providing documented evidence what it is intended to do. In other word the process of method validation ensures that the proposed analytical methodology is accurate, specific, reproducible and rugged for its intended use. Analytical techniques have different degrees of sophistication, sensitivity and selectivity, as well as, different cost and time requirements.

MATERIALS AND METHODS

Chemicals and Reagents

Water, Methanol, Acetonitrile of Analytical and HPLC grade purchased from Arti pharmaceuticals (Mumbai). Ammonium acetate buffer of AR grade purchased from Raj Chemicals (Latur).

Instrument

HPLC analysis was performed on Agilent 1220 Infinity LC with EZchrome software with variable wavelength detector. With made of Agilent technologies, A manually operating Rheodyne injector with 20µl sample loop was equipped with the HPLC system.,Zobrax Eclipse XDBC18 column (4.6×150×5µm), Electronic weighing balance BL-220 H

made of Shimatzu Corporation, Hot air oven made of Nisco Company, Sonicator made of the Ultrasonic's PCi Analytics sonicator.

METHODS:- Selection of Wavelength suitable wavelength for the HPLC analysis was determined by recording UV spectrum in the range of 200-400 nm for didanosine. Suitable wavelength selected was 246 nm (Figure 2).

Figure 1 Chemical structure of Didanosine

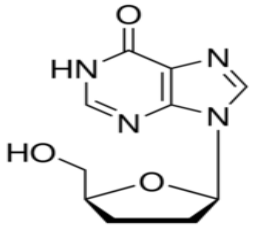


Figure 2 UV spectrum of Didanosine

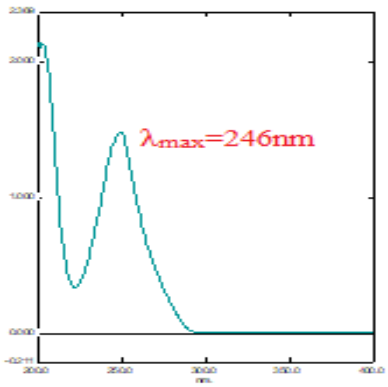


Figure 3 Typical Chromatogram of Blank solution

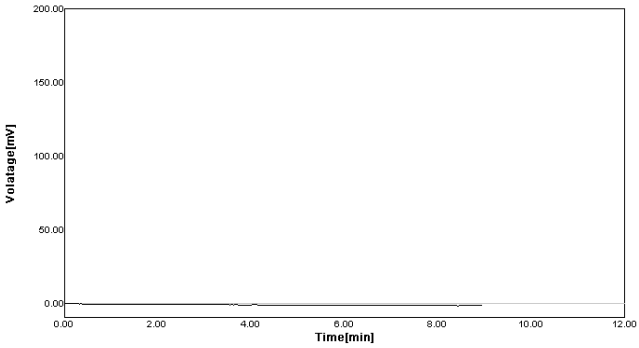


Figure 4 Typical chromatogram of the standard solution

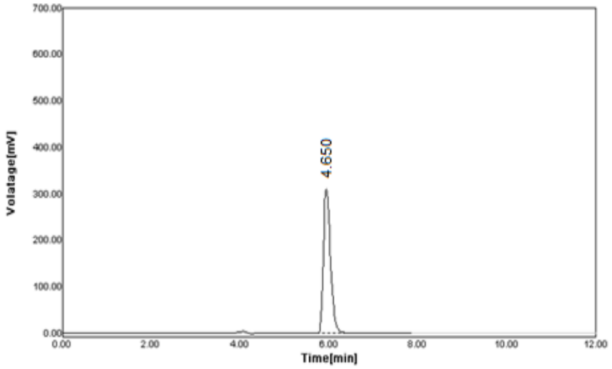


Figure5 Typical chromatogram for the tablet formulation

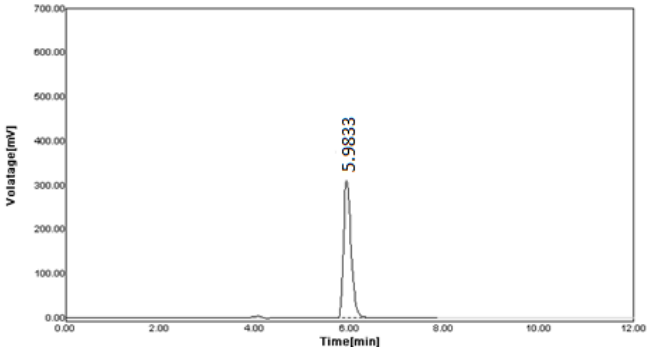
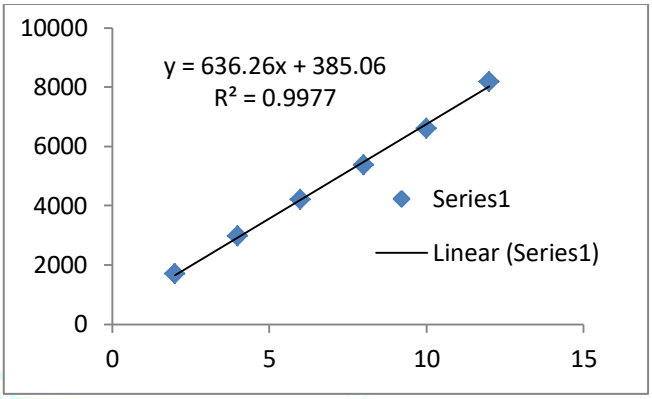


Figure 6 Calibration Curve



CHROMATOGRAPHIC CONDITIONS:

The developed method uses a reverse phase C18 column, Phenomena Gemini (C18 4.6×250×5µm), mobile phase consisting of a mixture of methanol: water in the ratio (30:70). The mobile phase was set at a flow rate of 1 ml/min and the volume injected was 20 µl for every injection. The detection wavelength was set at 246 nm.

Preparation Mobile Phase

A mixture of 30 volumes of Methanol, and 70 volumes of water was prepared (30:70). The mobile phase was sonicate for 10 min to remove gases.

Preparation of standard stock solution

Weigh accurately 10 mg of Didanosine drug in 100 ml volumetric flask and dissolve in 100 ml water.

Preparation of sample solution

20 Tablets (each tablet contains 10 mg of Didanosine) were weighed and taken into a mortar uniformly mixed. Test stock solutions of Didanosine (10µg/ml) and was prepared by dissolving weight equivalent to 10 mg of Didanosine and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter .after the filtration zsolicited for 5 min and dilute to 100 ml with mobile phase. Further dilutions are prepared in 5 replicates of 10µg/ml of Didanosine was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Calibration Curve

Appropriate aliquots of standard stock solution (1000µg/ml) was diluted to 100 mg/ml in 10ml volumetric flask and resultant solution was diluted up to the mark with mobile phase to obtain a final concentration of 2,4,6,8,10, and 12µg/ml. These solutions were injected into

chromatographic system. The chromatograms were obtained and peak area ratio was determined for each concentration of drug solution. Calibration curve of Didanosine were constructed by plotting peak area ratio vs applied concentration of Didanosine and regression equation was computed. The sample solution was chromatographic and concentration of didanosine in tablet samples was calculated using regression equation.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

A Reverse phase HPLC method was developed considering the system suitability parameters i.e. tailing factor (T), the number of theoretical plates (N), run time and the cost effectiveness. The optimized method developed resulted in the elution of Didanosine at 4.560 min. Figures 3&4 represent chromatograms of blank and standard solution (10µg/ml) respectively. The total run time is 10 minutes. System suitability tests are an integral part of method development and validation. System suitability tests are used to ensure adequate performance of the chromatographic system. System suitability parameters were evaluated for six replicate injections of the standard at working concentration. The results are given in Table 1.

Table 1 System suitability studies results

Parameters	Didanosine
Retention time (min)	4.650
Number Of Theoretical plates(N)	6245
Tailing factor (T)	1.6

Table 2 Calibration data for Didanosine

Sr. No	Concentration (µg/ml)	Area (µAU)
1	2	169062
2	4	298014
3	6	419805
4	8	537160
5	10	660275
6	12	818992

Table 3 Results of Accuracy studies for Didanosine

Recovery level	Accuracy Didanosine					
	Amount of taken	Area	Average Area	Amount recovered (mcg/ml)	% Recovery	Average % Recovery
80	2	169062	168764	1.94	96%	97%
	2	169072				
	2	168159				
100	6	419805	419310	5.94	98%	
	6	419710				
	6	418415				
120	10	660275	660750	9.8	97%	
	10	661165				
	10	660945				

Table 4 System precision results

Didanosine		
Injection No.	Rt	Area
1	4.631	670254
2	4.650	675148
3	4.550	670254
4	4.642	674545
5	4.551	676458
6	4.650	673654
AVG	4.612333	673385.5
SD	0.0484011	2591.362
%RSD	1.04938	0.4848

Table 5 Intraday precision results

Injection No.	Rt	Area
1	5	664257
2	5.01	664747
3	4.99	664346
4	4.99	664421
5	5.1	664237
6	5	664647
AVG	5.015	664452
SD	0.0423	210.216
%RSD	0.8623	0.31

Table 6 Inter day precision results

Injection No.	Rt	Area
1	4.655	664803
2	4.7	66472
3	4.95	66509
4	4.60	66429
5	4.90	66501
6	4.70	66519
AVG	4.7508	664850.5
SD	0.14072	326.039
%RSD	1.20	0.490

Table 7 Robustness results of Didanosine

Parameters		
Flow		
0.8mn/min	4.7283	672957
1.2ml/min	4.885	67296
Wavelength		
244	4.7866	67338.5
248	4.6866	674228.3

METHOD VALIDATION [15-16]

Authentification of the investigative method is the process that starts by laboratory studies in which the requirements of the performance properties of method are met for the intended analytical application. To the validation of analytical procedures RP-HPLC method developed was validated according to International Conference on Harmonization (ICH) and USP guidelines. Various parameters or criteria are used for the method of validification, such as linearity, accuracy, precision, system suitability, ruggedness, limit of Quantification (LOQ) and limit of Detection (LOD).

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of Didanosine. The solutions were injected six times and the parameters are calculated which are % RSD, peak tailing, resolution and USP plate count. The results are mentioned in Table 1. The standard chromatogram is shown in Fig. 4.

Specificity

Figures 3-5 are for the blank determination, the standard drug solution and sample chromatograph mixture discloses that the peaks acquired in the standard solution and sample solution at working concentrations are acquired only because of the drugs, this is the reason that blank determinations show no peak at the retention time of didanosine. Accordingly it can be concluded that, the method developed is said to be accurate and specific.

Linearity

Linearity the method was tested from 80-120 % of the targeted level of the assay concentration for analyte. Standard solutions contained 2-12 µg/mL of didanosine. Linearity solutions were injected in triplicate. The equations of the calibration curves for Montelukast sodium obtained were y = 02E+06X in the didanosine determination, the calibration graphs were found to be linear in the aforementioned concentrations with correlation coefficients 0.9978. The results are mentioned in the Table 2 & calibration curve Fig 6.

Accuracy

The precision was determined with the help of recovery experiments, by the verification with percentage mean recovery of sample at three different levels (80-120%). 20 blank tablets were powdered and mixed. This powder was then spiked with a quantity of didanosine corresponding to 80%, 100% and 120% of the labeled claim. Each of these powder mixtures was analyzed in triplicate and the quantity of Didanosine was determined using calibration equation. Accuracy was reported as 98 % of didanosine recovered. The results are mentioned in Table 3.

Precision

System precision

The value for %RSD (Relative Standard Deviation) was obtained less than 2 by six replicate injections of the standard solution at working concentrations, concerning the peak area for the drug. It designates the adequate reproducibility and hence the accuracy of the system. System precision results are tabulated in Table 4.

Method precision

The accuracy of procedure was determined by conducting assay of sample with the tests of (I) Repeatability (Intraday precision) and (II) Intermediate precision or ruggedness (Interday precision) completed within 3 successive daysbythree different analysts, at working concentration.

Repeatability (Intraday precision)

The value for %RSD (Relative Standard Deviation) less than 2 for six successive injections of the sample solution from the same homogenous mixture at working concentrations, concerning % assay for the drug which indicate that the method developed is method precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results (Table 5).

Intermediate Precision (Ruggedness / Inter day precision)

The value for %RSD (Relative Standard Deviation) less than 2 for six successive injections of the sample solution from the same homogenous mixture at working concentrations on three successive days by three unidentical analysts for % assay for the drug within and between days, which indicate the method developed is inter day precise / rugged (Table 6).

Robustness

The robustness of an analytical procedure is an estimate of its capacity to last unchanged by slight but intentional change in the analytical method parameters. To assess HPLC method robustness some measurable factors were intentionally changed. The factors comprise of variation of columns C8 (old & new), % of acetonitrile in the moving stage and acetonitrile of lots. Change in wavelength ± 2nm Change in flow rate ±0.2 ml/min. The results are mentioned in Table 7.

Limit of Detection (LOD)

The limit of detection (LOD) of methodological (analytical) process is defined as the concentration that produce instrument signal that is notably distinct from the blank determination. The IUPAC approach utilize the standard deviation of the intercept (Sa) for the spectroscopic techniques or other methods that depend upon a calibration curve for quantitative measurements, which may be related to LOD and the slope of the calibration curve, b. The limit of detection was found to be 5ng/ml

Limit of Quantification (LOQ)

The LOQ is the concentration that can be quantitate reliably with a specified level of accuracy and precision. The LOQ represent the concentration of analyte that would yield a signal-to-noise ratio of 10.The limit of quantification was found to be 15 mg/ml.

CONCLUSION

Method development and validation of model drug Didanosine was carried out under the circumstances of using mobile Water which give sharp peak and UV spectra also. A very few analytical method found in literature survey for the determination of Didanosine including FTIR, spectrophotometer. The develop UV and HPLC method was found to be simple, rapid, selective accurate, precise for the concurrent estimation of drug in dosage form. The method was evaluated in best condition, linear relation including coefficient of correlation, linearity, robustness, and accuracy, precision. The percent RSD for all parameter was found to be less than two which indicate the validity of method and assay result obtained by this method are in fair agreement. The force degradation studies performing in acidic, basic, neutral, Photo stability also performed.

All result obtained with proposed method confirm the suitability of these method for the analysis of pharmaceutical dosage form. The proposed method has been successfully applied for routine in process quality control.

ACKNOWLEDGMENT:

The authors take this opportunity thanks to Aarati Pharmaceuticals, Mumbai for providing gift sample. The authors would like to acknowledge Principal Dr. Vijayendra Swamy S. M., Principal, Channabasweshwar Pharmacy College, Latur for providing facilities for conducting research.

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