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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF DIDANOSINE IN BULKAND TABLET

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ABSTRACT
A simple, novel, accurate, precise, linear, rapid and economical HPLC method was developed for the estimation of Didanosine. The chromatographic separation was achieved using a Genesis 5µ C18 (100 x 4.60mm 5 µ) column and isoceatic elution, a mobile phase comprising of Methanol: 0.02MmAmmonium Formate (80: 20) at P^H2.8 was adjusted with water. The flow rate was 1.0 ml/min with detection at 240 nm using a UV detector and drug eluted with retention time of 2.60 min. The calibration curves were linear (r^2=0.999) in the concentration range of 0.2-1.4 µg/ml. The limit of detection and limit of quantitation were found to be 0.3548 and 1.0644µg/ml respectively. Thus the simple, novel, economical, accurate, precise and rapid HPLC method was developed for estimation of Didanosine and validated as per ICH guidelines. Hence the method holds good for routine analysis Didanosine in pure and pharmaceutical dosage form.

KEYWORDS: Didanosine, ICH guidelines, RP-HPLC, Validation.

INTRODUCTION
Didanosine is used for the treatment of infection with the human immunodeficiency virus (HIV). It is in class of drug called "reverse transcriptase inhibitor[1]. It works by decreasing the amount of HIV in the blood. It is used in combinations with other medication as part of highly active antiretroviral therapy[2] amount of HIV in the blood. It is used in combinations with other medication as part of highly active antiretroviral therapy[2].

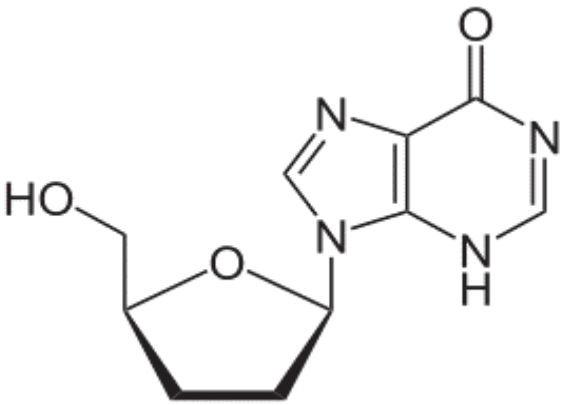

Figure. 1: Chemical structure of Didanosine.

DDI is chemically9-[(2R,5S)-5-(hydroxymethyl) oxolan-2-yl]-6, 9-dihydro-3H-purin-6-one. It has a molecular formula of $C_{10}H_{12}N_4O_3$ and molecular weight of 236.22728 g/mol. It has the structural formula (Fig.1).

DDI is a white crystalline powder which Sparingly soluble in water; slightly soluble in methanol and ethanol,Soluble in dimethylsulfoxide.

Literature Survey revealed that the drug has been estimated by UV[3-22] spectrophotometric, RP- HPLC[23-34] and HPTLC[35] methodhas been reported so far.

The aim of present work was to develop and validate a novel, rapid, simple, precise, and specific RP-HPLC method for estimation of Levofloxacin in its bulk and tablet dosage form.

MATERIAL AND METHODS
Instrumentation: Chromatographic separation was performed on a Shimadzu LC-20AD HPLC system comprising a PDA detector, Shimadzu LC-20AD pump and Genesis 5µ C18 (100 x 4.60mm, 5 µ) column.A manually operating Prominent auto sampler SIL-20 ACHT injector 20µl (20 µl injection valve) was used for injecting sample and standard solution. Data was compiled using Shimadzu LC Solution software.

Chemicals and reagents
- Didanosine pure form was obtained as gifted sample from pharma industryand its pharmaceutical dosage form Dedreto labelled claim 400mg(manufactured by Cipla Private limited)were purchased from local pharmacy, Mandya.

- Methanol, 0.02MmAmmonium formate and water are available in the Laboratory of Novetous pvt.limited, Bangolore.
- All the chemicals used in this investigation are HPLC grade.

Selection of mobile phase: Based on sample solubility, stability and suitability various mobile phase compositions were tried to get a good resolution and sharp peaks. The standard solution was run in different mobile phases. From the various mobile phases, Methanol:0.02MmAmmonium formate at P^H 2.8 (80:20 v/v) was chosen with detection wavelength 294nm, since it gave sharp peak with good symmetry within limits.

Preparation of mobile phase

Mobile phase was prepared by mixing Methanol: 0.02MmAmmonium formate at P^H 2.8 (80:20) This solution was sonicated for 10mins and filtered using a 0.2µ membrane filter.

Chromatographic conditions: The optimized parameters which were used as a final method for the estimation of Didanosine represented in the Table 1.

Preparation of standard stock solution

Weigh accurately about 100 mg of Didanosine pure drug and then transferred into 100ml volumetric flask, a portion of diluent is added and sonicated for 10 min to dissolve it completely. The volume is made up to the mark with diluent (stock solution-1). From the above solution pipette out 1.0 ml into 10 ml volumetric flask and made up to the mark with diluent (stock solution-2), From the above solution pipette out 1.0 ml into 10 ml volumetric flask and made up to the mark with diluent (stock solution-3), from this solution pipette out 0.2, 0.4, 0.6, 0.8 1.0,12,and14 ml individual volumetric flask and add diluent up to the mark, this gives0.2, 0.4, 0.6, 0.8 1.0,12,and14µg/ml concentrations.

Preparation of sample solution

Ten tablets of Didanosine 400 mg were weighed and powdered, the primary stock solution was prepared by dissolving a weight equivalent to 100 mg of Didanosine in 100ml volumetric flask by using HPLC grade methanol and the solution was sonicated for 10 minutes. The concentration of this solution gives (1000mg/ml). This solution was filtered using a 0.2µ membrane filter. The above solution 10ml was pipetted out into a 100ml volumetric flask and the volume was made up to the mark with Methanol, and used for the analysis in RP-HPLC method, all the dilutions are made by Methanol.

Flow rate selection: Different flow rates in between 0.98 to 1.02 ml/min were studied. A flow rate of 1.0 ml/min gave an optimal signal to noise ratio with a reasonable separation time.

RESULTS AND DISCUSSION

Resulted diagrams and tabular columns

Table. 1: Optimized chromatographic conditions.

Mobile phase	**Methanol: 0.02Mm Ammonium formate at P^H 2.8 with (80:20v/v)**
Stationary phase	Genisis 5µ C18 (100 x 4.60mm, 5 µ)
Wavelength	240nm
Run time	5min
P^H of mobile phase	2.60
Injector	(100 x 4.60mm, 5 µ)
Flow rate	1.0 ml per min
Injection volume	20 □l
Temperature	Ambient
Mode of operation	Isocreatic gradient elution

Table. 2: Results of System suitability studies.

System suitability parameters	**Acceptance criteria**	**Results**
Retention time(tR)	-	2.60
Number of theoretical plates(N)	N=≥ 2000	8859
Asymmetry	K=≤ 2.0	0.30
Area	-	94300
Tailing factor(T)	T=≤ 2.0	1.20

Table. 3: Linearity data.

Sl. No	**Concentation in µg/ml**	**Peak area**
01	2	61373.99
02	4	113108.2
03	6	161368.3
04	8	207338.9
05	10	256923.6
06	12	304166.6
07	14	352806.9

*indicates average of six determinations,

Table 4: Regression parameters.

Regression parameters	**Didanosine**
Regression Equation	Y=24142x+15017
Slope (b)	24142
Intercept (a)	15017
Correlation Coefficient (r^2)	0.999

Table. 5: Results of Precision studies.

Sl. No	Concentration (µg/ml)	Intraday precision (area)	Inter-day precision (area)
1.	4	82647.572	79881.230
2.	4	80537.068	79871.746
3.	4	79942.762	79061.621
4.	4	79782.281	79695.010
5.	4	79678.742	80647.532
6.	4	79869.748	79452.982
Mean		80409.7	76768.35
Std. dev.*		1136.82	529.281
%RSD		1.41	0.6894

*indicates average of six determinations, RSD indicates relative standard deviation

Table 6: Results of Accuracy studies

Level of recovery	Amount of formulation	Amount of Pure drug	Total amount of drug	%recovery	Mean
80	4	2.4	6.3	100.63	1.7230
100	4	4	8.0	100.73	1.9603
120	4	5.6	9.6	99.115	1.1315

Table. 7: Results of Robustness studies.

Concentration (0.6µg/ml)			
Parameters	**Factors**	**Mean area* ±Standard deviation**	**%RSD**
P^H	2.5	206880.9±3830.64	1.8516
	2.8	208444.1±2715.373	1.3026
	3.0	207468.8±2442.18	1.1771
Temprature	29	208209.7±3126.732	1.5017
	30	205832±2692.831	1.3082
	31	207512.3±3747.83	1.8060
Flow rate (ml/min)	0.98	209497.4±3175.316	1.5156
	1.00	208863.8±2980.602	1.4270
	1.02	208348.4±3865.903	1.8554

*indicates average of six determinations,

Table 8: Results of Ruggedness studies

Analysts	Mean area* ± Standard deviation	%RSD
Analyst 1	208770.54±2500.416	1.1976
Analyst 2	210565.73±1438.494	0.68312

*indicates average of six determinations.

Table. 9: Results of LOD and LOQ.

Parameters	Results
LOD (µg/ml)	0.3548
LOQ (µg/ml)	1.0644

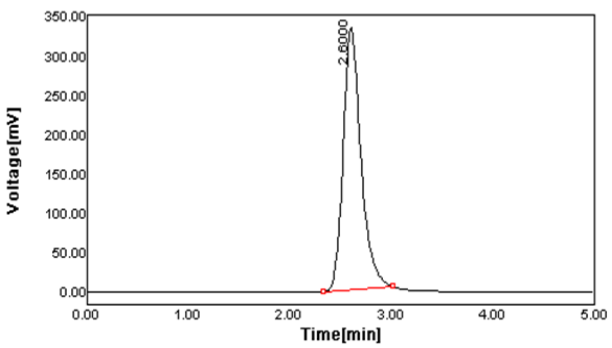

Fig. 2: Chromatogram of Didanosine.

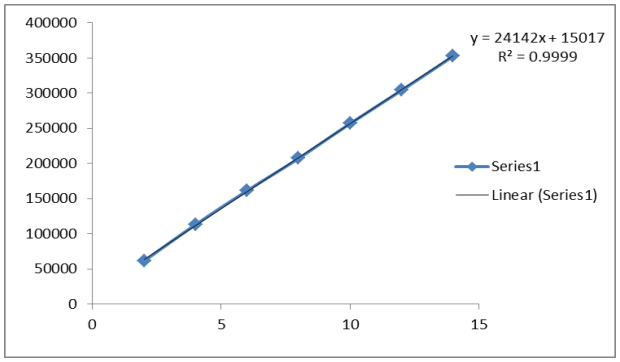

Fig. 3: Linearity graph of Didanosine.

System suitability

20 µl of the standard solution was injected under optimized chromatographic conditions to evaluate thesystem suitability. Parameters such as number of

theoretical plates (N), tailing factor (T), retention time (tR), asymmetry and area were determined. The obtained values indicate good performance of systemshown in Fig-2. The values of system suitability parameters were shown in Table- 2.

Method validation: The method is validated according to the ICH guidelines[36-38].

Specificity: Specificity of the HPLC method was checked for interference of impurities, degradants or excipients in the analysis of sample solution and was determined by injecting a volume of 20µl sample solution and the chromatogram was recorded. There is no interference of impurities, excipient on the peak of Didanosine indicating the high specificity of method.

Linearity and Range: Calibration curve was plotted for different concentrations of working standards prepared from standard drug solution of pure drug, shown in Fig-3 and showed linearity over a concentration range of 0.2-1.4µg/ml shown in Table-3, along with regression parameters in Table-4. Each calibration was injected six times. The calibration curve was performed in six replicates.

Precision: The precision of the analytical method was determined by intraday and interday precision. The sample solution was prepared as per the test method. In intraday precision, the same concentration of sample solution was injected 6 times in the same day and in interday precision, injecting six solutions of same concentration for six different days in a week. The results of precision were tabulated in table-5. The average and standard deviation of mean area were taken and %RSD was calculated and reported. %RSD values were within the limits and the method was found to be precise.

Accuracy: The accuracy of the method was determined by recovery studies by the determination of % mean recovery of the drug at three different levels (80%, 100% and 120%). At each level, three determinations were performed. A known amount of standard pure drug was added to preanalyzed tablet powder and the sample was then analysed by developed method. Results of recovery studies were reported in table-6. The observed data were within the range, which indicates good recovery values.

Robustness: The robustness of analytical method was carried by varying the parameters deliberately from the optimized chromatographic conditions like P^H of mobile phase (variation in ± 0.1 units), flow rate (variation in ± 0.02ml/min.), wavelength (variation in ± 2 nm).The observed results were within the limit. The results were shown in table-7.

Ruggedness: Ruggedness was determined between different analysts. The value of %RSD was found to be <2, showed ruggedness of developed analytical method. The values were shown in Table-8.

Limit of detection and Limit of quantitation

The LOD and LOQ of the present method were calculated based on standard deviation of the response and slope of linearity curve. LOD and LOQ values of Didanosine were shown in Table-9.

CONCLUSION

From the above it can be concluded that all validation parameters (precision, accuracy, linearity, LOD, LOQ and Ruggedness) met the predetermined acceptance criteria as mentioned in ICH guidelines. The developed HPLC method is simple, rapid, accurate, precise and shown good linearity. Hence it can be applied for routine analysis of Didanosine in bulk and its dosage forms.

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