DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DOLUTEGRAVIR IN BULK AND SOLID DOSAGE FORM

[1]CHANDRAMA DHERANGE, [2]KANCHAN CHAUHAN
[1] Department of Quality Assurance, [2] Department of Pharmaceutical Chemistry,
M.C. E. Society's Allana College of Pharmacy, Pune-411001 Maharashtra, India.

ABSTRACT:A new simple, precise, selective, accurate and rapid reverse phase high performance liquid chromatographic method has been developed and validated for drugdolutegravir in bulk and pharmaceutical dosage form. The column used for development was IntersilC-18, ODS-3, 5µm 4.6×250mm particle size and the mobile phase used aspH 3.6Phosphate buffer: Acetonitrile in ratio of (40:60) v/v with flow rate of 1ml/min. The wavelength used for detection was 258 nm. The limit of detection and limit of quantification was found to be 2.70 and 8.19 µg / mlwhich demonstrated that the method is sensitive. The method was found to be linear and correlation coefficient obtained was 0.9996.The system suitability parameters were found to be within the limits.

Key words: Dolutegravir, high performance liquid chromatography

INTRODUCTION

Dolutegravir chemically known as (4R,12aS)-N-(2,4-difluorobenzyl)-7-hydroxy-4-methyl-6,8-dioxo 3,4,6,8,12,12ahexahydro- 2H-pyrido[1',2':4,5]pyrazino[2,1-b][1,3]oxazine-9- carboxamide, with molecular formula is $C_{20}H_{19}F_2N_3O_5$.[1] It is antiretroviral drug and is used to treat HIV/AIDS.[2-4]It inhibits HIV integrase enzyme by binding to the active site and blocking the strand transfer step of retroviral DNA intergration in the host cell.[5]The strand transfer step is essential in the HIV replication cycle and results in the inhibition of viral activity.[6]The structure of Dolutegravir dug is shown in figure no.1.[7] Literature survey revealed that few methods were available for development and validation of dolutegravir alone or in combination.[4-13]The objective of the present study is to develop and validate a Reverse Phase High Performance Liquid Chromatographic method for the determination of Dolutegravir in pharmaceutical dosage form which requires less time and minimum solvent consumption.

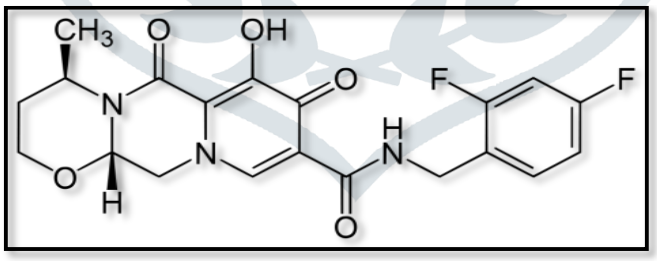

Figure no. 1: Structure of Dolutegravir

MATERIALS AND METHODS

Dolutegravir standard drug was kindly supplied by Emcure Pharmaceuticals Ltd., Pune as free gift sample. Dolutegravir tablet consist of 50mg drug, Batch no.E16DV18014 was purchased from local market. Throughout study HPLC grade solvents were used.

HPLC INSTRUMENTATION

HPLC system used for the study was equipped with UV detector (Jasco Model/PU 2080/UV2075 PLUS) Borwin software was used. The column used was Intersil ODS-3 C-18. Ultra SonicatorEnertech (Fast Clean Ultrasonic Cleaner) was used to dissolve the drug completely. pHAnalyzer of (Chemiline CL 180 µc based pH meter) was used to detect the accurate pH of the mobile phase. Prior to injection, the column was equilibrated for at least 30 min with mobile phase flowing through the system. The eluents were monitored at 258 nm.

PREPARATION OF STANDARD SOLUTION

Accurately weighed 10 mg standard dolutegravir drug was taken and dissolved in methanol and final volume was made up to 100 ml of methanol to produce a standard stock solution (100 µg / ml). Standard stock solution aliquots is pipetted out and diluted with methanol. Solutions were mixed well and filtered through a 0.45µ membrane filter.

PREPARATION OF SAMPLE SOLUTION

20 tablets were weighed and the average weight of each tablet was calculated, the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 3/4th volume of diluent added and sonicated for 30 min, further the volume was made up with diluent and the solution was filtered through 0.45µ filter paper.

DEVELOPMENT AND OPTIMIZATION OF METHOD

The mobile phase consists of Phosphate buffer : Acetonitrile in the ratio of 40:60% v/v with pH 3.6 was filter through 0.45µ of nylon membrane filter paper. It was degassed by ultrasonication and was pumped from solvent reservoir in the ratio of 40:60% v/v to the column in the flow rate of 1.0 ml/min whereas run time set was 10 min. The separation was performed on Intersil ODS-3 C-18 and the volume of each injection was 20 µl.

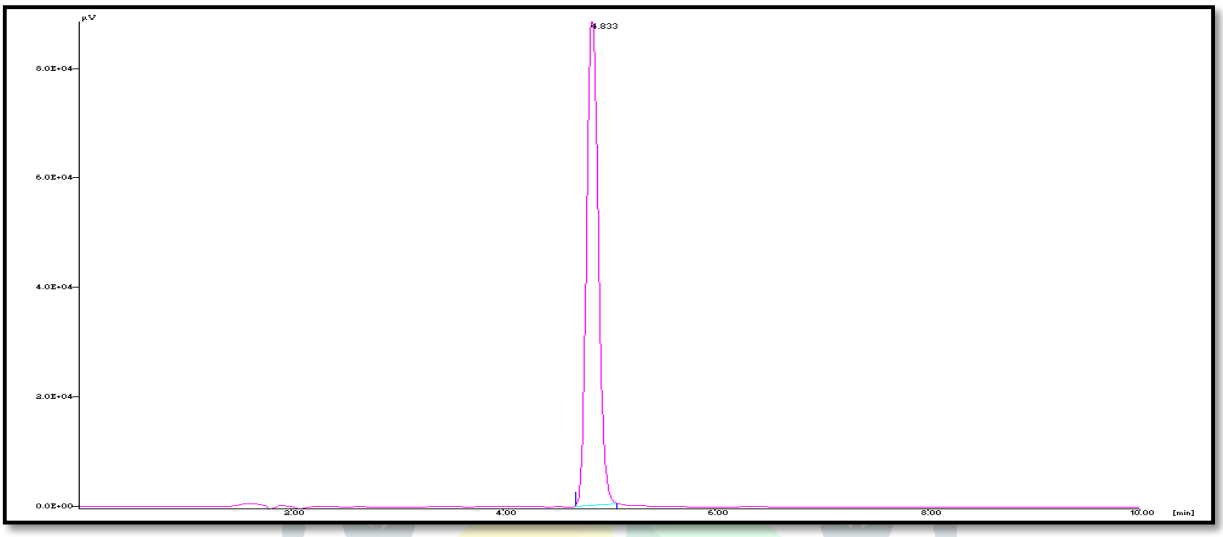

Figure no. 2: Chromatogram of Dolutegravir

VALIDATION OF PROPOSED METHOD

The developed and optimized method has been validated according to the guidelines of the ICH (International Conference on Harmonization)[14] concerning system suitability, precision, specificity, linearity, accuracy, detection limit of detection and limit of quantification. Optimized Chromatographic conditions and system suitability parameters of developed method for Dolutegravir are given in table 1.

Table no. 1: Chromatographic parameters of Dolutegravir

Parameters	Chromatographic Conditions
Column	Intersil ODS-3C-18 5µm 4.6×250mm particle size
Mobile Phase	Phosphate buffer : Acetonitrile (40:60) pH(3.6)
Concentration Of Standard Solution	30 µg/ml
Detection Of Wavelength	258nm
Flow Rate	1ml/min
Run Time	10min
Retention Time	4.833

RESULT AND DISCUSSION

Linearity

Linearity of drug was determined by taking 10 mg accurately weighed standard drugdolutergravir and it was transferred to 100ml volumetric flask and volume was madeup by methanol to get the concentrationof 100µg/ml. From the stock solution 10, 20, 30, 40, 50 µg/ml working solutions were pipetted out and volume was madeupto 10ml by mobile phase.For each concentration level, 20 µl of each sample was injected into the system three times, and calibration curve was constructed by plotting the peak area versus concentration

of the drug.The data of calibration curve were shown in Table no. 2 and calibration curve is shown in Fig. no. 4. The overlay chromatogram of linearity study is shown in Fig 3.

Table No 2: Linearity of drug (n=3)

Concentration µ/ml	Area	Mean	SD	% RSD
10	628528	628530	1.632	0.0002
	628530			
	628532			
20	1351952.25	1351952.25	597.438	0.0442
	1350762.1			
	1351275			
30	2004521.25	2023821.25	243.5058	0.0012
	2004431.25			
	2004061.75			
40	2766064.75	2766064.75	325.3793	0.0117
	2766387.2			
	2766715.5			
50	3415903.5	3415906.5	55.1573	0.0016
	3415906.5			
	3415909.5			

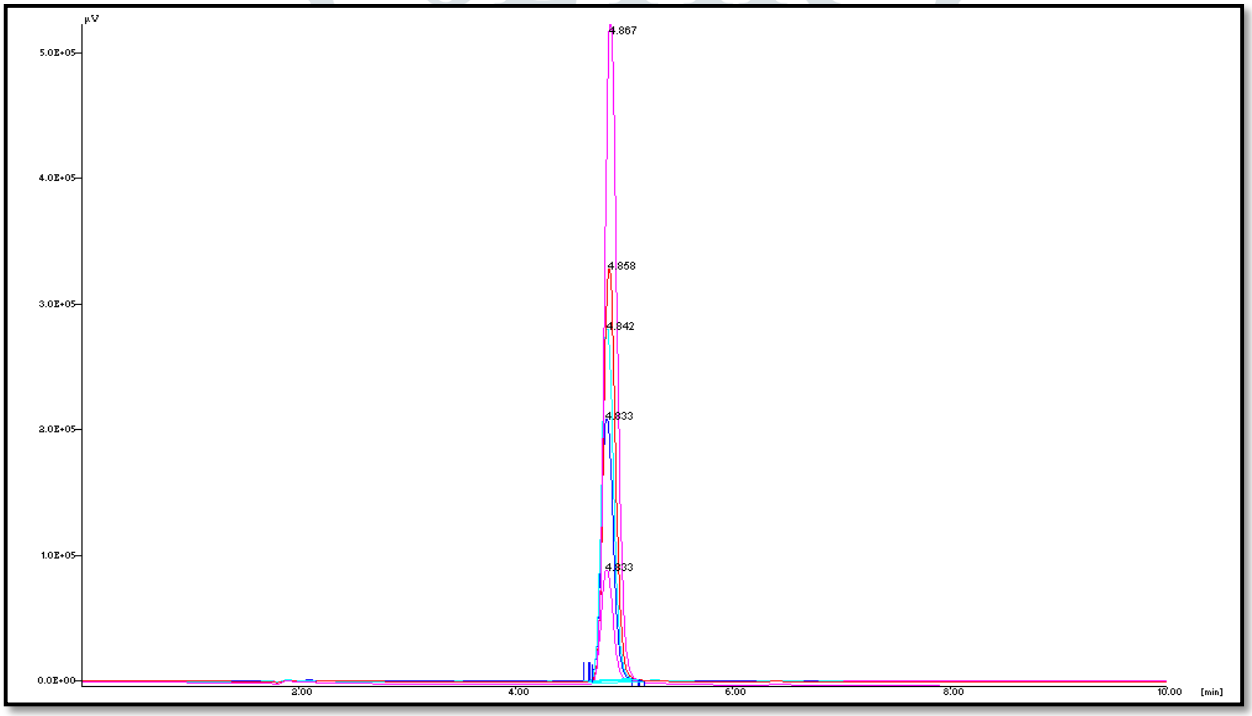

Figure no. 3: Dolutegravir linearity study

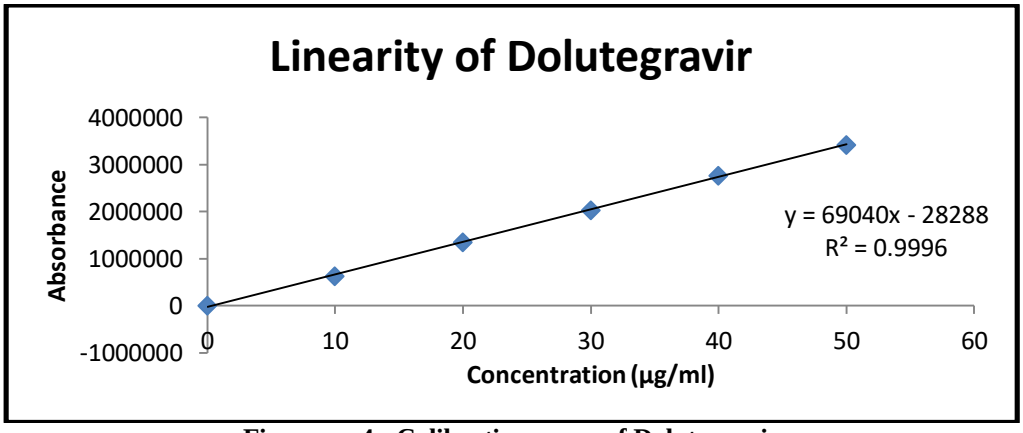

Figure no.4 : Calibration curve of Dolutegravir

Table No 3: Regressiondata of Dolutegravir

Slope	69040
Intercept	-28288
Regression	0.9996

Assay

Twenty tablets of dolutegravir were weighed; their average weight was determined and it is finely powdered. Powder equivalent to 50 mg Dolutegravir of was accurately weighed and dissolved in small amount of methanol. From the solution of concentration 100 µg/ml. Aliquot of 2 ml was diluted to 10 ml using methanol. The absorbance of sample solution was measured at wavelength 258nm. Results are shown in table no.4

Table No.4: Assay

Dolutegravir in tablet (mg)	**Amount injected (µg/mL)**	**Amount found (µg/mL)**	**% Recovery± SD**
50	20	19.95	99.75±234.76

Precision

For a number of measurements under the same analytical conditions, precision is the indicator of closeness of the data values to one another.The intraday and inter day precision study of Dolutegravir was carried out by estimating the corresponding responses six times on the same day and two different days. A solution of concentrations 20ppm, 30ppm and 40ppm for Dolutigravir were used. Results are shown in table no.5

Table no. 5: Precision Repeatability(n=3)

Precision Repeatability	**Concentration of drug (µg/ml)**	**Mean Area±SD**	**% RSD**
Intra-day	20	1351329.45±597.43	0.044211192
	30	2004338.083±243.50	0.012148939
	40	2766389.15±325.37	0.01176188
Inter-day	20	1351181.947±169.47	0.012542354
	30	2004037.237±58.26	0.002907282
	40	2766350.717±62.37	0.002254707

Accuracy

To determine the accuracy of sample preparation, standard addition method was used for measuring the recovery of drug. A fixed amount of sample was taken and standard drug was added 80%, 100% and 120% levels. The mean % recovery of Dolutegravir was found to be 99.6111%,100.066%and 99.7878%. The results were analyzed and were found within the limits. The accuracy results are shown in Table no.6

Table No. 6: Accuracy (n=3)

% level	**AmountAdded (µg/ml)**	**Amountrecovered (µg/ml)**	**%Recovery**
80	54	53.79	99.6111
100	60	60.04	100.066
120	66	65.86	99.7878

Limit of Detection and Limit Of Quantification

The quantitation limit is considered as the lowest concentration of an analyte in a sample that can be determined with the acceptable precision and accuracy under the stated operational conditions of the method. The LOD and LOQ values obtained for Dolutegravir are shown in table no.7

Table no. 7 :Method sensitivity

Analyte Name	**Signal to Noise ratio values**	
	LOD	**LOQ**
Dolutegravir	2.7042	8.1947

Robustness

Robustness is carried out bychanging the parameters from the optimized chromatographic conditions such as changes in flow rate, mobile phase and different column.Such small changes in the optimized method showsvery little change in the results. The degree of reproducibility of the results proven that the method is robust. The results are given in Table no.8

Table no. 8: Robustness Study (n=3)

Parameters	Level	% AREA	% RSD
Flow rate ml/min	0.8	2093949.25	1.874
	0.9	2090147.5	1.237
	1	2023821.25	1.548
Wavelength	256	2011827	0.333
	258	2023821.25	0.871
	260	2010801.72	0.426
Columns from different manufacturers	Inertsil	2023821.25	0.095
	Thermosil	2023821.25	0.071

SUMMARY AND CONCLUSION

The developed chromatographic method was simple and reliable for quantification of Dolutegravir from bulk and pharmaceutical dosage form which requires less time and less mobile phase consumption. %RSD values for accuracy and precision studies obtained were not more than 2.0% which revealed that developed method was accurate and precise. The validatedHPLC method was found to be robust and can be successfully applied to estimateDolutegravir in Bulk and Pharmaceutical dosage form in routine analysis.

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CONFLICT OF INTEREST: Declared None.

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