METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF DOLUTEGRAVIR IN BULK MATERIAL AND IN TABLET DOSAGE FORM

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Abstract: High Performance Liquid Chromatography (HPLC) is one of the effective separation analytical tools to determine and quantitate the impurities. By using HPLC, we can separate a mixture of compounds to identify and quantify into individual components. In literature survey, various quantification methods are available for the determination of dolutegravir in combined dosage form. The present research paper describes a simple, accurate and precise reversed-phase high performance liquid chromatography (RP-HPLC) method for quantification of dolutegravir in bulk and pharmaceutical dosage form (tablets). This method is validated for different analytical performance parameters like linearity, precision, accuracy, limit of detection, limit of quantification, and robustness were determined according to the International Conference of Harmonization (ICH) Q2B guidelines. Detection was carried out at 256 nm. The mobile phase used as pH-3.6 phosphate buffer:acetonitrile in ratio of (40:60) v/v with flow rate of 1 ml/min. All the parameters of validation were found in the acceptance range of ICH guidelines. 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. The proposed method was validated in terms of linearity, range, accuracy, precision, specificity, robustness and stability studies and the method is successfully applies to the estimation of dolutegravir in tablet dosage form. Hence the developed method can be adapted to regular quality control analysis.

Keywords: Reversed-Phase High-Performance Liquid Chromatography, dolutegravir, validation, linearity, accuracy.

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INTRODUCTION

Analytical chemistry is the science that seeks ever improved means of measuring the chemical composition of natural and artificial materials. Chemical composition is the entire picture (composition) of the material at the chemical scale and includes geometric features such as molecular morphologies and distributions of species within a sample as well as single dimensional features such as percent composition and species identity. The pharmaceutical analysis comprises the procedures necessary to determine the "identity, strength, quality and purity" of such compounds. It also includes the analysis of raw material and intermediates during manufacturing process of drugs[1-3].

Dolutegravir marketed name as Tivicay is an anti-retroviral medication used together with other medication to treat human immunodeficiency virus (HIV)-acquired immune deficiency syndrome. It may also be used, as part of post-exposure prophylaxis to prevent HIV infection following potential exposure. It is taken by mouth. Dolutegravir is an HIV integrase strand transfer inhibitor which blocks the functioning of HIC integrase, which is needed for viral replication[4,5].

Dolutegravir is an orally bio-available integrase strand-transfer inhibitor (INSTI). It inhibits HIV integrase by binding to the active site and blocking the strand transfer step of retroviral DNA integration in the host cell. The strand transfer step is essential in the HIV replication cycle and results in viral activity inhibition. Dolutegravir is an FDA approved drug for the treatment of HIV infection. If administered orally, it has a half-life of approximately 15 h[6-7].

The IUPAC name of dolutegravir is chemically (4R,12aS)-N-(2,4-difluorodolutegravirzyl)-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2H-pyridol [1',2',4,5] pyrazino [2,1-b][1,3] oxazine-9-carboxamide with molecular formula is

$C_{20}H_{19}F_2N_3O_5$. Dolutegravir is a prescription medicine approved by the U.S. Food and Drug Administration (FDA) for the treatment of HIV infection in adults and children 12 years of age and older and weighing at least 40 kilograms. Dolutegravir is always used in combination with other HIV medicines. It is a discovery of the second-generation integrase stand transfer inhibitor because of the collaborative efforts of scientists working for Shionogi (Japan) and GlaxoSmithKline (UK). Dolutegravir is a white to light yellow powder is slightly soluble in water and is freely soluble in methanol. In total, 34% of the dolutegravir dose is absorbed and excreted with the feces and urine; another 33-48% is involved in enterohepatic recirculation; and a further portion is secreted in bile[8-11]. The structure of dolutegravir was illustrated in Figure 1.

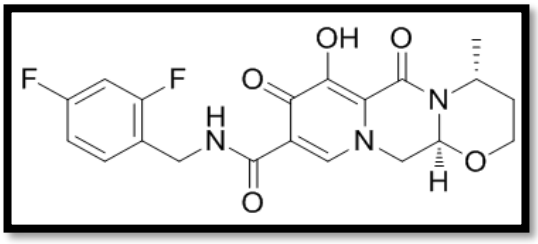

Figure 1: Molecular structure of dolutegravir

High Performance Liquid chromatography is one of the effective separation analytical tools to determine and quantitate the impurities. By using HPLC, we can separate a mixture of compounds to identify and quantify into individual components. In literature survey, various quantification methods are available for the determination of dolutegravir in combined dosage form. The main objective of the present research work is the development and validation of a method for the estimation of dolutegravir in bulk and tablet dosage form by RP-HPLC.

MATERIALS AND METHODS

Chemicals and reagents used

Dolutegravir standard drug (API) was supplied by Hetero Drugs Pvt. Ltd., Hyderabad, Telangana, India as gift sample. Dolutegravir tablet consist of 50mg drug, was purchased from local market near Uppal, Hyderabad, Telangana. And, throughout the study HPLC grade solvents were used.

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 Sonicator Enertech (Fast Clean Ultrasonic Cleaner) was used to dissolve the drug completely. pH Analyzer 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 256 nm.

Preparation of standard solution[11-15]

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 are 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.

Method development[16-20]

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.

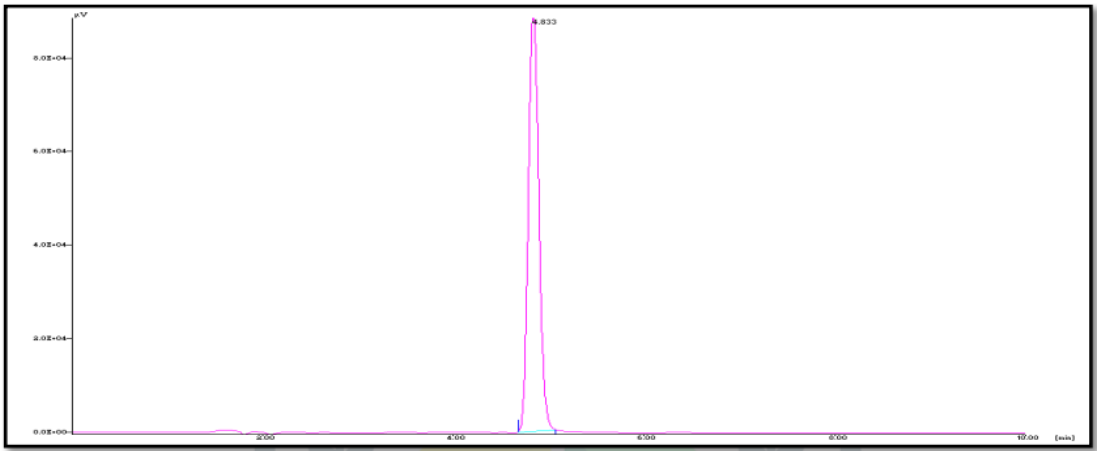

Figure 2: Chromatogram of dolutegravir

Method validation

System suitability

As per the test method, the standard solutions were prepared and injected into HPLC system from which the evaluated system suitability parameters are found to be within the limit.

Specificity

The specificity defined as the ability of the method to measure the analyte accurately and specifically in the presence of components present in the sample matrix was determined by analysis of chromatograms of drug free and drug-added placebo formulation.

Linearity

The ability of the method to produce results those are directly or indirectly proportional to the concentration of the analyte in samples within a given range.

Precision

The degree of closeness of the agreement among individual test results when the method is applied to multiple samplings of a homogeneous sample. It is a measure of either the degree of reproducibility (agreement under different conditions) or repeatability (agreement under the same conditions) of the method.

Accuracy

The closeness of results was obtained by a method to the true value. It is a measure of the exactness of the method.

Robustness

Robustness of the method was studied by slightly changes in experimental conditions such as flow rate and organic composition. This was performed by same analyst with same instrument.

Ruggedness

Ruggedness of the method was studied using different source of analysts, instruments, wavelengths, and columns with same experimental conditions.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The detection of limit and quantification limit for each analyte were determined based on a signal-to-noise concept, as the lowest concentration. The ICH indicates that LOD (which they call DL, the detection limit) can be calculated as LOD = 3.3Xσ/S, and the limit of quantification (which they call QL, the quantitation limit) LOQ = 10Xσ/S. Here σ is the standard deviation of the response and S is the slope of the calibration curve.

The developed and optimized method has been validated according to the guidelines of the ICH (International Conference on Harmonization) 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 illustrated in Table 1.

Table 1. Chromatographic parameters

Parameter	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
Wavelength	256 nm
Flow rate	1 ml/min
Run time	10 min
Retention Time	4.833

RESULTS AND DISCUSSION

Method validation: In this method, system suitability, linearity, precision, accuracy, robustness, limit of detection (LOD), limit of quantification (LOQ), and stability are validated for the selected dolutegravir drug.

Linearity: Linearity of drug was determined by taking 10 mg accurately weighed standard drug dolutegravir and it was transferred to 100ml volumetric flask and volume was made up by methanol to get the concentration of 100 µg/ml. From the stock solution 10, 20, 30, 40, 50 µg/ml working solutions were pipetted out and volume was made up to 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 2 and calibration curve is shown in Figure 4. The overlay chromatogram of linearity study is shown in Figure 3.

Table 2. Linearity of dolutegravir (n=3)

Conc. (µ/ml)	Area	Mean	SD	% RSD
10	628527	628530	1.632	0.0002
	628531			
	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			

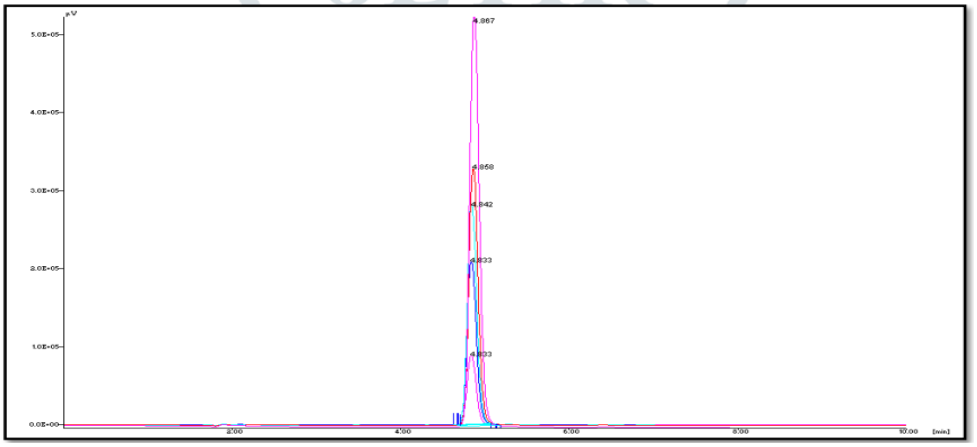

Figure 3. Linearity of dolutegravir

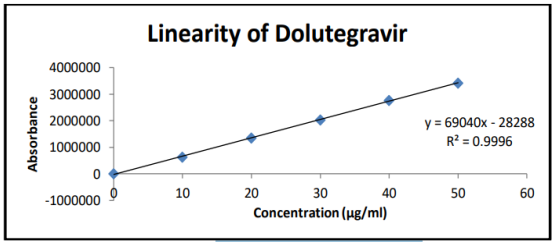

Figure 4: Calibration curve of dolutegravir

Table 3. Regression data of dolutegravir

Slope	69040
Intercept	-28288
Regression	0.9996

Assay of tablets

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 256 nm. Results are tabulated in Table 4.

Table 4: Assay of tablets

Dolutegravir in tablet	Amount injected	Amount found	% Recovery ± SD
50 mg	20 (µg/mL)	19.96 (µg/mL)	99.75 ± 234.76

Precision: For several 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 dolutegravir were used. Results are given in Table 5.

Table 5. Precision repeatability (n=3)

Precision repeatability	Concentration of drug (µg/ml)	Mean area ± SD	% RSD
Intraday	20	1351329.45±597.43	0.044211192
	30	2004338.083±243.50	0.012148939
	40	2766389.15±325.37	0.01176188
Interday	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 analysed and were found within the limits. The accuracy results are tabulated in Table 6.

Table 6. Accuracy (n=3)

% Level	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery
80	54	53.79	99.6111
100	60	60.04	100.066
120	66	65.86	99.7878

Robustness

Robustness is carried out by changing 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 shows very little change in the results. The degree of reproducibility of the results proven that the method is robust. The results are given in Table 7.

Table 7. Robustness (n=3)

Parameter	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	254	2011827	0.333
	256	2023821.25	0.871
	258	2010801.72	0.426
Columns from different manufacturers	Intersil	2023821.25	0.095
	Thermosil	2023821.25	0.071

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 8.

Table 8. LOD and LOQ

Drug	LOD	LOQ
Dolutegravir	2.7042	8.1947

CONCLUSION

The proposed RP-HPLC method was validated as per the International Conference on Harmonisation (ICH) Q2B Guidelines and was found to be applicable for routine quantitative analysis of dolutegravir by HPLC in pharmaceutical dosage form. The results of linearity, precision, accuracy, and specificity were proved to be within the limits. The method provides selective quantification of dolutegravir with no interference from other formulation excipients. The proposed method was highly reproducible, reliable, rapid, robust, and specific. 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 validated HPLC method was found to be robust and can be successfully applied to estimate dolutegravir in bulk and pharmaceutical dosage form in routine analysis. The present proposed methodology makes is cost effective which can be implemented for routine analyses in pharmaceutical industry.

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