Pharmacophore

ISSN-2229-5402

Journal home page: http://www.pharmacophorejournal.com

STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF RELATED SUBSTANCES FOR DOLUTEGRAVIR DISPERSIBLE TABLETS

Manikandan Velusamy[1]*, Srinivasan Nagarajan[1], Vinoth Rathinam[1], Murali[1]

1. *Department of Pharmacy, Faculty of Engineering and Technology, Annamalai University, Annamalai Nagar, Chidambaram, Tamilnadu, India.*

ARTICLE INFO

Received:
18 Feb 2022
Received in revised form:
10 Apr 2022
Accepted:
14 Apr 2022
Available online:
28 Apr 2022

Keywords: Dolutegravir, Method development, Validation, Related substances, RP-HPLC

ABSTRACT

A simple, rapid, and robust reverse phase HPLC method was developed and validated for the determination of related substances for Dolutegravir dispersible tablets 10mg. The primary goal of this research is to develop and validate a new RP-HPLC method for validating the amount of Impurity B (degradation impurity) as a related substance following USP guidelines. Dolutegravir and its impurities were separated using chromatographic conditions on a Phenyl-Hexyl (250 × 4.6 mm), 5µ column with a 45 % buffer (sodium dihydrogen phosphate dihydrate and EDTA): 49 % methanol: 6 % acetonitrile mixture and a pH of 2.5 ± 0.05 adjusted with orthophosphoric acid. The flow rate in isocratic elution mode was 1.2 mL/min. The column temperature was kept constant at 35°C, and the eluted compounds were measured at a wavelength of 258 nm using the PDA detector. According to USP guidelines, the developed method was tested and found to be stability-indicating, specific, rugged, precise, linear, accurate, and robust, with a high resolution and shorter retention time. The system suitability and other validation parameters were found to be within the limits. The method was sensitive because the LOD and LOQ demonstrate its sensitivity. The linearity curves for Dolutegravir and Impurity B were found to be linear, with a correlation coefficient of at least 0.997. The average percentage of impurities recovered ranged between 80 % to 120 %. As a result, the proposed method was found to be good and accurate for the quantitative determination of related substances associated with Dolutegravir dispersible tablets 10mg.

To Cite This Article: Velusamy M, Nagarajan S, Rathinam V, Murali. Stability Indicating RP-HPLC Method Development and Validation of Related Substances for Dolutegravir Dispersible Tablets. Pharmacophore. 2022;13(2):56-64. https://doi.org/10.51847/2MarzXSiL2

Introduction

Dolutegravir belongs to a class of HIV medications called "integrase inhibitors." Integrase inhibitors inhibit the activity of an enzyme in HIV called integrase. Integrase inhibitors prevent HIV replication by inhibiting the activity of integrase. This has the potential to reduce the amount of HIV in the body [1-4]. Dolutegravir is a prescription drug that, according to the US Food and Drug Administration (FDA), can be used in conjunction with Rilpivirine to treat HIV infection (brand name: Edurant). The chemical structure of Dolutegravir shown in **Figure 1**, and the chemical name for dolutegravir is sodium salt of (4R,12aS) – N – (2,4-difluorobenzyl) – 7 – hydroxy – 4 – methyl – 6,8 – dioxo – 3,4,6,8,12,12a – hexahydro – 2H pyrido [1',2':4,5] pyrazino [2,1-b] [1,3] oxazine – 9 – carboxamide. It has the molecular formula $C_{20}H_{19}F_2N_3O_5Na$ and a molecular weight of 419.38 g/mol.

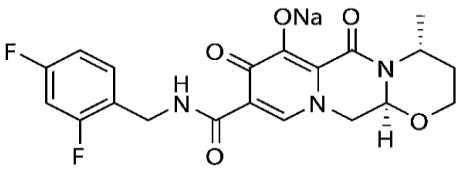


Figure 1. Structure of Dolutegravir Sodium

Corresponding Author: Manikandan Velusamy; Department of Pharmacy, Faculty of Engineering and Technology, Annamalai University, Annamalai Nagar, Chidambaram, Tamilnadu, India. E-mail: saravanamani481@gmail.com.

Dolutegravir (DTG), also known as Tivicay, is an antiretroviral drug used to treat HIV/AIDS in combination with other medications. It can also be used as part of post-exposure prophylaxis to protect against HIV after a possible exposure. Impurity profiling of drug ingredients and products is a critical regulatory activity in the pharmaceutical sector. Even trace levels of unknown impurities or unwanted solvents might alter the effectiveness of a drug and produce unwanted side effects [5-7]. To determine the impurity profile of the drug material and product, a stability-indicative analytical approach should be applied. High output rates one of the most effective methods for detecting impurities and determining their abundance is liquid chromatography. Using HPLC, we can separate a bunch of compounds into their constituent parts, allowing us to determine what each one is and how much of it. There is no literature available for the determination of impurities in Dolutegravir drug products. In this present research work, stability-indicating new RP-HPLC method for quantifying the related substances present in the Dolutegravir drug product. As a result, the proposed method can find to be good and accurate for the quantitative determination of related substances associated with Dolutegravir dispersible tablets 10mg.

Materials and Methods

Materials

Dolutegravir sodium standard and related impurities were procured from Jigs Chemicals, Ahmedabad. Methanol and Acetonitrile with HPLC grade procured from Rankem India Pvt. Limited. Dolutegravir dispersible tablets of 10mg and pure Milli-Q water are used in this research work.

Analytical Method Development

This analytical method development for related substances for Dolutegravir dispersible tablets 10 mg was developed as per USP guidelines. Many trials have been carried out to determine the best final chromatographic conditions for Dolutegravir dispersible tablets 10 mg with different pH of the buffer and composition of methanol and acetonitrile using C-18, C-8, and Phenyl-Hexyl as stationary phase columns [8]. To ensure great resolution between all known and unknown degradation compounds, the Phenyl-Hexyl column was used. In this case, the Phenyl-Hexyl column provided unique selectivity for aromatic compounds when compared to other columns such as C-18 and C-8. Unlike, the standard Phenyl-Hexyl column allowed much greater retention and separation of the aromatic compounds through its extended hexyl hydrocarbon functional group. In this case optimized chromatographic conditions such as mobile phase was constituted by the mixture of 45% of buffer (sodium dihydrogen phosphate dihydrate and EDTA): 49% of methanol: 6% of acetonitrile and pH adjusted to 2.5 ± 0.05 with Ortho-phosphoric acid (Isocratic elution). The flow rate of the mobile phase was 1.2 mL/min. The column temperature was maintained at 35°C, and the eluted compounds were monitored at the wavelength of 258 nm (PDA detector). The optimized chromatograms are given **Figure 2**.

Optimized Chromatographic Conditions

Column details	Kinetex® 5 µm Phenyl-Hexyl 100 Å, (250×4.6) mm. Make Phenomenex, Part No: 00G-4603-E0.
Flow rate	1.2 mL/min
Injection volume	20 µL
Column Oven Temperature	35°C
Auto sampler Temperature	25°C
Wavelength	258 nm
Run time	20 minutes
Wash vial	Water: Acetonitrile (1:1) % v/v Respectively.

Mobile Phase/Diluent Preparations

Buffer Preparation

Weighed and transferred 1.56gm Sodium dihydrogen orthophosphate dihydrate and 100 mg EDTA into 1000 mL of water and mix well. Filtered the solution through a 0.45µm membrane filter. Adjusted the pH 2.50 ± 0.05 with Ortho-phosphoric acid and mixed well. Filtered the solution through a 0.45µ nylon filter.

Mobile Phase Preparation

Mixed buffer solution, methanol, and acetonitrile in the ratio of 45:49:6, % v/v/v respectively. Sonicated to degas for 5 minutes.

0.1M Hydrochloric Acid Solution

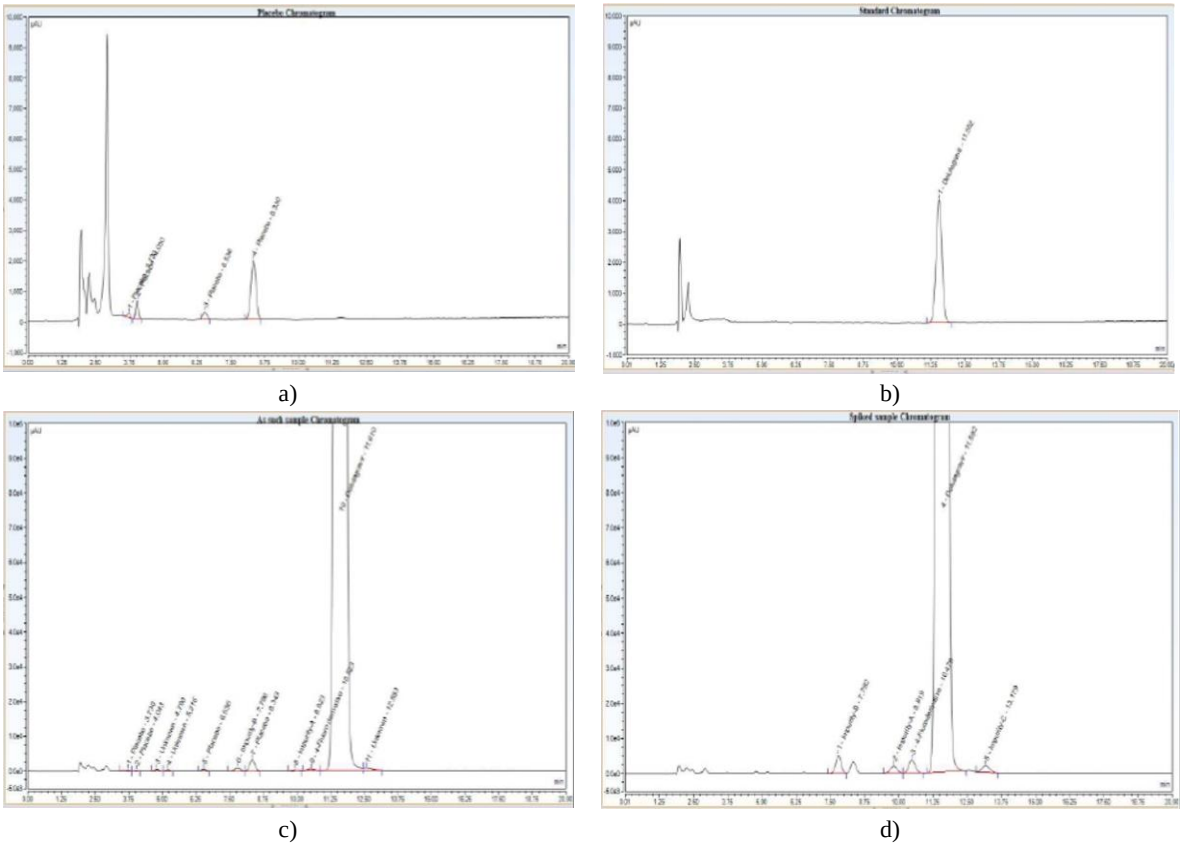
Transferred 8.5 mL of concentrated hydrochloric acid into a 1000 mL volumetric flask, containing 500 mL water and mixed well. Diluted to volume with water and mixed well.

Diluent Preparation

Mixed 0.1M hydrochloric acid and acetonitrile in the ratio of 1:1, % v/v respectively. Sonicated to degas for 5 minutes.

Blank

Used diluent as a blank solution.



a) b)

c) d)

Figure 2. Optimized Chromatograms

Standard Preparations

Preparation of Standard Stock 1 Solution

Weighed and transferred 20 mg of Dolutegravir sodium standard (Equivalent to 20 mg of Dolutegravir) into a 100 mL volumetric flask, added 70 mL of diluent, and sonicated (about 5 minutes) to dissolve. Diluted to volume with diluent and mixed well.

Preparation of Standard Stock 2 Solution

Further diluted 5.0 mL of standard stock 1 solution to 100 mL with diluent and mixed well.

Preparation of Standard Solution (1.0 ppm)

Diluted 5.0 mL of standard stock solution 2 to 50 mL with diluent and mixed well.

Impurity Standard Preparations

Impurity B Stock Preparation

Weighed 2.0 mg of Impurity B into 20 mL volumetric flask, added 10 mL of diluent and sonicated to dissolve, and made up to the volume with diluent.

Impurity B Standard Preparation

From the stock solution, diluted 0.5 mL to 50 mL with diluent and mixed well.

As Such Placebo Preparation

Weighed and transferred crushed powdered placebo sample (Equivalent to 5 tablets) into 100 mL volumetric flask and added about 50 mL of diluent and sonicated for 15 minutes then allowed to cool at room temperature then made up to volume with diluent and mixed well. And filtering the solution through a 0.45µm nylon filter by discarding 5 mL of the solution.

As Such Sample Preparation (10 mg)

Weighed and transferred crushed powdered sample Dolutegravir dispersible tablets 10mg (Equivalent to 5 tablets) into 100 mL volumetric flask and added about 60 mL of diluent and sonicated for 15 minutes then allowed to cool at room temperature then made up to volume with diluent and mixed well. And filtering the solution through a 0.45µm nylon filter by discarding 5 mL of the solution.

Spiked Sample Preparation (10 mg)

Weighed and transferred crushed powdered sample Dolutegravir dispersible tablets 10mg (Equivalent to 5 tablets) into 100 mL volumetric flask and added about 70 mL of diluent and added 1.0 mL of Impurity B individual stock and sonicated for 15 minutes then allowed to cool at room temperature then made up to volume with diluent and mixed well. And filtering the solution through a 0.45µm nylon filter by discarding 5 mL of the solution.

Procedure for Method Validation

The following parameters are considered for analytical method validation of related substances method in drug product Dolutegravir dispersible tablets 10 mg [9].

- System suitability
- Specificity
  - Blank, Placebo, and Impurity Interference
  - Filter compatibility study
  - Forced degradation
- Precision
  - System Precision
  - Method Precision
  - Intermediate Precision
- Stability in analytical solution and Mobile phase stability
- Linearity
- Limit of Detection and Limit of Quantitation
- Accuracy
- Range
- Robustness

System Suitability

It is necessary to make sure that the analytical system is functioning correctly before the analysis may proceed. The blank and standard solutions were prepared following the testing procedure.

Specificity

To be specific, an analytical technique has to be able to determine the analyte's existence with certainty, even when other components like contaminants, degradation products, and matrix components are present. The response to these solutions is given in **Table 1**.

Filter Compatibility Study

A spiked sample solution and a placebo solution were used in the filter compatibility investigation using a 0.45 nylon or PVDF filter, discarded the first 3.0 and 5.0 mL of filtrate from each solution. Dolutegravir area response and % difference of various filters are recorded.

Forced Degradation Study

To ensure that Dolutegravir and its known impurity are not harmed by any degradation products that may be detected during the stability research or the shelf life, a forced degradation study was conducted. As a result of the forced degradation research, it will be possible to determine the kind of degradation route (whether oxidative, acid hydrolysis, or neutral hydrolysis) for each degradant. As per the testing procedure, a blank, standard solution was prepared.

Precision

Individual test results may be compared to each other to determine an analytical method's precision. The standard deviation or relative standard deviation (coefficient of variation) of a sequence of data is often used to indicate the analytical method's precision.

System Precision

To confirm that the analytical system is functioning correctly, a standard solution is injected into the system. Six different measurements were made to determine the retention time and area response, and the relative standard deviation was computed for each. For six injections, the chromatograph was injected with Gradient, Blank, and Standard solutions. The chromatograph was recorded and calculated the relative standard deviation.

Method Precision

A single batch sample should be evaluated six times for technique precision. This tells us whether a procedure consistently produces the same results in different batches. Following the analytical procedure, tested the sample of Dolutegravir dispersible tablets 10 mg six times. Percentages of defined and unspecified degradation products, as well as the total degradation products, were calculated.

Intermediate Precision

Preserving analytical data from being tainted by changes in environmental circumstances such as those caused by changes in instrument, analyst, day and column were the primary goals of doing the intermediate precision analysis. Change one variation at a time and re-examine the intermediate precision of the method precision differs from the intermediate precision. Results were compared to the method precision established after making adjustments to the above-mentioned circumstances.

Following the analytical procedures, tested the sample of Dolutegravir dispersible tablets 10 mg six times. Degradant percentages for defined and nonspecific degradants and total degradants were calculated. Preparation of blanks, standard solutions, placebo solutions, and spiked sample solutions according to test procedures.

Linearity

Analytical methods' linearity refers to their capacity to provide test findings that are proportionate to the concentration of analyte in samples within a specified range, either directly or through a well-defined mathematical transformation. Dolutegravir and Impurity B were linearized from LOQ level (roughly) to 200 percent of the specified limit. A better degree of precision was achieved. Slope, intercept, correlation coefficient, and regression coefficient were determined for the area response **(Table 3)** at each level (R square). The statistical equivalency of zero was determined by calculating the intercept. On the X-axis, plotted the concentration (ppm) and on the Y-axis, plotted the area response under the curve.

LOD & LOQ

The lowest quantity of analyte in a sample that can be detected, but not necessarily quantified, under the provided experimental circumstances is the limit of detection (LOD). Limit of Quantitation (LOQ) refers to the smallest quantity of analyte in a sample that can be accurately and precisely quantified under specified experimental circumstances. The S/N ratio technique is used to calculate the detection limit (LOD) and the limit of quantitation (LOQ). LOD and LOQ concentrations were prepared and injected into the instrument for analysis, and area responses and S/N Ratios for Dolutegravir and Impurity B were recorded in the chromatograph.

Accuracy

Analysis at three separate levels in triplicate (3x3 levels), as well as LOQ concentration, should be carried out to verify the accuracy of an analytical technique throughout its range (in triplicate). At each accuracy level, prepared samples and injected. LOQ, 50%, 100%, 150%, and 200% levels of Dolutegravir and Impurity B, and these percentage recoveries were shown in **Table 4**.

Range

This is the distance between the higher and lower levels of the analyte that must be accurately and linearly measured by the analytical procedure. Dolutegravir linearity and Impurity B precision ranges have been calculated using RSD, as have the accuracy ranges, and precision ranges for both Dolutegravir and Impurity B.

Robustness

Reliability during typical use may be determined by the technique's ability to withstand changes in method parameters that are made with purposeful intent. This study has been performed with the effect of variation in flow rate (±10%); Column Temperature (±5°C); pH of Buffer (±0.2 units); Methanol in Mobile phase (±10%); Acetonitrile in Mobile phase (±10%). The system's suitability of robustness was recorded.

Results and Discussion

Method Validation

System Suitability

Injected the standard solution (6 injections). Recorded the area response and calculated the mean (62045.926), %RSD (0.9), theoretical plates (18302) and tailing factor (1.04) respectively.

Data Interpretation: According to the data shown above, it can be concluded that the system suitability parameters were within the acceptance criteria.

Specificity

Table 1. Response of placebo, sample, and spiked sample solutions

Sample Name	Peak Name	RT (Min)	RRT (about)	Peak Purity
Blank	Blank	1.941, 2.208	NA	NA
Standard solution	Dolutegravir	13.420	NA	999
Placebo solution	Placebo	2.459,2.939,3.113,3.946, 4.219,7.439,9.613	NA	NA
Sample as such -10 mg	Impurity B	8.846	0.65	998
	Dolutegravir	13.513	1.00	1000
	Impurity D	12.046	0.89	992
	Unknown	4.426,5.306,10.953,12.486, 14.873	NA	NA
Spiked sample solution-10mg	Impurity B	8.804	0.66	1000
	Dolutegravir	11.610		1000
	Impurity D	11.937	0.89	NA
	Impurity C	16.204	1.21	999
	Impurity A	11.937	NA	NA
	Unknown	4.424,5.277,5.817,10.884, 14.791	NA	NA

Data Interpretation: According to the data shown above, it can be concluded that there was no interference from the blank and placebo at the retention time of the Dolutegravir peak and Impurity peaks. The peak purity of desired peaks met the acceptance criteria. The method is specific for the determination of related substances for Dolutegravir dispersible tablets 10 mg.

Filter Compatibility Study

Recoreded the area response of spiked sample and placebo, as well as calculated the % difference of unfiltered sample, 3mL and 5mL discarded sample by using 0.45µm Nylon and 0.45µm PVDF filters.

Data Interpretation

According to the % difference, it can be concluded that both 0.45µm Nylon and 0.45µm PVDF filters are compatible for filtration (discarding 5 mL of filtrate) of sample solution of Dolutegravir dispersible tablets 10 mg.

Forced Degradation Study

Table 2. % Assay, peak purity, and % mass balance for forced degradation

S. No.	Condition (Sample)	Impurities from RS method	% Assay	Peak Purity	% Mass Balance
1	Sample as such-I	0.154	98.3	1000	-
2	Alkali stressed sample_5N NaOH_4 Hours at 80°C	0.167	95.0	1000	96.7
3	Neutral stressed sample_Water_4 Hours at 80°C	0.470	98.9	1000	100.9
4	Thermal stressed sample_105°C_1 Hour	0.204	97.7	1000	99.4
5	Peroxide stressed sample_5%v/v_H_2O_2_ 4 Hours at 80°C	0.275	98.0	1000	99.8
6	Sample as such-II	0.143	99.5	1000	-
7	Acid stressed sample_2N HCl_30 minutes at 80°C	10.485	89.8	1000	100.6
8	UV Stressed Sample_16 hours at UV Cabinet	0.154	97.3	1000	97.8
9	Photo stability stressed sample (Visible) LUX Stressed sample_1.2 million lux hours	0.701	97.5	1000	98.6

Data Interpretation: The degradation study was performed with Acid, Alkali, Peroxide, Neutral, Thermal, UV, and Visible conditions and it was observed **(Table 2)** that the molecule was sensitive to acid-stressed conditions. Mass balance was achieved in all the conditions. Hence, the method is stable indicating for determination of related substances for Dolutegravir dispersible tablets 10 mg.

Precision

System Precision

Injected the standard solution (6 injections). Recorded the area response and calculated the Mean for retention time and peak area were 11.424 and 62035.926 respectively. %RSD for retention time and peak area were 0.2 and 0.9 respectively.

Data Interpretation: According to the data shown above, it can be concluded that retention time & area response were consistent as evidenced by relative standard deviation. Hence, it was concluded that the system precision parameters met the acceptance criteria.

Method Precision and Intermediate Precision

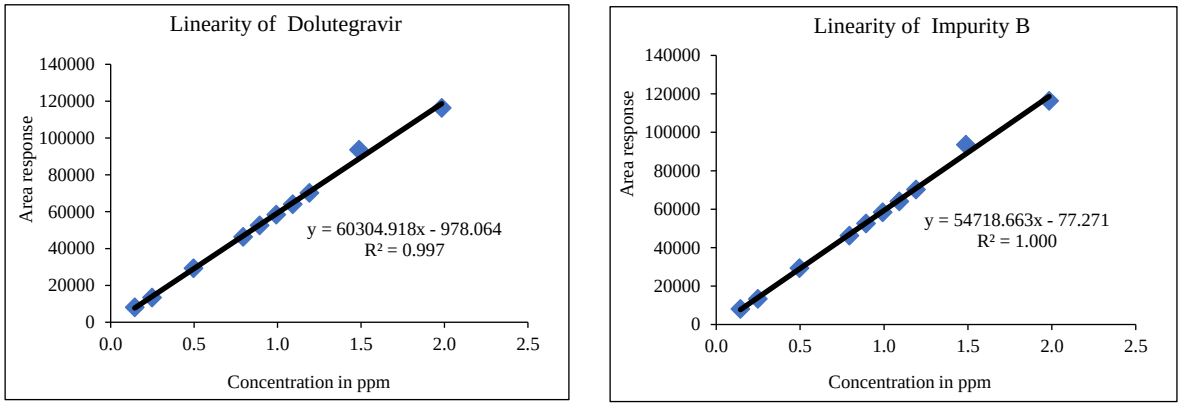
Calculated the % total impurities together with % Impurity B and % highest unknown. Their mean and % RSD were 0.29 and 1.5 respectively.

Data Interpretation: According to the data shown above, it can be concluded that the method is rugged for the determination of related substances for Dolutegravir dispersible tablets 10 mg.

Linearity

Table 3. Linearity of Dolutegravir and Impurity B

Linearity level in %	Concentration in ppm		Peak area	
	Dolutegravir	**Impurity B**	**Dolutegravir**	**Impurity B**
LOQ	0.0510	0.0523	3555.489	2806.664
10	0.1442	0.1544	8111.842	8337.053
25	0.2479	0.2558	13362.503	13703.251
50	0.4958	0.5115	29301.548	27605.915
80	0.7933	0.8185	46274.471	44277.477
90	0.8925	0.9208	52532.673	50506.496
100	0.9917	1.0231	58391.380	56894.493
120	1.1900	1.2277	70192.337	67637.253
150	1.4875	1.5346	93564.320	83786.201
200	1.9833	2.0462	116322.756	111442.089
	Dolutegravir		**Impurity B**	
Correlation coefficient ®	0.998		1.000	
Regression coefficient (r^2)	0.997		1.000	
Slope	60304.918		54718.663	
Intercept	-978.064		-77.271	
% Intercept	-1.7		-0.1	



a) b)

Figure 3. Linearity plot of Dolutegravir and Impurity B

Data Interpretation: According to the data shown above, it can be concluded that the response of Dolutegravir and Impurity B was linear between LOQ to 200% (Specification limit). The Correlation Coefficient and Regression Coefficient are more than 0.995 and 0.990. Plotted a graph of concentration (ppm) on the X-axis and area response under the curve on the Y-axis. **(Figure 3)**. Moreover, the value of the % intercept is within the ± 5.0 % of the area response at the 100 % level. Based on the linearity data, it was concluded that the detector response was directly proportional to concentration for Dolutegravir and Impurity B.

LOD & LOQ

Data Interpretation: According to the linearity data, the LOD and LOQ level were determined as 2% (0.02ppm) and 5% (0.05ppm) respectively, it can be concluded that Dolutegravir and Impurity B were visible and distinct at the LOD level. S/N ratio of LOD and LOQ solutions met acceptance criteria.

Accuracy

Table 4. Accuracy results

Sr. No.	Level	Area response	mg Added (actual)	mg recovered	% Recovery	Mean % recovery	% RSD
Accuracy for Dolutegravir standard							
1	LOQ	3615.189	0.0052	0.0062	119.4	121.7	1.9
2		3330.135	0.0052	0.0057	121.9		
3		3585.911	0.0052	0.0061	123.9		
1	50%	28926.182	0.0518	0.0513	99.0	97.9	1.6
2		28819.656	0.0518	0.0511	98.6		
3		28091.431	0.0518	0.0498	96.1		
1	100%	57965.946	0.1035	0.1028	99.3	98.7	0.7
2		57678.986	0.1035	0.1023	98.8		
3		57150.173	0.1035	0.1014	98.0		
1	200%	116172.218	0.2070	0.2060	99.5	99.4	0.2
2		115749.264	0.2070	0.2053	99.2		
3		116275.024	0.2070	0.2062	99.6		
Accuracy for Dolutegravir sample (spiked sample)							
1	LOQ	13458.061	0.0052	0.0040	92.0	92.0	1.4
2		13587.952	0.0052	0.0043	90.7		
3		13553.000	0.0052	0.0042	93.3		
1	50%	38966.179	0.0515	0.0506	98.3	98.1	0.2
2		38894.463	0.0515	0.0504	98.0		
3		38932.228	0.0515	0.0505	98.1		
1	100%	67419.720	0.1029	0.1030	100.1	99.4	0.6
2		66871.834	0.1029	0.1020	99.1		
3		66841.316	0.1029	0.1020	99.1		
1	200%	122252.274	0.2058	0.2041	99.2	99.3	0.2
2		122600.746	0.2058	0.2048	99.5		
3		122172.356	0.2058	0.2040	99.1		

Data Interpretation: According to the data shown above, it can be concluded that the analytical method meets the pre-established acceptance criteria for accuracy study as per protocol. Hence, the method is accurate for the determination of related substances of Dolutegravir dispersible tablets 10 mg.

Range

The range has calculated with levels of RSD, linearity and accuracy ranges (LOQ, 50%, 100%, 150%, 200%) for Dolutegravir and Imurity B from the linearity and accuracy data.

Data Interpretation: According to the data calculated, it can be concluded that the method is found to be linear and accurate in the range of LOQ to 200% of the specification limit for Dolutegravir and Impurity B.

Robustness

This study was performed under different changed conditions like, as such sample, flow rate (±10%), column temperature (±5°C), pH (±0.2 units), methanol (±10%), acetonitrile (±10%). Calculated the system suitability parameters like % RSD, theoretical plates, USP tailing factor, RRT of Imputiy B.

Data Interpretation: According to the system suitability parameter results, it can be concluded that the method is found to be robust with deliberate changes in flow rate, column temperature, variation in pH of mobile phase, variation in methanol in the mobile phase, and variation in acetonitrile in the mobile phase.

Conclusion

The proposed RP-HPLC method of related substances for Dolutegravir dispersible tablets 10mg was developed and validated as per USP guidelines and or specifications. The method found stability-indicating, specific, rugged, precise, linear, accurate, and robust with high resolution and shorter retention time. Hence, this developed and validated method can be used for its intended purpose.

Acknowledgments: None

Conflict of interest: None

Financial support: None

Ethics statement: None

References

1. Dolutegravir. AIDS Info, U.S. Department of Health and Human Services. 2022.
2. Venkatnarayana M, Siva Jyothi N. Development of Validation and Stability Indicating Method of Anti-HIV Dolutegravir Drug and its Related Impurities by Using RP-HPLC. J Chromatogr Sep Tech. 2020;11:426.
3. Ashok G, Mondal S. Development and Validation of Stability Indicating Method for the Simultaneous Estimation of Batcaver Sulfate, Lamivudine, and Dolutegravir Sodium in Pharmaceutical Dosage forms by RP-HPLC. Saudi J Med Pharm Sci. 2018;4(2):289-96.
4. Grease YN, Srinivas Rao S, Dipti Soni D. Development and Validation of Chiral RP-HPLC Method for Quantification of Optical Isomers in Dolutegravir Sodium. Pharm Lett. 2018;10(9):90-100.
5. Chandra Sekhar RK, Pavan Kumar KSR, Jagadeesh Kumar V, Sreenivas N, Sharma HK, Mukkanti K. Stability Indicating HPLC Method for the Quantification of (4S,12aR)-Enantiomer and (4R, 12aR) Diastereomer in Dolutegravir Sodium. Int J Pharm Pharm Res. 2017;9(2):52-63.
6. Satyadev TNVSS, Bhargavi Ch, Syam sunder B. Development and validation of High-performance Liquid Chromatographic method for the determination of Dolutegravir in human plasma. Pharm Sin. 2015;6(4):65-72.
7. Rao NM, Sankar DG. Development and validation of stability-indicating HPLC method for simultaneous determination of Lamivudine, Tenofovir, and Dolutegravir in bulk and their tablet dosage form. Futur J Pharm Sci. 2015;1(2):73-7.
8. Gorog S, Babjak M, Balogh G, Brlik J, Chi A, Dravecz F, et al. Drug impurity profiling strategies. Talanta. 2015;44(9):1517-26.
9. ICH guidelines for Method Validation Q2 (R1). USP NF 2021 Issue-1 <1225> Validation of compendia Methods.