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Analytical method development and validation for estimation of dolutegravir in tablet dosage form

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***Abstract*---**A simple, specific, accurate, and precise reverse phase high performance liquid chromatography method was developed and validated to analyse Dolutegravir Sodium bulk dosage form. Using Princeton C_{18} HPLC column separation was carried out. This was maintained at 25°C temperature. Shimadzu LC -20A prominence with LC solution softwarering separation mobile phase consist of Methanol: Water (0.1% OPA) in ratio of 80:20 v/v was delivered at a flow rate of 1mL/min. Using UV detector analysis was carried out at the wavelength 254 nm. RP-HPLC method was validated by using various parameter like system suitability, linearity, specificity, precision, limit

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of detection, limit of quantification and robustness. The RP-HPLC method was found to be linear over the concentration range from 10-60 µg/mL (r^2 =0.9998). Retention time for bulk Dolutegravir Sodium was found to be 4.383 min. LOQ of method was 1.3274 µg/mL and LOD 0.4380 µg/mL. Thus the developed RP-HPLC method was found to be robust which can be applied for the regular analysis of Dolutegravir Sodium in the bulk as well as pharmaceutical dosage form.

***Keywords*---**RP-HPLC, dolutegravir sodium, princeton C_{18}, acetonitrile, methanol.

Introduction

Dolutegravir (DTG) (fig1) is an antiretroviral drug. The U.S. Food and Drug Administration announced (in February 2013) that it would fast track Dolutegravir's approval process. The USA was approved the use of Dolutegravir in medical use (on August 13, 2013). It is used in treatment of HIV/AIDS in combination with other medicines. The another use of Dolutegravir is as a part of post exposure prophylaxis, to prevent HIV infection. Dolutegravir tablet is taken by oral route which is sold under the brand name Tivicay. The IUPAC name of Dolutegravir is (4R,12aS)-N-[(2,4-difluorophenyl)methyl]-7-hydroxy-4-methyl-6,8-dioxo-3,4,12,12a-tetrahydro-2H-pyrido[[1,2,4,5] pyrazino[[2,1-b]][1,3]oxazine-9-carboxamide.

Chemical formula of Dolutegravir is $C_{20}H_{19}F_2N_3O_5$ and molar mass is 419.385 g.mol^{-1}. Dolutegravir is listed as essential medicine in the list of World Health Organization. WHO recommends Dolutegravir as the first-line and second-line treatment of all persons with HIV. The use of Tivicay for children 12 years and older weighing at least 40 kg who are treatment but have not previously taken other integrase strand transfer inhibitor. Insomnia and headache are the common side effects of Dolutegravir. Allergic reaction and abnormal liver function in patients who were also infected with hepatitis B or C are reported as serious side effects of Dolutegravir.

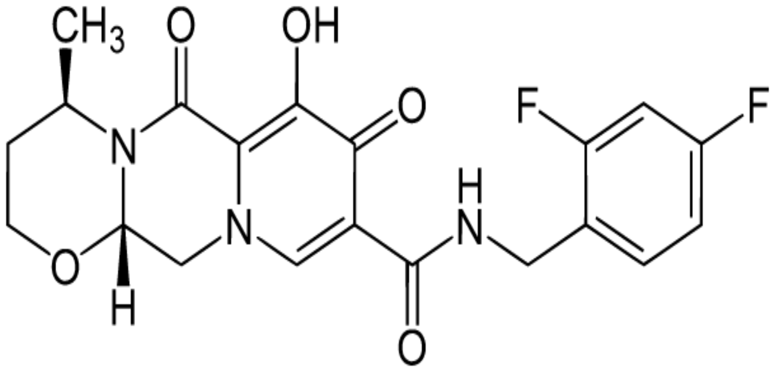

Fig 1-Chemical structure of Dolutegravir

Method And Materials

- Chemicals and Reagents
  Dolutegravir sodium was obtained as gift sample from Mylan Laboratories Limited, Jubilee Hills, Hyderabad, Telangana, India. All the chemicals used during the study were HPLC and AR grades and procured from Emerck, Qualigens, Rankem, S. D. fine chem Ltd, etc. Distilled water and Whatmann filter papar grade-1 (0.45 µm) were used throughout the experimental work.
- Instrumentation
  The analysis was performed by using chromatographic system, Shimadzu HPLC system Comprising of Shimadzu SPD-20A prominence PDA Detector Shimadzu DGU-20A3 prominence degasser .Rheodyne injector 7725 I with 20 µl loop Shimadzu LC -20A prominence with LC solution software Shimadzu HPLC comprised of a Model PU 2080 Plus Intelligent HPLC pump. The Rheodyne sample injector port with 20 µl loop and running on Shimadzu SPD-20A prominence PDA Detector prominence with LC solution software with princeton C_{18} Column . Mettler Toledo, Model No. AG135, sensitivity-1 mg was used for weighting purpose. Ultrasonicator: PCI Analytics, Mumbai was used for sonication purpose. Dissolution apparatus Electrolab Dissolution Tester, Model: PP50V, Electrolab India Pvt. Ltd., India. was use for dissolution purpose.
- Chromatographic conditions
  Dolutegravir was analysed with Princeton C_{18} column for the chromatographic separation and column was maintained at 25℃ temperature. The mobile phase was composed of a mixture of Methanol and water in the ratio of 80:20v/v and it was delivered at a flow rate of 1ml/min and detection was monitored at 254 nm with PDA detector. Injection volume was 20 µl loop. The run time of Dolutegravir was found to be 2.425min.

Table 1: Optimized chromatographic condition

Parameters	Conditions used
Column	Wates Xterra C_{18}
Mobile Phase	Methanol: Water(80:20)(0.1% OPA)
RT (retention time)	4.383 min
Flow Rate	1 ml/min
Sample Injector	20 µl loop
Detection Wavelength	254 nm
Column Temperature	25℃

Preparation of standard stock solution

I) Standard Stock Solution (Solution A) An accurately weighed quantity of Dolutegravir (10.0 mg) was dissolved in 10 ml of various solvents to obtain a final volume of 10.0 ml solution (1000 µg/ml) Called stock solution.

II) Working Standard Solution (Solution B) Standard stock solution (1.0 ml) was transferred to a 10.0 ml volumetric flask, and the volume is made up to mark with respective solvents so as to obtain a final volume of 10.0 ml (100 µg/ ml).

III) Working Standard Solution (Solution C) Solution B (1.0 ml) was further diluted up to 10.0 ml with the respective solvent so as to obtain the final volume of 10.0 ml solution (10 µg/ ml). This solution was used for spectral studies.

Selection of analytical wavelength

The final solution (Solution C) was scanned in the UV range (400-200 nm) using a 1.0 cm quartz cell against methanol as a blank. The λ max was found to be 254.0 nm which was used in further study. The absorption spectrum of the drug is depicted in (Figure 2)

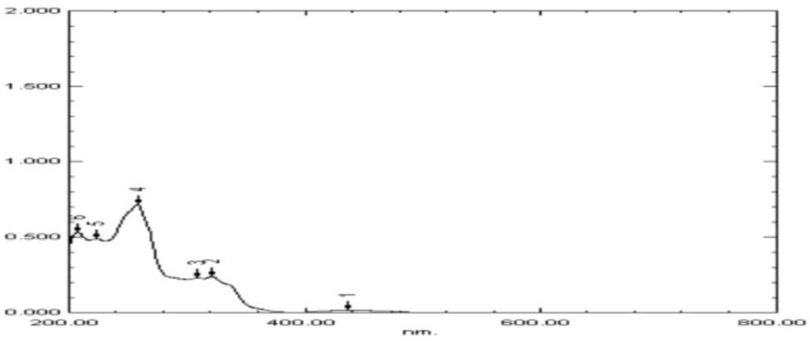

Figure 2: UV- Spectrum of Dolutegravir Sodium

Study of Beer-Lambert's Law

An accurately measured working stock solution (Solution C) was diluted with methanol to get a series of concentrations ranging from 5.0-25.0 µg/ml. The absorbance of each solution was measured at 254.0 nm against methanol as a blank. The plot of absorbance v/s concentration was found to be linear and is depicted in (Fig. 3). The results of regression analysis are depicted in Table 2.

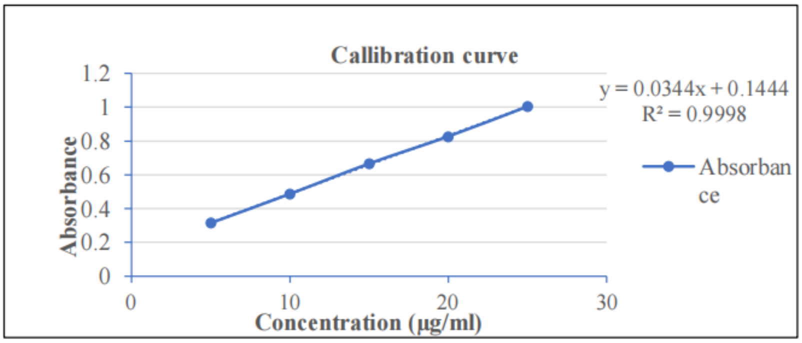

Fig. 3: Linearity Study of Dolutegravir Sodium

Table 2: Data for Regression Analysis

Sr. No.	Parameters	Dolutegravir sodium
1	Linearity range	10-60
2	Straight line equation	Y=0.334x = 0.1444
3	Slope	0.344
4	Correlation coefficient (r)	0.9998
5	LOD	0.4380
6	LOQ	1.3274

Mobile phase optimization

The chromatographic studies were performed on the C18 analytical column. For the selection of the mobile phase initially, different organic solvents alone and in combination were tried in order to obtain a sharp peak of Dolutegravir sodium with adequate retention. The mobile phase contains plain methanol, plain acetonitrile, a combination of various blends of methanol: water, ACN: water, methanol: ACN, and finally tried in a combination of Methanol: Water (0.1 % OPA) different ratios.

Trials for mobile phase of Dolutegravir

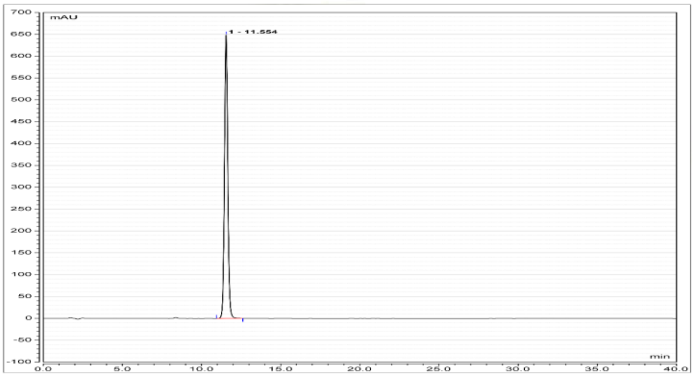

Fig.4.1: Chromatograms obtained for Trial 1

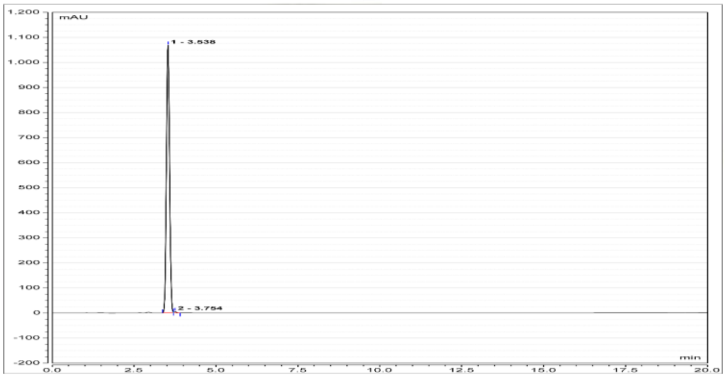

Fig.4.2: Chromatograms obtained for Trial 2

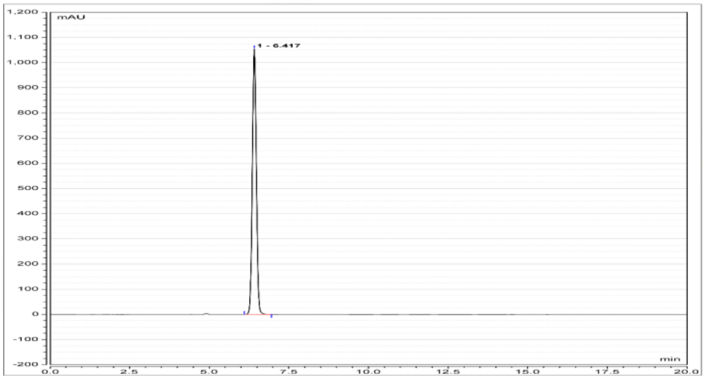

Fig.4.3: Chromatograms obtained for Trial 3

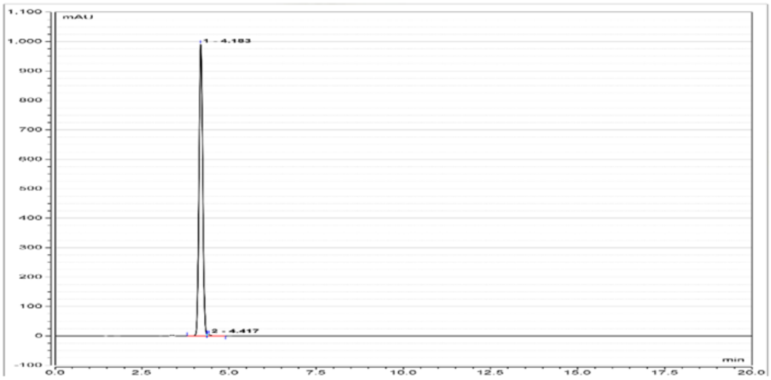

Fig.4.4: Chromatograms obtained for Trial 4

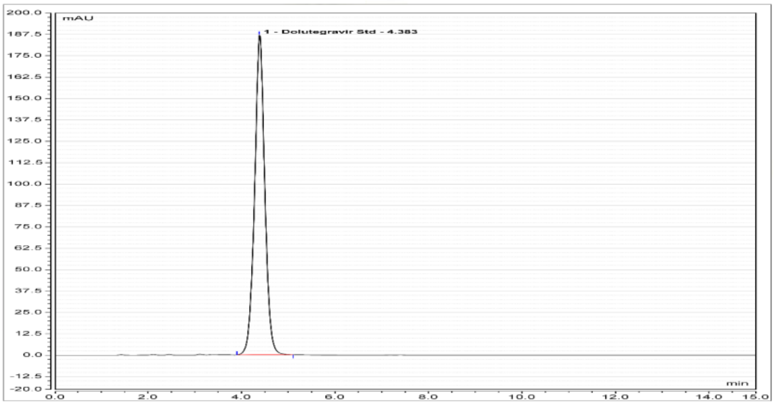

Fig.4.5: RP-HPLC Chromatogram of Standard Dolutegravir Sodium

Table 3: Trial 1-5 Chromatographic Parameters and specifications

Sr. No	Mobile Phase Composition	Retention Time	Remark
1	Methanol: Water (0.1 % OPA) (60:40)	11.55	Tailing
2	Methanol: Water (0.1 % OPA)	3.5	Tailing

	(65:35)		
3	Methanol: Water (0.1 % OPA) (70:30)	6.4	Peak splitting
4	Methanol: Water (0.1 % OPA) (75:25)	4.1	Tailing
5	Methanol: Water (0.1 % OPA) (80:20)	4.3	Sharp peak

Method validation

Validation of proposed analytical method involves linearity and range, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness study. It was validated according to ICH Q2 (R1) guideline.

System Suitability

System suitability is an integral part of chromatographic system. The calculation and comparison of verified resolution, capacity factor, tailing factor, theoretical plate count with standard specification of system.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The determination of the excipients effect and other additives which are present in formulation can be determined by using analytical method i.e. specificity.

Linearity

Linearity of an analytical method is carried out to demonstrate that concentration of an analyte is directly proportional to the peak area of analyte. The evaluation of linearity is done by visual inspection of plot of signal as a function of analyte concentration in sample. The linearity was determined by analysing six solutions over the concentration range of 10-60 µg/mL.

Accuracy

Accuracy of the analytical method is the capability of method to determine the correct assay value. Accuracy of analytical method can be determined by adding the known amount of standard drug to placebo preparation and then same shall be analysed by proposed method. The accuracy of the method was determined by calculating recovery of API by method of standard addition.

Precision

The closeness of agreement between a series of measurements obtained from multiple sampling of similar homogenous sample under the prescribed condition. Precision was determined by injecting the 6 injections of standard preparation.

Limit of detection and Limit of quantification

LOD is the lowest concentration of analyte that gives a detectable response where the LOQ is lowest concentration of analyte that can quantified with a specified level of accuracy and precision.

Robustness

The robustness of analytical method is a measurement of its capacity to remain unaffected by small changes but deliberate changes in procedure parameters and provides an indication of its reliability during normal usages.

Result And Discussion

Linearity studies

From the stock standard solution, aliquots portions were transferred into a series of 10 mL volumetric flasks and diluted up to the mark with the mobile phase to obtain a final concentration in the range of 10 – 60 µg/mL. A constant volume of each sample was injected. All measurements were repeated three times for each concentration a and calibration curve was constructed by plotting the peak area *versus* the concentration. The observations are shown in Table 4, while calibration curves are shown in Fig.5.

Table 4: Linearity Study of Dolutegravir

Sr. No	Concentration of [µg/mL]	Peak area (mAU*min) [Mean ± SD; n = 3]	% RSD
1	10	2.7086 ± 0.01	0.013275
2	20	5.2306 ± 0.05	0.04438
3	30	7.5816 ± 0.32	0.26328
4	40	10.2653 ± 0.002	0.002055
5	50	12.602 ± 0.008	0.006683
6	60	14.9833 ± 0.002	0.002055

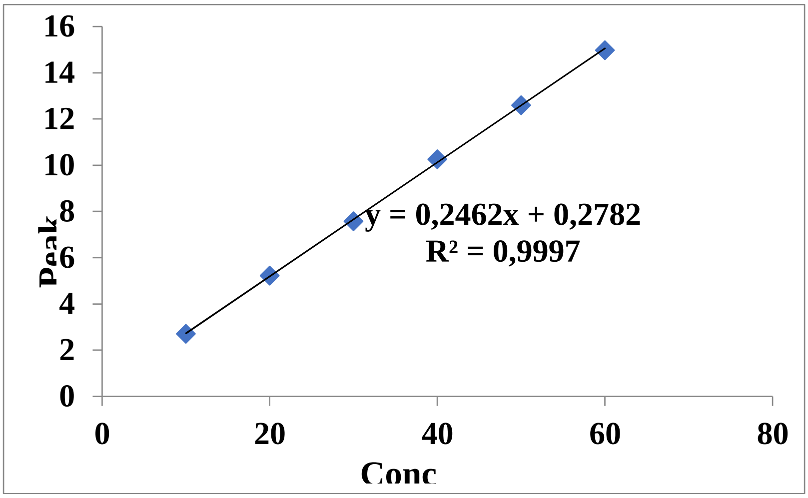

Fig.5 : Calibration Curve of Dolutegravir

Y = 0.2462 X + 0.2782;

Where, Correlation coefficient = 0.9997

Slope = 0.2462;
Intercept = 0.2782

Acceptance Criteria: Correlation coefficient should not be less than 0.999.

Conclusion

The Correlation coefficient is 0.9997. Therefore, the HPLC method for Dolutegravir is linear.

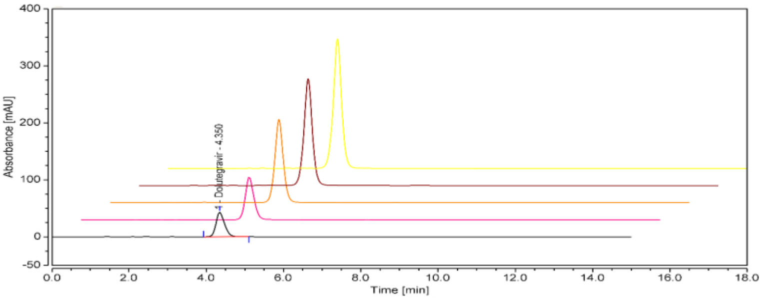

Fig.6 : RP-HPLC calibration graph of Dolutegravir (Linearity Studies)

Analysis of formulation

20 intact tablets of Dolutegravir were transferred into a 100-mL volumetric flask. About 70 mL of methanol was added, and the resulting solution was sonicated for 25 min with intermittent shaking, then cooled, and diluted up to the mark with methanol. The resulting solution was then allowed to settle for 15 min and then the solution was centrifuged at 5000 RPM. Further, 5 mL of the supernatant

solution was diluted to 100 mL with diluents and filtered through a 0.45-µ Nylon membrane syringe filter or equivalent.). The sample solutions were injected into column for six times. The concentrations were calculated from their linearity curve. % relative standard deviation should not be more than 2.0%. Results are shown in table 5.

Table 5: Analysis of formulation by HPLC

Drug	Label Claim (mg)	Amount Found mg ± SD [n = 6]	Percentage Amount found	% RSD
Formulation	50	49.81 ± 0.178	99.94 ± 0.051	0.1789

Accuracy

It was done by recovery study using standard addition method at 80%, 100% and 120 % level; known amount of standard Dolutegravir was added to pre-analyzed sample (10.0 □g/mL of Dolutegravir) and subjected them to the proposed HPLC method % recovery should be between 98% to 102% results are shown in table 6.

Table 6: Results of Recovery studies for Dolutegravir

Drug	Initial amount [µg/mL]	Excess drug added to the analyte [%]	Amount recovered □ S.D. [µg/mL]	Recovery [%]	%RSD [n = 3]
Dolutegravir	10	80	17.96 ± 0.052	99.77778	0.0432
	10	100	19.98 ± 0.025	99.91667	0.0205
	10	120	21.96 ± 0.015	99.83333	0.0124

Precision

Precision of the method was verified by repeatability and intermediate precision studies. Intra-day precision was studied by analyzing 10, 20, 30 µg/mL of Dolutegravir for three times on the same day. Inter-day precision was checked analyzing the same concentration for three different days over a period of week. Repeatability was measured by analyzing 10 □g/mL of Dolutegravir for six times. The % RSD should not be more than 2%. The results are shown in Table 6.7**,** and Repeatability shown in Table 7**.**

Table 7: Results of Precision study for Dolutegravir

Drug	Conc. [µg/mL]	Intra –day Amount Found [µg/mL] [n =3]		Inter- day Amount Found[µg/mL] [n = 3]	
		Mean	% RSD	Mean	% RSD
Dolutegravir	10	9.94 ± 0.047	0.0385	9.95 ± 0.075	0.0618
	20	19.93 ±	0.0205	19.97 ±	0.0216

		0.025		0.026	
	30	29.93 ± 0.045	0.0368	29.92 ± 0.098	0.0805

Table 8 : Results of Repeatability study for Dolutegravir

Sr. no.	Conc. (10 μg/mL)	Amount Found
1	10	9.98
2	10	9.99
3	10	9.97
4	10	10.1
5	10	9.97
6	10	9.99
Mean		10
S.D.		0.0497996
%R.S.D.		0.04546061

Ruggedness

From stock solutions, sample solution of 10 μg/mL of Dolutegravir was prepared and analyzed by two different analysts using similar operational and environmental conditions. Peak area was measured for same concentration solutions, six times; % amount found should be between 98% to 102%. % relative standard deviation should not be more than 2.0%. The results are shown in table 9.

Table 9: Results of Ruggedness study of Dolutegravir

Drug	Amount in μg/mL	Amount Found [n = 6] Mean ± SD		% Amount Found [n = 6]		% RSD	
		Analyst I	Analyst II	Analyst I	Analyst II	Analyst I	Analyst II
Dolutegravir	10	9.96 ± 0.031	9.93 ± 0.049	99.6 ± 0.316	99.31 ± 0.491	0.0288	0.0448

Robustness

Robustness of the method was studied by making deliberate changes in few parameters *viz*; change in mobile phase composition**,** acid concentration (pH modifiers)**,** and flow rate. The effects on the results were studied by injecting 10 μg/mL of Dolutegravir; one factor was changed at one time to estimate the effect; the results are shown in table 10.

Table10: Results of Robustness Studies Dolutegravir

Parameters	Dolutegravir

	R_t
Change in Acid Concentration for pH adjustment	
0.15 %	8.01
0.2 %	7.23
0.3 %	4.49
Change in Flow Rate	
0.9 ml/min	9.36
1.1 ml/min	3.69
Change in mobile phase composition	
(60 : 40 *v/v*)	11.36
(65 : 35 *v/v*)	9.65
(70 : 30 *v/v*)	6.4

Sensitivity

The quantitation limit is a parameter of quantitative assay for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. The limit of detection (LOD) and limit of quantitation (LOQ) were determined using following formulae. LOD = 3.3 (SD)/S; LOQ = 10 (SD)/S; Where SD = Standard Deviation of response, S = the slope of the calibration curve. LOD and LOQ were found to be 0.9076 mg and 2.7503 mg for Dolutegravir.

Specificity and Selectivity

The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix. The method is quite selective. There was no other interfering peak around the retention time of both the Dolutegravir; also the base line did not show any significant noise.

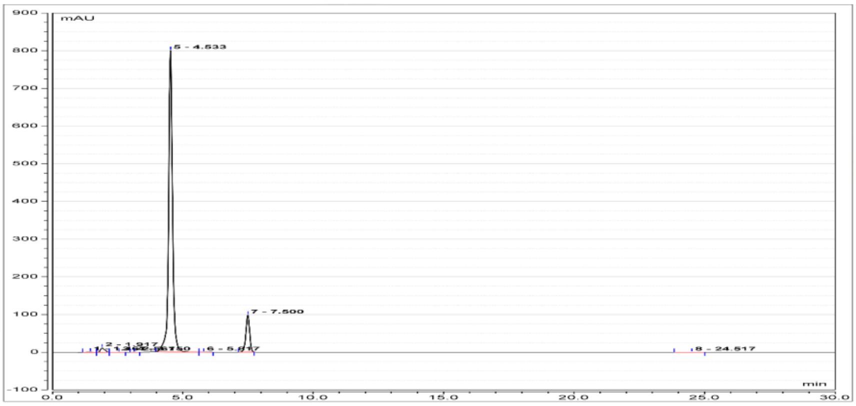

Fig.7 : RP-HPLC chromatogram for Formulation

Conclusion

The present work started with the development and optimization of mobile phase. This work describes the general technique of RP-HPLC method development and validation of developed RP-HPLC method of Dolutegravir sodium.

Acknowledgement

The authors are greatly thankful to Mylan Laboratories Limited, Jubilee Hills, Hyderabad, Telangana, India. All the chemicals used during the study were HPLC and AR grades and procured from Merck, Qualigens, Rankem, S. D. fine chem Ltd, Mumbai.

Conflict of interest

The authors declare no conflict of interest.

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