JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

RP-HPLC METHOD FOR ESTIMATION OF DOLUTEGRAVIR SODIUM IN BULK DRUG AND DOSAGE FORM INCLUDING SOLUTION STABILITY AND FILTRATION STUDY AS PER ICH Q2R1 GUIDELINE

1. Mr. Ravindra Motiram Amale *, 2. Dr. Sonali A. Waghmare

1.Student, Department of Pharmaceutical Chemistry S.N.D. College of Pharmacy, Nashik-422213, Maharashtra, India. 2.Professor ,Department of Pharmaceutical Chemistry S.N.D. College of Pharmacy, Nashik-422213, Maharashtra, India.

Abstract : Several spectrophotometric and HPLC methods have been described for the determination of dolutegravir in drugs and in pharmaceutical dosage forms. Therefore, a novel, sensitive, convenient, and robust reversed-phase high-performance liquid chromatography method for the determination of dolutegravir in bulk drug and tablet formulation was developed and validated in this study. In the RP-HPLC method, methanol and 0.025% TFA (80:20% v/v) were used as mobile phases, at a flow rate of 1.0 mL/min, on an HPLC system containing a UV detector with Openlab EZchrome and Kromasil C18 software. 250 mm x 4.6 mm, 5 µm. Detection was performed at 258 nm. The method gave a suitable retention time, i.e. 3.77 min for Dolutegravir. The results of the analysis in the method were validated in terms of Filtration study, Solution stability, specificity, Linearity, accuracy, precision (Repeatability and medium precision), limit of detection, limit of quantification and robustness. A simple and accurate method was developed for the determination of dolutegravir in the form of bulk drug and tablets. The method requires regular reagents to perform analysis and also less time consuming, it can be routinely performed in industry for routine analysis of bulk drug and marketed Dolutegravir.

Keywords: RP-HPLC, Dolutegravir Sodium, Methanol Method Development, Validation.

INTRODUCTION

In the pharmaceutical industry, analytical method validation is used to demonstrate that the method is fit for purpose; must follow a plan that includes ranges, performance characteristics, and acceptance limits. Analytical methods need to be validated or revalidated before being introduced into routine analyses. Chromatography is an analytical technique based on the separation of molecules due to differences in their structure and/or composition. Chromatography generally involves moving a sample through a system through a stationary phase. The molecules in the samples will have different affinities and interactions with the stationary support, leading to separation of the molecules. Sample components that exhibit a stronger interaction with the stationary phase will move through the column more slowly than components with a weaker interaction. Different compounds can be separated from each other as they move through the column. Chromatographic separation can be performed using different stationary phases. [1][2]

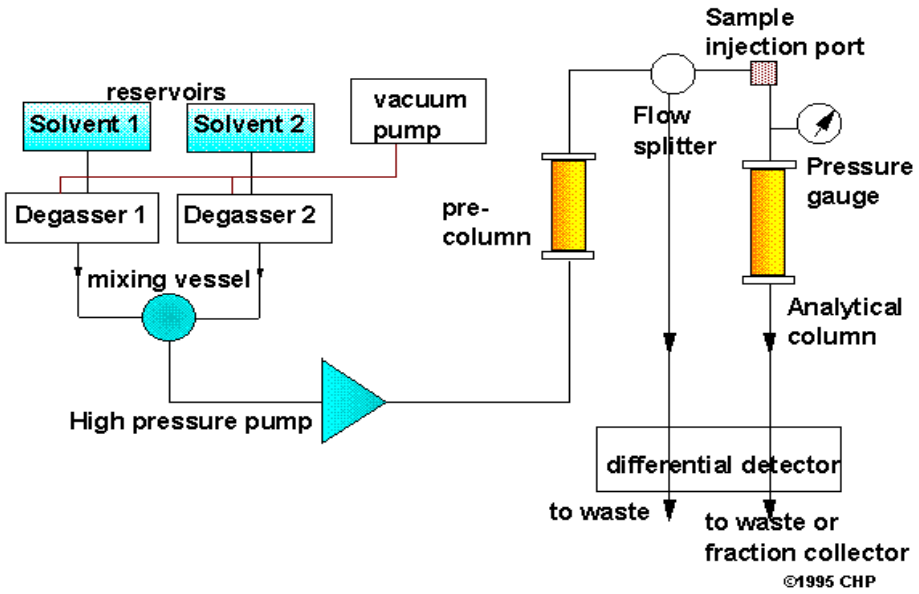


Figure-1: Schematic diagram of HPLC instrumentation

High performance liquid chromatography (HPLC) is a type of liquid chromatography used to separate and quantify compounds that have been dissolved in a solution. HPLC can be used to determine the amount of a particular compound in solution.
These different batches analyzed by different methods including crystal structure elution, polarimetry, UV, IR, HPLC, LCMS and similar other techniques are useful in different types of analysis in different dosage forms. However, the most important and better techniques in HPLC and GC are now being developed using HPLC with a simple reverse phase chromatographic method to determine the active content and relative impurities, and it is better to find a stability indicating method for the analysis. Since after some time the impurities present in the product increase more than its limit and if the impurities are washed very close to the main drug, there is a possibility of the impurities merging with the main drug in the watercress and this results in the failure of the method. [3][4]

Chemistry:

Dolutegravir sodium, chemical sodium salt (4r, 12as)-9-[(2,4-difluorobenzyl)carbamoyl]-4-Methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2h-pyrido[1',2':4,5]pyrazino[2,1b][1,3]oxazin-7 -olate, is a novel integrase growth inhibitor active against human immunodeficiency virus. The drug is effective against HIV type 1 (HIV -1) and also has some in vitro activity against HIV type 2 (HIV-2).

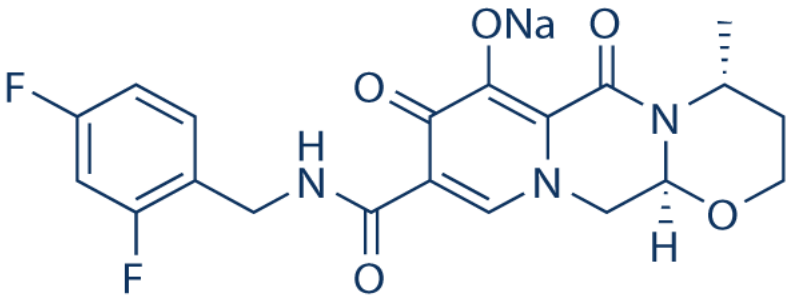


Figure-2: Dolutegravir Sodium Structure

A literature review revealed a liquid chromatography–tandem mass spectrometry method and a sensitive HPLC– MS/MS method for the determination of dolutegravir in human blood plasma. So far, no HPLC and HPTLC method is available for quantitative determination of dolutegravir in tablet formulation. Furthermore, no official or draft monograph of dolutegravir sodium has been published in any pharmacopoeia for compendium applications.
The aim of this work was to develop a simple, economical, accurate, precise, concrete,
A stability-indicating HPLC method and a simple HPTLC method for the determination of dolutegravir in bulk form and/or in pharmaceutical dosage form. The developed methods were validated according to the guidelines of the International Conference on Harmonization (ICH).[5][6]

AIM:

1. To develop a simple, accurate and precise RP-HPLC method for estimation of dolutegravir in bulk drug and dosage form including solution and filtration stability study.
2. Validate the developed method according to ICH Q2R1 guidelines.

Method Development by RP – HPLC

Preparation of standard stock solution for Chromatographic development:

Plagiarism Remover is a free online tool that easily removes plagiarism from any article and makes it 100% unique without changing its meaning. Just copy and paste your article into the box below and press the button to start the plagiarism removal process. Now wait few seconds to get your new plagiarism free content.

Selection of analytical wavelength for HPLC method development:

The analytical wavelength for the examination was selected from the wavelength of maximum absorption from the spectrophotometric analysis and was 258 nm.

Optimization of HPLC method

Following trials are taken for estimation of Dolutegravir .

Principle: Reversed Phase Liquid Chromatography with Isocratic elution and UV detection.

Preparation of System suitability test (Dolutegravir standard solution):

About 10.53 mg of dolutegravir sodium (equivalent to 10 mg of dolutegravir) was weighed and transferred to a 25 mL volumetric flask, 5 mL of DMSO was added, sonicated to dissolve, and made up to the mark with water. Pipette 0.25 ml from the standard stock solution and transfer to a 20 ml volumetric flask and make up to the mark with mobile phase (approximately 5 µ/ml = working concentration), chromatograms were recorded.

System suitability is a pharmacopoeial requirement and is used to verify that the chromatography system is suitable for performing the analysis. Tests were performed by collecting data from five repeated injections of a standard drug solution and the results were recorded.

Acceptance criteria

1. RSD should not be more than 2.0 % for five replicate injections of standard.
2. USP Tailing Factor/ Asymmetry Factor is not more than 2.0.
3. The column efficiency as determined for Plate Count should be more than 2000.

FILTRATION STUDY:

Filtration study of an analytical procedure checks the interference of extraneous components from filter, deposition on filter bedand compatibility of filter with sample.

Analysis of marketed Test sample:

Marketed test sample Having Name Auroteg 50 mg tablets are selected for analysis and for doing validation.

Average weight of test sample (Auroteg 50 mg):

Weighed the 20 tablets at a time and calculated average weight of tablet by following formula: Average weight (mg) = Weight of 20 tablets (mg) / 20

Sample preparation of Marketed test sample:

Weighed 20 tablets transferred to a grinding bowl and crushed to a fine powder. Mix the contents evenly with butter paper. The powdered material was weighed equivalent to 50 mg of dolutegravir and transferred into a clean and dried 50 mL volumetric flask. Add 10 ml of DMSO, sonicate for 10 minutes with intermittent shaking. After 10 minutes, the solution is allowed to cool to room temperature and the volume is made up to the mark with water. The solution was filtered through a suitable 0.45µ syringe filter, removing 3-5 ml of the initial filtrate. Next, 0.25 ml of the filtered stock solution is diluted to 50 ml with the mobile phase. (5 mcg of Dolutegravir), injected the resulting solution and the chromatograms were recorded and the results recorded. [7][8][9]

Table No.1 Summary of sample preparation as follows:

Sample	Sample (mg)	Diluted to (mL)	Volume taken	Diluted to (mL)
Sample 1	252.1	50	0.25	50
Sample 2	252.1	50	0.25	50

Formula for % Assay calculation:

$$\%\,Assay = \frac{\text{Dolutegravir } Spl\ area}{\text{Dolutegravir } Std\ avg\ area} X \frac{\text{Dolutegravir } STD\ wt\ (mg)}{25} X \frac{0.25}{20} X \frac{50}{Tablet\ sample\ weight\ (mg)} X \frac{50}{0.25} X \frac{Avg\ wt\ of\ sample\ (mg)}{Label\ claim\ of\ \text{Dolutegravir}} X\ Factor\ X\ 100$$

VALIDATION OF RP-HPLC METHOD

1) The developed method for estimation of dolutegravir was validated according to ICH guidelines for the following parameters.

2) This study was conducted with the test sample of Dolutegravir (tablet solution). The filtration study was performed with unfiltered and filtered test solution. During the filtration activity, 0.45 µm PVDF and 0.45 µm nylon syringe filters were used by removing a 5 mL aliquot.

STABILITY OF ANALYTICAL SOLUTION

A stability study was performed for the standard solution and the test sample solution. The stability study was performedunder normal laboratory conditions.

The solution was stored under normal light laboratory conditions and analyzed after 12 hours and 24 hours.

The stability study of the standard and test solution was performed by calculating the difference between the results of thetest solution at each stability time point and the initial one.

3) SPECIFICITY:

Specificity is the ability to access unequivocally the analyte in the presence of components which may be expected to bepresent. Following solution shall be prepared and injected to prove the specificity nature of the method. (Checked peak purity forstandard and test sample solution)

I. Blank (Mobile phase as a diluent)
II. Placebo
III. Dolutegravir Standard solution

IV. Tablet test sample solution

Analyzing marketed test sample contains excipients (additives) which are totally unknown. So Placebo prepared at lab levelby using formula as follows:

Table No.2 Formula for placebo preparation

Sr. No.	Ingredients	Role	Qty (mg)
1	Lactose	Filler	80
2	Starch	Binder	5
3	Magnesium stearate	Lubricant	5
4	Talc	Glidant	5
5	Crospovidone	Disintegrants	5
Total			100 mg

Total 10 gm of placebo prepared:

Placebo Sample solution preparation:

199.47 mg of placebo material (equivalent to 50 mg of Dolutegravir) was weighed and transferred to a clean and dried 50 mL volumetric flask. Add 10 ml of DMSO, sonicate for 10 minutes with intermittent shaking. After 10 minutes, the solution is allowed to cool to room temperature and the volume is made up to the mark with water. The solution was filtered through a suitable 0.45µ syringe filter, removing 3-5 ml of the initial filtrate. Next, 0.25 ml of the filtered stock solution is diluted to 50 ml with the mobile phase, the resulting solution is injected and the chromatograms are recorded. [10][11][12]

4) LINEARITY AND RANGE

Preparation of linearity solution

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional tothe concentration (amount) of analyte in the sample.

5 levels of Linearity was performed from 10% to 150% of working concentration.

Linearity Dolutegravir stock solution:

Weighed 10.53 mg of Dolutegravir sodium (Equivalent to 10 mg of Dolutegravir) and transferred in 20 ml volumetric flask, added 5 mL of DMSO, sonicated to dissolve it completely, made volume up to the mark with water. Further diluted 2.5 ml of stock solution to 50 mL with water (25 µg/mL).[13]

Table No.3 Linearity levels preparation:

Sr. No.	Level (%)	mL of stock solution	Diluted to with Mobile phase (mL)	Dolutegravir Concentration (µg/mL)
1	10	0.2	10	0.5
2	50	1.0	10	2.5
3	100	2.0	10	5.0
4	125	2.5	10	6.25
5	150	3.0	10	7.5

Determination

Each level injected in triplicate and mean area calculated. Calibration curve was plotted graphically as a function of analyte concentration in µg/mL on X-axis Vs mean area on y-Axis as given in results.

Acceptance criteria

Correlation Coefficient: NLT 0.98Intercept: To be report
Slope: To be report

5) Limit of Detection (LOD) and Limit of Quantitation (LOQ):Detection limit:

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detectedbut not necessarily quantitated as an exact value.

Quantitation limit:

The quantitative limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantified with appropriate precision and accuracy.

According to ICH Q2R1 guidelines, the LOD and LOQ were determined using a calibration curve approach in which the residual standard deviation of the regression line was calculated and the LOD and LOQ were determined using the following formula:

LOD = 3.3 σ / SLOQ = 10 σ / S

Where,

σ = residual standard deviation of a regression lineS = Slope of regression line

6) ACCURACY (% RECOVERY)

The precision of an analytical procedure expresses the closeness of agreement between a value that is accepted as either a conventional true value or an accepted reference value and the value of the observed value,

Accuracy will be carried out in the range from 50% to 150% of the working concentration. The solution of each precision level was prepared in triplicate. Calculated %Recovery for each sample, Mean %Recovery for each level and total recoveries as well as calculated %RSD for each level and %RSD for total recovery. [14][15]

Refer Following table for each sample:

Table No.4: Accuracy sample preparation

Level (%)	Dolutegravir Sodium Std (mg)	Placebo(mg)	Diluted to(mL)	Volume taken (mL)	Diluted to(mL)	Dolutegravir Concentartion (µg/mL)
50	26.3	199.5	50	0.25	50	2.50
	26.4	199.7	50	0.25	50	2.51
	26.5	199.4	50	0.25	50	2.52

100	52.8	199.6	50	0.25	50	5.02
	52.7	199.8	50	0.25	50	5.01
	52.7	199.5	50	0.25	50	5.01
150	79.3	199.4	50	0.25	50	7.53
	79.2	199.6	50	0.25	50	7.52
	79.1	199.6	50	0.25	50	7.51

Procedure for preparation of Accuracy sample solution:

Take a clean and dried 9 volumetric flask with a volume of 50 ml. Weigh approximately 199.47 mg of placebo and transfer to each 50 mL volumetric flask. Weighed Dolutegravir Sodium API according to level of accuracy and transferred to the same 50ml volumetric flask. Add 10 mL of DMSO and sonicate for 10 min with occasional shaking. The solution was allowed to cool to room temperature and the volume was made up to the mark with water. Filter the solution through a suitable 0.45 µ nylon syringe filter and discard 5 mL of the filtrate. Next, 0.25 ml of the filtrate is diluted to 50 ml with the mobile phase.

Acceptance criteria

1. % Recovery for each sample and Mean recovery should be in the range of 98-102%.
2. The Relative Standard Deviation should not be more than 2.0%.

PRECISION

Precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous test under the prescribed conditions. Precision is of two types, Repeatability and Intermediate precision. It is performed on tablet test sample.

I. Repeatibility:

Preparation of sample solution (6 Samples prepared):

20 tablets were weighed and average weight was calculated and transferred in a mortar and pulverized to a fine powder. Mix the contents evenly with butter paper. The powdered material was weighed equivalent to 50 mg of dolutegravir and transferred into a clean and dried 50 mL volumetric flask. Add 10 ml of DMSO, sonicate for 10 minutes with intermittent shaking. After 10 minutes, the solution is allowed to cool to room temperature and the volume is made up to the mark with water. The solution is filtered through a suitable 0.45 u nylon syringe filter, removing 3-5 ml of the initial filtrate. 0.25 ml of the filtered stock solution is further diluted to 50 ml with the mobile phase. (5 mcg of Dolutegravir), the resulting solution was injected and the chromatograms were recorded. Six samples prepared. [16][17][18]

Table No.5: Repeatability Precision sample preparation:

Sample No.	Test powder material (mg)	Diluted to(mL)	Volume taken (mL)	Diluted to(mL)
1	252.2	50	0.25	50
2	252.3	50	0.25	50
3	252.1	50	0.25	50
4	252.4	50	0.25	50
5	252.2	50	0.25	50
6	252.5	50	0.25	50

Acceptance criteria:

% Assay: 90-110% for each sample and mean assay value

% RSD for % assay of 6 samples: NMT 2%

II. Intermediate precision

It is performed by doing analysis on another day to check reproducibility of results. Samples prepared in same manner asthat of Repeatability parameter (6 Samples prepared). [19]

Table No.6: Intermediate Precision sample preparation

Sample No.	Test powder material (mg)	Diluted to(mL)	Volume taken (mL)	Diluted to(mL)
1	252.3	50	0.25	50
2	252.1	50	0.25	50
3	252.3	50	0.25	50
4	252.4	50	0.25	50
5	252.3	50	0.25	50
6	252.2	50	0.25	50

Acceptance criteria:

% Assay: 90-110% for each sample and mean assay value

% RSD for % assay of 6 samples of Intermediate precision**:** NMT 2

% RSD for Total 12 samples: NMT 2% for test results (6 of Repeatability and 6 of Intermediate precision)

7) ROBUSTNESS

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberatevariations in method parameters and provides an indication of its reliability during normal usage.

Determination: Blank and Standard solution were injected under different chromatographic conditions as shownbelow.[20]

a). Changes in flow rate by ±10%. (± 0.1ml/min)b). Change in column oven temperature. (± 2ºC)

c) Change in wavelength (± 3 nm)

RESULT AND DISCUSSION

8.1. PRELIMINARY CHARACTERIZATION AND IDENTIFICATION OF DRUG

8.1.1. Color, odour and appearance

Table No.6: Colour, odour and appearance of Drug

Sr. No	Name	Colour, odour and appearance of drug
1	Dolutegravir Sodium	White, odourless and Amorphous powder

8.1.2. Melting point determination

Table No.7: Melting point of Drug

Sr. No.	Name	Melting point std. value (°C)	Melting point observed (°C)
1	Dolutegravir Sodium	190-193 °C	190-192 °C

8.1.3. Solubility study

Table No.8: Solubility study of Dolutegravir Sodium

Sr.No.	Name ofSolvent	Observation	Conclusion	Summary
1	Water	Drug Particles seen after sonication	Drug was not found soluble in water.	DMSO used as a diluent for preparing stock solution.
2	Methanol	Drug Particles seen after sonication	Drug was not found soluble in methanol.	
3	Ethanol	Drug Particles seen after sonication	Drug was not found soluble in Ethanol.	
4	Acetonitrile	Drug Particles seen after sonication	Drug was not found soluble in Acetonitrile	
7	DMSO	No Drug Particles seen after sonication	Drug was found soluble in DMSO.	

8.2.1. Selection of solvent

DMSO was selected as the solvent for dissolving Dolutegravir Sodium

8.2.3. Selection of analytical wavelength

1) Dolutegravir STD solution: (20 PPM)

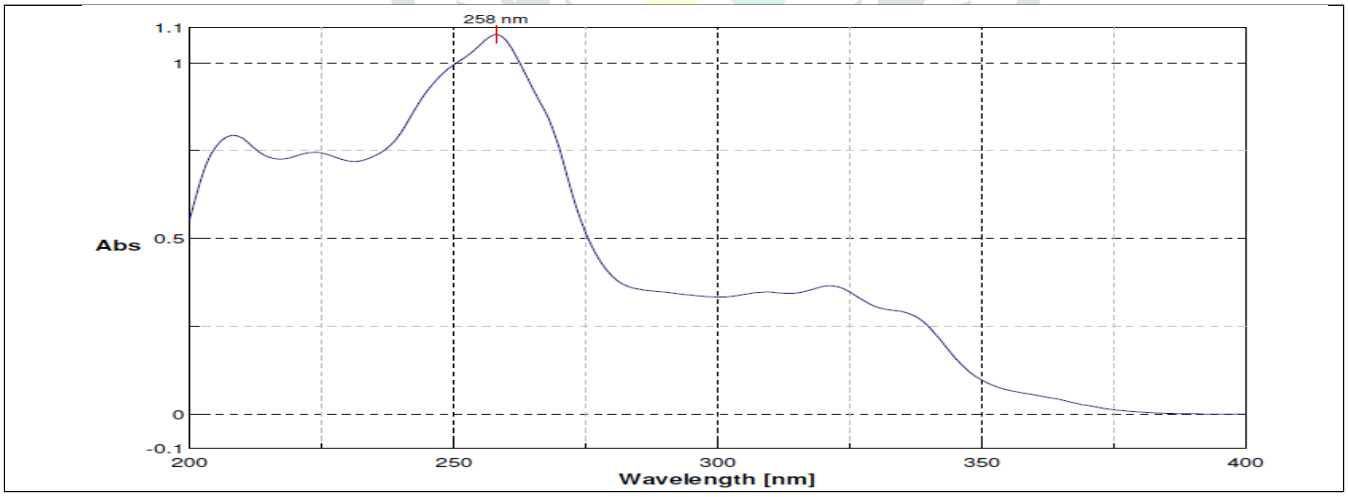


Figure-3: UV spectrum of Dolutegravir

Observation: The standard solution was scanned between 200 nm to 400nm. Wavelength of maximum absorption was determined for drug. Dolutegravir showed maximum absorbance at 258 nm. It is shown in **Figure No**. Therefore 258 nm considered as an analytical wavelength for further determination.

2) Method Development by RP – HPLC Optimization of HPLC method Chromatogram:

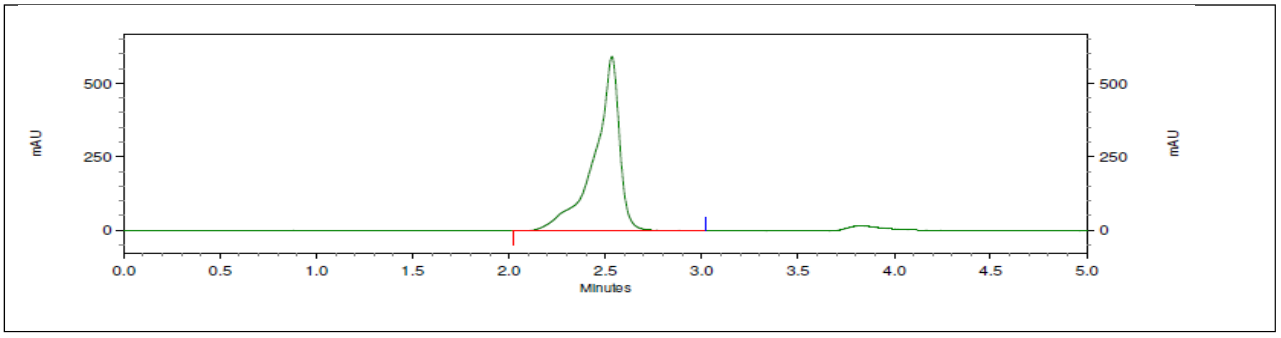
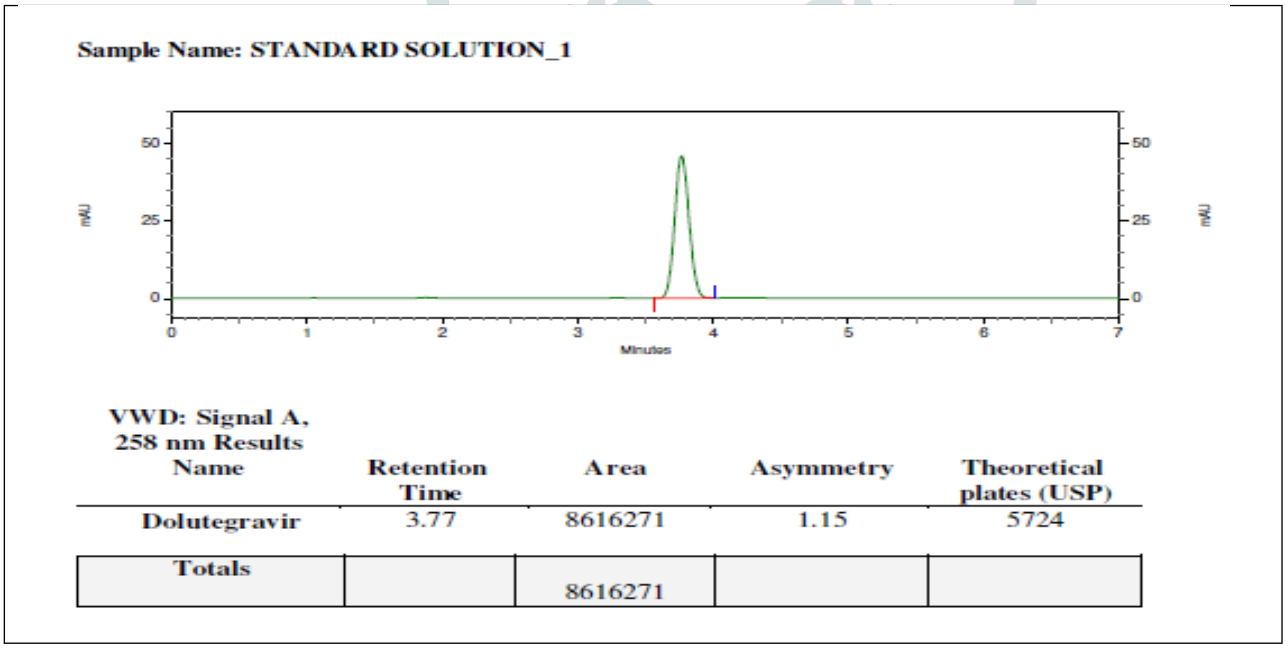


Figure-4: Typical chromatogram of Trial

System Suitability Acceptance Criteria:

1. Relative standard deviation of the area of analyte peaks in standard chromatograms should not be more than 2.0 %.
2. Theoretical plates of analyte peak in standard chromatograms should not be less than 2000.
3. Tailing Factor (Asymmetry) of analyte peaks in Standard Chromatograms should be less than 2.0

Data interpretation: It was observed from the data tabulated above; the method complies with system suitabilityparameters. Hence, it can be concluded that the chromatographic method is adequate for intended analysis.



VWD: Signal A, 258 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Dolutegravir	3.77	8616271	1.15	5724
Totals		8616271		

Figure-5: Typical chromatogram Standard solution 1 of system suitability solution.

8.4.1 Analysis of Marketed Test samples (Assay)Weight of 20 tablets: 5.0420 gm

Average weight = 5.0420 / 20 = 0.2521 gm = 252.1 mg

a) Auroteg 50 mg Tablet:

Table No. 9: Assay results of Auroteg 50 mg Tablet

Sample	Area	% Assay	Mean Assay
Sample 1	8540258	98.73	99.09
Sample 2	8602846	99.45	

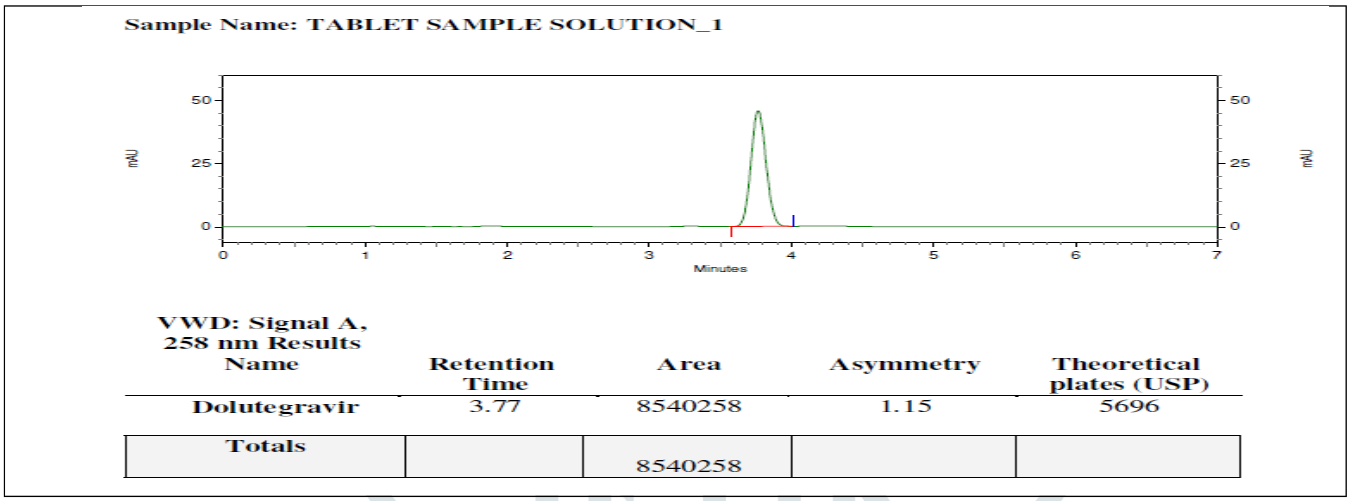


Figure-6: Typical chromatogram Auroteg 50 mg Tablet sample.

Acceptance criteria:

1) % Assay found should be in the range of 90-110%.

Data interpretation:

From the above results, it can be concluded that the assay result is within the limit for selected marketed test sample andsample can be used for validation.

8.5 VALIDATION OF RP-HPLC METHOD

1) FILTRATION STUDY:

Filtration study of an analytical procedure checks the interference of extraneous components from filter, deposition on filterbed and compatibility of filter with sample. Performed on tablet test sample.

Table No.10: Results of Filter study

Sample description	Area	% Absolute difference
Unfiltered	8649100	NA
0.45 µ PVDF filter	8620753	0.33
0.45 µ Nylon filter	8639720	0.11

Chromatograms:

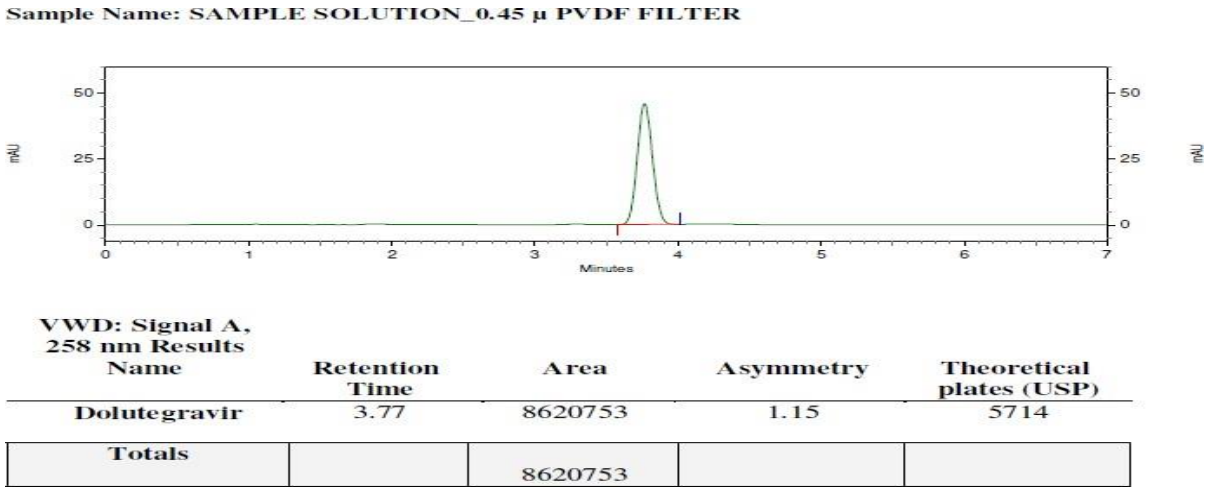


Figure-7: Typical chromatogram of sample filtered through 0.45µ PVDF filter

1) SOLUTION STABILITY: Stability study was conducted for Standard as well as Test Sample. Stability study was performed at normal laboratory conditions. The solution was stored at normal illuminated laboratory conditions and analyzed at initial, after 12 hours and 24 hours.

Table No.11: Results of Solution stability.

Sample solution			Standard solution		
Time point	**Area**	**% Absolute difference**	**Time point**	**Area**	**% Absolute difference**
Initial	8670541	NA	**Initial**	8681304	NA
12 Hours	8640145	0.35	**12 Hours**	8656014	0.29
24 Hours	8619716	0.59	**24 Hours**	8637289	0.51

Chromatograms:

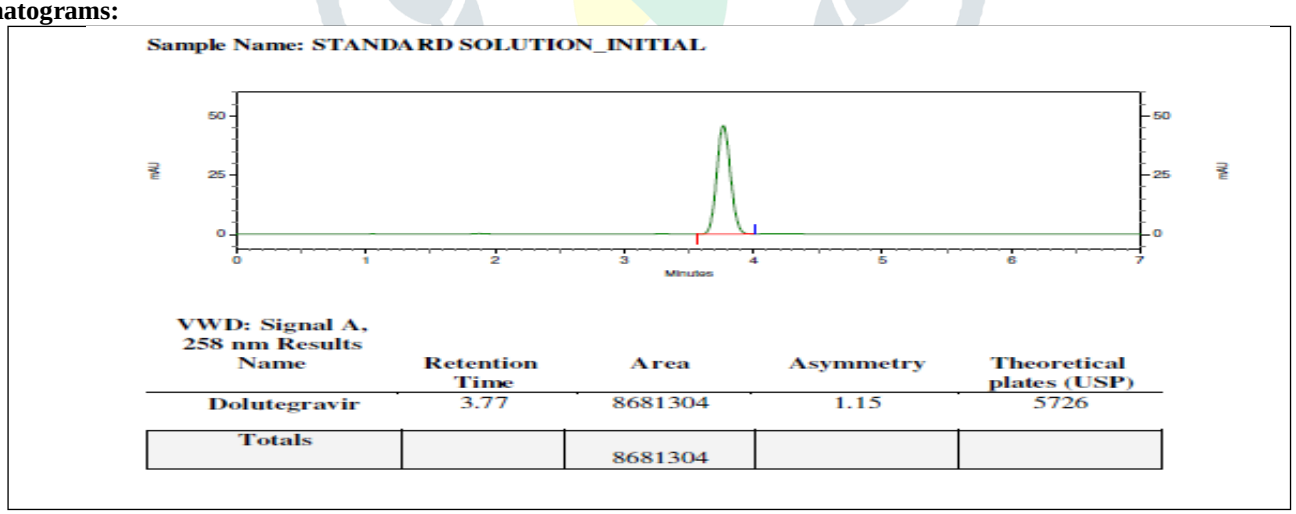
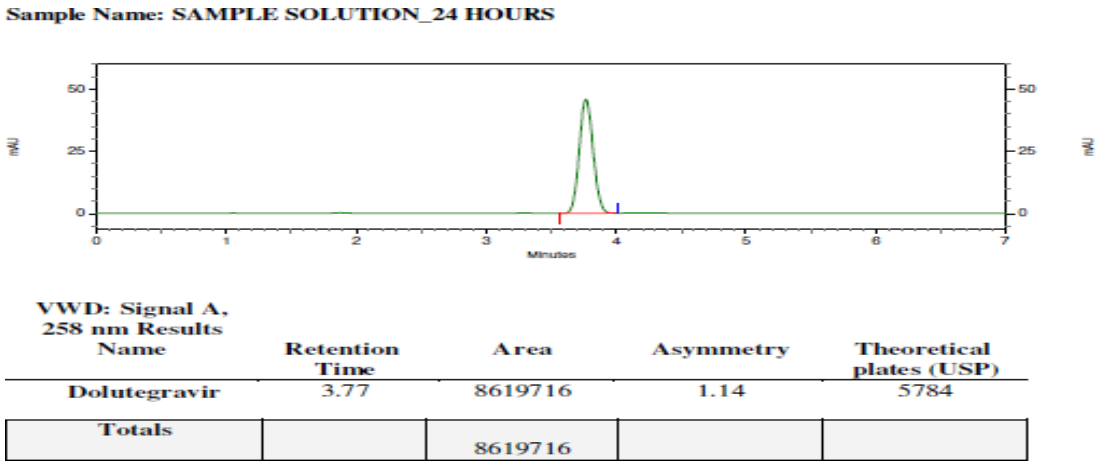


Figure-8: Typical chromatogram of Standard solution Initial.



VWD: Signal A, 258 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Dolutegravir	3.77	8619716	1.14	5784
Totals		8619716		

Figure-9: Typical chromatogram of Test solution After 24 Hrs.

Acceptance criteria: % Absolute difference of Stability solution: NMT 2.0 w.r.t. Initial solution.

Data interpretation: Standard and Test solution was found stable up to 24 Hrs. Hence both solutions can be used up to24 Hrs.

2) SPECIFICITY: Specificity is the ability to access unequivocally the analyte in the presence of components which may be expected to be present.

Blank, standard solution prepared and injected to check peak purity.

Table No.11: Results of Specificity.

Description	Observation
Blank	No interference at R.T. of Dolutegravir due to blank
Placebo	No interference at R.T. of Dolutegravir due to placebo
Standard solution	Peak purity was 0.997
Test Solution	Peak purity was 0.995

Chromatograms:

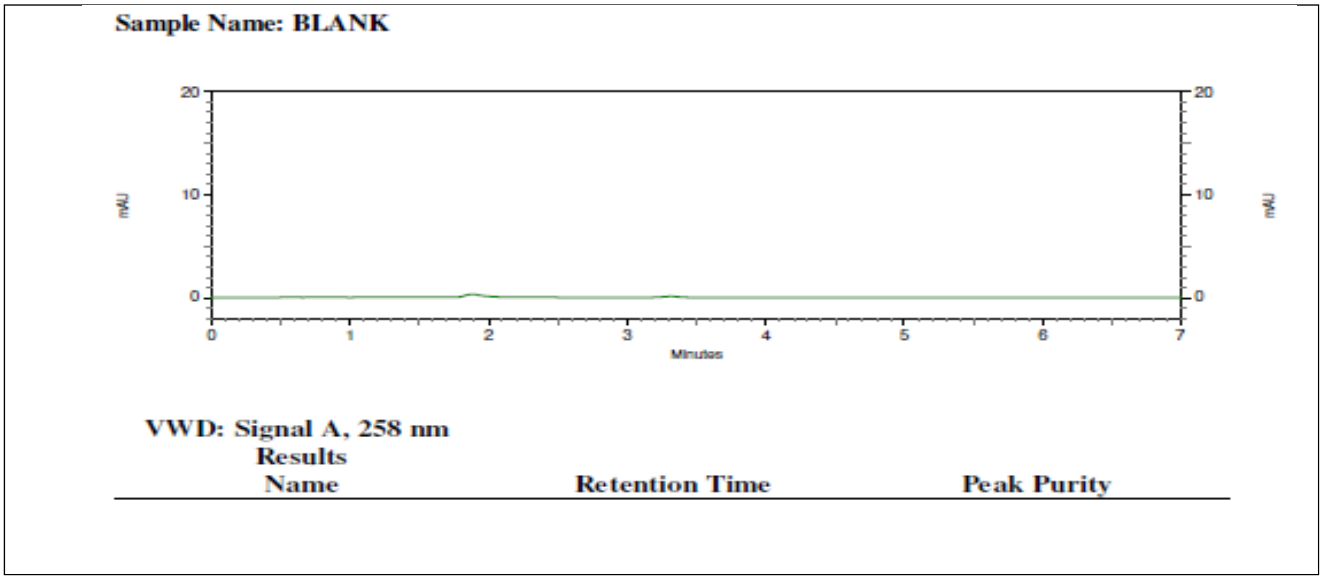


Figure-10: Typical chromatogram of Blank solution.

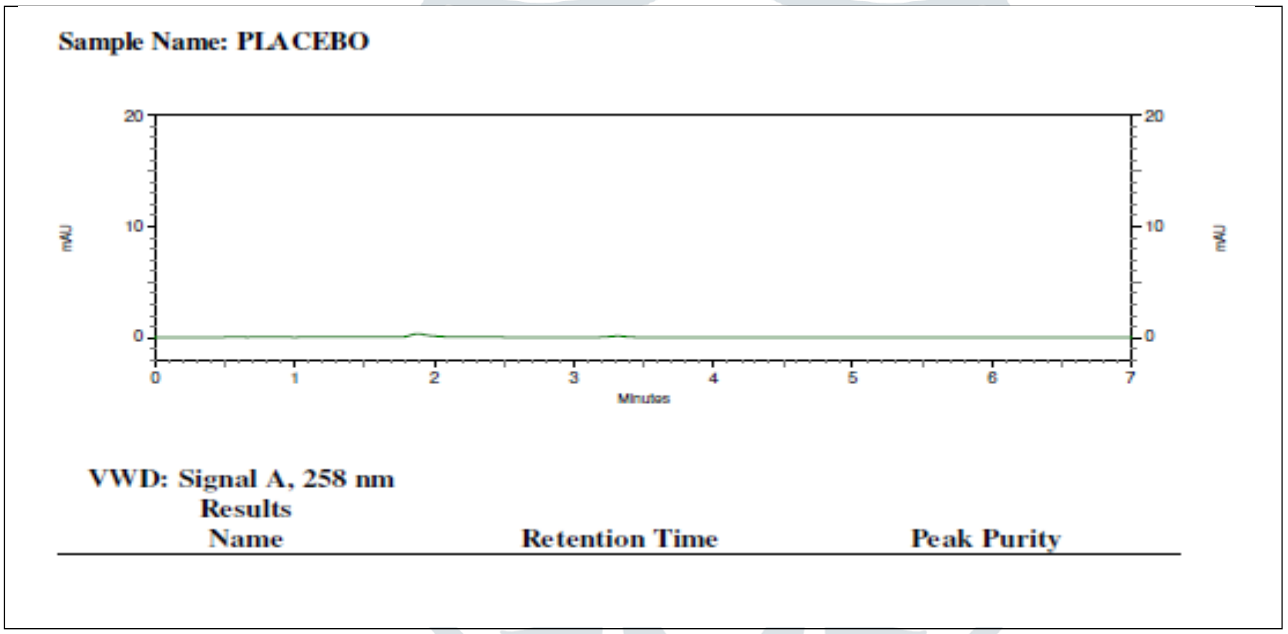


Figure-11: Typical chromatogram of Placebo solution.

1) Linearity and Range

Linearity of an analytical method is its ability to elicit test results that are proportional to the concentration of analyte insamples within a given range.

Table No.12: Linearity Data for Dolutegravir

Level	Conc (µg/mL)	Area	Mean	% RSD
10%	0.50	861091	860793	0.267
		858363		

		862926		
50%	2.50	4295456 4270469 4279410	4281778	0.296
100%	5.00	8630912 8589763 8646970	8622548	0.342
125%	6.25	10688640 10769403 10600685	10686243	0.790
150%	7.50	12946368 12897183 12867901	12903817	0.307

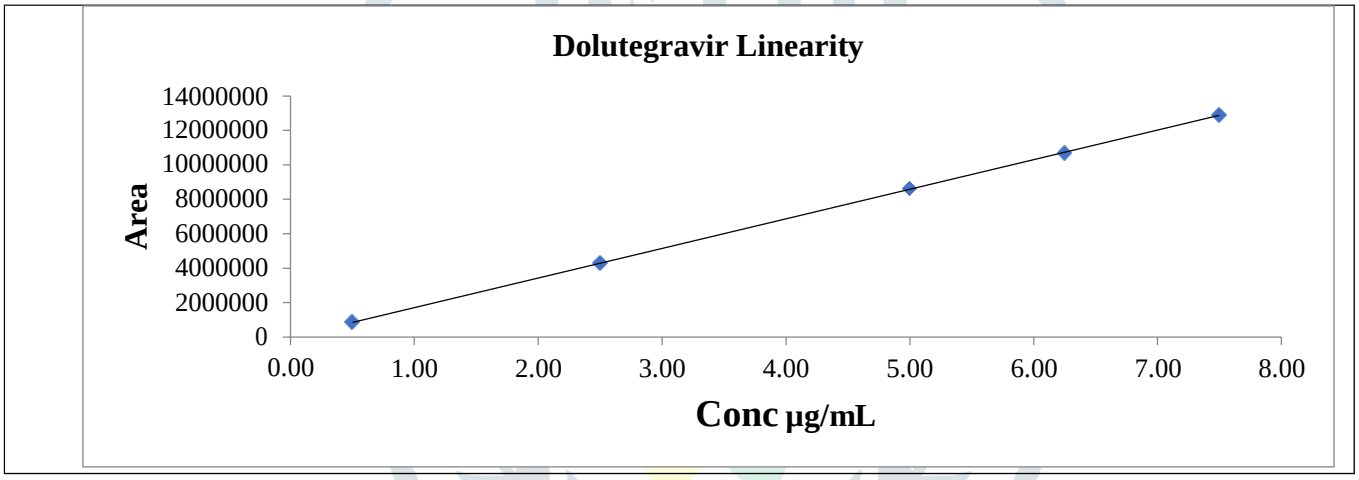


Figure-12: Calibration curve of Dolutegravir

1) Limit of Detection (LOD) and Limit of Quantitation (LOQ):

σ = 32377.17 (Residual standard deviation of a regression line)s = 1718018.606 (Slope)

Detection limit (LOD):

LOD = 3.3 σ / S

LOD = 3.3 x 32377.17 / 1718018.606

LOD = 0.062 µg/mL

Quantitation limit (LOQ):

LOQ = 10 σ / S

LOQ = 10 x 32377.17 / 1718018.606

LOQ = 0.188 µg/mL

2) ACCURACY (RECOVERY):

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method is determined by applying the method to analyzed samples to which known amounts of analyte have been added.

Table No.13: Result and statistical data of Accuracy of Dolutegravir

Level(%)	Area	Recovered conc(µg/mL)	Added conc (µg/mL)	% Recovery	Mean R
50	4310972	2.49	2.50	99.60	99.
	4269731	2.47	2.51	98.41	
	4386792	2.53	2.52	100.40	
100	8650746	5.00	5.02	99.60	99.
	8524821	4.93	5.01	98.40	
	8694304	5.02	5.01	100.20	
150	13089509	7.56	7.53	100.40	99.
	12897540	7.45	7.52	99.07	
	12942705	7.48	7.51	99.60	

Overall Recovery: 99.52 %

% RSD for Overall Recovery: 0.772

Chromatograms:

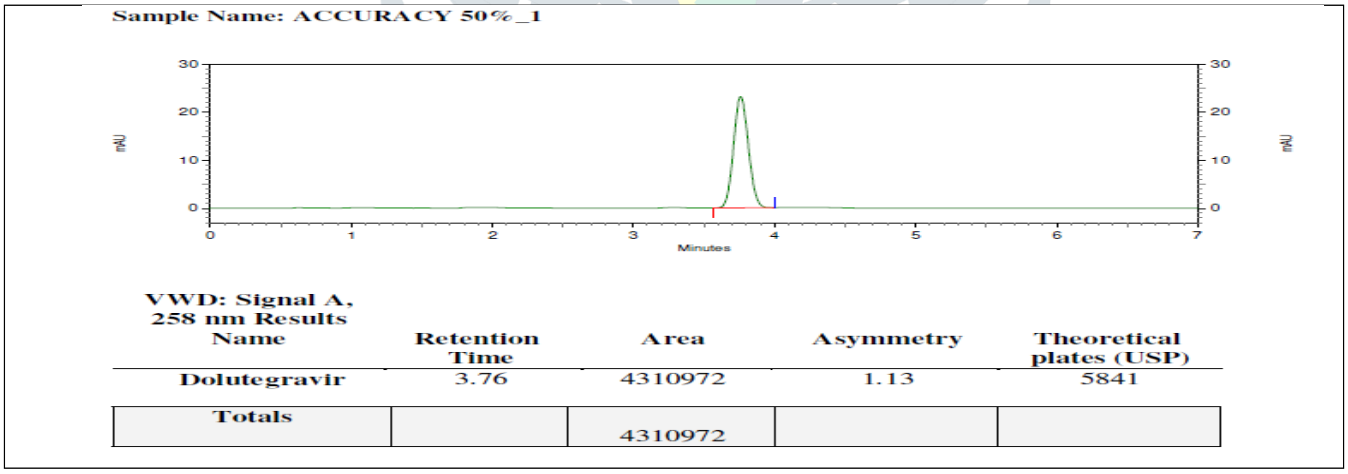


Figure-13: Typical chromatogram of Accuracy 50%.

3) PRECISION

Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation. Precision was performed on Test sample.

Table No.15: Result of Intra- day and Inter- Day Precision for Dolutegravir :

	Sample	Test Sample(mg)	Area	% Assay
Repeatability	Sample 1	252.2	8542013	98.71
	Sample 2	252.3	8517196	98.38
	Sample 3	252.1	8471289	97.93
	Sample 4	252.4	8609148	99.41
	Sample 5	252.2	8625716	99.68
	Sample 6	252.5	8640276	99.73
	Mean			**98.97**
	STD DEV			**0.744652**
	% RSD			**0.752**
Intermediateprecision (Inter-Day)	Sample 1	252.3	8512451	98.33
	Sample 2	252.1	8579130	99.18
	Sample 3	252.3	8517105	98.38
	Sample 4	252.4	8602475	99.33
	Sample 5	252.3	8679580	100.26
	Sample 6	252.2	8621793	99.63
	Mean			**99.19**
	STD DEV			**0.742125**
	% RSD			**0.748**
RepeatabilityPlus Inter-day	**Mean**			**99.079**
	STD DEV			**0.71736**
	% RSD			**0.724**

Chromatograms:

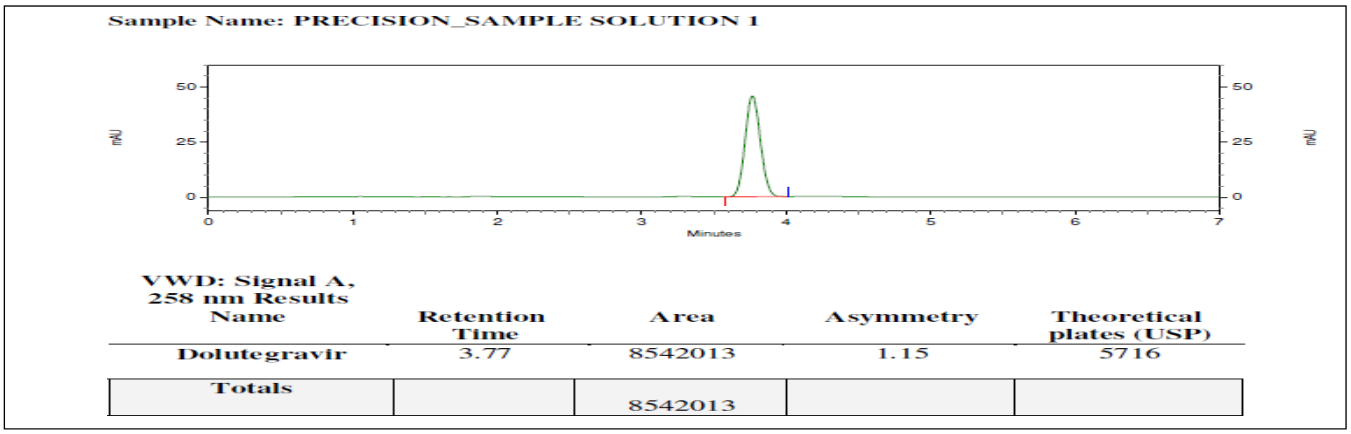


Figure-14: Typical chromatogram of Repeatability precision (Sample 1).

4) ROBUSTNESS:

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations inmethod parameters and provides an indication of its reliability during normal usage.

Following changes made under Robustness:

- Change in Wavelength
- Change in flow rate
- Change in column oven temperature

Table No.16: Result of Robustness study of Dolutegravir

Change in Parameter	R.T.	Standard area	Asymmetry	Theoretical plates
Wavelength by +3 NM (261 NM)	3.76	8306531	1.16	5678
Wavelength by -3 NM (255 NM)	3.76	8494566	1.15	5694
Flow rate by +10% (1.1mL/min)	3.42	8420357	1.14	5498
Flow rate by -10% (0.9mL/min)	4.17	8720143	1.16	5579
Column oven temp by +2ºC (42 ºC)	3.77	8607927	1.14	5568
Column oven temp by -2ºC (38 ºC)	3.77	8579027	1.17	5601

Chromatograms:

A. Change in Wavelength by +3 NM:

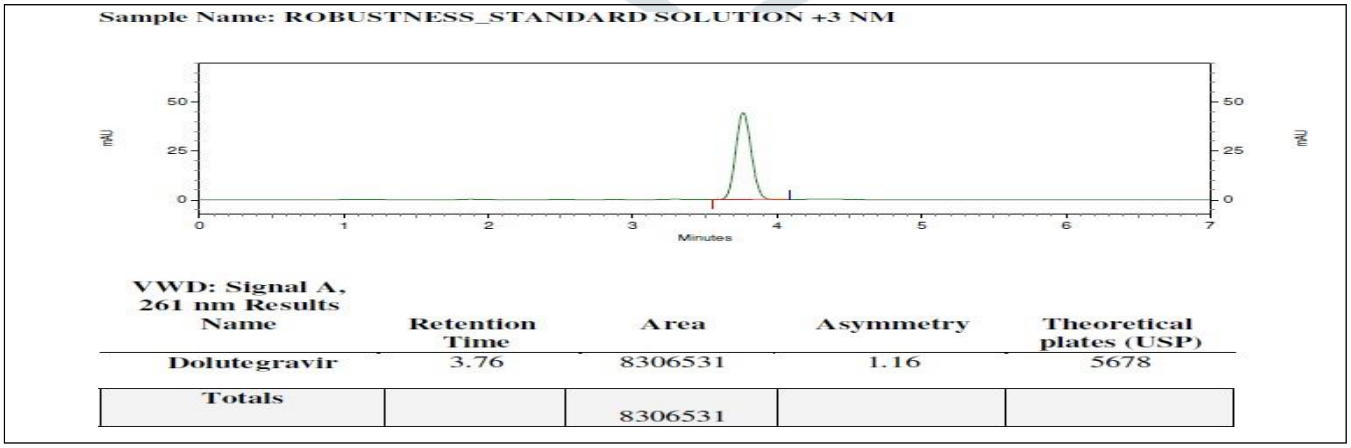


Figure-14: Typical chromatogram of Standard +3 NM.

CONCLUSION

The present work involved the development of simple, accurate, precise and suitable RP-HPLC method. Literature survey revealed that several methods have been reported for determination of Dolutegravir in bulk drug or in pharmaceutical dosage forms. Hence, in the present study, a new, sensitive and suitable reversed-phase high performance liquid chromatography method was developed and validated for the determination of Dolutegravir in bulk drug and pharmaceutical dosage form. In developed RP-HPLC method, the analyte were resolved by using isocratic program and mobile phase was used Methanol : 0.025% TFA in Water (80:20 % v/v) at a flow rate of 1.0 ml/min, on HPLC system containing UV- visible detector with Openlab EZ-Chrome Software and Kromasil C18, 250 mm X 4.6 mm, 5 µm. The detection was carried out at 258 nm. The results of analysis in the developed method were validated in terms of linearity, accuracy, precision, robustness, limit of detection and limit of quantification.The developed method has several advantages, including reproducibility of results, rapid analysis, simple sample preparation and improved selectivity as well as sensitivity. The regression coefficient (r2) for each analyte is not less than 0.999 which shows good linearity. The % recovery was in the acceptable range in tablet dosage form. The %RSD was also less than 2% showing high degree of precision of the proposed method. Since the developed method is robust and reproducible and also less time consuming, it can be performed for routine analysis in pharmaceutical industry for bulk drug of Dolutegravir and also in pharmaceutical dosage form.

REFERENCES:

1. Beckett A.H, Analytical Chemistry and Practical Chemistry. 1996; 14th edi. –Vol-2.: 275-95

2. Skoog D.A, Holler F.J, Timothy A, Nieman N.W, Principle of instrumental Analysis. Eastern Press, Bangalore 2004; 5th edi.: 1-4,729-35

3. Willard H.H, Merritt L.L, Dean J.A, Settle Jr. F.A, Instrumental Methods of analysis. CBS Publishers and distribution, Delhi, 2001; 7th edi.: 118-19

4. Christan G.D, Analytical chemistry. John Wiley and sons. 2003; 6th Edn:1-3

5. Chatwal G.R, Sharma A, Instrumental Method of chemical Analysis. Himalaya Publishing House Delhi 2004; 5th edi: 1.1-1.5

6. Jeffery G.H, Bassat J, Mendham J, Denny R.C., Vogel's textbook of Quantitative Chemical Analysis. Elbs with Longman Publication Harlow,1989; 5th edi:1-6

7. Sharma B.K, Instrumental Methods of Chemical Analysis. Goal Publishing House, 2005; 24th edi.:3-12

8. Snyder, L.R, Joseph J.K, Joseph L.G, Practical HPLC Method Development. A Wiley-Interscience publication, New York; 2nd edi: 3-35

9. Ojeda C.B, Rojas F.S, Analytical Chemistry Actta. 2004;1-24

10. ICH, Q2A, Text on validation of analytical Procedures International Conference on harmonization, Geneva, 1994October,1-5

11. ICH, Q_2B, Validation of analytical Procedure: Methodology, International conference on Harmonization, Geneva, 1996 November,1-8

12. ICH, Q2R1, Text on validation of analytical Procedures International Conference on harmonization, Geneva, 1994 October,1-13

13. Chandrama Dherange *et al*. Development and Validation RP-HPLC Method for Dolutegravir in Bulk and Solid Dosage form, Journal of Emerging Technologies and Innovative Research (JETIR) Vol 7, Issue 9, 2020; 811-816

14. Venkatnarayana M *et al*. Development of Validation and Stability Indicating Method of Anti-HIV Dolutegravir Drug and its Related Impurities by Using RP-HPLC, Journal of Chromatography & Separation Techniques Vol 11, Issue 426, 2020; 1-7.

15. Veeraswami B et al. Development and validation of rp-hplc method for the estimation of dolutegravir and rilpivirine in bulk and pharmaceutical dosage form and its application to rat plasma, Asian journal of pharmaceutical and clinical research Vol 12, Issue 2, 2019; 267-271.

16. Kanchipogu Usha Rani et al. A new analytical method for determination of dolutegravir and rilpivirine in pharmaceutical formulations by RP-HPLC method, International journal of research in Pharmaceutical chemistry and analysis, 1(2), 2019; 68-74.

17. Ponnamalla Jasmine Carey et al. Method development and validation for simultaneous estimation of abacavir, lamivudine & dolutegravir by rp-hplc method, World jounal of pharmaceutical and medical research vol 6, issue 7, 2020; 229-235.

18. Bhavar Girija Balasaheb et al. Development and validation of uv spectrophotometric method for estimation of dolutegravir sodium in tablet dosage form, Malaysian journal of analytical sciences Vol 19, Issue 6, 2015; 1156-1163.

19. K.Bhavyasri et al. Method Development, Validation and Forced Degradation Studies of Dolutegravir, An Anti-Retroviral Drug using Uv-Visible Spectroscopy, International Journal of Pharma Sciences and Scientific Research Vol 5, Issue 6,2019; 69-74.

20. Thaidala Sriveni et al. Development and Validation of Dolutegravir in Bulk and Formulation: An Anti-retroviral Drug using UV-spectroscopy, IJPQA, Vol 12, Issue 1, 2021; 58-60.