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Original Article

A New Stability Indicating RP-HPLC Method Development and Validation for the Simultaneous Estimation of Dolutegravir and Rilpivirine in Bulk and its Dosage Forms

Yusuff Ismail[a*], M.Vijaya Vara Prasad[b], S. M. Shaheedha[c], Mohammad Habeeb[d]

[a]*Department of Pharmaceutical Analysis, Crescent School of Pharmacy, B.S. Abdur Rahman Crescent Institute of Science and Technology, Chennai, Tamilnadu, India,* [b]*Department of Pharmaceutical Analysis, Crescent School of Pharmacy, B.S. Abdur Rahman Crescent Institute of Science and Technology, Chennai, Tamilnadu, India,* [c]*Department of Pharmacognosy, Crescent School of Pharmacy, B.S. Abdur Rahman Crescent Institute of Science and Technology, Chennai, Tamilnadu, India,* [d] *Department of Pharmaceutics, Crescent School of Pharmacy, B.S. Abdur Rahman Crescent Institute of Science and Technology, Chennai, Tamilnadu, India*

Abstract

The objective of the work is to develop and validate a new, simple, highly sensitive, stability indicating RP-HPLC method for simultaneous estimation of Dolutegravir and Rilpivirine in bulk and its dosage forms. The method was developed on a reversed-phase Thermosil C_{18} (4.6 × 150 mm, 5µm) column with an isocratic elution. The Mobile phase ratio was Acetonitrile: Phosphate Buffer pH 3.5 (45:55 % v/v). Detection was done by UV-Spectroscopy at a detection wavelength of 260 nm. The flow rate was 0.8 ml/min. The mobile phase was used as a diluent. The Injection volume was 10µl. The analytical procedure was validated as per ICH guidelines. The retention time for Dolutegravir and Rilpivirine in the standard solution having the concentration of 100 µg/ml of Dolutegravir and 50 µg/ml of Rilpivirine were observed to be around 2.427 min and 4.436 min. respectively. The purity percentage values of Dolutegravir and Rilpivirine were 99.22 % w/v and 99.81 % w/v respectively. System suitability parameters were calculated and found within the acceptance criteria. The proposed method was found to have a high degree of precision and reproducibility. Calibration plots were linear (r^2> 0.999) over the concentration range of 80 - 120 µg/ml for Dolutegravir and 30 - 70 µg/ml for Rilpivirine. The recovery percent were within the acceptance criteria of 98 – 102 % for Dolutegravir and Rilpivirine. The LOD was 0.044 µg/ml for Dolutegravir and 0.060 µg/ml for Rilpivirine. The LOQ was 0.134 µg/ml for Dolutegravir and 0.183 µg/ml for Rilpivirine. The method represents a fast-analytical procedure and stability indicating analytical method for the simultaneous estimation of Dolutegravir and Rilpivirine in bulk and its dosage forms. No interference from any component of pharmaceutical dosage form was observed. Validation studies revealed that the method is specific, rapid, reliable, and reproducible. The method is amenable to the routine analysis of large numbers of samples with good precision and accuracy.

Keywords: Dolutegravir, ICH, Reverse transcriptase inhibitor, Rilpivirine, RP-HPLC, Stability indicating method, UV-Spectroscopy.

Corresponding Authors: Yusuff Ismail, Department of Pharmaceutical Analysis, Crescent School of Pharmacy, B.S. Abdur Rahman Crescent Institute of Science and Technology, Chennai, Tamilnadu, India
Tel: +919700550331
E-Mail: ismailpharmacy786@gmail.com
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1. Introduction

Dolutegravir, with the chemical name (4R,12aS)-9-{[(2,4- difluorophenyl) methyl] carbamoyl}-4-methyl6,8-dioxo-3,4,6,8,12,12a-hexahydro-2H-pyrido[1',2':4,5] pyrazino [2,1-b][1,3]oxazin-7-olate (Figure 1) is an HIV-1 antiviral agent [1, 2]. It is a novel integrase strand transfer inhibitor active against Human immuno deficiency virus [3, 4].

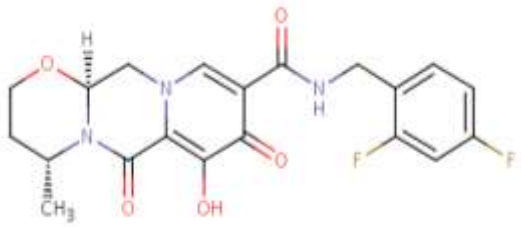

Figure 1. Chemical structure of dolutegravir.

Rilpivirine with the chemical name 4-{[4-({4-[(1E)-2-cyanoeth-1-en-1-yl] 2, 6 dimethylphenyl} amino) pyrimidin-2yl] amino} benzonitrile (Figure 2) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) which is used for the treatment of HIV-1 infections [5, 6]. It is a diarylpyrimidine, a class of molecules that resemble pyrimidine nucleotides found in DNA. Because of its flexible chemical structure, the resistance of Rilpivirine is less likely to develop than other NNRTI's. Rilpivirine is an NNRTI which binds to reverse transcriptase which results in a block in RNA and DNA-dependent DNA polymerase activities [7]. One such activity is HIV-1 replication. Intracellular phosphorylation is not necessary for its antiviral activity. Because the structure of Rilpivirine is flexible around the aromatic rings, the molecule can have multiple conformations so that can bind to residues in the reverse transcriptase enzyme which have a lower mutation rate [8].

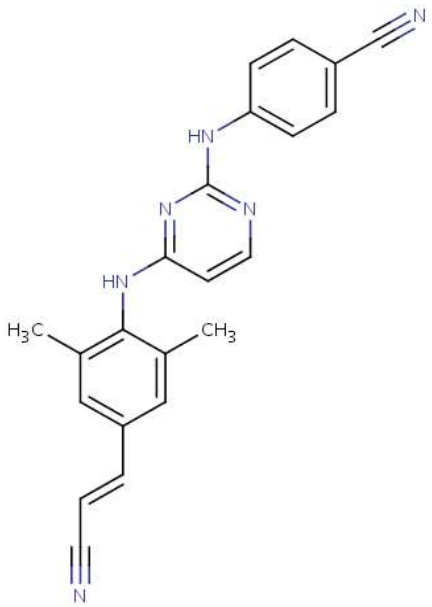

Figure 2. Chemical structure of rilpivirine.

It is imperative to have safety and efficacy in drug therapy, pharmacists must take into consideration, the stability of drugs and its therapeutic values [9, 10]. The stability of the drug formulations can be assessed by using stability indicating methods. A well-designed stress study is important to help develop and demonstrate the specificity of stability indicating methods. They are also useful for checking rapid and accurate drug quality during stability testing [11, 12].

Lack of in-depth study [13 – 17] prompted us to work on the stability indicating method for simultaneous determination of Dolutegravir and Rilpivirine in bulk and its dosage forms by RP-HPLC. The present work is aimed to develop novel, rapid, affordable, reliable, cost-effective, selective, sensitive, precise, accurate, reproducible, specific and stability indicating analytical method development and validation for the simultaneous estimation of Dolutegravir and Rilpivirine in bulk and its dosage forms by RP-HPLC.

2. Materials and Methods

2.1. Chemicals and Reagents

Pure samples of Dolutegravir and Rilpivirine were obtained from Pharma Train Lab, Hyderabad. The commercial samples of the tablets 'Juluca' containing Dolutegravir - 50 mg and Rilpivirine – 25 mg were provided by Janssen Therapeutics Pvt. Ltd, Mumbai. HPLC grade Acetonitrile was procured from Merck Ltd. (Mumbai, India).

2.2. Chromatographic Conditions

Waters e2695 Separation Module HPLC system and a UV-visible detector were used for Chromatographic separation. The chromatographic column utilized in the study was Thermosil C_{18} (4.6 × 150 mm, 5µm). Different mobile phases were tried, and the one containing Acetonitrile: Phosphate Buffer (pH 3.5 adjusted with Orthophosphoric acid) in the ratio of 45:55 % v/v was appropriate. Phosphate buffer was prepared by weighing 2.95 grams of KH_2PO_4 and 5.45 grams of K_2HPO_4, dissolved and diluted to 1000ml with HPLC water. The Orthophosphoric acid was added to maintain the pH level at 3.5. The mobile phase was subjected to vacuum filtration by using a 0.45 µm filter. The mobile phase was used as a diluent. The flow rate selected was 0.8ml/min [18, 19]. All the determinations were performed at a constant column temperature (Ambient) and an injection volume was 10µl. The mixed standard solution was scanned in the U.V range of 200-400 nm [20]. The overlay spectrum of Dolutegravir and Rilpivirine was obtained and the isosbestic point of Dolutegravir and Rilpivirine showed absorbance maxima at 260 nm (Figure 3).

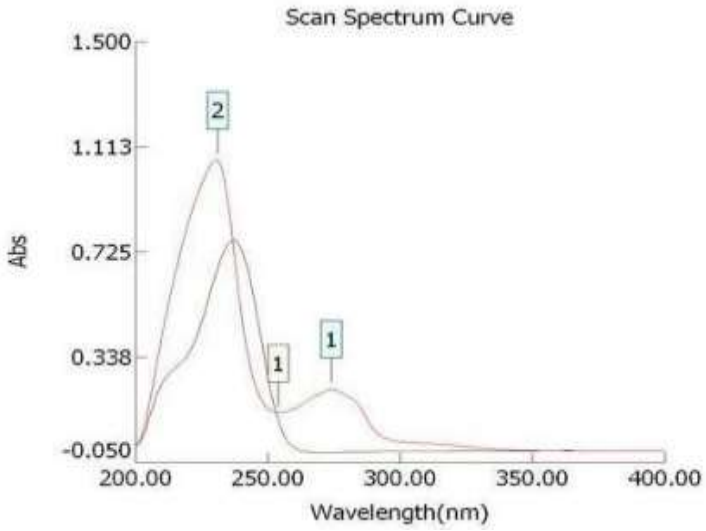

Figure 3. Overlay spectrum of dolutegravir and rilpivirine.

2.3. Preparation of Standard Solution Dolutegravir and Rilpivirine

50 mg Dolutegravir and 25 mg Rilpivirine working standard were placed into a 100 ml volumetric flask and about 20 ml of diluent was added. Then it was subjected for sonication and made up to the volume with diluent. 5 ml of this solution was placed into a 25 ml volumetric flask and made up to the volume with diluent to give the concentrations of 100 µg/ml Dolutegravir and 50 µg/ml Rilpivirine.

2.4. Preparation of Sample Solution Dolutegravir and Rilpivirine

The sample powder (Juluca tablets) equivalent to 50 mg of Dolutegravir and 25 mg Rilpivirine tablet was placed into a 100 ml volumetric flask and 20 ml of diluent was added and made up to the volume with diluent. 5 ml of this solution was placed into a 25 ml volumetric flask and made up with diluents to obtain a concentration of 100µg/ml Dolutegravir and 50 µg/ml Rilpivirine respectively.

2.5. Assay

10 µl of standard and sample solutions were injected into the injector of HPLC, and the peak areas of the drugs in standard and sample were compared and the assay was performed. Dolutegravir and Rilpivirine show the purity percentage values of 99.22 % w/v and 99.81 % w/v, respectively.

2.6. Method Validation

2.6.1. Specificity

It was confirmed by injecting the placebo (Talc, Magnesium Stearate, Starch, Hydroxypropyl methyl cellulose, Colloidal silicon dioxide and Sodium starch glycolate) and placebo spiked standard and observed that there was no shift in wavelength interference due to placebo. This confirms the specificity of the proposed method [21]**.**

2.6.2. Linearity and Range

50 mg of Dolutegravir and 25 mg of Rilpivirine working standard were placed into a 100 ml volumetric flask, about 20 ml of diluent was added. Then it was subjected for sonication and made up to the volume with diluent. Then it was further diluted to give a concentration of 80, 90, 100, 110 and 120 ppm of Dolutegravir and 30, 40, 50, 60 and 70 ppm of Rilpivirine respectively. Each level was injected into the chromatographic system and peak area was measured. A graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak was plotted and the correlation coefficient calculated [22].

2.6.3. Precision

2.6.3.1. System precision

The precision of the system was verified by six replicate injections of the mixed standard solution of Dolutegravir and Rilpivirine [23].

2.6.3.2. Method Precision

The precision of the method was determined by six replicate injections of mixed sample solution of Dolutegravir and Rilpivirine [24].

2.6.3.3. Inter-day Precision

To check the inter-day variations of the method, the solutions containing 100 µg/ml Dolutegravir and 50 µg/ml Rilpivirine

respectively, were subjected to the proposed HPLC method of analysis on different days and the results obtained were noted.

2.6.4. System Suitability

Six replicate injections of the mixed standard solution of Dolutegravir and Rilpivirine was injected. System suitability parameters such as resolution, tailing factor, no. of theoretical plates were calculated [25].

2.6.5. Accuracy

Recovery studies were used to evaluate the accuracy of the analytical method. It was verified by recovery studies. Dolutegravir and Rilpivirine working standards were spiked with Placebo and made up with diluent to give the target concentrations of 80 µg/ml, 100 µg/ml and 120 µg/ml of Dolutegravir and 30 µg/ml, 50 µg/ml and 70 µg/ml of Rilpivirine.10 µl of the above- mentioned solutions was injected into HPLC. The amount obtained for Dolutegravir and Rilpivirine samples were calculated. The recovery percent and mean recovery values were calculated.

2.6.6. Robustness

It was performed by changing the HPLC pump flow rate variations ± 0.2 ml/min and organic composition of the mobile phase ±5%.

2.6.7. Ruggednes

Ruggedness was determined by carrying out the assay under a variety of conditions, such as different analysts. There was reproducibility of test results under normal, expected operational conditions from laboratory and from analyst to analyst. The mixed standard solution of Dolutegravir and Rilpivirine was prepared by using a diluent as per test method separately by Analyst – I and Analyst – II. 10 µl of these solutions was injected six times and the % RSD for the Peak area of six replicate injections was calculated for Analyst – I and Analyst - II.

2.6.8. Limit of Detection:

LOD's can be calculated using the response standard deviation (σ) and the calibration curve slope (S).

Formula:

$$\text{LOD} = 3.3 \text{ X } \frac{\sigma}{S}$$

2.6.9. Limit of Quantification

LOQ's can be calculated using the response standard deviation (σ) and the calibration curve slope (S).

Formula:

$$\text{LOQ} = 10 \text{ X } \frac{\sigma}{S}$$

2.6.10. Degradation Studies

2.6.10.1. Acidic Degradation

The sample solution was prepared with 10 ml of 0.01 M Hydrochloric acid. Reflux under heat at 60°C for one hour. The sample solution was neutralised using 0.01 M Sodium hydroxide and diluted with diluent to make the final concentration as per test method [26-27].

2.6.10.2. Basic Degradation

The sample solution for basic degradation studies was prepared with 10 ml of 0.1 M

Sodium hydroxide. Reflux under heat at 60°C for one hour. The sample solution was neutralised using 0.1 M Hydrochloric acid and diluted with diluent to make the final concentration as per test method [28].

2.6.10.3. Neutral Degradation

The sample solution was prepared with 10 ml of water. Reflux under heat at 60°C for one hour. It was further diluted with diluent to make the final concentration as per test method [29].

2.6.10.4. Oxidative Degradation

The sample solution for oxidative degradation studies was prepared with 10 ml of 3 % Hydrogen peroxide. Reflux under heat at 60°C for one hour. It was further diluted with diluent to make the final concentration as per test method [30].

2.6.10.5. Photolytic Degradation

The sample solution for photolytic stability studies was prepared with diluent as per test method. The resultant solution was exposed to natural sunlight during the daytime for 8 hours [31].

10 µl of the acidic, basic, neutral, oxidative and photo stability solutions were injected. The peak area for the Dolutegravir and Rilpivirine were measured and % degradation calculated from % Assay.

3. Results and Discussion

In this study, attempts were made to develop a simple, accurate, rapid, precise and stability indicating method and validate for determination of Dolutegravir and Rilpivirine in bulk and its combined pharmaceutical formulations by HPLC using a Thermosil C_{18} (4.6 x150 mm, 5 µ) column with a solvent mixture of Acetonitrile: Phosphate Buffer pH 3.5 (45 : 55 % v/v) as a mobile phase. The flow rate was 0.8 ml/min. The mobile phase was used as a diluent. The Injection volume was 10 µl. The isocratic mode was used for the separation of Dolutegravir and Rilpivirine. From the overlay spectrum of Dolutegravir and Rilpivirine, 260 nm was selected for quantification. Because 260 nm was the isoabsorptive point for both the drugs and both drugs showed good absorbance at 260 nm. When compared to other reported methods [32-34], the flow rate in this developed method was 0.8 ml/min. So, the developed method benefits from the low volume of mobile phase and short analytical run time. Hence the developed method is economical and cost-effective. The retention times of Dolutegravir and Rilpivirine were found to be around 2.427 min and 4.436 respectively. Dolutegravir and Rilpivirine showed the purity percentage values as 99. 22 % w/v and 99.81 % w/v, respectively. All parameters of this proposed method were validated.

3.1. Specificity

No elution of the interfering peak takes place, which shows that the peak of the analyte was pure and excipients in the combination should not interfere with the analyte. No interference was observed at the retention time for the respective drug (Figure 4). Therefore, the method is selective for the determination of Dolutegravir & Rilpivirine. This confirms the specificity of the proposed method**.**

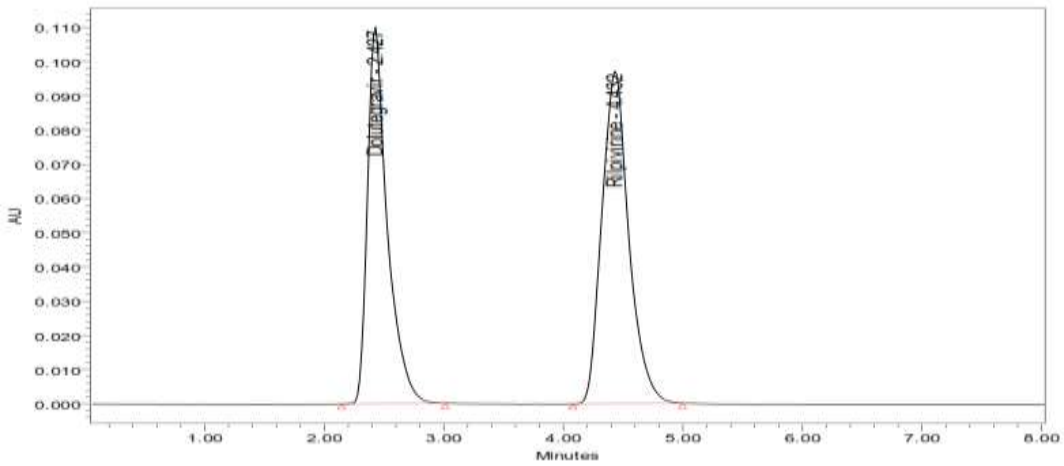

Figure 4. Chromatogram showing specificity of dolutegravir and rilpivirine.

3.2. Linearity and Range

It was evaluated by visual inspection of the plot of peak area as a function of analyte concentrations for Dolutegravir & Rilpivirine. The results are reported in table 1.

The calibration curves are shown in (Figure 5 and Figure 6). From the linearity studies, the specified range was determined for Dolutegravir & Rilpivirine as 80 - 120 µg/ml. and 30 - 70 µg/ml respectively. The Correlation Coefficient for Dolutegravir & Rilpivirine was found to be within the acceptance criteria of 0.999. The linearity results for Dolutegravir & Rilpivirine in the specified concentration range are found satisfactory.

Table 1. Linearity results for dolutegravir and rilpivirine.

Parameters	Dolutegravir	Rilpivirine
Linear Dynamic Range	80 – 120 µg/ml	30 – 70 µg/ml
Correlation Coefficient	0.999	0.999
Slope (m)	91548	87638

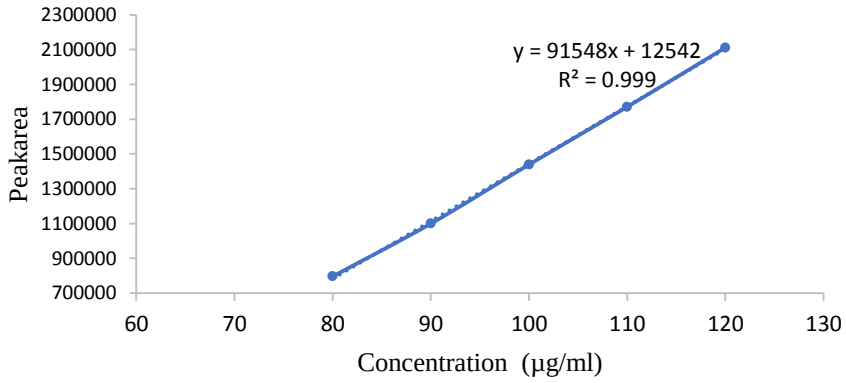

Figure 5. The Linearity curve of (80-120 µg/ml) dolutegravir.

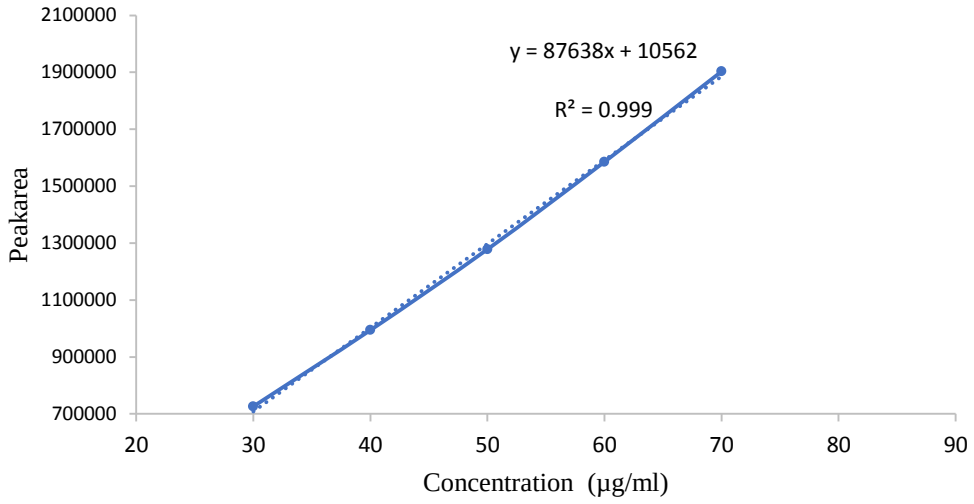

Figure 6. The Linearity curve of (30-70 µg/ml) rilpivirine.

3.3. Precision

3.3.1. System Precision

The % R.S.D of peak area was 0.097, retention time was 0.565, U.S.P Plate count was 1.280 and U.S.P tailing was 0.5050 for Dolutegravir. The % R.S.D of peak area was 0.8445, retention time was 0.2868, U.S.P Plate count was 0.2813 and U.S.P tailing was 0.7054 for Rilpivirine. It showed that the drug was having good precision and the % R.S.D of peak area, retention time, U.S.P Plate count and U.S.P tailing were present within the Acceptance criteria of R.S.D < 2 %. The results are reported in table 2 and table 3. The chromatograms are shown in (Figure 7).

Table 2. System precision data for dolutegravir.

Injection	R_t	Peak Area	USP Plate count	USP Tailing
1	2.462	1270846	2760	1.61
2	2.427	1268766	2750	1.61
3	2.427	1271255	2735	1.62
4	2.437	1271744	2673	1.62
5	2.429	1272393	2763	1.61
6	2.428	1271100	2765	1.63
MEAN	2.435	1271017.333	2741	1.6166
SD	0.01375	1231.3959	35.1055	0.0082
% RSD	0.565	0.097	1.280	0.5050

Table 3. System precision data for rilpivirine.

Injection	R_t	Peak Area	USP Plate count	USP Tailing
1	4.436	1519139	2750	1.71
2	4.436	1521543	2754	1.71
3	4.432	1519606	2744	1.73
4	4.466	1489639	2752	1.72
5	4.450	1501376	2762	1.73
6	4.449	1506026	2740	1.70
MEAN	4.4448	1509554.833	2750.333	1.7166
SD	0.01275	12748.2135	7.7373	0.0121
% RSD	0.2868	0.8445	0.2813	0.7054

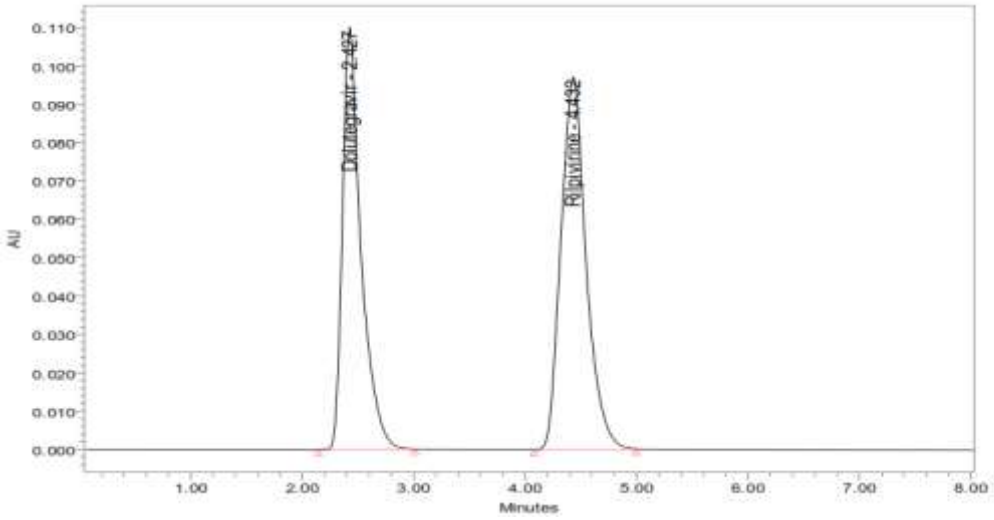

Figure 7. Chromatogram showing system precision of dolutegravir and rilpivirine.

3.3.2. Method Precision

The R.S.D % of peak area was 0.1183, retention time was 0.0432 and assay was 0.1185 for Dolutegravir. The R.S.D % of peak area was 0.1054, retention time was 0.0286 and assay was 0.1055 for Rilpivirine. It showed that the method was having good precision and the R.S.D % of the peak area, retention time and assay were present within the acceptance criteria of R.S.D < 2 %. The results are reported in Table 4. The chromatograms are shown in (Figure 8). Thus, the proposed method was found to have a high degree of precision and reproducibility.

3.3.3. Inter-day Precision

The R.S.D % of peak area was 0.186 and retention time was 0.242 for Dolutegravir. The R.S.D % of peak area was 0.187 and retention time was 0.144 for Rilpivirine. A statistical evaluation revealed that R.S.D % of peak area and retention time were within the acceptance criteria of R.S.D < 2 %. The results are reported in Table 5. Thus, the proposed method was found to have a high degree of precision and reproducibility.

Table 4. Method precision data for dolutegravir and rilpivirine

S.No	Dolutegravir			Rilpivirine		
	Rt	Peak Area	% Assay	Rt	Peak Area	% Assay
1.	2.424	1272041	100.08	4.421	1522117	100.8
2.	2.423	1272919	100.14	4.421	1524942	101.01
3.	2.421	1274872	100.3	4.421	1522873	100.88
4.	2.422	1271014	99.99	4.42	1521304	100.77
5.	2.423	1271656	100.05	4.419	1520215	100.70
6.	2.422	1270803	99.98	4.418	1521816	100.81
Mean	2.4225	1272217.5	100.09	4.420	1522211.167	100.828
SD	0.0010	1505.591	0.1186	0.00126	1604.2525	0.1065
% RSD	0.0432	0.1183	0.1185	0.0286	0.1054	0.1055

***Average of six injections**

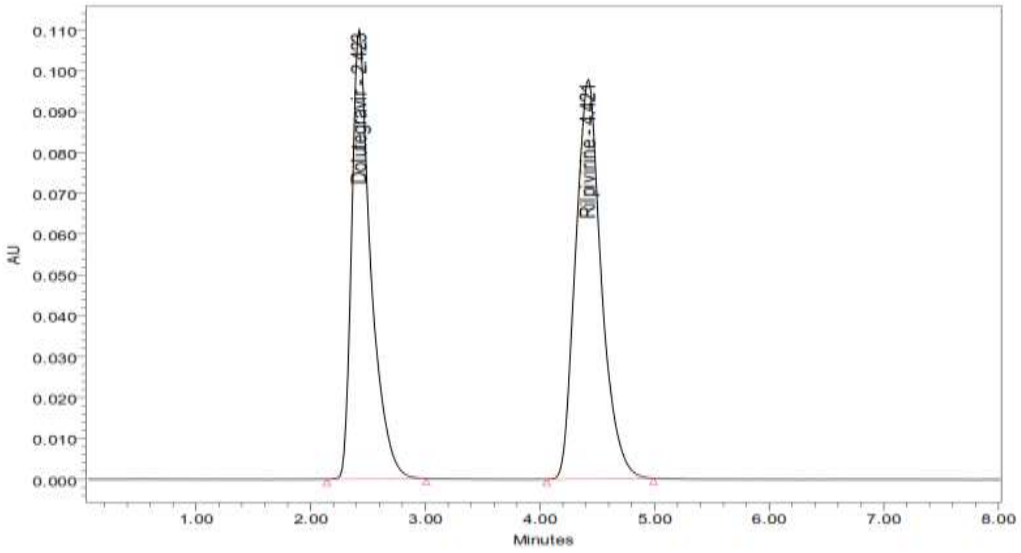

Figure 8. Chromatogram showing method precision of dolutegravir and rilpivirine.

Table 5. Inter-day precision data of the proposed method for dolutegravir and rilpivirine.

Days	Dolutegravir		Rilpivirine	
	R_t	Peak Area	R_t	Peak Area
Day 1*	2.436	1268124	4.460	1519248
Day 2*	2.428	1272458	4.452	1518342
Day 3*	2.429	1274362	4.448	1521373
Day 4*	2.439	1270152	4.462	1524682
Day 5*	2.426	1272586	4.452	1525184
Day 6*	2.424	1269235	4.446	1520175
MEAN	2.430	1271152.833	4.453	1521501
SD	0.0058	2362.615	0.006	2846.514
% RSD	0.242	0.186	0.144	0.187

3.4. System Suitability

The parameters including resolution, tailing factor, no. of theoretical plates mainly used to assess system suitability. The Tailing factor for Dolutegravir & Rilpivirine was found to be 1.61 and 1.71 respectively. The Theoretical plates per unit for Dolutegravir and Rilpivirine was found to be 2741 and 2750 respectively. The resolution value of Dolutegravir & Rilpivirine was found to be 5.08. The resolution value of more than 2 indicates satisfactory results in quantitative work and the higher resolution value obtained indicates the complete separation of the drugs. The tailing factor values for Dolutegravir & Rilpivirine indicated the symmetrical nature of the peak. The no. of theoretical plates was high indicating the efficient performance of the column. The resolution value of more than 2 indicates satisfactory results in quantitative work and the high-resolution value obtained indicates the complete separation of the drugs. System suitability results are presented in table 6. The chromatograms are shown in figure 9.

Table 6. System suitability parameters of dolutegravir and rilpivirine.

Parameters	Dolutegravir	Rilpivirine
Tailing factor	1.61	1.71
Resolution	5.08	
Retention time	2.435	4.444
Theoretical plates per unit	2741	2750

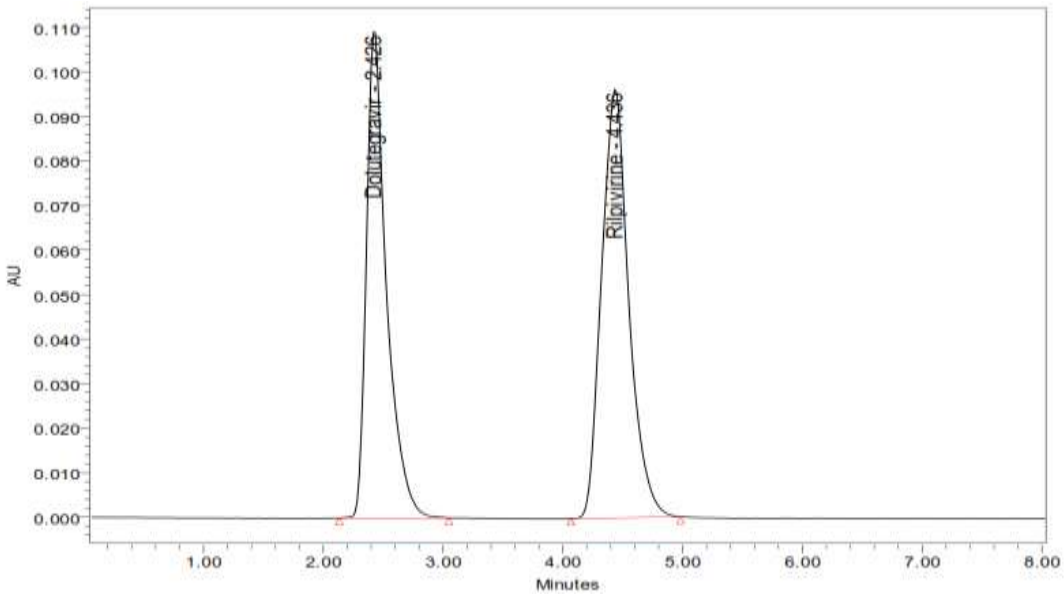

Figure 9. Chromatogram showing system suitability of dolutegravir and rilpivirine.

Table 7. Accuracy data for dolutegravir.

Concentration	Sample Peak Area	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Mean recovery
	1016342	80	80.02	100.02	
80 µg/ml	1015468	80	79.93	99.91	99.87 %
	1013186	80	79.75	99.69	
	1271348	100	99.00	99.00	
100 µg/ml	1274872	100	100.16	100.16	99.68 %
	1273652	100	99.88	99.88	
	1524617	120	120.04	100.03	
120 µg/ml	1523164	120	119.88	99.90	99.93 %
	1522348	120	119.82	99.85	

Table 8. Accuracy data for rilpivirine.

Concentration	Sample Peak Area	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Mean recovery
	912637	30	29.94	99.80	
30 µg/ml	914548	30	30.04	100.13	100.12 %
	918426	30	30.13	100.43	
	1522894	50	50.02	100.04	
50 µg/ml	1523415	50	50.08	100.16	99.96 %
	1519827	50	49.84	99.68	
	2131425	70	69.92	99.88	
70 µg/ml	2133682	70	70.03	100.04	99.91 %
	2130148	70	69.88	99.82	

3.5. Accuracy

The Mean recovery of 80 µg/ml, 100 µg/ml and 120 µg/ml for Dolutegravir were found to be 99.87 %, 99.68 % and 99.93 % respectively. The Mean recovery of 30 µg/ml, 50 µg/ml and 70 µg/ml of Rilpivirine were found to be 100.12 %, 99.96 % and 99.91 % respectively. The obtained mean recovery values were within the acceptance criteria of 98 - 102 %. The results are reported in table 7 and table 8. This serves as a good index of the accuracy and reproducibility of the proposed method. This shows that the method was accurate.

3.6. Robustness

U.S.P Plate count and U.S.P tailing were found to be 2706.8 and 1.6 for 0.6 ml/min flow rate of Dolutegravir. U.S.P Plate count and U.S.P tailing were found to be 2748.5 and 1.6 for 1.0 ml/min flowrate of Dolutegravir. U.S.P Plate count and U.S.P tailing were found to be 2722.6 and 1.7 for 0.6 ml/min flow rate of Rilpivirine. U.S.P Plate count and U.S.P tailing were found to be 2698.6 and 1.7 for 1.0 ml/min flow rate of Rilpivirine. U.S.P Plate count and U.S.P tailing were found to be 2700 and 1.6 for 5% less organic composition in mobile phase for Dolutegravir.

U.S.P Plate count and U.S.P tailing were found to be 2765.3 and 1.6 for 5% more organic composition in mobile phase for Dolutegravir. U.S.P Plate count and U.S.P tailing were found to be 2722 and 1.7 for 5% less organic composition in mobile phase for Rilpivirine. U.S.P Plate count and U.S.P tailing were found to be 2778 and 1.7 for 5% more organic composition in mobile phase for Rilpivirine. The method was robust even by the change in the Mobile phase ratio ±5 % and flow rate ± 0.2 ml/min. The obtained U.S.P Plate count and U.S.P tailing factor values are very close with actual flow rate and mobile phase composition. On the evaluation of the results, it can be concluded that the variation in ± 5% of organic composition in the mobile phase and flow rate ± 0.2 ml/min affected the method significantly. Hence it indicates that the method is robust even by the change in the mobile phase ±5% and flow rate ±0.2 ml/min. The chromatograms are shown in (Figure 10 – 13) and results are reported in table 9.

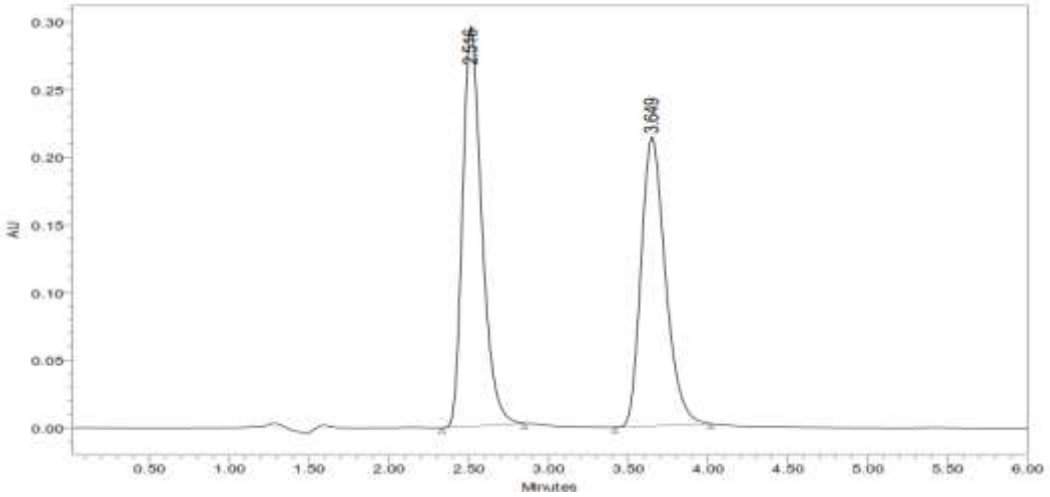

Figure 10. Chromatogram showing robustness solution of increased mobile phase flowrate (1.0 ml/min) of Dolutegravir and Rilpivirine.

Table 9. Results of the robustness study for dolutegravir and rilpivirine.

S.No	Parameters	Dolutegravir		Rilpivirine	
		USP Plate Count	USP Tailing	USP Plate Count	USP Tailing
1.	Actual method	2741.0	1.61	2750.0	1.71
2.	Changes in the flow rate (0.6 ml/min)	2706.8	1.60	2722.6	1.70
3.	Changes in the flow rate (1.0 ml/min)	2748.5	1.60	2698.6	1.70
4.	Changes in the Organic phase composition in the mobile phase (5 % less)	2700.0	1.60	2722.0	1.70
5.	Changes in the Organic phase composition in the mobile phase (5 % more)	2765.3	1.60	2778.0	1.70

3.7. Ruggedness

The R.S.D % of Peak area for Analyst I and Analyst II was found to be 0.001 and 0.048 for Dolutegravir. The R.S.D % of Peak area for Analyst I and Analyst II was found to be 0.026 and 0.024 for Rilpivirine. The R.S.D % of peak area was present within the acceptance criteria of R.S.D < 2 %. RSD % values for ruggedness indicated that the method is rugged and does not show variations in the results when performed by different analysts. The results are reported in table 10.

Table 10. Ruggedness data for dolutegravir and rilpivirine.

Parameters	% RSD Peak Area-Dolutegravir	% RSD Peak Area-Rilpivirine
Analyst 1	0.001	0.026
Analyst 2	0.048	0.024

3.8. Limit of Detection

The LOD of Dolutegravir and Rilpivirine were 0.044 µg/ml and 0.060 µg/ml. The obtained LOD values were very less when compared to the reported method. As per the analytical point of view, the method could detect very least quantity. So, when compared to the reported methods [35-36], the developed method is most suitable to detect less quantity.

3.9. Limit of Quantification

The LOQ of Dolutegravir and Rilpivirine were 0.1345 µg/ml and 0.183 µg/ml. The obtained LOQ values were very less when compared to reported methods. As per the analytical point of view, the method could detect very least quantity. So, when compared to the reported methods [37-38], the developed method is most suitable to quantify less quantity.

3.10. Degradation Studies

Degradation studies were carried out as per ICH guidelines. The sample solutions were subjected to acidic, basic, peroxide, water, and light. Whereas in acidic, basic the % degradations were found to be - 12 %, - 11 % and -3 %, -9 % for Dolutegravir and Rilpivirine respectively. The % degradation by peroxide was found to be -5 % and -4 % for Dolutegravir and Rilpivirine respectively. The % degradation by water was found to be -4 % and -9 % for Dolutegravir and Rilpivirine respectively. The % degradation by photolytic was found to be -15 % and -2 % for Dolutegravir and Rilpivirine respectively. Thus, Dolutegravir is not stable in photolytic, acidic and basic conditions and stable in oxidative and neutral degradation. Rilpivirine is slightly not stable in basic and neutral degradation and stable in acidic, oxidative and photolytic degradation. So far there is no stability indicating analytical method reported for the determination of Dolutegravir and Rilpivirine in bulk and its dosage forms. The proposed method was helpful in the separation of the two compounds without the interference of degradants, estimate the active contents. Hence the developed method can be used for quality control analysis and accelerated stability studies. The obtained data are reported in table 11. The chromatograms are shown in (Figure 14 – 18).

Table 11. Results of degradation studies for dolutegravir and rilpivirine.

S. No.	Name	Sample weight	% Assay-Dolutegravir	% Assay-Rilpivirine	% Degradation-Dolutegravir	% Degradation-Rilpivirine
1	Acid	217	87	96	- 12	-3
2	Base	217	88	90	-11	-9
3	Peroxide	217	94	95	- 5	-4
4	Water	217	95	90	- 4	-9
5	Light	217	84	97	-15	-2

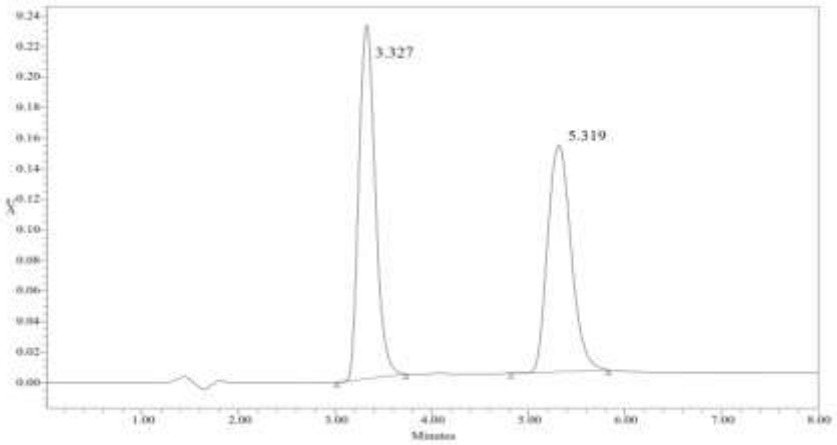

Figure 11. Chromatogram showing robustness solution of decreased mobile phase flowrate (0.6 ml/min) of dolutegravir and rilpivirine.

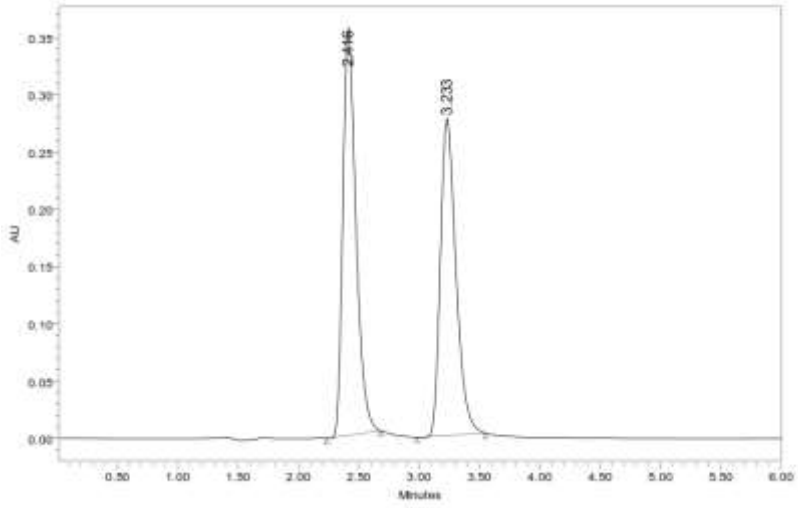

Figure 12. Chromatogram showing robustness solution of more organic phase ratio of mobile phase (+5 %) of dolutegravir and rilpivirine.

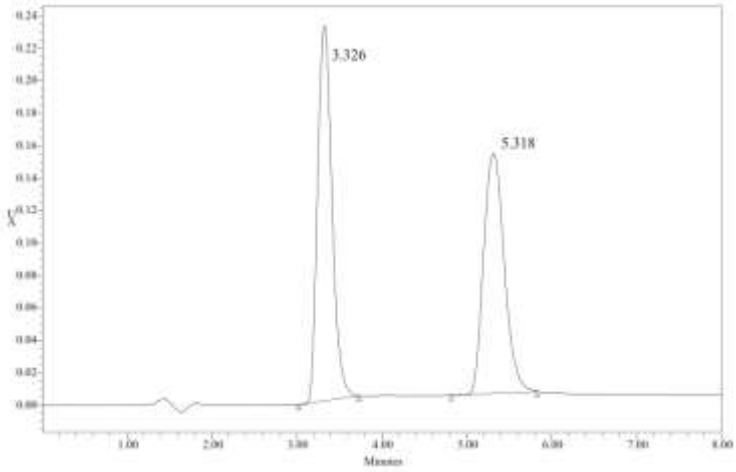

Figure 13. Chromatogram showing robustness solution of less organic phase ratio of mobile phase (-5 %) of dolutegravir and rilpivirine.

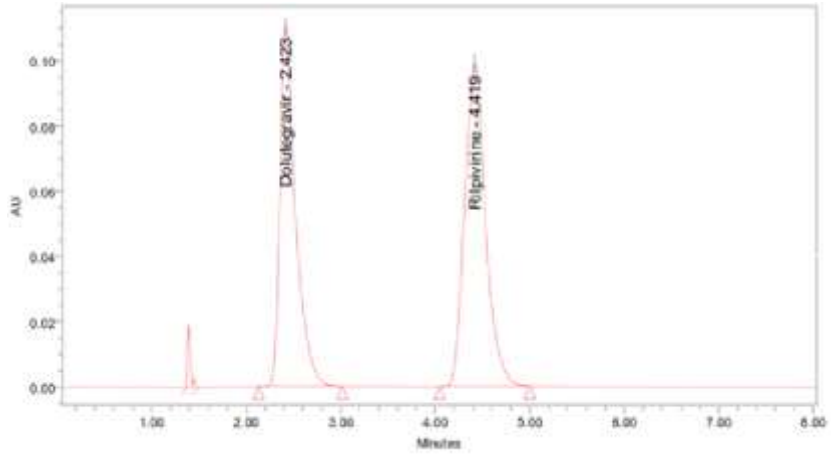

Figure 14. Chromatogram showing hydrolytic degradation by acid for dolutegravir and rilpivirine.

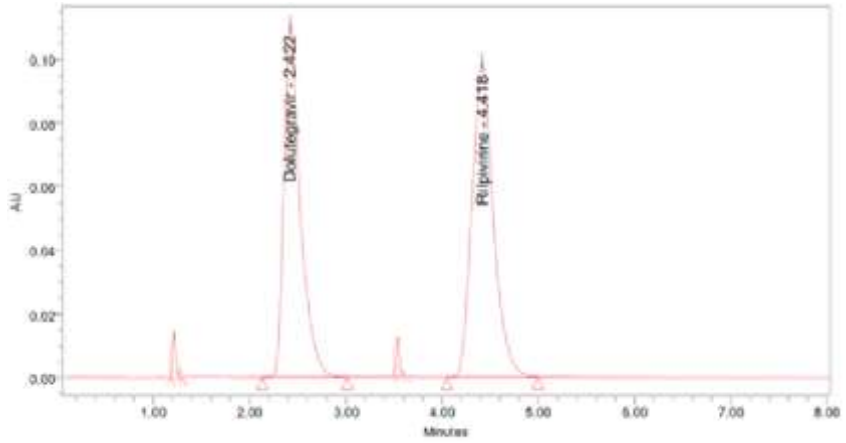

Figure 15. Chromatogram showing hydrolytic degradation by alkali for dolutegravir and rilpivirine.

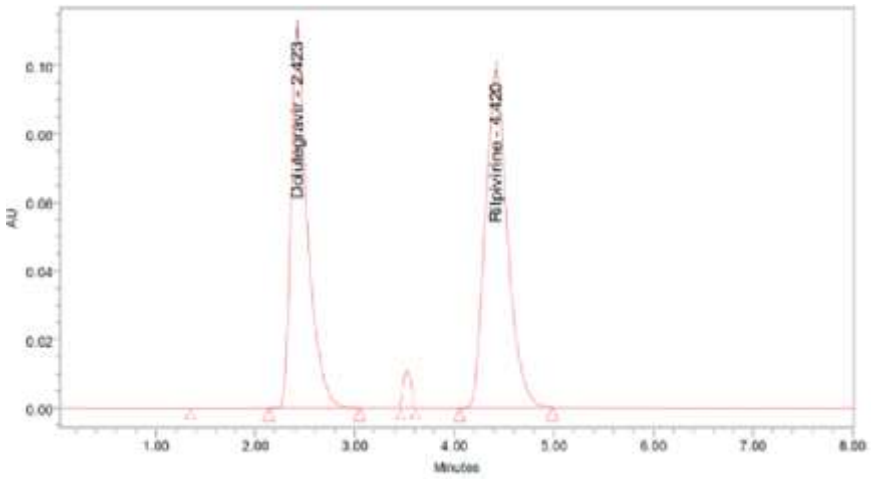

Figure 16. Chromatogram showing neutral degradation by water for dolutegravir and rilpivirine.

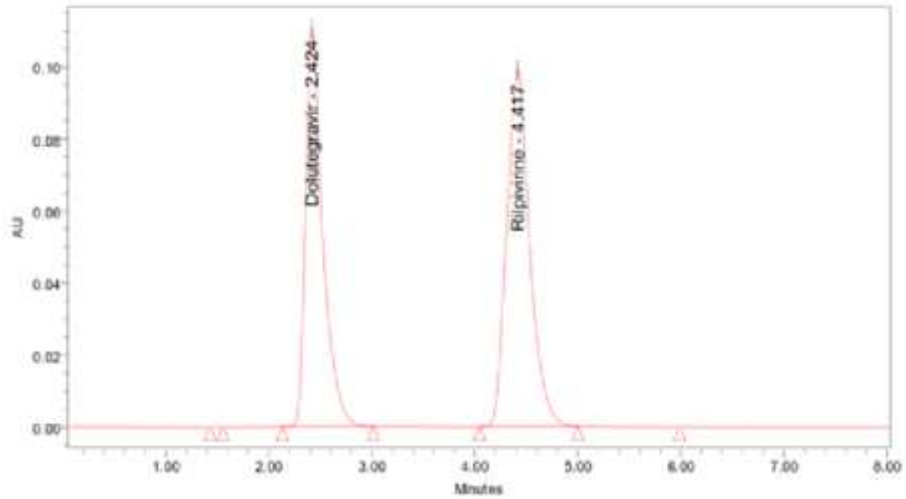

Figure 17. Chromatogram showing oxidative degradation by 3% hydrogen peroxide for dolutegravir and rilpivirine.

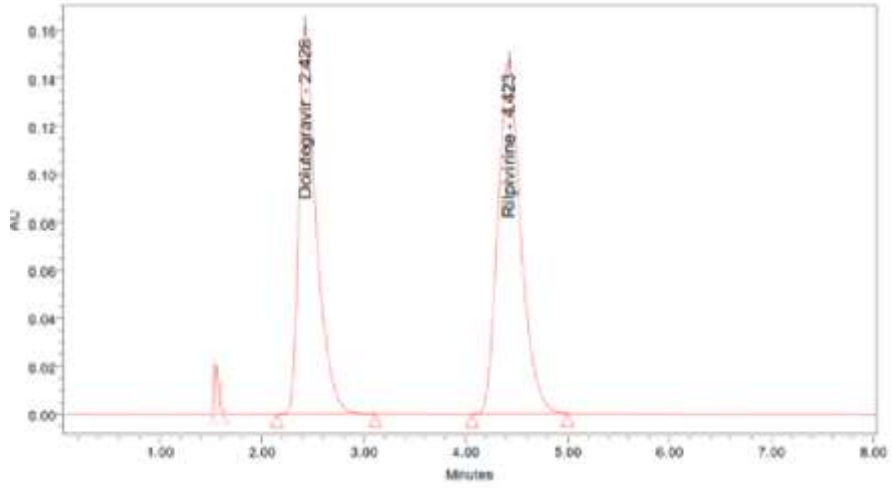

Figure 18. Chromatogram showing photolytic degradation for dolutegravir and rilpivirine.

4. Conclusion

A new stability indicating analytical method was developed and validated by RP-HPLC technique. The sample preparation is simple, consumes less amount of mobile phase and the required time for analysis is very short, the information given in the study will be very useful for the quality monitoring of Dolutegravir and Rilpivirine in bulk and its dosage forms. The method was applied for the determination of potency of the commercial product of Dolutegravir and Rilpivirine and potency was found within the limit. The results of assay analysis of two drugs from a combined dosage form using this developed method were found to be close to 100 %. Recovery studies were satisfactory which shows that there is no interference of excipients.

The developed analytical procedure has shown satisfactory results for all the validation parameters. The proposed method was specific as no interference of excipients was found. The information given in the study will be very useful in quality control, content uniformity test, in-vitro dissolution of the combination of Dolutegravir and Rilpivirine drug products. The proposed method can be efficiently applied for the separation of the drugs from its excipients and its degradation components in the pharmaceutical formulation. It can be used to check rapid and accurate drug quality during stability testing. The method can be used in the analysis of stability studies, as no interference was found with the degradants formed under various stress conditions.

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