Development of Stability Indicating Analytical Methods for Some Drugs in Bulk and Pharmaceutical Dosage Forms

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Abstract: Rilpivirine and Dolutegravir can be determined simultaneously in bulk and pharmaceutical dose forms using a quick, accurate, and stable HPLC technique. An analytical technique called a stability indicating method (SIM) is used to quantify the loss of active pharmaceutical ingredient (API) in a drug product as a result of deterioration. A stability indicating method is a quantitative analytical technique that has been approved for use in determining how changes in the stability of drug ingredients and drug products occur over time. On a Denali C18 column (150 mm 4.6 mm, 5 m), the chromatographic separation was carried out using mobile phase Methanol: Phosphate buffer (pH 4.0). All peaks were overplayed after various concentrations of DOL (15-45 g/ml) and RIL (2.5-7.5 g/ml) were synthesised and injected individually. The findings demonstrated a linear connection between peak area and DOL (15–45 g/ml) and RIL (2.5–7.5 g/ml) concentrations. It was crucial to check the Rt time for a specific medication at every concentration. The results show that Rt was achieved for DOL between 7.08 and 1.10 minutes, whereas RIL was acquired between 3.19 and 3.19 minutes across all five concentration ranges. RSD is less than 2% for both drugs combined. This demonstrates the precision of the suggested method. The value of Recovery shows that the suggested approach is reliable. It was discovered that LOD and LOQ yield the lowest concentration for a given drug that the suggested method can quantify as well as the lowest amount of drug that can be identified but not necessarily quantified. LOD for DOL and RIL were discovered to be 1.046 and 0.809 g/ml, respectively. So starting from the LOD concentration that has been obtained, the suggested approach can detect drugs. For DOL and RIL, the LOQ was discovered to be 3.234 and 2.241 g/ml, respectively. The development of diverse degradation products was shown by the acid, alkali, neutral, dry heat, UV, and photo-degradation experiments. The suggested analytical approach was shown to be appropriate for the routine simultaneous examination of both medication formulations for tablets and in bulk.

Keywords: Rilpivirine, Dolutegravir, Stability study, Simultaneous, High-performance liquid chromatography, Method validation, etc.

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Introduction

Chemical stability is a major concern for pharmaceutical molecules since it directly affects the drug's efficacy and safety. Stress testing is meant to determine the expected degradation products, according to the ICH

guidelines, which further aids in determining the inherent stability of the molecule, defining degradation routes, and validating the stability indicating processes used. Studies on forced degradation are both a legal mandate and a necessity for science. Before submitting a registration dossier, stability tests of novel drug moieties are now required. The stability indication method that will later be used for the analysis of samples produced from accelerated and long-term stability investigations can be developed using forced degradation research. In order to analyse stability samples, titrimetric, spectrophotometric, and chromatographic techniques have frequently been used [1].

Chemically known as 4-[4-(4-[(E)-2-cyanovinyl]-2,6-dimethylphenyl] amino] pyrimidin-2-yl] amino benzonitrile, rilpivirine is a type of anti-HIV medication [2]. Only a few analytical techniques, such as liquid chromatography tandem mass spectrometry [3], HPLC [4], and a Spectrophotometric analysis [5], have been established for its assessment in pharmaceutical formulations and in biofluids, either alone or in combination with other medications.

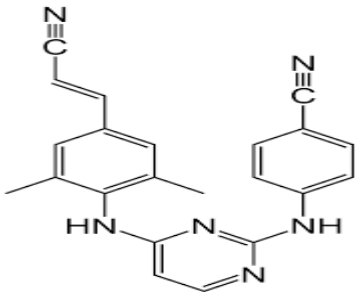

Fig. Structure of Rilpivirine

According to data gathered from the literature, only one method [6-10] has been described for the determination of rilpivirine, and of those methods, it uses HPLC.

Dolutegravir is chemically called as (3S, 7R)-N-[(2, 4-difluorophenyl) methyl]-11-hydroxy-7-methy l-9, 12-dioxo-4-oxa- 1, 8-diazatricyclo [8.4.0.03, 8] tetradeca-10, 13- diene-13-carboxamide.

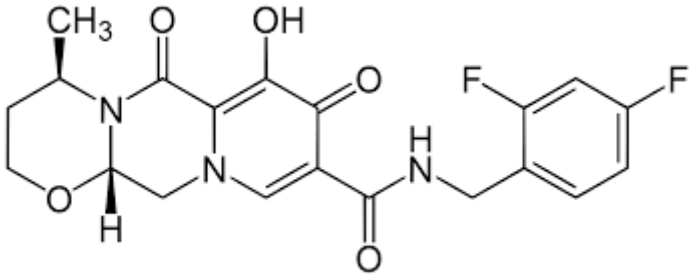

Fig. Structure of Dolutegravir

Antiretroviral medications in the dolutegravir class have been authorised for the treatment of the human immunodeficiency virus (HIV). The antiretroviral drug class that dolutegravir belongs to targets the viral integrase. Only other antiretroviral medications can be taken in conjunction with dolutegravir. It has no discernible impact on acute liver injury, but is associated with a low risk of serum aminotransferase increases during treatment [11].

The simultaneous measurement of Rilpivirine and Dolutegravir in pharmaceutical dosage forms and biological fluids is described using analytical techniques such as UV spectrophotometric and HPLC. For the stability suggesting simultaneous determination of such medicines by HPLC technique, no references have, however, been recorded to yet. It is necessary to establish drug substance and drug product degradation pathways as well as to ascertain a drug substance's intrinsic stability in formulation. Understanding the molecular

characteristics of medicinal molecules is also essential. In order to simultaneously estimate the abovementioned medications in combined dosage form, a simple, quick, and exact stability indicating HPLC approach was developed and validated. The International Conference on Harmonization (ICH) parent drug stability test recommendations (Q1A) mandate that analytical test techniques for stability samples be extensively validated and the tests be stability indicated [12-16].

Only a small number of documented analytical methods were available for the simultaneous determination of the drug combination and stability indicating High Performance Liquid Chromatography (HPLC) approach, according to a literature search through standard databases. This study is being done with the understanding that a simple, reproducible, exact, accurate, and economical approach must be created in order to overcome the current challenge for the routine analysis of the quality attributes of the combination in pure and pharmaceutical dosage form. The purpose of the current research is to provide an HPLC method that can simultaneously estimate Rilpivirine and Dolutegravir in pharmaceutical dosage forms while also providing stability indicators.

Material and Method

Material

Rilpivirine of purity 99% w/w procured from Hetero Drugs Ltd as a gift sample. Dolutegravir was received as gift samples from Mylan Laboratories Limited, Hyderabad, India. Methanol, Sodium Hydroxide, Conc. HCl, 1% Glacial acetic acid obtained from Mumbai, India.

Method

Method Validation of DOL & RIL

Linearity

The linear response of DOL & RIL was determined by analysing five independent levels of the calibration curve in the range of 15-45 µg/ml for DOL and 2.5-7.5 µg/ml for RIL.

Precision

The meticulousness of an investigative technique is frequently articulated by means of the customary nonconformity, relative standard deviation of coefficient of variance of a succession of dimensions.

Repeatability

It was performed by preparing the standard liquid mixture of DOL (30 µg/ml) and RIL (5 µg/ml) for six times and analysed as per the proposed method. Percentage relative standard deviation (RSD) should be less than 2%.

Intermediate Precision

DOL (30µg/ml) in addition RIL (5µg/ml) aimed at six stints and analysed by way of a piece in the format of the concerning that can apiece with qualitative analytical experiments. Measurement by means of analytical approaches relative standard deviation ought to be situated a lesser amount of than 2%.

Reproducibility

The intra-day meticulousness intended for highly performing liquidises chromatographical representation technique be present strong-minded meant intended for three deliberation of DOL and RIL explanation designed for the three stretches taking place the equivalent day.

Accuracy

The recovery experiments for HPLC method were carried out in triplicate by spiking previously analysed samples of DOL and RIL liquid mixture with three different

concentrations of standards at 80%, 100% and 120% for DOL and RIL respectively.

20 tablets weighed and average weight calculated. Tablets are crushed and weigh equivalent to DOL 30 mg and RIL 5mg tablet powder, transfer in 100 ml accurately calibrated flask and add 70 ml methyl alcohol and sonicate for 30 min (DOL test= 0.3mg/ml and RIL- 0.05 mg/ml).

Liquid Mixture A: 300 µg/ml of DOL standard

Liquid Mixture B: 50 µg/ml of RIL standard

Mix the specified volumes of liquid mixture A dilute and liquid mixture B as shown in Table dilute to 10 ml with liquid mobile phase.

Table: Preparation of Test liquid mixture for Accuracy study for HPLC

Level (%)	ml of test liquid mixture from tablet	ml of Liquid mixture A dilute	ml of Liquid mixture B dilute	Volume upto with Liquid mobile phase
80	1	0.8	0.8	10
100	1	1	1	10
120	1	1.2	1.2	10

Limit of Detection

Limit of detection is the lowest concentration of the analyte detected by the method.

LOD= (3.3xSD)/S

Where,

S=Slope of curve,

SD=Standard deviation

Limit of Quantification

Limit of Quantification (LOQ) is the minimum quantifiable concentration in the sample.

LOQ= (10 x SD)/S

Where,

SD= Standard deviation,

S= Slope

Robustness

The robustness was determined by variation in flow rate.

Forced Degradation Studies

Alkali Hydrolysis

Forced degradation in basic medium was performed by pipette out 1ml stock liquid mixture.

Each of Dolutegravir (DOL) and Rilpivirine (RIL) in separate 10ml accurately calibrated flasks, add 1ml of 1 N NaOH to each flask. Flasks were heated at 50^0C for 1 hr. and allowed to cool at room temperature. Liquid mixtures were neutralized with 1 N HCL and volume was adjusted to the mark with mobile phase to obtain final concentration of 5µg/ml of DOL and 30µg/ml RIL respectively. The final liquid mixtures were analysed. The amounts of drug remain un-degraded were computed using regression equation. Same procedure was carried out for DOL and RIL in mixture as per above forced degradation condition.

Acid Hydrolysis

Involuntary squalor in low pH environment was performed dropping out 1ml liquid mixture each of DOL and RIL in separate 10ml accurately calibrated flasks, add 1ml of 1 N HCL to each flask. Flasks were heated at 50^0C for 2 hrs. and permissible to lower its temperature at normal condition. Liquid mixtures were adjusted to pH 7 with hydroxide buffer and volume was adjusted to the mark with mobile phase to obtain final concentration of 5 µg/ml of DOL and 30 µg/ml of RIL respectively. The final liquid mixture

were analysed. The amounts of drugs remain un-degraded were computed using regression equation. Same procedure was carried out for DOL and RIL in mixture as per above condition.

Oxidation Degradation

To perform oxidative stress method, Pipette 1 ml stock liquid mixture each of DOL and RIL in separate 10 ml accurately calibrated flasks and add 5 ml of 6% H2O2. Flasks were heated at 50^0C for 2 hrs. and allowed to cool at room temperature and volume was adjusted to the mark with mobile phase to obtain final concentration of 5 µg/ml of DOL and 30 µg/ml RIL respectively. The final liquid was analysed. The amounts of drugs remain under graded were computed using regression equation. Same procedure was carried out for DOL and RIL in mixture as per above condition.

Photo Degradation

To study photo degradation study, 50 mg each of DOL and 300 mg of RIL were weighed, moved in separate Petri dish. The solid drugs were exposed to sunlight for 12 hrs. and 72 hrs. the solids were allowed to cool and dissolved in few ml of methyl alcohol and transfer in 100 ml accurately calibrated flask at last volume was made up to the mark of 100ml with the methanol. Aliquot of 0.1 ml from above liquid mixtures were moved to separate 10 ml accurately calibrated flasks and volume was adjusted to the mark with mobile phase to obtain final concentration of 5 µg/ml of DOL and 30 µg/ml RIL respectively. The final liquid was analysed. The amounts of under graded drugs were computed using regression equation. Same procedure was carried out for DOL and RIL in mixture as per above condition.

Dry heat Degradation

To study dry heat degradation, 50 mg each of DOL and 300 mg of RIL were weighed and moved in separate 100 ml accurately calibrated flasks. The solid drugs were exposed in oven at 50^0C for 2 hrs. The solids were allowed to cool and dissolved in few ml of methanol and transfer in 10 ml accurately calibrated flask at last volume was made up to the mark of 100 ml with the methanol. Aliquot of 0.1 ml from above liquid mixture were moved to separate 10 ml accurately calibrated flasks and volume was adjusted to the mark with mobile phase to obtain final concentration of 5 µg/ml of DOL and 30 µg/ml RIL respectively. The final liquid mixture was analysed. The amounts of under graded drugs were computed using regression equation. Same procedure was carried out for DOL and RIL in mixture as per above condition

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Result and Discussion

Preformulation study

Infra-Red Spectroscopy (IR)

A] Dolutegravir

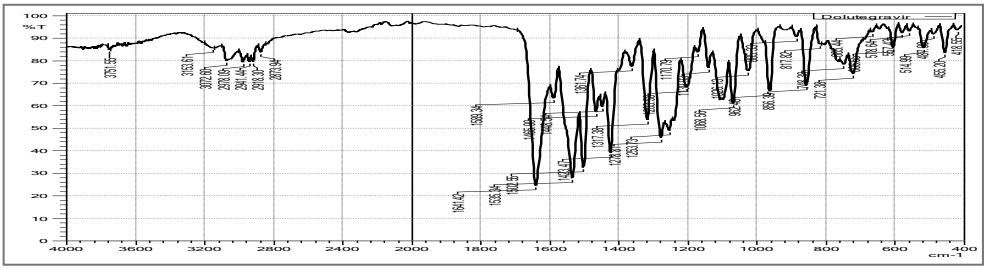

Figure: Spectrum of Infra-Red Spectroscopy (Dolutegravir)

B] Rilpivirine

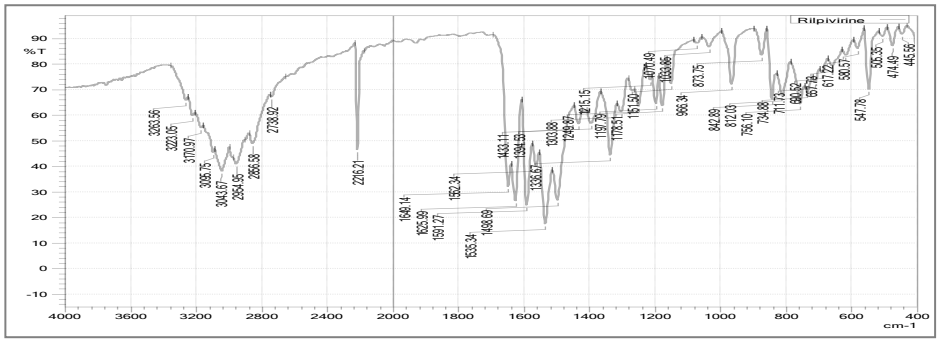

Figure: Spectrum of Infra-Red Spectroscopy (Rilpivirine)

The IR Spectrum of test sample was found to be concordant with that of standard spectrum.

UV-Spectroscopy

A] Dolutegravir

Preparation of Standard Stock Solution

Accurately weighed of Dolutegravir sodium working standard equivalent to 10 mg of DTG was transferred into a 100 ml volumetric flask. It was dissolved in 20 ml methanol by sonication for 10 minutes. Final volume was made up to 100 ml with methanol to give the solution containing 100µg/ml of DTG.

Selection of Maximum Wavelength for Analysis

The standard stock solution was further diluted with water to obtain concentration level of DTG at 10 µg/ml. the solution was scanned between 200 and 400 nm using water as blank. The UV spectrum of DTG has shown maximum absorbance at the wavelength 259.80 nm.

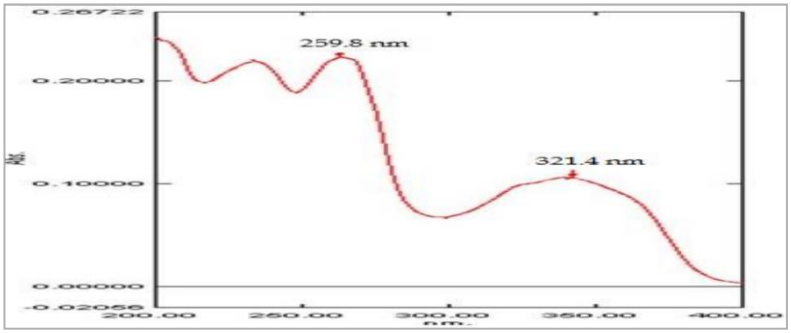

Fig. UV spectra of Dolutegravir

Preparation of Calibration Curve

An aliquot of standard stock solution were further diluted with water to get the solutions of concentration within range 5-40 µg/ml. the absorbance was measured at 259.80 nm against water as blank. All measurements were repeated three times for each concentration.

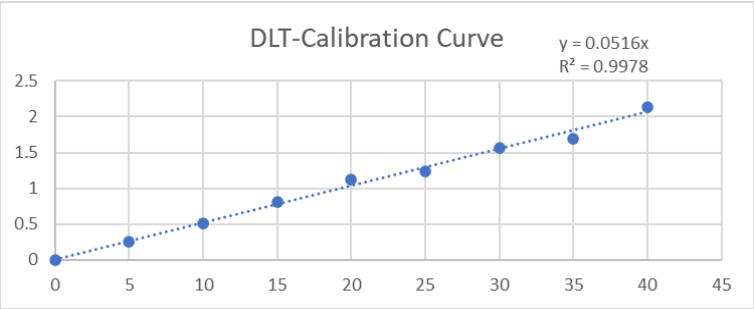

Fig. Calibration curve of Dolutegravir

B] **Rilpivirine**

Preparation of Standard Stock Solution

Standard stock solution of rilpivirine hydrochloride (1 mg/ml) was prepared by transferring 10 mg of rilpivirine hydrochloride into a 10ml volumetric flask containing 4ml of (8:2) methanol and water. It was then sonicated for 15 minutes and solution was diluted up to the volume by methanol and water. From these, further dilutions were made using (8:2) methanol and water to produce solution of rilpivirine hydrochloride (100 µg/ml).

Selection of Maximum Wavelength for analysis

0.1 ml of standard stock solution of rilpivirine hydrochloride was transferred into a 10 ml volumetric flask and diluted to a mark with methanol: water (8:2) to give concentration of 1 µg/ml. The resulting solution was scanned in the UV range (200-400 nm). The λmax was found at 305nm.

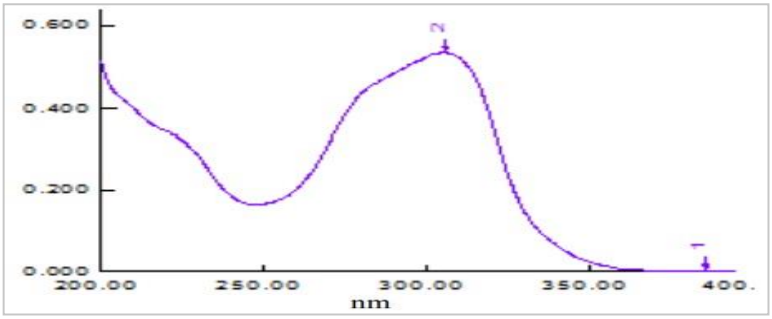

Fig. UV spectra of rilpivirine

Preparation of Calibration Curve

An aliquot of standard stock solution was further diluted with methanol: water (8:2) to get the solutions of concentration within range 0.5-3.5 µg/ml. The absorbance was measured at 305 nm against water as blank. All measurements were repeated three times for each concentration.

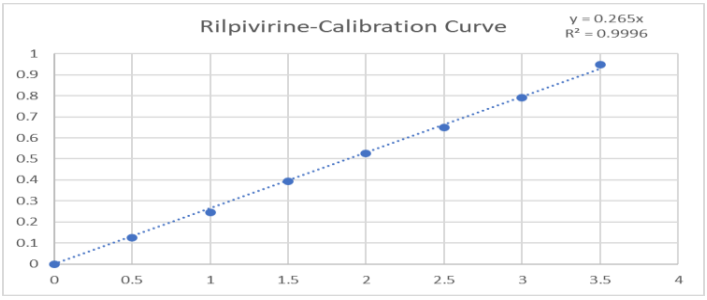

Fig. Calibration curve of Rilpivirine

Melting Point

The melting point of the pure drug was measured adopting the capillary test. The substance was contained in a modest quantity in a side sealed capillary tube attached to the thermometer at its mercury bulb. The thermometer was placed into the Thieles tube containing liquid paraffin in such a way that the upper, open end of the capillary tube remained above the oil layer. The side arm of the Thieles tube was then heated with a burner until the solid drug melts, and the melting temperature was measured.

Sr. No.	Drug	Melting Point (^{0}C)
1.	Dolutegravir	191
2.	Rilpivirine	239

Solubility

A] **Dolutegravir**

Dolutegravir was checked in water, acetonitrile and methanol. It was found to be freely soluble in methanol, slightly soluble in water but insoluble in acetonitrile. Methanol was selected as the solvent for dissolving the drug.

B] Rilpivirine

In dimethyl formamide (DMF), rilpivirine was soluble. The solvent was chosen based on its selectivity. In the solution made using acetonitrile as a secondary diluent, the medicines demonstrated enhanced absorption.

HPLC

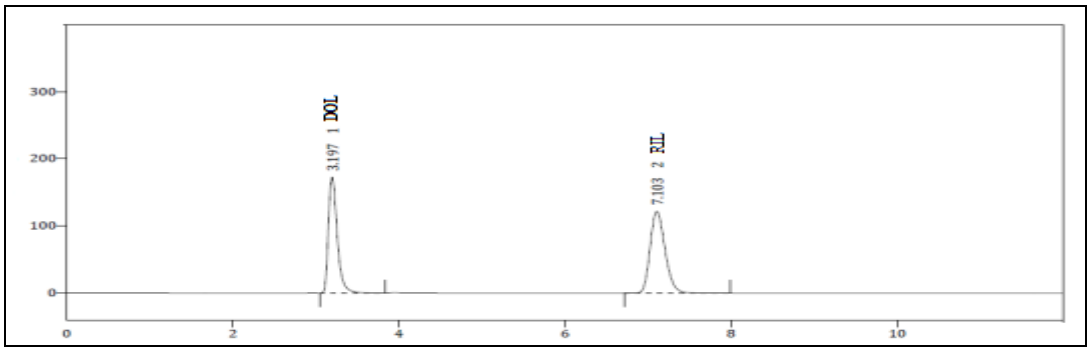

Figure: HPLC chromatogram of standard liquid mixtures of DOL (30 µg/ml) and RIL (5 µg/ml)

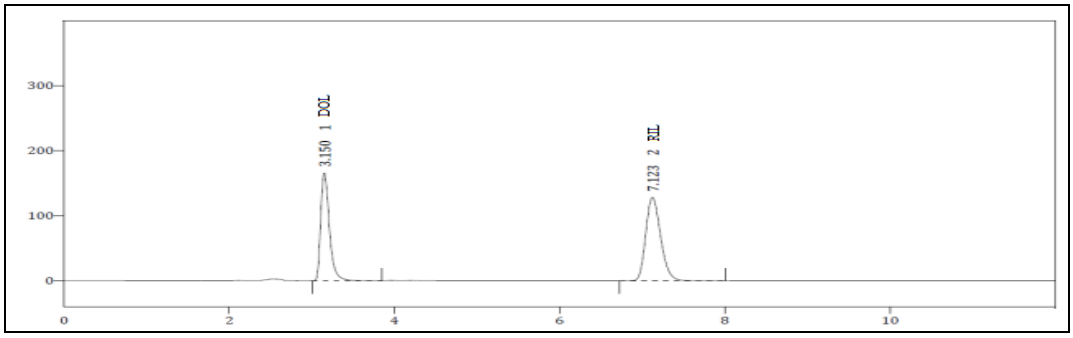

Figure: HPLC chromatogram of test liquid mixtures of DOL (30 µg/ml) and RIL (5 µg/ml)

System Suitability Parameters for HPLC

Statistical analysis of parameters required for system suitability testing of the HPLC method

Table: System Suitability Parameters for HPLC

System Suitability Parameters	Dolutegravir		Rilpivirine	
	Result ± SD	% RSD	Result ± SD	% RSD
Tailing Factor	1.265±0.021	1.42	1.424±0.051	2.61
Theoretical Plates	7164±114.15	1.52	4281±8.624	0.19
Retention Time (min.)	7.084±0.041	0.14	3.468±0.041	0.07
Resolution	14.48±0.046	0.58	-	-
Area	1540.74±14.84	0.81	1274±12.4	1.07

System suitability parameter indicates proper and precise working of HPLC instrument which ensure that method development was not affected by instrument parameter.

Tailing factor less than 1.5 ensures proper shape of drug peak and drug not bound to stationary phase which cause damage to solid phase containing cylinder. Theoretical plate more than 5000 indicates proper separation of drug from each other. Resolution more than 2 indicates two drugs were separated from each other with proper distance and not merge to each other.

From result, it was concluded that all the parameter fulfilled by proposed method with used opposite phase excellent performing fluid-based separation technique instruments.

Calibration Curve (Linearity)

Varying concentration of DOL (15-45 µg/ml) and RIL (2.5-7.5 µg/ml) were prepared and injected separately and then all peaks were overplayed. The result showed that linear correlation was obtained between peak area and concentration of DOL (15-45 µg/ml) and RIL (2.5-7.5 µg/ml). It was important to check Rt time for particular drug for all concentration. The same data are provided in table. The result data shoes that for DOL, Rt obtained at 7.08-1.10 minute where as for RIL obtained at 3.19 minute at all five concentration range. Further as concentrations were increasing, the area were also obtained increasing indicating good relation.

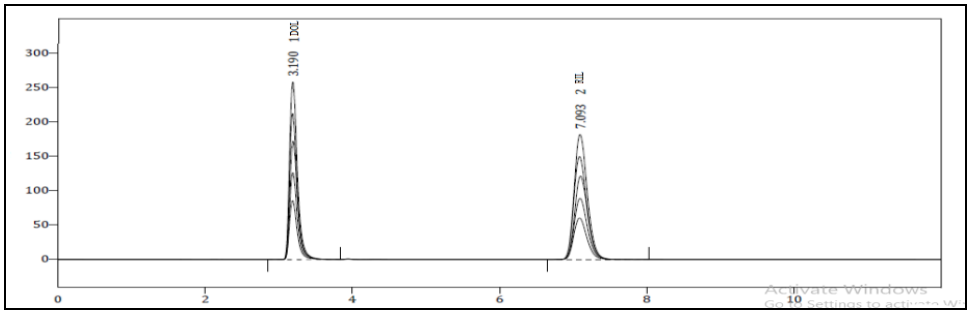

Figure: Chromatogram of Linearity of DOL (15-45 µg/ml) and RIL (2.5-7.5 µg/ml)

Table: Linearity data of DOL and RIL for HPLC

Sr. No.	DOL	Area	Rt. (Min.)	RIL	Area	Rt.(Min.)
1	15	755.7	7.15	2.5	641.8	3.190
2	22.5	1115.2	7.094	3.75	944.62	3.187
3	30	1523.06	7.091	5	1291.4	3.191
4	37.5	1875.07	7.086	6.25	1489.54	3.191
5	45	2280.54	7.097	7.5	1936.29	3.191

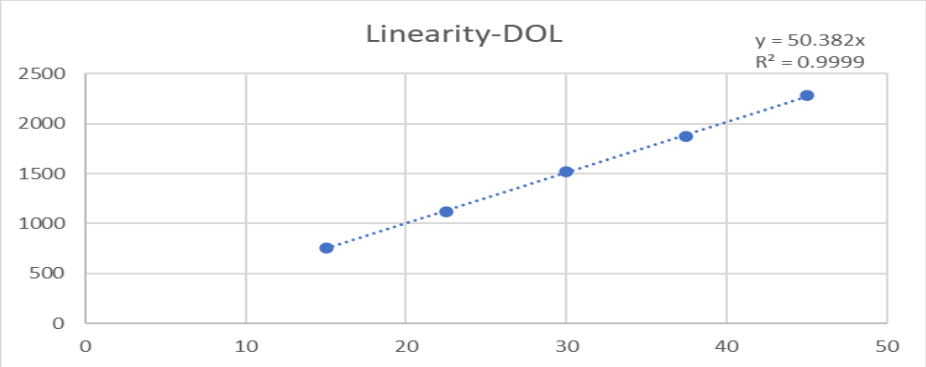

Figure: Calibration Graph for Dolutegravir (Area vs Concentration)

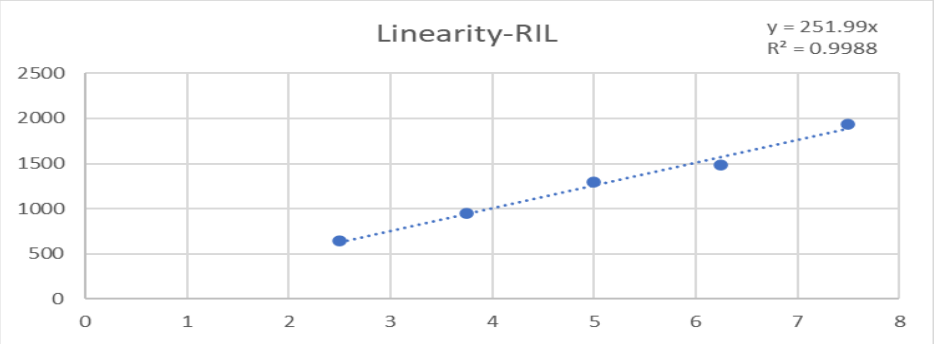

Figure: Calibration Graph for Rilpivirine (Area vs Concentration)

DOL and RIL were studied at five different concentrations at regular increasing interval and area were recorded. Result were shown in table and graph were plotted for area vs. concentration for both DOL and RIL. From regression equation, correlation coefficient was found to be 0.994 and 0.9894 for DOL and RIL respectively which indicates that both the drugs were linear in their given concentration range. Proposed method gives linear results for both the drugs. So, it can be used for estimation of drugs in given concentration range for combination of both the drugs.

Repeatability

Data of system precision is given in table. Percentage RSD for both the drug is less than 2%. This clearly indicates that proposed method is precise.

Table: Data of Repeatability in standard preparation of DOL and RIL

Sr. No	Dolutegravir	Rilpivirine
1	1412.006	1382.04
2	1416.172	1354.21
3	1580.995	1387.32
4	1422.57	1389.29
5	1414.456	1383.468
6	1417.235	1385.497
Average	1642.512	1345.28
Std Dev	13.9	12.15
% RSD	0.97	1.01

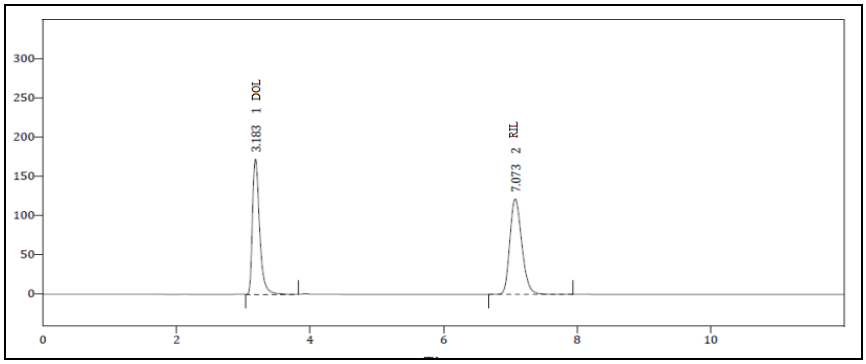

Figure: Chromatogram of Repeatability study of DOL (30 µg/ml) and RIL (5 µg/ml)- DATA 1

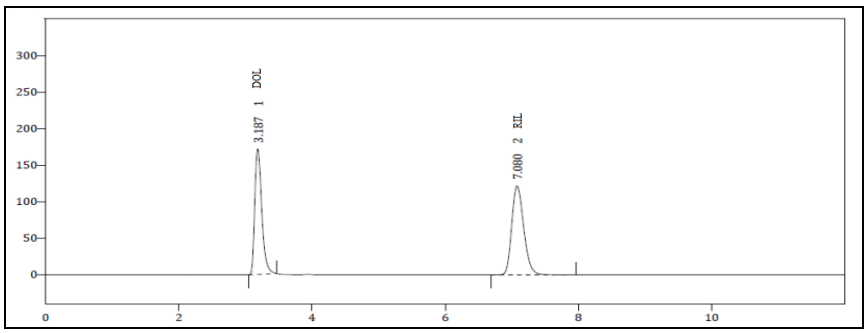

Figure: Chromatogram of Repeatability study of DOL (30 µg/ml) and RIL (5 µg/ml)- DATA 2

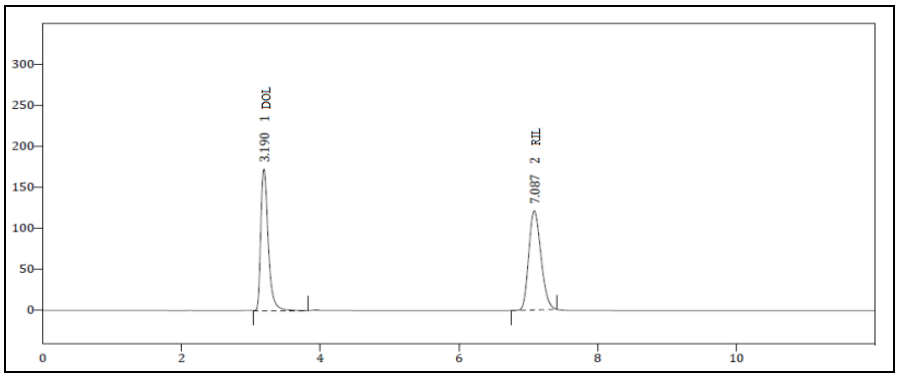

Figure: Chromatogram of Repeatability study of DOL (30 µg/ml) and RIL (5 µg/ml)- DATA 3

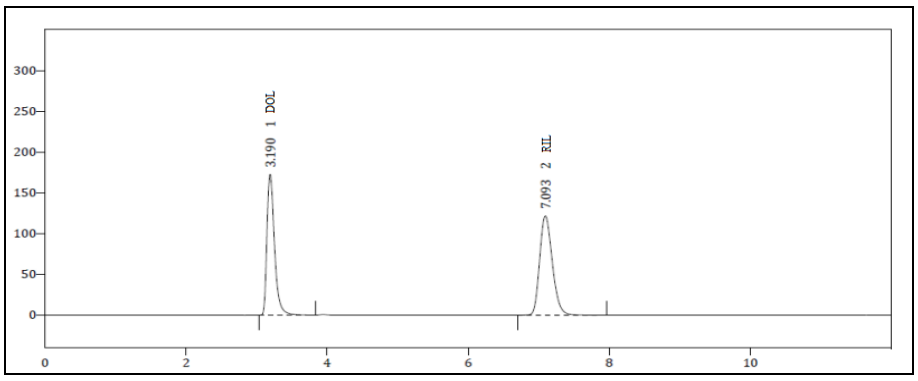

Figure: Chromatogram of Repeatability study of DOL (30 µg/ml) and RIL (5 µg/ml)- DATA 4

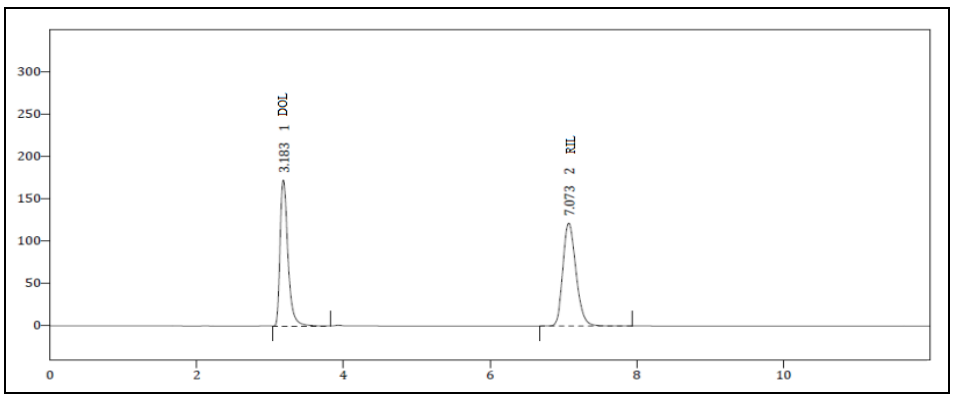

Figure: Chromatogram of Repeatability study of DOL (30 µg/ml) and RIL (5 µg/ml)- DATA 5

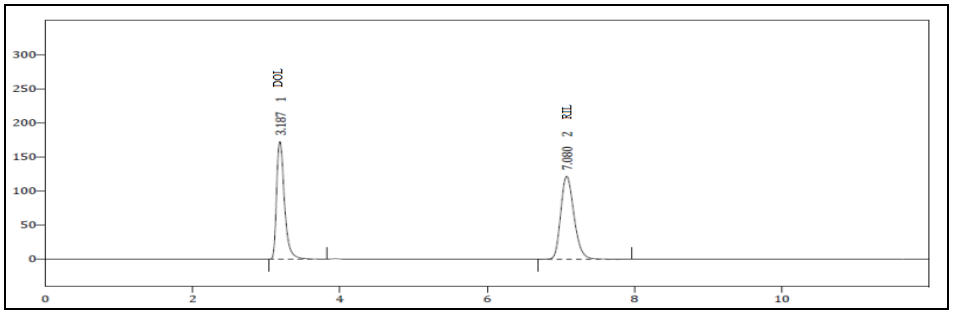

Figure: Chromatogram of Repeatability study of DOL (30 µg/ml) and RIL (5 µg/ml)- DATA 6

Figure are six images for peaks generated each after injecting DOL (30 µg/ml) and RIL (5 µg/ml), repetitively for 6 times in opposite phase excellent performing fluid-based separation technique, to check repeatability. The study was performed to get same result on repeat study of same drugs by same method and using same instrument. The result was shown in form of coefficient of variance which was less than 1% for both the drugs which indicates the method gives same result on repeat study. So, method can be called precise.

Intermediate Precision: The low % RSD values of intra-day and inter-day precision reveals that the proposed method is precise.

Table: Intraday Precision data for HPLC

Time (hr)	**Dolutegravir**			**Rilpivirine**		
	Test Area			**Test Area**		
% Level	50 (15 µg/ml)	100 (30 µg/ml)	150 (45 µg/ml)	50 (2.5 µg/ml)	100 (5 µg/ml)	150 (7.5 µg/ml)
2	756.51	1494.46	2269.45	621.45	1274.14	1894.26
4	745.65	1505.21	2255.47	625.48	1264.23	1915.45
6	751.23	1514.67	2261.46	624.15	1274.24	1921.14
Average	745.41	1503.46	2245.68	645.15	1247.12	1918.45
Std Dev	3.87	15.78	14.86	3.64	11.68	16.45
% RSD	0.54	1.09	0.67	0.62	0.84	0.95

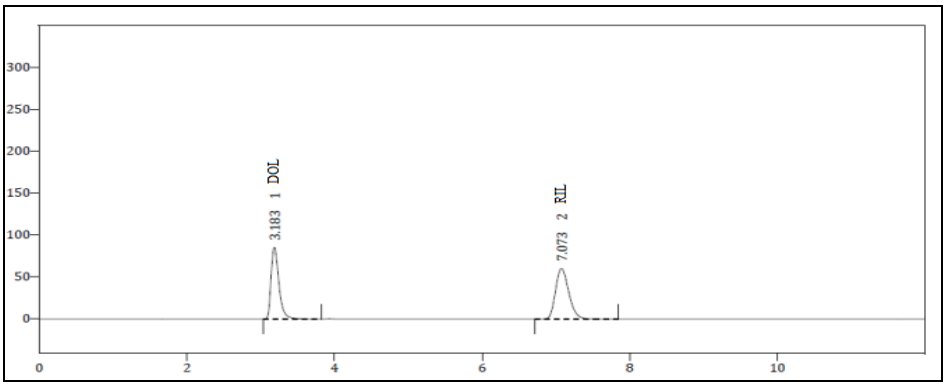

Figure: Chromatogram of Intraday Precision study of DOL (45 µg/ml) and RIL (7.5 µg/ml) at 50% Level- HOUR 2

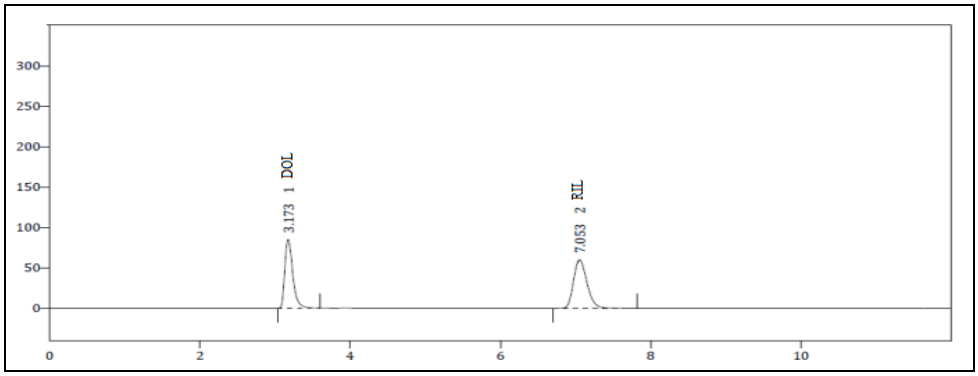

Figure: Chromatogram of Intraday Precision study of DOL (45 µg/ml) and RIL (7.5 µg/ml) at 50% Level- Hour 4

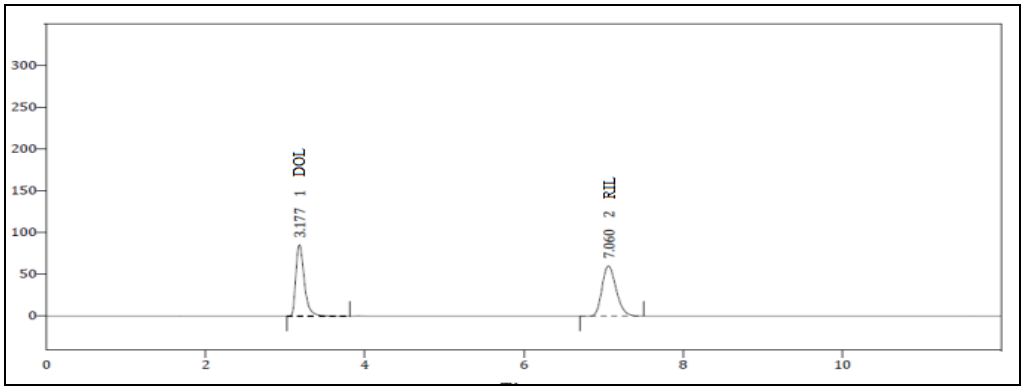

Figure: Chromatogram of Intraday Precision study of DOL (45 µg/ml) and RIL (7.5 µg/ml) at 50% Level- HOUR 6

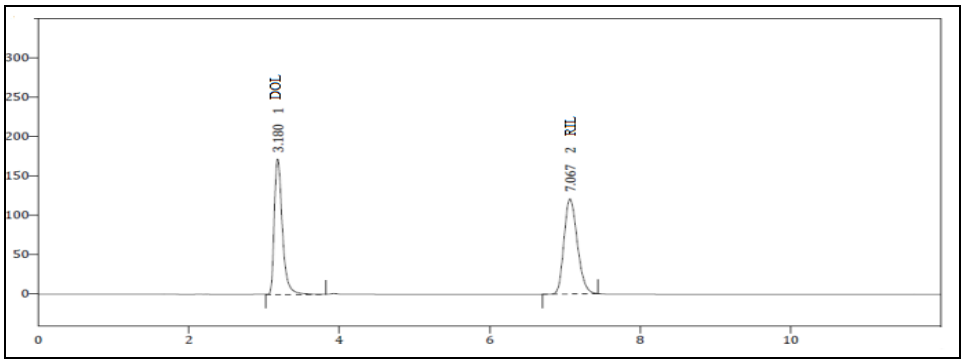

Figure: Chromatogram of Intraday Precision study of DOL (45 µg/ml) and RIL (7.5 µg/ml) at 100% Level- HOUR 2

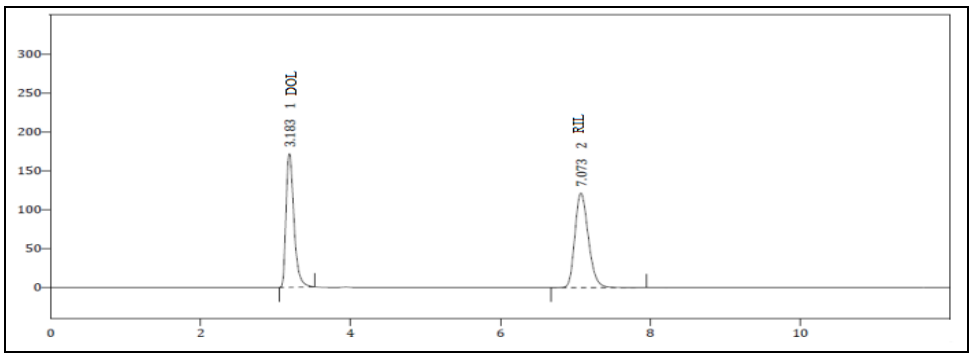

Figure: Chromatogram of Intraday Precision study of DOL (45 µg/ml) and RIL (7.5 µg/ml) at 100% Level- HOUR 4

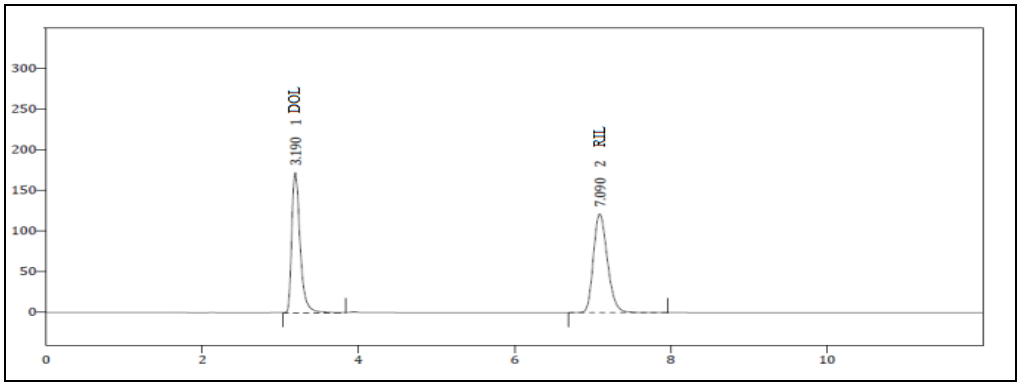

Figure: Chromatogram of Intraday Precision study of DOL (45 µg/ml) and RIL (7.5 µg/ml) at 100% Level- HOUR 6

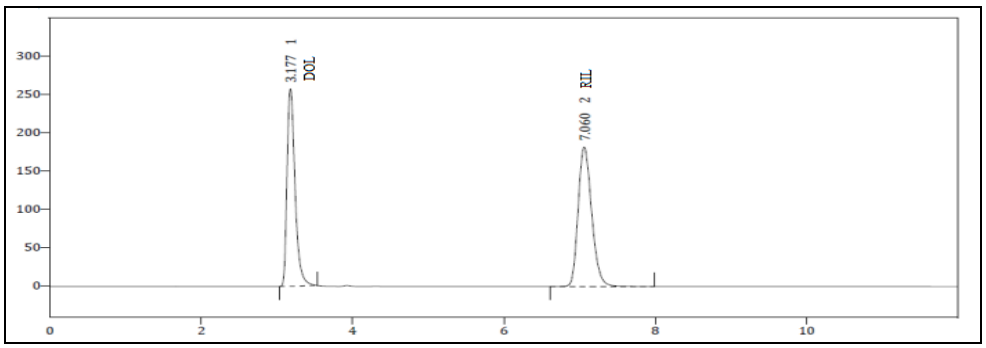

Figure: Chromatogram of Intraday Precision Study of DOL (45 µg/ml) and RIL (7.5 µg/ml) at 150% Level- HOUR 2

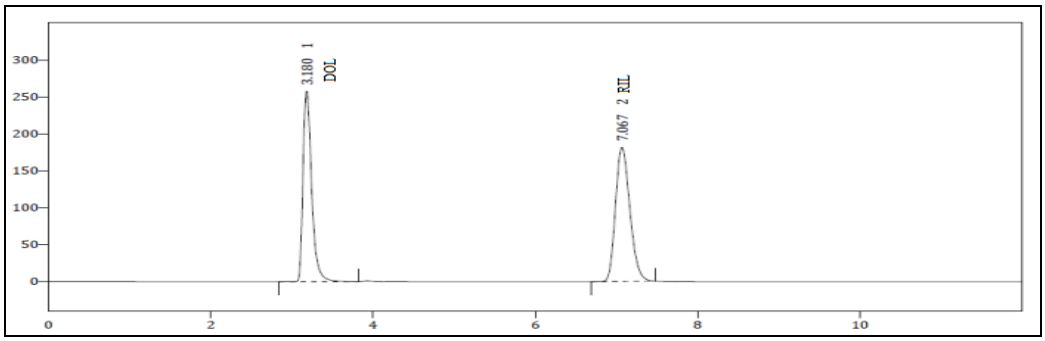

Figure: Chromatogram of Intraday Precision study of DOL (45 µg/ml) and RIL (7.5 µg/ml) at 150% Level- HOUR 4

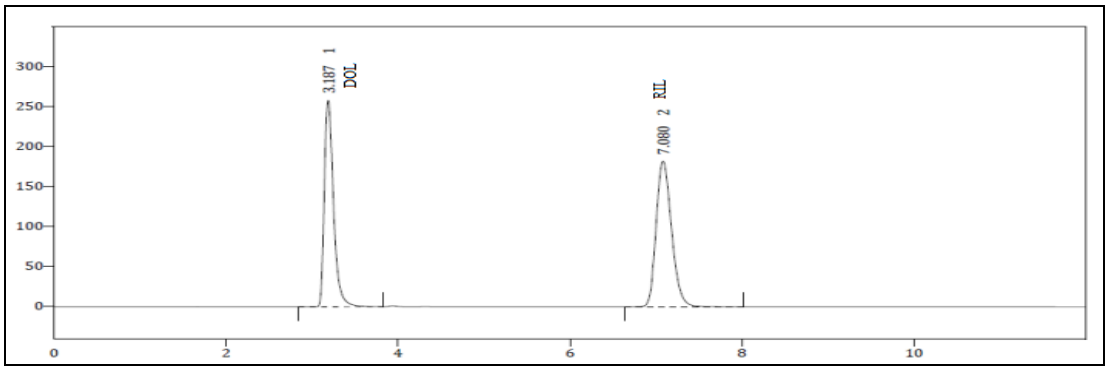

Figure: Chromatogram of Intraday Precision study of DOL (45 µg/ml) and RIL (7.5 µg/ml) at 150% Level- HOUR 6

Figure shows Intraday precision study for DOL (45µg/ml) and RIL (7.5µg/ml) at 50% 2,4 and 6 hours, 100% at 2,4,6 hours and 150% at 2,4, and 6 hour respectively. The study was performed to get same result on repeat study of same drugs by same method and using same instrument on different time of the same day. The result was shown in form of %RSD which was less than 2% for both the drugs which indicates the method gives same result on repeat study in different time of the same day so method was found precise.

Table: Interday Precision data for HPLC

DAY	**Dolutegravir**			**Rilpivirine**		
	Test Area			**Test Area**		
% Level	50 (15 µg/ml)	100 (30 µg/ml)	150 (45 µg/ml)	50 (2.5 µg/ml)	100 (5 µg/ml)	150 (7.5 µg/ml)
1	758.45	1545.15	2246.60	626.28	1246.48	1849.72
2	752.76	1489.94	2267.45	631.46	1249.54	1946.50
3	746.55	1542.34	2261.01	633.47	1274.69	1943.46
Average	738.46	1501.46	2256.48	629.97	1274.46	1937.49
Std Dev	9.41	14.48	12.84	5.47	10.49	12.49
% RSD	1.24	0.94	0.54	0.84	0.84	0.73

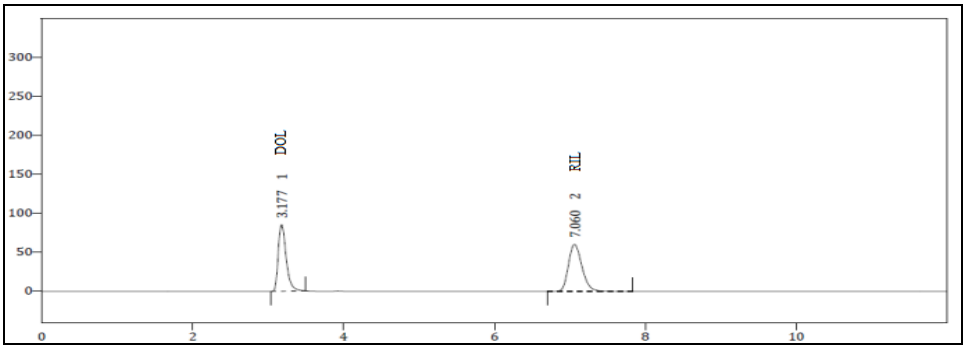

Figure: Chromatogram of Interday Precision of DOL (15 µg/ml) and RIL (2.5 µg/ml) at 50% Level- DAY 1

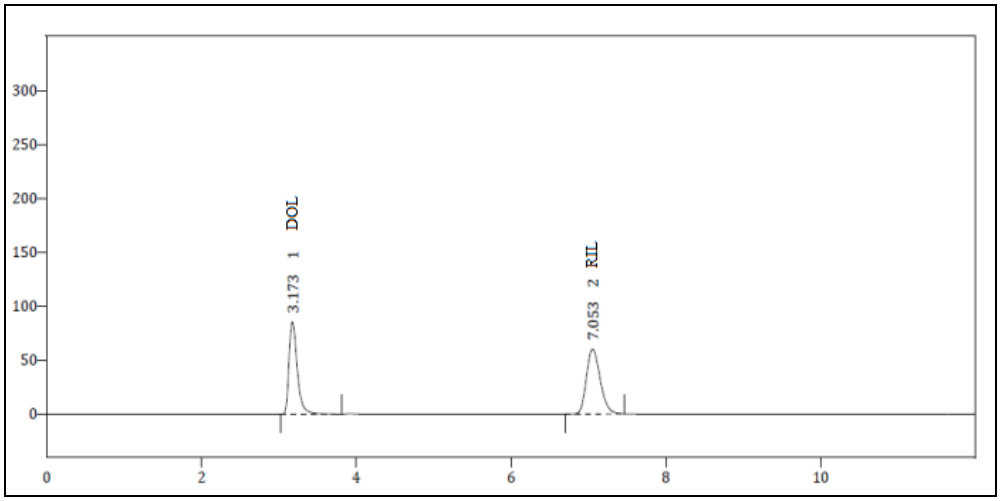

Figure: Chromatogram of Interday Precision study of DOL (15µg/ml) and RIL (2.5 µg/ml) at 50% Level- DAY 2

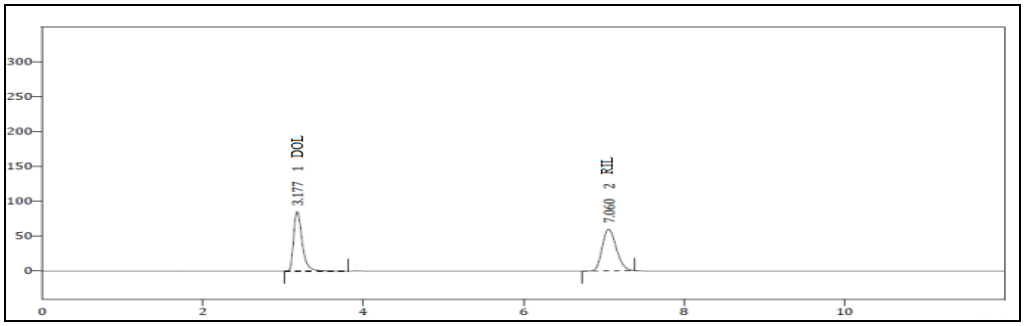

Figure: Chromatogram of Interday Precision study of DOL (15µg/ml) and RIL (2.5 µg/ml) at 50% Level- DAY 3

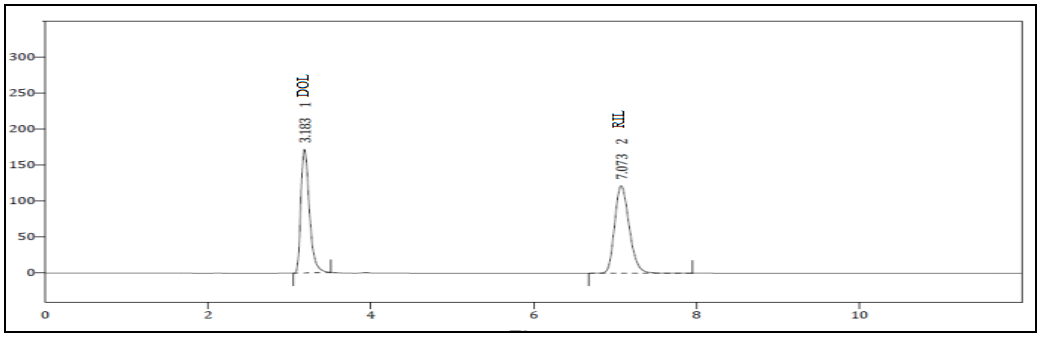

Figure: Chromatogram of Interday Precision study of DOL (30 µg/ml) and RIL (5 µg/ml) at 100% Level- DAY 1

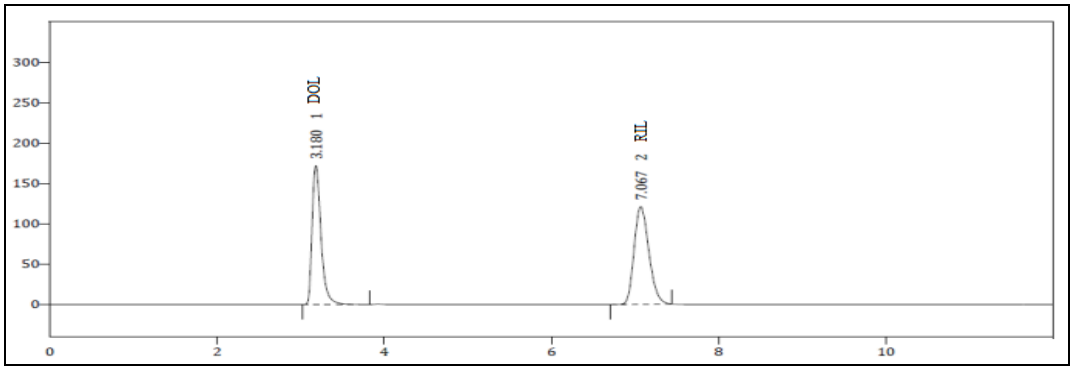

Figure: Chromatogram of Interday Precision study of DOL (30 µg/ml) and RIL (5 µg/ml) at 100% Level- DAY 2

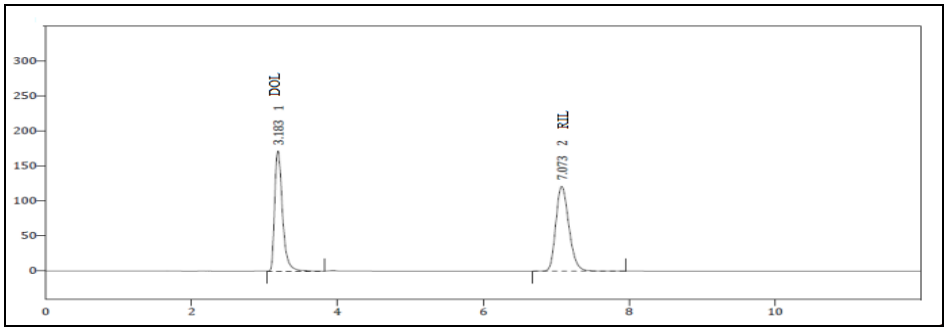

Figure: Chromatogram of Interday Precision study of DOL (30 µg/ml) and RIL (5 µg/ml) at 100% Level- DAY 3

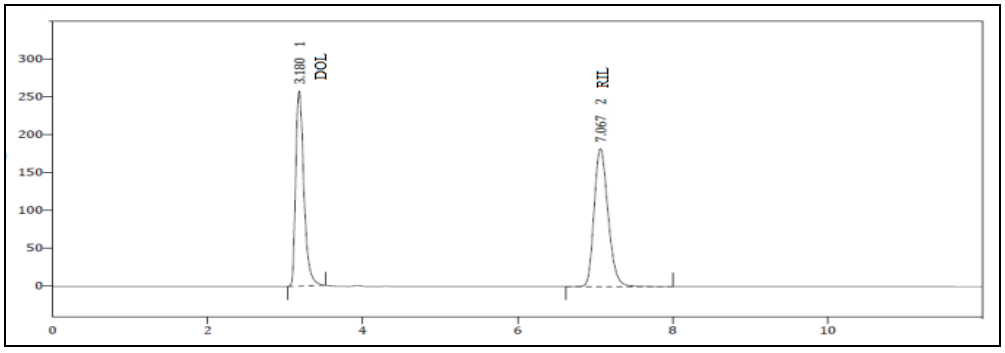

Figure: Chromatogram of Interday Precision study of DOL (45 µg/ml) and RIL (7.5 µg/ml) at 150% Level- DAY 1

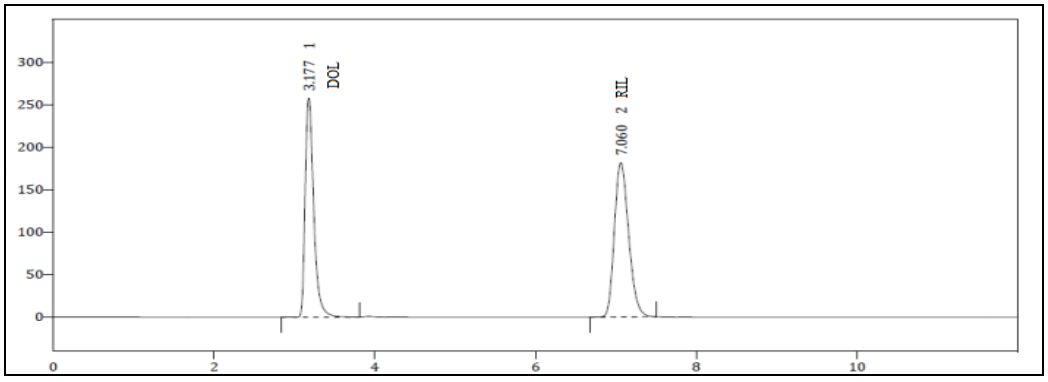

Figure: Chromatogram of Interday Precision study of DOL (45µg/ml) and RIL (7.5 µg/ml) at 150% Level- DAY 2

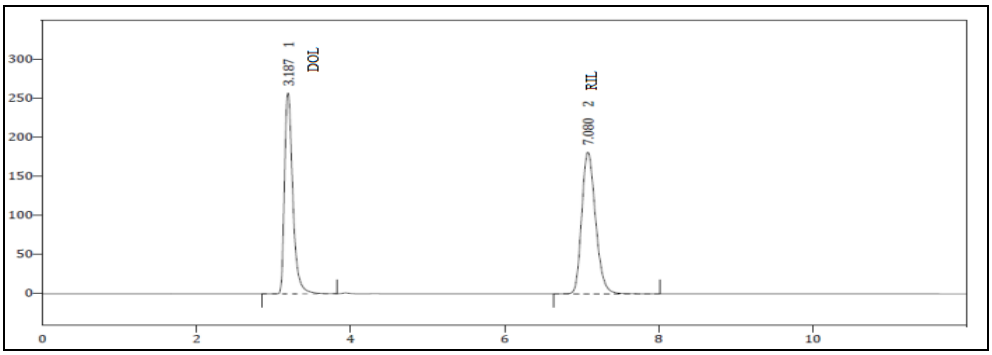

Figure: Chromatogram of Interday Precision study of DOL (45µg/ml) and RIL (7.5 µg/ml) at 150% Level- DAY 3

Figure shows validating for Interday precision study was performed to get same result on repeat study of same drugs by same method and using same instrument on different day. The result was shown in form of % RSD which was less than 2% for both the drugs which indicates the method gives same result on repeat study in different day so method was found precise.

Accuracy (% Recovery)

The recovery experiments were carried out at 80%, 100%, and 120%. The results of recovery studies are given in Table. The value of Recovery indicates that the proposed method is accurate.

Table: Data for Recovery study of DOL

Sr. No.		**Dolutegravir (15 µg/ml)**		**Rilpivirine (2.5 µg/ml)**	
Test Area (n=3)		776.14		591.57	
Dolutegravir					
% Level	**Area Found**	**Area of recovered standard**	**Conc. Found (µg/ml) of DOL**	**% Recovery**	**Avg. % Recovery ±SD, % RSD**
80% (12 µg/ml)	1416.24	641.15	12.10	99.46	100.06±0.73,0.73
	1413.61	635.45	12.04	99.31	
	1404.74	639.72	11.92	100.50	
100% (15 µg/ml)	1569.16	775.43	14.84	99.38	99.56±0.35,0.32
	1565.47	784.46	14.91	99.98	
	1563.99	781.41	14.89	99.84	
120% (18 µg/ml)	1721.81	934.71	17.49	99.64	99.51±0.18,0.19
	1719.46	936.43	17.51	99.49	
	1723.84	939.62	17.42	99.52	

Table: Data for Recovery study of Rilpivirine

% Level	Area Found	Area of recovered standard	Conc. Found (µg/ml) of Rilpivirine	% Recovery	Avg. Recovery ± SD, %RSD
80% (2 µg/ml)	1089.46	491.03	1.94	99.94	100.05±0.99,0.94
	1096.51	503.21	2.03	100.31	
	1091.23	499.64	2.04	100.23	
100% (2.5 µg/ml)	1209.23	619.24	2.49	99.16	99.72±0.61,0.59
	1212.34	618.46	2.45	100.29	
	1210.12	621.13	2.48	99.84	
120% (3 µg/ml)	1339.36	741.35	3.01	100.16	99.64±0.46,0.42
	1334.51	739.62	2.99	99.46	
	1336.40	740.34	2.87	99.52	

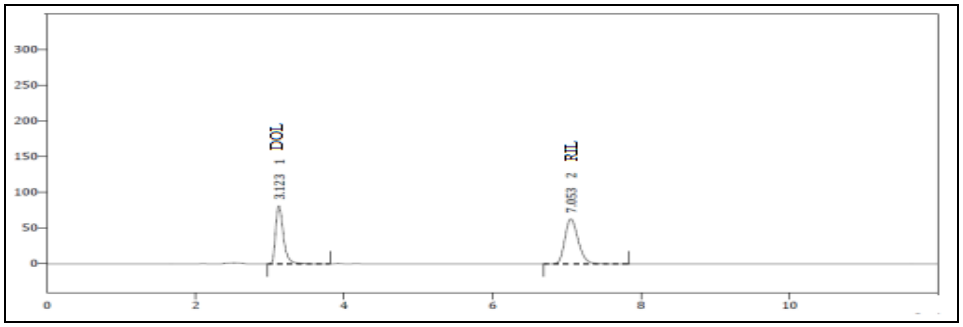

Figure: Chromatogram of Accuracy study of test DOL (15 µg/ml) and RIL (2.5 µg/ml)

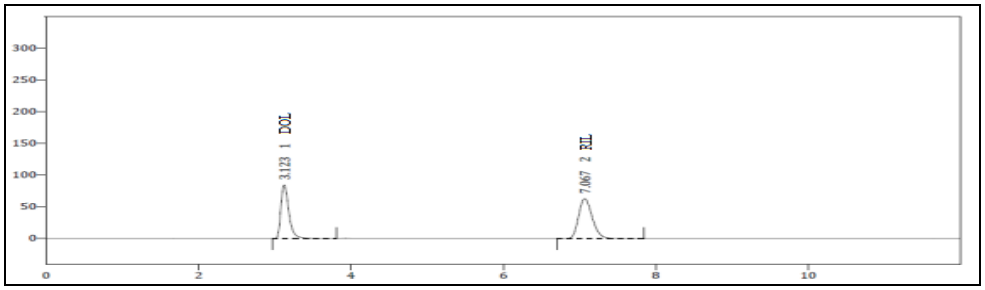

Figure: Chromatogram of Accuracy study of standard DOL (15 µg/ml) and RIL (2.5 µg/ml)

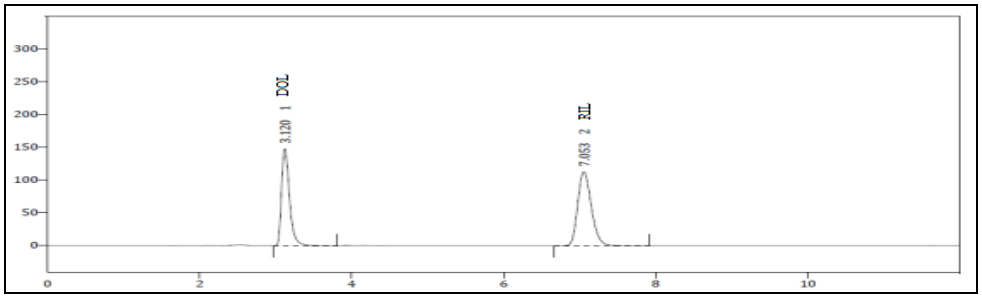

Figure: Chromatogram of Accuracy study at 80% for DOL and RIL DATA 1

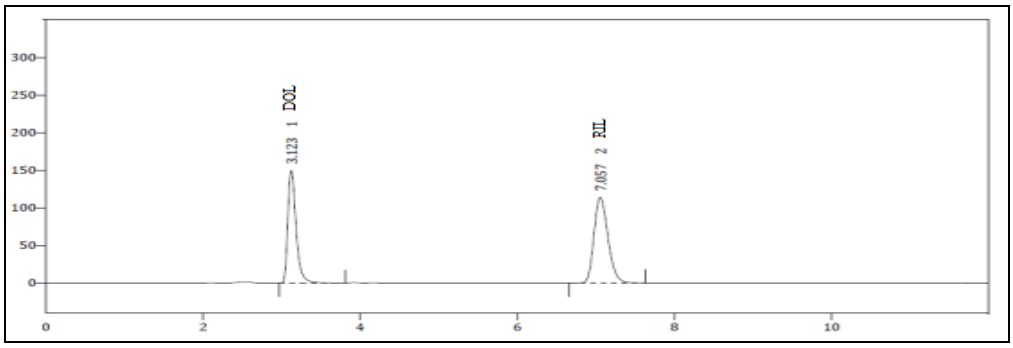

Figure: Chromatogram of Accuracy study at 80% for DOL and RIL DATA 2

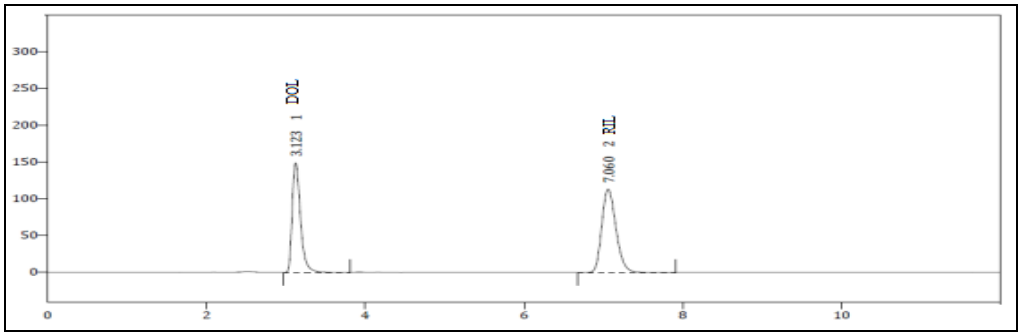

Figure: Chromatogram of Accuracy study at 80% for DOL and RIL DATA 3

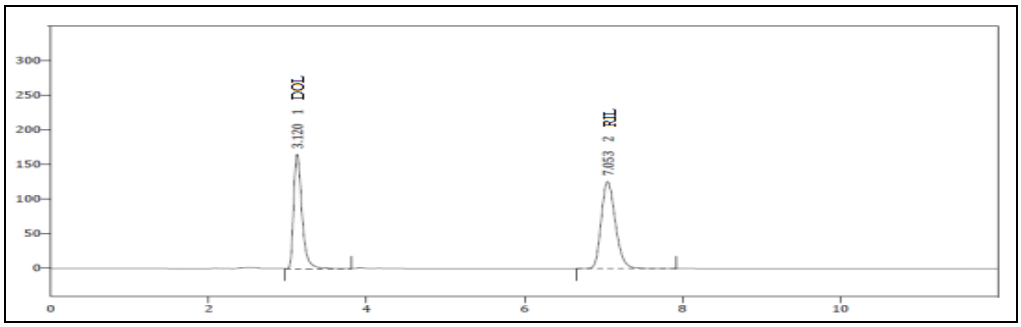

Figure: Chromatogram of Accuracy study at 100% for DOL and RIL DATA 1

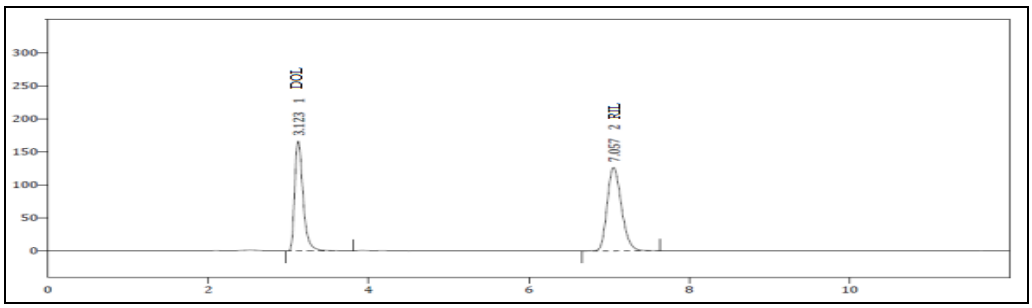

Figure: Chromatogram of Accuracy study at 100% for DOL and RIL DATA 2

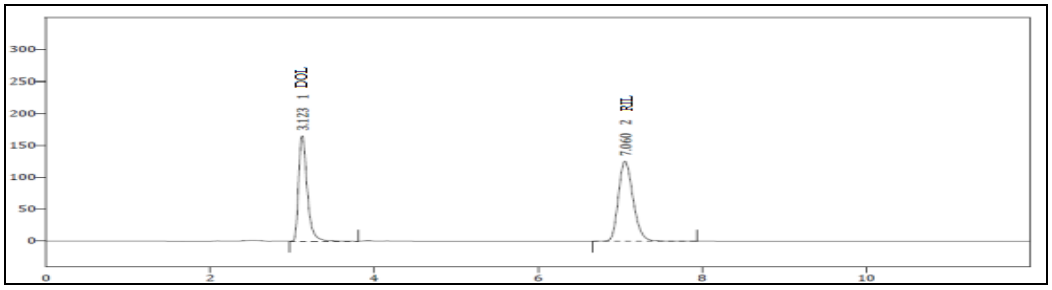

Figure: Chromatogram of Accuracy study at 100% for DOL and RIL DATA 3

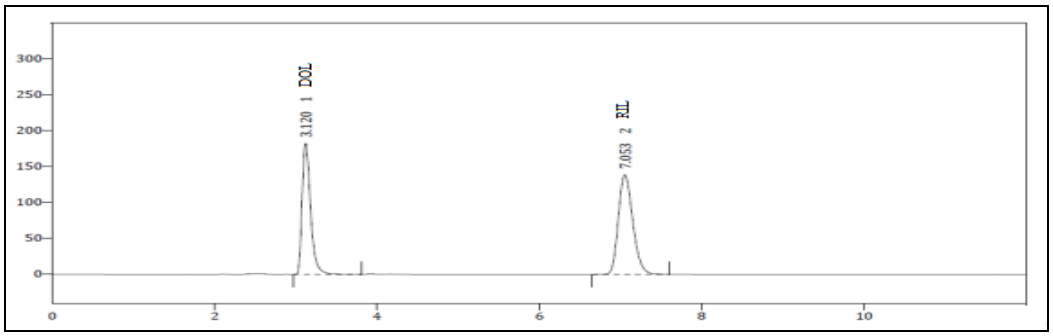

Figure: Chromatogram of Accuracy study at 120% for DOL and RIL DATA 1

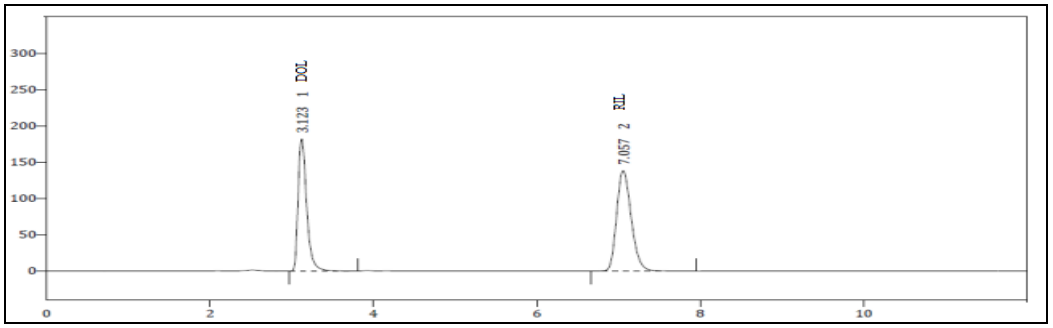

Figure: Chromatogram of Accuracy study at 120% for DOL and RIL DATA 2

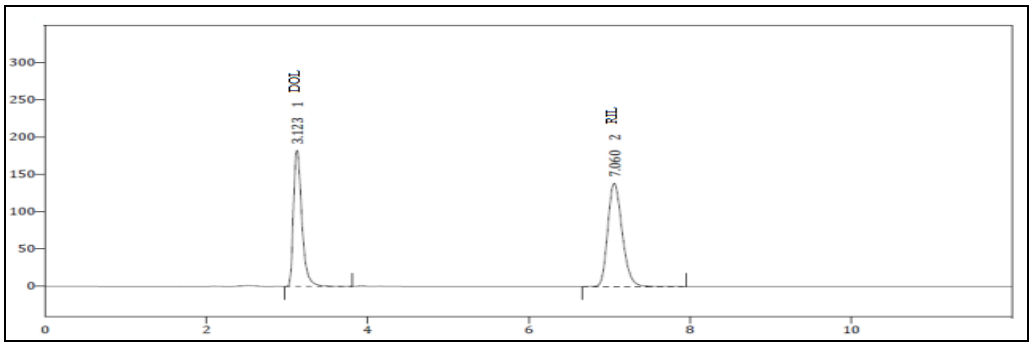

Figure: Chromatogram of Accuracy study at 120% for DOL and RIL DATA 3

Accuracy study was performed to get same result which is close to each other on repeat study of same drugs by same method and using same instrument. The result was shown in form of % RSD which was less than 2% for both the drugs and 5 recoveries was found between 98-102 % which

indicates the method gives same result on repeat study of drugs in presence of test. So method was found accurate.

LOD and LOQ

Based on calibration curve LOD and LOQ was calculated for DOL and RIL

Table: LOD and LOQ for DOL and RIL

Drug	Std. Dev	Slope	LOD (µg/ml)	LOQ (µg/ml)
DOL	15.461	49.72	1.046	3.234
RIL	57.145	245.02	0.809	2.241

LOD and LOQ was found to get lowest concentration that proposed method can quantify for given drug and lowest amount of drug that can be detected but not necessarily quantify. LOD were found to be 1.046 and 0.809 µg/ml for DOL and RIL respectively. So proposed method can detect drugs starting from found LOD concentration. LOQ were found to be 3.234 and 2.241 µg/ml for DOL and RIL respectively. So proposed method can quantify drugs starting from found concentration.

Robustness

Variation in Flow Rate

Results of Robustness of variation in flow rate 0.8 ml/min (-0.2 ml)

Table: Results of Robustness

Sr. No.	Dolutegravir		Rilpivirine	
	Std Area	Test Area	Std Area	Test Area
1	1503.04	1561.32	1284.13	1346.75
2	1509.26	1559.12	1279.15	1334.08
3	1499.64	1572.46	1289.53	1342.31
Average	1502.34	1565.13	1282.61	1330.95
Std Dev	19.51	8.004	18.334	11.649
% RSD	1.271	0.503	1.512	0.871

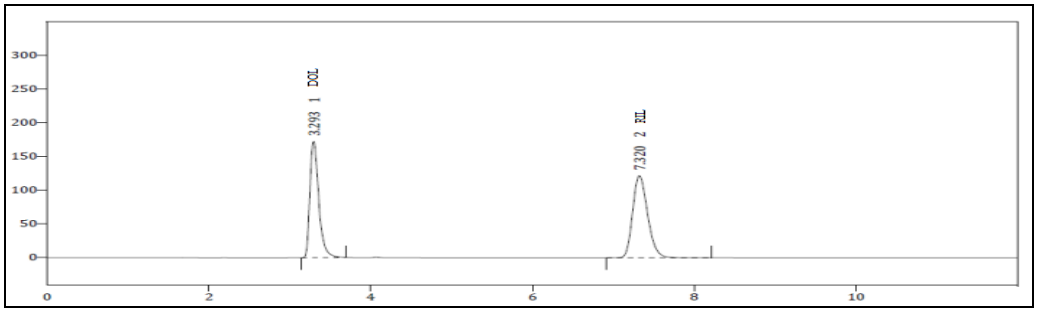

Figure: Chromatogram of Robustness study at 0.8 ml/min for DOL (30 µg/ml) and RIL (5 µg/ml) DATA 1

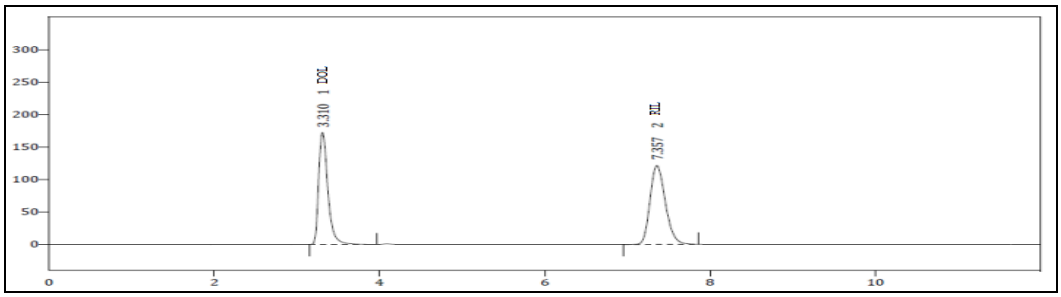

Figure: Chromatogram of Robustness study at 0.8 ml/min for DOL (30 µg/ml) and RIL (5 µg/ml) DATA 2

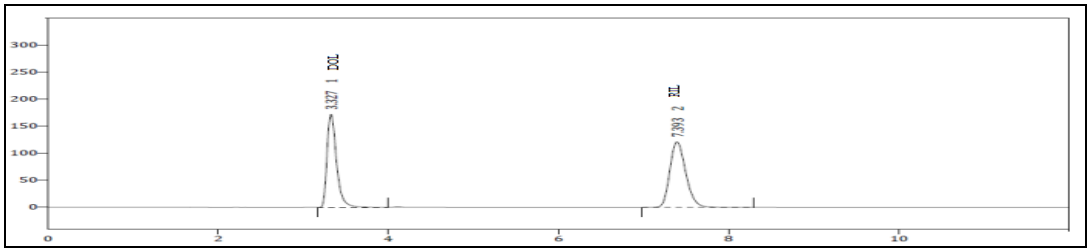

Figure: Chromatogram of Robustness study at 0.8 ml/min for DOL (30 µg/ml) and RIL (5 µg/ml) DATA 3

Table: Results of Robustness of variation in flow rate 1.2 ml/min (+0.2 ml)

Sr. No.	Dolutegravir		Rilpivirine	
	Std area	Test area	Std area	Test area
1	1509.03	1465.99	1254.07	1234.46
2	1513.19	1471.59	1281.21	1254.51
3	1516.94	1492.27	1282.24	1264.35
Average	1504.26	1476.84	1273.19	1251.024
Std Dev	19.513	13.6049	18.629	16.852
% RSD	1.284	0.972	1.481	1.353

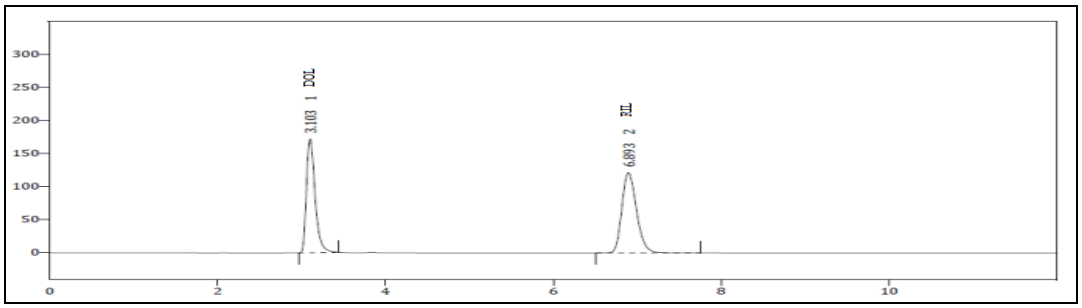

Figure: Chromatogram of Robustness study at 1.2 ml/min for DOL (30 µg/ml) and RIL (5 µg/ml) DATA 1

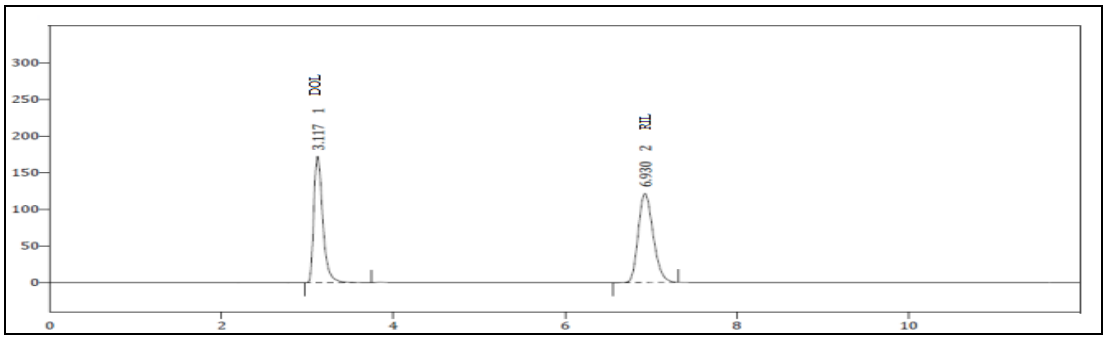

Figure: Chromatogram of Robustness study at 1.2 ml/min for DOL (30 µg/ml) and RIL (5 µg/ml) DATA 2

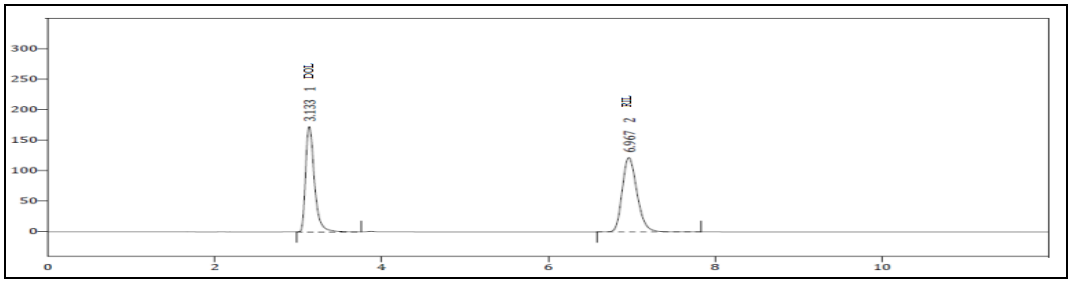

Figure: Chromatogram of Robustness study at 1.2 ml/min for DOL (30 µg/ml) and RIL (5 µg/ml) DATA 3

Robustness study was performed to check whether result was change or not on small change in proposed method. In this study, flow rate was changed ±0.2 ml from finalized condition i.e 1 ml/min. Result was shown in form of % RSD which was found less than 2% for both the drugs. The result indicates that method shows negligible change on change in flow rate. So method was found to be robust.

Forced Degradation Study

Acid Stress Study

Table: Acid Stability study data for Dolutegravir and Rilpivirine by HPLC

Stressed Conditions	Rilpivirine		Dolutegravir	
	Area Found	% Degradation	Area Found	% Degradation
0 Hr	1634.02	0	1319.06	0

2 Hr	1394.56	7.64	1233.46	5.64
6 Hr	508.47	68.46	503.46	62.46

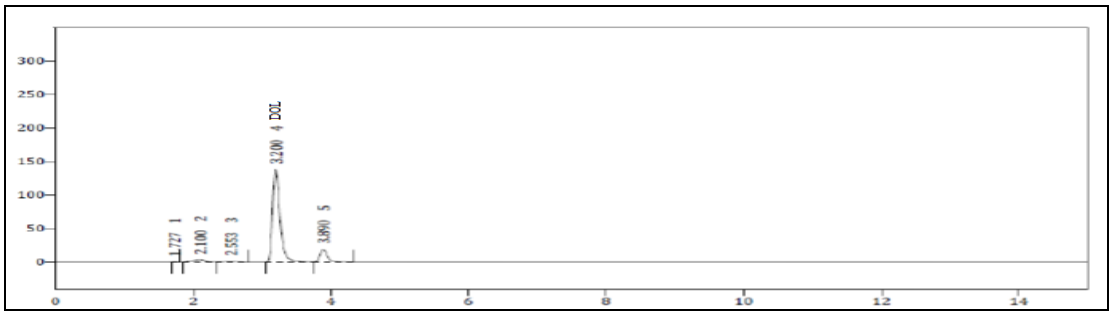

Figure: Chromatogram of DOL in acid stress condition

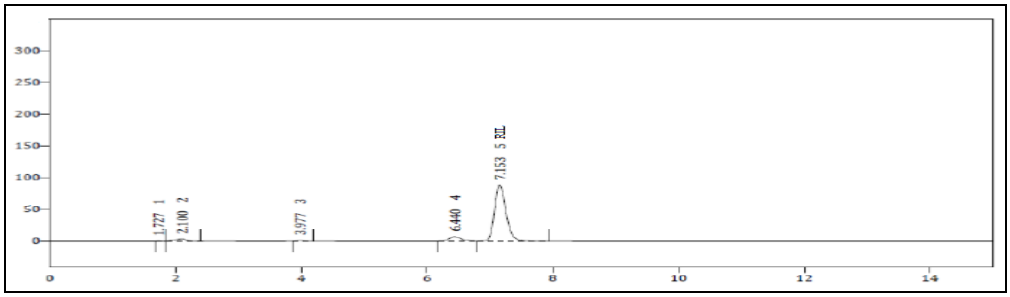

Figure: Chromatogram of RIL in acid stress condition

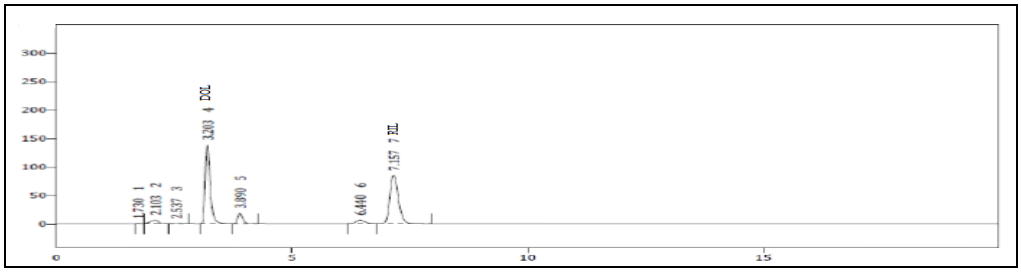

Figure: Chromatogram of DOL and RIL in acid stress condition

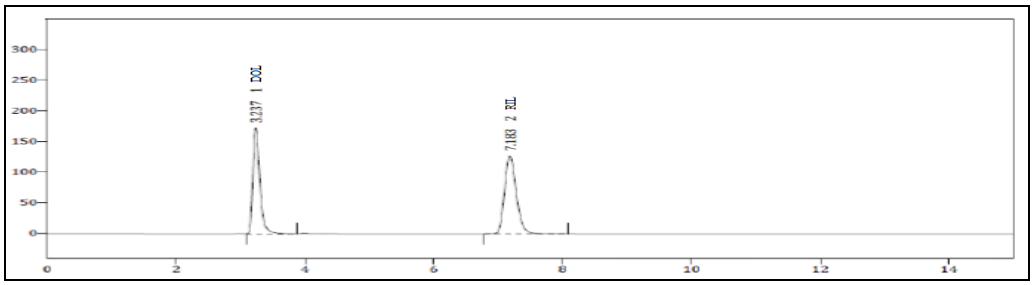

Figure: Chromatogram of DOL and RIL test in acid stress condition after 0 hr.

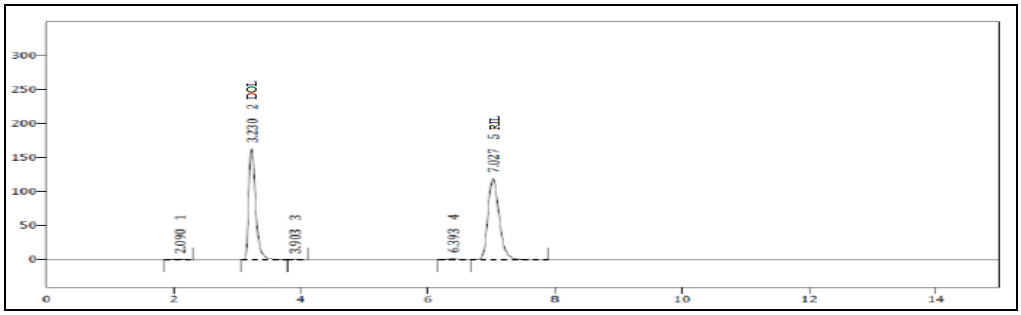

Figure: Chromatogram of DOL and RIL test in acid stress condition after 2 hr.

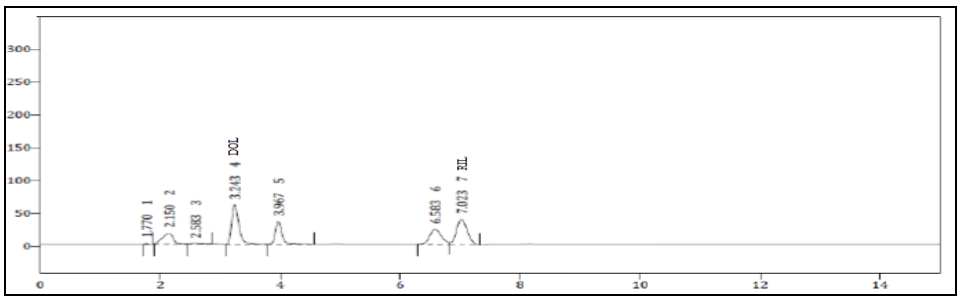

Figure: Chromatogram of DOL and RIL in acid stress condition after 6 hr.

Base Stress Study

Table: Base Stability study data for Rilpivirine and Dolutegravir by HPLC

Stressed Conditions	Rilpivirine		Dolutegravir	
	Area Found	% Degradation	Area Found	% Degradation
0 Hr	1525.62	0	1264.06	0
2 Hr	1434.58	4.06	1182.16	6.46
6 Hr	501.09	70.56	461.34	69.46

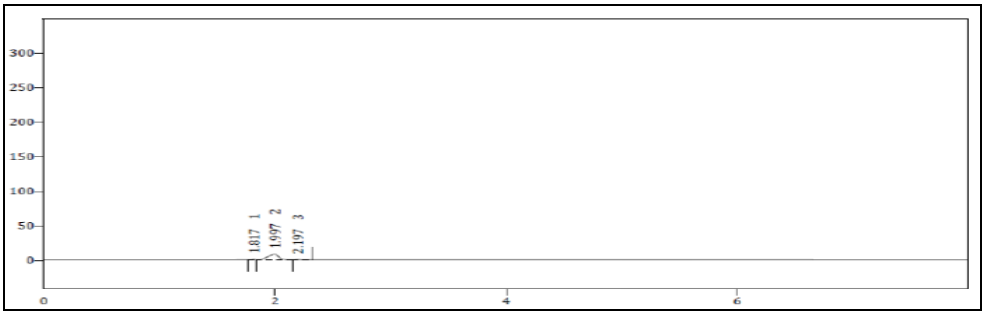

Figure: Chromatogram of base blank

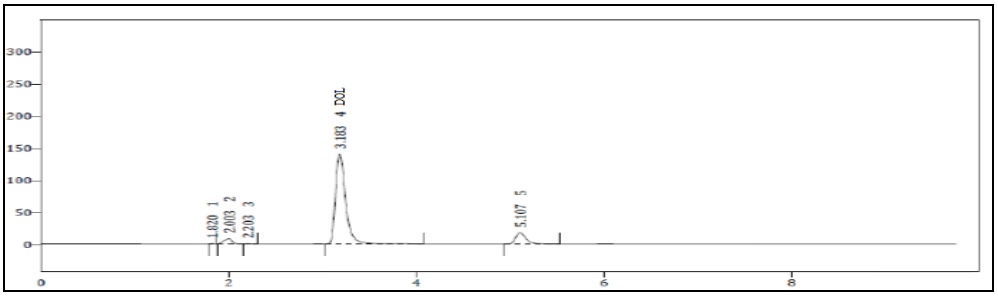

Figure: Chromatogram of DOL in base stress condition

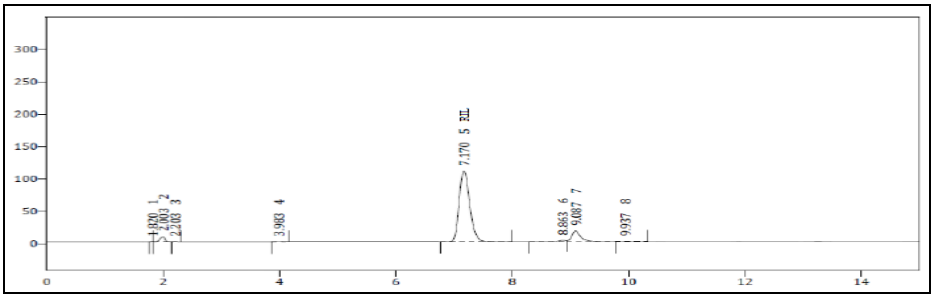

Figure: Chromatogram of RIL in base stress condition

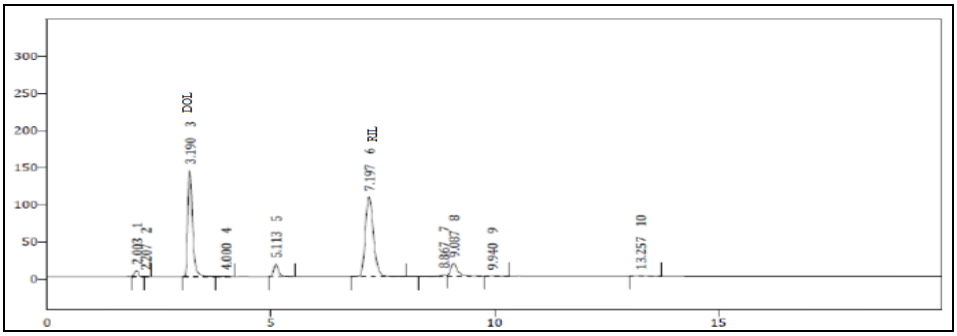

Figure: Chromatogram of DOL and RIL test in base stress condition

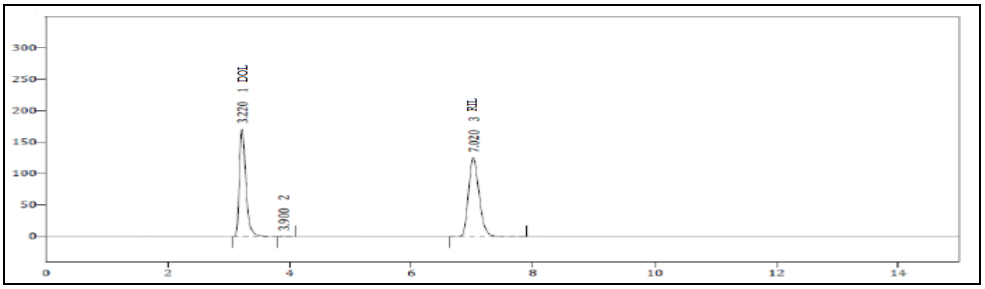

Figure: Chromatogram of DOL and RIL test in base stress condition after 0 Hr.

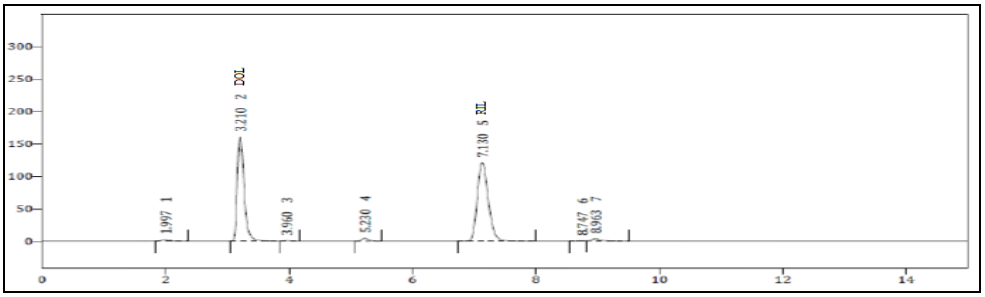

Figure: Chromatogram of DOL and RIL test in base stress condition after 2 Hr.

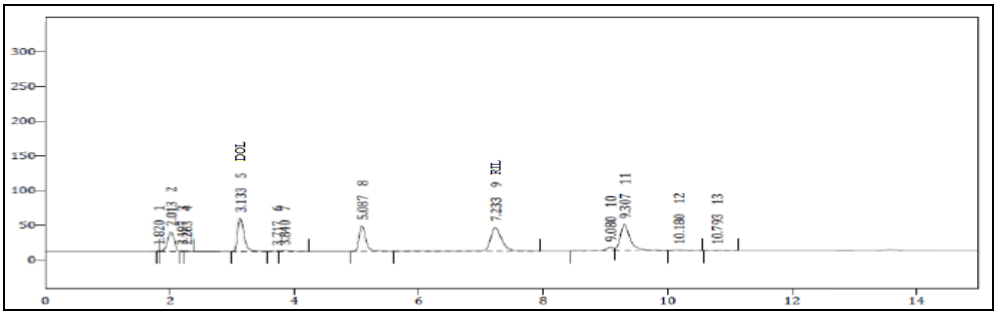

Figure: Chromatogram of DOL and RIL test in base stress condition after 6 Hr.

Oxidative Stress Study

Table: Oxidative Stability study data for Rilpivirine and Dolutegravir by HPLC

Stressed Conditions	Rilpivirine		Dolutegravir	
	Area Found	% Degradation	Area Found	% Degradation
0 Hr	1561.46	0	1284.46	0
2 Hr	1491.25	8.41	1204.92	7.14
6 Hr	506.12	69.31	503.05	64.13

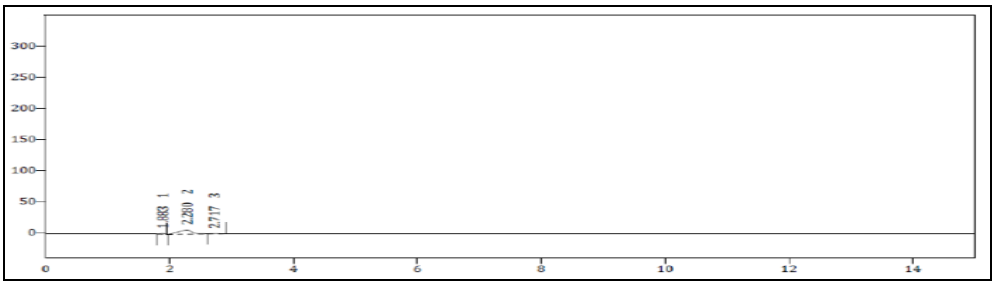

Figure: Chromatogram of Oxidative blank

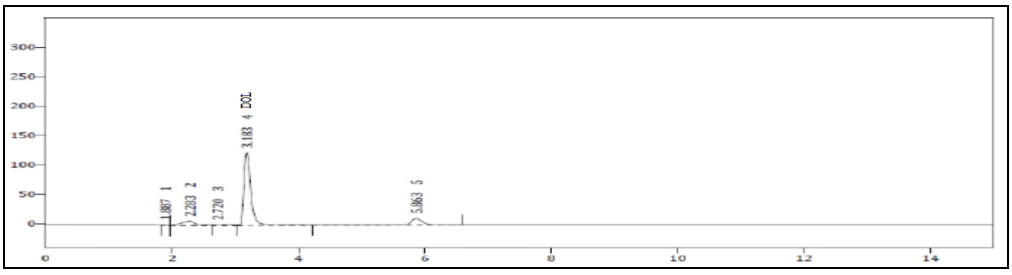

Figure: Chromatogram of DOL in Oxidative stress condition

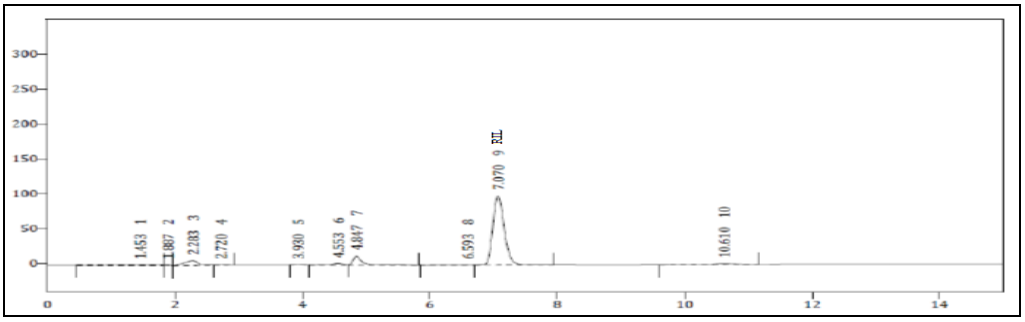

Figure: Chromatogram of RIL in Oxidative stress condition

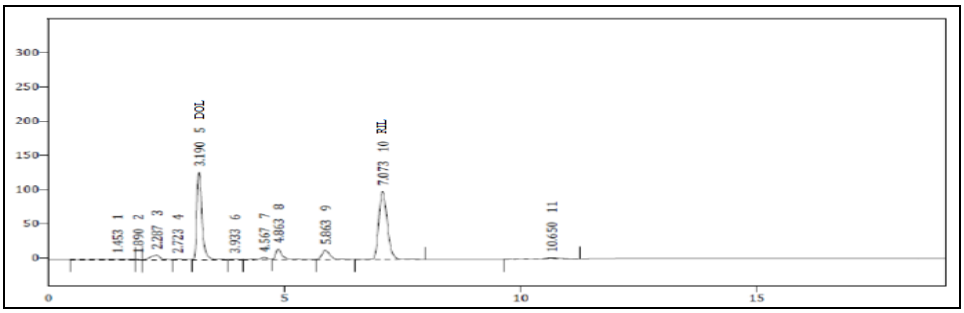

Figure: Chromatogram of DOL and RIL test in Oxidative stress condition

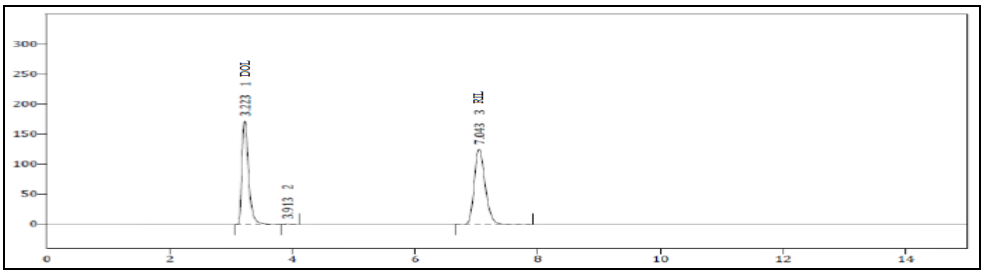

Figure: Chromatogram of DOL and RIL test in Oxidative stress condition after 0 Hr.

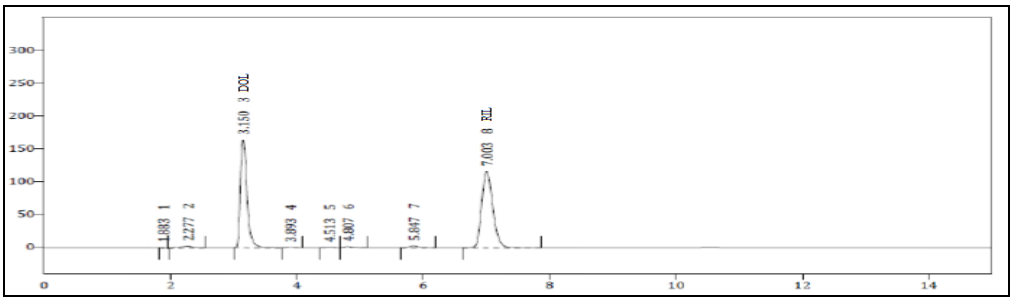

Figure: Chromatogram of DOL and RIL in Oxidative stress condition after 2 Hr.

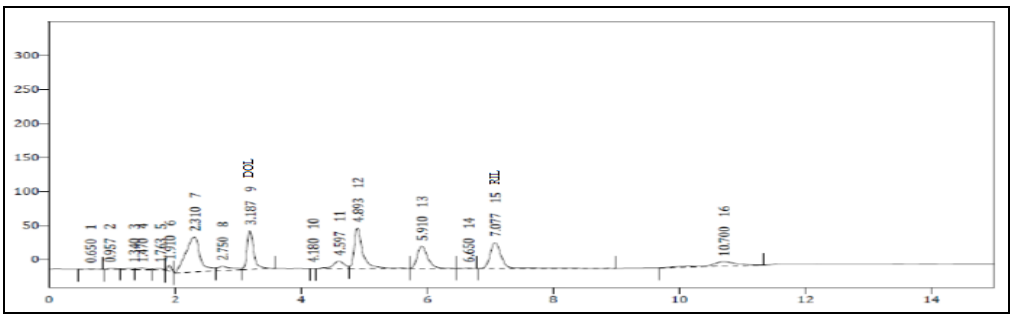

Figure: Chromatogram of DOL and RIL test in Oxidative stress condition after 6 Hr.

Photo Stability Study

Table: Photo Stability study data for Dolutegravir and Rilpivirine

Stressed Conditions	Rilpivirine		Dolutegravir	
	Area Found	% Degradation	Area Found	% Degradation
0 Hr	1561.26	0	1294.46	0
2 Hr	1492.42	2.61	1206.77	8.16
6 Hr	720.58	56.26	551.64	62.09

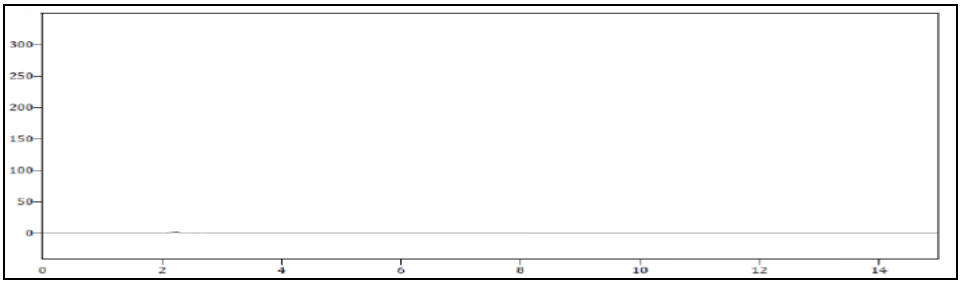

Figure: Chromatogram of Blank

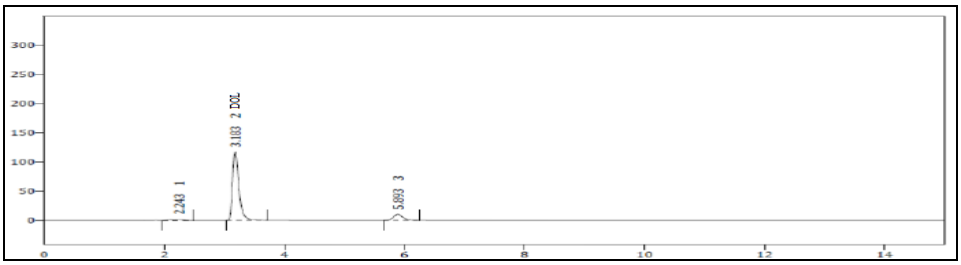

Figure: Chromatogram of DOL in Photo Stress Condition

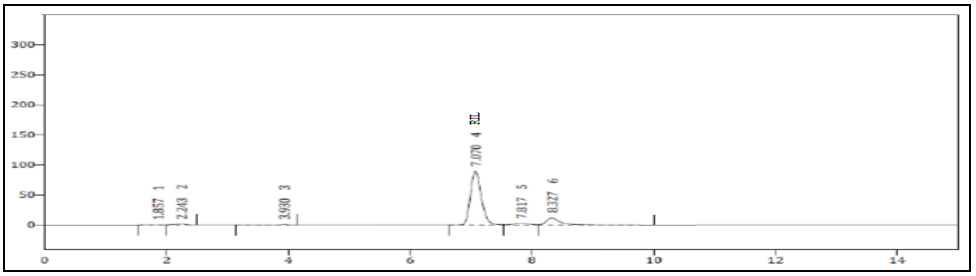

Figure: Chromatogram of RIL in Photo Stress condition

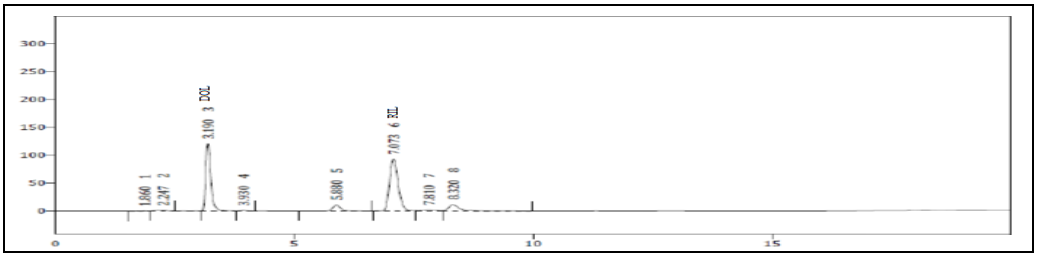

Figure: Chromatogram of DOL and RIL test in Photo stress condition

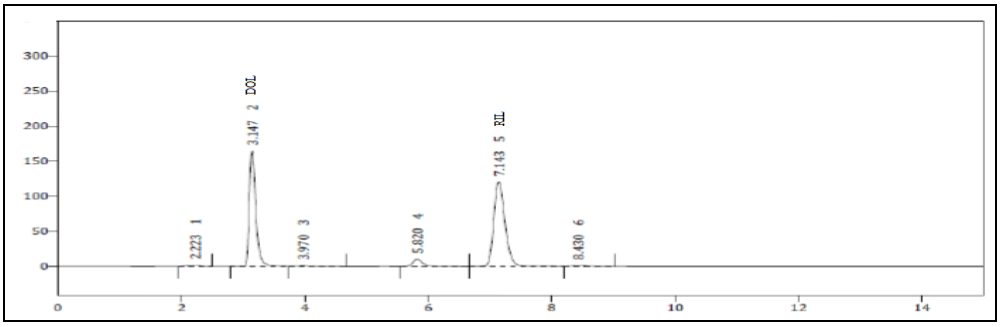

Figure: Chromatogram of DOL and RIL test in UV radiation after 12 Hr.

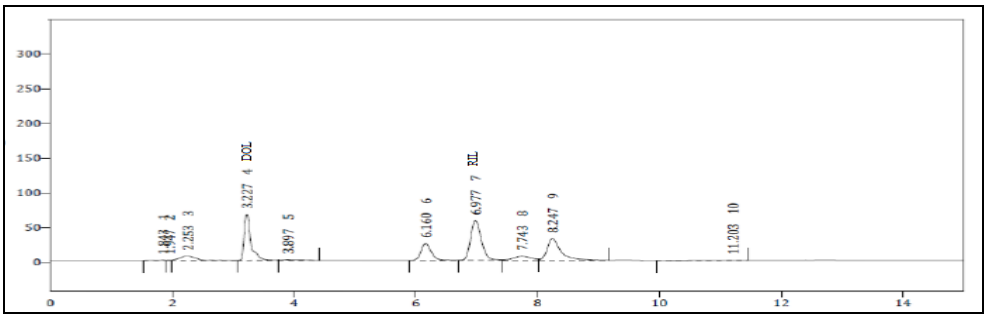

Figure: Chromatogram of DOL and RIL test in UV radiation after 72 Hr.

Thermal Stress Study

Table: Thermal Stability study data for Dolutegravir and Rilpivirine by HPLC

Stressed Conditions	Rilpivirine		Dolutegravir	
	Area Found	% Degradation	Area Found	% Degradation
0 Hr	1643.78	0	1164.64	0
2 Hr	1389.39	7.90	1052.37	8.32
6 Hr	361.45	79.54	301.46	78.95

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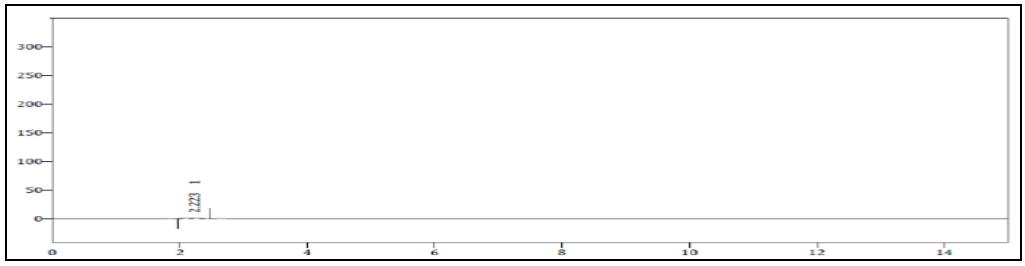

Figure: Chromatogram of sunlight exposure (Thermal stability) Blank

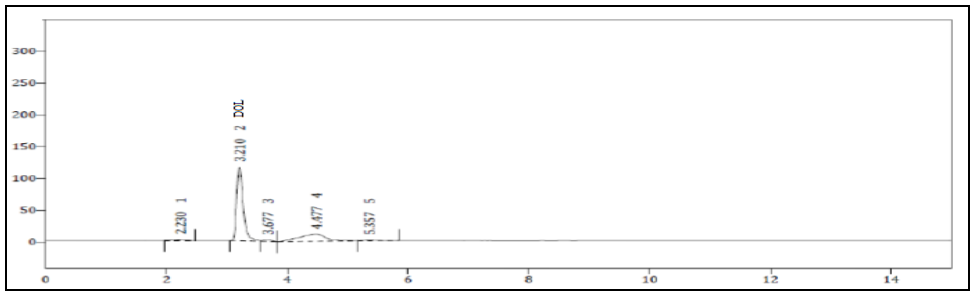

Figure: Chromatogram of DOL in Thermal stress condition

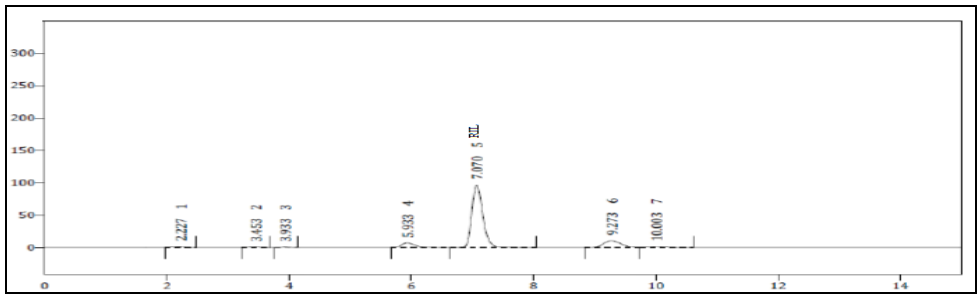

Figure: Chromatogram of RIL in Thermal stress condition

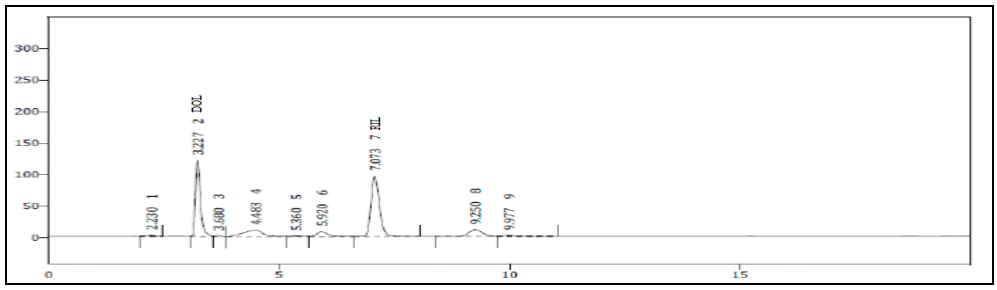

Figure: Chromatogram of DOL and RIL test in Thermal stress condition

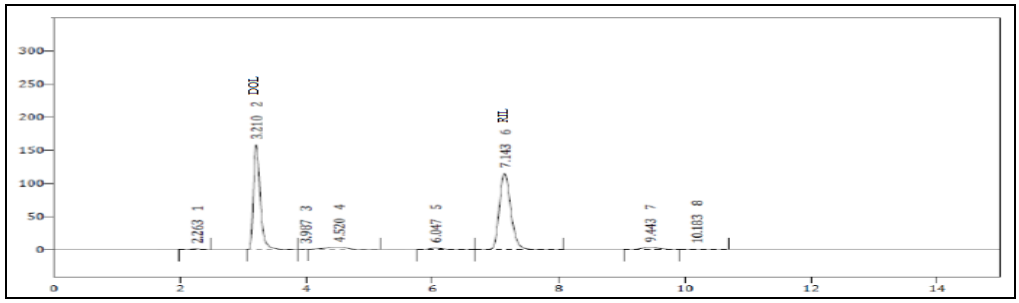

Figure: Chromatogram of DOL and RIL test in Thermal stress condition after 2 Hr.

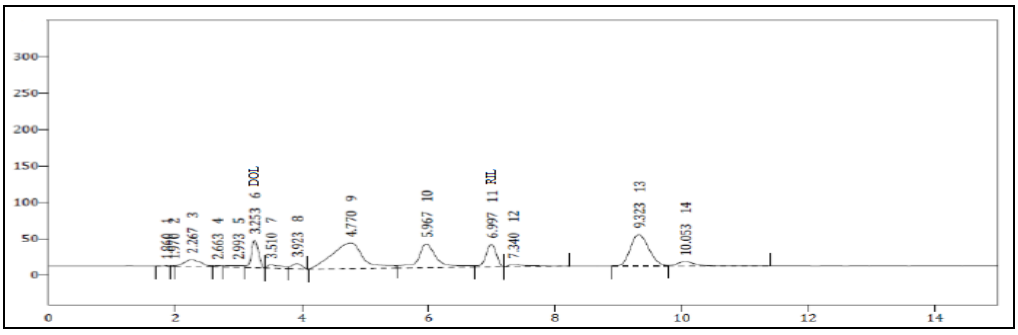

Figure: Chromatogram of DOL and RIL test in Thermal stress condition after 6 Hr.

Table: Stability study data for Rilpivirine standard by HPLC

Stressed Conditions	Standard		Sample	
	Area Found	% Degradation	Area Found	% Degradation
Acid	1015.14	31.9614	1064.24	31.4951
Base	1454.73	13.7164	1254.12	15.3795
Thermal	1121.34	22.6487	1127.26	24.1072
Oxidation	1361.01	22.3794	1261.72	22.6138
Photo	1140.61	28.3415	1294.84	25.6134

Upon study of Rilpivirine in different stress condition, it was found that Rilpivirine having highest stability in basic condition for standard as well as in sample while least stable in acid and on exposure of UV light.

Table: Stability study data for Dolutegravir by HPLC

Stressed Conditions	Standard		Sample	
	Area Found	% Degradation	Area Found	% Degradation
Acid	1161.54	19.46	1161.48	20.31
Base	1094.38	20.68	1142.91	18.46
Thermal	901.01	30.73	918.46	25.43
Oxidation	905.24	26.81	972.61	34.63
Photo	901.46	29.51	910.75	30.46

Upon study of Dolutegravir in different stress condition, it was found that Dolutegravir having highest stability in basic and acid condition for standard as well as in sample while least stable in thermal exposure and on exposure of UV light i.e., photo stability.

Conclusion

For the simultaneous determination of Rilpivirine and Dolutegravir in tablets, the established stability indicating RP-HPLC method offers a straightforward, accurate, affordable, precise, quick, and reproducible quantitative analysis. During the process of developing the approach, a new mobile phase was discovered. The technique was approved in accordance with ICH principles and is suitable for routine analysis.

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