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Original Article

Method development and validation for simultaneous estimation of rilpivirine and doultegravir by using RP – HPLC method in bulk dosage form

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Dolutegravir and Rilpivirine in Tablet dosage form. Chromatogram was run through Agilent C18 (4.6 x 150mm, 5µm) Mobile phase containing Buffer 50% OPA: 50% Acetonitrile was pumped through column at a flow rate of 1 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 257 nm. Retention time of Dolutegravir and Rilpivirine were found to be 2.399 min and 2.853 min. %RSD of the Dolutegravir and Rilpivirine were and found to be 0.8 and 1.6 respectively. %Recovery was obtained as 99.06% and 100.16% for Dolutegravir and Rilpivirine respectively. LOD, LOQ values obtained from regression equations of Dolutegravir and Rilpivirine were 1.08, 3.26 and 0.56, 1.69 respectively. Regression equation of Dolutegravir is y = 11916x + 4431., and y =208758x + 2773. of Rilpivirine. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: Rilpivirine, HPLC, ICH Guidelines

INTRODUCTION

The advancement in therapy for human immune virus (HIV) led the patients to survive longer periods and offering progressively gainful lives. HIV is a ribonucleic acid virus spread through certain body fluids that attacks the body's immune system, specifically destroys a type of defense cells in the body called CD4 helper lymphocytes (T cells), which help the immune system fight off infections[6,7] The use of multiple drug therapy, i.e., at least three or more drugs alone or in combination daily is in practice to treat the HIV effectively. However, extensive research on multiple drug therapy revealed that a two-drug regimen consisting of Lamivudine and Dolutegravir controls the HIV disease effectively[(4,5).] Rilpivirine is non-nucleoside reverse transcriptase inhibitor (NNRTI)

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which is used for the treatment of HIV-1 infections in treatment-naive patients. It is a diarylpyrimidine, a class of molecules that resemble pyrimidine nucleotides found in DNA and Dolutegravir chemically, (3S, 7R)-N-[(2,4-Difluorophenyl) methyl]- 11-hydroxy- 7-methyl-9,1 2-dioxo-4-oxa-1, 8-diazatricyclo]tetradeca-10, 13-diene- 13-carboxamide, is a novel integrase inhibitor used in the treatment of HIV and was approved by FDA. It works by blocking integrase and prevents HIV from Replicating and lowers the amount of HIV in the blood[8,9,10]. Literature survey revealed that there were few analytical methods reported for Dolutegravir and Rilpivirine such as UV methods and RP-HPLC methods[13-20]. An extensive literature search revealed the retention times are long for Dolutegravir and Rilpivirine in API and Pharmaceutical dosage form. Therefore an attempt has been made to develop and validate simple, precise, accurate economical RP-HPLC method as per ICH guidelines for the simultaneous estimation of Dolutegravir and Rilpivirine in API and Pharmaceutical dosage form.

MATERIALS AND METHODS

Chemicals and Reagents: Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem, india. All active pharmaceutical ingredients (APIs) of Dolutegravir and Rilipirine reference standards were procured from Spectrum Pharma labs, Hyderabad, India.

Instruments and Chromatographic Conditions: Electronics Balance-Denver, P^H meter -BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC Acuitysystem equipped with quaternary pumps, UV detector and Auto sampler integrated with Empower 2 Software was used for LC peak integration and Data processing. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV-win 6 Software was used for measuring absorbance of Dolutegravir and Rilipivirine solution. The mobile phase used was 0.1%OPA: Acetonitrile (60:40A) at a flow rate of 1.0ml/min, samples were analyzed at 257 nm detector wavelength and at an injection volume of 10 µL using Agilent C_{18}150 x 4.6 mm, 5µ with run time of 5 min.

Method

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50.

Buffer: 0.1%OPA Buffer (1ml of Ortho phosphoric acid was diluted to1000ml with HPLC grade water.)

Standard Stock Preparation: Accurately weighed 25mg of Dolutegravir, 12.25mg of Rilpivirine and transferred to 50ml flasks and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (500µg/ml of Dolutegravir and 250µg/ml Rilpivirine).

Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (50µg/ml of Dolutegravir and 25µg/ml of Rilpivirine)

Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100ml volumetric flask, 5 ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (500µg/ml of Dolutegravir and 250µg/ml of Rilpivirine)

Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (50µg/ml of Dolutegravir and 25µg/ml of Rilpivirine)

Method Validation

As per ICH guidelines the method was validated and the parameters like Linearity, Specificity, Accuracy, Precision, Limit of Detection (LOD) and Limit of Quantitation (LOQ) were assessed.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity: Stock solutions is taken into 6 different volumetric flasks and diluted to 10ml with diluents to get For dolutegravir- 12.5ppm, 25ppm, 37.5ppm, 50ppm, 62.5ppm, 75ppm, and for Rilpivirine-6.25ppm, 12.5ppm, 18.75ppm, 25ppm, 31.25ppm, 37.5ppm. Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5ml from the from standard Stock solution.

Accuracy: Accurately weighed 25mg of Dolutegravir, 12.25mg of Rilpivirine and transferred to 50ml flasks and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and

labeled as Standard stock solution. (500 µg/ml of Dolutegravir and 250 µg/ml Rilpivirine).

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Dolutegravir, Rilpivirine, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Dolutegravir, Rilpivirine, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Dolutegravir (50ppm) and Rilpivirine (25ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should be not more than 2%.

Assay: Rhodes pharmaceuticals, bearing the label claim Dolutegravir 50mg, Rilpivirine 25mg. Assay was performed with the above formulation. Average % Assay for Dolutegravir and Rilpivirine obtained was 99.06% and 100.16% respectively

RESULTS AND DISCUSSIONS

Optimization of Chromatographic Conditions: To develop and establish a suitable RP-HPLC method for Simultaneous estimation of Dolutegravir and Rilpivirine in bulk and tablet dosage forms, different preliminary tests were performed and different chromatographic conditions were tested and optimized chromatographic conditions were developed which were given in Table-1.The final analysis was performed by using 50% Ortho phosphoric acid:50% Acetonitrile at a flow rate of 1ml/min, samples were analyzed at 257 nm detector wave length and at an injection volume of 10µL using AgilentC18 4.6 x 150mm, 5µm with run time of 5min. The proposed method was optimized to give sharp peak with good resolution and minimum tailing effect for Dolutegravir and Rilpivirine, the optimized chromatogram was obtained as shown in (Figure-3).

Validation: Linearity was established (Dolutegravir-12.5-75µg/ml & Rilpivirine-6.25-37.5µg/ml) at six different concentrations each were injected in a duplicates and average areas were determined and linearity equations were obtained as for Dolutegravir is y = 11916x + 4431., and y = 208758x + 2773 of Rilpivirine. correlation coefficient (R^2) was determined as 0.999. The Linearity calibration curves were plotted as shown in (Figure-4&5). Retention time of Dolutegravir and Rilpivirine was 2.396 and 2.859 minutes. where no interfering peaks in blank and placebo were found in this method. So this method holds its specificity. Three levels of Accuracy samples 50%, 100%, 150% were prepared and triplicates of injections were given for each level of accuracy and mean% Recovery was obtained as For dolutegravir 99.46% and for Rilpivirine 99.34% was shown in (Table-2 & 3). % RSD was calculated from the corresponding peaks obtained by injecting six times a known concentrations of Dolutegravir and Rilpivirine were and found to be 0.8 and 1.6 respectively and the % RSD for Repeatability was obtained as for Dolutegravir is 0.4% and 0.6% for Rilpivirine , Low % RSD values indicates that the method developed was precise as shown in (Table-4). The LOD and LOQ values were evaluated based on Relative standard deviation of response and slope of the calibration

curve Dolutegravir and Rilpivirine. The detection limit value was obtained as 1.08, 0.56 and Quantitation limit was found to be 3.26, 1.69 as given in (Table-5).Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (55:45), mobile phase plus (45:55), temperature minus (25°C) and temperature plus (35°C) were maintained and samples were injected in duplicate manner(Table -6). System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit (Table -7). Dolutegravir and Rilpivirine pure drug (API) was obtained from Spectrum Pharma research solutions, (JULUCA) bearing the label claim 250mg. Assay was performed with the above formulation. Average % Assay obtained was 99.06% For dolutegravir & 100.16% for Rilpivirine the result was shown in (Table-8) and the chromatogram of standard drugs and pharmaceutical dosage forms were shown in (Figure-5, 6) respectively. Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation (Table 9).

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Dolutegravir and Rilpivirine in Tablet dosage form. Retention time of Dolutegravir and Rilpivirine were found to be 2.399 min and 2.853 min. %RSD of the Dolutegravir and Rilpivirine were and found to be 0.8 and 1.6 respectively. %Recovery was obtained as 99.06% and 100.16% for Dolutegravir and Rilpivirine respectively. LOD, LOQ values obtained from regression equations of Dolutegravir and Rilpivirine were 1.08, 3.26 and 0.56, 1.69 respectively. Regression equation of Dolutegravir is y = 11916x + 4431., and y = 208758x + 2773. of Rilpivirine. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

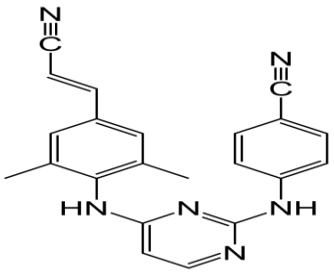

Figure-1: Chemical Structure of Rilpivirine

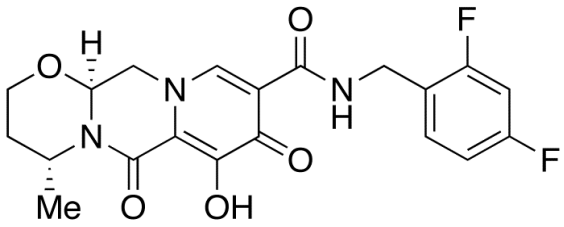

Figure-2: Chemical Structure of Dolutegravir

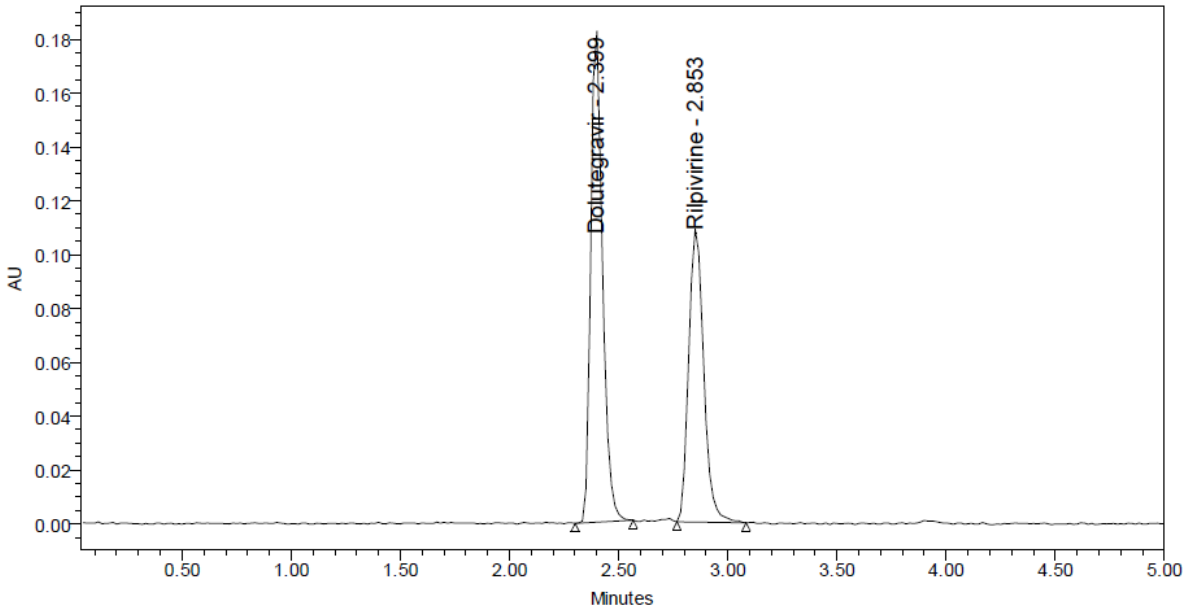

Figure-3: Optimized Chromatogram of Dolutegravir and Rilpivirine

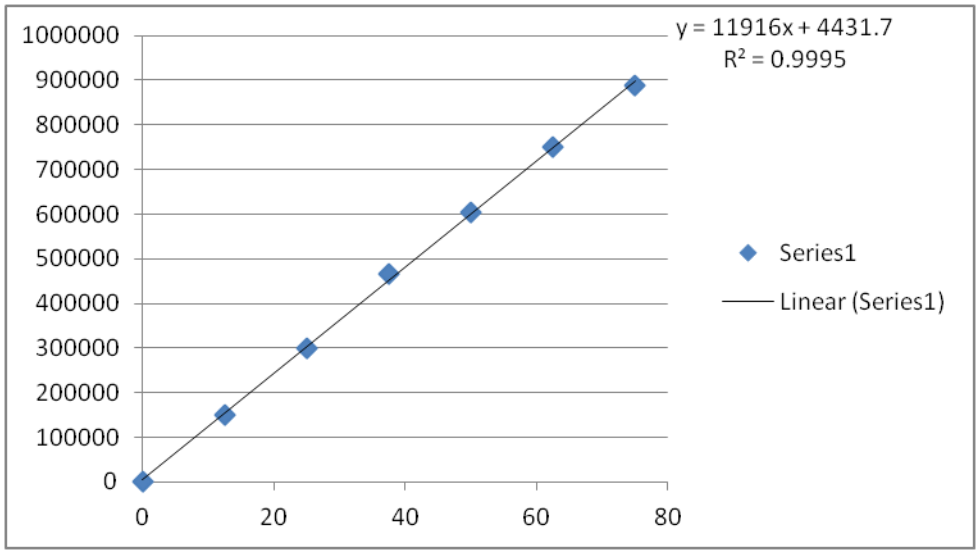

Figure-4: Linearity Curve of Dolutegravir

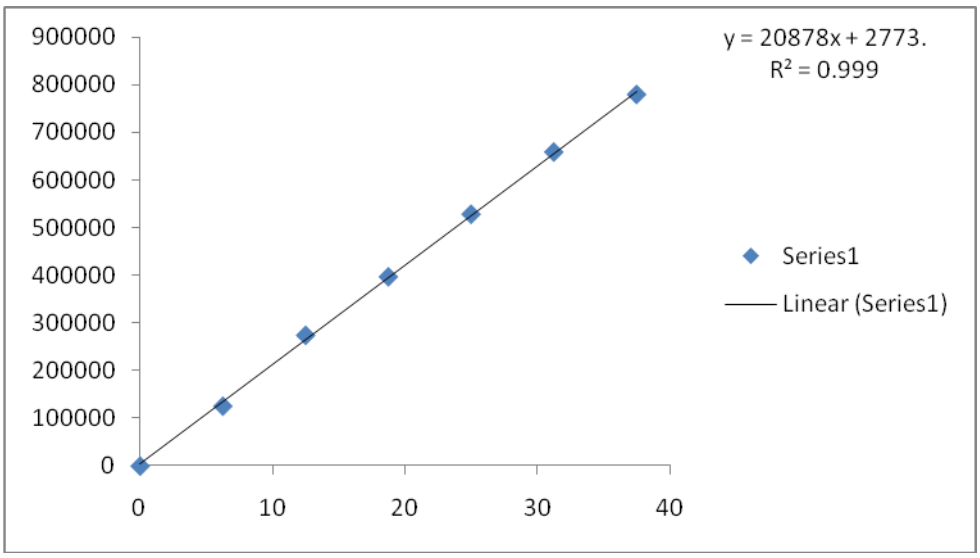

Figure-5: Linearity Curve of Rilpivirine

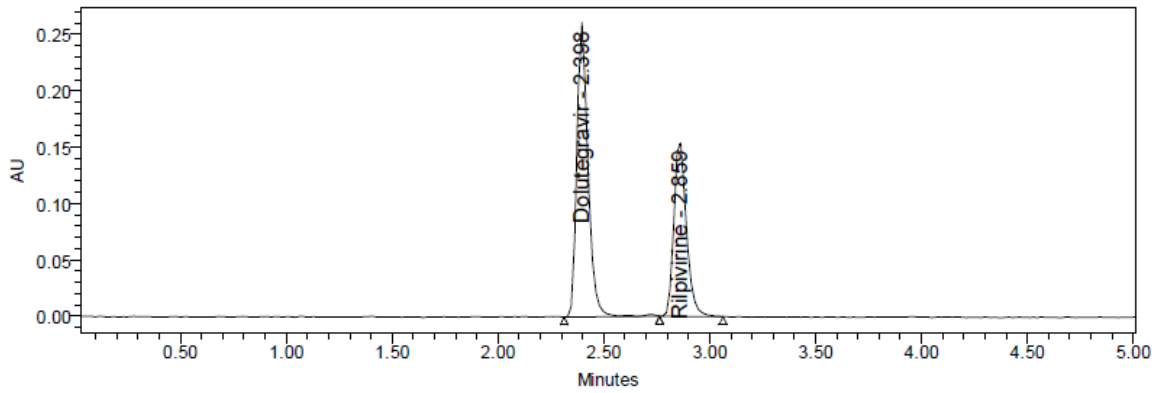

Figure-6: Standard Chromatogram of Dolutegravir and Rilpivirine

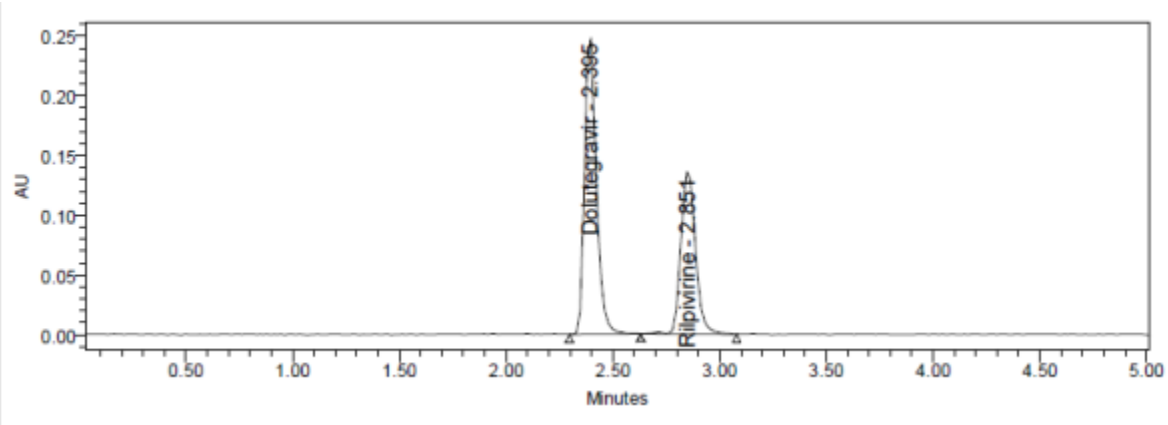

Figure-7: A Sample Chromatogram of Dolutegravir and Rilpivirine

Table-1: Optimized Chromatographic Conditions

Parameter	Condition
RP-HPLC	WATERS HPLC SYSTEM equipped with quaternary pumps with PDA detector
Mobile phase	50% OPA: 50% Acetonitrile
Flow rate	1ml/min
Column	Agilent C18 (4.6 x 150mm, 5µm)
Detector wave length	257nm
Column temperature	30°C
Injection volume	10µL
Run time	5 min
Diluent	Water and Acetonitrile in the ratio 50:50
Retention Time	Dolutegravir-2.396min, Rilpivirine- 2.859
Theoretical Plates	Dolutegravir -9504, Rilpivirine- 11065

Table-2: Accuracy results of Dolutegravir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	25	24.9	99.7	99.46%
	25	24.9	99.8	
	25	25.0	99.8	
100%	50	49.6	99.2	
	50	49.7	99.4	
	50	49.5	99.1	
150%	75	74.6	99.5	
	75	74.6	99.5	
	75	74.2	99.0	

Table-3: Accuracy results of Rilpivirine

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	12.5	12.36	98.88	99.34%
	12.5	12.40	99.18	
	12.5	12.41	99.32	
100%	25	24.75	99.01	
	25	24.80	99.22	
	25	24.75	98.98	
150%	37.5	37.50	99.99	
	37.5	37.40	99.74	
	37.5	37.40	99.74	

Table-4: Precision Result of Dolutegravir and Rilpivirine

	Precision		Repeatability precision	
S.no	Dolutegravir	Rilpivririne	Dolutegravir	Rilpivririne
1.	828954	839954	828545	828545
2.	835428	815428	825372	835372
3.	842353	832353	830782	830782
4.	833958	853958	834181	834181
5.	847017	827017	832622	839622
6.	836784	826784	830976	839976
Mean	837416	832582	830413	834746
S.D	6394.8	13192.3	3112.3	4604.5
%RSD	0.8	1.6	0.4	0.6

Table-5: LOD and LOQ values of Dolutegravir and Rilpivirine

Molecule	LOD	LOQ
Dolutegravir	1.08	3.26
Rilpivirine	0.56	1.69

Table-6: Robustness Data of Dolutegravir and Rilpivirine

S.no	Condition	%RSD of Dolutegravir	%RSD of Rilpivirine
1	Flow rate (-) 0.9ml/min	0.7	0.7
2	Flow rate (+) 1.1ml/min	0.4	0.8
3	Mobile phase (-) 70B:30A	0.7	0.7
4	Mobile phase (+) 60B:40A	0.5	0.6
5	Temperature (-) 25°C	0.6	0.9
6	Temperature (+) 35°C	0.6	0.7

Table-7: System Suitability Parameters Result of Dolutegravir and Rilpivirine

S.no	Dolutegravir			Rilpivirine			
Inj	RT(min)	USPPlate Count	Tailing	RT(min)	USPPlate Count	Tailing	Resoluton
1	2.389	8436	1.26	2.852	11137	1.25	4.2
2	2.397	9798	1.26	2.859	10488	1.28	4.4
3	2.397	9542	1.27	2.859	11205	1.20	4.5
4	2.398	10001	1.26	2.860	10658	1.21	4.5
5	2.398	9787	1.26	2.862	11188	1.19	4.5
6	2.399	9465	1.22	2.862	11718	1.20	4.5

Table -8: Assay Results of Dolutegravir and Rilpivirine

	Dolutegravir	Rilpivirine
S.no	**% Assay**	**% Assay**
1	98.84	99.42
2	98.46	100.23
3	99.11	99.68
4	99.51	100.09
5	99.33	100.74
6	99.13	100.79
Avg	99.06	100.16
Stdev	0.37	0.55
%RSD	0.4	0.55

Table - 9. Degradation Data of Dolutegravir and rilpivirine

S.NO	Degradation Condition	%Drug Degraded	
		Dolutegravir	**Rilpivirine**
1	Acid	2.93	2.60
2	Alkali	2.85	5.67
3	Oxidation	3.29	2.66
4	Thermal	3.10	5.41
5	UV	2.85	5.17
6	Water	2.65	5.17

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