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METHOD DEVELOPMENT AND VALIDATION OF RILPIVIRINE IN PHARMACEUTICAL FORMULATION BY RP-HPLC ANALYSIS

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ABSTRACT

This study aimed to develop and validate a stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method for the quantification of Rilpivirine in its formulations. The analysis was performed using an HPLC system (Waters - Alliance 510) equipped with UV-484 detector and controlled by Data Ace software (Instrument I.D: AL-011). A hypersil BDS C18 analytical column (250 X 4.6 mm X 5) was employed. A novel mobile phase composed of buffer and acetonitrile in a 20:80 (V/V) ratio was utilized at a flow rate of 1.2 ml/min. Detection was conducted at 300 nm. The stability-indicating nature of the method was evaluated through stress testing, including hydrolytic degradation under acidic, basic, and neutral conditions, UV degradation, and thermal degradation. The method exhibited a linear relationship over the concentration range of 12.50-37.50 ppm, described by the regression equation y = 21.58x + 135.1 (r^2 = 0.999). The limits of detection (LOD) and quantification (LOQ) were found to be 0.2 and 0.6 g/ml, respectively. Rilpivirine demonstrated exceptional stability under conditions of heat, oxidative stress, acidity, basicity and neutrality. The developed method was validated for robustness, linearity, specificity, accuracy, and precision. It displayed excellent specificity, accuracy, speed, precision, reliability, and reproducibility, making it suitable for the analysis of commercial dosage forms as recommended by ICH guidelines.

Keywords: Forced Degradation Studies, ICH Guidelines, Rilpivirine, RP-HPLC, Stability Studies

INTRODUCTION:

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are crucial in both treating and preventing HIV infections. These inhibitors function by binding to and inhibiting HIV reverse transcriptase, an enzyme vital for the virus's replication process. HIV employs reverse transcriptase to convert its RNA into DNA through a process known as reverse transcription. By blocking this enzyme and the reverse transcription process, NNRTIs like rilpivirine effectively hinder HIV replication **[1, 2]**. Rilpivirine, a second-generation NNRTI, has been approved for the treatment of HIV-1 in adult patients who have not received previous antiretroviral therapy. It is administered in combination with other antiretroviral agents **(Figure 1)**. The chemical name for rilpivirine hydrochloride is 4-{[4-({4-[(E)-2-cyano ethenyl]-2,6-di methyl phenyl}amino)-pyrimidinyl] amino}benzo nitrile mono hydro chloride **[3, 4]**.

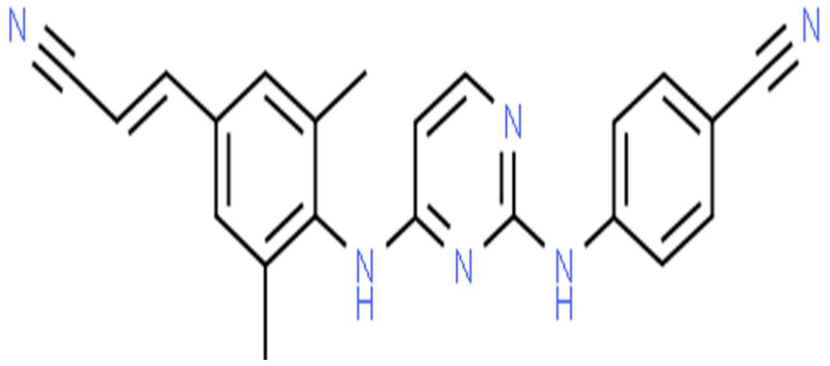


Figure 1: Chemical Structure of Rilpivirine

The literature review on Rilpivirine analytical methods revealed various approaches, including Spectrophotometry **[5]**, HPLC **[6]**, and HPTLC **[7]** techniques, individually. Some studies focused on simultaneous determination of Dolutegravir and Rilpivirine using RP-HPLC in single and combined dosage forms, employing methods such as UV Spectrophotometry **[8-15]**, HPTLC **[16]**, UPLC and LC-MS **[17-18]** and UPLC **[19-20]**. Additionally, there were reports of Rilpivirine analysis in bulk and pharmaceutical forms using HPLC **[21]** and UV Spectrophotometry for pure bulk Dolutegravir and Rilpivirine **[22]**. RP-HPLC methods were employed for simultaneous quantification of Dolutegravir and Rilpivirine, extending their application to quality control and plasma analysis **[23-25]**. RP-HPLC techniques were also utilized for the simultaneous determination of Rilpivirine with Lopinavir **[26]** and Cabotegravir **[27]**, as well as quantitative analysis of Dolutegravir and Rilpivirine in pharmaceutical formulations **[28-29]**. However, these methods posed limitations, such as high costs, column blockage due to strong buffers, occasional solvent requirements, and less precise yield findings due to high flow rates **[30]**.

In the present study, the application of Quality by Design in analytical method development and validation offered significant advantages, including time savings, result accuracy, and cost-effectiveness. Previous research using Quality by Design emphasized qualitative and quantitative analysis **[31]**. Despite the available literature, limited information was found on RP-HPLC methods for determining Rilpivirine in pharmaceutical formulations. Therefore, this study aimed to develop a stability-indicating RP-HPLC method for Rilpivirine determination in pharmaceutical formulations, focusing on speed, precision, time liness, and cost-efficiency. The methodology employed a short retention time with excellent resolution and separation on the column's stationary phase. The developed RP-HPLC method was thoroughly validated according to ICH guidelines **[32]**. This approach, characterized by its novelty, simplicity, precision, and conciseness, resulted in reduced retention times for both drugs, making it an economical and efficient choice for routine analysis.

Instruments and Equipment used:

The analysis was performed using an HPLC system, specifically the Waters Alliance 510, with UV-484 Data Ace software (Instrument I.D: AL-011). An HPLC

Analytical column of Hypersil BDS C18 (250 X 4.6mm X 5µ) was employed. Analytical weighing was conducted using a Mettler Toledo B204S balance. Millipore membrane with a pore size of 0.45µm was utilized, along with various laboratory accessories.

Chemicals utilized:

The analysis employed Rilpivirine HCl working standard with a purity of 99.9% on a dry basis, Rilpivirine HCl Tablets, Ammonium Acetate (AR grade), Tri ethylamine (AR grade), HPLC-grade Water, Acetic Acid (AR grade), and Acetonitrile (AR grade).

Analytical Method:

The quantitative analysis was carried out utilizing an HPLC system equipped with a UV/VIS detector.

Chromatographic conditions:		
Column	:	Hypersil BDS C18, 250 X 4.6 mm, 5µ.
Buffer	:	Weigh accurately and transfer 5.0 gm of ammonium acetate to a 1000 ml volumetric flask. Add about 980 ml of water, dissolve and dilute to volume with water. Adjust the pH to 6.0 ± 0.05 with diluted tri ethylamine (or) acetic acid. Filter the solution through 0.45µm porosity membrane filter.
Mobile Phase	:	For isocratic system, prepare a mixture of buffer and acetonitrile in the proportion 55:45 respectively. Filter through 0.5 µ Nylon membrane filter paper and degas prior to use.
Wavelength	:	300 nm.
Flow Rate	:	1.2 ml / minute.
Run time	:	25 minutes.
Column oven Temp	:	30^0C.
Sample cooler temp	:	5^0C.
Blank solution	:	Use diluent as blank.
Diluent	:	Prepare a mixture of Buffer and acetonitrile in the proportion 20:80 respectively. Filter through 0.5 µ Nylon membrane filter paper and degas prior to use.

Preparation of Rilpivirine HCl Standard solution:

Accurately weigh 50 mg of Rilpivirine HCl working standard and transfer it to a 100 ml volumetric flask. Add 70 ml of diluent and sonicate to dissolve. Make up the volume with diluent and mix thoroughly. Take 5.0 ml of this solution and transfer it to another 100 ml volumetric flask. Dilute to volume with diluent and mix again. Filter the solution through a 0.45 µm nylon filter.

Preparation of Test solution:

Determine the average weight of 10 tablets, powder them, and accurately weigh 260 mg of Rilpivirine HCl sample powder (equivalent to 50 mg of Rilpivirine HCl). Transfer it to a 100 ml volumetric flask, add 70 ml of diluent, and sonicate to dissolve. Dilute to volume with diluent and mix thoroughly. Take 5.0 ml of this solution and transfer it to another 100 ml volumetric flask. Dilute to volume with diluent and mix again. Filter the solution through a 0.45 µm nylon filter.

System Suitability solution: Utilize the Rilpivirine HCl standard working solution as the system suitability solution.

Procedure:

Inject equal volumes of blank, six replicate injections of the system suitability solution (Rilpivirine HCl standard working solution). Then inject two test solution injections and record the chromatograms. Disregard any peaks due to the blank in the test solution. Calculate the % Relative standard deviation (% RSD) of the six replicate injections of the system suitability solution (Rilpivirine HCl standard working solution). Verify that the tailing factor is less than 2.0 and the theoretical plates are not less than 3000 for the peak in the chromatogram obtained with the 5th injection of the system suitability solution (Rilpivirine HCl standard working solution).

VALIDATION PARAMETERS AND RESULTS:

The validation of the HPLC method was conducted in accordance with the ICH guidelines Q2A and Q2B of CPMP/ICH/281/95, with a focus on various validation parameters. The evaluation included monitoring system suitability parameters throughout the study, and the findings have been documented in the validation report. The summary of validation results is provided below: The system suitability parameters were monitored throughout the validation study and are recorded in the validation report.

Specificity/Selectivity:

To assess selectivity, injections of diluent blank solution, excipient blend, system suitability solution, and test solution were made. The acceptance criteria required the Rilpivirine HCl peak to be well resolved from any other peak and from each other. Additionally, the diluent blank solution and excipient blend solution were not supposed to exhibit any peak at the retention time of Rilpivirine HCl. The system suitability criteria were rigorously evaluated and were found to meet the pre-established acceptance criteria as outlined in the analytical method. Detailed results are tabulated in **Table 1** of the validation report.

Table 1: System suitability - Selectivity

S. No.	Area of Rilpivirine HCl
1	2143.80
2	2162.76
3	2174.21
4	2170.70
5	2164.25
Mean	2163.14
Standard Deviation (±)	11.78
(%) Relative Standard Deviation	0.54

The injections were conducted using the specified wavelength as outlined in the method. No interference was detected from the diluent blank solution, nor from the placebo containing Rilpivirine HCl peak.

FORCED DEGRADATION:

Forced degradation studies are conducted to validate the stability indicating nature of the assay method and to identify any degraded compounds. In these studies, Rilpivirine HCl WS and Sample are exposed to various stress conditions, including 5N HCl, 5N NaOH, thermal degradation, and UV degradation, as detailed in **Table 3**. Chromatographic analysis is performed on all the solutions, and the resulting chromatograms for Rilpivirine forced degradation are presented in **Figures 2-5**, depicting the relationship between time in minutes and area percentage. The obtained data is summarized in **Tables 2** and **4**, providing a comprehensive overview of the degradation outcomes under the specified stress conditions.

Table 2: System suitability – Forced degradation

S. No.	Area of Rilpivirine HCl
1	2191.37
2	2176.57
3	2171.20
4	2161.23
5	2166.80
Mean	2173.43
Standard Deviation (±)	11.51
(%) Relative Standard Deviation	0.53

Table 3: Conditions – Forced degradation

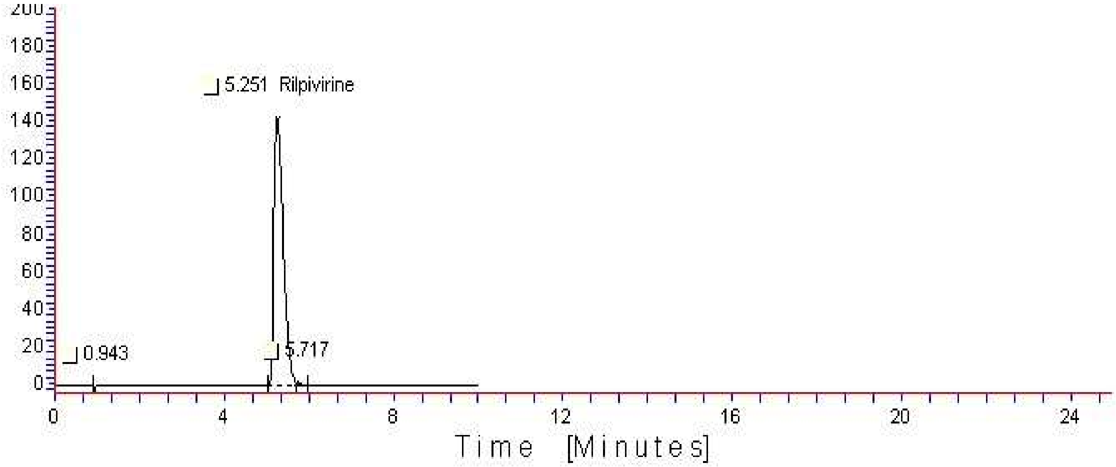
Sample stress condition	Description of stress condition
Acid degradation	5N HCl heated at about 60^0C for 10 min on a water bath
Alkali degradation	5N NaOH heated at about 60^0C for 10 min on a water bath
Thermal degradation	105^0C for 12 hours
UV degradation	expose to UV-radiation for 7 days

Observations:

- In the acid degradation of the standard preparation, two distinct degradation peaks were observed. This pattern was also mirrored in the acid degradation of the sample preparation, as illustrated in **Figure 2**.
- Similarly, during alkali degradation, two degradation peaks were evident in the standard preparation. In contrast, the sample preparation displayed only one degradation peak, as depicted in **Figure 3**.
- Upon subjecting the samples to thermal degradation at 105^0C for 12 hours, both the standard and sample preparations exhibited two distinct degradation peaks, as shown in **Figure 4**.
- In the case of UV degradation, the standard preparation displayed a single degradation peak after exposure to UV radiation for 7 days. However, the sample preparation exhibited two degradation peaks under the same conditions, as given in **Figure 5**.

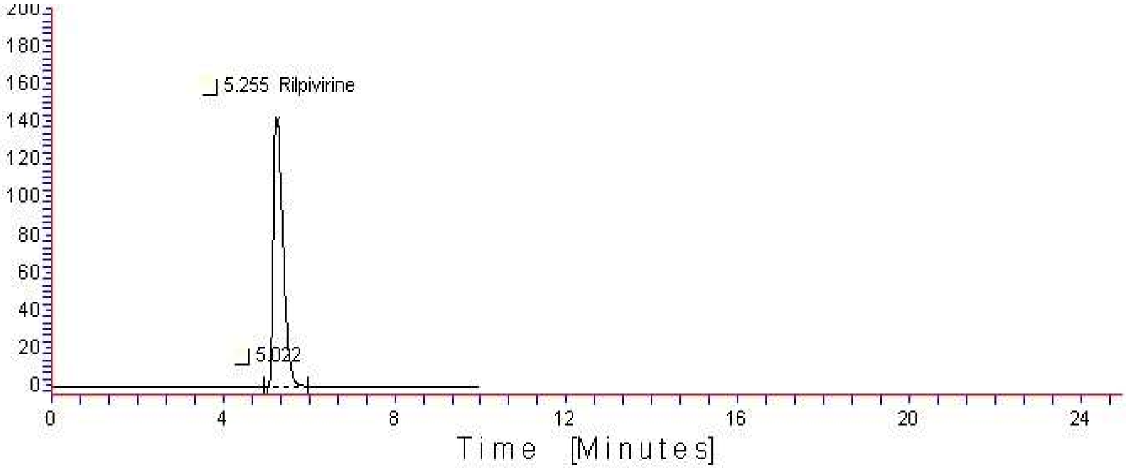
Table 4: % of Degradation by applying different conditions

Acid Stress	% Degradation
Standard	0.116
Sample	0.382
Alkali Stress	% Degradation
Standard	0.101
Sample	0.012
Thermal Stress	% Degradation
Standard	0.039
Sample	0.405
UV Stress	% Degradation
Standard	0.365
Sample	0.034



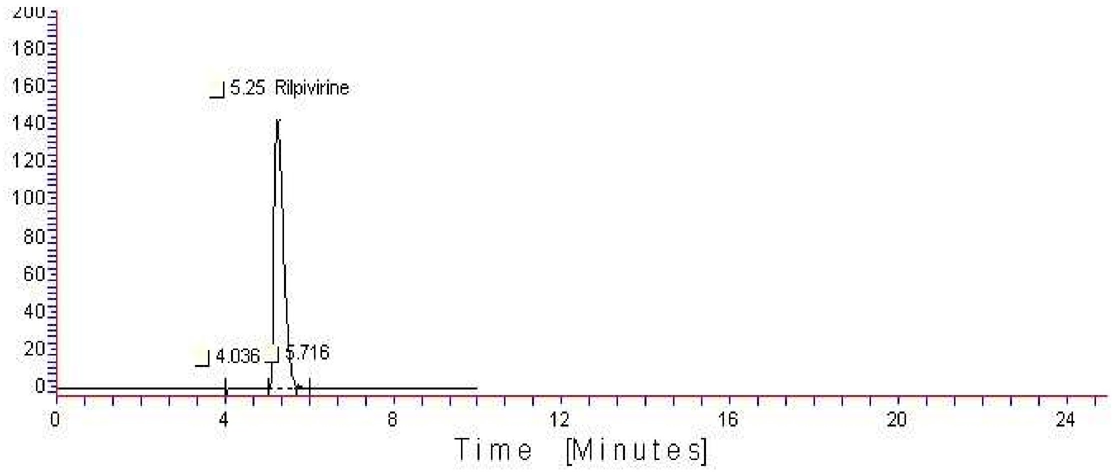
Peak No	Retn. Time	Area	Height	Area %	Height %	Width@50%	
1	0.943	0.078	0.052	0.003	0.035	0.033	
2	5.251	2246.703	143.792	99.618	98.792	0.266	
3	5.717	8.532	1.706	0.378	1.172	0.083	
Total		2255.313	145.55	100	100		

Figure 2: Chromatogram of Rilpivirine HCl sample in Acid degradation



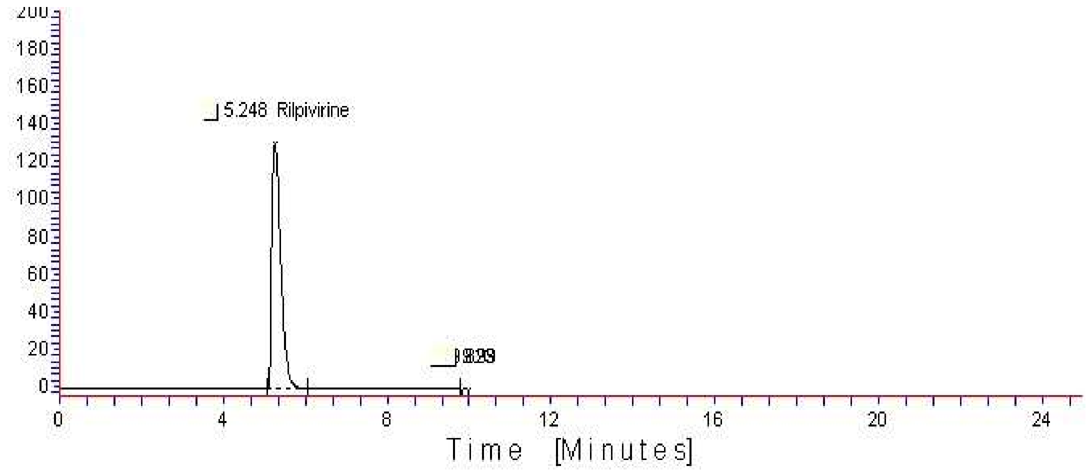
Peak No	Retn. Time	Area	Height	Area %	Height %	Width@50%
1	5.022	0.272	0.06	0.012	0.042	0.083
2	5.255	2181.555	143.374	99.988	99.958	0.25
Total		2181.827	143.434	100	100	

Figure 3: Chromatogram of Rilpivirine HCl sample in Alkali degradation



Peak No	Retn. Time	Area	Height	Area %	Height %	Width@50%	
1	4.036	0	0	0	0	0.033	
2	5.251	2173.035	143.627	99.595	98.841	0.25	
3	5.716	8.839	1.684	0.405	1.159	0.083	
Total		2181.874	145.311	100	100		

Figure 4: Chromatogram of Rilpivirine HCl sample in Thermal degradation



Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	5.249	2001.491	131.645	99.966	99.803	0.25
2	9.823	0.481	0.13	0.024	0.099	0.066
3	9.99	0.195	0.13	0.01	0.099	0.033
Total		2002.167	131.905	100	100	

Figure 5: Chromatogram of Rilpivirine HCl sample in UV degradation

Acceptance Criteria: Ensure that the degradation peaks exhibit clear separation from one another. The peak purity of the Rilpivirine HCl peak must meet the specified standards.

Conclusion: The chromatograms of the degradation preparations do not show any interference between the peaks. Under forced degradation conditions, the degradation peaks are distinctly separated from each other. The Rilpivirine HCl peak exhibits satisfactory peak purity. Consequently, the method is highly precise, selective, and specific for quantifying the Assay of Rilpivirine HCl in Rilpivirine HCl Tablets 25 mg using HPLC. Moreover, this method is stability-indicating, as it effectively separates the degraded products from Rilpivirine HCl and all adjacent peaks.

LINEARITY:

Linearity and Range for sample:

In the linearity study, five standard solutions of Rilpivirine HCl were prepared, ranging from 50% to 150% of the theoretical concentration of the assay preparation. These solutions, along with the system suitability solution, were injected following the prescribed protocol. A concentration versus peak response graph was plotted, and the correlation coefficient was calculated to assess linearity. The acceptance criterion for the correlation coefficient was set at ≥ 0.999.

The injection sequence for the linearity study is detailed in **Table 5**. The linearity of Rilpivirine HCl was determined, yielding a correlation coefficient of 0.999. The mean area of Rilpivirine HCl, calculated from **Table 5**, was found to be 2180.43. Additionally, the standard deviation and % RSD of the five test solutions were 0.50, indicating the precision of the measurements. It was observed that the system suitability criteria met the pre-established acceptance criteria outlined in the analytical method (refer to **Table 5** for system suitability results).

Table 5: System suitability - Linearity of sample

S. No.	Area of Rilpivirine HCl
1	2170.02
2	2188.60
3	2182.27
4	2168.43
5	2192.83
Mean	2180.43
Standard Deviation (±)	10.91
(%) Relative Standard Deviation	0.50

The average peak area corresponding to Rilpivirine HCl at various concentration levels was calculated, and a linearity graph was constructed by plotting these values against the sample concentration represented as a percentage. The outcomes of the linearity study are presented in **Table 6**.

Table 6: Results of Linearity of sample

Linearity Level	Sample Concentration (in %)	Sample Concentration (in ppm)	Peak Area	Correlation Coefficient
Level – 1	50	12.50	1219.27	0.999
Level – 2	75	18.75	1755.41	
Level – 3	100	25.00	2276.27	
Level – 4	125	31.25	2843.52	
Level – 5	150	37.50	3373.18	

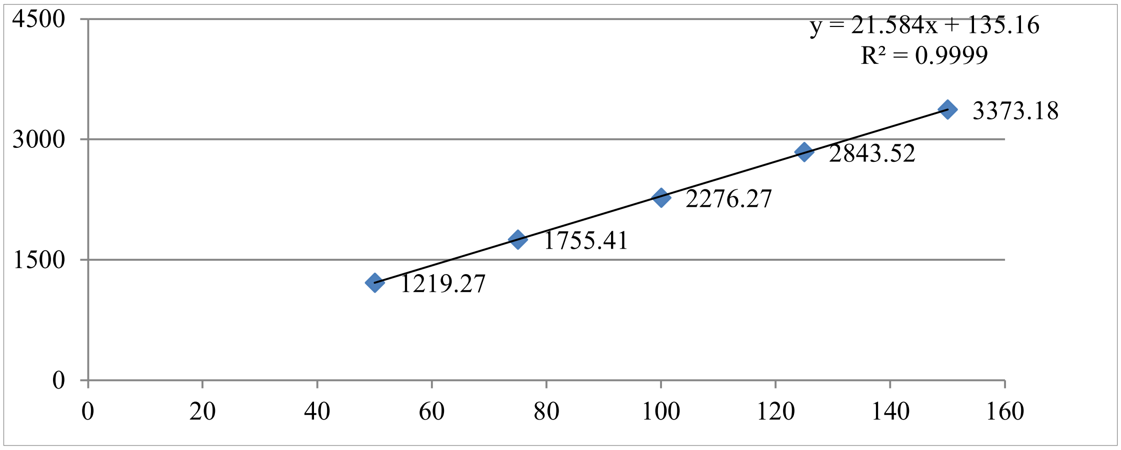
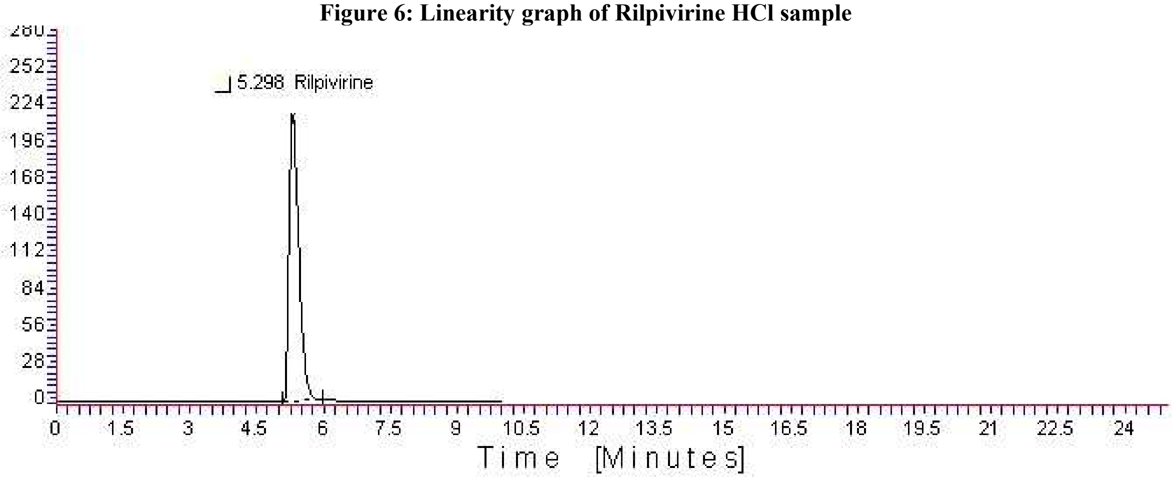


Figure 6: Linearity graph of Rilpivirine HCl sample



Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	5.299	3418.447	218.22	100	100	0.266
Total		3418.447	218.22	100	100	

Figure 7: Chromatogram of Rilpivirine HCl sample

A linearity graph was created by plotting the average area at each concentration level (%) in **Figure 6**. Remarkably, the graph formed a straight line. The regression equation for the calibration curve was Y=21.58x+135.1, with an impressive R^2 value of 0.999. Additionally, the correlation coefficient was found to be 0.999. The results demonstrating the linearity of the Rilpivirine HCl sample are summarized in **Table 6**. The analytical method's effective range spanned from 50 ppm to 150 ppm, confirming its linearity within 50% to 150% of the working concentration. **Figure 7** displays the chromatogram results of the Rilpivirine HCl sample, revealing a clear peak at 5.29 retention time that falls well within acceptable limits.

PRECISION AND METHOD PRECISION:

Procedure:

The analytical procedure involved the preparation of six test solutions of Rilpivirine HCl in 25 mg Rilpivirine HCl Tablets, following the prescribed method. The % RSD (Relative Standard Deviation) of the % assay for these six test solutions was calculated. The acceptance criteria stipulated that the % RSD of the results from these test solutions should not exceed 2.0%. The system suitability criterion was confirmed to meet the predetermined acceptance standards outlined in the analytical method. The assay results obtained from the preparation of six test solutions are detailed in **Table 7**.

Table 7: Results of method precision

Test Solution	% Assay of Rilpivirine HCl
1	99.53
2	99.45
3	100.33
4	100.38
5	98.35
6	99.54
Mean	99.60
Standard Deviation (±)	0.74
(%) Relative Standard Deviation	0.74

Based on the data presented in **Table 7**, the average area is calculated to be 99.60, with a relative standard deviation (RSD) of 0.74 percent. The percentage RSD for the six assay results falls below the 2.0% threshold, aligning with the predetermined acceptance criteria. Therefore, it can be concluded that the method demonstrates precision.

INTERMEDIATE PRECISION:

Procedure:

Six test solutions of Rilpivirine HCl Tablets 25 mg were meticulously prepared following the prescribed analytical method on different days. Different analysts analyzed these test solutions using distinct HPLC columns of the same make, each with a unique serial number, and varied HPLC systems. The % RSD (Relative Standard Deviation) of % assay results from twelve test solutions (comprising six samples from method precision and six from intermediate precision) was calculated. The predetermined acceptance criteria stipulated that the % RSD of these twelve test solutions (combining results from method precision and intermediate precision) should not exceed 2.0%. System suitability criteria were rigorously evaluated and met the predefined acceptance criteria outlined in the analytical method (refer to **Table 9** for system suitability results). The assay results from the six test solutions are detailed in **Table 8**. The % RSD of assay results obtained from both method precision and intermediate precision (totaling 12 results) is summarized in **Table 9**.

Table 8: Results of Intermediate precision

Test Solution	% Assay of Rilpivirine HCl
1	99.43
2	98.84
3	98.77
4	98.83
5	99.75
6	98.66
Mean	99.05
Standard Deviation (±)	0.44
(%) Relative Standard Deviation	0.44

Table 9: Results of twelve test solutions of Rilpivirine HCl in (Six of Method precision & Six of Intermediate precision)

Analysis performed during method precision study By Analyst 1 on system 1 and on column 1 on day 1	
Same column	% Assay of Rilpivirine HCl
1	99.53
2	99.45
3	100.33
4	100.38
5	98.35
6	99.54

Analysis performed during intermediate precision study By Analyst 2 on system 2 and on column 2 on day 2	
Column sr. no.	**022322030142 01**
Test Solution	**% Assay of Rilpivirine HCl**
7	**99.43**
8	**98.84**
9	**98.77**
10	**98.83**
11	**99.75**
12	**98.66**
Mean of twelve samples	**99.32**
Standard Deviation (±)	**0.65**

The analysis was conducted on six test solutions from the same batch of the drug product. Two different analysts used distinct equipment in the same laboratory, employing two different columns of the same brand but with different serial numbers, on separate days. The % RSD of the twelve assay results (six from method precision and six from intermediate precision) was determined to be below 2.0%. Consequently, the method was deemed both rugged and precise.

ACCURACY (% Recovery):

Procedure:

In the accuracy study, Rilpivirine HCl test solutions were analyzed. These solutions were created by mixing Rilpivirine HCl API with an excipient blend. Different concentrations of the test solutions were prepared, ranging from 50% to 150% of the desired concentration by adding varying amounts of Rilpivirine HCl API to the excipient blend. The acceptance criteria for this study were set at a mean recovery between 98.0% and 102.0% at each concentration level. The results of the study indicated that the system suitability criteria met the predetermined acceptance criteria outlined in the analytical method (refer the **Table 10** for system suitability results). The accuracy study results are summarized in **Table 11**.

Table 10: System suitability - Accuracy (% Recovery)

S. No.	Area of Rilpivirine HCl
1	2183.20
2	2184.45
3	2184.45
4	2178.72
5	2190.50
Mean	2184.26
Standard Deviation (±)	4.21
(%) Relative Standard Deviation	0.19

Table 11: Accuracy (% Recovery) – results

Level of addition	Amount of Rilpivirine HCl added in mg	Amount of Rilpivirine HCl found in mg	Recovery (%)
First Level (Rec-50 %)	27.1	26.90	99.26
Second Level (Rec-75 %)	39.6	39.75	100.38
Third Level (Rec-100 %)	50.7	50.79	100.18
Fourth Level (Rec-125 %)	62.6	62.61	100.02
Fifth Level (Rec-150 %)	75.7	75.50	99.74
Mean			99.92
Standard Deviation (±)			0.44
(%) Relative Standard Deviation			0.44

The Rilpivirine HCl recovery levels consistently fall within the range of 98.0% to 102.0%. Additionally, the percentage relative standard deviation (% RSD) for each recovery level remains below 2.0%. Referencing **Table 11**, it is evident that the average % recovery is 99.92%, and the % RSD is just 0.44%. This data demonstrates that the analytical method aligns with the predetermined acceptance criteria for recovery studies as outlined in the protocol. Consequently, we can confidently assert that the method is indeed accurate. The established criteria for acceptance encompass passing the system suitability requirements outlined in the analytical method. Furthermore, when comparing results obtained under altered conditions with the method's average precision results, the % RSD should not exceed 2.0%.

ROBUSTNESS-Experiment:

Prepare two test solutions using the same lot of Rilpivirine HCl found in sections 7.0.a and 7.0.b of the analytical method for Rilpivirine HCl Tablets 200 mg. Inject these solutions, along with a diluent blank solution and a system suitability solution, under various chromatographic conditions outlined below:

Change in Column lot: Utilize different lots of columns for chromatographic analysis.

Change in Flow rate (± 0.2 ml/minute): Vary the flow rate within a range of ± 0.2 ml/minute during the analysis.

Change in Wavelength (± 2 nm): Alter the detection wavelength within a range of ± 2 nm for the analysis.

Change in Mobile phase composition (± 0.2): Modify the composition of the mobile phase within a range of ± 0.2 for the analysis.

Change in Column lot:

The system suitability results for the normal experimental condition (Hypersil BDS C_{18} column, 250 X 4.6mm X 5μ) met the predetermined acceptance criteria outlined in the analytical method. Please refer to **Table 12** for detailed system suitability data.

Table 12: System suitability - Robustness with change in Column

S. No.	Area of Rilpivirine HCl	
	Same column	Different column
1	2188.71	2117.78
2	2190.04	2111.75
Mean	2189.37	2114.77
Standard Deviation (±)	0.94	4.26
(%) Relative Standard Deviation	0.04	0.20

The results of the assay conducted under various flow rate conditions are outlined in **Table 13**.

Table 13: Results for change in column

Flow rate →	Same column	Different column
Sample	% Assay	
Test solution	99.53	98.95
Average assay result from method precision	99.60	99.60
Mean	100.61	99.28
Standard Deviation (±)	0.05	0.46
(%) Relative Standard Deviation	0.05	0.46

Change in Flow rate (± 0.2 mL/minute):

The experimental conditions in the normal mode, with a flow rate of 1.2 mL per minute, were evaluated to ensure they conformed to the predefined acceptance criteria outlined in the analytical method. Please consult **Table 14** for the system suitability results.

Table 14: System suitability - Robustness with change in flow rate

S. No.	Area of Rilpivirine HCl	
	1.0ml/minute	1.4 ml/minute
1	2242.40	2247.90
2	2282.19	2265.27
Mean	2262.29	2256.58
Standard Deviation (±)	28.14	12.28
(%) Relative Standard Deviation	1.24	0.54

The results of the assay conducted under various flow rate conditions are presented in **Table 15**.

Table 15: Results for change in flow rate

Flow rate →	1.0ml/minute	1.4 ml/minute
Sample	% Assay	
Test solution	100.40	98.89
Avg. assay result from method precision	99.60	99.60
Mean	100.00	99.25
Standard Deviation (±)	0.57	0.50
(%) Relative Standard Deviation	0.57	0.51

Change in Wavelength (± 2 nm)

Under the normal experimental condition at 300nm, the system suitability criteria were evaluated and found to align with the predetermined acceptance criteria outlined in the analytical method. Please refer to **Table 16** for the detailed system suitability results.

Table 16: System suitability - Robustness with change in wavelength

S. No.	Area of Rilpivirine HCl	
	298 nm	302 nm
1	2162.00	2185.34
2	2154.32	2174.32
Mean	2158.16	2179.83
Standard Deviation (±)	5.43	7.79
(%) Relative Standard Deviation	0.25	0.36

The assay results, acquired under varying wavelength conditions, are documented in **Table 17**.

Table 17: Results for change in wavelength

Wavelength →	298 nm	302 nm
Sample	% Assay	
Test solution	100.89	99.01
Avg. assay result from method precision	99.60	99.60
Mean	100.25	99.31
Standard Deviation (±)	0.91	0.42
(%) Relative Standard Deviation	0.91	0.42

Change in change in composition of mobile phase:

In the standard experimental setup with a buffer composition of Buffer: Acetonitrile = 55:45, the system suitability parameters were determined to adhere to the predefined acceptance standards outlined in the analytical method (please refer to **Table 18** for specific system suitability results).

Table 18: System suitability - Robustness with change in mobile phase composition

S. No.	Area of Rilpivirine HCl	
	Buf:ANC-53:47	Buf:ANC-57:43
1	2188.71	2246.90
2	2182.29	2257.21
Mean	2185.50	2252.05
Standard Deviation (±)	4.54	7.29
(%) Relative Standard Deviation	0.21	0.32

The assay results, acquired under varying mobile phase composition, are given in **Table 19**.

Table 19: Results for Change in change in composition of mobile phase

Mobile phase composition	Buf:ANC-53:47	Buf:ANC-57:43
Sample	% Assay	
Test solution	99.61	99.31
Avg. assay result from method precision	99.60	99.60
Mean	99.61	99.46
Standard Deviation (±)	0.01	0.21
(%) Relative Standard Deviation	0.01	0.21

The analysis of a consistent batch of Rilpivirine HCl Tablets 25 mg was conducted under various conditions, including different column lots, flow rates, wavelengths, and mobile phase compositions. In all cases, the system suitability tests conformed to the predetermined standards, and the percentage relative standard deviation (% RSD) between results obtained under altered conditions and the method's average precision result did not exceed 2.0%. As per the protocol, the analytical method satisfies the predefined criteria for robustness evaluation. Therefore, it can be concluded that the method demonstrates robustness.

Stability of Analytical solution:

Procedure:

Throughout the experiment, System Suitability Solution and Test Solution for Rilpivirine HCl Tablets were prepared at intervals of 0th, 12th, 24th, 36th, and 48th hours. These solutions were stored at room temperature until the 48th hour, when they were analyzed alongside a freshly prepared test solution. The System Suitability Solution was prepared freshly right before the analysis. The objective was to calculate the assay of Rilpivirine HCl Tablets (25 mg) in the sample. Stability of the analyte was determined by monitoring any significant changes in % assay. The results obtained from the solution stability experiment are summarized in **Table 20**.

Table 20: Results for solution stability

% Assay results calculated against the freshly prepared system suitability standard	
Sample	**% Assay of Rilpivirine HCl**
0th hr	**100.65**
12th hr	**96.94**
24 hr	**97.28**
36 hr	**99.59**
48 hr	**97.18**
Mean	**98.33**
Standard Deviation (±)	**1.68**
(%) Relative Standard Deviation	**1.71**

The mean average area of **Table 20** is 98.33, and its percent Relative Standard Deviation (RSD) stands at 1.71, well within accepted limits. The system's suitability was confirmed against predefined criteria, and there is a minimal RSD difference of less than 2.0% between the assay results from freshly prepared test solutions and those from stored test solutions. Furthermore, the assay level for the test solution has exhibited no significant change over a 48 hour period at room temperature, indicating its stability under these conditions for up to two days.

CONCLUSION:

Following ICH guidelines, we have successfully developed and validated a reversed-phase high-performance liquid chromatography (RP-HPLC) method for the quantitative analysis of Rilpivirine HCl in its various forms. The method's validation studies have demonstrated its exceptional qualities, including speed, simplicity, accuracy, precision, specificity, selectivity, and cost-effectiveness. During force degradation studies, it was observed that no degradation peaks were detected under thermal and UV exposure. However, under acidic and basic conditions, two distinct degradation peaks were identified. This method proves highly efficient in separating the medication from its degradation products, as well as any associated chemicals and excipients found in tablet formulations, making it particularly valuable for analyzing samples obtained during accelerated stability testing.

The limits of detection (LOD) and quantification (LOQ) were determined to be 0.2 and 0.6 g/ml, respectively. The range for the analytical method is 50 ppm to 150 ppm, and the linearity of this method was determined to be linear in the range of 50% to 150% of the working concentration. The method's precision and accuracy were within acceptable bounds. The HPLC approach that has been developed thus far is deemed appropriate. It was discovered that the analytical solution remained stable for 48 hours at room temperature. It was acknowledged that the recovery levels for Rilpivirine HCl are constantly between 98.0% and 102.0 %, with a percentage RSD of less than 1.0% for each recovery level. The mean average % of recovery was 99.92.

The validation results affirm the suitability of this analytical approach for routine analysis and stability research. Its ability to effectively distinguish the medication from its degradation products underscores its utility for quantitative quality control and stability assessment in the pharmaceutical industry. This validated approach stands as a valuable tool for future research endeavors and quality assurance practices within the pharmaceutical industries.

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REFERENCES

[1] Jayaweera D, Dilanchian P, 2012. New therapeutic landscape of NN RTIs for treatment of HIV: a look at recent data. Exp Opin Phar coth.; 13(18): 2601-12.

[2] Sanford M, 2012. Rilpivirine: Adis Drug Profile. Drugs; 72(4):525-41.

[3] Schafer JJ, Short WR, 2012. Rilpivirine-a novel non-nucleoside reverse transcriptase inhibitor for the management of HIV-1 infection: a systematic review. Antivir Ther.; 17(8): 1495- 502.

[4] Sharma M, Saravolatz L.D, 2013. Rilpivirine: a new non-nucleoside reverse transcriptase inhibitor. J Anti microb Chemother.; 68(2):250-56.

[5] Bhavar G. B, Pekamwar S. S, Aher K. B, and Chaudhari S. R, 2013. Development and validation of UV Spectrophotometric method for estimation of Rilpivirine hydrochloride in bulk and pharmaceutical formulations. Amer Jou of Phar Tech Res.; 3(1): 450–58.

[6] Somsubhra G, Sowjanya B, Laxmi P.V, Vidyadhar S, David B and Subhadip R, 2013. Method development and validation of Rilpivirine in bulk and tablet doses form by RP-HPLC method. Res Jou of Phar & Tech.; 6(3): 240–43.

[7] Sudha T, Shanmuga sundram P, 2012. Reverse phase high performance and HPTLC methods for the determination of Rilpivirine bulk and in tablet dosage form. World Jou Phar Res.; 1(4):1183–196.

[8] Tempestilli M, Ammassari A, D'Avolio, 2018. A: Development and validation of an HPLC-UV method for quantification of elvitegravir and two other new antiretrovirals, Dolutegravir and Rilpivirine, in the plasma of HIV-positive patients. Bio med Chrom.; 4274.

[9] Venkatesan S, Kannappan N, Mannemala S.S, 2014. Stability-Indicating HPLC method for the simultaneous determination of HIV tablet containing emtricitabine, tenofovir disoproxil fumarate, and Rilpivirine hydrochloride in pharmaceutical dosage forms. Int Sch Res Notic.; 2(14): 849149.

[10] Patel S, Nagappan K, Santhosh G. R, 2018. A new quantitative reverse phase high-performance liquid chromatographic method for the quantification of Rilpivirine hydrochloride in bulk and dosage form. Jou of Appl Pharma Sci.; 8(11): 57-162.

[11] Ashok G, Mondal S, 2018. Development and validation of stability indicating method for the simultaneous estimation of batcaver sulfate, Lamivudine and

Dolutegravir sodium in pharmaceutical dosage forms by RP-HPLC. Saudi J Med Pharm Sci.; 4(2): 289-96.

[12] Veera swami B, Naveen V.M.K, 2019. Development and validation of RP-HPLC method for the estimation of Dolutegravir and Rilpivirine in bulk and pharmaceutical dosage form and its application to rat plasma. Asian J Pharm Clin Res.; 12(2): 267-71.

[13] Ismail Y, Prasad MVV, Shaheedha SM and Habeeb M, 2019. A new stability indicating RP-HPLC method development and validation for the simultaneous estimation of Dolutegravir and Rilpivirine in bulk and its dosage forms. Iranian J of Pharma Sci.; 15(4): 53-72.

[14] Venkata Narayana M, Siva J.N, 2020. Development of validation and stability indicating method of anti-HIV Dolutegravir drug and its related impurities by using RP-HPLC. J Chromatogr Sep Tech.; 11: 426-31.

[15] Reddy K.S, Shirisha S.S Kumar K.P, 2020. Validated stability indicating RP-HPLC method for the simultaneous estimation of Rilpivirine and Dolutegravir in bulk form. Int J Pharm Sci & Res.; 11(10): 4991-97.

[16] Bhavar G.B, Pekamwar S.S, Aher K.B, Thorat R.S, Chaudhari S.R, 2016, High-Performance liquid chromatographic and high-performance thin-layer chromatographic method for the quantitative estimation of Dolutegravir sodium in bulk drug and pharma dosage form. Sci Pharm.; 84(2): 305-20.

[17] Grégoire M, Deslandes G, Renaud C, 2014. A liquid chromatography-tandem mass spectrometry assay for quantification of Rilpivirine and Dolutegravir in human plasma. J Chromatogr B Analyt Techno Biomed Life Sci.; 971: 1-9.

[18] Prathipati P.K, Mandal S, Destache C.J, 2016. Simultaneous quantification of tenofovir, emtricitabine, Rilpivirine, elvitegravir and Dolutegravir in mouse biological matrices by LC-MS/MS and its application to a pharmacokinetic study. J Pharm Bio Med Anal.; 129: 473-81.

[19] Simiele M, Ariaudo A, De Nicola, 2017. A: UPLC-MS/MS method for the simultaneous quantification of three new antiretroviral drugs, Dolutegravir, Elvitegravir and Rilpivirine, and other thirteen antiretroviral agents plus cobicistat and ritonavir boosters in human plasma. J Pharm Bio Med Anal.; 138: 223-30.

[20] Khaleel N, Abdul Rahaman S.K, 2019. Stability-indicating RP-UPLC method for the simultaneous determination of Dolutegravir and Rilpivirine in Bulk and Pharmaceutical Dosage Form. Der Phar Let.; 11(2): 29-39.

[21] Katla S.R, Kumar V.R, Thota S, 2014. Estimation of Rilpivirine in bulk and pharmaceutical dosage form. Der. Pharm Let.; 6: 146–51.

[22] Thoke S.T, Jadhao U.T, Dhembre G.N, 2022. Development and Validation of UV Spectrophotometric Methods for Simultaneous Estimation of Dolutegravir Sodium and Rilpivirine Hydrochloride in Pure Bulk Form. Asian J. Pharm. Anal.; 12: 181–86.

[23] Yusuf I, Vara Prasad M.V, Shaheedha S.M, Habeeb M, 2019. A New Stability Indicating RP-HPLC Method Development and Validation for the Simultaneous Estimation of Dolutegravir and Rilpivirine in Bulk and its Dosage Forms. Iran. J. Pharm. Sci.; 15: 53–72.

[24] Sivagami B, Sharmil K, Chandrasekhar L.M, Niranjan R, Babu M, 2022. Development and Validation for the Simultaneous Estimation of Rilpivirine and Dolutegravir in Bulk and Pharmaceutical Dosage Forms by RP-HPLC Method. Res. J. Pharm. Technol.; 15: 5302–306.

[25] Veeraswami B, Naveen V.M.K, 2019. Development and Validation of RP-HPLC Method for the Estimation of Dolutegravir and Rilpivirine in Bulk and Pharmaceutical Dosage Form and Its Application to Rat Plasma. Asian J. Pharm. Clin. Res.; 12: 267–71.

[26] Srinivas L, Jain N, 2021. Stability Indicative and Cost Effective Creation and Validation of Analytical Method of Lopinavir and Rilpivirine by High Performance Liquid Chromatography. Int. J. Res. Pharm. Sci.; 12:786–92.

[27] Prasanna A, Rani S.S, 2022. A Validated Stability Indicating RP-HPLC Method Development for Simultaneous Estimation of Cabotegravir and Rilpivirine in Pharmaceutical Dosage Form. World J. Pharm. Sci.; 10:22–29.

[28] Cozzi V, Charbe N, Baldelli S, Castoldi S, Atzori C, Cattaneo D, Clementi E, 2016. Development and validation of a chromatographic ultraviolet method for the simultaneous quantification of Dolutegravir and Rilpivirine in human plasma. Ther Drug Monit.; 38: 407–13.

[29] Rao P.V, Rao N.S, 2019. A new analytical method for determination of Dolutegravir and Rilpivirine in pharmaceutical formulations by RP-HPLC method. Int. J. Res. Pharm. Chem. Anal.; 1: 68–74.

[30] John C, Chainulu C, Ghosh P, Srivastava S, Shukla S, Satyanarayana S, 2011. Validated High Performance Thin Layer Chromatography Method for the Simultaneous Determination of Amitriptyline, Atenolol and Propranolol. Asian J. Res. Chem.; 4: 1059–63.

[31] Peraman R, Bhadraya K, Padmanabha Reddy Y, 2015. Analytical quality by design: A tool for regulatory flexibility and robust analytics. Int. J. Anal. Chem.; 2(5): 868727.

[32] ICH Guideline. Validation of analytical procedures: Text and methodology. In Proceedings of the International Conference on Harmonization, Topic Q2 (R1), Geneva, Switzerland, November 2005, 1–13.