Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Rilpivirine Hydrochloride in Tablet Dosage Form

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Abstract

A simple, sensitive, rapid and reproducible HPLC Method was developed and validated for estimation of Rilpivirine in the presence of degradation products generated from forced decomposition studies. The analysis was carried out on Hypersil BDS C18, 250 X 4.6mm, 5μ column using a mixture of ammonium acetate Buffer (pH to 6.0 ± 0.05) and Acetonitrile in the proportion 55:45 respectively as a mobile phase at a flow rate of 1.2 mL/minute. The wavelength selected for the analysis was 300 nm. The peak for Rilpivirine HCl was observed at 10.33 minute. A linear response was observed in the range of 12.5 - 62.5 μg/mL with a correlation coefficient of 0.999. The method was validated for specificity, linearity, precision, accuracy and robustness. The obtained results were indicating that the method is selective in analysis of Rilpivirine in the presence of degradation products formed under various stress conditions.

Keywords: Rilpivirine, HPLC, Stress studies

1. Introduction

Rilpivirine hydrochloride (REL) is a di-amino pyrimidine derivative. Chemically, it is 4-[[4-[[4-[(E)-2-cyanoethenyl]- 2,6-dimethylphenyl] amino]-2-pyrimidinyl] amino] benzonitrile monohydrochloride (Figure 1).[1,4] It is a human immunodeficiency virus type 1 (HIV-1) specific, non-nucleoside reverse transcriptase inhibitor.[3-6] It is a white to almost white powder. . It is practically insoluble in water over a wide pH range. It is soluble in N,N-dimethylformamide (DMF) and N,N-dimethylacetamide, slightly soluble in methanol, propylene glycol and 1-methoxy-2-propanol. The active substance does not have chiral centres and is not considered hygroscopic.[6-9] Rilpivirine was approved by the European Medicines Agency, UK and Therapeutic Goods Administration, (TGA) Australia.[7-8] Rilpivirine HCl Tablets are available for oral administration in strength of 130 mg of Rilpivirine HCl (equivalent to approximately 25mg of Rilpivirine).

Earlier publications have described high-performance liquid chromatography (HPLC) methods useful for the quantification of REL in pharmaceutical dosage forms.[9-11] However; these methods involve arduous sample preparation and long chromatographic run times. It was felt necessary to develop a simple, precise, and rapid HPLC method for the quantitative determination of REL.[12] The current research work deals with the development of HPLC method and its validation as per International Conference on Harmonisation (ICH) guidelines[13]. The developed method was found to be selective, accurate, precise, reliable, and economical.

Figure 1: Structure of Rilpivirine HCl
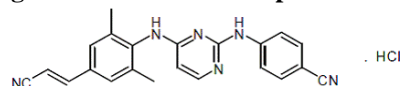
4-[[4-[[4-[(E)-2-cyanoethenyl]-2,6-dimethylphenyl]amino]-2-pyrimidinyl]amino]benzonitrile monohydrochloride

2. Materials and Methods

2.1. Chemicals and reagents

Rilpivirine HCl working standard was procured from Matrix laboratories pvt. Ltd., Hyderabad (India). Acetonitrile and water used were of HPLC grade (Qualigens, Mumbai). Analytical reagent grade Ammonium Acetate, Triethylamine, Acetic Acid was used.

2.2. Equipments

HPLC system: Waters - Alliance 510 with UV- 484 Data Ace software
Column: Hypersil BDS C18, 250 X 4.6mm, 5μ
Balance – Mettler Toledo 205
Ultrasonicator, ENERTECH Electronics Pvt. Ltd.

2.3. Preparation of solutions

2.3.1. Diluent

A mixture of ammonium acetate buffer (pH 6.0 ± 0.05) and Acetonitrile in the proportion 20:80 respectively, filtered through 0.5 μ Nylon membrane filter was used as diluent.

2.3.2. Rilpivirine HCl Standard solution

An accurately weighed 50 mg of Rilpivirine HCl working standard was transferred to a 100 mL volumetric flask and dissolved in diluent by sonication. The solution was diluted to mark with the diluent to give the stock solution of concentration 500 μg/mL. Aliquot of 5.0 mL from the stock solution

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was diluted to 100 mL with the diluent to give the working standard solution of concentration 25 µg/mL. The solution was then filtered through 0.45 µ nylon filter.

2.3.3. Preparation of Test Solution

Twenty tablets were accurately weighed, their average weight was determined and they were finely powdered. The powder equivalent to 50 mg of Rilpivirine HCl was transferred to a 100 mL volumetric flask. About 70 mL of diluent was added and sonicated for 10 minutes. The solution was diluted to volume with Diluent. The aliquot of 5.0 mL from this solution was transferred into a 100 mL of volumetric flask and diluted to volume with the diluent. The solution was filtered through 0.45µ nylon filter.

2.4. Chromatographic Conditions

Analysis was carried out on Hypersil BDS C18, 250 X 4.6 mm, 5µ column using a mixture of ammonium acetate Buffer (pH to 6.0 ± 0.05) and Acetonitrile in the proportion 55:45 respectively as a mobile phase at a flow rate of 1.2 mL/minute. The column temperature was set at 30 °C. The detection was carried out at 300 nm using a PDA detector.

2.5. Calibration Curve

From the standard stock solution of Rilpivirine HCl aliquots were transferred to series of 100 mL volumetric flasks and volume was made up to the mark with diluents to give solutions of concentrations in the range of 12.5 - 62.5 µg/mL. The chromatograms and peak areas of these solutions were measured at 300 nm and a calibration curve was constructed, by plotting the area against the corresponding drug concentration.

2.6. Forced Degradation

The forced degradation studies were performed to establish the stability indicating nature and specificity of the assay method and to observe any degraded compounds. Rilpivirine HCl working standard and Sample (Rilpivirine HCl Tablets 50 mg) were subjected to stress with 5N HCl, 5N NaOH, 3% H_2O_2

and Thermal degradation at 60°C in presence of 80 %RH (**Table 1**). Chromatograms were recorded for all the above solutions.

Table 1: Forced Degradation Conditions

| Stress condition	Description of stress condition
Acid degradation	5N HCl heated at about 60 °C for 10 min on water bath.
Alkali degradation	5N NaOH heated at about 60 °C for 10 min on a water bath.
Oxidation degradation	3%v/v H_2O_2 heated at about 60 °C for 10 min on a water bath.
Thermal degradation in presence of humidity	60°C @ 80 %RH for 7 days

2.7. Validation of the Method
2.7.1. Linearity

For the linearity study standard solutions of Rilpivirine HCl were prepared in the range starting from 50 % to 250 % of the theoretical concentration of assay preparation (12.5 - 62.5 µg/mL). The linearity graph of concentration against peak response was plotted and the correlation coefficient was determined**.**

2.7.2. Specificity

The analyte should have no interference from other extraneous components and be well resolved from them. To determine the specificity of the method, the mixture of reference standard Rilpivirine HCl and the degradation products was injected and chromatogram was recorded. The sample solution was then injected and the chromatogram was obtained. The sample chromatogram was compared with the standard chromatogram.

Figure 2: Typical chromatogram of Standard Rilpivirine HCl
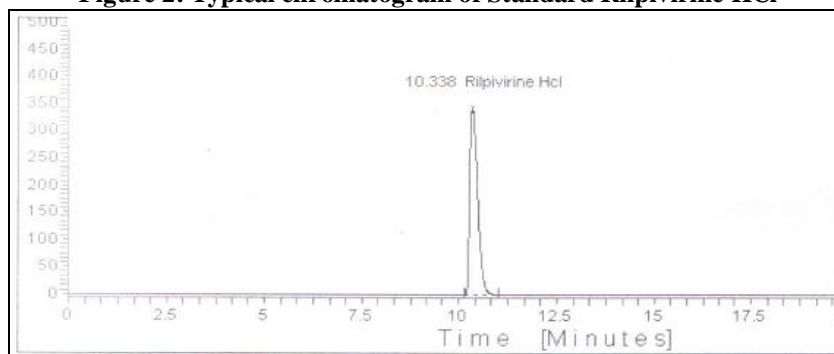
2.7.3. Precision

Precision of the method was studied in terms of method precision and intermediate precision.

Method Precision

Six test solutions of Rilpivirine HCl tablets were prepared as per the analytical method. The % RSD of assay of six test solutions was calculated.

Intermediate precision

Six test solutions of Rilpivirine HCl Tablets were prepared as per the analytical method on different day. These test solutions were analyzed by a different analyst. The % RSD of assay results of twelve test solutions (six samples from method precision and six samples from intermediate precision) was calculated.

2.7.4. Accuracy (Recovery)

Accuracy study was performed by analyzing Rilpivirine HCl test solutions spiked with a quantity of Rilpivirine HCl standard to produce three different concentration solutions equivalent to 80 %, 100 % and 120 % of test concentration.

2.7.5. Robustness

To determine the robustness of the method the experimental conditions were deliberately altered and peak area was evaluated. Three test solutions of the same lot of Rilpivirine HCl from tablets were prepared as per analytical method. These solutions were injected with different chromatographic conditions as Change in flow rate (± 0.2 mL/minute), Change in Column Oven Temp 300C (±5^{0}C), Change in wavelength (± 2 nm) and Change in pH of mobile phase (± 0.2). When the effect of altering one set of conditions was tested, the other conditions were held constant at the optimum values.

2.7.6. Limit of Detection and Quantitation

Limit of Detection and Quantitation is established by injecting six times very low concentration of Rilpivirine HCl standard preparation i.e. 1.0ppm and 2.0ppm.

2.8 Assay of Rilpivirine HCl in tablets

Six replicate injections of test solution were injected and chromatogram and peak area were recorded. The concentration of tablet solution was determined using linear regression equation of calibration graph and amount of drug in tablet was determined.

2.9. System Suitability Parameters

Five replicate injections of system suitability solution (Rilpivirine HCl standard working solution) were injected. The retention time, areas, theoretical plates, peak asymmetry and resolution were calculated for standard solutions

3. Result and Discussion

A new HPLC method was developed and validated for estimation of Rilpivirine HCl in the presence of degradation products using a mixture of ammonium acetate Buffer (pH to 6.0 ± 0.05) and Acetonitrile in the proportion 55:45 respectively as a mobile phase. The average retention time for Rilpivirine HCl was found to be 10.33 minute. The details of findings are as below.

3.1. Forced Degradation

The number of degradation peaks observed in different stress condition was as follows:

Acid Degradation

One degradation peak was observed in acid degradation of standard preparation (**Figure 3**) as well as sample preparation.

Alkali Degradation

One degradation peak was observed in alkali degradation of standard preparation (**Figure 4**) and two degradation peaks were observed in alkali degradation of sample preparation.

Peroxide Degradation

Three degradation peaks were observed in peroxide degradation of standard preparation (**Figure 5**) and five degradation peaks were observed in peroxide degradation of sample preparation.

Thermal With Humidity Degradation (60°C & 80%RH)

Four degradation peaks were observed in thermal with humidity degradation of standard Preparation (**Figure 6**) and three degradation peaks were observed in thermal with humidity degradation of sample.

The percent degradation of Rilpivirine HCl found is shown in **Table 2**

Table 2: Percent Degradation of Rilpivirine HCl

	% Degradation			
	Acid Degradation	**Alkali Degradation**	**Peroxide Degradation**	**Thermal With Humidity Degradation**
API	0.048	0.002	0.029	0.085
Tablet	0.002	0.099	0.008	0.296

Figure 3: Acid degradation of standard Rilpivirine HCl
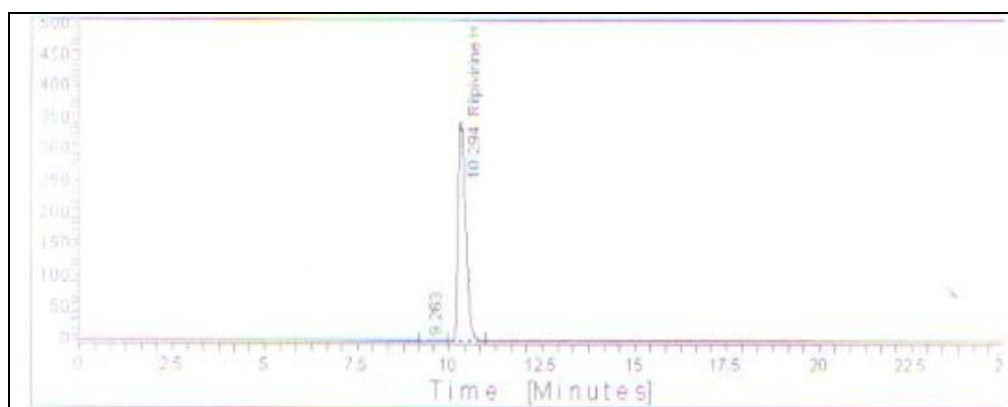
Figure 4: Alkali degaradation of standard Rilpivirine HCl
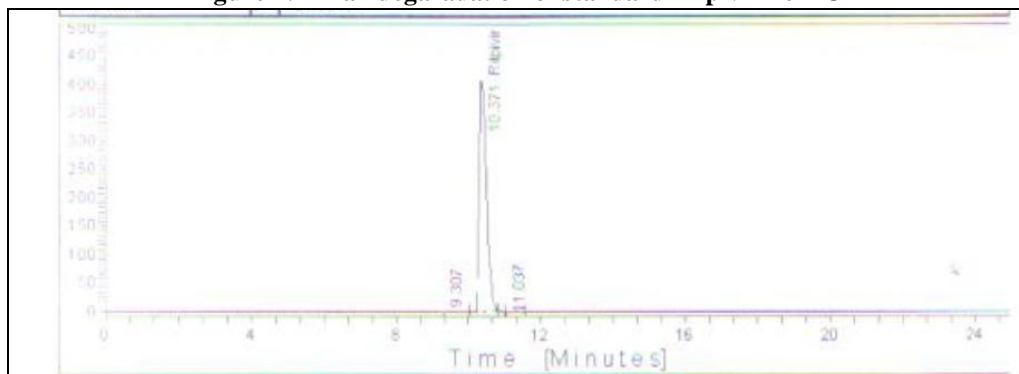

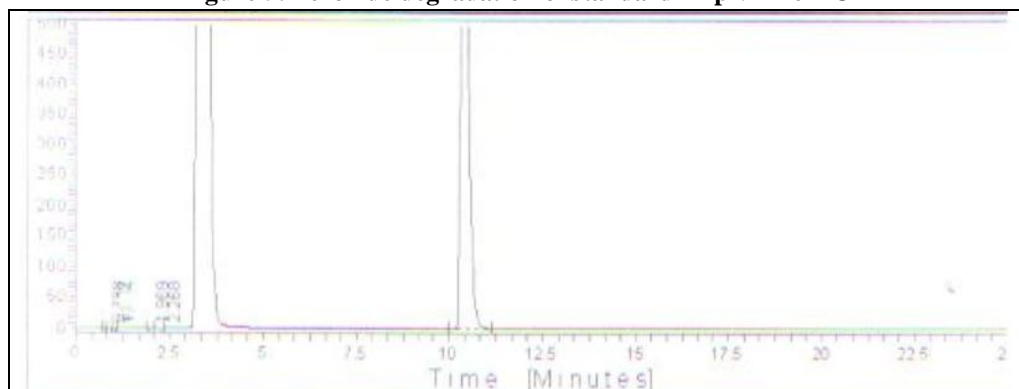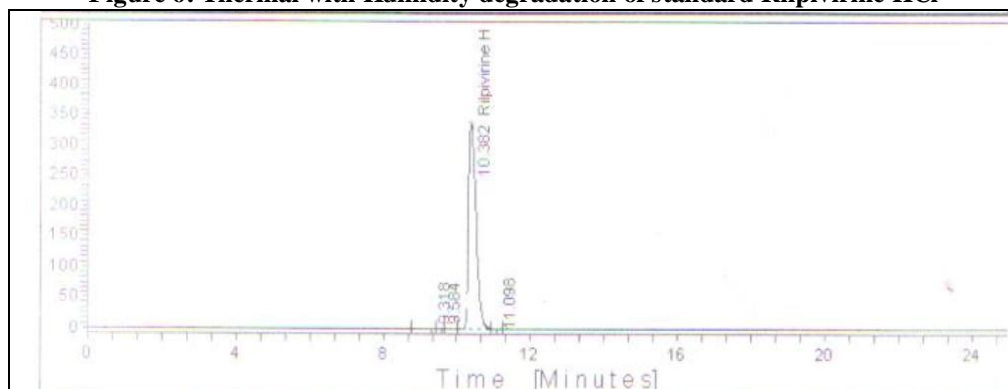**Figure 5: Peroxide degradation of standard Rilpivirine HCl**

Figure 6: Thermal with Humidity degradation of standard Rilpivirine HCl


3.2. Linearity

The average peak area of Rilpivirine HCl peak at each concentration level was determined and the linearity graph was plotted over the range of 12.5 - 62.5 μg/mL. The results of linearity study are as given in **Table 3.** The linearity plot of peak area of Rilpivirine HCl Vs. standard concentration is shown in **Figure 7**.

Table 3: Linearity Data for Rilpivirine HCl

Linearity range (μg/mL)	12.5 – 62.5
Slope	221.5
Y-intercept	-805
Correlation coefficient (r^2)	0.999

Figure 7: Linearity plot of standard Rilpivirine HCl
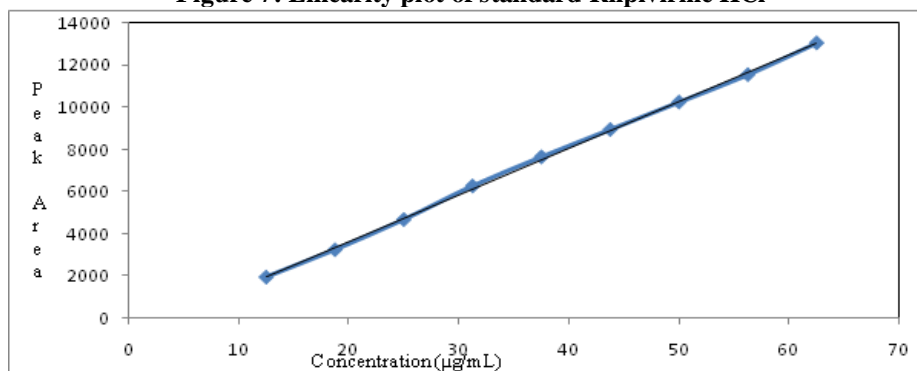
3.3. Specificity

The peaks due to degradation products were found to be well separated from the peak due to Rilpivirine HCl. The peak purity criteria of Rilpivirine HCl were found to pass at each condition of degradation.

3.4. Precision

Precision of the method was studied in terms of method precision and intermediate precision. Precision was expressed in terms of % R.S.D. All values for precision were within recommended limits.

Method precision

Analysis of six separate solutions of Rilpivirine HCl showed the repeatability of the method. The results of assay obtained from six test solutions preparations are given in **Table 4.**

Table 4: Results of Method Precision

% Assay of Rilpivirine HCl (Mean ± SD)*	101.22 ± 1.33
(%) Relative Standard Deviation	1.31

*-Mean of six determinations

Intermediate precision

The % RSD of assay results of analysis carried out on two different days as summarized in **Table 5** indicated that the method is precise and reproducible.

Table 5: Results of Intermediate Precision

% Assay of Rilpivirine HCl (Mean ± SD)*	100.54 ± 1.33
(%) Relative Standard Deviation (n=12)	1.33

*-Mean of twelve determinations

3.5. Accuracy (Recovery)

The results of recovery studies showed the accuracy of the method. The recoveries were ranged between 99.76 – 101.44 %, Results obtained are given in **Table 6.**

Table 6: Results of Recovery Studies

Level	Amount added (µg/mL)	Amount found (µg/mL)	%Recovery* (Mean ± SD)
80%	20	20.08	100.38 ± 1.04
100%	25	25.36	101.44 ± 1.85
120%	30	29.93	99.76 ± 1.35
		Mean ± SD	100.53 ± 0.85
		% RSD	0.85

*-Mean of three determinations

3.6. Robustness

The robustness of a method is its capacity to remain unaffected by small changes in conditions. Robustness of the method was indicated by the small % RSD observed for the assay of Rilpivirine HCl sample at deliberately altered experimental conditions. The optimum mobile phase flow rate was 1.2 mL min-1. This was changed by 0.2 units to 1.0 and 1.4 mL min-1 and the effect was studied. Similarly, the effect of change in column temperature was studied at 25 and 35°C. The effect of change in wavelength was studied at 298nm and 302 nm. The effect of change in pH of mobile phase was studied at pH 5.8 and pH 6.2. For all changes of conditions the sample was assayed in triplicate.

Assay of Rilpivirine HCl for all deliberate changes of conditions was within 99.80– 101.02%. The complete results are shown in **Table 7.**

Table 7: Results of Robustness Studies

Condition	% Assay*	%RSD
Flow rate (optimum flow rate ± 0.2 mL/min)		
1.0 mL/min	99.91	0.25
1.4 mL/min	100.11	1.81
Column temperature (optimum temperature ± 5°C)		
25°C	100.77	0.1
35°C	101.02	0.95
Wavelength (optimum Wavelength ± 2 nm)		
298 nm	100.64	0.13
302 nm	100.85	0.22
pH (optimum pH ± 0.2)		
pH 5.8	100.40	1.06
pH 6.2	99.80	0.47

*-Mean of three determinations

3.6. Limit of Detection and Quantitation

Limit of Detection and Quantitation was observed to be 1.0ppm and 2.0ppm respectively

3.7. Assay of Rilpivirine HCl in tablets.

The concentration of tablet solution was determined using linear regression equation (using slope and Y-intercept) and amount of drug in tablet was determined. The results of assay in tablets are summarized in **Table 8.**

Table 8: Results of Assay of Rilpivirine HCl in tablets

Labeled claim (mg)	25
Amount found (mg) mean ± SD*	24.96 ± 0.20
% labeled claim	99.85
% RSD	0.79

*-Mean of six determinations

3.8. System Suitability Parameters

System suitability parameters were tested for the chromatographic conditions and results are as shown in Table 9.

Table 9: System Suitability Parameters

Parameter	Average	%RSD
Retention time	10.33 minute	0.1
Peak Area	4678.42	0.90
HETP	5482	
Tailing Factor	1.31	

4. Conclusion

A simple, rapid and reliable HPLC method has been developed and successfully validated for estimation of Rilpivirine HCl in the presence of degradation products. The results of the validation tests indicated that the method was accurate, precise, robust, and stability indicating. This method is suitable for the routine quality control of the tablet dosage form.

Acknowledgements

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