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Stability Indicating High-Performance Thin-Layer Chromatographic Method Development and Validation for Estimation of Rilpivirine in Tablet Dosage Form

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ABSTRACT

The present work describes the development and validation of a stability-indicating high-performance thin-layer chromatographic method for estimation of Rilpivirine as bulk drug and in the tablet dosage form. Chromatographic resolution of the drug was achieved on pre-coated silica gel G60 F_{254} aluminum-backed plates using Toluene: Methanol (8: 2, v/v) as the mobile phase with densitometric detection at 299 nm. Acceptable peak parameters were obtained at the retention factor of 0.37 ±0.01. Stress degradation studies were conducted to check the stability of the drug as per ICH guidelines. Rilpivirine was found susceptible to all the analyzed stress conditions except photolysis. Linear regression analysis showed good linearity concerning peak area in the concentration range of 50-250 ng $band^{-1}$. The developed method has been successfully applied for the estimation of the drug in the tablet dosage form.

1.0 INTRODUCTION

Rilpivirine, chemically, [4-{[4-({4-[(E)-2-cyanovinyl]-2,6-dimethylphenyl} amino) pyrimidin-2-yl] amino} benzonitrile] is a second-generation non-nucleoside reverse transcriptase inhibitor (NNRTI) with higher potency, longer half-life, and reduced side effect profile as compared with older NNRTIsand used for the treatment of HIV/AIDS.[1]

An extensive literature review about the determination of Rilpivirine revealed that few methods were published for Rilpivirine involving spectrophotometric methods as a single drug and in combination with other drugs.[2-6] Analytical methods representing analytical method development and validation of Rilpivirine in pharmaceutical dosage form by high-performance liquid chromatography (HPLC) were also published in the literature. [7-9]Stability indicating RP-HPLC methods for determination of Rilpivirine in combination with dolutegravir and emtricitabine, tenofovir disoproxil fumarate was also reported in the literature. [10-13]

To the best of our knowledge, no reports were found in the literature for the determination of Rilpivirine in tablet formulation by stability-indicating high-performance thin-layer liquid chromatographic (HPTLC) method. Hence the current work was undertaken to develop simple, precise, accurate, and economic stability-indicating HPTLC procedure for determination of Rilpivirine as bulk drug and in tablet dosage form following International Conference on Harmonisation Guidelines.[14, 15]

2.0 MATERIALS AND METHODS

2.1 Chemicals and reagents

Rilpivirine working standard was obtained as a gift sample. The pharmaceutical dosage from Eudrant tablets containing 25 mg of Rilpivirine was procured from a local pharmacy. Toluene, Methanol (both AR grade) were obtained from LOBA Chemie Pvt. Ltd. Mumbai, India.

2.2 Instrumentation and chromatographic conditions

Chromatographic separation of the drug was performed on Merck TLC plates precoated with silica gel 60 F_{254} (10 cm × 10 cm) from E. MERCK, Darmstadt, Germany, using a CAMAG Linomat V sample applicator (Switzerland). Samples were applied on the plate as a band

under nitrogen stream with a 6 mm bandwidth using Camag 100 µL sample syringe (Hamilton, Switzerland).

Linear ascending development was carried out in a 10 ×10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using Toluene: Methanol (8:2, v/v/v) as mobile phase. The mobile phase was saturated in the (CAMAG) twin trough TLC chamber for 20 min before chromatogram development at room temperature. After development, TLC plates were removed and dried. A Camag TLC scanner with winCATs software version 1.4.2was used for densitometric evaluation. The densitometric evaluation was carried out at 299 nm.

2.3 Preparation of standard stock solution

Accurately weighed 10 mg of the drug was dissolved in 10 mL of methanol get the solution having a concentration of 1000 ng μL^{-1}which was further diluted with methanol to furnish the final working standard solution 50 ng μL^{-1}.

2.4 Tablet formulation analysis

Commercial brand of tablets Eudrantcontaining 25 mg of drug was used to estimate the amount of Rilpivirine in existing tablet formulation. For this, 20 tablets were weighed accurately and powdered. Powder quantity equivalent to 10 mg of was weighed and transferred to the 10 mL volumetric flask and 5 mL methanol was added and sonicated for 10 min. The solution was filtered using Whatman filter paper No. 41, and the volume was made up to the mark with methanol. The resulting solution was diluted further with methanol to get a final concentration of 50 ng μL^{-1}. Two microliter volume of this solution was applied to a TLC plate to provide a final concentration of 100 ng $band^{-1}$. After chromatographic development, the peak areas of the bands were measured at 299 nm and the amount of drug present in the sample was estimated from the respective calibration curve. The procedure was repeated six times for the analysis of the homogenous sample.

2.5 Forced degradation studies

Forced degradation studies were carried out to check the stability of the drug by subjecting the bulk drug to physical stress conditions. The study was carried out at a concentration of 1000 ng μL^{-1}. The hydrolytic studies were carried out by treatment of stock drug solution separately with 0.1 N HCl and 0.1 N NaOH at room temperature for 1 h and 2 h, respectively. The acid and alkali stressed samples were neutralized with NaOH and HCl, respectively to

furnish the final concentration of 200 ng band^{-1}. The drug was treated with water at room temperature for 24 h for neutral hydrolysis. Oxidative degradation was performed by treating standard drug solution with 3 % H_2O_2 at room temperature for 1 h and was diluted with methanol to obtain 200 ng band^{-1} solution. Thermal degradation was performed by keeping the solid drug in the oven at 60ºC for 6 h. The solid drug powder was exposed to UV light up to 200-watt h square meter^{-1} to check photolytic degradation. Thermal and photolytic samples were diluted with methanol to get a concentration of 200 ng band^{-1}.

3.0 RESULTS AND DISCUSSION

3.1 Method optimization

Initially, many method trials were performed using different mobile phases to achieve better separation of the drug. The mobile phase which consisted of Toluene: Methanol (8: 2, v/v) was selected as most favorable for getting a well-resolved drug peak. The retention factor of Rilpivirine was 0.37 ± 0.01. A representative densitogram of a standard solution of Rilpivirine is represented in Figure.

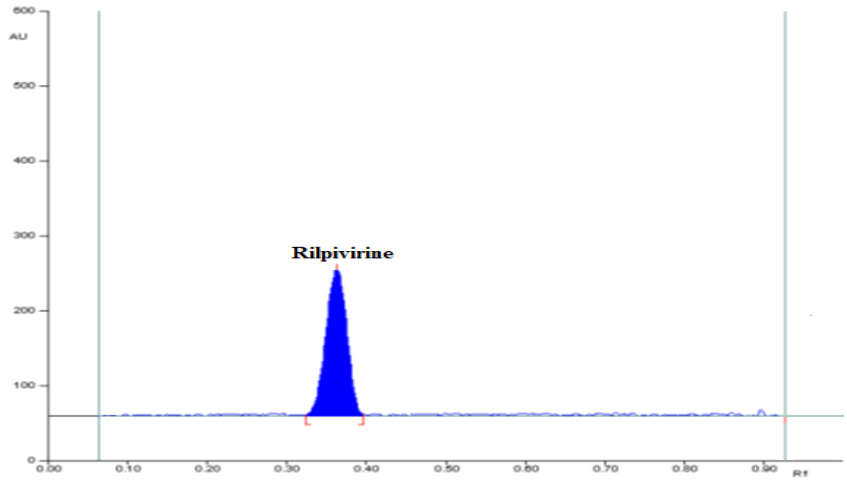
1. 

Figure No 1: Representative densitogram of standard solution

(150 ng band^{-1}, Rf= 0.37 ± 0.01)

3.2 Result of forced degradation studies

The forced degradation results revealed the susceptibility of the drug to hydrolytic, oxidative, thermal stress conditions but stability under photolytic stress conditions. The degradation products formed during forced degradation were well resolved from the active drug which indicated the specificity of the developed method. Figures 2 and 3 represent the densitograms

of acid and alkali hydrolytic degradation while Figures 4 and 5 indicates the densitograms of oxidative and thermal degradation, respectively. The results of degradation studies along with % degradation and % recovered are denoted in Table1.

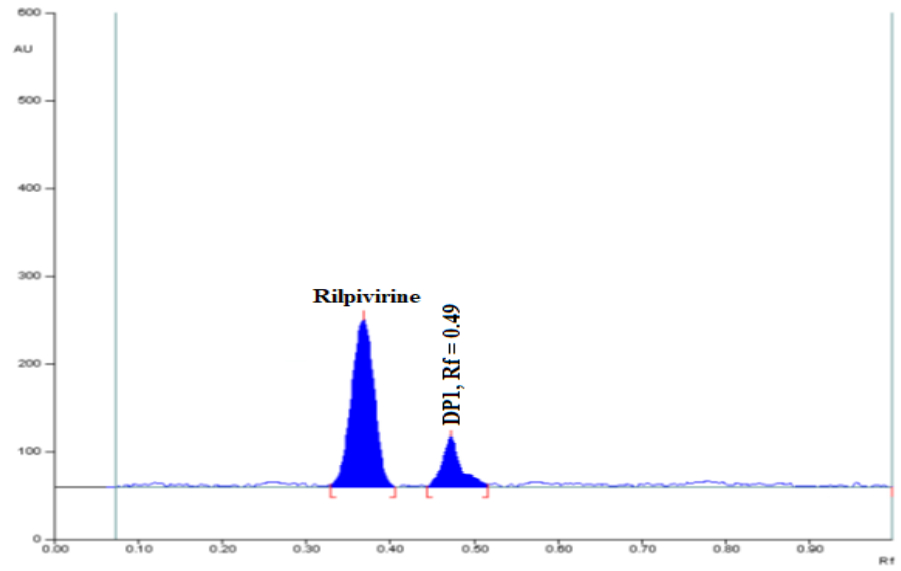


Figure No. 2:Densitogram of acid-treated rilpivirine with degradation product (DP1, Rf = 0.49)

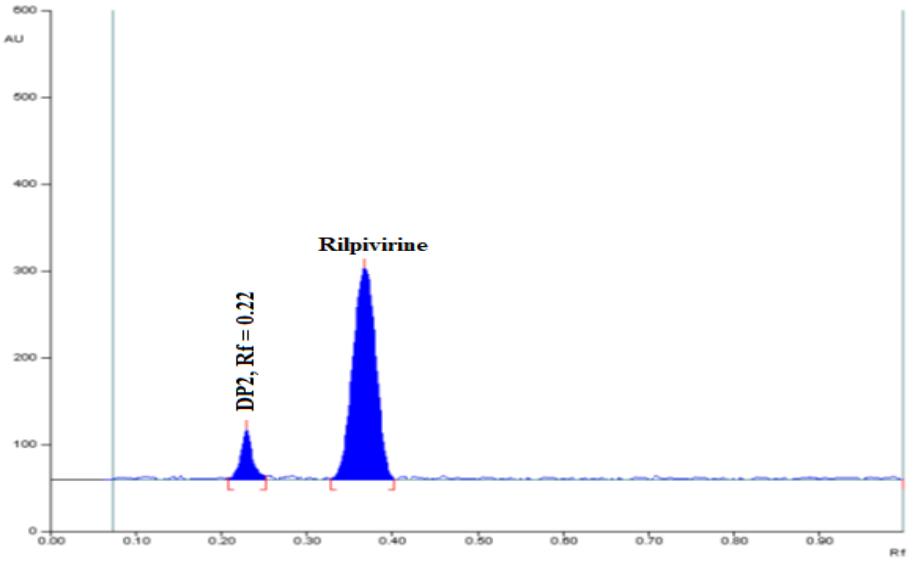


Figure No. 3:Densitogram of base treated sample with degradation product (DP2, Rf = 0.22)

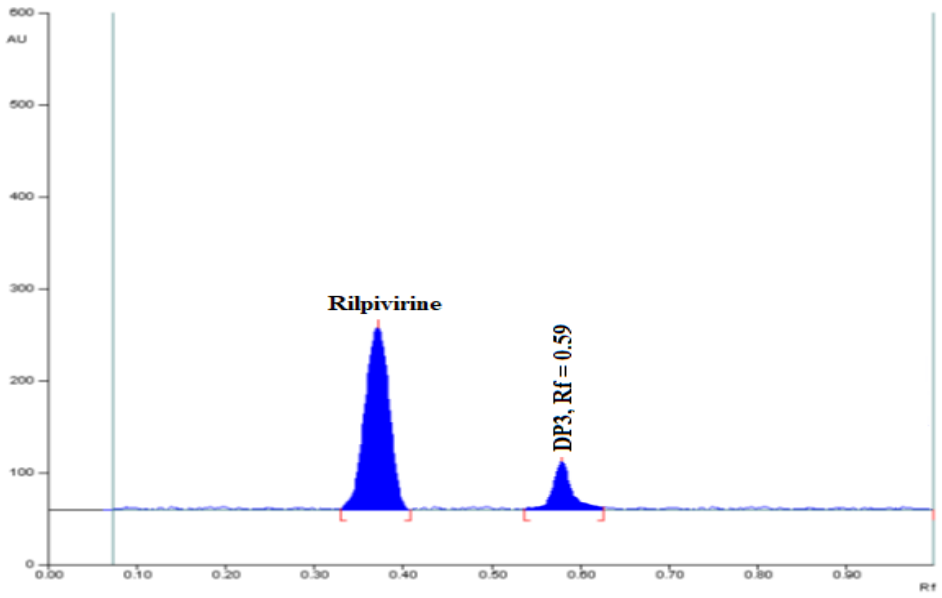


Figure No. 4: Densitogram of peroxide treated sample with degradation product (DP3, Rf = 0.59)

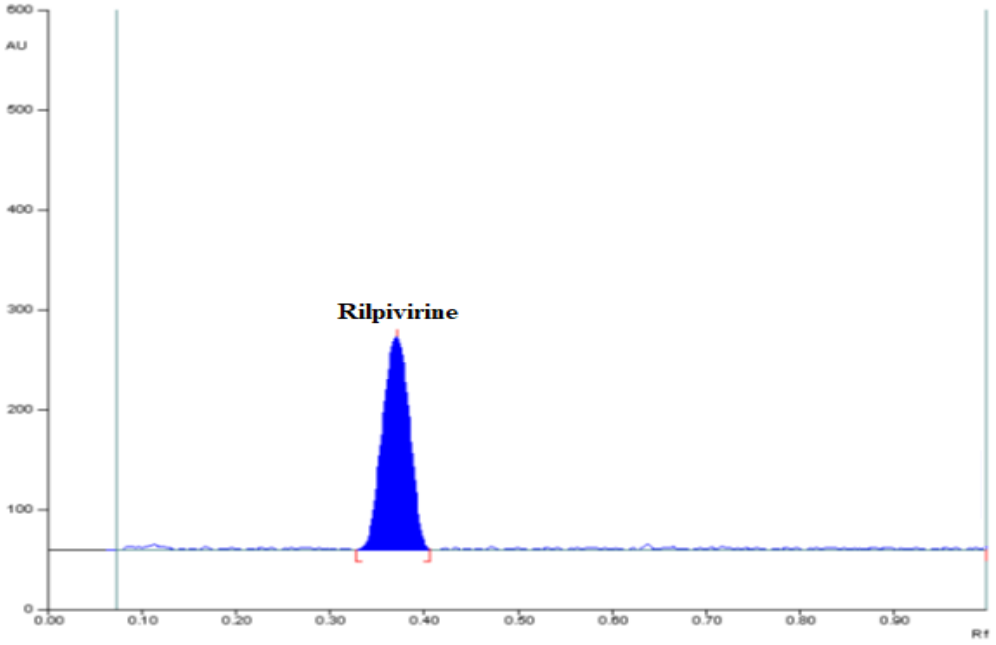


Figure No. 5:Densitogram obtained after thermal stress (60ºC for 6 h)

Table no 1: Summary of forced degradation studies

Sr. No.	Stress conditions	% Recovery	% Degradation
1.	Acid/ 0.1 N HCL/ Kept at RT for 1 h	82.55	17.44
2.	Alkali/ 0.1 N NaOH/ Kept at RT for 2 h	81.75	18.24
4.	Oxidative/ 3% H_2O_2/ Kept at RT for 1h	89.70	11.29
5.	Thermal Degradation/ 60ºC for 6h	82.35	17.64
6.	Photolytic degradation	99.30	----

3.3 Method validation

The developed method has been validated for different validation parameters such as linearity, the limit of detection (LOD), the limit of quantification (LOQ), precision, accuracy, and robustness as per ICH guidelines.

3.3.1 Linearity

The linearity of the method was accessed by spotting volumes 0.5, 1, 1.5, 2, and 2.5 mL from standard solution (1000 µg mL^{-1}) on TLC plate, developed and scanned under optimized chromatographic conditions. The established method was found to be linear in the concentration range 50-250ng $band^{-1}$with a high correlation coefficient. The linear regression equation was found to be y =3.906x + 2150.5 with a correlation coefficient (R^2) value of 0.9973. A 3D densitogram of linearity is shown in Figure 6 and the calibration curve achieved by the plot of concentration vs peak area is depicted in Figure 7.

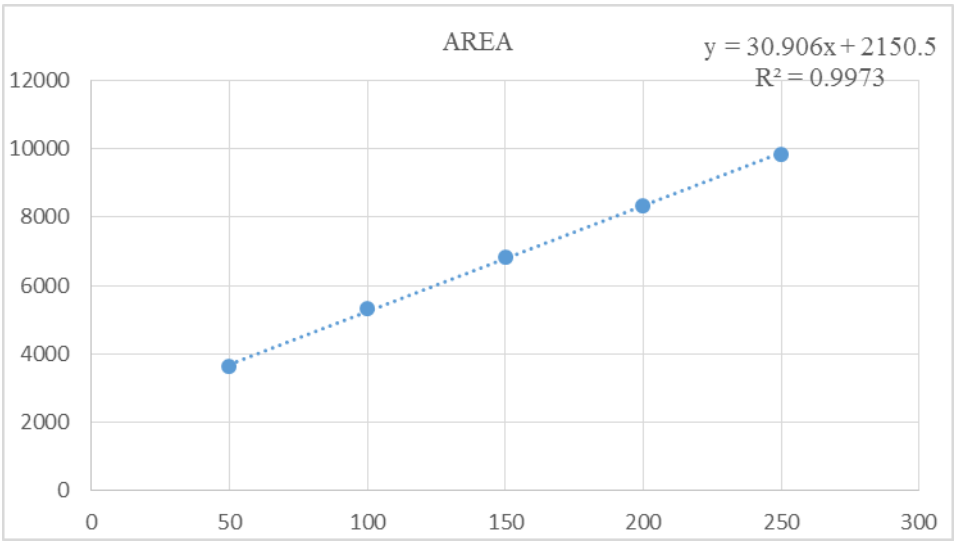


Figure No 6: Calibration curve for Rilpivirine (50-250 ng $band^{-1}$)

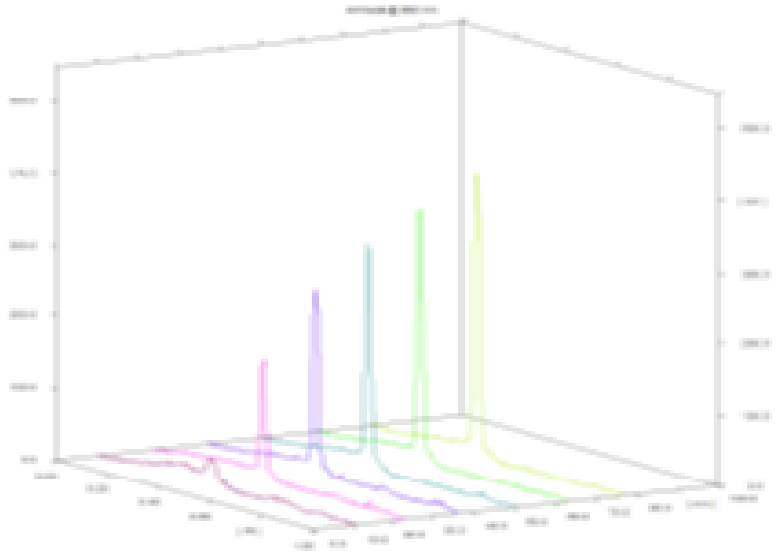


Figure No. 7: 3D densitogram of linearity in concentration range (50-250 ng band^{-1})

3.3.2 Precision

The method was subjected to intra-day and inter-day precision studies. Intra and inter-day deviations in results were examined by recording the values of peak area after application of three different concentrations in three replicates (100ng band^{-1}, 150 ng band^{-1}, 200 ng band^{-1})in linearity range on same and on three consecutive days. The % R.S.D. values were in the range of 0.71 to 1.01 for intraday and 0.65 to 0.81 for interday variation. The lesser % R.S.D. values (< 2) obtained indicated the precision of the developed method.

3.3.3 Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ values were found to be 16.23ng band^{-1}and 41.19ng band^{-1}, respectively.

3.3.4 Accuracy

The accuracy of the developed method was checked by performing recovery studies by the standard addition method. It involved the addition of standard drug solution to pre-analyzed sample solution at three different levels 80%,100%, and 120%. The basic concentration of the sample chosen was 100ng band^{-1}from tablet solution. The drug concentrations were calculated from the linear regression equation. The results of the recovery studies indicated the accurateness of the developed method for estimation of the drug in tablet formulation.

Table no 2: Recovery studies

Drug	Concentration taken (ng band^{-1})	Concentration added (ng band^{-1})	Concentration found (ng band^{-1})	% Recovery±R.S.D.*
Rilpivirine	100	80	179.17	99.54±0.70
	100	100	199.55	99.77±0.87
	100	120	220.99	100.45±0.83

***Average of three determinations

3.3.5 Robustness

Robustness was carried out by doing small and deliberate changes to optimized method parameters such as a change in mobile phase composition(± 1% methanol), saturation time (± 10 min), and wavelength(± 1 nm). The areas of peaks of interest remained unaffected by small changes of the operational parameters which indicated the robustness of the method.

4.0 CONCLUSION

The developed method describes a simple, sensitive, and selective stability-indicating HPTLC method for estimation of rilpivirine as bulk and in the tablet dosage form. The developed method is specified as the degradation products were well resolved from the active drug. The suggested method was found to be less time-consuming and cost-effective and may be more advantageous for routine analysis of drugs in marketed formulation.

5.0 ACKNOWLEDGEMENTS

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