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Estimation of Rilpivirine in bulk and pharmaceutical dosage form

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ABSTRACT

A simple and sensitive stability indicating reverse phase high performance liquid chromatography method with simple UV detection was developed for the estimation of rilpivirine. The HPLC analysis was carried out on Waters ODS (C_8) Column, 250 mm x 4.6 mm in isocratic mode with a mobile phase of acetonitrile-0.01M potassium dihydrogen orthophosphate (40:60 v/v). The method was validated according to ICH guidelines. The values obtained were found to be within acceptable limits. The method was found to be linear over a concentration range of 5- 30 µg/ml. The detection wavelength was selected from the UV spectra as 282 nm. The limit of detection and limit of quantitation was found to be as 0.05µg/ml and 0.15 µg/ml respectively. Forced degradation studies were performed for both the drugs. The drugs were degraded in acidic, basic and oxidative conditions. The peaks of degraded products were well resolved from the actual drug. As the method could effectively separate the drug from its degradation products, it can be employed as a stability indicating method. The method was successfully applied for the estimation of marketed formulation (tablet dosage form).

Keywords: Rilpivirine, RP-HPLC, Method development, Validation

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INTRODUCTION

A stability indicating method is a quantitative analytical procedure used to detect a decrease in the amount of the active pharmaceutical ingredient (API) present due to degradation. According to FDA guidelines, a stability indicating method is defined as a validated analytical procedure that accurately and precisely measures active ingredients (drug substance or drug product) free from potential interferences like degradation products, process impurities, excipients, or other potential impurities, and the FDA recommends that all assay procedures for stability studies be stability indicating. [1]. During stability studies, liquid chromatography (LC) is used routinely to separate and quantitate the analytes of interest. There are four components necessary for implementing a stability indicating method: sample generation, method development, method optimization and method validation [2]. Rilpivirine, 4-{[4-({4-[(1E)-2-cyanoeth-1-en-1-yl]-2,6dimethylphenyl}amino)pyrimidin-2-yl]amino}benzonitrile (**Fig 1**) is non-nucleoside reverse transcriptase inhibitor (NNRTI) which is used for the treatment of HIV-1 infections. It is a second-generation non-nucleoside reverse transcriptase inhibitor (NNRTI) with higher potency, longer half-life and reduced side-effect profile compared with older NNRTIs, such as efavirenz. It is a diarylpyrimidine, a class of molecules that resemble pyrimidine nucleotides found in DNA. It binds to reverse transcriptase which results in a block in RNA and DNA- dependent DNA polymerase activities [3- 4].

Very few methods were published for the estimation of rilpivirine involving HPLC techniques and spectrophotometric methods. Very few HPLC techniques available in literature discussed the stability indicating method development and validation [5-8]. Hence we made an attempt to develop and validate a stability indicating method for the estimation of rilpivirine an alternative method.

MATERIALS AND METHODS

1.1. *Chemicals:*
Rilpivirine was obtained as gift sample from Mylan Laboratory, Hyderabad. Methanol, acetonitrile of HPLC grade were obtained from Loba Chemicals limited, Mumbai. Potassium dihydrogen orthophosphate was of analytical reagent grade purchased from S. D. fine Chem., Mumbai.

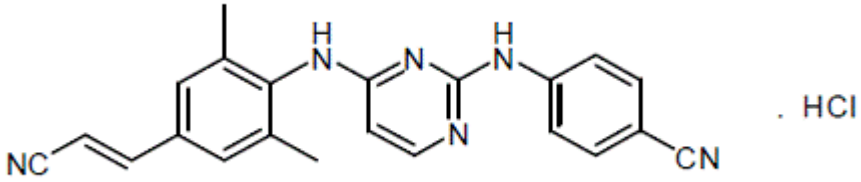

Fig 1: Structure of Ripivirine

1.2. *HPLC system:*
The chromatographic apparatus was of HITACHI L2130 model and software used was of D Elite 2000 and UV-VIS detector L 2400. The chromatographic separation was carried out on Waters ODS (C_8) RP Column, 250mm x 4.6mm. The mobile phase was acetonitrile-potassium dihydrogen orthophosphate (pH-3; 0.01M) (40:60, v/v) was run at a rate of 1 ml/min.

1.3. *Preparation of standard stock solutions:*
Standard stock solutions were prepared by transferring 100 mg of rilpivirine into 100 ml volumetric flask and the volume was made up to the mark using mobile phase. The solution was sonicated to dissolve the drug. From these stock solutions working standards were prepared.

1.4. *Preparation of sample solution:*
Twenty tablets were weighed and powdered. Tablet powder equivalent to 10 mg of rilpivirine was accurately weighed and transferred into 100 ml volumetric flask. To this 10 ml of mobile phase was added and mixed well. To this 80 ml of mobile phase was added and sonicated for about 5min. The volume was made upto the mark using mobile phase. This is then filtered through 0.45µ membrane filter to obtain clear solution. This stock sample solution was diluted quantitatively with mobile phase to obtain the suitable working sample solutions for chromatographic measurements.

1.5. *Method validation:*
The method was validated according to ICH guidelines and the results obtained were within the limits [9-10]. Specificity of the method was determined by analyzing the blank and the sample solutions. Specificity was demonstrated by the absence of interfering peaks at the retention time of rilpivirine.

Linearity was tested at different concentrations over a range of 5-30µg/ml. The calibration curves were established and the correlation coefficient values were calculated.

The limit of quantitation and limit of detection values for both the drugs were calculated by using the following formulae,

$$\text{LOD} = \frac{3.3 \times SD}{slope} \qquad \text{LOD} = \frac{10 \times SD}{slope}$$

The accuracy and precision of the method were determined by using analytical samples in three replicates of three concentrations for three days.

Robustness of the method was determined by making deliberate changes in parameters like flow rate (± 0.2ml), wavelength (±2nm) and column temperature (±5 ^{0}C).

RESULTS AND DISCUSSION

3.1. *Method development*:
Chromatographic method development was performed with the selection of wavelength and optimization of mobile phase composition.

3.1.1.Selection of wavelength:
The sample of rilpivirine was prepared in the concentration of 10µg/ml. This sample was scanned in the range of 200-400nm using UV-visible spectrophotometer. From the spectra [**Fig 2**] the detection wavelength was selected as 282 nm.

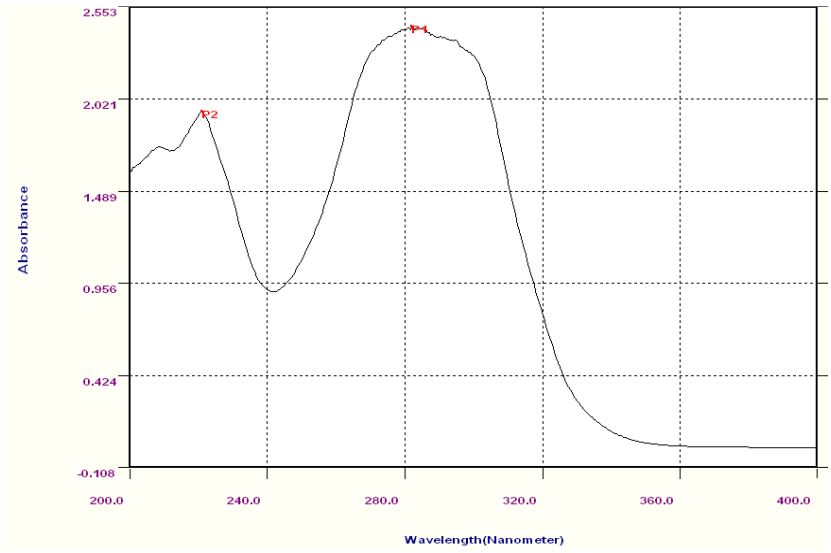

Fig 2: UV-Spectra of rilpivirine

*3.1.2.*Optimization of mobile phase:
Different compositions, pH and flow rate of the mobile phase were tried during method development. Several mixtures of solvent such as methanol and acetonitrile using different buffers such acetate, phosphate with variable pH range 3-5 was tested. Waters ODS (C_8) RP Column, with mobile phase acetonitrile-potassium dihydrogen orthophosphate (pH-3; 0.01M) (40:60, v/v) resulted in peak with good shape and symmetry (**Fig 3**).

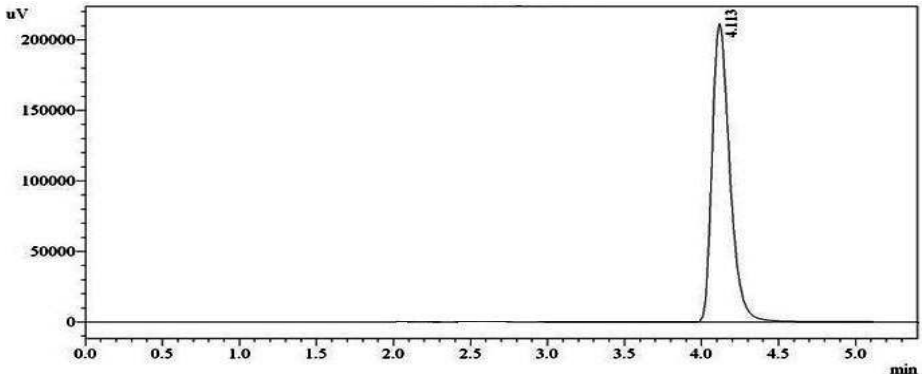

Fig 3: Chromatogram showing estimation of rilpivirine (Rt 4.2)

3.2 Method validation:
3.2.1 Specificity and Sensitivity:
Chromatograms of drug sample and blank were analysed and no interferences were found at the retention time of rilpivirine. LOD and LOQ values are determined and reported in **Table-1.**

3.1.3.Linearity:
Calibration standards were prepared in the concentration of 5-30 µg/ml. These samples were injected into the chromatographic system and the peak areas were recorded. The calibration curve was plotted. The calibration curve [**Fig 4**] was linear over the concentration range of 5-30 µg/ml. The correlation coefficient was found to be 0.992. The regression equation and linearity details were reported in **Table 1**.

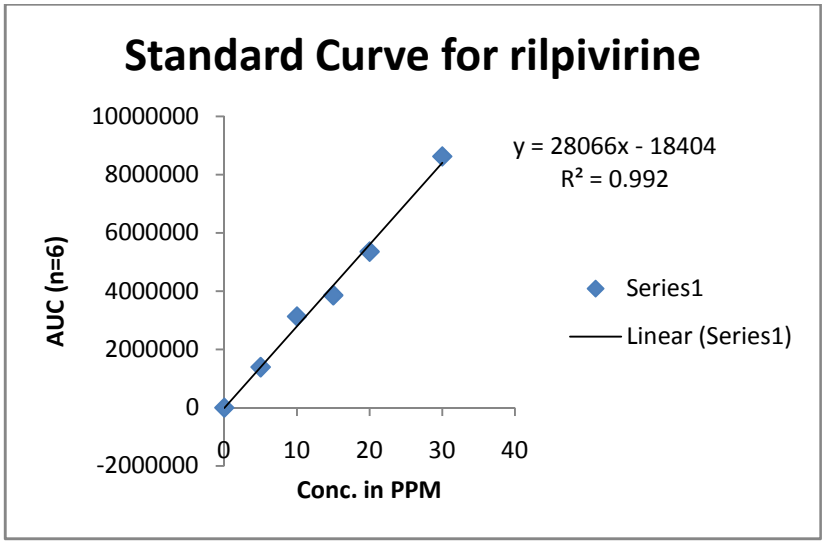

Fig 4: Calibration curve of rilpivirine

Table 1: linearity, LOD and LOQ Results

Parameter	Rilpivirine
Linear range	5 – 30 µg/ml
Slope	28066
R^2 value	0.992
LOQ	30 µg/ml
LOD	5 µg/ml

3.1.4.Precision:

Precision of the method was determined by performing intraday and interday precision. Intraday precision was determined by three replicate analyses of three concentrations within a day. Interday precision was performed for three days by three replicate injections of three samples in a day. Intraday and interday precision values are presented in **Table 2.**

3.1.5.Accuracy:

Accuracy of the method was determined by recovery studies (standard addition method) recovery was performed at three levels i.e. 80%, 100%, 120%. The samples were analysed for three repeated times and the peak areas were recorded. Percentage recovery values are presented in **Table 2.**

Table 2: Intraday, Inter day precision and accuracy results

Sample	Concentration (µg/ml)	Precision		Accuracy (% recovery)
		Intraday	Interday	
Rilpivirine	10	0.3	0.1	99.95
	20	1.1	0.3	99.98
	30	0.9	0.7	99.89

3.1.6.Robustness:

Robustness of the method is determined by making deliberate changes in the parameters like flow rate, wavelength, column temperature. The samples are analysed by changing the parameters and the peak areas are recorded. The values of robustness are presented in **Table 3.**

Table 3: Robustness values of rilpivirine

Change in parameter	% RSD
Flow (1.1 ml/min)	0.07
Flow (0.9 ml/min)	0.02
Temperature (27^0C)	0.09
Temperature (23^0C)	0.13
Wavelength of Detection (284 nm)	0.04
Wavelength of detection (278 nm)	0.01

3.2. *Forced degradation studies:*
Stability studies of both the drugs were carried out in acidic, basic, oxidative, photolytic and thermal conditions.

3.2.1.Acid degradation studies:
Accurately weighed sample of about 100 mg of rilpivirine was weighed and transferred into 100 ml volumetric flasks. To these samples mobile phase was added to dissolve the samples. To these samples 5 ml of 1N HCl was added and the volume was made up to the mark using mobile phase. These samples are kept at room temperature for seven days and analysed at every 24 h. The sample was degraded in 24 h and the degraded peak was well resolved from analyte peak

3.2.2.Base degradation studies:
Accurately weighed sample of about 100 mg of rilpivirine was weighed and transferred into 100 ml volumetric flasks. To these samples mobile phase was added to dissolve the samples. To these samples 5 ml of 1N NaOH was added and the volume was made up to the mark using mobile phase. These samples were kept a side for seven days and analysed at every 24H. The sample was degraded in 24 h and the degraded peak was well resolved from analyte peak.

3.2.3.Oxidative degradation:
Accurately weighed sample of about 100 mg of rilpivirine was weighed and transferred into separate 100 ml volumetric flasks. To these samples mobile phase was added to dissolve the samples. To these samples 5 ml of 3% H_2O_2 was added and the volume was made up to the mark using mobile phase. These samples are kept at room temperature for seven days and analysed at every 24 h. The sample was degraded in 24 h and the degraded peak was well resolved from analyte peak

3.2.4.Thermal degradation:
Accurately weighed sample of about 100 mg of rilpivirine was transferred into separate vials. These vials are kept in hot air oven at 60^0c for about seven days. These samples are analysed for every 24 h for seven days until degradation has been found. The sample was stable in thermal stress conditions.

3.2.5 Photolytic degradation:
Accurately weighed sample of about 100 mg of rilpivirine was placed in a UV cabinet at 254nm wavelength for 24H without interruption. Accurately 1mg of the UV exposed drug was weighed and transferred to a clean and dry 10ml volumetric flask. First the UV exposed drug was dissolved in methanol and made up to the mark. From this required concentration was made and analysed. The sample was stable in photolytic conditions. The forced degradation studies data are summarized in **Table 4.**

Table 4 : Summary of forced degradation studies

Stress condition	Time (hours)	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24	94.73	04.35	99.08
Basic Hydrolysis (0.I M NaOH)	24	93.93	06.37	100.30
Thermal Degradation (50 ^{0}C)	24	99.35	----------	99.35
UV (254nm)	24	98.31	---------	99.31
3 % Hydrogen peroxide	24	94.37	05.46	99.83

CONCLUSION

A simple, sensitive, accurate and precise stability indicating RP-HPLC method was developed and validated for the routine analysis of bulk and tablet dosage form. The results of forced degradation studies reveal that the method is stability indicating. The proposed method is able to separate the analyte degradation products and excipients found in tablets. The method can be employed for the routine analysis of rilpivirine.

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