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Research Article

Analytical Method Development and Validation of Rilpivirine by RP-HPLC Method

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ABSTRACT
A new a rapid, accurate and precise method was developed and validated for the estimation of Rilpivirine in pharmaceutical dosage form by reverse phase high performance liquid chromatographic. The Separation was achieved by Agilent Zorbax Bonus-RP (250 x 4.6 mm, 5 µ) column and using Buffer : ACN (20:80) as mobile phase. The flow rate was 1 mL/min and the detection wavelength was 304 nm. The limit of detection (LOD) for Rilpivirine was found be 2.35 µg/ml and the limit of quantification (LOQ) for Rilpivirine was found be 7.13 µg/ml.

Keywords: Rilpivirine, RP-HPLC, Agilent Zorbax Bonus-RP column, LOD and LOQ.

INTRODUCTION

Rilpivirine (RLP) sold under the brand name Edurant, is a medication developed by Tibotec for the HIV-1 infection in antiretroviral treatment naive patients. It is a second generation non-nucleoside reverse transcriptase inhibitor (NNRTL) with higher potency, longer half life and reduced side effects, is safe and tolerable as compared to other NNRTIs like nevirapine, efavirenz and etravirine.(1) It shows in-vitro activity against wild type HIV-14 NNRTI resistant mutants including K103N. Industrialized world treatment guidelines recommend Rilpivirine as initial or alternative therapy in patients whose baseline HIV RNA is less than 100000 copies/ml.(5)

It is a diaryl pyrimidine derivative, 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. It gives very high potency due to internal conformational flexibility and plasticity of Rilpivirine compared to other NNRTIs (2)

Rilpivirine is a poorly soluble drug with intermediate in-vitro permeability. Logarithm of partition coefficient [log P(octanol/water)] value of Rilpivirine is 5.47 and it comes under BCS class II drug.(6) The solubility and systemic absorption as pH dependant and an increased bioavailability is demonstrated in an acidic environment.

Rilpivirine has a long terminal half life allowing for once daily dosing and is therefore a suitable choice for treatment. Rilpivirine 25mg once daily is the only dose licensed by USFDA due to suspected QT interval problems with higher doses in phase I and II studies (3)

After oral administration the maximum plasma concentration is achieved within 4-5 hours, with a terminal half life of 34-55 hours. Rilpivirine is eliminated through the urine as unchanged form and it is inducer of of cytochrome P450, (CYP) 3A4. CYP3A enzyme metabolized Rilpivirine and inhibition of this enzyme affect clearance of Rilpivirine.(4)

MATERIAL AND METHOD

Instrument: Agilent 1260 infinity II with diode array detector

Chemicals and Reagent: Water, and acetonitrile of HPLC Grade were used for the work and Rilpivirine was provided as gift sample from Chiral Biosciences LTD. All the chemicals were analytical grade.

Chromatographic Condition:

a) Oven Temp: 30°C
b) Flow rate: 1 ml/min.
c) Mobile Phase: Buffer : ACN (20:80)
   i. Buffer: 10 Mmol/L Sodium Dihydrogen Phosphate.

Preparation of Buffer:

1.2 gms of Sodium Dihydrogen Phosphate was dissolved in 1000 ml HPLC Water and the pH was adjusted to 3.0 with ortho-phosphoric acid. Filtered twice through 0.45 μ nylon Membrane filter and degased for 15 min.

a. Runtime: 09 minutes
b. Injection Volume: 20μl
c. Wavelength: 304 nm
d. Diluent: 50%-Buffer 50%-ACN
e. Column : Agilent Zorbax Bonus-RP (250 x 4.6 mm, 5 μ)

Standard Preparation:

a. Standard Stock Solution-I (SSS-I):

Initially Prepare a Standard Stock Solution (SSS-I) of by adding 5 mg of Rilpivirine in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Rilpivirine = 500 μg/ml).

b. Then add 1.0 ml of SSS-I in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Rilpivirine =50 μg/ml).

Tablet Sample Preparation for Assay:

a. Tablet Sample Solution (TSS):

i. 10 tablets were weighed and average weight was calculated. And tablets were crushed in mortar and pestle.

ii. Powder Weight equivalent to 5mg Rilpivirine was weighed into 10 ml volumetric flask & add 5 ml diluent, Sonicate for 10 minutes and make the volume to 10 ml with diluent. (Conc. of Rilpivirine = 500 μg/ml).

iii. Pipette out 1.0 ml from above solution in a 10 ml Volumetric flask and dilute it upto the mark with diluent. (Conc. of Rilpivirine = 50 μg/ml).

Method Validation:

a. Specificity & Assay:

i. Individual samples of Rilpivirine were prepared of 50μg/ml, respectively and peaks were for identified from Retention Time.

ii. Blank was injected to ensure there is no blank peak interfering with the main analyte peaks.

b. Instrument Precision & System Suitability:

i. A single sample was prepared as described and 5 injections were made from same sample and checked for system suitability.

ii. System suitability parameters are as below:

1. Retention Time,
2. Theoretical plates,
3. Asymmetry (Tailing factor),

c. Linearity & Range:

i. 5 samples of varying concentrations ranging from 80-120% were made.

ii. The concentrations are given below

% Level	Rilpivirine Conc. (μg/ml)
80	40
90	45
100	50
110	55
120	60

iii. The sample preparations are given as below;

iv. X ml of Rilpivirine were added to 10 ml diluent to make up the concentrations given above:

d. Accuracy:

i. Samples were prepared of 80%, 100% and 120% concentration by spiking the same amount of concentration given above in table for Rilpivirine.

ii. Samples were injected in duplicate to calculate % RSD.

iii. % recovery was also calculated.

e. LOD/ LOQ:

i. Was calculated for both drugs by using ANOVA technique.

ii. Formula:

$$LOD = \frac{3.3 \times Std.Error\ of\ Intercept}{Coefficients\ of\ X\ Variable\ 1}$$

$$LOQ = \frac{10 \times Std.Error\ of\ Intercept}{Coefficients\ of\ X\ Variable\ 1}$$

RESULT AND DISCUSSION

Method Development

The selection of mobile phase was based on peak parameters like asymmetry, theoretical plates, and Retention time. reverse phase Zorbax Bonus RP (250 X 4.6 mm, 5μ) column. The mobile phase containing 10 Mmol/L Sod. Dihydro. Phosphate pH 3.0 with OPA: ACN (20:80) was used at a flow rate of 1ml/min. The optimum wavelength for detection and quantification was at 304 nm, at which good detector response was obtained

Table 1: Method development

Sr. No.	MP	Ratio	Diluent	Column	Wavelength (nm)	RT (min.)	TP	Asymmetry
1	10 Mmol/L Sod. Dihydro. Phosphate pH 3.0 with	50-50	50% 10Mmol/L Sod. Dihydro. Phosphate pH 3.0	Zorbax Bonus RP (250 X 4.6 mm, 5μ)	280	8.24	3131	1.38

	OPA: ACN		with OPA: 50% ACN					
2	10 Mmol/L Sod. Dihydro. Phosphate pH 3.0 with OPA: ACN	40-60	50% 10Mmol/L Sod. Dihydro. Phosphate pH 3.0 with OPA: 50% ACN	Zorbax Bonus RP (250 X 4.6 mm, 5μ)	280	6.97	6789	1.11
3	10 Mmol/L Sod. Dihydro. Phosphate pH 3.0 with OPA: ACN	30-70	50% 10Mmol/L Sod. Dihydro. Phosphate pH 3.0 with OPA: 50% ACN	Zorbax Bonus RP (250 X 4.6 mm, 5μ)	280	4.82	8588	1.05
4	10 Mmol/L Sod. Dihydro. Phosphate pH 3.0 with OPA: ACN	20-80	50% 10Mmol/L Sod. Dihydro. Phosphate pH 3.0 with OPA: 50% ACN	Zorbax Bonus RP (250 X 4.6 mm, 5μ)	280	4.11	9864	1.08
5	10 Mmol/L Sod. Dihydro. Phosphate pH 3.0 with OPA: ACN	20-80	20% 10Mmol/L Sod. Dihydro. Phosphate pH 3.0 with OPA: 80% ACN	Zorbax Bonus RP (250 X 4.6 mm, 5μ)	280	4.11	9484	1.06
6	10 Mmol/L Sod. Dihydro. Phosphate pH 3.0 with OPA: CAN	20-80	50% 10Mmol/L Sod. Dihydro. Phosphate pH 3.0 with OPA: 50% ACN	Zorbax Bonus RP (250 X 4.6 mm, 5μ)	304	4.11	9874	1.08

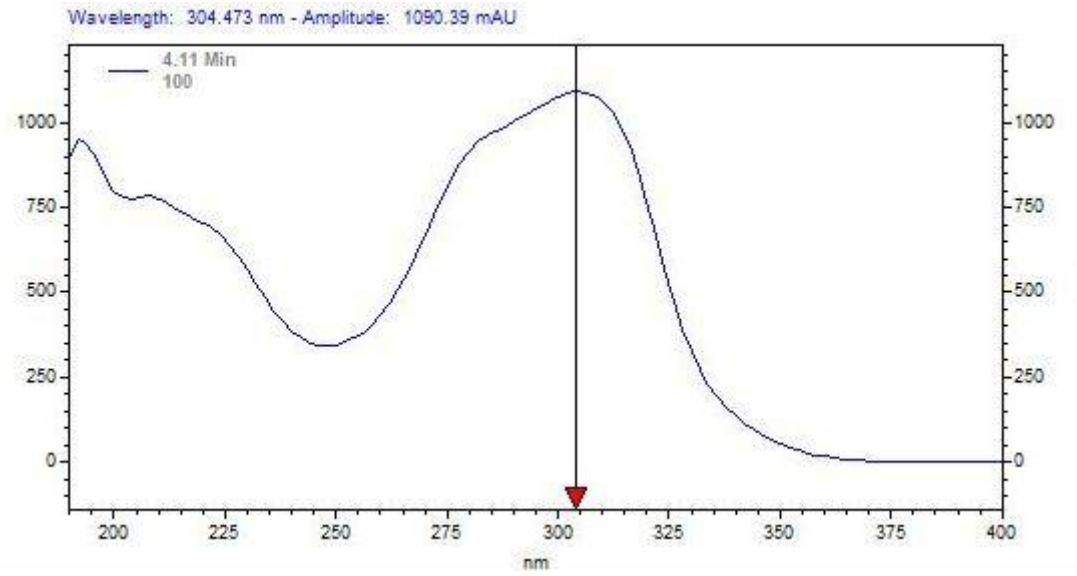

Fig 1: Selection of Waveleght

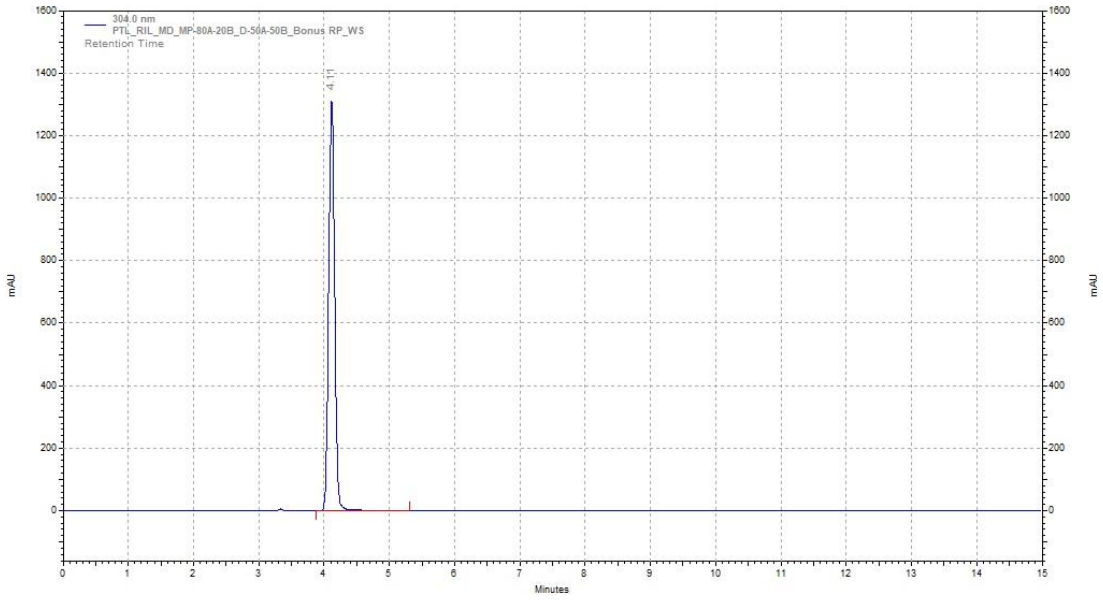

Fig 2: Chromatogram of Rilpivirine

Method validation

The method was validated as per ICH guidelines, using parameters specificity, assay, linearity, range, precision, accuracy, limit of detection and limit of quantification.

Specificity & Assay:

Specificity was used to check whether there is any interference of any impurities in retention time of analytical peak. The assay of Rilpivirine was found to be 99.99%.

Table 2: Assay of Rilpivirine

Sample ID	Area	% Assay
WS	11950109	-
DP	11948330	99.99

Linearity and range

The calibration curves were linear over the concentration range of 40-60µg/ml. Graph was plotted of Peak area against concentration. The linear regression equation is Y=23977x-13948

and Correlation coefficients were found to be 0.999. The results are given in Table

Table 3: Linearity of Rilpivirine at various level

% Level	Conc (ug/ml)	Area
80	40	9578141
90	45	10763515
100	50	11951410
110	55	13250963
120	60	14328660

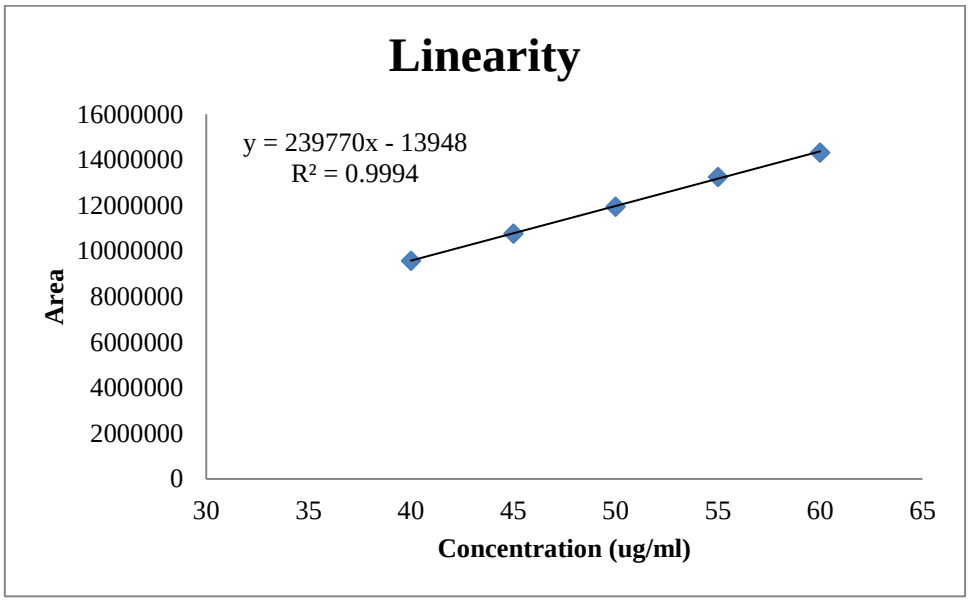

Fig 3: Graph of Linearity

Instrument Precision & System Suitability:
The precision of the analytical method was studied by multiple sampling of the homogenous sample. The %RSD values were found to be 0.04 and getting low RSD values indicate that the method is precise. The results are given in Table

Table 4: Precision of Rilpivirine

Sample ID	Area
100%_Rep 1	11951410
100%_Rep 2	11943827
100%_Rep 3	11949697
100%_Rep 4	11948791
100%_Rep 5	11956818
Average	11950109
Std Dev.	4692.379
% RSD	0.04

System suitability parameters like number of theoretical plates (N), peak asymmetry factor (As), tailing factor etc., were studied. The results are given in Table

Table 5: System suitability parameter

Sample ID	RT	TP	Asymmetry
100%_Rep 1	4.11	9918	1.08
100%_Rep 2	4.11	9951	1.05
100%_Rep 3	4.11	9903	1.10
100%_Rep 4	4.11	9971	1.07
100%_Rep 5	4.11	9970	1.06
Average	4.11		
Standard Deviation	0		

Accuracy:
Recovery studies were performed out by determining level of Rilpivirine sample with addition of standard concentration of Rilpivirine at 80%, 100% and 120% of label claim. At each level of the amount three determinations were performed. The results are given in Table

Table 6: Recovery Studies

Sample ID	Reps	Conc (ug/ml)	Area	Amount Recovered (ug/ml)	% Recovery	Avg	STDEV	% RSD
80%	Rep 1	39.99	9578141	40.06	100.19	100.17	0.02252	0.02
	Rep 2	39.99	9575096	40.05	100.16			
100%	Rep 1	49.99	11951410	49.99	100.01	99.98	0.04487	0.04
	Rep 2	49.99	11943827	49.96	99.95			
120%	Rep 1	59.98	14328660	59.93	99.92	99.92	0.00601	0.01
	Rep 2	59.98	14327441	59.93	99.91			

LOD/ LOQ:
LOD and LOQ decide about the sensitivity of the method. LOD is the lowest detectable concentration of the analyte by the method while LOQ is the minimum quantifiable concentration. LOD and LOQ were calculated by standard calibration curves. The results are given in Table

Table 7: LOD & LOQ of Rilpivirine

LOD	2.35	ug/ml
LOQ	7.13	ug/ml

CONCLUSION

The new, simple, reliable method was developed for the estimation of Rilpivirine in bulk and its oral dosage form. The chromatographic conditions were successfully developed by Zorbax Bonus RP (250 X 4.6 mm, 5μ) column and mobile phase containing 10 Mmol/L Sod. Dihydro. Phosphate pH 3.0 with OPA: ACN (20:80). The new method is accurate, precise, economical with lower limit of detection and quantification. The good recovery study suggests that the method could be applied efficiently for the estimation of Rilpivirine. This method can be used for routine analysis of the Rilpivirine.

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Conflict of interest:
Nil

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