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RP-HPLC method development & validation of rilpivirine pharmaceutical dosage form

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ABSTRACT

A simple, rapid, precise, accurate and sensitive reverse phase liquid chromatographic method has been developed for the determination of Rilpivirine in bulk and pharmaceutical dosage form dosage form. The chromatographic method was standardized using Develosil ODS HG-5 RP C18, 5µm, 15cm x 4.6mm i.d. column with UV detection at 205 nm and 0.1% Ortho phosphoric acid and Acetonitrile with 65:35 ratio at a flow rate of 1.0 ml/ min. The proposed method was successfully applied to the determination of Rilpivirine in bulk and pharmaceutical dosage form. The method was linear over the range of 20-70µg/ml. The recovery was in the range of 98% to 102% and limit of detection was found to be 0.8 µg/ml and quantification was found to be 2.4 µg/ml. Different analytical performance parameters such as precision, accuracy, limit of detection, limit of quantification and robustness were determined according to International Conference on Harmonization (ICH) guidelines.

Keywords: RP-HPLC, Rilpivirine, Method development and validation, ICH Guidelines.

INTRODUCTION

Rilpivirine is non-nucleoside reverse transcriptase inhibitor (NNRTI) which is used for the treatment of HIV-1 infections in treatment-naive patients.5 It is a diarylpyrimidine derivative, a class of molecules that resemble pyrimidine nucleotides found in DNA. [1-6] The internal conformational flexibility of rilpivirine and the plasticity of it interacting binding site gives it a very high potency and an unlikely generation of resistance compared to other NNRTI's. [7-10] Rilpivirine was developed by Tilbotec, Inc. and FDA approved on May 20, 2011.10 On November 21, 2017, Rilpivirine, in combination with dolutegravir, was approved as part of the first complete treatment regimen with only two drugs for the treatment of adults with HIV-1 named Juluca.11 [11-16]

The IUPAC Name of Rilpivirine is 4-{[4-({4-[(1E)-2-cyanoeth-1-en-1-yl]-2,6-dimethylphenyl}amino)pyrimidin-2-yl]amino}benzonitrile [17-21]

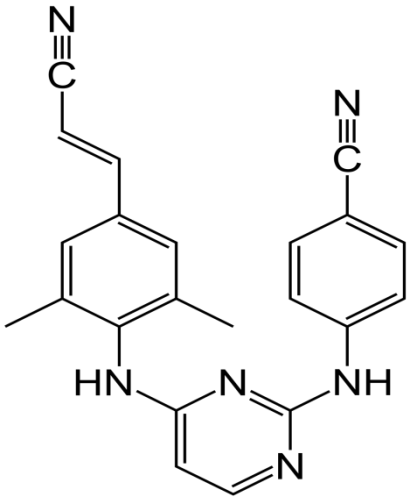


Fig 1: Chemical Structure of Rilpivirine [22-25]

MATERIALS AND METHODS

HPLC Instrumentation & Conditions

The HPLC system employed was HPLC with Empower2 Software with Isocratic with UV-Visible Detector.

Standard & sample preparation for UV-spectrophotometer analysis

25 mg of Rilpivirine standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.2 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase. The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Rilpivirine, so that the same wave number can be utilized in HPLC UV detector for estimating the Rilpivirine. While scanning the Rilpivirine solution we observed the maxima at 205nm. The UV spectrum has been recorded on T60-LABINDIA make UV – Vis spectrophotometer model UV-2450.

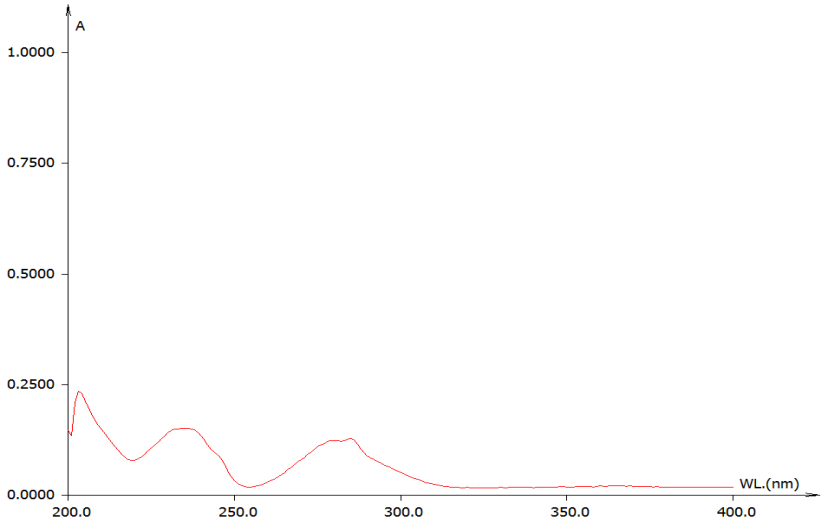


Fig 2: UV Spectrum

Optimized Chromatographic Conditions

Column: Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.
Mobile Phase: 0.1% Ortho phosphoric acid and Acetonitrile (65:35 v/v).
Flow Rate: 1.0ml/minute
Wave length: 205 nm
Injection volume: 20µl
Run time: 08 mins.
Column temperature: Ambient
Sampler cooler: Ambient

MOBILE PHASE PREPARATION

Mobile phase was prepared by taking 0.1% Ortho phosphoric acid and Acetonitrile (65:35 v/v). Mobile phase was filtered through 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min.

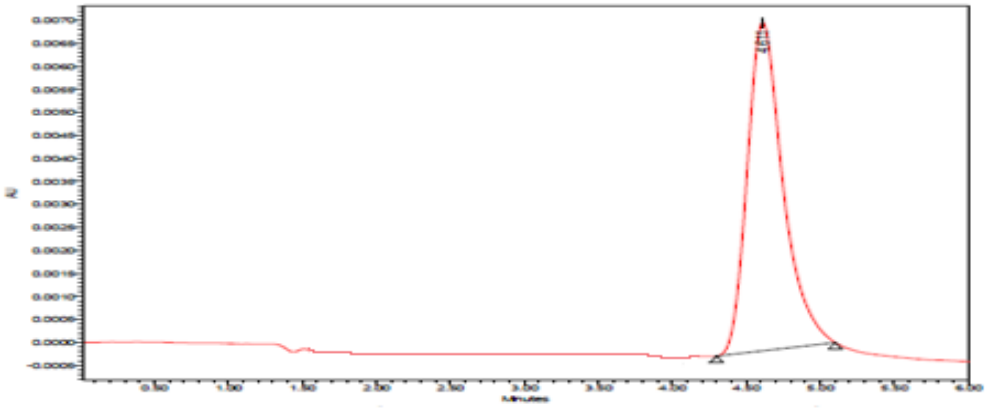
SAMPLE & STANDARD PREPARATION FOR THE ANALYSIS

25 mg of Rilpivirine standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

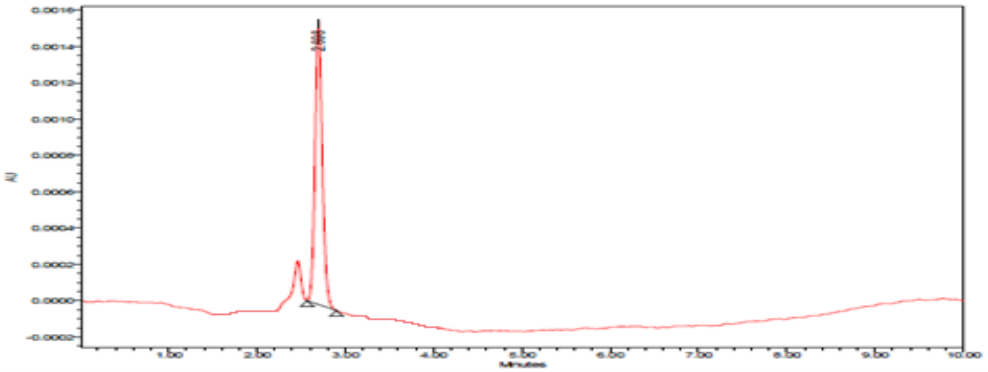
RESULT AND DISCUSSION

Table-1: Trials for method development

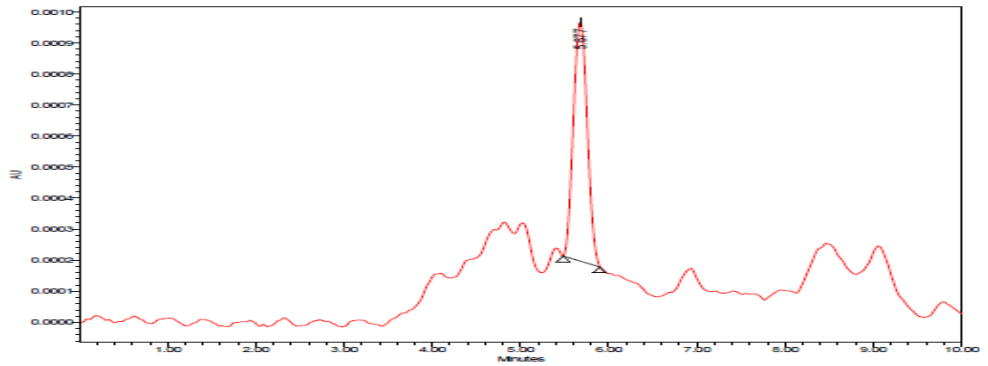
Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.	ACN : Water = 20 : 80	1.0 ml/min	205 nm	Low response	Method rejected
Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.	Methanol: water = 30 : 70	1.0 ml/ min	205 nm	Tailing peak	Method rejected
Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.	ACN: phosphate buffer (pH=3.8)= 50:50	1.0 ml/min	205 nm	Broad Peak	Method rejected
Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.	ACN : phosphate buffer (pH=3.5) = 40:60	1.0 ml/min	205 nm	Tailing peak	Method rejected
Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.	0.1% Ortho phosphoric acid and Acetonitrile = 65:35	1.0 ml/min	205 nm	Nice peak	Method accepted



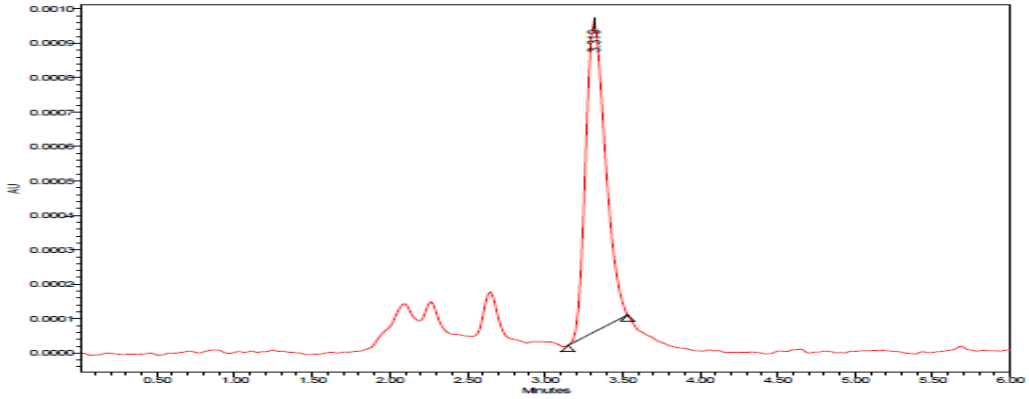
Trial-1



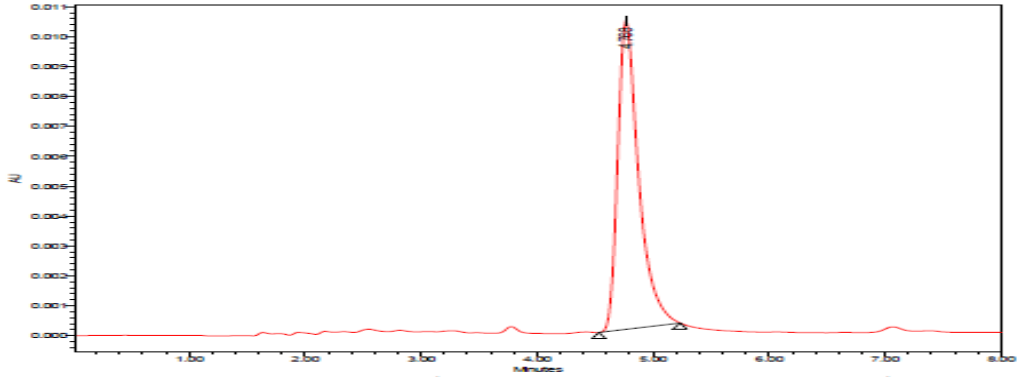
Trial-2



Trial-3



Trial-4



Trial-5

Table 2: Peak results

RT	Peak Area	Tailing Factor	Plate count
4.768	1026862	1.15	3652

METHOD VALIDATION

Accuracy: Recovery study

To decide the exactness of the proposed strategy, recuperation thinks about were completed by including diverse sums (80%, 100%, and 120%) of unadulterated medication of Rilpivirine were taken and added to the pre-dissected detailing of fixation 50µg/ml. From that rate recuperation esteems were ascertained. The outcomes were appeared in Table-3.

Table-3: Accuracy Readings

S. No.	Pure drug	Peak Area	Conc. Found	% Recovery of Pure drug	Statistical analysis
S_1 : 80 %	40	3595426	39.42	98.55	Mean= 98.515
S_2 : 80 %	40	3623514	39.73	99.325	S.D. = 0.828055
S_3 : 80 %	40	3563483	39.07	97.67	R.S.D.= 0.840537%
S_4 : 100 %	50	4629039	50.81	101.62	Mean= 99.5267
S_5 : 100 %	50	4471363	49.07	98.14	S.D. = 1.844487
S_6 : 100 %	50	4501884	49.41	98.82	R.S.D.= 1.853259%
S_7 : 120 %	60	5384304	59.12	98.533	Mean= 99.79967
S_8 : 120 %	60	5484934	60.23	100.383	S.D. = 1.098104
S_9 : 120 %	60	5490235	60.29	100.483	R.S.D. = 1.100309%

PRECISION

Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Rilpivirine (API) the percent relative standard deviations were calculated for Rilpivirine is presented in the Table-4.

Table-4: Repeatability Results of Precision

HPLC Injection Replicates of Rilpivirine	Retention Time	Peak Area
Replicate – 1	4.765	1024568
Replicate – 2	4.767	1025433
Replicate – 3	4.768	1024578
Replicate – 4	4.768	1032541
Replicate – 5	4.773	1021023
Replicate – 6	4.768	1047812
Average	4.768166667	1029326
Standard Deviation	0.002639	9811.684
% RSD	0.055355	0.953215

Intra day & Inter day

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Rilpivirine revealed that the proposed method is precise.

Table-5: Results of Intra day & Inter day

Conc. Of Rilpivirine (API) (µg/ml)	Observed Conc. Of Rilpivirine (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
40	39.46	0.82	60.28	0.98
50	49.26	0.42	49.53	0.23
60	60.51	0.13	49.59	0.33

Linearity and Range

Linearity range was found to be 20-70µg/ml for Rilpivirine. The correlation coefficient was found to be 0.999, the slope was found to be 90803 and intercept was found to be 15229 for Rilpivirine.

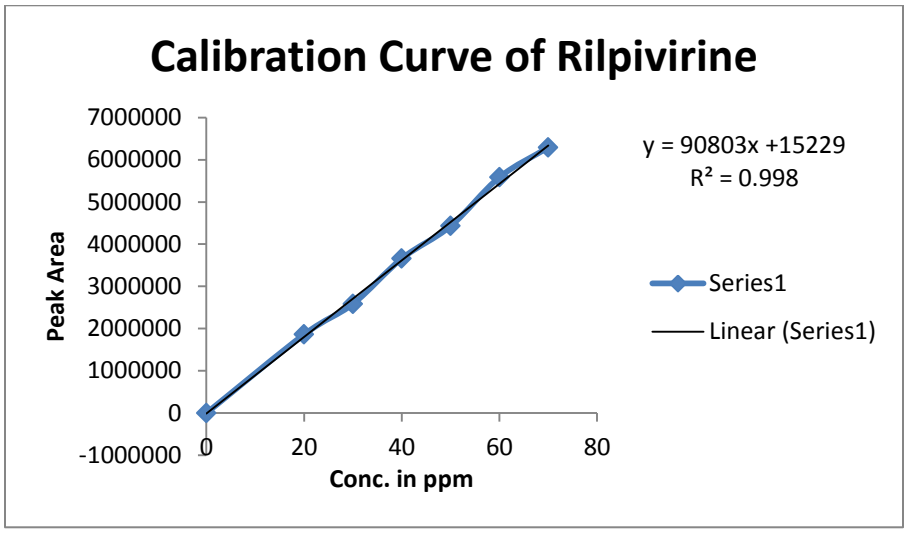


Fig-3: Calibration curve of Rilpivirine (API)

Table-6: Linearity Results of Rilpivirine

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
20	1861111
30	2584922
40	3659543
50	4429039
60	5584304
70	6291175

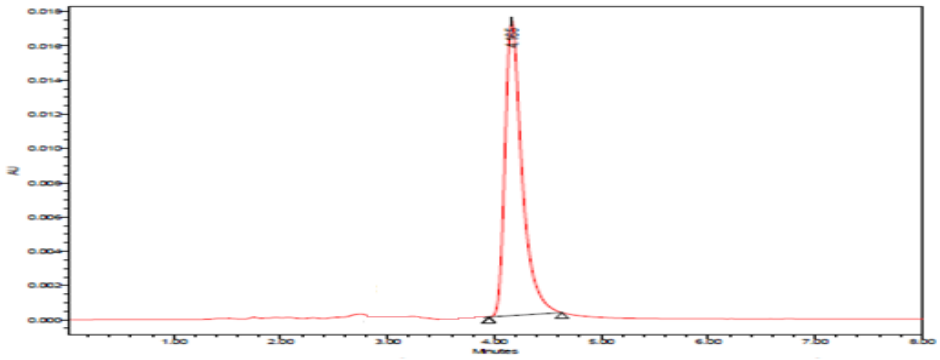


Fig 4: Calibration of Rilpivirine concentration in 20 ppm

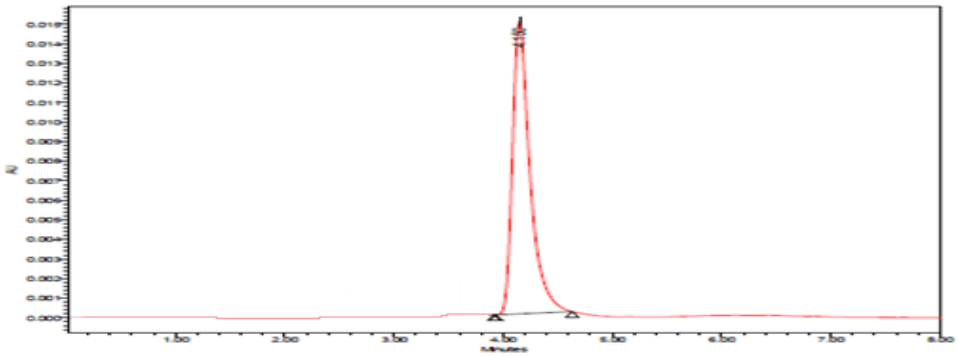


Fig 5: Calibration of Rilpivirine concentration in 30 ppm

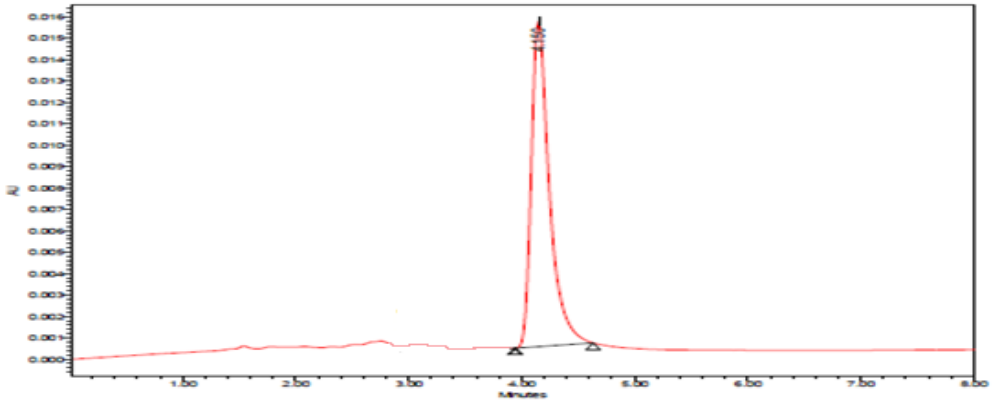


Fig 6: Calibration of Rilpivirine concentration in 40 ppm

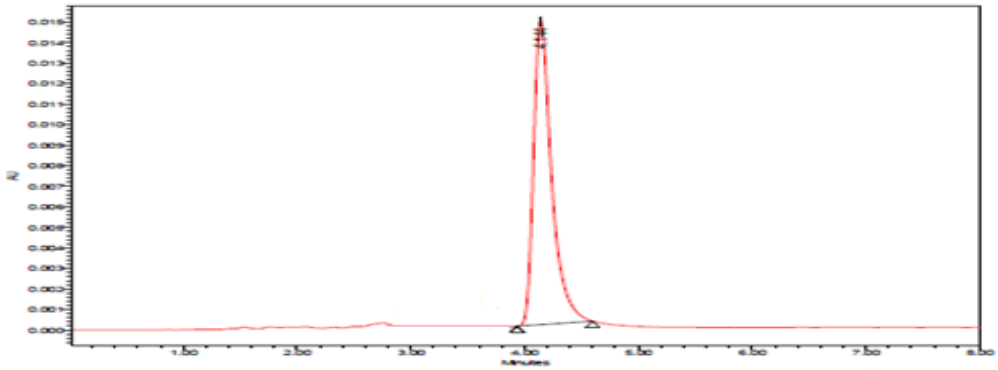


Fig 7: Calibration of Rilpivirine concentration in 50 ppm

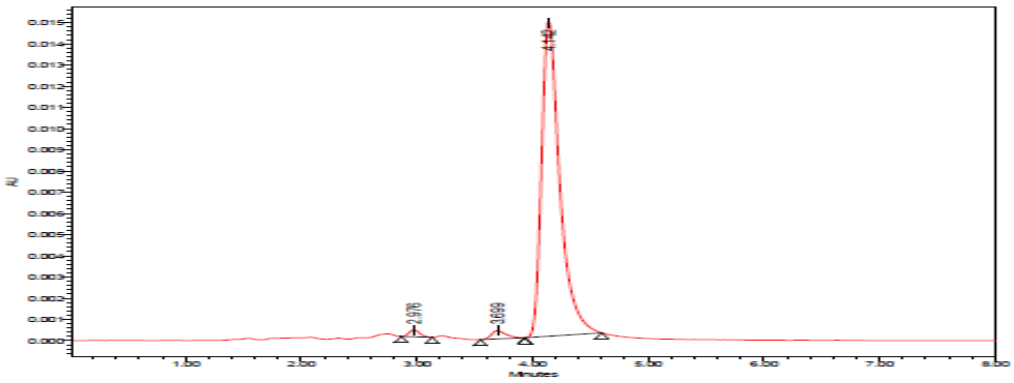


Fig 8: Calibration of Rilpivirine concentration in 60 ppm

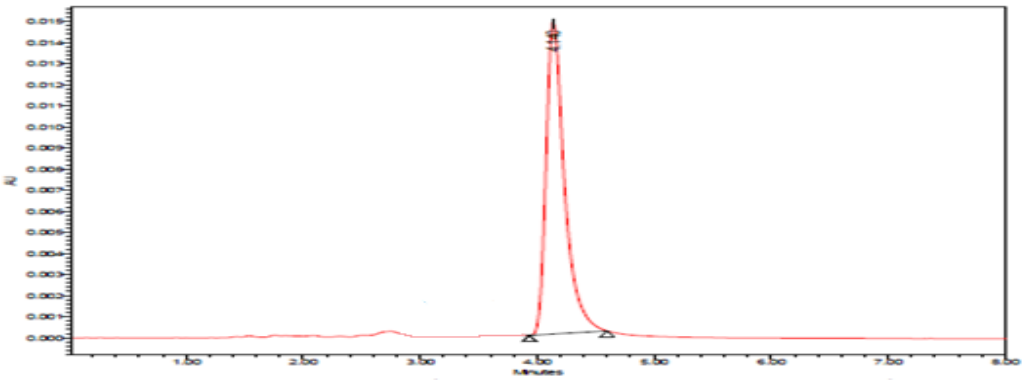


Fig 9: Calibration of Rilpivirine concentration in 70 ppm

LOD & LOQ

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.8 & 2.4 µg/ml respectively.

CONCLUSION

A delicate and specific, sensitive RP-HPLC strategy has been created and approved for the investigation of Rilpivirine API. Facilitate the proposed RP-HPLC strategy has amazing affectability, exactness and reproducibility.

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