INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN:2320-2831

IJPAR |Vol.6 | Issue 1 | Jan - Mar -2017
Journal Home page: www.ijpar.com

Research article *Open Access*

RP-HPLC method development and validation of Rilpivirine

Dr.A.Yasodha*[1], J. Rani[1], G.Venkataih[1], A.Sivakumar[2]

[1]*Dhanvanthri College of Pharmaceutical Sciences, Mahabubnagar- 509002, Telangana, India.*
[2]*AurobindoPharma Limited, Unit –VII, Jadcherla, Hyderabad.*

***Corresponding Author: Dr.A.Yasodha**
Email: yyasodhasivakumar@gmail.com

ABSTRACT

A simple, accurate, rapid, and stability-indicating RP-HPLC method for a Rilpivirine has been developed and subsequently validated in commercial tablets. The proposed HPLC method utilizes Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm and mobile phase consisting of ACN : Acetate buffer (pH=4.0) = 65:35 (v/v) at a flow rate of 1.0 ml/min. Quantitation was achieved with UV detection at 260nm. The method was validated in terms of accuracy, precision, linearity, limits of detection, limits of quantitation, and robustness. This optimized method has been successively applied to pharmaceutical formulation and no interference from the tablet excipients was found. Rilpivirine drug products were subjected to acid, base, neutral hydrolysis, oxidation, dry heat, and photolytic stress conditions and the stressed samples were analyzed by the proposed method. As the proposed RP-HPLC method could effectively separate the drugs from its degradation products, it can be employed as stability-indicating method for the determination of instability of these drugs in bulk and pharmaceutical dosage form.

Keywords: HPLC, Method development, Validation, Reverse Phase, Rilpivirine.

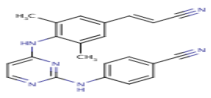
INTRODUCTION

The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one [1]. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs [2-5].

Chromatographic techniques [6-8] are dynamic processes wherein two mutually immiscible phases are brought into contact; one phase is stationary and the other mobile phase. A liquid mobile phase is pumped under pressure through a stainless steel column containing particles of stationary phase

with a diameter of 3-10 µm. The analyte is loaded onto the head of the column via a loop valve and separation of a mixture occurs according to the relative lengths of time spent by its components in the stationary phase. Components with the least affinity for the stationary phase emerge or elute first whereas the components with greater affinity for stationary phase elute last. Monitoring of the column effluent can be carried out with a variety of detectors. The aim of the proposed method is to develop simple and accurate methods for the determination of Rilpivirine by RP-HPLC method in pharmaceutical dosage forms. This new method was successfully developed and validated as per ICH guidelines [9-10], can be utilized for the validation of Rilpivirine in pharmaceutical dosage forms.

Rilpivirine is non-nucleoside reverse transcriptase inhibitor (NNRTI) which is used for the treatment of HIV-1 infections in treatment-naive patients. It is a diarylpyrimidine, a class of molecules that resemble pyrimidine nucleotides found in DNA. Because of its flexible chemical structure, resistance of rilpivirine is less likely to develop than other NNRTI's. FDA approved on May 20, 2011.



IUPAC Name	4-{[4-({4-[(1E)-2-cyanoeth-1-en-1-yl]-2,6dimethylphenyl}amino)pyrimidin-2-yl]amino} benzonitrile
Chemical Formula	C22H18N6

MATERIALS AND METHODS

Table 2.1: List of chemicals and equipemnets

Chemicals	Equipments
Ammonium acetate	Analytical Balance
Dimethyl Sulfoxide(DMSO)	Sonicator
HPLC grade Water	HPLC
	UV-spectrophotometer

Standard & sample preparation for UV-spectrophotometer analysis

25 mg of Rilpivirine standard was transferred into 25 ml volumetric flask, dissolved in mobile phase & make up to volume with mobile phase. Further dilution was done by transferring 4 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 absorption maxima was 260 nm. The UV spectrum has been recorded on Elico, corp. make UV – Vis spectophotometer model UV-2450. The scanned UV spectrum is attached in

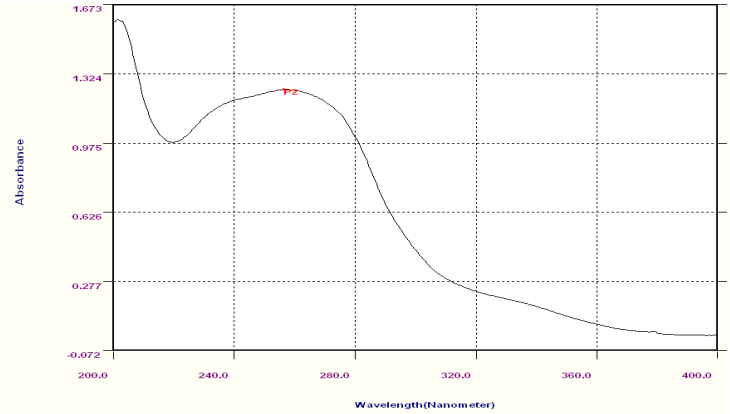


Fig -2.1.1: UV spectrum of Rilpivirine

Mobile Phase Preparation

The mobile phase used in this analysis consists of a mixture of acetate Buffer (pH adjusted to 4.2 with Glacial acetic acid) and Acetonitrile in a ratio of 40:60.

400 ml of this buffer solution was added and properly mixed with 600 ml of acetronitrile and a homogenous solution is achieved. This mobile phase was filled and sonicated for 15 minutes before using in the experiment.

Sample & Standard Preparation for the Analysis

25 mg of Rilpivirine standard was transferred into 25 ml volumetric flask, dissolved in DMSO & make up to volume with mobile phase. Further dilution was done by transferring 4 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

Method Development

Trails

Table no. 2.4.1.1:- Different trails used in 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 = 60 : 40	1.0 ml/min	260 nm	Did nt get any Peaks	Method rejected
Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.	ACN: water = 70 : 30	1.0 ml/min	260 nm	Pseudo peaks interfering main peak	Method rejected
Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.	ACN: phosphate buffer = 50 : 50	1.0 ml/ min	260 nm	Low response & Broad Peak	Method rejected
Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.	ACN : phosphate buffer (pH=2.2) = 40:60	1.0 ml/min	260 nm	Peak broadening	Method rejected
Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.	ACN : Acetate buffer (pH=4.0) = 65:35	1.0 ml/min	260 nm	Nice peak	Method accepted

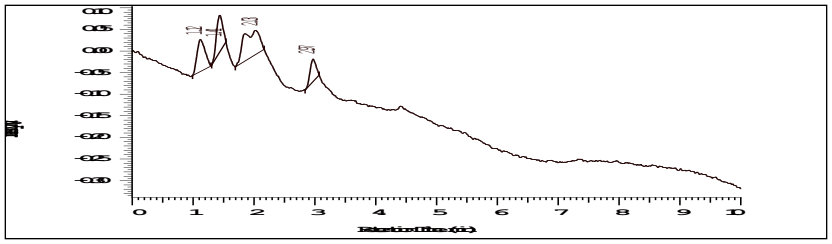


Fig -2.4.1.1:- Chromatogram of Trial-1

Table 2.4.1.2 Results of Trial-1

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.12	2153	2125	1.91
2	1.44	2586	2823	1.97
3	2.03	2453	2456	1.92
4	2.97	2354	1956	1.94

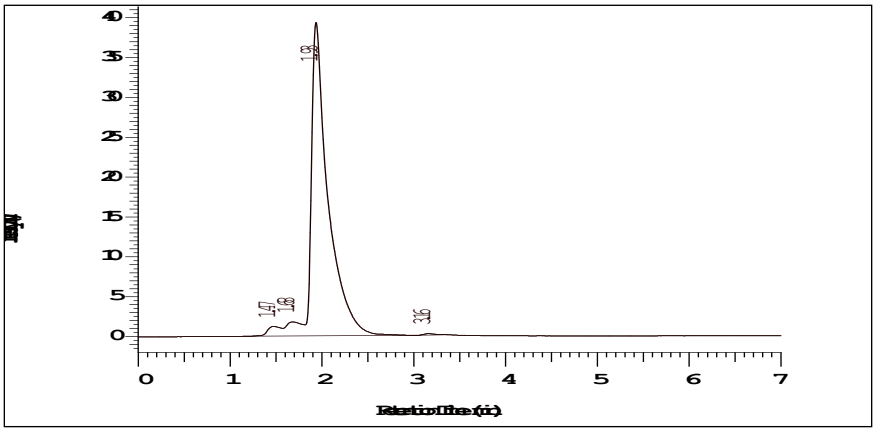


Fig - 2.4.1.2:- Chromatogram of Trial-2

Table 2.4.3.: Results of Trial-2

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.47	2365	2546	1.94
2	1.68	2658	2752	1.68
3	1.93	4745	3862	0.89
4	3.16	2214	1358	1.76

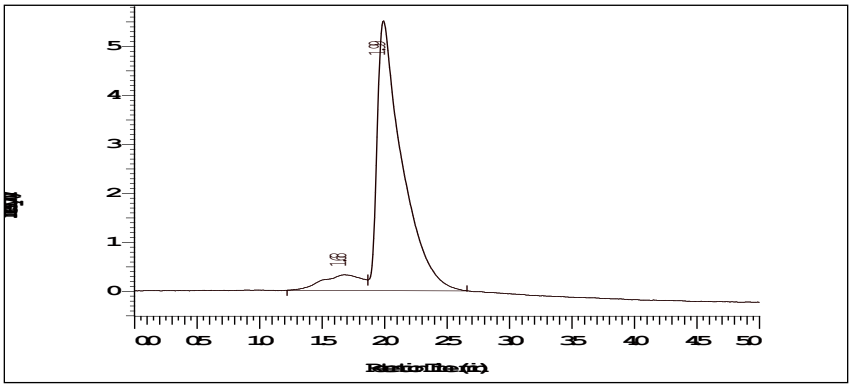


Fig- 2.4.1.3:- Chromatogram of Trial-3

Table 2.4.1.4: Results of Trial-3

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.68	2568	3254	1.91
2	1.99	2764	6587	0.95

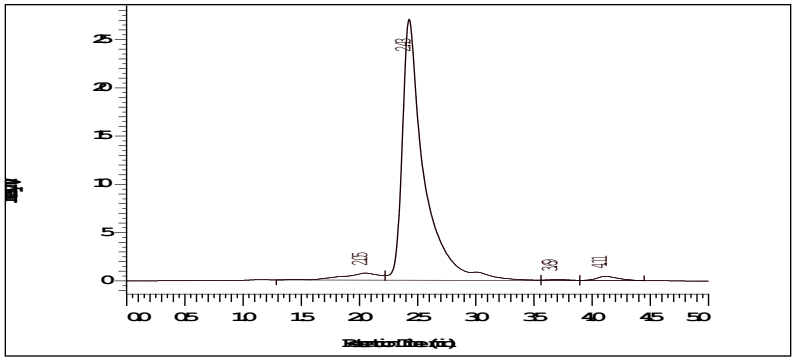


Fig- 2.4.1.4:- Chromatogram of Trial-4

Table 2.4.1.5: Results of Trial-4

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.47	2365	2365	1.84
2	1.68	6681	5462	0.74
3	1.93	2847	2684	1.28
4	3.16	2354	2364	1.59

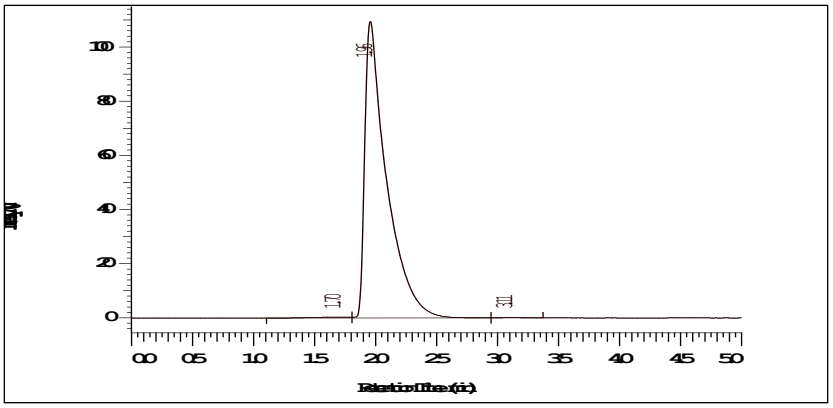


Fig- 2.4.1.5:- Chromatogram of Trial-5

Table 2.4.1.6: Results of Trial-5

S. no	Name	RT	Area
1	Rilpivirine	1.96	1678995

Optimized Chromatographic Conditions

Column : Develosil ODS HG-5 RP 150mm x 4.6mm 5µm particle size
Flow Rate : 1.0ml/minute
Wave length : 260nm
Injection volume : 20µl
Run time : 05minutes
Column temperature : 25^{0}C
Sampler cooler :Ambient

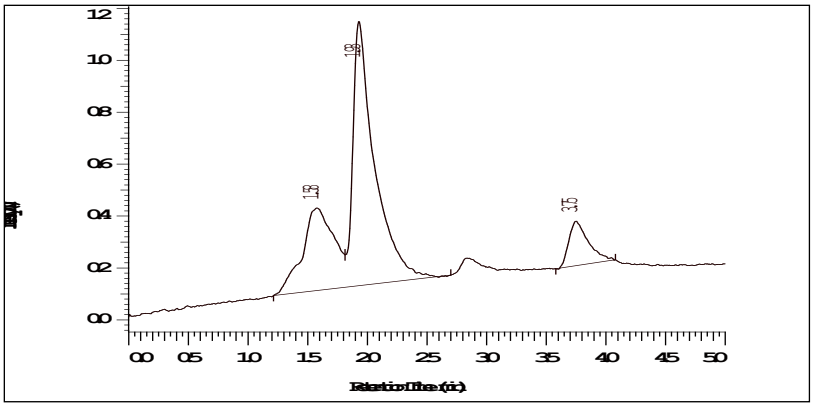


Fig -2.4.2.1:- Chromatogram for blank

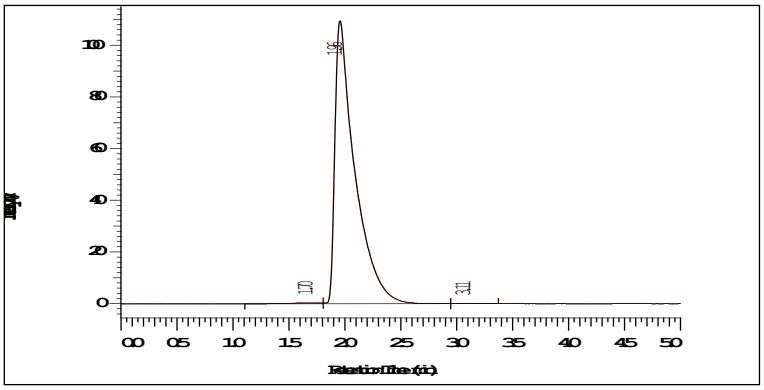


Fig-2.4.2.2: HPLC spectrum of Rilpivirine (40 ppm) in optimized conditions (RT 1.96 min.)

Table no 2.4.2.1: Peak results of optimised chromatogram

S. no	Name	RT	Area
1	Rilpivirine	1.96	1678995

Evaluation of system suitability

Perform the blank run by injecting 5µl of diluent and ensure that there is no interference with the main peak retention time. Inject 5µl of the standard solution for two times into the chromatograph and measure the peak responses. Calculate the % RSD for replicate injections of the standard solution and it should be less than 2.0%.

Procedure

5µl of placebo solution is injected and ensured that there is no interference. Then 5µl of sample solution is injected into the chromatograph and recorded the chromatograms. Now peak area responses are measured and the average peak responses are taken.

Calculations

Content of the drug =

$$At\backslash As \; x \; Sc\backslash Tc \; x \; LC\backslash 100 \; x \; Potency$$

Where,

At = Avg area responses of Rilpivirine obtained from the Sample preparation

As = Avg area responses of Rilpivirine obtained from the Standard preparation

Sc = Working standard concentration

Tc = Test sample concentration

LC = Label claim

RESULTS

Forced Degradation Studies

Following protocol was strictly adhered to for forced degradation of Rilpivirine Active Pharmaceutical Ingredient (API). The API (Rilpivirine) was subjected to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body.

This is one type of accelerated stability studies that helps us determining the fate of the drug that is likely to happen after along time storage, within a very short time as compare to the real time or long term stability testing.

The various degradation pathways studied are acid hydrolysis, basic hydrolysis, thermal degradation and oxidative degradation.

Acid hydrolysis

An accurately weighed 25 mg. of pure drug was transferred to a clean & dry 25 ml volumetric flask. To which 0.1 N Hydrochloric acid was added & make up to the mark & kept for 24 hrs. from that 0.1 ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase, then injected into the HPLC system against a blank of HCl (after all optimized conditions).

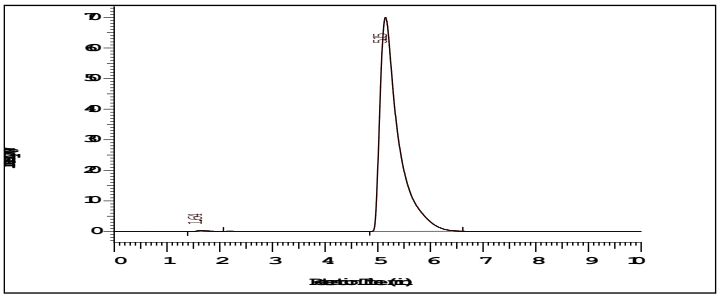


Fig -3.2.1: Chromatogram showing degradation for Rilpivirine in 0.1 N HCl

Table-3.2.1: Results of acid hydrolysis of Rilpivirine in 0.1 N HCL

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.64	1745	2548	1.59
2	5.15	2934	5687	0.17

Basic Hydrolysis

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 0.1 N Sodium hydroxide was added & make up to the mark & kept for 24 hrs. from that 4s ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase, then injected into the HPLC system against a blank of NaOH (after all optimized conditions).

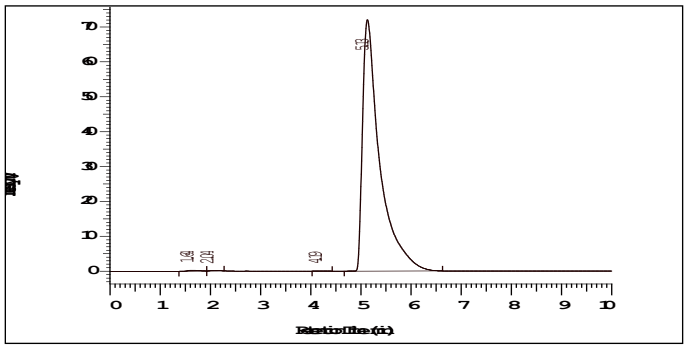


Fig -3.3.1: Chromatogram showing degradation related impurity in 0.1 N NaOH

Table-3.3.2: Results of basic hydrolysis of Rilpivirine in 0.1 N NaOH

Sl. No	**Rt**	**Theoretical Plates**	**Area**	**Tailing factor**
1	1.64	1685	2365	1.68
2	2.04	2156	2658	1.29
3	4.19	2654	2864	1.75
4	5.15	5698	4985	0.59

Thermal Degradation

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 100 ml volumetric flask, make up to the mark with mobile phase & was maintained at 50 0C. for 24 hrs then injected into the HPLC system against a blank of mobile phase (after all optimized conditions).

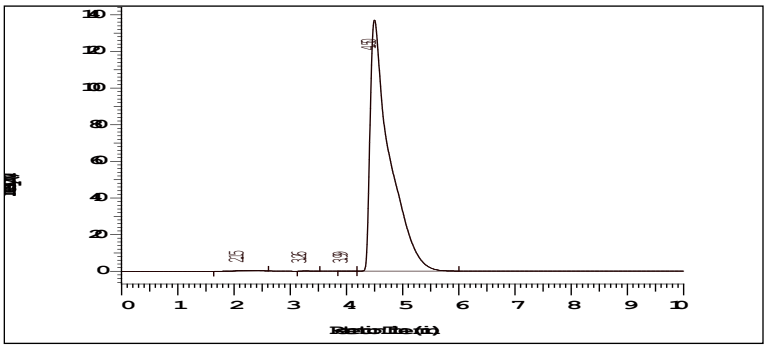


Fig -3.4.1: Chromatogram showing thermal degradation studies

Table-3.4.2: Results of thermal degradation of Rilpivirine

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	2.15	2416	2365	1.37
2	3.26	2568	2484	1.28
3	3.99	2654	2657	1.49
4	4.50	6598	6254	0.73

Photolytic degradation

Approximately 10 mg. of pure drug was taken in a clean & dry Petridis. It was kept in a UV cabinet at 254 nm wavelength for 24 hours without interruption. Accurately weighed 1 mg. of the UV exposed drug was transferred to a clean & dry 10 ml. volumetric flask. First the UV exposed drug was dissolved in methanol & make up to the mark than injected into the HPLC system against a blank of mobile phase (after all optimized conditions).

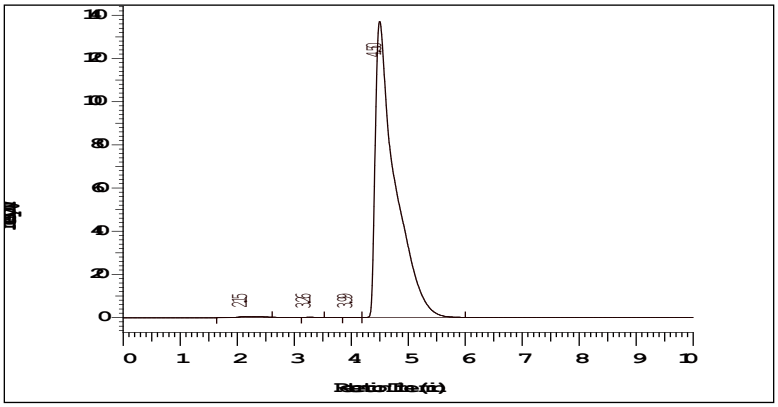


Fig -3.5.1: Chromatogram showing photolytic degradation.

Table-3.5.1: Results of photolytic degradation of Rilpivirine

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	2.15	2145	2365	1.38
2	3.26	2365	2654	1.49
3	3.99	2564	2846	1.53
4	4.50	6954	5942	0.81

Oxidation with (3%) h_2o_2

Accurately weighed 10 mg. of pure drug was taken in a clean & dry 100 ml. volumetric flask. 30 ml. of 3% H2O2 and a little methanol was added to it to make it soluble & then kept as such in dark for 24 hours. Final volume was made up to 100 ml. using water to prepare 100 ppm solution. The above sample was injected into the HPLC system.

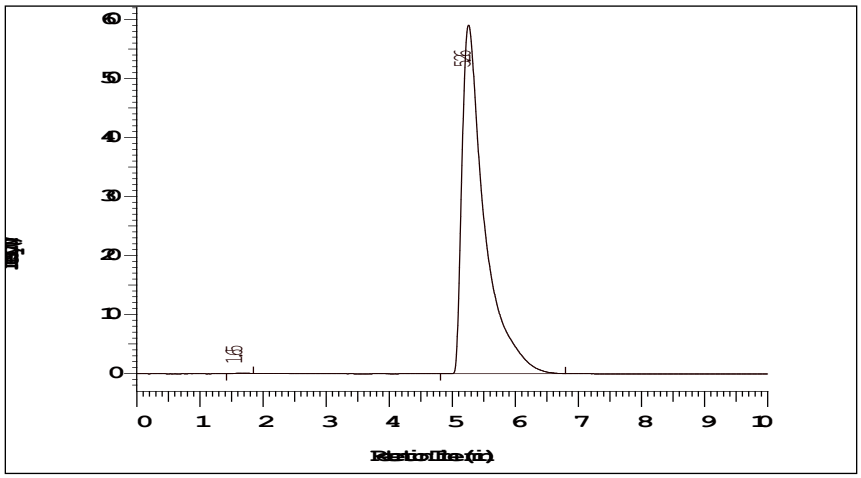


Fig -3.6.1: Chromatogram showing oxidative degradation.

Table-3.6.1: Results of oxidative degradation of Rilpivirine

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	2.15	2365	2564	1.85
2	3.26	5687	4685	0.73

Results of degradation studies

The results of the stress studies indicated the specificity of the method that has been developed. Rilpivirine was stable in photolytic & temperature stress conditions. The result of forced degradation studies are given in the following table-15.

Table-3.7.1: Results of force degradation studies of Rilpivirine API.

Stress condition	**Time**	**Assay of active substance**	**Assay of degraded products**	**Mass Balance (%)**
Acid Hydrolysis (0.1 M HCl)	24Hrs.	40.73	59.27	100
Basic Hydrolysis (0.I M NaOH)	24Hrs.	80.93	19.07	100
Thermal Degradation (50 ^{0}C)	24Hrs.	99.35	----------	99.35
UV (254nm)	24Hrs.	98.31	---------	99.31
3 % Hydrogen peroxide	24Hrs.	91.37	08.46	99.83

METHOD VALIDATION

Accuracy: *Recovery study*

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of RILPIVIRINE were taken and added to the pre-analysed formulation of concentration 10µg/ml. From that percentage recovery values were calculated. The results were shown in table-16.

Table-3.8.1: Accuracy Readings

STD			**Spike-1**			
Conc.	AUC		Conc	AUC	Diff	% Recovery
8	506881	1	18	1905481	1398600	99.14396
8	506881	2	18	1900430	1393549	98.7859
8	506881	3	18	1902331	1395450	98.92066
						98.95017
						0.180843
						0.182762

STD			**Spike-2**			
Conc.	AUC		Conc	AUC	Diff	% Recovery
10	1426346	1	20	2832999	1406653	99.71482
10	1426346	2	20	2834395	1408049	99.81378
10	1426346	3	20	2833499	1407153	99.75026
						99.75962
						0.050139
						0.05026

STD			**Spike-3**			
Conc.	AUC		Conc	AUC	Diff	% Recovery
12	1999858	1	22	3401595	1401737	99.36633
12	1999858	2	22	3400499	1400641	99.28864
12	1999858	3	22	3403358	1403500	99.49131
						99.38209
						0.10225
						0.102885

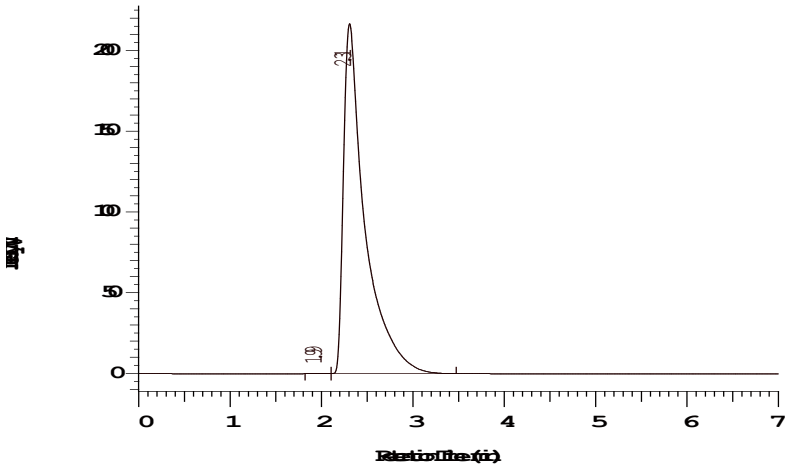


Fig -3.8.1:-Chromatogram for accuracy-1 Spike-1

Table-3.8.2: Accuracy Readings of accuracy-1 Spike-1

Sl. No	**Rt**	**Theoretical Plates**	**Area**	**Tailing factor**
1	1.99	2156	2654	1.59
2	2.31	4512	3984	0.73

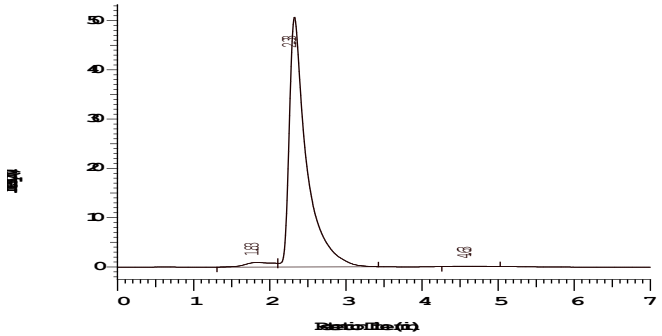


Fig-3.8.2:-Chromatogram for accuracy-1 Spike-2

Table-3.8.3: Accuracy Readings of accuracy-1 Spike-2

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.83	2641	2365	1.65
2	2.33	6954	5654	1.97
3	4.63	2021	2345	0.36

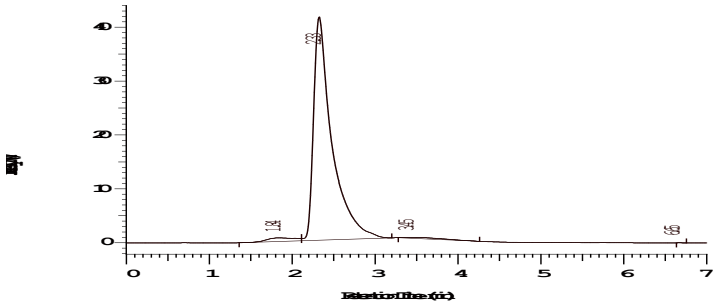


Fig-3.8.3:-Chromatogram for accuracy-1 Spike-3

Table-3.8.4: Accuracy Readings of accuracy-1 Spike-3

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.84	2364	2325	1.28
2	2.33	5461	4564	0.36
3	3.45	2021	2684	1.59
4	6.65	2465	2643	1.49

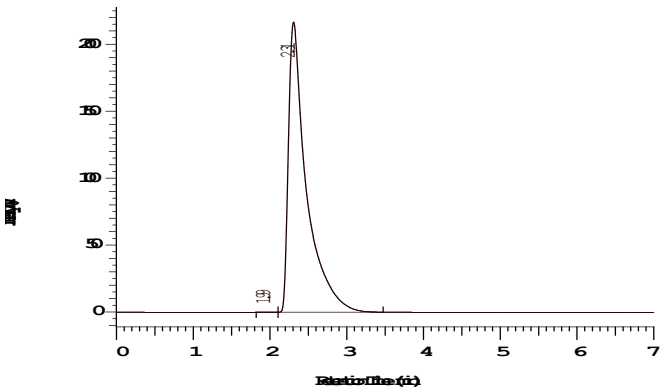


Fig-3.8.4:-Chromatogram for accuracy-2 Spike-1

Table-3.8.5: Accuracy Readings of accuracy-2 Spike-1

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.99	2685	2354	1.28
2	2.31	5684	4561	0.57

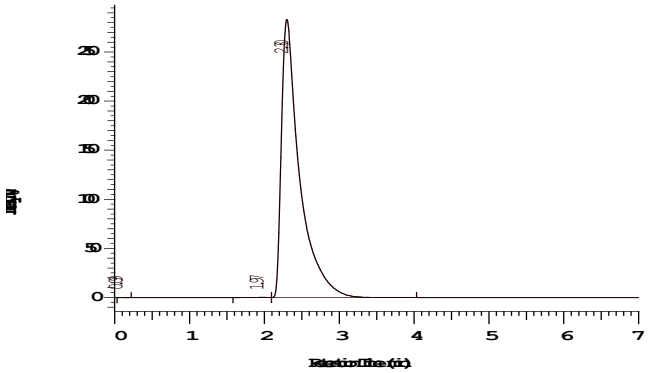


Fig-3.8.5:-Chromatogram for accuracy-2 Spike-2

Table-3.8.6: Accuracy Readings of accuracy-2 Spike-2

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	0.09	2657	2365	1.28
2	1.97	2634	2546	1.37
3	2.30	5478	4857	0.76

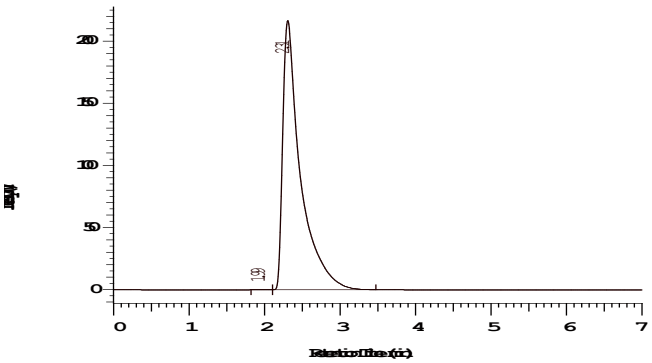


Fig-3.8.6:-Chromatogram for accuracy-2 Spike-3

Table-3.8.7: Accuracy Readings of accuracy-2 Spike-3

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.99	2654	2364	1.59
2	2.31	6587	5698	0.76

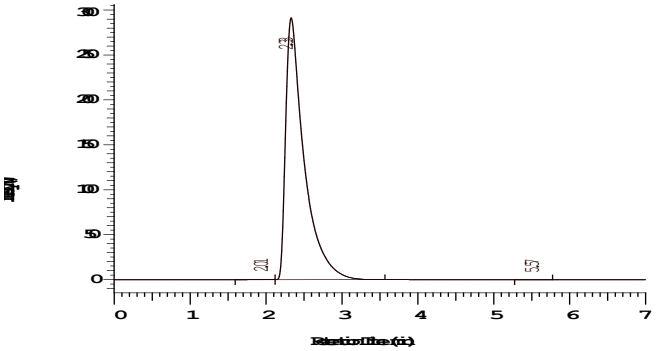


Fig-3.8.7:-Chromatogram for accuracy-3 Spike-1

Table-3.8.8: Accuracy Readings of accuracy-3 Spike-1

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	2.01	2354	2546	1.59
2	2.33	6356	5762	0.71
3	5.57	2021	2014	1.43

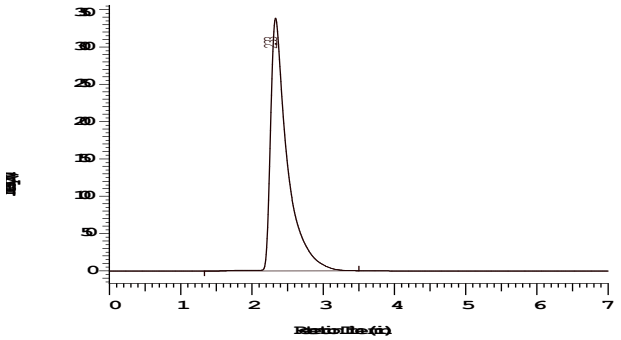


Fig-3.8.9:-Chromatogram for accuracy-3 Spike-2

Table-3.8.10: Accuracy Readings of accuracy-3 Spike-2

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	2.33	6514	5642	0.89

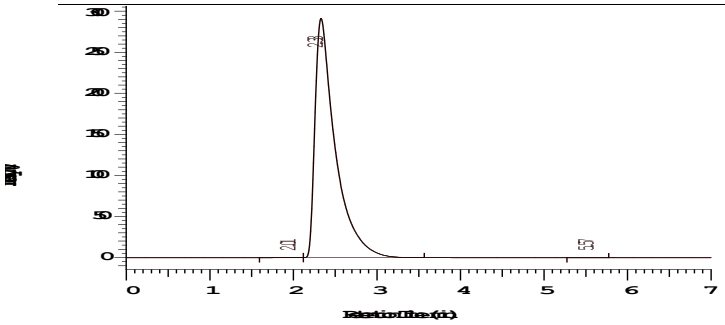


Fig-3.8.10:-Chromatogram for accuracy-3 Spike-3

Table-3.8.11: Accuracy Readings of accuracy-3 Spike-3

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	2.01	2461	2645	1.27
2	2.33	7546	6421	1.49
3	5.57	3164	3024	0.53

Precision

Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of five replicates of a fixed amount of drug. Rilpivirine (API). The percent relative standard deviations were calculated for Rilpivirine are presented in the table-26.

Table-3.8.2.1: Shows results of repeatability.

HPLC Injection Replicates of Rilpivirine	Retention Time	Area
Replicate – 1	2.08	833769
Replicate – 2	2.08	835768
Replicate – 3	2.08	855929
Replicate – 4	2.07	833458
Replicate – 5	2.07	848232
Average	2.076	841431.2
Standard Deviation	0.005477226	10133.98
% RSD	0.263835529	1.204374

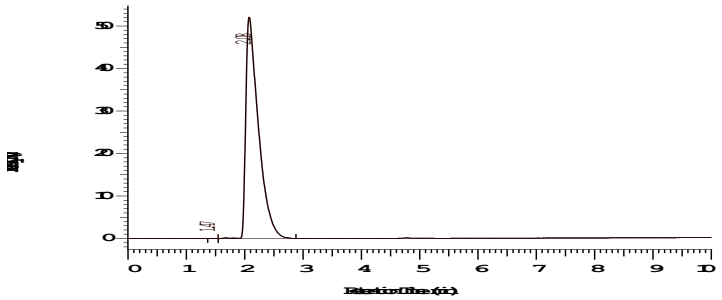


Fig-3.8.2.1: chromatography for Replicate – 1

Table-3.8.2.2: Readings of Repeatability-1

Sr no	Name	RT	Area
1	Rilpivirine	2.08	3293280

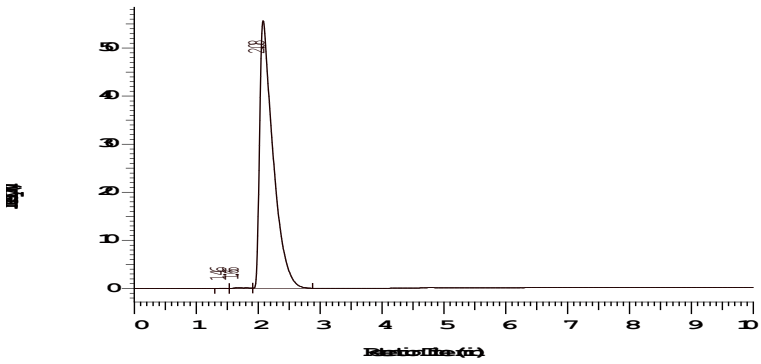


Fig-3.8.2.2: chromatography for Replicate – 2

Table-3.8.2.3: Readings of Repeatability-2

Sr no	Name	RT	Area
1	Rilpivirine	2.08	3293280

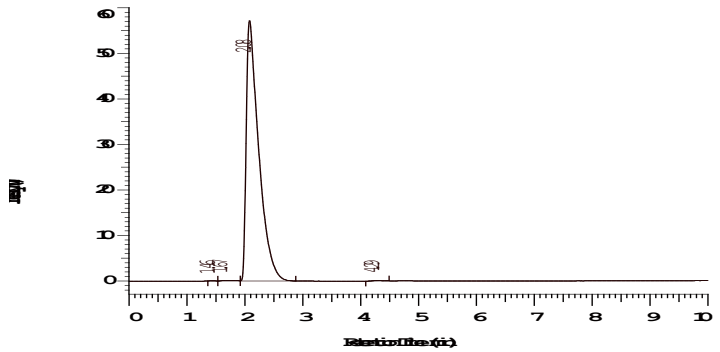


Fig-3.8.2.3: chromatography for Replicate – 3

Table-3.8.2.4: Readings of Repeatability-3

Sr no	Name	RT	Area
1	Rilpivirine	2.08	3293280

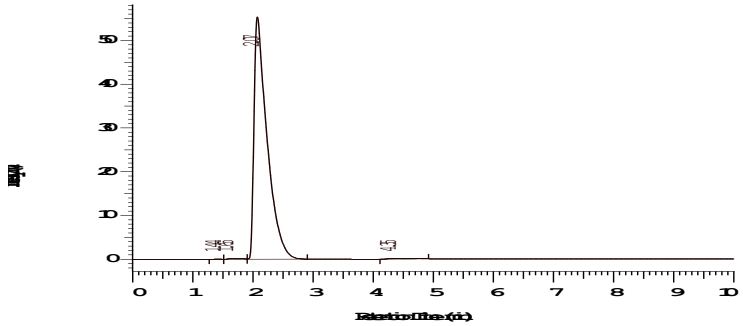


Fig-3.8.2.4: chromatography for Replicate – 4

Table-3.8.2.5: Readings of Repeatability-4

Sr no	Name	RT	Area
1	Rilpivirine	2.07	3293280

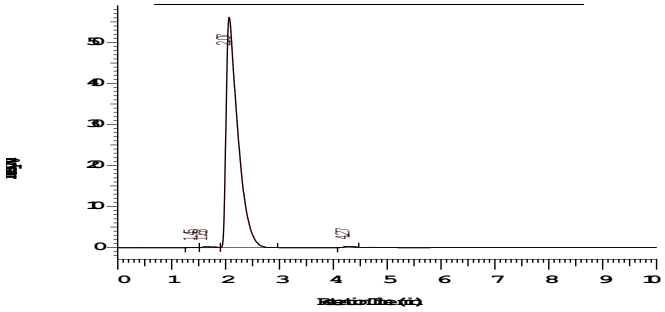


Fig-3.8.2.5: chromatography for Replicate – 5

Table-3.8.2.6: Readings of Repeatability-5

Sr no	Name	RT	Area
1	Rilpivirine	2.07	3293280

Intra-assay & inter-assay

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–3.8.2.7: Results of intra-assay & inter-assay

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
10	9.93	0.14	10.32	0.95
30	30.63	0.78	30.14	0.16
100	99.21	0.96	99.78	0.73

Linearity & Range

The calibration curve showed good linearity in the range of 0-25 µg/ml, for Rilpivirine (API) with correlation coefficient (r^2) of 0.994 (Fig-39). A typical calibration curve has the regression equation of y = 6780x + 65596 for Rilpivirine.

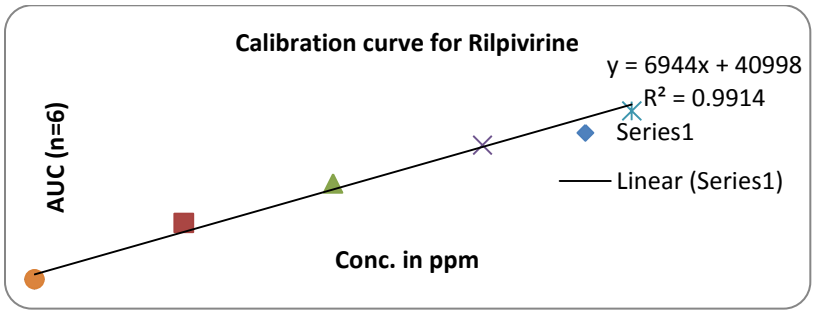


Fig-3.8.3.1: Calibration curve of Rilpivirine (API).

Table-3.8.3.1: Linearity Results

CONC. in ppm	AUC (n=6)
0	0
50	460548
100	783769
150	1096795
200	1376884

Fig-3.8.3.2: Chromatogram for 50ppm

Table-3.8.3.2: Readings of Linearity-50 ppm

Sr no	Name	RT	Area
1	Rilpivirine	2.07	3293280

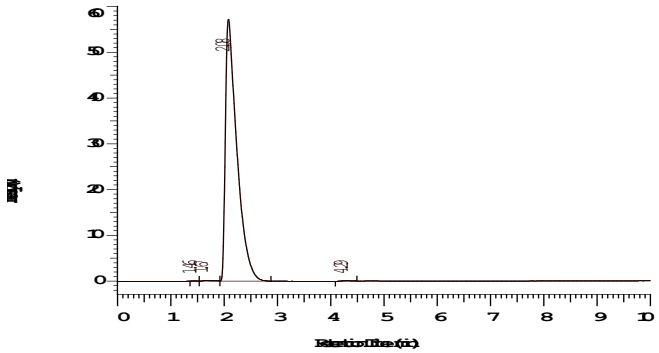


Fig-3.8.3.3: Chromatogram for 100ppm

Table-3.8.3.3: Readings of Linearity-100 ppm

Sr no	Name	RT	Area
1	Rilpivirine	2.08	3293280

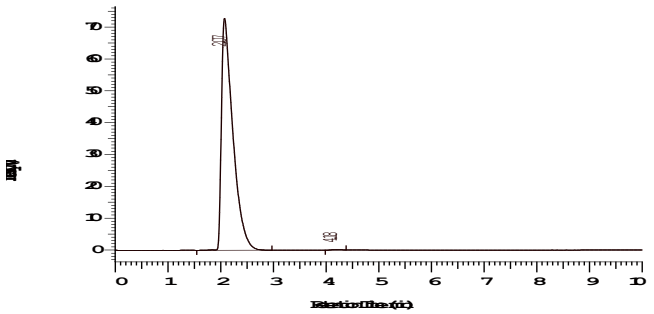


Fig-3.8.3.4: Chromatogram for 150ppm

Table-3.8.3.4: Readings of Linearity-150 ppm

Sr no	Name	RT	Area
1	Rilpivirine	2.07	3293280

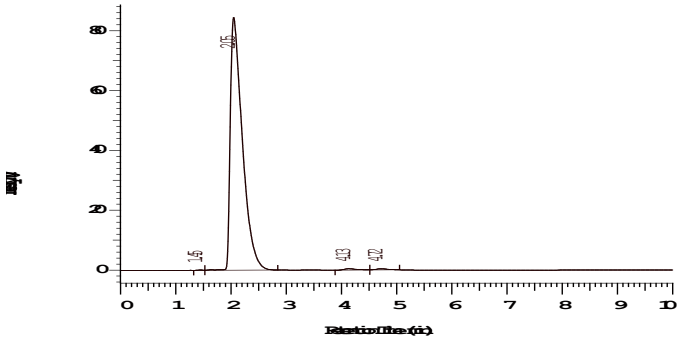


Fig-3.8.3.5: Chromatogram for 200ppm

Table-3.8.3.5: Readings of Linearity-200 ppm

Sr no	Name	RT	Area
1	Rilpivirine	2.05	3293280

Method Robustness

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) & acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table-38, % RSD < 2%) the developed RP-HPLC method for the analysis of Rilpivirine(API).

Table-3.8.4.1: Result of method robustness test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.06
Flow (0.9 ml/min)	0.04
Temperature (27^{0}C)	0.08
Temperature (23^{0}C)	0.11
Wavelength of Detection (202 nm)	0.03
Wavelength of detection (209 nm)	0.02

LOD & LOQ

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.03 & 0.09 µg/ml respectively.

System Suitability Parameter

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table-39.

Table-3.8.6.1: Data of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Resolution	Rs > 2	9.15
2	Asymmetry	T ≤ 2	Rilpivirine=0.12
3	Theoretical plate	N > 2000	Rilpivirine=3246

SPECIFICITY

Preparation and running of Rilpivirine

The performance test of the method has been conducted on market sample. As per the label claim, each tablet contains 50mg of Rilpivirine. To estimate this powder of the tablet equivalent to 25mg of Rilpivirine has been dissolved in 25 ml of the mobile phase. Further dilution was done by taking 1ml of this solution in 10ml volumetric flask, dissolve and making up the volume upto the mark with mobile phase by which 100ppm solution was prepared. Again same process is repeated to make 10ppm from 100ppm solution. To extract the drug in the solution, it has been sonicated for 5 minutes followed by cyclo-mixing for 5 minutes. Resulting solution was filtered by using Millipore syringe filter (0.45 micron). Resulting clear solution was injected in HPLC in duplicate as per the above mentioned HPLC method. Chromatogram obtained for the injection is shown below with Rt of 2.69 mins.

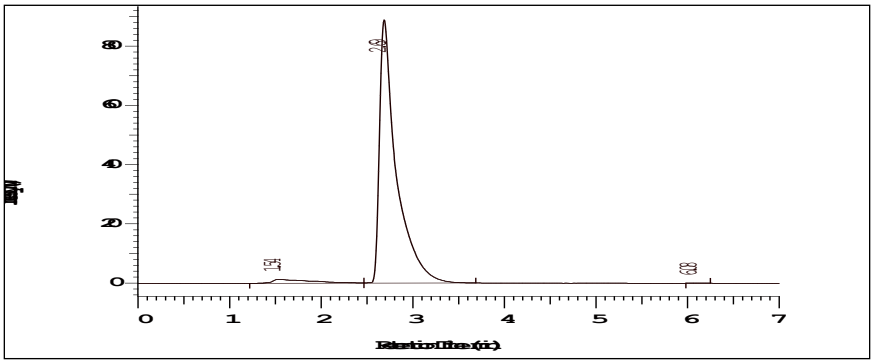


Fig-3.8.7.1: Chromatogram for specificity sample

Table-3.8.7.1: Results for specificity sample

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.54	2143	2354	1.28
2	2.69	5658	4689	1.74
3	6.08	2654	2541	0.49

Assay of Rilpivirine in dosage form

Estimation of rilpivirine in tablet dosage form rilpivirine 25 mg

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 100 mg of drugs were transferred to 100 ml volumetric flask, and 70 ml of Hplc grade methanol was added and solution was sonicated for 15 minutes, there after volume was made up to 100 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with hplc grade methanol. The solution was filtered through a membrane filter (0.45 □m) and sonicated to degas. From this stock solution (3.5 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system.

The solution prepared was injected in five replicates into the HPLC system and the observations were recorded.

A duplicate injection of the standard solution was also injected into the HPLC system and the peak areas were recorded. The data are shown in Table-41.

ASSAY

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:

AT = Peak Area of Test obtained with test preparation

AS = Peak Area of Standard obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

Assay was performed as described in previous chapter. Results obtained are tabulated below:

Table-3.8.8.1 Assay of Rilpivirine tablets

Brand name of tablets	Labelled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
EDURANT (Janssen Inc.)	25	25.31 (±0.06)	100.07 (±0.48)

The assay of EDURANT tablets containing Rilpivirine was found to be 100.07 %.

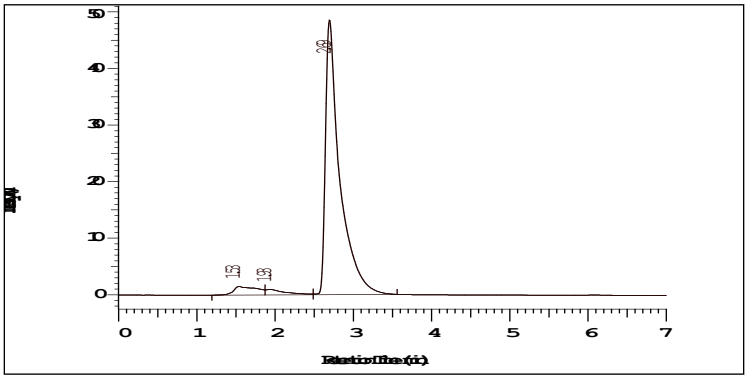


Fig-3.8.8.1: Chromatogram for assay sample-1

Table-3.8.8.1: Assay chromatogram results of Rilpivirine sample-1

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.53	2153	2133	1.91
2	1.93	2586	2852	1.97
3	2.69	5642	5293	0.98

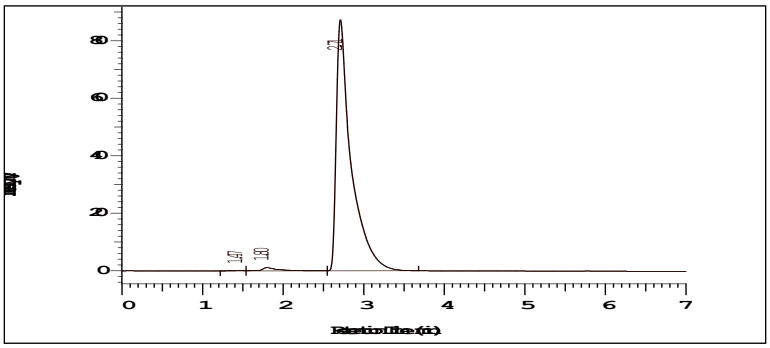


Fig-3.8.8.2: Chromatogram for assay sample-2

Table-3.8.8.2: Assay chromatogram results of Rilpivirine sample-2

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.47	2654	2635	1.57
2	1.80	2745	2843	1.29
3	2.71	6846	5684	0.37

DISCUSSION AND CONCLUSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Rilpivirine, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here develosil C_{18}, 5µm, 150 x 4.6 mm i.d. column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluents for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl).

The drug was found to be highly soluble in acetonitrile & soluble in dichloromethane and methanol. Drug was soluble in water. Using these solvents with appropriate composition newer methods can be developed and validated. Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Rilpivirine it is evident that most of the HPLC work can be accomplished in the wavelength range of 210-2500 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20 µl were found to be the best analysis.

The result shows the developed method is yet another suitable method for assay and purity which can help in the analysis of Rilpivirine in different formulations. A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Rilpivirine API.

Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The result shows the developed method is yet another suitable method for assay, purity which can help in the analysis of Rilpivirine in different formulations.

REFERENCE

[1]. Ravi Shankar,Instrumental method of analysis, 18-6, 18-3

[2]. A.Prabhakar Reddy, U. Chandra Teja, SK. Ashraf Sultana, M. Vijayalakshmi, Buchi N. Nalluri*,Development and validation of rp-hplc-pda method for the simultaneous estimation of emtricitabine, tenofovir disoproxil

fumarate and rilpivirine hydrochloride in bulk, pharmaceutical dosage forms and in dissolution samples, Indo American Journal of Pharmaceutical analysis, ISSN NO 2231-6876.

[3]. Uttam Prasad Panigrahy1and A. Sunil Kumar Reddy2, A novel validated RP-HPLC method for the simultaneous estimation of Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine in bulk and pharmaceutical tablet dosage forms, Scholars Research Library Der Pharmacia Lettre, 7(1), 2015, 303-314, ISSN 0975-5071 USA CODEN: DPLEB4.

[4]. Kavitha, k. y.; geetha, g.; hariprasad, r.; venkatnarayana, r.; subramanian, g., Development and validation of rp-hplc analytical method for simultaneous estimation of emtricitabine, rilpivirine, tenofovir disoproxil fumarate and its pharmaceutical dosage forms, Pharmacie Globale; 1(1), 2013, 1.

[5]. Masthanamma sk* and alekhya gottumukkala, Development and validation of uv spectrophotometric methods for estimation of rilpivirine in bulk and pharmaceutical formulation, international journal of pharmaceutical sciences and research, ISSN (Online): 0975-8232, ISSN (Print): 2320-5148.

[6]. M. E. Swartz, journal of liquid chromatography, 28(7/8), 2005, 1253-1263

[7]. Biomedical chromatography : bmc 22(5), 2008, 469-477

[8]. Journal of chromatography .b, analytical technologies in the biomedical and life sciences. 1, 2008, 863(2), 2008, 258-265.

[9]. Ich q2b: validation of analytical procedure; methodology (international conferences on harmonization of technical requirements for the registration of drugs for human use, Geneva, Switzerland, may 1997

[10]. Ich q2b: validation of analytical procedure; methodology (international conferences on harmonization of technical requirements for the registration of drugs for human use, Geneva, Switzerland, 2003.