ISSN: 2230-7346

B. Raj Kumar et al. / JGTPS/ 5(3)-(2014) 1822 –1826

(Research Article)

Journal of Global Trends in Pharmaceutical Sciences

Journal home page: www.jgtps.com

A VALIDATED STABILITY-INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF RILPIVIRINE

B. Raj Kumar[1]*
Dr. K. V. Subrahmanyam[2]

[1]Assistant Professor Department of Pharmaceutical Analysis, Dr. K.V.Subba Reddy Institute of Pharmacy, Kurnool, A.P,India

[2]Principal & Professor Department of Pharmaceutical Analysis, Samskruthi College of Pharmacy, Ghatkeskar, Hyderabad.

ABSTRACT

Rilpivirine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) of human immunodeficiency virus type 1 (HIV-1). A novel, stability-indicating reversed-phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative determination of Rilpivirine in active pharmaceutical ingredients and in its Pharmaceutical dosage form by using a symmetry Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm was used with a mobile phase containing a mixture of Acetonitrile and Potassium dihydrogen phosphate buffer adjusted to pH2.8 with ortho phosphoric acid in the ratio of 40:60. The flow rate was 1.0 ml/min and effluent was monitored at 282 nm and a peak eluted at 4.50 min and column oven temperature was maintained ambient. Calibration curve was plotted with a range from 0-30 µg/ml. Stress degradation conditions were established for Rilpivirine by subjecting it to acid, base, oxidation and thermal stress. The stress samples were assayed against a qualified reference standard and the mass balance was close to 99.31%. The developed RP-HPLC method was validated according to the current International Conference on Harmonization (ICH) guidelines for specificity, LOD, LOQ, linearity, accuracy, precision, intermediate precision and robustness. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Rilpivirine in bulk drug and in its pharmaceutical dosage form

Keywords: Rilpivirine, RP-HPLC, ODS, ICH, LOD, LOQ

INTRODUCTION:

EDURANT (rilpivirine) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) of human immunodeficiency virus type 1 (HIV-1). 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. Treatment of HIV-1 infections in treatment-naive patients with HIV-1 RNA ≤100,000 copies/mL in combination with at least 2 other antiretroviral agents. Each tablet contains 27.5 mg of rilpivirine hydrochloride, which is equivalent to 25 mg of rilpivirine.The chemical name for rilpivirine hydrochloride is 4-[[4-[[4-[(E)-2-cyanoethenyl]-2,6-dimethylphenyl]amino]2-pyrimidinyl]amino]benzonitrile monohydrochloride. Its molecular formula is $C_{22}H_{18}N_6$ • HCl and its molecular weight is 402.88. Rilpivirine hydrochloride has the following structural formula[1-3]

Address for correspondence

B. Raj Kumar*
Dr. K.V.Subba Reddy Institute of Pharmacy, Kurnool, A.P,India
E-mail: rajpharma2631@yahoo.com
Contact: 9000004806

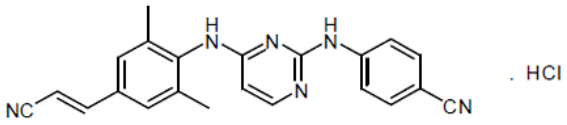

Fig 1: Showing the structure of Rilpivirine hydrochloride

Rilpivirine hydrochloride is a white to almost white powder. Rilpivirine hydrochloride is practically insoluble in water over a wide pH range. Each EDURANT tablet also contains the inactive ingredients croscarmellose sodium, lactose monohydrate, magnesium stearate, polysorbate 20, povidone K30 and silicified microcrystalline cellulose. The tablet coating contains hypromellose 2910 6 mPa.s, lactose monohydrate, PEG 3000, titanium dioxide and triacetin.[3-4]

MATERIALS AND METHODS[5-9]

Materials used: HPLC grade water, Dipotassium hydrogen orthophosphate, Ortho phosphoric acid (Sd fine-Chem ltd; Mumbai), Methanol and Acetonitrile (Loba Chem; Mumbai).

Instruments used: HITACHI L2130 with D Elite 2000 Software with Isocratic with UV-Visible Detector (L-2400), ELICO SL-159 UV – Vis spectrophotometer, Electronic Balance, Ultra Sonicator (Wensar wuc-2L), Triple Quartz Distillation Unit **(BOROSIL)**, Thermal

Oven, Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm, P^HAnalyzer(ELICO)

Chromatographic conditions:

Preparation of mobile phase

A mixture of above buffer 600ml (60%) and 400 ml of Acetonitrile HPLC (40%) were mixed and degassed in ultrasonic water bath for 15 minutes and filtered through 0.45 µm filter under vacuum filtration.

Preparation of Phosphate buffer:

6.8 grams of Potassium dihydrogen orthophosphate was Weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC Grade water. pH was adjusted to 2.2 with Orthophosphoric acid. The mobile phase was also filtered through a 0.45-µ (MILLIPORE, Germany) membrane filter prior to use. A Symmetry C_{18} Develosil ODS HG-5 RP 150mm x 4.6mm 5µm particle size column was used for determination. The flow rate was 1.0 ml/ min and the column was operated at ambient temperature (~25^{o}C). The volume of sample injected was 20 µl. Prior to injection of the solutions, column was equilibrated for at least 30min with mobile phase flowing through the system. The UV detector was set at wavelength of 282 nm.

Diluent: Mobile phase.

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 0.1 ml of the above solution into a 10 ml volumetric flask and make up to volume with mobile phase. The sample was analysed by HPLC by using the above method and a very nicely resolved peak has been obtained at a Retention Time of about 4.9 min. The respective chromatogram is attached.

Optimization of Chromatographic Conditions:

The chromatographic conditions were optimized by different trails. (Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc

The Optimum conditions obtained from experiments can be summarized as Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm I.D was used for analysis at column temperature 45°C. The mobile phase was pumped through the column at a flow rate of 1.0 mL/ min. The sample injection volume was 20 µL and the sample temperature was maintained at Ambient. The wavelength of UV-282 nm was set for Rilpivirine and Chromatographic Gradient programme runtime was 10minutes.

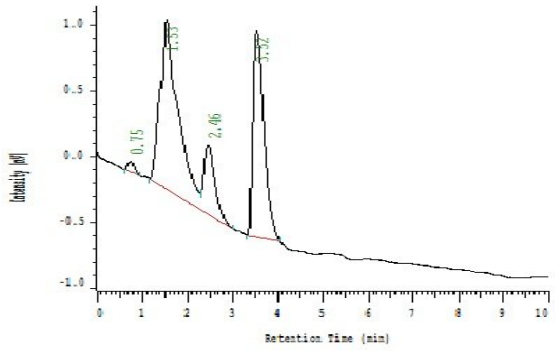

Fig 2: HPLC spectrum of Rilpivirine (blank)

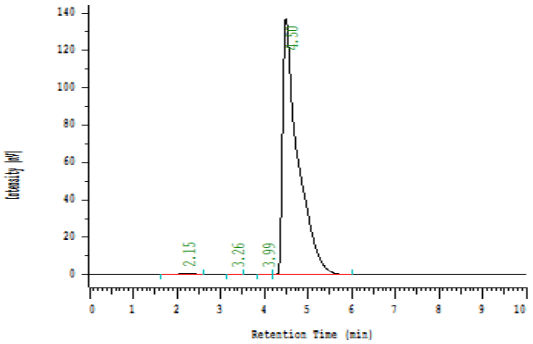

Fig 3: HPLC spectrum of Rilpivirine in optimized conditions (RT 4.50 min.)

RESULTS AND DISCUSSIONS:

To develop a suitable and robust LC method for the determination of Rilpivirine in different mobile phases were employed to achieve the best separation and resolution. The method development was started with Symmetry C18; 250 mmx4.6 mm I.D; particle size 5 µm with the flow rate of 1.0ml/min. Mobile phase was Buffer and Acetonitrile in the ratio of 60:40%, Column temperature was Ambient and the wavelength was 210nm. The retention time of Maraviroc is 2.6 minutes and the peak shape was broad. For better peak shape the mobile phase pH and Composition was changed, the trial-5 shown a sharp peak with good resolution on replacing the column with C_{18} Develosil ODS HG-5 RP 150mm x 4.6mm 5µm particle size with Rt of 4.50 minutes

METHOD VALIDATION:

Accuracy:

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-analyzed formulation of concentration 10µg/ml. From that percentage recovery values were calculated. The results were shown in table 1.

Sample ID	Concentration (µg/ml)		%Recovery of Pure drug	Statistical Analysis
	Pure drug	Formulation		
S_1 : 80 %	8	10	99.18	Mean= 98.97667%
S_2 : 80 %	8	10	98.78	S.D. = 0.200083
S_3 : 80 %	8	10	98.97	% R.S.D.= 0.202152
S_4 : 100 %	10	10	99.87	Mean= 99.54%
S_5 : 100 %	10	10	99.54	S.D. = 0.33
S_6 : 100 %	10	10	99.21	% R.S.D.= 0.331525
S_7 : 120 %	12	10	99.32	Mean= 99.567%
S_8 : 120 %	12	10	99.65	S.D. = 0.33
S_9 : 120 %	12	10	99.98	% R.S.D. = 0.331159

Table 1: Showing the Results of Accuracy

Precision:

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 Shown in Table 2.

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	10.08	0.96	10.03	0.97
20	20.04	0.4	30.03	0.42
40	39.97	0.33	39.95	0.14

Table 2: Showing the Results of 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 3.

HPLC Injection Replicates of Rilpivirine	Retention Time	Area
Replicate – 1	4.43	3418301
Replicate – 2	4.5	3293280
Replicate – 3	4.47	3404122
Replicate – 4	4.49	3435014
Replicate – 5	4.5	3429838
Average	4.478	3396111
Standard Deviation	0.026381812	58697.8
% RSD	0.58914274	1.72838

Table 3: Showing the Results of Repeatability

Linearity & Range:

The calibration curve showed good linearity in the range of 0 – 30 µg/ml, for Rilpivirine (API) with correlation coefficient (r^2) of 0.992 (Fig.04). A typical calibration curve has the regression equation of y = 28066x + 18404 for Rilpivirine.

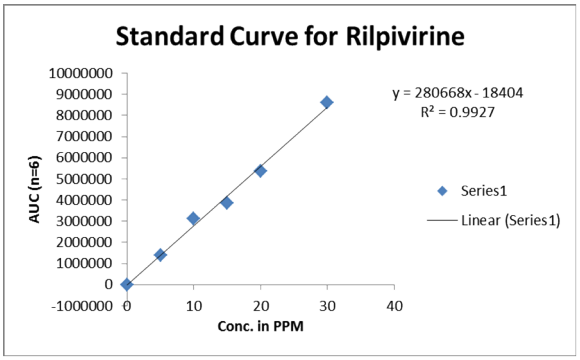

Fig. 4: Calibration curve of Rilpivirine (API)

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
5	1388562
10	3129838
15	3855797
20	5351271
30	8617536

Table 4: Showing the Results of Linearity

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-05 , % RSD < 2%) the developed RP-HPLC method for the analysis of Rilpivirine (API).

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

Table 5: Showing the Results of Robustness

LOD & LOQ:

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.05 & 0.15 µg/ml respectively.

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 results shown in table No: 06

Acid Hydrolysis

An accurately weighed 25 mg of pure drug was transferred to a clean & dry 25 ml volumetric flask. To which 0.1 M 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) results shown in table No: 06

Basic Hydrolysis

An accurately weighed 10 mg of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 0.1 M 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) results shown in table No: 06

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 ^{0}C for 24 hrs then injected into the HPLC system against a blank of mobile phase (after all optimized conditions) results shown in table No: 06

Photolytic Degradation

Approximately 10 mg of pure drug was taken in a clean & dry Petri dish. 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 and then injected into the HPLC system against a blank of mobile phase (after all optimized conditions) results shown in table No: 06

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 results shown in table 6.

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	8.46	99.83

Table 6: Showing the Results of Stability studies

CONCLUSION:

The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of Rilpivirine and can be reliably adopted for routine quality control analysis of Rilpivirine in Bulk and its pharmaceutical formulations

ACKNOWLEDGEMENT:

The authors are thankful to Aravinda Patnaik, Research analyst, COMPRIME Labs, Hyderabad for his help for providing all facilities to complete the work. Dr. Reddy Labs for providing the drugs.

REFERENCES:

1. http://www.drugbank.ca/drugs/DB08864
2. http://www.rxlist.com/edurant-drug/indications-dosage.html
3. AIDS info Drug Database http://aidsinfo.nih.gov/drugs/426/edurant/0/patient
4. http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/202022Orig1s000TOC.cfm
5. Practical HPLC Method Development; Second Edition, Lloyd R. Snyder, Joseph J. Kirkland, Joseph I. Glajch, (1997).
6. United States Pharmacopeia USP 34-NF 29, (2011).
7. ICH Guidelines on Validation of Analytical procedure: Text and Methodology Q 2 (R1), (2011).
8. ICH Q2B: Validation of analytical procedures; methodology,may (1997).8.
9. International Conference on Harmonization of technical requirements for the registration of pharmaceutical for human use (ICH) Q2B (1996). Validation of analytical procedures, methodology.
10. Method validation guidelines International Conference on harmonization; GENEVA; 1996
11. Berry RI, Nash AR. Pharmaceutical Process Validation, Analytical method validation,
12. Marcel Dekker Inc. New work. 1993; 57:41; 28.15.
13. P.N. Arora, P.K. Malhan. Biostatistics, Himalaya Publishers House, India, PP 113,139-140,154

How to cite this article:

B. Raj Kumar[1]*Dr. K. V. Subrahmanyam[2]: a validated stability-indicating RP-HPLC method for the determination of rilpivirine: 5(3): 1822-26. (2014)