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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF RILPIVIRINE TABLETS BY RP-HPLC

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ABSTRACT

A novel, accurate, simple, rapid, sensitive and precise RP-HPLC method was developed and validated for the estimation of Rilpivirine in bulk and Pharmaceutical tablet dosage form. The RP-HPLC separation was performed on Symmetry C_{18}, 5µm, 25cmx4.6mm i.d. by using mobile phase consists of Acetonitrile: Phosphate buffer in the ratio of 65:35 % v/v and the pH-5.20 was adjusted with the dil. ortho-phosphoric acid solution and maintained at a flow rate of 1.0 ml/min at room temperature (Ambient). The retention time was found to be 5.79minutes for Rilpivirine. Calibration curve was linear over the concentration range of 0-80ppm for Rilpivirine. The separation was achieved with UV detection at 266 nm. The proposed method was statistically validated and applied successfully for the estimation of Rilpivirine in API and tablet dosage form. The validation studies exposed that the developed method is rapid, accurate and reproducible. The high recovery and low relative standard deviation confirms that the suitability of the method for the routine determination of Rilpivirine in bulk and pharmaceutical tablet dosage forms. The developed method was validated according to the guidelines of ICH and USP.**S**

INTRODUCTION:

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[1,2] 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[3,4] 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. Rilpivirine is an NNRTI which binds to reverse transcriptase which results in a block in RNA and DNA- dependent DNA polymerase activities. One such activity is HIV-1 replication. Intracellular phosphorylation is not necessary for its antiviral activity. Because of the structure[5,6] of Rilpivirine is flexible around the aromatic rings, the molecule can have multiple conformations so that can bind to

residues in the reverse transcriptase enzyme which have a lower mutation rate. The IUPAC Name[7] of Rilpivirine is 4-{[4-({4-[(1E)-2-cyanoeth-1-en-1-yl]-2,6-dimethylphenyl}amino) pyrimidin-2-yl] amino}benzonitrile. The molecular formula[8,9] for Rilpivirine is $C_{22}H_{18}N_6$.The Chemical Structure of Rilpivirine is shown in Fig 1.

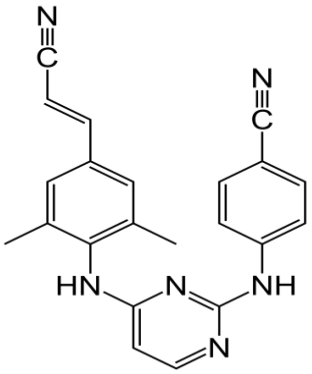

Fig-1: Chemical Structure of Rilpivirine

MATERIALS AND METHODS

INSTRUMENTATION

The HPLC system used was Waters-717 series, equipped with UV-detector, and automatic sampling system having Symmetry C_{18}, 5µm, 25cmx4.6mm i.d.

Chromatographic Conditions

The analysis was carried on HPLC Symmetry C_{18}, 5µm, 25cmx4.6mm i.d. column with detection wavelength of 266nm. Injection volume of 20.0 µL and maintaining a flow rate at 1ml/min.

Buffer Preparation

Weigh accurately around 6.8 grams of Potassium dihydrogen orthophosphate[10] and transferred into 1-liter beaker, dissolved and diluted to 1000ml (1 liter) with HPLC water. The pH was adjusted to 5.20 with diluted ortho-phosphoric acid solution. The solution is then filtered and degassed on a Sonicator for about 30 minutes to remove air bubbles.

Mobile Phase

The mobile phase can be prepared by taking the potassium dihydrogen orthophosphate buffer having molarity 0.01M and pH- 5.20 can be adjusted by using diluted orthophosphoric acid and Acetonitrile in the ratio of 65:35 v/v. The mobile phase can be filtered through the 0.45 µm filter membrane and degassed by using ultra sonication process. The prepared mobile phase is pumped through the stationary phase maintained at a flow rate of 1.0 ml/min.

Diluent: Mobile Phase was used as diluent.

Preparation of Standard Solution:

Weighed exactly and transferred 10mg of Rilpivirine Standard into a 10ml clean dry volumetric flask, add 6ml of diluent, sonicated for 15 minutes and volume make up to the mark with the same diluent. From the above prepared stock solution, 1ml was pipetted out in to a 20ml volumetric flask and then make up to the volume with the diluent(50ppm).

Preparation of Test Solution

10 tablets were weighed and powdered. Then the weight tablet powder equivalent to 100mg of Rilpivirine was weighed and transferred into a clean and 100ml volumetric flask, 50ml of diluent added and sonicated for 30 minutes, further the volume made up with the diluent preparation and filtered. From the resulted filtered solution 1ml was pipette out into a 20ml volumetric flask and made up the volume up to mark with the 20ml of diluent.

RESULTS AND DISCUSSION

System suitability:

All the system suitability test parameters are said to be within range and acceptable as per ICH guidelines. System

suitability tests are an integral part of chromatographic method which is used to verify reproducibility of the chromatographic system. To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the freshly prepared standard stock solutions at the concentration level 50ppm to check the reproducibility of the system.

Linearity:

Six linear concentrations of Rilpivirine (0-80ppm) are prepared and injected. Regression equation of the Rilpivirine are found to be, y = 2826.x + 1333 and regression co-efficient was 0.999.

Precision:

Intraday Precision was performed and % RSD for Rilpivirine was found to be 0.4%. Inter day precision was performed with 24hrs time lag and the %RSD Obtained for Rilpivirine was 0.3%.The results were shown in table 2.

Accuracy: To ascertain the accuracy of the method, recovery studies were carried out in triplicate by spiking different concentrations of pure drug in the pre analyzed samples with three different concentrations of standard containing 80%, 100% and 120% of the pure drug. The mean recovery percentage obtained was 100.20% for the assay (Table-3).

Limit of detection (LOD): LOD is referred as lowest concentration of the analyte present in the solution that can be used to detect the sample, but not quantified, by using experimental conditions. Based on signal to noise ratio: A signal to noise ratio between the 3 or 2:1 is generally considered acceptable for determining the limit of detection.

Limit of quantification (LOQ): LOQ is defined as the minimum concentration of the analyte that can be used to determine with the respect to the accuracy and precision by the analytical method by using experimental conditions. Based on signal to noise ratio: Generally the signal to noise ratio between10:1 is considered.

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio and temperature are made but there were no recognized change in the result and are within range as per ICH Guidelines. Robustness of the method was determined by deliberately changing some operating conditions such as flow rate and wavelength. It was observed that there were no remarkable changes in chromatogram, the tailing factor and plate counts. The results were shown in table 5.

Assay[10]:

Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected as six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The average %assay was calculated and found to be 99.57% for Rilpivirine. The standard HPLC chromatogram of Rilpivirine was shown in Fig 3.

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AT = Peak Area of Sample obtained with test the preparation, AS = Peak Area of Standard (Reference) obtained with standard the preparation, WS = Working standard weight taken in mg, WT = Sample weight taken in mg, DS = Standard solution dilution, DT = Sample solution dilution, P=Working standard percentage purity

Specificity: The specificity was carried out to determine whether there are any interference of any impurities (presence of components may be unexpected to present) in retention time of analytical peak[11].

Table-1: Calibration data of Rilpivirine method.

S. No.	Concentration Rilpivirine (ppm)	Area
1	0	0
2	30	86795
3	40	115302
4	50	143783
5	60	171237
6	70	198765
7	80	226134

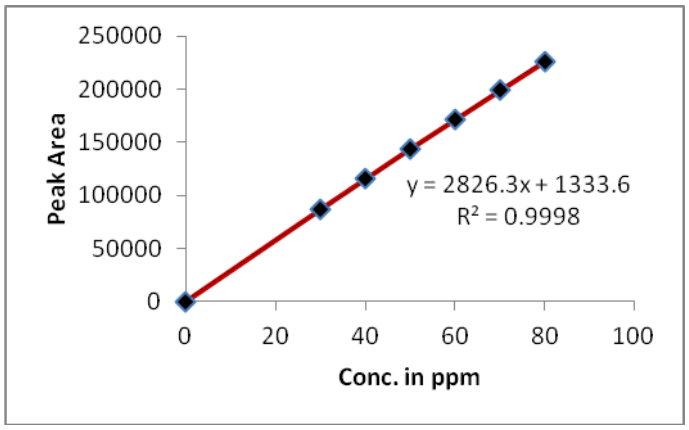

Fig-2: Calibration curve of Rilpivirine

Table-2: Precision results for Rilpivirine.

Sr. No.	Intraday Precision		Inter day precision	
	Retention Time (Mins)	Area	Retention Time (Mins)	Area
1	5.798	143562	5.798	143651
2	5.767	143658	5.781	144512
3	5.785	143652	5.785	143654
4*	5.777	143783	5.780	143586
5	5.781	144521	5.792	145687
6	5.756	142564	5.786	143658
Mean	5.777	143623	5.787	144124
Std. Dev.	0.01	625.9	0.01	841.9
% RSD	0.3	0.4	0.1	0.6

Table-3: Readings for Accuracy of Rilpivirine

% Level	Amount Injected (ppm)	Amount recovered (ppm)	Peak Area	% Recovery	Mean % Recovery
80%	40	40.276	115154	100.69	100.20%
	40	40.782	116584	101.955	
	40	40.459	115672	101.147	
100%	50	49.629	141587	99.258	
	50	49.967	142541	99.934	
	50	49.770	141985	99.54	
120%	60	59.592	169741	99.32	
	60	59.765	170231	99.608	
	60	60.230	171543	100.383	

Table-4: Robustness data of Rilpivirine

S.No.	**Robustness condition**	**Rilpivirine % RSD**
1	Flow minus	0.8
2	Flow Plus	1.3
3	Mobile phase minus	0.7
4	Mobile phase Plus	0.6

The specificity of the method was determined by checking the interference of any of the possible degradation products generated during the forced degradation study of the drugs.

CONCLUSION:

A new simple, accurate, precise, selective method was developed for the estimation of the Rilpivirine in bulk and pharmaceutical tablet dosage form. The Retention time of Rilpivirine was found to be 5.798mins. The Percentage Standard Deviation (%RSD) of the Rilpivirine was and found to be 0.4%. The %assay was obtained as 99.57% for Rilpivirine. LOD, LOQ values are obtained from regression equations of Rilpivirine was 0.09ppm and 2.70ppm respectively. Regression equation of Rilpivirine is y = 2826.x + 1333. Retention time is decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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