Development and Validation of Rilpivirine in Pharmaceutical Formulation by RP-HPLC

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ABSTRACT

In the present study a simple isocratic reverse phase HPLC method was developed for the estimation of rilpivirine in pharmaceutical formulation. The separation was carried out using a column of Zorbax Eclipse XDB-C18, 250x4.6mmi.d with 5micron particle size. The mobile phase comprises of 0.03M di potassium hydrogen orthophosphate with pH adjusted to 2.5 using dilute ortho-phosphoric acid (mobile phase solvent-A) and acetonitrile (mobile phase solvent-B) in the ratio of 15: 85 (v/v). The flow rate was 1.0 ml/min and the effluents were monitored at 284 nm. The retention time was 7.19 min. The detector response was linear in the concentration range of 100-300µg/ml. The respective linear regression equation being Y= 28817.742X-14741.2. The limit of detection (LOD) and limit of quantification (LOQ) for rilpivirine were found to be 0.05µg/ml and 0.15 µg/ml respectively. The assay was found to be 99.85%. The method was validated by determining its accuracy, precision and system suitability. 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 its pharmaceutical dosage form.

Keywords: Rilpivirine, Anti HIV agent, RP-HPLC, system suitability, linearity, recovery studies

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Received 04 October 2018, Accepted 10 October 2018
INTRODUCTION

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) play an important role in the treatment and prevention of HIV infections. NNRTIs bind and block HIV reverse transcriptase (an HIV enzyme). HIV uses reverse transcriptase to convert its RNA into DNA (reverse transcription). Blocking reverse transcriptase and reverse transcription prevents HIV from replicating [1,2]. Rilpivirine is a second generation non-nucleoside reverse transcriptase inhibitor (NNRTI) that is approved for HIV-1 treatment-naive adult patients in combination with other antiretroviral agents (Figure 1). The chemical name for rilpivirine hydrochloride is 4-{{4-((E)-2-cyanoethenyl)-2,6-dimethylphenyl}amino}-pyrimidinyl]amino]benzonitrile monohydrochloride[3,4].

![Figure 1: Structure of rilpivirine](image)

Literature survey reveals few chromatographic methods for the estimation of Rilpivirine individually and in combined form [5-15]. The aim of the study was to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of Rilpivirine in pharmaceutical dosage form.

MATERIALS AND METHOD

Materials:

Rilpivirine was obtained as a gift sample from Hetero Drugs Ltd Hyderabad. Acetonitrile used was of HPLC grade (Qualigens), potassium dihydrogen phosphate and ortho-phosphoric acid were of analytical grade (Rankem). Commercially available Rilpivirine tablets (Edurant ®-25 mg) were procured from local market.

Instrument:

Quantitative HPLC was performed on Waters Alliance 2695 Separations Module is a high performance liquid chromatographic system with a quaternary, low-pressure mixing pump and inline vacuum degassing powered with -2 Empower Software. A Zorbax Eclipse XDB-C18, 250x4.6mm.i.d of particle size 5micron column was used. The detector used is a photodiode array (model 2996) with a wavelength range of 190-800 nm.

HPLC Conditions:
The contents of the mobile phase A were prepared by dissolving 3.48 gm of di potassium hydrogen orthophosphate (0.03M) in 1000 ml of water and adjusting the pH to 2.5 with dilute orthophosphoric acid. These are mixed with acetonitrile (mobile phase solvent-B) in an isocratic mode in the ratio of 15: 85 (v/v) of separation was used. They were filtered before use through a 0.45 μm membrane filter and degassed by sonication.

The run time was set at 25 minutes and the column temperature was ambient. Prior to the injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluents were monitored at 284 nm.

**Preparation of Standard Stock solution:**
A standard stock solution of the drug was prepared by dissolving 250 mg of rilpivirine working standard in 100ml of the diluent. The contents were sonicated for 15 minutes to obtain 2500μg/ml.

**Working Standard solution:**
5ml of the primary standard stock solution of 2500μg/mL was taken in 50 ml volumetric flask and thereafter made up to 50 ml with mobile phase to get a concentration of 250μg/ml.

**Preparation of Sample solution:**
20 Tablets of rilpivirine (Edurant ®-25 mg) were powdered. A sample of the blended tablet powder, equivalent to 250 mg of the active ingredient, was mixed with 70 ml of mobile phase in 100 ml volumetric flask. The mixture was allowed to stand for 1 hour with intermittent sonication for complete solubility of the drug, and then filtered through a 0.45 μm membrane filter, followed by addition of mobile phase up 100 ml to obtain a stock solution of 2500μg/ml. The resultant solution was further diluted by taking 5 ml of the stock solution with 50 ml of mobile phase to get the concentration of 250μg/ml.

**RESULTS AND DISCUSSION:**
Validation for the method was carried out as per ICH Q2 (R1) guidelines [16]. Various parameters such as selectivity, linearity, lower limit of quantification (LLOQ), limit of detection (LOD), accuracy and precision and recovery were evaluated for the method validation. The specificity of the method was evaluated to confirm that components of analytical matrices did not interfere with analysis of rilpivirine sample and standard.

Linearity was evaluated by visual analysis of graphs, calculation of the correlation coefficient, back-calculation of concentrations of the calibration curve samples and analysis of the response factor (ratio between the response and nominal concentration of each calibration curve sample).
Accuracy and precision were determined for all the analytical matrices using three quality control samples. The relative standard deviation (RSD) for intra- and inter-day assays determined the precision, whereas the measured concentrations yielded accuracy. The percentage recovery was calculated by comparing the concentrations of the spiked samples with the concentration of the non-extracted samples.

**System Suitability:**

The system suitability tests were carried out on freshly prepared standard stock solution of rilpivirine. The system was suitable for use, the tailing factors for rilpivirine were 1.23 and USP theoretical plates were found to be significantly high around 16305. (Figure 2, 3, 4)

![Figure 2: Typical System suitability Chromatogram of rilpivirine](image)

![Figure 3: Typical Chromatogram of rilpivirine standard](image)
Figure 4: Typical Chromatogram of rilpivirine sample (Edurant ®-25 mg tablets)

**Linearity:**

Aliquots of standard rilpivirine stock solution were taken in different 10 ml volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of rilpivirine are in the range of 100-300 μg/ml. Each of these drug solutions (10 μL) was injected three times into the column, and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 284 nm and a Calibration graph was obtained by plotting peak area versus concentration of rilpivirine (Figure 5)

The plot of peak area of each sample was found to be linear in the range of 100-300μg/ml with correlation coefficient of 0.999. Linear regression least square fit data obtained from the measurements are given in Table 1 and Table 2 . The respective linear regression equation being

\[ Y = 28817.742X - 14741.2 \]

Figure 5: Calibration curve of rilpivirine
Table 1: Standard calibration values of rilpivirine

| Concentration of drug (µg/ml) | Peak Area   |
|------------------------------|-------------|
| 100                         | 2896589     |
| 150                         | 4596874     |
| 200                         | 6012589     |
| 250                         | 6998568     |
| 300                         | 7896523     |

Table 2: Optical & Regression Characteristics of HPLC method

| Parameter                                      | Results of HPLC Method         |
|------------------------------------------------|---------------------------------|
| Detection wavelength (nm)                      | 284                             |
| Linearity range (µg/ml)                        | 100-300                         |
| Regression Equation (y=mx + c)                 | Y=28817.742X-14741.2.           |
| Slope (m)                                      | 28817.742                       |
| Intercept (c )                                 | -14741.2                        |

**Precision:**

Intraday precision was performed at three different concentration levels of rilpivirine (200µg/ml, 250µg/ml and 300µg/ml) within the same day at three different times session 1, session 2 and session 3.

Inter day precision was carried by conducting at different concentration 200 µg/ml, 250 µg/ml and 300 µg/ml of rilpivirine on three different days, using same homogeneous samples. The % RSD values for both inter day and intra-day precision were found within acceptable limit. Results tabulated in Table 3 and Table 4.

Table 3: Intra day precision data of Rilpivirine sample:

| Level | 80%          | 100%         | 120%         |
|-------|--------------|--------------|--------------|
|       | Concentration (µg/ml) | 200 | 250 | 300 |
| Peak area Session 1 | 6012589 | 6998457 | 7896523 |
| Session 2          | 6023598 | 6985478 | 7902365 |
| Session 3          | 6100238 | 6995248 | 7914589 |
| Avg. peak area     | 6045475 | 6993061 | 7904492.33 |
| SD                | 47744.52 | 6760.23  | 9218.96    |
| %RSD              | 0.78      | 0.096     | 0.12       |

Table 4: Inter day precision data of Rilpivirine sample

| Level | 80%          | 100%         | 120%         |
|-------|--------------|--------------|--------------|
|       | Concentration(µg/ml) | 200 | 250 | 300 |
| Peak area day 1 | 6056891 | 6998457 | 7863594 |
| day 2   | 6123598 | 6999865 | 7902158 |
| day3    | 6115846 | 6895627 | 7888963 |
| Avg. peak area | 6098778.333 | 6964649.667 | 7884905 |
| SD      | 36481.98 | 59779.52 | 19599.64 |
| %RSD    | 0.59      | 0.85       | 0.24        |
Assay and recovery studies:
Recovery studies were conducted by analyzing pharmaceutical formulation in the concentration of 80% (200 µg/ml), 100% (250 µg/ml) and 120% (300 µg/ml) of the working standard solution by the proposed method. Each concentration was injected 3 times and the peak area was recorded. Three samples of standard (80%, 100% and 120%) were added to each 3 previously analyzed formulation and the total amount of the drug was once again determined by the proposed method (each concentration was again injected 3 times) after keeping the active ingredient concentration within the linearity limits(Table 5).

**Table 5: Recovery Peak areas of rilpivirine by Accuracy studies**

| S. No | Recovery at 80% dilution level Peak areas | Recovery at 100% dilution level Peak areas | Recovery at 120% dilution level Peak areas |
|-------|------------------------------------------|------------------------------------------|------------------------------------------|
|       | Standard Spiked | Standard Spiked | Standard Spiked | |
| 1     | 6078549 6883692 | 7137688 8171910 | 8693037 9402205 | |
| 2     | 6094909 6936077 | 7254913 8123507 | 8737102 9487382 | |
| 3     | 6117299 6939025 | 7199150 8025701 | 8581219 9334452 | |
| Avg   | 6096919 6919598.0 | 7197250.3 807039.3 | 8670452.7 9408013.0 | |
| SD    | 19453.0 31130.4 | 58635.6 74482.6 | 80358.1 76630.3 | |
| %RSD  | 0.3 0.4 | 0.8 0.9 | 0.9 0.8 | |
| Recovery | 102.0% 119.10% | 93.50% | |

Robustness:
A method is robust if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at two different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of the mobile phase flow rate was varied by ±10%) and different column had no significant effect on the retention time and chromatographic response of the method, indicating that the method was robust. When the chromatographic conditions were deliberately altered, system suitability results remained within acceptance limits and selectivity for individual substance was not affected. The results of the study prove the robust nature of the method. (Table 6)
Table 6: Robustness study of rilpivirine Standard solution at 100 % level (250 μg/ml)

| Parameter          | Peak areas of rilpivirine in Flow increase study | Peak areas of rilpivirine in Flow decrease study | Peak areas of rilpivirine in Variable column Study |
|--------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Injection-1        | 6589635                                      | 7896589                                      | 7023697                                      |
| Injection-2        | 6569852                                      | 7887965                                      | 7023894                                      |
| Injection-3        | 6558942                                      | 7902569                                      | 7105682                                      |
| Mean               | 6572809.667                                  | 7895707.667                                  | 7051091                                      |
| % RSD              | 15558.78                                     | 7341.78                                      | 47277.29                                     |
| Std. Dev           | 0.24                                         | 0.099                                        | 0.67                                         |

Limit of Detection [LOD] and Limit of Quantification [LOQ]:
The detection limit of the method was investigated by injecting standard solutions rilpivirine into the HPLC column. By using the signal-to-noise method the peak-to-peak noise around the analyte retention time is measured, and subsequently, the concentration of the analyte that would yield a signal equal to certain value of noise to signal ratio is estimated. A signal-to-noise ratio (S/N) of 3 is generally accepted for estimating LOD and signal-to-noise ratio of 10 is used for estimating LOQ. This method is commonly applied to analytical methods that exhibit baseline noise. The limit of detection (LOD) and limit of quantification (LOQ) for rilpivirine were found to be 0.05μg/ml and 0.15 μg/ml respectively. (Table 7)

Table 7: Performance & Detection Characteristics of HPLC method

| Parameter                      | Results of the proposed HPLC method |
|--------------------------------|-------------------------------------|
|                                | Rilpivirine Standard | Rilpivirine Sample |
| Retention time (min)           | 7.185                                     | 7.197                        |
| Theoretical plates (n)         | 16633.23                                 | 16304.73                     |
| Plates per meter (N)           | 66532.8                                  | 65218.92                     |
| HETP                           | 1.5030x10^{-5}                         | 1.5333 x10^{-5}             |
| Peak asymmetry (T)             | 1.23                                    | 1.23                         |

CONCLUSION:
The author has developed a sensitive, accurate and precise HPLC for the estimation of rilpivirine in pharmaceutical formulation. The typical chromatogram of rilpivirine shows that the retention time was 7.19±0.15 min. The contents of the mobile phase were Buffer: Acetonitrile in ratio of 15:85 (v/v). Solvent-A (Buffer) is 3.48 gm of di Potassium hydrogen ortho-phosphate (0.03M) in 1000 ml of water and by adjusting the pH to 2.5 with dilute orthophosphoric acid and solvent-B is acetonitrile. A flow rate of 1.0 ml/min maintained and eluents were monitored at 284 nm, was found to be most suitable to obtain a peak well defined and free from tailing. A good linear relationship (r²=0.999) was observed between the concentration range of 100-300 μg/ml. The %
RSD of inter and intraday precision studies vary between 0.09-0.85%. From the recovery studies it was found that about 119.10 % on average of rilpivirine was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible.

REFERENCES:

1. Jayaweera D, Dilanchian P. New therapeutic landscape of NNRTIs for treatment of HIV: a look at recent data. Expert Opin Pharmacother 2012; 13(18): 2601-12.
2. Sanford M. Rilpivirine: Adis Drug Profile. Drugs 2012; 72(4):525-41.
3. Schafer JJ, Short WR. Rilpivirine-a novel non-nucleoside reverse transcriptase inhibitor for the management of HIV-1 infection: a systematic review. AntivirTher 2012; 17(8): 1495-502.
4. Sharma M, Saravolatz LD. Rilpivirine: a new non-nucleoside reverse transcriptase inhibitor. J Antimicrob Chemother 2013; 68(2) :250-6.
5. Else L, Watson V, Tjia J, Hughes A, Siccardi M, Khoo S, Back D. Validation of a rapid and sensitive high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) assay for the simultaneous determination of existing and new antiretroviral compounds. J Chromatogr B Analyt Technol Biomed Life Sci 2010 ; 878(19): 1455-65.
6. Mathias A, Menning M, Wiser L, Wei X, Dave A, Chuck S, Kearney BP. Bioequivalence of the emtricitabine/rilpivirine/tenofovir disoproxil fumarate single tablet regimen. J Bioequiv Availab 2012; 4: 100–105.
7. Aouri M, Calmy A, Hirschel B, Telenti A, Buclin T, Cavassini M, Rauch A, Decosterd LA. A validated assay by liquid chromatography-tandem mass spectrometry for the simultaneous quantification of elvitegravir and rilpivirine in HIV positive patients. J Mass Spectrom 2013; 48(5): 616-25.
8. Burugula L, Pilli NR, Makula A, Lodagala DS, Kandhagatla R. Liquid chromatography-tandem mass spectrometric assay for the non-nucleoside reverse transcriptase inhibitor rilpivirine in human plasma. Biomed Chromatogr 2013; 27(2): 172-8.
9. Mohanareddy C, Hussain Reddy K, Narayanareddy P, Venkataramana M. Degradation pathway for rilpivirine hydrochloride by validated stability indicating UP-LC method. Int J Pharmacol clin Toxicol 2012; 1(1) :1-8.
10. Bhavar G. B, Pekamwar S. S, Aher K. B, Chaudhari S. R. Development and validation of UV Spectrophotometric method for estimation of rilpivirine hydrochloride in bulk and pharmaceutical formulations. Am. J. PharmTech Res. 2013; 3(1):450–458.

11. Somsubhra G, Sowjanya B, Laxmi P.V, Vidyadhar S, David B, Subhadip R. Method development and validation of rilpivirine in bulk and tablet doses form by RP-HPLC method. Res J Pharm Technol 2013; 6(3):240–243.

12. Sudha T, Shanmugasundram P. Reverse phase high performance and HPTLC methods for the determination of rilpivirine bulk and in tablet dosage form. World J Pharm Res 2012; 1(4):1183–196.

13. Kavitha K. Y, Geetha G, Hariprasad R, Venkatnarayanan R, Kaviarasu M. Development and validation of RP-UPLC analytical method for simultaneous estimation of the emtricitabine, tenofovirdisoproxilfumarate and rilpivirine and its pharmaceutical dosageform. Int J Pharm Res 2013; 4(1):150–155.

14. Venkatesan S, Kannappan N. Simultaneous Spectrophotometric Method for Determination of Emtricitabine and Tenofovir Disoproxil Fumarate in Three-Component Tablet Formulation Containing Rilpivirine Hydrochloride. Int Sch Res Notices. 2014:541727.

15. Date AA, Shibata A, Bruck P, Destache CJ. Development and validation of a simple and isocratic reversed-phase HPLC method for the determination of rilpivirine from tablets, nanoparticles and HeLa cell lysates. Biomedical chromatography : BMC 2015; 29(5):709-715.

16. International Conference on Harmonization, ICH Guidelines, Validation of Analytical Procedures Technical Requirements for Registration of Pharmaceuticals for Human Use: Text and Methodology Q 2 (R1), International Conference on Harmonization, Geneva, Switzerland, November 2005.