Spectrofluorimetric quantification of Rilpivirine using ethyl acetoacetate and sulfuric acid as fluorigenic reagent in bulk and tablet dosage form

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Abstract:

Background: Rilpivirine is also known as pentyl 4-{[4-({4-[(E)-2-cyanovinyl]-2,6-dimethylphenyl} amino) pyrimidin-2-yl] amino} benzonitrile. It is a non-competitive NNRTI which binds to reverse transcriptase, results in the blockage of RNA and DNA- dependent DNA polymerase activities, like HIV-1 replication.

Methods: New, sensitive and precise spectrofluorimetric method have been developed for the determination of rilpivirine in tablets. As rilpivirine does not exhibit any native fluorescence, it has been derivatized using ethyl acetoacetate reagent and sulphuric acid as a highly sensitive fluorogenic reagent. Fluorescence intensity of rilpvirine was measured at excitation wavelength λ_{ems} 457nm and emission wavelength λ_{exc} 483nm.

Results: The fluorescence intensities were directly proportional to the concentration over the range 5-25 µg mL-1, correlation coefficient being $r^2 = 0.9995$. The %RSD for inter-day and intra-day precisions was in a range of 0.23-1.05% and the accuracy results were in the range of 98.12% - 101.5%.

Conclusion: The method was validated in accordance with ICH guidelines, and the results for validation parameters—including linearity, precision, accuracy, limit of detection, limit of quantification, and assay—demonstrate its suitability for routine quality control of the drug in both bulk and pharmaceutical formulations.

Key words: Rilpvirine, Ethyl Acetoacetate, Sulfuric acid, Spectrofluorometry, Validation.

1. INTRODUCTION

Rilpivirrine is also known as pentyl 4-{[4-({4-[(E)-2-cyanovinyl]-2,6-dimethylphenyl} amino) pyrimidin-2-yl] amino} benzonitrile (Figure 1). It is a non-competitive NNRTI that binds to

reverse transcriptase. Binding results in the blockage of RNA and DNA- dependent DNA polymerase activities, like HIV-1 replication.

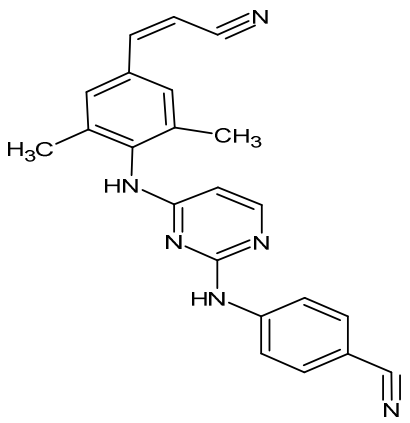

Figure 1. Structure of Rilpivirine

An extensive literature review on Rilpivirine revealed numerous analytical methods for its quantification, either as a single agent or in combination with other drugs. Reported methods include ultraviolet (UV) spectrophotometric techniques using various solvents[13-16], high-performance liquid chromatography (HPLC) methods employing different combinations of stationary and mobile phases[17–26], bioanalytical techniques using HPLC[27, 28] LC-MS/MS methods[29] and bioequivalence studies[30].

Although numerous instrumental techniques were available till date, to the best of our knowledge, no spectrofluorimetric method has been reported for Rilpivirine using any fluorophore or fluorescent tagging agent. Spectrofluorimetry has gained prominence in drug analysis due to its high specificity and sensitivity. Unlike spectrophotometry, spectrofluorimetry enables analysis at both excitation and emission wavelengths[10, 31] Considering these advantages, a simple, extraction-free, and sensitive spectrofluorimetric method was developed for the quantification of Rilpivirine using ethyl acetoacetate and sulfuric acid. The method was validated in accordance with International Conference on Harmonisation (ICH) guidelines[12, 32] and was successfully applied to the analysis of Rilpivirine in a marketed dosage form.

2.MATERIALS AND METHODS:

2.1. Chemicals and reagents:

All chemicals and reagents used were of analytical grade. Pure Rilpivirine (99.50% purity) was obtained as a gift sample from Mylan Laboratories Limited, Hyderabad, India. Commercial

Rilpivirine tablets (Edurant) were procured from local pharmacies. Ethyl acetoacetate, sulfuric acid, ethanol, and methanol were purchased from Research-Lab Fine Chem Industries, Mumbai, India.

2.2. Instrumentation:

Fluorescence intensity measurements were carried out using a Shimadzu RF-5301 PC Spectro fluorophotometer (Japan), equipped with a 150 W xenon arc lamp and operated via RFPC software. A 1 cm path length non-fluorescent quartz cell was used for all measurements. The instrument was operated at both low and high sensitivity settings, with excitation and emission slit widths set at 5 nm. Additional equipment included an analytical balance (Shimadzu AUX 220, Japan), a dissolution apparatus (Electro Lab TDT-08L, India), a pH meter (Elico, India), a hot air oven, and a UV cabinet (Bio-Technics, India).

2.3. Preparation of standard stock solutions:

An accurately weighed 10.00 mg of Rilpivirine was dissolved in 10.00 mL of methanol to prepare a standard stock solution with a concentration of 1000 µg/mL. From this stock, 1.00 mL was pipetted and further diluted to 10.00 mL with methanol to obtain a working standard solution with a concentration of 100 µg/mL.

2.4. Construction of the calibration graph:

A series of standard solutions of Rilpivirine in the concentration range of 5–25 µg/mL were prepared by transferring appropriate volumes (0.5, 1.0, 1.5, 2.0, and 2.5 mL) of the working stock solution (100 µg/mL) into separate 10.0 mL volumetric flasks. To each flask, 0.1 mL of 2% ethyl acetoacetate reagent and 2.5 mL of sulfuric acid were added. The mixtures were then heated in a water bath for 20 minutes. After cooling to room temperature, the volumes were adjusted to the mark with distilled water. The solutions were allowed to stand undisturbed for 10 minutes before fluorescence intensity was measured using a Spectro fluorophotometer, scanning from 400 to 700 nm

2.4. Determination of Rilpivirine in tablet dosage form (assay)

Twenty-five tablets of the marketed formulation (Edurant®), each containing 25 mg of Rilpivirine, were accurately weighed, and the average tablet weight was determined. The tablets were then finely powdered. A portion of the powder equivalent to 5.0 mg of Rilpivirine was accurately weighed and transferred to a 10.0 mL volumetric flask. The sample was dissolved in methanol and sonicated for 15 minutes to ensure complete extraction. The mixture was shaken and diluted to volume with distilled water. The resulting solution was filtered through Whatman No. 41 filter paper. An appropriate volume of the filtrate was further diluted to obtain a final concentration suitable for analysis using the proposed spectrofluorimetric method.

3. RESULTS AND DISCUSSION

3.1. Selection of wavelength:

"To develop a sensitive and specific spectrofluorimetric method for the quantification of Rilpivirine, various solvent systems—including methanol, dimethyl sulfoxide (DMSO), ethanol, dimethylformamide (DMF), and glacial acetic acid—were evaluated. Among these, methanol provided the highest fluorescence intensity and was therefore selected as the optimal solvent. Rilpivirine exhibited maximum fluorescence at an emission wavelength of 483 nm.

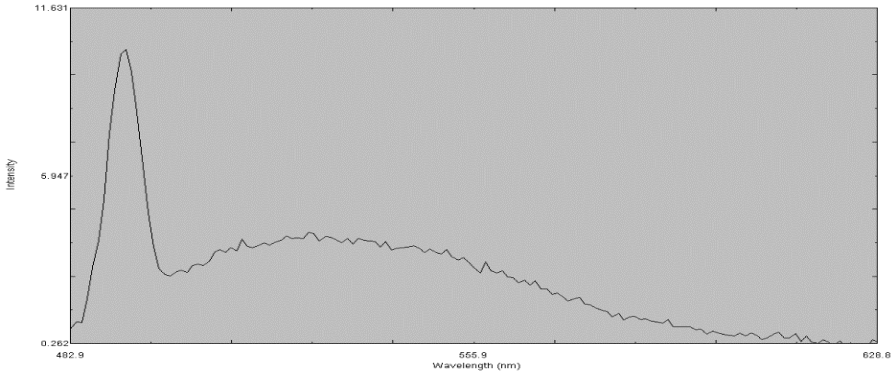

Figure 2. Emission spectrum (483 nm) of rilpivirine in methanol as solvent.

Parameter	Value
Excitation wavelength (nm)	457
Emission wavelength (nm)	483
Linearity range (µg/mL)	5-25
Limit of detection (µg/mL)	0.124
Limit of quantification (µg/mL)	0.378
Correlation coefficient (r^2)	0.9995
Regression equation	Y=0.6271x+0.1372

Table 1. Optimized parameters for the proposed method

3.2. Analytical method validation:

3.2.1. Linearity and range:

Linearity was qualitatively assessed by plotting the fluorescence intensity at 483 nm against several concentrations of Rilpivirine. The plot showed a gradual increase in fluorescence intensity, providing clear evidence of the method's suitability for analysis (Figure 3). The linearity was further confirmed by the regression equation derived from the calibration curve. As shown in Table 1 and Figure 3, the response for Rilpivirine at 483 nm was linear within the concentration range of 5–25 µg/mL, with a correlation coefficient (r^2) of 0.9995.

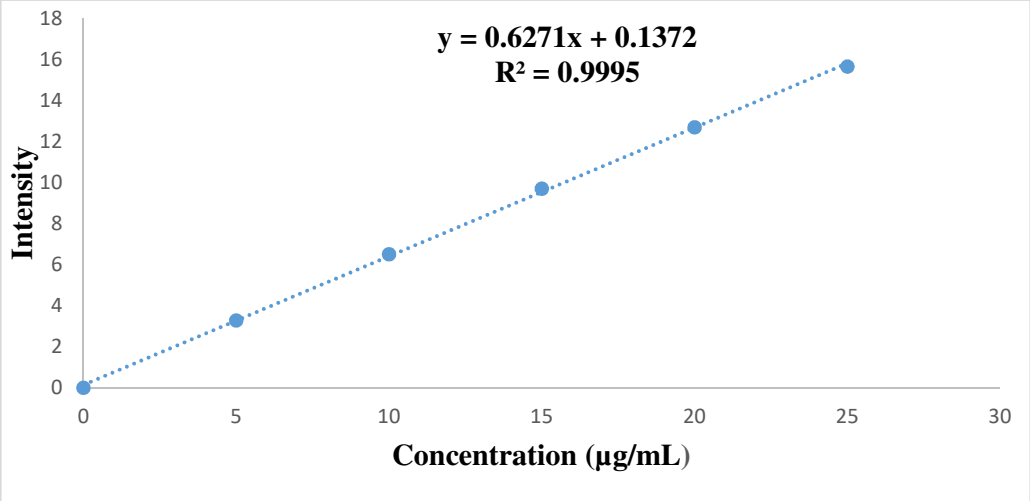

Figure 3. Linearity plot of rilpivirine with ethylacetoacetate and sulphurc acid.

3.2.2. Accuracy (recovery studies)

Accuracy was evaluated using the standard addition method at three different concentration levels (80%, 100%, and 120%) by spiking commercial tablets with the respective standard solutions in triplicate. The mean percentage recoveries and the relative standard deviation (% RSD) values were calculated and are presented in Table 2. The % RSD values at the relevant concentration levels were found to be 2, and the percentage recoveries of Rilpivirine ranged from 99% to 101%, demonstrating the accuracy of the method

Table 2: Accuracy (% recovery) of proposed method.

Formulation	Recovery level(%)	Theoritical content (µg/mL)	Concentration found (µg/mL) (mean ± SD)	% Amount recovered (mean ± SD)	% RSD
	80	14	8.76± 0.01	98.2	0.11

Edurant®	100	16	10.23± 0.3	98.12	0.1
	120	18	11.598± 0.05	101.5	0.43

SD: Standard deviation, RSD: Relative standard deviation

3.2.3. Precision:

Intra-day and inter-day precision were evaluated according to ICH guidelines. Samples containing Rilpivirine at concentrations of 5, 15, and 25 µg/mL were analyzed six times on the same day (intra-day precision) and across three consecutive days (inter-day precision). The % RSD values were then calculated. The results of intra-day and inter-day precision are presented in Table 3, which show no significant difference between the % RSD values for intra-day and inter-day analyses. This indicates that the proposed method exhibits high precision.

Table 3: Precision data of the proposed analytical method.

Theoritical content (µg/mL)	Interday		Intraday	
	Concentration (mean[a] ± SD)	% RSD	Concentration (mean[b] ± SD)	% RSD
5	3.23± 0.034	1.05	3.23± 0.026	0.8
15	9.77± 0.11	1.1	9.79± 0.0988	1.001
25	15.613± 0.049	0.3	15.57± 0.036	0.23

a: Mean values of six different standards for each concentration, b: Inter-day reproducibility was quantified from six different standards of each concentration for three consecutive days, SD: Standard deviation, RSD: Relative standard deviation

3.2.4. Limit of detection and limit of quantitation:

The limit of detection and limit of quantitation were discretely appraised based on the standard calibration curve, and the results are presented in Table 1.

3.3. Applications:

3.3.1. Assay:

The proposed method was applied to assay commercial tablets (Edurant®) containing 25 mg of Rilpivirine. The results were compared with the labeled amounts, as shown in Table 4. The assay yielded a peak value of 98.4% with a % RSD of less than 2, indicating the accuracy of the proposed method.

Table 4. Assay results of marketed tablets of rilpivirine.

Formulation	Rilpivirine Label claim(mg)	Amount found (mg) (mean ± SD) (n=3)	% Assay	% RSD
Edurant®	25	24.6± 0.15	98.4	0.609

SD: Standard deviation, RSD: Relative standard deviation

4. CONCLUSION:

The proposed method was used to assay commercial tablets (Edurant®) containing 25 mg of Rilpivirine. The results were compared to the labeled amounts, as presented in Table 4. The assay showed a recovery of 98.4% with a % RSD of less than 2, confirming the accuracy of the method..

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Conflict of Interest: No conflict of interest was declared by the authors.

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