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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF RILPIVIRINE IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT: In the present research work, four simple, precise, accurate and economical methods of UV spectroscopy have been developed for the estimation of Rilpivirine in bulk and pharmaceutical formulation. Method A involves estimation of Rilpivirine using low cost solvent, 0.01N HCl by zero order spectroscopy at an absorption maximum of 280nm. Method B involves Area under Curve method which involved calculation of integrated value of absorbance with respect to wavelength between two wavelengths selected, 275nm and 285nm respectively. Method C involves first order derivative technique for the same at 264nm. Method D involves second order derivative technique for the same at 237nm. The developed methods were found to be linear in the concentration range of 0.5-7.5µg/mL with correlation coefficient (R^2) of 0.9998. The mean percentage label claim of tablets of Rilpivirine estimated by proposed methods was within the acceptable range. The developed methods were validated by following the analytical performance parameters suggested by ICH. All the validation parameters were within the acceptable range. As economical solvent is used, these methods can be used for routine analysis of Rilpivirine in bulk and pharmaceutical formulation.

INTRODUCTION: Rilpivirine (TMC278; formerly known as R278474) is non-nucleoside reverse transcriptase inhibitor (NNRTI), it had been developed for treatment of ARV naïve HIV-I infected patients to have better safety/ tolerability profile compared to other NNRTIs (such as nevirapine, efavirenz and etravirine) ¹. Chemically Rilpivirine is 4-[[4-[[4-(E)-2-cyanoethenyl]-2,6-dimethylphenyl] amino]-2-pyrimidinyl] amino] benzonitrile ².

The chemical structure was shown in **Figure 1** ³. It is a di-amino pyrimidine derivative. It acts by binding to reverse transcriptase which results in block in RNA and DNA- dependent DNA polymerase activities. One such activity is HIV-I replication ^{1,4}.

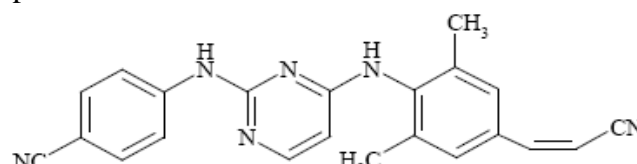


FIGURE 1: CHEMICAL STRUCTURE OF RILPIVIRINE

It is an E-isomer. As the structure of Rilpivirine is flexible around aromatic rings, the molecule can have multiple conformations and so it can bind to residues in reverse transcriptase enzyme which have a lower mutation rate.

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It is white to almost white powder. It is practically insoluble in water and is soluble in N, N- dimethyl acetamide, N, N-DMF, slightly soluble in methanol, propylene glycol and 1-methoxy-2-propanol⁵. Rilpivirine was approved by European Medicines Agency, UK and Therapeutic Goods Administration (TGA), Australia⁶.

Literature survey revealed that two simple UV spectroscopic methods^{7,8}, few RP-HPLC methods⁹, HPTLC method¹⁰, LC-MS method¹¹, stability indicating UPLC method¹² were reported and for quantitative estimation of Rilpivirine in bulk and formulation have been developed.

However these methods include arduous sample preparation, long retention times and expensive solvents. To our knowledge, AUC and derivative UV methods are not available.

The objective of present investigation deals with development of simple, accurate, precise and more economical UV spectroscopic method using AUC and derivative methods for estimation of Rilpivirine in bulk and formulation and their validation as per ICH guidelines¹⁸.

EXPERIMENTAL SECTION:

Instrumentation:

- A double beam UV spectrophotometer: Shimadzu (1800) with UV probe software (2.31) and 10mm matched quartz cells.
- pH meter : Elico
- Weighing balance: Shimadzu (220h)

Materials, reagents and chemicals: Rilpivirine (99.7%) working standard drug was obtained from MYLAN labs ltd India, commercial tablet formulation of Rilpivirine (Edurant 25mg) were bought from pharmacy, methanol and hydrochloric acid which are of analytical grade were purchased from Merck Fine Chemicals(Mumbai, India). Double distilled water was used to prepare 0.01N HCl. Whatman filter paper no. 41 was used.

METHOD DEVELOPMENT:

Selection of solvent: Solubility of drug was performed in solvents like methanol and HCl and UV spectra of drug in these solutions were recorded. Absorbance values of drug were higher at distinct λ_{\max} with HCl as solvent. Then drug solutions were prepared using different normality solutions of HCl. The drug showed good absorbance value at that distinct λ_{\max} even with 0.01N HCl solution. Hence, 0.01N HCl was selected as solvent for further investigation as it is more economical.

Preparation of standard stock solution: The standard stock solution of Rilpivirine was prepared by dissolving accurately weighed 10mg of working standard taken in 10mL volumetric flask in 7mL methanol and the volume was made up to the mark with methanol.

Preparation of solutions of analytical concentration range: The working standard solution of Rilpivirine was prepared by transferring 1mL stock solution into 10ml volumetric flask and made up the volume with 0.01N HCl (concentration 100 μ g/ml). Appropriate aliquots were pipetted out from the working standard solution in to a series of 10 ml volumetric flasks. The volume was made up to the mark with 0.01N HCl to obtain solutions of concentration range, ranging from 0.5-7.5 μ g/mL of Rilpivirine.

Construction of calibration curve:

Method A: Zero order spectroscopic method: The analytical wavelength was selected by preparing a solution of concentration 3.0 μ g/mL by dilution of standard stock solution with 0.01N HCl solution. The solution was scanned in the wavelength range of 200-400nm using 0.01N HCl as blank. The UV spectrum of Rilpivirine showed λ_{\max} at 280nm in 0.01N HCl as shown in **Figure 2**.

The calibration curve was prepared in the concentration range of 0.5-7.5 μ g/mL at 280nm by measuring the absorbance of each concentration using 0.01N HCl as blank and plotting the absorbance v/s respective drug concentration. The regression equation was calculated.

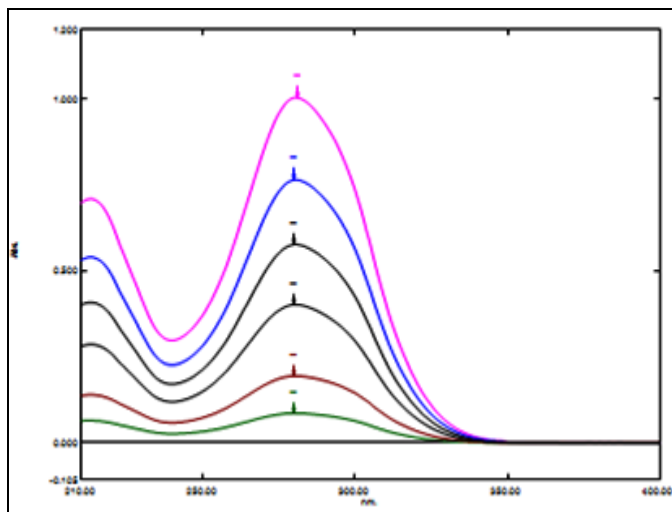


FIGURE 2: ZERO ORDER SPECTRA OF RILPIVIRINE AT 280nm

Method B: Area under Curve method¹³: The AUC method is applicable where there is no sharp peak or when broad peak is obtained. It involves the calculation of integrated value of absorbance with respect to wavelength between two selected wavelengths λ_1 and λ_2 . Area calculation processing item calculates area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area had to be calculated¹³.

The wavelength range is selected based on repeated observations so as to get the linearity between AUC and concentration. The solutions having analytical concentration range of 0.5-7.5 $\mu\text{g/mL}$ were scanned in the spectrum mode from the wavelength range 200-400nm and the AUC spectra were measured between wavelengths, 275nm and 285nm as shown in **Figure 3**. The calibration curve was prepared by plotting concentration v/s AUC.

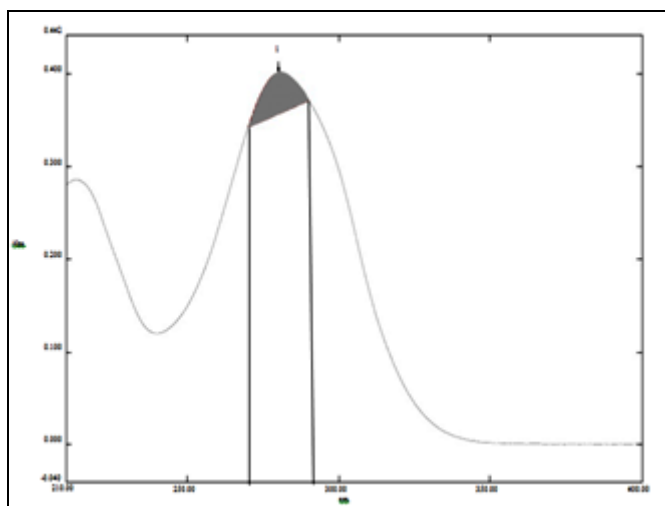


FIGURE 3: AUC SPECTRUM OF RILPIVIRINE

Method C: First Order Derivative Spectroscopic method^{14, 15}: It involves the conversion of normal spectrum into first derivative spectrum. These derivative spectra have narrow spectral band width. Because of this resolution is better and it reveals overlapping bands that were lost in original spectra. Thus it is advantageous for selection of accurate wavelength. In addition, concentration measurements of an analyte in the presence of interference or of two or more analytes in a mixture can sometimes be made more easily or more accurately using derivative methods^{14, 15, 16}.

For the selection of analytical wavelength, a solution of concentration 3.0 $\mu\text{g/mL}$ of Rilpivirine was prepared and scanned in the spectrum mode in the λ range 200-400nm. The absorption spectrum thus obtained was derivatized in first order. First order derivative spectrum showed a sharp peak at λ_{max} at 264nm and λ_{minima} at 306nm. The absorbance difference at $n=1$ ($dA/d\lambda$) was calculated by inbuilt software of the instrument. The amplitude of absorbance was measured for all the solutions of concentration range 0.5-7.5 $\mu\text{g/mL}$ at 264nm and was plotted against concentration to give calibration curve as shown in **Figure 4** and regression equation was calculated. The concentration of the drug present in the solution was determined against the calibration curve in quantitation mode.

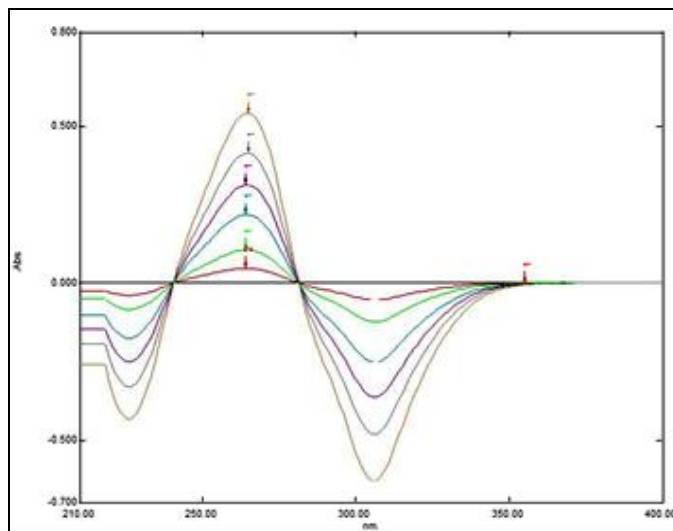


FIGURE 4: FIRST ORDER DERIVATIVE SPECTRA OF RILPIVIRINE AT 264nm

Method D: Second Order Derivative Spectroscopic method¹⁷: The original spectra were derivatized in the second order to get second order derivative spectra.

The spectra showed λ_{\max} at 237nm. The $d^2A/d\lambda^2$ of corresponding crests were measured at 237nm and plotted against concentrations to give calibration curve as shown in **Figure 5** which is linear in the concentration range of 0.5-7.5 $\mu\text{g/mL}$ and regression equation was calculated.

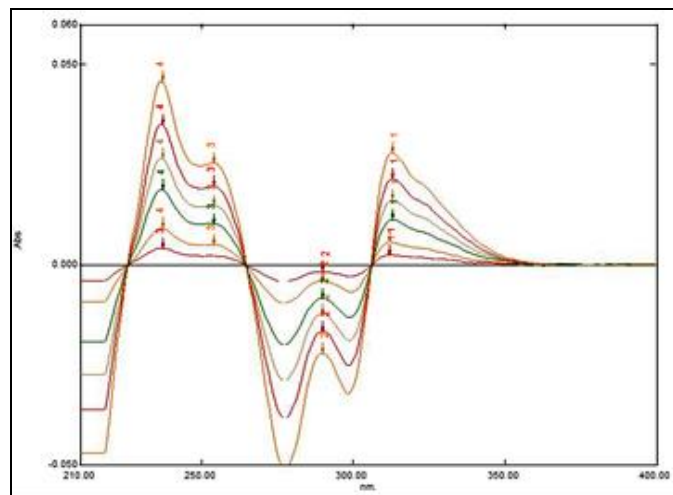


FIGURE 5: SECOND ORDER DERIVATIVE SPECTRA OF RILPIVIRINE at 237nm

TABLE 1: ASSAY RESULTS OF RILPIVIRINE BY FOUR METHODS

Analysis methods	Label claim (mg/tablet)	Amount found (mg) (n=6)	% amount found	%RSD
A	25 mg	2.94	98.16	0.094
B	25 mg	3.05	101.6	0.064
C	25 mg	3.02	100.7	0.038
D	25 mg	3.01	100.3	0.011

The assay values determined by four methods were given in the table and from the above values it was clear that of all the developed methods second derivative method i.e., method D was accurate as the assay value was 100.3 and can conclude that on derivatization accuracy is increased.

METHOD VALIDATION: The methods were validated according to ICH guidelines¹⁸ to study linearity, precision and accuracy.

1. **Linearity:** The linearity of the proposed UV spectroscopic methods were evaluated by analyzing different concentrations of standard solutions of Rilpivirine and by plotting

Estimation of Rilpivirine in tablet formulation:

For the estimation of Rilpivirine in the commercial formulations, 5 tablets each containing 25 mg of Rilpivirine were weighed and the average weight was calculated. The tablets were crushed and powdered in glass mortar. For the analysis of drug, quantity of powder equivalent to 10mg of Rilpivirine was transferred to 10 mL volumetric flask and dissolved in sufficient quantity of methanol and the volume made upto the mark with methanol to obtain a stock solution of 1000 $\mu\text{g/mL}$ of Rilpivirine.

Then, the solution was filtered through Whatmann filter paper no. 41. Further dilutions of the stock solution were made in 0.01N HCl to get required concentration of 3.0 $\mu\text{g/mL}$. The concentration of Rilpivirine in formulation was determined by above developed methods. Results of tablet analysis are shown in **Table 1**. The assay procedure was repeated six times (n=6) for each method.

absorbances of analyte against concentrations of the analyte. Beer's law was obeyed for all four methods in the concentration range of 0.5-7.5 $\mu\text{g/mL}$.

A good linear relationship ($R^2=0.999$) was observed between the concentrations of Rilpivirine and the corresponding absorbance. The regression analysis was made for slope, intercept and correlation coefficient values. The slope, intercept and the correlation coefficient of the drug were shown in **Table 2**. All the values were within the acceptable range.

TABLE 2: LINEARITY STUDIES OF THE PROPOSED METHODS

S. No.	Parameter	Method A	Method B	Method C	Method D
1	Linearity ($\mu\text{g/mL}$)	0.5 - 7.5	0.5 - 7.5	0.5 - 7.5	0.5 - 7.5
2	Slope	0.1295	1.3017	0.0717	0.0061
3	Intercept	0.0082	0.0039	0.0027	0.0002
4	Correlation coefficient	0.9992	0.9993	0.9994	0.9996

2. Accuracy: Accuracy is expressed as degree of closeness of experimental value to the true value. To study the accuracy of the proposed method and to check the interferences from excipients used in the dosage forms, recovery experiments were carried out by standard addition method. This parameter is evaluated by percent recovery studies at concentration levels of 80,100 and 120% which includes addition of known amounts of Rilpivirine working standard to a pre-quantified sample

solution. Each of the dilution was observed six times. The samples were reanalyzed by proposed methods. The amount of Rilpivirine was estimated by applying obtained values to regression equation. The percentage recovery of the drug was calculated. The results were shown in the **Table 3**. All the values were within the acceptable range and the %recovery values show that there is no interference of additives and excipients.

TABLE 3: ACCURACY STUDIES OF RILPIVIRINE BY PROPOSED METHODS

Concentration taken ($\mu\text{g/mL}$)	Spiked level (%)	Amount added (mg)	Amount found (mg) (n=6)				%Recovery			
			A	B	C	D	A	B	C	D
3	80	2.4	5.34	5.48	5.45	5.42	98.8	101.4	100.9	100.3
3	100	3	5.96	6.07	6.04	6.02	99.3	101.16	100.6	100.1
3	120	3.6	6.55	6.69	6.69	6.63	99.2	101.3	100.5	100.4

3. Precision: Precision is the level of repeatability of results as reported between samples analyzed on the same day (Intra- day) and samples run on three different days (Inter-day). To check the intra-day and inter-day variation of the methods, solutions containing 3.0, 4.5 and 6.0 $\mu\text{g/mL}$ concentrations of Rilpivirine were subjected to the proposed spectrophotometric methods of analysis and the recoveries obtained were noted.

The precision of the proposed method i.e. the intra and inter –day variations in the absorbance of the drug solutions was calculated in terms of % RSD and the results were presented in the **Table 4**. Statistical evaluation revealed that relative standard deviation of drugs at different concentration levels for three times was less than 2.0. (Intra-day -0.48, inter-day- 0.473). The values were shown in **Table 4**.

TABLE 4: PRECISION STUDIES OF RILPIVIRINE

Concentration taken($\mu\text{g/ml}$)	Intra-day precision		Inter-day precision	
	*mean \pm SD	%RSD	*mean \pm SD	%RSD
3	0.429 \pm 0.004	0.93	0.412 \pm 0.005	1.3
4.5	0.585 \pm 0.003	0.60	0.579 \pm 0.004	0.86
6	0.782 \pm 0.005	0.57	0.776 \pm 0.005	0.70

*= mean of 3 readings

RESULTS AND DISCUSSION:

TABLE 5: SUMMARY OF OPTICAL CHARACTERISTICS AND VALIDATION PARAMETERS (TABLET)

Parameter	Method A	Method B	Method C	Method D
λ_{max}	280nm	275-285nm	264nm	237nm
Beer's limit($\mu\text{g/mL}$)	0.5 – 7.5	0.5 – 7.5	0.5 – 7.5	0.5 – 7.5
Sandell's sensitivity($\mu\text{g/mL}$)	0.7×10^{-2}	0.66×10^{-3}	0.1×10^{-1}	0.16
Linearity indicated by correlation coefficient	0.9992	0.9993	0.9994	0.9996
Precision indicated by %RSD	1.1	0.96	0.84	0.73
Accuracy indicated by %recovery	99.1	101.28	100.6	100.2

The summary of validation parameters was shown in **Table 5**. All the values were within the acceptable range. All the proposed methods for estimation of Rilpivirine were found to be simple, precise, accurate and economical. For method A, the absorption maxima was found to be at 280 nm,

for method B area under the curve in the range of 275-285 nm was selected for the analysis, for method C, the absorption maxima of first derivative spectra was found to be 264nm and finally for method D, the absorption maxima for second derivative spectra was found to be 237nm.

The calibration curve was linear in the concentration range 0.5-7.5µg/mL as shown in **Table 2**. The % assay by the four methods was found to be in the range 98.75-100.15% for Rilpivirine as shown in **Table 1**. No interference was observed from the pharmaceutical excipients. The recovery studies showed that these methods were accurate and reproducible. The results revealed that any change in drug concentration could be accurately determined by the proposed method. Accuracy and reproducibility of the proposed methods were further confirmed by percent recovery values, which were close to 100 with low values of standard deviation as shown in **Table 3**. Repeatability results indicated the precision under the same operating conditions over a short interval time and inter-assay precision. Intermediate precision study expresses within laboratory variation in different days.

In both intra and inter-day precision study for the method %RSD is not more than 2.0 indicate good intermediate precision which were shown in **Table 4**. Hence, the proposed methods were validated in terms of linearity, precision and accuracy. Characteristic parameters and summary of validation parameters for all the four methods were given in **Table 5**. By observing the validation parameters, the methods were found to be simple, accurate and precise. Hence these methods can be employed for the routine analysis of Rilpivirine in tablet formulations.

CONCLUSION: The four spectrophotometric methods were developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are within acceptance limits, indicating high degree of precision of the methods. The results of the recovery studies performed indicate the methods to be accurate and of these methods, second order derivative method is more accurate. Hence, it can be concluded that the developed spectrophotometric methods are simple, accurate, precise and economical and can be employed successfully for the estimation of Rilpivirine in bulk and formulation. There is a good scope for estimation of Rilpivirine by these methods to carry out their spectrophotometric analysis excluding the use of costlier and unsafe organic solvents by using low cost and easily available HCl as solvent.

Thus, it can be conveniently adopted for routine quality control analysis.

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