http://www.e-journals.in
DOI:10.7598/cst2015.935

Chemical Science Transactions
2015, **4(3),** 799-805

RESEARCH ARTICLE

UV-Visible Spectrophotometric Method for the Estimation of Rilpivirine Hydrochloride in Pharmaceutical Dosage Form by Using Multivariate Technique

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Received 20 August 2014 / Accepted 5 September 2014

Abstract: To develop a simple, precise and accurate UV-method with multivariate calibration technique for estimation of rilpivirine hydrochloride in pharmaceutical dosage form. This technique is based on the use of the linear regression equations by using relationship between concentration and absorbance at five different wavelengths like 301, 303, 305, 307 and 309 nm. The results were treated statistically and were found highly accurate, precise and reproducible. The rilpivirine hydrochloride shows absorption maxima at 305 nm and obeyed Beer's law in the range of 0.5-3.0 µg/mL. The % recovery of tablets was found to be in the range of 101.1-101.7%. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.358 and 1.086 µg/mL, respectively. The low % RSD values are indicates the accuracy and precise of the method. The proposed methods can be successfully applied for method development, validation and multivariate analysis of rilpivirine hydrochloride.

Keywords: Rilpivirine hydrochloride, UV spectroscopy, Development, Validation, Multivariate technique

Introduction

Rilpivirine hydrochloride is a di-amino pyrimidine derivative. Chemically, it is 4-[[4-[[4-[(*E*)-2-cyanoethenyl]-2,6-dimethylphenyl]amino]-2-pyrimidinyl]amino]benzonitrile monohydrochloride structure is shown in Figure 1. Rilpivirine hydrochloride is a human immunodeficiency virus type 1 (HIV-1) specific non-nucleoside reverse transcriptase inhibitors (NNRTI) indicated for used in single and combination of other drugs like tenofovir, emcitribine and effarveniz. Rilpivirine (TMC278) is NNRTI, which was approved[1-3] by the FDA in May 2011. It is a basic, white, amorphous powder which is readily soluble in methanol, dichloromethane and insoluble in water. Rilpivirine hydrochloride is not yet official in I.P and B.P. A thorough literature survey revealed that UV spectroscopy[4-6], HPLC[7-9] method for rilpivirine hydrochloride with combination of other drugs, UPLC[10], LC-MS[11], for its estimation in bulk, pharmaceutical dosage forms and biological samples.

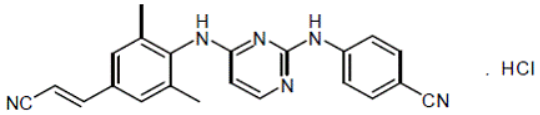

Figure 1. Chemical structure of rilpivirine hydrochloride

There is no reported method for multivariate technique in UV studies for the estimation of rilpivirine hydrochloride. Multivariate calibration refers to the process of constructing a mathematical model that relates a property such as content or identity to the absorbance of a set of known reference samples at more than one wavelength[12]. If the absorbance of an analyte (Z) is measured at five wavelengths set, straight line equation can be written as; $A_\lambda = aX(Cz+k)$ where A_λ represent the absorbance of the analyte, A is the slope and k is the intercept of the linear regression function of the analyte. C_Z represents the concentration of analyte. At five selected wavelengths, the equation system can also be summed as; $A_T = aX\ (C_Z + b)\ X\ (C_Z + c)\ X\ (C_Z + d)\ X\ (C_Z + e)\ X\ (C_Z + K_T)$, which can be simplified to $A_T = C_Z\ (a+b+c+d+e) + K_T$ where a, b, c, d, e are the slopes, A_T and K_T represents the sum of absorbance obtained and sum of intercepts of regression equations at five-wavelength set respectively. The concentration of the Z analyte in a mixture can be calculated by using the Eqn. $C_Z = A_T - K_T / (a+b+c+d+e)$. The present research work on the application of UV spectral multivariate calibration technique having simple mathematical content for the quantitative determination of rilpivirine hydrochloride in pharmaceutical formulation. UV Spectrophotometric methods of analysis are more economic and simpler, compared to methods such as chromatography and electrophoresis.

Experimental

Rilpivirine hydrochloride was gift sample from Hetero Laboratories Ltd. (Hyderabad, A.P, India). Methanol was purchased from Merck Chemical Company, India. The commercially tablets are not available in Indian market, hence we have manufactured immediate release tablet containing 25 mg, acacia, micro crystalline cellouse, lactose, magnesium sterate and talc from S D Fine Chem. Ltd. (Mumbai, India).

Instruments

The multivariate technique was performed in double beam UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe was employed with spectral bandwidth of 1 nm and wavelength accuracy of ±0.3 nm with a pair of 10 mm matched quartz cells and sonicator.

Preparation of standard stock solution of rilpivirine hydrochloride

Standard stock solution of rilpivirine hydrochloride (1 mg/mL) was prepared by transferring 10 mg of rilpivirine hydrochloride into a 10 mL volumetric flask containing 4 mL of (8:2) methanol and water. It was then sonicated for 15 minutes and solution was diluted up to the volume by methanol and water. From these, further dilutions were made using (8:2) methanol and water to produce solution of rilpivirine hydrochloride (100 µg/mL).

Selection of wavelength for analysis of rilpivirine hydrochloride

0.1 mL of standard stock solution of rilpivirine hydrochloride was transferred into a 10 mL volumetric flask and diluted to a mark with methanol: water (8:2) to give concentration of 1 µg/mL. The resulting solution was scanned in the UV range (200-400 nm).

Preparation of sample solution

Twenty tablets are analysed for their drug content by UV spectrophotometric methods. The tablet contents were crushed into a fine powder and suitably diluted in methanol: water (8:2) to yield a concentration of 1.0 mg/mL for rilpivirine hydrochloride. The mixture was sonicated to dissolve, make up the volume with methanol: water (8:2). The above solutions were filtered through Whatman filter paper and the solution was transferred into volumetric flask and was made up to the mark with methanol: water (8:2) to obtain a final concentration of 1.5 µg/mL. The spectrum was recorded at 305 nm against blank solution of methanol: water (8:2).

Validation of proposed method

The method was validated according to ICH guidelines in order to determine the linearity, precision, accuracy and ruggedness of the method.

Linearity

Linearity was evaluated by seven point standard curve in concentration range of 0.5-3.5 µg/mL (0.5, 1, 1.5, 2, 2.5, 3 and 3.5 µg/mL) of rilpivirine hydrochloride. The calibration curve was obtained by plotting absorbance against concentration (µg/mL) for five different wavelengths. Each set was analyzed to plot a calibration curve. Standard deviation (SD), slope, intercept and correlation coefficient of determination (r^2) of the calibration curves were calculated to ascertain the linearity of the method.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solution (n=6) for rilpivirine hydrochloride (1.5 µg/mL) without changing the parameter of the proposed UV method. The %RSD was calculated.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses on the same day and next day for three different concentration of standard solution of rilpivirine hydrochloride (0.5, 1.5 and 2.5 µg/mL). The result was reported in terms of relative standard deviation (%RSD).

Accuracy

Accuracy of the proposed method was determined using recovery studies by standard addition method. The recovery studies were carried out by adding different amounts (50, 100 and 150%) of the pure drug to the pre-analysed formulation. The solutions were prepared in triplicates and the % recovery was calculated.

Limit of detection and limit of quantitation

The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. The limit of detection (LOD) and the limit of quantitation (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, ***i.e.***, 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$$LOD = 3.3 \times \sigma/S,\ LOQ = 10 \times \sigma/S$$

Where σ = the standard deviation of the response and S = slope of the calibration curve

Ruggedness studies

Ruggedness studies were performed by preparing three replicates of 1.5 µg/mL, analysing by two different analyst and on two different instruments and the results are reported as %RSD.

Results and Discussion

In spectrum, rilpivirine hydrochloride in pharmaceutical dosage form shows maximum absorbance at 305 nm shown in Figure 2.

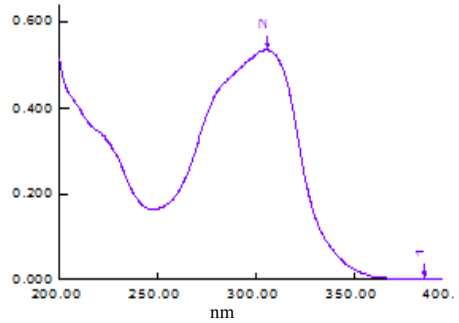

Figure 2. Absorbtion spectra of rilpivirine hydrochloride in pharmaceutical formation

Method validation

The proposed method was validated as per ICH guidelines in order to determine the linearity, precision, accuracy and ruggedness of the method.

Linearity

Standard solutions of rilpivirine hydrochloride in the concentration range of 0.5 to 3.5 µg/mL were observed in UV spectroscopy at 305 nm. A graph of absorbance (on Y-axis) *versus* concentration (on X-axis) was plotted (overlay of absorbance) was shown in Figure 3. In order to improve this correlation and minimize instrumental fluctuations, absorbances of these solutions were measured over a range surrounding 305 nm *i.e.*, 301, 303, 307, 309 nm are shown in Table 1. The calibration curves of rilpivirine hydrochloride at different wavelengths are shown in Figure 4.

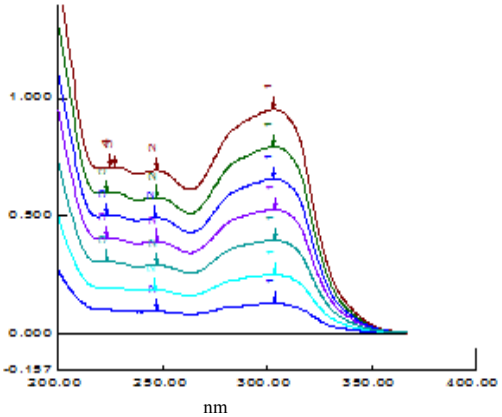

Figure 3. Overlay spectra of rilpivirine hydrochloride in pharmaceutical formation

Table 1. Calibration data of proposed method by using multivariate technique

Concentration µg/mL	Absorbance at 301 nm	Absorbance at 303 nm	Absorbance At 305 nm	Absorbance At 307 nm	Absorbance At 309 nm
0.5	0.124	0.125	0.125	0.124	0.122
1	0.247	0.247	0.247	0.245	0.242
1.5	0.392	0.393	0.395	0.392	0.387
2	0.524	0.525	0.526	0.524	0.516
2.5	0.648	0.649	0.650	0.647	0.641
3	0.789	0.790	0.791	0.783	0.773
3.5	0.947	0.948	0.949	0.941	0.926

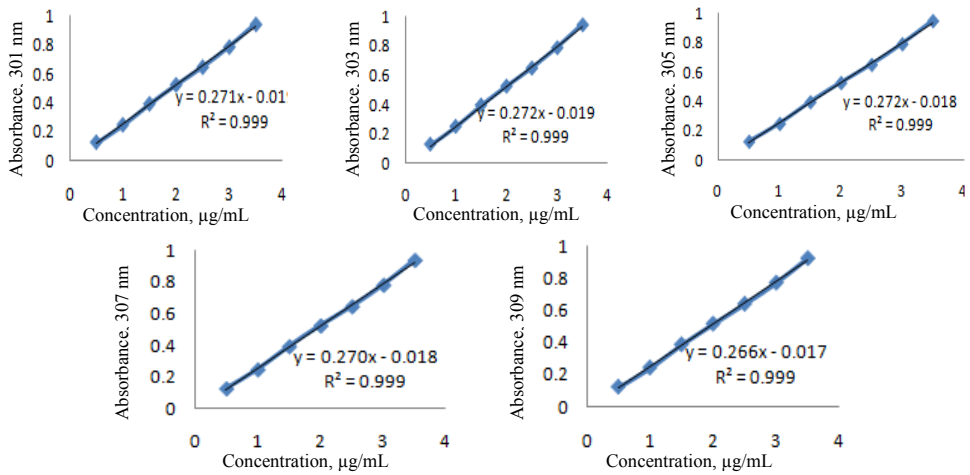

Figure 4. Calibration curves of rilpivirine hydrochloride showing maximum absorbance at 301, 303, 305, 307 and 309 nm

Method precision (repeatability)

Repeatability was determined by analyzing 1.5 µg/mL concentration of rilpivirine hydrochloride for six times and calculated the % RSD was found to be < 2 which shown in Table 2.

Table 2. Repeatability studies of rilpivirine hydrochloride

Concentration µg/mL	Absorbance at 305nm	Absorbance Mean ±SD	%RSD
1.5	0.382		
1.5	0.392		
1.5	0.385	0.386±0.004	1.03
1.5	0.389		
1.5	0.385		
1.5	0.382		

Intermediate precision (reproducibility)

The precision of the developed method was expressed in terms of percent relative standard deviation (% RSD). These results show reproducibility of the assay. The % RSD values were found to be less than 2 that indicate this method precise for the determination of the pure form. The interday and intraday precision results were mentioned in Table 3.

Table 3. Intraday and Interday precision of rilpivirine hydrochloride

Concentration µg/mL	Absorbance mean± S.D. (n=3)	%RSD	Absorbance mean± S.D. (n=3)	%RSD
0.5	0.125±0.002	1.60	0.127±0.002	1.55
1.5	0.384±0.005	1.23	0.385±0.005	1.19
2.5	0.650±0.003	0.47	0.655±0.009	1.44

Accuracy

Accuracy is determined by performing recovery studies at 3 levels in which known amount of analyte shall be added and recovery shall be carried out in three replicates of each concentration level and the % recovery was calculated. The mean recovery was found between 100.1-100.7% and % RSD between 0.3-0.7. The accuracy results are shown in Table 4.

Table 4. Recovery studies of rilpivirine hydrochloride

Spiked level, %	Formulation Conc, µg/mL	Pure Drug conc µg/mL	Amount Conc recovered µg/mL	% Recovery	% Mean recovery± S.D	%RSD
50	1	0.5	1.50	100.2	100.7±0.339	0.336
	1	0.5	1.51	100.9		
	1	0.5	1.51	100.4		
100	1	1	2.01	100.7	100.3±0.604	0.603
	1	1	2.00	100.2		
	1	1	1.99	99.5		
150	1	1.5	2.51	100.4	100.1±0.652	0.651
	1	1.5	2.52	100.7		
	1	1.5	2.49	99.4		

Limit of detection and limit of quantitation

The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. LOD and LOQ values are 0.358 and 1.086.

Ruggedness studies

This study was performed by analyzing 1.5 µg/mL by two different analysts and on two instruments, results of the study were given in Table 5 and % RSD obtained was less than 2 which is within the acceptance limits.

Table 5. Ruggedness of rilpivirine hydrochloride

Parameter	Concentration, µg/mL	Absorbance	Absorbance mean ± S.D. (n=3)	%RSD
Different Analyst	1.5	0.382 0.386 0.391	0.386±0.005	1.17
Different instrument	1.5	0.383 0.388 0.392	0.388±0.005	1.16

Application of the proposed method for pharmaceutical formulation

The proposed method was able to remove the interferences of the other excipients present in the pharmaceutical formulations (tablets) are assessed with a high percent of recovery. The percentage recovery for tablet formulation was found to be 99 -101% enlisted in Table 6. The results for assay are within acceptable limits.

Table 6. Results of Assay

Labeled amount, mg	Amount taken for assay, µg/mL	Amount found µg/mL	% Recovery
25	1.0	1.0	100
25	1.0	1.01	101
25	1.0	0.99	99.2

Conclusion

The above proposed UV method is very simple, precise, accurate, rapid and cost effective for the quantification of rilpivirine hydrochloride from its pharmaceutical dosage forms by the multivariate spectrophotometric method. The method requires only the wave length scan and the solvent is also very cheaper when compared the other methods. Hence it can be utilized for routine analysis in bulk and pharmaceutical dosage forms.

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