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Development and validation of UV Spectrophotometric Method for Estimation of Rilpivirine Hydrochloride in Bulk and Pharmaceutical Formulations

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ABSTRACT

A simple, rapid, precise, and economical spectrophotometric method has been developed for quantitative analysis of Rilpivirine hydrochloride (RILH) in manufactured tablet formulations. The initial stock solution of RILH was prepared in dimethyl formamide: acetonitrile solvent and subsequent dilution were done in acetonitrile. The standard solution of RILH in acetonitrile showed absorption maxima at 281.6 nm. The drug obeyed Beer–Lambert's law in the concentration range of 1–16 µg/mL with coefficient of correlation (R^2) was 0.9999. It showed coefficient of variation below 2 % in intra-run and inter-run precision. The results of analysis have been validated as per ICH guidelines. The method can be adopted in routine analysis of RILH in bulk and tablet dosage form and it involves relatively low cost solvents and no complex extraction techniques.

Keywords: ICH guidelines, Method validation, Rilpivirine Hydrochloride, UV Spectrophotometric

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INTRODUCTION

Rilpivirine hydrochloride (TMC278; formerly known as R278474) is a di-amino pyrimidine derivative. Chemically, it is 4-[[4-[[4-[(E)-2-cyanoethenyl]- 2,6-dimethylphenyl] amino]-2-pyrimidinyl] amino] benzonitrile monohydrochloride (Figure 1).[1,4] Rilpivirine hydrochloride (REL) is a novel non-nucleoside reverse transcriptase inhibitor (NNRTI) with in vitro activity against wild-type HIV-1 (human immunodeficiency virus) and NNRTI-resistant mutants.[3-6] It has been developed for treatment of ARV naïve HIV-1 infected individuals with the aim to have a better safety/tolerability profile compared to other NNRTIs (such as nevirapine and efavirenz and etravrine). rilpivirine hydrochloride in combination with other antiretroviral medicinal products, is indicated for the treatment of human immunodeficiency virus type 1 (HIV-1) infection in antiretroviral treatment naïve adult patients with viral load ≤ 100,000 copies/mL at baseline.[5,6]

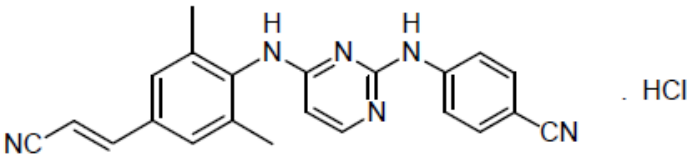

Figure 1: Chemical structure of RILH

REL is an E-isomer. It contains no chiral centre but cis/trans isomerism is possible. It is a white to almost white powder. It is soluble in N,N-dimethylformamide (DMF) and N,N-dimethylacetamide, slightly soluble in methanol, propylene glycol and 1-methoxy-2-propanol. The active substance does not have chiral centres and is not considered hygroscopic.[6-9] Rilpivirine was approved by the European Medicines Agency, UK and Therapeutic Goods Administration, (TGA) Australia. [7-8]

Earlier publications have described high-performance liquid chromatography (HPLC) methods useful for the quantification of REL in pharmaceutical dosage forms.[9-11] However, these methods involve arduous sample preparation and long chromatographic run times.

Surprisingly there is no UV spectrophotometric analytical method is available in the literature for analyzing REL in pharmaceutical tablet dosage form or bulk drug samples. It was felt necessary to develop a simple, precise, and rapid spectrophotometric method for the quantitative determination of REL.[12] The current research work deals with the development of spectrophotometric method and its validation as per International Conference on Harmonisation (ICH) guidelines[13]. The developed method was found to be selective, accurate, precise, reliable, and economical.

MATERIALS AND METHODS

Materials

RILH bulk drug was obtained from Hetero Labs Ltd, (Hyderabad, India), dimethyl formamide (DMF) and acetonitrile (HPLC grade) from Merck Fine Chemicals (Mumbai, India), and Whatman filter paper number 1 from Qualigens Fine Chemicals (Glaxo, Mumbai, India). The commercially tablets of RILH are not available in Indian market; hence we have manufactured RILH immediate release tablet containing 25 mg rilpivirine hydrochloride. The other ingredients are PVP K-30, lactose monohydrate, croscarmellose sodium, microcrystalline cellulose, and magnesium stearate. Other chemicals used were analytical or HPLC-grade and glassware used were Class A grade.

Instruments

Shimadzu UV - 1700 UV/VISIBLE spectrophotometer with UV probe 2.10 software and 1 cm matched quartz cells were used for absorbance measurements. Analytical balance used for weighing standard and sample was Make-Mettler Toledo, Model-XP 105.

Preparation of standard stock solution

Accurately weighed 100 mg of RILH working standard was transferred into a 100 mL volumetric flask and dissolved in 10 mL of DMF. The volume was made up to 100 mL with acetonitrile to give the solution containing 1000 µg/mL of RILH.

Selection of λ_{max}

The standard stock solution was further diluted with Acetonitrile to get a 10 µg/mL of concentration. The solution was scanned between 200 and 400 nm using acetonitrile as blank. The UV spectrum of RILH in acetonitrile had shown λ_{max}, at 281.6 nm. Hence, it was selected for the analysis of RILH [Figure 2].

Preparation of the calibration curve

Aliquots of standard stock solution were further diluted with acetonitrile to get the solutions of concentration 1–15 µg/mL. The absorbances were measured at 281.6 nm against acetonitrile as blank. All measurements were repeated three times for each concentration. The calibration curve was constructed by plotting mean of absorbance against corresponding concentration.

Preparation of the sample solution

The tablets of RILH are not available in Indian market; hence tablets manufactured in laboratory were assayed. These were labeled to contain 25 mg of RILH as an active substance per tablet. Twenty tablets containing 25 mg of RILH were accurately weighed and powdered. The powder

equivalent to 100 mg of RILH was weighed and transferred to a 100 mL volumetric flask; 10 mL DMF was added and sonicated for 10 min. The volume was adjusted to 100 mL with acetonitrile. The solution was filtered through Whatman filter paper No. 01. From this filtrate, 1 mL was transferred to a 100 mL volumetric flask and diluted with acetonitrile to 100 mL in order to obtain the final concentration of 10 µg/mL. The absorbance was measured at 281.6 nm using acetonitrile as blank. This procedure was repeated for six times. The amount of RILH present in formulation was calculated by comparing it with standard absorbance.

Method validation

The developed method was validated as per ICH guidelines for following parameters.[13]

Linearity

The linearity was studied in the concentration range of 1–16 µg/mL at 281.6 nm.

Specificity and selectivity

The spectra obtained from tablet solutions were identical with that obtained from standard solution containing an equivalent concentration of RILH. This showed that there was no any interference from excipients. Therefore, it could be said that developed method is highly selective.

Recovery studies

To ensure accuracy of the method, recovery studies were performed by standard addition method at 80%, 100%, and 120% level to preanalyzed samples and subsequent solutions were reanalyzed. At each level, three determinations were performed. The absorbances were measured at 281.6 nm using acetonitrile as blank and the amount of drug recovered from the formulation were calculated, and the results obtained are shown in Table 3.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision of the method was determined in terms of repeatability and intraday and interday precisions.

Repeatability

Repeatability of the method was determined by analyzing six samples of same concentrations of drug. Graphs were recorded, and the area of each graph was measured.

Intraday and interday precision

Intraday precision was determined by analyzing the drugs at three different concentrations and each concentration for three times, on the same day. Interday precision was determined similarly,

but the analysis being carried out daily, for three consecutive days.

Robustness

The robustness of a method is its capacity to remain unaffected by small changes in conditions. To determine the robustness of the method, the experimental conditions were deliberately altered and assay was evaluated. The effect of detection wavelength was studied at ±2 nm. For changes of conditions, the sample was assayed in triplicate. **Ruggedness**

To determine ruggedness, two different analyst performed assay on manufactured tablets of the drug in similar operational and environmental conditions using developed method.

Solution stability

The stability of the standard solution was tested at intervals of 1, 6, 10, and 24 h. The stability of solutions was determined by comparing absorbance of RILH. The absorbance values were within 0.5% after 24 h.

RESULTS AND DISCUSSION

The overlay UV spectra of standard and tablet solutions of RILH in acetonitrile were found to be same. The UV spectrum of RILH in acetonitrile has maximum absorption (λ_{max}), at 281.6 nm. The absorbance of excipients in tablet solution did not interfere with RILH at 281.6 nm. As a result, the wavelength was selected for quantitative analysis. The developed method was applied for estimation of RILH in tablet formulation. The results obtained are shown in Table 1.

The drug showed linearity in the concentration range of 1–16 µg/mL. Linear regression data is shown in Table 2 and Figure 3.

Table 1: Assay of tablet formulations

Labeled claim (mg)	Amount Found (mg)	% Assay*	%RSD*
25	24.88	99.59	0.73

*Mean of six determinations.

Table 2: Linear regression data

Sr. No.	Parameters	Results
1	λmax (nm)	281.6
2	Beer's law limit (µg/mL)	1–16
3	Correlation coefficient	0.9999
4	Regression equation	y = 0.1312x - 0.0024
5	Slope (*m*)	0.1312
6	Intercept (*c*)	-0.0024
7	Detection limit (µg/mL)	0.1
8	Quantification limit (µg/mL)	0.3

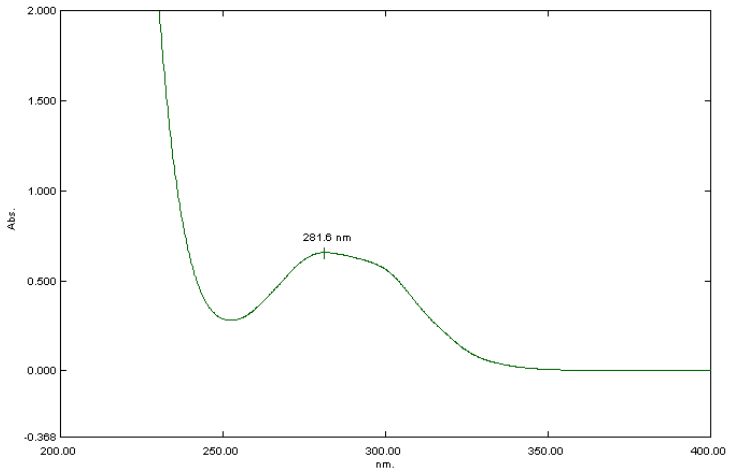

Figure 2: UV spectrum of pure drug RILH in acetonitrile

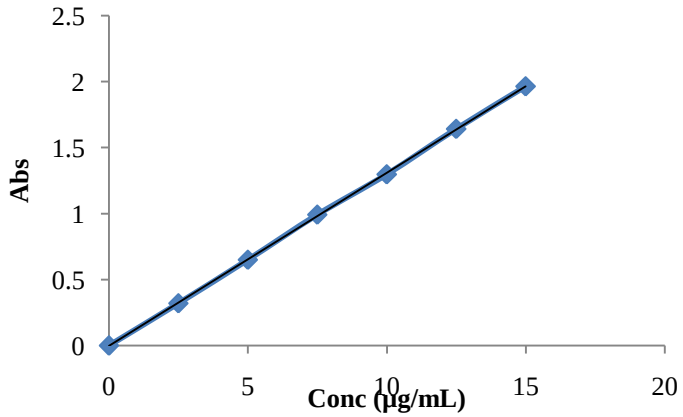

Figure 3: Linearity plot

The developed method was found to be accurate, indicated by mean % recoveries ranging from 99.66 to 100.51% in table 3.The method was also found to be precise as the %RSD values for intraday and interday precision were found to be less than 2%. The results are summarized in Table 4. Assay of RILH for all deliberate changes of conditions was within 98.0–102.0 % as shown in Table 5, which indicates robustness of the method. The ruggedness of the method is indicated by the results summarized in Table 6. These results of stability studies indicate that the solution was stable for 24 h at ambient temperature. The %RSD of assay was 1.27 % after 24 h. The results are shown in Table 7.

Table 3: Results of recovery studies

Level of addition (%)	Amount of std drug added (µg/mL)	Amount recovered (µg/mL)*	% Recovery ± SD*
80	4	3.99	99.66 ± 0.50
100	5	5.03	100.51 ± 0.84
120	6	6.01	100.19 ± 0.90

*Mean of three determinations.

Table 4: Result of Repeatability, Intraday, and Interday precision studies

Intraday		Interday		Repeatability	
% Labeled claim ± SD *	% RSD	% Labeled claim ± SD *	% RSD	% Labeled claim ± SD *	% RSD
99.48 ± 0.50	0.51	99.21 ± 0.42	0.43	99.97 ± 0.025	0.26

*Mean of six determinations.

Table 5: Result of robustness studies

Method wavelength (nm)	Condition (nm)	% Assay*	% RSD
281.6	279.6	99.67	0.67
	283.6	99.02	0.56

*Mean of three determinations.

Table 6: Result of ruggedness studies

Parameter	Analyst I	Analyst II
Label claim (mg)	25	25
% Assay*	99.78	99.63
% RSD	0.76	0.96

*Average of six determinations.

Table 7: Stability data

Sr. No.	Ingredient	Time (h)	% Assay*	% RSD
1	RILH	1	99.37	0.34
		6	99.21	0.79
		10	99.37	0.65
		24	98.77	1.27

*Average of three determinations.

CONCLUSION

The developed UV spectrophotometric method for the determination of RILH has the advantage of being fast, simple, inexpensive, and applicable over a wide concentration range with high precision and accuracy. The method was validated as per the guidelines laid by ICH. The results of the validation tests were found to be satisfactory and therefore this method can be applied successfully to analyze drug formulations.

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REFERENCES

1. Drug monograph- rilpivirine hydrochloride. Available from: http://www.drugs.com/ppa/rilpivirine-hydrochloride.html.

2. Product Monograph, EDURANT, rilpivirine tablets, Janssen Inc. 19 Green Belt Drive, Toronto, Ontario, 2011 July 20. Available from: http://www.ama-assn.org/resources/doc/usan/rilpivirine-hydrochloride.pdf
3. Miller CD, Crain J, Tran B et al. Rilpivirine: a new addition to the anti-HIV-1 armamentarium. Drugs Today (Barc) 2011;47:5-15.
4. Ripamonti D, Maggiolo F. Rilpivirine, a non-nucleoside reverse transcriptase inhibitor for the treatment of HIV infection. Curr Opin Investig Drugs. 2008;9:899-12.
5. Arasteh K, Rieger A, Yeni P, Pozniak A, Boogaerts G, van Heeswijk R, de Béthune MP, Peeters M, Woodfall B. Short-term Randomized Proof-of-Principle Trial of TMC278 in Patients with HIV Type-1 who have Previously Failed Antiretroviral Therapy. Antivir Ther.2009:14(5):712-22.
6. Azijn H, Tirry I, Vingerhoets J, de Béthune MP, Kraus G, Boven K, Jochmans D, Van Craenenbroeck E, Picchio G, Rimsky LT. TMC278, a Next-Generation Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI), Active Against Wild-Type and NNRTI-Risistant HIV-1. Antimicrob Agents Chemother. 2010;54(2):718-27.
7. Committee for Medicinal Products for Human Use (CHMP) assessment report, Edurant, International non-proprietary name: rilpivirine, Procedure No. EMEA/H/C/002264, 22 September 2011, European Medicines Agency, 2011. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002264/WC500118872.pdf
8. Australian Public Assessment Report for Rilpivirine, Proprietary Product Name: Edurant; Sponsor: Janssen-Cilag Pty Ltd., Therapeutic Goods Administration, Australia, March 2012.
   Available from: http://www.tga.gov.au/pdf/auspar/auspar-rilpivirine-120327.pdf
9. Venkata Reddiah CH, Rama Devi P, Mukkanti K. Effective estimation of rilpivirine by hplc method in tablet dosage forms and its in-vitro dissolution assessment. Int J Pharm Pharm Sci.2012;4(3):595-9.
10. Else L, Watson V, John 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.

11. Mohanareddy C, Hussain Reddy K, Narayanareddy PP, Venkataramana M. Degradation Pathway for Rilpivirine Hydrochloride by Validated Stability Indicating UP-LC Method. Int J Clin Pharmacol Toxicol.2012;1:1.
12. Aher KB, Bhavar GB, Joshi HP, Chaudhari SR. Economical spectrophotometric method for estimation of zaltoprofen in pharmaceutical formulations. Pharma Methods.2011;2(2):152-6.
13. International Conference on Harmonization, Validation of analytical procedures:Text and methodology. In ICH Harmonized Tripartite Guidelines Q2 (R1), November 2005.