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Research Article

EFFECTIVE ESTIMATION OF RILPIVIRINE BY HPLC METHOD IN TABLET DOSAGE FORMS AND ITS INVITRO DISSOLUTION ASSESSMENT

CH VENKATA REDDIAH*[a], P RAMA DEVI[b], K MUKKANTI[c]

[a]Analytical Development, Unit-III, E. O. U, Block-B, Hetero Drugs Ltd., Jeedimetla, Hyderabad-500055, [b]Analytical Research and Development, HRF, Balanagar, Hyderabad-500018, [c]Center for Chemical Science, Institute of Science and Technology, J. N. T. University, Hyderabad-500072, India. Email: venkat.re2007@gmail.com

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ABSTRACT

A simple, sensitive, rapid and reproducible HPLC Method has been developed and validated for estimation of Rilpivirine simultaneously and also the comparative study of invitro data in tablet formulation. The solvent used was Acetonitrile: buffer (55:45) %v/v and the λmax or the absorption maxima of the drug was found to be 280 nm. A linear response was observed in the range of 5.5-41.25µg/ml with a regression coefficient of 0.99. The invitro release of various test units was compared for their similarity using the f2 test which limits were found within the acceptance criteria. All the validation parameters were within the acceptance range according to ICH norms. The described method was successfully employed for quality control assay of the component simultaneously and dissolution data helpful in generating the further information regarding invivo absorption rate in tablet dosage form.

Keywords: Rilpivirine, In vitro dissolution study, HPLC.

INTRODUCTION

Rilpivirine Hydrochloride chemical name is Benzonitrile, 4-[[4-[[4-[(1E)-2-Cyanoethenyl]-2,6-dimethylphenyl]amino]-2-pyrimidinyl] amino]-, hydrochloride and molecular formula is C22H18N6•HCl as well as CAS Number: 700361-47-3 and Class: Nonnucleoside Reverse Transcriptase Inhibitors[1]. This drug is Antiretroviral; nonnucleoside reverse transcriptase inhibitor (NNRTI)[2-4].It is treated with Treatment of HIV-1 infection in conjunction with other antiretrovirals[5].

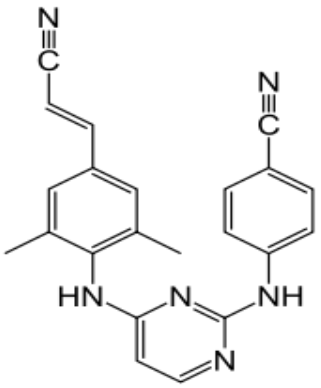


Fig. 1: Structure of Rilpivirine

MATERIAL AND METHODS

Chemicals and Materials

Hetero Labs Ltd supplied Rilpivirine Hydrochloride working standard respectively. Ammonium Acetate, Acetic acid (AR grade), Acetonitrile (HPLC grade) Sodium hydroxide (AR grade) and Hydrochloric acid, Tween 20 (Polyoxyethylene sorbitan monolaurate) AR grade, POE10LE (Poly ethoxy 10 lauraly ether) was purchased from Spectrochem and E-Merck Limited respectively. In-house purified water (USP grade) was used throughout the study.

Chromatographic Conditions

Ammonium acetate buffer was prepared by dissolving 0.77 g of ammonium acetate into 1000 mL of water, and adjusting the pH to 4.0 with dilute acetic acid solution. Filtered and degassed mixtures of acetonitrile and buffer (different volume fractions) were tested as mobile phases for Rilpivirine HCl analysis. Different flow rates (1.0, 1.2, and 1.5 mL/min) were also tested. Column temperature was 35°C and UV detection was performed at 280 nm and injection volume was 10 µL. For elute purpose used HPLC column is Symmetry C18, 75 x 4.6mm, 3.5µ or equivalent.

Instrumentation

The chromatographic separations were performed using Shimadzu LC 2010C integrated system equipped with quaternary gradient pump, 2010C UV-VIS detector, 2010C Column Oven and 2010C programmable auto sampler controlled by CLASS-VP software. The Symmetry C18, 75 x 4.6mm, 3.5µ or equivalent was used as a stationary phase. The system suitability results displayed in Table 1 were evaluated throughout the study. Electrolab TDT-08L auto sampler dissolution apparatus were used for comparative dissolution study.

Dissolution

Preparation of 1N HCl

Dilute 85 mL of Hydrochloric acid to 1000 mL with water and mix.

Preparation of 1N NaOH

Weigh and transfer about 4 g of sodium hydroxide pellets into 1000 mL Volumetric flask, dilute to volume with water and mix.

Preparation of dissolution medium

Accurately weigh and transfer about 5 g of Tween 20 into beaker containing 1000 mL Of 0.01 N HCl solution and mix. Verify the pH is 2.0±0.05. If necessary, adjust the pH of the solution to 2.0±0.05 with 1N HCl or 1N NaOH.

Preparation of Diluent

Prepare a degassed mixture of water and acetonitrile in the ratio of 30:70 % v/v

Dissolution parameters

Medium: 0.5% Tween 20 in 0.01N Hydrochloric acid, pH 2.0

Volume: 900 ml

Apparatus: Paddle (USP-II)

Speed: 75 rpm

Temperature: 37.0± 0.5°c

For Single point: 45 minutes

For profile: 5, 15, 30, 45 & 60 minutes

Standard preparation

Accurately weigh and transfer about 60 mg of Rilpivirine HCl working standard into a 100 ml volumetric flask, add about 60 ml of diluent and sonicate to dissolve. Dilute to volume with diluent. Transfer 5.0 ml of the above solution into a 100 ml volumetric flask. Dilute to volume with dissolution medium and mix.

Sample preparation

Place 1 tablets each in six different vessels and operate the instrument as mentioned above. Withdraw about 10 mL of the sample solution, filter.

Applied method to compare dissolution profiles

The description of the in vitro dissolution profiles was calculated by using model-independent method. In this study, as model-independent approaches, two fit factors were applied to the dissolution data that compare the dissolution profiles. These fit factors directly compare the difference between the percent drug dissolved per unit time for a test and reference product. The fit factors are f2 (similarity factor).

The specification of dissolution method is set by considering the solubility, permeability, dissolution and pharmacokinetics of the drug substance. A model-independent method was used for the comparison of in vitro dissolution profiles. In this study f2 (similarity factor) was calculated. The use of these factors was also recommended for dissolution profile comparison in the FDA's guides for industry.

For Assay

Standard preparation

Accurately weigh and transfer about 60 mg of Rilpivirine HCl working standard into a 100 ml volumetric flask, add about 60 ml of diluent and sonicate to dissolve. Dilute to volume with diluent. Transfer 5.0 ml of the above solution into a 100 ml volumetric flask. Dilute to volume with diluent and mix.

Sample preparation

Weigh accurately tablets powdered equivalent to about 25 mg of Rilpivirine HCl in to 100-mL volumetric flask. Add about 60-mL diluent and sonicate it for 30 minute to dissolve. Dilute to volume with diluent. Transfer 5.0 ml of the above solution into a 50 ml volumetric flask. Dilute to volume with diluent and mix. Filtered it through 0.45 µ HVLP nylon filter.

RESULTS

Table 1: Method Precision

Compound	Concentration (µg/mL) (n=6)	% Assay Mean (n=6)	%RSD of Assay
Rilpivirine HCl	25	99.10	0.50

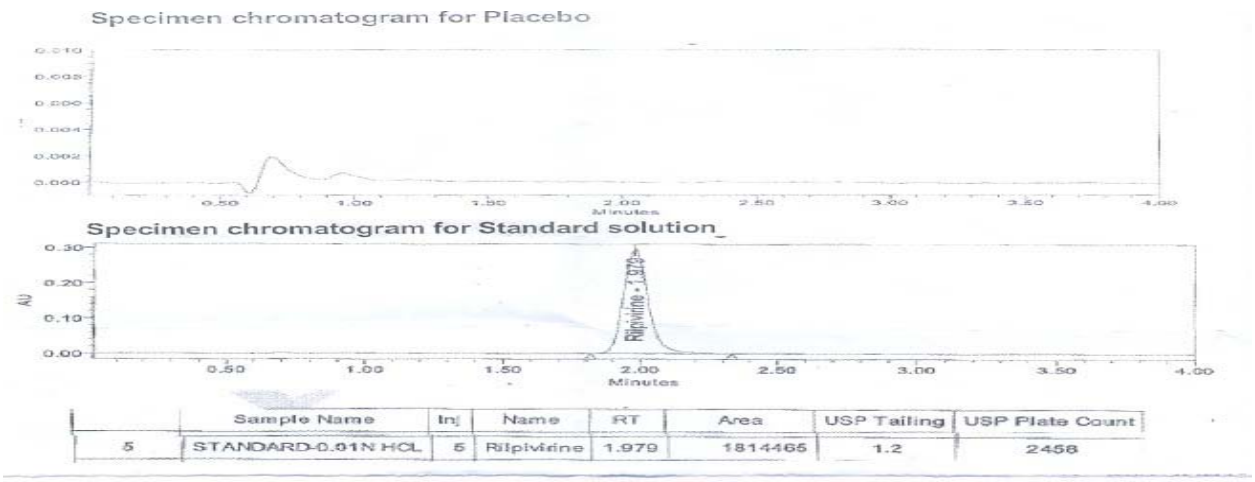


Fig. 1: Standard Solution

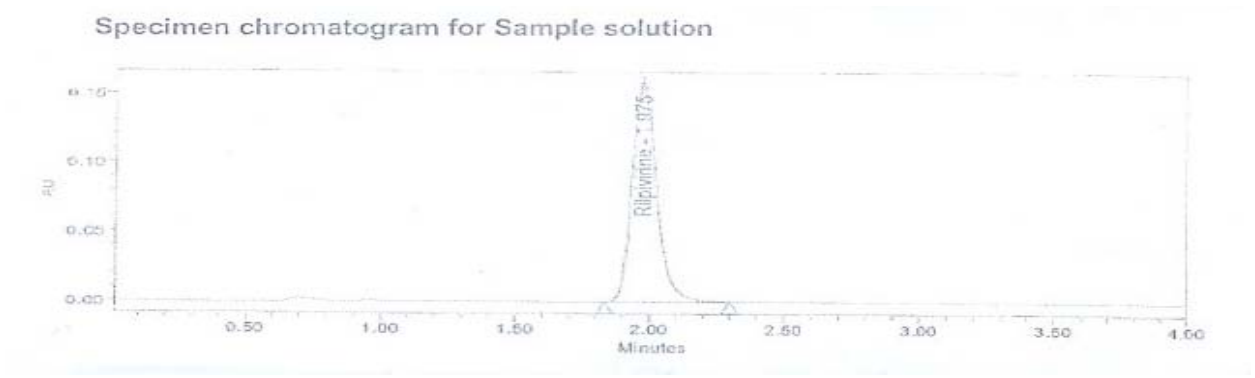
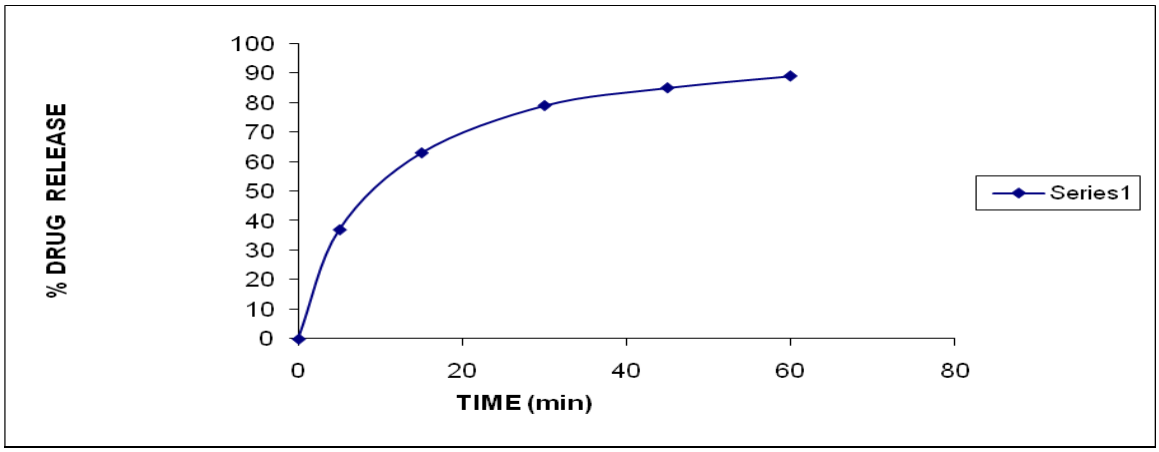


Fig. 2: Test Solution

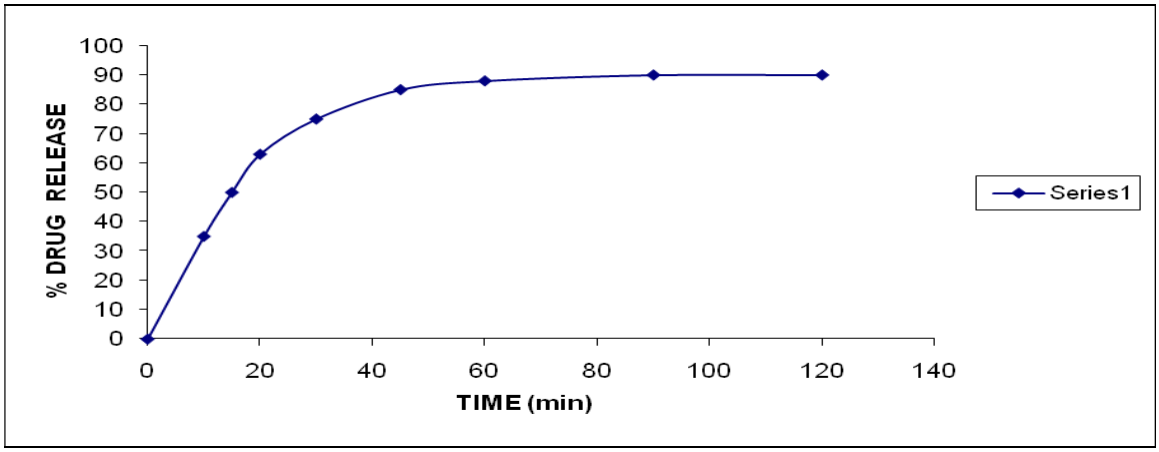
Table 2: Method Accuracy

Drug	Level	Drug Added	Drug recovered	%Assay	% Assay(n=3)
Rilpivirine	50%	30.62	30.89	100.88	0.5
HCl %	100	60.12	60.10	99.96	0.7
%	150	90.23	90.92	100.74	0.5

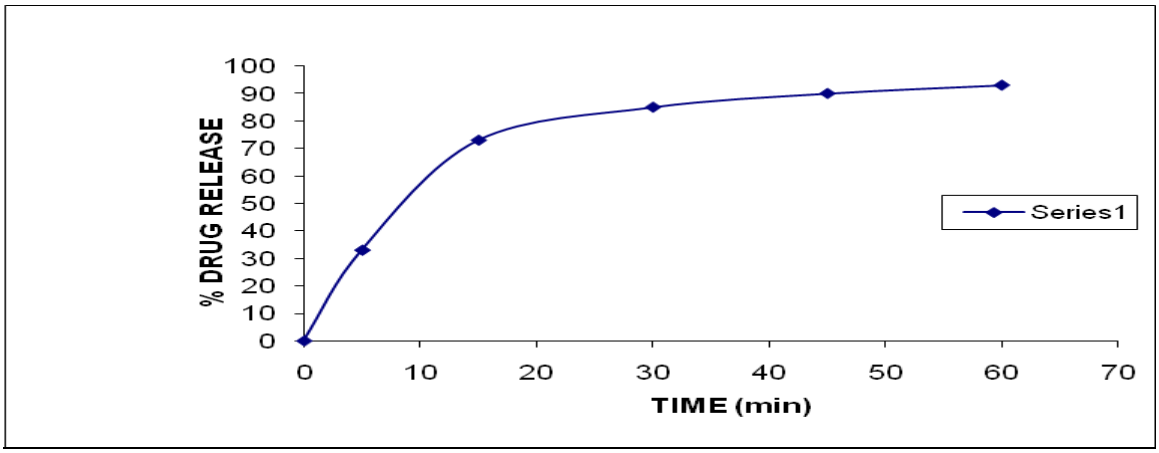
Comparative Dissolution Data



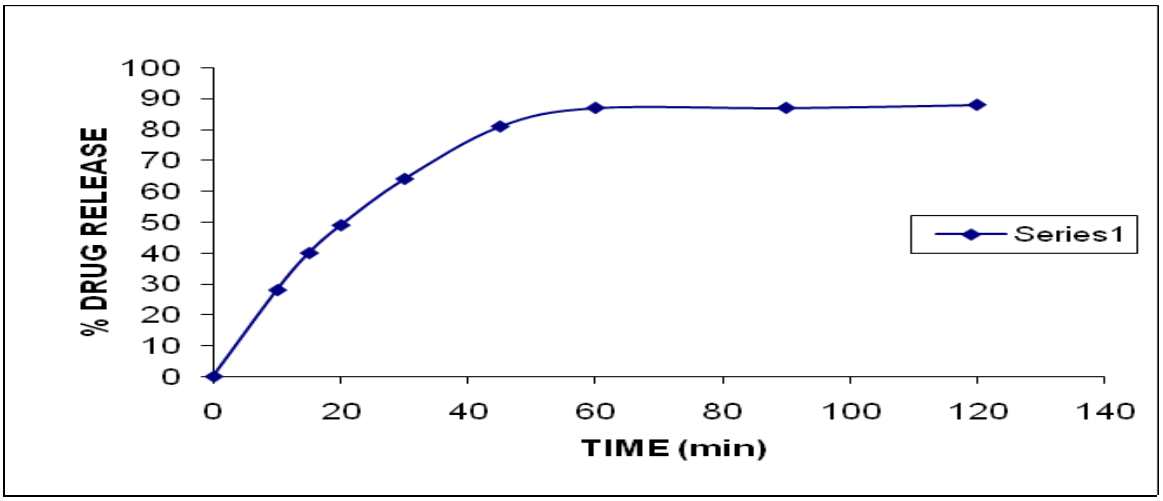
EDURANT tablets 25 mg: 0.5% Tween 20 in 0.01N Hydrochloric acid, pH 2.0



EDURANT tablets 25 mg: 25mM POE10LE in pH 4.5 Acetate Buffer



Hetero (Rilpivirine) Tablets 25 mg: 0.5% Tween 20 in 0.01N Hydrochloric acid, pH 2.0



Hetero (Rilpivirine) Tablets 25 mg: 25mM POE10LE in pH 4.5 Acetate Buffer

Table 3: Method Ruggedness

Day	Compound	% Assay Mean	%RSD
Day 1	Rilpivirine HCl	99.56	0.6
Day 2	Rilpivirine HCl	98.45	0.5

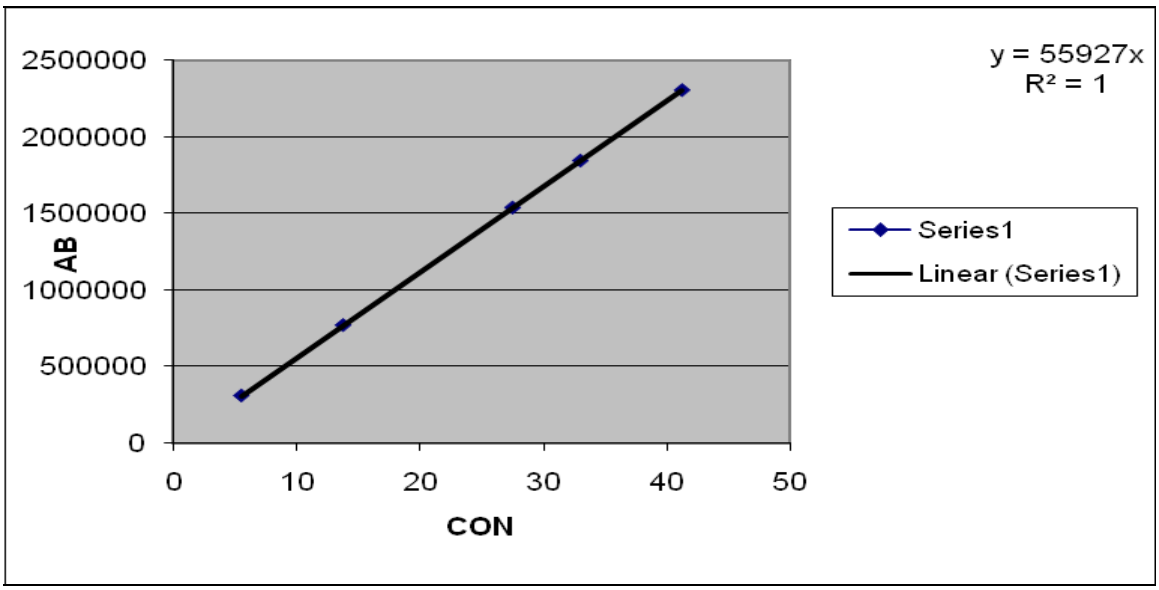


Table 4: Linearity

Manufactured by	**Reference (EDURANT)**	**Test**
	Tibotec Therapeutics, NJ 08869	**Hetero**
Apparatus	USP-II Paddle	
RPM	75	
Dissolution Media	900 ml, **0.5% Tween 20 in 0.01N Hydrochloric acid, pH 2.0**	
% of Drug release for Rilpivirine		
Time in minutes	Reference	test
5	37	33
15	63	73
30	79	85
45	85	90
60	89	93
Inf	93	94
F2(similarity factor)	61.93	

Table 5: Comparative Dissolution Profile for Rilpivirine tablets

Dissolution Media	**1000 ml,Basket,100rpm, 25mM POE10LE in pH 4.5 Acetate Buffer**	
% of Drug release for Rilpivirine		
Time in minutes	**Reference**	**test**
10	35	28
15	50	40
20	63	49
30	75	64
45	85	81
60	88	87
90	90	87
120	90	88
F2(similarity factor)	53.67	

Standard and sample solution stability

Standard and sample solution stability was evaluated at room temperature for 48 h. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution was stable up to 24 h at room temperature.

Specificity

There was no interference from Standard, sample, placebo and the values of f2 were calculated for the dissolution in three different Medias. As can be seen in Table 5 data obtained for f2 were found to be within the acceptable criteria.

Method precision

The relative standard deviation for six replicate injections was less than 1.0 %, which met the acceptance criteria established for the method. The results obtained were presented in Table 1.

Accuracy/recovery

The data presented in Table 2 show excellent recoveries at all levels. The average recoveries for triplicate determinations at 50,100, and 150% levels were within the acceptable criteria. Excellent recovery and low relative standard deviation value showed that the method is suitably accurate for potency assay of Rilpivirine simultaneously in the drug substances.

Linearity

The plot of peak area responses against concentration. It can be seen that plot is linear over the concentration range of 5.5 to 41.25 µg/mL of Rilpivirine respectively with a correlation coefficient (r2) 0.999. The results of linearity, limit of detection and limit of quantification were presented in Table 4.

Method Ruggedness

Ruggedness test was determined between two different analysts, instruments and columns. The value of percentage RSD was below 2.0%, showed ruggedness of developed analytical method. The results of ruggedness were presented in Table 3

DISCUSSION

Considering the efficiency of RP-HPLC, attempt has been made to develop simple, accurate, precise, rapid and economic method for simultaneous estimation of Rilpivirine in a tablet dosage form. Thus method described enables to the quantification of Rilpivirine. The advantages lie in the simplicity of sample preparation and the low costs of reagents used. Dissolution testing is very important invitro test to evaluate drug product. This data from the part of the pharmaceutical development report, but can also be included in the bioequivalence study report. Results from statistical analysis of the experimental results were indicative of satisfactory precision and reproducibility. Hence, this RP-HPLC method can be used for analysis of commercial formulation and dissolution data provides useful information for in vivo studies.

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