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REVERSE PHASE HIGH PERFORMANCE AND HPTLC METHODS FOR THE DETERMINATION OF RILPIRIVINE BULK AND IN TABLET DOSAGE FORM

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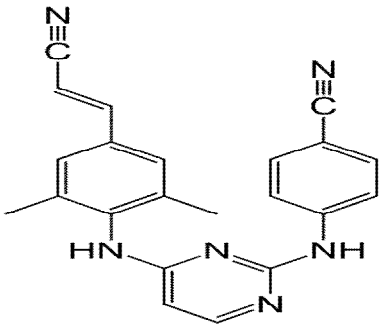
ABSTRACT

Two simple, rapid, sensitive and economic chromatographic methods have been developed for the determination of Rilpirivine. First method depends on reverse phase high performance liquid chromatography. The mobile used consists of using mixed phosphate buffer: acetonitrile (60:40%v/v) with pH 6.8 and flow rate of 1.0ml/min in isocratic mode. The separation was carried out by UV detector at wavelength 272nm. A concentration range from 1-10 µg/ml was used for calibration curve. The percent recovery of rilpirivine was found to be 100.53%. Second method depends on high performance thin layer liquid chromatography. The mobile phase used consists of ethyl acetate: methanol: chloroform (8.0:1.0: 1.0%v/v/v).Densiometric analysis was carried out at wavelength 254nm. The Rf value for rilpirivine was found to be 0.33. A concentration range from 5-30 µg/spot was used for calibration curve. The percent recovery of rilpirivine was found to be 100.17%. HPLC and HPTLC methods are widely employed in quality control assessment of drugs because of their sensitivity, repeatability and specificity. The proposed method was validated statically and recovery study for the determination of rilpirivine in bulk and in tablet dosage form was performed.

Key words: Rilpirivine, RP-HPLC, HPTLC, validation, second generation.

INTRODUCTION

Rilpirivine (RPV) is chemically known as 4[(4[(4-(1E) -2 cyanothenyl] -2, 6- dimethyl phenyl] amino] 2 pyrimidimyl] amino benzonitrile mono hydrochloride [1].fig-1. Rilpirivine is the second generation of non nucleoside reverse transcriptase inhibitors (NNRTIS) recently marketed for the treatment of HIV infection. Rilpivirine is superior to first generation NNRTIS in that it is active against NNRTI resistant HIV-I [2-5]. Literature survey revealed analytical method for the determination of rilpivirine by HPLC method in dosage forms and its invitro dissolution assessment [6]. Simultaneous determination of existing and new antiretroviral compound is done by HPLC-MS/MS [7]. Literature survey reveals that, Rilpivirine is not official in any of the pharmacopeias like IP, BP, USP and European pharmacopeia. Hence an attempt has been made to develop a simple, efficient and selective method for the determination of Rilpirivine in pharmaceutical dosage forms.



"Fig.1" Chemical Structure of Rilpirivine

MATERIALS AND METHODS

Instrumentation

The HPLC grade system consisted of a LC-10ADVP shimandzu pump, photodiode detector and a plus auto sampler used as an ambient temperature. Empower 2.0 was used for data acquisition and processing. HPTLC Cagmag with precoated silica gel plate 60F254 (20cmX 10cm), 250µm thickness was used as stationary phase. Same application was done by using 100 µl syringe and Cagmag Linomat V applicator. Linear ascending development was carried out in 20cmX10CM twin trough glass chamber. The densiometric scanning was performed by using cagmag TLC scanner III supported by Win cats soft ware. Evaluation of chromatogram was done by using peak areas.

Chemicals

Rilpirivine was received with a certificate of 99.80% purity. It was used as such with out checking their purity. The HPLC grade methanol, acetronitrile and water were purchased

from qualigens fine chemicals Mumbai, India. Analytical grade potassium dihydrogen phosphate, dipotassium hydrogen phosphate and ortho phosphoric acid were used.

Preparation of stock solution and working standard solution

Preparation of phosphate buffer

7.0 gm of Potassium dihydrogen phosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted to get 1000ml with HPLC water. pH value is adjusted to 6.8 with sodium hydroxide solution.

Preparation of mobile phase

Phosphate buffer pH (6.8) 600ml and 400 ml of acetonitrile HPLC (40%) are mixed and degassed in ultrasonic water for 5 minutes and filtered through 0.45 µ filter under vaccum filtration.

Preparation of standard stock solution

10mg of Rilpirivine pure was accurately weighed and transferred into 100ml volumetric flask. About 7ml of mobile phase was added and sonicated to dissolve it completely and the volume was made upto the mark with the same mobile phase to obtain a working standard solution with (100µg/ml) for RP-HPLC and HPTLC method.

Preparation of working sample solution

10 tablets of Edurant (containing 25mg of RLP) were weighed and powdered, the tablet powder equivalent to 10 mg of RLP was transferred to 100ml standard flask and 50 ml of mobile phase was added. The solution was sonicated for 15 minutes and the final volume was made with the same to obtain the concentration (100µg/ml). The above solution was suitably diluted with mobile phase to obtain the final dilution of RLP (5µg/ml) for HPLC method and (15 µg/ml) for HPTLC method.

Calibration curve (or) linearity

Calibration curve was constructed by plotting peak area concentration of RLP solutions. Aliquots of standard stock solution of RLP (0.1 to 1.0ml of 100mg/ml) was transferred into 10 ml standard flask and made upto the volume with mobile phase to get a concentration range of (1 to 10 µg/ml) for HPLC method. Then aliquots of standard stock solution of RLP (0.5 to 3.0ml) was transformed into 10ml standard flask and made upto the volume with mobile phase to get a concentration range of (5 to 30µg/ml) for HPTLC method. Each

concentration 20µl of the standard solutions was injected and the Chromatogram was recorded. The calibration graph was done by external standard calibration method.

Limit of detection and limit of Quantification

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. The quantification limit of an individual of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy.

The detection limit (LOD) for the proposed was calculated using the following equation

LOD=3.3S/K.

Where 'S' is the standard deviation of replicate determination values under the same conditions as for sample analysis in absence of the analyte and 'K' is the sensitivity namely the slope of the calibration graph. The limit of quantification (LOQ) was defined as LOQ=10S/K**.**

Quantification of formulation (Assay)

In HPLC method, from the prepared sample stock solution 0.5 ml was pipetted out and transferred into six separate 10ml volumetric flasks and made upto the volume with mobile phase (5µg/ml). In HPTLC method, from the prepared sample stock solution 1.5 ml was pipetted out and transferred into six separate 10ml volumetric flasks and made upto the volume with mobile phase (15µg/ml). 20µl of each solution was injected and the chromatograms were recorded. The peak area was determined and the procedure was repeated for three times. The standard solution was prepared in the same manner. By using the following formula the percentage purity of RLP was calculated.

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:

AT = Peak Area of RLP obtained with test preparation

AS = Peak Area of RLP obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

Accuracy

Accuracy was determined for standard quality samples (in addition to calibration standard) prepared in triplicates at different concentration levels (30, 60, 90% for HPLC method and 40, 80, 120 % for HPTLC method.) within the range of linearity of the drug. The results of analysis of recovery studies were obtained by method validation by statistical evaluation.

Precision

Precision is the degree of repeatability of an analytical method under normal operational condition. The precision of the assay was determined by repeatability (intra day) intermediate precision (interday) and reported as % RSD for a statistically significant number of replicate measurements. Repeatability and intermediate precision of the method were determined by analyzing 6 samples of the test concentration (5µg/ml for HPLC method and 15µg/ml for HPTLC method.)

Robustness

Robustness was established by varying the chromatographic condition with respect to flow rate and organic composition of mobile phase. Standard and sample solutions were injected and the chromatograms were recorded.

Specificity

The specificity of the assay method is established by injecting blank (diluent), placebo, standard and sample of RLP HCL preparation into the HPLC and HPTLC chromatograph.

RESULT AND DISCUSSION

Method development and optimization

For HPLC method

The optimization was done by changing the mobile phase, mobile phase ratio, flow rate and column. Different ratios of mixed phosphate buffer and acetonitrile (pH 6.8) were tried. The mobile phase was filtered through 0.45 µm Teflon membrane filter and degassed by sonication prior to use. The different flow rates (1.0ml/min, 1.5ml/min and 2.0ml/min) were tried. Finally stationary phase Ymc C_{18} short column with mobile phase containing mixed

phosphate buffer adjusted to pH 6.8 and acetonitrile in the ratio of 60:40%v/v with flow rate 1.0ml/min. The retention time was found to be 3.137 min using the above chromatographic conditions resulted in the development of an efficient reproducible method for the determination of RLP in bulk and tablet dosage form and the observation was shown in table-1.

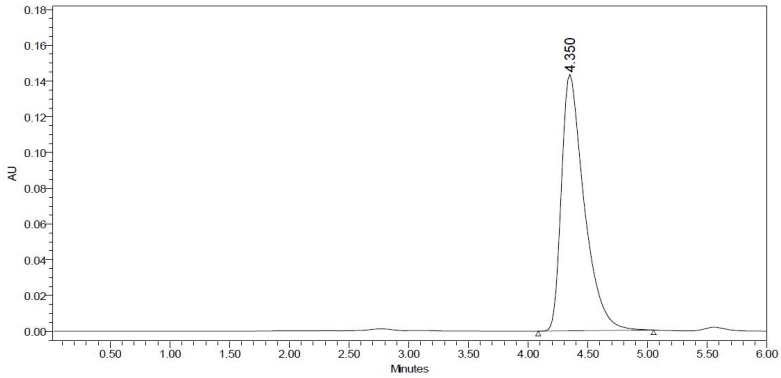


Fig. 2 Optimized HPLC Chromatogram of Rilpirivine

HPTLC Method

For HPTLC initially plane solvents like methanol, toluene, chloroform and ethyl acetate were tired. The spots were developed with methanol and ethyl acetate but tailing was observed. Then methanol and ethyl acetate in the ratio (8:2 %v/v) were used but the distance traveled by the developed spots was high at solvent front and tailing was also observed. Then methanol and ethyl acetate in the ratio (2:8%v/v) was tired. In this condition RF values were reduced. The proportion of methanol was increased and the Rf value of drug was satisfactory but peak was not symmetrical and tailing was observed. The tailing was reduced by addition of chloroform .The symmetrical peak was observed. Ultimately mobile phase consists of ethyl acetate: methanol: chloroform (8:1:1%v/v/v) which gave good peaks at 254nm.

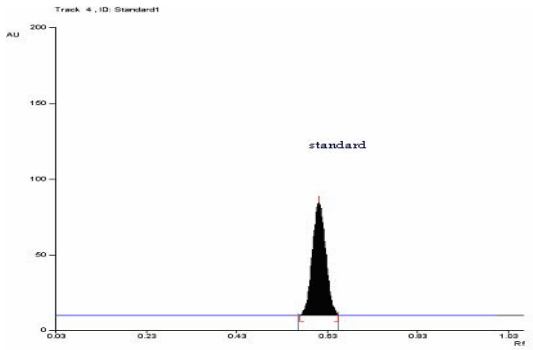


Fig .3 Shows the Densitogram of RLP

The Rf values for RLP 0.33. Well defined spots were obtained when plate was activated at 110˚C for 20min and the chamber was saturated with the mobile phase for 20min at room temperature. Trials results were shown in Table-2.

Method validation

When method has been optimized it must be validated before practical use. By following the ICH guidelines for analytical method validation Q2 (R1), the validation characteristics were addressed [8-9].

System suitability

In HPLC method the system suitability parameters like tailing factor, number of theoretical plates, %RSD, HETP and capacity factor were calculated and these values were compared with the standards limit as per USP. In HPTLC method The results were shown in Table-3.

Calibration curves

Calibration curve was constructed by plotting the peak area vs concentration. For HPLC method the mean equation of calibration curve consisting of ten points y=14701.67X+91351.25 where 'Y" represents the peak area and x represents the concentration of RLP. Correlation coefficient 0.9990 confirmed that the calibration curve was linear over the concentration range of 1-10µg/ml. For HPTLC method, correlation coefficient was found to be 0.9992 and linearity was found to be over the concentration range of 5-30 µg/ml. Regression equation for stand curve was y=27865.03X+22950.3 for HPTLC method. The results were shown in table-4.

Limit of detection and limit of Quantification

For HPLC method, the LOD and LOQ values were found to be 0.0427µg/ml and 0.724 µg/ml respectively. For HPTLC method the LOD and LOQ values were found to be0.317 µg/ml and 0.513µg/ml respectively. The reports of analysis were shown in table -4

Quantification of formulation (Assay)

The tablet formulation of Edurant was selected for analysis and the percentage purity was found to be 100.4% for HPLC method and 99.8% for HPTLC method. The procedure was repeated for six times to validate the method. The method was validated according to ICH guidelines. The %RSD [10-11] was found to be less than 2% which indicates that the method had good precision.

Accuracy

Accuracy was determined with standard quality samples (in addition to calibration standards) prepared in triplicates at different concentrations levels covering the entire linearity range and reported as %RSD for a statistically significant number of replicate measurements. For HPLC method the %RSD value was found to be 0.6397 and for HPTLC method the %RSD values was found to be 0.8692. The analytical reports were shown in table 6-7.

Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision of the assay was determined by repeatability (intraday) and intermediate precision (interday) and reported as %RSD for a statistically significant number of replicate measurements. The intermediate precision was studied by comparing the assays on 3 different class and the limits documented on standard deviation and %RSD. The reports of analysis were shown in table-8-9.

Table-1 HPLC Method development Trials

Mobile phase ratio(%v/v)	Flow rate (mL/min)	Column	Observation
Acetonitrile: Water(50:50)	1 mL/min	YMC C_{18}	Efficiency was less
Mixed phosphate buffer: Acetonitrile (55:45)	1mL/min	YMC C_{18}	Tailing factor value was less
Ammonium phosphate buffer: Acetonitrile (40:60)	1.2mL/min	YMC C_{18}	Tailing factor value was less
Methanol: Water(50:50)	1.2mL/min	BDS Hypersil C_{18}	Retention time was more
Mixed phosphate buffer: Acetonitrile (55:45)	1.2mL/min	BDS Hypersil C_{18}	Retention time was more
Mixed phosphate buffer: Acetonitrile (60:40)	1.0mL/min	BDS Hypersil C_{18}	Optimized chromatogram

Table-2 HPTLC Method development Trials

Mobile phase ratio(%v/v)	Observation
Methanol: ethyl acetate (8:2 %v/v)	Distance traveled by the developed spots was high at solvent front
Methanol: ethyl acetate (2:8 %v/v)	RF value was reduced
Ethyl acetate: Methanol (8:2 %v/v)	Peak was not symmetrical and tailing was observed
Ethyl acetate: methanol: chloroform (8:1:1%v/v/v)	The tailing was reduced by addition of chloroform
Ethyl acetate: methanol: chloroform (8:1:1%v/v/v)	.The symmetrical peak was observed
	Optimized chromatogram was obtained

TABLE 3. System Suitability Parameters for RPV for the Proposed RP- HPLC & HPTLC

Parameters	Rilpirivine	Limits as per USP
Tailing factor	1.057	Less than 2
Asymmetric factor	0.654	Less than 2
Theoretical plates	2653	More than 2000
Capacity factor	1.45	1 to 10
HETP	0.4567	-
Theoretical plate per unit length	231.12	-
Rf value	0.33	-
Area average	391027.2	-

TABLE.4.Regression Analysis of the Calibration Curve for RPV for the Proposed RP-HPLC and HPTLC

Parameters	Rilpirivine	
	RP-HPLC	HPTLC
Concentration range	1-10µg/ml	5-30µg/spot
Slope	91351.25	22950.3
Intercept	14701.67	27865.03
Correlation coefficient	0.9990	0.9992
Regression equation	y=14701.67X+91351.25	y=27865.03X+22950.3
LOD	0.0427	0.317
LOQ	0.724	0.513

TABLE.5 Analysis of RPV Formulation by the Proposed RP-HPLC and HPTLC

S.no	Rilpirivine	HPLC(5 µg/ml)	HPTLC(15µg/ml)
1		425713	394102
2	Standard	426112	390230
3		425543	389901
	Average	425789.3	391411
	Edurant Tablet	429005	391245
1	(25mg)	428937	390231
2	Sample	427705	389843
3	Average	428549	390439.6
	%Assay	99.36	100.24

Table-6 Accuracy studies for HPLC method

Concentration (%)	Mean area	Amount added(µg/ml)	Amount found (µg/ml)	Mean % Recovery	SD	%RSD
30	241070.3	1.56	1.52			
60	492788.6	3.05	3.04	100.53	0.6429	0.6397
90	736384	4.54	4.53			

Table-7 Accuracy studies for HPTLC method

Concentration (%)	Mean area	Amount added(µg/ml)	Amount found (µg/ml)	Mean % Recovery	SD	%RSD
40	344917.6	3.01	3.02			
80	432001	6.32	6.22	100.17		0.8692
120	603513	9.10	9.10		0.8711	

Table-8 Precision Studies for HPLC and HPTLC method

S. No	HPLC Peak area	HPTLC Peak area
1	426526	392147
2	422003	391521
3	422028	389421
4	428139	390132
5	422393	391915
Average	424217.8	391027.2
SD	2904.05	1190.298
%RSD	0.6845	0.3044

Table-9 Intermediate Precision for HPLC and HPTLC method

S.no	HPLC Peak area	HPTLC Peak area
1	431013	381456
2	431517	382368
3	431577	380634
4	431612	384942
5	438790	379942
Avg	432901.8	381868.4
SD	3300.56	1943.029
%RSD	0.7624	0.5088

Table-10 Robustness Study for HPLC method

Parameters		USP plate count	USP tailing
Variation in flow rate	0.8ml/min	2562.2	1.8
	1.2ml/min	2609.6	1.7
Change in organic Composition of mobile phase	10% less	2557.4	1.8
	10% more	2670.7	1.7

Robustness

The robustness of the method was evaluated by analyzing the system suitability standards and evaluating system suitability parameter data after varying individually, the HPLC pump flow rate and organic composition of the mobile phase. The results were shown in table-10.

Specificity

The method specificities was assessed by comparing the chromatograms (HPLC and HPTLC) obtained from the drug and the most commonly used excipients mixture with those obtained from blank(excipients solution in water without drugs). It was observed that there was no interference from the peaks obtained for the chromatogram of blank and placebo with that of

RPV HCL peaks obtained for the chromatogram of sample and standard of RPV HCL. Hence the proposal method is lightly selective and specific.

CONCLUSION

A new, accurate and selective gradient RP-HPLC and HPTLC methods were proposed for the determination of Rilpirivine bulk and in tablet dosage form validated as per the ICH guidelines. The methods were found to be simple, selective, precise, accurate and robust. Therefore, these methods can be used as routine testing as well as stability analysis of Rilpirivine bulk and in tablet dosage. All statistical results (Percentage, Mean, RSD, Percentage difference and recovery %) were within the acceptance criteria.

REFERENCES

1. http:// www.drugs.com/monograph/rilpivirine-hydrochloride.html/.
2. Tibotec therapeutics, Edurant [rilpirivine] tablets prescribing information, Raritan, N; 2011.
3. Ripamonti D, Maggiolo F, Rilpivirine, a non –nucleoside reverse transcriptase inhibitor for the treatment of HIV infection. Curr Opin Investig Drugs, 2008; 9:899-912.
4. Miller CD, Crain J, Tran B. Rilpivirine, a new addition to the anti HIV -1 armamentarium. Drugs Today, 2011; 47:5-15.
5. Chen X, Zhan P, Li D. Recent advances in DAPYs and related analogues as HIV-1 NNRTIs. Curr. Med chem., 2011; 18: 359-76.
6. Laura Else, Victoria Watson, John Tjia, Andrew Hughes, Macro Siccandi, Saye Khoo, David Back. Validation of rapid and sensitive high performance liquid chromatography tandem mass spectrometry (HPLC-MS) assay for the simultaneous determination of existing and new antiretroviral compounds. J. of chromatography B. Analytical technologies in the biomedical and life sciences, 2010; 878 (19): 1455-65.
7. Venkata Reddiah CH, Rama Devi P, Mukkanti K. Effective estimation of Rilpirivine by HPLC method in tablet dosage forms and its invitro dissolution Assessment. Inter. J. Pharmacy and pharmaceutical sciences,2012; 4(3): 595-99
8. International Conference on Harmonization. ICH.Validation of analytical procedures: Definition and terminology Q2A. Switzerland: 1994, pp.1-4.
9. International conference on Harmonization guidance for Industry In: Q2B Text on validation of Analytical methods. Switzerland: IFPMIA, 1996, pp.1-8.

10. Gupta SP. Statistical methods. New Delhi; Sultan Chand and Sons: 1991, pp. E10.1 - 10.61.
11. Beckett AH, Stenlake J B. Practical Pharmaceutical Chemistry. Vol-II 4th Edn., New Delhi; CBS Publishers and Distributors: 2007, pp.85-92.