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A VALIDATED STABILITY INDICATING HPLC ASSAY METHOD FOR RILPIVIRINE HCL IN BULK DRUG

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ABSTRACT

A reverse phase stability indicating high performance liquid chromatographic (HPLC) assay method was developed and validated for quantitative determination of Rilpivirine HCl in bulk drugs. A reverse phase HPLC method was developed to separate the drug from the degradant products, using a Geminiphenomenex C18 (250x4.6) mm, 5µ column and the mobile phase containing 15mM potassium dihydrogen phosphate anhydrous and pH adjusted to pH=3.0with H_3PO_4 (was used as mobile phase A). The method used methanol as mobile phase B and the gradient programme is 0/30, 35/85, 40/85, 41/30, 45/stop. The detection was carried out at the wavelength 290nm. The developed method was validated with respect to linearity, accuracy, precision, system stability, selectivity, robustness to prove the stability indicating ability of the method.

KEY WORDS

Rilpivirine HCl, Stability indicating, Rp-Hplc, Geminiphenomenex C18 and Validation

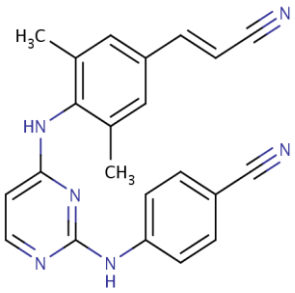
1. INTRODUCTION

Rilpivirine is a prescription medicine approved by the U.S. Food and Drug Administration (FDA) for the treatment of HIV infection in adults who have never taken HIV medicines before and who have a viral load (number of HIV RNA copies per mL of blood) of 100,000 copies/ mL or less at the start of treatment. Rilpivirine is always used in combination with other anti-HIV medicines. Rilpivirine is a type of anti-HIV medicine called a non-nucleoside reverse transcriptase inhibitor (NNRTI). NNRTIs work by binding to and blocking HIV reverse transcriptase, an HIV enzyme. This prevents HIV from replicating and lowers the amount of HIV in the blood. Rilpivirine does not cure HIV/AIDS. It is not known if rilpivirine reduces the risk of passing HIV to other people. A few chromatographic methods have appeared in the literature for the quantification of Rilpivirine hydrochloride in 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 [1-7].

No stability-indicating HPLC method for the quantitative estimation of Rilpivirine hydrochloride in bulk drug samples in the presence of degradation products along with its potential impurities has been reported. The purpose of the present research work is to develop a single stability-indicating HPLC method for the determination Rilpivirine hydrochloride and its related impurities and to establish the degradation pathway for Rilpivirine hydrochloride along with its six potential impurities. The developed LC method is validated with respect to specificity, LOD, LOQ, linearity, precision, accuracy and robustness. Accordingly, the aim of the present study is to establish degradation pathway of Rilpivirine hydrochloride through stress studies under a variety

of ICH recommended test conditions. We now report stability, indication method for the analysis and separation of the drug from its degradation products formed under ICH suggested conditions hydrolysis, oxidation and thermal stress. The present article reports a reverse phase method for the separation of Rilpivirine HCl in bulk drug and the impurities formed from its forced degradation under stress conditions, like acid hydrolysis; base hydrolysis, oxidation, heat.

Rilpivirine hydrochloride



4-[[4-[[4- [(E)-2-cyanoethenyl]-2, 6-dimethylphenyl] amino-2- pyrimidinyl] amino] benzonitrile monohydrochloride.

(Molecular weight: 402.88)(Molecular formula: C22H18N6)

2. EXPERIMENTAL:

2.1 Material and reagents:

Rilpivirine HCl bulk drug was made available from larous laboratories (purity 99.8) potassium-dihydrogen phosphate and H_3PO_4, actonitrile and methanol and water were obtained from Rankem Laboratories, India.

2.2 Chromatographic conditions:

System: SHIMADZU prominence High performance liquid chromatograph with binary pumping, PDA system.

Column: Gemini phenomenexC18 (250x4.6) mm, 5µm

2.3. Mobile phase:

Mobile phase A: 15mm KH_2PO_4 in water P^H=3.0 with H_3PO_4

Mobile phase B: Methanol

Gradient elution (T/%B):

0.01/30,35/85,40/85,41/30,45/stop.

Flow rate: 1.20ml/min Oven: 30c

Diluent: MPA: ACN (40:60) v/v

Sample conc.: 0.8mg in 10 ml of diluents.

Inj Vol: 20.0µl.

2.4 Preparation of standard stock solution:

20mg of Rilpivirine HCl was weighed accurately into a 50 ml volumetric flask 10 ml of diluent were added and sonicated for 5minutes and shaken well to get a clear solution and then made up to volume with the diluent.

2.5 Preparation of sample solution:

2 ml of above stock solution were taken into a 10 ml volumetric flask and made up to a volume to get a solution containing 80µg/ml.

2.6 Selectivity:

Selectivity is the ability of the method to assess unequivocallty the analyte in presence of components, which may be expected to be present. The might include degradent, matrix etc. The selectivity of the developed HPLC method for Rilpivirine HCl was carried out in presence of it s degradation products. Stress studies were performed for Rilpivirine HCl bulk drug to provide an indication of the stability indicating property and selectivity of the proposed method. Intentional degradation was attempted to stress condition exposing it with acid (5N HCL) **Fig-4**, alkali (1N NaOH) **Fig-3**, Hydrogen peroxide (30%) **Fig-5**, heat (60^0C) **Fig-6**, to evaluate the ability of the proposed method to separate Rilpivirine HCl from its degraded products. For heat study period was 7days where as for acid, oxidation and base the study period was 48 hours. Assay studies were carried out for stress samples against Rilpivirine HCl reference standard and the mass balance (%assay +%of sum of impurities +%of sum of degraded products) was calculated.

3. RESULT AND DISCUSSION

3.1. Optimization of chromatographic conditions:

The main target for the development of chromatographic method was to get the reliable method for the bulk drug and which will be also applicable products. Initially we took the effort for the development of HPLC method quantification of

Rilpivirine HCl from bulk. For this purpose we have used inertsil ODS (250x4.6) mm, 5µ and unison (250x4.6) mm, 5µ column but peak shape was not good. Severe tailing was observed.

Then we used Gemini phenomenon (250x4.6) mm, 5µ column with mobile phase combination of 10mm KH_2PO_4 and pH adjusted to 3.0 and the organic modifier was acetonitrile. Peak shape is good but Imp-D and Imp-E merged with Rilpivirine HCl peak. For this we changed the organic modifier to a mixer of ACN and methanol (50:50) Imp-D to separate from the major peak but imp-E merge with major analyte peak. Again we changed the organic modifier to only methanol. All impurities are separated from the major analyte peak and peak shape of the Rilpivirine HCl has slightly fronting. Because of this we again increased the strength of buffer to 15mm KH_2PO_4 and pH adjusted to 3.0. Then the peak shape was s good and all impurities were well resolved from the major analyte peak. Finally the method was optimized in Gemini phenomenan (250x4.6) m, 5µ column with buffer of 15mm KH_2PO_4 and pH adjusted to 3.0 with H_3PO_4 and the organic modifier was methanol. The gradient elution programme is 0.01/30, 35/85, 40/85, 41/30, 45/stop and flow rate is 1.20 mi/min.

4. METHOD VALIDATION

4.1. System suitability:

For system suitability studies, two replicate injections of imp D, and imp E are spiked[0.15%] with Rilpivirine HCl were used and the RSD of peak area , Resolutions, tailing factor and number of theoretical plates of the peak were calculated. The system suitability results are shown in **Table II & III**.

4.2. Precision:

The precision of the method was studied by determining the concentrations of the drug Rilpivirine hydrochloride for six times[10]. The results of the precision study (**Table V**) indicate the reliability of the method (RSD %< 2).

4.3. Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the method was studied by preparing three different concentrations of the analyte in the of test concentration range. These concentrations were 80%, 100% and 120%. The solutions were then analyzed, and the percentages of recoveries were calculated from the calibration curve. The recovery values for Rilpivirine hydrochloride ranged from 99.64% to 100.62% (**Table VI**). The average recoveries of three levels, nine determinations for Rilpivirine hydrochloride were 99.75- 100.25%.

4.4. Calibration and linearity:

For Linearity studies test solutions for the method were prepared from Rilpivirine hydrochloride stock solutions at six concentration levels from tested from 80% to 120% of the targeted level of the assay concentration Rilpivirine hydrochloride. Standard solutions containing 60-100 µg/ml of Rilpivirine hydrochloride in each linearity level were prepared. Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area verses the concentration. Data was treated by least-squares linear regression analysis, the calibration graphs were found to be linear in the mentioned concentrations and slopes and correlation coefficients are shown in **Table –V**.

4.5. Robustness:

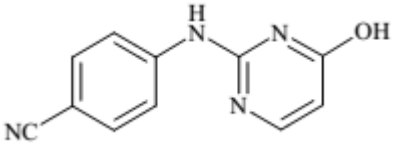
To determine the robustness of the developed method experimental condition were purposely altered and the resolution between Rilpivirine hydrochloride and imp4 and imp5 were evaluated. The flow rate of the mobile phase was 1.2 ml/min. To study the effect of flow rate on the resolution, it was changed by 0.2 units from 1.0 to 1.4ml/min while the other mobile phase components were held as stated in chromatographic conditions. The effect of percent organic strength on resolution was studied by varying Methanol from –5 to +5 % while other mobile phase components were held constant as stated in chromatographic condition. The effect of column temperature on resolution was studied at 25 and 35°C instead of 30°C, while the other mobile phase components were held constant stated in

chromatographic condition. The results are shown in **Table-VII**.

Table I: Summary of Forced degradation results

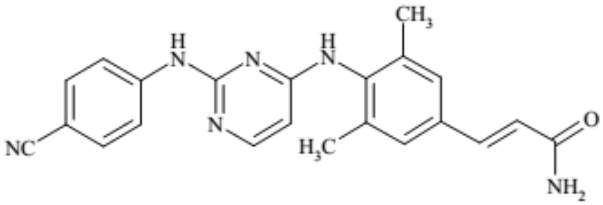
Stress condition	Time	Assay of active Substance %	Remarks
Acid Hydrolysis (5.0 N HCl)	48hrs	99.81	No Degradation
Base Hydrolysis (1 N NaOH)	48hrs	93.57	Degradation
Oxidation (30% H2O2)	48hrs	96.67	Degradation
Thermal (60°C)	7days	99.71	No Degradation
Photolytic degradation	1.2 Lux million Hrs	99.59	Negligible degradation

IMPURITY-1



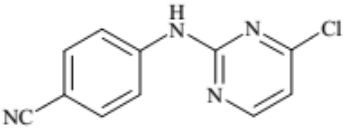
4-(4-Hydroxypyrimidin-2-ylamino) benzonitrile (molecular weight 212.21) $C_{11}H_8N_4O$

IMPURITY -2



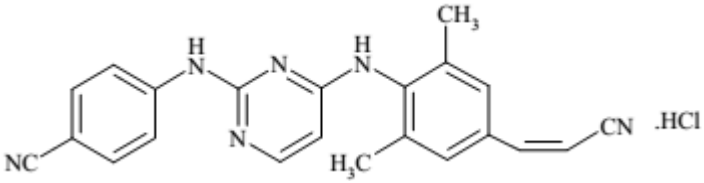
(E)-3-(4-(2-(4-Cyanophenylamino) pyrimidin-4-ylamino)-3, 5-dimethylphenyl) acrylamide
(Molecular weight 384.43) $C_{22}H_{20}N_6O$

IMPURITY-3



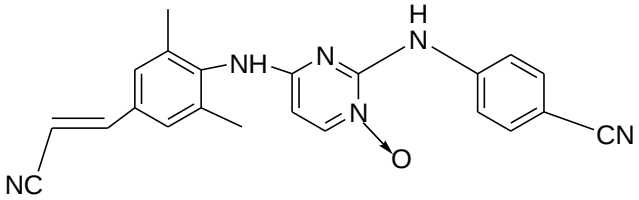
4-[(4-Chloro-2-pyrimidinyl) amino] benzonitrile (molecular weight 230.65) $C_{11}H_7ClN_4$.

IMPURITY-4



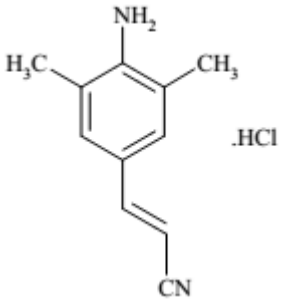
4-{[4-({4-[(Z)-2-Cyanovinayl]-2, 6-dimethylphenyl} amino) pyrimidin-2-yl] amino} benzonitrile
Hydrochloride (molecular weight 402.88) $C_{22}H_{18}N_6$ HCl.

IMPURITY-5



4-[[4-[[4-[(E)-2-cyanoethenyl]-2,6-dimethylphenyl] amino-2-pyrimidinyl] amino] benzonitrile monohydrochloride.

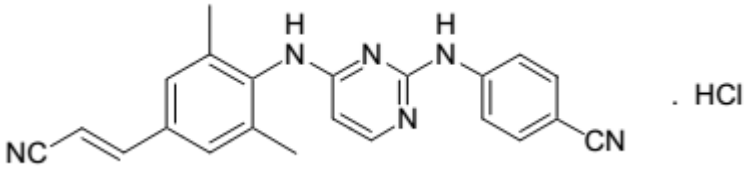
IMPURITY-6



(2E)-3-(4-Amino-3,5-dimethylphenyl) acrylonitrile hydrochloride (molecular weight 208.69)

$C_{11}H_{12}N_2$ HCl

Rilpivirine HCl



4-[[4-[[4-[(E)-2-cyanoethenyl]-2,6-dimethylphenyl] amino-2-pyrimidinyl] amino] benzonitrile monohydrochloride.

Table: II Summary of Relative Retention time of Impurities.

S.NO	Compound	RT	RRT
1	IMPURITY-1	11.90	0.49
2	IMPURITY-2	14.93	0.61
3	IMPURITY-3	19.19	0.79
4	IMPURITY-4	22.44	0.92
5	IMPURITY-5	23.53	0.97
6	IMPURITY-6	27.12	1.12
7	Rilpivirine HCl	24.27	1.00

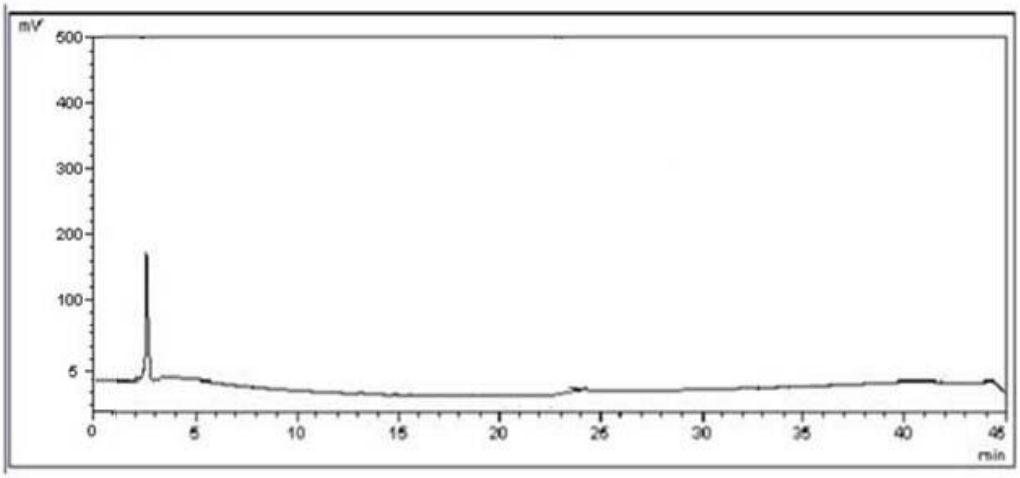


Fig-1 A Blank chromatogram of the Rilpivirine hydrochloride.

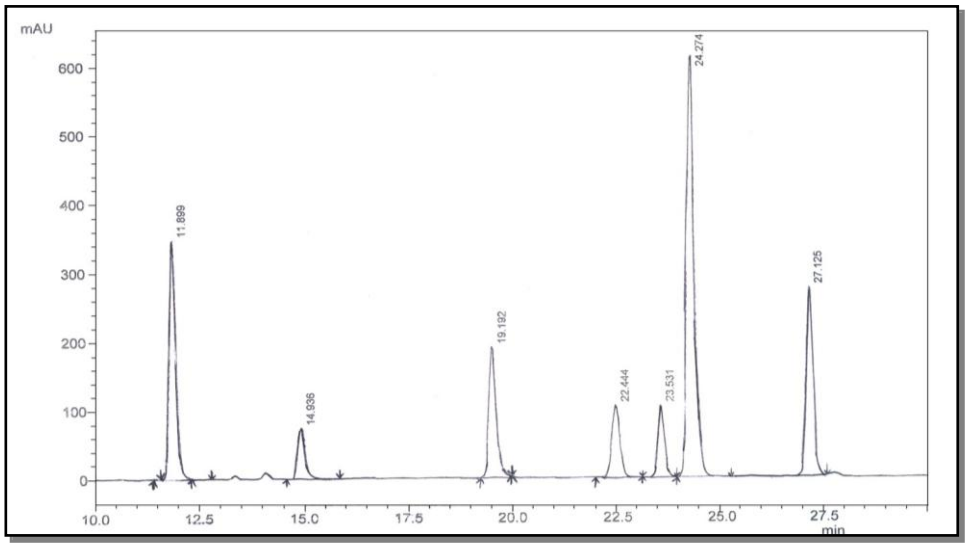


Fig-2 A chromatogram of the Rilpivirine hydrochloride for selectivity.

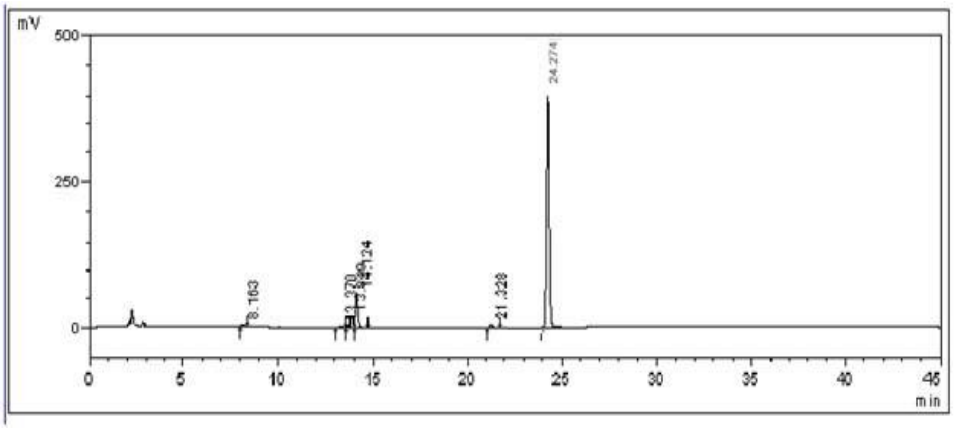


Fig-3 A chromatogram of Rilpivirine HCl in Alkali degradation.

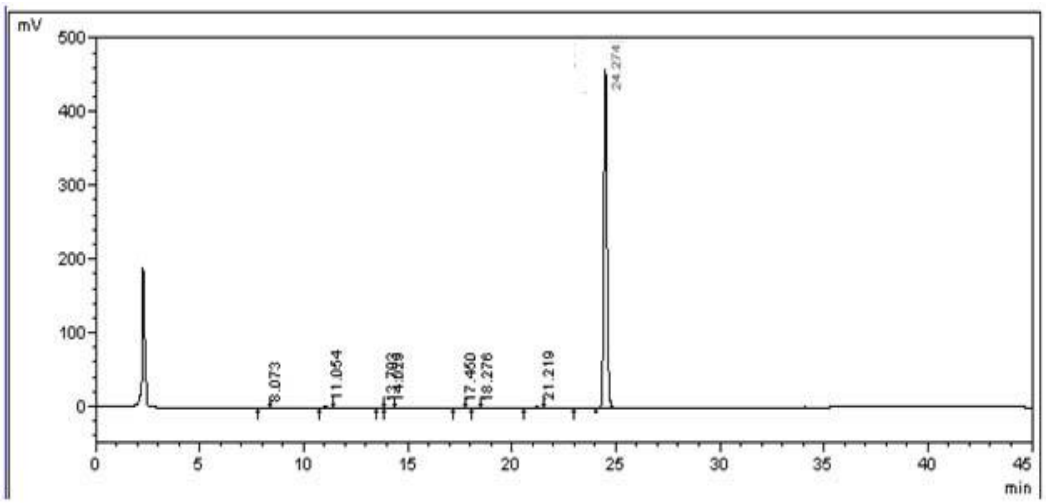


Fig-4 A chromatogram of Rilpivirine HCl in Acid hydrolysis degradation.

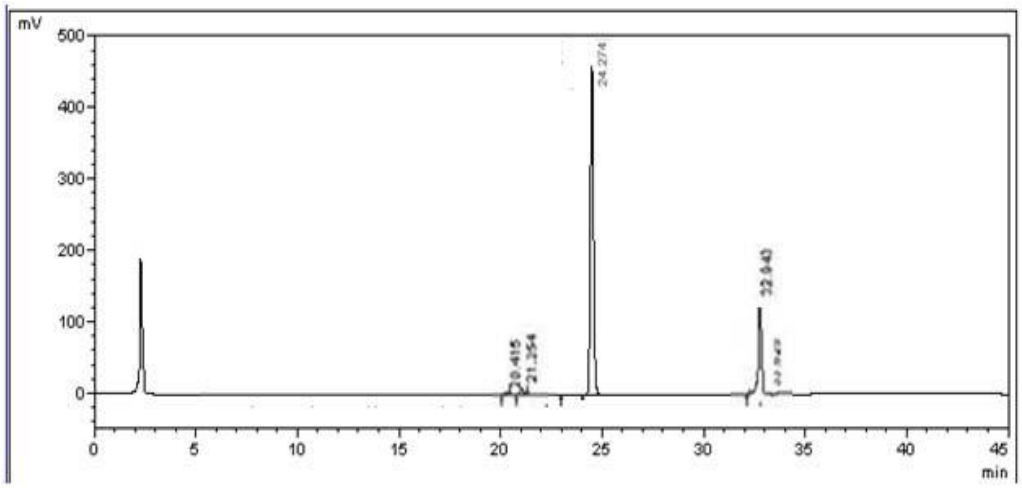


Fig-5 A chromatogram of Rilpivirine HCl in Peroxide degradation

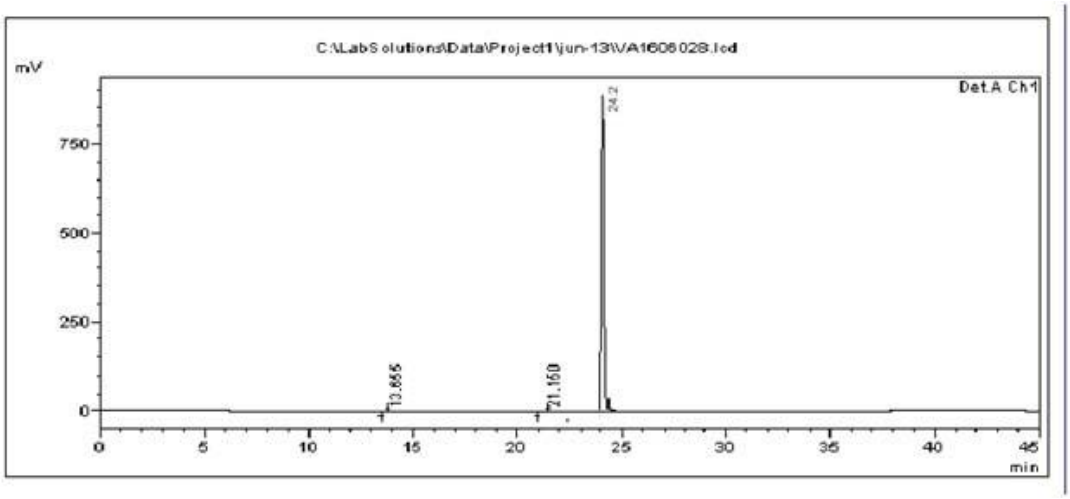


Fig-6 A chromatogram of Rilpivirine HCl under heating.

Table III System suitability reports.

Compound	Retention Time	% RSD	USP tailing	Theoretical plates
Rilpivirine HCl	24.27	0.44	1.02	17432

Table –IV Results of the LOD and LOQ

Name	%LOD	%LOQ
Impurity A	0.05	0.16
Impurity B	0.08	0.14
Impurity C	0.06	0.13
Impurity D	0.05	0.12
Impurity E	0.04	0.13
Rilpivirine HCl	0.04	0.10
Impurity F	0.12	0.28

Table-V: Results of the Linearity study and Precision

Ingredient	Precision (% RSD)	Linearity (µg/ml)	Slopes (n= 3)	Coefficients of correlations
Rilpivirine HCl	0.44	60-100	4465.65	0.99984

Table-VI Results of the Recovery Tests for the Irinotecan HCL

Level of Addition (%)	Amount added (n = 3) (ppm)	% Recovery	% Average recovery^
80	60	**99.64**	**99.75**
100	80	**99.85**	**99.90**
120	100	**100.62**	**100.25**

^ Average recovery = the average of three levels, nine determinations

Table-VII Results of robustness study

Sr. No	Parameters	Variations	Resolution between Rilpivirine HCl and Impurity D
1	Temperature	at 25c	2.74
		at 35c	2.55
2	Flow rate	1.0 ml/min	2.68
		1.4ml/min	2.48
3	Changes in % of Organic solvent in gradient elution	MPA:MPB [65:35]	2.38
		MPA:MPB [75:25]	2.63
4	Changes in PH of buffer	3.5	2.51
		2.5	2.76

4.6. LOD and LOQ (Sensitivity):

A series of solutions in the range 0.1–0.5% of the assay concentration (80 µg mL−1) were prepared by dilution of the standard solutions. Each solution (20 µL) was injected three times, the areas were measured for the drug peak, and the standard deviation for the three injections was Calculated for each concentration. On the basis of data obtained, the standard deviation at each concentration was calculated and this value was used for calculation of the LOD and LOQ. The results are shown in **Table-IV**

4.7. Stability of analytical solution:

The stability of the standard solutions and the sample solutions was tested at intervals of 24, 48 and 72 h. The stability of solutions was determined by comparing results of the assay of the freshly prepared standard solutions. The RSD for the assay results determined up to 72 h, for Rilpivirine hydrochloride was 0.27 %. The assay values were within ± 2 % after 72 h. The results indicate that the solutions were stable for 72 h at ambient temperature.

5. CONCLUSION

The method developed for quantitative determination of Rilpivirine hydrochloride is rapid, precise, accurate and selective. The method was completely validated showing satisfactory data for all method-validated parameters tested. The developed method is stability indicating and can be used for assessing the stability of Rilpivirine hydrochloride as bulk drug. The developed method can be conveniently used for the assay determination of Rilpivirine hydrochloride in bulk drugs.

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