

Method Development and Validation of Rilpivirine by Rp-Hplcmethod

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ABSTRACT:

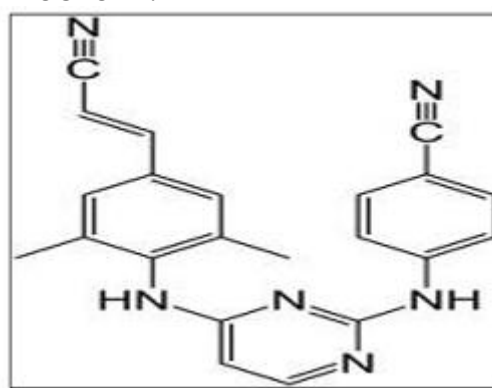
An HPLC method was developed and validated for the Rilpivirine in bulk drug. The chromatographic system was equipped RP C18 column (150 mm x 4.6 mm, 5 μ m) mobile phase consisted of Orthophosphoric acid (0.1%) and Methanol in the ratio 40:60 v/v delivered at a flow rate of 0.8 mL/min. The detection wavelength was set at 218 nm, and the injection volume was 10 μ L. The drug exhibited a well-resolved and sharp peak with a retention time of 7.5 minutes. The separation was performed at ambient temperature. The injection volume was 10ul. Linearity was assessed using a fixed concentration of 10 μ g/mL, with injection volumes of 5, 7.5, 10, 12.5, and 15 μ L, showing excellent correlation (r^2 0.999). The Percentage recoveries were found in the range of 98.12-99.16%. The proposed method was validated in accordance with ICH parameters the method is precise, accurate, selective and rapid.

key words: RP-HPLC, Rilpivirine, Validation, and ICH.

I. INTRODUCTION:

Rilpivirine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) used to treat HIV-1 infections in patients who have not previously received treatment. It falls within the diary pyrimidine class of drugs, which have a distinct chemical structure from the pyrimidine nucleotides present in DNA. The flexible nature of Rilpivirine's structure contributes to a reduced risk of resistance development compared to some other NNRTIs. Its unique binding site on the reverse transcriptase enzyme differs from those of natural nucleotides and other NNRTIs, which helps minimize the risk of cross-resistance with other medications in its class. The drug received FDA approval On may 20, 2011.

STRUCTURE:



IUPAC NAME:4-((4-((4-(2-cyanovinyl)-2,6-dimethylphenyl)amino)pyrimidin-2-yl)amino)benzonitrile.

MECHANISM OF ACTION:

Rilpivirine binds directly to HIV-1 reverse transcriptase at a site different from the active site. This binding causes a conformational change in the enzyme. As a result, reverse transcriptase activity is inhibited. This prevents conversion of viral RNA into DNA. Without viral DNA formation, HIV cannot integrate into the host genome, stopping viral replication.

II. MATERIALS AND METHODS:

Drug sample used:

RILPIVIRINE bulk drug was gifted by Med Reich Limited, Bengaluru.

Chemicals and solvents used:

All the chemicals used were of analytical grade procured from Great scientific, Tiruvannamalai & Delhi, the chemicals used for the study were,
Ethanol (Analytical grade)
Methanol (Analytical grade)
Ortho phosphoric acid (Analytical grade)
Milli-Q water (HPLC grade)

Instrument employed for the study were,

Digital Balance
SHIMADZU HPLC-DLC20AD
Sonica Ultra sonic cleaner- model 2200MH
Melting point apparatus.

Preparation of Mobile Phase

0.1% of Ortho Phosphoric Acid is mixed with 1000ml of Milli-Q-Water were measured and mixed well. Then the solution was degassed using ultrasonic water bath for 5 minutes. The resultant solution was filtered through 0.45 μ filter under vacuum filtration.

Preparation of Standard Stock Solution

About 10 mg Rilpivirine was accurately weighed and transferred into a 10 ml volumetric flask. To this, 7 ml of mobile phase was added and sonicated to dissolve it completely and made volume up to the mark with the mobile phase. The concentration of resultant solution was 1000 μ g/ml.

Linearity:

To study the linearity, an aliquot of stock solution of Rilpivirine (5ml, 7.5ml, 10ml, 12.5ml and 15ml of 1000 μ g/ml) were transferred into a five separate 10 ml volumetric flask and made up to the mark with mobile phase. The solutions were injected and the chromatograms were recorded at 218 nm. It was found that the above concentration range was linear, within the concentration range of 5-15 μ g/ml. The peak area was plotted against concentration and the calibration curve was constructed.

Recovery Study:

An equivalent to 10 mg of Rilpivirine tablet powder was accurately weighed and transferred into three separate 10 ml volumetric flasks. Then 8 mg, 10 mg and 12 mg (80%, 100% and 120% respectively) of standard were accurately weighed and added to each

respectively. 7 ml of methanol was added and sonicated to dissolve it completely. Then it was made volume up to the mark with the same solvent. From which 1 ml was pipetted out from each flask and transferred to separate 10 ml volumetric flask. Then the solution was made volume up to the mark with the same solvent. Then from each prepared solution 10 μ l solution was injected into the chromatographic system. The percentage recovery was calculated by using peak area.

Precision:

1 ml of the stock solution was pipetted out into a 10 ml volumetric flask and diluted up to the mark with mobile phase. Mixed well and filtered through 0.45 μ m filter. Therefore, the concentration of the resultant solution was 10 μ g/ml. The solution was taken and injected for five times within the same day and the chromatogram was recorded. The peak area was measured to calculate the % Relative Standard Deviation value.

Intermediate Precision:

1 ml of the stock solution was pipetted out into a 10 ml volumetric flask and diluted up to the mark with mobile phase. Mixed well and filtered through 0.45 μ m filter. Therefore, the concentration of the resultant solution was 10 μ g/ml. The solution was taken and injected for five times between days and the chromatogram was recorded. The peak area was measured to calculate the % Relative Standard Deviation value.

Robustness:

As per ICH guidelines, a deliberate change in the flow rate and mobile phase composition were made and studied to evaluate the impact of the method.

➤ Organic composition in the mobile phase was varied 40-60%

III. Result and Discussion:

Initial separation conditions

Mode of operation	: Isocratic
Stationary phase	: C ₁₈ Column (150 mm x 4.6 mm i.d., 5 μ)
Mobile phase	: 0.1% of Ortho Phosphoric Acid: Methanol
Ratio	: 50:50 % v/v
Detection wavelength	: 218 nm
Flow rate	: 1 ml/min
Temperature	: Ambient
Sample volume	: 10 μ l

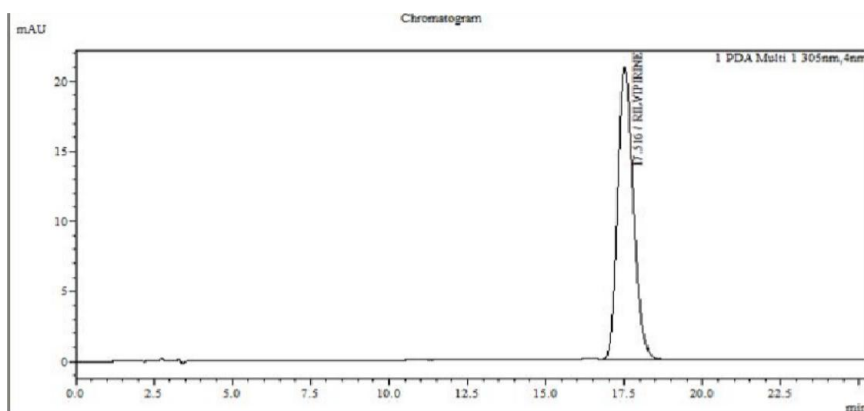


Figure:01 Trial-1 0.1% of Ortho phosphoric acid: Methanol (50:50%V/V)

Retention Time	Area (μV sec)	Height(μV)	USP Plate Count
17.156	736106	20825	5619

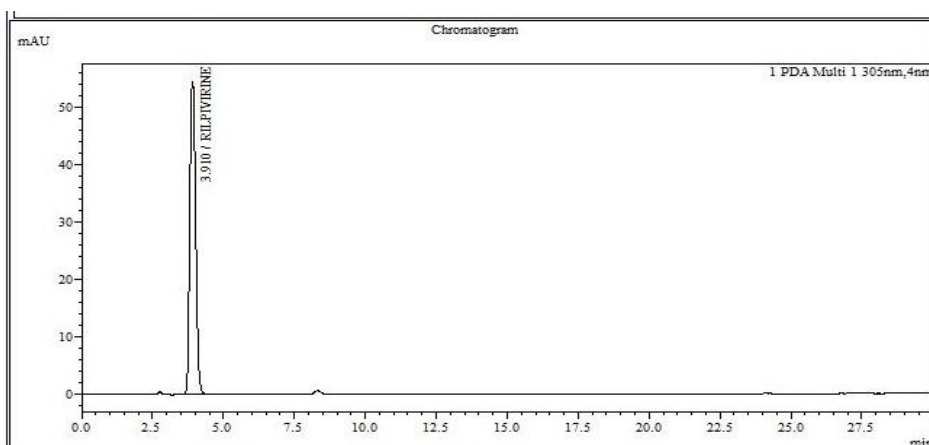


Figure:02 Trial-2 0.1% of Ortho phosphoric acid : Methanol(30:70%V/V)

Retention Time	Area(μV sec)	Height(μV)	USP Plate Count	USP
3.910	778981	54513	1639	17690

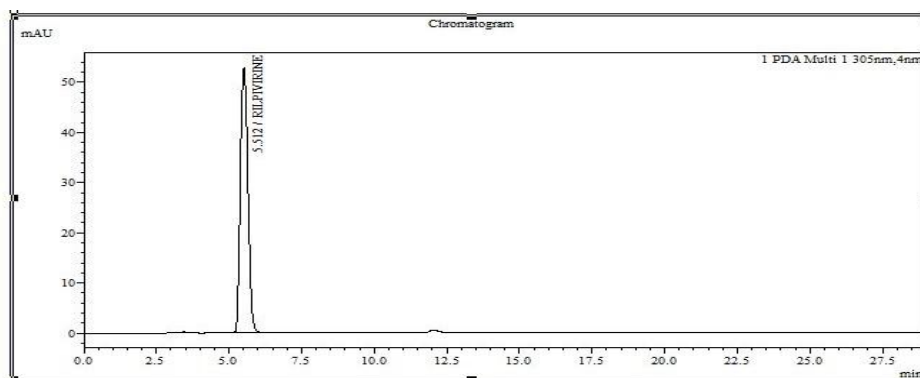


Figure:18 Trial-3 0.1% of Ortho phosphoric acid : Methanol(25:75%V/V)

Retention Time	Area(μ V sec)	Height(μ V)	USP Plate Count	USP
5.512	956566	52770	2013	17690

OPTIMIZED CHROMATOGRAM CONDITIONS:

Optimized chromatogram:

Mode of operation : Isocratic
 Stationary phase : C₁₈ Column (150 mm x 4.6 mm i.d., 5 μ)
 Mobile phase : 0.1% of Ortho Phosphoric Acid: Methanol
 Ratio : 40:60 % v/v
 Detection wavelength : 218 nm
 Flow rate : 0.8 ml/min
 Temperature : 30 °c
 Sample volume : 10 μ l

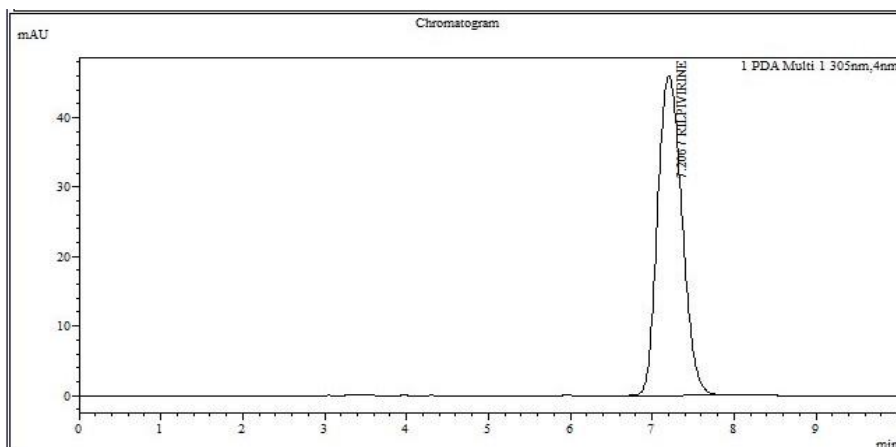


Figure:04 Optimized chromatogram 0.1% of Ortho phosphoric acid : Methanol (40:60%V/V)

Retention Time	Area(μ V sec)	Height(μ V)	USP Plate Count	USP
7.206	955332	45951	2653	17690

LINEARITY:

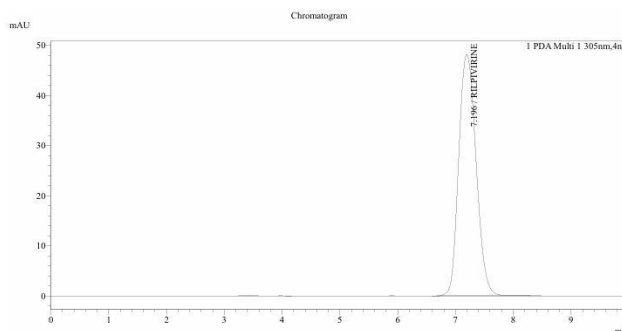
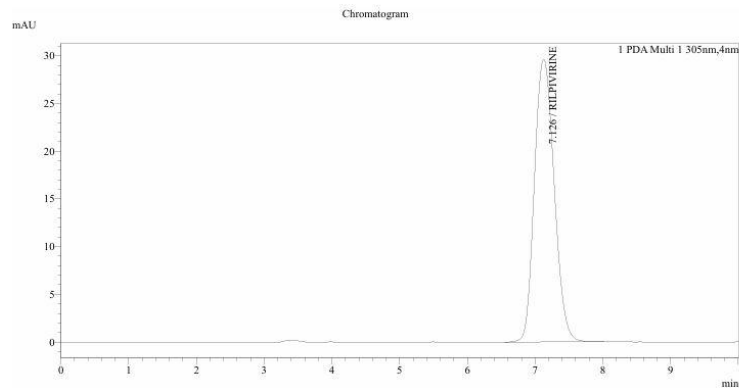


FIG.NO : 05 Linearity Chromatogram of 5 μ g/ml



Linearity injection 02
FIG.NO : 06 Linearity Chromatogram of 7.5 µg/ml

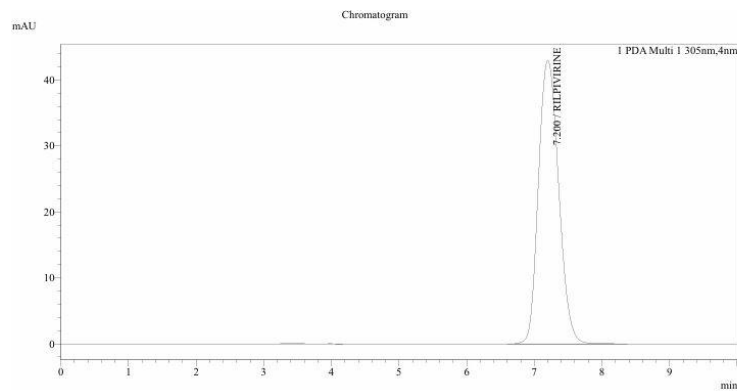


FIG.NO : 07 Linearity CHROMATOGRAM OF 10 µg/ml

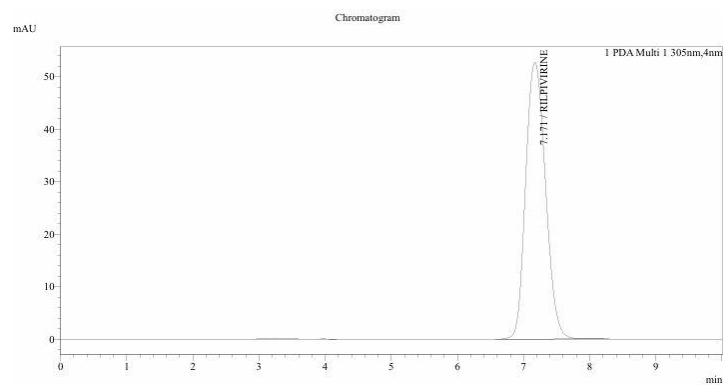


FIG.NO : 08 Linearity CHROMATOGRAM OF 12.5 µg/ml

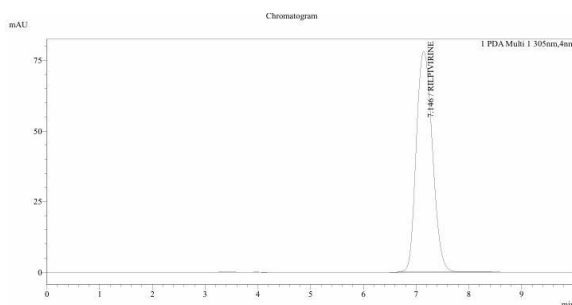


FIG.NO : 09 Linearity Chromatogram of 15 µg/ml

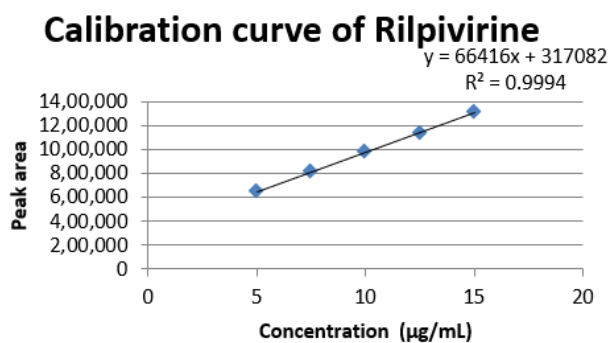
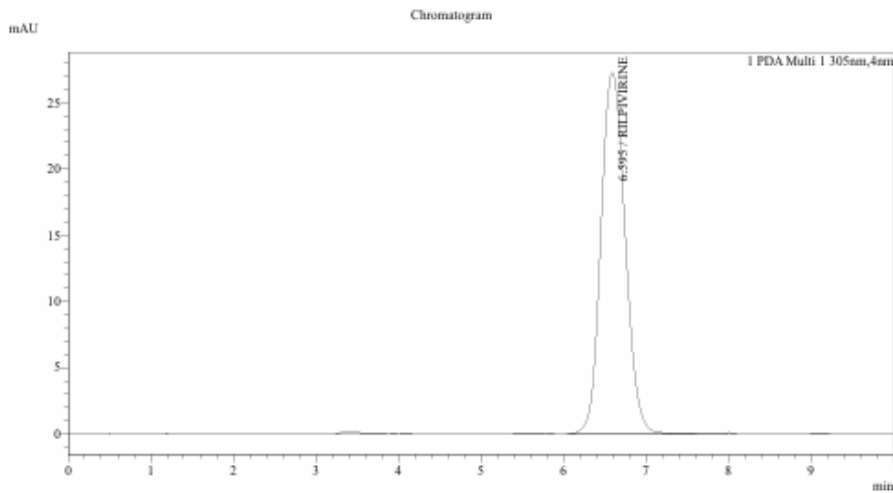
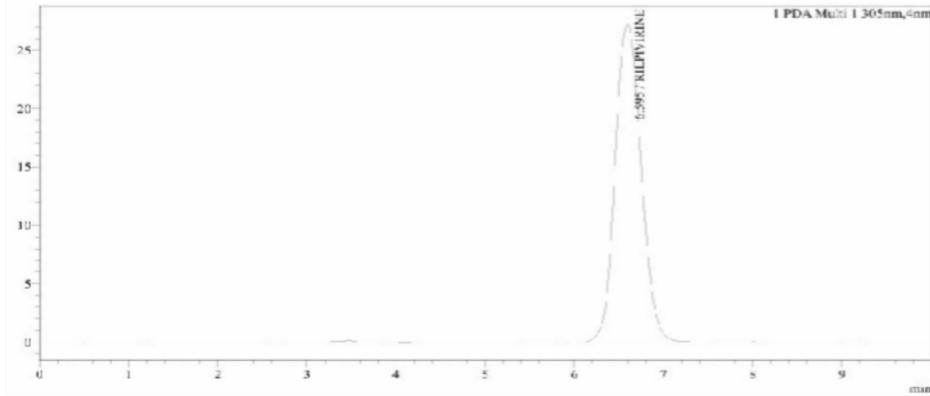


FIG.NO : 10 LINEARITY CURVE

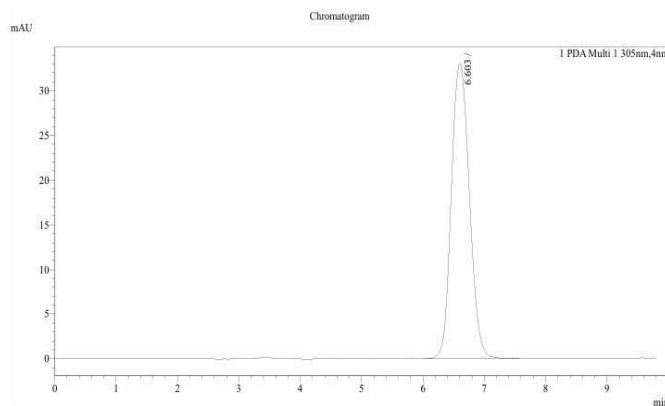
S. No	Concentration	Average peak area	Correlation coefficient	LOD	LOQ	Slope	Intercept
1	5	653563	0.9994	1.064793	3.226645	66416	317082
2	7.5	814704					
3	10	976844					
4	12.5	1139985					
5	15	1321125					

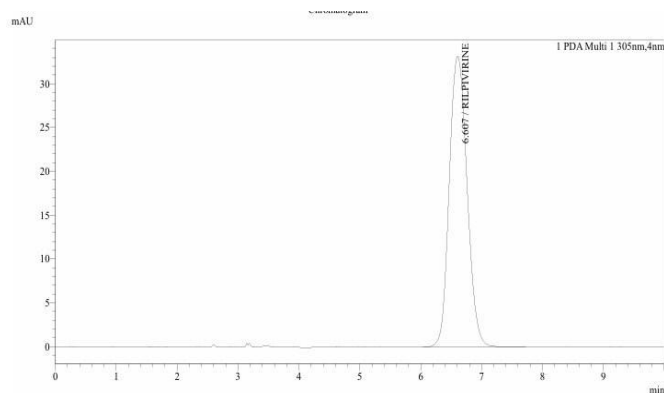
RECOVERY STUDY:



RT	Injection	Area
6.595	1	553189
6.615	2	553743

FIG.NO:11 RECOVERY 80%





RT	Injection	Area
6.607	1	67447
6.603	2	674848

FIG.NO:12 RECOVERY 100%

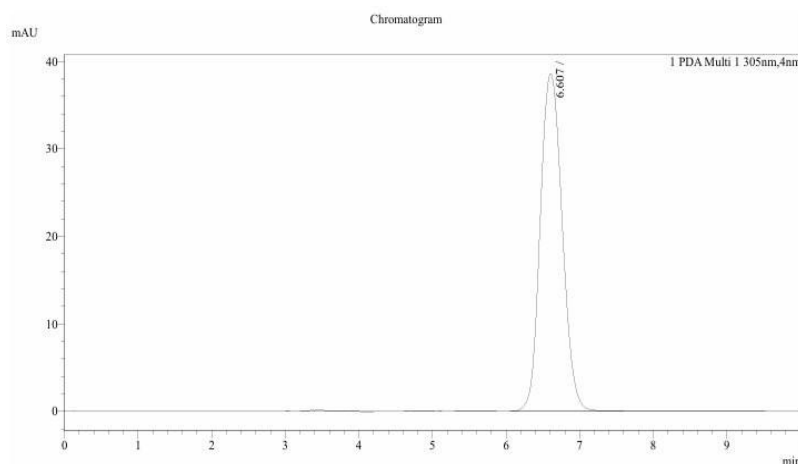
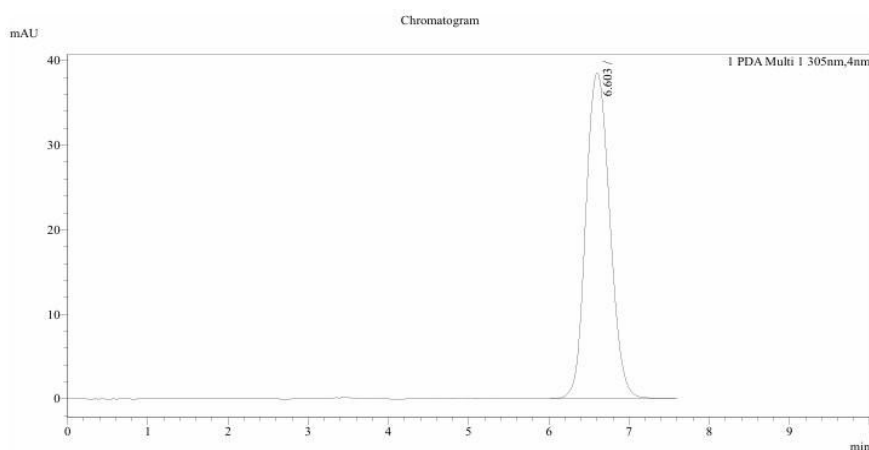


FIG.NO : 13 RECOVERY 120%

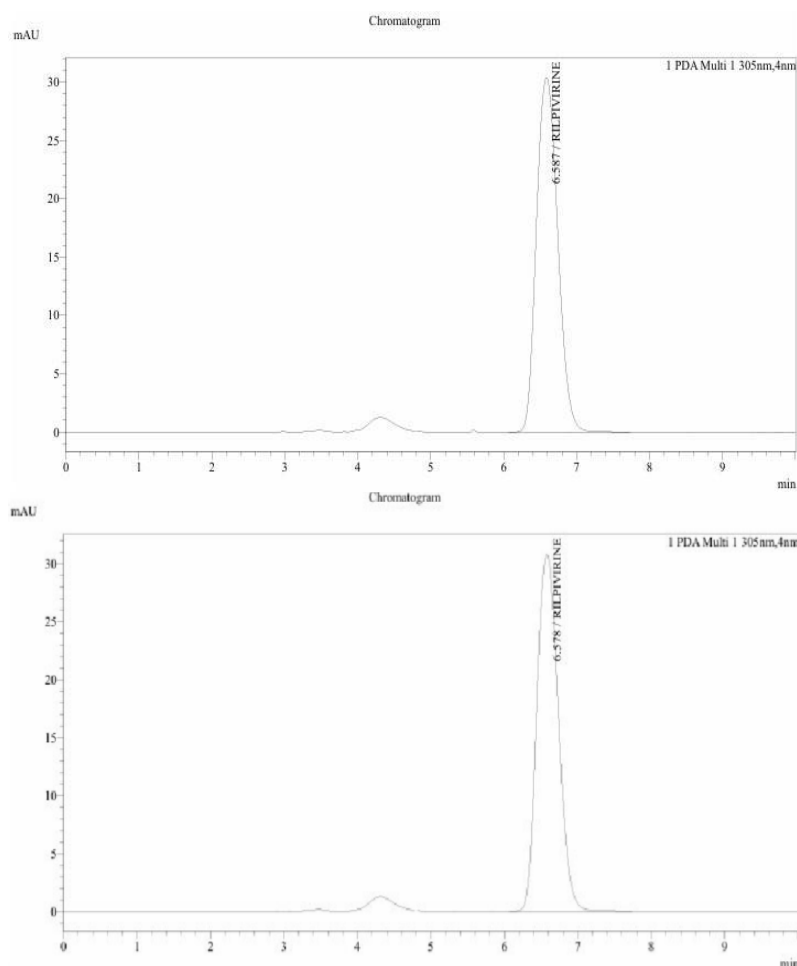
RT	Injection	Area
6.603	1	799138
6.607	2	799356

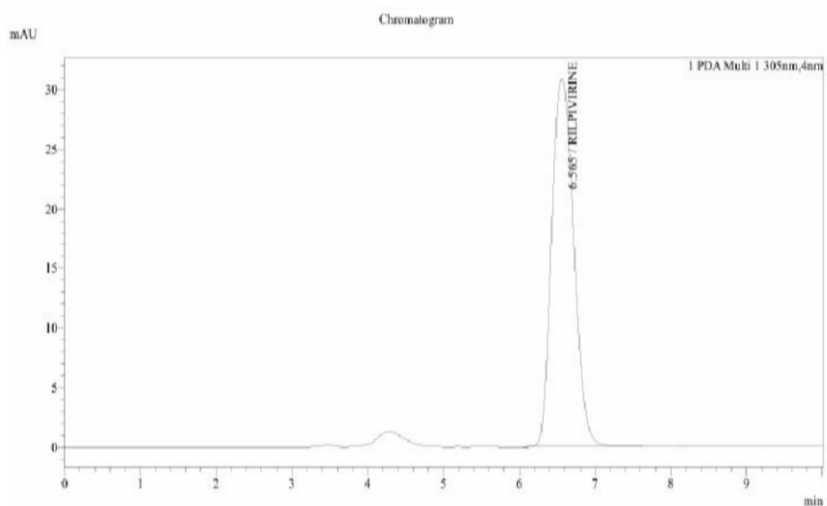
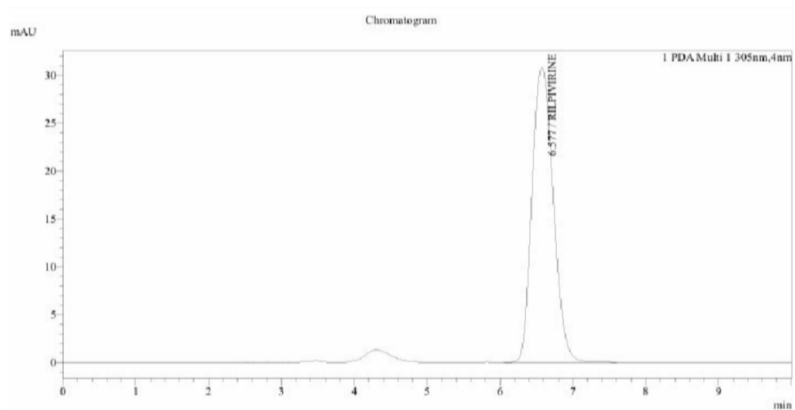
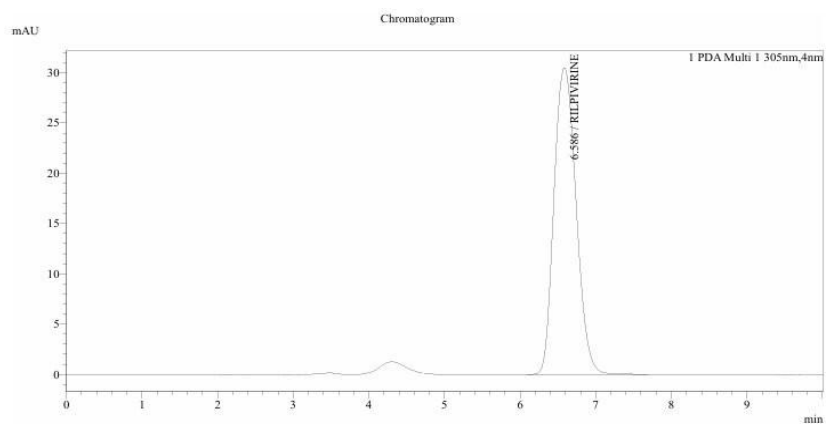
RECOVERY STUDY DATA

S.no	% concentration	Average Area	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery	SD	%RSD
1	80%	553441	8	7.85	98.12%	98.67%	0.523	0.53
2	100%	687538	10	9.87	98.74%			
3	120%	818323	12	11.91	99.16%			

The %RSD value less than 2% was found.

PRECISION:

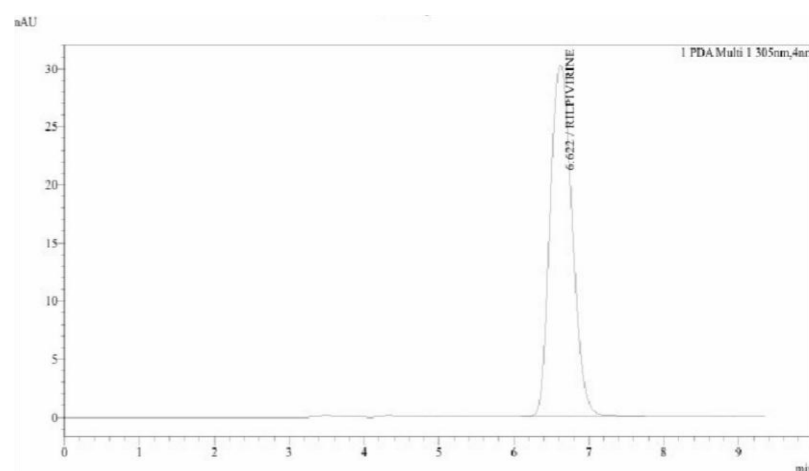
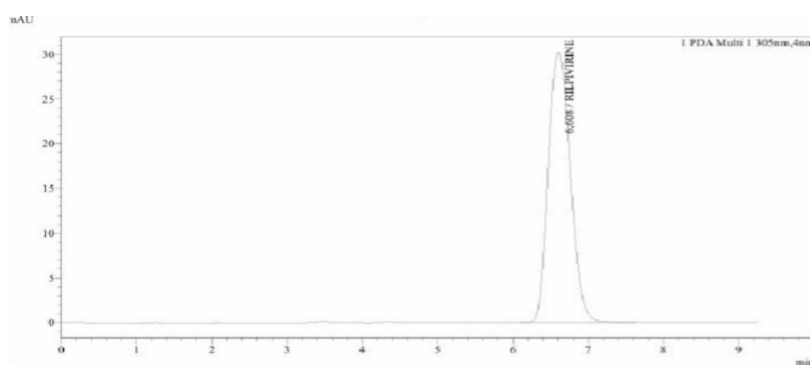
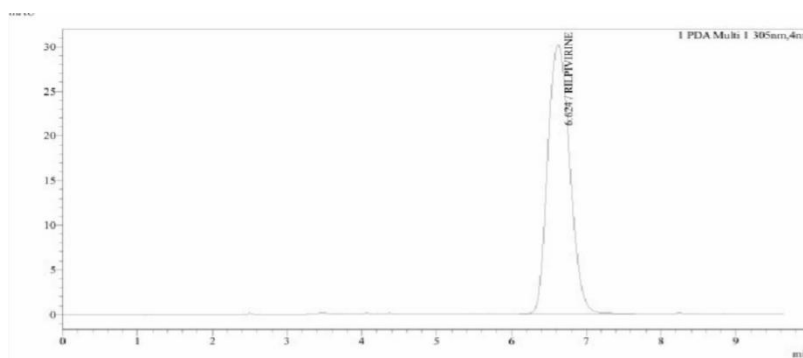


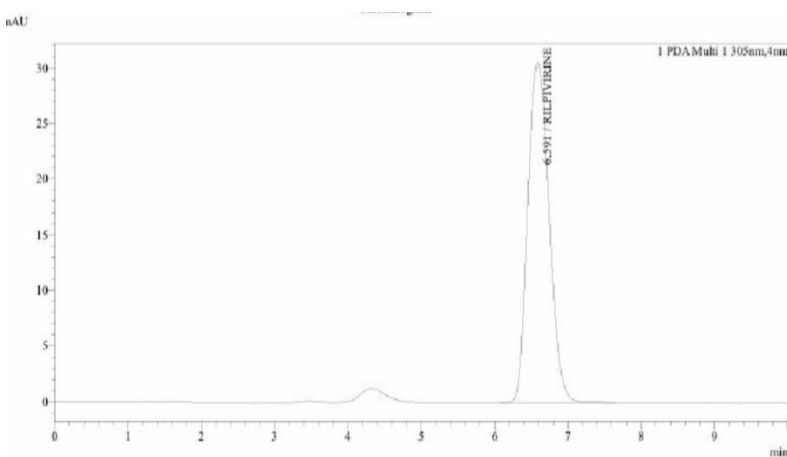
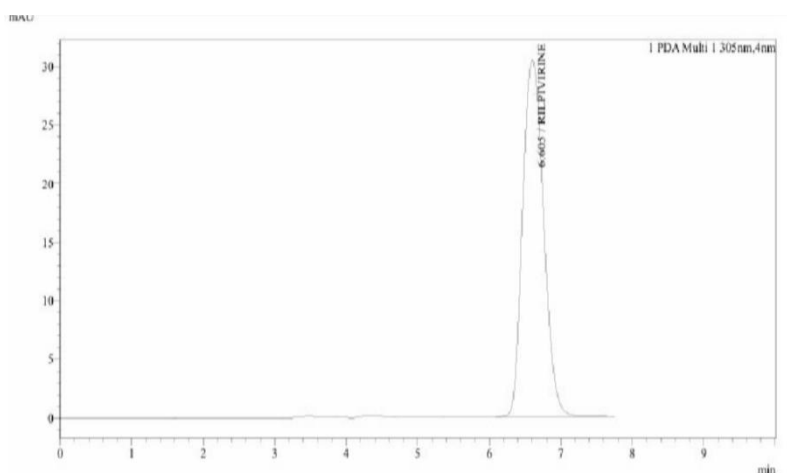


RETENTIONTIME	INJECTION	AREA
6.586	1	618360
6.587	2	617253
6.578	3	624391
6.577	4	623893
6.591	5	622923

S.NO	PEAKAREA	AVERAGE	SD	%RSD
1	618360	621364	3313.35	0.53324
2	617253			
3	624391			
4	623893			
5	622923			

INTERMEDIATE PRECISION:



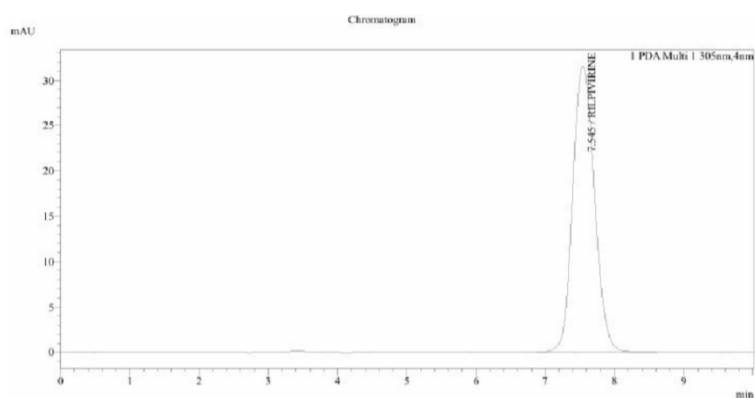
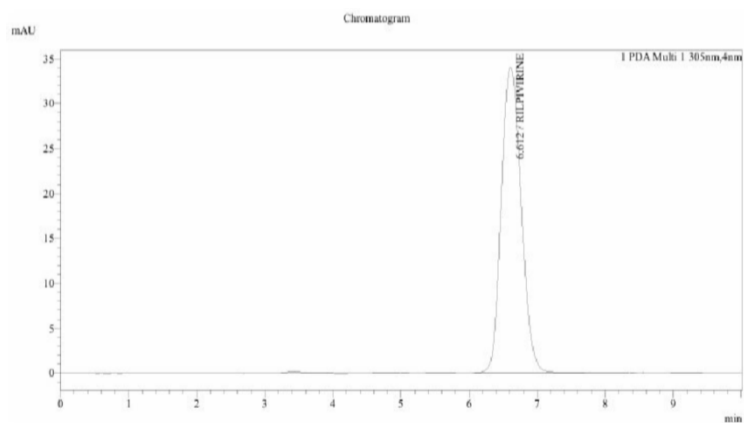


RETENTIONTIME	INJECTION	AREA
6.586	1	615814
6.587	2	615722
6.578	3	616615
6.577	4	617499
6.591	5	618799

INTERMEDIATE STUDY DATA:

S.NO	PEAKAREA	AVERAGE	SD	%RSD
1	615814	616889.8	1285.76	0.2084
2	615722			
3	616615			
4	617499			
5	618799			

ROBUSTNESS:

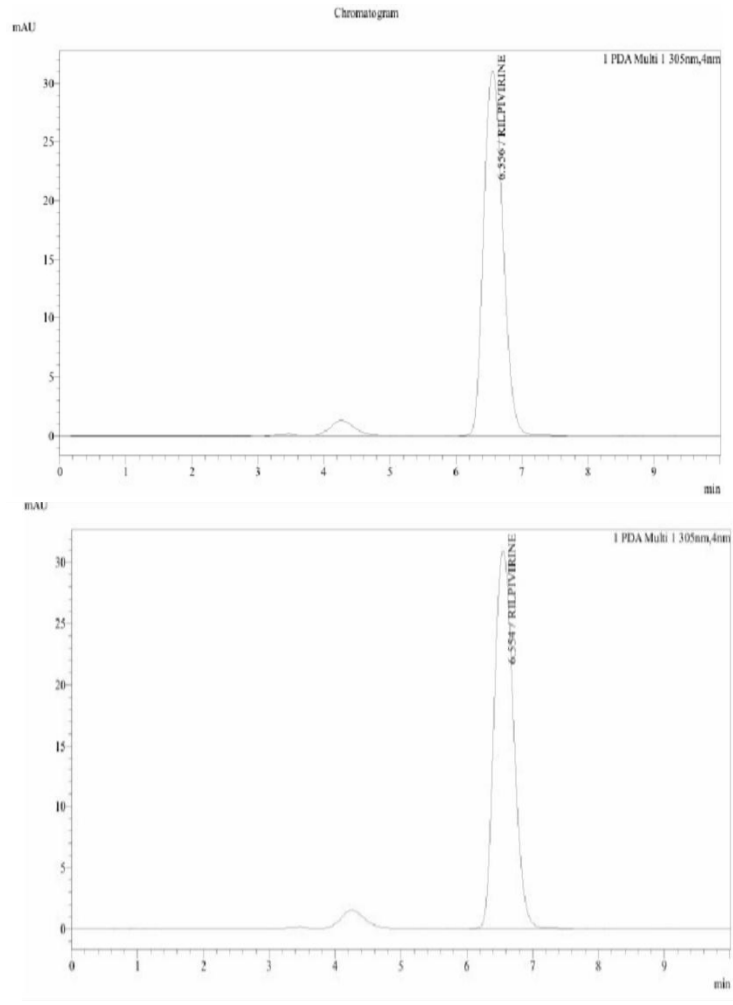


RETENTIONTIME	INJECTION	AREA	TAILINGFACTOR
6.612	1	690121	1.141
7.545	2	686696	1.127

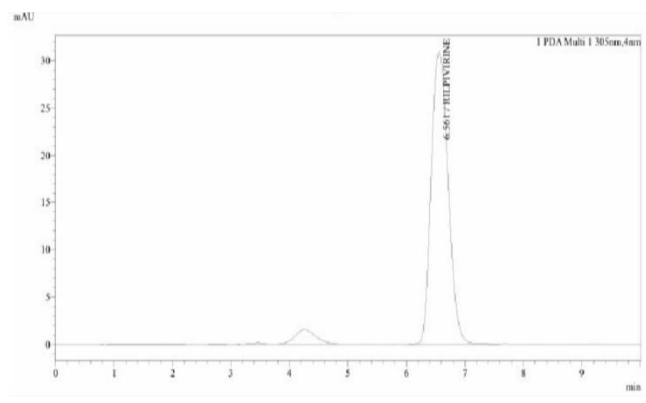
ROBUSTNESS - CHANGE IN ORGANIC COMPOSITION IN MOBILE PHASE

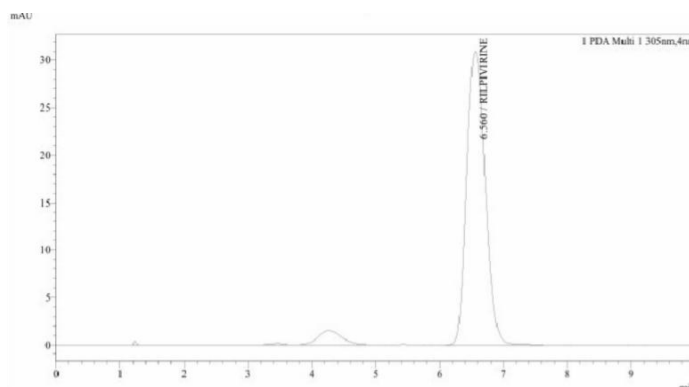
S.NO	COMPOSITION OF MOBILE PHASE	RETENTION TIME	PEAKAREA	SD	%RSD
1	38:62	6.612	625263 623113	1520.28	0.243
2	42:58	7.545	624611 626708	1482.80	0.236

RUGGEDNESS:



RETENTIONTIME	INJECTION	AREA	TAILINGFACTOR
6.556	1	625263	1.165
6.554	2	625611	1.165





RETENTIONTIME	INJECTION	AREA	TAILINGFACTOR
6.561	1	625235	1.165
6.560	2	623079	1.162

RUGGEDNESS STUDY DATA:

S.NO	ANALYST	CONC	PEAKAREA	SD	%RSD
1	Analyst-1	10	690121	2421.841	0.351803
		10	686696		
2	Analyst-2	10	625235	2461.732	0.39344
		10	623079		

IV. CONCLUSION

Analytical methods using RP-HPLC were successfully developed for the estimation of Rilpivirine in bulk. The developed methods were validated with various parameters like accuracy, precision, linearity, robustness, range, intermediate precision, ruggedness, specificity etc., as per ICH Q2 guidelines. The results obtained were within the limits of Indian Pharmacopoeia (IP)

From the results obtained, the developed method were found to be accurate, simple, rapid, precise, reliable, sensitive, reproducible and economical for the determination of Rilpivirine in bulk. The RP-HPLC method development of Rilpivirine gives good, accurate, results with low sample volume and less solvent consumption. The statistical parameters and the recovery data reveal good accuracy and precision of the methods. These methods can be used as general methods for the determination of Rilpivirine in bulk form.

REFERENCES

- [1]. Danish Sayyad, Ashok Pingle, Sarfaraz Shaikh, Shoeb Sayyed, Amol Mhaske, Tohid Sayyad. Application of Quality by Design Approach for Development and Validation of Stability Indicating RP-HPLC Method for Rilpivirine Hydrochloride in Bulk. International Journal Of Pharmacy and Pharmaceutical Research 2020, 17 (4), 473-509.
- [2]. Sivagami B, Sharmil Kumar. L.M, Chandrasekar. R, Niranjan Babu. M. Development and Validation for the Simultaneous Estimation of Rilpivirine and Dolutegravir in Bulk and Pharmaceutical Dosage Forms by RP-HPLC Method. Research Journal of Pharmacy and Technology. 2022, 15 (11), 5302-6. doi: 10.52711/0974-360X.2022.00893.



- [3]. Patel S, Nagappan K, Santhosh GR. A new quantitative reverse phase high-performance liquid chromatographic method for the quantification of Rilpivirine hydrochloride in bulk and dosage form. *Journal of Applied Pharmaceutical Science*. 2018, 8 (11), 157-162. DOI: 10.7324/JAPS.2018.81122.
- [4]. Ismail Y, Vara Prasad M, Shaheedha S. M., Habeeb M. A New Stability Indicating RP-HPLC Method Development and Validation for the Simultaneous Estimation of Dolutegravir and Rilpivirine in Bulk and its Dosage Forms. *Iranian Journal of Pharmaceutical Sciences*. 2019, 15 (4), 53-72.