ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24SEP033

Method Development and Validation of Assay of Rilpivirine in Rilpivirine Tablets by RP-HPLC

Shriya. N*[1]; Dr. S. Shobha Rani[2]; Karthik Manda[3]; Mohammad Naseeruddin[4]; Dr. Y. Sridhar Reddy[5]
[1]Research Scientist, Department of Pharmaceutical Analysis, CPS, IST, Jawaharlal Nehru Technological University, Hyderabad.
[2]Professor, Pharmaceutical Sciences, CPS, IST, Jawaharlal Nehru Technological University, Hyderabad.
[3]Analytical Scientist, Analytical Research and Development, Dr. Reddy's Laboratories Ltd., Hyderabad.
[4]Senior Executive, Analytical Research and Development, Aizant Drug Research Solutions Pvt. Ltd., Telangana.
[5]Senior Research Scientist, Department of Clinical Pharmacology and Therapeutics, Nizam's Institute of Medical Sciences, Hyderabad.

Correspondence to Author:- Shriya. N*[1]

Abstract:- A method was developed and validated for determining the Assay of Rilpivirine in Rilpivirine Tablets by using WATERS HPLC 2695 with Autosampler, PDA Detector and Empower 3 Software. ACE Excel 3 C18 AR (250 x 4.6 mm, 3 µm) column was used along with a mobile phase combination Acetate Buffer: Methanol: Acetonitrile in ratio 60: 30: 10 % v/v/v. Isocratic flow with flowrate of 1.5 mL/minute, Injection volume 12 µL was employed. Column temperature of 30°C was maintained. Detection wave length of Rilpivirine was 258 nm. Run time was optimised to 5 min based on retention time. The retention time of Rilpivirine was 2.29 minutes. The method was found to be specific, linear with a square of correlation coefficient (R^2) 1.000, accurate over the concentration range of 25 % to 200 %, precise with % RSD of six samples 1.1 and robust. Thus a simple, quick and economical method was developed and validated.

Keywords:- *Method Development, Validation, Assay, Rilpivirine, RP-HPLC.*

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 diarylpyrimidine 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.

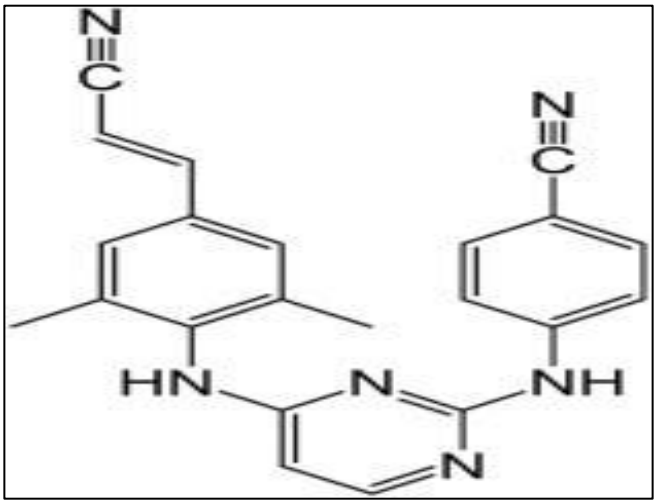

Fig 1 Structure of Rilpivirine

II. MATERIALS AND METHODS

➢ *Chemicals and Reagents:*

Rilpivirine Hydrochloride API, EDURANT Tablets containing 25 mg of Rilpivirine, Sodium Acetate Trihydrate, Glacial Acetic Acid, Acetonitrile and Methanol, Milli Q Water.

➢ *Instrumentation:*

Electronic Weighing Balance, pH Meter, Ultra sonicator, WATERS HPLC 2695 with Autosampler, PDA Detector and Empower 3 Software.

➢ *Chromatographic Conditions:*

- **Column:** ACE Excel 3 C18 AR (250 x 4.6 mm, 3 µm)
- **Mobile phase:** Acetate Buffer: Methanol: Acetonitrile in ratio 60: 30: 10 % v/v/v
- **Flow rate:** 1.5 mL/minute.
- **Flow:** Isocratic
- **Injection volume:** 12 µL
- **Detector wave length:** 258 nm
- **Column temperature:** 30°C
- **Run time:** 5 min

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24SEP033

- **Preparation of Solutions:**

➢ *pH 4.5 Acetate Buffer Preparation:*

3 g of Sodium acetate trihydrate and 1.6 mL of Glacial acetic acid were dissolved in 1 Litre Milli Q water by sonicating in Ultra sonicator and pH was adjusted to 4.5 with Glacial acetic acid.

➢ *Diluent Preparation:*

pH 4.5 Acetate Buffer and Acetonitrile taken in ratio 50: 50 % v/v and mixed well.

➢ *Mobile Phase Preparation:*

pH 4.5 Acetate Buffer, Methanol, Acetonitrile taken in ratio 60: 30: 10 % v/v/v and mixed well.

➢ *Standard Solution Preparation:*

Weighed and transferred 27.5 mg of Rilpivirine Hydrochloride API equivalent 25 mg of Rilpivirine into 100 mL volumetric flask, 30 mL of diluent was added, sonicated for 5 minutes in sonicator. Volume was made up with the diluent and mixed thoroughly. 5 mL of the above solution was pipetted into 50 mL volumetric flask and volume was made up with diluent and mixed thoroughly. (Concentration of Rilpivirine was 25 ppm).

➢ *Sample Solution Preparation:*

One EDURANT Tablet which contained 25 mg of Rilpivirine was added to 100 mL volumetric flask, 30 mL of diluent was added, sonicated for 20 minutes in sonicator. Volume was made up with the diluent and mixed thoroughly. 5 mL of the above solution was pipetted into 50 mL volumetric flask and volume was made up with diluent and mixed thoroughly. (Concentration of Rilpivirine was 25 ppm).

➢ *Validation:*

Method Validation was performed by analysing validation parameters (i.e. System Suitability, Specificity, Linearity, Accuracy, Precision, Robustness) as per ICH guidelines and ensuring that the method has met acceptance criteria as given by ICH Q2 guidelines for all the validation parameters.

III. RESULTS AND DISCUSSION

➢ *System Suitability:*

The tailing factor was 1.0, Plate count was 6050 and % RSD for peak areas was 0.5 for five replicate injections of Rilpivirine Standard. From the results it was concluded that system was meeting the acceptance criteria for System Suitability.

Table 1 Results of System Suitability of Rilpivirine

Parameter	Observed value
Tailing factor	1.0
Plate count	6050
%RSD (peak areas)	0.5

➢ *Specificity:*

At the retention time of Rilpivirine no interference from impurity, blank and placebo peaks was detected thus specificity of the method was confirmed. (Refer Figures 2, 3, 4, 5).

Table 2 Results of Specificity of Rilpivirine

Sample Name	Inference
Blank	No interference of Blank peaks observed at retention time of Rilpivirine Peak
Placebo	No interference of Placebo peaks observed at retention time of Rilpivirine Peak
Impurities	No interference of Impurities peaks observed at retention time of Rilpivirine Peak

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24SEP033

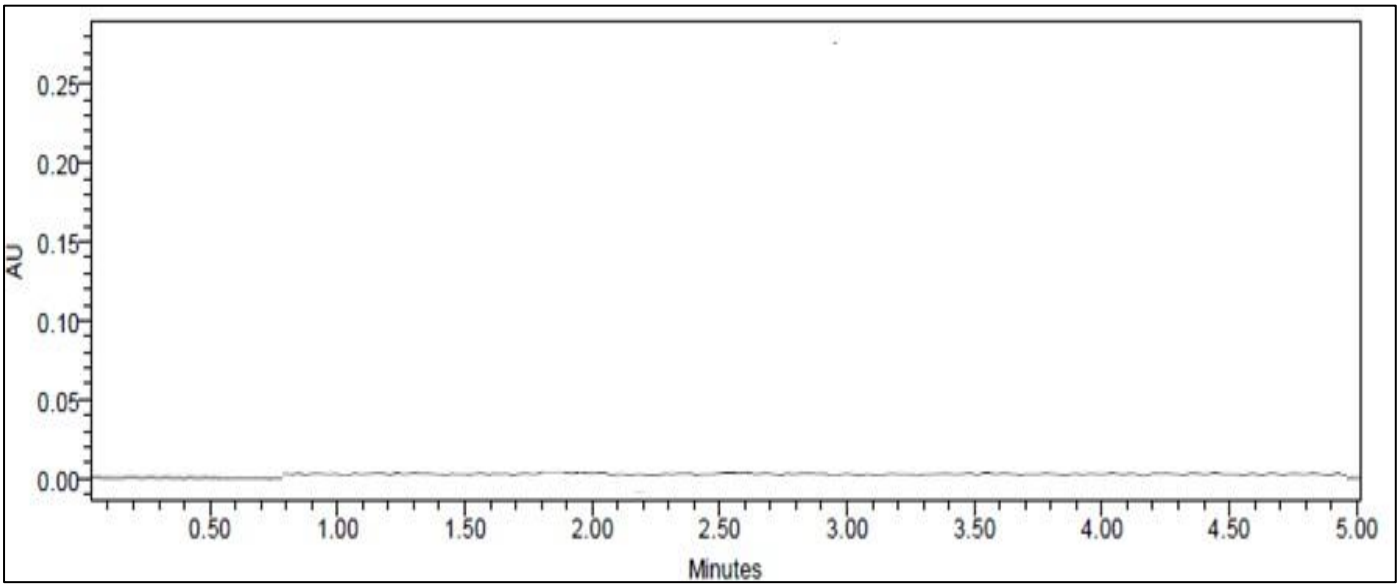

Fig 2 Chromatogram of Blank

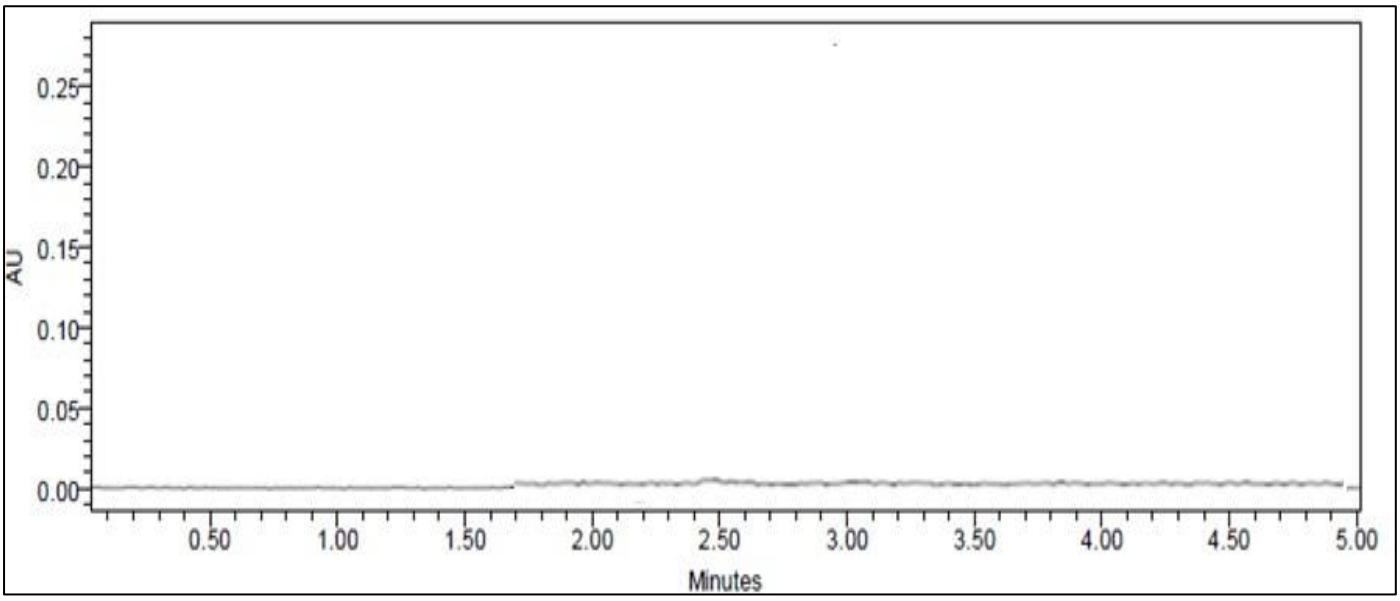

Fig 3 Chromatogram of Placebo

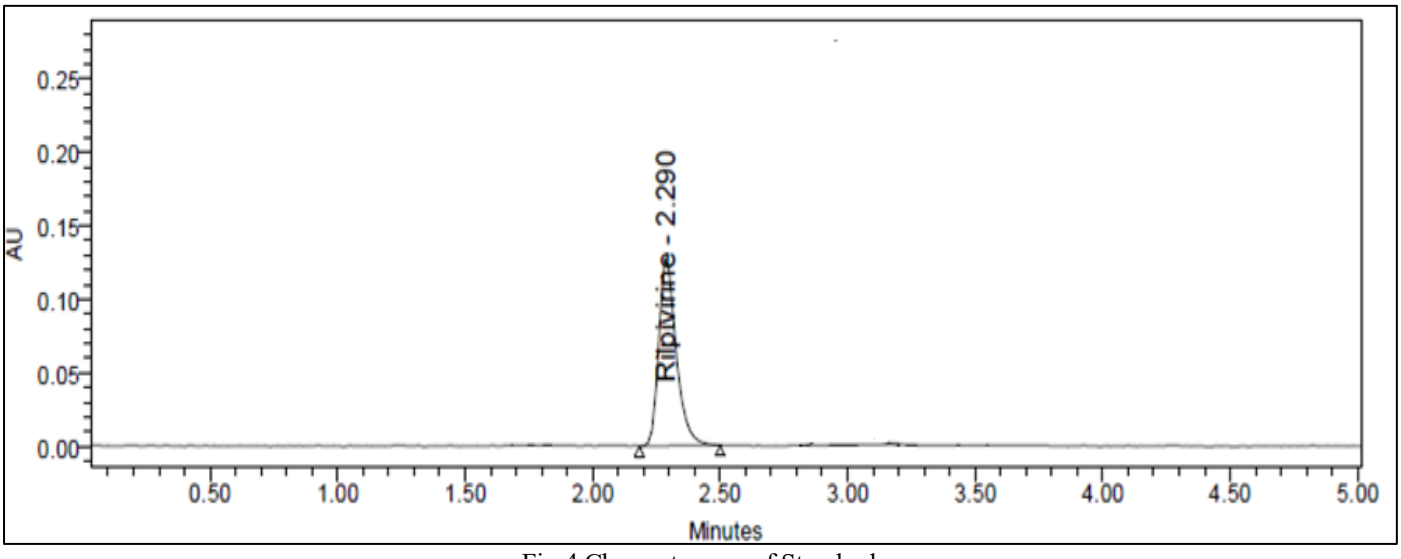

Fig 4 Chromatogram of Standard

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24SEP033

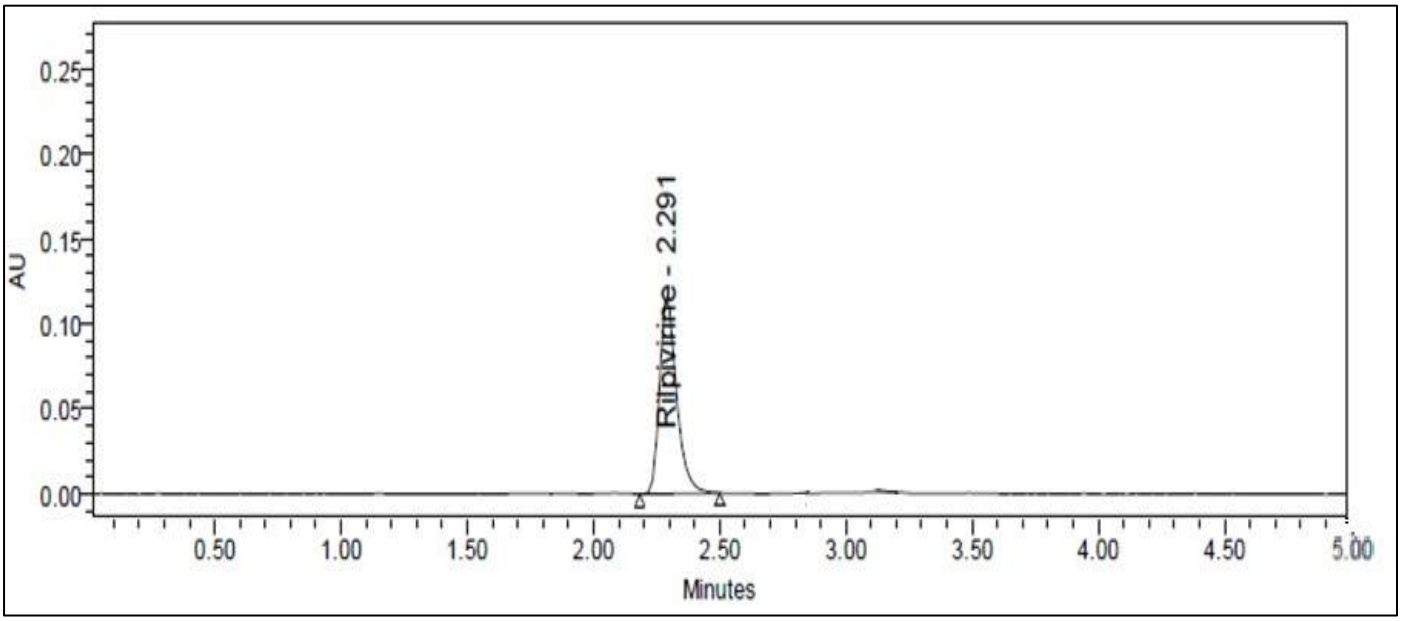

Fig 5 Chromatogram of Sample

➢ *Linearity:*

The method expressed desired linearity over the concentration level of 25 % to 200 % with a Correlation Coefficient (R^2) 1.000.

Table 3 Results of Linearity of Rilpivirine

Concentration (µg/mL) of Rilpiverine	**Peak Areas of Rilpiverine**
6.253	75693
12.521	152060
25.123	302999
37.549	453000
50.110	604362
Square of correlation coefficient (R^2) of Rilpiverine	**1.000**
% Y-intercept at 100% response of Rilpiverine	**0.1**

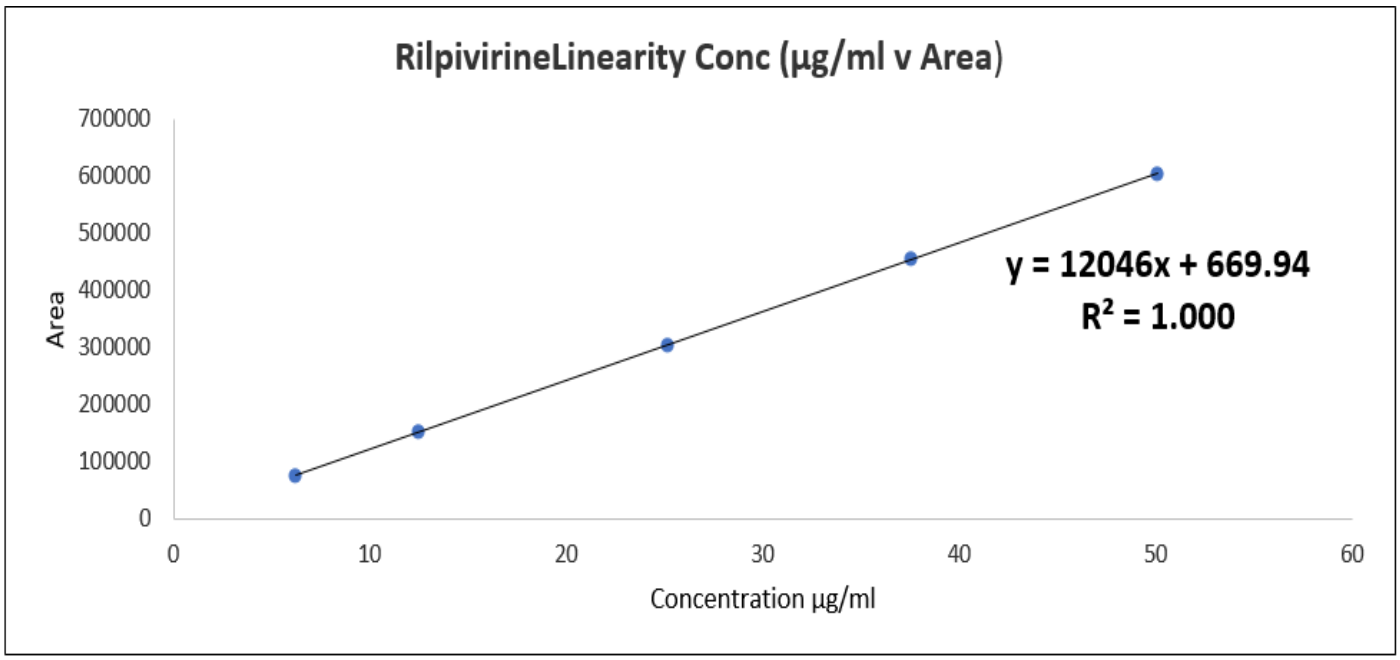

Fig 6 Linearity Graph of Rilpiverine

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24SEP033

➢ *Accuracy:*

The Recovery results ranged from 98.0 % to 102.0 % and % RSD values did not exceed 2.0 over the three concentration levels confirming the Accuracy of method.

Table 4 Results of Accuracy of Rilpivirine

% Level	Rilpivirine Added (mg)	Rilpivirine Found (mg)	% Recovered	Mean % Recovered	% RSD
25	6.27	6.26	99.8	99.9	0.4
	6.28	6.30	100.3		
	6.24	6.21	99.5		
100	25.30	25.26	99.8	100.0	0.2
	25.51	25.46	99.8		
	25.21	25.27	100.2		
200	50.31	50.45	100.3	99.9	0.3
	50.12	50.01	99.8		
	50.20	50.03	99.7		

➢ *Precision:*

The % RSD of Rilpivirine six samples was found to be 1.1 and Mean % Assay was 99 confirming the precision of method.

Table 5 Results of Method Precision of Rilpivirine

Sample Number	% Assay of Rilpivirine
1	99.1
2	98.6
3	99.0
4	98.5
5	100.3
6	101.2
Mean	**99**
% RSD	**1.1**

➢ *Robustness:*

The system suitability parameters remained unaffected by the small changes that were made in chromatographic conditions of the method thus confirming the Robustness of the method.

IV. CONCLUSION

Pharmaceutical analysis has a critical role in ensuring quality of drugs, thus ensuring safety of patients. Well developed and validated methods are important for testing of medicines to ensure their efficacy and safety. The current method "Method Development and Validation of Assay of Rilpivirine in Rilpivirine Tablets by RP-HPLC" was developed with high quality standards and rightly validated as per ICH Q2 guidelines. The method was found to be specific, linear with a square of correlation coefficient (R^2) 1.000, accurate over the concentration range of 25 % to 200 %, precise with % RSD of six samples 1.1 and robust. The method was simple and run time was less, hence the method was quick can be utilized in industries for Assay of Rilpivirine in Rilpivirine Tablets.

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 Researc*h 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.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24SEP033

[5]. Reddy BS, Harish G, Md. Ul-Haq F. Formulation and in-vitro Characterisation of Solid - Self Nanoemulsifying Drug Delivery System (SSNEDDS) of Rilpivirine. *Int J Pharm Sci Res* 2016, 7 (7), 3117-29.doi: 10.13040/IJPSR.0975-8232.7(7).3117-29.

[6]. Reddiah. V C, Ramadevi. P, Mukkanti. K. Effective Estimation Of Rilpivirine By HPLC Method In Tablet Dosage Forms And Its In-vitro Dissolution Assessment. *International Journal of Pharmacy and Pharmaceutical Science*. 2012, 4 (3), 595-599.

[7]. Putcharoen O, Kerr SJ, Ruxrungtham K. An update on clinical utility of rilpivirine in the management of HIV infection in treatment-naive patients. HIV AIDS Auckl. 2013, 16 (5), 231-41. doi: 10.2147/HIV.S25712.

[8]. Burugula L, Pilli NR, Makula A, Lodagala DS, Kandhagatla R. Liquid chromatography-tandem mass spectrometric assay for the non-nucleoside reverse transcriptase inhibitor Rilpivirine in human plasma. *Biomed. Chromatogr*. (2013), 27 (2), 172–178. DOI: 10.1002/bmc.276.

[9]. S. Venkatesan, N. Kannappan, Sai Sandeep Mannemala. Stability-Indicating HPLC Method for the Simultaneous Determination of HIV Tablet Containing Emtricitabine, Tenofovir Disoproxil Fumarate, and Rilpivirine Hydrochloride in Pharmaceutical Dosage Forms. *Int Sch Res Notices.* 2014:849149. doi: 10.1155/2014/849149.

[10]. ICH Validation of Analytical Procedures: Text and Methodology Q2 (R1). International Conference on Harmonisation.