EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 8
Issue: 10
286-292
Year: 2021

DEVELOPMENT AND VALIDATION OF LIQUID CHROMATOGRAPHIC METHOD FOR ESTIMATION OF RILPIVIRINE AND ITS APPLICATION IN SOLUBILITY ENHANCEMENT STUDIES

Kanchan Chauhan*[1] and Saloni Abdul Shikur Shaikh[2]

[1]Department of Pharmaceutical Chemistry, [2]Department of Quality Assurance,
M.C. E. Society's Allana College of Pharmacy, Pune-411001 Maharashtra, India.

***Corresponding Author: Kanchan Chauhan**
Department of Pharmaceutical Chemistry, M.C. E. Society's Allana College of Pharmacy, Pune-411001 Maharashtra, India.

Article Received on 10/08/2021 **Article Revised on 31/08/2021** **Article Accepted on 21/09/2021**

ABSTRACT
Aim of the present study is to develop a precise, accurate and linear RP-HPLC method for the assessment of rilpivirine. The chromatographic system employs Inertsil C18 column using mobile phase Acetonitrile: phosphate buffer in the ratio of 80:20 v/v, at a flow rate of 1 ml/min with the detection wavelength of 280 nm. The retention time of rilpivirine was found to be 4.16 minutes. Rilpivirine exhibited a linear relationship (r^2 > 0.9997) over the analytical range of 10–50 µg/ml. The percentage recovery was observed in the range of 99.00% to 99.96%, indicating the accuracy of the study. The detection limit and quantification limit were found to be 1.52 µg/ml and 4.61 µg/ml respectively stating method is sensitive. Relative standard deviation of the test results of the selected parameters at different condition was calculated and found within the ICH limit indicating that the method is sufficiently robust. Further the extension of the developed and validated method was successfully carried out for identification and quantification of rilpivirine solid dispersion.

KEYWORDS: Rilpivirine, RP-HPLC, Method Development and Validation.

INTRODUCTION
Rilpivirine, a second-generation non-nucleoside reverse transcriptase inhibitor (NNRTI) is commonly used for the treatment of HIV infection in humans. Rilpivirine, chemically denoted as 4-{[4-({4-[(1E)- 2-Cyanoethenyl]-2, 6 dimethylphenyl} amino)-2 pyrimidinyl] amino} benzonitrile with a molecular formula of $C_{22}H_{18}N_6$.[1] It is mainly used to treat HIV-1 infection in combination with other antiretroviral agents.[2] (Fig. 1) Literature survey revealed that there are several methods reported for estimation of rilpivirine alone or in combination with other NNRTIs.[3-9] The present study aims to develop and validate a simple, rapid, precise, accurate and sensitive RP-HPLC method which is compatible for LC-UV and can be routinely used for quality control studies. UV spectrophotometric methods are commonly used methods for the estimation of drug concentrations during solubility enhancement studies. HPLC methods are more sensitive than spectrophotmetric methods. The present study focuses on development of a simple liquid chromatographic method for the estimation of rilpivirine in bulk and its dosage form. Further this method is used for determination and quantification of rilpivirine solid dispersion.

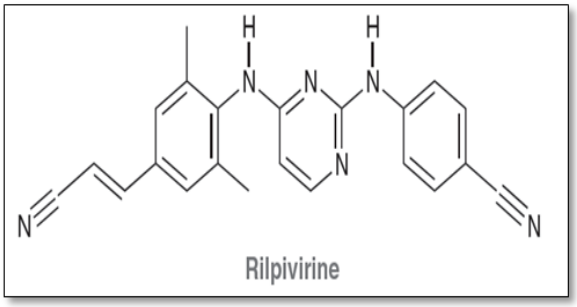

Fig.1: Structure of Rilpivirine.

MATERIALS AND METHODS
Chemicals and reagents
Rilpivirine was obtained from Emcure pharmaceuticals, Pune India. HCl, Acetonitrile was procured from Merck Specialites Pvt Ltd, Mumbai, India and Citric Acid Monohydrate, disodium hydrogen phosphate from Sigma Aldrich, Mumbai, India. All solvents used were of HPLC grade.

Instrument
HPLC Jasco Model/PU 2080/UV2075 PLUS with Borwin software for data collection and analysis was used. C18 (5µm, 150 x 4.6 mm i.d.) inertsil column was

used for separation of analyte. Ultra Sonicator Model Enertech Fast Clean was used to dissolve the drug completely. Microwave oven (LG solo intellowave technology) was used for preparation of solid dispersion.

Preparation of Standard and Sample Solution
Standard Solution preparation was done by taking 10 mg of rilpivirine drug was transferred into 10 ml 0.1N HCl in volumetric flask and sonicated for 30 minutes followed by filtration through 0.45 membrane filter. 1 ml solution was withdrawn from this solution and diluted to 10 ml with mobile phase to form 100 µg/ml. Similarly, for sample preparation 20 tablets were weighed, the average weight of each tablet was calculated and the quantity equivalent to 10 mg of rilpivirine was transferred to 10 ml of 0.1N HCl. The solution was sonicated for 30 minutes and filtered through 0.45µ filter paper. Further dilutions were made using mobile phase.

Selection of wavelength
Detection wavelength was selected after scanning UV spectrum of the rilpivirine solution over the range of 200 to 400 nm.

Development and optimization of method
HPLC method was optimized by injecting drug in different solvent systems. Initially methanol and water and acetonitrile and water in different ratios were tried. Further to improve the peak symmetry the drug was injected in mobile phase consisting of phosphate buffer and acetate buffer in different ratios. The presence of phosphate buffer in mobile phase resulted in excellent overall chromatography with appropriate peak symmetry and complete base line resolution. Finally the mobile phase consisted of Acetonitrile : phosphate buffer (80:20 v/v) with pH 5.2 was selected for analysis. It was filtered through 0.45µ nylon membrane filter paper, sonicated and was pumped from solvent reservoir to the column at a flow rate of 1.0 ml/min with a run time of 10 min. The separation was performed on C18 column with injection volume of 20 µl.

System suitability parameter
The chromatogram of standard drug rilpivirine was recorded in HPLC system. The system suitability parameters of the method were evaluated in terms of retention time, peak area, tailing factor, resolution and theoretical plate.

Method validation[10-11]
The developed RP-HPLC method was validated in terms of the parameters such as linearity, sensitivity, precision and accuracy of solutions. The validation was carried out according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures.

Specificity
The specificity of the developed method was established by analyzing the sample solutions containing drug rilpivirine in pharmaceutical formulation and observed for any unresolved excipient peaks as well as for any coelution with analyte peaks.

Linearity
To study the linearity of analytical procedure, five working standard solutions at different concentration levels (10-50 µg/ml) were injected into HPLC system in triplicates. Calibration curve was constructed by plotting concentration of rilpivirine on X-axis and average peak area on Y-axis. From the calibration curve, regression equation and correlation coefficient was calculated.

Method Sensitivity
The sensitivity of the analytical method was determined in terms of limit of detection (LOD) and limit of quantification (LOQ). LOD and LOQ were defined based on signal to noise ratio of 3:1 and 10:1, respectively.

Precision and accuracy
For precision study samples at three different concentration levels were analyzed during three consecutive days (inter-day precision) and three times during the same day (intra-day precision). The precision of proposed method was obtained by calculating the relative standard deviation (RSD) values for intra-day and inter-day analysis with acceptance criteria of not more than 2%.

The recovery studies were performed by spiking three different known quantity of pure standard drug into the sample solution. The sample was spiked with standard at levels 80%, 100% and 120% of test concentration. The resulting spiked sample solutions were assayed in triplicate and the results were compared with the expected results; % RSD (relative standard deviation) and the mean recovery were calculated.

Robustness
Robustness of the proposed method was evaluated by deliberate alterations of the analytical parameters. The analytical parameters such as change in wavelength, composition of organic solvent, columns from different manufacturer was studied and the response was recorded and the % RSD was calculated.

Assay of Rilpivirine tablet
Twenty tablets were weighed and triturated into fine powder and their average weight was calculated. Weight equivalent to10 mg of drug was transferred to 10 ml volumetric flask containing 0.1N HCl and the solution was sonicated for 15 minutes, volume was made up to 10 ml with same solvent. Further 1 ml of the above solution was diluted to 10 ml with buffer solution. The solution was filtered through a membrane filter (0.45 µ). From this stock solution 20 µg/ml solution was prepared and was injected in three replicates into the HPLC system.

Preparation of rilpivirine solid dispersion (SD)

Solid dispersions of rilpivirine were prepared by microwave irradiation method. Required quantity of the drug and polymer in 1:3 ratio was weighed and was taken in petry dish. In this process, the drug along with the polymer is kept in the microwave for 7 minutes at 360 Watt. Proper mixing of the mixture was carried out after every 30 seconds. Only one beaker at a time was kept inside the microwave oven. The beaker was removed from the oven after 7 minutes and the sample was cooled at room temperature. The product was sieved via sieve no. 44 and stored in desiccators.

Analysis of rilpivirine solid dispersion by HPLC[12-14]

Sample solution was prepared in triplicate by transferring drug equivalent to 10 mg of rilpivirine solid dispersion in 10 ml 0.1N HCl in volumetric flask and sonicated for 30 mins followed by filtration through 0.45 membrane filter. Further dilutions were made with mobile phase to form 100 µg/ml and 10 µg/ml. Prepared solutions were injected into HPLC system and were analyzed as per the developed method. From the chromatograms, peak areas were recorded and % purity was calculated.

RESULTS AND DISCUSSION

Selection of Wavelength

The drug rilpivirine has good absorption characteristics in UV region and the absorption maximum was found to be 280 nm. This wavelength was selected as a detection wavelength in HPLC- UV detector for the determination of drug. The UV spectrum of rilpivirine is shown in Fig.2.

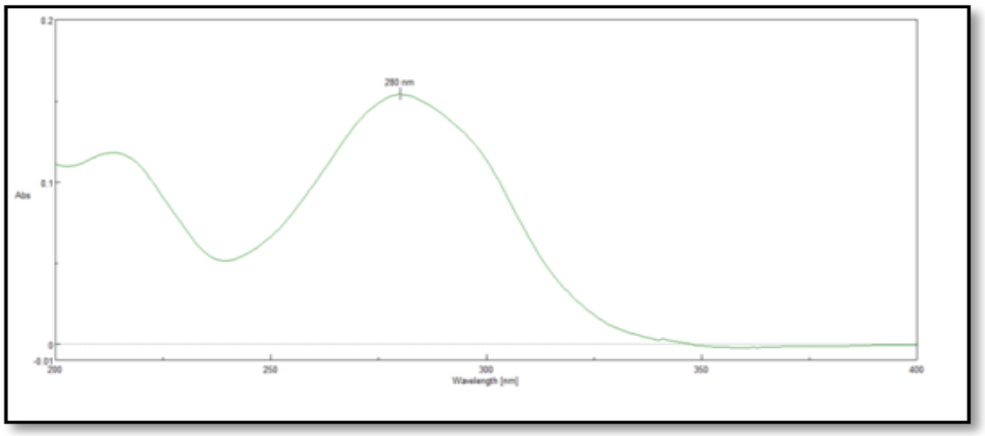

Fig.2: UV Spectrum of rilpivirine in 0.1N HCl.

Method Development and Optimization

The mobile phase Acetonitrile: phosphate Buffer (80:20 v/v) with pH 5.2 was selected for analysis. At this pH peak shape, peak tailing and theoretical plate count were found to be satisfactory. The chromatographic conditions are shown in Table 1 and Optimized Chromatogram is shown in Fig.3. The system suitability parameters were recorded and are presented in Table 2.

Table 1: Optimized Chromatographic Conditions.

Parameters	Chromatographic Conditions
Column	Inertsil, C18, (5µm, 150 x 4.6mm i.d.)
Column Temperature	Ambient
Mobile Phase	Acetonitrile : Phosphate Buffer (80:20)
Mobile Phase pH	5.2
Wavelength	280 nm
Flow Rate	1ml/min
Run Time	10 min
Injection Volume	20 µl
Retention Time	4.160

Table 2: System suitability parameters.

Name of drug	Rilpivirine
Retention Time (t_R)	4.160
Area (A)	2481167.887
Theoretical plates (N)	8428.371
Asymmetry	1.249

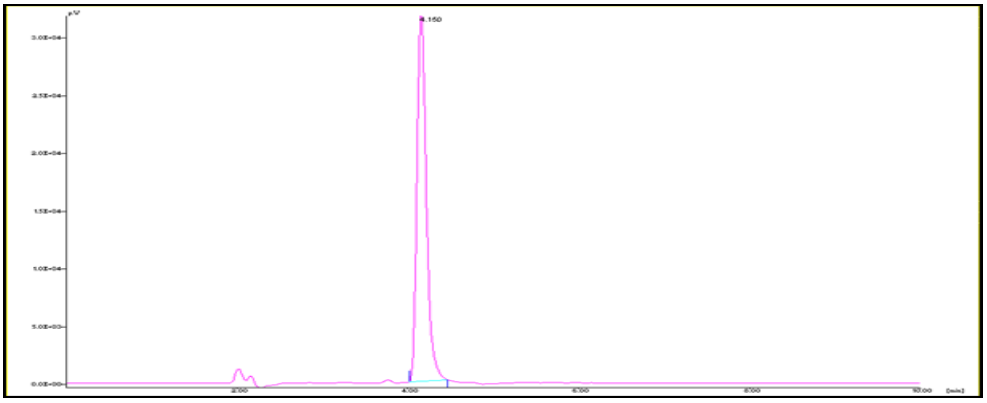

Fig.3: Optimized Chromatogram of rilpivirine.

METHOD VALIDATION

Linearity and Method Sensitivity

Calibration curve was obtained for rilpivirine from which linear regression equation was computed and a correlation coefficient was obtained. The results were found to be linear in the range of 10 to 50µg/ml. The correlation coefficients values were found to be 0.9997. The LOD and LOQ values were found to be 1.52µg/ml and 4.61 µg/ml respectively. The results are presented in Table 3. Calibration curve and overlay of peaks are shown in Fig.4 and Fig.5 respectively.

Table 3: Linearity data of rilpivirine.

Concentration (µg/ml)	Peak Area
10	837736
20	1646297
30	2581167
40	3437373
50	4267159

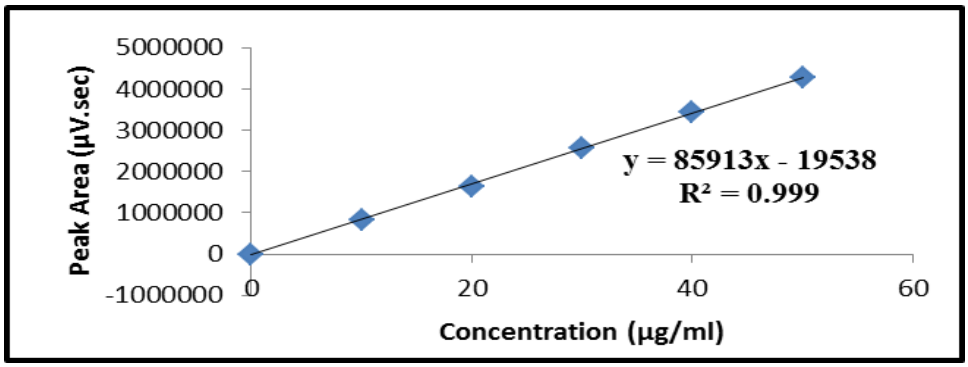

Fig.4: Calibration curve of rilpivirine.

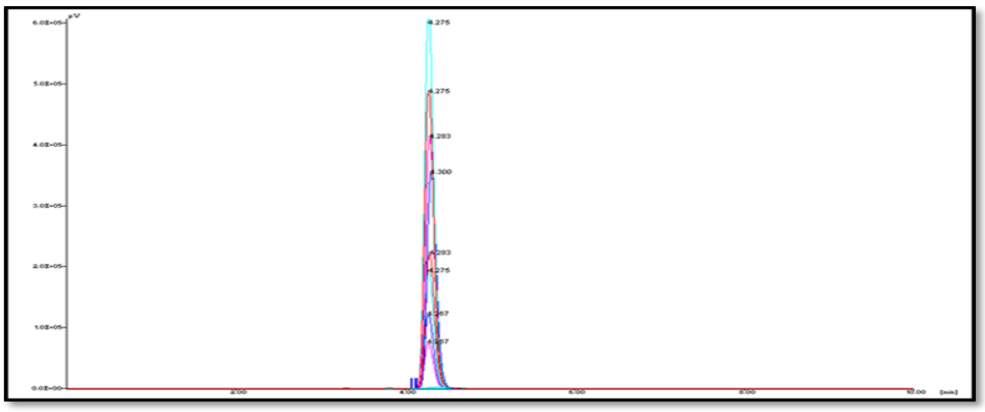

Fig.5: Overlay chromatogram of rilpivirine.

Precision

Precision of the method was determined by repeatability and reproducibility. It was expressed in terms of relative standard deviation (% RSD) of the peak area. % RSD of repeatability study was found to be in the range of 0.005% to 0.01% and for reproducibility study shows 0.005 % to 0.016 %. The results are shown in Table 4 and 5.

Table 4: Intraday Precision (Repeatability)

Conc. (µg/ml)	Peak Area				Std Deviation	% RSD
	I	II	III	Mean		
20	1646297.7	1646139.7	1645783.5	1646073.6	263.4289191	0.016003471
30	2581167.8	2581427.0	2581246.8	2581280.5	132.8038902	0.005144884
40	3437373.5	3436562.1	3436719.4	3436885.0	430.2614682	0.012518937

Table 5: Interday Precision (Reproducibility).

Conc. (µg/ml)	Peak Area				Std Deviation	% RSD
	I	II	III	Mean		
20	1646073.6	1646347.7	1646017.0	1646146.1	176.882394	0.01074524
30	2581280.5	2581199.5	2581719.7	2581399.9	279.909640	0.01084332
40	3436885.0	3436642.1	3435879.0	3436468.7	524.948646	0.01527581

Accuracy

The recovery studies were carried out at 80 %, 100 % and 120 % of the test concentration as per ICH guidelines. The % recovery was calculated and the values of % RSD was found to be less than 1 which proves the proposed method is accurate. The result is shown in Table 6.

Table 6: Recovery studies.

Level (%)	Base Level Conc. (µg/ml)	Spiked Conc. (µg/ml)	Total Conc. (µg/ml)	Obs. Conc. (µg/ml)	% Recovery
80	30	24	54	53.98	99.96
100	30	30	60	59.94	99.0
120	30	36	66	65.97	99.95

Assay of rilpivirine tablet

The proposed method was applied for the determination of rilpivrine in tablet dosage form. The result of these assay yielded 99.87 % of label claim of the tablets. The assay results of rilpivirine HCl indicate that the method is selective without interference of the excipients used in these tablets. The result is shown in Table 7.

Table 7: Assay of rilpivirine (n=3).

Conc. (µg/ml)	Avg.Area	% Assay	% RSD
20	1735186.66	99.87	0.54

Robustness

The study results indicated that areas of peaks of interest and retention time remained unaffected by small changes of the operational parameters. % RSD of the test results of the selected parameters at different condition was calculated and found within the ICH limit indicating that the method is robust to analyze rilpivirine in pharmaceutical dosage form. The result of robustness study is shown in Table 8.

Table 8: Robustness study.

Parameters	RT (min)	Peak Area	% Assay	% RSD
Flow Rate (±1ml)	4.35	2489693.4	97.33	1.990
	4.54	2499318.7	97.7	
	4.3	2581167.8	100.9	
Wavlength (±1nm)	4.26	2499562.7	97.5	1.735
	4.3	2581167.8	100.9	
	4.28	2526768.2	98.76	
Column; Inertsil	4.28	2581167.8	100.9	0.161
Thermosil	4.3	2587905.7	101.13	
Composition of Organic Solvent (±1ml)	4.29	2570056.7	98.4	1.715
	4.3	2581167.8	100.9	
	4.3	2550912.5	97.66	

Quantitative analysis of solid dispersions

Analysis of solid dispersions was carried out by developed RP-HPLC method. The assay data is given in Table 9 and chromatogram of assay is shown in Fig 6.

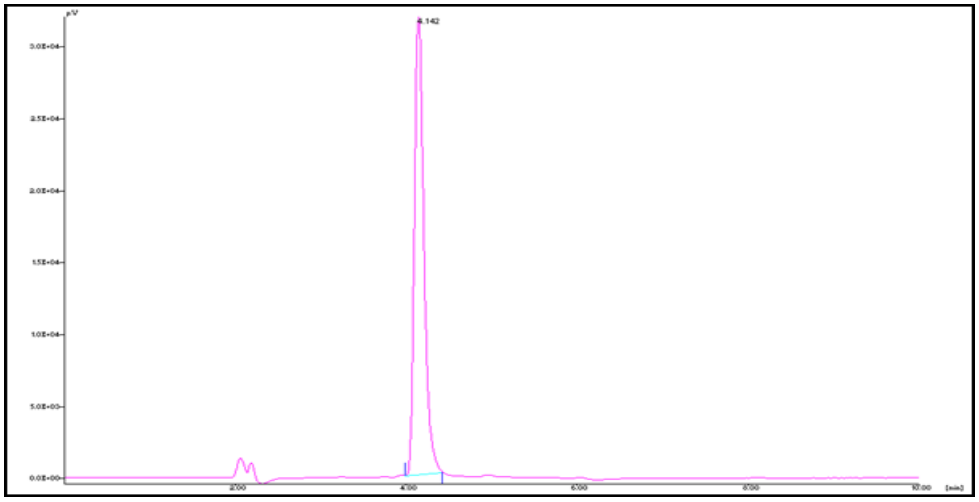

Fig. 6: HPLC Spectra of rilpivirine solid dispersion.

Table 9: Assay of rilpivirine Solid dispersion (1:3) by HPLC.

Sr. No	Rilpivirine		
	Peak Area	% Assay	% RSD
1	169551.23	99.12	0.982
2	174551.58	98.85	
3	171641.71	99.05	

CONCLUSION

A precise, linear, accurate, rapid and sensitive RP-HPLC method has been developed and validated for rilpivirine as per ICH guidelines. The validated method can be used for routine analysis of rilpivirine in various pharmaceutical industries. Poorly water soluble drug rilpivirine is having the problem of less bioavailability. Solid dispersion technique adopted in this investigation has been found to increase the dissolution rate of drug rilpivirine which shows improved solubility of rilpivirine. For monitoring the enhancement in solubility, most of the solubility enhancement studies were done by UV method. In this study the developed HPLC method was further used for estimation of prepared rilpivirine solid dispersion and the peak area was calculated. Thus it can be concluded that the proposed method can be used for estimation of rilpivirine solid dispersion.

ACKNOWLEDEMENT

Authors are thankful to Emcure pharmaceuticals, Pune India for providing gift sample. Authors are also thankful to the Management and Principal of M.C.E. Society's Allana College of Pharmacy, for providing the facility and infrastructure for carrying out the research work.

REFERENCES

1. DHHS Panel on Antiretroviral Guidelines for Adults and Adolescents. Recommendations for NNRTI use in Antiretroviral Treatment-Naïve Patients with HIV-1 Infection. Supplemental Information Regarding the Role of Rilpivirine (RPV) as Initial Therapy- August 16, 2011. Accessed on line on October 1, 2011.
2. Patel S, Nagappan K, Reddy GS. A new quantitative reverse phase high-performance liquid chromatographic method for the quantification of Rilpivirine hydrochloride in bulk and dosage forms. J App Pharm Sci, 2018; 8(11): 157–162.
3. Joseph J, Hepsebah NJR and Deepthi K: Analytical method development and validation for the simultaneous estimation of dolutegravir and rilpivirine using RP-HPLC method in both Bulk and Pharmaceutical Dosage Form. European Journal of Biomedical and Pharmaceutical Sciences, 2016; 22-27.
4. Nagaraju P, Fatima SKS, Mounika V, Priyadarshini GI and Naik V: Stability-indicating method development and validation of RP-HPLC method for simultaneous estimation of Lamivudine, Abacavir, Dolutegravir in Pharmaceutical Dosage Forms. Indo American Journal of Pharmaceutical Sciences, 2017; 4(02): 359-67.
5. Ghosh S, Sowjanya B, Prasanna VL, Vidyadhar S and David B: Method Development and Validation of Rilpivirine in Bulk and Tablet dosage form by RP-HPLC method. Research Journal Pharmacy and Technology, 2013; 6(3): 240-43.
6. Kavitha KY, Geetha G, Hariprasad R and Subramaniam G: Development and validation of RP-HPLC method for simultaneous estimation of Emitricitabine, Rilpivirine, Tenofovir Disproxil Fumarate and its pharmaceutical dosage forms. International Journal of Comprehensive Pharmacy, 2012; 4(1): 1-5
7. Nitin C, Valeria C, Sara B, Simone C, Chiara A, Dario C and Emilio C: Development and validation of a Chromatographic UV method for the simultaneous quantification of Dolutegravir and Rilpivirine in human plasma. Article in Therapeutic drug monitoring, 2016; 212- 16
8. Damle MC, Amita N and Pardeshi: Development and validation of stability indicating HPTLC method for Rilpivirine and Dolutegravir sodium in combination. Indo American J of Pharmaceutical Res, 2017; 7(5): 8830-37

9. Raju AV and Raju NA: Development and validation of a LC-MS/MS method for the determination of Rilpivirine in Sprague Dawley Rat serum and its application to pharmacokinetic study. Asian Journal of Biomedical and Pharmaceutical Sciences, 2013; 3(21): 23-29.
10. ICH Guidelines for Stability Testing of New Drug Substances and Products Q1A (R2), 2004; 1-24. 18.
11. ICH Guidelines for Validation of Analytical Procedures: Text and Methodology Q2 (R1), 2005; 1-17
12. Chaudhary et al. Enhancement of solubility and bioavailability of poorly soluble drugs by physical and chemical modifications: A recent review. Journal of advanced Pharmacy Education and research, 2012; 2(1): 32-67.
13. Serajuddin A. Solid dispersion of poorly water soluble drugs early promises, subsequent problems, and recent breakthroughs J Pharm Sci, 1999; 88(10): 1058–66.
14. Kumar SD, Bihari GV, Purohit S. Solubility improvement using solid dispersion; Strategy, mechanism and Characterization responsiveness and Prospect way outs. Int Res J Pharm, 2011; 1: 50-66.