IJPSR (2020), Volume 11, Issue 10 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 23 July 2019; received in revised form, 10 September 2020; accepted, 19 September 2020; published 01 October 2020

VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF RILPIVIRINE AND DOLUTEGRAVIR IN BULK FORM

K. Srinivas Reddy [*1], S. Sai Shirisha [2] and K. Praveen Kumar [2]

Pharmacognosy & Phytochemistry [1], Department of Pharmaceutical Analysis [2], Vaagdevi College of Pharmacy, Ramnagar, Hanamkonda, Warangal - 506001, Telangana, India.

Keywords:

Rilpivirine, Dolutegravir, RP-HPLC, Simultaneous Estimation, Stability studies, ICH Guidelines

Correspondence to Author:
K. Srinivas Reddy

Head of Department, Pharmacognosy & Phytochemistry, Vaagdevi College of Pharmacy, Ramnagar, Hanamkonda, Warangal - 506001, Telangana, India.

E-mail: seenukaruka@gmail.com

ABSTRACT: New simple, sensitive, and validated stability-indicating RP-HPLC method has been developed for the simultaneous estimation of Rilpivirine and Dolutegravir in its bulk form. Chromatographic separation was achieved on a Hypersil ODS (250mm × 4.6mm i.d., 5µm) maintained at ambient temperature by a mobile phase consisted of methanol and water (80:20v/v) and a flow rate of 1.0mL/min with a load of 20µL. The detection wavelength was set at 282nm. The retention time for the drugs was found to be Rilpivirine (5.14min), Dolutegravir (6.72min). The eluted compounds were detected using a UV detector. The drugs were subjected to stress degradation as per ICH Q1A. There was the interference of degradant at RT of Rilpivirine and Dolutegravir. The developed method was successfully validated according to ICH guidelines. The calibration curve was found to be linear over a range of 10-100µg/mL. The accuracy of the method is indicated by a good recovery in the range of Rilpivirine 99-102% and of Dolutegravir 99-102%. The limit of detection and limit of quantification of Rilpivirine was found to be LOD-0.844µg/mL and LOQ-2.557µg/mL, and for Dolutegravir was found to be LOD-0.082µg/mL and LOQ-0.249µg/mL.

INTRODUCTION: HIV [1, 2] stands for Human Immunodeficiency Virus. It is the virus that can lead to Acquired Immunodeficiency Syndrome, or AIDS, if not treated. Unlike some other viruses, the human body can't get rid of HIV completely, even with treatment. HIV is a ribonucleic acid virus spread through certain body fluids that attacks the body's immune system, specifically destroys a type of defense cells in the body called CD4 helper lymphocytes (T cells), which help the immune system fight off infections. Untreated, HIV reduces the number of CD4 helper lymphocytes (T cells) in the body, making the person more likely to get other infections or infection-related cancers.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(10).4991-97

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(10).4991-97

Dolutegravir [3, 4] chemically, (3S, 7R)-N-[(2,4-Difluorophenyl)methyl]- 11-hydroxy- 7-methyl-9,12-dioxo-4-oxa-1, 8-diazatricyclo]tetradeca-10, 13-diene- 13- carboxamide, is a novel integrase inhibitor used in the treatment of HIV and was approved by FDA. It works by blocking integrase and prevents HIV from Replicating and lowers the amount of HIV in the blood.

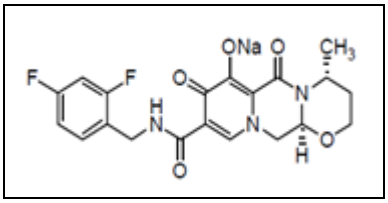

FIG. 1: STRUCTURE OF DOLUTEGRAVIR

Rilpivirine is chemically known as 4-[[4-[4-[(E)-2-Cyanoethenyl]-2, 6-dimethylanilino] pyrimidin- 2-yl] amino] benzonitrile was shown in **Fig. 2**. It is the second generation of non-nucleoside reverse transcriptase inhibitors (NNRTIS) recently marketed for the treatment of HIV infection. It is a diaryl-

pyrimidine, a class of molecules that resembles pyrimidine nucleotides found in DNA. Because of its flexible chemical structure, resistance to Rilpivirine is less likely to develop than other NNRTI's. The Literature review revealed that the methods for Rilpivirine and Dolutegravir by simultaneous estimation with stability studies by RP-HPLC [5-11], Spectrophotometer [12-14], UPLC [15] and HPTLC [16, 17], LC-MS/MS [18] are the reported analytical methods for compounds either individually or in combination with other dosage forms with the usage of Buffers.

The present work is aimed to develop and validate a method without using Buffers and to perform Stability studies in combined form. A combination of Dolutegravir and Rilpivirine can be a very promising two-drug regimen for HIV patients. Hence stability-indicating method has been developed for this combination as per ICH Q1A R2 [19, 20] guidelines, and the method was validated.

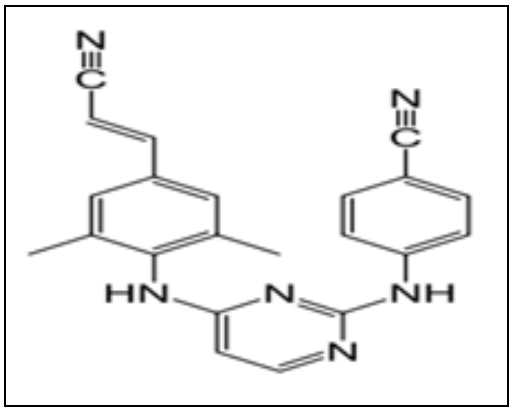

FIG. 2: STRUCTURE OF RILPIVIRIN

MATERIALS AND METHODS:

TABLE 1: LIST OF STANDARDS USED

S. no.	Name	Supplier
1	Dolutegravir	Gift sample from Pharma Train Lab, Hyderabad, Telangana.
2	Rilpivirine	Gift sample from Pharma Train Lab, Hyderabad, Telangana.

TABLE 2: LIST OF CHEMICALS USED

S. no.	Name	Grade	Supplier
1	Methanol	HPLC	Fine Chem Industries
2	Water	HPLC	Avantor Performance Materials India Limited
3	Hydrochloric acid	LR	Fine Chem Industries
4	Hydrogen peroxide	LR	Fine Chem Industries
5	Sodium hydroxide	LR	Fine Chem Industries

TABLE 3: LIST OF INSTRUMENTS USED

S. no.	Instrument	Model	Make
1	HPLC	SPD-20A	SHIMADZU
2	UV-VIS spectrophotometer	UV-1800	SHIMADZU
3	Analytical balance	AUY220	SHIMADZU
4	pH meter	MK V1	DIGITAL
5	Bath Ultra Sonicator	1.5L50	ULTRASONICS

Selection of Analytical Wavelength: 10µg/mL individual solutions of Rilpivirine and Dolutegravir was prepared separately and were scanned in the range of 200-400nm to determine the absorption maximum for the drugs for which the overlay spectra were shown in **Fig. 3**.

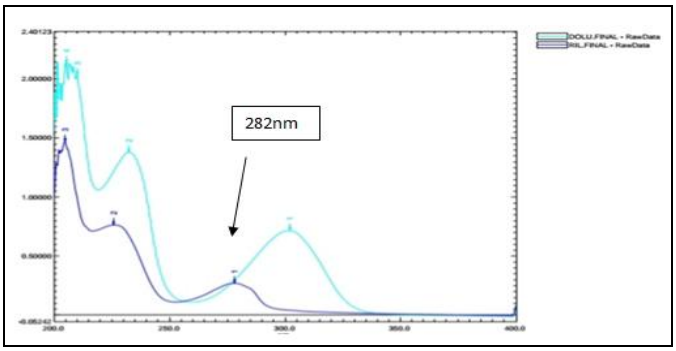

FIG. 3: OVERLAY SPECTRA OF RILPIVIRINE AND DOLUTEGRAVIR

Optimized Chromatographic Conditions: The mobile phase consisted of Methanol and Water. The chromatograph was operated in the isocratic mode, starting at a mobile phase of Methanol: Water (80:20v/v). The eluent was delivered at a flow rate of 1mL/min. Absorbance was monitored at λ_{max} = 282nm. The column was kept at ambient temperature.

Preparation of Mobile Phase: 80mL of Methanol and 20mL of Water HPLC grade were mixed and degassed in an ultrasonic water bath for 15 min. Then it was filtered through a 0.2µ filter under vacuum filtration before injection.

Vehicle: Mobile phase used as vehicle.

Preparation of Standard Stock Solution: Accurately weigh and transfer 10mg of each Rilpivirine and Dolutegravir standard drugs into a 100mL of the clean and dry volumetric flask, add about 30mL of mobile phase and sonicate to dissolve it completely, and make volume up to the mark with the mobile phase (100µg/mL).

Acid Degradation: Accurately weighed 1mg of each Rilpivirine and Dolutegravir were taken into a volumetric flask, add 10 ml of 0.1N of hydrochloric acid, and it is kept aside for 24 h at room temperature. Take 1ml of the above solution, dilute with a 10ml mobile phase and inject it on to analytical column of HPLC.

Base Degradation: Accurately weighed 1mg of each Rilpivirine and Dolutegravir were taken into a volumetric flask, add 10 ml of 0.1N of sodium hydroxide, and it is kept aside for 24 h at room temperature. Take 1ml of the above solution, dilute with a 10ml mobile phase and inject it on to analytical column of HPLC.

Oxidative Degradation: Accurately weighed 1mg of each Rilpivirine and Dolutegravir were taken into a volumetric flask, add 10 ml of 0.1N of hydrogen peroxide, and it is kept aside for 24 h at room temperature. Take 1ml of the above solution, dilute with a 10ml mobile phase and inject it on to analytical column of HPLC.

Thermal Degradation: Accurately weighed 1gm of Rilpivirine and Dolutegravir were taken into a two separate Petri dishes and kept in a hot air oven at 60 °C for 24 h. 1mg of this sample was taken into a 10 mL volumetric flask, dissolved with the mobile phase, and inject on to analytical column of HPLC.

RESULTS AND DISCUSSION:

Method Development: The method development was initiated in the isocratic mode of HPLC with a composition of the mobile phase consisting of Methanol and Water using different concentrations. Based upon trials, a ratio of mobile phase consisting of Methanol: Water (80:20% v/v) using Hypersil ODS (250mm × 4.6 mm i.d., 5µm) column and UV detection at 282nm was proved the most suitable of all combinations since the chromatographic peaks were better defined was finalized for the evaluation of Rilpivirine and Dolutegravir. The standard chromatogram was represented in **Fig. 4**.

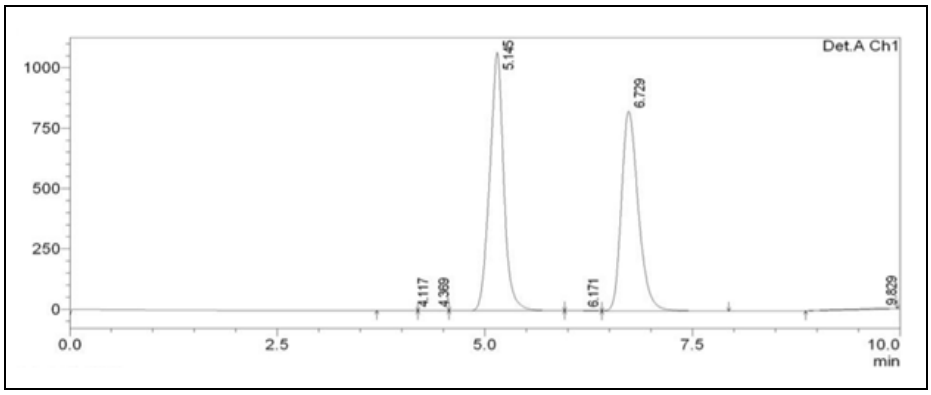

FIG. 4: CHROMATOGRAM OF OPTIMIZED METHOD

TABLE 4: OPTIMIZED METHOD RESULTS

Peak Name	R_t (min)	Peak area	Height	Tailing Factor	Theoretical Plates
Rilpivirine	5.1min	12552459	1070051	28781.364	1.045
Dolutegravir	6.7min	11671969	826982	1.325	34969.139

Method Validation: The developed and optimized HPLC method was validated according to ICH guidelines for the following parameters:

Linearity: In this method, the aliquots of the stock solution of Dolutegravir and Rilpivirine (10 - 100 ml of 100µg/ml) were transferred into seven 10 ml volumetric flasks and made up to the mark with the mobile phase. The solutions containing 10 - 100 µg/ml of Dolutegravir and Rilpivirine in the mobile phase were injected, and the chromatograms were recorded at 282 nm. It was found that the above concentration range was linear. The peak area was plotted against concentration, and the calibration curve was constructed. Linearity results were presented in **Table 5**.

Precision: The precision of the method was determined by intraday studies. Prepare 10µg/mL solution from a standard solution and injected five times in a day on to the analytical column. The percentage relative standard deviation (%RSD) was calculated, and lower %RSD indicates that there is

less variation, and there is high precision in the values.

$$\% \text{ RSD} = (\text{S.D} \times 100) / \text{mean}$$

Results for the Intra-day precision of Rilpivirine and Dolutegravir presented in **Table 6**.

Limit of Detection: The limit of detection (LOD) is the smallest concentration of the analyte that gives a measurable response. LOD was calculated using the following formula:

$$\text{LOD} = 3.3 \times (\text{Standard deviation} / \text{Slope of calibration curve})$$

Limit of Quantification: The limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the formula:

$$\text{LOQ} = 10 \times (\text{Standard deviation} / \text{Slope of calibration curve})$$

LOD and LOQ results were presented in **Table 7**.

Accuracy: The accuracy of the method was determined by recovery experiments. The recovery studies were performed by the regular addition method. At 50%, 100%, 150% level, the percentage recovery was calculated. For both drugs, recovery was performed in the same way. The recovery studies were performed in triplicate. The results were presented in **Table 8**.

Robustness: Robustness of the method was studied by making slight changes in chromatographic conditions, such as mobile phase ratio and mobile phase flow rate. The results were presented in **Table 9**.

Forced Degradation Study: Forced degradation of Rilpivirine and Dolutegravir in various conditions like acidic, basic, oxidation, and thermal degradation was observed. Chromatograms for these studies were represented in **Fig. 7-11**. Results for the Forced degradation study of Rilpivirine and Dolutegravir and presented in **Table 10**.

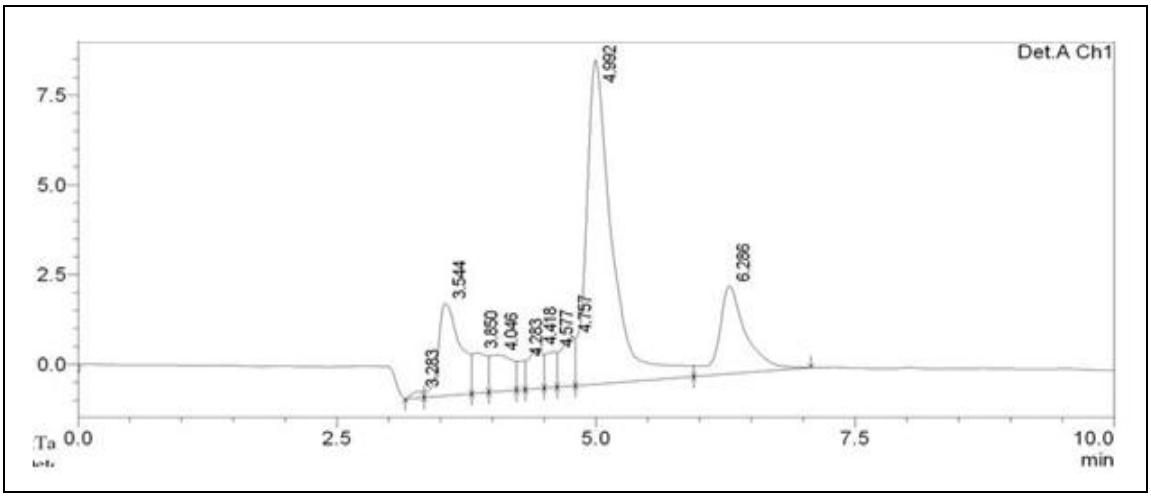

FIG. 7: CHROMATOGRAM OF RILPIVIRINE AND DOLUTEGRAVIR ACID DEGRADATION AFTER 1 DAY

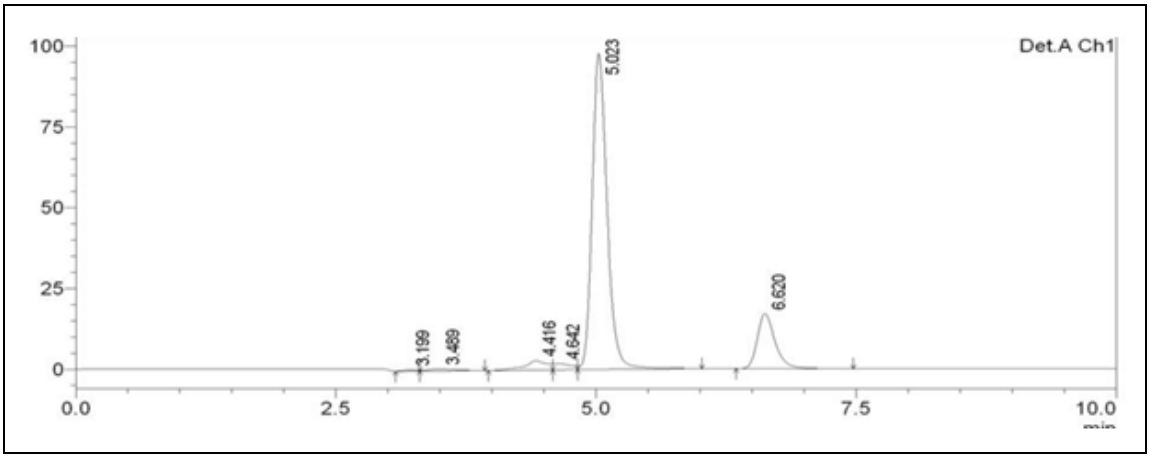

FIG. 8: CHROMATOGRAM OF RILPIVIRINE AND DOLUTEGRAVIR ALKALI DEGRADATION AFTER 1 DAY

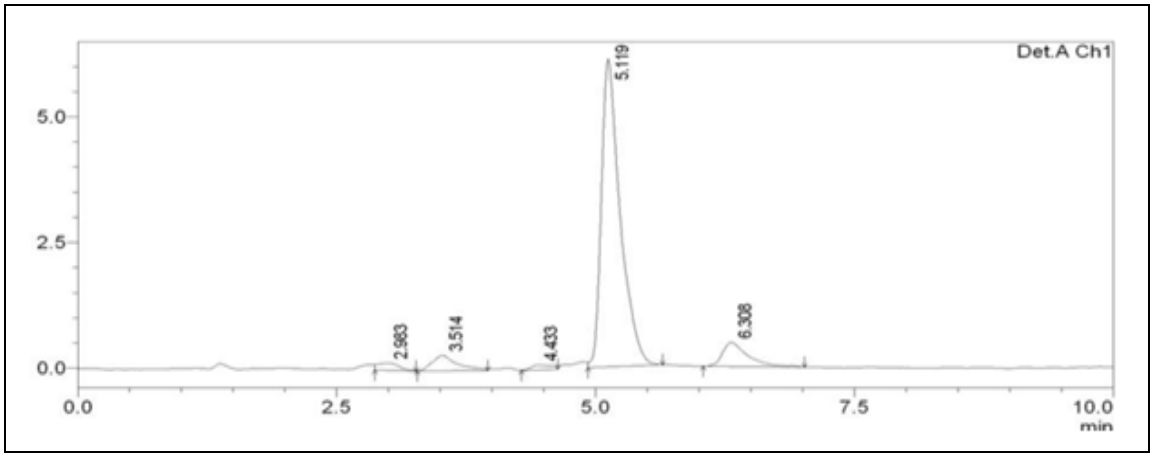

FIG. 9: CHROMATOGRAM OF RILPIVIRINE AND DOLUTEGRAVIR OXIDATION STRESS AFTER 1 DAY

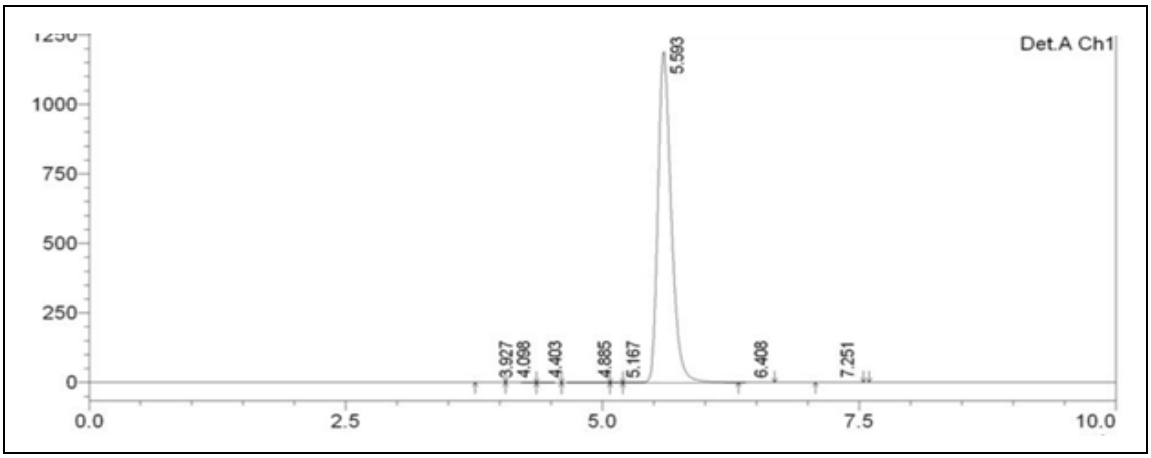

FIG. 10: CHROMATOGRAM OF RILPIVIRINE THERMAL DEGRADATION IN BULK AFTER 5 DAYS

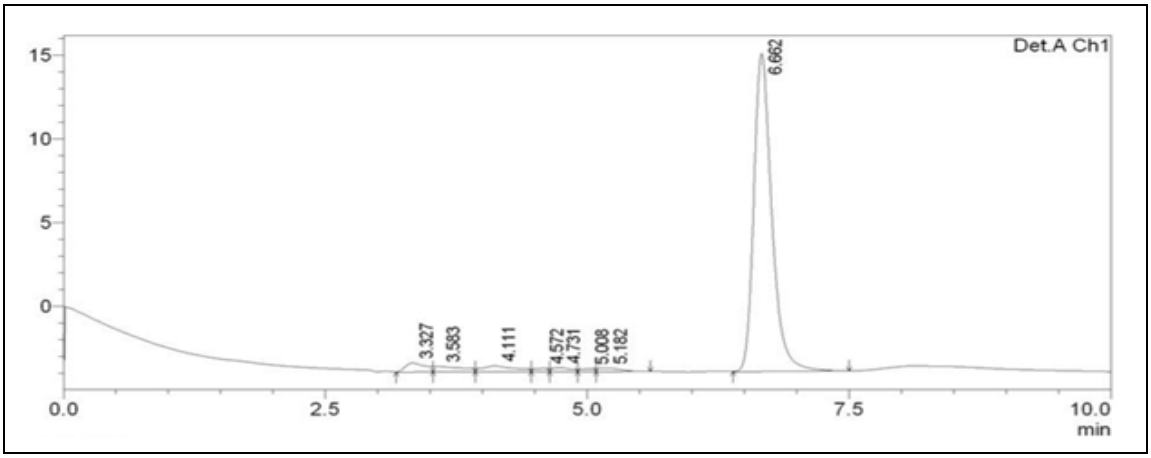

FIG. 11: CHROMATOGRAM OF DOLUTEGRAVIR THERMAL DEGRADATION IN BULK AFTER 5 DAYS

TABLE 5: LINEARITY RESULTS FOR RILPIVIRINE AND DOLUTEGRAVIR

S. no.	Concentration (µg/mL)	Peak Area of Rilpivirine	Peak Area of Dolutegravir
1	0	0	0
2	10	1355461	1309313
3	20	2457489	2365377
4	30	3758686	3363858
5	40	5697085	387627
6	50	7246650	489594
7	70	8516363	556206
8	100	10641025	724949
	Correlation coefficient (r^2)	0.9977	0.9954

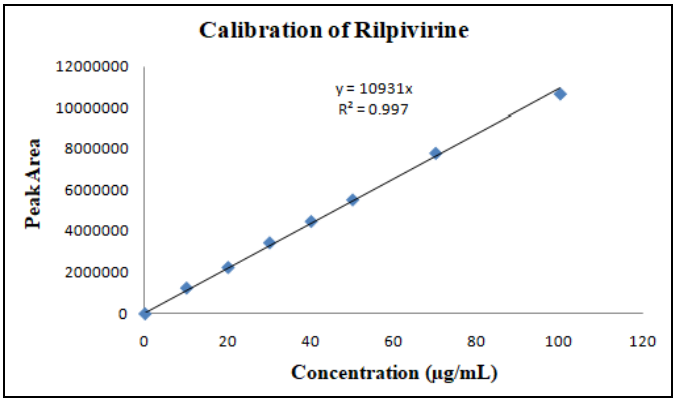

FIG. 5: STANDARD GRAPH OF RILPIVIRINE

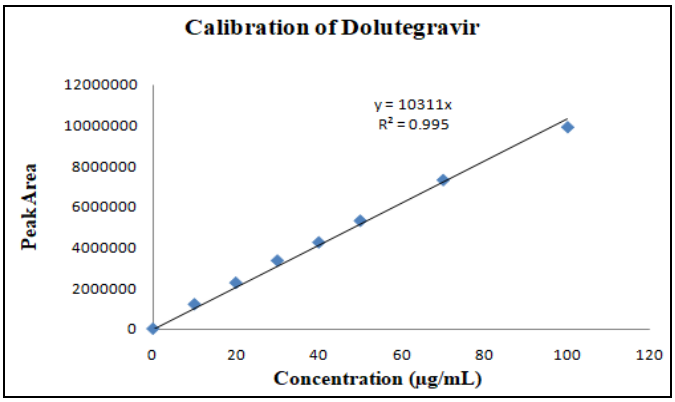

FIG. 6: STANDARD GRAPH OF DOLUTEGRAVIR

TABLE 6: INTRA-DAY PRECISION DATA OF RILPIVIRINE AND DOLUTEGRAVIR

S. no.	Peak Area of Rilpivirine	Peak Area of Dolutegravir
1	1855461	1709313
2	1595854	1472256
3	183980	175741
4	1831683	1687670
5	1610578	152079
Mean	1415511.2	1313051.8
SD	2576.01	27957.99
%RSD	1.96	1.97

TABLE 7: LOD AND LOQ DATA OF RILPIVIRINE AND DOLUTEGRAVIR

S. no.	Drug	LOD	LOQ
1	Rilpivirine	0.844 µg/mL	2.557 µg/mL
2	Dolutegravir	0.082 µg/mL	0.249 µg/mL

TABLE 8: ACCURACY DATA OF RILPIVIRINE AND DOLUTEGRAVIR

Concentrations (%)	% Recovery of Rilpivirine	%Recovery of Dolutegravir
50	99±3	101±3
100	99±3	102±2
150	101±2	102±2

TABLE 9: RESULTS OF ROBUSTNESS

S. no.	Mobile phase (%v/v)	Flow rate (mL/min)	%RSD of Rilpivirine	%RSD of Dolutegravir
1	80:20	0.9	0.36	0.58
2	80:20	1.1	1.02	0.44
3	85:15	1	0.41	0.49
4	75:25	1	0.43	1.34

TABLE 10: RESULTS FOR FORCED DEGRADATION STUDY

Degradation type	Sampling time	Bulk
Acid degradation	1,3,5 days	1 day
Base degradation	1,3,5 days	1day
Oxidation stress	1,3,5 days	1 day
Thermal degradation	1,3,5 days	-

CONCLUSION: A simple, fast, precise, accurate, robust, economical and stability-indicating reversed-phase high performance liquid chromatographic method was developed and validated according to ICH guidelines for the estimation of Rilpivirine and Dolutegravir in bulk. Forced degradation of Rilpivirine and Dolutegravir in various conditions like acidic, alkaline, oxidation, and thermal degradation was performed. Drug degradation was confirmed by observing the RT improper and multiple peaks formation in the chromatogram of Rilpivirine and Dolutegravir. Rilpivirine and Dolutegravir were degraded under acidic, alkali, and oxidation. No degradation of both individual drugs in bulk was observed in Thermal condition. So this method is used to monitor the stability of Rilpivirine and Dolutegravir.

ACKNOWLEDGEMENT: Authors are thankful to the principal and the management of Vaagdevi College of Pharmacy, Hanamkonda, for providing the necessary facilities for research work and to Pharma Train Lab, Hyderabad, for providing Rilpivirine and Dolutegravir.

CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Tripathi KD: Essentials of Medical Pharmacology. Jaypee Brothers Medical Publisher Pvt Ltd, Sixth Edition 2013.
2. Rang HP, Dale MM, Ritter JM, Flower RJ and Henderson G: Rand and Dale's pharmacology, Seventh edition 2012.
3. Pardeshi AN and Damle MC: Stability Indicating HPLC method for Rilpivirine and Dolutegravir Sodium. European Journal of Biomedical and Pharmaceutical Sciences 2017; 4(7): 454- 59.
4. 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.
5. 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.
6. SyamSundar B, Satyadev T.NVSS and Bhargavi C: Development and validation of high performance liquid chromatographic method for the determination of Dolutegravir in human plasma. Pelagia Research Library Der Pharmacia Sinica 2015; 6(4): 65-72.
7. Rao NM and Sankar DG: Development and validation of stability-indicating HPLC method for simultaneous determination of Lamivudine, Tenofovir, and Dolutegravir in bulk and their tablet dosage form. Future Journal of Pharmaceutical Sciences 2015; 73-77.
8. 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.
9. 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.
10. Girija BB, Kiran BA, Subhash TR and Sachin JK: Development and Validation of UV Spectrophotometric Method for Estimation of Dolutegravir sodium in tablet dosage form. Malaysian Journal of Analytical Sciences 2015; 19(6): 1156-63.
11. 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.
12. Masthanamma SK, Sridhar TA and Saidulu P: A novel UV-Spectrophotometric method development and validation of Dolutegravir in bulk and its laboratory synthetic mixture by using 8M urea as hydrotropic solubilising agent. International Journal of Pharamaceutical Sciences and Drug Research 2015; 7(4): 370-75.
13. Xinzhu W, Dilly PS, Amara ALE, Myra M and Marta B: A validated method for quantification of Dolutegravir Using Ultra Performance Liquid Chromatography coupled with UV detection. Therapeutic Drug Monitoring 2016; 38(3): 328-34.
14. 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
15. Kiran B, Aher, Ravindra S, Thorat, Sanjay R and Chaudhari: High-Performance Liquid Chromatography and High-Performance Thin Layer Chromatographic method for the Quantitative Estimation of Dolutegravir sodium in bulk and pharmaceutical dosage form. Scientia Pharmaceutica 2015; 84: 305-20.
16. 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.
17. ICH Guidelines for Stability Testing of New Drug Substances and Products Q1A (R2) 2004; 1-24.
18. ICH Guidelines for Validation of Analytical Procedures: Text and Methodology Q2 (R1) 2005; 1-17.

How to cite this article:
Reddy KS, Shirisha SS and Kumar KP: Validated stability indicating RP-HPLC method for the simultaneous estimation of rilpivirine and dolutegravir in bulk form. Int J Pharm Sci & Res 2020; 11(10): 4991-97. doi: 10.13040/IJPSR.0975-8232.11(10).4991-97.