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Stability-Indicating RP-UPLC Method for the Simultaneous Determination of Dolutegravir and Rilpivirine in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

Specific stability-indicating reversed-phase ultra-performance liquid chromatography (UPLC) method has been developed and validated for the simultaneous quantification of dolutegravir and rilpivirine in bulk drugs and pharmaceutical dosage forms. The optimized conditions for the developed UPLC method are SB C8 column (100 × 3 mm, 1.8 mm) maintained at 30°C with mobile phase consisting of 0.1% ortho phosphoric acid and acetonitrile in the ratio 55:45% v/v on isocratic mode at flow rate of 1 ml/min and detection wavelength 260 nm. The retention time of dolutegravir and rilpivirine was found to be 1.25 min, and 1.69 min with linearity in the concentration range from 12.5-75.0 µg/ml and 6.25-37.5 µg/ml respectively. The mean percentage recoveries of dolutegravir and rilpivirine were found to be 99.04%-99.79% and 99.20%-99.92%, respectively. The percent relative standard values were less than 2 at all the levels indicates a satisfactory accuracy and precise. Performed the robustness found to meet the acceptance criteria. The stress study against qualified working standards of dolutegravir and rilpivirine, which indicated that the developed UPLC method was stability-indicating, conducted as per ICH requirements.

Keywords: RP-UPLC, Stability indicating method, Dolutegravir, Rilpivirine.

INTRODUCTION

Dolutegravir sodium (DOL) is chemically designated as (4R, 12aS)-N-(2, 4-difluorobenzyl)-7-hydroxy-4-methyl-6, 8-dioxo- 3,4,6,8,12,12a-hexahydro-2H-pyrido [1',2':4,5] pyrazino [2, 1-b] [1,3] oxazine-9-carboxamide (Figure 1). Dolutegravir is a HIV-1 intergrase inhibitor that blocks the strand transfer step of the integration of the viral genome into the host cell. The effect of this drug has no homology in human host cells which gives it an excellent tolerability and minimal toxicity and rilpivirine (RIL) is non-nucleoside reverse transcriptase inhibitor (NNRTI) which is used for the treatment of HIV-1 infections in treatment-naive patients [1]. It is a diarylpyrimidine derivative, a class of molecules that resemble pyrimidine nucleotides found in DNA (Figure 2).

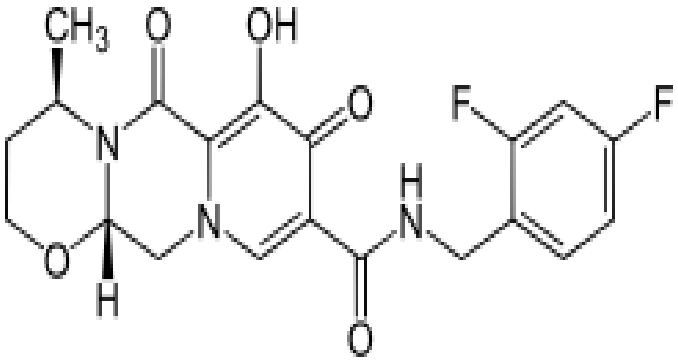

Figure 1: Structural formula of dolutegravir.

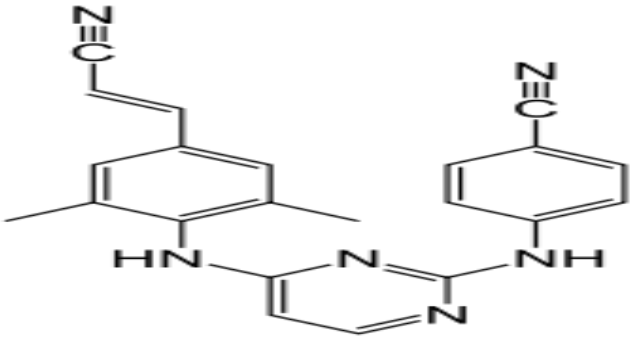

Figure 2: Structural formula of rilpivirine.

A literature survey reveals the spectrophotometer, HPLC UPLC-MS/MS and HPTLC methods for compounds either individually or in combination with other dosage forms. Based on the literature review does not show any stability-indicating UPLC method for simultaneous quantification of DOL and RIL. Hence, it was felt that there is need of new analytical method by UPLC. The present research work was to develop a single, simple, fast, rapid suitable stability-indicating UPLC method for the determination of DOL and RIL. The developed method was validated and forced degradation studies were performed on the drug product solution to show the stability-indicating nature of the method and to ensure its compliance in accordance with International Conference on Harmonization (ICH) guidelines [2-13].

MATERIALS AND METHODS

Chemicals and reagents

Samples of DOL and RIL pure drugs were received from Mylan Laboratories Limited (Hyderabad, India). HPLC-grade Acetonitrile were purchased from Merck (Mumbai, India). Ortho-phosphoric acid was purchased from Qualigens Fine chemicals (Mumbai, India). HPLC-grade water was prepared by using a Millipore Milli-Q plus purification system.

Equipments

The UPLC method development, validation and forced degradation (stress) studies were conducted using UPLC-waters acquity system consisting of an in-built auto sampler, a column oven and PDA detector. The data was acquired through Empower-2-software. Meltronics sonicator, Elico pH meter and Sartorius balance (model AE-160) were used for this work.

Preparation of standard solutions

For DOL, an accurately weighed quantity of 12.5 mg of dolutegravir was dissolved in 10 ml of diluent. The volume was made up to 25.0 ml with diluents (The diluent composed of water and acetonitrile (50:50% v/v)). A sample (1 ml) of the resultant solution was pipetted into a 10.0 ml volumetric flask. The volume was made up to the mark with diluent (concentration: 50.0 µg/ml). For RIL, an accurately weighed quantity of approximately 6.25 mg of RIL was dissolved in diluent and diluted to 25.0 ml with diluent. A sample (1 ml) of the resultant solution was pipetted in to a 10.0 ml volumetric flask. The volume was made with diluent (concentration: 25.0 µg/ml). For the mixed standard stock solution, accurately weighed quantities of DOL (12.5 mg) and RIL (6.25 mg) were transferred to a 25.0 ml of volumetric flask and dissolved in approximately 10.0 ml diluent, and the volume was made with diluent.

Method validation

The following parameters were considered for the validation of method for title analytes. The method is validated according to ICH Q2 (R1) guidelines [13].

System suitability

System suitability was performed by injecting a blank (diluent) solution followed by a standard blend solution of drugs six times into the UPLC system and chromatograms were recorded.

Specificity

Specificity of developed method was appraised through the analysis of drug solution, sample and placebo solutions in order to check if any extraneous component could spawn a chromatographic response similar to the drugs. The placebo solution was prepared with the GRAS excipients (lactose, starch, microcrystalline cellulose, magnesium stearate, titanium dioxide and talc) of the pharmaceutical formulation.

Linearity

Aliquots of about 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 ml were taken from mixed standard stock solution concurrently transferred into different volumetric flasks of 10 ml capacity. These solutions were diluted up to the mark with diluents to get the final concentrations as 12.5-75 µg/ml and 6.25-37.5 µg/ml for dolutegravir and rilpivirine, respectively. Volume of 10 µl of

sample was injected in six times for each concentration level and calibration curve was constructed by plotting the peak area versus drug concentration. A linear relationship between peak responses vs. concentration was observed in the range of study.

Precision

Standard solution of dolutegravir (50 µg /ml) and rilpivirine (25 µg/ml) was prepared and injected the same solution for six times to check the system precision. Method precision study was carried out by estimating corresponding responses of drug solutions prepared for six times for the concentration of dolutegravir (50 µg/ml) and rilpivirine (25 µg/ml) when analyzed through proposed method. The retention time and area of the determinations were measured and % RSD was calculated.

Accuracy

Accuracy of proposed method was ascertained based on recovery study performed by standard addition method. The known amount of dolutegravir/rilpivirine was added to the pre-analyzed sample according to 50/100 and 150% levels of labelled claim, further subjected to contemplated analytical method, anon percentage recovery and relative standard deviation (%RSD) were calculated for each concentration.

Sensitivity

Limit of detection (LOD) and limit of quantification (LOQ) parameters were discretely appraised based on signal to noise ratio values obtained through waters empower software.

Robustness

Robustness study was carried out by varying three parameters from the optimized chromatographic conditions such as making small changes in flow rate (± 0.2 ml/min), mobile phase organic composition (± 2%) and column temperature (± 5°C). Blank and the standard solutions were injected for three times and analyzed using varied flow rate (0.8 ml, 1.2 ml), organic mobile phase (43% and 47% v/v) and temperature (25°C, 35°C) along with proposed method flow rate, mobile phase and temperature conditions. The% RSD value of peak area for triplicate injections was calculated.

Assay of dosage forms

The commercial pharmaceutical formulation of Juluca tablets containing 50 mg of dolutegravir and 25 mg of rilpivirine were selected for analysis. Twenty tablets were weighed and crushed into fine powder. The tablet powder weight equivalent to 25 mg of dolutegravir/12.5 mg of rilpivirine was taken and transferred into 50 ml volumetric flask. To this 25 ml of methanol was added, sonicated to dissolve the drugs and diluted to volume with diluent. Further the solution was filtered through 0.45 µm filter paper. Filtrate of 10 ml was diluted to 100 ml with mobile phase. This sample solution was injected into the UPLC system, analyzed by the proposed method and calculated the % assay value.

Forced degradation studies

Forced degradation studies were performed to demonstrate the optimized method was stability indicating. To prove the method which can be able to measure accurately active pharmaceutical ingredient in presence of degradants which are expected to be formed during different types of degradations applied to the drug sample. In order to check the selectivity of the proposed method towards the drugs, degradation studies were carried out acid (2 N HCl), basic (2 N NaOH), oxidation (20% v/v hydrogen

peroxide) neutral (water), thermal treatment (dry heat at 105°C for 6 h) and photodegradation studies (the drug sample was exposed to light for 24 hours in a UV chamber).

For UPLC studies, above prepared stressed samples were diluted to obtain 50 µg/ml and 25 µg/ml concentration of dolutegravir and rilpivirine, respectively using diluent. These solutions were injected into the system and the chromatograms were recorded to assess the stability of drugs. Concurrent injection of controlled sample (mixed binary standard solution of same concentration) was done along with stressed samples [14-18].

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

To achieve better efficiency of the chromatographic system several solvents were tested using different proportions of acetonitrile-phosphate buffer, a satisfactory separation for two drugs and its degradation products was achieved with mobile phase consisting of phosphate buffer (0.1% ortho-phosphoric acid) and acetonitrile (55:45% v/v) at a flow rate of 1.0 ml/min on the C8 (100 × 3 mm, 1.8 m) column in an isocratic elution mode. Mixture of water and acetonitrile (1:1) was used as diluent. An injection volume of 0.3 µl and column temperature of 30°C was afforded the best separation of these analytes. Under these conditions, the analyte peaks were well resolved and were free from tailing with a reasonably short runtime of 3 minutes. This rapid analysis time makes the contemporary method as economical method where consumption of organic solvent was squat.

The detection wavelength was optimized using photo diode array detector (PDA) and found that dolutegravir and rilpivirine have maximum response at 260 nm wavelength. Therefore, detection was carried out at 260 nm and retention times were found to be 1.25 minutes and 1.68 minutes, respectively for dolutegravir and rilpivirine. The peak shapes of all the analytes were symmetrical and the asymmetry factor was lesser than 2.0. Chromatogram obtained under optimized conditions was reported in Figure 3.

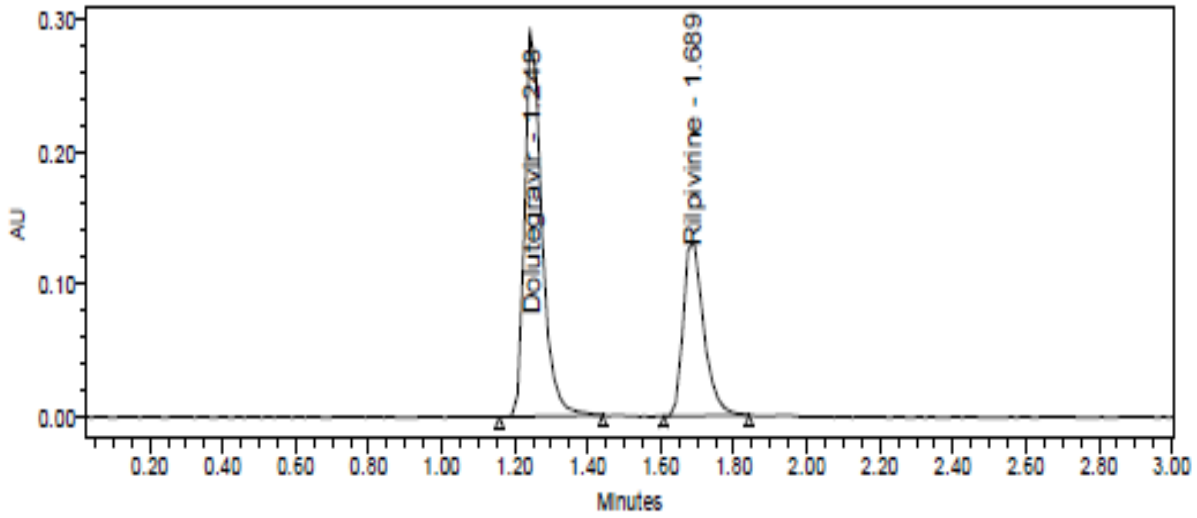

Figure 3: Optimized chromatogram of dolutegravir and rilpivirine.

Method validation

To prove that the above developed method can be useful for routine quality control of these drugs, the method is validated according to ICH guidelines as follows. For system suitability study, a replicate injection of mixed standard solution was injected under optimized chromatographic conditions. Results were summarized in Table 1 and chromatogram obtained was shown in Figure 4. It was found that determined parameters were in relation with the pre-determined system suitability parameters of test method. The observed RSD values (peak area), theoretical plates, tailing factor and peak resolution were found to be well within the acceptable limits. The results of system suitability test convey the optimized chromatographic conditions were adequate for simultaneous determination of dolutegravir and rilpivirine. For specificity study, standard, sample, blank (mobile phase) and placebo solutions were injected into the UPLC system as per the test procedure. The chromatograms were examined for any interference at the retention time of title entities and observed for changes in the retention time values of drugs. Chromatograms obtained were reported Figure 5, revealed that there was no interference due to blank and placebo throughout the analysis of drugs in standard and sample solutions. The elution order and retention times obtained from individual standard solution and binary blend solution were comparable. These results substantiate that the test procedure has discriminate power for analysis of dolutegravir and rilpivirine.

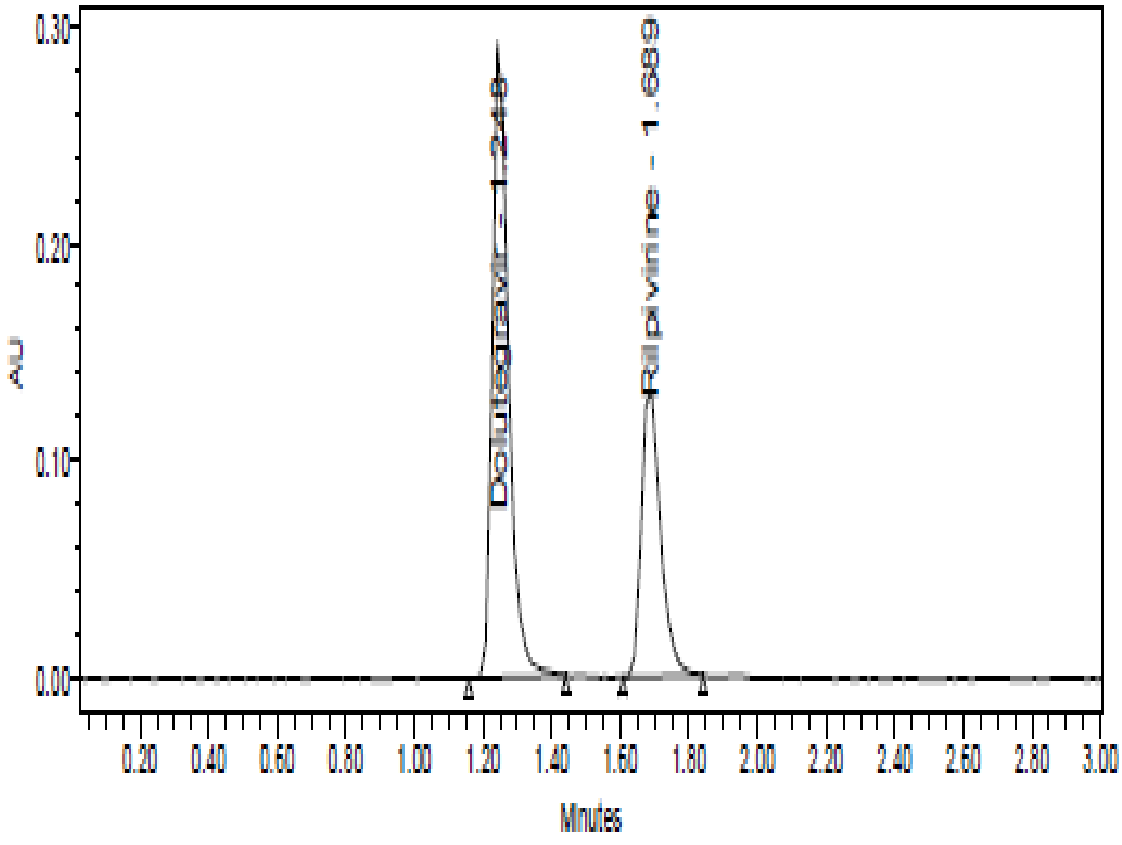

Figure 4: Chromatogram of mixed standard solution (dolutegravir-50 µg/ml and rilpivirine-25 µg/ml).

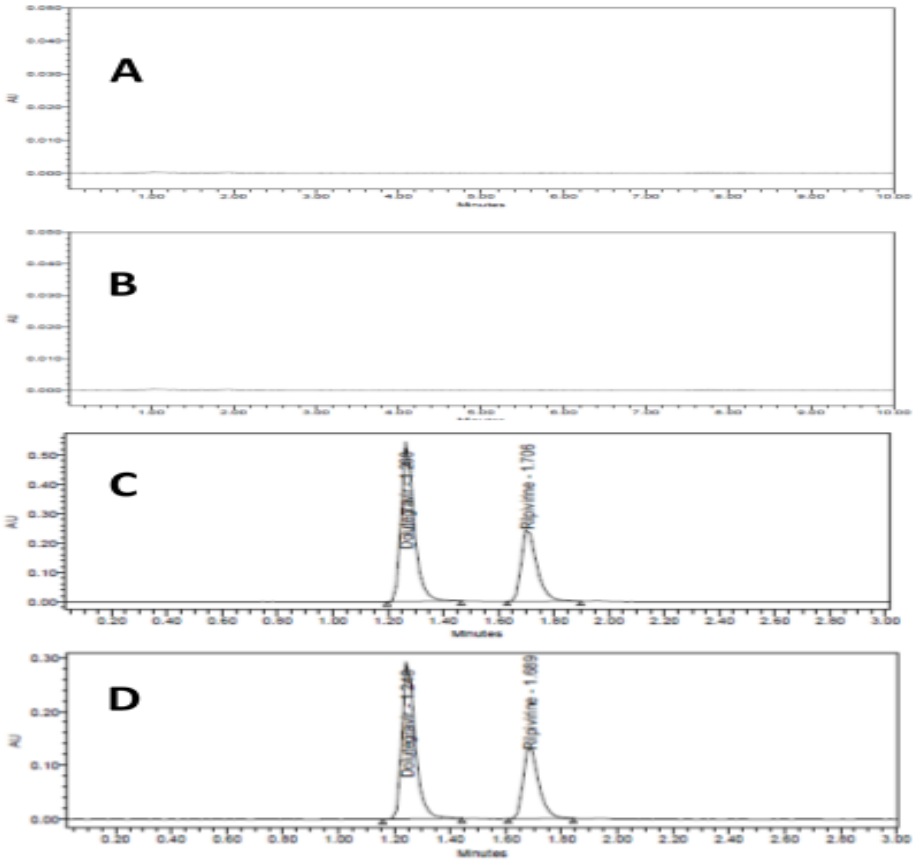

Figure 5: Typical chromatogram of blank (A), placebo (B), sample (C) and mixed ternary standard solution (D).

Method sensitivity was appraised based on the LOD and LOQ values, obtained through signal to noise ratio method. For these discrete injections of diluted standard drug solution was analyzed through test method and LOD/LOQ values were calculated using waters empower software. The results were reported in Table 2, revealed the ability of proposed method to detect/quantify very low concentration of title analytes. Hence the method was found to be sensitive for estimation of title analytes.

Table 1: Results of system suitability study.

System suitability parameters	**Observed value**		**Acceptance criteria**
	Dolutegravir	**Rilpivirine**	
Retention time	1.24	1.68	NLT 1.0 minutes
%RSD for area count of six replicate injection of standard	0.65	0.52	NMT 2.0
Tailing factor	0.9	1.0	NMT 2.0
Theoretical plates	4580	6520	NLT 2000
Resolution	2.1	NLT 2.0	

Table 2: Results of LOD and LOQ.

S. no.	Analyte	LOD (µg/ml)	LOQ (µg/ml)
1	Dolutegravir	0.281	0.852
2	Rilpivirine	0.152	0.493

In order to study the linearity of detector response, aliquot standard solutions were prepared and injected into UPLC system in triplicate, which corresponded to 25%, 50%, 75%, 100%, 125% and 150% of test solution concentration. Linearity was evaluated by linear-regression analysis wherein calibration curves were constructed for drug concentration versus chromatographic peak area. A linear response was willful over the examined concentration range of 12.5-75 µg/ml and 6.25-37.5 µg/ml for dolutegravir and rilpivirine, respectively and calibration plots obtained were demonstrated in Figures 6 and 7, reveals the line of best fit for concentration versus peak area of analytes.

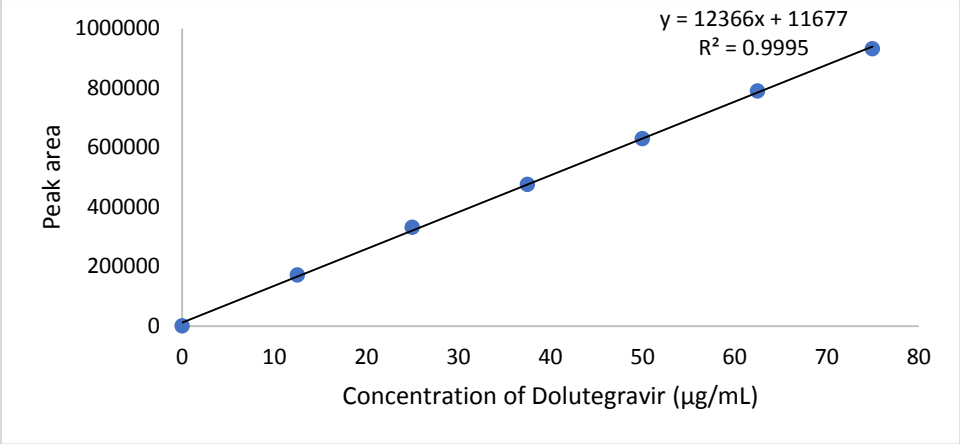

Figure 6: Linearity graph of dolutegravir.

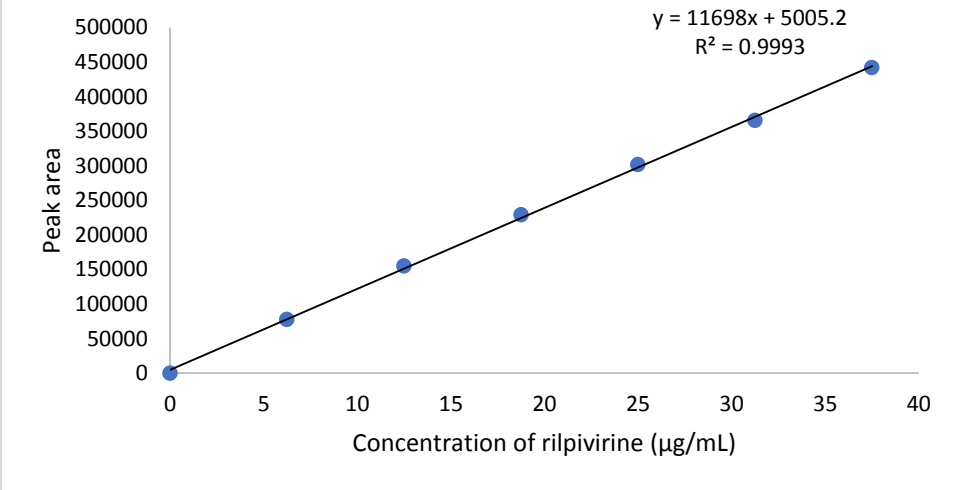

Figure 7: Linearity graph of rilpivirine.

The correlation coefficient value was found to be 0.999 for three drugs under estimation, indicated that the proposed method has good linearity throughout the concentration range of study. The% RSD value obtained was found to be within acceptance limit, indicated that the proposed method was able to produce reproducible results. The mean percentage recoveries of dolutegravir and rilpivirine were found to be 98.55-99.79% and 99.20-99.96%, respectively and percent relative standard values were less than 2 at all the levels. Results of recovery were found to be within the limits, indicated that there is no interference with matrix also showed an agreement between the standard values and ascertained values along these lines contemplated UPLC method was accurate for determination of title analytes. The robustness results shown that, the method is specific and unaffected by variable conditions applied during study. This signifies the endure performance method under unreliable conditions. The degree of reproducibility of results proven that the method is robust for regular quality analysis of dolutegravir and rilpivirine.

Assay of tablet dosage form

The contemplated method was employed to the assay of commercial binary solid dosage form (Juluca tablets) containing 50 mg of dolutegravir and 25 mg of rilpivirine. The chromatogram obtained for sample solution of marketed formulation has dearth of additional peaks, indicated no interference of the formulation excipients used in the dosage form, denoted the selectivity of proposed method towards dolutegravir and rilpivirine. The peak area of two drugs at their respective retention time was measured and their concentrations in the sample were determined using linear regression equation. The assay results obtained were compared with consequent labeled amounts and proclaimed in Table 3. The amount of drug in sample was found to be agreed with respective label claimed. The % assay values were found to be within the limits (98-102%) and low % RSD values indicated the suitability of the method for routine quality control of dolutegravir and rilpivirine in binary pharmaceutical dosage form.

Table 3: Analysis of commercial tablets containing DOL and RIL.

Formulation	Drug	Label claim (mg)	Amount found ± SD	Assay (%)	%RSD
Juluca tablets	Dolutegravir	50 mg	49.7 ± 0.15	99.4	0.301
	Rilpivirine	25 mg	24.9 ± 0.29	99.6	1.16

Stress degradation studies

Forced degradation studies were performed to demonstrate the optimized method is stability indicating. The details of degradation were recorded, and results of the analysis were shown in Table 4, revealed that% net decomposition of was within the acceptance limits (2-30%) denotes the stability of these drugs under stress conditions. All above samples were analyzed in PDA detector and evaluated peak purity for three drugs. Stress degradation study results of dolutegravir revealed its stability under UV and neutral hydrolysis condition. Significant degradation of dolutegravir and rilpivirine was observed upon exposure to acid/ base hydrolysis condition. Chromatographic peaks of interested drugs were well estranged from the degradation product peaks and the resolution was found to be more than 2.

Table 4: Results of forced degradation study.

S. no	Degradation condition (DOL and RIL)	Retention times of analyte		%Assay		%Degradation		Peak purity
		DOL	RIL	DOL	RIL	DOL	RIL	
1	Untreated sample	1.25	1.691	99.43	99.62	--	--	Pass
2	Acid degradation	1.255	1.711	94.79	94.87	4.64	4.75	Pass
3	Base degradation	1.253	1.714	95.73	95.51	3.7	4.11	Pass
4	Peroxide degradation	1.257	1.713	96.75	96.34	2.68	3.28	Pass
5	Thermal degradation	1.257	1.712	97.97	96.1	1.46	3.52	Pass
6	UV degradation	1.256	1.712	98.72	98.43	0.71	1.19	Pass
7	Water degradation	1.253	1.714	99.41	99.24	0.02	0.38	Pass

CONCLUSION

There is currently no stability indicating UPLC method available for the simultaneous quantification of dolutegravir and rilpivirine in dosage forms and in the presence degradation samples. As the developed method the analysis time is short and sensitive, specific, selective, accurate and robust proved by validation results. The behaviour of dolutegravir and rilpivirine under various stress conditions was studied and degradation peaks were well separated from the sample peaks. The proposed method was successfully employed for simultaneous quantification of dolutegravir and rilpivirine in dosage forms and in the presence degradation samples.

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