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OPTIMIZATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF DOLUTEGRAVIR AND RILPIVIRINE IN BINARY MIXTURE BY USING DESIGN OF EXPERIMENTS

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ABSTRACT

The present study describes the simultaneous assessment of the antiretroviral drugs in the binary mixture with the help of design of experiments for enhancing the robustness. The column employed was Kromosil 250 ×2.1mm, 1.7µ with temperature 30°C. The ranges of the independent variables used for the optimization were flow rate 0.9 to 1.1, wavelength 255 to 265 nm and composition of buffer in the mobile phase is 55 to 65 %. The influence of these independent variables on the output responses: retention time, peak area and resolution were evaluated. The three responses were simultaneous optimized by using central composite design. Optimum conditions chosen for the assay were flow rate 0.998ml/min, wavelength 257 nm and buffer and Acetonitrile taken in the ratio 57.4: 42.6 respectively. The retention time of Dolutegravir and Rilpivirine are 3.962 and 2.977 minutes respectively by employing the optimum conditions given by the design experiments. All the system suitability parameters were satisfied. Further the method has been validated by the regulatory guidelines framed by the ICH. The limit of detection and the limit of quantification were found to be 0.24 and 0.72 for Rilpivirine and 0.10 and 0.30 for Dolutegravir respectively. The method was found to be simple, linear, accurate, precise and robust. Hence the proposed method can be used for routine quality control of Dolutegravir and Rilpivirine in its tablet dosage forms.

INTRODUCTION

Dolutegravir sodium chemically, (4R,12aS)- 9- {[(2,4-difluorophenyl) methyl] carbamoyl} - 4-methyl-6,8-dioxo- 3,4,6,8,12,12a- Hexahydro-2H-pyrido [1',2':4,5] pyrazino [2,1-b][1,3]oxazin-7-olate, is a novel integrase stand transfer inhibitor active against Human Immunodeficiency Virus as shown in Figure 1. Dolutegravir (DTG) promoted name as Tivicay is an antiretroviral prescription utilized together with other drug to treat human immunodeficiency infection (HIV)- acquired immune deficiency syndrome. The drug is active against HIV type 1 (HIV-1) and also has some in vitro activity against HIV type 2 (HIV-2) (drug bank DB08930). It is taken through rally. DTG is a HIV integrase

strand exchange inhibitor which hinders the working of HIC integrase which is required for viral replication (Steigbigel et al., 2008; Takao M, 2011).

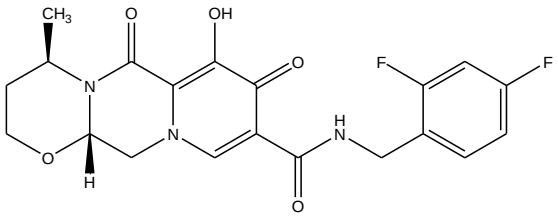

Fig 1. Structure of Dolutegravir

Rilpivirine is chemically known as 4-{[4-({4-[(1E)-2- cyanoeth-1-en-1-yl]-2, 6-dimethylphenyl} amino) pyrimidin-2-yl] amino} benzonitrile was shown in fig 2 (drug bank DB08864). It is non-nucleoside reverse transcriptase inhibitor (NNRTI), it had been developed for treatment of ARV naïve HIV-I infected patients to have better safety/ tolerability profile compared to other NNRTIs (such as nevirapine, efavirenz and etravirine) (Goebel F et al.,2006 ; Poznaik A et al., 2009). It is a di-amino pyrimidine derivative. It acts by binding to reverse trancriptase which results in block in RNA and DNA-dependent DNA polymerase activities. One such activity is HIV-I replication. It is an E-isomer (M.Sharma et al., 2013; R. Schrijversa et al., 2011).

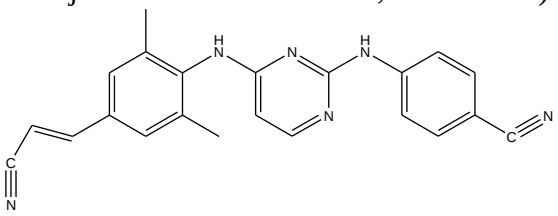

Fig 2. Structure of Rilpivirine

Previously, the normal practice to build up an analytical method in liquid chromatography was performed by a trial and error approach, for instance by shifting one-factor-at- a- time (OFAT) and examine at the resolution of peaks until the best technique was found. This methodology was tedious and required a huge amount of manual data elucidation. It regularly came about in a non-robust performance when moved into another lab since interactions between factors were not considered. The OFAT approach should possibly be utilized if the client needs to comprehend selectivity changes, for example in the event that everything is fixed and just one factor is shifted and if the factor of interest is known not to have an intuitive impact with any other factor studied. Hence chemometric technique have been progressively seen as valuable complements to high performance liquid chromatographic practices, since an extensive number of factors can be at the same time controlled to accomplish the ideal separations. A fruitful implementation of experimental design in HPLC can be executed through four common stages; choosing the conventional design, suitable software, experimental trails, data analysis and interpretation (Sahu et al., 2018). This design of experiments was applied for the quality analysis of the antiviral drugs in standards and in dosage for m after a vast literature survey. Either individually or in combination with other dosage forms there is some spectrophotometer (Masthanamma Sk et al., 2014), HPLC (A.Yasodha et al., 2017; Raj Kumar et al., 2014; T.Sreenivasulu reddy et al., 2013; S. Venkatesan et al., 2014; Sridhar Thota et al., 2014; Uttam Prasad Panigrahy et al., 2015; Somsubhra Ghosh et al., 2013; Saidulu et al., 2016; Sonam Patel et al., 2018) or bioanalytical methods (Raju et al., 2013; Nitin Charbe et al., 2016; Veeraswami et al., 2019) HPTLC (Bhavar et al., 2016) methods are reported. But so far to our present knowledge by the literature review does not show any methods for Rilpivirine and Dolutegravir by using RP-HPLC with the help of chemometric optimization. Hence, it was felt that there is a need to develop more robust method along with the stability studies. The developed method has been validated with the help of ICH guidelines (ICH Q2 (R1), 2005).

Experimental

Material and reagents

Dolutegravir reference standard is procured from Dr.Reddy's Laboratory and Rilpivirine reference standard were procured as gift sample from Hetero, Hyderabad, India. HPLC grade reagents were purchased from Rankem chemicals.

HPLC equipment and chromatographic conditions

The chromatographic separation was carried out by Waters Acquity HPLC with binary solvent manager, equipped with PDA detector and auto sampler. The Empower 2 software was used for signal monitoring, data collection and data processing. In addition, an electronic balance (BL-220H; Shimadzu Corporation), a pH meter (ELICO® LI 120), a sonicator (PCi, Mumbai) has been employed in this study.

Optimization of analytical variables by employing software

After initial screening of the conditions like stationary phase, mobile phase the method has been gone for optimization by using Design Expert 10.0.0.8 trail version software. In this software, Central composite design under the category of Response surface methodology (RSM) have been employed to design a set of experimental runs by concerning the three independent variables i.e., flow rate, wavelength and % of buffer in the mobile phase. Here the independent variables ranges were entered along with their actual levels. The levels used were tabulated in (Table.1). In the experimental design central composite design was followed with three factors, 2 levels, responses and 20 experimental runs. These three factors have an effect on the dependent variables i.e., retention time, resolution and peak area for both Rilpivirine and Dolutegravir.

Chromatographic conditions: The final conditions optimized by the software include 0.99ml/min. flow rate, 257.06nm of wave length and buffer ratio 57.4% in the mobile phase. The HPLC analysis was performed on reverse phase high performance liquid chromatographic systems with using a mobile phase of Acetonitrile and buffer (0.01N Potassium dihyrogen ortho phosphate buffer) on Kromosil 250 X 2.1mm, 1.7m with flow rate 0.998ml/min at 257 nm using TUV detector. The column temperature has been maintained at 30°C with an injection volume 10 mL. flowed by the run time of 7 minutes, the comparison of initial HPLC method and optimized HPLC method from Design Expert experiments are drawn in the (Table.2). Design- Expert 10 trail version have been used for the experimental design, data analysis and desirability function calculations. The rest of the calculations for the analysis were performed with the help of Microsoft Excel 2007 software (Microsoft, USA).

Standard solutions preparations: Accurately weighed and transferred 25mg of Dolutegravir and 12.5 mg of Rilpivirine working Standards into a 50ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

Mobile phase preparation: The mobile phase used was 0.01N Potassium dihydrogen ortho phosphate and Acetonitrile in the ratio of 57.4: 42.6 respectively. The buffer have been prepared by accurately weighed 1.36 gm of Potassium dihydrogen ortho phosphate in a 1000ml of volumetric flask add about 900ml 0f milli-Q water and sonicate to degas and finally make up the volume with water then add 1ml of Triethylamine then pH adjusted to 3.0 with dil. Orthophosphoric acid solution.

Assessment of validation parameters: As per ICH guidelines, analytical method was known to be validated if it has been evaluated through characteristics such as accuracy, precision, linearity, limit of detection, limit of quantification and robustness. The characteristics of analytical method should be within prescribed limit and defined standards to confirm its accuracy and authenticity. Spiking, mean, standard deviations and relative standard deviation are the terms used to measure the characteristics for a validation of an analytical method.

Accuracy as recovery : The accuracy of the technique was dictated by computing percentage recovery of Dolutegravir and Rilpivirine. By applying standard addition strategy for both the drugs recovery studies have been carried out at three unique concentrations such as 50, 100 and 150% of label claim. At each dimension ICH recommendation for performing accuracy was minimum triplicate at each concentration and results acquired were compared.

Table1. Variables selected in Central Composite Design

Factors	Levels used		
	Low(-1)	Medium(0)	High(+1)
Independent variables			
Flow rate	0.9	1	1.1
Buffer in mobile phase	55	60	65
Wavelength	255	260	265
Dependent variables			
t_R- Dolu	3.07	4.06	4.9
t_R- Ril	2.53	3.09	3.6
Area-Dolu	2.77679E+006	3.58319E+006	4.66929E+006
Area-Ril	532918	668730	898096
Resolution	3.5	5.16025	6.4

Table2. A comparison of initial HPLC method, optimized HPLC method from Design Expert Experiments

Parameter	Initial method	Optimized method
Column	Kromosil 250 X 2.1mm, 1.7m	Kromosil 250 X 2.1mm, 1.7m
Injection volume	10µL	10µL
Column temperature	30°C	30°C
Flow rate	1 ml/min	0.998ml/min
Detection	257 nm	257.06 nm
Mobile phase	40:60 ACN & 0.01N Potassium dihyrogen ortho phosphate	42.6:57.4 ACN & 0.01N Potassium dihyrogen ortho phosphate
Run time	7 minutes	7 minutes

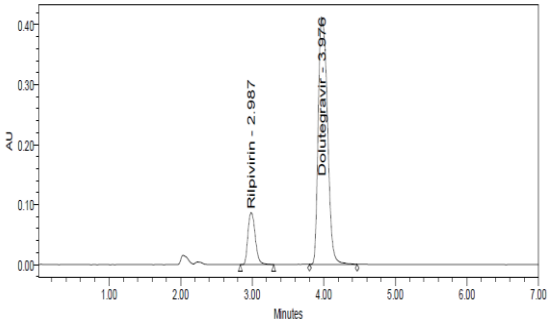

Fig 3. Chromatogram of standard drug

Table3. Experimental factors and responses by using Central Composite Design data matrix

Design point	Factor 1 Flow rate ml/min	Factor 2 Buffer %	Factor 3 Wavelength nm	Response 1 t_R Dolu	Response 2 t_R- Rilp	Response 3 Area- Dolu	Response 4 Area- Rilp	Response 5 Resolution
1	0.9	65	265	3.774	2.962	3166485	649616	5.2
2	0.9	65	255	3.772	3.107	3970276	711927	4.4
3	1	51.591	260	4.9	3.5	3552648	674074	6.4
4	1	68.409	260	3.2	2.7	3664394	700268	3.5
5	1.1	55	255	3.9	2.9	3304412	588815	5.7
6	1	60	260	3.7	2.9	3535505	674041	5
7	1	60	260	3.78	2.97	3547858	674697	4.9
8	1	60	260	3.78	2.94	3571523	680631	4.9
9	1	60	260	3.79	2.97	3559023	678365	4.9
10	1.1	55	265	3.94	2.93	2783926	580472	5.6
11	1.1	65	265	3.08	2.54	2846777	584659	3.8
12	1	60	260	3.79	2.97	3633533	690714	4.7
13	1.16818	60	260	3.23	2.57	3093037	589500	4.1
14	0.831821	60	260	4.52	3.53	4669293	898096	4.8
15	0.9	55	265	4.86	3.6	3464634	716025	5.3
16	0.9	55	255	4.85	3.59	3476003	705644	5.1
17	1	60	260	3.79	2.98	3601976	684113	4.8
18	1	60	251.591	3.78	2.96	3445156	575147	4.7
19	1	60	268.409	3.79	2.97	2776791	532918	4.7
20	1.1	65	255	3.07	2.53	3371198	602934	3.7

Precision: Precision is the closeness agreement between series of measurement obtained from multiple sampling of homogenous sample under prescribed conditions. Precision includes repeatability and intra laboratory repeatability; both were performed for six replicates at concentration of and 50 ppm Dolutegravir and 25 ppm Rilpivirine, respectively.

Linearity and calibration range: Linearity was established from 25 to 150 % of working standard concentration using minimum of six calibration levels (25, 50, 75, 100, 125 and 150 %) having a range of 12.5 to 75 ppm for Dolutegravir and 6.25 to 37.5 ppm for Rilpivirine. The calibration curve was plotted as the concentration of the reference standard of substance against peak area and linearity of the method was evaluated by regression analysis.

Limit of detection and Limit of quantification: The detection limit and quantification limit of method were obtained from the following formulae:

$$\mathbf{LOD = 3.3\times (\sigma/S)}$$

$$\mathbf{LOQ = 10\times (\sigma/S)}$$

Where

σ = standard deviation of response and

S = slope obtained from calibration curves of linear study.

Robustness: A few parameters like flow rate, percentage of organic phase and temperature were changed deliberately for the robustness evaluation using HPLC method. One factor was changed at one time to estimate the effect. Each factor selected was changes at three levels (-1, 0, +1) with respect to the optimized parameters. Robustness of the method was done at the concentration level 50 ppm and 25 ppm Dolutegravir and Rilpivirine, respectively.

Stability studies: The stability studies have been performed to know how long the drug is stable at the accelerated conditions. This was done with the help of ICH guidelines. The stability studies were performed for acid, alkaline, oxidation, photolytic, hydrolytic and thermal studies.

Acid degradation: For the determination of acid stress conditions 1 ml of stocks solution Rilpivirin and Dolutegravir 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60°C .The resultant solution was diluted to obtain (25ppm&50ppm) solution and 3 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkaline degradation: To 1 ml of stock solution Rilpivirin and Dolutegravir 1 ml of 2 N Sodium hydroxide was added and refluxed for 30mins at 60°C. The resultant solution was diluted to obtain (25ppm&50ppm) solution and 3 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Peroxide degradation (oxidation): To 1 ml of stock solution of Rilpivirin and Dolutegravir 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60°C. For HPLC study, the resultant solution was diluted to obtain (25ppm&50ppm) solution and 3 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Photolytic degradation (photo stability studies): The photochemical stability of the drug was also studied by exposing the (250ppm&500ppm) solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain (25ppm&50ppm) solutions and 3 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Thermal degradation (Dry heat degradation studies): The standard drug solution was placed in oven at 105°C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to (25ppm&50ppm) solution and 3µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Hydrolytic degradation (neutral degradation): Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to (25ppm&50ppm) solution and 3 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Analysis in the marketed dosage form: About 20 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to one tablet was transferred into a 100mL volumetric flask, 50mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipetted out into a 10 ml volumetric flask and made upto 10ml with diluent.

RESULTS AND DISCUSSION

Optimization of Central composite design (CCD): The method optimization was performed by employing design expert software. Three factors and five responses were selected for randomized response surface central composite quadratic design using 20 experimental runs tabulated in (Table.3). Various factors optimized were flow rate, wavelength and % buffer in the mobile phase. The limits of these variables were set to yield specific desired numerical conditions for retention time, peak area and resolution. The effects of independent variables on the responses for the 20 experimental runs are summarized in (Table.4). The polynomial equations for the response generated by ANOVA are depicted below: Retention time (t_R Dolu) = +3.77 -0.40 * A -0.49* B +5.771E-003 * C +0.059 * AB +4.750E-003 * AC -4.750E-003 * BC

+0.035 * A2 +0.097 * B2 +3.539E-003 * C2

Retention time (t_R Ril) = +2.96 -0.29 * A -0.24 * B -5.725E-003 * C +0.045 * AB +0.022 * AC -0.022 * BC +0.029 * A2 +0.046 * B2 -1.426E-003 * C2

Peak area (Dolu)= +3.582E+006 -3.238E+005 * A +37614.42 * B -2.185E+005 * C -8310.87 * AB -28718.37 * AC -99544.62 * BC +59094.27 * A2 -37299.94 * B2 -2.132E+005 * C2

Peak area (Rilp) = +6.810E+005 -69219.93 * A +163.50 * B -10951.89 * C +9804.00 *AB +3164.00* AC -10328.00 * BC +18602.92* A2 -1417.75 * B2 -48489.31* C2. Resolution = +4.81 -0.17 * A -0.69 * B +0.073 * C -0.38 * AB -0.12 * AC +0.100 * BC

The above equations in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels of the factors are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

With the help of design expert software the model was obtained and it was validated by using ANOVA. The results are depicted in (Tables.5, 6&7). By using a lack of fit test, the model was examined which indicated a significant lack of fit value corresponding with a lower p-value as compared to the model F-value. From the ANOVA results, the model F-value of D and R implied that the model was significant.

Table 4. Summary results for responses in Quadratic model

Response	Models	Adjusted R^2	Predicted R^2	SD	PRESS	% CV	Adequate precision
t_R- Dolu	Quadratic	0.9965	0.9928	0.032	0.040	0.83	79.005
t_R- Rilp	Quadratic	0.9908	0.9755	0.031	0.049	1.03	48.534
Area-Dolu	Quadratic	0.7413	-0.0556	2.155E+005	3.601E+012	6.24	11.039
Area- Rilp	Quadratic	0.8837	0.5393	26856.61	5.430E+010	4.07	17.093
Resolution	2FI	0.8780	0.7396	0.24	2.38	5.04	17.138

Table 5. Summary results of ANOVA statistical analysis for models and response (t_R) for finally suggested Quadratic model

Source	Sum of Squares		Degree of freedom		Mean Square		F- Value		P-Value	
	Dolu	**Rilp**	**Dolu**	**Rilp**	**Dolu**	**Rilp**	**Dolu**	**Rilp**	**Dolu**	**Rilp**
Model	5.64	1.98	9	9	0.63	0.22	602.41	228.43	<0.0001	<0.0001
Flow rate	3.30	1.16	1	1	3.30	1.16	2080.02	1198.57	<0.0001	<0.0001
% of Buffer	2.16	0.76	1	1	2.16	0.76	3172.68	790.24	<0.0001	<0.0001
Wavelength	4.549E-004	4.476E-004	1	1	4.549E-004	4.476E-004	0.44	0.46	0.5233	0.5112
Residual	0.010	9.646E-003	10	10	1.040E-003	9.646E-004				
Lack of Fit	4.117E-003	5.096E-003	5	5	8.235E-004	1.019E-003	0.66	1.2	0.6730	0.4521

Table 6. Statistical analysis for factors and response (Peak area) by ANOVA for response surface quadratic model

Source	Sum of Squares		df		Mean Square		F- Value		P-Value	
	Dolu	**Rilp**	**Dolu**	**Rilp**	**Dolu**	**Rilp**	**Dolu**	**Rilp**	**Dolu**	**Rilp**
Model	2.947E+012	1.107E+011	9	9	3.274E+011	1.230E+010	7.05	17.05	0.0026	<0.0001
Flow rate	1.432E+012	6.544E+010	1	1	1.432E+012	6.544E+010	30.83	90.72	0.0002	<0.0001
% of Buffer	1.932E+010	3.651E+005	1	1	1.932E+010	3.651E+005	0.42	5.061E-004	0.5335	0.9825
Wavelength	6.521E+011	1.638E+009	1	1	6.521E+011	1.638E+009	14.04	2.27	0.0038	0.1627
Residual	4.645E+011	7.213E+009	10	10	4.645E+010	7.213E+008				
Lack of Fit	4.577E+011	7.015E+009	5	5	9.155E+010	1.403E+009	68.14	35.55	0.0001	35.55

Table7. Statistical analysis for factors and response (Resolution) by ANOVA for response surface quadratic model

Source	Sum of Squares	Degree of freedom	Mean Square	F- Value	P-Value
Model	**8.39**	**6**	**1.40**	**23.80**	**<0.0001**
Flow rate	**0.41**	**1**	**0.41**	**7.04**	**0.0199**
% of Buffer	**6.58**	**1**	**6.58**	**111.87**	**<0.0001**
Wavelength	**0.073**	**1**	**0.073**	**1.25**	**0.2846**
Residual	**0.76**	**13**	**0.059**		
Lack of Fit	**0.71**	**8**	**0.089**	**8.33**	**0.0159**

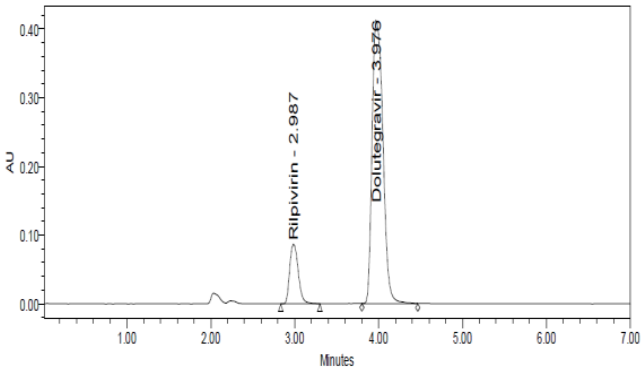

Fig 4.Chromatogram of drug product

Table8. Optimized method- Report card

Response Variable	Initial method	Optimized method	
		Predicted by DE	Experimental values
Retention time- Ril	2.973	3.096	2.970
Retention time- Dolu	3.802	4.062	3.958
Peak Area- Rilp	670712	668730	661335
Peak Area- Dolu	3595513	3583196	3625220
Resolution	5.5	5.16	5.2

Table9. Linearity

Concentration (ppm) Rilpivirine	Concentration (ppm) Dolutegravir	Linearity Range
6.25	12.5	25
12.5	25	50
18.75	37.5	75
25	50	100
31.25	62.5	125
37.5	75	150

Table10. Validation summary and SST parameters

Parameter	Rilpivirine	Dolutegravir
Linearity range	25- 150%	25- 150%
Correlation coefficient	0.999	0.999
Slope	26403	70991
Intercept	2860	5343
LOD(ppm)	0.24	0.10
LOQ (ppm)	0.72	0.30
Accuracy (Recovery %)	100.19	99.86
Precision (% RSD)		
Repeatability	0.335	0.361
Intermediate precision	0.4509	0.499
Robustness	Robust	Robust
Retention time (min)	2.977	3.962
USP Resolution	_	5
USP Plate Count	4640	5293
Asymmetry factor	1.21	1.23

Table11. Results of Robustness studies

Robustness parameter	% RSD for Dolutegravir	% RSD for Rilpivirine
Flow rate (0.898mL/min)	1.32	0.76
Flow rate (1.198mL/min)	1.5	1.6
Mobile phase (- 5%)	0.9	0.6
Mobile phase (+5%)	1.2	1.2
Temperature (28°C)	1.42	0.60
Temperature (32°C)	1.89	0.92

Table12. Summary of forced degradation results

Stress conditions	Dolutegravir			Rilpivirine		
	Degradation (%)	Purity of angle	Purity of Threshold	Degradation (%)	Purity of angle	Purity of Threshold
Acid	6.11982	1.041	1.251	5.96	0.224	0.428
Alkali	5.55613	0.932	1.265	5.65	0.193	0.430
Peroxide	3.15966	0.778	1.010	3.95	0.209	0.406
Neutral	1.05	0.199	0.390	1.93	1.185	1.139
Thermal	0.81	0.231	0.3851	2.18	0.979	1.175
Photolytic	0.14	0.212	0.413	0.49	0.931	1.134

Table13. Assay results obtained by the proposed method for the drugs in pharmaceutical preparations

Parameter	Rilpivirine	Dolutegravir
Mean peak area	667360	3595957
Recovery (%)	100.26	99.59
RSD (%)	0.36	0.39

Table14. System suitability parameters of Drug product

Peak name	Retention time (min)	Peak area	USP tailing	USP plate count	USP resolution
Rilpivirine	2.987	3718205	1.29	4916	4.9
Dolutegravir	3.976	616699	1.24	4403	-

The perturbation plots are constructed to evaluate the effect of the factors on the retention time, peak area of each drug and resolution. The chromatogram of standard Dolutegravir and Rilpivirine at optimized conditions obtained by the design is shown in (fig 3). The results of system suitability parameters like resolution, plate count, peak area and asymmetric factor were measured to verify optimum conditions are within the limit. The comparison of initially developed method and optimized method predicted by the design and experimental values are reported in (Table.8).

Results of validation parameters: The optimized method was validated in compliance with ICH guidelines. Average percentage recovery for Dolutegravir and Rilpivirine were found to be 99.86 and 100.19% respectively, while % RSD values are less than 1% indicating accuracy of the repeated method. The calibration curve was found to be linear over the range of 25- 150% with concentration range of Dolutegravir was 12.5-75 ppm and of Rilpivirine was 6.25-37.5 ppm respectively (Table.9). The limit of detection and limit of quantification for Dolutegravir was found to be 0.10 and 0.30 ppm and for Rilpivirine 0.24 and 0.72 ppm, respectively. The precision data representing both repeatability and intermediate precision are summarized in (Table.10). The % RSD values for both repeatability and intermediate precision were less than 2% which indicates that the proposed method is precise. The results for validation and system suitability parameters are shown in (Table.10). The results for robustness are presented in (Table.11), which shows that change in conditions like flow rate, mobile phase ratio and column temperature did not significantly affects the recoveries, peak area and retention time of the drugs indicating that the proposed method was

robust. The % RSD was calculated, which was found to be in permissible limit.

Stability studies results: The sample was subjected to various stress conditions and the stability of the method was observed. The results of stability studies (Table.12) revealed that the method is stability indicating.

Analysis of tablet formulation: The validated method conditions has been employed for the estimation of the Dolutegravir and Rilpivirine content in a commercially available brand of the tablet containing 50 mg of Dolutegravir and 25 mg of Rilpivirine (Juluca). The potency of the tablet formulation sample was found to be 99.47 % of Dolutegravir and 99.62% of Rilpivirine, respectively (Table.13). and the system suitability parameters of drug product is shown in (Table.14). The amount measured was in good agreement with the label claims. The results of the assay indicated that the method was selective for analysis of Dolutegravir and Rilpivirine without interference from the excipients (fig 4).

CONCLUSION

A fruitful, simple, robust chemometric approach for RP-HPLC method development technique has been utilized for Dolutegravir and Rilpivirine. Comparison of experimental results with predicted results illustrated the preciseness of the design. The stability of the developed technique was shown by exposing the drug to different stress conditions. The acquired results revealed that the stability indicating power of the strategy. Further validation was performed in consistence with ICH guidelines and robustness of the method was checked by changing three chromatographic parameters. The validation result indicates strategy meets the regulatory aspects; henceforth concerning the method done, it very well may be utilized in the routine quality control examination of the pharmaceutical formulation.

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