J. Pharm. Res. 2021;20(3):18–23

Print ISSN: 0973-7200
Online ISSN: 2454-8405

Journal of Pharmaceutical Research

RESEARCH ARTICLE

Development and Validation of RP-HPLC Method for the Estimation of Dolutegravir and Rilpivirine in Bulk and its Tablet Dosage form

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ARTICLE INFO

Article history:
Received 06.04.2021
Revised 12.08.2021
Accepted 04.09.2021
Published 25.11.2021

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https://doi.org/10.54839/v20i3.ms21040C

ABSTRACT

An accurate and precise method was developed and validated for the simultaneous estimation of the Dolutegravir and Rilpivirine in Tablet dosage form. Chromatogram was run using Agilent C_{18} column (4.6x150mm, 5μm) with mobile phase containing KH_2PO_4 buffer of pH 3.5 and Acetonitrile in the ratio of 45:55 v/v was pumped through column at a flow rate of 1mL/min. Temperature was maintained at 30°C. Selected wavelength was 240.0 nm. Retention time of Dolutegravir and Rilpivirine was found to be 2.239 min and 2.899 min respectively. %RSD of the Dolutegravir and Rilpivirine for system precision was found to be 0.9 and 0.6 respectively. Accuracy was performed in triplicate and the % Recovery was obtained as 99.33% and 100.5% for Dolutegravir and Rilpivirine respectively. LOD, LOQ values for Dolutegravir was 0.2 μg/mL, 0.6 μg/mL and for Rilpivirine was 0.02, 0.06 μg/mL respectively. So, the method developed was simple, accurate and reproducible can be adopted in regular Quality control test in pharmaceutical Industry.

Keywords: Dolutegravir; Rilpivirine; RPHPLC; Method development and Validation

INTRODUCTION

Dolutegravir[1] is 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) and the selected drugs inhibit HIV integrase by binding to the active site and blocks the retroviral DNA integration. Rilpivirine[2] is a non-nucleoside reverse transcriptase inhibitor used especially for treating HIV-1 infections in treatment-naive patients. It is a derivative of diarylpyrimidine, a class of molecules that resemble pyrimidine nucleotides found in DNA. Chemically rilpivirine is 4-[[4-[(E)-2-cyanoethenyl]-2,6-dimethyl-anilino]pyrimidin-2-yl]amino]benzonitrile).[3] The structures of the two drugs are shown in Figures 1 and 2. These drugs are used in combination for the treatment of HIV-1 infections in treatment of native patients.

There are several methods available for estimation of dolutegravir individually by UV[4], HPTLC[5] and UPLC[6] method. Rilpivirine was estimated by UV[7] method. But there are also methods reported for simultaneous estimation of dolutegravir and rilpivirine by UV[8], HPLC[9,10] and UPLC[11] method in a combination formulation. The aim of the present work is to develop and validate RP-HPLC method which will be specific, precise, accurate, robust and sensitive for simultaneous determination of dolutegravir and rilpivirine in bulk drug and formulations.

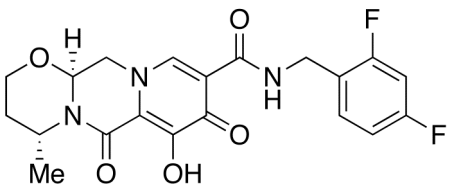
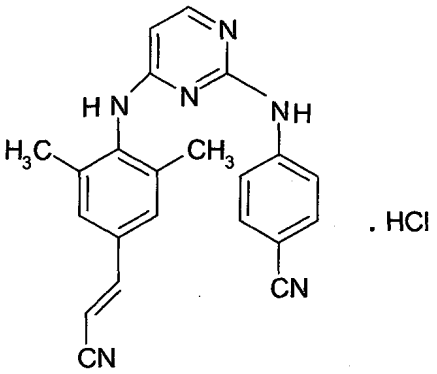


Fig. 1: Structure of Dolutegravir

Dolutegravir and Rilpivirine pure drugs were obtained from BMR chemical private limited, Combination dolutegravir and rilpivirine tablets (Juluca), containing dolutegravir 50mg and rilpivirine 25mg were obtained from local pharmacy. Water, methanol and acetonitrile were of HPLC grade. Potassium dihydrogen orthophosphate and ortho phosphoric acid were procured from Rankem Chemicals. All



Rilpivirine hydrochloride (I)

Fig. 2: Structure of Rilpivirine

the dilutions were done in standard volumetric flask.

MATERIALS AND METHODS

HPLC Waters 2695 separation mode with T-60 UV-Visible spectrophotometer and a Rheodyne injector valve fitted with 10μL sample loop was used. Chromatographic separation was performed on Agilent C_{18} column (4.6 x150mm, 5μm). The column effluent was monitored with PDA detector set at 240nm and column temperature at 30°C.

Chemicals and Reagents

Preparation of Buffer

Accurately weighed 1.36 gm of potassium dihydrogen ortho phosphate in a 1000 mL of Volumetric flask add about 900 mL of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1 mL of triethylamine then PH adjusted to 3.5 with dil. orthophosphoric acid solution

Preparation of standard solution

Accurately weighed 25mg of Dolutegravir and 12.5mg of Rilpivirine were transferred to 50mL flasks separately and 3/4th of diluents was added to the flask and sonicated for 10min. Volume was made up to the mark with diluent and labelled as standard stock solution (500μg/mL of dolutegravir and 250μg/mL of rilpivirine).

Preparation of working standard solutions (100% solution)

1mL from each stock solution was pipetted out and taken into a 10mL volumetric flask and volume was made up to the mark with diluent (50μg/mL of dolutegravir and 25μg/mL of rilpivirine).

Preparation of Sample solution

20 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred in to a 100mL volumetric flask, 5mL of diluents was added and sonicated for 25 min, further the volume was made up to the mark using diluent and filtered using HPLC filter of 0.45μm pore size.

Preparation of Sample working solutions (100%solution)

1 mL from the above stock solution was pipetted out and was transferred into a 10 mL volumetric flask and made up to the mark using diluent to give 50μg/mL of Dolutegravir and 25μg/mL of Rilpivirine respectively.

METHOD OPTIMIZATION

The separation was performed on Kromasil C8 column (4.6 x 150 mm, 5 μm) using 0.1% OPA and acetonitrile in the ratio of 50:50 v/v as mobile phase, both peaks were eluted but peak splitting was observed. Then the mobile phase composition was varied using 0.1% OPA: Methanol in the ratio of 50:50 v/v using column of Agilent C_{18} column (4.6 x 150 mm, 5 μm) gave one retention peak due to rilpivirine. Then KH_2PO_4 buffer of pH 3.5 and Acetonitrile in the ratio of 45:55 v/v was selected as mobile phase and injected at a flow rate of 1mL/min. The column temperature was maintained at 30°C and the detection wavelength was 240 nm. The retention times were about 2.239 min and 2.899 min for rilpivirine and dolutegravir respectively and optimised chromatogram was shown in Figure 3.

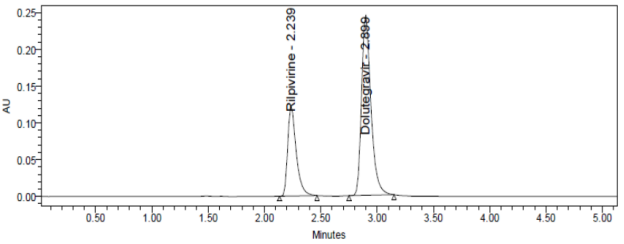


Fig. 3: Chromatogram of Dolutegravir and Rilpivirine for standard solution

RESULTS

METHOD VALIDATION PARAMETERS

The method was validated for parameters like stability, linearity, specificity, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ).

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of dolutegravir (50μg/mL) and

Table 1: System suitability parameters of Dolutegravir and Rilpivirine

S. No	Dolutegravir			Rilpivirine			
	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	2.217	5009	1.32	2.210	3745	1.42	4.2
2	2.740	4528	1.34	2.124	3154	1..42	4.1
3	2.894	5763	1.34	2.201	4233	1.44	4.1
4	2.899	5321	1.34	2.239	3916	1.42	4.6
5	2.937	5864	1.32	2.271	4409	1.43	4.4
6	2.987	5901	1.34	2.306	4440	1.43	4.5

rilpivirine (25μg/mL) and the solutions were injected six times and the parameters like peak tailing, resolution and plate count were determined. The results were depicted in Table 1.

Specificity

Specificity of the method was determined by injecting blank and placebo to check whether peaks in the blank and placebo are eluting with drugs peaks and the chromatogram was shown in Fig.4 and 5.

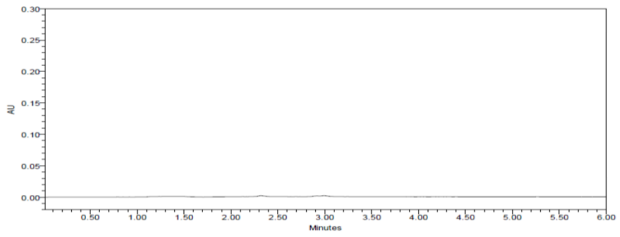


Fig. 4: Chromatogram of blank

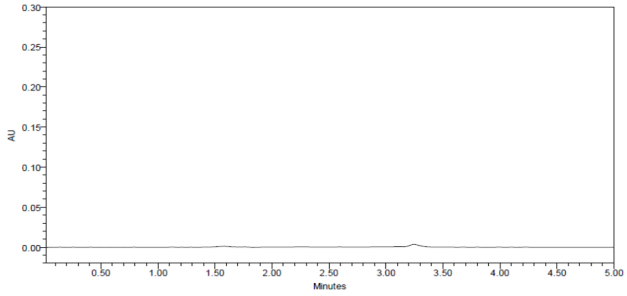


Fig. 5: Chromatogram of placebo

Linearity

The linearity of dolutegravir and rilpivirine was in the concentration range of 12.5 -75 μg/mL and 6.25-37.5 μg/mL respectively with correlation coefficient (r^2) of 0.9999 and 0.9997 for dolutegravir and rilpivirine respectively. The results are given in Table 2 and calibration curves are shown Figures 6 and 7.

Table 2: Linearity data for Dolutegravir and Rilpivirine

Dolutegravir		Rilpivirine	
Conc (μg/mL)	Peak area	Conc (μg/mL)	Peak area
0	0	0	0
12.5	103359	6.25	102055
25	200439	12.5	216049
37.5	297910	18.5	305830
50	396270	25	415891
62.5	496039	31.5	525961
75	601434	37.5	622606

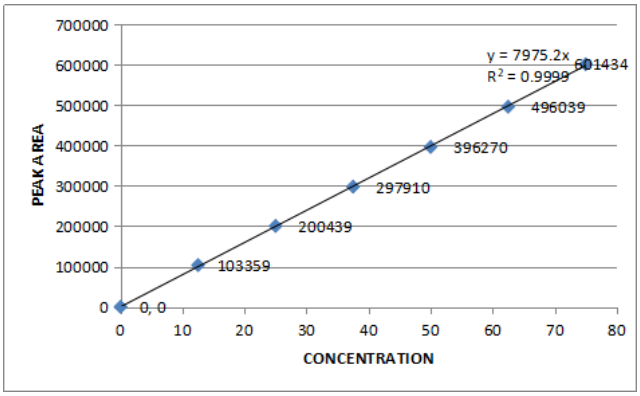


Fig. 6: Calibration curve of Dolutegravir

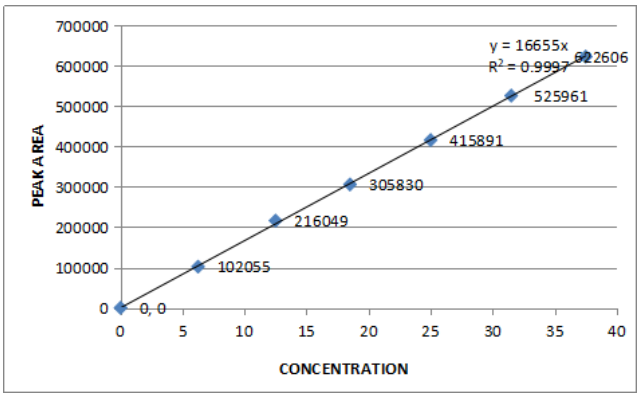


Fig. 7: Calibration curve of Rilpivirine

Accuracy

The accuracy of an analytical method is the closeness of the test results obtained by that method to the true value. It is done by measuring the amount of pure drug recovered at three different concentrations (50%, 100% and 150%) in triplicate. The results are given in Tables 3 and 4.

Table 3: Accuracy data of Dolutegravir

% Level	Amount taken	Amount Spiked (μg/ mL)	Amount recovered (μg/ mL)	% Recovery	Mean % Recovery
50%	(50 μg/mL)	25	74.7	99.6	99.46
		25	74.4	99.2	
		25	74.7	99.6	
100%	(50 μg/mL)	50	99.2	99.2	99.26
		50	99.4	99.4	
		50	99.2	99.2	
150%	(50 μg/mL)	75	124.83	99.86	99.85
		75	124.81	99.84	
		75	124.81	99.86	

Table 4: Accuracy data of Rilpivirine

% Level	Amount taken (μg/mL)	Amount Spiked (μg/mL)	Amount recovered (μg/mL)	% Recovery	Mean % Recovery
50%	(25 μg/mL)	12.5	24.8	99.2	99.73
		12.5	25	100	
		12.5	25	100	
100%	(25 μg/mL)	25	49.6	99.2	99.2
		25	49.6	99.2	
		25	49.6	99.2	
150%	(25 μg/mL)	37.5	62	99.2	99.25
		37.5	62	99.2	
		37.5	62.1	99.36	

Precision

The precision of an analytical procedure is usually expressed as relative standard deviation (coefficient of variation) of a series of measurements.

System precision

It was performed by injecting a standard solution of Dolutegravir and Rilpivirine at working concentration of 100% six times that is 50μg/mL of Dolutegravir and 25μg/mL of Rilpivirine). The % RSD was calculated for peak area and R_t and results are given in Table 5.

Table 5: Precision data of Dolutegravir and Rilpivirine

S. No	Area of Dolutegravir	Area of Rilpivirine
1.	1327652	616251
2.	1337270	613005
3.	1329689	615445
4.	1329926	617843
5.	1357152	615461
6.	1321429	623139
Mean	1333853	616857
S.D ±(n=6)	12490.7	3452.1
%RSD	0.9	0.6

LOD and LOQ

For determining the limit of detection (LOD) and limit of quantitation (LOQ), the method based on the standard deviation and slope was adopted.

The limit of detection for dolutegravir and rilpivirine was 0.2μg/mL and 0.6μg/mL respectively and limit of quantitation (LOQ) was 0.02μg/mL, 0.06μg/mL for dolutegravir and rilpivirine respectively as shown in Table 6.

Table 6: Sensitivity data of Dolutegravir and Rilpivirine

Molecule	LOD	LOQ
Dolutegravir	0.2μg/mL	0.6μg/mL
Rilpivirine	0.02μg/mL	0.06μg/mL

Robustness

Small deliberate changes in method like flow rate, mobile phase ratio, and temperature were made. Robustness conditions like flow rate of +0.1mL/min, mobile phase ratio of + 5v/v, temperature +5°C was maintained and samples were injected in triplicate manner. There was no recognized change in the peak area and were within range as per ICH Guide lines as shown in Table 7.

Assay of marketed formulation of Dolutegravir and Rilpivirine

The validated RP-HPLC method was successfully applied for the assay of dolutegravir and rilpivirine in marketed formulations. The chromatogram for sample was shown in Figure 8. Assay results were represented in Tables 8 and 9.

DISCUSSION

A new HPLC method was developed for estimation of dolutegravir and rilpivirine by trial and error method i.e., by using Kromasil C8 column, peak splitting was observed. Then the mobile phase composition was changed to 0.1%

Table 7: Robustness data for Dolutegravir and Rilpivirine.

Sl.no	Conditions	% RSD of Dolutegravir	% RSDof Rilpivirine
1	Flow rate (-) 0.9 mL/min	1.3	0.6
2	Flow rate (+) 1.1 mL/min	1.2	0.9
3	Mobile phase (-) 55 B:45 A	1.0	0.7
4	Mobile phase (+) 60 B:40 A	1.2	0.8
5	Temperature (-) 25°C	1.1	0.7
6	Temperature (+) 35°C	1.3	1.1

Table 8: Assay Data of Dolutegravir

Sl.no	Standard Area	Sample area	% Assay
1	1327652	1328520	99.50
2	1337270	1328860	99.53
3	1329689	1346299	100.83
4	1329926	1335282	100.01
5	1357152	1327674	99.44
6	1321429	1339273	100.31
Avg	1333853	1334318	99.93

Table 9: Assay Data of Rilpivirine

Sl.no	Standard Area	Sample area	% Assay
1	616251	619629	100.35
2	613005	621117	100.59
3	615445	614870	99.58
4	617843	621588	100.67
5	615461	614001	99.44
6	623139	615660	99.71
Avg	616857	617811	100.05

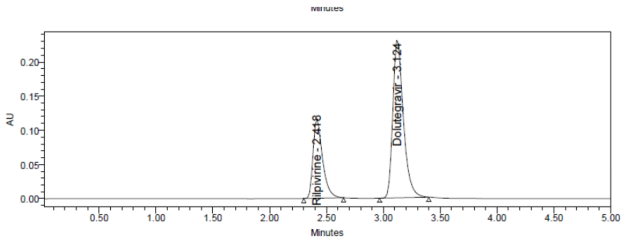


Fig. 8: Sample chromatogram of Dolutegravir and Rilpivirine

OPA: Methanol in the ratio of 50:50 v/v using column of Agilent C_{18} column (4.6 x 150 mm, 5 μm) gave one retention peak due to rilpivirine. Then by keeping the column constant, different mobile phase was used to give satisfactory separation, well resolved and good symmetrical peaks which was obtained with the mobile phase of KH_2PO_4 buffer of pH 3.5: Acetonitrile in the ratio of 45:55 v/v. UV overlain spectra of Dolutegravir and Rilpivirine shows that both the drugs absorb appreciably at 240 nm, So 240 nm was selected as the detection wavelength in liquid chromatography. Optimization of mobile phase was done based on considering factors like resolution, asymmetric factor and peak area.

Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase (potassium dihydrogen phosphate: acetonitrile) in the ratio of 45:55 v/v. The retention time of dolutegravir was found to be 2.239 min and that of rilpivirine was found to be 2.899 min, respectively. Resolution between dolutegravir and rilpivirine was found to be 4.583 which indicate good separation of both the compounds. The asymmetric factor for dolutegravir and rilpivirine was found to be 1.420 and 1.350 respectively. The calibration curve for dolutegravir and rilpivirine was obtained by plotting the respective peak areas versus their concentration over the range of 12.5-75 μg/mL and 6.25-37.5 μg/mL with correlation coefficient (r^2) of 0.9999 and 0.9997 for dolutegravir and rilpivirine respectively which indicates good correlation exist between concentration and response. Detection of limit for dolutegravir and rilpivirine was 0.2 and 0.02 μg/mL and quantitation limit was 0.6 μg/mL and 0.06 μg/mL respectively; which suggest that the method is sensitive. The % recovery of dolutegravir and rilpivirine was found to be in the range of 99.26-99.85 and 99.2-99.73% respectively, which shows that the developed method is accurate. The % RSD was found to be less than 2, which shows that the method was precise. The proposed RP-HPLC method was applied for the determination of dolutegravir and rilpivirine in tablet formulations and the assay values for dolutegravir and rilpivirine were comparable with the corresponding labelled amount.

Therefore the developed RP-HPLC method was suitable for quantification of the raw materials and formulation in combined dosage form.

ACKNOWLEDGEMENTS

We are very thankful to the Management of Malla Reddy Pharmacy College for the support during the execution of work and also for providing the facilities and equipment i.e. HPLC (High Performance Liquid Chromatography).

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