Research Article

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

B. Raj Kumar*[1], K. V.Subrahmanyam[2]
[1]Research Scholar & Asst.Prof, Jawaharlal Nehru Technological University Hyderabad, Telangana, India.
[2]Professor, Siddhartha Institute of Pharmacy, Ghatkeskar Mandal, Hyderabad, Telangana, India.
***Corresponding author's E-mail:** rajpharma2631@gmail.com**

Received: 04-03-2020; **Revised:** 15-05-2020; **Accepted:** 22-05-2020.

ABSTRACT

A Simple Rapid, precise and accurate High Performance Liquid Chromatography method was Developed and Validated for Determination of Dolutegravir and Rilpivirine in Bulk and Pharmaceutical Dosage Form. The Method Development was carried out by using X Bridge C18, (250mm x 4.6mm, 5µm), using a mixture 5g/l Octane-1-sulfonic acid added 7mL of TEA, pH 2.8; with OPA/ ACN (5:5) %v/v as mobile phase. The flow rate is 1.0 ml/min, with a detection wavelength of 243 nm. The retention time of Dolutegravir was found to be 4.6 min and Rilpivirine was 8.5 min. The percentage purity of Dolutegravir and Rilpivirine was found to be 99.14 % and 100.08 % respectively. The analytical method was validated according to ICH guidelines (ICH, Q2, (R1)). The method was found to be linear in the concentration ranges of 37.5µg/ml to 112.5µg/ml for Dolutegravir and 17.5µg/ml to 52.5µg/ml for Rilpivirine and correlation coefficient (r2) value was found to be 0.9996 and 0.9982, The precision study was precise, robust, and repeatable. The LOD values are 2.00µg/ml and 2.02µg/ml and LOQ values are 6.67 and 6.75 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Dolutegravir and Rilpivirine in API and Pharmaceutical Dosage Form.

Keywords: DTG, RPV,RP-HPLC, precision, API, Pharmaceutical Dosage Form, LOD. LOQ, TEA.

INTRODUCTION

More than 37 million people are living with HIV infection worldwide, with about 2 million new infections occurring every year, and, in USA, about 40,000 new infections occur annually, adding to 1.2 million individuals already living with HIV infection[1-4]. DTG/RPV is a two-drug combination of DTG, an INSTI, and RPV, an HIV-1 NNRTI, and is approved as a regimen for the maintenance treatment of HIV-1 infection for select individuals[5]. DTG is a second-generation integrase inhibitor with chiral, non racemic structure with a molecular weight of 419 g/mol. The chemical name of DTG sodium is sodium (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. The empirical formula is $C_{20}H_{18}F_2N_3NaO_5$. This medication is a white light yellow powder that is slightly soluble in water. Each coated tablet of 52.6 mg of DTG sodium is equivalent to 50 mg of DTG.

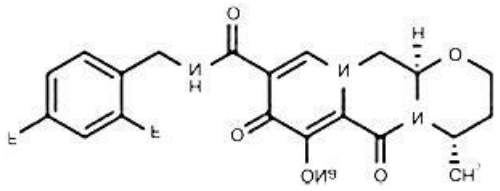

Figure: 1 Chemical structure of Dolutegravir.

In addition, the tablet includes d-mannitol, microcrystalline cellulose, povidone K29/32, sodium starch 343 glycolate, and sodium stearyl fumarate as well as iron oxide yellow, macrogol/polyethylene glycol (PEG), polyvinyl alcohol-part hydrolyzed, talc, and titanium dioxide.[6-7]. RPV is a diarylpyrimidine derivative and considered a second-generation NNRTI. The molecular formula for RPV is $C_{22}H_{18}N_6$ HCl with a molecular weight of 402.88 g/mol. The chemical name for RPV is 4-[[4-[[4-[(E)-2-cyanoethenyl]–2, 6-dimethylphenyl]amino]-2-pyrimidinyl]amino]benzo-nitrile monohydrochloride. Each tablet contains the inactive ingredients such as croscarmellose sodium, lactose monohydrate, magnesium stearate, polysorbate 20, povidone K30, and silicified microcrystalline cellulose. The tablet coating contains hypromellose 29,106 mPa.s, lactose monohydrate, PEG 3000, titanium dioxide, and triacetin.[9-10]

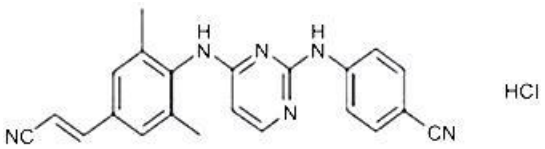

Figure 2: Chemical structure of RPV hydrochloride.

The literature survey shows that there are few methods related to simultaneous estimation of Dolutegravir and Rilpivirine had revealed that less HPLC analytical method development was reported in the articles. Majority of the research was done on bio analysis estimation of drug in human plasma[10]. The detailed study of literature related to Rilpivirine had revealed that majority of HPLC methods reported were focused on bio-analysis and estimation of drug in the pharmaceutical dosage form. But none of the reported method was based on forced degradation studies and not developed as stability indicating assay

method. There is a simultaneous estimation method for both Dolutegravir and Rilpivirine by UPLC& UPLC-MS/MS method but in human plasma. Most of the simultaneous methods involve binary mixture estimation[13] and by using dual wavelengths[14] for estimation of Dolutegravir and Rilpivirine[11-12]. Hence, there is need for development of a stability indicating RP-HPLC method for simultaneous estimation of Dolutegravir and Rilpivirine which has the capability of separating both analytes with its impurities.

METHODS

Chemicals and reagents

Acetonitrile (HPLC grade), orthophosphoric acid (HPLC grade), and water (HPLC grade) were purchased from Merck (India) Ltd., Worli, Mumbai, India. DTG and RPV reference standards were produced from Glenmark Pharmaceuticals Limited, Mahape, Navi Mumbai, India.

Optimized Chromatographic conditions

After various experimental trials and with reference to the acceptance criteria for various system suitability parameters, the following conditions were optimized for the Simultaneous Estimation of Dolutegravir and Rilpivirine in bulk API and its pharmaceutical preparation. The optimized method includes using X Bridge C18, 250mm x 4.6mm, dimension chromatographic column with 5µm internal diameter and ambient column temperature. Mobile phase was 7mL of Tri-ethylamine and 0.5 g of 1-octane sulfonic acid sodium salt in to a 1000mL of water and sonicate to dissolve, adjust to pH 2.0 (±0.05) with ortho-phosphoric acid and flow rate of 1.0mL/min. Detection of the analyte separated was done with UV detector at 280nm.

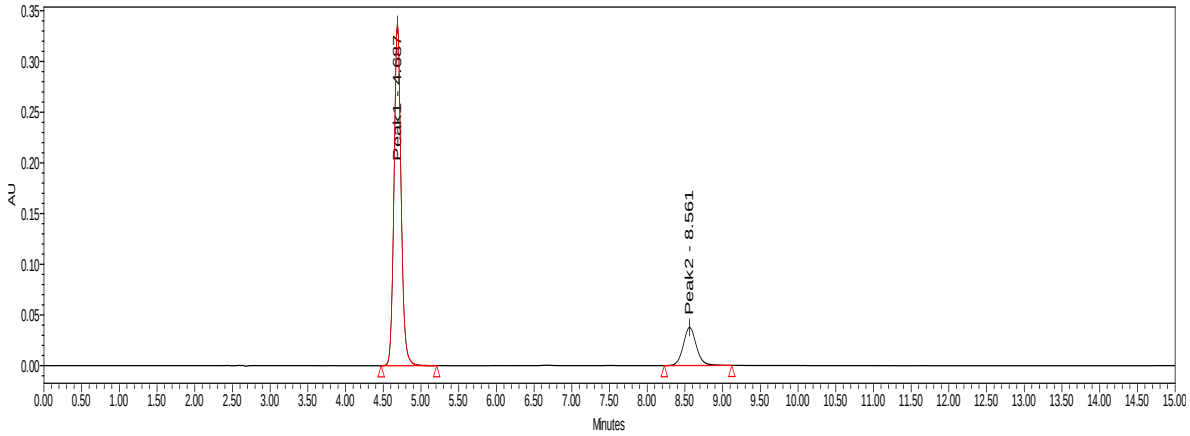

Figure 3: Optimized method Chromatogram

Preparation of Standard Solution

Weighed about 75 mg of Dolutegravir and 35mg of Rilpivirine into 100mL individual volumetric flask, dissolved & make up to volume with mobile phase. Further diluted 5.0 ml of Dolutegravir standard and 5.0 ml of Rilpivirine standard into a 100ml volumetric flask and make up to volume with mobile phase to give a concentration of 75µg/ml of each Dolutegravir and 35µg/ml of Rilpivirine.

Preparation of Sample

10 tablets of combination tablets were weighed and powdered. The tablet powder equivalent to 150mg of Dolutegravir and 75mg of Rilpivirine were weighed accurately and transferred into 200mL volumetric flask. 150ml of mobile phase was added to the flask, sonicated for 10min and made up to the mark with water. Later, it was filtered through 0.45µm. 5mL of this solution was taken in 50mL volumetric flask and made up to the volume with mobile phase and analysed.

Table 1: Showing the Assay Results of Dolutegravir and Rilpivirine 50mg/ 25mg tablet.

Formulation	Component	Label Claim (mg)	Amount Found (mg)	% Assay
Dolutegravir and Rilpivirine 50mg/ 25mg tablet	Dolutegravir	50	49.57	99.14
	Rilpivirine	25	25.02	100.08

Method Validation

The optimised methods for all the selected APIs were found to be satisfactory. To gain confidence on the method, all the proposed methods were validated as per ICH Q2 guidelines for Precision, Specificity, Linearity, Accuracy, LOD, LOQ and Robustness. Even though the individual approach for each API during validation might differ, the generalized approach is depicted below.

Precision

Repeatability

The precision of proposed method was ascertained by analysing the sample assay prepared 5 times and from

the obtained peak areas & retention times of analyte from each sample, percent relative standard deviation were calculated and presented in the Table No's 02 & 03

Table 2: Repeatability of the method for Dolutegravir

	RT	Peak Area	% Assay	USP Tailing	USP Resolution
Dolutegravir	4.687	2289643	99.8	9224.0	1.5
	4.68	2284254	99.6	9245.0	1.4
	4.689	2285846	99.6	9251.0	1.5
	4.688	2278542	99.3	9265.0	1.5
	4.687	2279651	99.4	9356.0	1.4
Average		2283587	99.5		
STD Dev		4559.803	0.19875		
%RSD		0.20	0.20		

Table 3: Repeatability of the method for Rilpivirine

	RT	Peak Area	% Assay	USP plate count	USP Tailing
Rilpivirine	8.563	412545	99.5	6555	1.3
	8.566	412322	99.4	6552	1.2
	8.565	412252	99.4	6585	1.2
	8.565	412875	99.6	6522	1.2
	8.568	414254	99.9	6586	1.2
Average		412849.6	99.6		
STD Dev		821.7745	0.198208		
%RSD		0.20	0.20		

Intermediate Precision

The intra & inter day variation of the method was carried out by calculating the amount of analyte in the formulation from six preparations to calculate %RSD within a day and day to day variation of the proposed method. Results were calculated and reported in Table No's 04, 05, 06& 07

Table 4: Intermediate precision for Dolutegravir Day-1

	RT	Peak Area	% Assay	USP Tailing	USP Resolution
Dolutegravir	4.625	2285522	99.6	9058.0	1.5
	4.665	2285447	99.6	9316.0	1.5
	4.658	2287874	99.7	9295.0	1.4
	4.657	2287441	99.7	9521.0	1.4
	4.639	2289554	99.8	9025.0	1.5
	4.708	2288558	99.8	9107.0	1.5
Average		2287168	99.7		
STD Dev		1727.507	0.075298		
%RSD		0.08	0.08		

Table 5: Intermediate precision for Dolutegravir Day-2

	RT	Peak Area	% Assay	USP Tailing	USP Resolution
Dolutegravir	4.582	2302542	100.4	9152.0	1.5
	4.539	2301125	100.3	9115.0	1.5
	4.609	2300225	100.3	9524.0	1.5
	4.612	2299853	100.2	9427.0	1.5
	4.877	2315526	100.9	9622.0	1.5
	4.695	2309178	100.7	9745.0	1.5
Average		2303854	100.4		
STD Dev		6606.474	0.287962		
%RSD		0.29	0.29		

Table 6: Intermediate precision for Rilpivirine Day-1

	RT	Peak Area	% Assay	USP plate count	USP Tailing
Rilpivirine	8.552	420102	101.3	5269.7	1.5
	8.547	417805	100.8	5100.5	1.4
	8.508	415289	100.2	5127.6	1.4
	8.958	418635	101.0	5269.7	1.4
	8.405	413251	99.7	5048.8	1.5
	8.709	412847	99.6	5997.2	1.4
Average		417016.4	100.6		
STD Dev		2735.18	0.659711		
%RSD		0.66	0.66		

Table 7: Intermediate precision for Rilpivirine Day-2

	RT	Peak Area	% Assay	USP plate count	USP Tailing
Rilpivirine	8.26	418236	100.9	5200	1.4
	8.54	416556	100.5	5118	1.5
	8.536	417589	100.7	5265	1.4
	8.205	417222	100.6	5224	1.4
	8.658	418462	100.9	5365	1.4
	8.455	416995	100.6	5244	1.4
Average		417613	100.7		
STD Dev		771.3034	0.186034		
%RSD		0.18	0.18		

Specificity

Specificity was performed by subjecting the individual API to various stress conditions like acid hydrolysis, base hydrolysis, thermal degradation, UV degradation and oxidative degradation. The solutions were then injected in the proposed method for analysis against their respective stress subjected blank. The amount of degradation each analyte undergone, the % impurities formed and the total mass balance achieved were tabulate in Table-No's 08 & 09

Table 8: Results showing the forced degradation of Dolutegravir

Stress condition	Condition	Assay of active substance	% Impurities	Mass Balance (%)
Control	NA	100.1	0.05	100.15
Acid Hydrolysis	1N HCl/24Hrs.	98.5	1.98	100.48
Basic Hydrolysis	1N NaOH/24Hrs.	99.4	0.18	99.58
Thermal Degradation	60°C/24Hrs.	99.53	0.13	99.66
Photo Degradation	UV-254nm/24Hrs.	95.5	4.85	100.35
Oxidative Degradation	1% H2O2/24Hrs.	99.22	0.28	99.5

Table 9: Results showing the forced degradation of Rilpivirine

Stress condition	Condition	Assay of active substance	% Impurities	Mass Balance (%)
Control	NA	99.82	0.15	99.97
Acid Hydrolysis	1N HCl/24Hrs.	95.48	3.51	98.99
Basic Hydrolysis	1N NaOH/24Hrs.	91.02	7.25	98.27
Thermal Degradation	60°C/24Hrs.	98.17	1.92	100.09
Photo Degradation	UV-254nm/24Hrs.	99.75	0.16	99.91
Oxidative Degradation	1% H2O2/24Hrs.	97.11	2.08	99.19

Linearity

Based on the concentration of the test solution proposed, linearity was performed for the concentration 50% to 150% of test concentration covering 5 concentrations. Linearity curves were plotted for each method with sample concentration to the AUC and determined y-intercept and slope of the curve.

Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug to the non-active placebo mimicking the Formulation. Accuracy of the optimized method was determined by recovery studies. The recovery studies was carried out at three replicates at each level (50%, 100% and 150%), the % recovery was in between 99.0-101.0% and %RSD was found to be less than 2.0. Sample solution.

Table 10: Linearity data for Dolutegravir

Dolutegravir Linearity Plot		
Concentration Level (%)	**Concentration in µg/ml**	**Peak Area**
50	37.5	1105942
75	56.25	1726597
100	75	2294221
125	93.75	2865912
150	112.5	3424587
Slope		30808.56
y-Intercept		-27190.2

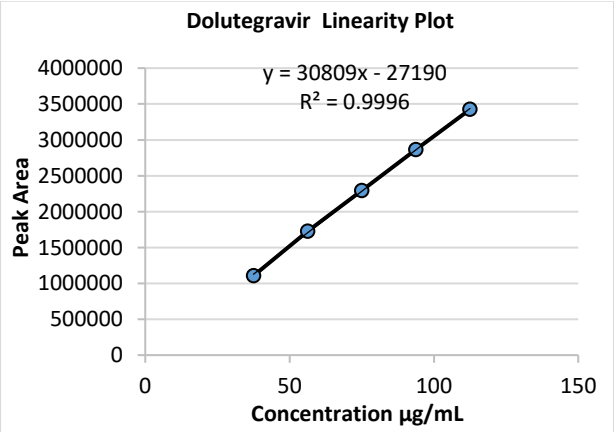

Figure 4: Showing the linearity plot for Dolutegravir

Table 11: Linearity data for Rilpivirine

Rilpivirine Linearity Plot		
Concentration Level (%)	**Concentration in µg/ml**	**Peak Area**
50	17.5	215674
75	26.25	321953
100	35	414603
125	43.75	530273
150	52.5	646715
Slope		12233.17
RSD		0.9982
y-Intercept		-2317.2

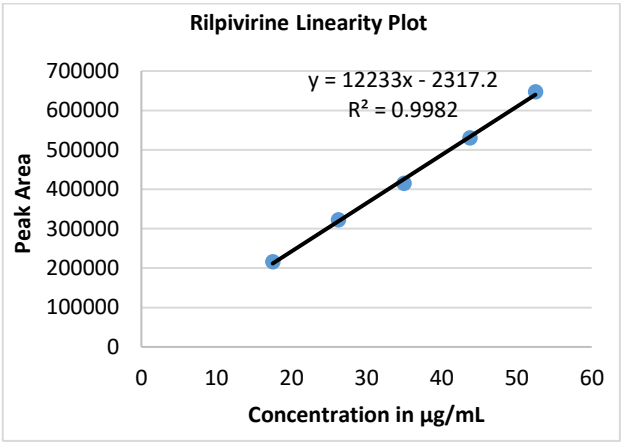

Figure 4: Showing the linearity plot for Rilpivirine

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ values of Dolutegravir and Rilpivirine were calculated based on the formula proposed by ICH Q2 guidelines. The LOD values were found to be 2.00µg/ml and 2.02µg/ml for Dolutegravir and Rilpivirine respectively. The LOQ values were found to be 6.67µg/ml and 6.75µg/ml Dolutegravir and Rilpivirine respectively.

Method Robustness

Influence of small changes in chromatographic conditions such as change in flow rate (±0.1ml/min), Temperature (±2°C) and Wavelength of detection (±2nm) studied to determine the robustness of the method is performed. The %RSD of retention time of Dolutegravir and Rilpivirine

for n=6 in each condition was found to be less than 2.0%. The Results are tabulated in table 12.

Table 12: Results showing the Robustness parameter for Dolutegravir and Rilpivirine

Change in parameter	% RSD for Dolutegravir	% RSD for Rilpivirine
Flow (1.1 ml/min)	0.15	0.11
Flow (0.9 ml/min)	0.12	0.23
Temperature (27^{0}C)	0.17	0.10
Temperature (23^{0}C)	0.25	0.23
Wavelength of Detection (278 nm)	0.28	0.11
Wavelength of detection (282 nm)	0.05	0.15

CONCLUSION

Based on the interpretation of the results and outcome of the HPLC method developed for Simultaneous estimation of Dolutegravir and Rilpivirine, it can be concluded that the method is highly precise, reproducible, linear and stability indicating method. The forced degradation studies that were carried out prove that the method is efficient in quantifying the quality of APIs in the presence of degradation products.

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Source of Support: Nil, **Conflict of Interest:** None.