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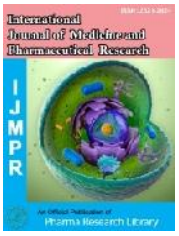

Research Article **Open Access**

Analytical Method Development and Validation for Rilpivirine and Dolutegravir in Combine Dosage Forms by RP- HPLC

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A B S T R A C T

The chromatographic conditions were successfully developed for the separation of Rilpivirine and Dolutegravir by using ACE C18 column (4.6×150mm) 5µ, flow rate was 1.2 ml/min, mobile phase ratio was (70:30 v/v) methanol: Phosphate buffer pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 240nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2690, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.344 mins and 3.284 mins. The % purity of Rilpivirine and Dolutegravir was found to be 101.27% and 99.97% respectively. The system suitability parameters for Rilpivirine and Dolutegravir such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Rilpivirine and Dolutegravir was found in concentration range of 50µg-250µg and 5µg-50µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.2 and 0.2, % RSD for intermediate precision was 0.2 and 0.1respectively. The precision study was precise, robust, and repeatable. LOD value was 3.17 and 5.68, and LOQ value was 0.0172 and 0.2125 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Rilpivirine and Dolutegravir in API and Pharmaceutical dosage form.

Keywords: ACE C18 column, Rilpivirine and Dolutegravir, RP-HPLC

A R T I C L E I N F O

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1. Introduction

Rilpivirine (TMC278, trade name Edurant) is a pharmaceutical drug, developed by Tibotec, for the treatment of HIV infection. It is a second-generation non-nucleoside reverse transcriptase inhibitor (NNRTI) with higher potency, longer half-life and reduced side-effect

profile compared with older NNRTIs, such as efavirenz. Dolutegravir is approved for use in a broad population of HIV-infected patients. It can be used to treat HIV-infected adults who have never taken HIV therapy (treatment-naïve) and HIV-infected adults who have previously taken HIV therapy (treatment-experienced), including those who have been treated with other integrase strand transfer inhibitors. Tivicay is also approved for children ages 12 years and older weighing at least 40 kilograms (kg) who are treatment-naïve or treatment-experienced but have not previously taken other integrase strand transfer inhibitors

2. Materials and Methods

Materials: Ortho phosphoric acid, KH2PO4, Water, Methanol, Acetonitrile, K2HPO4

Methodology

HPLC Method Development

Selection of Mobile Phase

Phosphate buffer: Methanol (30: 70% v/v)

Buffer pH should be between 2 to 8.

Below 2: siloxane linkages are cleaved.

Above 8: dissolution of silica.

pH selected: 3 ±0.05

pH controls the elution properties by controlling the ionization characteristics.

Reasons:

To decrease the retention and improve separation. Good Response, Area, Tailing factor, Resolution.

2. Selection of wavelength

10 mg of Rilpivirine and Dolutegravir was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Rilpivirine and Dolutegravir. The isobestic point was taken as detection wavelength.

Trial -5 (optimized method) Chromatographic conditions

Column: ACE C18 (4.6×150 mm) 5.0 µ m

Column temperature : Ambient

Wavelength : 240 nm

Mobile phase ratio : 70:30 Methanols: Phosphate buffer

Flow rate : 1.2 ml/min

Auto sampler temperature: Ambient

Injection volume : 10µ l

Run time : 10.0 minutes

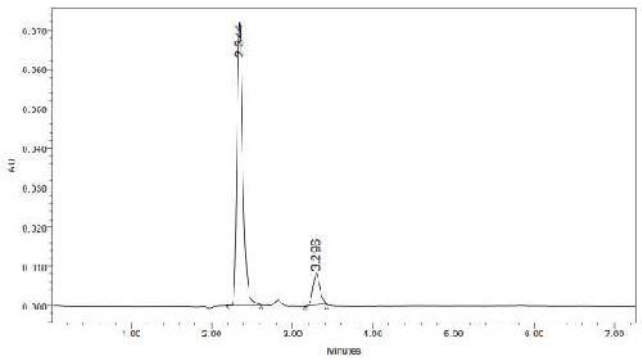

Figure 1

3. Results and Discussion

Specificity: The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

Linearity: 10 mg of Rilpivirine & 1 mg of Dolutegravir working standard were accurately weighed and were transferred into a 10ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Range

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 50µ g/ml-250µ g/ml and 5µ g/ml-25µ g/ml of Rilpivirine and Dolutegravir respectively.

Accuracy: 10mg of Rilpivirine and 1mg of Dolutegravir working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent

Precision

10 mg of Rilpivirine and 1 mg of Dolutegravir working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Intermediate Precision/Ruggedness

To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different make column of same dimensions. Limit of detection (LOD)

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula.

Limit of quantification (LOQ)

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Robustness: As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

- The flow rate was varied at 0.4ml/min to 0.6 ml/min. Standard solution 150 ppm of Rilpivirine and 15 ppm of Dolutegravir was prepared and analysed using the varied flow rates along with method flow rate.
- The organic composition in themobile phase was varied from 65% to75 %standard solution 150 µ g/ml of Rilpivirine and15 µ g/ml of Dolutegravir were prepared and analysed using the varied mobile phase composition along with the actual mobile phase composition in the method.

System suitability: 10 mg of Rilpivirine and 1 mg of Dolutegravir working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

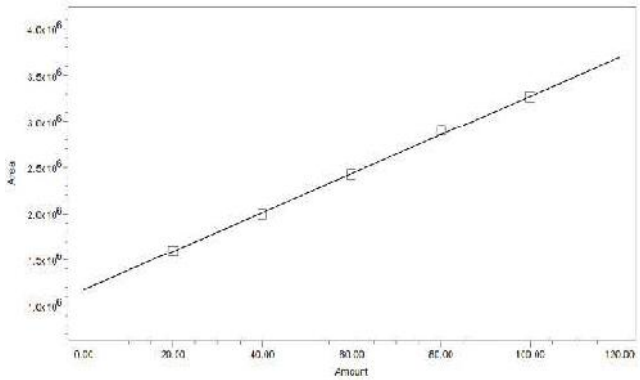

Figure 2: Calibration graph Rilpivirine

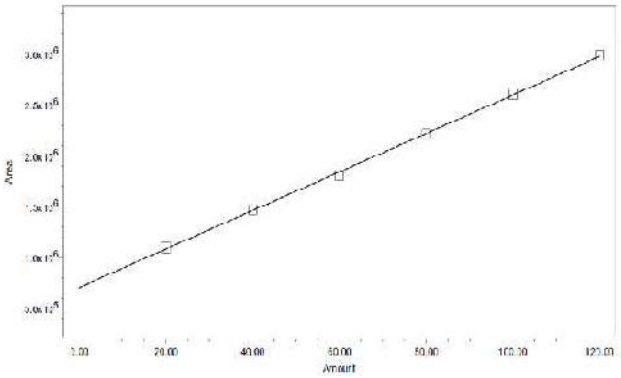

Figure 3: Calibration graph for dolutegravir

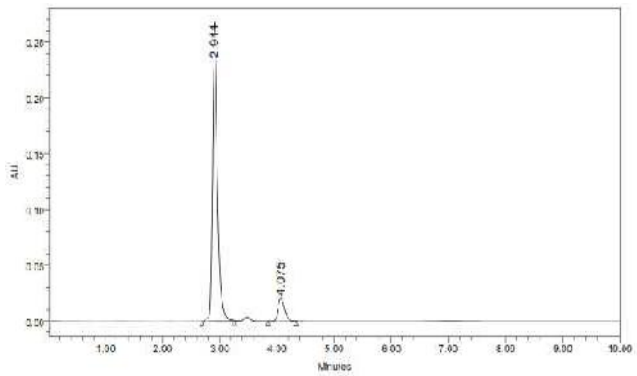

Figure 4: Chromatogram showing less flow rate 0.8ml/min

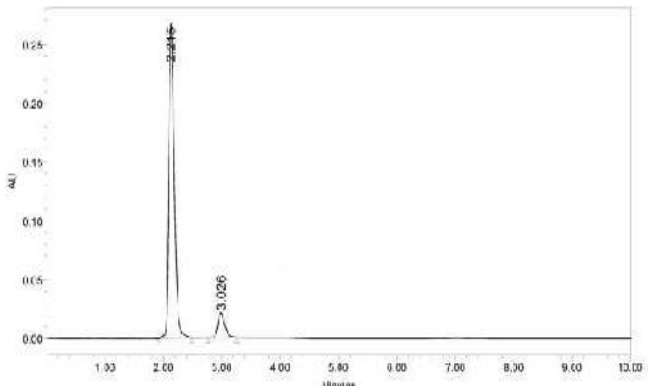

Figure 5: Chromatogram showing less flow rate 1.2 ml/min

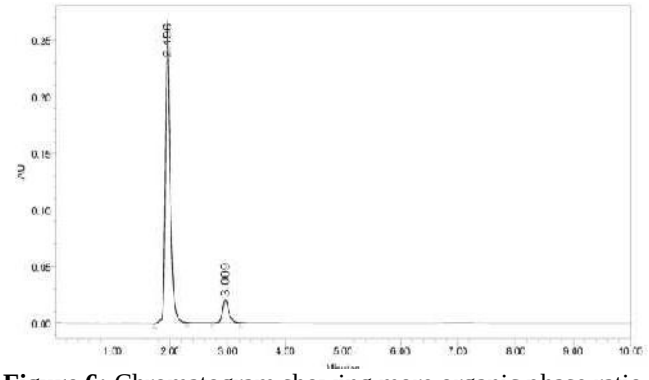

Figure 6: Chromatogram showing more organic phase ratio

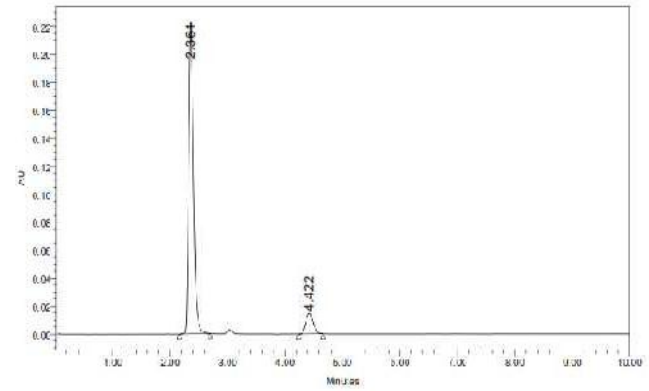

Figure 7: Chromatogram showing less organic phase ratio

Table 1: Linearity Results for Rilpivirine

S.No	Linearity Level	Concentration	Area
1	I	50 ppm	471543
2	II	100 ppm	656277
3	III	150 ppm	794999
4	IV	200 ppm	946124
Correlation Coefficient			0.999

Table 2: Linearity Results for dolutegravir

S.No	Linearity Level	Concentration	Area
1	I	5 ppm	56472
2	II	10 ppm	73841
3	III	15 ppm	92655
4	IV	20 ppm	111541
Correlation Coefficient			0.999

Table 3: Showing accuracy results for Rilpivirine

% Conc. (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	656659	5	4.96	99.91%	99.56%
100%	1304258	10	9.98	99.18%	
150%	1854608	15	15.02	99.60%	

Table 4: Showing accuracy results for dolutegravir

% Conc. (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	65312	0.5	0.99	99.53%	99.47%
100%	124509	1.0	1.05	99.38%	
150%	178517	1.5	1.495	99.52%	

Table 5: Showing% RSD results for Rilpivirine

Peak Name: Rilpivirine

	Peak Name	RT	Area (µV*sec)	Height (µV)
1	Rilpivirine	2.343	1302729	248455
2	Rilpivirine	2.344	1309759	248699
3	Rilpivirine	2.344	1302947	249526
4	Rilpivirine	2.345	1303977	246695
5	Rilpivirine	2.345	1303236	250012
Mean			1304529.8	
Std. Dev.			2961.1	
% RSD			0.2	

Table 6: Showing %RSD results for dolutegravir

Peak Name: dolutegravir

	Peak Name	RT	Area (µV*sec)	Height (µV)
1	dolutegravir	3.285	124263	19458
2	dolutegravir	3.287	124487	19634
3	dolutegravir	3.287	124175	19600
4	dolutegravir	3.288	124894	19327
5	dolutegravir	3.288	124495	19540
Mean			124462.7	
Std. Dev.			278.6	
% RSD			0.2	

Table 7: Showing results for intermediate precision of Rilpivirine

Peak Name: Rilpivirine

	Peak Name	RT	Area (µV*sec)	Height (µV)
1	Rilpivirine	2.342	1305937	247870
2	Rilpivirine	2.343	1306476	246764
3	Rilpivirine	2.344	1304520	245696
4	Rilpivirine	2.344	1300148	247140
5	Rilpivirine	2.345	1308271	247280
Mean			1305070.2	
Std. Dev.			3061.8	
% RSD			0.2	

Table 8: Showing results for intermediate precision of dolutegravir

Peak Name: dolutegravir

	Peak Name	RT	Area (µV*sec)	Height (µV)
1	dolutegravir	3.278	122962	19165
2	dolutegravir	3.281	122487	19115
3	dolutegravir	3.281	122632	19073
4	dolutegravir	3.281	122626	19003
5	dolutegravir	3.283	122702	19123
Mean			122681.8	
Std. Dev.			174.8	
% RSD			0.1	

Table 9: Showing results for Limit of Detection

Drug name	Standard deviation (σ)	Slope (s)	LOD (µg)
Rilpivirine	382625.50	572175863	3.17
dolutegravir	5862	467579210	0.0172

Table 10: Showing results for Limit of Quantification

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Rilpivirine	381727.80	583265980	5.8
dolutegravir	5681	469828490	0.21

4. Conclusion

The chromatographic conditions were successfully developed for the separation of Rilpivirine and Dolutegravir by using ACE C18 column (4.6×150mm) 5µ, flow rate was 1.2 ml/min, mobile phase ratio was (70:30 v/v) methanol: Phosphate buffer pH 3 (pH was adjusted with ortho phosphoricacid), detection wavelength was 240nm. The instrument used was WATERS HPLC auto Sampler, Separation module 2690, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.344 mins and 3.284 mins. The % purity of Rilpivirine and Dolutegravir was found to be 101.27% and 99.97% respectively. The system suitability parameters for Rilpivirine and Dolutegravir such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Rilpivirine and Dolutegravir was found in concentration range of 50µ g-250µ g and 5µ g-50µ g and correlation coefficient (r2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.2 and 0.2, % RSD for intermediate precision was 0.2 and 0.1respectively. The precision study was precise, robust, and repeatable.LOD value was 3.17 and 5.68, and LOQ value was 0.0172 and 0.2125 respectively.

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