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Analytical Method Development and Validation for Dolutegravir and Rilpivirine in API and Combined Pharmaceutical Dosage Forms by RP-HPLC

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ABSTRACT

New method was established for simultaneous estimation of Rilpivirine and Dolutegravir by RP-HPLC method. 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 orthophosphoric acid), detection wavelength was 240nm. The retention times were found to be 2.344 mins and 3.296 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 (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. 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

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1. Introduction

Rilpivirine 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.

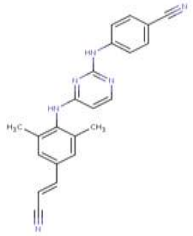

Fig 1: Chemical structure of Rilpivirine

Dolutegravir sold under the brand name Tivicay, is an antiretroviral medication used, together with other medication, to treat HIV/AIDS. It may also be used, as part of post exposure prophylaxis, to prevent HIV infection following potential exposure. It is taken by mouth.

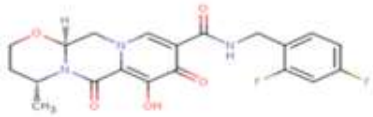

Fig 2: Chemical structure of Dolutegravir

2. Materials and Methods

Instrumentation:

HPLC Auto Sampler : Shimadzu Model number SPD20A, Software LC Solutions, Detector: Photo diode array detector, Thermosil C18 Column (4.0×1.25mm, 5µ), Sonicator: Model number SE60US Enertech , U.V double beam spectrophotometer: PG Instrument Model number T60 Software UV Win5, pH meter: ADWAModel number AD102U, Digital Weighing machine:a Model number ER200A .

Chemicals:

Rilpivirine and Dolutegravir, KH2PO4, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid, K2HPO4.

Optimized Chromatographic conditions:

Column : ACE C18 (4.6×150 mm) 5.0 µm
Column temperature : Ambient
Wavelength : 240 nm
Mobile phase ratio : 70:30 Methanol: Phosphate buffer
Flow rate : 1.2 ml/min
Auto sampler temperature : Ambient
Injection volume : 10µl
Run time : 10.0 minutes

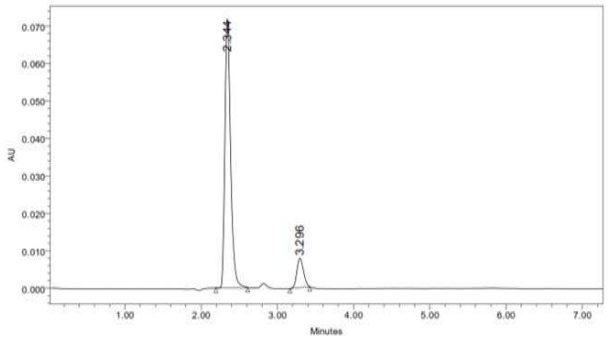

Fig 3: Optimized Chromatogram

Sample solution preparation:

10 mg of Rilpivirine and 1 mg Dolutegravir tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation

10 mg Rilpivirine and 1 mg 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 (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Method Validation

- Linearity
- Accuracy
- Precision
- Intermediate Precision
- Limit of Detection
- Limit of Quantification
- Robustness
- System suitability testing

3. Results and Discussion

Table1: 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
5	V	250 ppm	1002139
Correlation Coefficient			0.999

Table 2: Linearity Results for Dolutegravir

S.No	Linearity Level	Concentration	Area
1	I	5ppm	56472
2	II	10 ppm	73841
3	III	15ppm	92655
4	IV	20ppm	111541
5	V	25ppm	130567
Correlation Coefficient			0.999

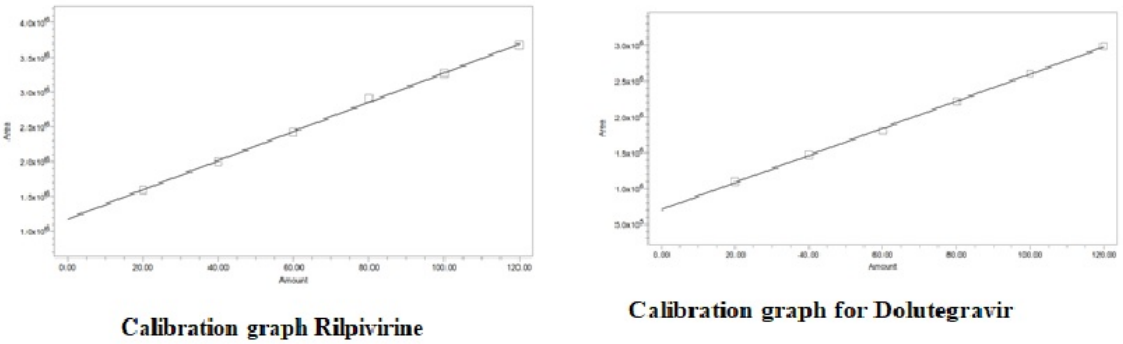

Fig 4: Calibration Curves

Table 3: Accuracy results for Rilpivirine

%Concentration (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: Accuracy results for Dolutegravir

%Concentration (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: % RSD results for Rilpivirine

	Peak Name	RT	Area	Height
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: %RSD results for Dolutegravir

	Peak Name	RT	Area	Height
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: Intermediate precision of Rilpivirine

	Peak Name	RT	Area	Height
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: Intermediate precision of Dolutegravir

	Peak Name	RT	Area	Height
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: Results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Rilpivirine	382625.50	572175863	3.17
Dolutegravir	5862.40	467579210	0.0172

Table 10: Results for Limit of Quantitation

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Rilpivirine	381727.80	583265980	5.80
Dolutegravir	5681.30	469828490	0.212

Table 11: System suitability results for Rilpivirine

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	5339	1.4
2	1	4668	1.3
3	1.2	5216	1.4

Table 12: System suitability results for Dolutegravir

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	7036	1.3
2	1	6089	1.2
3	1.2	6998	1. 3

Table 13: System suitability results for Rilpivirine

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	6232	1.4

2	*Actual	4668	1.3
3	5 % more	6387	1.4

Table 14: System suitability results for Dolutegravir

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	85 % less	5437	1.3
2	*Actual	6089	1.2
3	5 % more	4817	1.2

4. Conclusion

The developed RP–HPLC method was found to be suitable for the analysis of Rilpivirine and Dolutegravir in bulk form, and was found to be simple, reliable, sensitive, economical and precise.

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