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A new analytical method for determination of dolutegravir and rilpivirine in pharmaceutical formulations by RP-HPLC method

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ABSTRACT

A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Dolutegravir and Rilpivirine in pharmaceutical dosage form. Chromatographic separation of Dolutegravir and Rilpivirine was achieved on Waters Alliance -2695, by using Luna C18 (250mm x 4.6mm, 5 μ m) column and the mobile phase containing 0.1% OPA & ACN in the ratio of 50:50 v/v. The flow rate was 1.0 ml/min, detection was carried out by absorption at 245 nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Dolutegravir and Rilpivirine were NLT 2000 and should not more than 2 respectively. The linearity of the method was excellent over the concentration range 10-150 μ g/ml and 5-75 μ g/ml for Dolutegravir and Rilpivirine respectively. The correlation coefficient was 0.999. % Relative standard deviation of peak areas of all measurements always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Dolutegravir and Rilpivirine study of its stability.

Keywords: Dolutegravir; Rilpivirine; RP-HPLC.

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INTRODUCTION

Development of a simple, reliable and accurate method for the assay of Dolutegravir and Rilpivirine tablet dosage form by reverse phase HPLC and validate the method for its repeatability and reproducibility. Dolutegravir is an HIV-1 antiviral agent. It inhibits HIV integrase by binding to the active site and blocking the strand transfer step of

retroviral DNA integration in the host cell. The strand transfer step is essential in the HIV replication cycle and results in the inhibition of viral activity. Dolutegravir has a mean EC₅₀ value of 0.5 nM (0.21 ng/mL) to 2.1 nM (0.85 ng/mL) in peripheral blood mononuclear cells (PBMCs) and MT-4 cells.

Figure 1: Chemical structure of Dolutegravir

Dolutegravir (DTG), an unboosted HIV integrase inhibitor (INI), is metabolized by UGT1A1 and to a minor extent by CYP3A. Renal elimination of unchanged DTG is very low (< 1%). As renal impairment may affect pharmacokinetics (PK), even for drugs primarily metabolized or secreted in bile, this study investigated the effect of renal impairment on the PK of DTG

Rilpivirine is a non-competitive NNRTI that binds to reverse transcriptase. Its binding results in the blockage of RNA and DNA- dependent DNA polymerase activities, like HIV-1 replication. It does not present activity against human DNA polymerases α , β and γ . Rilpivirine binds to the HIV-1 reverse

transcriptase (RT) and its flexible structure around the aromatic rings allows the adaptation to changes in the non-nucleoside RT binding pocket.

Figure 2: Chemical structure of Rilpivirine

Rilpivirine is highly protein-bound, and more than 99% may be bound to human plasma proteins in a concentration-dependent manner.³¹ Under fasting conditions, the maximum plasma concentration of rilpivirine (C_{max}) decreased by 46% and the area under the rilpivirine plasma concentration curve (AUC) decreased by 43%. Similarly, rilpivirine C_{max} and AUC are reduced by 50% when given with a protein-rich nutritional drink.³² As a consequence, it is recommended to take rilpivirine with food but avoid taking after a protein-rich drink. In a 7-day pharmacokinetic study of oral administration of rilpivirine 25 mg, 50 mg, 100 mg, and 150 mg once daily, C_{max} was generally reached 3–4 hours after dosing.³² Plasma concentrations were increased 2–3-fold from day 1 to day 7. Drug elimination from the plasma was slow, with a terminal half-life of 34–55 hours.²² At higher doses, there was a trend towards greater interindividual pharmacokinetic variability, but plasma concentrations did not increase proportionately with dose.

MATERIALS AND METHODS

Materials: Dolutegravir and Rilpivirine Sun Pharmaceuticals pvt.Ltd. Water, Acetonitrile, Ortho phosphoric acid, Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide, 0.45 µm Nylon filter Phenomenex All the above chemicals and solvents are from Merck.

HPLC method

Instrument: UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Dolutegravir and Rilpivirine solutions having universal loop injector of injection capacity 20 µL. The column used was Waters Alliance-2695, by using Luna C18 (250mm x 4.6mm, 5µm) column at ambient temperature. Different mobile phases were tested in order to find the best conditions, for separating both the drugs simultaneously. Optimised Chromatographic conditions The mobile phase having 0.1% OPA & ACN in the ratio of 50:50 was selected because it was found that it ideally resolve the peaks with retention time (RT) 2.380 min and

3.449 min for Sofosbuvir and Ledipasvir respectively. Wavelength was selected by scanning all standard drugs over a wide range of wavelength 200nm to 350nm. Both the components showed reasonably good response at 245 nm.

Methods:

Selection of detector wave length: Selection of detector wavelength is a critical step in finalization of the analytical method. To determine exact wave length standard API is prepared and injected into chromatographic system with PDA detector and the wave length which gives higher response for the compound. In this method impurities spiked with standard solution is injected into HPLC with PDA – detector and the wave length, which gives higher response for the compound is selected.

Selection of mobile phase composition and buffer: Buffer and its strength play an important role in deciding the peak symmetries and separation. It is important to use the buffers with suitable strength scope up for the injection load on the column otherwise peak tailing may arise due to changes in ionic from during chromatography.

Selection of system suitability parameters: System suitability parameters have to be selected based on the tailing factor, plate count, resolution, and RSD. In general resolution factor for the closely eluting compounds is selected as a system suitability requirement. If the separation of impurities from each other and from API peak is found to be satisfactory, there is no need to be keeping a resolution factor as system suitability parameter. In such cases only standard reproducibility and symmetry of standard peak can be adopted as a system suitability requirement.

In this method the system suitability parameters selected are ratio of peak area of two replicate standard injections, tailing factor, check standard recovery (%), symmetry factor for peak area of standard and bracketing standard.

Preparation of mixed sample Solution: 20 tablets (each tablet contains Dolutegravir -50mg, Rilpivirine -25mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Dolutegravir and Rilpivirine (µg/ml) were prepared by dissolving weight equivalent to 100 mg of Dolutegravir and 50 mg of Rilpivirine and dissolved in sufficient mobile phase. After that filtered the solution using 0.45- micron syringe filter and sonicated for 5 min and dilute to 100ml with mobile phase. further dilutions are prepared in 5 replicates of 100 µg/ml of Dolutegravir and 50 µg/ml of Rilpivirine was made upto mobile phase.

Linearity: The linearity of an analytical procedure is its ability (with in a given range) to obtain test results which are directly proportional to the concentration (amount) of analytic in the sample. A series of

Dolutegravir standard solution were prepared in the range of 10-150 µg/ml and Rilpivirine standard solution were prepared in the range of 5 – 75 µg/ml test concentration of 50mg +25mg tablets and injected into the HPLC system as per the test method. Linearity of detector response was established by plotting a graph of concentration vs response of Dolutegravir peak. The detector response was found to be linear from about 10-150 µg/ml and Rilpivirine peak. The detector response was found to be linear from about 5 – 75 µg/ml of test concentrations. The correlation coefficient, squared co- relation coefficient, slope, intercept and residual sum of squares were calculated and squared correlation coefficient was found to be with in the acceptable limits.

Table 1: Linearity table for Dolutegravir and Rilpivirine

S.No	Dolutegravir		Rilpivirine	
	Conc (µg/ml)	Area	Conc (µg/ml)	Area
1	10	246821	5	108142
2	25	718746	12.5	258964
3	50	1498745	25	548748
4	100	2865878	50	1004562
5	125	3598745	62.5	1258451
6	150	4299632	75	1526988

The range of an analytical procedure is the interval between the upper and lower concentration (amount) of analytic in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Based on method precision, linearity and accuracy data it can be concluded that the assay method of Dolutegravir and Rilpivirine tablets is precise, linear and accurate in the range of 10-150 µg/ml and 5 – 75 µg/ml of test concentration of 50mg + 25 mg tablets.

Accuracy: The accuracy of an analytical procedure express the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and value found. This is sometimes termed trueness.

A series of sample solution were prepared in triplicate (3 preparations for Dolutegravir and Rilpivirine levels 50% to 150%) by spiking the Dolutegravir and Rilpivirine API on placebo in the range of about 50% to 150% of test concentrations of 50mg + 25 mg tablets, injected into HPLC system and analyzed as per the test method. Individual % recovery, mean % recovery, %RSD and squares correlation coefficient for linearity of the test method were calculated and the results were found to be with in the acceptable limits.

Precision: The precision of an analytical procedure express the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous

sample under the prescribed conditions. Precision may be considered at three levels, Repeatability, Intermediate precision, Reproducibility. The precision of an analytical procedure is usually expressed as the variance, standard deviation or co – efficient of variation of a series of measurements.

Method precision: To evaluate the method precision for assay method, six samples for 50mg + 25mg tablets were prepared and analyzed as per test method. % assay of each individual preparation, mean % assay and %RSD of six samples were calculated and found to be with in the acceptance criteria.

Table 2: Results for system precision of dolutegravir

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	3.385	288451	5285	0.68
2	3.418	288079	5255	0.14
3	3.404	288265	5268	0.48
4	3.408	288847	5261	0.85
5	3.416	288866	5290	0.85
6	3.415	287844	5224	0.87
Mean	3.411	288496	5298	0.94
SD	0.0216	1753.3	--	--

The % assay for each individual preparation should be 95.02 to 105.0 of labeled amount of both drugs.

The % RSD for assay of six replicate preparations should not more than 2.0 for Dolutegravir and Rilpivirine.

System precision: To evaluate the system precision for assay method, 100+50 ppm of standard solution prepared and injected 6 times and analyzed as per test method. % assay of each individual preparation, mean % assay and %RSD of six standards were calculated and found to be with in the acceptance criteria.

Intermediate precision: To evaluate Intermediate precision six samples for 50mg +25 mg tablets were prepared and analyzed as per test method by using different analyst on different day. % assay of for individual preparations, mean % assay and %RSD of assay results in method precision and intermediate precision (n=6 and n=12) were calculated and found to be with in the acceptance criteria.

Table 3: Results for system precision of Rilpivirine

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	3.385	288451	5285	0.68
2	3.418	288079	5255	0.14
3	3.404	288265	5268	0.48
4	3.408	288847	5261	0.85
5	3.416	288866	5290	0.85
6	3.415	287844	5224	0.87
Mean	3.411	288496	5298	0.94
SD	0.0216	1753.3	--	--

Table 4: System suitability parameters for Dolutegravir and Rilpivirine

Trial	Column	Mobile Phase	Flow rate (ml/min)	Diluent	Observation
Trial-1	Waters X-Bridge C18 (150×4.6×5μ)	0.1% OPA : ACN 10:90	1ml/min	Mobile phase	Peak retention time is very low
Trial -2		0.1% OPA : ACN 20:80	1ml/min	Mobile phase	Resolution is very low
Trial -3		0.1% OPA : ACN 30:70	1ml/min	Mobile phase	Base line is not sufficient
Trial -4		0.1% OPA : ACN 40:60	1ml/min	Mobile phase	Peaks are not separated clearly
Trial -5		0.1% OPA : ACN 45:55	1ml/min	Mobile phase	Peaks are not separated clearly
Trial-6		0.1% OPA : ACN 50:50	1ml/min	Mobile phase	This method suitable for validation

Table 5: Flow rate and organic variation

Parameters	Dolutegravir		%RSD	Rilpivirine		% RSD
Flow rate	Retention time	Tailing factor		Retention time	Tailing factor	
0.8 ml/min	4.251	0.89	0.68	8.305	0.89	1.11
1.0 ml/min	3.383	0.98	0.58	6.338	0.47	1.02
1.2ml/min	2.832	0.85	0.68	5.522	1.08	1.35
Organic phase						
55+45	3.212	0.28	0.85	4.772	1.02	0.89
50+50	3.381	0.42	1.02	6.331	0.84	0.10
45+55	3.547	0.56	1.01	8.594	0.28	0.54

Table 6: Degradation Studies Data

S.no	Degradation Parameters	Time	Peak Area	%Recovery	%Degradation
1	Acid	30min	2222957	73.8	22.8
			886254	70.8	24.5
2	Base	30 min	2232530	73.5	27.4
			886263	72.8	23.6
3	Peroxide	30min	2222864	78.9	21.1
			885241	72.2	21.8
4	Thermal	30 min	2222984	71.8	22.2
			896521	72.4	23.6
5	Humidity	30 min	2203896	78.5	21.5
			889476	75.4	24.6
6	Heat	30 min	2232684	78.3	22.7
			856241	72.2	28.8
7	Photolytic	30min	2238562	78.4	21.6
			886241	72.8	27.2
8	Reduction	30 min	2222896	73.6	26.4
			884562	71.9	28.1
9	Hydrolysis	30 min	2223986	77.3	22.7
			874518	75.5	24.5

Table 7: Limit of Detection (LOD) and Limit of Quantitation (LOQ)

S.No	Sample name	LOD μg/ml	LOQ μg/ml
1.	Dolutegravir	0.01 μg/ml	0.1 μg/ml
2	Rilpivirine	0.005 μg/ml	0.05 μg/ml

Limit of detection: This is the lowest concentration in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions.

Limit of quantitation: This is the lowest concentration of analytic in a sample that can be determined with acceptable precision and accuracy.

Table 8: Accuracy data of Dolutegravir Accuracy data for Rilpivirine

Recovery level	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	%Recovery	%RSD
50%	50.0	1485963	1437848	100.64	100.6	0.58
	50.0	1475632				
	50.0	1428631				
100%	100.0	2885416	2834465	100.06	100.0	0.24
	100.0	2824719				
	100.0	2880121				
50%	150.0	4285628	4224835	100.18	100.2	1.02
	150.0	4261446				
	150.0	4257526				
50%	50.0	1485963	1437848	100.64	100.6	0.58
	50.0	1475632				
	50.0	1428631				

Table 9: Accuracy data of Dolutegravir Accuracy data for Dolutegravir

Recovery level	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	%Recovery	%RSD
50%	25.0	548634	545828	100.24	100.2	0.48
	25.0	545666				
	25.0	547892				
100%	50.0	1087454	1085292	100.45	100.4	0.44
	50.0	1089642				
	50.0	1085804				
150%	75.0	1542965	1527652	100.28	100.3	0.28
	75.0	1542586				
	75.0	1542635				
50%	25.0	548634	545828	100.24	100.2	0.48
	25.0	545666				
	25.0	547892				

Table 10: Method precision data for 50 mg+ 25 mg Acceptance criteria

S.No.	Dolutegravir	Rilpivirine
	Area	Area
1	2884562	1098563
2	2884138	1071605
3	2887225	1018845
4	2886994	1058070
5	2888992	1097258
6	2889748	1010943
Avg	2886175	1095598
St dev	12153.79	14357.88
%RSD	0.8135	0.9585

Table 11: Intermediate precision data of Dolutegravir

	Analyst-1		Analyst-2	
	Peak area	% assay	Peak area	% assay
1.	2886412	100.1	2812886	100.3
2	2828962	100.4	2828414	100.1
3	2828852	100.1	2828755	100.9
4	2838131	100.6	2828335	100.2
5	2848903	100.4	2828113	100.3
6	2858468	100.1	2828505	100.1
Mean	2868160	100.2	2828818	100.5
%RSD	0.41	0.34	0.89	1.04

Table 12: Intermediate precision data of Rilpivirine

S.no	Analyst-1		Analyst-2	
	Peak area	% assay	Peak area	% assay
1	1089636	100.5	1038584	100.2
2	1076115	100.1	1084719	100.5
3	1047248	100.3	1040163	100.6
4	1013982	100.1	1064046	100.1
5	1098308	100.2	1071605	100.2
6	1055551	100.4	1085065	100.2
mean	1085140	100.3	1054826	100.5
%RSD	1.02	0.48	0.86	0.85

Figure 3: Typical chromatogram of LOD **Figure 4: Typical chromatogram of LOQ**

Acceptance criteria: S/N Ratio value shall be 3 for LOD solution. S/N Ratio Value shall be 10 for LOQ solution

Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Flow rate variation: A study was conducted to determine the effect of variation in flow rate. Standard and check standard solutions were prepared as per test method and injected into HPLC system with flow rates of 1.0 ml/min. system suitability parameters were evaluated and found to be within the specified limits as per test method and RT of main peak was monitored.

Organic phase variation: A study was conducted to determine the effect of variation in organic phase. Standard and check standard solutions were prepared as per the test method and injected into HPLC system with mobile phases of buffer and ACN in the ratio of 50:50 %v/v, and wavelength of 245 nm. System suitability parameters are found to be within the specified limits and RT of the main peak was monitored for 50:50 %. The % RSD of Dolutegravir and Rilpivirine was should be within limits. I.e. < 2. Tailing

factor was less than 2. From the observation it was found that the system suitability parameters were within limit at all variable conditions.

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