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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, 5µm) 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 µg/ml and 5-75 µg/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.

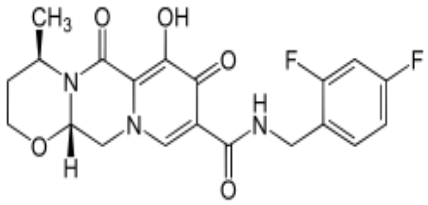


**Figure 1: Chemical structure of Dolutegravir**

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.

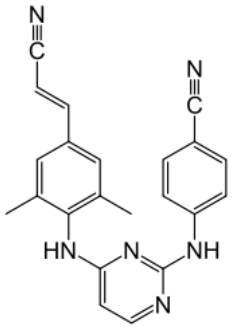


**Figure 2: Chemical structure of Rilpivirine**

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 (Cmax) decreased by 46% and the area under the rilpivirine plasma concentration curve (AUC) decreased by 43%. Similarly, rilpivirine Cmax 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, Cmax 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**

| Trials | 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 |

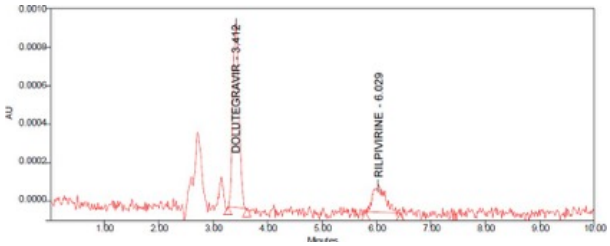


**Figure 3: Typical chromatogram of LOD**

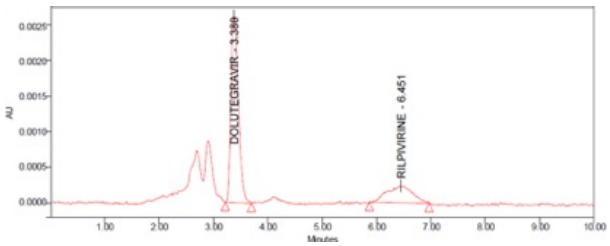


**Figure 4: Typical chromatogram of LOQ**

**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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