Validated stability indicating RP-HPLC method for the determination of dolutegravir and rilpivirine in bulk and pharmaceutical dosage forms

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ABSTRACT

For simultaneous analysis of Dolutegravir and Rilpivirine utilizing RP-HPLC, an accurate, rapid, economical, quick and reliable assay technique was developed and tested. Successful chromatographic detachment using acetonitrile and 0.1 percent tri ethyl amine in the water of pH-2.5 adjusted with 0.1% orthophosphoric acid in 40:60 v/v as a movable phase with a flow of 1 ml/min and UV observation at 230 nm. Chromatography at ambient temperature was performed isocratically, and the run time was 10 min. By injecting the norm six times, device suitability parameters were studied and the findings were far below the acceptance criteria. The linearity analysis was performed at levels ranging from 10% to 150% and the R² value was found to be 0.999. Precision has been found to be 0.8 for repeatability and 1.2 for intermediate precision. Assay of the commercialized formulation was performed by using the above method, and we get 100.01 percent was present. For routine analysis in drug testing, this chromatographic approach can be effectively implemented. By using the above technique, an assay of the marketed formulation was performed and found to be within the limit. Degradation studies were carried out on Dolutegravir and Rilpivirine, with a purity threshold greater than purity angle in all conditions and within the acceptable range. The above-mentioned technique was validated according to ICH guidelines.

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INTRODUCTION

Dolutegravir (DTG) and Rilpivirine (RLP) are used to monitor HIV infection (Sepkowitz, 2001; Krämer et al., 2010; Kirch, 2008). This helps to reduce the amount of HIV in your body so that your immune system (Beck and Habicht, 1996) can function preferable. This decreases your risk of having complications of HIV (such as HIV infection, cancer) and enhances your quality of life. DTG is an antiretroviral medication (Moore and Chaisson, 1999) used, together with other medication, to treat HIV/AIDS (BNF, 2015). It may also be used as part of post display prophylaxis to prevent HIV infection following potential exposure (Dolutegravir, 1975). It is taken by mouth. Usual side effects involve trouble sleeping, feeling tired, diarrhea (WHO, 2013), high blood sugar (Diabetes Care, 2013) and headache. Critical consequences may include allergic reactions (Kay, 2000) and liver
problems (NIH, 2016). It is unclear if used through pregnancy (Pregnancy, 2013) or breastfeeding (Ballard and Morrow, 2013) is safe. DTG is an HIV integrates strand shift inhibitor which blocks the work of HIV integrase (Masuda, 2011), which is needed for viral replication (Roberts, 2002).

Rilpivirine is a pharmaceutical medicine (Europea, 2004) evolved by Tibotec (Johnson & Johnson, 2002) for the nursing of HIV infection (Stellbrink, 2007). It is a second-generation non-nucleoside reverse transcriptase inhibitor (NNRTI) (Clercq, 1998) with big potency, longer half-life (McNaught and Wilkinson, 1997) and reduces side effect profile (Guideline for good clinical practice, 1996) compared with older NNRTIs such as Efavirenz (Goebel et al., 2006; Pozniak et al., 2007). Rilpivirine entered phase III clinical trials in April 2008 (ClinicalTrials.gov, 2012; ClinicalTrials.gov, 2012) and was approved for use in the United States in May 2011 under the brand name Edurant. The chemical representation of Dolutegravir and Rilpivirine was shown in Figure 1.

The literature survey revealed that there is only a few methods reported HPLC (Joseph et al., 2016; Damel and Prdeshi, 2017) and in UV spectrophotometric method (Cozzi et al., 2016), two reports for only DTG (Satyadev and CH, 2018; Balasaheb et al., 2015). Most of the methods are cost-effective, and run time is high. The author has therefore tried to develop a more sensitive HPLC approach for the testing of the selected drugs in pharmaceutical formulations. In terms of accuracy, precision, linearity, Limit of detection, Limit of quantification, robustness and forced degradation, the established technique was validated.

MATERIALS AND METHODS

Reagents and Chemicals

Acetonitrile, Tri ethyl amine (TEA), Ortho phosphoric acid (OPA) and water (HPLC) were purchased from Merck (India) Ltd. Worli, Mumbai, India. All APIs of DTG and RLP (purity-99.9%) as reference standards were procured from Glenmark pharmaceutical private Ltd, Andheri (E), Mumbai, India.

Instrumentation and chromatographic conditions

The Waters Alliance e-2695 chromatographic system consisting of the quaternary pump, the PDA detector 2996 with a chromatographic software Empower-2.0 was used. Chromatographic separation was achieved in isocratic mode at room temperature using a waters X-bridge C8 (150x4.6mm, 3.5μ) column. The mixture of 0.1% TEA in water change pH-2.5 with OPA: acetonitrile (60:40 v/v) at a flow rate 1.0 ml/min was used as a mobile process. The amount of injection was 10μl and the eluents were monitored at 230 nm using a PDA detector. 7 minutes of run time was used in the separation of Dolutegravir and Rilpivirine. The peaks were recognized by retention time. A typical chromatogram was shown in Figure 2.

Preparation of standard

Carefully weigh and transfer 100 mg of DTG and 50 mg of RLP working standards in a volumetric flask of capacity 100 ml, app. add 70 ml of diluents and sonicate it for 15 min. to dissolve the components, and made up to the mark with diluents. Further, dilute 5 ml of the above solution to a 50 ml volumetric flask and diluted to volume with diluents.

Preparation of sample solution

Carefully weigh 20 tablets and take the average of one tablet weight. Powder the tablets using mortar and pestil, and take 250 mg of sample into a flask of capacity 100 ml, app. add 70 ml of diluents and sonicated for 30 min to dissolve the components and then diluted up to the mark with diluents. Further, dilute 5 ml of the above solution to 50 ml diluents and it was filtered through a 0.45 μ nylon syringe filter.

RESULTS AND DISCUSSION

Different trials were conducted to achieve good resolution between dolutegravir and rilpivirine. Several types of buffers and movable phases were used for developing the method. None of this movable phase was able to achieve a good resolution between dolutegravir and rilpivirine. After a selected mobile phase slightly raised the resolution and gave better resolution between dolutegravir and rilpivirine. The selected mobile phase was 0.1% TEA and acetonitrile in 60:40 v/v. Development trials were performed with C8, C18, cyano, phenyl columns, but the separation of the selected drugs were achieved with an X-bridge C8 column (150x4.6 mm, 3.5 μ) connected to a PDA detector. The movable phase flow rate was maintained at 1 ml/min and the PDA detector was observed at 230 nm. The final chromatographic conditions provide better resolution between dolutegravir and rilpivirine. The chromatographic, standard, method and intermediate precision results were represented in Table 1.

Validation of the method

The method was confirmed by using ICH guidelines for precision, accuracy, linearity, specificity, ruggedness, robustness and forced degradation.
Table 1: Outcomes of System Suitability studies and validation

<table>
<thead>
<tr>
<th>Method characteristics</th>
<th>Dolutegravir</th>
<th>Rilpivirine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretical plates</td>
<td>6368</td>
<td>10858</td>
</tr>
<tr>
<td>Resolution</td>
<td>-</td>
<td>5.82</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.02</td>
<td>1.11</td>
</tr>
<tr>
<td>Standard % RSD</td>
<td>0.71</td>
<td>0.68</td>
</tr>
<tr>
<td>Accuracy % RSD*</td>
<td>0.36</td>
<td>0.30</td>
</tr>
<tr>
<td>Method precision (% RSD)**</td>
<td>1.26</td>
<td>0.87</td>
</tr>
<tr>
<td>Intermediate precision (% RSD)**</td>
<td>0.44</td>
<td>1.12</td>
</tr>
</tbody>
</table>

*Results are average of three sample preparations with three different drug concentration levels
**Results are average of six sample preparations

Table 2: Linearity study results

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Linearity Range</th>
<th>Equation of calibration curve</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTG</td>
<td>10.0-150.0 µg/ml</td>
<td>Y=28760x+121.8</td>
<td>0.999</td>
</tr>
<tr>
<td>RLP</td>
<td>5.0-75.0 µg/ml</td>
<td>Y=10064x+10930</td>
<td>0.999</td>
</tr>
</tbody>
</table>

Figure 1: Chemical representation of (A) Dolutegravir and (B) Rilpivirine

System precision

Six replicate injections of the standard solution containing 100 µg/ml of DTG and 50 µg/ml of RLP were assessed to check the system suitability. The values obtained for tailing factor, resolution, plate count were well under the acceptance criteria.

Linearity

A standard solution containing 100 µg/ml of DTG and 50 µg/ml of RLP was established to assess the linearity of the process. (100% of the amount of the assay targeted concentration). Sequential dilutions were carried out at 10, 25, 50, 100, 125 and 150% of the target concentration to plot calibration curves. This was injected and the peak areas were used against the concentration. The correlation coefficient values of these analytes were 0.9997 for DTG and 0.9992 for RLP, slope values of DTG and RLP were 28760, 10064 and the intercept values are 121.8, 10930 respectively. Linearity results were shown in Table 2, and linearity plots of DTG and RLP were shown in Figure 3, Figure 4 respectively.

Limit of detection and quantification

Limit of detection and quantification minimum concentration levels at which the analyte can be reliably detected, quantified by using the standard formulas (3.3 times σ/s and 10 times σ/s for LOD and LOQ respectively. LOD values for DTG and RLP were 0.01 µg/ml, 0.005 µg/ml and s/n values are 3 and 4 respectively.
Table 3: Outcomes of Accuracy

<table>
<thead>
<tr>
<th>% level</th>
<th>µg/ml added</th>
<th>Dolutegravir µg/ml found</th>
<th>% Accuracy</th>
<th>µg/ml added</th>
<th>Rilpivirine µg/ml found</th>
<th>% Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>50</td>
<td>50.1</td>
<td>100.28</td>
<td>25</td>
<td>25.3</td>
<td>100.38</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
<td>100.2</td>
<td>100.36</td>
<td>50</td>
<td>25.1</td>
<td>100.69</td>
</tr>
<tr>
<td>150</td>
<td>150</td>
<td>150.2</td>
<td>100.15</td>
<td>75</td>
<td>25.4</td>
<td>100.47</td>
</tr>
</tbody>
</table>

Table 4: Outcomes of Robustness

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Flow plus (1.2 ml/min)</th>
<th>Flow minus (0.8 ml/min)</th>
<th>Org Plus (45:55)</th>
<th>Org minus (35:65)</th>
<th>pH plus (3.0)</th>
<th>pH minus (2.0)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dolutegravir</td>
<td>0.22</td>
<td>0.16</td>
<td>0.28</td>
<td>0.35</td>
<td>0.48</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>Rilpivirine</td>
<td>1.01</td>
<td>0.28</td>
<td>0.39</td>
<td>1.21</td>
<td>0.68</td>
<td>0.36</td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Outcomes of Forced Degradation

<table>
<thead>
<tr>
<th>Degradation</th>
<th>DTG (% Assay)</th>
<th>% Degradation</th>
<th>RLP (% Assay)</th>
<th>% Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100.58</td>
<td>0.00</td>
<td>100.36</td>
<td>0.00</td>
</tr>
<tr>
<td>Acid</td>
<td>92.36</td>
<td>8.22</td>
<td>95.18</td>
<td>5.18</td>
</tr>
<tr>
<td>Alkali</td>
<td>88.78</td>
<td>11.8</td>
<td>92.36</td>
<td>8.00</td>
</tr>
<tr>
<td>Peroxide</td>
<td>90.36</td>
<td>10.22</td>
<td>96.45</td>
<td>3.91</td>
</tr>
<tr>
<td>Reduction</td>
<td>98.68</td>
<td>1.9</td>
<td>97.25</td>
<td>3.11</td>
</tr>
<tr>
<td>Thermal</td>
<td>95.36</td>
<td>5.22</td>
<td>93.68</td>
<td>6.68</td>
</tr>
<tr>
<td>Photolytic</td>
<td>93.36</td>
<td>7.22</td>
<td>92.14</td>
<td>8.22</td>
</tr>
<tr>
<td>Hydrolysis</td>
<td>91.36</td>
<td>9.22</td>
<td>90.87</td>
<td>9.49</td>
</tr>
</tbody>
</table>

For the quantification of DTG and RLP, the method was found to be precise with six sample preparations. The precision was also evaluated on different days. Percent RSD values for DTG and RLP were found to be less than 2.0 percent in precision and intermediate precision.

Accuracy

Accuracy was determined by recovery experiments which were performed at three different levels of concentration (50 percent, 100 percent and 150 percent levels). As per the test method the test solution was injected to three preparations each spike level and the assay was performed. Accuracy outcomes were given in Table 3.

Robustness

Robustness of the method was found to be % RSD should be less than 2.0%. Slightly variations were done in optimized method parameters like flow rate (±0.2%), organic content in the mobile phase (±5%), pH of the buffer variations (±0.5). The results are given in Table 4.

Stability

Stability of standard and sample solutions were pre-
pared from initial to 24 hrs in stored at RT and 2-8°C. They are injected at different time intervals and the difference between initial to 24 hrs % of the assay was not more than 2%. There is no effect in storage conditions for DTG and RLP drugs. The solutions were stable up to 24 hrs.

**Forced degradation**

Forced degradation conditions such as acidic, basic, peroxide, reduction, thermal, hydrolysis and photolytic stress were attempted as per ICH guidelines. The outcomes of forced degradation were shown in Table 5.

**CONCLUSION**

This method described the quantification of dolutegravir and rilpivirine in bulk and pharmaceutical formulation as per ICH guidelines. The evolved technique was found to be accurate, precise, linear and reliable. The advantage lies in the simplicity of sample preparation and reproducibility data are satisfactory. The evolved chromatographic method can be effectively applied for regular investigation in drug research.

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**Conflict of Interest**

The authors declare that they have conflict of interest for this study.

**REFERENCES**


