Development And Validation Of Stability Indicating Rp-HPLC Method For The Estimation Of Valganciclovir In Bulk And Pharmaceutical Dosage Form

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Abstract- A simple, Precise, Accurate method was developed for the estimation of Valganciclovir by RP-HPLC technique. Chromatographic conditions used are stationary phase Ascentis 150mm x 4.6 mm, 5.0µ, Mobile phase 0.01N potassium dihydrogenphosphate: Methanol in the ratio of 65:35 and flow rate was maintained at 0.8ml/min, detection wave length was 254nm, column temperature was set to 30oC, retention time 2.180min. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to150 % levels, R2 value was found to be as 0.999. Precision was found to be 0.8 for repeatability and 0.4 for intermediate precision. LOD and LOQ are 0.0542µg/ml and 0.1643µg/ml respectively. By using above method assay of marketed formulation was carried out 99.8% was present. Degradation studies of Valganciclovir were done, in all conditions purity threshold was more than purity angle and within the acceptable range. This method can be used for routine analysis of Valganciclovir.

Index Terms- HPLC Valganciclovir, Method development. ICH Guidelines

I. INTRODUCTION

Valganciclovir is an anticoagulant and the first orally active direct factor Xa inhibitor. Unlike warfarin, routine lab monitoring of INR is not necessary. However there is no antidote available in the event of a major bleed. Only the 10 mg tablet can be taken without regard to food. The 15 mg and 20 mg tablet should be taken with food. FDA approved on July 1, 2011[3].

The chemical name of Valganciclovir is 5-chloro-N-{{[(5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl]methyl}thiophene-2-carboxamide. The molecular formula of Valganciclovir C19H18ClN3O5S.

The main objective of this proposed method is to develop a new rapid, simple, precise, accurate and economical analytical method for the estimation of Valganciclovir [3].

II. MATERIALS AND METHODS

Pharmaceutical grade Valganciclovir was purchased from Spectrum Labs. The solvents used for the procedure are of analytical grade. The HPLC grade chemical used is methanol and double distilled water and they were obtained from SDFCL. All the solutions were filtered through vacuum filter and sonicated. The marketed formulation of Valganciclovir(Valgan) is obtained from Cipla Pharma India Limited [3].

Apparatus:
U.V. Visible double beam spectrophotometer shimadzu along with two matched cuvetts was used. Stock solutions of the samples were prepared in AR grade methanol and used for analysis. The HPLC system used is waters HPLC model 2695. The column used was sunsil C18 (150mm X 4.6mm, 5µ). Auto sampler 171 Plus and the detector consisting of waters dual λ absorbance detector operated at 254nm. Software used for HPLC is empower 3.0.

Chromatographic conditions:
As the drug is soluble in methanol, the experimentation was started with the mobile phase 0.01N Potassium dihydrogen Ortho phosphate : methanol water with 65:35, and tried at different levels of combination containing these solvents. The optimal composition of mobile phase was determined as 0.01N Potassium dihydrogen Ortho phosphate : methanol water with 65:35. The mobile phase was filtered through 0.45µm nylon filter, and then sonicated for at least 10min[4].

Figure 1: structure of Valganciclovir
III. STANDARD STOCK SOLUTION:

Accurately weighed and transferred 45mg Valganciclovir working Standard into a 100 ml clean dry volumetric flask, add 70ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution, 1 ml was pipetted out into a 10ml Volumetric flask and then make up to the final volume with diluent. 10 tablets were weighed 45μg/ml the average weight of each tablet is equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 5ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 0.5ml was pipette out into a 50 ml volumetric flask and made up to 50ml with diluent. Inject the samples by changing the chromatographic conditions and record the chromatograms [5].

Validation of RP-HPLC method for Valganciclovir in bulk drug:

Preparation of solutions:

Preparation of 11.25μg/ml solution:
0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (11.25μg/ml of Valganciclovir)

Preparation of 22.5μg/ml solution:
0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (22.5μg/ml of Valganciclovir)

Preparation of 33.75μg/ml solution:
0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (33.75μg/ml of Valganciclovir).

Preparation of 45μg/ml solution:
1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (45μg/ml of Valganciclovir)

Preparation of 56.25μg/ml solution:
1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (56.25μg/ml of Valganciclovir)

Preparation of 67.5μg/ml solution:
1.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (67.5μg/ml of Valganciclovir)

Calibration curve (linearity):

The standard solutions were prepared by dilution of stock solution with methanol in concentration range 11.25μg/ml, 22.5μg/ml, 33.75μg/ml, 45μg/ml, 56.25μg/ml and 67.5μg/ml with concentration on X- axis and absorbance on Y- axis at 225nm. The correlation coefficient for Valganciclovir was found to be 0.999 [6].

Precision:

Precision of the analytical method was determined by taking 1ml of Valganciclovir stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (45 μg/ml of Valganciclovir) This solution of Valganciclovir was analyzed in HPLC for six replicates at the selected wavelength 254nm [7].

Accuracy:

Accuracy of the method was determined by recovery experiments. To the formulations the reference standard were added at the level 50%, 100%, and 150%. Accurately weighed 45mg of Valganciclovir transferred 100ml and volumetric flasks, 3/4 Th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (450μg/ml of Valganciclovir). 0.25ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent to get 50%. 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent to get 100%. 0.75ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent to get 150%. The recovery studies were carried out three times and the percentage recovery and percentage standard deviation of the recovery for Valganciclovir was calculated [8].

Robustness:

It 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. Standard 10g/ml solution was prepared by taking 1ml from solution B and transferred into 10ml volumetric flask and the volume was made up to the mark with methanol(10µg/ml) and this solution was scanned at two different flow rates i.e., 0.7ml and 0.9ml [9].

Limit of detection (LOD) and Limit of quantification (LOQ):

The LOD and LOQ were separately determined and calculated based on the calibration curve of standard solution [10].

Degradation studies:

Oxidation:

To 1 ml of stock solution of Valganciclovir, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 600c. For HPLC study, the resultant solution was diluted to obtain 45μg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:

To 1 ml of stock solution of Valganciclovir, 1ml of 2N hydrochloric acid was added and refluxed for 30mins at 600c . The resultant solution was diluted to obtain 45µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 1 ml of stock solution of Valganciclovir, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain 45µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 1050c for 1 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 45µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies:

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The photochemical stability of the drug was also studied by exposing the 450µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1hrs or 200 Watt hours/m² in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 45µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 1hrs at a temperature of 60°C. For HPLC study, the resultant solution was diluted to 45µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

IV. RESULTS AND DISCUSSIONS:

The present study was performed to develop a rapid precise and accurate method of Valganciclovir using RP-HPLC in bulk drug. The optimized chromatographic conditions were maintained using sunsil C18 column (250 X 4.6mm, 5µm) and mobile phase 0.01N potassium dihydrogenphosphate: Methanol in the ratio of 65:35 with a flow rate of 1ml/min at UV detection 254nm. The retention time of Valganciclovir was found to be 2.180 min\(^{11}\).

1. Precision:

   Intraday precision:

   ![](image1.png)

   **Fig-1.1: intra precision injection 1**

   ![](image2.png)

   **Figure 1.2: intraday precision injection 2**

   ![](image3.png)

   **Figure 1.3: intraday precision injection 3**
Table 1.1: Intraday precision results

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name</th>
<th>Rt</th>
<th>Peak area</th>
<th>Theoretical plate count</th>
<th>USP tailing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Valganciclovir(45µg/ml)</td>
<td>2.179</td>
<td>766317</td>
<td>4506</td>
<td>1.4</td>
</tr>
<tr>
<td>2</td>
<td>Valganciclovir(45µg/ml)</td>
<td>2.180</td>
<td>763941</td>
<td>4674</td>
<td>1.3</td>
</tr>
<tr>
<td>3</td>
<td>Valganciclovir(45µg/ml)</td>
<td>2.177</td>
<td>752415</td>
<td>4549</td>
<td>1.2</td>
</tr>
<tr>
<td>4</td>
<td>Valganciclovir(45µg/ml)</td>
<td>2.178</td>
<td>766353</td>
<td>4349</td>
<td>1.3</td>
</tr>
<tr>
<td>5</td>
<td>Valganciclovir(45µg/ml)</td>
<td>2.179</td>
<td>769865</td>
<td>4503</td>
<td>1.2</td>
</tr>
<tr>
<td>6</td>
<td>Valganciclovir(45µg/ml)</td>
<td>2.180</td>
<td>759417</td>
<td>4680</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>%RSD</td>
<td></td>
<td></td>
<td></td>
<td>0.8</td>
</tr>
</tbody>
</table>

Precision interday:
Fig 1.7: interday precision injection 1

Fig 1.8: interday precision injection 2

Fig 1.9: interday precision injection 3

Fig 1.10: interday precision injection 4

Fig 1.11: interday precision injection 5
Table 1.2: precision interday results

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name</th>
<th>Rt</th>
<th>Peak area</th>
<th>Theoretical plate count</th>
<th>USP tailing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Valganciclovir(45µg/ml)</td>
<td>2.1</td>
<td>79</td>
<td>747245</td>
<td>4506</td>
</tr>
<tr>
<td>2</td>
<td>Valganciclovir(45µg/ml)</td>
<td>2.1</td>
<td>84</td>
<td>745461</td>
<td>4674</td>
</tr>
<tr>
<td>3</td>
<td>Valganciclovir(45µg/ml)</td>
<td>2.1</td>
<td>81</td>
<td>740508</td>
<td>4549</td>
</tr>
<tr>
<td>4</td>
<td>Valganciclovir(45µg/ml)</td>
<td>2.1</td>
<td>83</td>
<td>743757</td>
<td>4349</td>
</tr>
<tr>
<td>5</td>
<td>Valganciclovir(45µg/ml)</td>
<td>2.1</td>
<td>85</td>
<td>739776</td>
<td>4503</td>
</tr>
<tr>
<td>6</td>
<td>Valganciclovir(45µg/ml)</td>
<td>2.1</td>
<td>85</td>
<td>740304</td>
<td>4680</td>
</tr>
</tbody>
</table>

% RSD = 0.4

2. Linearity:

\[ y = 16750x + 2558.2 \]

\[ R^2 = 0.999 \]
Figure 2.1: linearity 11.25µg/ml

Figure 2.2: linearity 22.5µg/ml

Figure 2.3: linearity 33.75µg/ml

Figure 2.4: linearity 45µg/ml
Figure 2.5: linearity 56.25 µg/ml

Table 2.1: linearity results

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name</th>
<th>Linearity Level (%)</th>
<th>Rt</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Valganciclovir(11.25µg/ml)</td>
<td>25</td>
<td>2.1</td>
<td>189457</td>
</tr>
<tr>
<td>2</td>
<td>Valganciclovir(22.5 µg/ml)</td>
<td>50</td>
<td>2.1</td>
<td>383064</td>
</tr>
<tr>
<td>3</td>
<td>Valganciclovir(33.75µg/ml)</td>
<td>75</td>
<td>2.1</td>
<td>570179</td>
</tr>
<tr>
<td>4</td>
<td>Valganciclovir(45µg/ml)</td>
<td>100</td>
<td>2.1</td>
<td>761587</td>
</tr>
<tr>
<td>5</td>
<td>Valganciclovir(56.25µg/ml)</td>
<td>125</td>
<td>2.1</td>
<td>935600</td>
</tr>
<tr>
<td>6</td>
<td>Valganciclovir(67.5µg/ml)</td>
<td>150</td>
<td>2.1</td>
<td>1135097</td>
</tr>
</tbody>
</table>

3. Accuracy:

Figure 3.1: accuracy 50% injection 1
Table 3.1: accuracy results

<table>
<thead>
<tr>
<th>% Level</th>
<th>Amount Spiked (µg/mL)</th>
<th>Amount recovered (µg/mL)</th>
<th>% Recovery</th>
<th>Mean % Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>22.5</td>
<td>22.57</td>
<td>100.32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22.5</td>
<td>22.54</td>
<td>100.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22.5</td>
<td>22.29</td>
<td>99.06</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>45</td>
<td>45.38</td>
<td>100.85</td>
<td>100.26%</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>44.96</td>
<td>99.91</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>45.04</td>
<td>100.10</td>
<td></td>
</tr>
<tr>
<td>150%</td>
<td>67.5</td>
<td>67.57</td>
<td>100.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>67.5</td>
<td>68.66</td>
<td>101.72</td>
<td></td>
</tr>
<tr>
<td></td>
<td>67.5</td>
<td>67.56</td>
<td>100.09</td>
<td></td>
</tr>
</tbody>
</table>
4. Robustness:

[1] Figure 4.1: Flow minus Chromatogram of Valganciclovir injection 1

Figure 4.2: Flow plus Chromatogram of Valganciclovir injection 2

Figure 4.3: Mobile phase plus Chromatogram of Valganciclovir injection 1

Figure 4.4: Mobile phase minus Chromatogram of Valganciclovir injection 2
Figure 4.5: Temperature plus Chromatogram of Valganciclovir injection 1

![Temperature plus Chromatogram of Valganciclovir injection 1](image1)

Figure 4.6: Temperature minus Chromatogram of Valganciclovir injection 1

![Temperature minus Chromatogram of Valganciclovir injection 1](image2)

Table 4.1: Robustness flow rate 0.9ml/min results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Minus</th>
<th>Actual</th>
<th>Plus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>0.7ml/min</td>
<td>0.8ml/min</td>
<td>0.9ml/min</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>70B:30A</td>
<td>65B:35A</td>
<td>60B:40A</td>
</tr>
<tr>
<td>Temperature</td>
<td>25°C</td>
<td>30°C</td>
<td>35°C</td>
</tr>
</tbody>
</table>

5. Limit of detection (LOD):

\[
LOD = 3.3 \times \frac{\sigma}{s}
\]

\[
= 3.3 \times \frac{16754}{2752.8}
\]

\[
= 0.542 \mu g/ml
\]

\(\sigma = \text{standard deviation, } s = \text{slope of calibration curve}\)

5. Limit of quantification (LOQ):

\[
LOQ = 10 \times \frac{\sigma}{s}
\]

\[
= 10 \times \frac{16745}{2363}
\]

\[
= 0.164 \mu g/ml
\]

\(\sigma = \text{standard deviation, } s = \text{slope of calibration curve}\)

Degradation Studies:

![Degradation Studies](image3)

Figure 4.7: Acid degradation chromatogram of Valganciclovir
Figure 4.8: Base degradation chromatogram of Valaganciclovir

Figure 4.9: Peroxide degradation chromatogram of Valaganciclovir

Figure 4.10: Thermal degradation chromatogram of Valaganciclovir
V. CONCLUSION

Chromatographic conditions used are stationary phase Ascentis (150mm*4.6mm5.0m), Mobile phase 0.01N potassium dihydrogenphosphate: Methanol in the ratio of 65:35 and flow rate was maintained at 0.8ml/min, detection wave length was 254 nm, column temperature was set to 30C and diluent was mobile phase. Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to 150% levels, R2 value was found to be as 0.999. Precision was found to be 0.8 for repeatability and 0.4 for intermediate precision. LOD and LOQ are 0.0542µg/ml and 0.1643µg/ml respectively. By using above method assay of market formulation was carried out 100.52% was present. Degradation studies of Valganciclovir were done, in all conditions purity threshold was more than purity angle and within the acceptable range. Full length method was performed if it is done this method can be used for routine analysis of Valganciclovir.

A good sharp peak was eluted at 2.1min using potassium dihydrogenphosphate: Methanol (65:35) v/v as eluting solvents. All the system suitability parameters were found to be within limits. Therefore this method can be employed for routine laboratory analysis.

Extended study for the drug may include degradation studies by HPLC. Characterization by various hyphenated techniques using bio analytical methods.

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