Research articles

Development of forced degradation studies of favipiravir by RP-HPLC

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ABSTRACT

Forced degradation studies and stability indicating method were developed for the estimation of Favipiravir by reverse phase High performance liquid chromatography in active Pharmaceutical ingredient and its tablet dosage form. The method was achieved by using C18 column (250 X 4.6mm X 4µm) with mobile phase mixture ortho phosphoric acid and acetonitrile in the ratio 60:40. The mobile phase was allowed to pump with the flow rate 1ml/min by maintaining detection wavelength at 324nm using ultra-violet detector. Favipiravir drug was subjected to various stress conditions according to International Conference of Harmonization Q1A(R2) guidelines to establish stability indicating method. Favipiravir drug was found to be sensitive at peroxide degradation. The impurity peak was characterized by mass spectral studies. The method was validated for analytical standards such as linearity, accuracy, Precision, sensitivity and robustness. A rapid and sensitive method was developed for the estimation of favipiravir which indicates its stability indicating behavior.

Keywords: Favipiravir, HPLC, forced degradation studies and Stability indicating method.

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INTRODUCTION

The IUPAC name of favipiravir is 6-fluoro-3-hydroxypyrazine-2-carboxamide and chemical structure of favipiravir shown in figure 1.

Figure 1: Chemical structure of favipiravir (Wiki)

Favipiravir, has been reported to exert broad spectrum activity against RNA viruses, and is permitted for use in Japan as an oral anti-influenza treatment [1-3]. Within patients receiving FAV as an influenza healing, a high genetic barrier of struggle has been observed but deployment is complicated by concerns about teratogenicity. [4] Ibrahim Bulduk had studied HPLC method for the quantification of favipiravir in pharmaceutical formulations. Favipiravir and its related substances quantification by were done by HPLC and is reported in Patent [5-6]. Rama Rao Nadendla with his coworkers developed a HPLC method for quantification of favipiravir using PDA detection [7]. Literature review reveals that there is no reported work on Stability indicating nature of favipiravir. The present study focuses on forced degradation on favipiravir and to establish stability indicating nature RP-HPLC [8-9].

MATERIAL AND METHOD

Equipment’s

HPLC used for development was shimadzu LC-2010 HT with an ultra-fast auto sampler and UV-Visible detector integrated with LC solutions software. UV–Visible spectrophotometer used was Nicolet evolution integrated with vision pro software. Mettler Toledo was used as Digital electronic balance for weighing. A mass spectrometer detector for characterizations achieved with waters maker Quattro premier XE model controlled by Open lab software. Electro spray Ionization (ESI) and Triple quadrupole detector is capable for recording ions up to 1000 m/z.

Drug sample

Favipiravir pure drug (API) procured from Natco Pharma and Favivir 200 mg tablet dosage form was purchased from local pharmacy in Hyderabad.

Reagent and solutions

HPLC grade distilled water procured from martin Synge pharma science Pvt. Ltd. HPLC grade methanol and acetonitrile were procured from Avant or Performance materials Ltd. Potassium Dihydrogen Phosphate procured from Thermo Fischer scientific Pvt. Ltd.

Preparation of Mobile Phase

Preparation of 1000 ml Mobile Phase Composition contains
600 ml of ortho phosphoric acid and 400 ml of acetonitrile filtered separately by using 0.45-micron pore size membrane filter. The filtered 600 ml ortho phosphoric acid & 400 ml of acetonitrile transferred in 1000 ml of volumetric flask.

**Preparation of Standard Stock Solution**
Accurately weighed 10 mg of favipiravir reference transferred in 10 ml of volumetric flask and dissolved in methanol and make up to 10 mL with methanol. The resulting solution concentration is 1000 µg/ml. This solution is considered as standard stock. From above standard stock solution transfer 1 mL solution in to 10 mL of volumetric flask and make up with methanol up to the mark. The resulting solution concentration is 100 µg/ml.

**Preparation of Sample Stock Solution**
Accurately weighed 10 mg of favipiravir API sample transferred in 10 mL of volumetric flask and dissolved in methanol and make up to 10 mL with methanol. The resulting solution concentration is 1000 µg/ml. This solution is considered as sample stock. From above sample stock solution transfer 1 mL solution in to 10 mL volumetric flask and make up with methanol up to the mark. The resulting solution conc. is 100 µg/ml.

**Marketed Formulation Sample Preparation**
Weighed 10 tablets and calculated average weight of each tablet and transferred 50 mg equivalent quantity of Tablet powder into 50mL volumetric flask dissolved & make up to the mark with methanol. Transferred 1mL of above solution to 10 mL volumetric flask diluted up to mark with methanol. Pipetted 1.2 mL from above solution into 10mL volumetric flask & made up to mark with methanol and sonicate well.

**Selection of wavelength maximum**
prepared stock solution was determined in ultraviolet spectroscopy range of 200-400nm. Wavelength maximum at 324 nm was observed & the UV spectrum of favipiravir was given in figure 2.

**RESULT AND DISCUSSION**

**Method Development**
Several trials were carried out to obtain optimized method with better separation and efficient theoretical plate. The method was achieved by using C18 column (250 X 4.6mm X 4µm) with mobile phase mixture ortho phosphoric acid and acetonitrile in the ratio 60:40. The number of theoretical plates and tailing factor for favipiravir was observed 82651(NLT 2000) and 1.265 (NMT 2). The optimized condition chromatogram was shown in figure 3.

**Method validation**
Linearity The concentration varies from 4µg/ml to 20µg/ml were analyzed. A linearity calibration graph is (Y=253.5x+1881; representing X as concentration and Y as peak area respectively) was calculated. The correlation coefficient was found to be 0.999. The linearity graph shown in figure 4. The linearity data shown in table 1.

**System Suitability**
Prepared 12 µg/ml samples from the standard stock solution and injected six replicates into the HPLC system. The system suitability data is shown in table 2.

**Method Precision (Repeatability)**
The six replicate injections for favipiravir is injected and chromatograms were recorded for same concentration (12 µg/mL). The relative standard deviation was found to NMT 2%, indicating method is repeatable.

**Intermediate Precision**
The intermediate precision was performed by the
favipiravir at 12 µg/ml concentration in six preparations. The intermediate precision study was carried out on different days. The method precision and intermediate precision data given in table 3.

Table 2: System suitability data of favipiravir

<table>
<thead>
<tr>
<th>Rt</th>
<th>Peak area</th>
<th>Theoretical plates</th>
<th>Tailing factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.453</td>
<td>5260.493</td>
<td>3391</td>
<td>1.526</td>
</tr>
<tr>
<td>4.453</td>
<td>5262.453</td>
<td>3385</td>
<td>1.452</td>
</tr>
<tr>
<td>4.430</td>
<td>5233.427</td>
<td>3483</td>
<td>1.595</td>
</tr>
<tr>
<td>4.430</td>
<td>5222.232</td>
<td>3483</td>
<td>1.595</td>
</tr>
<tr>
<td>4.437</td>
<td>5259.877</td>
<td>3366</td>
<td>1.500</td>
</tr>
<tr>
<td>4.463</td>
<td>5354.958</td>
<td>3406</td>
<td>1.526</td>
</tr>
</tbody>
</table>

Mean 5265.572
S. D 46.841
% RSD 0.89

Table 3: Intermediate Precision

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Method precision peak area</th>
<th>Intermediate precision peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>4255.073</td>
<td>4236.047</td>
</tr>
<tr>
<td>12</td>
<td>4184.798</td>
<td>4262.647</td>
</tr>
<tr>
<td>12</td>
<td>4280.981</td>
<td>4236.320</td>
</tr>
<tr>
<td>12</td>
<td>4262.854</td>
<td>4246.320</td>
</tr>
<tr>
<td>12</td>
<td>4286.183</td>
<td>4221.649</td>
</tr>
<tr>
<td>12</td>
<td>4259.877</td>
<td>4280.981</td>
</tr>
</tbody>
</table>

Mean 4259.877
S. D 36.500
% RSD 0.858

Accuracy

Accuracy was determined by using standard favipiravir transferred to calculated quantity of sample. By the use of three different concentrations equivalent to 50%, 100% and 150% of the sample accuracy was evaluated by calculating the recovery of favipiravir with % RSD. The percentage % RSD was found to be NMT 2.

Specificity

Specificity was evaluated by injecting standard solution and placebo solution individually into HPLC system. There is no interference observed in standard and placebo Retention time.

Robustness

The robustness is evaluated by deliberately different the chromatographic conditions such as the flow rate was changed to ± 0.2ml/min and wavelength to about ± 5nm. The robustness results given table 4.

Table 4: Robustness data of favipiravir

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Optimized condition</th>
<th>Changed condition</th>
<th>Peak area</th>
<th>Mean ± S. D</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate (ml/min)</td>
<td>1ml/min</td>
<td>0.8 ml/min</td>
<td>4614.433</td>
<td>4585.558 ± 40.83</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.2 ml/min</td>
<td>4556.682</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wave length (nm)</td>
<td>324 nm</td>
<td>322 nm</td>
<td>4318.383</td>
<td>4270.041 ± 68.43</td>
<td>1.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>326 nm</td>
<td>4221.649</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Assay of Marketed Formulation

The percentage purity of favipiravir was found to be 100.09%. The assay results data shown in table 5.

Table 5: Assay data of favipiravir

<table>
<thead>
<tr>
<th>S.N.O.</th>
<th>Standard</th>
<th>Peak area</th>
<th>Mean ± S. D</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4236.320</td>
<td>4230.67</td>
<td>4226.320</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>4256.047</td>
<td>4257.64</td>
<td>4260.493</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>4260.493</td>
<td>4260.09</td>
<td>4260.493</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>4246.320</td>
<td>4245.67</td>
<td>4246.320</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>4280.493</td>
<td>4280.98</td>
<td>4280.493</td>
<td>5</td>
</tr>
</tbody>
</table>

Mean ± S. D 4255.935 ± 16.591
% RSD 0.390
% RSD 100.09

Degradation Studies

Acid degradation study 10mg of favipiravir transferred in 10ml of volumetric flask and dissolve in methanol and make up to mark with methanol. The resulting solution concentration is 1000 µg/ml. Pipette out 1ml from above solution transferred in 10ml volumetric flask add 1ml of 1N HCl then kept at 60ºC for 5 hours & 80 ºC for 10 hours and neutralized with 0.1N NaOH solution.

Base degradation study

10mg of favipiravir transferred in 10ml of volumetric flask and dissolve in methanol and make up to mark with methanol. The resulting solution concentration is 1000 µg/ml. Pipette out 1ml from above solution transferred in 10ml volumetric flask add 1ml of 1N NaOH then kept in oven at 60ºC for 5 hours and 80ºC for 10 hours neutralized with 0.1N HCl solution and diluted with mobile phase. The solution is filtered through the 0.45µ membrane filter and injected in to HPLC system.

Peroxide degradation study

10mg of favipiravir transferred in 10ml of volumetric flask and dissolve in methanol and make up to mark with methanol. The resulting solution concentration is 1000 µg/ml. Pipette out 1ml from above solution transferred in 10ml volumetric flask add 5 ml of 10% hydrogen peroxide and make up to the mark with diluent then kept in oven at 60ºC for 5 hours and 80ºC for 10 hours. Degradation peak was observed during 10hours of peroxide stress study and chromatogram was shown in Fig 5.

Photolytic degradation study

10mg of favipiravir transferred in 10ml of volumetric flask and dissolve in methanol and diluted with methanol. The resulting solution concentration is 1000 µg/ml. Pipette out 1ml from above solution transferred in 10ml volumetric flask add 5ml of 10% hydrogen peroxide and make up to the mark with diluent then kept in oven at 60ºC for 5 hours and 80ºC for 10 hours.

Thermal degradation study

The sample was spread uniformly in the petri dish and wrapped in aluminum foil placed in oven at 60ºC for 5 hours & 80ºC for 10 hours in hot air oven. The sample was withdrawn after 5 hours & 10hours and the solution was introduced into the HPLC system. The degradation methods data is given in table 6.

Degradation Characterization

LC-MS/MS study for Degradation peak revealed that molecular weight of 113.016 gm/ml. The mass analysis of degradation peak exhibited 113.0.16 gm/mol molecular mass for parent ion along with 156.021 gm/mol molecular weight for fragment ion. Identical results were acquired for the favipiravir solution stressed with peroxide solution. The degradation peak mass spectra shown in figure 6 and degradation pathway shown in figure.
7. Figure 5: Degradation peak eluted during peroxide stress study (10hrs)

Figure 6: Mass spectra of peroxide degradation peak

Figure 7: Favipiravir degradation pathway

Table 6: Degradation methods data of favipiravir

<table>
<thead>
<tr>
<th>Type of degradation</th>
<th>Trial</th>
<th>Condition for degradation</th>
<th>% Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid degradation</td>
<td>1</td>
<td>1 N HCl, 60°C, 5 hours</td>
<td>No Degradation</td>
</tr>
<tr>
<td>Acid degradation</td>
<td>2</td>
<td>1 N HCl, 80°C, 10 hours</td>
<td>No Degradation</td>
</tr>
<tr>
<td>Base degradation</td>
<td>1</td>
<td>1 N NaOH, 60°C, 5 hours</td>
<td>No Degradation</td>
</tr>
<tr>
<td>Base degradation</td>
<td>2</td>
<td>1 N NaOH, 80°C, 10 hours</td>
<td>No Degradation</td>
</tr>
<tr>
<td>Oxidation degradation</td>
<td>1</td>
<td>10% H$_2$O$_2$, 60°C, 5 hours</td>
<td>No Degradation</td>
</tr>
<tr>
<td>Oxidation degradation</td>
<td>2</td>
<td>10% H$_2$O$_2$, 80°C, 10 hours</td>
<td>8.5</td>
</tr>
</tbody>
</table>

CONCLUSION
The present work is precise and validated for the estimation of favipiravir. The Rt of favipiravir was found to be 4.4 min. The favipiravir % Recovery was obtained as 100.09%. LOD and LOQ values obtained from regression equations of favipiravir 1.26µg/ml and 3.83µg/ml respectively. An Isocratic mode has employed for elution and it efficiently separated drug from its degradant. The Forced degradation study and Mass spectra reveal that the decomposed product is 5-Fluoropyrimidine-2-Ol Anion.

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CONFLICTS OF INTEREST
Authors were declaring that there is no conflict of interest.

REFERENCE