Fluorimetric Determination of Antiviral (COVID-19) Drug Favipiravir in Bulk and Pharmaceutical Dosage Forms

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Abstract

Favipiravir is a synthetic prodrug, which was first discovered while assessing the antiviral activity of chemical agents active against the influenza virus in the chemical library of Toyoma chemicals. It works by inhibiting RNA dependent RNA polymerase (RdRP), an enzyme required for RNA viral replication inside human cells. A simple, rapid, and economic method was developed for the quantitative determination of Favipiravir using spectrofluorometer. The Favipiravir standard drug solution and sample tablet solution was prepared using double distilled water as a diluent. The different concentrations of pure drug in the range 2-10 µg/ml and one sample solution were measured for the intensity at 432 nm in the spectrofluorometer. The calibration curve was plotted and the sample’s unknown concentration was calculated from the plot. The calibration curve was found to be linear with r² value obtained as 0.99. There are various other methods available for the quantification of Favipiravir which include RP-HPLC, UV-spectroscopic methods, FTIR, LC-MS with different extraction methods spiked in human plasma but not by using spectrofluorometer. Favipiravir shows fluorescence when dissolved in appropriate solvent hence this method was developed to quantify Favipiravir which is a simple and efficient method. This method developed is easy and can be used for routine quality control test for Favipiravir pharmaceutical formulations.

Keywords: Favipiravir, spectrofluorometer, Calibration curve.

INTRODUCTION:

Favipiravir (FAV) is an antiviral drug which was approved in Japan in 2014 for use in the outbreak of influenza viral infections, where use of other antiviral drugs was insufficiently effective in the treatment of influenza1,2. FAV was also used in the treatment of Ebola virus infections and for the post exposure prophylaxis3. When the pandemic hit the world in December 2019, there was no proper and approved specific drug to treat SARS-Cov-2. Few of the already approved antiviral drugs which have the safety data were tested for the activity against the covid-19 infection. The class of drugs which were preferably selected was the RNA dependent RNA polymerase (RdRP) inhibitors. One of the drugs belonging to that class is Favipiravir, which was then started to use to treat the Coronavirus infected patients.

Favipiravir chemically known as 6-fluoro-3-oxo-3,4-dihydro pyrazine 2-carboxamide as shown in Figure 1, acts by selectively inhibiting RNA dependent RNA polymerase. It is an enzyme which is required for the replication of RNA virus in the cells of humans4. It acts as a substrate for the RNA-dependent RNA-polymerase (RdRp) enzyme, which is mistaken by the enzyme as a purine nucleotide, thus inhibiting its activity leading to termination of viral protein synthesis5. Single molecule of FAV is enough to terminate the further extension of viral RNA6.

The reported methods for analysis of FAV include HPLC method with UV detection and UV-visible spectroscopic method7,8. There are no reported spectrofluorimetric methods has been developed for quantitation of FAV neither in pure form nor in pharmaceutical preparation with the use of simple solvent as a diluent. Favipiravir shows fluorescence activity when dissolved in appropriate solvent. The solutions were measured at 432 nm after excitation at 361 nm.

Figure 1: Chemical structure of Favipiravir.
MATERIALS AND METHODS:

**Apparatus:** Volumetric flasks, Pipette, Digital weighing balance.

**Instrument:** Spectrofluorometer.

**Reagents:** Favipiravir pure drug was a gift from pharmaceutical industry, Fabiflu (Favipiravir tablet-200mg).

**Standard stock solution:** Accurately weighed 10mg of Favipiravir pure drug was transferred into 10mL Volumetric flask and dissolved in Double distilled water. The volumetric flask was filled with the same up to the mark. This standard stock has a concentration of 1000μg/mL.

**Working Standard solution:** From the standard stock solution, 1mL solution was pipetted out and transferred in 10mL volumetric flask and filled up to the mark with Double distilled water. This working standard has a concentration of 100μg/mL.

**Standard Dilutions:** From the working standard solution, standard dilutions were made from concentrations 2μg/mL to 10μg/mL using 10mL volumetric flasks. The absorbance of these standard dilutions was checked in the fluorimeter. The readings were noted and a calibration curve was plotted.

**Tablet analysis:** 10 Fabiflutaeblets were accurately weighed and average weight was calculated. The equivalent weight of 10 mg was accurately weighed and dissolved in double distilled water and filled with the same up to the mark. From this solution a sample solution was prepared and checked for the absorbance value. From the calibration curve the actual concentration was calculated.

RESULTS AND DISCUSSION:

Figure 2 represents the calibration curve data of the Fluorescence activity of Favipiravir pure drug using double distilled water as solvent. The regression coefficient $r^2$ value was calculated to be 0.99 which is within limits. Table 1 shows the concentration and intensity data of the Favipiravir pure drug and that of sample. From the table, the concentration of Favipiravir in the Fabiflu tablet (sample) solution was found to be 4.89 μg/mL for a sample made with theoretical concentration of 5 μg/mL.

The other methods available for the analysis and quantification of Favipiravir include HPLC using UV detection, HPLC using DAD detection, HPLC using bioanalytical samples (drug spiked in human plasma) using different extraction methods. Addition to these, analysis of favipiravir was done by developing method and validating it using FTIR, NMR/LC-MS and invitro safety evaluation. Comparative studies of method developed for analyzing Favipiravir in Pharmaceutical preparations using HPLC and UV were also found. Concurrent Fluorescence based analysis of remdesivir and favipiravir are the two methods found for analysis of Favipiravir along with other drugs using Fluorescence. Since there were no reported methods found for individual analysis of Favipiravir using fluorescence, the present method was developed.

CONCLUSION:

The present method developed for the estimation of Favipiravir using spectrofluorometer is easy, rapid, simple without use of complex process or instrumentation. The method developed is according to ICH guidelines and results obtained fall under limits of the parameters set by the guidelines. This method developed can be used as a routine quality control test for Favipiravir pharmaceutical preparations.

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REFERENCES:


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<th>Table 1: Calibration curve data of Favipiravir</th>
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<td>Concentration</td>
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<td>Concentration of unknown sample</td>
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Figure 2: Calibration curve of Favipiravir.


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