STABILITY INDICATING HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF FAVIPIRAVIR IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT
Favipiravir \{FVP\}, is an antiviral drug C5H4FN3O2 that is administered orally or intravenously also and the drug inhibitits viral replication of RNA viruses by interfering with viral RNA polymerase function. The officially Method are not available in any pharmacopeia. The ultraviolet (UV) detection 323nm and 225nm, Scanning between 200 and 800 nm.

KEYWORDS: Favipiravir, antiviral, UV method, Development, Validation.

INTRODUCTION
Coronavirus disease (COVID-19) spread rapidly and became is an epidemic, there are affecting almost all countries and regions around the world. COVID-19 case and death rate ranges from 1% to 7% according to the reports of World Health Organization (WHO). It caused all people in the world to change their lifestyle. Since the outbreak of the COVID-19 began to affect the all world and countries have implemented different treatment methods. So far, there is no gold standard for the treatment of COVID-19 since there is not enough proof. Favipiravir (6-fluoro- 3-hydroxypyrazine-2-carboxamide) is an analog of pyrazine. Favipiravir (FVP) is an antiviral drug that was initially developed for influenza by Toyama Chemical. It selectively inhibits the RNA polymerase of RNA viruses, thus prevent viral reproduction. It displays antiviral activity against alpha-, filo-, bunya-, arena-, flavi-, and noroviruses as well as being active against the influenza virus.
According to the literature search, there are two published high performance liquid chromatography (HPLC) methods for determining FVP assay and impurities in active pharmaceutical ingredients. A UV mode was used for them. FVP the wavelength corresponding to maximum absorbance $\lambda_{\text{max}}$ was determined as 323 nm. FVP is not officially available in any pharmacopoeia and there is still a need for validated HPLC methods to determine FVP in pharmaceutical formulations.

**EXPERIMENTAL**

**Chemicals**

Analytical grade chemicals used without further purification in this study. Methanol (Merck ≥99.9%) were used. Acetonitrile (Merck, Sodium Hydroxide (Merck), Hydrochloric Acid (Merck), Ortho phosphoric acid (Merck), were used and Deionized water was purified by a Milli-Q (Millipore) system with conductivity lower than 18.2 μS cm$^{-1}$. FVP bulk powder and tablets (favicovir, 200 mg) were obtained from Atabay Pharmaceuticals and Fine Chemicals Inc (Istanbul, Turkey).

**Solution stability and solubility**

The stability of sample and standard solutions was monitored over a 24 h period. Standard solutions have been stored at ambient temperature (25°C) and protected from light. FVP API solubility in Methanol, ACN and water.

**Determination of $\lambda_{\text{max}}$**

Standard solution of Favipiravir (10 ppm) scanned between 200-400 nm using UV-visible spectrophotometer. 225 nm Wavelength was selected from the overlay spectra of above solutions.
Fig. 2: UV spectrum (standard solution, 10μg/mL) Standard Stock Solution.

**Preparation of Standard Stock Solutions**

Favipiravir standard stock solution (10μg/mL), Taken And accurately Weigh (10.0mg) Ten milligram of Favipiravir Active Pharmaceutical Ingredients (FVP API) into a 100 mL volumetric flask, add about Few mL of Acetonitrile and vigorously shaking with sonicate to dissolve it, cool the room temperature and makeup the volume up to the mark with ACN. Further Dilute 1mL of solution into 10mL of volumetric flask, makeup the volume up to the mark with ACN and mixed well.

**Sample Solution Preparation**

Accurately weighed 10 Tablets of Favipiravir and transferred to a dry and clean mortar, then crush into a fine powder. Taken tablet powder equivalent to 10 mg of Favipiravir was transferred to a 100 ml volumetric flask. Add 40 ml of mobile phase and shake for 15 minutes and made-up volume up to the mark with mobile phase. The solution was filtered through 0.45 micro meter filter paper and first few drops of filtrate were discarded. 1ml of this solution was diluted to 10 ml with mobile phase. The solution was injected. The areas of resulting peak were measured at 225 nm. (10ppm μg mL⁻¹ sample solution).

**Chromatographic Conditions**

Chromatographic analysis was performed on a column a stainless-steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 μm) (Such as Intersil ODS 3V), – mobile phase: A. 0.1 percent v/v solution of orthophosphoric acid, B. acetonitrile, – a gradient programme using the conditions given below, – flow rate: 1 ml per minute, – spectrophotometer set at 225 nm, – injection volume: 10 μl. The run time was 10 min under these conditions. The column has been thermostat at 30°C.
Method Validation

The analytical method validation has been performed as per ICH guideline of validation of analytical Procedure: Q2 (R1). The validation parameters such as system suitability, linearity, the limit of detection (LOD), the limit of quantification (LOQ), specificity, accuracy, precision, and robustness were addressed.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc.

Specificity was performed by injecting individual and combined solution of Favipiravir 10ppm and interference was checked with the chromatogram of blank.

2 Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. The linearity for Favipiravir analysis of standard solution in range of 10-30μg/ml respectively, 0.5mL, 1mL, 1.5mL, 2mL, 2.5mL, 3mL solutions were pipette out from the Stock solution of Favipiravir(10ppm). In term of slope, intercept and correlation co-efficient value, the graph of peak area obtained verses respective concentration was plotted. Good linearity (correlation coefficient $r^2 = 0.9989$) was observed for Favipiravir the Concentration range of 10-30μg/ml for each.

3 Precision

The precision of an analytical procedure expresses 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 and reproducibility.

1. Repeatability

The data for repeatability of peak area measurement for Favipiravir (10μg/ml) based on six measurements.

Intraday precision

Standard solution containing (10, 20, 30 μg/ml) of Favipiravir were analysed three times on the
sameday and % R.S.D was calculated.

**Interday precision**
Standard solution containing (10,20,30 µg/ml) of Favipiravir were analysed three times on the different day and % R.S.D were calculated.

## 4 Accuracy
Definition: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.10 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 50%, 100%, 150% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 225 nm. The amount of Favipiravir was calculated at each level and % recoveries were computed.

**LOD**
Definition: The limit of detection of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

**LOQ**
Definition: The limit of quantitation of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The limit of quantitation is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts were calculated. Then LOD and LOQ were calculated.

## 6 Robustness
Definition: 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.

The robustness of the proposed method was evaluated by slight modification in the mobile phase composition, Flow rate and pH values of the mobile phase. During these studies it was found that there was not much change retention time, area and symmetry of peak. The
developed method was used for the assay of commercially available tablets and six replicate
determinations were performed. The interference of excipients was studied by comparing the
chromatography of standards and formulations. The same shape and retention times of peaks
showed that there was no interference from excipients.

**Method development**

Taken sample equivalent to 10 mg of Favipiravir was transferred to a 100 ml volumetric flask. Add 40 ml of mobile phase and shake for 15 minutes and made-up volume up to the mark with mobile phase. The solution was filtered through 0.45 micro meter filter paper and first few drops of filtrate were discarded. 1 ml of this solution was diluted to 10 ml with mobile phase. The solution was injected. The areas of resulting peak were measured at 225 nm.

**Force Degradation Study**

- Acid Degradation
- Basic Degradation
- Oxidation Degradation
- Thermal Degradation
- Phytolytic Degradation

**Force Degradation Studies Preparation of stock solutions**

(A) **Favipiravir standard stock solution (100μg/mL)**

Taken And accurately Weigh (10.0mg) Ten milligram of Favipiravir Active Pharmaceutical Ingredients (FVP API) into a 100 mL volumetric flask, add about Few mL of Acetonitrile and vigorously shaking with soniccate to dissolve it, cool the room temperature and makeup the volume up to the mark with ACN. Further Dilute 1mL of solution into 10mL of volumetric flask, makeup the volume up to the mark with ACN and mixed well.

**Acid degradation**

Working Sample Solution for Acid Degradation: Acid degradation studies were performed by transferring 1 ml of stock solution to 10 ml of volumetric flask. Two ml of 0.1 N HCL solutions was added and mixed well and put for 1 hr. at RT. Then the volume was adjusted with diluent to get 10μg/ml for Favipiravir. Add 2ml of 2N NaOH to neutralize the solution. Then the volume was adjusted with diluent to get Favipiravir (10 μg/ml) And filtered through membrane filter paper and injected in to HPLC system.
Base Degradation
Working Sample Solution for Base Degradation: -Base degradation studies were performed by transferring 1ml of stock solution to 10 ml of volumetric flask 2ml of 0.1 N NAOH solutions was added and mixed well and put for 1hrs. at RT. Then the volume was adjusted with diluent to get 10μg/ml for Favipiravir Add 2ml of 2N HCL to neutralize the solution then the volume was adjusted with diluent to get Favipiravir (10 μg/ml) and filtered through membrane filter paper and injected in to HPLC system.

Working Sample Solution for Oxidative Degradation
Oxidation degradation studies were performed by transferring 1 ml of stock solution to 10 ml of volumetric flask. 2 ml of 3% H2O2 solutions was added and mixed well and put for 24 hrs. at RT. Then the volume was adjusted with diluent to get 10μg/ml for Favipiravir and filtered through membrane filter paper and injected in to HPLC system.

Thermal Degradation
Thermal degradation studies were performed by transferring 1 ml of stock solution to 10 ml of volumetric flask. The flask was kept for 2 hrs at 40°c Temperature then after volumetric flask was removed and cool at room temperature Then the volume was adjusted with diluent to get 10μg/ml for Favipiravir and injected in to HPLC system.

Photolytic degradation
Working Sample Solution for Photo Degradation: -Photo degradation studies were performed by transferring 1 ml of stock solution to 10 ml of volumetric flask. The flask was kept for 24hrs under UV light. Then the volume was adjusted with diluent to get 10μg/ml for Favipiravir and filtered through membrane filter paper and injected in to HPLC system.

CONCLUSION
A simple, accurate and precise stability indicating RP-HPLC method has been developed and validated as per ICH guideline for Stability Indicating HPLC Method Development and Validation for Estimation of Favipiravir in Pharmaceutical Dosage Form. 0.1 per cent v/v solution of orthophosphoric Acid and Acetonitrile (77:23) was used as mobile phase. Retention time of Favipiravir was found to be 5.6min, respectively with a flow rate of 1 ml/min. Forced degradation study of Favipiravir was performed by RP-HPLC method which includes acid, base, oxidative, photo and thermal degradation. Results of degradation were found within limit. Validation parameters like system suitability, linearity, accuracy,
precision, robustness, specificity, limit of detection and limit of quantitation were carried out on the proposed RP-HPLC method and validated as per ICH guideline. Observation of all these parameters leads to the point that developed RP-HPLC method is linear, accurate, precise, and robust. So, the developed method can be used for quality control, routine analysis and stability study of Favipiravir in Single component without any interference from common excipients and impurity.

![Fig. 3: Calibration curve (λ max 225nm).](image3)

**Fig. 3: Calibration curve (λ max 225nm).**

![Fig. 4: (A) Overlay chromatogram (standard solutions, 10 μg mL⁻¹).](image4a)

**Fig. 4: (A) Overlay chromatogram (standard solutions, 10 μg mL⁻¹).**

![Fig. 4: (B) chromatogram (standard solutions, 10 μg mL⁻¹).](image4b)

**Fig. 4: (B) chromatogram (standard solutions, 10 μg mL⁻¹).**
Fig. 4: (c) chromatogram (sample solutions, 10 μg mL⁻¹).

Fig. 4: (D) chromatogram (Blank solutions, 10 μg mL⁻¹)

Fig. 4: (E) chromatogram (Acid Degradation, 10 μg mL⁻¹).
Fig. 4: (F) chromatogram (Base Degradation, 10 μg mL−1).

Fig. 4: (F) chromatogram (Oxidative Degradation, 10 μg mL−1).

Fig. 4: (G) chromatogram (Thermal Degradation, 10 μg mL−1).
Fig. 4: (H) chromatogram (Photolytic Degradation, 10 µg mL⁻¹).

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13. ICH HARMONIZED TRIPARTITE GUIDELINE: Validation of Analytical procedures: Text and Methodology Q2(R1)
