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Analytical Method Development and Validation and Forced Degradation Stability-Indicating Studies of Favipiravir by RP-HPLC and UV in Bulk and Pharmaceutical Dosage Form

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To develop and validate a new, simple, rapid, precise, and accurate An Eco-friendly RP-HPLC and UV-Method Development and Validation for an estimation of Favipiravir in Bulk and pharmaceutical dosage form followed by Forced Degradation Studies.
Study Design: This was employed for UV-visible (200-400 nm and 400-800 nm respectively) and RP-HPLC method development using C 18 inertsil column and optimization of variables for Favipiravir estimation in bulk and formulations.
Place and Duration of the Study: The present work was carried out at Ali-allana College of Pharmacy, Akkalkuwa between the duration of November-2020 to February-2021.
Methodology: UV-Spectroscopic method was developed for the estimation of Favipiravir in the bulk and pharmaceutical dosage form. The solvent selected for the Favipiravir UV analysis was water, the solution in a range of 2-10µg/ml was scanned in the UV region from 200-400 nm and the λmax value was determined. The RP-HPLC method was developed on inertsil ODS-3V C18 150 mm x 4.6mm x 5µ column using buffer pH 3.5: acetonitrile [90:10] as mobile phase at flow rate 1.0 ml/min and PDA detection at 358 nm.

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Results: The maximum absorbance was observed at 358 nm. The wavelength 358 nm was selected for further analysis of Favipiravir. The calibration curve was determined using drug concentrations ranging from 2-10 µg/ml. The % recovery for accuracy was 100.50-100.76%. The method was to be precise with a % RSD value 0.51-1.37 and 0.77-1.78 for intraday and Interday respectively. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.0723 &0.219 µg/ml respectively by UV method. The RP-HPLC method was shown to be linear in the 50-250 µg/ml concentration range. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 2.186 & 6.626 µg/ml respectively. The method was to be precise with a % RSD value 0.25-1.53 and 0.86-1.68 for intraday and inter-day respectively.

Conclusion: Here we conclude that the developed UV and RP-HPLC methods are precise, accurate, sensitive, and reproducible for the quantitative estimation of Favipiravir bulk and its formulation. The developed method can be used by the pharmaceutical industries for the routine analysis of Favipiravir, in particular by UV and RP-HPLC. The main features of the proposed method are economic and eco-friendly with less retention time around 5.0 min.

Keywords: RP-HPLC; UV-visible spectroscopy; method development; validation; favipiravir; stability; ICH Q2R1.

ABBREVIATIONS

RP-HPLC: Reverse Phase High-Performance Liquid Chromatography
ICH : International Conference on Harmonization
LOD : Limit of Detection
LOQ :Limit of Quantification
RSD : Relative Standard Deviation
RNA : Ribonucleic Acid
SD : Standard Deviation

1. INTRODUCTION

Favipiravir (6-fluoro-3-hydroxypyrazine-2-carboxamide), a purine nucleic acid analog developed by Toyama Chemical in Japan for the treatment of viral infections, including influenza. This has recently been evaluated and found to be a promising option in the management of COVID-19. It works by inhibiting the RNA-dependent enzyme RNA polymerase (Rd-Rp), a key enzyme that prevents the replication of RNA viruses [1].

Favipiravir (T-705; 6-fluoro-3-hydroxy-2-pyrazine carboxamide) is an antiviral agent that selectively and potently inhibits RNA-dependent RNA polymerase (RdRp) of RNA viruses. Toyama Chemical Co. Ltd. discovered Favipiravir by examining a chemical library for antiviral activity against the influenza virus. Recognized as a substrate by RdRp and inhibits RNA polymerase activity [1,2]. As the catalytic domain of RdRp is conserved among various types of RNA viruses, this mechanism of action supports a broader spectrum of antiviral activities than Favipiravir. Favipiravir is effective against a wide range of influenza virus types and subtypes, including strains resistant to existing influenza drugs. Of note, Favipiravir exhibits antiviral activity against other RNA viruses such as arenavirus, bunyavirus, and filovirus, all of which are known to cause fatal hemorrhagic fever. These unique antiviral profiles will make Favipiravir a potentially promising drug for specifically intractable viral RNA infections [1, 3].

Favipiravir has great utility for treating patients with COVID-19. However, research examining the efficacy and safety of Favipiravir for COVID-19 patients is limited. Favipiravir induces viral spread after 7 days and contributes to clinical improvement in 14 days. The results indicated that Favipiravir has a strong ability to treat COVID-19, especially in patients with mild to moderate disease. Further well-designed studies, including examinations of dose and duration of treatment, are critical to reaching firm conclusions [3,4].

Jyothi and Kavya developed only and single UV method on Favipiravir and concluded that given spectrophotometric method for the estimation of new antiviral repurposing drug Favipiravir as there is no reported simple UV spectrophotometric method for estimation. The efforts were made for development and validation of Favipiravir as per ICH guidelines, because drug has a wide scope for formulations to be developed for treating different viruses [5].

Dikma Technologies developed a simple HPLC-UV method for simultaneous analysis of ivermectin, molnupiravir, remdesivir, favipiravir and ritonavir Diamonsil® Plus C18 Column.Varma *et al.*concluded 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.Bulduk developed HPLC-UV method and revealed he ultraviolet (UV) detection and column temperature were 323 nm, and308C, respectively. The run time was 15 min under these chromatographic conditions. Excellent linear relationship between peak area and Favipiravir concentration in the range of 10–100mg/ml has been observed [6].

Nadendla and Patchala developed HPLC method by PDA detector concluded that The proposed method was successfully applied for the marketed formulations of Favipiravir tablets. In addition the main features of the proposed method are economic and eco-friendly with less retention time around 4.622 min [7].

A stability indicator test method can be defined as a "validated quantitative analytical method capable of detecting the change over time in the chemical, physical or microbiological properties of the pharmaceutical substance and specific pharmaceutical products so that the content of active ingredients and degradation products can be accurately measured without interference. The goal of this work is to develop and validate analytical methods for stability indicators for selected drugs and bulk formulations with forced degradation studies along with characterization [8].

Although many methods have been reported on the quantitative and qualitative estimation of Favipiravir in bulk and its commercial formulation, very little work has been done on its estimation by RP-HPLC followed by methods with stability indicators. The advantage of proposed method over other methods is that given method give less retention time (eco-friendly) with good resolution.Therefore, in the present work, we have developed and validated the selective stability indicated by the RP-HPLC and UV methods. The proposed methods were validated according to the ICH guidelines [1, 8]. The structure of Favipiravir is depicted in Fig. 1.

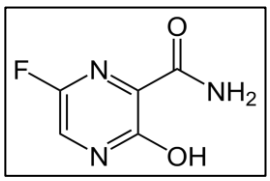


Fig. 1. The structure of favipiravir

2. MATERIALS AND METHODS

2.1 Instruments

Shimadzu HPLC system [LC-20AD Multi-solvent delivery system, SPD-20A, PDA Detector, LC solution software] UV-Visible Spectrophotometer [Shimadzu- 1800 double beam, with UV Probe 2.33].Labman sonicator was usedforsonication of the sample solution. Thermo scientific pH meter was used to measure pH. A vacuum pump filter was used for the filtration of mobile phase solvents.

2.2 Chemicals and Reagents

The drug was procured from Honour Lab, Hetero Limited with the certificate of analysis. The marketed formulation [Favipiravir tablet (Fabi flu) 200mg] was purchased from Blue Cross Laboratories Pvt. Ltd. Acetonitrile, HPLC grade water, Orthophosphoric Acid, Potassium Dihydrogen Phosphate, and Tri-ethylamine was procured from research lab fine chem industry.

3 SPECTROPHOTOMETRIC CONDITIONS

The stock solution was suitably diluted with distilled water, to get 10 µg/ml of Favipiravir. This solution was scanned in the UV region (200-400 nm) and found that Favipiravir exhibited maximum absorbance at about 358 nm as shown in Fig. 2.Hence 358 nm was selected for the proposed study [9, 10].

3.1 Methodby UV Spectroscopy

3.1.1 Preparation of standard stock solution

The spectral pattern and absorbance maxima of Favipiravir were thoroughly analysed. It was found that significant spectra of Favipiravir appeared in distilled water and this solvent was selected for determining Favipiravir content in formulation by UV spectroscopic method. A stock solution of Favipiravir was prepared by dissolving 100mg of drug in 100ml of distilled water to obtain the concentration of 1000µg/ml. And from this 0.1ml was taken and transferred to a 100ml volumetric flask and diluted with distilled water to get 10µg/ml solutions [10, 6].

3.1.2 Determination of standard calibration curve

Adequate dilutions were made from the stock solution to get a concentration ranging from 2-10

µg/ml for Favipiravir using distilled water. The absorbance of these solutions was measured at358nm. The absorbance values are tabulated. The measured absorbance was plotted against concentration. From the graph, it was found that the Beer's law concentration for Favipiravir lies between 2-10 µg/ml [6].

3.2 Method by RP-HPLC method

3.2.1 Chromatographic conditions

Chromatographic analysis was performed on a column of Inertsil ODS-3V C18. The mobile phase consisted of potassium dihydrogen phosphate 50 mM (pH 3.5) and acetonitrile (90:10, v/v). The mobile phase was filtered and degassed through a 0.45 mm membrane filter before use and then pumped at a flow rate of 1 ml/min.The run time was 10 min under these conditions as shown in fig.3 [11,12].

3.2.2 Preparation of buffer, mobile phase, and diluents

1.36gm of potassium dihydrogen phosphate and 2ml of triethylaminewere transferred into a beaker containing 1000ml of water and sonicated to dissolve the contents completely. The pH was adjustedto 3.5±0.05 with Orthophosphoric acid and mixed and filtered through 0.45µ filter paper [13].

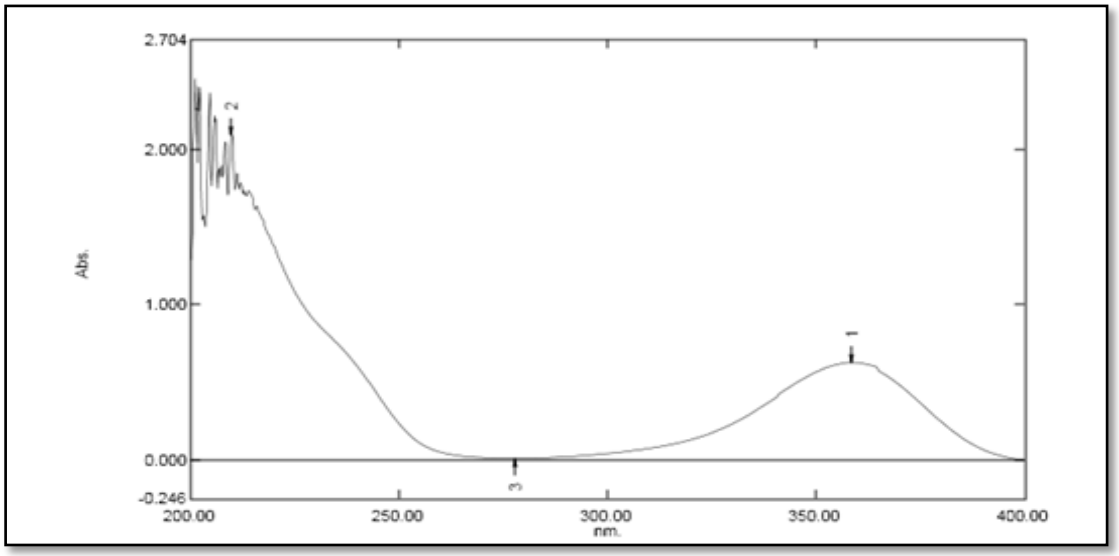


Fig. 2. UV spectra of Favipiravir for the selection of wavelength

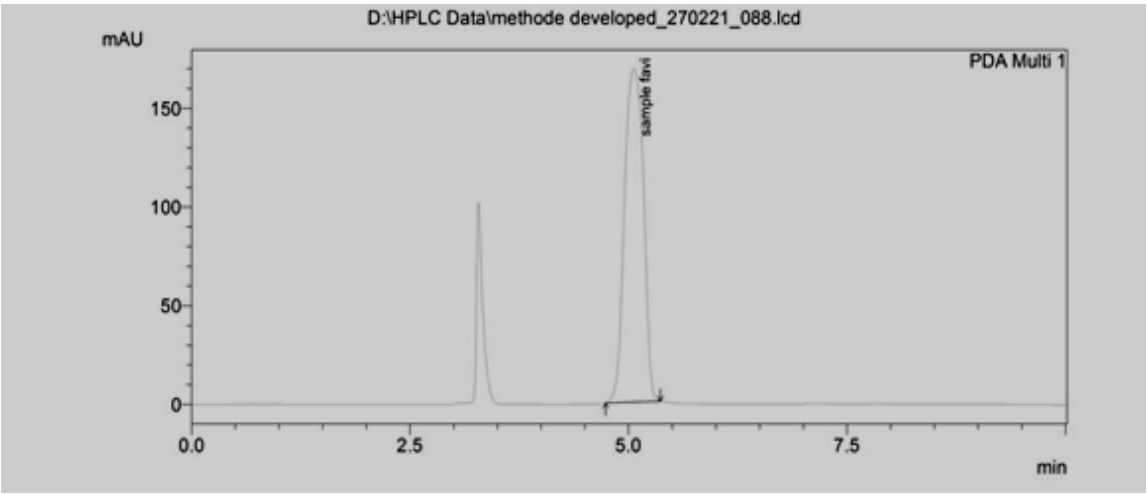


Fig. 3. The optimized chromatogram of Favipiravir in buffer pH 3.5: Acetonitrile (90:10), flow rate=1 ml/min

3.2.3 Preparation of diluent

The degassed mixture of buffer and acetonitrile in50:50%v/v ratio was prepared.

3.2.4 Mobile phase

Select the Binary method in HPLC and select the pump B ratio as 90.

3.2.5 Preparation of standard stock solution

One hundred milligrams pure drug was accurately weighed, dissolved in about 30 mL of diluent, and transferred to a 100 mL volumetric flask. Then the volume was completed to 100 mL with diluent to obtain1000 mcg/mL of stock solution. The resulting stock solution was sonicated and filtered through a 0.45 mm filter. . The stock solution was further diluted with diluent to obtain the required concentration of standard solutions [50–250 µg/m]) before being injected into the system [6,12,13].

3.3 Stability Study

3.3.1 Preparation of stock for the stability study

For forced degradation 10mg of Favipiravir standard drug were weighed and transferred to 100 ml volumetric flask containing 70 ml of diluents, sonicated for 5 min, and the volume was made up to the mark with diluents [10, 14-15].

4. ANALYTICAL METHOD VALIDATION

4.1 By UV Spectroscopy Methods

4.1.1 Linearity

Favipiravir was found to be linear in a concentration range of 2-10µg/ml. The absorbance of this solution was measured at 358 nm and a calibration graph was plotted using absorbance versus concentration.

4.1.2 Precision

The reproducibility was determined by repeating the above methods at different time intervals (morning, afternoon, and evening) on the same day (Intraday precision) and on three consecutive days (Interday precision). The intraday and inter-day variation for the estimation of Favipiravir was carried out at three different concentration levels of 2, 8, and 12μg/ml.

4.1.3 Accuracy

Accuracy was determined by performing recovery studies by spiking different concentrations of pure drug in a pre-analyzed sample solution of 4µg/ml. To pre-analyzed sample solution, a known amount of working standard solution of Favipiravir (0.33, 0.42, and 0.48 ml of 100 µg/ml) was added in 10 ml volumetric flask and made up to mark with diluent which was at different level i.e. 80%, 100%, and 120%. The solutions were analyzed by the proposed method. Calculate the mean % recovery from peak areas obtained.

4.1.4 Repeatability

It was determined by analyzing the same solution of 8 µg/ml of Favipiravir standard solution repeatedly.

4.1.5 Limit of detection (LOD) and limit of quantification (LOQ)

4.1.5.1 LOD

The LOD was estimated from the set of five calibration curves used to determine method linearity. The calibration curve was repeated for 6 times and the SD of the intercept was calculated then LOD was calculated as follow:

LOD= (3.3*SD)/slope.

Where,

SD= the standard deviation of the y-intercept of 5 calibration curves.
Slope= the mean slope of the 5 calibration curves

4.1.5.2 LOQ

The LOQ was estimated from the set of five calibration curves used to determine method linearity.

The LOQ may be calculated as:

LOQ = 10 × (σ/S).

Where,

σ = Standard deviation of the Y- intercepts of the five calibration curves.
S = Mean slope of the five calibration curves [14, 15].

4.2 Quantification of Favipiravir in Formulation

Ten Favipiravir tablets were accurately weighed and transferred to a dry and clean mortar, then grind into a fine powder, weighed tablet powder equal to 100mg transferred to 100ml of the volumetric flask containing distilled water of 30ml and sonicated for 15 min to dissolve the drug and volume made up to the mark with water (1000µg/ml), then 10ml from this solution was transferred to 100ml of volumetric flask and diluted up to the mark with water this gives 100 µg/ml. From the above solution, 2 ml of contents was transferred to 10ml of the volumetric flask to make 20µg/ml.

The data of the Favipiravir assay is tabulated, Beer-Lamberts law will be expressed as:

A= abc
Where,
A=specific absorbance =358nm
a=absorptivity or extinction coefficient
b=path length of radiation through the sample (cm)
c=concentration of solute in solution [15 , 2].

4.3 By RP-HPLC Method

4.3.1 Linearity

Standard calibration has been prepared using 5 standard solutions within the concentration range of 50-250 µg/ml. In optimized chromatographic conditions, each standard solution was chromatograph for 10 min three times. Least squares linear regression analysis of theaverage peak area versus concentration data were used to evaluate the linearity of the method.The linearity for Favipiravir was assessed by analysis of standard solution in a range of 50-250 µg/ml [15,2,16].

4.3.2 Precision

Precision was analyzed by calculating variations of the method in intraday (repeatability performed by analyzing standard solution on the same day) and inter-day (repeatability carried out by analyzing standard solution on three different days). A precision study was performed by injecting six times of standard solution at three different concentrations 50, 100, and 150µg/ml on the same day and three consecutive days. Results are expressed as relative standard deviation (RSD) or the coefficient of variance.

4.3.3 Accuracy

Accuracy was determined by performing recovery studies by spiking specific concentrations of pure drug in a pre-analyzed sample solution of 50µg/ml of Favipiravir. To pre-analyze the sample solution, a known amount of standard stock solution was added which was at different levels 50, 100, and 150%. The solutions were analyzed by the proposed method. The mean % recovery was calculated.

4.3.4 Repeatability

It was determined by analyzing the same solution of 150 µg/ml of Favipiravir standard solution repeatedly.

4.3.5 Robustness

In the robustness study, the following parameters have been changed one by one and observed their effect on system suitability test and assay.

i. Change mobile phase composition by ± 1.0mL of organic solvent.
ii. Change Wavelength ± 1nm
iii. Change flow rate ±0.1mL/min.

4.3.6 Change in mobile phase composition

Standard the working solution was injected three times by change in the mobile phase composition by ±1.0 mL of organic solvent (phosphate buffer pH 3.5: acetonitrile) (89:11v/v and 91:09v/v) of the developed method.

4.3.7 Change in pH

Standard the working solution was injected three times by change in the pH by ± 1 of the sample (3.4, 3.5 and 3.6) of the developed method. Calculate the % RSD of the mean area for change in the method parameter.

4.3.8 Change in flow rate

Standard the working solution was injected three times by change in the flow rate by ± 0.1 mL/min (0.9 mL/min and 1.1mL/min) of the developed method. Calculate the %RSD of mean area for change in method parameter [17-19].

5. ANALYSIS OF MARKETED FORMULATION USING DEVELOPED HPLC METHOD

5.1 Preparation of Standard Solution for Assay

Accurately weigh and transfer about 48mg of Favipiravir API into a 100ml volumetric flask.Add about 80ml of diluent and sonicate to dissolve. Dilute to volume with the diluent and mix. Transfer 5ml of above solution into a 50ml volumetric flask,dilute to volume with diluent, and mix.

5.2 Preparation of Sample Solution for Assay

Crush accurately weight 10 tablets of blend into fine powder. Take accurately weigh and transfer the powder equivalent to 600mg into a 200ml volumetric flask. Add about 160ml of diluent and sonicate for 15 min. Dilute to volume with diluent and mix. Transfer 4ml of the solution into 250ml volumetric flask dilute with diluent and mix. Filter the solution through a 0.45 µ filter.

$$The\ theoretical\ average\ weight\ of\ favipiravir\ \left(\frac{mg}{T}\right) = \frac{AT}{As} \times \frac{\mathrm{Ws}}{100} \times \frac{5}{50} \times \frac{200}{WT} \times \frac{250}{4} \times \frac{\mathrm{P}}{100} \times T --- [1]$$

Where,

AT= Area of Favipiravir peak in a sample solution
As = Average area of Favipiravir peak obtained from standard solution
Ws = weight of standard taken in mg
WT = Weight of sample taken in mg
P= % Purity of standard
P =% Purity of Favipiravir working standard used
T= Theoretical average weight of tablet sample
L= Label claimed of Favipiravir in mg
% Label Amount= Content of Favipiravir(mg/T)/Label claimed of Favipiravirx100

5.3 Solution Stability of Favipiravir

The stability of the analytical solution was verified by analyzing the standard and filtered sample solution initially and also at different time intervals as mentioned below by storing it in the sample compartment of the HPLC instrument at ambient conditions. Calculated the cumulative percentage RSD for peak areas of Favipiravir for both the sample and standard solution [10, 15, and 19,20,3,4].

5.4 Degradation Studies

5.4.1 Acid degradation

From the test stock solution, 1 ml was taken in a 10 ml volumetric flask, adds 1 ml of 1N HCl, and heated at 60^0C for 30 min on a water bath. The flask was removed from the water bath and allowed to cool at room temperature. Add 1 ml of 1N NaOH to neutralize the solution and diluted to volume with diluents and mix 10 ml solution were injected into the system and the chromatograms were recorded to assess the stability of the sample [10].

5.4.2 Alkali degradation

From the test stock solution, 1 ml was taken in a 10 ml volumetric flask, adds 1 ml of 1 N NaOH, and heated at 70^0C for 1 h on a water bath. The flask was removed from the water bath and allowed to cool at room temperature. Add 1 ml of 1N HCl to neutralize the solution and diluted to volume with diluents and mix 10 ml solution were injected into the system and the chromatograms were recorded to assess the stability of sample [10].

5.4.3 Peroxide (oxidation) degradation studies (3%v/v of H_20_2)

From the test stock solution, 1 ml was taken in a 10 ml volumetric flask add 1ml of 3% H_2O_2, and heated at 70^0C for1 an hour on a water bath. The flask was removed from the water bath and allowed to cool at room temperature and diluted to volume with diluents and mixed, 10 ml solution were injected into the system and the chromatograms were recorded to assess the stability of the sample [15].

5.4.4 Photolytic degradation

From the test stock solution, 1 ml was taken in a 10 ml volumetric flask, add 5 ml of water, and sonicated to disperse, dissolve, and heated at 70^0C for 3 h on a water bath. The flask was removed from the water bath and allowed to cool at room temperature and diluted to volume with diluents and mixed. 10 ml solution was injected into the system and the chromatograms were recorded to assess the stability of the sample [15].

5.4.5 Thermal degradation studies

For the Thermal Degradation 10 mg, Favipiravir, and drug samples were weighed accurately and transfer to the petridish Heat the sample in the oven for about 24h at 70^0C and transfer the sample into a 100 ml volumetric flask dissolve and dilute to volume with diluents. Filter the solution using a 0.45m Nylon filter. Transfer 1ml of above stock solution to 10 ml volumetric flask and make up the volume with diluents to get the concentration of 10 mcg Favipiravir solution was injected into the system and the chromatograms were recorded to assess the stability of the sample [10, 15].

5.4.6 UV degradation studies

Weighed 100mg sample and keep it in the UV chamber at 200-400nm for 24hrs and 48 hrs. From this weigh 10 mg sample and transfer it in 100 ml volumetric flask. Makeup volume up to the mark with diluent. Then take 1ml of the above solution and transfer it into a 10ml volumetric flask. Make volume with diluents, this solution injected into the system [10, 15].

6. RESULTS AND DISCUSSION

6.1 Optimization by UV Spectrophotometric Method

6.1.1 Linearity

The linearity studies have been performed by scanning the solution of different concentrations from 2-10 µg/ml. The absorbance, standard deviation, and % RSD were calculated as shown in Table 1. The linearity curve suggests the perfect variation in absorbance value as increased in concentration. A calibration curve was plotted using absorbance versus concentration [Fig. 4]. The correlation coefficient value was found to be 0.990 [10, 15].

6.1.2 Precision

A precision study has been performed to check the reproducibility of the results of the developed method. In precision, the solution of Favipiravir (2, 8, and 12 µg/ml) was scanned at 358 nm on the same day but at a different time and on different three days. The results obtained are tabulated in Table 2. From the precision study, it was confirmed that the method is reproducible and selective enough [6].

6.1.3 Accuracy

The accuracy of the method was checked by a recovery experiment performed at three different levels, i.e., 80%, 100%, and 120%. The % recovery was found to be in the range of 99.99–100.76%. The low values of % RSD are indicative of the accuracy and reproducibility of the method in Table 3.

6.1.4 Repeatability

The low values of % RSD are indicative of the accuracy and reproducibility of the method. The repeatability of the method was determined by analyzing the same solution of 8 µg/ml of Favipiravir standard solution repeatedly in Table 4 [6].

6.2 Limit of Detection [LOD] and Limit of Quantification [LOQ]

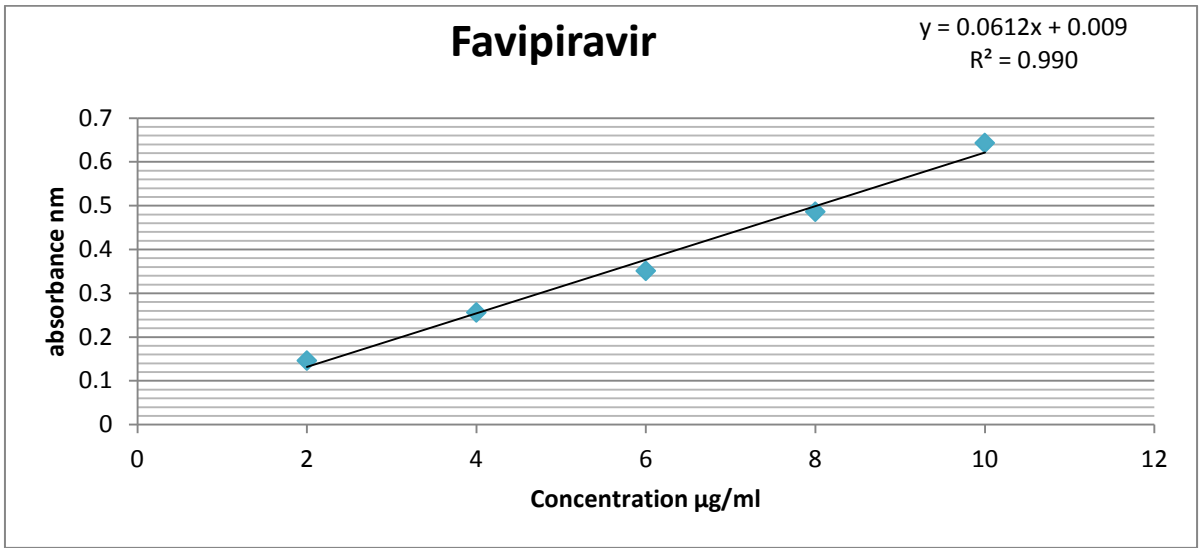
Limit of Detection [LOD] and Limit of Quantification [LOQ] of the developed method has been studied in Table 5. It was found in the permissible range i.e. LOD, 0.0723, and LOQ, 0.219µg/ml.

6.3 Quantification of Favipiravir in Formulation

The λmax was observed exactly at 358 nm and the % assay was found to be 102% [Fig. 5]. The label claim of the Favipiravir was 200 mg and we found it to be 204 mg in the tablet assay of Favipiravir using this developed method in Table 6 [6,2].

Table 1. The absorbance, %RSD, r^2, and regression equation of Favipiravir

Sr. No.	Concentration mcg/ml	Absorbance at 358nm	SD	RSD%
1	2	0.146	0.0014	1.01
2	4	0.256	0.0016	0.62
3	6	0.351	0.0011	0.32
4	8	0.486	0.0011	0.23
5	10	0.643	0.0015	0.24
Correlation coefficient (r^2)		0.990	Average SD=0.00134	
Regression equation		y=0.0612x+0.009		



Fi.g 4. Calibration curve of Favipiravir by UV

Table 2. The precision data of the Favipiravir UV method

Sr. No.	Conc.	Absorbance			Mean	SD	%RSD
		I	II	III			
Interday							
1	2	0.148	0.144	0.146	0.15	0.020	1.37
2	8	0.487	0.49	0.492	0.49	0.0025	0.51
3	12	0.648	0.649	0.66	0.65	0.0063	1.02
Intraday							
1	2	0.154	0.149	0.15	0.15	0.00267	1.78
2	8	0.491	0.485	0.492	0.49	0.00377	0.77
3	12	0.649	0.65	0.66	0.65	0.0061	0.93

Table 3. The accuracy data of the Favipiravir UV method

% conc.	Sample amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	% recovery	% Recovery means	S.D.	% RSD
80	7.2	3.2	3.23	100.31	100.72	0.48	0.48
	7.2	3.2	3.20	100.6			
	7.2	3.2	3.25	101.25			
100	8	4	3.96	101.25	100.50	1.09	1.08
	8	4	4.03	101			
	8	4	4.05	99.25			
120	8.8	4.8	4.83	101.83	100.76	0.76	0.76
	8.8	4.8	4.79	100.42			
	8.8	4,8	4,85	100.62			

Table 4. The repeatability data of the Favipiravir UV method

Sr. No.	Conc. (µgm/ml)	Absorbance	Mean	S.D.	% RSD
1	8	0.523	0.5224	0.0011	0.22
2		0.521			
3		0.522			
4		0.522			
5		0.524			

Table 5. The LOD and LOQ data Favipiravir UV method

Parameters	Result
SD of intercept	0.00134
Slope	0.0612
LOD(µgm/ml)	0.0723
LOQ(µgm/ml)	0.219

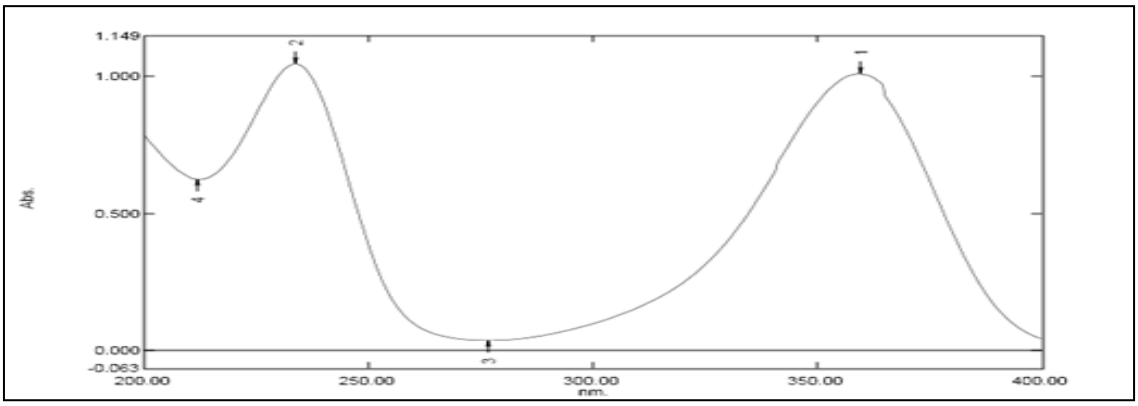


Fig. 5. UV graph of Favipiravir from tablet assay

Table 0.The data of Favipiravir assay

The label claimed in mg	The average weight of tablet (mg)	Absorbance at 358nm	The label claimed found in mg	% assay
200	272	0.628	204	102%

6.4 Optimization by HPLC Method

6.4.1 Linearity

The linearity has been performed by determining a standard calibration curve using a concentration range of 50-250 µg/ml.Least squares linear regression analysis of the average peak area versus concentration data were used to evaluate the linearity of the method. The values obtained were linear correlation co-efficient for calibration curve was found to be 0.996 in Fig. 6 and Table 7.

6.4.2 Precision

Precision was analyzed by calculating variations of the method in intraday (repeatability performed by analyzing standard solution on the same day) and inter-day (repeatability carried out by analyzing standard solution on three different days). The precision study showed the very closeness of the results in Table 8.

6.4.3 Accuracy

Accuracy was determined by performing recovery studies by spiking specific concentrations of pure drug in a pre-analyzed sample solution of 50µg/ml of Favipiravir. To pre-analyze the sample solution, a known amount of standard stock solution was added which was at different levels 50, 100, and 150%. The % recovery was found to be between 99.99-100.42% [Table 9], which is the acceptable limit as per the ICH guidelines.

6.4.4 Repeatability

The low values of % RSD are indicative of the accuracy and reproducibility of the method. The repeatability of the method was determined by analyzing the same solution of 150 µg/ml of Favipiravir standard solution repeatedly in Table 10.

6.5 Limit of Detection (LOD) and Limit of Quantification (LOQ)

Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method has been studied. It was found in the permissible range i.e. LOD, 2.186 and LOQ, 6.626µgm/ml tabulated in Table 11.

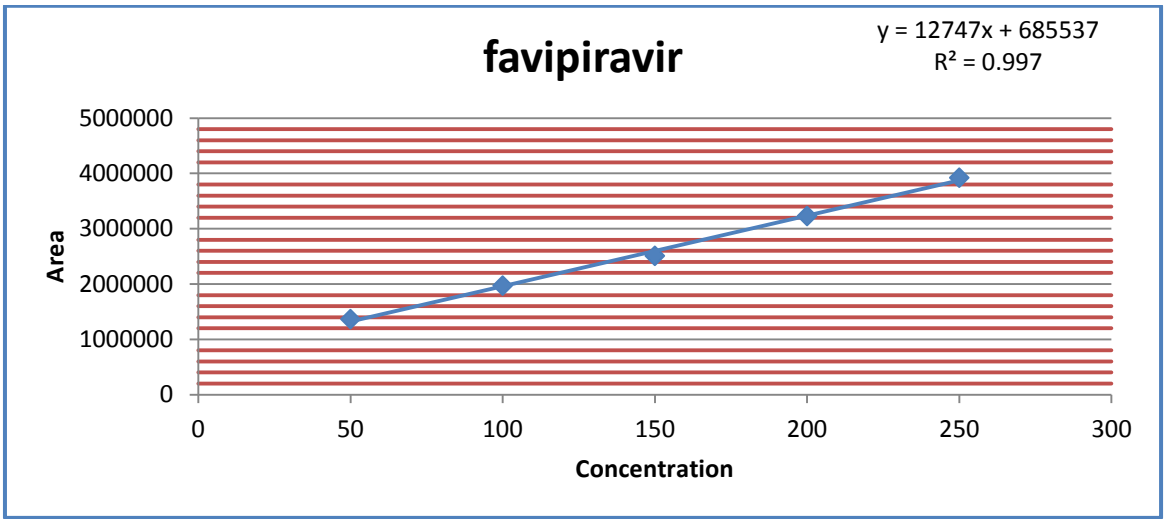


Fig. 6. Calibration curve of Favipiravir by HPLC

Table 7. The linearity data of the Favipiravir HPLC method

Sr. No.	Concentration (µgm/ml)	Area	SD	%RSD
1	50	1362884	2010.00	0.15
2	100	1969386	10111.00	0.51
3	150	2507427	8889.00	0.35
4	200	3227615	11111.00	0.34
5	250	3920467	10111.00	0.26
Regression coefficient (r^2)		0.997	AVG SD=8446.4	
Regression equation		y = 12747x + 68553		

Table 8. The precision data of the Favipiravir HPLC method

Sr. No.	Conc.	Absorbance			Mean	SD	%RSD
		I	II	III			
Interday							
1	50	1902159	1953257	1902543	1919319.67	29391.22	1.53
2	100	2507427	2535265	2486589	2509760.33	24421.74	0.97
3	150	4216871	4202589	4196548	4205336.00	10436.26	0.25
Intraday							
1	50	1551402	1546752	1504321	1546752.00	25944.28	1.68
2	100	1969386	2016743	1975462	1987197.00	25767.31	1.30
3	150	3920467	3986579	3940067	3949037.67	33956.65	0.86

Table 9. The accuracy data of the Favipiravir HPLC method

% Conc.	Sample amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Recovery means	S.D.	% RSD
50	12.5	7.5	7.45	99.33	100.26	1.01	1
	12.5	7.5	7.51	100.13			
	12.5	7.5	7.6	101.33			
100	20	15	14.8	98.66	100.21	1.38	1.37
	20	15	15.1	100.66			
	20	15	15.2	101.3			
150	27.5	22.5	22.4	99.5	100.42	0.92	0.91
	27.5	22.5	22.6	100.44			
	27.5	22.5	22.8	101.33			

Table 10. The repeatability data of the Favipiravir HPLC method

Sr. No.	Conc. (µgm/ml)	Area	Mean	S.D.	% RSD
1	150	2507427	2508631.2	8808.11	0.35
2		2507427			
3		2507427			
4		2507427			
5		2507427			

Table 11. The LOD and LOQ data of the Favipiravir HPLC method

SD of Intercept	8446.4
The slope of Calibration Curve	12747
LOD	2.186
LOQ	6.626

6.5.1 Robustness

The robustness of the developed method has been studied by changing mobile phase composition, altering the pH of the solvent system, and changing the flow rate. The % RSD of the robustness was found to be within permissible amount i.e. 0.28 to 1.46 in Table 12.

6.6 Analysis of Marketed Formulation using Developed HPLC Method

This developed RP-HPLC method has been applied to the marketed formulation of Favipiravir. The % of the assay was found to be 100.1% which is permissible as per the ICH guidelines in Table 13.The chromatogram is shown in Fig. 7 and 8.

Table 12. The robustness data of the Favipiravir HPLC method

Condition	Peak area mean	SD	% RSD
Change in ratio of mobile phase ±1ml	1673614.333	4829.76	0.29
	1672815.667	16402.25	0.98
Change in pH ±1	1695825	4774.77	0.28
	1668691.667	15418.54	0.92
Change in flow rate ±1ml	1627783.667	23809.49	1.46
	1635044.667	9395.67	0.57

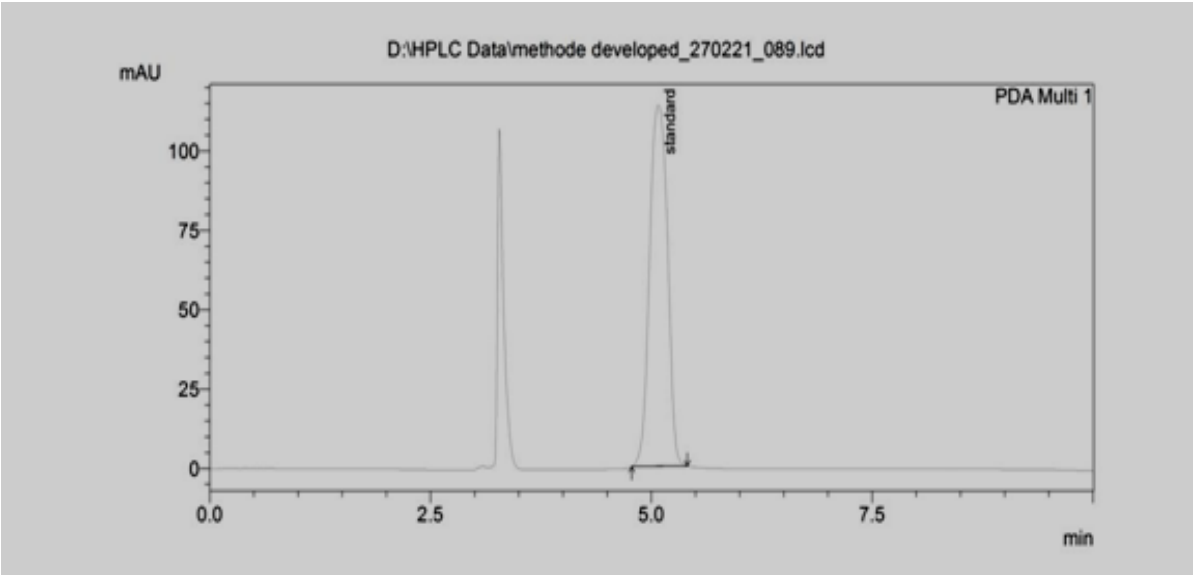


Fig. 7. The chromatogram of standard Favipiravir

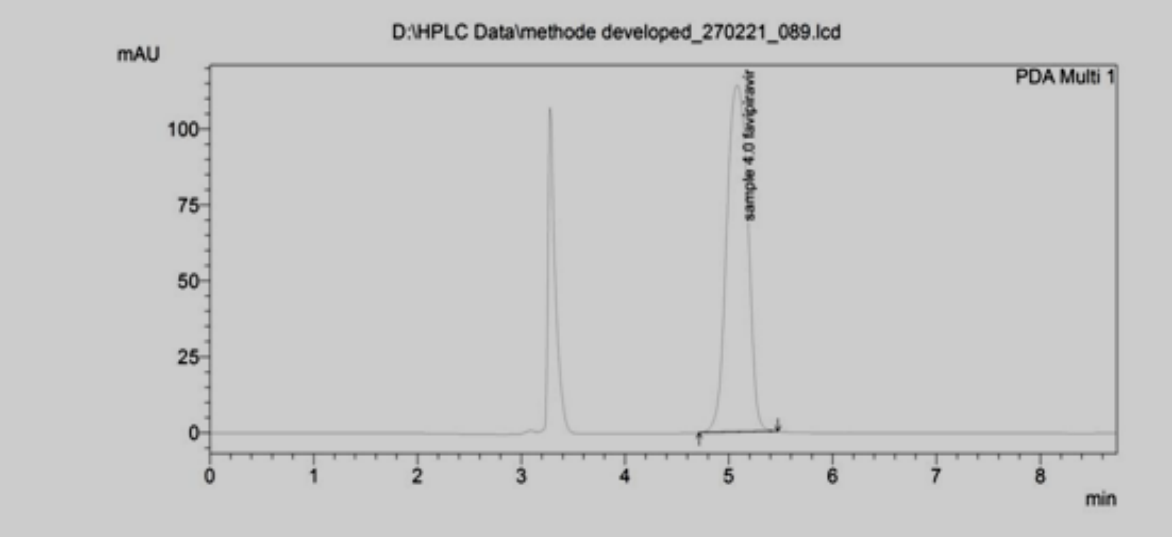


Fig. 8. The chromatogram of Favipiravir sample

Table 13. The assay data of marketed formulation of Favipiravir

Sr. No.	Area of standard	Area of sample	The label claimed the amount of tablet in (mg)	Amount Found	Assay amount found
1	1610139	1628765	200	200.21	100.1

6.7 Summary of validation Parameter

Table 14. The summary table for validation parameter

Validation Parameter	Uv spectroscopy Results	HPLC Results
Linearity and Range	2-10 µg/ml	50-250 µg/ml
Correlation coefficient	0.990	0.997
Precision (%RSD)		
Interday	0.51-1.37	0.25-1.53
Intraday	0.77-1.78	0.86-1.68
Accuracy(% Recovery)	0.48-1.08	0.91-1.37
Repeatability(%RSD)	0.22	0.35
LOD & LOQ	0.0723 &0.219µg/ml	2.186 & 6.626
Assay	102%	100.1

6.8 Degradation Studies

The stability-indicating methods have been studied for the developed method by applying forced degradation studies. The % of degradation is tabulated in Table 15. The lowest degradation of 3.57% was observed in water hydrolysis, whereas, for acid hydrolysis, 39.64% of degradation was caused. All degradation graphs were represented between Fig. 9 to 14 [6,11,12,13,14,15,2, 16,17,18,19,20,3,4].

For the development of the Favipiravir UV spectroscopic method, distilled water was selected as the solvent. The solution of appropriate concentration was tested in a UV range of 200 to 400 nm. The maximum absorbance was observed at 358 nm and the same value was selected for further analysis. The UV spectra have been reported in fig. 6. The Favipiravir calibration curve was determined using concentrations of 2-10 µg / mL. The calibration curve increased absorbance as we increased the concentration and a standard slope was given. This developed UV spectroscopic method was validated according to ICH guidelines in terms of linearity, precision, accuracy, and repeatability and stability studies [1, 6, and 2].

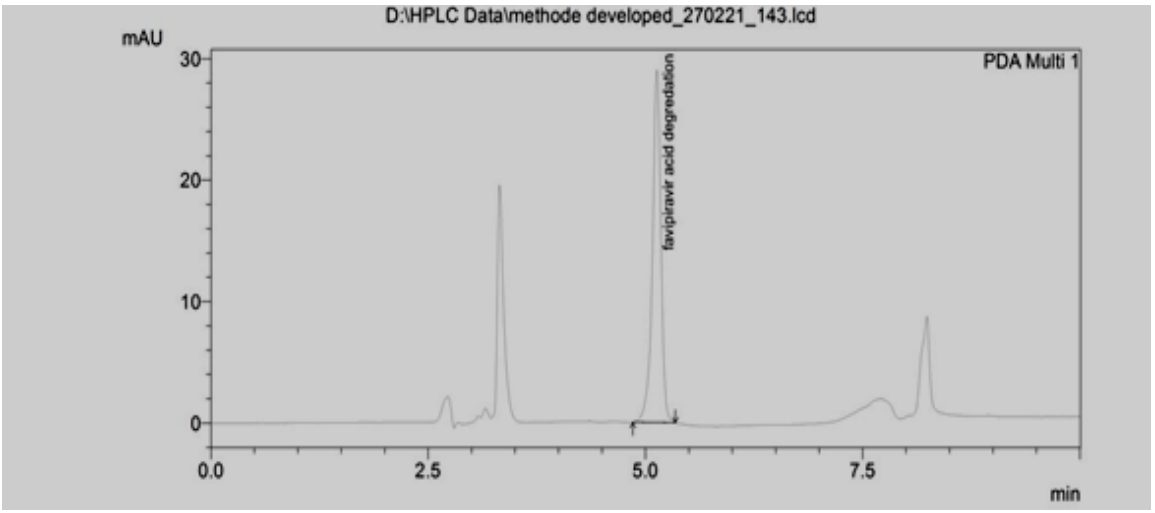


Fig. 9. The chromatogram of favipiravir in acid degradation

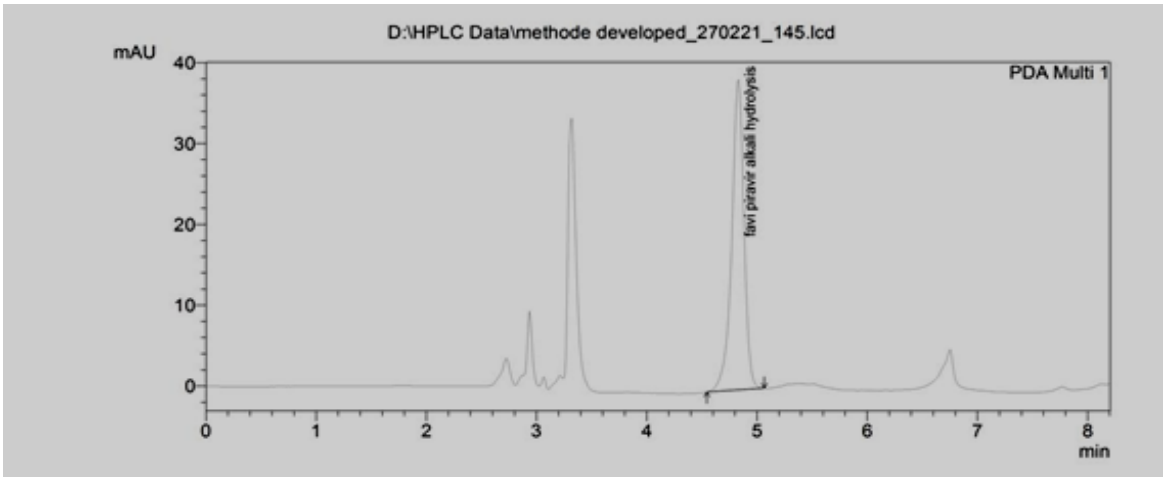


Fig. 10. The chromatogram of Favipiravir from alkali degradation

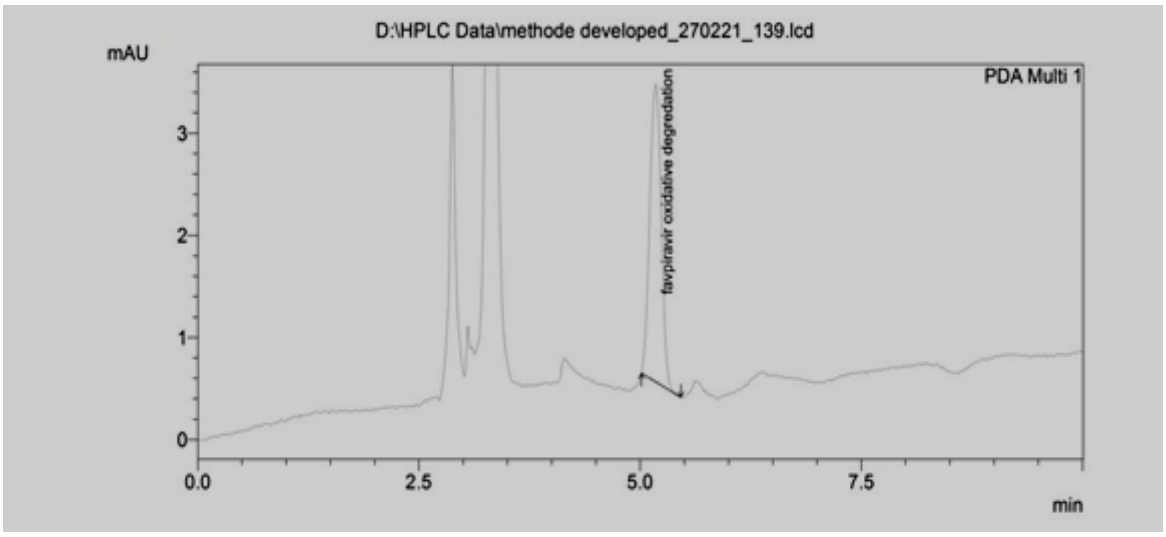


Fig. 11. The chromatogram of Favipiravir in oxidative degradation

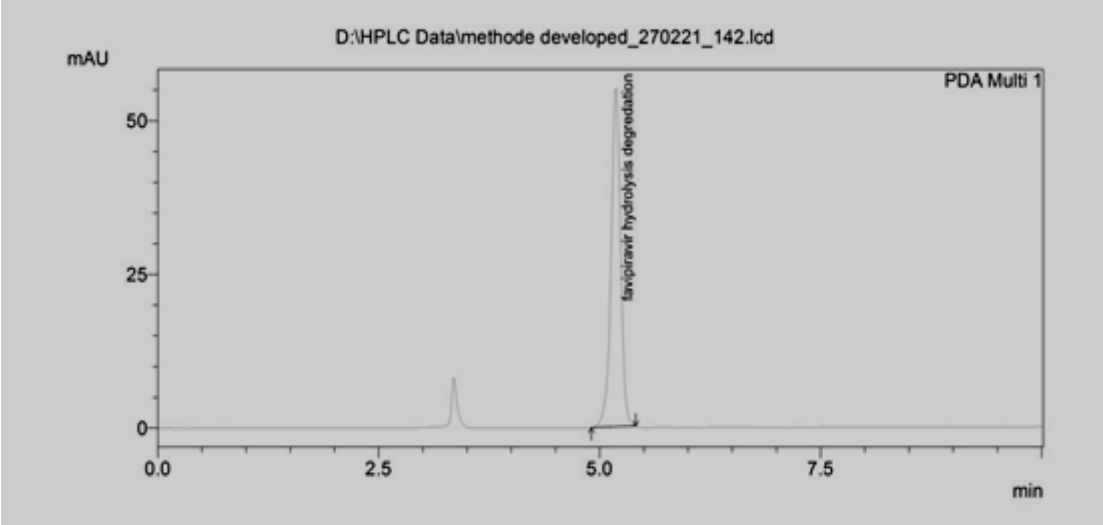


Fig. 12. The chromatogram obtained from photolytic degradation studies

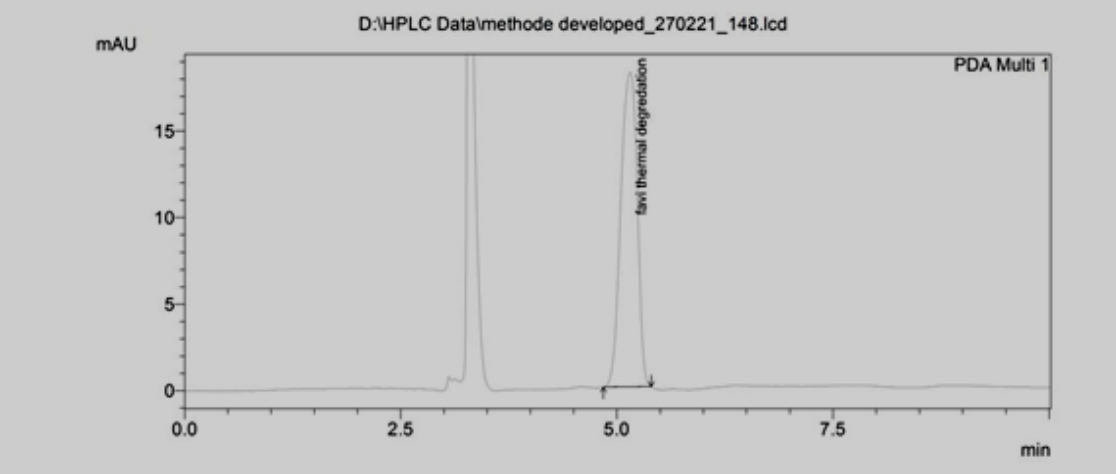


Fig. 13. The chromatogram obtained from thermal degradation studies

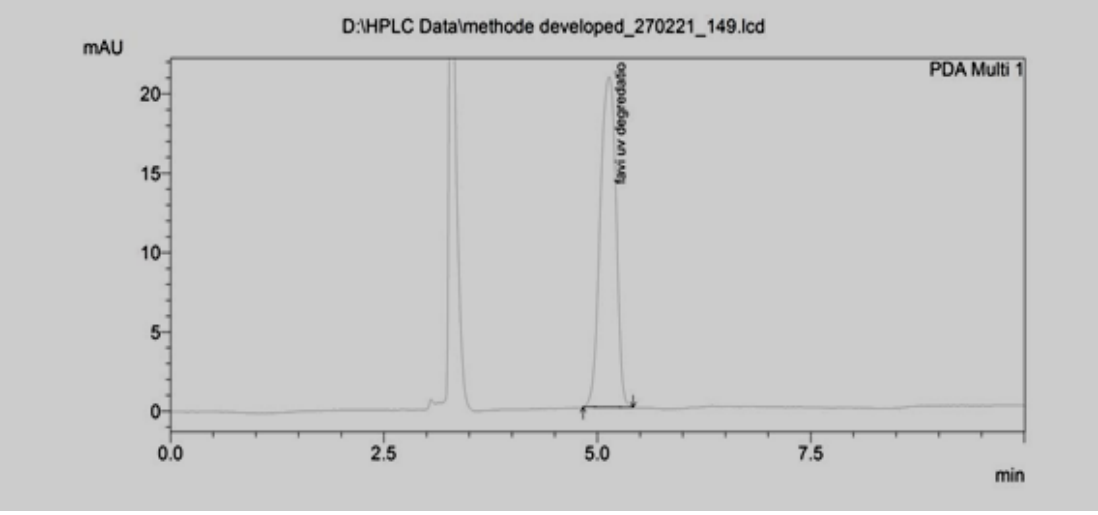


Fig. 14 (a). The chromatogram of Favipiravir obtained in UV degradation studies after 24 hrs

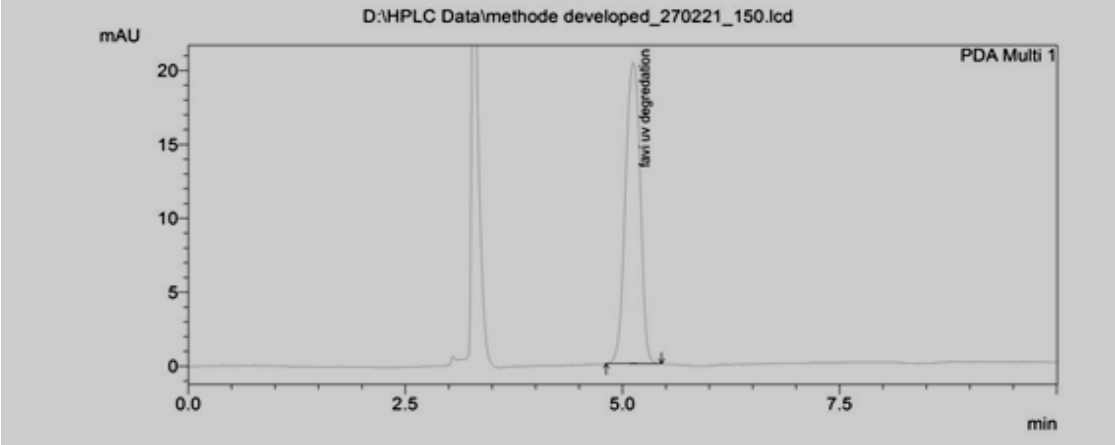


Fig.14 (b). The chromatogram of Favipiravir obtained in UV degradation studies after 48 hrs

Table 15. The peak areas and % degradation of the drug instability studies

Parameter	Area	% Degradation
Standard	325986	00
Acid	196748	39.64
Base	277070	15.01
Thermal	258328	20.76
Hydrolysis	314325	3.57
Photolytic UV 24hrs	269524	17.32
UV 48 Hrs	242240	25.69

Linearity studies were performed by scanning the solution at different concentrations of 2-10 µg / ml. Absorbance, standard deviation and% RSD were calculated. The linearity curve suggests the perfect variation of the absorbance value as the concentration increases. A precision study was carried out to verify the reproducibility of the results of the developed method. Precisely, the Favipiravir solution (2, 8, and 12 µg / mL) was scanned at 358 nm on the same day, but at different times and on three different days. The precision study confirmed that the method is sufficiently reproducible and selective. The accuracy of the method was verified by a recovery experiment performed at three different levels, namely 80%, 100%, and 120%. The recovery percentage was between 99.99 and 100.76% [2]. Low RSD% values are indicative of the accuracy and reproducibility of the method. The repeatability of the method was determined by repeatedly testing the same 80 µg / ml Favipiravir standard solution. The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were investigated. It was found in the allowable range, namely LOD, 0.0723, and LOQ, 0.219 µg/ml. The developed method was successfully applied for the estimation of Favipiravir in selected commercial formulations. The UV graph obtained is illustrated in Fig. 6. Λmax was observed at exactly 358 nm and the% dosage was found to be 102%. The label indication for Favipiravir was 200 mg and we found that it was 204 mg in the Favipiravir tablet study using this developed method [6].

A simple, accurate, precise, rapid, specific, sensitive, and selective method indicating the stability of RP-HPLC has been developed and validated for the estimation of bulk and tablet Favipiravir, according to ICH guidelines.

A wavelength of 358 nm was chosen for the development of the RP-HPLC method. The solvent system was optimized by testing different proportions of buffer pH 3.5: acetonitrile, after about 8 tests, the most optimized chromatogram buffer pH 3.5 was obtained: acetonitrile (90:10) at a flow rate of 1 ml/min with time retention of 5 minutes. The most optimized peak of Favipiravir is shown in Fig. 12. The optimized chromatographic conditions were the C18 column, with an injection volume of 20 µg/ml and a detection wavelength of 358 nm. This developed RP-HPLC method has been validated in terms of linearity, precision, accuracy, LOD, LOQ, and robustness.

Linearity was achieved by determining the standard calibration curve using a concentration range of 50-250 µg / mL. The precision was analyzed by calculating the variations of the method in intraday (repeatability carried out by analyzing the standard solution on the same day) and inter-day (repeatability carried out by analyzing the standard solution on three different days). The precision study showed a great closeness to the results. Accuracy was determined by performing recovery studies by adding a specific concentration of pure drug to a previously tested 50 µg/mL sample solution of Favipiravir. A known amount of standard stock solution was added to the previously analyzed sample solution which was at different levels 50, 100, and 150%. The recovery rate was found to be between 99.99 and 100.42%, which is an acceptable limit according to ICH guidelines. The LOD and LOQ were found to be 2,186 and 6,626 respectively. The robustness of the developed method was studied by modifying the composition of the mobile phase, altering the pH of the solvent system, and modifying the flow rate. The% RSD of robustness was found to be within the allowable amount, which is 0.28 to 1.46.

Solution stability studies were conducted with Favipiravir solution. The% RSD of the peak areas obtained was calculated against the standard and the Favipiravir sample solution. The% RSD of the standard solution was found to be between 0.44 and 0.87 and of the sample solution between 0.28 and 0.58. It indicates the stability of the analytical solution.

Stability indicator methods were studied for the developed method by applying forced degradation studies. Acid hydrolysis was performed using 1N HCl followed by neutralization using a 1N NaOH solution. 1N NaOH was used for alkaline hydrolysis and neutralized using a 1N HCl solution. The% degradation was calculated for forced degradation studies. Similarly, photolytic, thermal, and UV degradation was carried out. The lowest degradation of 3.57% was observed in water hydrolysis, while, for acid hydrolysis, it caused 39.64% degradation.

This developed RP-HPLC method was applied to the commercial formulation of Favipiravir. The% degradation of the study was found to be 100.1%, which is allowed under ICH guidelines. Therefore, we hereby conclude that the developed method is accurate, accurate, sensitive, and reproducible for the quantitative estimation of Favipiravir bulk and its formulation [6,11,12, 15,2,16].

7. CONCLUSION

This developed UV spectroscopic method was validated according to ICH guidelines in terms of linearity, precision, accuracy, and repeatability and stability studies. All validation parameters were found to be within the allowable limit according to ICH guidelines. The developed method was successfully applied for the estimation of Favipiravir in selected commercial formulations. Here we conclude that the developed UV and RP-HPLC methods are precise, accurate, sensitive, and reproducible for the quantitative estimation of Favipiravir bulk and its formulation. The developed method can be used by the pharmaceutical industries for the routine analysis of Favipiravir, in particular by UV and RP-HPLC.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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