IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
July 2022 Vol.:24, Issue:4
© All rights are reserved by Santosh V. Gandhi et al.

Development and Validation of UV-Spectrophotometric Method for Estimation of Favipiravir

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Santosh V. Gandhi*, Hanmant A. Bade

AISSMS College of Pharmacy, Kennedy Road, Near RTO, Pune-411001, India.

Submitted: 21 June 2022
Accepted: 26 June 2022
Published: 30 July 2022

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: Favipiravir, COVID-19, Spectrophotometry, ICH Q2 R1 guideline.

ABSTRACT

Favipiravir is an antiviral drug that is active against many RNA viruses, also known as favilavir. A simple UV-spectrophotometric method was developed for the estimation of Favipiravir in a bulk and pharmaceutical dosage form. The λmax was found to be 323 nm in 0.1 N HCl. Pure drug concentrations were prepared in the range of 5-30 µg/ml and the linear regression analysis data showed a good linear relationship with an R^2 value of 0.997. The method was validated according to International Conference on Harmonization (ICH Q2 R1) guidelines concerning linearity, range, precision, accuracy and robustness, the limit of detection, and the limit of quantitation. The limit of detection and limit of quantitation were found to be 1.49 and 4.53µg/ml, respectively. Recoveries were found to be in the range of 101.41% to 102.73% and % RSD less than 2 % which indicate that the developed method is suitable for the analysis of commercial samples.

INTRODUCTION:

Favipiravir is an antiviral drug that is active against many RNA viruses, also known as favilavir. Favipiravir (6-fluoro-3-hydroxypyrazine-2-carboxamide) is a pyrazine analog (Figure 1). The mechanism of action is linked to transcription inhibition and viral gene replication which finally prevents the synthesis of viral *RNA* inside infected cells.[1]

A literature survey revealed few methods are reported for estimation of Favipiravir which include bioanalytical HPLC[1-2] or LC-MS/MS[3-5]and analytical HPLC[6-10]. Few stability-indicating LC methods are also reported[11-13]. Reported methods were not much cost-effective in terms of time and solvent consumption. To the best of our knowledge, there is no simple UV method reported for the estimation of Favipiravir in bulk and tablet dosage forms. The present work was carried out with the view of establishing a simple, rapid, accurate, economic, precise, and robust UV method for the estimation of favipiravir in bulk and capsule dosage form, using methanol as the solvent.

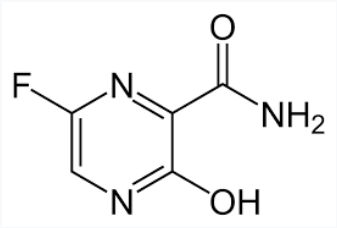

Fig. No. 1: Structure of Favipiravir

MATERIAL AND METHODS:

Chemicals and Equipment:

The pharmaceutical grade reference standard of Favipiravir was obtained as a gift sample from Lok-Beta Pharmaceuticals Pvt Ltd, Mumbai. Water (Type I) and HCl (AR Grade) were used in this project. The tablet formulation of Favipiravir was brought from a local Pharmacy. Shimadzu 1780 double beam UV- visible Spectrophotometer was used for UV analysis and for weighing purposes Shimadzu Analytical balance A120 was used.

Preparation of standard solutions

Preparation of the standard stock solution: Favipiravir standard stock solution (1000 µg/ml) was prepared by dissolving accurately weighed 10 mg of Favipiravir in 0.1 N HCl to

make up the volume to 10 ml.1 ml of this solution was diluted to 10 ml with 0.1 N HCl, to obtain a solution of concentration 100 µg/ml.

Selection of detection wavelength:

The standard stock solution was suitably diluted with 0.1 N HCl and scanned in a double beam UV spectrophotometer at a range of 200- 400 nm with 1.0 cm matched cell against 0. 1 N HCl as blank to the absorbance maxima was recorded. It was observed that the drug showed maximum absorbance at a wavelength of 323 nm. The representative UV spectrum of Favipiravir is shown in Fig. 2.

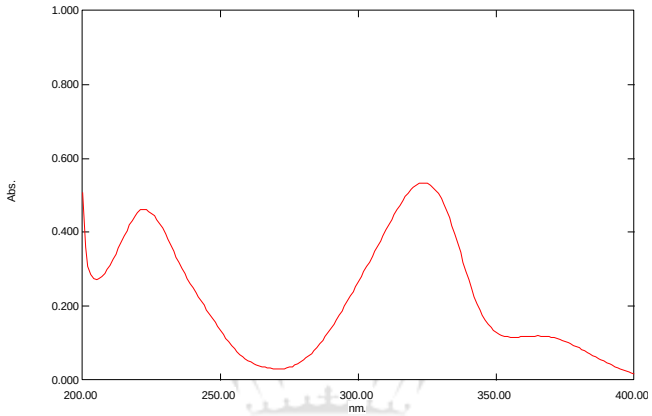

Fig. No. 2: UV Spectrum of pure Favipiravir of 10 µg/ml

Sample Solution (Marketed Formulation Analysis): For the preparation of the sample solution, twenty tablets were weighed and the average weight was determined. The tablets were crushed into a fine powder and tablet powder equivalent to 10 mg of Favipiravir (Label claim: 400 mg Favipiravir per tablet) was accurately weighed and transferred into a 10 ml volumetric flask and volume made up with 0.1N HCl, shook well, and filter it. Then 1 ml solution was pipette out and transferred into a 10 ml volumetric flask and make up volume up to mark with 0.1N HCl from which 1 ml was further diluted to get the solution of 10 µg/ml. The procedure was repeated six times.

METHOD VALIDATION: According to the ICH guidelines, the proposed method was validated to meet the validation acceptance criteria like linearity, accuracy, precision repeatability, the limit of detection, and the limit of quantification and robustness[14].

Linearity and Range: From the working solution, different aliquotes were taken to prepare solutions ranging from 5-30µg/ml of concentrations and the solutions were analyzed. The

spectras were measured at 200-400 nm, and a calibration curve was plotted of Absorbance Vs concentration of the drug.

Accuracy: The accuracy of the proposed technique was verified by spiking test samples in the entire calibration range with a known amount of pure drug at 50%, 100%, and 150% concentration. Their recovery of samples was calculated it showed a low percentage relative standard deviation.

Precision: The precision of the method was determined as Intra-day and Inter-day. The experiment was performed three times a day for intra-day precision and on 3 different days for inter-day precision at three different concentrations levels and results were reported as % RSD.

Limit of detection (LOD) and limit of quantification (LOQ): The sensitivity of the UV spectrophotometric methods was determined in terms of LOD and LOQ. LOD was calculated using equation 3.3 d/s and LOQ was calculated using 10 d/s where “d" is the standard deviation of the intercept and “s" is the slope of the calibration curve.

Robustness: The only variable parameter in the experiment is the wavelength. To confirm the robustness of the method, the absorbance of working standard solutions of Favipiravir was recorded at ±1 nm of absorbance maxima and analyzed. The % RSD was calculated.

RESULTS AND DISCUSSION:

The linearity of Favipiravir was found to be in the range of 5-30µg/ml with a linear correlation coefficient of 0.997. The data for linearity is given in Table 1 and the calibration curve of Favipiravir is shown in Fig 3. The % RSD was found to be in the range of 0.37-0.82 for intra-day precision and 0.68-2.00 for inter-day precision (Table 2a and Table 2b). Recovery was found to be 101.41–102.73 percent under the accuracy study (Table 3). The sensitivity of the method was assessed by determining the LOD and LOQ. The LOD and LOQ for Favipiravir were found to be 1.49 and 4.53µg/ml, respectively. The Assay was performed and the result was found to be 99.18 ± 0.64 (Table 4). Method robustness was tested by measuring the absorbance (± 1 nm) at 322, 323, and 324 nm, and the method was found to be robust having % RSD within limits (Table 5).

Table No. 1: Results of Linearity (Conc. Vs Absorbance)

Concentration (µg/ml)	1	2	3	4	5	6	Mean	SD	% RSD
5	0.183	0.189	0.186	0.18	0.184	0.179	0.184	0.004	2.032
10	0.388	0.392	0.390	0.391	0.382	0.380	0.373	0.001	0.277
15	0.615	0.635	0.612	0.628	0.612	0.625	0.572	0.004	0.697
20	0.764	0.77	0.741	0.774	0.777	0.777	0.767	0.014	1.790
25	0.979	0.978	0.982	0.996	0.958	0.965	0.976	0.013	1.369
30	1.199	1.197	1.167	1.166	1.159	1.153	1.174	0.020	1.675

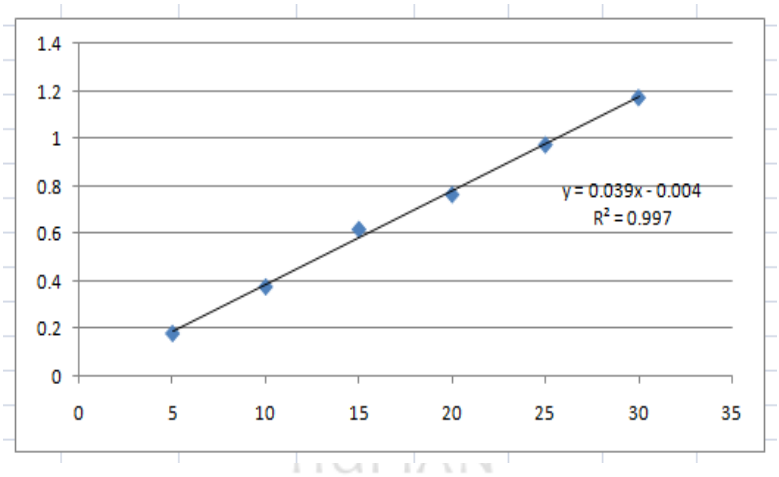

Fig. No. 3: Calibration Curve Plot of Standard Favipiravir

Table No. 2a: Results of Intraday precision

Concentration(µg/ml)	Absorbance	Amount Recovered (µg/ml)	% Recovery	S.D.	% RSD
10	0.388	10.05	100.51	0.82	0.82
	0.383	9.92	99.23		
	0.382	9.89	98.97		
20	0.764	19.69	98.46	0.44	0.44
	0.777	19.84	99.23		
	0.777	19.84	99.23		
30	1.174	30.20	100.68	0.37	0.37
	1.167	30.02	100.08		
	1.166	30	100		

Table No. 2b: Results of Interday precision

Concentration(µg/ml)	Absorbance	Amount recovered (µg/ml)	% Recovery	S.D.	% RSD
10	0.383	9.92	99.23	0.67	0.68
	0.384	9.94	99.48		
	0.388	10.05	100.51		
20	0.770	19.84	99.23	2.05	2.05
	0.774	19.94	99.74		
	0.777	20.02	102.56		
30	1.156	29.74	99.14	0.45	0.45
	1.153	29.66	98.88		
	1.196	30.76	102.56		

Table No. 3: Results of recovery studies for Favipiravir

Level	Sample Conc. µg/ml	Standard Conc. µg/ml	Absorbance	Theoretical Conc. µg/ml	Obtained Conc. µg/ml	% Recovery	Mean % Recovery ± %RSD
50%	10	5	0.605	15	15.41	102.73	102.39±0.44
	10	5	0.604	15	15.38	102.56	
	10	5	0.600	15	15.28	101.88	
100%	10	10	0.785	20	19.64	100.12	101.11±0.86
	10	10	0.795	20	19.82	101.41	
	10	10	0.798	20	19.89	101.79	
150%	10	15	0.985	25	24.94	101.64	101.43±0.72
	10	15	0.995	25	24.69	100.61	
	10	15	0.999	25	25.05	102.05	

Table No. 4: Assay of Favipiravir tablet formulation

Sr. No.	Conc	Absorbance	% Recovery	Average	SD	% RSD
1	10	0.388	98.46			
2	10	0.389	98.71			
3	10	0.391	99.23	99.18	0.63	0.64
4	10	0.395	100.25			
5	10	0.390	98.97			
6	10	0.392	99.48			

Table No. 5: Robustness results at three different wavelengths

Concentration (µg/ml)	Absorbance at Wavelength (nm)			% RSD
	322 nm	323 nm	324 nm	
10	0.383	0.382	0.384	0.26
10	0.384	0.383	0.381	0.26
10	0.381	0.381	0.380	0.15

CONCLUSION:

The proposed method was quantitatively evaluated in terms of linearity, accuracy, precision, assay, and robustness. All these factors lead to the conclusion that the proposed UV-Spectrophotometric method is simple, accurate, precise, sensitive, and cost-effective. The method was validated as per the ICH guidelines. The validated method can be successfully applied to the quantification and quality control of Favipiravir tablets.

ACKNOWLEDGMENT:

The authors are thankful to the principal and management of AISSMS College of Pharmacy, Pune, for providing all necessary equipment and facilities to carry out this research work.

REFERENCES:

1. Hailat M,Al-Ani I,Hamad,Z Zakareia M, Dayyih WA. Development and Validation of a Method for Quantification of Favipiravir as COVID-19 Management in Spiked Human Plasma. Molecules 2021; 26:1-11.
2. Duse PV, Baheti KG. Bioanalytical Method Development and Validation for the Determination of Favipiravir in Spiked Human Plasma by using RP-HPLC. Journal of Pharmaceutical Research International 2021:1-17.
3. Onmaz DU, Abusoglu S, OnmazM, Yerlikaya FH, Unlu A. Development and validation of a sensitive, fast and simple LC-MS / MS method for the quantitation of Favipiravir in human serum. Journal of Chromatography B 2021:1-18.
4. Morsya MI, Noumana EG, Abdallaha YM, Zainelabdeena MA, Darwisha MM, Hassana AY, Goudaa AS, Rezk MR, Abdel-Megiedc AM, Marzouk HM. A novel LC-MS/MS method for determination of the potential antiviral candidate favipiravir for the emergency treatment of SARS-CoV-2 virus in human plasma: Application to a bioequivalence study in Egyptian human volunteers. Journal of Pharmaceutical and biomedical analysis 2021; 199:2-29.
5. Rezk MR, Badr KA, Abdel-Naby NS, Ayyad MM. A novel, rapid and simple UPLC–MS/MS method for quantification of favipiravir in human plasma: Application to a bioequivalence study. Biomedical Chromatography 2021:130-40.
6. Nadendla R, Patchala A. A Validated High-Performance Liquid Chromatographic Method for the Quantification of Favipiravir by PDA Detector**.** International Journal of Life Science and Pharma Research 2021:181-188.
7. Bulduk I, HPLC-UV method for quantification of favipiravir in pharmaceutical formulations. Acta Chromatographica 2020; 33: 9-15.
8. Göktu O, Altas E, Kayar G, Gökalp M. The Development and the Validation of a Novel Dissolution Method of Favipiravir Film-Coated Tablets. Scientia Pharmaceutica 2021; 90:1-14.
9. Sharaf YA, Deeb SE, Ibrahim AE. Al-Harrasi A, Sayed RA. Two Green Micellar HPLC and Mathematically Assisted UV Spectroscopic Methods for the Simultaneous Determination of Molnupiravir and Favipiravir as a Novel Combined COVID-19 Antiviral Regimen. Molecules 2022; 27:1-15.
10. Mikhail IE, Elmansi H, Belal F, Ibrahim AE. Green micellar solvent-free HPLC and spectrofluorimetric determination of Favipiravir as one of the COVID-19 antiviral regimens. Microchemical Journal 2021:1-19.
11. Gökce S, Höl A, Bulduk I. Development and Validation of UPLC-MS/MS Method for Obtaining Favipiravir Tablet Dosage form and Evaluation of its Behavior Underforced Conditions. Journal of Pharmaceutical Research International 2021:1-12.
12. Ali SNS, Mobina L, Mehfuza M, Seema P, Ahmed A, Khan GJ. Analytical Method Development and Validation and Forced Degradation Stability-Indicating Studies of Favipiravir by RP-HPLC and UV in Bulk and Pharmaceutical Dosage Form. World Journal of Pharmaceutical Research 2021:1-19.
13. Patil AB, Mahaparale SP. Stability indicating RP HPLC method development for estimation of Favipiravir in the bulk and pharmaceutical dosage form. World Journal of Pharmaceutical Research 2021; 33: 1-22.
14. ICH Guideline, Q2 (R1), Validation of Analytical Procedures. Text and Methodology, November 2005.