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

Human Journals
Research Article
September 2022 Vol.:25, Issue:2

RP-HPLC Method for the Quantification of Favipiravir in Bulk and Formulations

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Submitted: 21 August 2022
Accepted: 27 August 2022
Published: 30 September 2022

Keywords: Favipiravir, RP-HPLC, Method Development, Validation

ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Favipiravir in bulk and tablet dosage form. Chromatogram was run through Std Kromasil C18 150 x 4.6 mm, 5µ. Mobile phase containing Acetonitrile: 0.1% OPA taken in the ratio 70:30 was pumped through column at a flow rate of 1.0ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 320nm. Retention time Favipiravir were found to be 2.164 min. %RSD of the Favipiravir were and found to be 0.5. % Recovery was obtained as 100.28% for Favipiravir. LOD and LOQ values for favipiravir were 0.38 and 1.15, respectively, according to regression equations. Favipiravir regression formula is y = 53530x + 20315. As a result of shorter retention times and shorter run times, the method was created to be straightforward and cost-effective, and it may be used for routine Quality Control Tests in Industries.

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INTRODUCTION:

The global coronavirus disease-2019 (COVID-19) outbreak has motivated researchers to work on developing medications or vaccinations to stop the progression of this illness [5,17]. The first oral favipiravir medicine in India to be authorized for the treatment of COVID-19 is FabiFlu [1]. The mechanism of action related to the drug is through selective inhibition of viral RNA-dependent RNA polymerase [2]. An analogue of a nucleoside is favipiravir-RTP. For the viral RdRP, it imitates both guanosine and adenosine. Two of these bases in a row prevent primer extension.[3] Prodrugs are used to give favipiravir. It has a high bioavailability (>94%), a modest volume of distribution, and a 54% protein binding rate (10–20 L). After a single dose, it reaches Cmax in less than 2 hours. Tmax and half-life both rise with additional dosages.[4,16] A respiratory infection called COVID-19 is brought on by Coronavirus-2 that causes severe acute respiratory syndrome (SARS-CoV-2). [1,2,6] The COVID-19 pandemic still has a negative impact on the worldwide socioeconomic system. [3] COVID-19 manifests in a variety of ways, from early, mild sickness that is primarily driven by virological response to late, severe illness that is primarily driven by a dysregulated inflammatory response.[4] In mild-moderate and severe cases, viral shedding can be seen 1-2 days before symptoms appear and can last for 1-2 weeks [7].

Both oral and injectable preparations of favipiravir-ribofuranosyl-5'-triphosphate (favipiravir-RTP) are available [8].

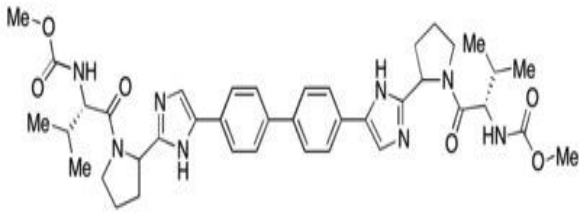

Fig. No. 1: Chemical Structure of Favipiravir

There are few RP-HPLC methods have been reported in the literature for the determination of Favipiravir in bulk and pharmaceutical dosage form by RP-HPLC [9,10,11,12,13]. An attempt has been made to develop an RP-HPLC method that is simple, specific, rapid, precise, and economical method for the quantitative determination of Favipiravir in bulk and pharmaceutical dosage form. This method has been validated as per International Conference on Harmonization (ICHQ2 (R1) guidelines.[14]

MATERIALS AND METHODS

Chemicals and reagents

Favipiravir pure drugs (API), Combination Favipiravir (FABIFLU) tablets, Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Instrumentation: The instrument used in the study was HPLC (Waters 2695 with PDA detector 2996) was monitored and integrated using Empower 2 software, electronic balance, sonicator, hot air oven, digital pH meter and UV-Visible chamber.

Preparation of Standard stock solution:

Accurately weighed 25mg of Favipiravir transferred to 50ml volumetric flask and ¾ th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (500µg/ml of Favipiravir).

Preparation of Standard working solution: 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (50µg/ml of Favipiravir)

Preparation of Sample stock solution:

10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to tablet was transferred into a 100ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (2000µg/ml of Favipiravir).

Preparation of Sample working solution: 0.25ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (50µg/ml of Favipiravir)

Chromatographic conditions:

Flow rate	**:** 1ml/min
Column	**:** Kromasil C18 (4.6 x 150mm, 5µm)
Wavelength	**:** 320.0 nm
Column temperature:	30°C
Injection volume	**:** 10.0µL
Run time	**:** 5.0minutes
Diluent	**:** Water and Acetonitrile in the ratio 50:50

Observation: Favipiravir eluted at 2.164 min respectively with good resolution (Fig. 2). Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

Degradation: According to ICH recommendations and standard industrial practice, forced deterioration is typically carried out in conjunction with a control sample under various stress conditions, including acid, alkali, peroxide, heat, and UV. Although there are no established standards for industrial degradation, it is recommended that 5 to 30 percent of degradation be reached under any of the applied stress conditions. The goal of the degradation to be accomplished by stress testing is to replicate the stability circumstances of the control room temperature[15]. To conduct the forced degradation experiment, standard stock solutions of Favipiravir was exposed to various stress conditions, including 1 mL of 20% H_2O_2 (for oxidative degradation), 1 mL of 2N HCl (for acidic degradation), and 1 mL of 2N NaOH (for acidic degradation) (for basic degradation). The produced solutions were refluxed for 30 minutes at 60^{o}C. To examine the descent, the standard solutions were also subjected to UV radiation and temperature conditions. The resulting solutions were diluted to yield 50µg/ml of Favipiravir for degradation studies. To examine sample stability, 10µl samples were fed into the system and chromatograms were obtained.

Method Validation: The method was validated in accordance with ICH recommendations Q2R1. System appropriateness, specificity, linearity, accuracy, precision, LOD& LOQ, and robustness are among the validation parameters.

RESULTS AND DISCUSSION

System suitability parameters: The system suitability parameters were assessed by making standard solutions of Favipiravir (20µg/ml) and injecting them six times. Peak tailing, resolution, and USP plate count were all determined. For three medications in combination, the USP Plate count exceeded 2000 and the tailing factor was less than 2. All of the system's appropriate parameters were passed and remained within the limitations. Table 1 shows the results.

Specificity: In the Optimized method, the interference is checked. Favipiravir, had retention time of 2.164 minutes. Method did not found any interfering peaks in the chromatograms of blank and placebo samples during the retention periods of the drug in our approach. As a result, this procedure was stated to be particular. Figures 3, 4, and 5 show the chromatograms for specificity.

Linearity: Six linear concentrations of Favipiravir (12.25-75µg/ml) were injected in triplicate manner. Correlation coefficients obtained was 0.999 for Favipiravir drug. The results were shown in table 2 and fig 6.

Precision:

Repeatability: Multiple samples were taken from a sample stock solution, and six working sample solutions of the same concentrations (50µg/ml Favipiravir) were created. Each injection was given from each working sample solution, and the results are shown in table 3. The average area, standard deviation, and % RSD for the medication were computed and found to be 0.3% for Favipiravir. The system precision was passed for this procedure since the precision limit was less than "2 %." Table 3 shows the information results.

Intermediate Precision: Multiple samples were taken from a sample stock solution, and six working sample solutions of the same concentrations (50µg/ml of Favipiravir) was prepared. Each injection from each working sample solution was given on the following day of the sample preparation, and the obtained areas are listed in table 4. The average area, standard deviation, and % RSD for the medication was computed and found to be 0.4% for Favipiravir. Because the precision limit was less than "2%" the intermediate precision was used for this procedure. Table 4 shows the information results.

Accuracy: The conventional addition procedure was used to create three levels of accuracy samples. Triplicate injections were administered at each degree of accuracy, and the mean % recovery for Favipiravir was found to be 100.28 %. Tables 5 show the outcomes. Because satisfactory recover values were achieved, the accuracy for this approach was passed.

Robustness: Robustness conditions such as flow minus (0.9ml/min), flow plus (1.1ml/min), mobile phase minus (65:35 v/v), mobile phase plus (75:25 v/v), temperature minus (27°C), and temperature plus (33°C) were maintained, and samples (20µg/ml Favipiravir) was injected in duplicate. The % RSD was computed and determined to be within the acceptable range. Table 6 shows the data.

Assay: Favipiravir tablets had a label claim of Favipiravir 25mg per unit formulation. The aforementioned formulation was used for the assay. The average % assay achieved for Favipiravir was 100.22%.

Degradation Studies: Degradation studies were performed with the stock standard solution and the degraded samples were analyzed using proposed method. Assay % of Favipiravir in the injected samples was calculated and all the samples passed the limits of degradation. The results were shown in table 7.

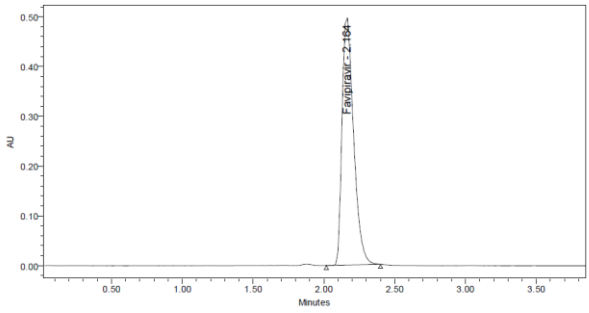

Fig. No. 2: Optimized Chromatogram

Table No. 1: System suitability parameters

Sr. No.	**Favipiravir**		
Inj	RT (min)	USP Plate Count	Tailing
1	2.126	3289	1.42
2	2.129	3278	1.41
3	2.129	3250	1.41
4	2.132	3161	1.43
5	2.136	3213	1.43
6	2.138	3209	1.42

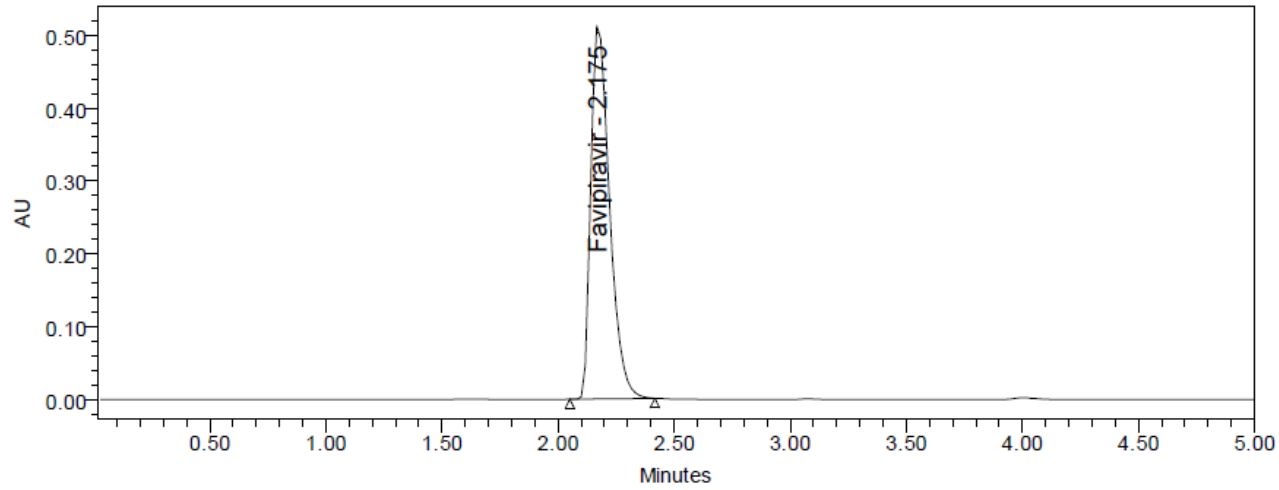

Fig. No. 3: Standard solution chromatogram

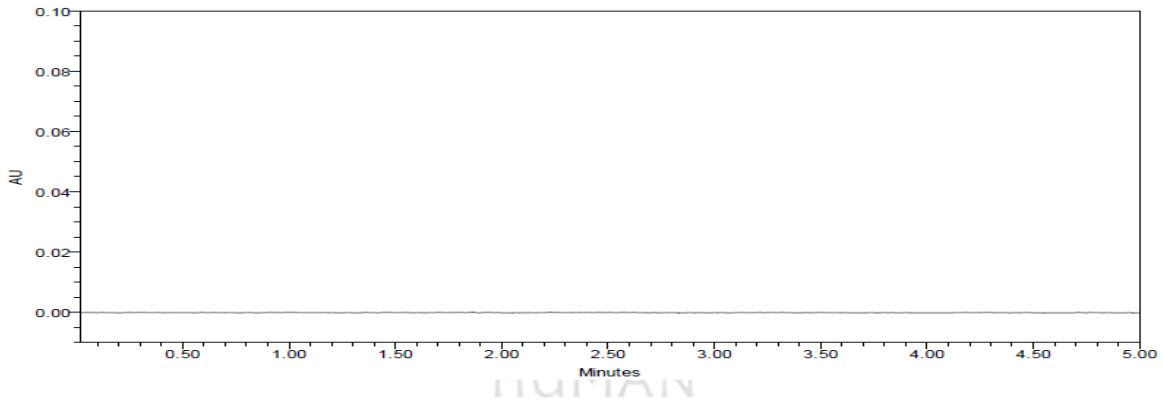

Fig. No. 4: Blank chromatogram

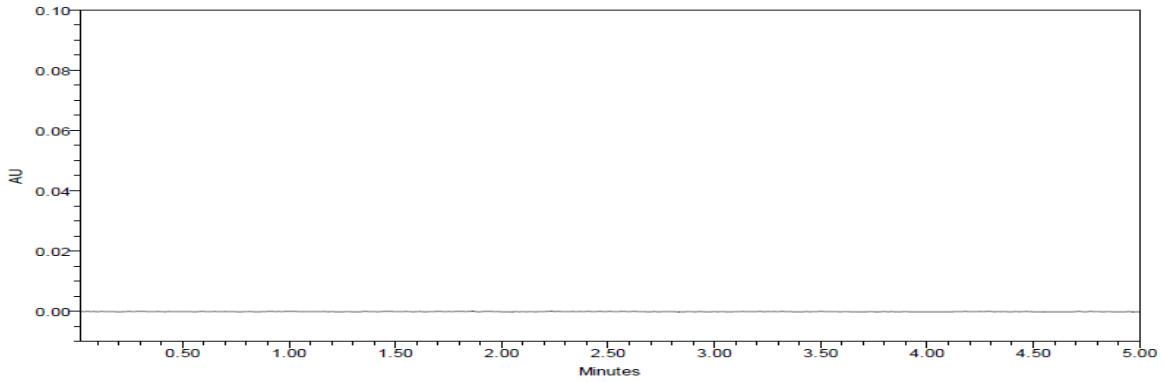

Fig. No. 5: Placebo chromatogram

Table No. 2: Linearity table for Favipiravir

Favipiravir	
Conc (µg/mL)	**Peak area**
12.5	666906
25	1395168
37.5	2076182
50	2675364
62.5	3351494
75	4028705

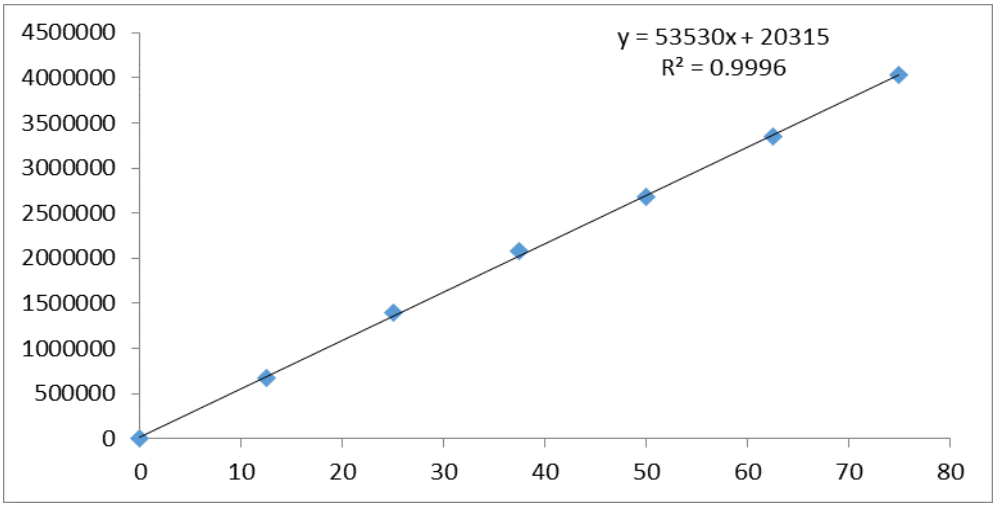

Fig. No 6: Calibration curve of Favipiravir

Table No. 3: Repeatability for Favipiravir

Sr. No.	Favipiravir
1	2709076
2	2686403
3	2686344
4	2697035
5	2701500
6	2696211
Mean	2696095
S.D	8804.9
% RSD	0.3

Table No. 4: Intermediate Precision for Favipiravir

Sr. No.	Favipiravir
1	2676515
2	2672523
3	2673007
4	2704804
5	2677958
6	2671880
Mean	2679448
S.D	12652.8
% RSD	0.5

Table No. 5: Accuracy for Favipiravir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	25	24.82	99.29	100.28%
	25	25.15	100.59	
	25	25.21	100.83	
100%	50	50.21	100.42	
	50	50.17	100.34	
	50	50.29	100.58	
150%	75	75.24	100.32	
	75	74.87	99.83	
	75	75.24	100.32	

Table No. 6: Robustness Data

Sr. No.	Condition	% RSD of Favipiravir
1	Flow rate (-) 0.9ml/min	0.2
2	Flow rate (+) 1.1ml/min	0.1
3	Mobile phase (-) 65B:35A	0.2
4	Mobile phase (+) 75B:25A	1.0
5	Temperature (-) 27°C	0.1
6	Temperature (+) 33°C	0.9

Table No.7: Degradation Data

Sr. No.	Condition	% Degraded	% Obtained
1	**Acid**	6.65	93.35
2	**Base**	4.39	95.61
3	**Oxidation**	4.98	95.02
4	**Dry heat**	1.65	98.35
5	**UV Light**	1.84	98.16

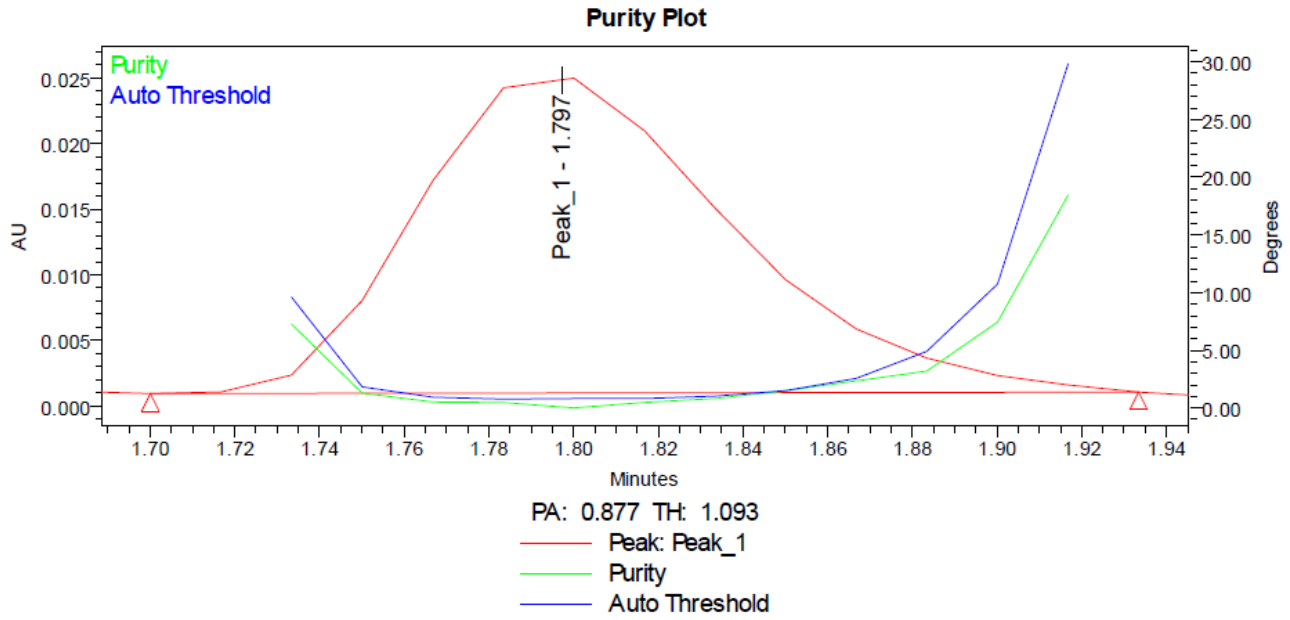

Fig. No. 7: Purity Plots

CONCLUSION:

For the identification and quantification of Favipiravir in pure and pharmaceutical formulations, a simple, quick, validated, and isocratic RP-HPLC technique with UV detection was devised. The approach was validated according to ICH requirements, and statistical results confirmed the suggested method's selectivity, linearity, sensitivity, precision, and accuracy. The provided method can also be used to investigate the stability of analytical solutions. The new method appears to be relevant as a quality control tool for Favipiravir assay in pharmaceutical businesses due to the necessity of low retention time in regular drug analysis.

ACKNOWLEDGEMENT: The author expresses sincere thanks to the Principal of Krishna Teja Pharmacy College, Tirupathi and Spectrum Pharma Research Solution, for providing Favipiravir drug as gift samples.

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