ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, S Iss 2 ,2022

Development and Validation of RP-HPLC Method for Estimation of Favipiravir in Bulk and Its Pharmaceutical Dosage Form

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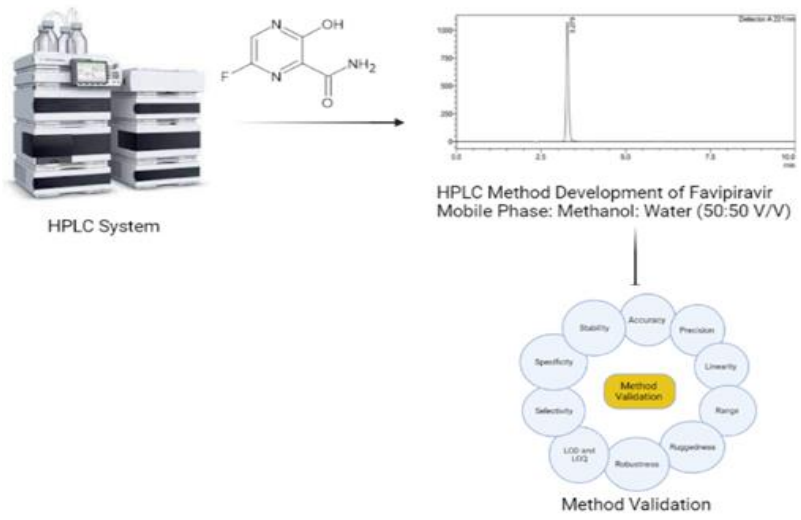
ABSTRACT:

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Favipiravir, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Kromasil 100-5-C18 column (300×3.9 mm, 5 µm) using a mixture of HPLC grade water and methanol (50:50v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 221 nm. The retention time of the drug was

3.325 ±0.25 min.The method produce linear responses in the concentration range of 20-100µg/ml of Favipiravir. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords*:* Favipiravir, RP-HPLC, method development, methodvalidation.

Graphical Abstract:



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INTRODUCTION

Favipiravir is an antiviral used to manage influenza, and that has the potential to target other viral infections, also known as favilavir.[1,2]Favipiravir (6-fluoro-3-hydroxypyrazine-2-carboxamide) is a pyra-zine analog. The mechanism of action is linked to transcription inhibition and viral gene replication which finally prevents the synthesis of viral RNA inside infected cells.[3] It has been shown that Favipiravir has an anti-viral effect on several RNA viruses, such as influenza-resistant viruses, bunyaviruses, filoviruses, arenaviruses, Yellow Fever, Rift Valley Fever, Western Equine Encephalitis, West Nile, Mouth and Foot Viruses, Avian Influenza and Norovirus.[4]The objective of the proposed study is to create a liquid chromatographic method and a validated, sensitive, and repeatable method for the determination of Favipiravir in bulk and pharmaceutical dose forms.[5]

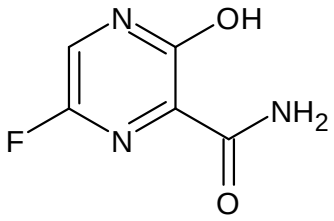

Figure 1: Chemical structure of Favipiravir

MATERIALS AND METHODS

Chemicals and reagents

Favipiravir, acetonitrile, methanol, HPLC grade water were obtained from Sigma Aldrich (St. Louis, MO, USA).

Chromatographic conditions

A prominence isocratic HPLC system (Shimadzu LC-2030Plus HighPerformance liquid chromatography with auto sampler Kromasil UV detector) column symmetry C18 (300×3.9 mm, 5µm).A 20µL injection syringe was used for sampleinjection. 2.3 pH HPLC grade Water and methanol in the ratio of (50:50 v/v) wereused for the preparing the mobile phase. A freshly preparedMethanol: Water (pH-2.3) (50:50 v/v) was used as the mobile phase. Thesolvents was filtered through a 0.45µ membrane filter andsonicated before use. The flow rate of the mobile phase wasmaintained at 1mL/min., column temperature wasmaintained at room temperature and detection was performed at 221 nm using a UV detector.

Preparation of mobile phase

Mix a HPLC grade water with PH 2.3 (50%) HPLC grade Methanol (50%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45µ filter under vacuum filtration.

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Diluent preparation:

Mobile phase as diluents

Standard solution preparation:

Accurately weigh and transfer 100mg of Favipiravir working standard in to 100mL volumetric flask add about 40 mL HPLC grade water and sonicated to dissolve it completely and the make volume up to the mark with the same solvent. (Stock solutions).Mix well and filter through 0.45 µm filter.(1000 µg/ml or PPM) Further 10 ml of stock solution transferred into 100 ml volumetric flask and diluted up to the mark with diluent and mixed well. (Concentration of Standard Solution: 100 µg/ml or PPM)

Sample solution preparation:

Weight 20 tables and calculate the average weight Triturate 20tablets to a fine powder. Weighed accurately equivalent to 100mg of Favipiravir, transfer into a 100ml volumetric flask, add 40ml of HPLC grade water, shake for 5min and sonicated for 30min and make up the volume with HPLC grade water, and filter through 0.45 micron membrane filter. Pipetted out 10 ml of filtrate, transfer in 100 ml volumetric flask and make up the volume with HPLC grade water.

METHOD VALIDATION

Specificity:

Blank, API and Drug product were injected. Chromatographs were observed.

Linearity:

The linearity of the method was demonstrated over the concentration range of 20-100 µg/ml of the target concentration. Aliquots of 20, 40, 60,80 and 100 µg/ml were prepared from above stock solution. Different concentrations of the pure drug were injected into the chromatographic system. Each concentration was evaluated five times and the corresponding mean peak area ratios (response factor) were plotted as a function of concentrations. The data was then statistically tested for its fitness in the linear regression model.

Precision method

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day and Interday studies, three repeated injections of 3 different concentrations (40, 60 and 80ug/ml)of standard solution was made and the response factor of drug peak and% RSD were calculated.

Accuracy

A study of recovery of Favipiravir from spiked placebo was conducted at three different spike levels i.e.80%, 100% and120% Samples were prepared with Favipiravir active

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pharmaceutical ingredients equivalent to about the target initial concentration of Favipiravir. Sample solutions were prepared in triplicate for each spike level and assayed as per proposed method.

LOD and LOQ:

According to ICH, Limit of Detection (LOD), and limit of quantification (LOQ) were calculated using the response's standard deviation and slope. Equation (1) was used to determine LOD, and Equation (2) was used to determine LOQ (ICH harmonized tripartite guideline, 2005)

Equation (1) – LOD = 3.3 σ/s

Equation (2) – LOQ = 10 σ/s

where σ corresponds to standard deviation of the y-intercepts of regression line and S is the slope of calibration curve.

System suitability

System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 40µg/ml.

RESULTS AND DISCUSSION

Development and Optimization ofthe HPLC Method

In HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. Satisfactory chromatographic separation was achieved with Kromasil 100-5-C18 column (300×3.9 mm, 5 µm) column. Optimized mobile phase consisted of a mixture of composed of methanol and water (50: 50 v/v). Above mobile phase ratio provided good resolution of Favipiravir. Flow rate of the mobile phase was set at 1.0 ml/min and injection volume 20 ml. Analytes were detected at a wavelength of 221 nm. Retention times of Favipiravir were found to be 3.325 ±0.25min, respectively. Total chromatographic run time was 6 min. Figure 2 represents the chromatogram of a standard preparation.

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Figure 2: chromatogram of a standard preparation

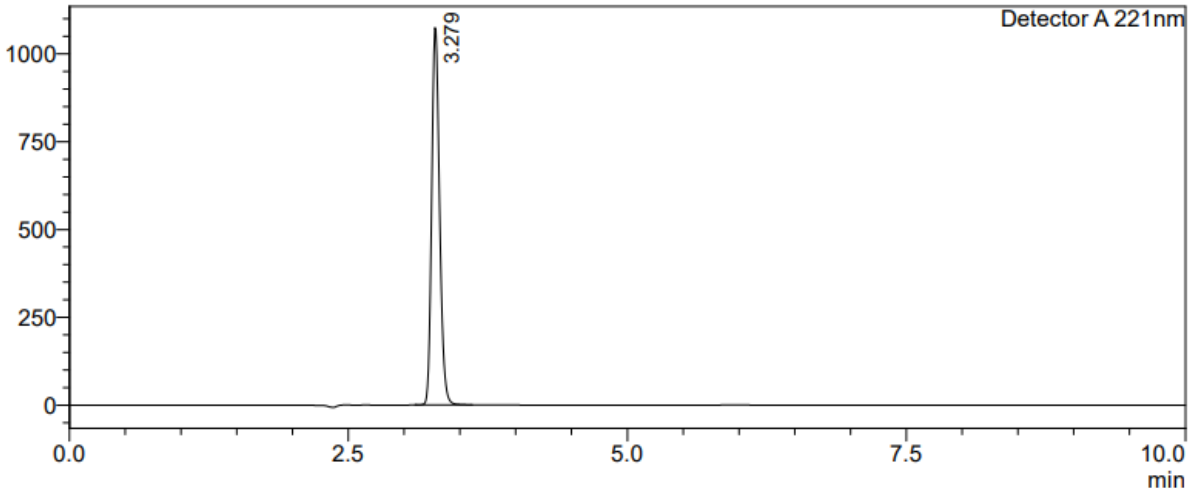

Table: Optimized chromatographic conditions

Parameter	**Conditions**
Type of system	Shimadzu LC-2030Plus High Performance liquid chromatography with auto sample
Mobile phase	Mixture of water and methanol (50:50v/v).
Column	C18 column (300×3.9 mm, 5 µm)
Detection wavelength	221.0 nm
Flow rate	1.0 ml/min
Volume of injection	20 µl
Temperature of column	Ambient (about 25°C)
Pump mode	Isocratic
Run time	06 minutes
Retention time	3.279

Method Validation

Specificity:

No interference from blank at retention time of Favipiravir peak, indicating that method is specific. Following figure show result of specificity:

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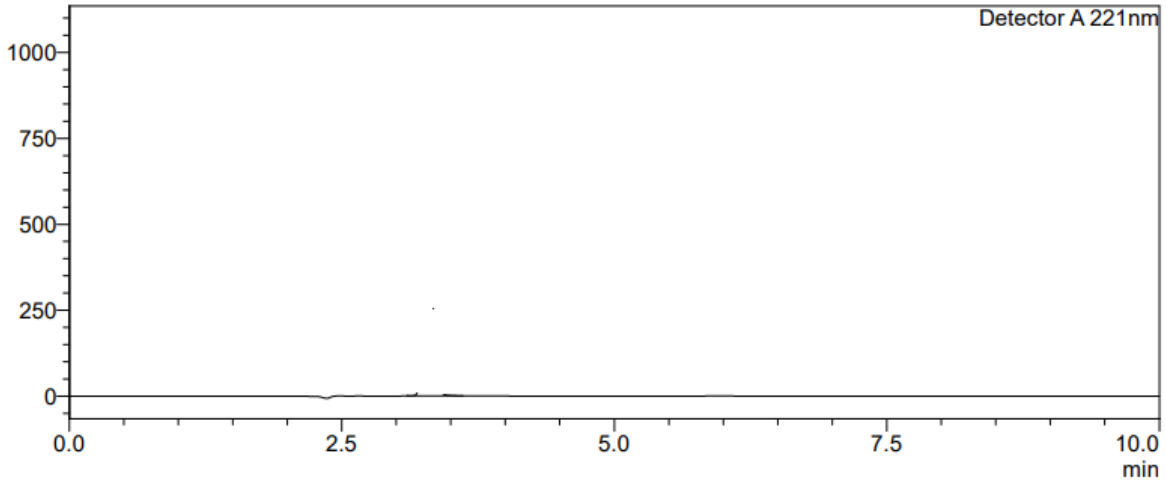
Figure 3: Chromatogram of Specificity



Linearity

The method is found to be linear in the concentration range of 20 – 100 µg/mL for Favipiravir. The regression coefficient was found to be 0.99978 (r^2<1), Hence the method was linear within given range. Linearity results for Favipiravir are shown in Table. The linearity graphs are shown in Figure.

Table 1: Linearity

Conc (µg / ml)	20	40	60	80	100
Avg.area	1076649	2142766	3220315	4267051	5276769
Regression coefficient (r2)	0.9998				

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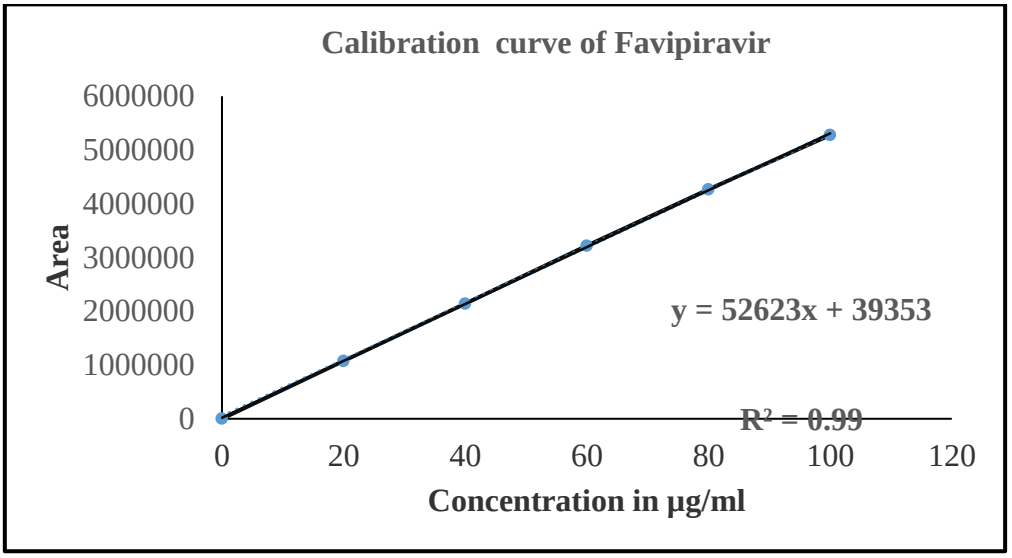
Figure 4: Linearity graph of Favipiravir



Precision

As per ICH guidelines the limit for precision is NMT 2% RSD, the above developed method shows the precision of 0.12% RSD and 0.61 % RSD which complies with the ICH guidelines. Hence the method was precise.

Table 2: Precision results

Sr. No.	Concentration(µg/ml)	Intraday precision (Mean area) n=3	SD	%RSD	Interday precision (Mean Area) n=3	S.D.	%RSD
1	40	2142707	855.92	0.04%	2117217	23876.17	1.13%
2	60	3219574	1963.37	0.06%	3193380	27875.92	0.87%
3	80	4267340	4962.77	0.12%	4213118	25608.21	0.61%

Accuracy:

The percentage recoveries of the results indicate that the recoveries are well within acceptance range (RSD<2) therefore method is accurate.

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Table 3: Accuracy results

Sample No.	Spike level	Amount (µg / ml) added	Amount (µg / ml) found	% Recovery	Mean % Recovery	SD	%RSD
1	80	48	48.60	101.25%	101.21	0.05	0.052
	80	48	48.55	101.15%			
	80	48	48.59	101.23%			
2	100	60	60.06	100.10%	101.04	0.05	0.051
	100	60	60.02	100.03%			
	100	60	60.00	100.00%			
3	120	72	72.51	100.71%	100.73	0.02	0.021
	120	72	72.54	100.75%			
	120	72	72.53	100.74%			

LOD and LOQ

LOD was found to be 0.15µg/ml, LOQ was found to be 0.44µg/ml. Hence; the developed method was validated for all the above parameters.

System suitability

Retention time, theoretical plate and tailing factor are within required limit.

Table 4: System suitability results

Property	**Values**	**Required limits**
Retention time	3.325 ±0.25	RSD ≤ 1%
Theoretical plates	8485	N > 2000
Tailing factor	1.2	T ≤ 1

For the proposed RP-HPLC method, characteristic parameters were shown in Table:

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Table 5: Characteristic parameters of Favipiravir for the proposed RP-HPLC method

Parameters	RP-HPLC
Calibration range	20 to 100 µg/ml
Detection wavelength	221 nm
Mobile phase	HPLC grade water and methanol (50:50v/v)
Retention time	3.325 ±0.25
Regression equation	Y= 52623x+39353
Slope	52623
Intercept	39353
Correlation coefficient (r2)	0.9998
Intraday precision (%RSD)	0.073%
Interday precision (%RSD)	0.87%
Limit of detection	0.15 µg/ml
Limit of quantitation	0.44 µg/ml

CONCLUSION:

For quantifying favipiravir in pharmaceutical formulations, the proposed method is easy, quick, precise, and sensitive. An easy mobile phase is used in this technique. Unlike several of the previously mentioned approaches, this one can be used without a buffer, needs no intricate sample preparation steps, and has a better sensitivity. In order to determine favipiravir, this proposed method can therefore be used widely in quality control laboratories.

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