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A Validated high Performance Liquid Chromatographic Method for the Quantification of Favipiravir by PDA Detector

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Abstract: Favipiravir is an antiviral agent showing activity for the treatment of various life threatening viruses such as Ebola virus, Lassa virus and also recent virus for COVID-19. It is a pyrazine carboxamide derivative with activity against RNA viruses which targets RNA-dependent RNA polymerase enzymes which are necessary for the transcription and replication of viral genomes. The lack of research work and no compendial methods available for the estimation of this drug influenced the current research investigation to give a simple, sensitive, rapid, precise, accurate and robust isocratic high performance liquid chromatographic and UV Spectroscopic method for the determination and quantification of Favipiravir. The elution was done by using SHIMADZU Prominence-i, LC-2030 C system equipped with Shim-Pack GIST C18 (250X 4.6 mm, 5µm) column with a mobile phase mixture of 10 mM potassium dihydrogen ortho phosphate buffer (pH 4.0) and acetonitrile in the ratio of 90:10 v/v at a flow rate of 1.0 ml/min. The ultraviolet detection was done at the wavelength of 315 nm by maintaining column temperature at 30°. The total run time was 8.0 min. Calibration plot showed best regression over the concentration range of 10-60 µg/ml of Favipiravir standard solutions. The LOD and LOQ was found to be 0.18 µg/ml and 0.53 µg/ml, respectively. The accuracy of the proposed method was determined by performing recovery studies and was found to be between 99.47-100.80%. The repeatability testing for both sample and standard solutions was found as %RSD<2.0% which is within the acceptable limits showing that the method is precise as well. 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.

Keywords: Favipiravir, Antiviral, HPLC, UV, method development, Validation

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1.   INTRODUCTION

Favipiravir is an established drug for the treatment of influenza and is being explored more for its role in the treatment of COVID-19. It is the first oral antiviral drug approved for mild to moderate COVID-19. The already completed studies in China, Japan, and Russia have shown Favipiravir to be a promising cure for this disease. Toyama Chemical of Japan has developed Favipiravir which is a pyrazine carboxamide derivative that acts against many RNA viruses. It was first described as a selective inhibitor of influenza virus replication with minimal cytotoxicity. It is believed that the drug Favipiravir directly target the RdRp catalytic site preventing virus replication in cells and inhibiting infection; it has been shown to have *in-vitro* and/or *in -vivo* antiviral activity against a wide array of human-infecting RNA viruses, including ss(–)RNA viruses. Oestereich et al. (2014) reported that T-705 has *in vivo* antiviral activity against Zaire EBOV in a mouse model. No cytotoxicity under the experimental conditions used was observed: the treatment was started 6-day post infection and resulted in 100% protection. Similar results were also obtained by Smither et al. (2014). The literature supports its wide therapeutic efficacy and safety profile. Notably, studies exhibit the absence of resistance to favipiravir and a broad spectrum antiviral activity, which is a driving force to pursue clinical studies for distressing coronavirus infections. It has a wide therapeutic safety margin for a high dose and is available as an oral formulation. As 80% of the patients infected with COVID-19 have mild to moderate severity, oral formulation is more convenient. [1-2, 8-9] The RNA polymerase inhibitor Favipiravir (T-705) is a broad spectrum antiviral drug with activity against a number of RNA viruses including arenavirus, bunyaviruses, flavivirus like west nile as well as influenza A and B viruses. Favipiravir has a strong binding affinity to RdRp with a docking score of −6.925. Hence, favipiravir targets the Achilles heel (RdRp complex) of SARS-CoV-2. Favipiravir is one such oral drug that was approved for new and reemerging pandemic influenza in Japan in 2014 and has shown potent *in-vitro* activity against severe acute respiratory syndrome coronavirus-2. It has a wide therapeutic safety margin indicated by a wide CC50/EC50 ratio for a high dose. From the clinical studies in COVID-19, it has shown rapid viral clearance as compared to lopinavir/ritonavir (LPV/RTV) and superior recovery rate than umifenovir. (Figure 1)
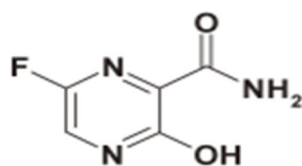

<div align="center">**Fig 1: Chemical structure of Favipiravir**</div>

Literature search reveals that there are fewer methods reported for the determination and quantification of Favipiravir and there is less research work done on this antiviral drug. No official pharmacopoeial method found for this particular antiviral drug. In the view of this criterion, the main aim and objective of this study is to develop and validate a sensitive method for the estimation of Favipiravir with applicability of Favipiravir in routine analysis.[1-2]

2.   EXPERIMENTAL

2.1   Chemicals

Potassium dihydrogen orthophosphate, ortho phosphoric acid, HPLC grade water, HPLC grade acetonitrile, Favipiravir active pharmaceutical ingredient, Favipiravir tablets (FVP 200mg).

2.2   Preparation of stock solution

10 mg of active pharmaceutical ingredient was weighed and transferred into 100 ml of volumetric flask and dissolved in a mixture of 50 ml methanol. The resultant solution was vortexed, sonicated and filtered and made up to the mark with water to obtain 100µg/ml solution. From the stock solution further dilutions were made by using mobile phase to get the concentration solutions from 10-60µg/ml. [3-5, 7,10-11]

2.3   Preparation Sample solution

10 tablets of Favipiravir were accurately weighed and transferred to the motor and ground to fine powder. A tablet powder equivalent to 250mg Favipiravir was weighed and transferred into 250 ml of volumetric flask. To that 50 ml of methanol and 50 ml of HPLC grade water was added and the flask was kept in a rotary shaker for 20 mins to disperse the excipients into the solution. The solution was sonicated for 30 min and made up to the mark using HPLC water to get 1000µg/ml solution then filtered by vacuum filtration unit using 0.45µ filter paper. [11]

2.4   Determination of wavelength

A standard solution containing 40 µg/ml was scanned between 200-800 nm using ultra-violet UV Spectrophotometer (Lab India, UV win 5 software) to determine the maximum wavelength and determined as 315 nm for the standard solution.

2.5   Chromatographic conditions

HPLC System: SHIMADZU Prominence-i, LC-2030 C
Column: Shim-Pack GIST C18 (250X 4.6 mm, 5µm) column
Mobile phase: Mixture of 10 mM potassium dihydrogen ortho phosphate buffer (pH 4.0) and acetonitrile in the ratio of 90:10 v/v
Flow rate: 1.0 ml/min.
Detection wavelength: 315 nm
Column temperature: 30° C
Run time: 8.0 min

2.6   Method validation

The analytical method validation was done according to ICH Q2 (R1) guidelines of validation of analytical methods for the parameters of specificity, system suitability, linearity, the limit of detection (LOD), the limit of quantification (LOQ), accuracy, precision and robustness were discussed below. [12-13]

2.7 Specificity

Specificity is the ability of the analytical method to produce a response for the analyte in the presence of other components present in the solution, technically they can be like impurities, degradants or matrices. In this method the specificity is tested for the standard solution and blank and found no interference in the blank injection. Tailing factor and theoretical plates were taken into consideration.

2.8 System suitability

System suitability was performed for the standard solution

and confirmed the method suitability by taking tailing factor, theoretical plates, % RSD and retention time parameters into consideration.

2.11 Limit of Quantification (LOQ)

LOQ is the parameter which explains the detection and quantification of the lowest amount. In the method, the values of LOQ were determined from the following formula.

$$LOQ = 10* \sigma / S$$

2.12 Precision

Precision is an analytical procedure that expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. In the current study, the % RSD for the sample solution was found below <2.0.

2.13 Accuracy

Accuracy can be defined as the closeness of agreement between accepted reference value and the value found. In this study, recovery was calculated by standard weighing method for 50%, 100% and 150%.

2.14 Robustness

A robustness method was performed to confirm whether the method is capable of reproducibility during the deliberate changes taken place in the proposed method.

2.9 Linearity

The linearity of a validation parameter, which confirms the ability of a method (within a given range) to obtain test results, will be directly proportional to the concentration of analyte in the sample. By giving different concentrations of sample solutions it is confirmed that the method is linear in the 10-60μg/ml range with 0.999 regression value.

2.10 Limit of Detection (LOD)

The detection limit 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. The values were determined by calculating from slope and regression line by following the equation.

$$LOD = 3.3* \sigma / S$$

2.15 Solution stability

The stability of the sample was determined by analysing the sample solutions which were stored at ambient temperature at different time levels. The average peak area and % RSD of solution were calculated.

3. RESULTS AND DISCUSSION

3.1 UV-Spectroscopic Method

To confirm the maximum wavelength of Favipiravir, a standard solution of 40 μg/ml was scanned between 200-800 nm in UV-Spectrophotometry using mobile phase as blank and it was found that the Λ_max was determined at 315 nm.

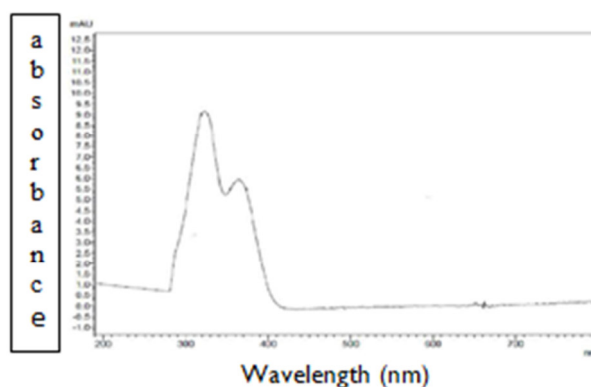
Fig 2: Λmax (315 nm) of UV Spectrum (standard solution, 40μg/ml)

3.2 HPLC Method development

The physical and chemical properties of Favipiravir were obtained from the literature. Several preliminary tests were conducted to optimize the chromatographic conditions for the quantification of Favipiravir. The analytical method was developed to select preliminary reversed phase HPLC chromatographic conditions including mobile phase, wavelength, flow rate, stationary phase and sample preparation. Mobile phases consisting of various buffers and organic solvents were tried and were unable to meet the expected and required results. Then the mobile phase with potassium dihydrogen orthophosphate was tried and got better results with higher retention time. and organic phases containing acetonitrile and methanol were tried along with the combination of potassium dihydrogen orthophosphate buffer. Finally it was concluded that the mobile phase containing 90:10 ratio of potassium dihydrogen

orthophosphate buffer (10mM) with 4.0 pH adjusted with ortho phosphoric acid and acetonitrile has given the giving best peak shape, required theoretical plates and symmetrical peak shape by using Shim-Pack GIST C18 (250X 4.6 mm, 5μm) column with 1.0 ml/mi. The UV detector was fixed at 315 nm. Column temperature was maintained at 30°C. The developed chromatographic conditions are much economical and eco-friendly with low column pressure, enhanced column efficiency with a good number of theoretical plates.[12-13]

3.3 Method Validation

3.3.1 Specificity

By injecting a blank solution it was confirmed that there was no inference found in the standard chromatogram when tailing factor and theoretical plates were taken into consideration.
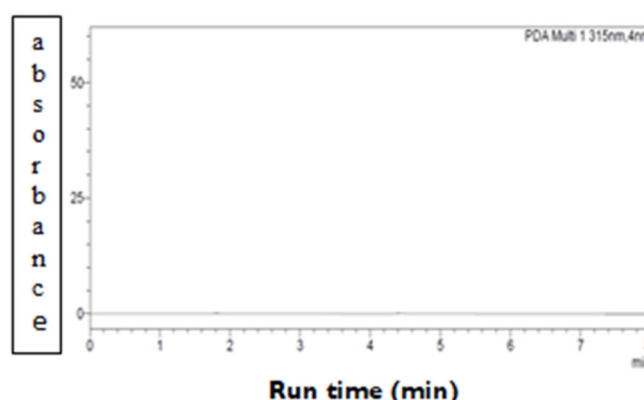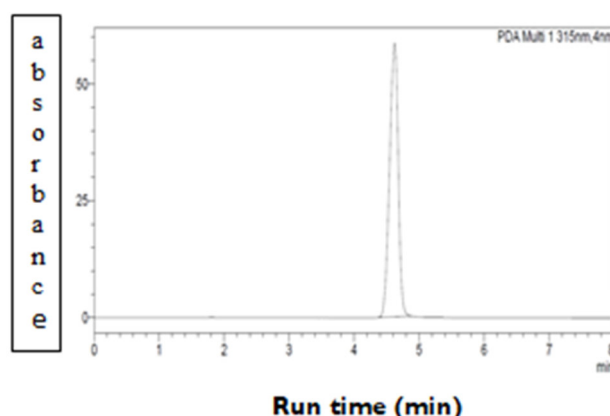

Fig 3: Chromatogram of blank

Fig 4: Chromatogram of standard

3.3.2 System suitability

Six replicate injections of sample were given for the test of system suitability and found % RSD was within limits (<2.0). Results were given in Table 1.[12-13]

Table 1: Results of System Suitability Parameters			
Sr. No	Retention time (m)	No. of theoretical plates	Peak area
1.	4.622	5100	542772
2.	4.621	5212	542323
3.	4.622	5078	543490
4.	4.623	5183	542175
5.	4.620	5234	542658
6.	4.624	5120	543159
Mean			542763
SD			497.1508
% RSD			0.09

3.3.3 Linearity

From the standard stock solution the concentrations from 10-60µg/ml were prepared and injected into the system under the mentioned chromatographic conditions. Linearity of the proposed method was estimated at 6 concentration levels within a range of 10-60µg/ml by regression analysis. By using least square methods correlation coefficient, slope and intercept were calculated.
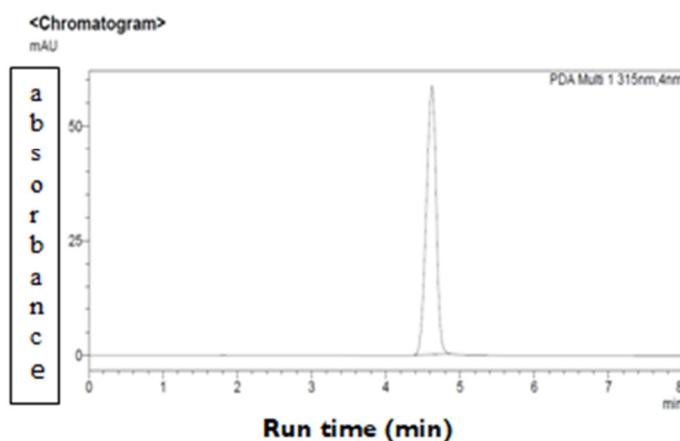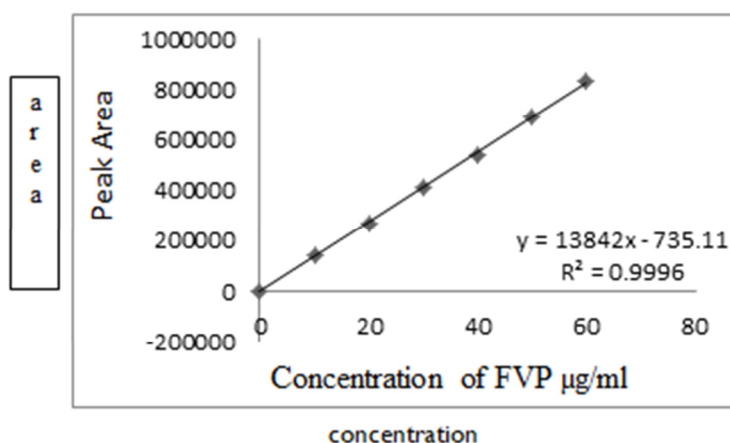

Fig 5: Chromatogram of Favipiravir

Fig 6: Calibration curve

3.3.4 Accuracy

Recovery studies were performed on the basis of standard weighing method for 50%, 100% and 150%. Results were given in Table 2. [12-13]

Table 2: Results of Recovery Studies				
Level	**Wt of sample taken (mg)**	**Peak area**		**% Recovery**
		Sample	**Standard**	
50%	72.88	276433	542763	100.76
100%	145.33	544219	542763	99.47
150%	217.4733	820999	542763	100.28
*All the readings were taken as a mean of 3 readings				

3.3.5 Precision

Precision was performed by estimating the drug concentration for intraday and inter day by calculating % RSD. The % RSD was found to be 0.12% for intraday and 0.18% for inter day precision. Results of precision were given in Table 3.

Table 3: Results of Precision			
Sr. No	**No of injections**	**Peak area**	
		Intraday precision	**Inter day precision**
1.	Injection 1	543772	542965
2.	Injection 2	541958	542454
3.	Injection 3	543152	542268

4.	Injection 4	542275	542369
5.	Injection 5	542268	543489
6.	Injection 6	542868	544891
	Mean	542716	543073
	SD	677.9504	1001.663
	% RSD	0.12	0.18

3.3.6 Robustness

To perform robustness in this method, deliberate changes were done in flow rate, wavelength, column temperature and mobile phase ratio. No significant changes were observed and % RSD was calculated which was within acceptance limits and shown in Table.4.

Table 4: Results of Robustness		
Condition	**Variation**	**% RSD**
Mobile phase flow(1.0 ml/min)	0.8 ml/min	0.17
	1.2 ml/min	0.18
Acetonitrile ratio (10%)	8%	0.09
	12%	0.10
Column temperature (30°C)	28°C	0.12
	32° C	0.12
Wavelength (315 nm)	312 nm	0.18
	317 nm	0.18

3.3.7 LOD and LOQ

LOD and LOQ were determined based on the standard deviation and calibration curve slope. The results were shown in Table 5.

Table 5: Results of LOD and LOQ	
Parameter	**Result**
LOD	0.18
LOQ	0.53

3.3.8 Solution stability

The stability of sample and standard solutions was monitored for 24h time period. For this, samples were injected into the system over 8 hrs time period. Then peak area and retention time were evaluated. No changes were observed in sample and standard over 24h, %RSD was found within limits (<2.0). Results were presented in Table 6.

Table 6: Results of Stability (40µg/ml)					
Time	**Peak area**	**Retention time**	**Mean**	**SD**	**% RSD**
8 hrs	542812	4.589	543183.3	621.643	0.114
	542837	4.572			
	543901	4.590			
16 hrs	542175	4.577	542948	857.8922	0.158
	542798	4.489			
	543871	4.482			
24 hrs	542612	4.478	543673	1102.072	0.202
	543595	4.479			
	542612	4.469			

3.4 Marketed formulation

The developed and validated method was successfully applied for the marketed Favipiravir formulations and estimated by using the formula followed. Results were placed in Table.7. [12-13]

$$\%Assay = \frac{Asam\ X\ Std\ wt\ X\ Sam\ DF\ X\ Avg.Wt\ X\ Potency\ X100}{Astd\ X\ Std.DF\ X\ Sam\ wt\ X\ LC\ X100}$$

Where,

A_{sam}	=	Sample area
A_{std}	=	Area of standard
Std wt	=	Weight of standard
C_{std}	=	Concentration of standard
Sam DF	=	Sample Dilution Factor
Std DF	=	Standard dilution factor
Avg. Wt	=	Average weight
LC	=	Labeled Claim

Table 7: Results of Assay Of Marketed Formulation			
Formulation	**Label claim**	**Amount of tablet powder (mg)**	**% Assay**
FVP	200	145.35	99.46

The proposed quantification method is very cost effective, precise and accurate for the determination of Favipiravir[1-6]. By giving the evidence of results through validation parameters (as per ICH Q2 guidelines) it is proved that this particular method is very much suitable for simple evaluation of Favipiravir which is currently used for the treatment of Covid-19 virus [7-10]. Phosphate buffer (pH 4.0) which is used in the present study is the best eco-friendly solvent that won't affect the efficiency of column. The optimized run time 8 minutes explains about the efficiency of method by taking less time for the analysis of this particular drug and also consumes less volume of mobile phase at 1.0 ml/min flow rate. LOD was reported as 0.18 µg/ml which proved the sensitivity of this proposed method by detecting less amount Remaining parameters like accuracy, linearity, system suitability, stability the results were falling within specified limits revealing the efficiency and advantages of this method.

4.   CONCLUSION

Present work was performed to give the best method for the estimation of Favipiravir. No official compendial method is available for the estimation of Favipiravir and very few methods were reported. The proposed method is more accurate and sensitive. In consideration of this criterion, it is confirmed that this given method can be applied for the routine analysis of Favipiravir in pharmaceutical industries also.Here, it is concluded that the proposed method is a simple, reproducible, sensitive and accurate method for the estimation of Favipiravir.

8.   REFERENCES

1. National library of Medicine, National center for Biotechnology information, PubChem. Compound summary. Favipiravir CID. PMID 492405. [cited on: 01.08.2005]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Favipiravir.[a1].
2. Acquavia MA, Foti L, Pascale R, Nicolò A, Brancaleone V, Cataldi TRI, Martelli G, Scrano L, Bianco G. Detection and quantification of Covid-19 antiviral drugs in biological fluids and tissues. Talanta. 2021 March 1;224. doi: 10.1016/j.talanta.2020.121862, PMID 121862.
3. Prajapat Manisha, Sarma Phulen, Shekhar Nishant, Avti Pramod, Sinha Shweta, Kaur Hardeep, Kumar Subodh, Bhattacharyya Anusuya, Kumar Harish, Bansal Seema, Medhi Bikash. Drug targets for coronavirus: A

4.1   ABBREVIATIONS

FVP	:	Favipiravir
RSD	:	Relative Standard Deviation
Mg	:	Milligrams
µg	:	Micrograms
ml	:	Millilitre
hrs	:	Hours
LOD	:	limit of detection

5.   AUTHOR CONTRIBUTION STATEMENT

6.   ACKNOWLEDGEMENT

7.   CONFLICT OF INTEREST

Conflict of interest declared none.

systematic review. Indian J Pharmacol. 2020;52(1):56-65. doi: 10.4103/ijp.IJP_115_20, PMID 32201449.
4. Bulduk İ. HPLC-UV method for quantification of favipiravir in pharmaceutical formulations. Acta Chromatographica. august 9 2020. doi: 10.1556/1326.2020.00828.
5. Dong Liying, Hu Shasha, Gao Jianjun. Discovering drugs to treat coronavirus disease 2019 (Covid-19). Drug Discov Ther. 2020;14(1):58-60. doi: 10.5582/ddt.2020.01012, PMID 32147628.
6. Madelain Vincent, Guedj Jérémie, Mentré France, Nguyen Thi Huyen Tram, Jacquot Frédéric, Oestereich Lisa, Kadota Takumi, Yamada Koichi, Taburet Anne-Marie, de Lamballerie Xavier, Raoul Hervé. Favipiravir pharmacokinetics in nonhuman Primates and insights for future efficacy studies of hemorrhagic fever viruses. Antimicrob Agents

Chemother. 2017;61(1):e01305-16. doi: 10.1128/AAC.01305-16, PMID 27736754.

7. China patent (CN104914185B). A kind of favipiravir has the HPLC assay method of related substance. 21.09.2016. Available from: https://patents.google.com/patent/CN104914185B/en

8. China patent (CN104914185A). HPLC method for measuring related substances in favipiravir. 16.09.2015. Available form: https://patents.google.com/patent/CN104914185A/en

9. Smee Donald F, Hurst Brett L, Egawa Hiroyuki, Takahashi Kazumi, Kadota Takumi, Furuta Yousuke. Intracellular metabolism of favipiravir (T-705) in uninfected and influenza A (H5N1) virus-infected cells.

10. Furuta Y, Takahashi K, Morrey J, et al. (Favipiravir) and related compounds: novel broad-spectrum inhibitors of RNA viral infections. (2). Antiviral Res. 2009 T-705;82(3):95-102.

11. ICH. validation of analytical procedures: text and methodology, in proceedings of the International conference on harmonization of technical requirements for registration of pharmaceuticals for human use. 2005;Q2:(R1). Harmonized tripartite guideline.

12. Center for drug evaluation and research (CDER). Reviewer guidance: validation of chromatographic methods; 1994.

J Antimicrob Chemother. 2009;64(4):741-6. doi: 10.1093/jac/dkp274, PMID 19643775.