Clinical and Experimental Health Sciences

Development and Validation of a Rapid HPLC-DAD Method for Determination of Favipiravir in Pharmaceutical Formulation

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ABSTRACT

Objective: The aim of this work was to develop and validate a rapid and simple high-performance liquid chromatography method with a diode-array detector (HPLC-DAD) for determination of favipiravir in bulk and tablet formulations.

Methods: The chromatographic analysis was performed at 30 °C with a Poroshell 120EC-C18 column (4.6 x 50 mm, 2.7 µm). The mobile phase was a mixture of 0.1% formic acid in water and 0.1% formic acid in acetonitrile (90:10, v/v). The run time was 5 min at a flow rate of 0.5 mL/min.

Results: The proposed method was successfully validated in terms of precision, accuracy, linearity, robustness, limits of detection (LOD) and quantification (LOQ) parameters. The calibration plot was linear over a concentration range of 10-100 µg/mL. The LOD and LOQ values were found to be 0.58 µg/mL and 2.03 µg/mL, respectively. The average recovery values were found to vary from 99.45 percent to 104.29 percent.

Conclusion: As a result, it was concluded that the developed method can be used successfully in the determination of favipiravir in pharmaceutical preparations.

Keywords: COVID-19 treatment, HPLC, validation, favipiravir, SARS-CoV-2

1. INTRODUCTION

The Chinese government notified the WHO in December 2019 of pneumonia hospitalized patients with an unknown etiology. These patients were eventually identified as COVID-19, with SARS-Cov-2 as the causal virus. WHO declared a pandemic of Coronavirus illness in March 2020 (1-3). By the end of July 2021, the total number of cases recorded globally has surpassed 200 million, with 4 million fatalities. There is currently no COVID-19 therapy that has been scientifically proved to be effective and safe. Off-label treatments have already been approved for the treatment of various illnesses in Europe, the United States (USA), and our nation and have been demonstrated to be efficacious in vitro in SARS-CoV. One of these drugs used in this treatment is favipiravir (FAV) (4-10). When the literature studies on the analysis of favipiravir by HPLC are examined, it is seen that there are two studies based on gradient elution, and the analysis time of favipiravir in these studies was 21 and 60 minutes, respectively (11,12). Another HPLC techniques for determining FAV in pharmaceutical dosage forms was recently published (1,13). The proposed study is more sensitive than the two previously reported methods (1.20/3.60 and 0.985/2.986 µg/mL), with lower LOD and LOQ (0.58/2.03 µg/mL). So, it is predicted that this validate, and rapid method because it is inexpensive and allows rapid and sensitive analysis. The proposed method is rapid, accurate, very simple, and sensitive for quantification of favipiravir in pharmaceutical formulations. This method employs a simple mobile phase without the need of a buffer, requires no complicated sample preparation procedures, and has higher sensitivity than some of the previously described methods.

2. METHODS

2.1. Chemicals and Reagents

Bulk favipiravir and its pharmaceutical formulation (Favimol labelled content of FAV 200 mg) were kindly supplied by Dr. Şükran Özdatlı. HPLC-grade acetonitrile and formic acid were purchased from Merck. Millipore's Milli-Q water purification

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technology was used to create HPLC-grade water. All the other reagents were of analytical grade.

2.2. Instruments

The Agilent 1260 Infinity HPLC system (Agilent Technologies, USA) was utilized for the chromatographic operations, which included a solvent pump, auto-sampler, column compartment, and DAD detector. The wavelength of detection was 323.0 nm. The chromatographic analysis was performed at 30 °C with a Poroshell 120EC-C18 column (4.6 x 50 mm, 2.7 µm) and a mobile phase A and B composed of 0.1% formic acid in water and 0.1% formic acid in acetonitrile, respectively. The flow rate was maintained at 0.5 mL/min.

2.3.Preparation of Stock and Working Solutions

Stock solutions of 1.0 mg/mL of FAV was prepared using an ultra-pure water in volumetric flask. The solution was warmed at 37 ℃ and shake it in the ultrasonic bath for a while for obtaining a higher solubility. Working standard solutions containing 10–100.0 µg/mL of FAV were generated by diluting this solution with ultra-pure water.

2.4. Method Validation

The ICH guidelines were followed for developing and validating the analytical method. System appropriateness, linearity, specificity, precision, accuracy, robustness, the limit of detection (LOD) and quantification (LOQ) were all addressed as validation parameters.

2.5. Specificity

In order to determine the specificity of the method, the presence of interference from the mobile phase was investigated with the help of a diode array detector. For this purpose, besides the chromatogram of the mobile phase, the UV spectrum and peak purity index values obtained from the FAV peak were examined.

2.6. Linearity

A six-point calibration plot for FAV was used to assess linearity. The standard solutions, ranging in concentration from 10 to 100 µg/mL, were used to prepare standard calibration. Under optimum chromatographic conditions, the standard solutions were injected three times into the HPLC system. The regression line's slope and Y-intercept values were calculated.

2.7. Limits of Detection and Quantitation (LOD and LOQ)

The LOD and LOQ values were calculated as 3.3 and 10 times the ratio of the calibration plot standard deviation to its slope, respectively.

2.8. Precision

Precision was determined at three distinct concentrations (n=5) by calculating intra-day and interday (repeatability was determined by examining the standard solution on five separate days) variations of the method. In precision research, on the same day and for five days, five repetitions of standard solution at three concentrations (30, 50, 70 µg/mL) were injected into the system. The relative standard deviation was used to get the accuracy value (RSD).

2.9. Accuracy

To test the accuracy of the suggested method, recovery studies were carried out using the spiking method. The sample solutions of known quantity (20 µg/mL) were spiked with 80, 100, and 120 percent of three different amounts of bulk and calculating favipiravir recovery for each concentration from equation of the calibration plot.

2.10. Robustness

A robustness study was conducted to assess the impact of certain modifications in chromatographic conditions. Column temperature, mobile phase flow rate and ratio are all variables. The system appropriateness parameters were verified after each change by injecting the sample solution into HPLC system and comparing the results to those obtained with the initial chromatographic settings.

3. RESULTS

3.1. Chromatographic Conditions

The analysis was performed in a C18 column (4.6 x 50 mm, 2.7 µm) using a solvent system of 0.1 % formic acid in water: 0.1 % formic acid in acetonitrile (90:10, v/v) by isocratic elution. The flow rates of the mobile phase were maintained at 0.5 mL/min. From the UV spectra of standard solution, the wavelength corresponding to maximal absorbance (max) was determined to be 323 nm (Figure 1). The column temperature was kept at 30 °C during the procedure. Table 1 presents the conditions of HPLC system.

Table 1. *Optimized chromatographic conditions*

Parameter	Chromatographic conditions
Instrument	Agilent 1260 Infinity HPLC system
Column	Poroshell 120EC-C18 4.6 x 50 mm, 2.7 µm
Mobile phase [A:B (90:10, v/v)]	Mobil-phase A: 0.1% formic acid in water Mobil-phase B: 0.1% formic acid in acetonitrile
Flow rate	0.5 mL/min
Detection wavelength (DAD)	323 nm
Runtime	5 min
Column temperature	30 °C
Volume of injection	10 µL
Retention time	2.40 min

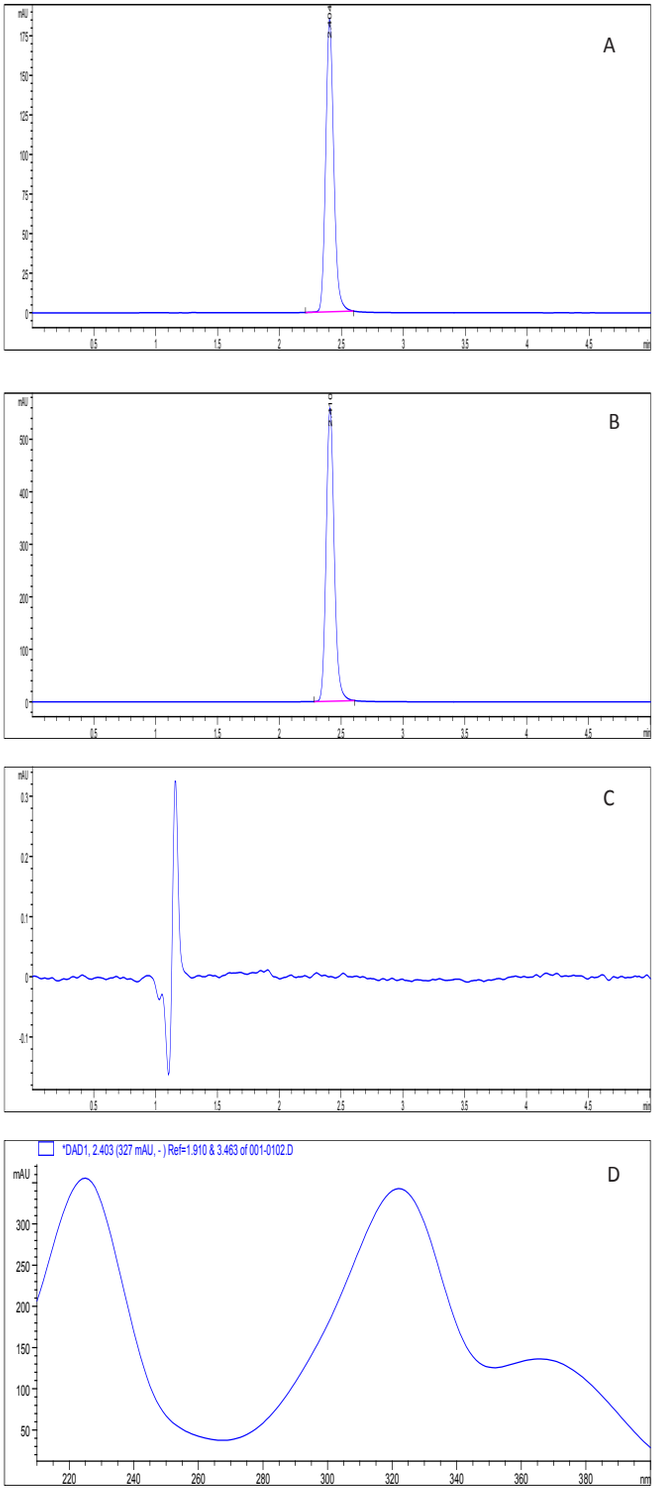

Figure 1. *(A) Chromatogram (standard solution, 40 µg/mL, λ:323 nm). (B) Chromatogram (sample solution, 100 µg/mL, λ: 323 nm). (C) Chromatogram (Blank solution, λ: 323 nm). (D) Absorbance spectra of FAV.*

3.2. Method Validation

Specificity

In the analysis, only the mobile phase injection was performed, and it was determined that there was no peak in the minutes when favipiravir was seen. Whether there was any interference with the favipiravir peak obtained when working with the developed method was determined with the help of a diode sequential detector. The spectra and peak purity index values show that there is no interference, and the peaks are pure (Figure 2).

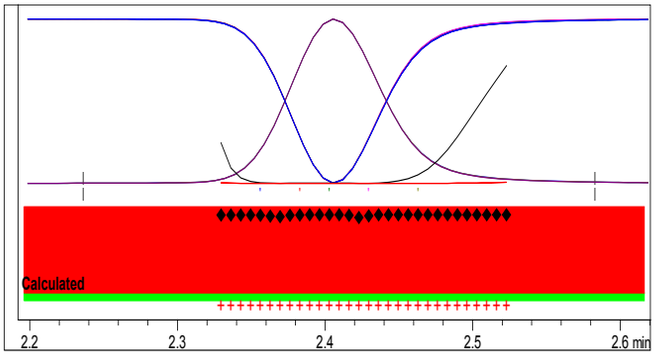

Figure 2. *Peak purity of FAV. (Purity factor: 999.963 threshold: 999.991 noise threshold: 0.016)*

3.3. Linearity

To prepare standard solutions, the stock standard solution of FAV was diluted appropriately with ultra-pure water. Under chromatographic working conditions, the standard solutions were injected three times into the chromatographic system. Regression analysis was used to assess the suggested method's linearity at six concentration levels ranging from 10 to 100 µg/mL. The parameters of the regression analyze of the calibration plot are given in the Table 2, and the calibration plot drawn between the indicated concentration values and the average peak areas is given in the Figure 3. The LOQ and LOD were calculated using a recommended formula (ICH Q2 (R1) as follows (14):

LOD = 3.3 SD / slope ; LOQ = 10 SD/slope

Table 2. *Regression analysis and LOD/LOQ values of the proposed method*

Parameter	Value
Linearity range (µg/mL)	10-100
Slope	23.375
Intercept	25.924
Correlation coefficient	0.9998
SE of intercept	10.0322
SD of intercept	4.0948
LOD/LOQ (µg/mL)	0.58/2.03

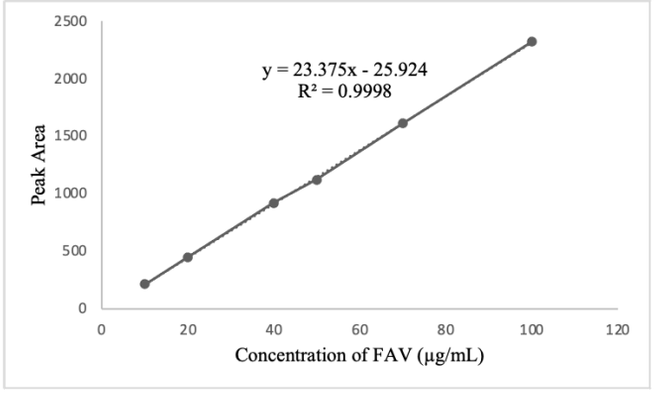

Figure 3. *Calibration curve of standard working solutions of favipiravir*

3.4. Precision

A sequence of FAV analyses was done for five consecutive days (n=5) in the precision trials. The intra-day and inter-day precision RSD were found to be <1%, indicating that the method was accurate enough. In the analyzes performed on the same day, the relative standard deviation was calculated between 0.06-0.14%. The relative standard deviation obtained in the analyzes performed on different days was found to be between 0.14-0.58%. Table 3 summarizes the findings.

Table 3. *Intra-day and Inter-day precision data of FAV*

Std. Conc.µg/ml	Taken conc. (µg/mL)	Found conc. (µg/mL) ± SD	Peak area RSD (%)
	30	30.0325±0.0371	0.1371
Intra-day	50	49.9796±0.0445	0.0909
	70	70.5806±0.0394	0.0574
	30	29.9302±0.0417	0.1394
Inter-day	50	49.4170±0.0627	0.1269
	70	69.0084±0.3973	0.5757

3.5. Accuracy

The method's accuracy was demonstrated using the standard addition technique. In this approach, a certain amount (20 µg/mL) of solution was mixed with 80, 100, and 120 percent of three distinct levels of bulk. Percentage recoveries for the FAV was computed. As a result of the trials, the recovery values were found in the range of 99.45-104.29%. Table 4 shows the findings of the recovery results.

Table 4. *Recovery data*

Spiked level (%)	Added conc. (µg/mL)	Found conc. (µg/mL)	Recovery%	Average Recovery %± SD	RSD%
	36	37.6877	104.6882		
80	36	37.5136	104.2045	104.2976	0.2766
	36	37.4400	104.0001		
	40	40.5147	101.2869		
100	40	40.4229	101.0572	101.0416	0.2048
	40	40.3123	100.7808		
	44	43.7792	99.4983		
120	44	43.7623	99.4599	99.4457	0.0499
	44	43.7561	99.3791		

3.6. Robustness

According to the data, flow rate and mobile phase concentration have little influence on FAV chromatographic separation behavior. FAV retention period is unaffected by changes in the flow rate or mobile phase ratio. The technique did not alter much when the column temperature was changed. Table 5 shows the findings of this investigation, given as a percent RSD.

Table 5. *Robustness of the proposed HPLC method for favipiravir**

Flow rate		Composition of mobile phase		Column temperature	
0.4 mL/min	0.6 mL/min	A:B (95:5)	A:B (85:15)	28 °C	32 °C
108.60±0.43	99.98±0.87	98.28±0.17	102.81±0.46	100.03±0.20	100.47±1.82

**The studies were performed with the 50 µg/mL standard solution and the results were provided in mean recovery%±RSD%*

3.7. Application of the Method to the Tablet Formulation

The developed method has been successfully used for the determination of favipiravir in pharmaceutical formulations. Table 6 shows the results of the favipiravir test on the commercially available tablet. The amounts mentioned on the pill labels are closely connected to the findings achieved. This demonstrates the utility of the content assessment approach.

Table 6. *Results of analysis of FAV*

Formulation	Original tablet solution (µg/mL)	Amount found±SD (µg/mL)	% RSD
FAV tablets	100	102.9175±0.1170	0.1137

Average of 6 determinations; SD: standard deviation; RSD: relative standard deviation

4. DISCUSSION

When the analyzes of favipiravir in pharmaceutical preparations were examined, it was seen that there were very few chromatographic analysis methods. The UV spectrum obtained from the FAV peak with a diode array detector was examined and it was decided that the wavelength was 323 nm. When operating under these conditions, the retention time for FAV was found to be 2.4 minutes. After the appropriate chromatographic conditions were determined, the method validation study was carried out. For this purpose, studies were carried out to determine the Selectivity, Linearity, Accuracy, Precision, LOD, LOQ and robustness parameters. The method was found to be linear in the concentration range of 10-100 µg/mL. In order to determine the reproducibility of the method, three different concentrations of samples were studied on the same day and on different days. As a result, the RSD values of the analyzes performed on the same day were determined as 0.06-0.14%, and the RSD values of the analyzes performed on different days were determined as 0.14-0.58%. To determine the accuracy of the developed method, the standard addition method was applied. In the study, increasing concentrations of standard FAV solutions were added to the capsule solution containing FAV. As a result of the trials, the recovery values were found in the range of 99.45-104.29%. LOD and LOQ values for the developed method were determined as 0.58 µg/mL and 2.03 µg/mL, respectively. After the validation process was completed, the developed method was used for FAV quantification in FAV capsules and as a result, the average FAV amount was found to be 102.92%.

When the validation findings were compared to the ICH limits, the suggested technique yielded good system appropriateness values. The precision values in terms of RSD percent were lower than 1% with high recoveries in the tablet analysis. The suggested approach was compared to selected previous research on the determination of FAV in tablet formulations by HPLC-UV method in the literature. The r^2 values for all the compared techniques were more than 0.999. The proposed technique has the least duration

of retention. The proposed study is more sensitive than the two previously reported methods [1.20/3.60 µg/mL (1) and 0.985/2.986 µg/mL (13)], with lower LOD and LOQ (0.58/2.03 µg/mL). So, this method is inexpensive and allows more rapid and sensitive analysis than two related methods in literature.

5. CONCLUSION

The proposed method is simple, rapid, accurate, and sensitive for quantification of favipiravir in pharmaceutical formulations. This method employs a simple mobile phase without the need of a buffer, requires no complicated sample preparation procedures, and has higher sensitivity than some of the previously described methods. Hence this proposed method can be widely used in quality control laboratories for determination of favipiravir.

Ethics Committee Approval: Ethics committee approval is not required for the study.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: D.T, Design: D.T., Analysis or Interpretation: D.T., Literature Search: D.T., Writing: D.T.

Conflict of Interest: No conflict of interest was declared by the author.

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REFERENCES

[1] Ibraam EM, Heba E, Fathalla B, Adel EI. Green micellar solvent-free HPLC and spectrofluorimetric determination of favipiravir as one of COVID-19 antiviral regimens Microchemical Journal 2021;165 (2021): 106189.

[2] Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease, (COVID-19): A review. JAMA 2020; 324 (2019): 782–793.

[3] Acquavia A, Foti L, Pascale R, Nicolo A, Brancaleone V, Cataldi TR. Detection and quantification of Covid-19 antiviral drugs in biological fluids and tissues. Talanta. 2021; 224 (2021): 121862.

[4] Delang L; Abdelnabi R, Neyts J. Favipiravir as a potential countermeasure against neglected and emerging RNA viruses. Antiviral Res. 2018; 153: 85–94.

[5] China patent (CN104914185A). HPLC method for measuring related substances in Favipiravir. 16.09.2015.

[6] Madelain JG, Nguyen THT, Jacquot F, Oestereich L, Kadota T. Favipiravir pharmacokinetics in nonhuman primates and insights for future efficacy studies of hemorrhagic fever viruses, Antimicrob. Agents Chemother.2017; 61 (1): (2017), https://doi.org/10.1128/AAC.01305-16.

[7] Smee DF, Hurst BL, Egawa H, Takahashi, K, Kadota T, Furuta Y. Intracellular metabolism of favipiravir (T-705) in uninfected and influenza A (H5N1) virusinfected cells, J. Antimicrob. Chemother. 2009;64 (2009): 741–746.

[8] Nguyen THT, Guedj J, Anglaret X, Laou´enan C, Madelain V, Taburet AM. Favipiravir pharmacokinetics in Ebola-Infected patients of the JIKI trial reveals concentrations lower than targeted, PLoS Negl. Trop. Dis. 2017;11 (2): e0005389.

[9] Gowen BB, Sefing EJ, Westover JB, Smee, DF, Hagloch J, Furuta Y, et al., Alterations in favipiravir (T-705) pharmacokinetics and biodistribution in a hamster model of viral hemorrhagic fever. Antiviral Res. 2015;121 (2015): 132–137.

[10] Safa MM, Ahmed A H, Sherin F H, Amira H. Kamal Experimental design approach for development of spectrofluorimetric method for determination of favipiravir; a potential therapeutic agent against COVID-19 virus: Application to spiked human plasma Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy.2021; 249 (2021): 119241

[11] Nguyen THT, Guedj J, Anglaret X, Laou´enan C, Madelain V, Taburet AM. Favipiravir pharmacokinetics in Ebola-Infected patients of the JIKI trial reveals concentrations lower than targeted, PLoS Negl. Trop. Dis. 2017;11 (2): e0005389.

[12] A kind of Favipiravir has the HPLC assay method of related substance, Google Patents, CN104914185B (2015) Chinese article.

[13] Bulduk I. HPLC-UV method for quantification of favipiravir in pharmaceutical formulations, Acta Chromatographica. 2020; 1-7, DOI: 10.1556/1326.2020.00828.

[14] ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology.; 2018 July 7. Available from: http://www.ema.europa.eu/docs/en_GB/document_library /Scientific_guideline/2009/09/WC500002662.pdf [Website].

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