UV SPECTROSCOPIC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF FAVIPIRAVIR

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ABSTRACT

A new, accurate, and easy-to-use UV-spectrophotometry method for analyzing favipiravir in both bulk and tablet forms has been developed. Favipiravir, an antiviral drug, is classified as a modified pyrazine analogue and is also known as 6-fluoro-3-hydroxypyrazine-2-carboxamide. The drug's concentration was determined by measuring zero-order derivative values at a wavelength of 323 nm. A linear plot was constructed, demonstrating linearity within the concentration range of 4-20 µg mL^{-1}, with an impressive correlation coefficient (r2) of 0.9997 for the zero-order spectrophotometry method. The method's limits of detection (LOD) and quantification (LOQ) were determined to be 0.08 g and 0.26 g, respectively. All suggested methods were rigorously tested to make sure they met the standards set by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use. The developed spectrophotometry method for analyzing favipiravir in both bulk and tablet forms are characterized by their linearity, accuracy, precision and sensitivity.

Keywords: UV spectroscopy, COVID-19, favipiravir, International Council for Harmonization guidelines, severe acute respiratory syndrome, zero order derivative

INTRODUCTION

Favipiravir (FAV), also referred to as 6-fluoro-3-hydroxypyrazine-2-carboxamide, is a well-known antiviral medication (Fig. 1). It falls under the category of modified pyrazine analogues and was initially approved for treating the cases of influenza that were resistant to other medications. The discovery of this compound can be credited to Toyama Chemical, a Japanese company[1, 2]. Favipiravir works by blocking an enzyme called RNA-dependent RNA polymerase (RdRP) from doing its job. This is an important enzyme that needs RNA to work. RdRP is what makes RNA viruses copy themselves in human cells. By acting as a purine analogue, favipiravir substitutes guanine and adenine in viral RNA. When a single molecule of favipiravir is incorporated, it stops the viral RNA from growing. Inside the cell, favipiravir becomes active and goes through a process called phosphorylation. This phosphorylated form is recognized by the viral RdRP as a substrate. Favipiravir can fight many RNA viruses, including the flu virus. It is important to note that SARS-CoV-2, the virus causing COVID-19, belongs to the same group of viruses as MERS-CoV and SARS-CoV, which are responsible for other respiratory diseases[2,3]. Favipiravir has been effective in treating people with Ebola virus infections and has also been used as a preventive measure after potential exposure[4].

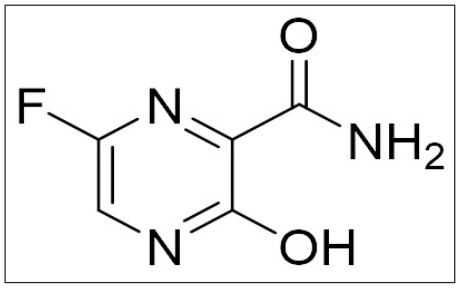

Fig. 1: Structure of favipiravir

A high CC50/EC50 ratio indicates a significant therapeutic safety margin, implying a favorable balance between effectiveness and safety. Clinical trials have shown a substantial increase in the prevalence of COVID-19. In these trials, umifenovir demonstrated superior performance compared to lopinavir/ritonavir (LPV/RTV) in terms of viral clearance and recovery rate[5].

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Favipiravir exists as a white to light yellow powder with slight solubility in water. Its melting point ranges from 187 °C to 193 °C. Approximately 54% of favipiravir binds to plasma proteins, with serum albumin accounting for 65 % of this fraction and 1-acid glycoprotein binding the remaining 6.5 %. The bioavailability of favipiravir is nearly complete, with a value of 97.6%[6]. Rapid metabolism of favipiravir occurs, and most of its metabolites are excreted in the urine[7].

Previous literature surveys have indicated that the only published methods for analyzing favipiravir involve using HPLC with UV detection[8,9]. There is currently no available spectrophotometric method for accurately quantifying favipiravir, whether in its pure form or in pharmaceutical preparations. The goal of this research was to develop a straightforward, speedy, and consistent spectrophotometric method for determining the amount of favipiravir in both it's pure form and in pharmaceutical formulations. This method can be used for regular quality control analysis and stability testing. Due to the absence of an official monograph for favipiravir in pharmacopoeias, it is crucial for researchers and analysts to develop and validate UV-spectrophotometric, HPLC and HPTLC analytical techniques independently. By doing so, they can ensure the accuracy, reliability and reproducibility of the results obtained for the quantitative analysis of favipiravir in both bulk samples and pharmaceutical formulations[10].

MATERIALS AND METHODS

Materials

Macleods Pharma, Mumbai, India, supplied a free sample of favipiravir for the study. The present research utilized chemicals and reagents that were of analytical grade and were acquired from Merck Chemicals, India. The favipiravir product used in the study, specifically the 'CODIFAB' brand with a strength of 200 mg, was obtained from a local Indian pharmacy.

Instrumentation

A UV-VIS dual beam spectrophotometer (UV-2450, Shimadzu, Japan) was employed in the study. A connection was established between the spectrophotometer and a computer through Spectra Manager® software and a UV Probe. Additionally, 10 mm quartz cells were utilized. The instrumental parameters for generating the spectra were as follows: medium scanning speed, 1.0 nm sample interval, and a wavelength range of 200-400 nm. The recording of all weights in the experiment was done using an electronic balance (Model Shimadzu AUX 120).

Preparation and selection of wavelength for stock standard solution

For the preparation of a 100 µg mL^{-1} concentration, a stock standard solution of 10 mg favipiravir was dissolved in 100 mL of methanol. Subsequently, this stock solution was further diluted to get the concentration of 10 µg mL^{-1}. The UV-visible scanning of the diluted solution was performed in the range of 200-400 nm. Among the scanned wavelengths, favipiravir exhibited the highest absorbance at 323 nm.

VALIDATION METHOD

Linearity curves

To establish a concentration range of 4-20 µg mL^{-1}, different volumes (ranging from 0.4-2.0 mL) were taken from the original standard solution and transferred into several 10 mL volumetric flasks. These flasks were then filled up to the mark using the same solvent, ensuring consistent solvent composition throughout the process. The resulting solutions contained concentrations within the desired range of 4-20 µg mL^{-1}. Afterwards, the solutions were subjected to spectrophotometric scanning within the wavelength range of 200-400 nm.

Recovery studies

The accuracy and precision of the analysis were assessed by adding known quantities of the stock standard solution to pre-analyzed sample solutions at three different levels: 80%, 100%, and 120% of the target concentration. The re-analysis of these solutions was conducted using the methods and techniques described earlier. This process allowed for the assessment of the accuracy and precision of the analytical approach by comparing the obtained results with the expected values.

Precision

The precision of the methods was evaluated by investigating the repeatability, as well as the intra-day and inter-day variations. Intra-day variation was evaluated by analyzing favipiravir solutions with concentrations of 8, 12, and 16 µg mL^{-1} within the same day, and inter-day variation was measured by analyzing the same solutions on different days. These experiments were performed to measure the precision of the methods and determine the consistency and trustworthiness of the analytical results.

Sensitivity

The sensitivity of the proposed methods for analyzing favipiravir was evaluated by determining the limit of

quantification (LOQ) and the limit of detection (LOD). The LOQ and LOD were calculated using the following equations:

$$LOD = 3.3\, x \frac{N}{B}$$

$$LOQ = 10\, x \frac{N}{B}$$

where 'N' represents the standard deviation and 'B' corresponds to the slope of the calibration curve.

These calculations were performed to quantify the LOD and LOQ of favipiravir using the developed analytical method.

Ruggedness

To evaluate the ruggedness of the method, aliquots were extracted from a uniform favipiravir sample with a concentration of 12 µg mL^{-1}. Two different analysts conducted the analysis using the same operational and environmental conditions as specified in the method. This evaluation aimed to determine the robustness and consistency of the proposed method when applied by different analysts under similar experimental conditions.

Analysis of tablet formulation

10 tablets were individually weighed and then crushed to obtain a powdered form. The powdered tablet equivalent to 200 mg of favipiravir was precisely weighed and transferred into a 100 mL volumetric flask. Following that, 15 mL of methanol was added to the flask, and thorough shaking was performed to ensure complete dissolution. To achieve a final volume of 100 mL, an additional 85 mL of methanol was added. Subsequently, 1 mL of this solution was withdrawn and transferred into a 10 mL volumetric flask. The volume was adjusted using an appropriate diluent to reach a final volume of 10 mL, resulting in a concentration of 10 µg mL^{-1}. This process was followed to prepare the required solution concentration for further analysis (Table I).

Table I: Analysis of tablet formulation

Drug	Amount taken [µg mL^{-1}] [n=6]	Amount found [µg mL^{-1}] ±SD	Amount found (%)	% RSD
FVP	12	12.06 ± 0.09	100.51	0.78

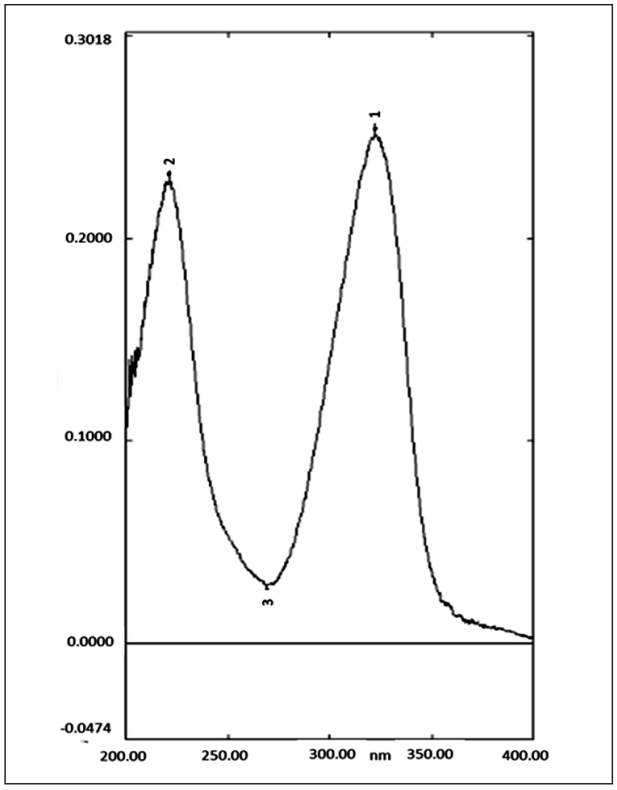

Fig. 2: Zero order UV- spectrum of FVP depicting λmax at 323 nm

Table II: Optical characteristics and linearity study of favipiravir

Parameter	Method
Linearity range (µg mL^{-1})	4-20 (µg mL^{-1})
Selected range (nm)	200-400 nm
Slope	0.0109
Intercept	0.0494
Correlation coefficient	0.999
Limit of detection (µg)	0.08
Limit of quantitation (µg)	0.26

RESULTS AND DISCUSSION

Selection of wavelength

The standard solution was scanned using a UV spectrophotometer in spectrum mode, covering the wavelength range of 200-400 nm. A blank solution containing only the diluent was used as a reference. The spectrum obtained revealed that favipiravir exhibited a maximum absorption wavelength (λ-max) at 323 nm, as depicted in Fig. 2. The selection of wavelengths used in this method was based on ensuring the reproducibility of the analytical results.

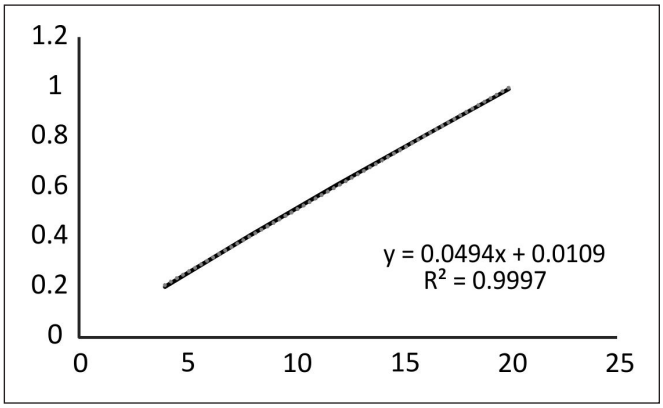

Fig. 3: Linearity curve for the zero order UV determination of FVP

Linearity study

A strong linear relationship was demonstrated within the concentration range of 4-20 µg mL^{-1}, as depicted by the calibration curves obtained through linear regression analysis of favipiravir (FVP). The overlain spectra and

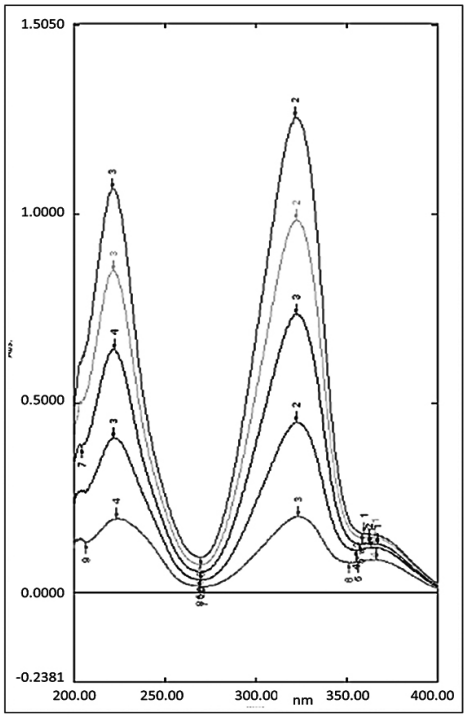

Fig. 4: Overlain spectra of FVP for UV – spectrophotometry analysis

linearity graph of FVP can be observed in Fig. 3 and Fig. 4, respectively. The analytical results are presented in Table II.

Accuracy

To assess the accuracy of the method, the usual addition method was employed at three levels. Standard quantities equivalent to 80%, 100%, and 120% of the target concentration were added to the sample. The obtained results indicated excellent recoveries, ranging from 99.34% to 99.95%, at each additional concentration. These findings demonstrate the accuracy of the method, as presented in Table III.

Table III: Accuracy

Drug	% Recovered	% RSD
FVP	99.34	1.41
	99.91	1.18
	99.95	1.18

Precision

The precision of the developed method was estimated by calculating the percent relative standard deviation (% RSD). The results, expressed in terms of % RSD, indicate the reproducibility of the assay. Notably, the % RSD values obtained were all below 2, demonstrating that the method was accurate for determining the drug content in the formulation. These findings are summarized in Table IV.

Table IV: Intra-day and Inter-day precision studies

Drug	Conc. [µg mL^{1}]	Intra-day		Inter-day	
		Amount found [n=3]	% RSD	Amount found [n=3]	% RSD
FVP	8	7.99	1.30	8.06	1.59
	12	12.06	1.31	12.10	1.07
	16	16.08	0.64	16.13	0.46

Repeatability

The method's repeatability was assessed by examining the favipiravir solution at a concentration of 12 µg mL^{-1}. The analysis was performed six times, and the % amount determined for each analysis was calculated. The obtained results showed that the % RSD was less than 2, indicating good repeatability of the method. These findings are summarized in Table V.

Table V: Repeatability

Drug	Conc. [µg mL^{-1}]	Amount found ± SD	%RSD [n=6]
FVP	12	12.05 ± 0.10	0.90

Sensitivity

The LOD and LOQ for favipiravir were determined to be 0.16 µg and 0.48 µg, respectively. These values represent

the lowest detectable and quantifiable concentrations of favipiravir using the developed method.

Ruggedness

To evaluate the method's precision, the peak area of the same concentration solutions was measured six times. The obtained results for both drugs were found to be within acceptable limits. The percentage relative standard deviation (% RSD) was determined, and the results demonstrated that the % RSD values were all below 2%. This indicates that the method exhibited good precision. The summarized results as presented in Table VI.

Table VI: Ruggedness studies

Drug	Conc. (µg mL^{-1})	Analyst	% Amount found	%RSD (n=6)
FVP	12	I	100.52 ± 0.86	0.85
	12	II	100.45 ± 1.00	0.99

CONCLUSION

Based on our current knowledge, there is a lack of documented UV spectrophotometric approaches for quantifying favipiravir. The developed analytical method has undergone the prescribed validation process and complies with the specific acceptance criteria outlined in the ICH Q2 (R1) guideline. The method is considered novel, specific, linear and precise. The analytical procedure presented in this study effectively fulfills its intended purpose.

REFERENCES

1. Hayden F. G. and Shindo N.: Influenza virus polymerase inhibitors in clinical development. **Curr. Opin. Infect. Dis.,** 2019, 32(2), 176.
2. Furuta Y., Komeno T., and Nakamura T.: Favipiravir (T-705), a broad spectrum inhibitor of viral RNA polymerase. **Proc. Jpn. Acad., Ser. B, Phys. Biol. Sci.,** 2017, 93(7), 449-463.
3. Megahed S. M., Habib A. A., Hammad S. F. and Kamal A. H.: 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. **Spectrochim. Acta A Mol. Biomol. Spectrosc.** 2021, 249, 119241.
4. Bai C. Q., Mu J. S., Kargbo D., Song Y. B., Niu W. K., Nie W. M., Kanu A., Liu W. W., Wang Y.P. and Dafae F.: Clinical and virological characteristics of Ebola virus disease patients treated with favipiravir (T-705)—Sierra Leone, 2014. **Clin. Infect. Dis.,** 2016, 63(10), 1288-1294.
5. Nadendla R. and Abhinandana P.: A validated high performance liquid chromatographic method for the quantification of favipiravir by PDA Detector. **Int. J. Life Sci. Pharma. Res**., 2021, 11(2), P181-188.
6. Venkataraman S., Prasad B. V. L. S., and Selvarajan R.: RNA dependent RNA polymerases: insights from structure, function and evolution. **Viruses,** 2018, 10(2), 76.
7. Madelain V., Nguyen T. H. T., Olivo A., De Lamballerie X., Guedj J., Taburet A. M. and Mentré F.: Ebola virus infection: review of the pharmacokinetic and pharmacodynamic properties of drugs considered for testing in human efficacy trials. **Clin. Pharmacokinet**., 2016, 55(8), 907-923.
8. Feng G., Ding W., Deng Y., Zhao R., Gong Y., Duan C. and Sun J.: A kind of favipiravir has the HPLC assay method of related substance, **Chinese Patent** CN104914185B, 2015.
9. Bulduk İ.: HPLC-UV method for quantification of favipiravir in pharmaceutical formulations. **Acta Chromatogr.** 2021, 33(3), 209-215.
10. Guideline I. C. H. H. T. Validation of analytical procedures: text and methodology. Q2 (R1). 2005, 1(20), 05.