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Original Article

Spectrophotometric Determination of Favipiravir in Bulk and Pharmaceutical Formulation Using Bromothymol Blue Reagent

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ABSTRACT

Background: The primary objective of this research was to establish a spectrophotometric technique to quantitatively analyze the concentration of favipiravir in both its pure form and pharmaceutical preparations using bromothymol blue reagent. **Materials and Methods:** In this method, a yellow-colored chromagen was developed when favipiravir reacted with bromothymol blue reagent. Acetonitrile was selected as a solvent and the colored complex was detected at a wavelength of 475 nm. **Results:** The validation of the developed method was conducted following the guidelines set by the International Council for Harmonisation (ICH). The results demonstrated a strong linear relationship within the concentration range of 10-50 µg/mL, exhibiting a correlation coefficient of 0.9995. Moreover, the developed method exhibited excellent accuracy, precision, specificity, and sensitivity **Conclusion:** For routine analysis purposes, this method can be readily utilized to determine the concentration of favipiravir in both bulk samples and pharmaceutical dosage forms.

Keywords: Favipiravir, Spectrophotometric method, Bromothymol blue, Method development, Validation.

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INTRODUCTION

Favipiravir (Figure 1) is an anti-viral drug developed for the treatment of various viral infections like influenza, and COVID-19.[1] Chemically it is designated as 6-fluoro-3-hydroxypyrazine-2-carboxamide, with molecular formula $C_5H_4FN_3O_2$ and molecular weight 157.104 g/mol. It is a colorless powder, soluble in organic solvents and slightly soluble in water, and has a pKa value of 5.1. It is an organic compound belonging to the pyrazine carboxamides class.[2] Favipiravir belongs to the anti-viral category where it acts by inhibiting RNA-dependent RNA polymerase enzyme which prevents viral transcription and replication.[3,4]

As per the literature survey, it was known that analytical methods were developed for the estimation of favipiravir formulations. The developed methods included spectroscopic methods such as UV spectroscopic methods,[5-10] Visible spectrophotometric methods,[11] Fourier Transform Infrared Spectroscopic (FTIR) method,[12] spectrofluorimetric method,[13,14] chromatographic methods such as RP-HPLC methods,[15-34] thin layer chromatography (TLC)[35] and hyphenated techniques such as UPLC-MS/MS methods,[36,37] LC-MS/MS methods[38-42] and electrical methods such as voltammetric methods.[43-45] But it was evident that only one method was developed for the estimation of favipiravir in pharmaceutical formulations using methyl orange and methyl red reagents in spectroscopy. Hence, this current study targets to validate a method using spectrophotometry for the estimation of favipiravir in bulk and in pharmaceutical formulation using bromothymol blue reagent.

MATERIALS AND METHODS

Reagents and chemicals

Favipiravir working standard was obtained as gift sample from the Hetero Labs, Hyderabad. The favipiravir tablets (fabiflu) were bought at a nearby pharmacy. All of the solvents required for the method's development came from Merck in Mumbai, India. Additionally, all of the chemicals used for the method's development were of the AR grade and came from Sigma Aldrich in Bangalore, India.

Instruments

The estimate of favipiravir in pharmaceutical formulations utilized a T60V UV-vis double-beam spectrophotometer. UV

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Win software was used to regulate every parameter. Other instruments utilized in the study included weighing digital balance, and ultrasonic bath sonicator.

Preparation of standard and sample solutions

An accurately weighed 100 mg of favipiravir working standard was made to dissolve in 100 mL of acetonitrile solvent constituting to a concentration of 1000 µg/mL. A volume of 10 mL was taken from the stock solution and diluted with 100 mL of distilled water (concentration 100 µg/mL). A mixture of 3 mL of the above solution and 1 mL of bromothymol blue reagent was diluted to 10 mL with distilled water to obtain a concentration of 30 µg/mL.

20 tablets of fabiflu were accurately weighed and an average weight was calculated. Weight equivalent to 100 mg of favipiravir was made to dissolve in 100 mL of acetonitrile solvent. The stock solution was subjected to sonication using an ultrasonic bath sonicator for 30 min. Later the solution was filtered and 10 mL of filtrate was diluted to 100 mL with distilled water. Finally, 3 mL of the above solution along with 1 mL of bromothymol blue reagent was diluted to 10 mL with distilled water.

Method validation[46]

Linearity

The linearity of this method was determined by preparing a serial dilution in the concentration ranges of 10-50 µg/mL and their absorbance was measured. A graph was plotted between concentration and absorbance values.

Precision

%RSD calculation was done by performing intraday and interday precision studies. Six replicates of 30 µg/mL concentration solution were produced, and their absorbance was assessed within the day (intraday precision studies) and for two days (interday precision studies).

Accuracy

Solutions in three levels 50%, 100% and 150% were prepared by std addition method and their absorbance was noted. From these values, % recovery was calculated at three levels.

Specificity

For the determination of specificity of this method, a blank solution was prepared and observed.

RESULTS

UV-visible spectrum for standard drug was shown in Figure 2.

The prepared standard solutions and sample solutions were placed in UV-visible spectrophotometer and their respective absorbance was measured and noted as presented in Table 1.

As illustrated in Figure 3, a linearity graph was created by plotting the concentration on the x-axis and the absorbance values on the y-axis. The results are reported in Table 2.

DISCUSSION

The present study aimed to develop and validate a simple, novel spectrophotometric method for favipiravir in bulk and pharmaceutical formulations using bromothymol blue as a reagent.

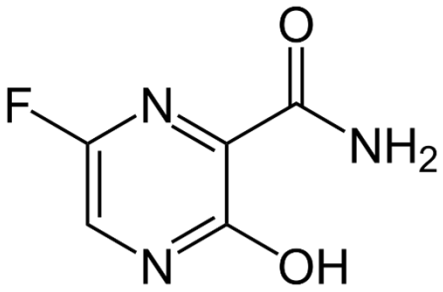

Figure 1: Chemical Structure of Favipiravir.

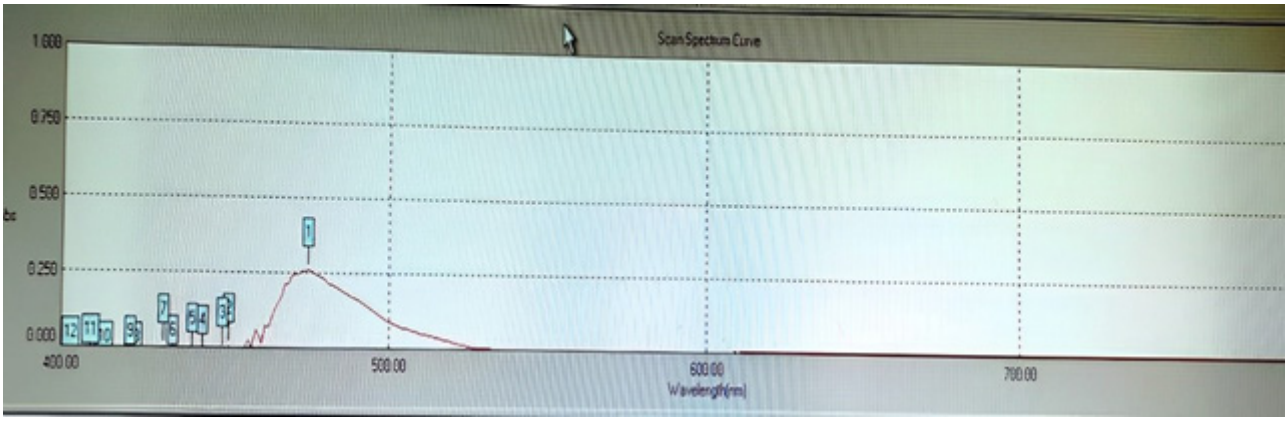

Figure 2: UV-visible spectrum of favipiravir.

Table 1: **Optical Characteristics.**

Sl. No.	Parameters	Results
1.	Absorption maximum	475 nm
2.	Linearity range	10-50 µg/mL
3.	Regression equation	y=0.0134x+0.0072
4.	Slope	0.0134
5.	Intercept	0.0072
6.	Correlation coefficient (r)	0.9995
7.	Molar extinction coefficient ($L.mol^{-1}$ cm^{-1})	2245
8.	Sandell's sensitivity (µg/cm^2-0.001 absorbance units	0.069
9.	Accuracy (% recovery)	99.84%-100.32%
10.	Precision (Intra-day) % RSD (Inter-day) % RSD	0.34 0.28
11.	LOD	1.60
12.	LOQ	4.85
13.	Standard error	0.0065

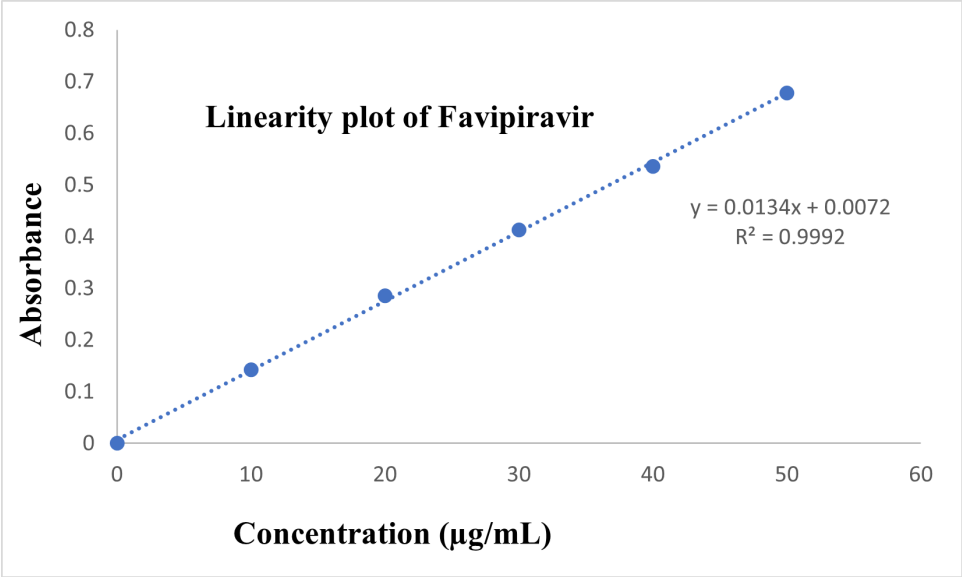

Figure 3: Linearity plot of favipiravir.

Solubility studies

Initially, for the development of this method, the standard drug favipiravir was subjected to solubility studies where the drug was made to dissolve in various solvents like methanol, acetonitrile, water, 0.1 N HCl, and 0.1 N NaOH.

Selection of solvent

From the above solubility assays, it was found that the drug was readily soluble in methanol and acetonitrile. For further study, acetonitrile solvent was used as a diluent for the preparation of solutions.

The medication was discovered to be easily soluble in methanol from the aforementioned solubility assays.

Selection of ion pair reagent

Different reagents were used such as phenol red, bromothymol blue, and thymol blue. When these reagents were individually mixed with a standard solution, a clear solution was found with bromothymol blue. Hence, it was selected as ion pair reagent in this study.

Table 2: Results of linearity.

Sl. No.	Concentration (µg/mL)	Absorbance
1	10	0.143
2	20	0.286
3	30	0.413
4	40	0.536
5	50	0.678
Regression coefficient (r^2)		0.9992
Correlation coefficient (r)		0.9995

The results for precision were presented in Tables 3a and 3b.

Table 3a: Intra-day precision results.

Sl. No.	Sample absorbance	% Assay
1	0.413	99.60
2	0.414	99.84
3	0.411	99.12
4	0.415	100.08
5	0.413	99.60
6	0.412	99.36
Average	0.413	99.60
%RSD	0.34	0.34

Table 3b: Inter-day precision results.

Sl. No.	Sample absorbance	% assay
1	0.416	99.60
2	0.415	99.36
3	0.418	100.08
4	0.417	99.84
5	0.417	99.84
6	0.418	100.08
Average	0.417	99.80
% RSD	0.28	0.28

The accuracy results were summarised in Table 4.

Selection of detection wavelength

In order to detect the wavelength for the measurement, a standard solution of concentration 10 µg/mL was prepared and scanned in the visible range of 400-800 nm in UV-visible spectrophotometer. Maximum absorbance was observed at a wavelength of 475 nm and it was utilised for further investigation.

For the determination of linearity of the method, serial dilutions in the range 10-50 µg/mL were prepared and absorbance was measured. Later, a graph between the concentration and absorbance values was established. From the graph, correlation coefficient values was determined and it was found to be 0.9995.

The %RSD for intraday precision studies was 0.34 and for interday precision studies, it was 0.28, indicating the method to precision.

Accuracy of the method was determined by calculating % recovery. The % recovery was found to be 99.84%-100.32% which indicates that the method was accurate.

The UV-visible spectrum of the standard solution when compared with that of the blank solution, there was no interference observed in the blank spectrum, indicating that the method was specific.

The approach was found to be sensitive with a limit of detection of 1.60 g/mL and a limit of quantification of 4.85 g/mL.

Table 4: Accuracy results.

Sl. No.	Level (in %)	Amount of Favipiravir added (mg)	Amount of Favipiravir found (mg)	% Recovery	Mean % Recovery
1	50	50.00	49.92	99.84	99.84
2	50	50.00	50.16	100.32	
3	50	50.00	49.68	99.36	
1	100	100.00	100.08	100.08	100.08
2	100	100.00	99.84	99.84	
3	100	100.00	100.32	100.32	
1	150	150.00	150.49	100.32	100.32
2	150	150.00	150.73	100.48	
3	150	150.00	150.24	100.16	

CONCLUSION

A simple, novel spectrophotometric method was developed for the determination of favipiravir in bulk and pharmaceutical dosage form using bromothymol blue reagent and validated in accordance with ICH guidelines. The developed method was found to be accurate, precise, linear, specific, and sensitive. This developed method can be easily applied for the estimation of favipiravir for routine analysis or for quality control in formulations.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

RNA: Ribonucleic Acid; **UV:** Ultra Violet; **FTIR:** Fourier Transform Infrared Spectroscopy; **RP-HPLC:** Reverse Phase High-Performance Liquid Chromatography; **TLC:** Thin Layer Chromatography; **UPLC-MS/MS:** Ultra-Performance Liquid Chromatography Tandem Mass Spectrometry; **LC-MS/MS**: Liquid Chromatography Tandem Mass Spectrometry; **AR:** Analytical Reagent; **RSD:** Relative standard deviation; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **ICH:** International Conference on Harmonization.

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