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Development of visible spectrophotometric methods for the analysis of favipiravir in pure drug and tablet formulation

Wrushali A. Panchale [1], Sonam B. Bisen [2], Jagdish V. Manwar [2,*], Ravindra L. Bakal [1], Tejaswini V. Tidke [1], Mitali N. Paithankar [1] and Aparna G. Vakhare [1]

[1] *IBSS's Dr. Rajendra Gode Institute of Pharmacy, Mardi Road, Amravati-444 602, MS, India.*
[2] *IBSS's Dr. Rajendra Gode College of Pharmacy, Mardi Road, Amravati-444 602, MS, India.*

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Abstract

Favipiravir is a synthetic prodrug, first discovered while assessing the antiviral activity of chemical agents active against the influenza virus. The present study is aimed at developing and validating a new colorimetric methods for its determination in both pure form and in tablet formulation. The developed method utilizes ion-pair spectrophotometry and is employed for the quantitative evaluation of the drug using the acidic dyes - methyl orange (MO) and methyl red (MR) as ion-pairing agent. The ion-pair complex of drugs with both dyes obeyed Beer's law in the range of 10 -50 µg/ml with a acceptable correlation coefficients. Recovery by the method was also good, with a relative standard deviation (%RSD) less than 2.5%. The developed method was successfully applied to determine favipiravir in tablet formulation and was demonstrated to be accurate, precise and reproducible. It is also sufficiently sensitive and specific for the determination of the drug in bulk and formulation with satisfactory results.

Keywords: Favipirvir; Visible spectrophotometric; Tablet formulation; Validation.

1. Introduction

In colorimetric analysis, organic reactions are specific for a particular substance that give color s for a small group of related substances only and because of this it is important to control the operational procedure so that the color is specific for the component being determined. This may be achieved by isolating the substance by the normal methods of analysis. The specificity of color in colorimetric reactions can be achieved by introducing other complex forming compounds. These are required to suppress the action of interfering substance by the formation of selective complexes [1-2]. In the present investigation we have had used some organic compound as complex forming compounds for the analysis of favipiravir in pure drug and tablet formulations. The coronavirus disease (COVID-19) eruption across the world encouraged most researchers to exert effort to develop treatment strategies to overcome the fast progression of this respiratory disease. Favipiravir (Fig. 1), is one among potential drugs that may possibly be used in the management of COVID-19 infection. It is a pyrazine carboxamide derivative, that showed effective antiviral activity against a variety of RNA viruses including influenza A virus, adenovirus, and SARS Corona virus [3-5]. In Feb., 2020 post the outbreak of novel corona virus (COVID-19). Favipiravir was studied in China and several other countries as an experimental treatment of Covid-19 [6].

* Corresponding author: Jagdish V. Manwar; Email: jvmanwar@gmail.com
IBSS's Dr. Rajendra Gode College of Pharmacy, Mardi Road, Amravati-444 602, MS, India.

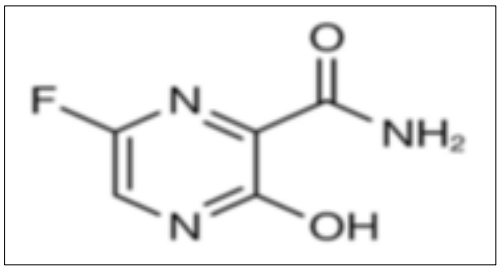

Figure 1 Chemical structure of favipiravir

There are several analytical methods for the analysis of various drugs from bulk and various formulations like tablets, capsules, injections, etc [7-63]. Literature survey revealed various analytical methods have been reported for estimation of favipiravir alone and in combination with other drugs [64-69].

No Visible Spectrophotometric was reported. Hence the author has attempted to develop two visible spectrophotometric methods with better sensitivity using several chromogenic reagents by exploiting the analytically useful functional groups present in the drug molecule.

2. Material and methods

2.1. Instrument

All absorption measurements were made using a Systronics model 119 digital spectrophotometer (Systronics Ltd, Ahmadabad, India) with 1 cm path length quartz cells.

2.2. Chemicals

2.2.1. Pure drug

Favipiravir API sample was gifted obtain from Glenmark Pharmaceuticals Ltd, Sikkim, India. Tablets containing drug Favipiravir (Fabiflu® 400mg) were procured from local market.

2.2.2. Solvents & reagents

All the Solvents, materials and reagents used for the assay of favipiravir were of analytical reagent grade and were prepared in double distilled water.

2.2.3. Methyl orange (50 µg/ml)

The indicators were prepared by dissolving 5.9 mg of methyl orange (S. D. Fine Chem. Ltd., Mumbai, India; dye content 85 %) in 100 ml water.

2.2.4. Bromate-Bromide mixture

About 1000 µg/ml KBrO3 solution containing a large excess of KBr was prepared by dissolving 100 mg of KBrO3 and 1 g of KBr in water and diluting to the mark in a 100 ml calibrated flask. This was diluted stepwise to get 10 µg/ml bromate solutions.

2.2.5. Sodium acetate (0.1 M)

Prepared by dissolving stated amount of the pure sodium acetate trihydrate (Merck Specialities Pvt Ltd, Mumbai, India) in 100 ml water.

2.2.6. Hydrochloric acid (HCl, 0.1M)

Concentrated hydrochloric acid (Merck, Mumbai, India; Sp. gr. 1.18) was diluted appropriately with water to get 0.1M and 5 M HCl.

2.2.7. Methyl red (0.0037M)

Methyl red (Honeywell Fluka) solution was prepared as 0.0037M ethanol solution.

2.2.8. Buffer solutions

Sodium acetate-hydrochloric acid buffer of pH 3.5 were prepared by mixing 50 ml of 0.1M sodium acetate solution with 50.50, of 0.1M HCl solution and diluted to 250 ml with doubly distilled water. The pH of solution was adjusted to an appropriate value with the aid of a pH meter.

2.2.9. Ethanolic KOH (1 %)

One gram of the pure KOH (S.D. Fine Chem Ltd, Mumbai, India) was dissolved in and diluted with ethanol to 100 mL.

2.2.10. Preparation of standard stock solution of drug

Eighty milligrams of favipiravir (FAVI) pure drug powder was accurately weighed and transferred into a 100 ml volumetric flask, dissolved and the volume was made up with distilled ethanol to obtain a final concentration of 100 µg/ml.

2.2.11. Selection of solvent

Various solvent alone and in combination were tried. Ethanol was selected as solvent as dissolve drug.

2.3. Method A: methyl orange based colorimetry

2.3.1. Selection of analytical wavelength

Different aliquots (0.0-2.5 ml) of 20 µg/ml favipiravir solution were accurately measured into a series of 10 ml calibrated flasks and the total volume was adjusted to 2.5 ml with ethanol. To each flask were added 1 ml each of bromate-bromide solution (10 µg/ml w. r. t. $KBrO_3$) and 5 M hydrochloric acid. The content was mixed well and let stand for 20 min with occasional shaking. Then 1 ml of 50 µg/ml methyl orange solution was added to each flask and diluted to the mark with water.

2.3.2. Selection of concentration range

Several concentration ranges were prepared and performed the reaction. But the most stable and detectable absorbance was observed with the concentration range from 10 to 50 µg/ml, prepared from the stock solution. To each flask were added 1 ml of drug solution and 1 ml of bromate-bromide solution (10 µg/ml w. r. t. $KBrO_3$) and 5 M hydrochloric acid. The content was mixed well and let stand for 20 min with occasional shaking. Then 1 ml of 50 µg/ml methyl orange solution was added to each flask and diluted to the mark with water. After 5 min, the absorbance of solution was measured at 477 nm against a reagent blank similarly prepared. From calibration curve concentration 20 µg/ml was selected for further analysis.

2.3.3. Study of spectra of blank solution

Blank solution was prepared without adding drug and vis- spectra was obtained by scanning solution in the range 400 to 800 nm against solvent ethanol.

2.4. Procedures

Into a of 100 ml volumetric flask, 1 ml of standard drug solution (20 µg/ml) of favipiravir was accurately transferred and the total volume was brought to 10 ml by adding water to each flask. To each flask were added 1 ml of drug solution and 1 ml of bromate-bromide solution (10 µg/ml w. r. t. $KBrO_3$) and 5 M hydrochloric acid. The content was mixed well and let stand for 20 min with occasional shaking. Then 1 ml of 50 µg/ml methyl orange solution was added to each flask and diluted to the mark with water. After 5 min, the absorbance of solution was measured at 477 nm against a reagent blank similarly prepared. The standard calibration curve was prepared or regression equation was derived to calculate the amount of drug in unknown samples.

2.4.1. Preparation of a calibration graph

A calibration graph of concentration versus absorbance was plotted. The drug colour complex has followed the Beer's Lamberts law in the concentration range of 10-50 µg/ml. Regression equation and correlation coefficient was determined. Absorbance of the above solutions was measured at 477 nm.

Effect of temperature on the absorbance of color complex

Effect of temperature on the stability of color complex formed was studied with increase in temperature. The absorbance values were measured at every 5 min interval maintaining the conditions.

Effect of concentration on the absorbance of color complex

The effect of the concentration of the MO reagent on color complex development was studied varying the concentrations from 0.01, 0.02, 0.03, 0.04, and 0.05% of reagent solution were studied.

Effect of variation in wavelength on the absorbance of color complex

The effect of the wavelength on color complex development was studied varying the wavelength by ±2nm were studied.

2.4.2. Assay of pharmaceutical formulation

The contents of 20 tablets were taken and finely powdered. Weighed accurate tablet powder equivalent to 20 mg of drug and transferred to 100 ml volumetric flask and diluents was added. The solution is sonicated for 15 min. The volume is made up with same diluents and mixed well. The solution was further diluted to get concentration 20 µg/ml. The drug-dye complex was produced by previously discussed procedure. The solution was filtered through a membrane filter of 0.45 µm. The amount of drug was calculated from the calibration curve.

2.4.3. Validation of Method

Validation of developed method was carried out in respect of accuracy, precision, robustness (Intra- and inter-day precision), limit of detection (LOD) and limit of quantitation (LOQ) as per ICH guidelines [70-73].

2.5. Method B: methyl red based colorimetric

2.5.1. Selection of analytical wavelength

About 200 mg of favipiravir was transferred into a 100 mL volumetric flask with 25 mL ethanol. The mixture was shaken and diluted to volume with ethanol. Resultant solution was further diluted to ethanol to yield final concentration 20 µg/ml. About 10 ml of above solution (20 µg/ml) was transferred to 25 ml volumetric flask. To this 1ml of 0.0037 M ethanolic solution of methyl red was added. The volume 25.0 mL was made up to the mark by adding ethanol. After 15 min solution was scanned in the visible range 400-800nm against blank solution. From spectra, wavelength 521.6 nm was selected for further analysis.

2.5.2. Selection of concentration range

Several concentration ranges were prepared in 25 ml volumetric flasks and performed the reaction. But the most stable and detectable absorbance was observed with the concentration range from 10 to 50 µg/ml, prepared from the stock solution. To each flask were added 10 ml of drug solution and 1 ml of 0.0037 M ethanolic solution of methyl red. Final volume was made upto 25 ml with ethanol. The content was mixed well and let stand for 20 min with occasional shaking. After 15 min, the absorbance of solution was measured at 521.6 nm against a reagent blank similarly prepared. From graph concentration 20 µg/ml was selected for further analysis.

2.5.3. Study of spectra of blank solution

Blank solution was prepared without adding drug and vis- spectra was obtained by scanning solution in the range 400 to 800 nm against solvent ethanol.

2.5.4. Preparation of a calibration graph

A calibration graph of concentration versus absorbance was plotted. The drug colour complex has followed the Beer's Lamberts law in the concentration range of 80-400 ng/ml. Regression equation and correlation coefficient was determined. Absorbance of the above solutions was measured at 521.6 nm.

Effect of temperature on the absorbance of color complex

Effect of temperature on the stability of color complex formed was studied with increase in temperature. The absorbance values were measured at every 5 min interval maintaining the conditions. The change in absorbance values and linearity with respect to the time.

Effect of concentration on the absorbance of color complex

The effect of the concentration of the MR reagent on color complex development was studied varying the concentrations from 0.01, 0.02, 0.03, 0.04, and 0.05% of reagent solution were studied.

Effect of variation in wavelength on the absorbance of color complex

The effect of the wavelength on color complex development was studied varying the wavelength by ±2nm were studied.

2.5.5. Assay of tablet formulation

The contents of 20 tablets were taken and finely powdered. Weighed accurate tablet powder equivalent to 80 mg of favipiravir and transferred to 100 ml volumetric flask and diluent was added. The solution is sonicated for 15 min. The volume is made up with same diluent and mixed well. The solution was further diluted to get concentration 20µg/ml. The drug-dye complex was produced by previously discussed procedure. The solution was filtered through a membrane filter of 0.45 µm. This is used for the assay by following the procedure. The amount of drug was calculated from the calibration curve

2.5.6. Validation of Method

Validation of developed method was carried out in respect of accuracy, precision, robustness (Intra- and inter-day precision), limit of detection (LOD) and limit of quantitation (LOQ) as per ICH guidelines.

3. Results and discussion

3.1. Method A- Methyl orange based colorimetry

After 5 min each solution was scanned in the visible range 400-800nm against blank solution. From spectra, wavelength 477 nm was selected for further analysis. Spectra of blank solution was also taken (Fig. 2&3). After comparing spectras, drug reacted with methyl orange and formed colored complex with wavelength maximum 477nm.

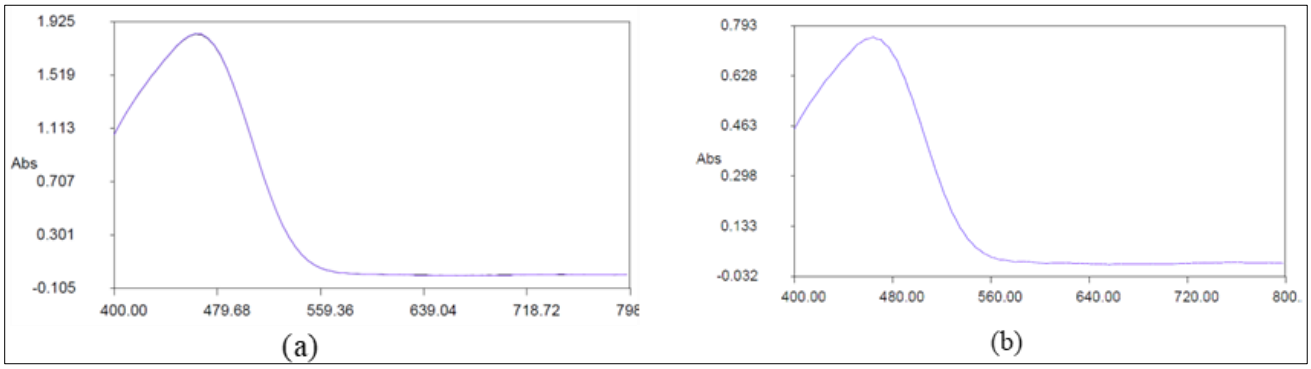

Figure 2 Spectrum of drug after color formation with Methyl Orange dye (λmax = 477 nm) (a) and blank solution of methyl orange (λmax = 462.2 nm) (b)

A calibration graph of concentration versus absorbance was plotted. The drug color complex has followed the Beer's Lamberts law in the concentration range of 10-50 µg/ml (Fig. 3).

Effect of temperature on the absorbance of color complex was studied at different temperatures with minute variation. Absorbance of the above solutions was measured at 477 nm. Effect of concentration and wavelength on the stability of color complex formed was studied (see Table 1).

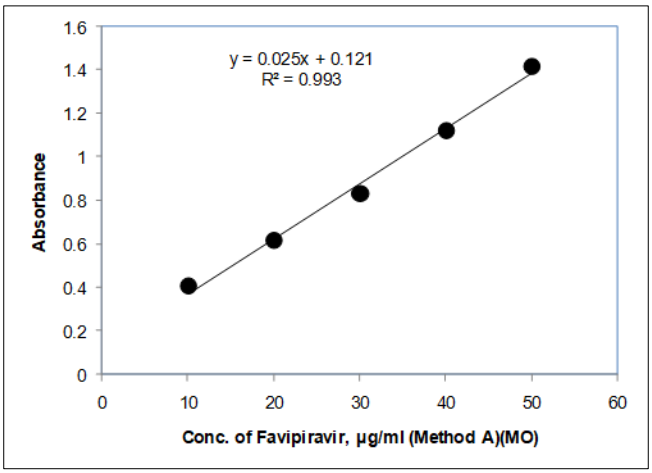

Figure 3 Calibration curve for Favipiravir (Method A)

Table 1 Effect of temperature and concentration on the absorbance of complex

Temp.*	Abs. at 477 nm	Conc. (µg/ml)	Abs. at 477 nm	WL±2nm	%RSD
28.01	0.615	20.01	0.619	625.2	2.19
28.02	0.618	20.02	0.620	629.2	2.51
28.03	0.618	20.03	0.614	-	-
28.04	0.614	20.04	0.615	-	-
28.05	0.618	20.05	0.619	-	-

Assay of marketed pharmaceutical formulation of favipiravir (Fabiflu® 400mg) was performed using developed method. The results of analysis and recovery study are given in Table 2.

Table 2 Results of assay of marketed formulation and validation

Parameters	**Result**	**Recovery study**		
Marketed formulation	Fabiflu® 400mg Tablet	% Level	%Recovery	RSD
Amount estimated (mg)	410 mg	80	100.23	2.27
%Purity	102.50%	120	100.58	2.41
Linearity range	10-50 µg/ml	**Precision**		
Slope	0.025	Day	Result	%RSD
Intercept	0.121	Intra day	100.43	2.28
Correlation coefficient	0.993	Inter day	100.51	2.33
Regression equation	y = 0.025x + 0.121	-	-	-
LOD	1.22 µg/ml	-	-	-
LOQ	3. 60 µg/ml	-	-	-

3.2. Method B- Methyl orange based colorimetry

Color complex of drug was prepared with 0.0037 M ethanolic solution of methyl red. From spectra, wavelength 521.6 nm was selected for further analysis. Spectra of blank solution was also taken (Fig. 4&3). After comparing spectras, drug reacted with methyl orange and formed colored complex with wavelength maximum 521.6 nm.

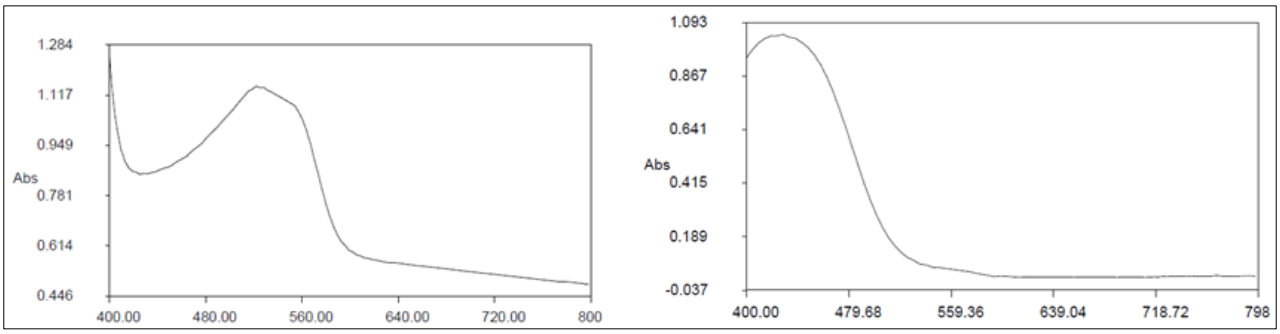

Figure 4 Spectrum of drug after color formation with Methyl red dye (λmax = 521.6 nm) (c) and blank solution of methyl orange (λmax = 419.2 nm) (d)

A calibration graph of concentration versus absorbance was plotted. The drug color complex has followed the Beer's Lamberts law in the concentration range of 10-50 µg/ml (Fig. 5).

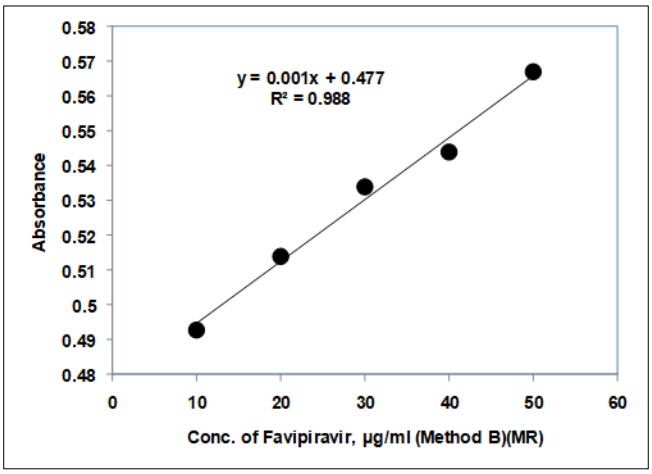

Figure 5 Calibration curve for Favipiravir (Method A)

Effect of temperature on the absorbance of color complex was studied at different temperatures with minute variation. Absorbance of the above solutions was measured at 521.6 nm. Effect of concentration and wavelength on the stability of color complex formed was studied (see Table 3).

Table 3 Effect of temperature and concentration on the absorbance of complex

Temp.*	Abs. at 521.6 nm	Conc. (µg/ml)	Abs. at 521.6 nm	WL±2nm	%RSD
28.01	0.515	20.01	0.514	519.6	2.12
28.02	0.515	20.02	0.515	523.6	2.48
28.03	0.517	20.03	0.515	-	-
28.04	0.518	20.04	0.517	-	-
28.05	0.518	20.05	0.517	-	-

Assay of marketed pharmaceutical formulation of favipiravir (Fabiflu® 400mg) was performed using developed method. The results of analysis and recovery study are given in Table 4.

Table 4 Results of assay of marketed formulation and validation

Parameters	**Result**	**Recovery study**		
Marketed formulation	Fabiflu® 400mg Tablet	% Level	%Recovery	RSD
Amount estimated (mg)	407 mg	80	98.31	1.28
%Purity	101.75%	120	100.21	2.31
Linearity range	10-50 µg/ml	**Precision**		
Slope	0.001	Day	Result	%RSD
Intercept	0.477	Intra day	100.13	2.3633
Correlation coefficient	0.9878	Inter day	100.15	2.36
Regression equation	y = 0.001x + 0.477	-	-	-
LOD	1.45 µg/ml	-	-	-
LOQ	3. 91 µg/ml	-	-	-

4. Conclusion

Methyl orange and methyl red based visible spectrophotometric methods for the determination of favipiravir were successfully developed and validated. The proposed methods involved simple reactions of favipiravir with various organic reagents, and subsequent measuring of the absorbances of the reaction products. The developed proposed method could be applied without prior extraction steps for pure samples of favipiravir respectively. Moreover, the proposed method is specific, accurate, reproducible, and highly sensitive which can be applied on the analysis of tablets. Since, no visible spectrophotometric methods are available, the proposed method is considered to be advantageous due to high sensitivity with reasonable precision and accuracy.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The author declares no conflict of interest.

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