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Research Article

ULTRAVIOLET SPECTROPHOTOMETRIC METHOD DEVELOPMENT FOR ESTIMATION OF NEW ANTIVIRAL REPURPOSING DRUG FAVIPIRAVIR

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ABSTRACT

Objective: A new, economical, precise, linear, sensitive, accurate, ultraviolet (UV) spectrophotometric method for the estimation of new antiviral repurposing drug favipiravir as there is no reported simple UV spectrophotometric method for estimation. The efforts were made for development and validation of favipiravir as per ICH guidelines, because drug has a wide scope for formulations to be developed for treating different viruses.

Methods: This method was developed using ethanol and water as a solvent. Favipiravir showed the absorption maxima at 234 nm. A Shimadzu UV–visible spectrophotometer (UV JAPAN 1801) was used to carry out spectral analysis.

Results: The developed method was linear for a range of 0–10 µg/ml and displayed a good correlation coefficient of 0.9995. Accuracy of the method was estimated using a recovery study. The amount of drug recovered was found to be in the range of 99.30–99.91%. The % relative standard deviation value of intraday precision was found to be 0.408% and interday precision was found to be in the range of 0.348–0.693%. The % relative standard deviation found to be <2 which are indicative of the precision and reproducibility of the method. Detection limit and quantitation limit were noticed as 0.095 and 0.290, respectively.

Conclusion: The developed UV spectrophotometric method was validated statistically for linearity, accuracy, precision, and sensitivity and results proved that the method can be employed for routine analysis of favipiravir.

Keywords: Favipiravir, Ultraviolet spectrophotometric method, Linearity, Relative standard deviation.

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INTRODUCTION

Favipiravir is a prodrug derived by chemical modification of the pyrazine moiety of T-1105. Favipiravir is quickly emerging as the top choice for COVID-19 treatment due to the absence of definitive treatment and a lot of emphasis on repurposing of existing drugs because developing a new drug would take years. Favipiravir (Fig. 1) is emerging as repurposing drug for the treatment of novel viruses. Toyama Chemicals, Japan, initially developed favipiravir for treating influenza. Chemically, it is 5-fluoro-2-oxo-1*H*-pyrazine-3-carboxamide [1].

Favipiravir is a viral RNA polymerase inhibitor which prevents replication of the viral genome. RNA polymerase is the enzyme which is essential for the virus to make copies of itself in the body. It selectively targets this enzyme, to inhibit the replication of the virus in the body [2].

Literature survey revealed that there were no simple ultraviolet (UV) spectrophotometric methods for favipiravir. Efforts were made for development and validation of favipiravir as per ICH guidelines. Favipiravir is effective antiviral drug and has a wide scope for formulations to be developed for treating different viruses.

METHODS

UV–visible spectrophotometer Shimadzu UV JAPAN 1801, electronic balance, Sonicator. Favipiravir is obtained as a gift sample from INCHEM Laboratories Pvt. Ltd., Hyderabad, India.

Development of UV spectrophotometric method for the estimation of favipiravir

Favipiravir with aqueous solvents produced no absorbance due to its poor solubility. Absorbance was produced with non-aqueous solvents [3]. In this method, absorbance was produced when favipiravir was first dissolved with non-aqueous solvent ethanol and made up to the volume with aqueous solvent water. Hence, water with ethanol was used as cosolvent for UV estimation of favipiravir.

Preparation of standard stock and working standard solutions

Accurately weighed 100 mg of favipiravir was transferred to a standard flask. Five milliliters of ethanol was added, sonicated and then volume was made up to 100 ml with water to give a solution 1 mg/ml. This solution is labeled as standard stock solution. From the standard stock solution, 10 ml was pipetted out into a 100 ml volumetric flask and diluted up to mark with distilled water to yield a solution of strength 100 µg/ml. This solution was labeled as working standard solution [4].

Determination of absorption maxima (λ_{max}) of favipiravir

From working standard solution, 1 ml was pipetted out into a 100 ml volumetric flask and diluted up to mark with distilled water to yield a solution of strength 10 µg/ml. The spectrum of this solution was scanned over 200–400 nm range in a UV spectrophotometer against distilled water as blank to estimate the absorption maxima (λ_{max}) of favipiravir.

Validation of proposed UV spectrophotometric method

UV spectrophotometric method was validated statistically for the following parameters.

Linearity

The linearity of the developed method was established by preparing fresh aliquots from working standard solution. Subsequent dilutions were made to produce concentration range 2–10 µg/ml by taking aliquots of 0.2, 0.4, 0.6, 0.8, and 1.0 ml to series of 10 ml volumetric flasks from working standard solution and diluted with distilled water.

Absorbance was recorded in triplicate for each concentration at 234 nm using distilled water as blank. Calibration curve was plotted by taking concentration on X-axis and absorbance on Y-axis [5].

Precision

Precision is a measure of the agreement among the values obtained when the same solution is repeatedly assayed. Repeatability also termed as intraday precision was obtained by evaluating the absorbance of six replicates of working standard solution 10 µg/ml of favipiravir on a single day. Interday precision was calculated in three different days at different time intervals. The % relative standard deviation (% RSD) serves as a measure of precision [6,7].

Accuracy

Standard addition method was employed to determine the accuracy of proposed UV spectrophotometric method. To 10 µg/ml of pre-analyzed sample solution, a known amount of favipiravir was added to yield a solution of concentration range 15, 20, and 25 µg/ml. In triplicate, absorbance was recorded and percentage recovery was calculated [8,9].

Detection limit and quantitation limit

Using the calibration curve, detection limit and quantitation limit for favipiravir can be determined [10]. The formulas used to measure are as follows:

$$\text{Detection limit} = 3.3 \times \text{S D/Slope}$$

$$\text{Quantitation Limit} = 10 \times \text{S D/Slope}$$

Where,

S.D = Standard deviation of response of least concentration of the calibration curve

S = Slope of calibration curve

RESULTS

Absorption maxima (λ_{max}) of favipiravir

The λ_{max} of favipiravir was found to be 234 nm (Fig. 2). Hence, reported λ_{max} of favipiravir was 234 nm.

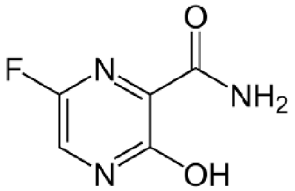

Fig. 1: Chemical structure of favipiravir

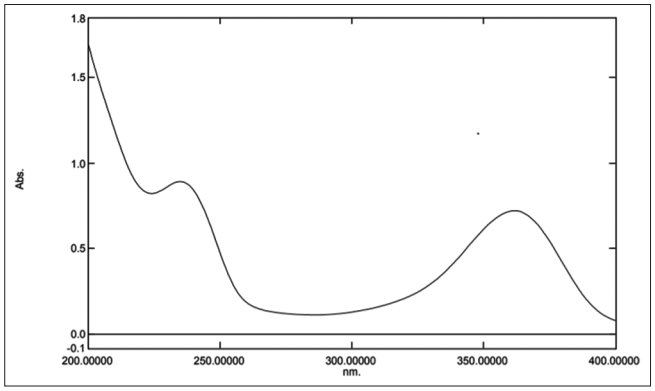

Fig. 2: *λmax* of favipiravir in distilled water at 234 nm

Linearity

The results obtained for the linearity of favipiravir are presented in Table 1. Calibration curve of favipiravir is unveiled in Fig. 3; it was evident that favipiravir obeys Beer's Lambert law with the concentration range of 0–10 µg/ml having line equation y=0.0853x +0.0089 with a correlation coefficient of 0.9995 which was very close to unity indicating positive correlation between concentrations of favipiravir.

Precision

Intraday and interday precision results are provided in Tables 2 and 3. RSD value of intraday precision was found to be 0.408% and interday precision was found to be in the range of 0.348–0.693%. RSD values obtained were within the acceptable limit, that is, RSD <2% which indicates that the developed method was precise with repeatability.

Accuracy

Accuracy results obtained by mean % recovery are presented in Table 4 which were acceptable which means that the established method was accurate.

Detection Limit and Quantitation Limit

Detection limit and quantitation limit of favipiravir were found to be 0.095 µg/ml and 0.290 µg/ml, respectively, indicating the sensitivity of above method (Table 5).

DISCUSSION

The spectrum of 10 µg/ml of favipiravir is shown in Fig. 2 indicated peak absorbance at 234 nm. Hence, 234 nm was considered as

Table 1: Concentration versus absorbance values for the estimation of favipiravir

Concentration of favipiravir (µg/ml)	Absorbance n=3± sd	RSD (%)
0	0	0
2	0.198±0.0025	1.262
4	0.344±0.0028	0.813
6	0.524±0.0076	1.450
8	0.693±0.0097	1.399
10	0.857±0.0110	1.283

RSD: Relative standard deviation

Table 2: Repeatability or intraday precision results for favipiravir

Concentration (µg/ml)	Absorbance	RSD (%)
10	0.859	0.408
	0.862	
	0.856	
	0.860	
	0.852	
	0.857	

RSD: Relative standard deviation

Table 3: Interday precision results for favipiravir

Day	Absorbance	RSD (%)
Day 1	0.872	0.693
	0.863	
	0.859	
Day 2	0.865	0.463
	0.858	
	0.867	
Day 3	0.860	0.348
	0.857	
	0.863	

RSD: Relative standard deviation

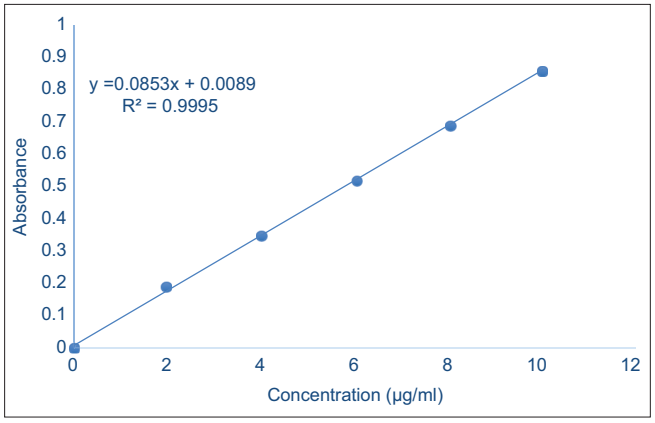

Fig. 3: Calibration curve for the estimation of favipiravir

Table 4: Accuracy results for favipiravir

Amount of sample (µg/ml)	Amount of drug added (µg/ml)	Amount recovered (µg/ml)	% recovered	Mean % recovered
10	5	14.94	99.60	99.42
	5	14.88	99.20	
	5	14.92	99.46	
10	10	20.10	100.5	99.91
	10	19.89	99.45	
	10	19.96	99.80	
10	15	24.80	99.2	99.30
	15	24.92	99.68	
	15	24.75	99.0	

Table 5: Detection limit and quantitation limit results for favipiravir

Detection limit	0.095 µg/ml
Quantitation limit	0.290 µg/ml

Table 6: Findings of favipiravir

Parameters	Findings of favipiravir
Working λ_{max} (nm)	234 nm
Beer's law limit (µg/ml)	0–10 µg/ml
Regression equation	y= 0.0853x + 0.0089
Correlation coefficient	0.9995
Intercept	0.0089
Slope	0.0853
Intraday precision	0.408%
Interday precision	0.348–0.693%
Accuracy	99.30–99.91%
Detection limit	0.095 µg/ml
Quantitation limit	0.290 µg/ml

absorption maxima, λ_{max}. The results of concentrations of favipiravir and the corresponding absorbances are shown in (Table 1) and calibration curve is represented in Fig. 3. The curve was obtained with satisfactory a correlation coefficient value of 0.9995, which indicated a positive correlation between concentrations of favipiravir and the corresponding absorbance values. The method obeyed Beer's law in the range of 0–10µg/ml. Repeatability or intraday precision of favipiravir was 0.408% and interday precision was within the range of 0.348–0.693%, as shown in Tables 2 and 3. Accuracy was within the range of 99.30–99.91% (Table 4). Detection limit and quantitation limit are 0.095 µg/ml and 0.290 µg/ml, respectively (Table 5). All the findings of favipiravir are mentioned in (Table 6).

CONCLUSION

It is concluded that the developed UV spectrophotometric method was found to be simple, economic, easy, accurate, precise, linear, specific, and highly sensitive and can be used for routine estimation of favipiravir.

AUTHORS' CONTRIBUTIONS

The present work was done in collaboration with the two authors, Jeevana Jyothi B and Venkata Kavya R. Both the authors were involved in collection of literature, conducting the experiments, preparation of the manuscript, and contributed equally.

CONFLICTS OF INTEREST

Declared none.

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