DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF FAVIPIRAVIR

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ABSTRACT:

A sensitive ultraviolet spectrophotometric method was developed and validated according to ICH guidelines for quantitative estimation of Favipiravir. The solvent used was 0.1 N HCl, and the analysis was performed at 323 nm. The calibration curve was linear over the concentration range 1 to 25 µg/mL. various validation parameters like accuracy, precision, LOD, LOQ, recovery study, range were determined The proposed method was simple, rapid, precise, accurate and sensitive, and can be used for the routine analysis of favipiravir.

Keywords: Favipiravir, UV spectrophotometry, validation

INTRODUCTION:

Favipiravir is an antiviral drug which is indicated for the treatment of patients with mild to moderate COVID-19 disease[1]. It is a RNA-dependent RNA polymerase inhibitor. It is activated in its phosphoribosylated form (Favipiravir-RTP) in cells, inhibiting viral RNA polymerase activity[2]. Chemically favipiravir is 6-fluoro-3-hydroxypyrazine-2-carboxamide (fig.1). The RNA-dependent RNA-polymerase enzyme uses this molecule as a substrate; however, the enzyme misinterprets it for a purine nucleotide, which inhibits its activity and stops the synthesis of viral proteins.

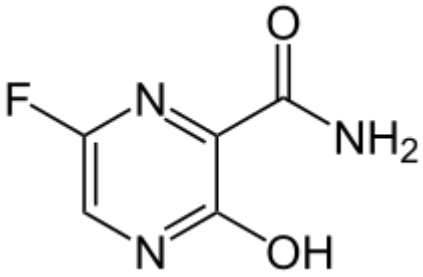

Fig 1: Structure of favipiravir

Literature survey revealed that various HPLC methods are available for estimation of favipiravir in pharmaceutical formulations. A HPLC-UV method for quantification of favipiravir in pharmaceutical formulations using the Mixture of 50 mM potassium dihydrogen phosphate (pH 2.3) and acetonitrile (90:10, v/v)as the mobile phase was reported [6]. A validated HPLC Method for Quantification of Favipiravir in Tablet using Water: methanol as the mobile phase has been reported [7]

Although there were various HPLC methods available, there was no simple UV spectroscopic method available for estimation of favipiravir. The present work describes the simple, accurate and precise UV spectroscopic method for estimation of favipiravir in tablets.

MATERIAL AND METHOD

Instrumentation:

SHIMADZU UV –1900 UV/Visible Spectrophotometer with UV Probe 2.10 Software and 1cm matched quartz cells were used for absorbance measurements.

Selection of solvent:

The solubility of favipiravir was checked in water and 0.1 N HCl. It was found to be soluble in 0.1 N HCl but insoluble in water. 0.1 N HCl was selected as the solvent for dissolving the drug.

Preparation of 0.1 N HCl:

Accurately measured 4.2 ml of concentrated HCL was transferred it into a 500 ml Volumetric flask and volume was adjusted to mark with the distilled water.

Preparation of standard stock solution:

Accurately weighed 0.01 g (10 mg) of Favipiravir standard was transferred into a 100 mL volumetric flask and dissolved in 0.1 N HCl. The volume was made up to 100 mL with the same solvent to give the solution containing 100 µg/mL of Favipiravir. The aliquot of 50 mL from this solution was transferred to 100 mL volumetric flask and diluted up to the mark with distilled water to give the solution of concentration 50 µg/mL.

Selection of Wavelength for Analysis (λ_{max}):

The standard stock solution was further diluted with distilled water to get a 10 µg/mL of concentration. The solution was scanned between 200 and 400 nm using 0.1 N HCl as a blank.

Preparation of the calibration curve:

Aliquots of standard stock solution (0.2, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 and 5 mL) were transferred to separate 10 mL volumetric flask and further diluted to the mark with distilled water to get the solutions of concentration 1–25 µg/mL. The absorbances were measured at 322 nm against 0.1N HCl as blank.

Assay of Favipiravir in Tablet:

Tablet were labelled to contain 400 mg of Favipiravir as an active substance per tablet. 10 tablets were accurately weighed and powdered. The powder equivalent to 0.2 g (200 mg) of Favipiravir was weighed and transferred to a 100 mL volumetric flask. To the flask, 50 mL of 0.1N HCL was added and sonicated for 15 min. The volume was adjusted to 100 mL with 0.1N Hcl. The solution was filtered through Whatman filter paper No. 01. From this filtrate, 2.5 mL was transferred to a 100 mL volumetric flask and diluted with water to 100 mL. The aliquot of 2 mL from this solution was further diluted to 10 mL with distilled water. The absorbance was measured at 323 nm using 0.1N HCl as blank. This procedure was repeated for six times. The amount of Favipiravir present in formulation was calculated using calibration curve equation.

Method Validation

The developed method was validated as per ICH guidelines for following parameters.

Linearity:

Aliquots of standard stock solution were further diluted with water to get the solutions of concentration within range from 1 to 25 μg/mL. The absorbance was measured at wavelength 322 nm. Linear calibration graph was obtained by plotting the absorbance versus concentration of Favipiravir.

Specificity:

The spectra obtained from tablet solutions was compared with that obtained from standard solution containing an equivalent concentration of Favipiravir.

Accuracy (Recovery studies):

To ensure accuracy of the method, recovery studies were performed by standard addition method at 80%, 100%, and 120% level to preanalyzed samples and subsequent solutions were reanalyzed. At each level, three determinations were performed. The absorbances were measured at 322 nm using and the amount of drug recovered from the formulation were calculated.

Precision:

Precision of the method was determined in terms of repeatability and intraday and interday precisions.

Repeatability:

Repeatability of the method was determined by analyzing six samples of same concentrations of drug (10 μg/mL). The absorbance of each solution was measured.

Intermediate (Intraday and Interday) precision:

The three concentrations of sample solution were analysed in triplicate on same day to determine intraday precision. The procedure was repeated for three consecutive days to determine the interday precision.

Robustness:

To determine the robustness of the method, the experimental conditions were deliberately altered and assay was evaluated. The effect of change in detection wavelength was studied at 324 nm. For changes of conditions, six standard solutions of concentration 10 µg/mL were analyzed.

Limit of detection and limit of quantitation:

Limit of detection (LOD / detection limit) and the limit of quantitation (LOQ / quantitation limit) are calculated using the formulae LOD = 3.3σ / S, and LOQ = 10σ / S. Here σ is the standard deviation of y-intercept of regression lineand S is the slope of the calibration curve.

RESULT AND DISCUSSION

Selection of Wavelength for Analysis (λmax):

The UV spectrum of favipiravir had shown λmax, at 322 nm. Hence, it was selected for the analysis of favipiravir. The typical UV spectrum of favipiravir is shown in figure 2.

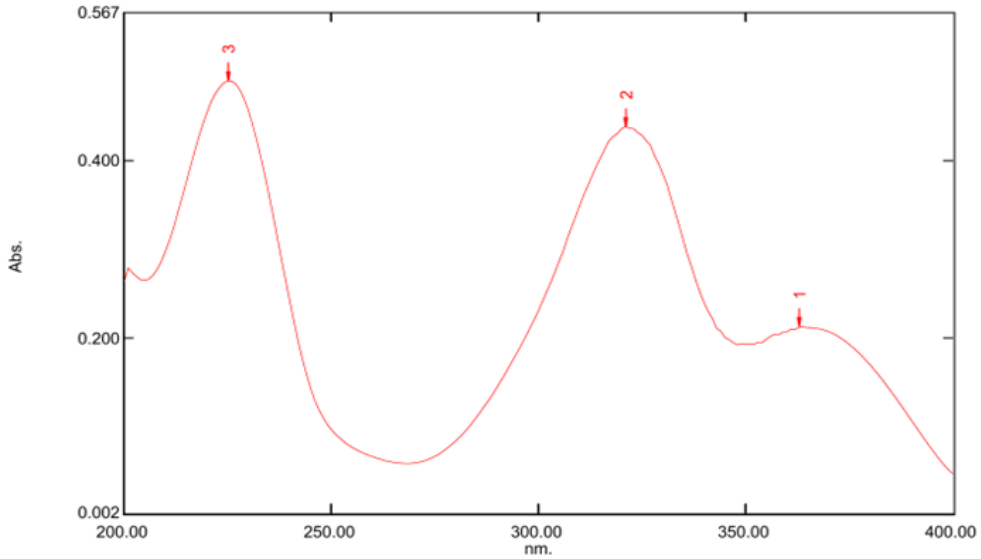

Figure 2: UV spectrum of Favipiravir in 0.1N HCl

Preparation of the calibration curve:

The calibration curve was constructed by plotting the absorbance against corresponding concentration. The calibration curve is shown in Figure 3.

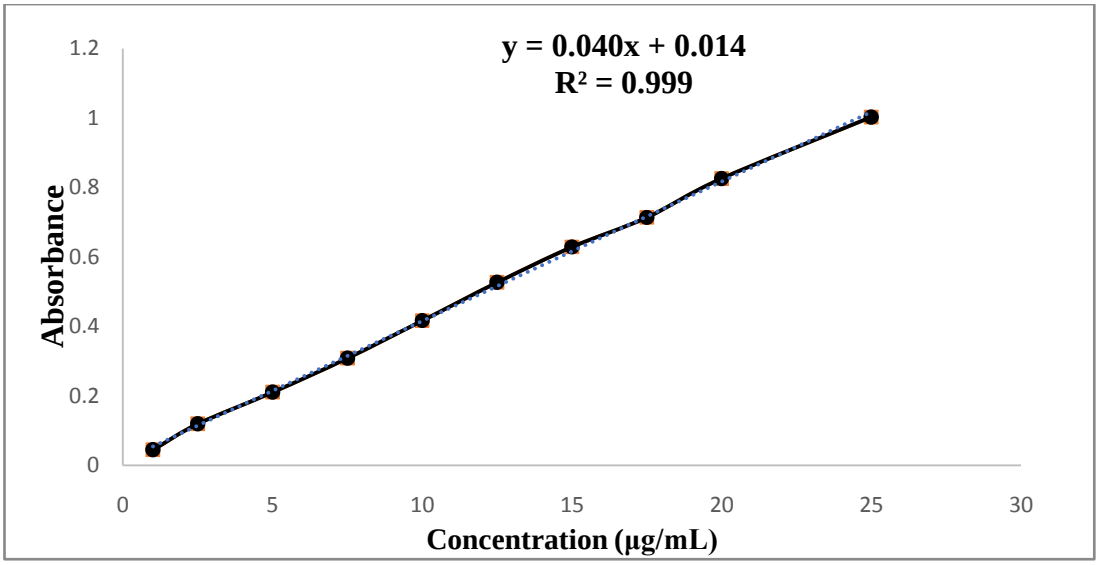

Figure 3: Calibration curve for favipiravir

Assay of Favipiravir in Tablet:

The absorbance of sample solution was measured at 323 nm using 0.1N HCl as blank. This procedure was repeated for six times. The amount of Favipiravir present in formulation was calculated using calibration curve equation. The results are shown in **Table 1.**

Table 1: Results of Assay of Favipiravir in Tablet

Labelled claim (mg)	**Amount found (mg) Mean ± SD n=6**	**% Assay Mean ± SD n=6**	% RSD **n=6**
400	356.19 ± 4.43	89.05 ± 1.11	1.24

n= number of repetitions

Linearity:

Favipiravir showed linear response in the concentration range of 1-25µg/mL (fig. 4). Linear regression data is shown in Table 2.

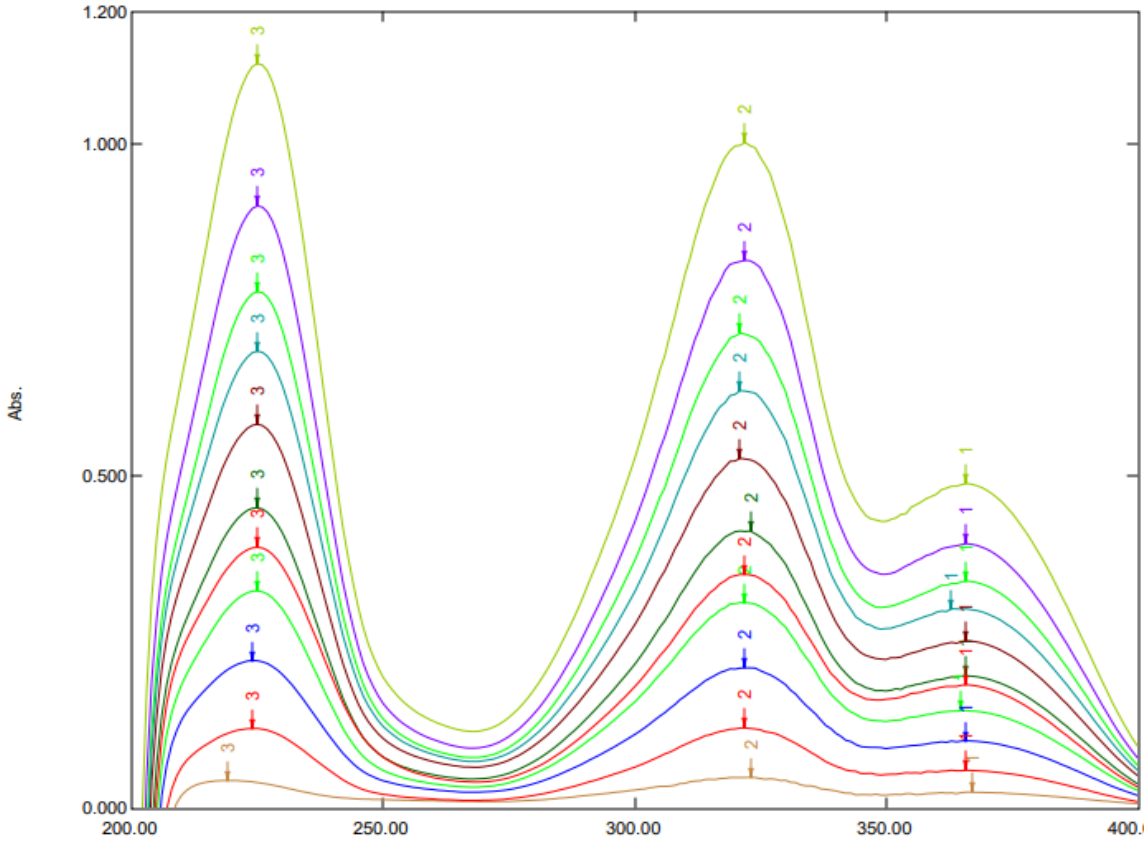

Figure 4: Linearity of Favipiravir

Table 2: Linear regression data

Parameter	Result
λmax (nm)	322 nm
Beer's law limit (µg/mL)	1-25 µg/mL
Correlation coefficient	0.9992
Regression equation	Y=0.0402x+0.0142
Slope (m)	0.0402
Intercept (c)	0.0142

Specificity:

The spectra obtained from tablet solutions were identical with that obtained from standard solution of favipiravir (Figure 5). This showed that there was no any interference from excipients. Therefore, it could be said that developed method is highly specific.

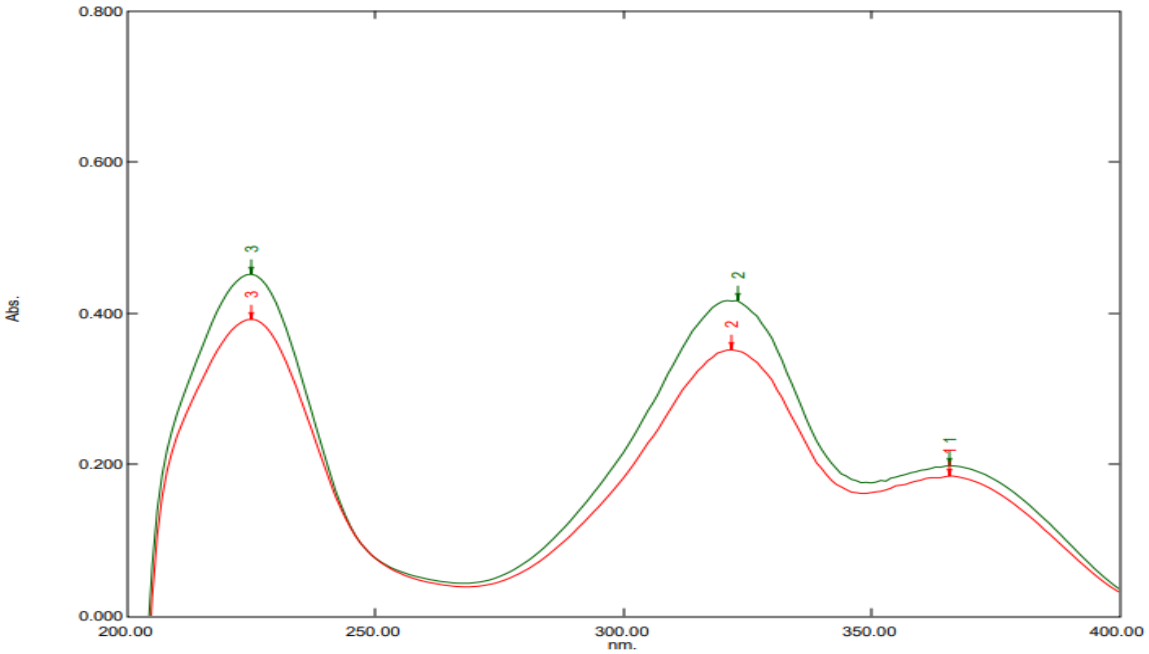

Figure 5: Overlain spectra of Standard and Sample

Accuracy (Recovery studies):

The developed method was found to be accurate, indicated by mean % between 98 and 102 % as shown in Table 3.

Table 3: Result of Recovery studies by UV method

Level (%)	Sample concentration (µg/ml)	Standard drug added (µg/mL)	% Recovery Mean ± SD n=3	Amount recovered (µg/mL) Mean ± SD n=3	% RSD n=3
80	10	8	99.09 ± 1.82	7.93 ± 0.15	1.84
100	10	10	100.92 ± 1.46	10.09 ± 0.15	1.44
120	10	12	100.13 ± 1.45	12.02 ± 0.17	1.45

n= number of repetitions

Precision:

The % RSD values for repeatability and intermediate precision were found to be less than 2%. The results are summarized in Table 4 and 5 respectively.

Table 4: Results of Repeatability studies

Concentration (µg/mL)	**Concentration found (µg/mL) Mean ± SD**	**% RSD n=6**
10	**9.85 ± 0.18**	**1.86**

n= number of repetitions

Table 5: Results of Intermediate precision

Concentration (µg/mL)	**Intraday precision**		**Interday precision**	
	Concentration found (µg/mL) Mean ± SD n=3	**% RSD n=3**	**Concentration found (µg/mL) Mean ± SD n=3**	**% RSD n=3**
5	5.25 ± 0.06	1.19	5.33 ± 0.09	1.64
10	10.29 ± 0.13	1.28	10.28 ± 0.13	1.22
15	14.78 ± 0.14	0.97	15.44 ± 0.21	1.38

n= number of repetitions

Robustness Assay of favipiravir for deliberate changes of conditions was within 98.0–102.0 % as shown in Table 6, which indicates robustness of the method.

Table 6: Result of Robustness Studies

Concentration (µg/mL)	**Concentration found (µg/mL)**	**% Assay Mean ± SD n=6**	**% RSD n=6**
10	**10.13 ±0.13**	**101.28 ± 1.26**	**1.24**

n= number of repetitions

Limit of detection and limit of quantitation

LOD and LOQ were found to be 0.47 µg/mL and 1.43 µg/mL respectively

SUMMARY AND CONCLUSION

A simple, rapid, precise and accurate spectrophotometric method has been developed for quantitative analysis of Favipiravir in tablet formulations. The initial stock solution of Favipiravir was prepared in 0.1 N HCl and subsequent dilution was done in water. The standard solution showed absorption maxima at 322 nm. The drug obeyed Beer–Lambert's law in the concentration range of 1–25 µg/mL with coefficient of correlation (R^2) was 0.9992. The method was validated as per the ICH guidelines. The % RSD values were below 2 % for precision

studies.The recoveries between 98 and 102 % indicated that the method was accurate. There was no interference from excipients of tablet.

The results of the validation tests indicated that the method was accurate, precise, robust, and specific. The method was successfully applied to pharmaceutical dosage form. The proposed UV is suitable for routine determination of Favipiravir in pharmaceutical formulation in quality control laboratories.

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