Evaluation and Development of a UV-Spectrophotometric Method for Favipiravir in Pure and Formula

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ABSTRACT

This study primarily focuses on technique development and validation for the analysis of favipiravir in pharmaceutical dosage forms and pure forms using UV spectroscopy. This procedure calls for the creation of working and standard solutions of the drug favipiravir, followed by the fabrication and analysis of working solution aliquots at various concentrations. Then, the linearity, precision, accuracy, robustness, ruggedness, limit of quantification, and limit of detection of this developed technique were all validated. The information received shows that the procedure was sensitive and accurate within the range of 4-24 g/ml. The correlation coefficient (R^2) was found to be 0.999. There is no interference observed with excipients in formulation. The project method may be duly applied for the analysis of Favipiravir in bulk for routine analysis.

Key Words: Ultraviolet Spectroscopy, validation, Favipiravir, method development, assay.

INTRODUCTION

Antiviral medications are frequently recommended to treat infections brought on by coronavirus-2 causing severe acute respiratory syndrome (SARS-CoV-2). A pyrazinecarboxamide derivative with efficacy against RNA viruses is favipiravir[Fig-1]. By specifically inhibiting the influenza virus RNA-dependent RNA polymerase, ribofuranosyltriphosphate derivative, which is formed when favipiravir is transformed to it by host enzymes. It has a melting point between 187 and 193 °C, is a white, crystalline powder with a molecular weight of 157.10 g/mol, and is just weakly soluble in water. It has the IUPAC name 5 -fluoro-2-oxo-1H-pyrazine-3-carboxamide.Favipiravir is an antiviral drug candidate that is now being investigated in COVID-19 patients in phase III trials. Favipiravir enhances the frequency of viral RNA alterations in animal models[1-3].

By an extensive literature review we found that very few studies have been reported for the determination of Favipiravirusing High-Performance Liquid Chromatography. Another study on, LC-MS/MS studies for determination ofFavipiravir and its metabolite in human plasma[7].Few UV spectroscopic methods for analysis of Favipiravir in pure and pharmaceutical dosage forms have been reported[6-11].

In this study, we tried to analyze Favipiravir in pure and in pharmaceutical formulation. We selected the pharmaceutical preparation to be tablet and the solvent preferred as acetonitrile. All the optimization parameters were also considered, after development of UV method. The developed method was successfully validated and can be used to estimate the total drug content in the commercially available formulations of Favipiravir.

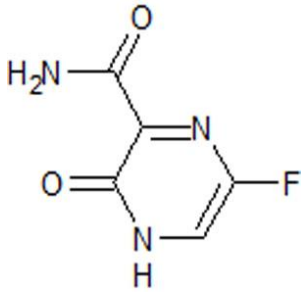

Fig-1: Chemical structure of Favipiravir

MATERIALS AND METHODS:

Instruments and Reagents:

A gift sample of Favipiravir with purity 99.98% wasobtained from a Local manufacturing unit at Visakhapatnam. LAB INDIA (T_{60}) double beam UV/Visible spectrophotometer and ELITE analytical balance were the instruments used. The chemicals and reagents used are of analytical grade. Favipiravir tablet formulation of 400 mg with a brand name of FABIFLU were purchased from the market.

Preparation of standard stock solution (1000µg/ml):

25mg of drug was added into a 25ml volumetric flask and dissolved using acetonitrile and finally made up to the mark with acetonitrile to get a concentration of 1000µg/ml, which is a standard stock solution of Favipiravir.

Preparation of working standard solution (100µg/ml):

2.5ml of sample, from the above standard stock solution was transferred to a 25ml volumetric flask and made up to mark with acetonitrile to get a concentration of 100µg/ml.

Construction of calibration curve:

The working standard was then further diluted to get 16µg/ml solution.It is scanned by a UV Spectrophotometer in the range of 200-400 using distilled water as blank. The maximum absorbance was found to be at wavelength 223 nm.Aliquots ranging from 4-24µg/ml solutions were prepared by using distilled water as solvent. These samples were then analysed at λ_{max}223nm to get respective absorbance. The values are then plotted to get a calibration curve.

Preparation of the Assay solution:

The proposed method was applied to analyze the commercially available "FABIFLU" capsule formulation.

RESULTS:

Method Validation:

Linearity:

Different aliquots of Favipiravir were prepared in the range of 4-24µg/ml from the working standard solution (100µg/ml). These solutions were scanned on a Double beam UV-VIS spectrophotometer in the range of 200-400nm using distilled water as the blank. The spectrum was recorded at 223 nm **(Fig-2)**. The calibration plot was constructed as concentration versus absorbance and can be shown in **Fig-3** and **Table-1.**This shows a perfect linearity has been established.

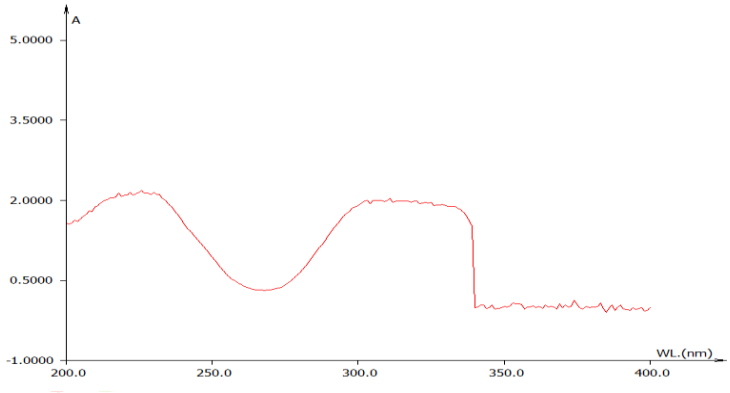

Fig-2: Spectrum obtained for Standard

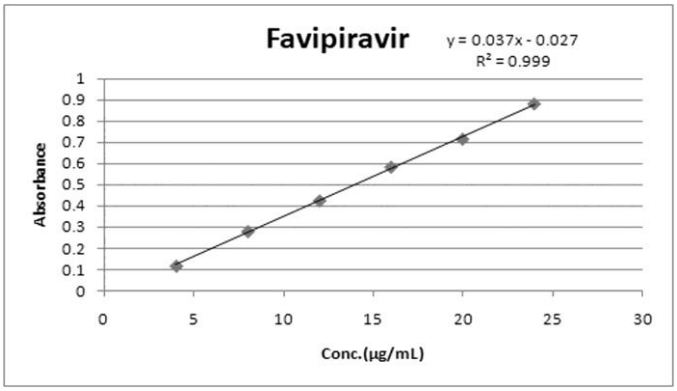

Fig-3: Calibration plot

Table-1: Linearity Data

Conc. (µg/ml)	Absorbance
4	0.118
8	0.2812
12	0.4261
16	0.5852
20	0.7171
24	0.8837
Regression equation	y = 0.037x - 0.027
Correlation coefficient(R^2)	0.999

Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra-day variation study, six different solutions of the same concentration 16µg/ml were prepared and analyzed twice a day (Morning, and Evening) and the % RSD was 1.09. In the inter-day variation study, the solutions of the same concentration 16µg/ml were prepared and analyzed six times, for two consecutive days and the absorbance was recorded **(Table-2)**. The percentage relative standard deviations are found to be <2%.

Table-2: Intermediate Precision

Conc. [µg/ml]	Absorbance	
	Analyst-1/Day-1	Analyst-2/Day-2
16	0.5828	0.5798
16	0.5765	0.5712
16	0.5898	0.5764
16	0.5722	0.5736
16	0.5822	0.568
16	0.5861	0.588
Mean	0.5816	0.576167
S.D	0.006382	0.007088
%RSD	1.097346	1.230284
%RSD(12 Determinations)	1.16	

Accuracy

The accuracy of the method was determined by standard addition method the percent recovery of Favipiravir was calculated. To the known amount of 16µg/mL formulation solution following level were added at 80,100, 120% working standard. The level solutions were prepared in triplicate and analyzed thrice. The total of nine determinations was performed for accuracy study. The accuracy was indicated by % recovery was calculated and reported in the **Table-**

3. The percent recovery was found to be good.

Table-3: Accuracy of method

Level of addition	Formulation amount	Amount added	Theoretical amount	Experimental amount [n=3]	% recovery	% mean recovery ±SD
80%	16	12.8	14.4	14.22	98.79	98.7±0.005
100%	16	16	16	15.88	99.29	99.29±0.19
120%	16	19.2	17.6	17.48	99.31	99.3±0.22

Robustness

The Robustness of the method was carried out by analyzing the sample of 16µg/ml using three different wavelengths (1ofλ_{max}) and respective absorbance were recorded. The results shown in **Table-4** indicate the method was robust.

Table-4: Robustness Study

Conc. (µg/ml)	Absorbance		
	222nm	223nm	224nm
16	0.5623	0.5828	0.5844
16	0.5722	0.5765	0.5985
16	0.5698	0.5898	0.5852
16	0.5632	0.5722	0.5985
16	0.5678	0.5822	0.5976
16	0.5612	0.5861	0.5998
AVG	0.566083	0.5816	0.594
SD	0.004487	0.006382	0.007165
%RSD	0.792571	1.097346	1.206262

Ruggedness

The ruggedness of the method was carried out by analyzing the sample using two different analysts with same equipment and two different cuvettes by same analyst and respective absorbance were recorded. The results of first Analyst showed %RSD 0.1672 and second analyst showed %RSD 0.2184, which indicate that the methodology employed was rugged, since there is no significant difference between different operators.

Sensitivity

Limit of detection (LOD) and limit of quantification (LOQ) of the drug was calculated from the calibration curve using regression analysis as 0.679µg/mland 2.059µg/ml as respectively.

DISCUSSION

The method was developed and validated as per ICH guidelines. The method was validated in terms of linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. Beers law obeyed over the concentration range of 4-24 µg/mL, using multivariate analysis the linear equationY=0.037x-0.027 with a correlation coefficient if R^2= 0.999. The precision results show % RSD less than 2 at each level which indicates clearly that the method was precise enough for the analysis ofFavipiravir.The accuracy of the method was checked by recovery studies. The high recovery with values indicates the accuracy of the developed methodology. The robustness and ruggedness studies reveal that the method is more sensitive. There was no interference ascertained from the excipients present in the formulation, indicated that the

method is specific. Determination of Favipiravir in formulation showed that the drug was very close to the label amount. The percentage RSD values and all the characteristics parameters of the method are represented in the (Table-3).

Table-3: Summary of Results

Parameters		Results
Absorption maxima(nm)	:	223nm
Linearity range(µg/ml)	:	4-24
Regression equation	:	Y=0.037x-0.027
Correlation coefficient(R^2)	:	0.999
Accuracy	:	98.7 -99.3%
LOD(µg/ml)	:	0.679
LOQ(µg/ml)	:	2.059
Intraday precision(%RSD)	:	1.09
Inter-day precision(%RSD)	:	1.16

CONCLUSION

The proposed method was simple and found to be accurate, precise, robust and easy to determine. The proposed method gave a lower range of the calibration plot. The sample recoveries were in good agreement. The apparatus and reagents used seem to be accessible even for simple laboratories. Therefore, developed method can be recommended for routine and QC analysis of Favipiravir. This methodology is also suitable for analysis of sample during accelerated stability studies, routine analysis of formulations and Active pharmaceutical ingredient.

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