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Establishment and Verification of a First-Order Derivative UV Spectrophotometric Technique for Quantifying Favipiravir in Bulk and Pharmaceutical Formulations

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Authors' contributions

This work was carried out in collaboration among all authors. Author JB designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors TJ, MK and PKVR managed the analyses of the study. Author KS managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The primary aim of this study was to establish and validate a UV spectrophotometric method for quantifying Favipiravir in both bulk and pharmaceutical formulations using a first-order derivative technique. In this approach, acetonitrile was chosen as the solvent, and measurements were taken at a wavelength of 323 nm. The methodology was developed and subsequently validated in compliance with the ICH guidelines. The analysis revealed a strong linear response within a concentration range of 5-25 µg/ml, with a correlation coefficient of 0.9995. Furthermore, the method exhibited a low detection limit of approximately 0.48 µg/ml and a quantification limit of about 1.44 µg/ml. A comprehensive assessment of the method's precision, accuracy, specificity, and sensitivity produced consistently positive results. Given its robust and reliable performance, this technique can be effectively utilized for the routine analysis of Favipiravir in both bulk and pharmaceutical forms.

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1. INTRODUCTION

Favipiravir, as depicted in Fig. 1, serves as an antiviral medication developed to address a range of viral infections, including influenza and COVID-19 [1]. It is chemically identified as 6-fluoro 3-hydroxypyrazine-2-carboxamide, with a molecular formula of C5H4FN3O2 and a molecular weight of 157.104 g/mol. This substance exists in the form of a colorless powder and exhibits solubility in organic solvents, while being slightly soluble in water, possessing a pKa value of 5.1. Favipiravir falls into the category of organic compounds known as pyrazine carboxamides [2]. Specifically, it belongs to the antiviral class of pharmaceuticals, functioning by inhibiting the RNA-dependent RNA polymerase enzyme, which plays a crucial role in preventing viral transcription and replication [3,4].

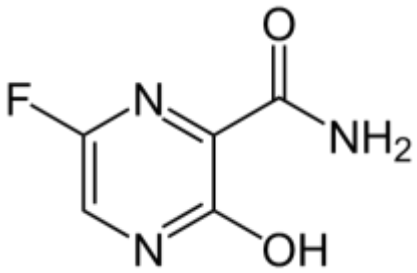

Fig. 1. Chemical Structure of Favipiravir

As per the literature survey, it was known that few methods were developed for the determination of favipiravir in pharmaceutical formulations. The developed methods included spectroscopic methods such as UV spectroscopic methods [5-10], Visible spectrophotometric methods [11], Fourier transform infra-red 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]. It was apparent that there was a notable absence of a UV first-order method for determining the presence of favipiravir in pharmaceutical formulations. Consequently, the objective of this investigation was to establish and validate a First-Order Derivative UV Spectrophotometric technique for the quantification of Favipiravir in both bulk and pharmaceutical formulations.

2. MATERIALS AND METHODS

2.1 Reagents Used and Chemicals Used

Favipiravir working standard was obtained as research support sample from the Hetero labs Pvt. Ltd, Hyderabad. The favipiravir tablets (fabiflu) were purchased in a pharmacy. The acetonitrile and methanol solvents used for the development of method were procured from Merck ltd, Mumbai-India. And all the chemicals utilized for the development of method were of AR grade and purchased from sigma Aldrich, Bangalore, India.

2.2 Instruments

T60V UV/VIS double beam spectrophotometer, weighing digital balance, ultrasonic bath sonicator.

2.3 Preparation Procedure for Standard and Sample Solutions

In the preparation of the favipiravir working standard solution, an accurately measured 100 mg of the compound was dissolved in 100 ml of acetonitrile solvent, resulting in a concentration of 1000 µg/ml. Subsequently, 10 ml of this stock solution was withdrawn and diluted with distilled water to attain a final concentration of 100 µg/ml within a 100 ml volume. For a subsequent dilution to reach a concentration of 15 µg/ml, 1.5 ml of the previously prepared solution was mixed with 10 ml of distilled water.

A total of 20 tablets of fabiflu were precisely weighed, and their average weight was determined. A quantity corresponding to 100 mg of favipiravir was solubilized in 100 ml of acetonitrile solvent. The resulting solution was subjected to sonication using an ultrasonic bath sonicator for a duration of 30 minutes. Subsequently, the solution was filtered, and 10 ml of the filtrate was diluted to a final volume of 100 ml with distilled water. Finally, a volume of 1.5 ml from the aforementioned solution was diluted with 10 ml of distilled water.

2.4 Method Validation [46]

2.4.1 Linearity

Linearity of this method was determined by preparing a serial dilutions with concentration

ranges of 5-25 µg/ml and their absorbance were measured. A graph was plotted between concentration and absorbance values.

2.4.2 Precision

%RSD was calculated by performing intra and inter day precision. A solution with concentration 15 µg/ml was prepared in six replicates and their absorbance was measured within a day and for two days.

2.4.3 Accuracy

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

2.4.4 Specificity

For the determination of specificity of this method, a blank solution was prepared and observed for any interference of absorbance of solvent with favipiravir absorbance.

2.4.5 Sensitivity

To ascertain the limits of detection (LOD) and quantification (LOQ), we employed the standard deviation of the response in conjunction with the slope of the regression equation.

3. RESULTS AND DISCUSSION

The present study targeted for the Establishment and Verification of a First-Order derivative UV Spectrophotometric technique for quantifying Favipiravir in bulk and Pharmaceutical formulations.

3.1 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 different solvents such as methanol, water. Acetonitrile, 0.1N HCl and 0.1N NaOH.

3.2 Selection of Solvent

The drug was soluble in methanol and acetonitrile. As per the literature review, it was found that existing methods were developed using methanol [5,8] and water [6,7]. For the further study, acetonitrile solvent was used as diluent for the preparation of solutions.

3.3 Selection of Detection Wavelength

In order to detect the wavelength for the measurement, a standard solution of concentration 10µg/ml was prepared and scanned in the UV range of 200-400nm in a UV spectrophotometer as shown in Fig. 2 and Fig. 3. Maximum absorbance was observed at a wavelength of 323nm and it was utilised for further investigation [5,10].

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

Table 1. Optical properties

S.No.	Parameters	Results
1	Absorption maximum	323 nm
2	Linearity Range	5 – 25 µg/ml
3	Regression Equation	y = 0.0275x + 0.0088
4	Slope	0.0275
5	Intercept	0.0088
6	Correlation coefficient (r)	0.9997
7	Molar extinction coefficient ($L.mol^{-1} cm^{-1}$)	4898
8	Sandell's sensitivity ($\mu g/cm^2$ -0.001absorbance units)	0.032
9	Accuracy (% recovery)	99.21 % - 101.61 %
10	Precision (Intra-day) % RSD	0.38
	(Inter-day) % RSD	0.39
11	LOD (µg/ml)	0.48
12	LOQ (µg/ml)	1.44
13	Standard error	0.0039
14	Specificity	Specific, No interference

Fig. 2. UV spectrum of favipiravir

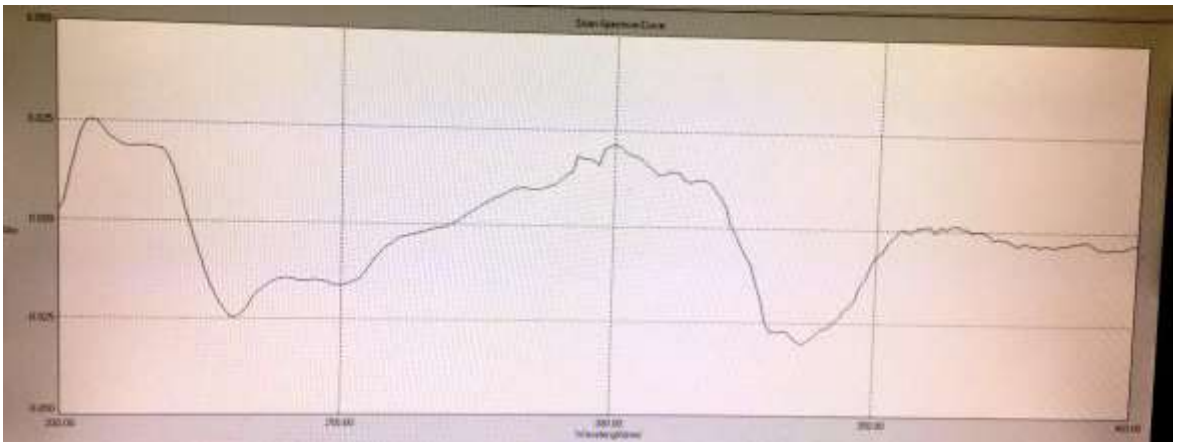

Fig. 3. 1st order derivative UV spectrum of favipiravir

For the determination of linearity of the method, serial dilutions in the range 5-25 µg/ml were prepared and absorbance was measured. Later A plot was created by representing concentration on the x-axis and absorbance values on the y-axis as shown in Fig. 4 and results were summarised in Table 2. From the graph, correlation coefficient values was determined and it was found to be 0.9997.

The %RSD (Relative Standard Deviation) for the intra-day precision analysis was determined to be 0.38, while for the inter-day precision analysis, it was found to be 0.39. These findings indicate that the developed method exhibits precision. Detailed precision results are available in Tables 3a and 3b.

The accuracy of the method was evaluated through the calculation of percentage recovery. The results revealed a range of 99.21% to 101.61% for percentage recovery, demonstrating the method's accuracy. A comprehensive summary of accuracy results is provided in Table 4.

The UV-Visible spectrum of standard solution when compared with that of blank solution, there was no interference observed in the blank spectrum, indicates that the method was specific. The blank spectrum was shown in Fig. 5.

Table 2. Results of linearity

S. No.	Concentrations (µg/ml)	Absorbance
1	5	0.156
2	10	0.283
3	15	0.425
4	20	0.561
5	25	0.692
Regression coefficient (r^2)		0.9994
Correlation coefficient (r)		0.9997

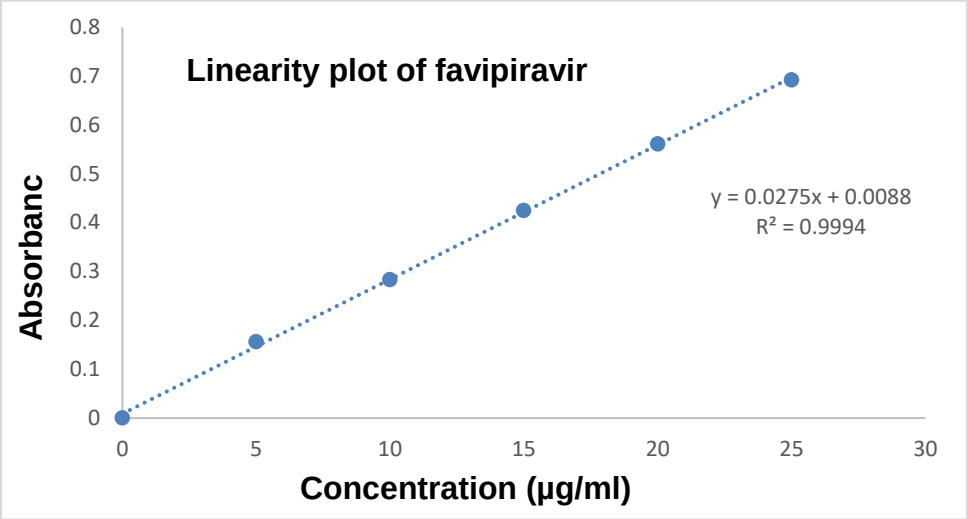

Fig. 4. Linearity plot of favipiravir

Table 3a. Intra-day precision results

Sample name	Sample absorbance	%Assay
1	0.425	99.83
2	0.426	100.07
3	0.423	99.36
4	0.427	100.30
5	0.423	99.36
6	0.424	99.60
Average	0.425	99.75
% RSD	0.38	0.38

Table 3b. Inter-day precision results

Sample name	Sample absorbance	% Assay
1	0.421	99.84
2	0.420	99.60
3	0.418	99.13
4	0.422	100.07
5	0.421	99.84
6	0.418	99.13
Average	0.420	99.60
% RSD	0.39	0.39

Table 4. Accuracy results

Sample No.	Level (in %)	Amount of Favipiravir added (mg)	Amount of Favipiravir found (mg)	% Recovery	Mean % Recovery
1	50	50.00	49.21	98.43	
2	50	50.00	49.68	99.37	99.21
3	50	50.00	49.92	99.83	
1	100	100.00	99.13	99.13	
2	100	100.00	99.13	99.13	99.21
3	100	100.00	99.37	99.37	
1	150	150.00	152.79	101.86	
2	150	150.00	152.33	101.55	101.61
3	150	150.00	152.09	101.39	

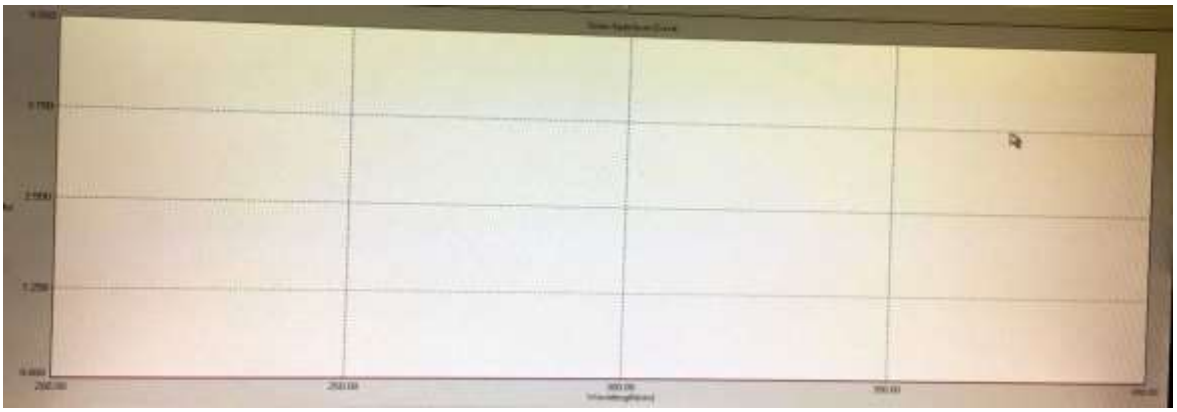

Fig. 5. Blank spectrum

LOD was found to be 0.48µg/ml and LOQ was found to be 1.44µg/ml, indicating the method to be sensitive.

4. CONCLUSION

A straightforward and innovative establishment and verification of a First-Order derivative UV Spectrophotometric technique for quantifying Favipiravir in Bulk and Pharmaceutical Formulations. The validation of the method was conducted in accordance with the guidelines prescribed by ICH. The developed method demonstrated accuracy, precision, linearity, specificity, and sensitivity. This method is readily applicable for routine analysis or quality control of favipiravir in formulations.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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