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Quantification Of Favipiravir By Analytical Methods In Bulk And Capsule Dosage Form

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ABSTRACT:

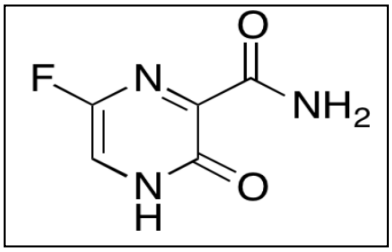
Favipiravir is an antiviral drug currently under development for the treatment of severe viral infections, including the coronavirus responsible for causing chronic diseases like COVID-19. In this study, we have developed and validated three different analytical methods, namely UV-Spectrophotometry (Method 1), NP-HPTLC (Method 2), and RP-HPLC (Method 3), for the quantitative estimation of Favipiravir in bulk and capsule dosage forms. These methods were found to be specific, sensitive, rapid, and cost-effective, making them suitable for routine analysis in pharmaceutical laboratories. Following the ICH guidelines, the developed methods were validated for linearity, accuracy, precision, ruggedness, and sensitivity.

KEYWORDS

Favipiravir, Covid-19, UV-Spectrophotometry, NP-HPTLC, RP-HPLC, Chronic & Antiviral**.**

INTRODUCTION

Favipiravir has emerged as a promising antiviral drug candidate in the treatment of COVID-19 and other viral infections. It is an isopropyl ester prodrug, which converts to an active nucleoside analogue (T-705-RTP or EIDD-1931) analogue of tent antiviral activity against various RNA viruses when metabolized in the plasma. The urgency of effective treatment options for COVID-19 has led to the Emergency Use Authorization (EUA) of Favipiravir by the U.S. Food and Drug Administration (FDA) for high-risk patients. Therefore, the development of reliable and efficient analytical methods for the quantification of Favipiravir in pharmaceutical formulations is crucial for quality control and therapeutic monitoring.



Favipiravir (DB12466) Structure:

Molecular Formula	$C_5H_4FN_3O_2$
Molecular Weight	157.104 $g \cdot mol^{-1}$
Chemical Name	6-fluoro-3-oxo-3,4-dihydropyridine-2-carboxamide
Description	Light yellow to yellow solid
Melting Point	187–193 °C
Solubility	Slightly soluble in water

Following the ICH guidelines, the developed methods were validated for linearity, accuracy, precision, ruggedness, and sensitivity agents

All chemicals and reagents were purchased from Merck Chemicals India's analytical grade. including Favipiravir standard, solvents, mobile phase components, and any other necessary materials.

2. Instrumentation

Mention the specific instruments used in each analytical method:

- UV-Spectrophotometry: SHIMADZU AUX – 120 (Weighing Balance) UV Shimadzu 2450 (PC Series) UV-visible double beam spectrophotometer.
- NP-HPTLC: HPTLC instrument and Camag TLC System with Linomat 5 Applicator used for the NP-HPTLC method.
- RP-HPLC: HPLC instrument (UFLC-LC 20 AD) equipped with a PDA detector used for the RP-HPLC method.

3. Standard Preparation

The stock standard solution was prepared by weighing 10 mg of Favipiravir. The weighed powder was transferred into a volumetric flask of 10 mL and dissolved and diluted to mark with methanol to obtain a concentration of n 1 mg/mL.

4. Method 1: Quantitative Estimation of Favipiravir by UV-Spectrophotometric Method

a. Zero Order UV-Spectrophotometric Method (Method A)

- The wavelength (235 nm) used for the analysis.
- The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in the sample within the range. Apprvolumese volumes in the range of 0.5-3.0 mL were transferred from the stock solution into a series of 10 mL volumetric flasks and volumes were made up to mark with the Methanol: Water (60:40 v/v) mobile phase to the concentration in the range of5-30 µg/mL. A constant volume of 20 µL for each sample was injected with the help of Hamilton Syringe. All measurements were repeated five times for each concentration and a calibration curve was constructed by plotting the peak area versus the drug concentration
- To assess the accuracy of the proposed method, recovery studies were carried out at three different levels i.e. 80, 100 and 120%. To pre-analyse sample solution a known amount standard rd drug solution was added at three different levels, and absorbance was recorded.

b. UV-Spectrophotometry using AUC (Method B)

- The wavelength range (228.00 – 243.40 nm) was used for AUC calculations.
- From the stock solutions, 1 mL of FVP was transferred to a 10 mL volumetric flask and the volume was adjusted to the mark with the same solvent to obtain concentrate ion 10 µg/mL. The solution was scanned in the UUV of ange 400 - 200 nm. AUC was selected in the wavelength range of 228.00 – 243.40 nm.

5. Method 2: Development and Validation of NP-HPTLC Method for Quantitative Estimation of Favipiravir in Bulk and Capsule Dosage Form

- NP-HPTLC aluminium plates precoated with silica gel 60-F254 TLC plates used.
- Mobile phase composition (Acetone: Chloroform: Formic acid, 4:6:0.1 v/v).
- The densitometric quantification for these drugs was carried out at 235 nm. Favipiravir obeyed linearity in the range of 500 – 3000 ng/band. The Rf of Favipiravir was found to be 0.55.

6. Method 3: RP-HPLC Method Development and Validation for Estimation of Favipiravir in Bulk and Capsule Dosage Form

- LC-GC Qualisil BDS C8 column (250 mm x 4.6 mm, 5 µm) used for separation.
- Mobile phase composition (Methanol: Water, 60:40 v/v) and the flow rate (1 ml/min).
- Wavelength (235 nm) at which the analyte was monitored.
- Retention time (4.1 min) of Favipiravir and the range of concentrations used for linearity (5 - 30 µg/mL).

7. Validation of Methods

Accuracy

To assess the accuracy of the proposed method, recovery studies were carried out a three different levels i.e. 80, 100 and 120%. Absorbapre-analyzeded to the pre - analyzed sample solution, a known amount standard drug solution was added at three different levels The results are reported in **Tables 4.1.4 and 4.1.5.**

Table 4.1.4: % Recovery Studies (Method A)

Drug	Initial Amount [µg/mL]	Amount added [µg/mL]	Amount Recovered [µg/mL, n=3]	% Recovered	% RSD
	15	12	11.99	99.97	0.23
FVP	15	15	14.90	99.34	0.23
	15	18	17.84	99.15	0.17

Table 4.1.5: % Recovery Studies (Method B)

Drug	Initial Amount [µg/mL]	Amount added [µg/mL]	Amount Recovered [µg/mL, n=3]	% Recovered	% RSD
	15	12	11.94	99.50	0.14
FVP	15	15	14.93	99.56	0.13
	15	18	17.93	98.85	0.37

Precision

Precision of the method is studied as repeatability, intra-day and inter-day precision. Repeatability was determined by analyzing FVP (15 µg/mL) for six times and the results are reported in **Table 4.1.6.** Intra-day precision was determined by analyzing the10, 15 and 20 µg/mL of FVP for three times in the same day.

Inter-day precision was determined by analyzing the same concentration of the solutions daily for three days, results are reported in **Table 4.1.7.**

Table 4.1.6: Repeatability Studies

Drug	Amount Taken [µg/mL]	Method A Amount found [µg/mL]	% Amount found [n=6]	Method B Amount found [µg/mL]	% Amount found
	15	15.06	100.46	15.09	100.65
	15	15.04	100.30	15.02	100.19
FVP	15	14.88	99.21	15.07	100.52
	15	15.21	101.42	15.06	100.46
	15	15.09	100.66	15.12	100.84
	15	14.91	99.46	15.14	100.95
	Mean ± SD	15.03 ± 0.12	100.2 ± 0.81	15.09 ± 0.03	100.60 ± 0.24
	% RSD	0.80	0.80	0.24	0.24

n-number of determinations

Table 4.1.7: Precision Studies [Intra-day and Inter-day]

	Method A			Method B		
Standard Concentration (µg/mL)	**Amount Found [µg/mL]**	**% Amount found**	**% RSD**	**Amount found [µg/mL]**	**% Amount found**	**% RSD**
Intra-day Precision						
10	10.1154	101.15	1.84	10.0432	100.43	0.073
15	14.7642	98.42	1.43	14.8212	98.80	1.30
20	19.9926	99.96	0.45	19.7255	98.62	1.068
Inter-day Precision						
10	9.7073	97.07	1.31	9.8297	98.29	1.69
15	15.0081	100.05	1.67	14.8921	99.28	0.71
20	19.1780	95.89	0.64	19.30	96.5	1.09

Sensitivity

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantification (LOQ). The LOD and LOQ were calculated by the use of the equation LOD = 3.3 X ASD/S and LOQ = 10 X ASD/S; where, 'ASD' is Average standard deviation of the peak height and areas of the drug (n = 3), taken as a measure of noise, and 'S' is the slope of the corresponding calibration curve.

Different volume of stock solution in the range 5-10 µg/mL was prepared. The procedure was repeated in triplicate. LOD and LOQ was found to be **1.1 µg** and **3.3 µg** (**Method A**), and **1.2µg** and **3.6 µg** (**Method B**), respectively.

Ruggedness

Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slot by two analysts using same operational and environmental conditions and the results are reported in **Table 4.1.8.**

Table 4.1.8: Ruggedness Studies

	Method A		**Method B**	
Analysts	**[%] Amount found ± SD [n= 6]**	**% RSD**	**[%] Amount found ± SD [= 6]**	**% RSD**
I	99.85 ± 0.40	0.30	100.36 ± 0.97	0.16
II	99.76 ± 0.43	0.34	100.20 ± 0.95	0.35

n-number of determinations

Method 2

Precision studies

Repeatability, Intra-day and Inter-day precisions were perceived using six repetitive measurements in target concentration level.

The precision of the proposed method was ascertained by actual determination of three replicates of 1000, 1500 and 2000 ng/band concentration of Favipiravir finding out the result by the proposed method. Repeatability expresses the precision under the same operating conditions over a short interval of time.

Repeatability is also termed intra-assay precision. So, the intra assay precision has been executed by analyzing samples 1500 ng/band of Favipiravir for six times. The precision of developed method was evaluated in terms of % RSD.

The results are shown in **Table 4.2.4 and 4.2.5.**

Table 4.2.4: Precision Studies [Intra and Inter-day]

Standard Concentration (ng/band)	Amount Found [ng/band] [n=3]	% Amount found	% RSD
Intra-day Precision			
1000	999.44	99.94	0.67
1500	1483.37	98.89	0.10
2000	1984.42	99.22	1.94
Inter-day Precision			
1000	990.84	99.08	1.58
1500	1459.03	97.26	1.89
2000	1957.99	97.89	0.69

n- number of determinations Table 4.2.5: Repeatability Studies

Drug	Amount taken [ng/band]	Amount found [ng/band]	% Amount found
FVP	1500	1493.32	99.55
	1500	1493.10	99.54
	1500	1492.19	99.47
	1500	1492.67	99.51
	1500	1492.94	99.52
	1500	1492.04	99.46
	Mean ± SD	1492.71 ± 0.51	99.51 ± 0.034
	% RSD	0.034	0.034

Accuracy

Accuracy study was executed by standard addition method using three different levels. Recovery experiment was evaluated by over spotting the drug standard at 80 %, 100 % and 120 % to the pre - analyzed sample and the results were re-analyzed by proposed HPTLC method. The experiment was repeated three times.

The results are shown in **Table 4.2.6.**

Table 4.2.6: Recovery Studies

Drug	Initial Amount [ng/band]	Excess drug added to the analyte	Total amount found ± S.D. [µg/mL]	Recovery [%] [n=3]	%RSD [n = 3]
FVP	1500	1200	2683.76 ± 48.27	98.64	0.38
	1500	1500	2993.03 ± 53.48	99.53	0.38
	1500	1800	3268.61 ± 50.28	98.25	0.33

n- number of determinationsRuggedness

The ruggedness of the method was performed by two different analysts using same operational and environmental conditions. The ruggedness of the proposed method was determined by 1500 ng/band concentration of Favipiravir.

The results are shown in **Table 4.2.7**.

Table 4.2.7: Ruggedness studies

Drug	Concentration[ng/band]	Amount Found (%) ± S.D.	
		Analysts- I [n=6]	Analysts- II [n=6]
FVP	1500	99.05 ± 0.59	98.76 ± 0.81

n-number of determinations

Method 3

Accuracy

To assess the accuracy of the proposed method, recovery studies were carried out three different levels i.e. 80, 100 and 120%. To the pre - analyzed sample solution a known amount standard drug solution was added at three different levels, absorbance was recorded. The results are reported in Table 4.1.5.

Drug	Initial Amount [µg/mL]	Excess Drug Added to the Analyte	Total Amount Found ± SD [µg/mL]	Recovery [%] [n=3]	% RSD [n=3]
FVP	15	12	26.77 ±3169.56	98.09	0.18
	15	15	29.73 ± 2158.95	98.25	0.11
	15	18	32.86 ± 1089.69	99.24	0.05

Precision

The precision of the method was studied as repeatability, intra-day and inter-day variations. The precision of the proposed method was ascertained by actual determination of three replicates of 10, 15 and 20 µg/mL concentrations of FVP. The precision of the developed HPLC method was found to be precise as the RSD values for repeatability and intra-day and inter-day precision studies were < 2%, respectively as recommended by ICH guidelines.

Repeatability was measured by analyzing 15 µg/mL of Favipiravir for six times. The results are shown in **Table 4.3.6** and **Table 4.3.7.**

Table 4.3.6: Precision Studies [Intra and Inter-Day]

Standard Concentration [µg/mL]	Amount Found [µg/mL]	% Amount Found [µg/mL] [n=3]	% RSD
Intra-day Precision			
10	9.72	97.24	0.92
15	14.88	99.21	0.24
20	19.42	97.10	1.50
Inter-day Precision			
10	9.76	97.69	0.81
15	14.86	99.10	0.17
20	19.25	96.28	1.55

n=number of determinations

Table 4.3.7: Repeatability Studies

Drug	Amount Taken [µg/mL]	Amount Found [µg/mL] [n=6]	% Amount Found
FVP	15	14.91	99.44
	15	14.85	99.02
	15	14.85	99.02
	15	14.84	98.95
	15	14.84	98.95
	15	14.99	99.99
	Mean ± SD	14.88 ± 0.06	99.23 ± 0.41

% RSD	0.41	0.41

Sensititvity

The sensitivity measurements of Favipiravir by use of the proposed methods were estimated in terms of the Limit of Detection (LOD) and Limit of Quantification (LOQ). The LOD and LOQ were calculated using equation LOD = 3.3 x N/B and LOQ = 10 x N/B, where 'N' is the standard deviation of the results of the drugs (n=3),taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve. Different concentrations i.e. 5, 6, 7, 8, 9 and 10 µg/mL were selected for the method. The Average Standard Deviation (A.S.D.) of peak areas was determined. For determination LOD and LOQ slope of the corresponding calibration curve was considered. LOD and LOQ were found to be **0.44 µg** and **1.34 µg** for Favipiravir.

Ruggedness

An appropriate concentration of Favipiravir (15 µg/mL) were prepared and analyzed by two different analysts using similar operational and environmental conditions.

Peak area was measured for same concentration solutions, six times. The results are shown in **Table 4.3.8.**

Table 4.3.8: Ruggedness Studies

Drug	**Concentration [µg/mL]**	**Amount Found (%) ± SD**	
		Analyst-I [n=6]	**Analyst-II [n=6]**
FVP	15	98.87 ± 0.179	98.18 ± 1.294

n= number of determinations

10. Data Analysis

Method 1: UV-Spectrophotometry

Data Analysis:

- The data obtained from the UV-Spectrophotometer involved measuring the absorbance of Favipiravir at a specific wavelength (235 nm).
- Linearity was established by plotting a calibration curve using different concentrations of Favipiravir standard solutions. The data points were fitted to a linear regression equation to determine the relationship between concentration and absorbance.
- The correlation coefficient (r^2) was calculated to assess the goodness of fit of the calibration curve.

Software Used for Data Processing and Calculations:

- Microsoft Excel: Excel was used to organize the raw data, perform calculations for the calibration curve, and plot the graphical representation of the calibration curve.

Method 2: NP-HPTLC

Data Analysis:

- The data obtained from NP-HPTLC involved recording the peak areas of separated spots for Favipiravir in the samples.
- Linearity was established by plotting a calibration curve using different concentrations of Favipiravir standard solutions. The data points were fitted to a linear regression equation to determine the relationship between concentration and peak area.
- The correlation coefficient (r^2) was calculated to assess the goodness of fit of the calibration curve.

Software Used for Data Processing and Calculations:

- Camag HPTLC Software: The Camag HPTLC Software was used to analyze the raw data obtained from NP-HPTLC, calculate peak areas, construct the calibration curve, and visualize the data graphically.

Method 3: RP-HPLC

Data Analysis:

- The data obtained from RP-HPLC involved chromatographic peaks corresponding to Favipiravir at a specific retention time (4.1 min).
- Linearity was established by plotting a calibration curve using different concentrations of Favipiravir standard solutions. The data points were fitted to a linear regression equation to determine the relationship between concentration and peak area or height.
- The correlation coefficient (r^2) was calculated to assess the goodness of fit of the calibration curve.

Software Used for Data Processing and Calculations:

- HPLC Data Processing Software: The HPLC Data Processing Software associated with the UFLC-LC 20 AD HPLC system was utilized for data processing, peak integration, calibration curve construction, and visualization of the results.

Overall Data Interpretation:

For all methods, the results obtained from the data analysis were interpreted to validate the linearity of the calibration curves and the accuracy of the methods for quantifying Favipiravir in bulk and capsule dosage forms. The correlation coefficients were used to assess the goodness of fit, and high correlation values close to 1 indicated excellent linearity between the concentrations and the corresponding response (absorbance, peak area, or height).

RESULTS

Method 1

Quantitative Estimation Of Favipiravir By UV- Spectrophotometric Method

Two simple, specific, sensitive, rapid and economical UV-Spectrophotometric methods have been established for the determination of Favipiravir in bulk and capsules. **Method A** is zero order UV-Spectrophotometry using absorbance, and**Method B** is zero order UV-Spectrophotometry using AUC. The anticipated methods were effectively applied for the determination of Favipiravir in bulk and capsules.In water, Favipiravir showed maximum absorbance at **235 nm**. In **Method A**, the absorbance was recorded at 235 nm, while in **Method B**, AUC was selected in the wavelength range of **228.00 – 243.40 nm**. In all methods, Favipiravir followed linearity in the concentration range of 5-30 µg/mL with (r^2 >0.999). All these developed methods were applied for the estimation of Favipiravir capsules. All these methods were validated for linearity and range, accuracy, precision, ruggedness and sensitivity.

The summary of all these methods are given in **Table 5.1 Table 5.1:**

Summary of developed method

		Methods	
Parameters		A	B
Accuracy [n=3]	80%	99.97	99.50
	100%	99.34	99.56
	120%	99.15	98.85
Precision (% RSD)	Intra-day (n=3)	1.24	0.81
	Inter-day (n=3)	1.20	1.16
Repeatability (%RSD)		0.80	0.24
Ruggedness (%RSD)	Analyst-I	0.30	0.16
	Analyst-II	0.34	0.35
LOD (µg)		1.1	3.3
LOQ (µg)		1.2	3.6

Method 2

Development and Validation of NP-HPTLC Method for Quantitative Estimation of Favipiravir in Bulk and Capsule Dosage Form

A NP-HPTLC method has been developed for the determination of Favipiravir pharmaceutical formulation. The separation of Favipiravir was performed on NP- HPTLC aluminum plates precoated with silica gel 60-F254 TLC plates using **Acetone: Chloroform: Formic acid (4:6:0.1 *v/v*)** as mobile phase. The densitometric quantification for these drugs was carried out at 235 nm. Favipiravir obeyed linearity in the range of 500 – 3000 ng/band. The Rf of Favipiravir was found to be 0.55. The proposed method was applied for pharmaceutical formulation and % amount found for Favipiravir was found to be 99.45 ± 0.042. The method was validated for accuracy, precision, and ruggedness. Accuracy of the method was checked by recovery studies at three different levels i.e. 80 %, 100 %, and 120 %. The % recovery of Favipiravir was found to be in the range of 98.25-99.53 %; the % RSD values were less than 2 indicate the accuracy of the method. The method was found to be precise as indicated by the inter-day, intra-day and repeatability analysis; showing % RSD less than 2. The results did not show any statistical difference between operators suggesting that method developed was rugged. The results of the developed method are shown in **Table 5.2.**

Method 3

The RP-HPLC method was developed and validated for the estimation of Favipiravir in bulk and capsule dosage form. The separation was achieved using a LC-GC Qualisil BDS C8 column (250 mm x 4.6 mm, 5 µm). During separation mobile phase consist of Methanol and Water (60:40 v/v) was delivered at a rate of 1 ml/min. The analyte was monitored with PDA detector at 235 nm. The method was found to be linear in the range of 5 to 30 µg/ml. The retention time for analyte was found 4.1 min. The linear response is observed in the range of 5 - 30 µg/mL. A same optimized method has successively been applied for the determination of Favipiravir in the capsules formulation. The drug content for Favipiravir was found to be 99.25 ± 0.18%. Accuracy of the method was studied by the recovery studies at three different levels i.e. 80 %, 100 %, and 120 % level. The % recovery was found to be within the limits of the acceptance criteria within range of 98.09– 99.24%. The precision of the method was studied in terms of repeatability, intra-day, and inter-day precision. The results were examined as % RSD values of concentration of drug determined. The low value of %RSD (less than 2) indicates high precision of the method. The method proved to be adequately sensitive as indicated by low values of DL and QL. The Summary of the developed Method shown in **Table 5.2.**

Table 5.2: Summary of developed methods

Parameters	NP-HPTLC	RP-HPLC
Drug	FVP	FVP
Linearity range	500 – 3000 (ng/band)	5 - 30 (µg/ml)
Correlation coefficient	0.999	0.999
LOD	47.51 ng/band	0.44 µg
LOQ	143.99 ng/band	1.34 µg
% Recovery (n=3)	98.80	98.52
Precision (%RSD)		
Intra-Day(n = 3)	0.90	0.88
Inter-Day(n = 3)	1.38	0.84
Repeatability (n=6)	0.034	0.41
Ruggedness (%Amount Found)		
Analyst-I	99.05	98.87
Analyst-II	98.76	98.18

CONCLUSION:

Three methods RP-HPLC, NP-HPTLC and UV-Spectrophotometry (Zero order spectroscopy and zero order AUC) have been developed for Favipiravir in bulk and capsules. RP-HPLC and HPTLC methods are found to be accurate, precise, rugged and robust. Both these methods are adequately sensitive. Two UV-Spectrophotometric methods using absorbance and Area Under Curve techniques has been developed for estimation of Favipiravir in bulk and capsules. UV-Spectrophotometry methods are simple, accurate and economical and least calculations are involved for estimation of concentrations of Favipiravir in bulk and capsules. All these methods may regularly be used for estimation of Favipiravir in its pharmaceutical formulation

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