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Green Analytical Approach: Quantitative Estimation of Favipiravir In Bulk and Pharmaceutical Dosage form using Fourier Transform-Infrared Spectroscopy

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Abstract

The quantitative analysis of favipiravir was conducted on its solid form, employing the KBr pellet method. To ensure the robustness and reliability of the method, it was validated by the guidelines of the International Conference on Harmonization (ICH). The calibration curve was constructed by plotting the concentration of favipiravir against the corresponding absorbance intensity. This curve demonstrated a linear relationship within the concentration range of 5-15 mg, characterized by a correlation coefficient exceeding 0.999. Limits of detection and quantification were found to be 0.30µg and 0.90µg respectively. The method was applied to assess the % assay of a marketed dosage form of favipiravir, yielding a value of 99.6%. One of the distinguishing features of this method is its economy and eco-friendliness. It avoids using organic solvents, making it an environmentally conscious choice, while proving to be a cost-effective analytical tool.

Keywords: FT-IR Spectroscopy, Favipiravir, ICH, Validation, Antiviral

Full length article **Corresponding Author*, e-mail: : achaiah_g@yahoo.co.in

1. Introduction

Fourier Transform Infrared Spectroscopy (FTIR) has emerged as a powerful analytical technique used in pharmaceutical sciences. The term Fourier transformation originated from a mathematical operation demonstrated by 'Jean Fourier' which converts the frequency domain into the time domain [1]. FTIR serves as a non-destructive, highly sensitive, highly specific, and robust analytical technique by which almost any solid, liquid, or gas sample can be analyzed with little or no sample preparation procedure without using any expensive, toxic solvents and reagents, so it is economical as well as environmentally friendly technique. FTIR is a rapid technique compared to HPLC, it consumes much less time, and bypasses the mobile phase preparation, running, and column washing time [2]. FT-IR is an excellent technique for pharmaceutical analysis that offers many advantages since it is easy to use, sensitive, selective, green, and fast (the total analysis time including making the pellets, measurement, identification, and report generation is lower than 10 minutes) [3]. For many drugs not containing the chromophoric group, it is very difficult to develop a method by UV or HPLC due to its molecular structure, but the method can be easily developed by using IR for such drugs.

Favipiravir, a well-established antiviral drug used in the treatment of influenza, has gained significant attention in the ongoing battle against COVID-19. Notably, it stands as the first approved oral antiviral drug for the treatment of mild to moderate COVID-19 cases. Favipiravir is a novel broad-spectrum antiviral drug against RNA viruses that has been approved for the treatment of seasonal and pandemic influenza [4]. Favipiravir is a prodrug and its active form, favipiravir ribofuranosyl-triphosphate (FTP), inhibits viral replication by targeting the RNA-dependent RNA polymerase (RdRp). Favipiravir has a chemical structure of 6-fluoro-3-hydroxy-2-pyrazine carboxamide.

Importantly, favipiravir triphosphate is a broad-spectrum antiviral agent and shows inhibitory activities against the RNA polymerases present in influenza A viruses. Highly pathogenic H5N1 viruses and many other positive- and negative-sense RNA viruses also belong to the group of influenza A virus and hence favipiravir is active against them. Recently, favipiravir was found effective in curing patients infected with Ebola virus (EBOV). It also inhibits the growth of human norovirus and human arenaviruses [5]. It is a light yellow to yellow solid with a molecular formula of C5H4FN3O2 and a molecular weight of 157.104 g/mol. Its chemical structure is depicted in Figure 1.

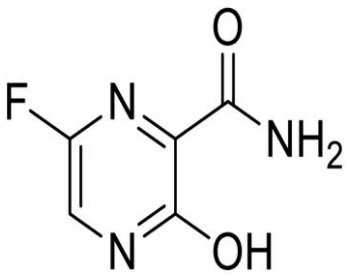

Figure 1: Chemical structure of Favipiravir

There are few published research studies on the quantitative estimation of favipiravir using different analytical methods, including Spectrophotometric [6], RP-HPLC [7-11], and LCMS/MS[12]. The FTIR spectroscopy method presents itself as a promising, simple, faster, direct, sensitive, selective, and relatively cost-effective alternative for determining the content of favipiravir in pharmaceutical formulations while ensuring sufficient reliability. However, it is worth noting that not many methods for the analysis of favipiravir using FTIR have been reported. Nithila et al. (2022) did report an FTIR method for favipiravir, selecting the peak at 3353 cm^{-1}, with LOD and LOQ values of 5.8 and 17.8 µg/mg, respectively [13]. The primary objective of this study is to develop and validate a method for the quantitative estimation of favipiravir in bulk and pharmaceutical dosage forms, specifically tablets. This newly developed method boasts significantly improved sensitivity, as reflected in LOD and LOQ values that are notably lower than the previously reported figures of 5.8 and 17.8 µg/mg.

2. Materials and Methods

Chemicals and reagents:

The Favipiravir (FVP) sample was procured from Chandra Laboratories, Hyderabad, India. Fabiflu® tablets, each containing 200 mg of Favipiravir (manufactured by Glenmark), were purchased from a local drugstore. Potassium bromide (KBr) of IR grade was sourced from Merck, Mumbai, India. All other chemicals and reagents utilized in the study were of analytical grade.

Instrumentation

The infrared spectra of solid samples were recorded across a spectral range spanning from 4000 to 650 cm^{-1} using a Perkin Elmer FT-IR spectrophotometer, equipped with Opus software. The baseline technique was employed for the analysis of FT-IR spectra of FVP in absorbance mode.

Analysis of solid samples (using KBr pellet)

The infrared spectrum of favipiravir, spanning a frequency range of 4000–650 cm^{-1}, was acquired using the KBr pellet method to identify the characteristic absorption peaks associated with the stretching vibrations of various functional groups present in favipiravir. The values of the amount of light incident on the sample (P_0) and the amount of light transmitted through the sample (P) were determined using the infrared spectra of solid samples obtained through the KBr pellet technique. The quantitative analysis was conducted utilizing the regression method.

a) Blank Preparation:

KBr was ground into a fine powder using a mortar and pestle. From this powder, a 400 mg KBr pellet was prepared using a hydraulic press. This pellet was then placed into a KBr holder and inserted into an IR Spectrophotometer, where it underwent scanning in the spectral range from 4000 cm^{-1} to 650 cm^{-1} to enable quantitative measurement. A resolution of 4 cm^{-1} was chosen, and the scanning process involved 16 scans to generate the FTIR spectrum.

b) Standard Preparation:

Approximately 780 mg of KBr powder and 20 mg of FVP Working Standard were finely powdered using a mortar and pestle. The mixture was subsequently dried in a hot air oven for 15 minutes at 60 °C. The resulting FVP-KBr blend was used to create a homogenous pellet, weighing 400 mg, with the help of a hydraulic press. This pellet was then transferred to a KBr holder and introduced into the IR Spectrophotometer. It underwent scanning within the spectral range from 4000 cm^{-1} to 650 cm^{-1} for quantitative measurements to identify the characteristic absorption peaks corresponding to the stretching vibrations of various functional groups present in favipiravir. The FTIR spectrum for the FVP standard is depicted in Figure 2. The characteristic absorption peaks associated with the stretching vibrations of various functional groups in favipiravir are detailed in Table 1. The prominent and characteristic peak of FVP observed at 1434 cm^{-1} due to C-F stretching vibrations, was chosen for analytical purposes. The optimized FTIR conditions are outlined in Table 2.

c) Sample Preparation

Ten tablets of FABIFLU® were weighed, and crushed into a powder, and the average weight was calculated. A portion of the powder, equivalent to 32 mg of FVP, was weighed and then added to 668 mg of KBr powder in a mortar. The mixture was thoroughly blended with the aid of a pestle. The FVP-KBr combination was subsequently dried in a hot air oven for 15 minutes at 60 °C. From the resulting FVP-KBr mixture, a homogenous pellet weighing 400 mg was prepared using a hydraulic press. This pellet was then transferred to a KBr holder and inserted into an IR Spectrophotometer, where it underwent scanning in the spectral range spanning from 4000 cm^{-1} to 650 cm^{-1} for quantitative measurements. The FTIR spectrum for the sample was recorded and depicted in Figure 3.

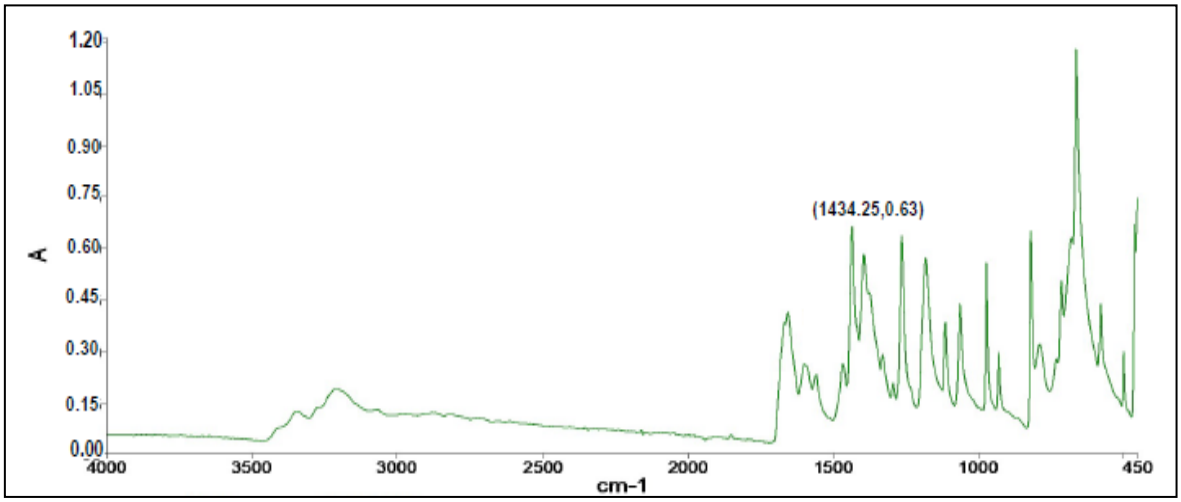

Figure 2: Standard FTIR spectrum of favipiravir (FVP)

Table 1: IR spectral analysis of Favipiravir

Wavenumber (cm^{-1})	Functional group
3259.19	N-H stretch
1692.86	C=O stretch
1646.03	C=C stretch
1434.25	C-F stretch

Table 2: Optimized FTIR conditions for Favipiravir

S.No	Parameters	Optimized conditions
1	Method of making pellets	Direct mixing method
2	Modes of Measurement	Absorbance
3	Weight of the final pellet	400 mg
4	Number of scans	16
5	Peak selection	1434 cm^{-1}

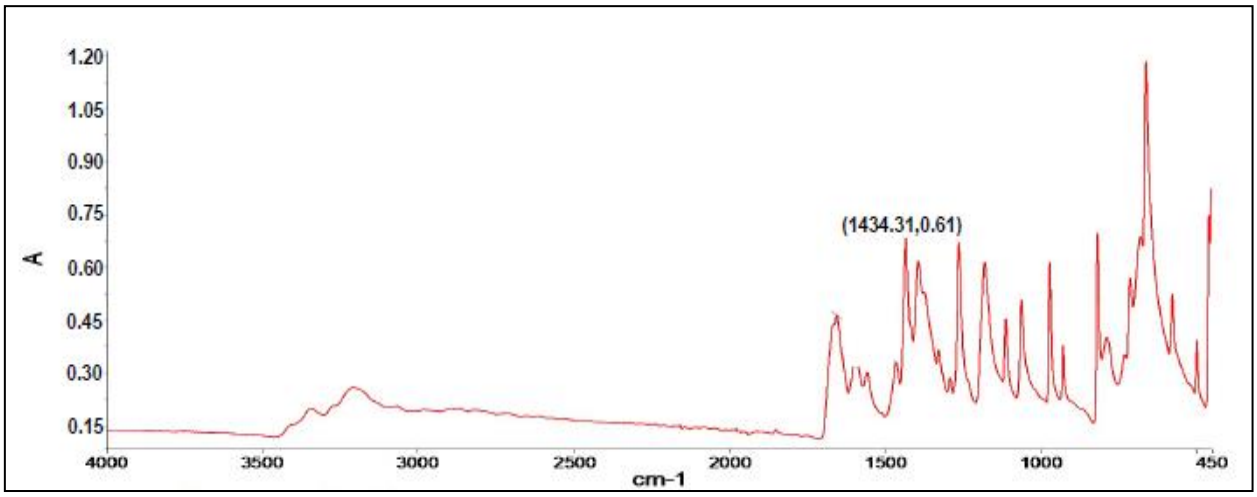

Figure 3: Sample (tablet) FTIR spectrum of favipiravir (FVP)

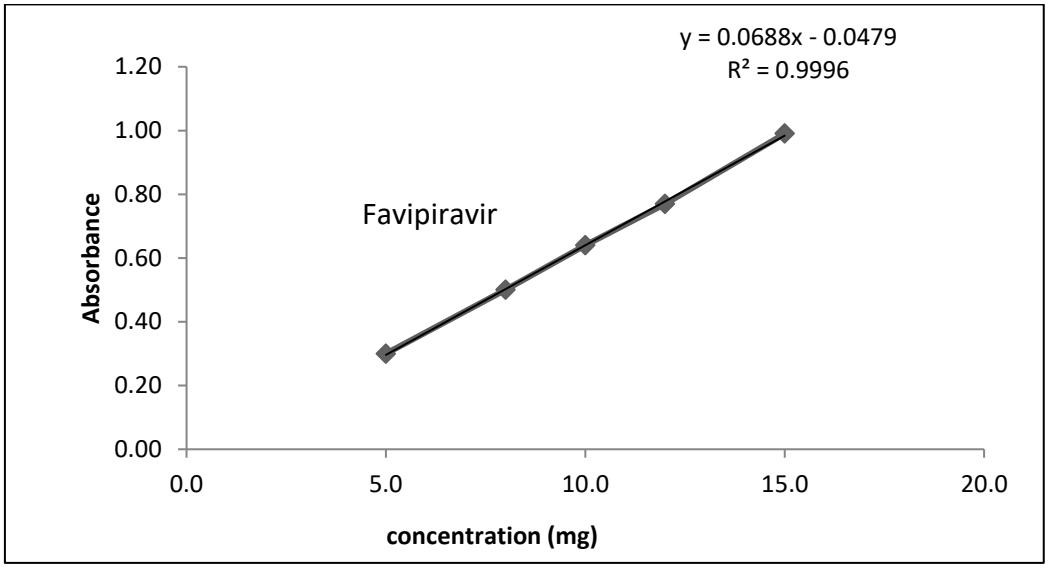

Figure 4: Standard calibration curve of favipiravir

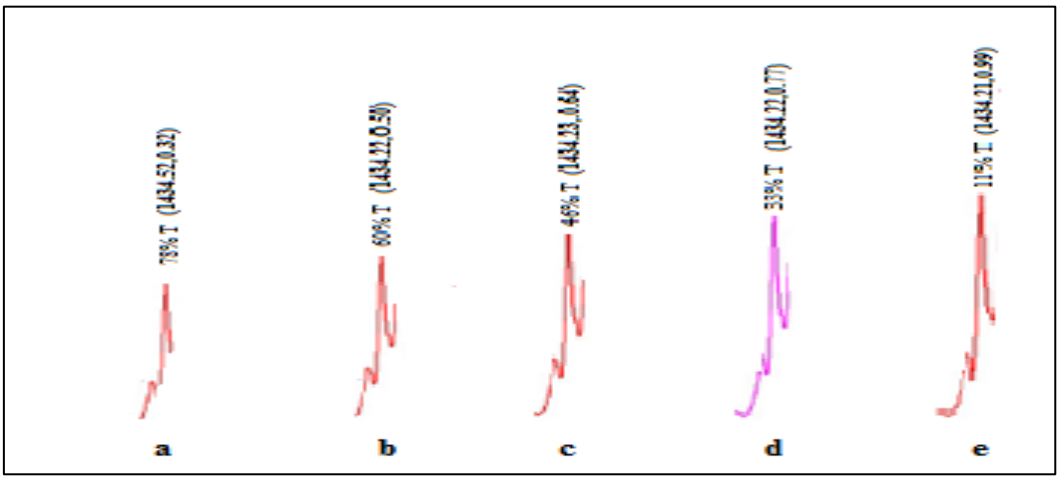

Figure 5: C-F stretching vibrations of favipiravir at various concentrations
a) 5 % b) 8 % c) 10 % d) 12 % and e) 15 % w/w

Table 3: Linearity data results

S.No	Concentration (mg)	Absorbance
1.	5.0	0.30
2.	8.0	0.50
3.	10.0	0.64
4.	12.0	0.77
5.	15.0	0.99

Table 4: Standard calibration data of favipiravir

Parameter	Conditions
Linearity range	50-150% w/w
Wavenumber	1434 cm^{-1}
Regression equation	y = 0.068x-0.047
R^2	0.999
Slope	0.068

Table 5: Accuracy results of Favipiravir

Spiked level	Amount added (mg)	Amount recovered (mg/g)	% recovery	Mean % recovery
50%	5	4.92	98.40	98.13
	5	4.91	98.20	
	5	4.89	97.80	
100%	10	9.94	99.40	98.87
	10	9.89	98.90	
	10	9.83	98.30	
150%	15	14.69	97.93	98.09
	15	14.76	98.40	
	15	14.69	97.93	

Table 6: Method precision results of FVP

Conc. (%w/w)	% Assay of FVP
10	99.71
10	99.21
10	99.52
10	100.28
10	99.24
10	99.51
Average	99.58
S.D	0.392
% R.S.D	0.394

Table 7: Results of Ruggedness

Conc. (%w/w)	%Assay	
	Analyst-01	Analyst-02
10	99.71	99.67
10	99.21	98.12
10	99.52	101.34
10	100.28	98.12
10	99.24	99.73
10	99.51	101.34
Average	99.58	99.72
S.D	0.392	1.439
%RSD	0.394	1.443

Table 8: Limit of detection and Limit of quantification results

S.No.	Parameter	Measured values (µg/g)
		Favipiravir
1.	Limit of detection	0.2977
2.	Limit of quantification	0.9020

Table 9: Robustness results of Favipiravir

	Absorbance	
	Pressure applied	
Conc. (%w/w)	8 tons	10 tons
10	0.55	0.44
10	0.54	0.45
10	0.53	0.44
10	0.53	0.43
10	0.54	0.44
10	0.53	0.45
Average	0.54	0.44
S.D	0.008	0.008
% R.S.D	1.521	1.704

Table 10: Assay results of FVP (Fabiflu®)

Label claim (mg)	Amount found mg)	% Assay
200	199.44	99.72

4.4 Method validation

Linearity:

The linearity of the method developed was elucidated by linear regression analysis and is measured using the least square method. A series of standard concentrations of FVP was prepared and analyzed with FTIR at six different concentration levels (50%, 75%, 100%, 125%, and 150%) i.e., 5-15 mg. It was observed that the absorbance value of a particular concentration is also affected by a change in pellet weight (> 5 %). Therefore, 400 mg of the freshly prepared homogenous fine powder was taken each time and a force of 10 tons was applied to prepare the pellet. Calibration curves for the standard solutions were plotted against respective concentrations (w/w) on the x-axis with their absorbance values on the y-axis and are depicted in Figure 4. Slope-a, intercept-b, and correlation coefficient (R2) were determined by applying a linear regression equation. It was observed that with an increase in the concentration, the % transmittance value of the selected peak decreased, whereas the intensity and absorbance values of the selected peak increased as depicted in Figure 5. The peak areas of FVP exhibited linearity as concentrations increased within the specified range, and the correlation coefficient was greater than 0.99, confirming the linearity of the method.

Accuracy:

The method's accuracy was assessed through recovery studies. At three concentration levels (50%, 100%, and 150%), known quantities (5 mg, 10 mg, and 15 mg) of standard favipiravir were added to a known quantity of a placebo (formulation blank). Pellets of spiked concentrations were then prepared, and their absorbance was measured. The percentage recovery and mean percentage recovery of FVP at each level were calculated, and the results are presented in Table 5. The mean % recovery at all levels fell within the acceptable limits of 98% to 102%, signifying the accuracy of the method.

Method precision

Method precision was ascertained by preparing six replicate working sample pellets, each at a concentration of 100% (10 mg). The FTIR spectra of these six preparations were recorded. Subsequently, the %RSD of the assay results for the six preparations was calculated and is presented in Table 6. The %RSD value obtained falls within the acceptable criteria, confirming the precision of the method.

Intermediate Precision (Ruggedness) / interday precision

Intermediate Precision (Ruggedness) was evaluated by different analysts on two separate days. This involved preparing six replicate working sample pellet preparations, each at 100% concentration (10 mg), and recording their FTIR spectra. The %RSD of the assay results for the six preparations, obtained by different analysts on two different days, has been calculated and is presented in Table 7. The % Assay and %RSD values obtained fall within the acceptance criteria, indicating that the method is rugged.

Limit of detection (LOD) and limit of quantification (LOQ):

LOD and LOQ represent the minimum detectable and quantifiable amounts of the analyte. The LOD and LOQ values for FVP were determined using the proposed method, based on the signal-to-noise ratio, and are presented in Table 8.

Robustness:

Robustness testing involved minor systematic variations in the optimized spectroscopic conditions, such as altering the applied pressure for pellet preparation from 10 tons to 8 tons. The %RSD of absorbance values from six preparations under each varied pressure condition was calculated and is presented in Table 9. The slight variation of 2 tons in the pressure applied for pellet preparation did not have a significant impact on the absorbance. The %RSD of FVP remained within the acceptable limits (not more than 2), even with this minor pressure variation, confirming the robustness of the method.

ASSAY

The commercial tablet (Fabiflu®) was analyzed using FTIR with six replicate translucent pellet preparations of both standard and sample working concentrations (100%), and their spectra were recorded. The amount of the drug present in the marketed tablets and the percentage of favipiravir content was calculated using the following equations.

$$Ca = Aa \times Cb / Ab$$

$$\% Ca = Ca/Ct \times 100$$

Were,

Ca: concentration of favipiravir in the sample (%w/w)

Aa: absorbance intensity of the sample pellet

Cb: concentration of favipiravir in the standard

Ab: absorbance intensity of the standard pellet

Ct: theoretical concentration of favipiravir in the sample

The assay values of the formulation were found to be within the acceptance criteria, indicating that the interference of the excipient's matrix is insignificant in the estimation of FVP by the proposed method.

4. Conclusions

In conclusion, the developed method presents a compelling alternative to existing techniques. Its exceptional time efficiency, non-destructiveness, sensitivity, environmental friendliness, and cost-effectiveness collectively position it as a superior analytical tool. This method not only streamlines the analytical process but also aligns with sustainable and responsible laboratory practices,

underscoring its potential to advance scientific research and quality control efforts across various applications. Hence, this method can be of use in routine quantitative analysis of Favipiravir in the pharmaceutical industry.

Acknowledgement

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Conflict Of Interest

The authors declare that they have no conflict of interest.

Abbreviations

FTIR: Fourier Transform Infrared Spectroscopy; FVP: Favipiravir; ICH: International Conference on Harmonisation; SD: Standard Deviation; RSD: Relative Standard Deviation; LOD: Limit of Detection; LOQ: Limit of Quantification

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