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Original Article

NEW FTIR METHOD DEVELOPMENT AND VALIDATION FOR QUANTITATIVE ANALYSIS OF FAVIPIRAVIR IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Objective: A simple spectrophotometric method has been proposed for quantitative analysis of favipiravir in bulk and pharmaceutical dosage form.

Methods: New Fourier transform infrared (FTIR) spectroscopic method has been developed for the estimation of favipiravir by using solid pellet technique.

Results: Results were linear over the 20–100µg/mg concentration range, with correlation values exceeding 0.999. The approach was thoroughly validated in accordance with the recommendations of the International Conference on Harmonization, demonstrating acceptable levels of accuracy, precision, selectivity, robustness, and linearity.

Conclusion: The statistical comparison between this method and HPLC revealed that the newly developed method was significantly distinct. Thus, it proves to be applicable. It met all validation standards over a variety of concentrations and can be used as a substitute for the official procedures.

Keywords: Favipiravir, Infrared spectroscopy, Method validation, HPLC

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INTRODUCTION

Favipiravir [1, 2] is a pyrazine carboxamide derivative that possesses anti-RNA viral action. Favipiravir is transformed to ribofuranosyl triphosphate by host enzymes and inhibits influenza viral RNA-dependent RNA polymerase selectively. It was initially approved for the treatment of influenza resistant to treatment. RNA-dependent RNA polymerase (RdRp) enzymes are required for the transcription and replication of viral genomes and serve as antiviral targets [3]. In addition to inhibiting the replication of influenza A and B, favipiravir has showed promise in the treatment of avian influenza and may be a viable alternative for influenza strains resistant to neuramidase inhibitors. Favipiravir has been studied for the treatment of life-threatening infections, including Ebola, Lassa, and recently COVID-19. Favipiravir's IUPAC name is 6-flouro-3-hydroxy-pyridine-2-carboxamide. It was determined that its chemical formula [4] and weight are $C_5H_4FN_3O_2$ and 157.10 g/mol g. mol^{-1}, respectively [fig. 1]

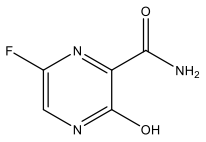

Fig. 1: Chemical structure of favipiravir

Review of the literature reveals that there are HPLC, UV, HPLC-UV, and spectrophotometric methods for estimation of favipiravir [5-14]. There is no FTIR spectrophotometric method for the estimation of favipirvair. Hence the present work was an attempt to develop a new sensitive method for the quantitative estimation of favipiravir in its pure and pharmaceutical dosage forms by using FTIR.

MATERIALS AND METHODS

Instrument

A FTIR spectrophotometer Shimadzu 8400S with IR solutions software, UV-Visible spectrophotometer Shimadzu LC 20AT with UV probe 2.43 software, HPLC Shimadzu 8400S with LC solutions software were used for all the spectral measurements.

Reagents

Potassium bromide is of IR grade and manufactured by SD Fine Chemicals Limited (SDFCL), Mumbai. Methanol of HPLC grade was used to produce all of the solutions manufactured by Merck Specialities Private Limited, Mumbai. The pharmaceutical preparation of favipiravir in the form of tablets, viz., Fabiflu (SUN Pharmaceuticals Ltd, Hyderabad), is procured from the local market. Pure favipiravir was obtained as a gift sample from Hubert Drugs Pvt. Ltd., Hyderabad.

Standards stocks of favipiravir in KBr

Using geometric mixing, 20 mg of the favipiravir were precisely weighed with 100 mg of dried KBr. This produces the 200 µg/mg stock. It is important to mix thoroughly so that the drug is dispersed evenly throughout each pellet that is produced.

Preparation of working standard

5, 10, 15, 20, and 25 mg of favipiravir were carefully measured from the stock (200 µg/mg of favipiravir) and diluted to 50 mg with dried KBr to create the final concentration of 20, 40, 60, 80, and 100 µg/mg of favipiravir, respectively. The drug and dry KBr were appropriately mixed to ensure homogeneous mixing.

Calibration curve

To produce a calibration curve, six different favipiravir standard concentrations between 20 and 100 µg/mg were employed. A sufficient amount of favipiravir was diluted with potassium bromide and triturated to ensure sample homogeneity in order to obtain each concentration. The drug's reaction was demonstrated to be linear in the 20–100 µg/mg concentration range used in the experiment. The calibration curve was found to be linear with an R2 value of 0.9994 and a regression equation of y = 0.005x-0.233. The O-H stretching and C-O stretching-related peaks in the favipiravir IR spectra are located at 3353 cm^{-1}, 3200 cm^{-1}, 1658 cm^{-1}, 1602 cm^{-1}, and 1265 cm^{-1}, respectively. The 3353 cm^{-1} group among them displayed a distinct, strong peak that rose linearly as concentration increased. Correlation coefficient values shouldn't be less than 0.999. Favipiravir's response was found to be linear within the specified

concentration range of 20-100 µg/mg, with a coefficient correlation of 0.9994.

Validation of the method

The developed FTIR method was validated [15] by specificity, linearity, limit of detection (LOD), the limit of quantification (LOQ), precision, and accuracy.

Linearity

Each of the favipiravir working standards (20, 40, 60, 80, and 100 µg/mg) was generated and assessed in FTIR. The absorbance of the peaks at 3353 cm^{-1} was determined for the standard solutions. Concentration and absorbance were displayed on typical calibration curves. To establish linearity, regression analysis was conducted; the regression equation and coefficient of determination were reported.

Limit of detection and limit of quantitation

Limit of detection (LOD) and limit of quantification (LOQ) was assessed by calculation from the regression curve.

LOD and LOQ was calculated by the formula

$$LOD=3.3\sigma/S \quad (1.5)$$

$$LOQ=10\sigma/S \quad (1.6)$$

Where σ = the standard deviation of the response

S = the slope of the calibration curve

Sandell's sensitivity

The lowest concentration of favipiravir (20µg/mg). Calculate the Sandell's sensitivity using the following formula.

Sandell's sensitivity (JJ) = Concentration (µg/100 mg) ×0.001/absorbance value

Precision

The precision of the approach was assessed using repeatability and intermediate precision. By thoroughly analysing the favipiravir standard at 100% w/w six times on the same day, the repeatability was put to the test. Experiments were conducted repeatedly to evaluate the method's inter-day accuracy (three different days).

Accuracy

For drug

Using the traditional addition procedure, the percent recovery of favipiravir was calculated at three different concentrations (80, 100, and 120 percent). Favipiravir in a known quantity was added to the tablet sample preparation. By measuring absorbance and fitting these results into the regression equation for the calibration curve, the percent recovery was calculated. The % relative standard deviation (RSD) was calculated for each level.

Assay of favipiravir tablets

Twenty tablets of the drug Fabiflu were triturated after estimating their average weight. Next, one tablet's worth of powder was added to an Eppendorf tube and dissolved in methanol. Prior to being centrifuged for 10 min at 5000 rpm, it was vortexed for 2 min. The resulting supernatant was then gathered and allowed to evaporate overnight. They collected the remainder. Following that, a 120 g/mg pellet made from the entire residue was triturated with 50 mg of KBr before being scanned in absorbance mode. The quantity of two medications included in the tablet is calculated using the formula below.

Assay = Concentration µg/mg X Dilution factor X Average weight of the tablet (mg)

Weight of the tablet powder taken (mg) X Label claim of the drug * 100

RESULTS AND DISCUSSION

The drug's response was demonstrated to be linear in the 20–100 µg/mg concentration range used in the experiment. The calibration curve was found to be linear with an R2 value of 0.9994 and a regression equation of y = 0.005x-0.233. The resulting R2 value for these investigations was deemed suitable for proving the linearity of the strategy (table 1).

Correlation coefficient values shouldn't be less than 0.999. Favipiravir's response was discovered to be linear within the specified concentration range of 20-100µg/mg, with a coefficient correlation of 0.9994 (fig. 2).

Table 1: Standard calibration curve data for favipiravir

S. No.	Concentration (µg/mg)	Absorbance* at 3353 cm^{-1}
1	20	0.352
2	40	0.451
3	60	0.564
4	80	0.682
5	100	0.795

*Average of three determinations.

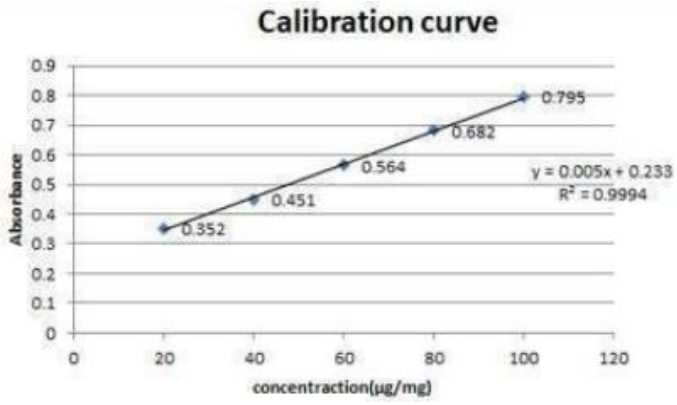

Fig. 2: Standard calibration curve of favipiravir

The LOD and LOQ of favipiravir were found to be 5.8 µg/mg and 17.8 µg/mg, respectively. This demonstrates how sensitive the technique is. According to Sandell's study, the lowest dose of favipiravir (20µg/mg) produced a sensitivity of 0.05 µg/cm^2. The developed analytical techniques reported precision in terms of repeatability and accuracy. The repeatability results for six duplicates of the same favipiravir pellets are shown in the table 2 below. The readings for the percentage RSD were discovered to be within limits. The strategy that was developed as a result was precise.

Table 2: Repeatability data of favipiravir

Concentration µg/mg	Absorbance	Mean±standard deviation (n=6)	% RSD
60	0.580	0.582±0.005	0.86
60	0.584		
60	0.589		
60	0.586		
60	0.573		
60	0.584		

Fig. 3: Repeatability overlay spectrum of favipiravir

Interday precision results obtained for three replicates of each concentration of favipiravir (40µg/mg, 60µg/mg, and 80µg/mg) are shown in the following (table 3). The results revealed that % RSD values were within limits. Hence the developed method was precise.

Table 3: Interday precision data of favipiravir

Concentration µg/mg	Absorbance*			Mean±standard deviation n=3	% RSD
	Day 1	Day 2	Day 3		
40	0.427	0.439	0.442	0.436±0.00793	1.6
60	0.563	0.565	0.566	0.564±0.00152	0.17
80	0.70	0.70	0.71	0.70±0.00577	0.71

*Average of three determinations

Accuracy study was carried out by calculating % Recovery of the Favipiravir by standard addition method, respectively. The % recovery was calculated by measuring absorbance and fitting these values into the regression equation of the calibration curve. The results obtained for recovery data of favipiravir are shown in the following (table 4).

Table 4: Recovery data for FVP drug product

Spike level	Absorbance*	Concentration recovered (µg/mg)	% Recovery
80%	0.427	48	80%
100%	0.521	60	96%
120%	0.664	72	119.7%

*Average of three determinations, Assay was performed for marketed Fabiflu tablets and the % purity was found to be 98% (table 5).

Fig. 4: FTIR spectrum of assay of marketed formulation

Table 5: Assay results of marketed tablets

Brand name	Name of the drug	Functional groups	Absorbance*	Label claim (mg)		% Purity
				Actual	found	
Fabiflu	Favipiravir	O-H (3353 cm^{-1})	1.66	200	198	98%

*Average of three determinations

Comparison of results of FTIR and HPLC method

We then used acetonitrile and water (60:40) as the mobile phase in an RP-HPLC test for favipiravir. The extracted tablet residue was dissolved in methanol (100µg/ml) and spiked in 10 ml methanol to obtain the standard stock solutions of 100µg/ml and the chromatogram was obtained after injecting these solutions into RP-HPLC (fig. 5).

Statistical analysis

Student *t*-test

Assay results of favipiravir were calculated by both methods. Statistical analysis of the results of two techniques showed a significant difference between the methods at a significance level (α) of 5% (tcalculated<tcritical) (table 6).

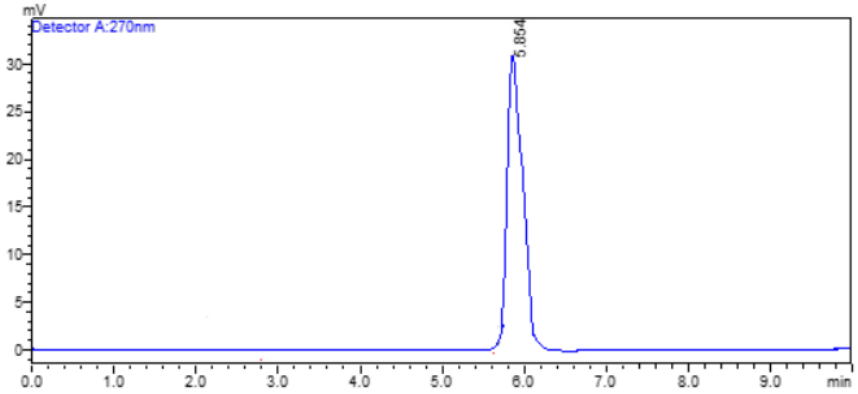

Fig. 5: HPLC spectrum for assay of marketed formulation

Table 6: Statistical data for t-test of favipiravir assay

Method	Method of assay of favipiravir	Standard deviation of favipiravir	Size of samples
FTIR	X_1 = 98.7	S_1^2 = 0.94	n_1= 3
RP-HPLC	X_2= 96.83	S_2^2 = 1.00	n_2= 3

Hypothesis: The two analytical methods to determine linearity are not significantly different.

$$H_0: \mu = \mu_0$$

$$\text{Against } H_1: \mu \neq \mu_0$$

Since variances of the population were not known and the size of the samples was small, t-test for the difference in means was adopted, assuming the populations to be normal and the test statistic t were worked out under the given formula:

$$t = \frac{\underline{x_1} - \underline{x_2}}{\sqrt{\frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{(n_1+n_2-2)}} \times \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

P value (Probability of rejection) = 0.05 (two–tailed)

$t_{calculated}$ = 2.21

$t_{critical}$ (0.05) = 1.85

Degrees of freedom (df) = n_1+n_2-2; (3+3−2) = 4

As our hypothesis was two-sided, we applied a two-tailed test for determining the rejection regions at 5 percent level, which came to as under, using table of t-distribution for 4 degrees of freedom:

$$R: |t| < 1.85$$

The observed value of t falls in the region of rejection of our hypothesis. So, we reject our hypothesis of both methods not being significantly different and conclude that the two methods to determine the assay of favipiravir differ significantly.

CONCLUSION

The quantitative determination of favipiravir in bulk and dosage forms was successfully accomplished in this work using an analytical IR spectroscopy approach. Its simplicity, affordable conditions, and lack of polluting reagents make it superior to other existing procedures. Favipiravir was analysed using the FTIR spectrophotometric method, which was created using the solid pellet process. When this was statistically compared to HPLC, the findings showed that the newly devised approach was considerably different. As a result, its applicability is good. It can be used as an alternative to the official procedures because it met all validation standards in a variety of concentrations. It is appropriate for quality control of both pure and commercial solid dosage forms, and comparable techniques can be developed for additional drug categories to estimate them in formulations.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

Declared none

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