Bioanalytical Method Development and Validation for the Determination of Favipiravir in Spiked Human Plasma by using RP-HPLC

Pallavi V. Duse[1*] and Kamalkishor G. Baheti[2]

[1]Y. B. Chavan College of Pharmacy, Rauza Bagh, Aurangabad-431003, Maharashtra, India.
[2]Department of Pharmaceutical Chemistry, Y. B. Chavan College of Pharmacy, Rauza Bagh,
Aurangabad-431003, Maharashtra, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Original Research Article

ABSTRACT

A precise, simple and reproducible reverse phase liquid chromatography (RP-HPLC) method was developed and validated for determination of Favipiravir by using Carbamazepine as internal standard in spiked human plasma. A chromatographic separation was accomplished with Cromasil C18 (250mm x 4.6ID, Particle size: 5 micron) column using mobile phase consists of methanol: water in the ratio (35:65, %v/v), at pH 3.0 with binary gradient system-maintained flow rate at 0.8ml/min. The detection wavelength of drug sample was at 225 nm. Extraction was done by using ethyl acetate as extracting solvent. The retention time of Favipiravir was found to be 6.62 min. The method was found to be linear in the concentration range of 0.2-3.2 µg/ml. Limit of quantitation (LOQ) value was found to be 0.72. The intra- and inter day precision and accuracy lies within the specified range. The recovery studies were found to be in the range of 97.6 to 100.2%. %Relative standard deviation (RSD) was found to be in the range of 0.07-2.80%. All parameters were found to be validated from spiked human plasma. The proposed RP-HPLC method is highly accurate and rapid for the determination of favipiravir in human plasma and can be applied for pharmacokinetic studies and Therapeutic drug monitoring.

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*Corresponding author: E-mail: palaskarpallavi@gmail.com;

1. INTRODUCTION

Favipiravir, 6-fluoro-3-hydroxypyrazine-2-carboxamide (Fig. 1), a promising antiviral agent for the treatment of covid-19 infection as the clinical trials studies showed its antiviral effect on corona virus. It is also classified as anti influenza drug for the treatment of influenza virus [1]. Several medications being initiated scientific trials globally for its assessment and control to triumph over Covid-19 outbreaks. The rising effect of Covid-19 pandemic began to affect the global health, countries have undergone different active therapeutic medication [2]. There are numerous drugs become examined and attempted for the treatment of SARS-COV-2 [3-7]. So far, there's loss of proof for the remedy of covid-19 pandemic for its protection and effectiveness. Favipiravir is analogue of purine nucleotides and inhibit RNA dependant RNA polymerase. Its use is being initiated and evaluated to deal with one of a kind viral mass in lots of countries [8-9]. Favipiravir suggests large spectrum pastime with the aid of using incorporating into viral RNA strand and save the extension. It's also lively in opposition to 53 varieties of one of a kind traces of virus.
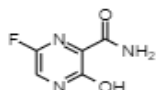

Fig. 1. Structure of Favipiravir

The literature review discovered that there are three methods of HPLC developed and published for determination of assay and impurity in active pharmaceutical ingredients (API) [10-12] and few LC-MS methods developed and validated for determination of favipiravir [13-15].

The proposed method needs to produce simple, accurate and economical bioanalytical method for determination of favipiravir in spiked human plasma by using HPLC. The method describes the estimation of favipiravir (FVP) in spiked human plasma using carbamazepine as internal standard. Internal standard (IS) compensates the various analytical errors in method by improving the accuracy, precision and robustness of bioanalytical method.

2. METHODS

2.1 Instrumentation

Chromatographic separation was performed on HPLC binary gradient system 3000 series with reciprocating pump of pressure 40MPA. The data was processed using HPLC workstation software and analysis was carried out at 40^0 C column oven temp. A chromatographic separation was accomplished with Cromasil C18 (250mm x 4.6ID, Particle size: 5 micron) column with binary gradient system-maintained flow rate at 0.8ml/min. Detection wavelength selected was at 225 nm and run time was 9.39 min.

2.2 Chemicals and Reagents

The drug sample favipiravir and internal standard (IS) carbamazepine obtained from Abbott healthcare, Mumbai, India. Methanol, HPLC grade water and ortho-phosphoric acid were received from RAP Analytical lab, India. The frozen human plasma was obtained from Arpan blood bank.

2.3 Standard Stock Solution and Internal Standard Preparation

Standard stock solution of favipiravir (1.0 mg/ml) was prepared by dissolving 25 mg of FVP in 25 ml of solvent. Standard working solution was prepared by diluting above stock solution to desired concentration (10µg/ml). Quality control (QC) samples were prepared at three levels namely LQC (lower), MQC (middle) and HQC (higher) concentrations. Linearity standards were prepared to get final concentration range from 0.2-3.2 µg/ml.

Internal standard of carbamazepine was prepared by dissolving 10 mg of solution into 10ml of volumetric flask and volume made up with the solvent. From the stock solution pipette out 0.3 ml of solution into 10ml volumetric flask and volume made up with the solvent to produce 30 µg/ml.

2.4 Plasma Extraction Procedure

Initially freezed plasma thawed. 2 ml of plasma was withdrawn and transferred to the centrifuge tube. Stock solution was added equivalent to the required concentration. The mixture was vortexed for 3 to 4 mins. Then 2ml of ethyl acetate were added as extracting solvent. Again, the mixture was vortexed for 3 to 4 mins. Finally, the mixture was centrifuged and a clear supernatant of non-polar solvent was withdrawn to isolate the extracted sample. The non-polar solvent (ethyl acetate) was allowed to vaporize and mobile phase was added as a solvent in the remaining of the sample and the formed solution was injected for further analysis.

2.5 Validation Parameter

2.5.1 Linearity

Calibration method was performed on different standard solution within the concentration range of 0.2-3.2 µg/ml of favipiravir. Least square method was used to evaluate the linearity of the proposed method. A standard curve was found to be validated if coefficient of correlation (r^2 value) should be near to one.

2.5.2 System suitability parameters

System suitability parameters were used to compare the results with approximate standard values. It includes resolution, theoretical plates and asymmetry factor. All the required values found to be 4.0(Resolution), 4000 (theoretical plates), 1.2(asymmetry factor) within specifications.

2.5.3 Specificity

Specificity is the method which used to assess the response obtained from screening of different lots of spiked human plasma (standard blank). This method was used to check the response in presence of interfering substances. Carryover was performed immediately after the ULOQ sample injection to check the signal in blank sample in the form of chromatogram.

2.5.4 Precision and accuracy

Precision was described as closeness of agreement between the repeated individual that measures of the analyte. It is expressed in the form of coefficient of variation (CV).

Accuracy was defined as the closeness of the actual value spiked with known amount of analyte. It is expressed in percentage (%). It can be demonstrated by spiking the sample matrix with known concentration of analyte. Percent deviation should be less than 15% for all three concentrations (LQC, MQC and HQC).

2.6 Stability Studies

Stability studies were analysed at different conditions. Quality control samples after storage at -4^0 C in deep freeze for long term stability can be assessed against freshly prepared stock solutions.

3. RESULTS AND DISCUSSION

3.1 Optimization Conditions

The HPLC analysis was performed by using methanol: water in the ratio (35:65, %v/v), pH 3.0 as mobile phase found to give good resolution and better separation. Selection of wavelength was accomplished by scanning the solution using UV spectrophotometer, confirmed maximum absorbance at 225 nm. Carbamazepine used as internal standard in spiked human plasma. The bioanalytical method using internal standard by using HPLC was developed and validated. Fig.3-5 represents chromatogram of Plasma, chromatogram of plasma spiked with drug sample favipiravir and chromatogram of favipiravir and internal standard (IS).

3.2 Method Validation

3.2.1 Linearity

Calibration samples was prepared by spiking the drug favipiravir and internal standard in plasma at different concentration over the range of 0.2 - 3.2 µg/ml and validated. Calibration curve was plotted as concentration on X -axis and Area on Y-axis respectively (Fig.2). The correlation coefficient was found to be linear as shown in Fig.2.

3.2.2 Specificity and sensitivity

Blank chromatogram (Fig.3) showed no interference occurs with at the retention time of drug as well as internal standard. Sensitivity results were found to be precise and accurate. Limit of detection (LOD) and limit of quantitation (LOQ) was found to be 0.23 and 0.72. The method was confirmed that no carryover effect observed in the blank plasma. Chromatogram was obtained consists of time on X -axis and area on Y-axis respectively.

3.3 Accuracy and Precision

Accuracy and precision studies were performed for the QC samples of LQC, MQC and HQC respectively. Percent SD and RSD was found to be as per specification (Table 2). Percentage mean accuracy was found to be in the range of 98 to 101.2%.

Table 1. Linearity curve data of favipiravir

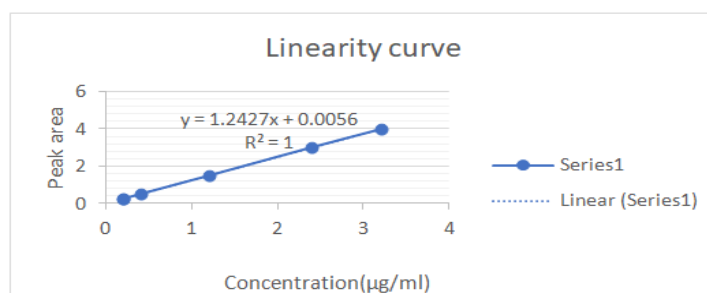| Ratio of Conc.(µg/ml)	Ratio of area
0.2	0.2512
0.4	0.4978
1.2	1.4974
2.4	3.0038
3.2	3.9707
Slope	1.2427
intercept	0.0056
r^2	1

Fig. 2. Linearity curve of Favipiravir using internal standard
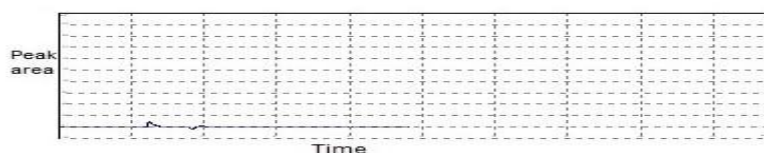

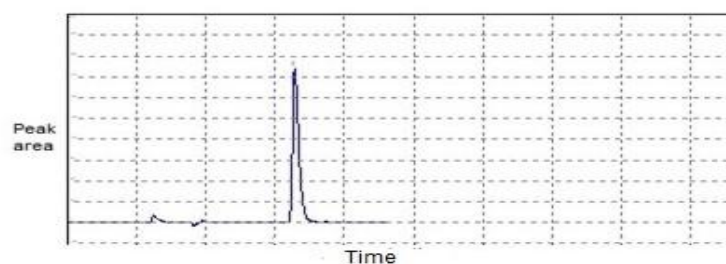**Fig. 3. Chromatogram of blank plasma**
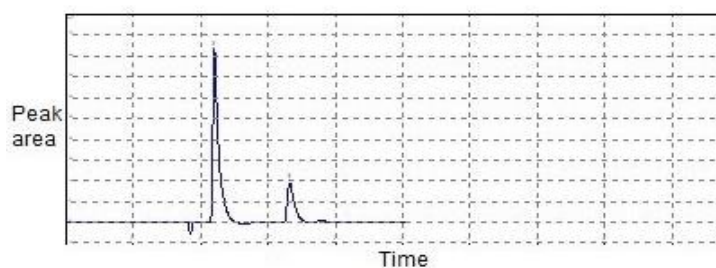

Fig. 4. Chromatogram of plasma spiked with favipiravir

Fig. 5. Chromatogram of favipiravir and IS carbamazepine

Table 2. Accuracy and precision method for determination of favipiravir

Sr No.	Conc.	Intra Assay (n=5)		Accuracy	Precision
		Conc. added in µg/ml	Conc. found in µg/ml	%	%RSD
1	LQC	12	10.8	98	0.09
2	MQC	60	61.03	101.03	0.44
3	HQC	120	119.3	99.97	0.07

Table 3. Recovery studies for determination of favipiravir from spiked human plasma

Sr. NO.	QC samples	Area of Standard	Area of Sample	% Recovery
1	LQC	248841	242891	97.60
2	MQC	2884550	2859411	100.87
3	HQC	3850969	3838581	100.32

Table 4. Long term stability studies of Favipiravir

Sr no.	Concentration in µg/ml (QC Samples)	Measured Conc.± SD (µg/ml)	% RSD
1	0.2	0.180±0.001	1.56
2	0.4	0.372±0.003	2.80
3	1.2	1.220±0.190	1.94

Recovery studies was performed and measured by extracted blank plasma spiked with standard containing known amount of Favipiravir. The recovery studies (HQC, MQC and LQC) was found to be 97.6 to 100.32%. The results are given in Table 3.

3.4 Sample Stability

Long term matrix sample stability of favipiravir was determined for three QC samples at -4^0C in deep freeze for period of 60days. The percent RSD of favipiravir was found within acceptable range. Results are summarized in Table 4.

4. CONCLUSION

Quantitative analysis of favipiravir spiked in plasma using by HPLC is reported. Compared to published method, The proposed method makes the procedure more simple, sensitive, reliable and accurate by using internal standard which is readily available and economical. Analyte showed good stability study during storage condition. The developed method showed easy sample preparation and less retention time used for chromatographic procedure that makes the method suitable for Therapeutic drug monitoring and pharmacokinetic studies as well. The method was validated and lies within specification as per given by USFDA guidelines.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and procedure of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of the knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the author.

CONSENT

It is not applicable

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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