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RESEARCH ARTICLE

Method development and validation of Favipiravir by RP-HPLC

Vishal Bansi Ghune, Amit Suryakant Tapkir*

Department of Pharmaceutical Quality Assurance, PES, Modern College of Pharmacy, Pune, Maharashtra, India

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ABSTRACT

Favipiravir (FVP) shows antiviral activity to tackle many viruses. It is very important for further analytical tests as a potential drug for COVID-19. A simple, accurate, precise, rapid, and gradient reverse phase high-performance liquid chromatography method developed for FVP pharmaceutical formulation. Analysis was carried out by Cosmosil C18 (250 mm × 4.6ID, Particle size: 5 micron). The mobile phase consisted of Methanol: Water (75:25, v/v). pH of Mobile Phase: 3 (pH is adjusted with o-phosphoric acid) The mobile phase was filtered and degassed through a 0.45 mm membrane filter before use and then pumped at a flow rate of 0.8 mL/min. The ultraviolet (UV) detection and column temperature were 227 nm, and 30°C. The retention time of FVP was found to be 4 min. The run time was 9 min under these chromatographic conditions. Linearity for FVP concentration 10–50 ppm has been observed with coefficient of determination of 0.9995. The recovery (%) was in the range of 98.94–99.12%, while the mean RSD (%) is 0.23%. The developed method was found to be sensitive (LOD and LOQ was found to be 0.2236 and 0.6776 resp.) Proposed method has been successfully applied for method development and validation of FVP in pharmaceutical formulations.

Keywords: Favipiravir, High performance liquid chromatography, Method, Development, Validation, Antiviral

INTRODUCTION

In Wuhan, China, a new coronavirus (COVID-19) was discovered in 2019 which was never seen in people before. Coronaviruses are a broad family of viruses that may cause everything from a normal cold to more serious illnesses such as Middle East Respiratory Syndrome and Severe Acute Respiratory Syndrome (SARS). Since the COVID-19 pandemic began to spread over the world, countries have devised several treatment strategies. Several treatments are currently being tested in clinical studies around the world to assess and control COVID-19 outbreaks. As the impact of the COVID-19 pandemic grew, it began to have an impact on world health, and countries began to use various active therapeutic medications.[1]

***Corresponding Author:**
Dr. Amit Suryakant Tapkir,
E-mail: amittapkir.8@gmail.com

As a result of the developing COVID-19 epidemic and its potential impacts on world health, active treatment solutions are desperately needed.[1] For the treatment of SARS-CoV-2, variety of medicines are being tested and used.[2-6] Different drugs, including chloroquine, arbidol, remdesivir, and favipiravir (FVP), are conducting clinical studies in several countries to determine their efficacy and safety in treating coronavirus illness.[3,7] FVP is an antiviral medication created by Toyama Chemical for the treatment of influenza. It prevents viral replication by specifically inhibiting the RNA polymerase of RNA viruses. It has antiviral efficacy against alpha, filo, bunya, arena, flavi, and noroviruses, as well as influenza. FVP is deemed to be worth further exploration as a prospective candidate treatment for COVID-19 after a pilot study by Zhongnan Hospital of Wuhan University demonstrated a superior recovery rate in COVID-19 patients in the FVP group compared to the arbidol group.

FAV, a nucleoside analogue, is rapidly metabolized in host cells, disrupting viral production and causing mutagenesis. Clinical trials are becoming available that show the benefits of using both medications at the same time in mild-COVID-19 patients. Simultaneously, adverse effects such as QTc prolongation or teratogenicity raise concerns about widespread use in the population. Figure 1 shows the structure of FVP.

EXPERIMENTAL

Chemicals and reagents

Without purification analytical grade chemicals were used for study. Ortho-phosphoric acid and high performance liquid chromatography (HPLC)-grade methanol were received from aRAP analytical research India was used. Deionized water was purified by a Milli-Q system (Millipore) with conductivity lower than 18.2 mS cm1. FVP bulk powder and tablets (FaviBluz, 400 mg) were obtained from BlueCross Pharmaceuticals.

Stock solution preparation

Tablet weighing 620.85 mg contains API dose of 400 mg. Hence, to get 10 mg of API, we take 15.52 mg powder. Then after that 15.52 mg was weighed and dissolved into 10 mL of deionized water which gave 1000 ppm of solution. The stock solution was further diluted with deionized water to obtain the required concentration of standard solution (10–50 ppm) and further dilutions were prepared.

Sample solution preparation

Standard Stock solution preparation of 1000 ppm of individual drug. 10 mg of pure drug dissolved in 10 mL of solvent (solvent was used as mobile phase); this gives 1000 ppm solution. Samples are prepared according to Table 1:

Table 1: Sample solution preparation data

S. No.	Concentration (ppm)	FVP (conc.)		Final Solution Volume
		Volume of Stock (mL)	(ppm)	(mL)
1.	10	0.1	10	10
2.	20	0.2	20	20
3.	30	0.3	30	30
4.	40	0.4	40	40
5.	50	0.5	50	50

Determination of λ max

On an UV spectrophotometer, a standard solution of 20 ppm was scanned between 190 and 1100 nm (Analytical Technologies Ltd. Double Beam spectrophotometer). From the UV spectra of standard solution, λ max was discovered to be 227 nm.

Chromatographic conditions

Chromatographic analysis was performed on a column of Cosmosil C18 (250 mm × 4.6ID, Particle size: 5 micron). The mobile phase consisted of Methanol: Water (75:25, v/v). pH of Mobile Phase: 3 (pH is adjusted with o-phosphoric acid). The mobile phase was filtered and degassed through a 0.45 mm membrane filter before use and then pumped at a flow rate of 0.8 mL/min. The run time was 7–8 min under these conditions.

METHOD VALIDATION PARAMETERS

The analytical method validation has been performed as per ICH guidelines of Validation of Analytical Procedure: Q2(R1).[8,9] The validation parameters linearity, system suitability, the limit of detection, the limit of quantification, precision, accuracy, specificity, and robustness are included in this study.

Linearity

Each standard solution was chromatographed and a standard calibration was constructed using five standard solutions in the concentration range of 10–50 ppm under optimum chromatographic conditions. The method's linearity was assessed using a least squares linear regression analysis of average peak area against concentration data.

System suitability parameters

Suitability of the system parameters is the benchmarks against which you can compare your results to the estimated standard values. The following are some of them:

1. Resolution: The resolution should be at least 1.75. This parameter is only applicable when two samples are mixed together. In the event of a single sample, the value will be zero.
2. Theoretical Plates: There should be at least 2000 theoretical plates. It shows how effective a column is.
3. Asymmetry/Tailing Factor: The asymmetry factor should be smaller than 2.

All three parameters are within the standard parameters according to study.

Specificity

Selectivity refers to an analytical procedure's ability to generate a response for the analyte in the face of extra interference. By comparing the chromatograms produced for FVP standard, tablet, and blank solutions, the method's selectivity was determined. To demonstrate that the method adopted was specific, the variables retention time and tailing factor were estimated. UV– Spectra of FVP has shown in Figure 2.

Precision

Precision was determined by calculating variations of the approach in intraday (repeatability was determined by analyzing a standard solution on the same day) and interday (repeatability was determined by analyzing a standard solution on the same day) (repeatability carried out by analyzing standard solution on two different days). The precision investigation was carried out by injecting a standard solution 3 times at a concentration of 30 ppm on the same day and 2 days in a row.

Accuracy

To confirm the accuracy of the suggested method, recovery studies were carried out using the standard addition technique. FVP recovery was estimated for each concentration by adding 50%, 100%, and 150% of three distinct levels of pure drug to the previously assessed sample solutions.

Robustness

To analyze the effects of modest but systematic changes in chromatographic parameters, a robustness study was done. Wavelength and pH are among the variables. System suitability parameters were evaluated after each change by injecting the sample solution into the chromatographic system and comparing the findings to those obtained under the original chromatographic settings.

RESULTS AND DISCUSSION

Determination of λ max

The wavelength corresponding to maximum absorbance (λmax) was determined as 227 nm from the UV spectrum of standard solution.

Method development

For the Method Development of FVP, several preliminary investigations were undertaken to optimize the chromatographic conditions. To find the best circumstances, several methanol solution ratios were tested. Because the FVP peak was nicely formed and symmetrical, the methanol and water ratio was measured using an o-phosphoric acid buffer solution (pH 3). The mobile phase of methanol and water (75:25, v/v) was eventually discovered to yield stronger theoretical plates (>2,000) and peak tailing factor (1.0). Mobile phases including combinations of organic solvents and phosphate buffers, with varying ionic strengths and pH ranges, were examined at flow rates of 0.8 mL/min. The optimum chromatographic conditions were obtained on Cosmosil C18 (250 mm × 4.6ID, Particle size: 5 micron) employing a gradient mobile phase of methanol and water (75:25, v/v) at a flow rate of 0.8 mL/min. The experiment was carried out at 28°C, which has a number of advantages, including superior chromatographic peak shape, increased column efficiency, and reduced column pressure, as

well as being cost-effective. A UV detector tuned at 227nm was used to monitor the eluate. The tablet solution was analyzed for 60 min to confirm that no matrix components remained in the column under the prescribed conditions for much longer. Continuing the study for another 9 min, on the other hand, will reduce both the analysis time and the cost. In samples from sample analyses put into the system consecutively with up to 9 min of analysis time, overlapping peaks were not seen to overlap. Due to these factors, the analysis time/run time was set to 9 min; however, we discovered that the medication elutes at 4.6 (± 0.2) min, therefore a run period of 5 min should enough.

METHOD VALIDATION

Linearity

To obtain standard solutions in the concentration range of 10–50 ppm, the stock standard solution of FVP was diluted accordingly with deionized water. Under the chromatographic working conditions described above, each standard solution was injected three times into the HPLC system. Regression analysis was used to determine the linearity of the suggested technique at five concentration levels ranging from 10 to 50 ppm. Plotting average peak area versus standard produced the calibration curve. Figure 3 shows the linearity curve of FVP and Figure 4 shows the standard chromatogram of FVP.

Precision

The precision investigation was carried out by injecting the standard solution 6 times at 30 ppm on the same day and 2 days in a row. Table 2 contains the precision data. For specified FVP concentrations, all RSD values were <2.0%. The approach is precise in this circumstance and can be used for our intended purpose.

Limit of detection and limit of quantification

These values were determined using the standard deviation and slope of the regression line (m). Limit of detection was found to be 0.2236 and limit of quantification was found to be 0.6776.

Accuracy study

A known amount of standard solution was added to the sample solutions that had previously been analyzed at three levels (50%, 100%, and 150%). The amount of FVP recovered has been calculated at three concentrations. Table 3 summarizes the recovery information.

Robustness

The findings revealed that changes in pH and wavelength concentration had no impact on FVP's chromatographic behavior. The retention time of FVP is affected only slightly by modest changes in pH and wavelength. Tables 4 and 5 present the findings of this investigation, reported as % RSD.

Table 2: Precision data

Conc.	Interday		Conc.	Intraday	
30 ppm	Day 1 (Area)	Day 2 (Area)	30 ppm	Morning (Area)	Evening (Area)
	1141088	1129711		1141088	1113309
	1123425	1114131		1123425	1120630
	1134054	1125775		1134054	1126575
Mean	1123205.667		Mean	1126513.5	
%RSD	0.82%		%RSD	0.88%	

Table 3: (%) Recovery data

S. No.	% Composition	Area of Standard	Area of Sample	% Recovery
1.	50% Recovery	1141088	1131095	99.1242
2.	100% Recovery	1498952	1491369	99.4941
3.	150% Recovery	1852436	1832821	98.9411

Table 4: Robustness data of change in pH

Conc.ppm	Area	Mean	SD	%SD
20	739949	735883	4290.87	0.5830
20	736302			
20	731398			

Table 5: Robustness data of change in wavelength

Conc.ppm	Area	Mean	SD	%SD
20	739949	741584	3173.28	0.4279
20	745241			
20	739561			

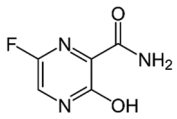

Figure 1: Structure of FVP

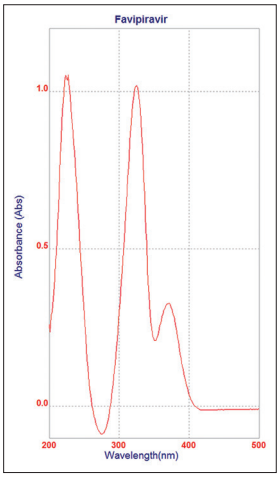

Figure 2: UV Spectra of FVP

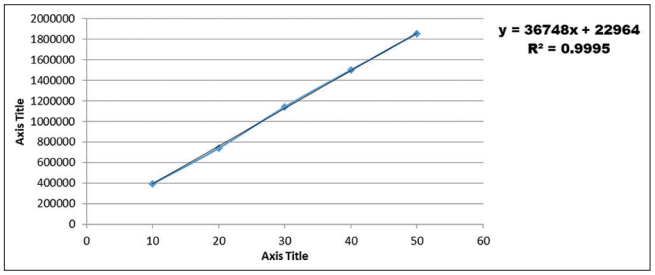

Figure 3: Linearity curve of FVP

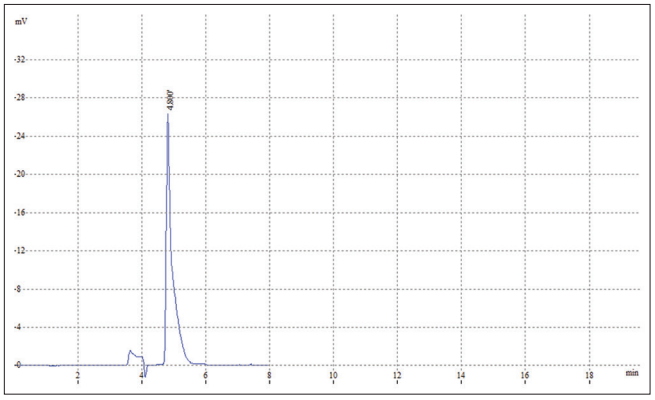

Figure 4: Chromatogram of FVP

Change in pH

For robustness study the change in pH has been studied where change in pH was studied in three levels pH 2.8, pH 3, pH 3.2 and the % RSD was found to be 0.58%.

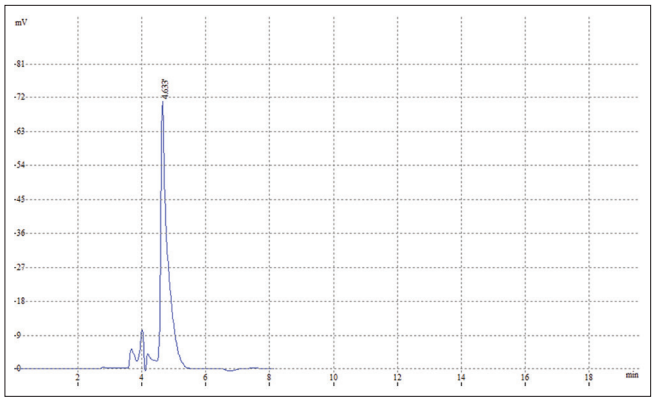

Figure 5: FVP 50 ppm treated with 0.1N HCl at 60C for 30 min

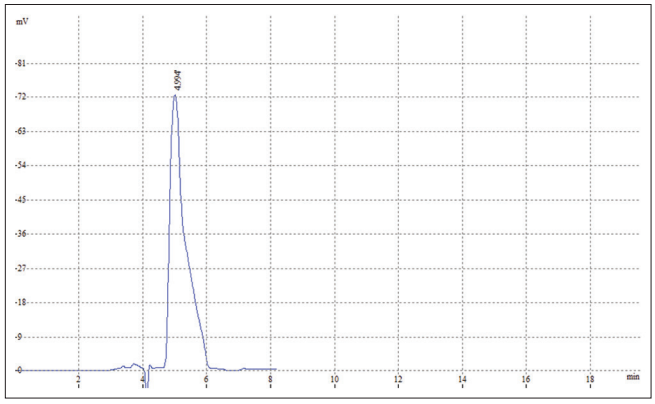

Figure 6: FVP 50 ppm treated with 0.1N NaOH at 60C for 30 min

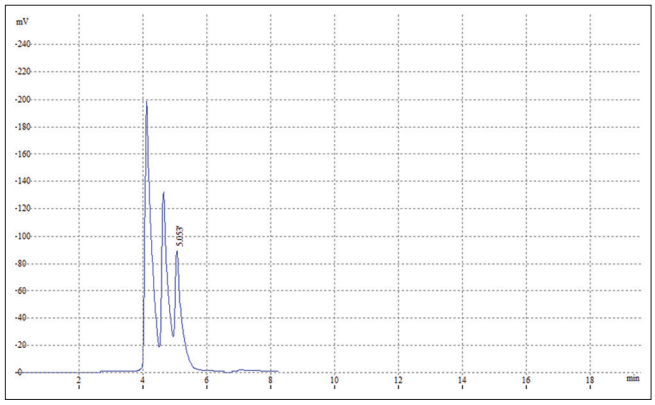

Figure 7: FVP 50 ppm treated with 3% H_2O_2 at RT for 3 h

Change in wavelength

For robustness study the change in wavelength has been studied where change in wavelength was studied in three levels 225nm, 227nm, 229nm and the % RSD was found to be 0.42%.

Table 6: Forced degradation data

S. No.	Degradation	Area of Standard	Area of degraded sample	Degraded up to %	Actual %degradation
1.	Acid Degradation	1852436	1627088	87.8350	12.1649
2.	Base Degradation	1852436	1564733	84.4689	15.5310
3.	H2O2 Degradation	1852436	1739389	93.8973	6.1026
4.	Photolytic Degradation	1852436	1804271	97.3999	2.6000
5.	Thermal Degradation	1852436	1815332	97.9970	2.0029

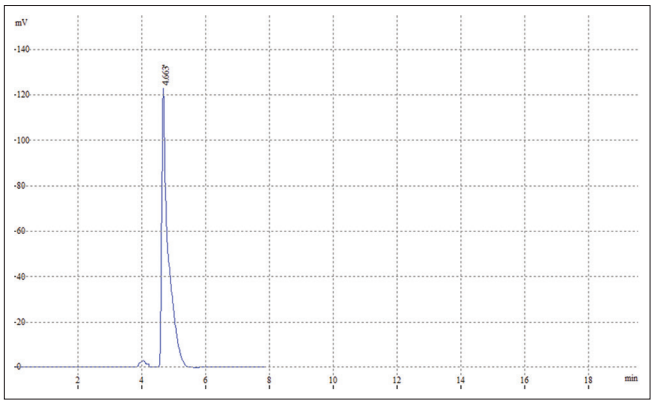

Figure 8: FVP 50 ppm treated photolytically for 24 h

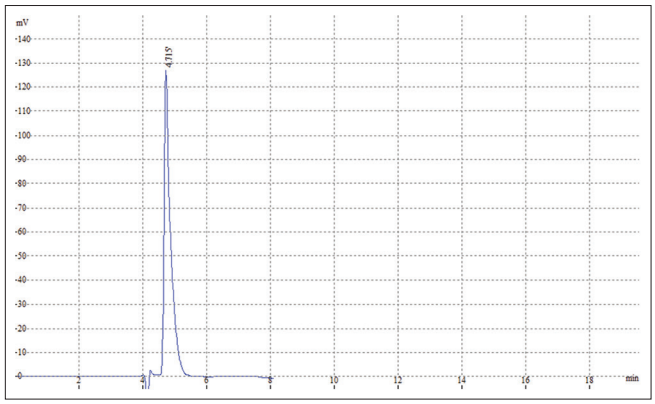

Figure 9: FVP 50 ppm treated thermally at 60C for 24 h

Forced degradation

Forced degradation experiments are used to purposely degrade the active pharmaceutical drug. These experiments are used to see if an analytical approach can accurately assess an active component and its breakdown products. Acid, base, oxidizing agent, photolytic, and thermal conditions are applied to samples, drug products, and drug substances. The approach was then used to examine the deteriorated samples to see if there were any interference with the active. As a result, the stability-indicating property was assessed.

Acid Degradation

In a water bath, 0.1N HCl was added to 10 ml of stock solution and maintained at 60 °C for about 1 hour. After cooling, 100 ml of mobile phase was added. A 0.22 µ membrane filter was used to filter the solution.12.16 % FVP has been degraded in acid condition. Figure 5 shows the degradation chromatogram of FVP in acid conditions.

Base Degradation

In a water bath, 10 ml of 0.1N NaOH was added to 10 ml of stock solution and maintained at 60 °C for about 1 hour. After cooling, 100 ml of mobile phase was added. A 0.22 µ membrane filter was used to filter the solution. 15.53 % FVP has been degraded in base condition. Figure 6 shows the degradation chromatogram of FVP in base conditions.

Oxidative Degradation

5 ml of 3% H2O2 was added to 10 ml of stock solution and maintained at room temperature for 24 hours, volume make up of 100 ml with mobile phase was done. A 0.22 µ membrane filter was used to filter the solution. 6.10 % FVP has been degraded in oxidative conditions Figure 7 shows the degradation chromatogram of FVP in oxidative conditions.

Photolytic Degradation

A 10 ml stock solution was degraded photolytically for about 24 hours, then cooled made up the amount of 100 ml with mobile phase. A 0.22 µ membrane filter was used to filter the fluid. 2.60 % FVP has been degraded in photolytic conditions. Figure 8 shows the degradation chromatogram of FVP in photolytic conditions.

Thermal Degradation

A 10 ml stock solution was kept at 60°C for about 24 hours, then cooled made up the amount of 100 ml with mobile phase. A 0.22 µ membrane filter was used to filter the solution. 2.00 % FVP has been degraded into thermal conditions. Figure 9 shows the degradation chromatogram of FVP in thermal conditions.

APPLICATION OF THE METHOD TO THE MARKETED TABLETS

FVP in pharmaceutical formulations has been effectively determined by using established and verified method. Table 6 shows the results of an assay of a marketed FVP tablet. The acquired results are closely connected to the amount specified on the tablet labels. This demonstrates the effectiveness of the content evaluation method.

CONCLUSION

Using an internal standard that is commonly available and inexpensive, the suggested technique makes the operation more simple, sensitive, dependable, and accurate. The new approach demonstrated simple sample preparation and chromatographic process retention duration, making it appropriate for therapeutic medication monitoring and pharmacokinetic research. The approach has been verified and found to be within the USFDA's criteria.

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