FORCE DEGRADATION STABILITY INDICATING STUDIES AND ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF FAVIPIRAVIR BY HPLC- UV IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

In the current work, a simple, economical, accurate and prices HPLC method with UV detection was developed quantify favipiravir (FVIR) in spiked human plasma using acyclovir (ACVIR) as an international standard in the covid-19 pandemic time both FVIR and ACVIR were well separated resolved on the C18 inertsil column and optimization of variable for FVIR estimation in bulk and pharmaceutical dosage forms. UV visible (200-400nm and400-800nm respectively). UV spectroscopic method was developed for the estimation of FVIR in the bilk and pharmaceutical dosage forms. The solvent selected for the FVIR UV analysis was water, the solution in a range of 2-10µg/ml was scanned in the UV region from 200-400nm and the λmax value was determine. The RP-HPLC method was developed on inertsil ODS-3V C18 150mm x 4.6mm x 5micron using buffer pH 3.5; acetonitrile [90; 10] as a mobile phase at flow rate 1.0 ml/min.

Keywords: *Favipiravir, method development, validation, HPLC*

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1. **INTODUCTION:** Favipiravir (6-Fluoro-3-hydroxypyrazine-2-carboxamide), a purine nucleic acid analog that has been developed by Toyama Chemical in Japan for a treatment of viral infections including influenza.3 This has recently been evaluated and was found to be a promising choice in management of COVID-19. It works by inhibiting RNA dependent RNA polymerase (RdRp) enzyme, a key enzyme impeding replication of RNA viruses [1-2]. According to the literature search, there are very few published HPLC method for determining FVIR assay and impurities in active pharmaceutical ingredients [3-4]. In

those method a gradient HPLC modes was used for chromatographic separation FVIR is not official available in any pharmacopeia and there is still a need for validated HPLC method to determine FVIR in pharmaceutical dosage forms chemical stability of pharmaceutical molecules is a matter of grade concern as it affect the safety and efficacy of the drug product. A stability indicator test method can be defined as a "validated quantitative analytical method capable of detecting the change over time in the chemical, physical or microbiological properties of the pharmaceutical substance and specific pharmaceutical products so that the content of active ingredients and degradation products can be accurately measured without interference. The goal of this work is to develop and validate analytical methods for stability indicators for selected drugs and bulk formulations with forced degradation studies along with characterization [5].

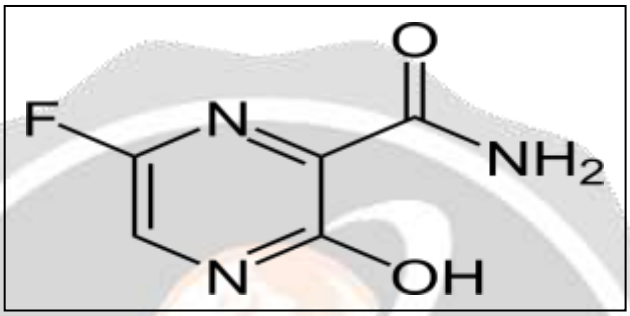

Fig-1. Favipiravir

2. MATERIAL AND METHODS

2.1 Instruments

Shimadzu HPLC system [LC-20AD Multi-solvent delivery system, SPD-20A, PDA Detector, LC solution software] UV-Visible Spectrophotometer [Shimadzu- 1800 double beam, with UV Probe 2.33].Labman sonicator was usedforsonication of the sample solution. Thermo scientific pH meter was used to measure pH. A vacuum pump filter was used for the filtration of mobile phase solvents.

2.2 Chemicals and Reagents

The drug was procured from Honour Lab, Hetero Limited with the certificate of analysis. The marketed formulation [Favipiravir tablet (Fabi flu) 200mg] was purchased from Blue Cross Laboratories Pvt. Ltd. Acetonitrile, HPLC grade water, Orthophosphoric Acid, Potassium Dihydrogen Phosphate, and Tri-ethylamine was procured from research lab fine chem industry.

2.3 SPECTROPHOTOMETRIC CONDITIONS

The stock solution was suitably diluted with distilled water, to get 10 µg/ml of Favipiravir. This solution was scanned in the UV region (200-400 nm) and found that Favipiravir exhibited maximum absorbance at about 358 nm as shown in Fig. 2.Hence 358 nm was selected for the proposed study [6-7].

3. PREPARATION OF SOLUTION

3.1 Preparation of Buffer and Other Solutions

1.36 gm of potassium dihydrogen phosphate and 2 ml of triethylamine was transferred into a beaker containing 1000 ml of water and sonicated to dissolve the contents completely. The pH was adjusted to 3.5±0.05 with orthophosphoric acid and mixed and filtered through 0.45µ filter paper.

3.2 Preparation of Diluent

The degassed mixture of buffer and acetonitrile in 50:50% v/v ratio was prepared.

3.3 Mobile Phase

Select the Binary method in HPLC and select pump B ratio as 90.

3.3 Standard Stock Solution

One hundred milligram pure drug was accurately weighed, dissolved in about 30 mL of diluent and transferred to a 100 mL volumetric flask. Then the volume was completed to

100 mL with diluent to obtain1000 mcg/mL of stock solution. The resulting stock solution was sonicated and filtered through a 0.45 mm filter. The stock solution was further diluted with diluent to obtain the required concentration of standard solutions (50–250 mcg/mL) before being injected into the system.

3.4 Sample Solution

Weigh and transfer equivalent to100mg powdered tablet into a 100ml volumetric flask. Add 30ml of diluent and sonicate to dissolve then make up to the volume with diluent, pipette out 5ml of this solution into a 100ml volumetric flask and dilute up to the mark with mobile phase. Filter through 0.45µ membrane filter.

4. METHOD VALIDATION

The developed method was validated as per the ICH guidelines in terms of linearity, precision, accuracy, repeatability, and stability studies.

4.1 linearity

Favipiravir was found to be linear in a concentration range of 2-10µg/ml. The absorbance of this solution were measured at 358 nm and a calibration graph was plotted using absorbance verses concentration. The correlation co-efficient value was found to be 0.990.

4.2 **Precision**

The reproducibility was determined by repeating the above methods at different time intervals (morning, afternoon and evening) on the same day (Intraday precision) and on three consecutive days (Interday precision). The intraday and interday variation for the estimation of favipiravir was carried out at three different concentration levels of 2, 8 and12 μgm/ml.

4.2 Accuracy

Accuracy was determined by performing recovery studies by spiking different concentration of pure drug in pre-analyzed sample solution of 4µg/ml. To pre analyzed sample solution, a known amount of working standard solution of Favipiravir (0.33, 0.42 and 0.48 ml of 100 µg/ml) was added in 10 ml volumetric flask and made up to mark with diluent which was at different level i.e. 80%, 100% and 120%. The solutions were analyzed by proposed method.

4.3. Repeatability

It was determined by analyzing same solution of 80 µgm/ml of favipiravir standard solution repeatedly.

4.4. Limit of Detection (LOD) and Limit of Quantification (LOQ)

1) LOD

The LOD was estimated from the set of five calibration curves used to determine method linearity. The calibration curve was repeated for 6 times and the SD of the intercept was calculated then LOD was calculated as follow:

$$LOD = (3.3*SD)/slope.$$

Where,

SD= the standard deviation of y-intercept of 5 calibration curves.Slope= the mean slope of the 5 calibration curves.

2) LOQ

The LOQ was estimated from the set of five calibration curves used to determine methodlinearity. The LOQ may be calculated as

$$LOQ = 10 \times (\sigma/S).$$

Where,
σ = Standard deviation of the Y- intercepts of the five calibration curves.
S = Mean slope of the five calibration curves.

5. RESULTS

5.1 Optimized Chromatographic Conditions and Mobile Phase

Column:	C18
Mobile phase:	Phosphate buffer pH 3.5: Acetonitrile (90:10)
Flow rate:	1ml/min
Detection Wavelength:	358nm Run time: 10min
Injection volume:	20µ/ml
Diluent:	Buffer 3.5: Acetonitrile (50:50)

5.2 Determination of Standard Calibration Curve

Adequate dilutions were made from stock solution to get a concentration ranging from 2- 10 µgm/ml for Favipiravir using distilled water. Absorbance of these solutions were measured at 358 nm. The absorbance values are tabulated in table-1. The measured absorbance was plotted against concentration. From the graph it was found that the Beer's law concentration for Favipiravir lies between 2-10 µgm. The calibration graph are shown in FIG-2.

Table-1: The absorbance of favipiravir at different concentration

Concentration (µ/ml)	Absorbance(nm)
2	0.146
4	0.256
6	0.351
8	0.486
10	0.643

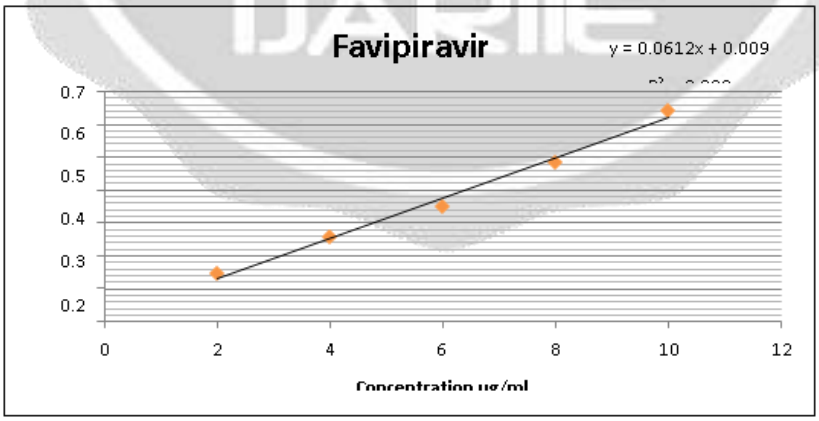

Fig-2: The calibration curve of favipiravir

5.3 Linearity

Standard calibration has been prepared using 5 standard solutions within the concentration range

of 50-250 µgm/ml. In optimized chromatographic conditions, each standard solution was chromatographed for 10 min three times. Least squares linear regression analysis of the average peak area versus concentration data were used to evaluate the linearity of the method. The linearity for Favipiravir were assessed by analysis of standard solution in range of 50-250 µg/ml .Correlation co-efficient for calibration curve was found to be 0.996. The linearity graph has been depicted in fig-4. The %RSD and absorbance are tabulated in table-2.

Table-2 The linearity data of favipiravir HPLC method

Sr. No.	**Concentration (µgm/ml)**	**Area**	**SD**	**% RSD**
1	50	1362884	2010.00	0.15
2	100	1969386	10111.00	0.51
3	150	2507427	8889.00	0.35
4	200	3227615	11111.00	0.34
5	250	3920467	10111.00	0.26
Regression coefficient (r^2)		0.997	AVG SD=8446.4	
Regression equation		y = 12747x + 68553		

5.4 Precision

Precision was analyzed by calculating variations of the method in intraday (repeatability performed by analyzing standard solution on the same day) and inter-day (repeatability carried out by analyzing standard solution on three different days). Precision study was performed by injecting six times of standard solution at three different concentrations 50, 100, and 150 µg/ml on the same day and three consecutive days. Results are expressed as Relative standard deviation (RSD) or coefficient of variance. The precision data is tabulated in table-5

Table-5 The precision data of favipiravir HPLC method

Sr. No.	**Conc.**	**Absorbance**			**Mean**	**SD**	**% RSD**
		I	**II**	**III**			
Interday							
1	50	1902159	1953257	1902543	1919319.67	29391.22	1.53
2	100	2507427	2535265	2486589	2509760.33	24421.74	0.97
3	150	4216871	4202589	4196548	4205336.00	10436.26	0.25
Intraday							
1	50	1551402	1546752	1504321	1546752.00	25944.28	1.68
2	100	1969386	2016743	1975462	1987197.00	25767.31	1.30
3	150	3920467	3986579	3940067	3949037.67	33956.65	0.86

5.5 Accuracy

Accuracy was determined by performing recovery studies by spiking specific concentration of pure drug in pre analyzed sample solution of 50µg/ml of Favipiravir. To pre-analyzed sample solution, a known amount of standard stock solution were added which was at different level 50, 100 and 150%. The solutions were analyzed by proposed method. Mean % recovery was calculated. The accuracy data of favipiravir is tabulated in table -6

Table-6 The accuracy data of favipiravir HPLC method

% Conc.	Sample amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Recovery mean	S.D.	% RSD
50	12.5	7.5	7.45	99.33	100.26	1.01	1
	12.5	7.5	7.51	100.13			
	12.5	7.5	7.6	101.33			
100	20	15	14.8	98.66	100.21	1.38	1.37
	20	15	15.1	100.66			
	20	15	15.2	101.3			
150	27.5	22.5	22.4	99.5	100.42	0.92	0.91
	27.5	22.5	22.6	100.44			
	27.5	22.5	22.8	101.33			

5.6 LOD and LOQ

The LOD and LOQ were estimated from the set of 5 calibration curves used to determine method linearity. It may be calculated as

LOD = 3.3 x (SD / Slope) LOQ = 10 x (SD / Slope)

Where,

SD = the standard deviation of Y- intercept of 5 calibration curves.Slope = the mean slope of the 5 calibration curves.

The LOQ and LOQ data is tabulated in table-7.

Table-7 The LOD and LOQ data of favipiravir HPLC method

S.D. of Intercept	8446.4
Slope of Calibration Curve	12747
LOD	2.186

LOQ	6.626

5.7 Robustness

In robustness study, the following parameters have been changed one by one andobserved their effect on system suitability test and assay.

- Change mobile phase composition by ± 1.0mL of organic solvent.
- Change Wavelength ± 1nm
- Change flow rate ±0.1mL/min.

Change in mobile phase composition: Std. working solution was injected three times by change in the mobile phase composition by ±1.0 mL of organic solvent (phosphate bufferpH 3.5:acetonitrile) (89:11v/v and 91:09v/v) of developed method.

Change in pH: Std. working solution was injected three times by change in the wavelength by ± 1 PH of sample (3.4 and 3.6) of developed method. Calculate the % RSD of mean area for change in method parameter.

Change in flow rate: Std. working solution was injected three times by change in the flow rate by ± 0.1 mL/min (0.9 mL/min and 1.1mL/min) of developed method. Calculate the %RSD of mean area for change in method parameter. The robustness data is illustrated in table-8.

Table-8 The robustness data of favipiravir HPLC method

Condition	Peak area mean	SD	% RSD
Change in ratio of mobile phase ±1ml	1673614.333	4829.76	0.29
	1672815.667	16402.25	0.98
Change in pH ±1	1695825	4774.77	0.28
	1668691.667	15418.54	0.92
Change in flow rate ±1ml	1627783.667	23809.49	1.46
	1635044.667	9395.67	0.57

5.8 UV Degradation Studies

Weighed 100mg sample and keep it in UV chamber at 200-400nm for 24 hrs and 48 hrs. From this weigh 10 mg sample and transfer it in 100 ml volumetric flask. Make up volume up to the mark with diluent. Then take 1ml of above solution and transfer it in to 10ml volumetric flask. Make volume with diluents, this solution injected in to system. The chromatogram obtained are given in fig-3 and 4 The peak area and % degradation is tabulated in table-9.

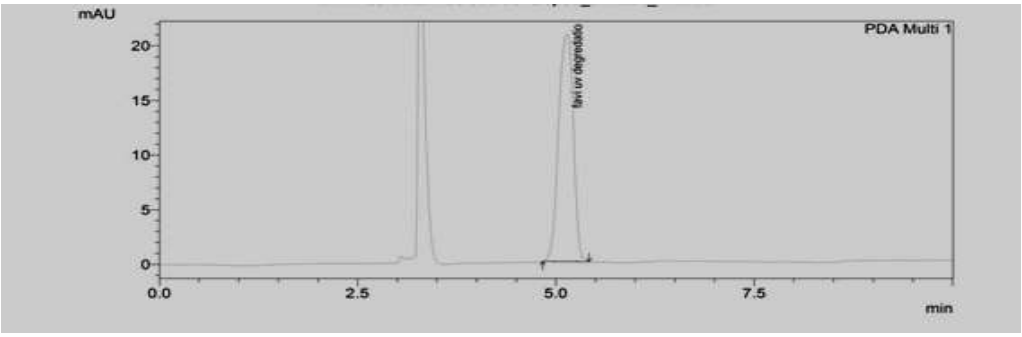

Fig-3 The chromatogram of favipiravir obtained in UV degradation studies after 24 hrs

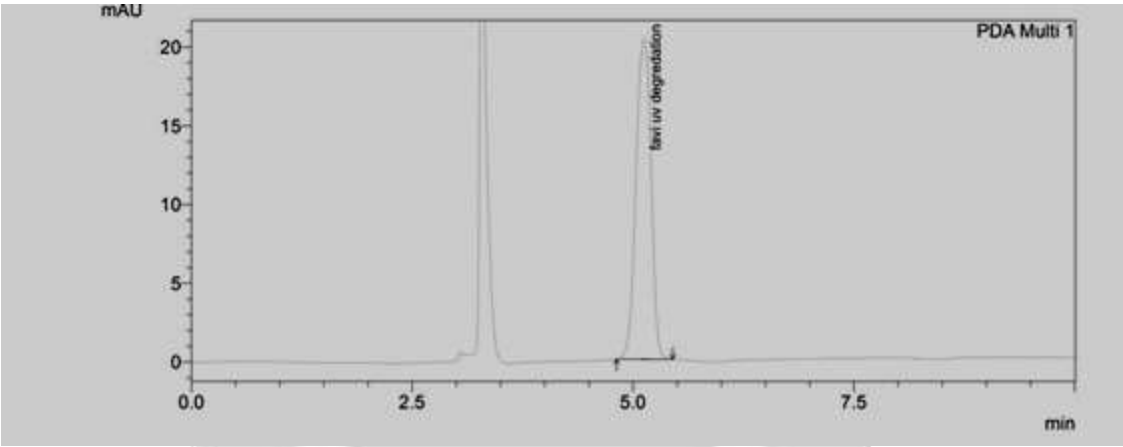

Fig-4 The chromatogram of favipiravir obtained in UV degradation studies after48 hrs

Table-9 The peak areas and % degradation of drug in stability studies

Parameter	**Area**	**% Degradation**
Standard	325986	00
Acid	196748	39.64
Base	277070	15.01
Thermal	258328	20.76
Hydrolysis	314325	3.57
Photolytic UV 24hrs	269524	17.32
UV 48 Hrs	242240	25.69

6. CONCLUSION

The aim of present work is to develop and validate the stability indicating analytical methods for favipiravir in bulk and its formulations with the forced degradation studies. Although, there are many methods have been reported on the quantitative and qualitative estimation of favipiravir in bulk and its marketed formulation, but very few work has been done on its estimation by RP-HPLC followed by stability indicating methods. Therefore, in present work we have developed and validated the selective stability indicating RP-HPLC and UV methods. the development of UV Spectroscopic method of favipiravir, distilled water was selected as solvent. The solution of appropriate concentration was analyzed in UV range from 200-400 nm. The maximumabsorbance was observed at 358 nm and the same value was selected for further analysis. Calibration curve resulted in increase in absorbance as we increased the concentration

and given a standard slope. This developed UV Spectroscopic method was validated as per the ICH guidelines in terms of linearity, precision, accuracy, repeatability and stability studies. All the validation parameters were found to be within permissible limitas per the ICH guidelines. The developed method has been successfully applied for the estimation of favipiravir on selected marketed formulation. The λmax was observed exactly at 358 nm and the % assay was found to be 102%. The label claim of the favipiravir was 200 mg and we found it to be 204 mg in the tablet assay of favipiravir using this developed method.

7. ACKNOWLEDGEMENT

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