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ORIGINAL ARTICLE

Development and Validation of A Stability-Indicating RP-HPLC Method for the Estimation of Favipiravir in Bulk and Pharmaceutical Formulation

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ABSTRACT
Favipiravir is a broad-spectrum antiviral agent widely investigated for the treatment of influenza and emerging viral infections, including COVID-19. The present study aimed to develop and validate a simple, accurate, precise, and stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method for the quantitative estimation of favipiravir in bulk drug and pharmaceutical formulations. Chromatographic separation was achieved on a Zodiac C18 column (150 × 4.6 mm, 5 µm) using a mobile phase consisting of 15 mM ammonium acetate and acetonitrile (80:20, v/v) at a flow rate of 0.8 mL/min, with detection at 230 nm. The developed method exhibited excellent linearity over the concentration range of 3.125–100 µg/mL with a correlation coefficient (R^2) of 0.9999. The limits of detection and quantitation were found to be 2.06 µg/mL and 6.87 µg/mL, respectively. Validation studies performed according to ICH guidelines demonstrated satisfactory accuracy, precision, repeatability, and system suitability, with %RSD values below 2%. Forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic conditions confirmed the stability-indicating nature of the method and revealed that favipiravir is particularly susceptible to alkaline and photolytic degradation. The assay of the marketed formulation showed 100.6% of the labeled claim. The proposed RP-HPLC method is reliable, sensitive, and suitable for routine quality control and stability assessment of favipiravir in pharmaceutical dosage forms.
KEYWORDS: *Favipiravir, RP-HPLC, Method Validation, Stability-Indicating Method, Forced Degradation, Pharmaceutical Analysis.*

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INTRODUCTION

Favipiravir (FVP), commercially known as Avigan, is a broad-spectrum antiviral drug originally developed for the treatment of influenza infections in Japan. Chemically, it is a pyrazinecarboxamide derivative (6-fluoro-3-hydroxypyrazine-2-carboxamide) that exhibits potent antiviral activity against a wide range of RNA viruses (Figure 1) [1–3]. Favipiravir was developed and manufactured by Toyama Chemical Co., a subsidiary of Fujifilm Holdings Corporation, and received approval for medical use in Japan in 2014. Owing to its promising antiviral properties, the drug has subsequently been investigated for the treatment of several viral diseases, including Ebola virus disease, influenza, and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections [4–6].

The emergence of coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2, rapidly evolved into a global public health emergency, affecting millions of individuals worldwide and causing significant morbidity and mortality [7,8]. The pandemic dramatically altered healthcare systems, economies, and daily life across the globe. Although substantial progress has been made in understanding and managing the disease, COVID-19 continues to pose a threat due to the emergence of new variants and ongoing transmission in several regions. During the early stages of the pandemic, the absence of specific antiviral

therapies prompted the urgent search for effective treatment options to reduce disease severity and improve patient outcomes [7,8].

Several existing antiviral agents, including chloroquine, remdesivir, arbidol, and favipiravir, were repurposed and evaluated through clinical trials to determine their efficacy and safety against COVID-19 [9,10]. Despite extensive research efforts, no universally accepted gold-standard antiviral treatment was initially available, highlighting the need for further investigation of promising therapeutic candidates.

Favipiravir exerts its antiviral action by selectively inhibiting the RNA-dependent RNA polymerase (RdRp) enzyme, which is essential for the replication of RNA viruses. After intracellular conversion to its active ribofuranosyl triphosphate form, favipiravir interferes with viral RNA synthesis, thereby preventing viral replication and proliferation. This mechanism enables its activity against a diverse range of RNA viruses, including influenza viruses, flaviviruses, arenaviruses, bunyaviruses, filoviruses, noroviruses, and coronaviruses [11–13].

Clinical studies conducted during the COVID-19 pandemic suggested that favipiravir could accelerate viral clearance and improve clinical recovery in infected patients. Notably, a pilot study conducted at Zhongnan Hospital of Wuhan University reported improved recovery rates in patients treated with favipiravir compared with those receiving arbidol, indicating its potential therapeutic value against COVID-19. These findings have encouraged continued research into favipiravir as a promising antiviral agent for the management of emerging viral infections and pandemic diseases.

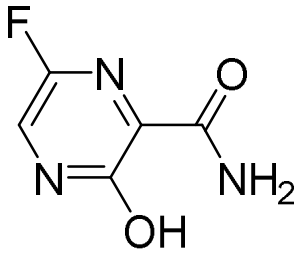

Figure 1: Structure of Favipiravir

MATERIAL AND METHODS

Chemicals and Reagents

Commercial Favipiravir tablets (Avigen®, 200 mg) were purchased from the local market and used for the study. HPLC-grade methanol (Rankem), ammonium acetate, acetonitrile (Avra Synthesis Ltd.), 0.45 µm nylon membrane filters (Axiva Sichem Pvt. Ltd.), and Milli-Q water were used throughout the analysis.

Instrumentation

The HPLC system consisted of a quaternary pump, autosampler, PDA detector, and Empower-2 software. A Zodiac C18 column (150 × 4.6 mm, 5 µm) was used for separation. A UV-Visible spectrophotometer (WATERS Alliance 2695) was used for wavelength selection. All weighing was done using a Sartorius analytical balance, and pH adjustments were performed with a digital pH meter.

Preparation of Mobile Phase and Diluent

The diluent was prepared using 15 mM ammonium acetate–acetonitrile– (80:20 v/v). For the mobile phase, 15 mM ammonium acetate–acetonitrile (80;20 v/v) was filtered through a 0.45 µm membrane filter and sonicated before use [14,15].

Selection of Detection Wavelength

A solution of favipiravir (10 µg/mL) was scanned in the UV range of 200–400 nm. Maximum absorbance was observed at 230 nm, which was selected as the detection wavelength [16,17].

Preparation of Standard Solution

About 10 mg of favipiravir was dissolved in diluent and sonicated to obtain a stock solution (1000 µg/mL). Appropriate dilutions were made to obtain working concentrations.

Chromatographic Conditions

The chromatographic separation of favipiravir was achieved using a Zodiac C18 column (150 × 4.6 mm, 5 µm) with an isocratic mobile phase consisting of 15 mM ammonium acetate and acetonitrile in the ratio of 80:20 (v/v). The mobile phase was delivered at a flow rate of 0.8 mL/min, and the analyte was detected at a wavelength of 236 nm. A sample injection volume of 10 µL was employed for all analyses. The total run time was maintained at 20 min, and favipiravir was eluted at a retention time of 11.7 min under the optimized chromatographic conditions [14,15].

Validation Parameters

The developed method was validated according to ICH guidelines for linearity, accuracy, precision, sensitivity (LOD, LOQ), system suitability, robustness, and assay of marketed formulation.

Forced Degradation Studies

Stress testing was performed by subjecting 100 µg/mL solutions of favipiravir to different conditions.

Acidic and Basic, oxidative Degradation

Using 10 mL of 0.1N hydrochloric acid and sodium hydroxide, respectively, 100 µg/mL of favipiravir was refluxed and evaporated for one hour at 70 °C in a water bath. The same concentration was then allowed to cool at room temperature in HCL. Following this time frame, each solution was filtered, neutralised, and its volume was reconstituted using diluent to achieve a concentration of 40 µg/mL [18,19].

Photolytic degradation

10 mL of 100 ppm solution of favipiravir was kept under the UV light for 24 hours. After that, 40 µg/mL of concentration was made with diluent and injected for analysis.

RESULTS AND DISCUSSION

Method Development and Optimization

The RP-HPLC method for the estimation of favipiravir was optimized by evaluating different chromatographic parameters, including stationary phase, mobile phase composition, flow rate, and detection wavelength, to obtain adequate peak resolution and sensitivity. The optimized chromatographic conditions consisted of a Zodiac C18 column (150 × 4.6 mm, 5 µm) with a mobile phase comprising 15 mM ammonium acetate and acetonitrile (80:20, v/v) delivered at a flow rate of 0.8 mL/min under isocratic elution. Detection was carried out at 230 nm at room temperature.

Under the optimized conditions, favipiravir produced a sharp, symmetrical, and well-resolved peak with a retention time of 2.981 min. The chromatogram exhibited minimal baseline noise and no interference from solvent or excipient peaks, indicating the specificity of the method. As shown in Figure 2, favipiravir was effectively separated from other minor peaks observed at retention times of 0.60 and 0.69 min. The principal favipiravir peak accounted for 98.54% of the total chromatographic area, demonstrating excellent selectivity and purity.

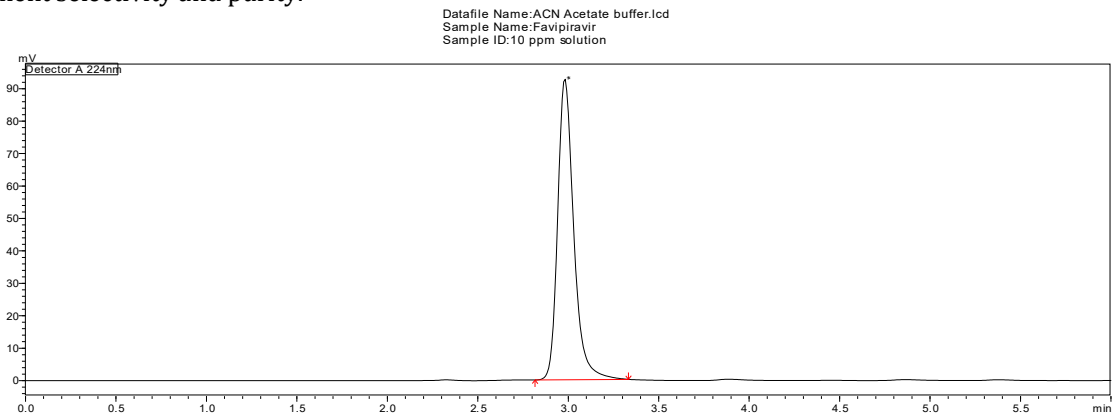

Figure 2: Optimized RP-HPLC chromatogram of favipiravir showing a sharp and symmetrical peak at a retention time of 2.981 min under the selected chromatographic conditions.

The chromatographic performance data summarized in Table 1 revealed that the favipiravir peak exhibited a theoretical plate count of 567.166, indicating acceptable column efficiency under the selected conditions. The tailing factor was found to be 1.469, confirming good peak symmetry. Furthermore, a resolution value of 5.323 and a capacity factor (k′) of 3.652 were obtained, indicating efficient retention and separation of the analyte from neighboring peaks. The separation factor of 24.382 further demonstrated the excellent resolving capability of the developed method. Collectively, these results confirm that the optimized chromatographic conditions are suitable for the accurate and reliable estimation of favipiravir in bulk drug and pharmaceutical formulations.

Table 1: method development of favipiravir at wavelength 230 nm

Peak#	Ret. Time	Area	Area%	T. Plate#	Tailing F.	Resolution	k'	Separation
1	0.6	39241	0.5539	0.003	--	--	0	0
2	0.69	64226	0.9065	74.066	--	0.004	0.15	0
favipiravir	2.981	6981293	98.5396	567.166	1.469	5.323	3.652	24.382

Method validation

The method was validated according to ICH guidelines.

Linearity and Sensitivity

The calibration curve was linear across the concentration range of 3.12–100 µg/mL with a correlation coefficient (R^2) of 0.9999, indicating excellent linearity (Figure 3). The regression equation (y = 7151x + 3095.6) showed proportionality between peak area and drug concentration (Table 2).

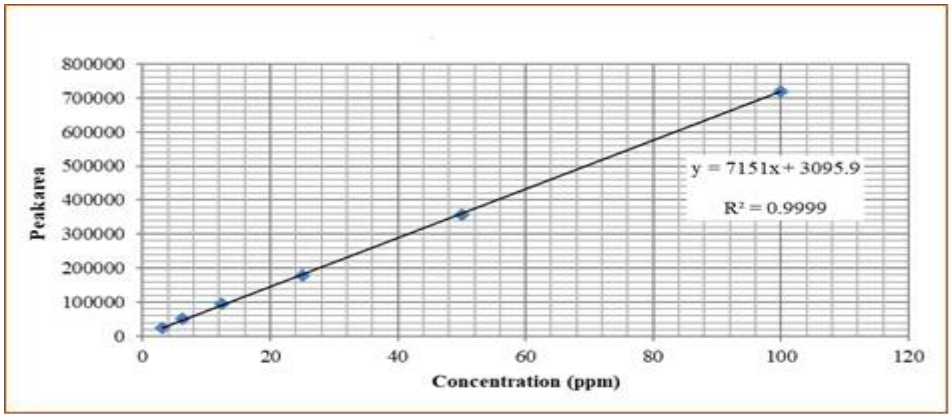

Figure 3: Calibration curve of favipiravir showing excellent linearity over the concentration range of 3.12–100 µg/mL with a correlation coefficient (R^2) of 0.9999 using the developed RP-HPLC method.

Table 2: Linearity and sensitivity parameters and results

Concentration (µg/mL)	**Area**
100	720107
50	358015
25	177301
12.5	95693
6.25	50544
3.125	24777
Regression Equation	y=7151x + 3095.6
Correlation coefficient (R^2)	0.9999
Std. error of intercept	2006.300268
Std. Dev. of intercept	4914.411927
LOQ	6.87 µg/ml
LOD	2.06 µg/ml

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of the developed method was determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD and LOQ were determined to be 2.06 µg/mL and 6.87 µg/mL, respectively, confirming the sensitivity of the method.

Repeatability study of favipiravir

Implementing the procedure mentioned under experimental section (5.3), the favipiravir was tested for six injections within the same day. Furthermore, their RSD values in percentage were calculated and as observed it was 0.59 for favipiravir. Thus, calculated RSDs for all selected drugs in simultaneous analysis were less than 2% which are quite significant in accordance with the ICH guidelines.

Table 3: Repeatability study of favipiravir

S. No.	**Drug Name: favipiravir**
	Peak Area: Conc. 100 ppm
1	6981293
2	6911241
3	6994443
4	6898812
5	6944063
6	6904918
Mean	**6939128**
STD. DEV.	**41074.02926**
RSD (%)	**0.59**

Precision

The intraday and interday precision studies further confirmed the reliability of the developed method (Table 4). For intraday analysis, six replicates of favipiravir at 100 µg/mL were evaluated within the same day, and the %RSD values ranged from 0.31 to 1.34. Similarly, interday precision performed over three consecutive days yielded %RSD values between 0.30 and 0.71. All results were well within the ICH

acceptance limit of not more than 2%, confirming that the method is both reproducible and consistent across time. The low variability in peak area and retention time demonstrates excellent instrument performance and robustness of the chromatographic conditions, establishing the suitability of the method for routine quality control analysis.

Table 4: Intraday and interday precision data from developed method

Sr. No.	Concentration (ppm)	Area	Average	Std. Deviation	%RSD
Intraday					
1	100 ppm	603596	612541	8179.43	1.34
		619639			
		614388			
2	100 ppm	609301	610415	1892.39	0.31
		612600			
		609344			
3	100 ppm	613144	610417	4306.19	0.71
		612655			
		605453			
	Range of % RSD				**0.31-1.34**
Interday					
Day 1	100 ppm	613144	610417	4306.19	0.71
		612655			
		605453			
Day 2	100 ppm	619035	618156	1880.81	0.30
		615997			
		619437			
Day 3	100 ppm	619127	613571	4853.23	0.79
		610159			
		611427			
	Range of % RSD				**0.30 -0.71**

Accuracy

Accuracy was assessed by recovery studies at three concentration levels (100%, 120%, and 150% of the target concentration (Table 5). The percentage recovery of Favipiravir was found to range between 99.9% and 102.5%, with mean recovery values close to 101%. The very low standard deviation and %RSD values (<0.3) indicate minimal variation among replicates, confirming the method's accuracy. These findings clearly demonstrate that the proposed RP-HPLC method can reliably quantify molnupiravir without interference from excipients present in the formulation, thereby proving its applicability for assay of both bulk drug and finished product.

Table 5: Accuracy studies

% Level	Std spiked (mL)	Amount recovered (%)	% Recovery	Mean % recovery	±SD	% RSD
100	0	101.18	100%	100%	0.0252	0.0252
100	0	101.14	99.99%			
100	0	101.19	101.02%			
120	4	123.27	101.72	101.06%	0.2276	0.2276
120	4	123.56	102.5%			
120	4	123.83	102.08%			
150	8	133.75	101.9	101.42%	0.1799	0.1799
150	8	133.42	101.62			
150	8	133.65	101.89			

System suitability studies

System suitability test for favipiravir reveals the factors such as, theoretical plate (N), capacity factor (k'), resolution (R), separation factor (α), tailing factor (*T*), Mean±SD and RSD% which should be in the acceptable range for at least 6 successive injections of same analytes (Table 6).

Table 6: System suitability of favipiravir

System suitability parameters	Results	Acceptable Values
Theoretical plates (N)	33502	> 2000
Capacity Factor (K')	3.652	> 0.5 - <10
Resolution (R)	---	≥ 2
Selectivity/Separation factor (α)	1.157	> k'
Asymmetry/Tailing factor (T)	0.694	> 2
Retention time (t_R)	2,981 min	> k'
Wavelength of Detection (nm)	236 nm	> 200 nm
Repeatability (%RSD)	0.71	< 2
Intra-Day Precision (%RSD)	0.31-1.34	< 2
Inter-Day Precision (%RSD)	0.30 -0.71	< 2
Linearity range	3.13 – 100 µg/ml	NA
Regression equation	y=7151x + 3095.6	NA
SE of intercept (S_e)	2006.300268	NA
SD of intercept (S_a)	4914.411927	NA
Correlation Coefficient (r^2)	0.9999	NA
LOQ (µg/mL)	6.87 µg/ml	NA
LOD (µg/mL)	2.06 µg/ml	NA

Force degradation studies

The oxidative degradation study using 3% H_2O_2 demonstrated that favipiravir underwent slight degradation, producing an additional degradation peak at a retention time of 3.544 min (Figure 4). The parent drug peak was observed at 2.757 min with an area percentage of 6.60%, while the degradation product accounted for 0.72% of the total area (Table 7). Adequate separation between the drug and degradation product was achieved with a resolution value of 3.74, confirming the stability-indicating capability of the developed RP-HPLC method. These findings indicate that favipiravir is relatively stable under oxidative stress conditions, with only minimal degradation observed.

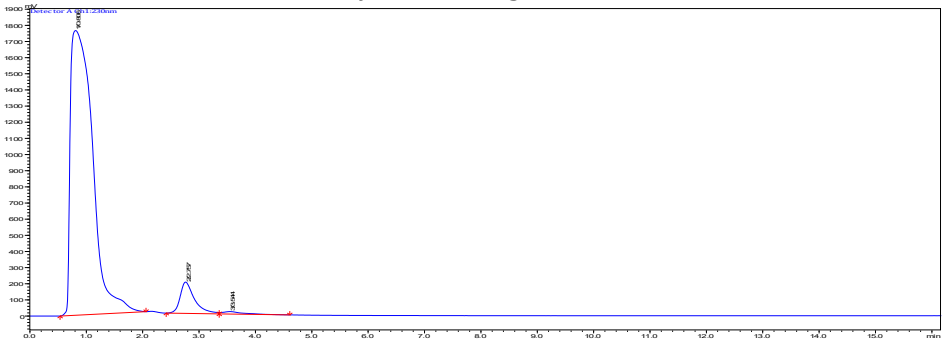

Figure 4: effect of 3% H2O2 on favipiravir

Table 7: effect of 3% H_2O_2 on favipiravir

Peak#	Ret. Time	Area	Area%	T. Plate#	Tailing F.	Resolution	k'	Separation
1	0.806	48225301	92.6811	27.863	3.221	--	0	0
favipiravir	2.757	3432311	6.5963	648.843	1.703	3.74	2.422	0
3	3.544	376002	0.7226	527.289	--	1.499	3.399	1.403

The photolytic degradation study demonstrated that favipiravir was susceptible to degradation upon exposure to sunlight, resulting in the formation of multiple degradation products (Figure 5). The parent drug peak was observed at a retention time of 2.639 min with an area percentage of 61.86%, while four additional degradation peaks appeared at retention times of 0.768, 1.054, 1.738, and 2.138 min (Table 8). The appearance of these degradation peaks indicates significant photodegradation of favipiravir under sunlight exposure. Adequate separation between the drug and degradation products was achieved, with the favipiravir peak exhibiting acceptable chromatographic characteristics, including a tailing factor of 2.0 and a resolution value of 1.39 (Table 8). The observed degradation of approximately 10% under photolytic stress suggests that favipiravir is sensitive to sunlight and should be protected from light during storage and handling. The successful separation of degradation products from the parent drug further confirms the stability-indicating nature of the developed RP-HPLC method.

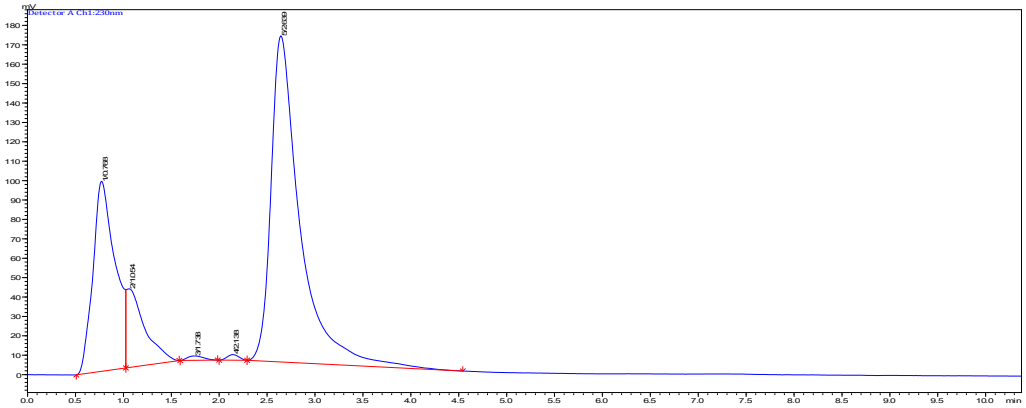

Figure 5: Effect of sunlight on favipiravir

Table 8: Effect of sunlight on favipiravir

Peak#	Ret. Time	Area	Area%	T.Plate#	Tailing F.	Resolution	k'	Separation
1	0.768	1587897	27.8161	49.044	--	--	0	0
2	1.054	538584	9.4347	10.046	--	0.324	0.374	0
3	1.738	26590	0.4658	473.35	1.307	0.828	1.264	3.383
4	2.138	23917	0.419	1301.269	1.061	1.438	1.785	1.412
favipiravir	2.639	3531576	61.8645	475.141	2	1.39	2.438	1.366

The alkaline degradation study using 0.1 N NaOH revealed that favipiravir undergoes noticeable degradation under basic conditions, resulting in the formation of two additional degradation peaks at retention times of 3.186 and 3.585 min (Figure 6). The parent drug peak was observed at a retention time of 2.614 min with an area percentage of 60.09%, while the degradation products contributed 0.31% and 4.21% of the total chromatographic area, respectively (Table 9). The favipiravir peak exhibited acceptable chromatographic performance with a tailing factor of 1.135 and a resolution value of 3.936, indicating effective separation from the degradation products (Table 9). The observed degradation of approximately 4.5% under alkaline stress demonstrates that favipiravir is susceptible to basic conditions. The clear separation of degradation peaks from the parent drug confirms the stability-indicating capability of the developed RP-HPLC method and its suitability for stability assessment of favipiravir.

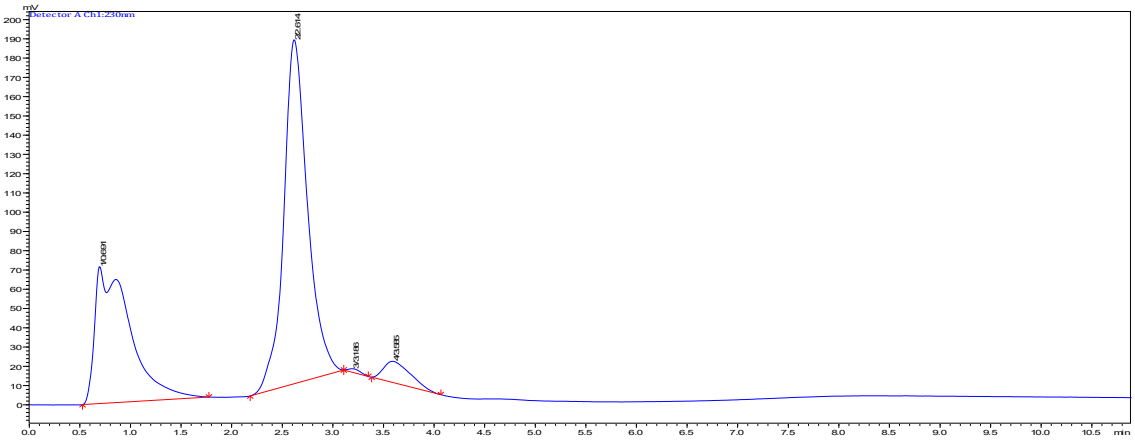

Figure 6: Effect of 0.1N NaOH on favipiravir

Table 9: Effect of 0.1N NaOH on favipiravir

Peak#	Ret. Time	Area	Area%	T. Plate#	Tailing F.	Resolution	k'	Separation
1	0.691	1726668	35.3956	24.148	3.688	--	0	0
favipiravir	2.614	2931086	60.0854	636.273	1.135	3.936	2.783	0
3	3.186	15051	0.3085	3213.486	1.545	1.791	3.611	1.298
4	3.585	205394	4.2104	721.144	1.618	1.051	4.188	1.16

The acid degradation study was carried out using 0.1 N HCl to evaluate the stability of favipiravir under acidic conditions. The chromatogram showed the parent drug peak at a retention time of 2.63 min along with four minor degradation peaks at retention times of 0.656, 0.945, 1.716, and 3.575 min, indicating limited degradation of the drug under acidic stress (Figure 7). The favipiravir peak accounted for 89.61% of the total chromatographic area, while the degradation products represented only a small fraction of the total area (Table 10). The parent drug peak exhibited satisfactory chromatographic characteristics with a tailing factor of 1.287 and a resolution value of 2.628, demonstrating adequate separation from the degradation products (Table 10). The overall degradation observed under acidic conditions was approximately 2.84%, suggesting that favipiravir is relatively stable in an acidic environment. The successful resolution of the degradation products from the drug peak confirms the stability-indicating nature of the developed RP-HPLC method and its suitability for stability studies of favipiravir.

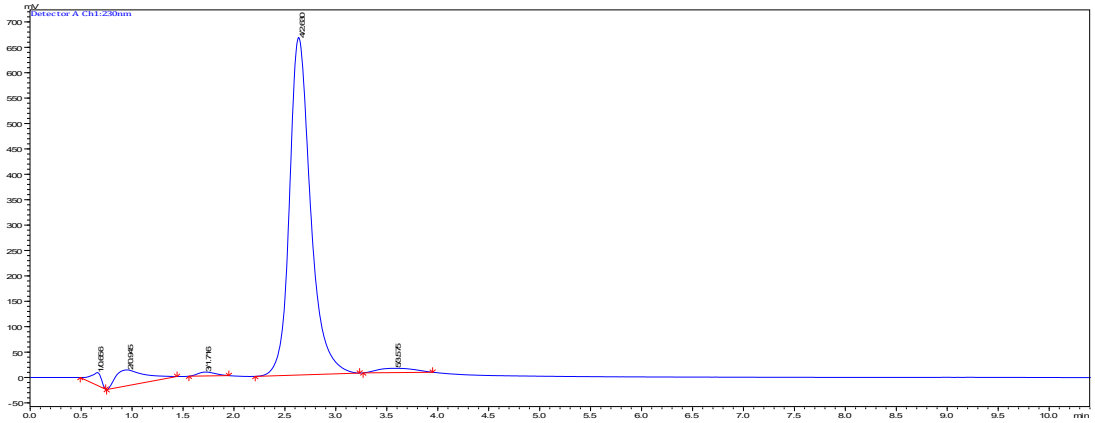

Figure 7: Effect of 0.1N HCl on favipiravir

Table 10: Effect of 0.1N HCl on favipiravir

Peak#	Ret. Time	Area	Area%	T. Plate#	Tailing F.	Resolution	k'	Separation
1	0.656	170823	1.6027	175.023	0.755	--	0	0
2	0.945	631478	5.9246	58.398	1.801	0.835	0.441	0
3	1.716	90542	0.8495	422.487	1.255	1.862	1.618	3.667
Favipiravir	2.63	9551277	89.6112	846.688	1.287	2.628	3.012	1.862
5	3.575	214453	2.012	488.959	1.121	1.873	4.453	1.478

Forced degradation studies showed that favipiravir was most susceptible to photolytic degradation, exhibiting 10% degradation and three degradant peaks under sunlight exposure (Table 11). Moderate degradation was observed under alkaline conditions (4.50%), while acidic and oxidative stress resulted in 2.84% and 0.72% degradation, respectively. No degradation was detected under thermal conditions, indicating good thermal stability. These results confirm that the developed RP-HPLC method is stability-indicating and capable of effectively separating favipiravir from its degradation products (Table 11).

Table 11: Summary of forced degradation studies of favipiravir under acidic, alkaline, thermal, oxidative, and photolytic stress conditions.

Conditions: Favipiravir	Retention time	Retention time (after degradation)	No. of degradants	% degradation
Acid (0.1N/M HCl)	2.79	2.63	2 degradants	2.84%
Base (0.1N/M NaOH)	2.79	2.61	2 degradants	4.50%
Thermal (45˚C) + 12 Hrs.	2.79	2.67	0 degradants	0%
Oxidation (3-6% H_2O_2)	2.79	2.75	1 degradant	0.72%
Sunlight exposed + 3 days	2.79	2.63	3 degradant	10%

Assay of Marketed Formulation

The assay of the commercial formulation (200 mg) was performed using the developed method. The amount of favipiravir present was calculated to be 100.6% of the label claim, which is within pharmacopeial acceptance limits (±2%). The chromatograms showed no interfering peaks at the retention time of favipiravir (Figure 8), thereby confirming the specificity of the method. This result not only supports the accuracy of the method in real pharmaceutical matrices but also underscores its suitability for routine batch analysis in quality control laboratories.

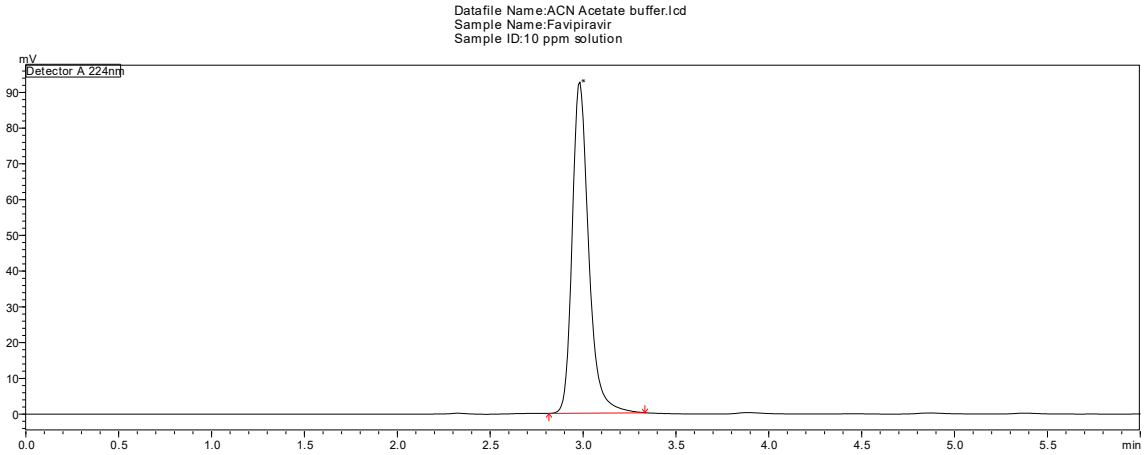

Figure 8: The chromatogram of favipiravir obtained from marketed formulation by applying developed method

The developed RP-HPLC method provides a reliable approach for quantitative estimation of favipiravir in both bulk and capsule formulations. The linearity and sensitivity data confirm that the method is suitable for detecting low concentrations, making it applicable for trace-level impurity profiling. The accuracy and precision results validate reproducibility and reliability, which are critical for quality control. Forced degradation studies revealed that favipiravir is stable under acidic and photolytic conditions but highly susceptible to degradation under alkaline and oxidative stress. This information is crucial for designing appropriate storage and formulation strategies. Compared to previously reported analytical techniques such as LC-MS/MS, this method is cost-effective, less resource-intensive, and practical for routine laboratory use.

CONCLUSION

In conclusion, a simple, accurate, precise, and stability-indicating RP-HPLC method was successfully developed and validated for the quantitative estimation of favipiravir in bulk drug and pharmaceutical dosage forms. The method exhibited excellent linearity over the concentration range of 3.125–100 µg/mL (R^2 = 0.9999), with satisfactory sensitivity, accuracy, precision, repeatability, and system suitability parameters in compliance with ICH guidelines. The assay of the marketed formulation yielded 100.6% of the labeled claim, confirming the applicability of the method for routine pharmaceutical analysis. Forced degradation studies demonstrated that the method could effectively separate favipiravir from its degradation products, confirming its stability-indicating nature. Favipiravir showed maximum degradation under photolytic stress, followed by alkaline, acidic, and oxidative conditions, while exhibiting good thermal stability. Overall, the developed RP-HPLC method is reliable, cost-effective, and suitable for routine quality control, assay determination, and stability studies of favipiravir in pharmaceutical industries and research laboratories.

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