RESEARCH PAPER

Development and Validation of a RP-HPLC Method for Favipiravir in Bulk Form

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ABSTRACT

Background

To develop and validate a simple, sensitive, accurate, and reproducible reversed-phase high-performance liquid chromatography (RP-HPLC) method for the quantitative estimation of favipiravir in bulk drug substance and its marketed tablet formulation.

Materials and Methods

Chromatographic separation was achieved on a SunQSIL C18 column (250 × 4.6 mm, 5 µm) using a mobile phase consisting of methanol and water in a ratio of 80:20 (v/v), pumped isocratically at a flow rate of 1.0 mL/min. Detection was carried out at 323 nm using a PDA detector with an injection volume of 20 µL. The method was validated in compliance with ICH Q2(R1) guidelines, covering linearity, precision, accuracy, specificity, robustness, limit of detection (LOD), and limit of quantitation (LOQ).

Results

Favipiravir eluted at a retention time of 5.23 min, with a theoretical plate count of 4657.25 and an asymmetry factor of 1.16. The method demonstrated linearity over the concentration range of 5–30 µg/mL ($R^2 = 0.9993$). Intra-day and inter-day precision studies yielded %RSD values well below 2%. Mean percentage recovery from the assay was 100.17 ± 0.52%, and accuracy studies at 50%, 100%, and 150% spiking levels gave mean recoveries of 99.72%, 99.97%, and 99.90%, respectively. LOD and LOQ were determined to be 0.85 µg/mL and 2.58 µg/mL, respectively. Robustness testing confirmed the method's stability under deliberate minor variations in flow rate, detection wavelength, and mobile phase composition.

Conclusion

The validated RP-HPLC method proved to be accurate, precise, linear, robust, and specific. It is suitable for routine quality control and quantitative analysis of favipiravir in pharmaceutical dosage forms.

KEYWORDS: Favipiravir; RP-HPLC; Method Development; ICH Q2(R1); Validation; Analytical Method.

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1. INTRODUCTION

Favipiravir (T-705; 6-fluoro-3-hydroxy-2-pyrazinecarboxamide) is a broad-spectrum antiviral agent that functions as a selective inhibitor of viral RNA-dependent RNA polymerase (RdRp). Originally developed and approved in Japan for the treatment of novel or re-emerging influenza virus infections, favipiravir gained renewed global attention during the COVID-19 pandemic owing to its demonstrated in vitro activity against SARS-CoV-2 and several other RNA viruses including arenaviruses, bunyaviruses, flaviviruses, and paramyxoviruses [1,2]. Unlike neuraminidase inhibitors, which primarily prevent viral entry and release, favipiravir exerts its antiviral effect through a distinct mechanism: once intracellularly phosphoribosylatedpale to its active triphosphate form (favipiravir-RTP), it mimics purine nucleotides and competes for incorporation into the nascent viral RNA strand by RdRp, thereby halting chain elongation and ultimately suppressing viral replication [3,4]. The molecular formula of favipiravir is $C_5H_4FN_3O_2$, with a molecular weight of 157.10 g/mol. It appears as a white to pale yellow crystalline powder. Its pyrazinecarboxamide core structure carries a fluorine substituent at the 6-position and a hydroxyl group at the 3-position, features that are integral to its biological activity. The drug is commercially available in India under brand

names such as Favipil and Fabiflu, primarily in 200 mg and 400 mg tablet strengths [1,2].

Given the wide clinical deployment of favipiravir, particularly during the pandemic, the need for reliable and validated analytical methods to quantify it in pharmaceutical formulations is of considerable importance for quality assurance and pharmacokinetic research. High-performance liquid chromatography (HPLC), and in particular its reversed-phase variant (RP-HPLC), remains the most widely employed technique for pharmaceutical analysis owing to its superior sensitivity, specificity, reproducibility, and the ability to separate polar as well as non-polar analytes under physiologically relevant mobile phase conditions [5,6]. Several analytical methods for favipiravir have been reported in the literature, employing different chromatographic conditions, mobile phase systems, and detection wavelengths [3,4,] However, the method conditions described in these reports vary considerably, and many have not been validated under the current ICH Q2(R1) framework with a comprehensive parameter set including robustness evaluation. The present study was therefore undertaken to develop a new, simple, cost-effective RP-HPLC method for quantitative estimation of favipiravir in bulk drug and its marketed tablet formulation (Favipil-400, Terrace Pharmaceuticals Pvt. Ltd.), and to validate it fully as per ICH Q2(R1) guidelines [20]. The method employs a readily available SunQSIL C18 stationary phase with a binary mobile phase of methanol and water, avoiding the need for buffer preparation or pH adjustment, which simplifies routine laboratory application.

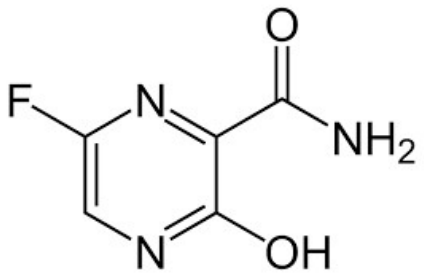

Figure.1 Structure of Favipiravir

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Favipiravir working standard was procured as a gift sample from Glenmark Pharmaceuticals (Mumbai, India). The marketed tablet formulation Favipil-400 (each tablet labeled to contain 400 mg of favipiravir, manufactured by Terrace Pharmaceuticals Pvt. Ltd.) was obtained from a local pharmacy. HPLC-grade methanol was purchased from Loba Chemie Pvt. Ltd., Mumbai. HPLC-grade water was obtained from an in-house ELGA PURELAB UHQ-II water purification system. All chemicals used were of analytical or HPLC grade, and solvents were filtered through a 0.45 µm membrane filter before use.

2.2 Instruments and Equipment

The following instruments were used throughout the study:

HPLC System: Jasco HPLC system comprising PU-2080 Plus intelligent HPLC pump, MX-2080-31 solvent mixing module, Rheodyne manual injector fitted with a 20 µL loop, and MD-2010 Plus multi-wavelength PDA detector, operated through Borwin-PDA software (Version 1.50).

Column: SunQSIL C18 reversed-phase column (250 × 4.6 mm, 5 µm particle size).

UV-Visible Spectrophotometer: Shimadzu UV-1780, used for wavelength selection.

Analytical Balance: Shimadzu ATX-224R electronic balance.

Membrane Filtration Unit: 0.45 µm pore size membrane filter (BIOMEDIA/JETVAC-JI).

Sonicator: Prama Solutions ultrasonic bath.

2.3 Selection of Analytical Wavelength

A solution of favipiravir at a concentration of 10 µg/mL was prepared in methanol from the standard stock solution and scanned over the wavelength range of 200–400 nm using the Shimadzu UV-1780 spectrophotometer. The UV spectrum revealed two absorption maxima at 227 nm and 323 nm. Considering the specificity and absence of potential interferents at the longer wavelength, 323 nm was selected as the analytical detection wavelength for all subsequent HPLC analyses.

Fig.2: UV absorption spectrum of favipiravir (10 µg/mL in methanol).

2.4 Preparation of Standard Stock Solution

An accurately weighed quantity of 10 mg of favipiravir working standard was transferred to a clean, dry 10 mL volumetric flask. The drug was dissolved in methanol with gentle swirling, and the volume was made up to the mark with the same solvent to give a stock solution of concentration 1000 µg/mL. This solution was stored at room temperature and used within the day of preparation.

2.5 Preparation of Working Standard Solutions

From the stock solution (1000 µg/mL), 1.0 mL was pipetted into a 10 mL volumetric flask and diluted to volume with methanol to obtain an intermediate solution of 100 µg/mL. Further serial dilutions were prepared from this intermediate solution to cover the concentration range of 5 to 30 µg/mL. All working solutions were freshly prepared for each experiment. Prior to injection, solutions were filtered through 0.45 µm nylon membrane syringe filters.

2.6 Mobile Phase Optimization and Chromatographic Conditions

The mobile phase was optimized through a systematic evaluation of different solvent systems and column types. Multiple trial combinations were examined, including acetonitrile–water on a Rubitas C18 column and on the SunQSIL C18 column, before arriving at the final optimized system. The selection criteria were chromatographic resolution, peak symmetry, baseline stability, and retention time suitability.

Table 1: Summary of mobile phase and column optimization trials for favipiravir

Sr. No.	Column and Mobile Phase	Observation
1	Rubitas C18, 5 µm (250 × 4.6 mm) ACN:Water (50:50, v/v)	Broad, asymmetric peaks; baseline instability; presence of multiple noise peaks
2	SunQSIL C18, 5 µm (250 × 4.6 mm) ACN:Water (70:30, v/v)	Baseline drift; late, broad peaks; excessive noise
3	SunQSIL C18, 5 µm (250 × 4.6 mm) Methanol:Water (80:20, v/v)	Well-resolved, sharp peak with good retention, minimal tailing, and stable baseline

The finalized chromatographic conditions are summarized in Table 2. The mobile phase (methanol:water, 80:20 v/v) was filtered through a 0.45 µm nylon membrane filter and degassed by sonication for 15 minutes before use. The HPLC system was equilibrated for at least 30 minutes under the selected mobile phase prior to injection.

Table 2: Optimized chromatographic parameters for favipiravir analysis

Parameter	Details
Column	SunQSIL C18 (250 × 4.6 mm, 5 µm)
Mobile Phase	Methanol : Water (80:20, v/v)
Flow Rate	1.0 mL/min
Detection Wavelength	323 nm (PDA)
Injection Volume	20 µL
Retention Time	5.23 min
Run Time	10 min
Diluent / Solvent	Methanol (HPLC grade)

2.7 Method Validation

The developed RP-HPLC method was validated in accordance with ICH Q2(R1) guidelines [20]. The validation parameters evaluated were: system suitability, linearity and range, precision (intra-day and inter-day), accuracy, specificity, assay, LOD, LOQ, and robustness.

2.7.1 System Suitability

System suitability was assessed by injecting a standard solution of favipiravir (10 µg/mL) six times under the optimized chromatographic conditions and evaluating retention time, theoretical plate count (N), and asymmetry (tailing) factor from the resulting chromatograms.

2.7.2 Linearity and Range

Working solutions covering the concentration range 5–30 µg/mL were prepared by serial dilution from the 100 µg/mL intermediate solution. Six concentration levels (5, 10, 15, 20, 25, and 30 µg/mL) were analyzed in six replicates each. A calibration curve was constructed by plotting the mean peak area against the corresponding concentration. The regression equation and correlation coefficient (R^2) were calculated.

2.7.3 Precision

Precision was evaluated at both intra-day and inter-day levels at three concentration levels: 10, 20, and 30 µg/mL, each analysed in triplicate (n = 3). Intra-day precision was assessed by analysing all concentrations within a single day. Inter-day precision was assessed by repeating the same analysis on a different day under identical conditions. Results were expressed as %RSD. An acceptance criterion of %RSD ≤ 2.0% was applied.

2.7.4 Accuracy

Accuracy was determined by the standard addition method. Known amounts of favipiravir standard were spiked into a solution of the marketed tablet formulation at three concentration levels corresponding to 50%, 100%, and 150% of the target analytical concentration (10 µg/mL). Each level was analysed in triplicate (n = 3). The percentage recovery was calculated and compared against the acceptance range of 98.0%–102.0%.

2.7.5 Assay

Twenty tablets of Favipil-400 were weighed, and the average weight was determined. A tablet powder quantity equivalent to 10 mg of favipiravir was accurately weighed and transferred to a 10 mL volumetric flask. The drug was extracted in methanol with sonication for 10 minutes, and the volume was made up to 10 mL to give a stock solution of 1000 µg/mL. From this, serial dilutions were carried out to obtain a final analytical concentration of 10 µg/mL. The assay was performed in six replicates (n = 6) and expressed as mean % recovery ± %RSD.

2.7.6 LOD and LOQ

The limit of detection (LOD) and limit of quantitation (LOQ) were determined using the calibration curve approach as prescribed in ICH Q2(R1) [20]. LOD = 3.3 × σ / S and LOQ = 10 × σ / S, where σ is the standard deviation of the y-intercept of the regression line and S is the slope of the calibration curve.

2.7.7 Specificity

Specificity was evaluated by comparing the chromatograms of the blank (mobile phase), pure favipiravir standard solution, and the sample solution (tablet formulation extract) under the optimized conditions. The retention time and peak purity of favipiravir in the sample were compared against the standard to confirm that no interfering peaks from excipients co-eluted at the drug's retention time.

2.7.8 Robustness

Robustness was assessed by introducing deliberate minor variations in three critical method parameters, one at a time, while holding all others constant: (i) flow rate (±0.05 mL/min), (ii) detection wavelength (±2 nm), and (iii) mobile phase composition (±2 mL). Standard solutions of favipiravir (10 µg/mL) were analysed in triplicate under each modified condition. The %RSD of peak areas across each variation was calculated and compared against the acceptance threshold of %RSD ≤ 2.0%.

3. RESULTS AND DISCUSSION

3.1 Preformulation Study:

3.1.1 Physical Appearance

Favipiravir is a White to off-white crystalline powder. It is odourless in nature and possesses a slightly bitter taste. The drug exists as a solid crystalline form at room temperature and is stable under normal storage conditions. Its molecular formula is $C_5H_4FN_3O_2$ with a molecular weight of 157.10 g/mol [1,2]. The melting point of Favipiravir is reported to be in the range of 180–200°C, which is characteristic of its crystalline nature [1,2].

3.1.2 Solubility

Favipiravir exhibits moderate to good aqueous solubility across the physiological pH range (1.2–6.8), with reported in vitro solubility values of approximately 4.48–8.5 mg/mL at 37°C, which exceed the BCS high-solubility threshold [19]. It exhibits good solubility in organic solvents such as dimethyl sulfoxide (DMSO) and methanol, which are commonly used as co-solvents in analytical and pharmaceutical preparations. Based on in vitro solubility and Caco-2 permeability studies, favipiravir is classified as **BCS Class III**, characterised by high aqueous solubility and low intestinal permeability, suggesting that oral absorption is rate-limited by membrane permeation rather than dissolution [19].

3.1.3 Differential Scanning Calorimetry (DSC) Analysis of Favipiravir

The thermal behavior of Favipiravir was assessed by DSC using a STARe system. Approximately 5.0 mg of the sample was weighed into a standard aluminum pan (40 µL) and analyzed from 25°C to 350°C at a heating rate of 10°C/min under an air atmosphere.

The DSC thermogram revealed a single sharp endothermic peak with an onset at 189.26°C, a peak temperature of 190.59°C (extrapolated peak: 190.58°C), and an endset at 194.43°C, corresponding to the melting point of Favipiravir. The FWHM of 2.85°C indicates a narrow, well-defined melting transition. The enthalpy of fusion was −171.74 J/g (total integral: −858.69 mJ), with a peak height of 43.84 mW and an area distribution of 63.96% (left) and 36.04% (right). The sharp, symmetrical nature of the endothermic event confirms the high purity and crystallinity of the sample, consistent with literature-reported values for pure Favipiravir, thus validating the drug's identity prior to formulation [1,2].

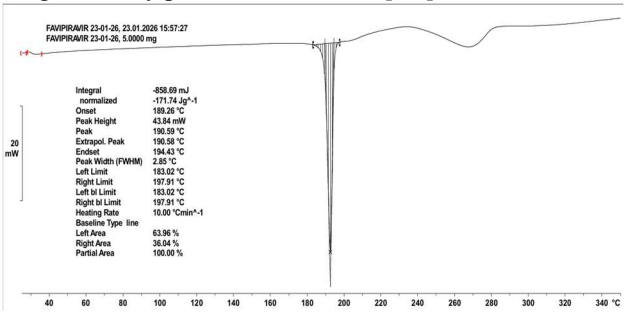

Figure 2. DSC of Favipiravir

3.1.4 FTIR Spectral Analysis

The FTIR spectrum of Favipiravir was recorded using an ATR PRO ONE accessory on a JASCO FT/IR-4600 spectrometer (45° incident angle, TGS detector, 16 accumulations, 4 cm^{-1} resolution) over 650–4000 cm^{-1}. A broad band at 3679.51 cm^{-1} corresponds to O–H/N–H stretching of the amide group, while C–H stretching appeared at 2980.45 and 2843.52 cm^{-1}. The strong band at 1654.62 cm^{-1} confirms C=O stretching of the carboxamide group, with the amide II band (N–H bending/C–N stretching) at 1558.20 cm^{-1}. Bands at 1434.78 and 1395.25 cm^{-1} are attributed to pyrazine ring C–H/C=C bending and C–F/C–N stretching, respectively. In the fingerprint region, C–F stretching vibrations at 1264.11 and 1181.19 cm^{-1} confirm the fluorine substituent, while bands at 1112.73, 1060.66, 1032.69, and 1013.41 cm^{-1} correspond to C–O and C–N stretching of the heterocyclic framework. Out-of-plane C–H deformations appeared at 970.98 and 928.56 cm^{-1}, aromatic C–H out-of-plane bending at 816.71 and 786.82 cm^{-1}, and ring deformation/skeletal bending at 712.57 and 660.50 cm^{-1}. The overall spectral profile is consistent with the reported structure of Favipiravir (6-fluoro-3-hydroxypyrazine-2-carboxamide), confirming compound identity and integrity [1,2].

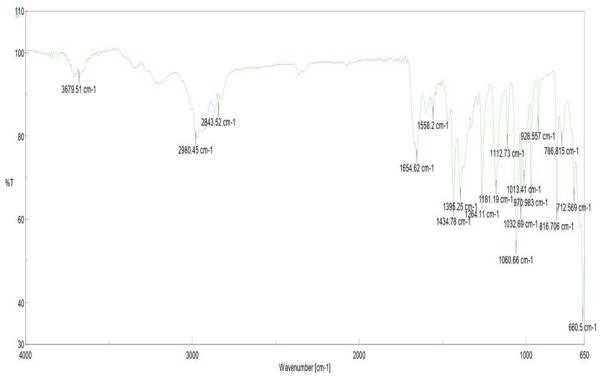

Figure 3. FTIR Analysis of Favipiravir

3.1.5 UV Spectral Analysis and Wavelength Selection

The UV scanning of favipiravir (10 µg/mL in methanol) over 200–400 nm revealed two characteristic absorption maxima at 227 nm and 323 nm. The wavelength of 323 nm was selected for chromatographic detection because this region offers greater specificity, reducing the likelihood of interference from co-extracted excipients that may absorb at shorter UV wavelengths. Detection at 323 nm is also consistent with the chromophoric system of favipiravir, which contains a fluoropyrazinecarboxamide ring system [1,2].

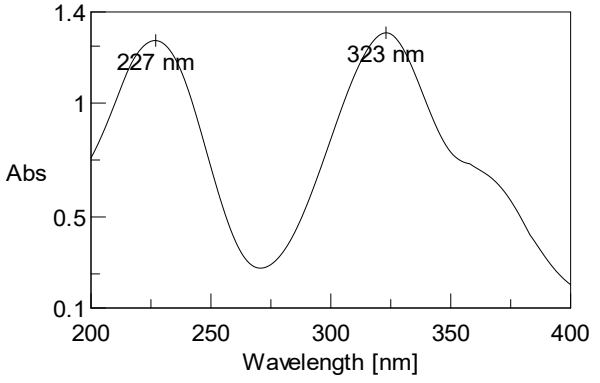

Figure 4. UV spectroscopy of Favipiravir

3.2 Preparation of Mobile Phase

The mobile phase was composed of methanol and water in the ratio of 80:20 (v/v). Prior to use, both solvents were filtered through a 0.45 µm membrane filter and degassed by sonication to remove dissolved gases. The mobile phase was pumped at a flow rate of 1.0 mL/min through the SunQSil C18 column (250 × 4.6 mm, 5 µm particle size). The system was equilibrated with the mobile phase before injection of samples to ensure a stable baseline and reproducible chromatographic conditions.

3.3 Standard Stock Solution Preparation

Accurately weighed 10 mg of Favipiravir was transferred into a 10 mL volumetric flask, dissolved in methanol, and the volume was made up with methanol to prepare a stock solution of concentration 1000 µg/mL.

3.4 Standard Solution Preparation (Secondary Dilution)

From the stock solution (1000 µg/mL), 1 mL was pipetted into a 10 mL volumetric flask and diluted with methanol to obtain a working standard solution of 100 µg/mL. Further serial dilutions were prepared from this working solution to obtain final concentrations in the range of 5–30 µg/mL, which were used for the construction of the calibration curve.

3.5 Sample Solution Preparation (Tablet)

The assay was performed on the marketed formulation Favipil-400 (Terrace Pharmaceuticals Pvt. Ltd.), each tablet containing 400 mg of Favipiravir. Twenty tablets were weighed, and their average weight was calculated. Tablet powder equivalent to 10 mg of Favipiravir was accurately weighed, transferred into a 10 mL volumetric flask, dissolved in methanol, and sonicated for 10 minutes to ensure complete dissolution, yielding a stock solution of 1000 µg/mL. From this, serial dilutions were performed to obtain an intermediate concentration of 100 µg/mL, and further dilution with mobile phase gave a final working concentration of 10 µg/mL for chromatographic analysis.

3.6 Chromatographic Method Optimization and Development

The chromatographic conditions were optimized by systematically evaluating different columns and mobile phase compositions. Initial trials with a Rubitas C18 column using acetonitrile: water (50:50 v/v) and a SunQSil C18 column with acetonitrile: water (70:30 v/v) yielded unsatisfactory results due to poor peak symmetry and baseline instability. Finally, the SunQSil C18 column (250 × 4.6 mm, 5 µm) with methanol: water (80:20 v/v) at a flow rate of 1.0 mL/min and detection at 323 nm produced a well-resolved peak with a retention time of 5.23 min, theoretical plate count of 4657.25, and asymmetry factor of 1.16, confirming satisfactory system suitability [20].

Validation Parameters

3.7 System Suitability

System suitability parameters were evaluated from a standard injection (10 µg/mL). The theoretical plate count of 4657.25 confirms adequate column efficiency, while the asymmetry factor of 1.16 (well within the accepted limit of ≤2.0) indicates a symmetrical peak profile. These results confirm that the system was performing appropriately before sample analysis [20]. The parameters are summarized in Table 3.

Table 3: System suitability parameters for favipiravir (10 µg/mL, n = 6)

Sr. No.	Parameter	Result
1	Retention Time (min)	5.23

2	Theoretical Plates (N)	4657.25
3	Asymmetry Factor (Tailing Factor)	1.16

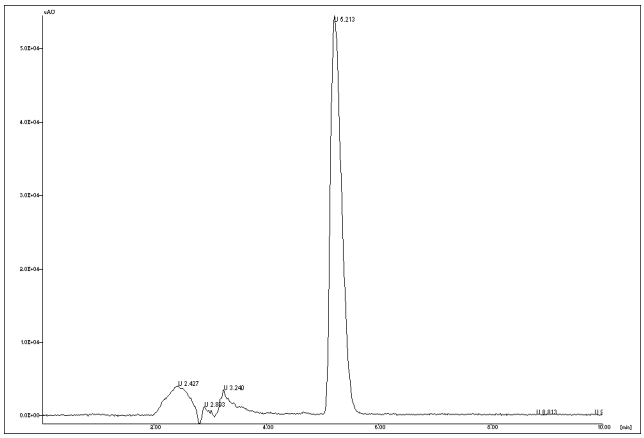

Figure 5: Chromatogram of favipiravir standard

3.4 Linearity and Calibration

Favipiravir demonstrated a linear relationship between peak area and concentration over the studied range of 5 to 30 µg/mL. Six replicate injections were performed at each of the six concentration levels. The mean peak areas at each concentration, along with their standard deviations and %RSD values, are presented in Table 4. The regression equation derived from the calibration curve was:

$y = 30473x - 9977.1$ ($R^2 = 0.9993$)

where y represents the peak area and x is the concentration in µg/mL. The high correlation coefficient ($R^2 = 0.9993$) confirms excellent linearity across the validated range [20]. All %RSD values for peak area at each concentration level were below 2%, indicating good reproducibility of the response.

Table 4: Linearity data for favipiravir (5–30 µg/mL, n = 6 per level)

Conc. (µg/mL)	5	10	15	20	25	30
Inj. 1	150628.46	282523.43	447448.60	586844.10	775710.17	935230.66
Inj. 2	153992.40	286782.24	433175.40	587852.49	761042.52	899095.56
Inj. 3	145168.20	297061.44	443742.48	594321.24	776608.56	905784.60
Inj. 4	151180.43	293827.45	430974.17	591239.64	744196.95	893650.82
Inj. 5	149931.00	295511.40	433107.36	600621.12	760382.76	886263.96
Inj. 6	151859.04	292778.40	432570.24	616812.84	754179.36	896464.32
Mean	**150459.92**	**291414.06**	**436836.37**	**596281.90**	**762020.05**	**902748.32**
SD	2941.62	5601.14	6930.90	11222.69	12514.15	17156.05
%RSD	1.96	1.92	1.59	1.88	1.64	1.90

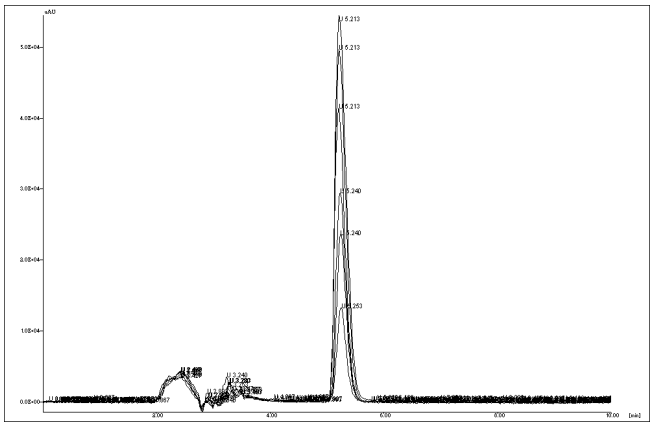

Figure 6. Overlay of HPLC chromatograms at linearity

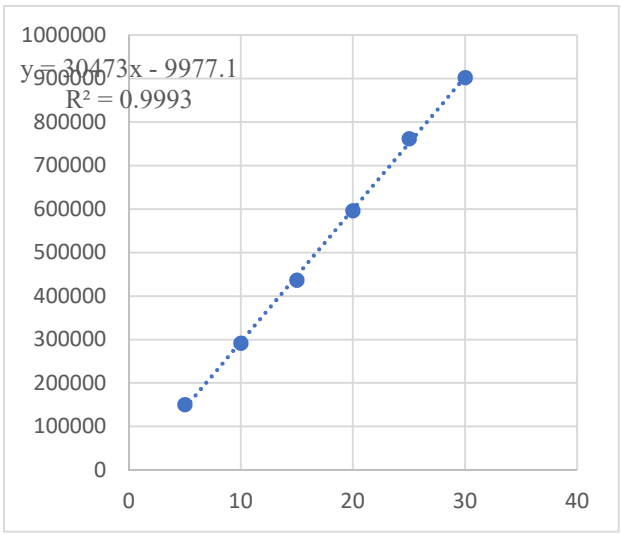

Figure.7 Calibration curve of favipiravir

3.5 Precision

The precision of the method was evaluated at three concentration levels (10, 20, and 30 µg/mL) for both intra-day and inter-day studies. The results are presented in Tables 5 and 6, respectively.

Table 5: Intra-day precision results for favipiravir

Theoretical Conc.	Peak Area	Amount Recov	% Recovery	Mean ± SD	SD	%RSD

(µg/mL)		ered (µg/mL)				
	297449.85	10.09	100.89			
10	295511.42	10.02	100.25	100.27 ± 0.61	0.61	0.61
	293746.85	9.97	99.67			
	599235.03	19.99	99.96			
20	597666.43	19.94	99.70	99.95 ± 0.24	0.24	0.24
	600570.72	20.04	100.18			
	907023.10	30.09	100.31			
30	898558.02	29.81	99.38	100.15 ± 0.70	0.70	0.70
	911068.17	30.22	100.75			

Table 6: Inter-day precision results for favipiravir

Theoretical Conc. (µg/mL)	Peak Area	Amount Recovered (µg/mL)	% Recovery	Mean ± SD	SD	%RSD
	2940997	9.98	99.79			
10	295266.97	10.02	100.17	99.92 ± 0.22	0.22	0.22
	294143.10	9.98	99.80			
	594109.27	19.82	99.12			
20	595807.72	19.88	99.40	99.51 ± 0.46	0.46	0.46
	599532.84	20.00	100.01			
	903487.96	29.98	99.92			
30	894790.54	29.69	98.97	99.44 ± 0.48	0.48	0.48
	898991.40	29.83	99.43			

All %RSD values for both intra-day and inter-day precision studies were within the acceptance criterion of ≤2.0% [20], demonstrating that the method delivers consistent and reproducible results regardless of minor temporal variation in laboratory conditions.

3.6 Assay of Marketed Tablet Formulation

The validated method was applied to estimate the favipiravir content in the commercial tablet formulation Favipil-400 (Terrace Pharmaceuticals Pvt. Ltd.). The sample solution (10 µg/mL) was injected in six replicates. The results, presented in Table 7, indicate a mean % recovery of 100.17 ± 0.52%, which falls well within the accepted range of 98.0%–102.0%, confirming the suitability of the method for routine assay of favipiravir tablets [20].

Table 7: Assay results for Favipil-400 tablet formulation (n = 6)

Injection No.	Peak Area	Amount Recovered (µg/mL)	% Recovery
1	295848.98	10.04	100.36
2	294630.35	10.00	99.96
3	296631.98	10.06	100.62
4	297283.46	10.08	100.83
5	293276.90	9.95	99.52
6	293881.53	9.97	99.71
Mean	**295258.87**	**10.02**	**100.17**
SD	1584.48	0.05	0.52
%RSD	0.54	0.52	0.52

3.7 Accuracy

The accuracy of the method was established through the standard addition technique. Pure favipiravir standard was spiked into the tablet formulation solution at three levels—50% (5 µg/mL added), 100% (10 µg/mL added), and 150% (15 µg/mL added) of the target concentration—each analysed in triplicate. The results are presented in Table 8.

Table 8: Accuracy results (standard addition method, n = 3 per level)

Level	Formulation Conc. (µg/mL)	Spiked Conc. (µg/mL)	Peak Area	Amount Recovered (µg/mL)	% Recovery	Mean	%RSD
	10	5	445196.15	14.94	99.58		
50%	10	5	445264.02	14.94	99.59	99.72	0.23
	10	5	447061.08	15.00	99.99		
	10	10	596101.76	19.89	99.45		
100%	10	10	599104.80	19.99	99.94	99.97	0.53
	10	10	602609.98	20.10	100.51		
	10	15	756136.56	25.14	100.56		
150%	10	15	746766.50	24.83	99.33	99.90	0.62
	10	15	750391.39	24.95	99.81		

Mean percentage recoveries at all three spiking levels (99.72%, 99.97%, and 99.90%) were well within the accepted range of 98.0%–102.0% [20]. The %RSD values at all levels were below 1.0%, confirming the method's high accuracy and the absence of matrix-related interference from tablet excipients.

3.8 Limit of Detection and Limit of Quantitation

LOD and LOQ were computed from the calibration curve data using the ICH Q2(R1) formula, based on the standard deviation of the y-intercept (σ = 7851.25) and the slope of the regression line (S = 30472.60). The calculated values are presented in Tables 9 and 10.

Table 9: LOD and LOQ based on calibration curve (y-intercept SD method)

Parameter	SD of Y-Intercept (σ)	Slope (S)	Value (µg/mL)
LOD	7851.25	30472.60	0.85
LOQ	7851.25	30472.60	2.58

Table 10: LOD and LOQ based on alternate regression (SD of lowest response)

Parameter	SD of Lowest Response (σ)	Slope (S)
LOD	2941.62	30472.60
LOQ	2941.62	30472.60

The LOD of 0.85 µg/mL and LOQ of 2.58 µg/mL (based on the y-intercept approach) confirm that the method possesses sufficient sensitivity for detecting trace levels of favipiravir in pharmaceutical matrices [20], comfortably below the lowest concentration in the validated linear range (5 µg/mL).

3.9 Robustness

Robustness was assessed by intentionally varying three chromatographic parameters—flow rate, detection wavelength, and mobile phase composition—one at a time while keeping all others constant. Standard solutions (10 µg/mL, n = 3 per condition) were analysed and %RSD of peak areas computed. Results are presented in Table 11.

Table 11: Robustness study results for favipiravir

Parameter Varied	Condition	Mean Peak Area	SD	%RSD
Flow Rate (mL/min)	0.95	292784.38	2005.55	0.68
(Nominal: 1.0 mL/min)	1.00	289514.51	5028.06	1.74
	1.05	296407.27	2218.21	0.75
Detection Wavelength (nm)	321	287373.60	5150.30	1.79
(Nominal: 323 nm)	323	295135.94	4695.50	1.59
	325	284321.42	4213.66	1.48
Mobile Phase MeOH:Water (v/v)	78:22	288340.45	3620.94	1.26
(Nominal: 80:20)	80:20	295024.78	5693.36	1.93

	82:18	291232.74	5065.21	1.74

Across all tested conditions, the %RSD values for peak area remained below the acceptance threshold of 2.0% [20], confirming that the method is robust and can withstand small unintentional deviations in operating parameters during routine laboratory use without compromise to analytical performance.

3.10 Specificity

Specificity was evaluated by comparing the chromatogram of the blank (mobile phase), a standard favipiravir solution, and a sample solution prepared from the Favipil-400 tablet formulation. No extraneous peaks were observed in the blank chromatogram at the retention time of favipiravir (5.23 min). In the sample solution, favipiravir eluted at the same retention time as the reference standard, and no co-eluting peaks attributable to tablet excipients were detected. This confirms that the method is specific for favipiravir and that the presence of formulation components does not interfere with its quantitative determination [20].

3.11 Summary of Validation Parameters

Table 12: Summary of ICH Q2(R1) validation results for the RP-HPLC method

Sr.	Parameter	Result
1	Linearity Range	5–30 µg/mL
	Regression Equation	y = 30473x − 9977.1
	Correlation Coefficient (R^2)	0.9993
2	Intra-day Precision (%RSD) at 10/20/30 µg/mL	0.61 / 0.24 / 0.70
3	Inter-day Precision (%RSD) at 10/20/30 µg/mL	0.22 / 0.46 / 0.48
4	Assay (Mean % Recovery ± %RSD)	100.17 ± 0.52%
5	Accuracy at 50% level	99.72 ± 0.23%
	Accuracy at 100% level	99.97 ± 0.53%
	Accuracy at 150% level	99.90 ± 0.62%
6	LOD	0.85 µg/mL
7	LOQ	2.58 µg/mL
8	Robustness	Robust (%RSD < 2.0% under all varied conditions)
9	Specificity	Specific; no interference from excipients

4. CONCLUSION

A simple, accurate, precise, and reproducible RP-HPLC method has been successfully developed and validated for the quantitative estimation of favipiravir in bulk drug substance and its pharmaceutical tablet formulation. The method employs an isocratic mobile phase of methanol:water (80:20, v/v) on a
SunQSIL C18 column with UV detection at 323 nm. The total run time of 10 minutes and the simplicity of the mobile phase—requiring no buffer preparation or pH adjustment—make it particularly well suited for routine quality control laboratories.

Validation results in accordance with ICH Q2(R1) guidelines [20] demonstrated excellent linearity (R^2 = 0.9993) over the range of 5–30 µg/mL, with all precision, accuracy, and specificity parameters meeting the prescribed acceptance criteria. The LOD and LOQ values of 0.85 and 2.58 µg/mL, respectively, confirm adequate sensitivity, while the robustness study established that minor variations in operational parameters do not significantly affect the method's performance. The mean assay recovery of 100.17 ± 0.52% from the commercial tablet formulation confirms that the method can reliably quantify favipiravir in the presence of common pharmaceutical excipients.

This validated method can be adopted for routine quality control and batch release testing of favipiravir-containing pharmaceutical products [3,4,8,9] and may also serve as a reference procedure for stability studies and pharmacokinetic investigations [13,14].

5. CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest

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