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Analytical Method Development and Validation of RP-HPLC Method for Estimation of Favipiravir in Bulk and Pharmaceutical dosage form

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ABSTRACT

Objective: To develop and validate a simple, accurate, and cost-effective RP-HPLC method for the estimation of Favipiravir in bulk and pharmaceutical dosage forms.

Methods: An RP-HPLC method was developed using a C18 column with methanol:water (35:65, v/v) as the mobile phase, pH 3.0, flow rate 0.8 mL/min, and UV detection at 225 nm. The method was validated as per ICH Q2(R1) guidelines for specificity, linearity, accuracy, precision, LOD, LOQ, and robustness. Forced degradation studies were conducted as per ICH Q1A(R2).

Results: Favipiravir showed a retention time of **6.62 min**. The method was linear over **0.2–3.2 µg/mL** with **R^2 > 0.999**, **LOD: 0.38 µg/mL**, and **LOQ: 1.15 µg/mL**. Recovery ranged between **97.6–100.2%** and %RSD was within **0.07–2.80%**. Significant degradation occurred only under **alkaline stress**.

Conclusion: The developed RP-HPLC method is accurate, precise, robust, and stability-indicating. It is suitable for routine analysis and quality control of Favipiravir in pharmaceutical dosage forms.

Keywords: *Favipiravir, RP-HPLC, Method Validation, ICH Guidelines, Stability-Indicating, Forced Degradation.*

1. INTRODUCTION

A new antiviral drug called favipiravir(T-705; 6-fluoro-3-hydroxy-2-pyrazinecarboxamide) effectively and selectively inhibits influenza and numerous other RNA viruses' RNA-dependent RNA polymerase (RdRP) [(1)]. It has been shown to suppress all influenza A, B, and C virus strains and serotypes that it has been tested against, even those that are resistant to currently licensed neuraminidase inhibitors, as explained below. Additionally, it exhibits strong in vitro activity against members of the alphavirus, paramyxovirus, and norovirus families as well as a variety of arena, bunya, and flavi viruses in both in vitro and rodent models. The current understanding of favipiravir's antiviral mechanism of action and the range of RNA viruses it inhibits both in vitro and in vivo is reviewed in this research. [(2)]

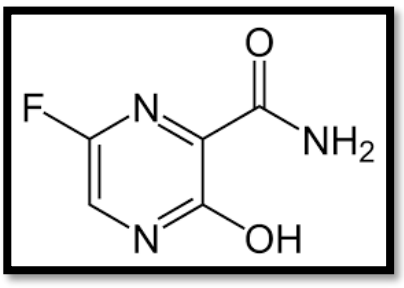

Fig. 1: Structure of Favipiravir

Mechanism of action:

Favipiravir's mode of action is different from that of other influenza antivirals, which mainly stop the virus from entering and leaving cells. By specifically inhibiting RNA polymerase, the active favipiravir-RTP stops the viral genome from replicating. [3] The interaction between favipiravir-RTP and RNA dependent RNA polymerase (RdRp) is the subject of various theories. According to certain research, favipiravir-RTP inhibits viral growth and RNA strand elongation when it is integrated into a developing RNA strand. Purine analogs have also been shown to decrease favipiravir's antiviral activity, indicating that purine nucleosides and favipiravir-RTP compete for RdRp binding. [4]

Despite being initially created to treat influenza, favipiravir's key target, the RdRp catalytic domain, is anticipated to function similarly for other RNA viruses. The broad-spectrum coverage of favipiravir is facilitated by this conserved RdRp catalytic domain.

2. MATERIALS AND METHODS

Favipiravir and its marketed formulation (Fabiflu 400 mg) were obtained from Vidisha Analytical and Glenmark Pharmaceuticals, respectively. HPLC-grade methanol, acetonitrile, and water were used as solvents, along with 0.45 µm nylon membrane and PVDF syringe filters. The RP-HPLC analysis was performed using a C18 column with a mobile phase of methanol:water (35:65, v/v), adjusted to pH 3.0, at a flow rate of 0.8 mL/min, and detection at 225 nm. The method was validated as per ICH Q2(R1) guidelines for specificity, [8] linearity, accuracy, precision, LOD, LOQ, and robustness. Forced degradation studies were carried out under acidic, alkaline, oxidative, photolytic, and thermal conditions to establish the stability-indicating nature of the method. [5]

- **Chromatographic condition**

The RP-HPLC method was performed using a **C18 column** with a mobile phase of **methanol:water (35:65, v/v)** at **pH 3.0**. The flow rate was set at **0.8 mL/min**, and detection was carried out at a **wavelength of 225 nm**. The **injection volume** was appropriate for analytical scale, and the column oven temperature was maintained as per method optimization. [6]

3. CHARACTERISATION OF DRUG SUBSTANCE.

- **Selection of solvent**

Table 1: Drug Solubility Summary

Sr. No.	Name of Solvent	Observation	Conclusion
1	Water	Drug Particles seen after sonication	Drug was not found soluble in water.
2	Methanol	Drug Particles seen after sonication	Drug was not found soluble in methanol.
3	Ethanol	Drug Particles seen after sonication	Drug was not found soluble in Ethanol.
4	Acetonitrile	Drug Particles seen after sonication	Drug was not found soluble in Acetonitrile
5	0.1 N HCl	Drug Particles seen after sonication	Drug was not found soluble in 0.1 N HCl.
6	0.1 N NaOH	No Drug Particle seen after sonication **but solution turns yellow** which may indicate degradation of Favipiravir	Drug was found soluble in 0.1 N NaOH but can not used as diluent because Favipiravir not stable in this diluent.
7	DMSO	No Drug Particles seen after sonication	Drug was found soluble in DMSO.

- **Final Conclusion:** Used Dimethyl sulfoxide (DMSO) as a diluent**.**

❖ **Preparation of Mobile phase:**

Prepare mixture of Water, methanol and Orthophosphoric acid in the ratio of 35:65:0.1 v/v respectively, mix well. Filter through 0.45µ nylon membrane disc filter. Sonicated for 15 min to degas the mobile phase.

✓ **Preparation of Diluent:**

Prepare mixture of Dimethyl sulfoxide (DMSO) and Methanol in the ratio of 10:80 v/v respectively, mix well.

✓ **Preparation of Blank:**

Use diluent as blank.

❖ **Preparation of Standard solution:**

Weighed and transferred accurately about 60 mg of Favipiravir working standard into 50 mL clean and dry volumetric flask. Added about 30 mL of diluent, sonicate to about 30 minutes to dissolve and dilute up to the mark with diluent and mix. Further dilute above stock 5.0 mL of this stock solution to 50 mL with diluent and mix well. Filter the sample solution through 0.45µ membrane PVDF filter. Discard first 4.0 mL of filtrate and then collected the sample. [7]

(Concentration of Favipiravir standard solution: 120 ppm)

❖ **Preparation of Favipiravir standard stock solution:**

Weighed 30 mg Favipiravir and dissolved in 100 mL of diluent (300 PPM of Favipiravir). Final Favipiravir solution: Pipette out 2 mL of Favipiravir stock solution and diluted up to 20 mL with diluent. (30 PPM of Favipiravir) .The API standard solutions were scanned separately between 400nm to 200nm.From the spectrum show high absorbance that select as a wavelength of drug. Selected wavelength was used for estimation of drugs. Diluent used as a Blank.

❖ **Preparation of Sample solution:**

Weighed and transferred Favipiravir tablets in to 500 mL clean and dry volumetric flask. Added about 150 mL of diluent, sonicate for 60 minutes with intermittent shaking, at control room temperature and make volume up to mark with diluent and mix. Further diluted above stock solution3.0 mL of this sample stock solution to 100 mL volumetric flask make up with Diluent and mixed well. [7] Filter the sample solution through 0.45µ membrane PVDF filter. Discard first 4.0 mL of filtrate and then collected the sample.

(Concentration of Sample Solution: 120 ppm)

❖ **Analytical Method Validation:**

The developed RP-HPLC method for Favipiravir was validated according to **ICH Q2(R1)** guidelines. Validation parameters included system suitability, specificity, linearity, accuracy, precision, robustness, LOD, and LOQ to ensure the method's reliability for routine quality control. [6]

1. **System Suitability Test:**

System suitability was confirmed by injecting standard solutions and evaluating parameters like **retention time (6.62 min)**, **theoretical plates**, **tailing factor**, and **%RSD**, which were all within acceptable limits, ensuring the system's performance before sample analysis.

2. **Specificity:**

The method demonstrated excellent specificity by showing no interference from excipients or degradation products. **Peak purity** was confirmed using diode-array detection, and all analyte peaks were well-resolved, indicating selective identification of Favipiravir in the presence of other components.

3. **Linearity:**

The method showed linear response over a concentration range of **0.2–3.2 µg/mL**, with a correlation coefficient ($R^2 > 0.999$), indicating strong proportionality between peak area and concentration.

4. **Accuracy (Recovery):**

Accuracy was assessed through recovery studies at different concentration levels. The % recovery values ranged from **97.6% to 100.2%**, confirming the method's ability to measure the true content of Favipiravir in the formulation.

5. **Precision:**

✓ **Method Precision:** Six replicates of Favipiravir were analyzed at 100% concentration level. The %RSD was found between **0.07% to 2.80%**, demonstrating good repeatability.

✓

✓ **Intermediate Precision:** Performed on different days and by different analysts, results remained consistent with acceptable %RSD, confirming the method's reproducibility under variable conditions.

6. **Robustness:**

The method remained robust under small deliberate variations in flow rate, pH, mobile phase composition, and column type. These changes did not significantly affect retention time or peak shape, confirming method stability during routine use.

4. RESULTS AND DISCUSSION

- **Reverse Phase High Performance Liquid Chromatography Method Development and Optimization**

➢ **Chromatographic Condition:**

After several trials with the different combination and ratio of solvents and sharp peak.

Table 2:

Column	Phenomenex C18, 5µ, 4.6 x 150mm
Mobile Phase	Water: Methanol: Orthophosphoric Acid (35:65:0.1 v/v/v)
Flow Rate	1.5 mL/min
Injection Volume	10 µL
Wavelength	239 nm
Column Temp	40ºC
Sample Temp	25ºC
Run Time	7.0 minutes
Seal Wash	Water: Methanol(90:10) v/v
Needle Wash	Water: Methanol(10:90) v/v

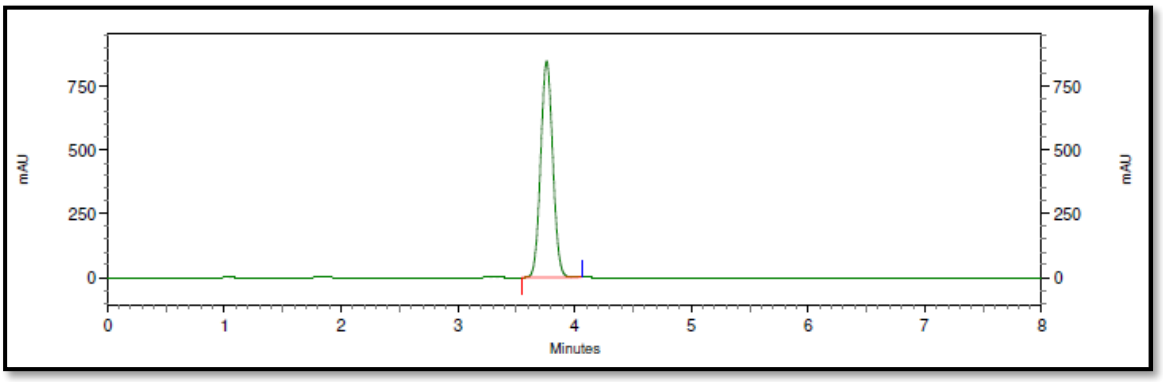

Fig 2: Typical chromatogram for Favipiravir

➢ **Observation:** Favipiravir eluted at 3.76 minutes with acceptable chromatography (Asymmetry: 1.10 and Theoretical plates 6253)

➢ **Conclusion:** Method can be used for further analysis and subjected for validation.

❖ **Analytical Method Validation of RP-HPLC**

1. **System Suitability:**

Table 3: System Suitability Test ofFavipiravir

Tailing Factor	**1.08**
Theoretical plates	**6628**
Injection No.	**Area**
1	6082514

2	6072582
3	6086529
4	6079114
5	6075968
Mean	**6079341**
%RSD	**0.1**

Conclusion:

- The data demonstrates that the system suitability is within the acceptance criteria, thus the system is suitable.

Specificity: (Identification, Interference & Peak Purity)

Inject Blank (Diluent), standard solution, placebo solution and sample solution. The data obtained is summarized in Table

Table 4: Specificity (Identification and Interference)

Solution	**Specificity data**		
	Retention time (min)	**Purity Match**	
Blank solution	NA	NA	
Placebo solution	NA	NA	
Standard solution	3.77	Purity angle	Purity threshold
		2.65	3.88
Sample solution	3.76	2.41	3.76

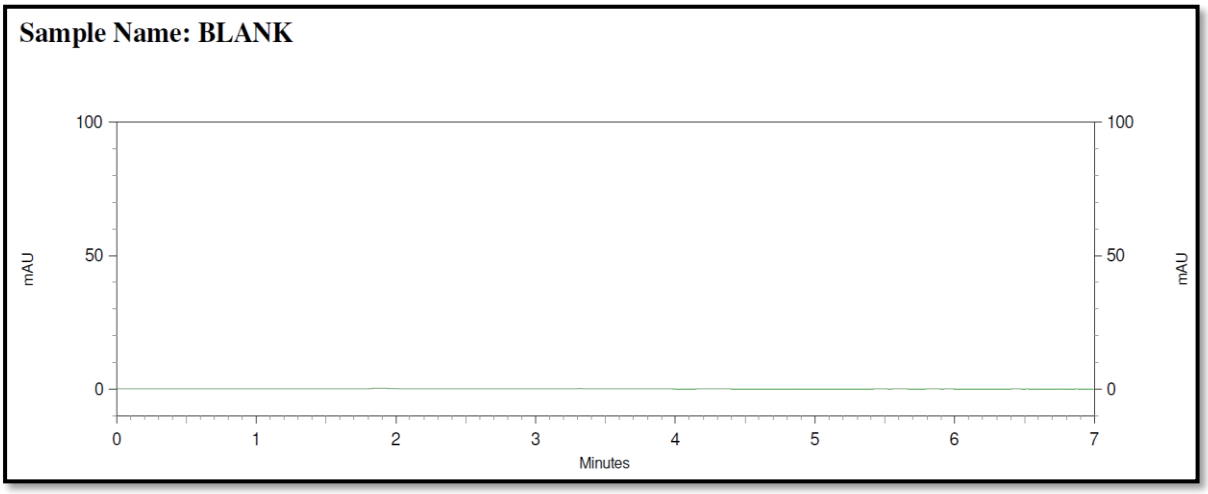

Fig 3: Chromatogram of Blank

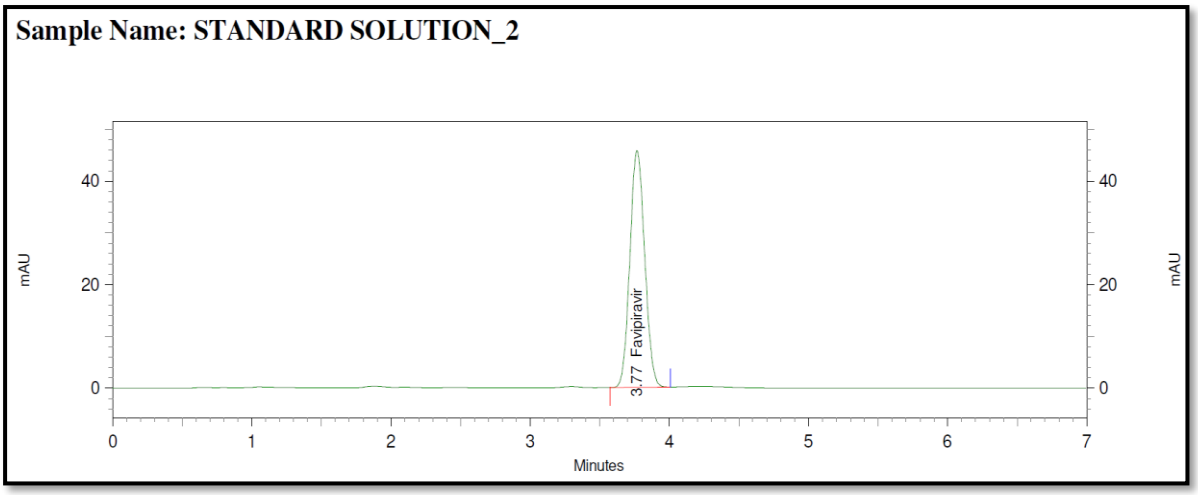

Fig 4: Chromatogram of Standard

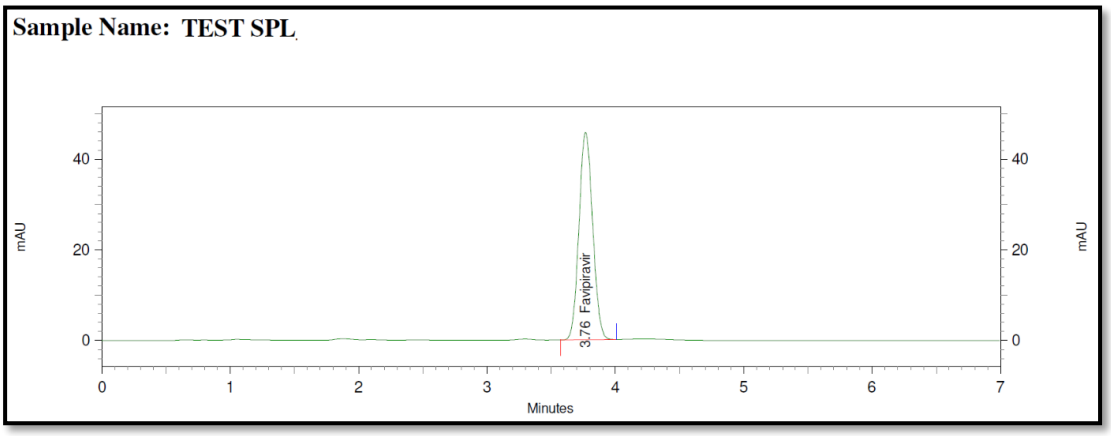

Fig 5: Chromatogram of Sample

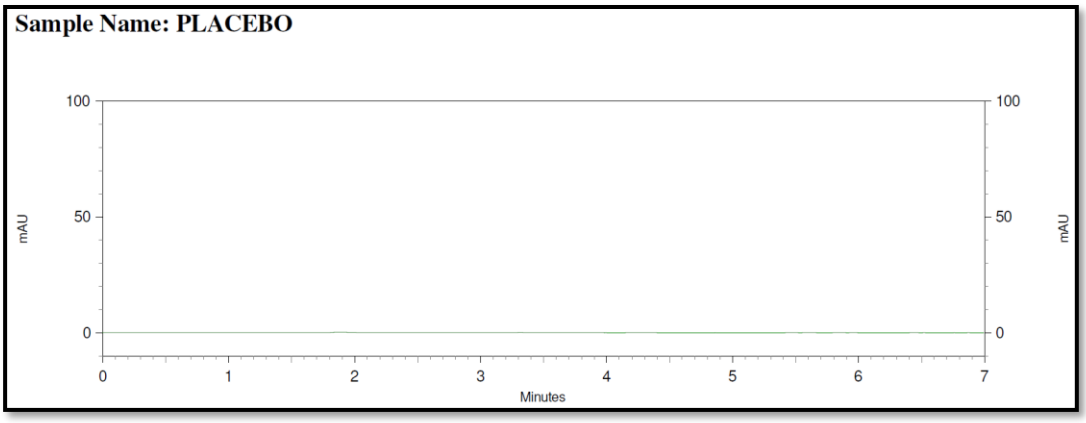

Fig 6: Chromatogram of Placebo

❖ **Conclusion:**

- The data demonstrates that retention time in standard and sample is same for Favipiravirpeak.
- The data demonstrates that there is no interference in blank and placebo at the retention time of Favipiravirpeak. Peak Purity match in both chromatograms obtained from Standard and Sample solution.

2. Linearity:

Table 5: Linearity of Favipiravir

Level	Conc (µg/mL)	Area	Mean
50%	60	2996854	2993253
		2985490	
		2997415	
75%	90	4512635	4516423
		4520106	
		4516529	
100%	120	6075291	6079137
		6079524	
		6082596	
125%	150	7565291	7565228
		7559864	
		7570529	
150%	180	9212524	9209204
		9216524	
		9198564	
Corr. Coeff			0.9997
Intercept			18468
Slope			50076
% Y-intercept			0.30

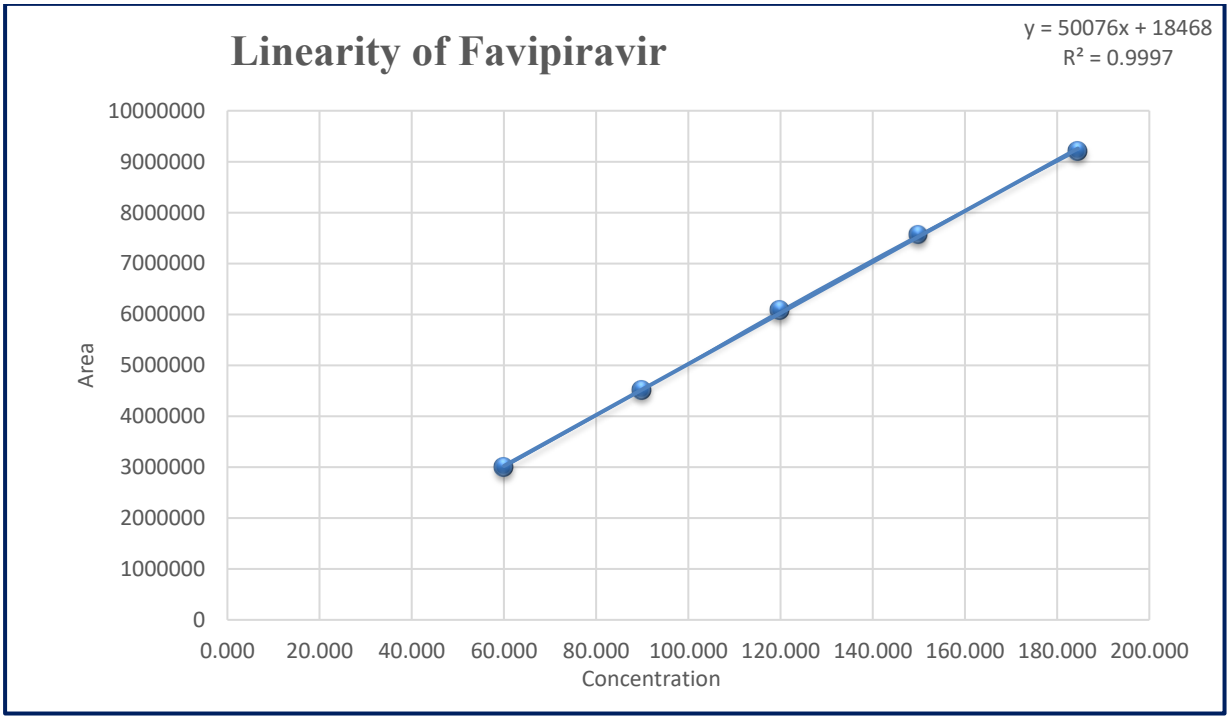

Fig 7: Linearity plot of Favipiravir

❖ **Conclusion:**

- The data shows that system suitability is fulfilled.
- The data shows that the response is found to be linear.
- Co-relation coefficient ($r^2$was found 0.9997.

3. Accuracy (Recovery):

Table 6: % Recovery for Favipiravir

Level (%)	Favipiravir Added Conc (µg/mL)	Favipiravir Recovered conc	Area	% Recovery	Mean % Recovery
50	60.11	60.52	3063501	100.38	99.91
	60.36	59.49	3025634	98.95	
	60.10	60.52	3058416	100.42	
100	120.01	117.96	6025968	98.92	100.43
	119.99	120.25	6126964	100.99	
	120.03	121.34	6201053	101.39	
150	180.51	180.57	9205106	100.88	100.09
	180.50	180.54	9172055	100.65	
	180.52	178.42	9023517	98.76	

❖ **Conclusion:**

The data shows that the Mean recovery for 50% to 150% is in the range of 98.0%-102.0% and individual recovery for 50% to 150% is in the range of 95.0% - 105.0%.

4. Precision:

Method Precision:

Table 7: Method precision

Sample	Area	% Assay
Sample 1	5882519	96.37
Sample 2	5965241	97.94
Sample 3	6025399	98.63
Sample 4	6025960	99.16
Sample 5	5985128	98.05
Sample 6	5869521	96.73
Mean		**97.81**
STD DEV		**1.0792**
% RSD		**1.103**

Conclusion:

- The data shows that system suitability is fulfilled.
- The data shows that % RSD for % Assay is within the acceptance criteria and hence the method is precise.

Intermediate Precision:

Table 8: Intermediate Precision

Sample	**Area**	**% Assay**
Sample 1	5926531	97.02
Sample 2	6025419	98.42
Sample 3	5942513	98.01
Sample 4	5963201	98.13
Sample 5	6029854	99.00
Sample 6	5920659	96.99
Mean		**97.93**
STD DEV		**0.7929**
% RSD		**0.810**

Table 9: Intermediate Precision Pool Data

Parameter	**Method Precision (Analyst-I)**	**Intermediate Precision (Analyst-II)**
HPLC NO.	**AD/HPLC-31**	**AD/HPLC-19**
Column No.	**HPLC- 12**	**HPLC-09**
Sample No.	**%Assay**	
1	96.37	97.02
2	97.94	98.42
3	98.63	98.01
4	99.16	98.13
5	98.05	99.00
6	96.73	96.99
Mean	**97.81**	**97.93**
Mean of Precision % Assay	**97.87**	
Absolute Mean difference % assay	**1.0**	

Conclusion:

- The data shows that system suitability is fulfilled.
- The data shows that % Assay is of six samples is not more than 2.0

- The data shows that % Assay is within the acceptance criteria and hence the method is rugged.

6) Robustness:

Table 10: Robustness for Favipiravir

Change in parameter	Condition	Area	Absolute difference of % Assay
Control	As per method	6025960	NA
Change in flow rate1.0 ml/min (±0.1 ml/min)	1.6 ml/min	5996500	-0.5
	1.4 ml/min	6060294	0.6
Change in wavelength (±2 nm)	241 nm	6129892	1.7
	237 nm	6004209	-0.4

Conclusion:

- System suitability criteria were fulfilled.
- The difference of % assay value in each modified condition is within acceptance criteria.

5. CONCLUSION

RP-High Performance Liquid Chromatography (HPLC) Method:

HPLC is a widely used analytical technique known for its specificity, sensitivity, and suitability for analyzing complex samples. In the present study, it was applied for the estimation of Favipiravir in tablet formulations. The analysis was carried out using an Agilent 1260 Infinity II HPLC system equipped with a Phenomenex C18 column (150 × 4.6 mm, 5 µm) and a UV/PDA detector, operated through Openlab EZ Chrome software. Standard and sample solutions were prepared in an appropriate diluent. Various solvent combinations were tested to develop an optimal mobile phase. The best results were obtained using a mobile phase of Water, Methanol, and Orthophosphoric Acid in the ratio 35:65:0.1. The selected wavelength for detection was 239 nm, based on UV scanning. The method provided good resolution, proper retention time, and a tailing factor less than 2. The validated method showed accurate and consistent results. It was successfully extended for routine estimation of Favipiravir in pharmaceutical tablet formulations.

REFERENCES

[1] *Furuta Y., Gowen B.B., Takahashi K., Shiraki K., Smee D.F., Barnard D.L. Favipiravir (T-705), a novel viral RNA polymerase inhibitor. Antivir Res. 2013 Nov;100:446–454. doi: 10.1016/j.antiviral.2013.09.015.* [DOI] [PMC free article] [PubMed] [Google Scholar]

[2] Hsieh HP, Hsu JT: Strategies of development of antiviral agents directed against influenza virus replication. Curr Pharm Des. 2007;13(34):3531-42. [Article]

[3] Pharmaceuticals and Medical Devices Agency: Avigan (favipiravir) Review Report [Link]

[4] Furuta Y, Komeno T, Nakamura T: Favipiravir (T-705), a broad spectrum inhibitor of viral RNA polymerase. Proc Jpn Acad Ser B Phys Biol Sci. 2017;93(7):449-463. doi: 10.2183/pjab.93.027. [Article]

[5] Chatwal g. R., anand s. K.; instrumental methods of chemical analysis; himalaya publishing house, mumbai; 11th edition, 2005, 1.1-1.2, 2.108-2.109, 2.151-2.153.

[6] Skoog d., holler f., timothy a., nieman n.; principles of instrumental analysis; saunders college publications, london; 4th edition, 1992; 1-2, 338-340.

[7] British pharmacopoeia, 1993, volume ii, 180-190.

[8] ICH Q2(R1). Validation of Analytical Procedures: Text and Methodology. International Conference on Harmonisation, 2005.