**World Journal of Pharmaceutical Sciences**
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: https://wjpsonline.com/
**Research Article**

# METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF FAVIPIRAVIR BY USING RP-HPLC

Koredi Nandini,[1] Dr. Aneesa[2]

[1]M. Pharmacy, Department of Pharmaceutical Analysis, Malla Reddy College of Pharmacy, Maisammaguda, Secunderabad, Hyderabad, 500100, Medchal District

[2]HOD and Professor, Department of Pharmaceutical Chemistry, Malla Reddy College of Pharmacy, Maisammaguda, Secunderabad, Hyderabad, 500100, Medchal District

***Received: 29-11-2025 / Revised Accepted: 01-12-2025 / Published: 03-12-2025***

## ABSTRACT:

A robust and precise analytical method was developed and validated through RP – HPLC for the estimation of Favipiravir by using a Discovery 250mm x 4.6 mm, 5m column with Acetonitrile: 0.01N Kh2po4 (70:30 v/v) as mobile phase. The method demonstrated excellent linearity in the concentration ranges of and 10–60 µg/ml for Favipiravir with correlation coefficients ($R^2$) of 0.999 for both drugs, confirming strong linearity. The regression equations obtained were and y = 63434x + 13743 for Favipiravir. The assay results indicated high accuracy with mean assay values of 99.46% for Favipiravir. Specificity studies confirmed the method's ability to distinctly quantify both analytes without interference. System suitability and method precision showed %RSD values within acceptable limits (≤2%), ensuring reproducibility. Accuracy studies yielded recoveries of 99.54% for Favipiravir. The sensitivity of the method was demonstrated by low limits of detection (LOD) and quantification (LOQ), observed at 0.03 µg/ml and 0.10 µg/ml for Favipiravir, respectively. Robustness studies further confirmed method reliability under small variations in method parameters

**Key Words:** Favipiravir, RP-HPLC, validation, Method Development.

## Introduction:

Favipiravir (T-705, 6-fuoro-3-hydroxypyrazine-2-carboxamide) is a new influenza medication that specifically inhibits the virus's RNA-dependent RNA polymerase.[1] Favipiravir is one of the prospective medications that might be employed in the treatment of COVID-19 infection.[2] Favipiravir was initially used against SARS-CoV-2 in Wuhan, the pandemic's epicenter. Then, when the epidemic expanded to Europe, this medicine got permission for emergency use in Italy and is now in use in other Countries.[3] Favipiravir begins as a prodrug and is ribosylated and phosphorylated intracellularly to form the active favipiravir-RTP.[4,5] Favipiravir-RTP binds to and inhibits RNA dependent RNA polymerase (RdRp), effectively stopping viral transcription and replication.[4,6]

Favipiravir is chemically known as 6-fluoro-3-hydroxypyrazine-2-carboxamide7 and The bioavailability of favipiravir is almost complete at 97.6%. It is converted inside cells into an active form known as favipiravir-ribofuranosyl-5'-triphosphate (favipiravir-RTP). [8,9] This active form is recognized by viral RNA-dependent RNA polymerase (RdRp), an enzyme required for viral replication, and functions as a substrate, limiting the polymerase's activity. By disrupting the production of viral RNA, favipiravir prevents the virus from reproducing.[10, 11]

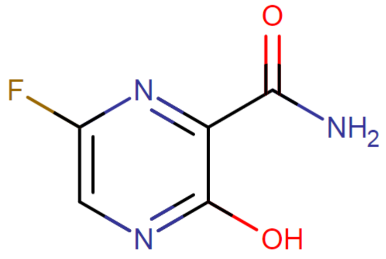

**Figure 1: Structure of Favipiravir**

High-performance liquid chromatography (HPLC) is critical in the analysis, method development, and validation of Favipiravir because it provides a dependable, selective, and sensitive platform for quantification in bulk drugs and pharmaceutical formulations. HPLC, particularly RP-HPLC, provides the effective separation of Favipiravir from its contaminants, excipients, and degradation

**Address for Correspondence**: Koredi Nandini, M. Pharmacy, Department of Pharmaceutical Analysis, Malla Reddy College of Pharmacy, Maisammaguda, Secunderabad, Hyderabad, 500100, Medchal District, Telangana, 502313.; **E-Mail:** nandinirao2916@gmail.com

**How to Cite this Article:** Koredi Nandini, Method Development and Validation for Estimation of Favipiravir by using RP-HPLC. World J Pharm Sci 2025; 13(04): 137-146; https://doi.org/10.54037/WJPS.2022.100905

products, making it critical for developing stability-indicating procedures. HPLC achieves precise peak resolution and repeatable retention characteristics by adjusting chromatographic settings such as mobile phase composition, pH, stationary phase, flow rate, and detection wavelength. During method validation, HPLC is essential for routine quality control, stability testing, impurity profiling, and confirming the safety and efficacy of Favipiravir drugs.

Extensive literature research has unearthed a multitude of recorded analytical procedures, including the discovery of more economically efficient ways[12-20]. Hence, a reliable and cost-effective approach is suggested for assessing the stability of Favipiravir, and their medicinal dose form using RP-HPLC. must be validated and developed as per ICH guidelines

## Materials and Methods:

Favipiravir (API), Favipiravir tablets (Fabiflu), Acetonitrile, Methanol, Ortho Phosphoric Acid, Distilled water. All of the solvents and chemicals were of HPLC quality and obtained from Rankem Chemicals Pvt Ltd.

### Instrumentation:

The Method Development and Validation was performed by Waters HPLC Model 2695 equipped with PDA Detector and Empower 3 Software. Analytical weighing Balance, Ultrasonicator, pH Meter, Hot air oven.

### Chromatographic Condition:

An Isocratic Elution carried out by using **Acetonitrile and 0.01N Kh2po4 30:70 v/v** as the Mobile Phase, Diluent used was Combination of Acetonitrile and Water in 1:1 ratio. **Discovery C18** (4.6 x 250mm, 5µm) column was used to determine the Method at a flow rate of 1ml/min, by maintaining the column Temperature at 30 0C. In addition, with an injection volume of 10µL and the wavelength detected at 320nm.

### API Formulation

**Preparation of Standard stock solutions and Working Solution:** Accurately weighed 20mg of Favipiravir transferred to 50ml volumetric flask and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (400µg/ml of Favipiravir). Moreover, from this 1ml from stock solution was pipetted out, taken into a 10ml volumetric flask, and made up with diluent to make 40µg/ml of Favipiravir.

### Sample Formulation

**Preparation of Sample stock solutions and Working Solution:** 10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100ml volumetric flask, 80ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters to make 2000µg/ml of Favipiravir solution. From this 0.2ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent to make 40µg/ml of Favipiravir.

## Method Validation

The established technique is validated in accordance with ICH criteria for the purpose of validating analytical methods. The validation metrics were: system appropriateness, accuracy, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, robustness, specificity, and degradation studies.

### System suitability parameters:

The chromatographic analysis was performed in accordance with the developed and optimized parameters after the working standard solution was introduced into the HPLC system six times. By calculating the % RSD of retention times, theoretical plates, and peak areas from six duplicate injections, the system suitability parameters were established.

**Specificity:** Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

### Precision:

The procedure and system accuracy were demonstrated by creating a standard solution with a known concentration and providing six repeating injections to ensure the suggested approach's consistency. Intermediate precision was also attained by producing six working sample solutions and injecting them six times. The area was measured, and the mean, standard deviation, and % RSD were calculated. The results were favourable, falling below the 2% limit.

### Linearity:

To test the drug's linearity, serial dilutions from 10 to 60 µg/ml were prepared. A graph was used to demonstrate the link between peak area response and medication concentration. It was found to be linear at the indicated drug concentration. Dilution were as follows.

- 25 µg/mL: Take 0.25 mL of stock solution and dilute to 10 mL
- 50 µg/mL: Take 0.5 mL of stock solution and dilute to 10 mL

- 75 µg/mL: Take 0.75 mL of stock solution and dilute to 10 mL
- 100 µg/mL: Take 1.0 mL of stock solution and dilute to 10 mL
- 125 µg/mL: Take 1.25 mL of stock solution and dilute to 10 mL
- 150 µg/mL: Take 1.5 mL of stock solution and dilute to 10 mL

**Accuracy:**

Accuracy was performed in triplicate for various concentrations of Favipiravir equivalent to 50%, 100% and 150% of the standard amount were injected into the HPLC system per the test procedure. Dilution were as follows.

- 50 µg/mL: Take 0.1 mL of stock solution and dilute to 10 mL
- 100 µg/mL: Take 0.2 mL of stock solution and dilute to 10 mL
- 150 µg/mL: Take 0.3 mL of stock solution and dilute to 10 mL

**Sensitivity:**

**Limit of detection and Limit of Quantification**

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve as per ICH guidelines.

Based on the response’s standard deviation and calibration curve’s slope, the LOD and LOQ can be estimated. The formulae given below can be used to calculate LOD and LOQ:

$$LOD = 3.3\sigma/S$$

$$LOQ = 10\sigma/S$$

where S is calibration curve of the slope and σ is the response of the standard deviation.

**Sensitivity Stock solution:** Take 0.25 mL of stock solution and dilute to 10 mL

- **LOD:** From above take 0.3 ml solution and dilute to 10 mL
- **LOQ:** From above take 0.9 ml solution and dilute to 10 mL

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guidelines.

**Assay:**

The assay and % purity were calculated for brand FabiFlu with label claim 200g. The observed value was compared with that of standard value without interference from the excipients used in the tablet dosage form

**Degradation Studies**

These investigations are carried out under various stress situations to describe the stability of the pure pharmacological material and are useful in establishing the best storage settings. These research cover base, peroxide, acid, neutral hydrolysis, photo, and heat degradation.

**Oxidation:**

After adding 1ml of a stock solution to 10ml of a 20% volume fraction of $H2O2$ and allowing it to sit in an oven at 60°C for 30 minutes, a chromatogram was produced by injecting a 40 µg/ml solution at 10 µl into HPLC

**Acid Degradation Studies:**

1 ml of hydrochloric acid was added to 10 ml of vf with 1 ml stock and refluxed for 30 minutes at 60 oC. A 40 µg/ml solution was injected at 10 µl into the system, resulting in the formation of a chromatogram.

**Alkali Degradation Studies:**

A mixture of 1 ml of stock and 1 ml of NaOH in 10 ml of vf was refluxed for 30 minutes at 60°C. A 40 µg/ml solution was injected at 10 µl into HPLC, resulting in the production of a chromatogram.

**Dry Heat Degradation Studies:**

The stock solution was allowed to undergo thermal deterioration in an oven set at 105oC for 6 hours. Subsequently, a chromatogram was prepared by injecting a 40 µg/ml solution at 10 µl into HPLC.

**Photo Stability studies:**

The stock underwent degradation by exposure to UV radiation in the laboratory for a duration of 7 days. Upon injecting a 40 µg/ml solution at a volume of 10 µl into HPLC, a chromatogram was developed.

**Neutral Degradation Studies:**

After refluxing the stock for 6 hours at 60 degrees Celsius, a chromatogram was prepared by injecting a 40 µg/ml solution at 10 µl into HPLC.

## RESULT AND DISCUSSION

**Table 1 Optimized Condition**

| ***Parameter*** | ***Condition*** |
|---|---|
| ***Mobile phase*** | *Acetonitrile: 0.01N* $KH_2PO_4$ *(70:30 v/v)* |
| ***Flow rate*** | *1 ml/min* |
| ***Column*** | *Discovery C18 (4.6 x 250mm, 5µm)* |
| ***Detector wave length*** | *320nm* |
| ***Column temperature*** | *30°C* |
| ***Injection volume*** | *10µL* |
| ***Diluent*** | *Water and Acetonitrile in the ratio 50:50* |

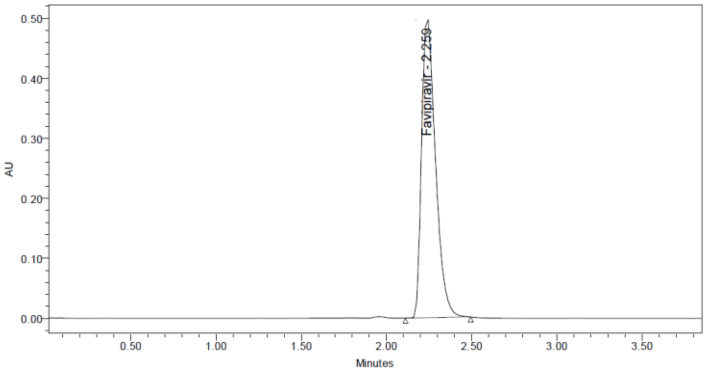

**Figure 2: Optimized Chromatogram**

**System Suitability:**

**Table 2: System Suitability**

| ***S no*** | ***Favipiravir*** | | | |
|---|---|---|---|---|
| ***Injection*** | ***RT*** | ***area*** | ***Plate Count*** | ***Tailing*** |
| ***Injection-1*** | *2.226* | *2563952* | *3289* | *1.42* |
| ***Injection-2*** | *2.229* | *2562960* | *3278* | *1.41* |
| ***Injection-3*** | *2.229* | *2560444* | *3250* | *1.41* |
| ***Injection-4*** | *2.232* | *2582241* | *3161* | *1.43* |
| ***Injection-5*** | *2.236* | *2565395* | *3213* | *1.43* |
| ***Injection-6*** | *2.238* | *2569317* | *3209* | *1.42* |
| ***Mean*** | | *2567385* | | |
| ***Std ev*** | | *7847.8* | | |
| ***RSD*** | | *0.3* | | |

Six injections of the standard Favipiravir solution were performed, and the chromatograms that corresponded to each injection were acquired. Observations showed that the percent RSD was less than 2%, the USP tailing was less than 2, and the theoretical plate count surpassed 2,000. Every condition for system appropriateness was satisfied and falls within permissible bounds.

**Linearity:**

Six concentrations ranging from 10 to 60 µg/ml were prepared and linearity was estimated in a duplicate manner. The linearity equation for Favipiravir was y = 63434x + 13743. For the calibration curve over the concentration range, the data have shown a good correlation.

**Table 3: Linearity Data**

| *Concentration (ppm)* | **Peak area* |
|---|---|
| ***0*** | *0* |
| ***5*** | *646906* |
| ***10*** | *1285168* |
| ***15*** | *1926182* |
| ***20*** | *2565364* |
| ***25*** | *3205994* |
| ***30*** | *3787705* |
| ***y :*** | *63434x+13743* |
| $R^2$ | *0.999* |
| ***Slope*** | *63434* |
| ***Intercept*** | *13743* |
| ***LOD*** | *0.03µg/ml* |
| ***LOQ*** | *0.10 µg/ml* |

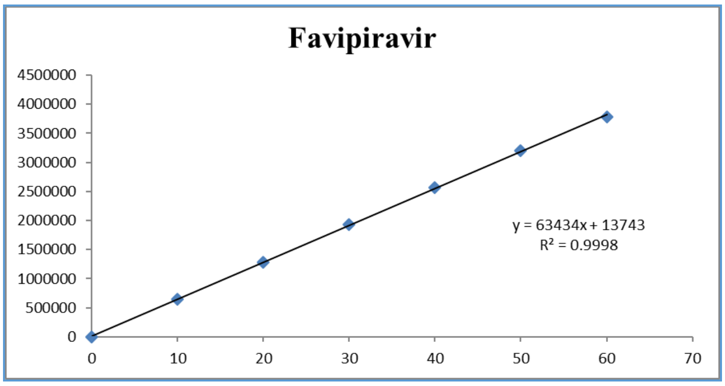

**Figure 3: Calibration Curve of Favipiravir**

**Accuracy:**

Three doses were given at each level, and the mean % recovery was calculated. Favipiravir's recovery rate was observed to be between 99% and 100.%, which is within the acceptable ranges

**Table 4: Accuracy Data**

| *% Level* | *Amount Spiked (µg/mL)* | *Amount recovered (µg/mL)* | *% Recovery* | *Avg %* | *Mean %Recovery* |
|---|---|---|---|---|---|
| ***50%*** | ***20*** | *19.93* | *99.67* | *99.62* | ***99.54%<br>%RSD: 0.33*** |
| | | *19.89* | *99.47* | | |
| | | *19.94* | *99.71* | | |
| ***100%*** | ***40*** | *39.94* | *99.85* | *99.87* | |
| | | *39.90* | *99.76* | | |
| | | *40.00* | *100.01* | | |
| ***150%*** | ***60*** | *59.48* | *99.14* | *99.14* | |
| | | *59.49* | *99.15* | | |
| | | *59.48* | *99.14* | | |

**Precision:**

**Table 5: Precision Data**

| S. No | Day 1 | Day 2 |
|---|---|---|
| **Injection-1** | 2546513 | 2544421 |
| **Injection-2** | 2553840 | 2546924 |
| **Injection-3** | 2563781 | 2533857 |
| **Injection-4** | 2554472 | 2530194 |
| **Injection-5** | 2568937 | 2518236 |
| **Injection-6** | 2563648 | 2534963 |
| **Mean** | 2558532 | 2534766 |
| **S.D** | 8306.0 | 10354.2 |
| **%RSD** | 0.3 | 0.4 |

**Robustness:**

**Table 6: Robustness data**

| condition | Optimized condition | Condition applied | area | RT | Plate Count | Tailing factor | RSD |
|---|---|---|---|---|---|---|---|
| **Flow rate (-0.1ml/min)** | **1ml/min<br>30:70<br>30$^{0}$c** | 0.9ml/min | 2594426 | 2.363 | 3570 | 1.41 | **0.3** |
| **Mobile phase (-5%v/v)** | | 35: 65 | 2600711 | 2.330 | 3468 | 1.44 | **0.3** |
| **Column temp (-3$^{0}$c)** | | 27$^{0}$c | 2493810 | 2.335 | 3335 | 1.44 | **0.5** |
| **Optimized** | | | **2560334** | **2.259** | **3375** | **1.4** | **-** |
| **Flow rate (+0.1ml/min)** | | 1.1ml/min | 2467774 | 2.088 | 3338 | 1.39 | **0.6** |
| **Mobile phase (+5%v/v)** | | 25: 75 | 2535038 | 2.094 | 3207 | 1.42 | **0.5** |
| **Column temp (+3$^{0}$c)** | | 33$^{0}$c | 2608858 | 2.214 | 3392 | 1.44 | **0.3** |

**Assay**

**Table 7: % Assay Purity Data**

| Formulation | Label claim(mg) | % Assay* |
|---|---|---|
| Fabiflu | Favipiravir 200 mg | 99.46 %w/w |

**Degradation studies:**

**Table 8: Force Degradation Studies**

| S.No | % Degradation | Peak area | Stress Conditions | Peak Purity |
|---|---|---|---|---|
| 1 | 6.24 | 2411441 | Acid | Passes |
| 2 | 3.88 | 2472137 | Base | Passes |
| 3 | 4.50 | 2456247 | Oxidation | Passes |
| 4 | 2.96 | 2495913 | Thermal | Passes |
| 5 | 1.21 | 2540792 | Photolytic | Passes |
| 6 | 0.85 | 2550111 | Hydrolytic | Passes |

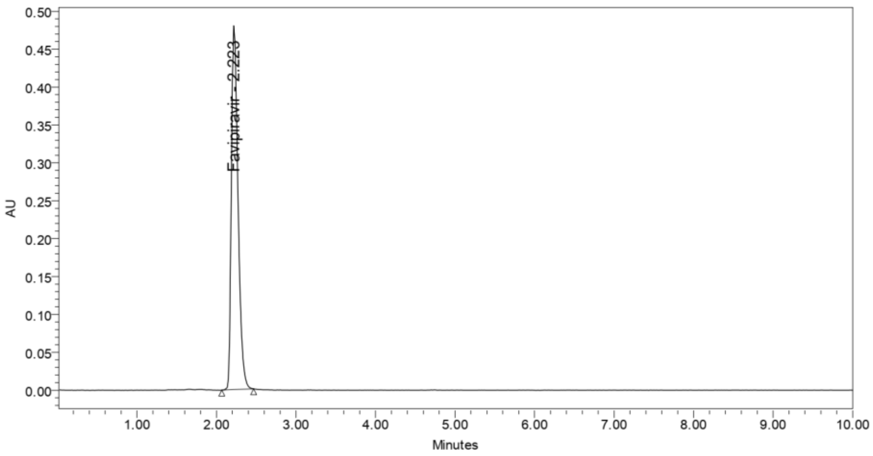

Figure 4: Acid Chromatogram

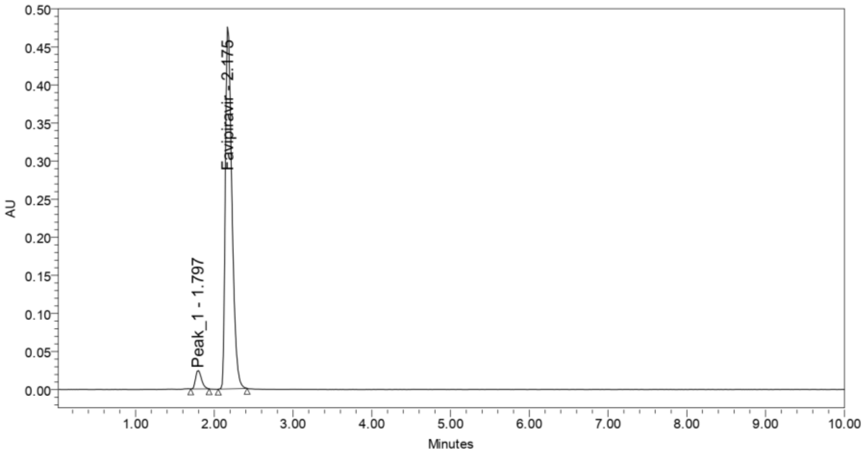

Figure 5: Base Chromatogram

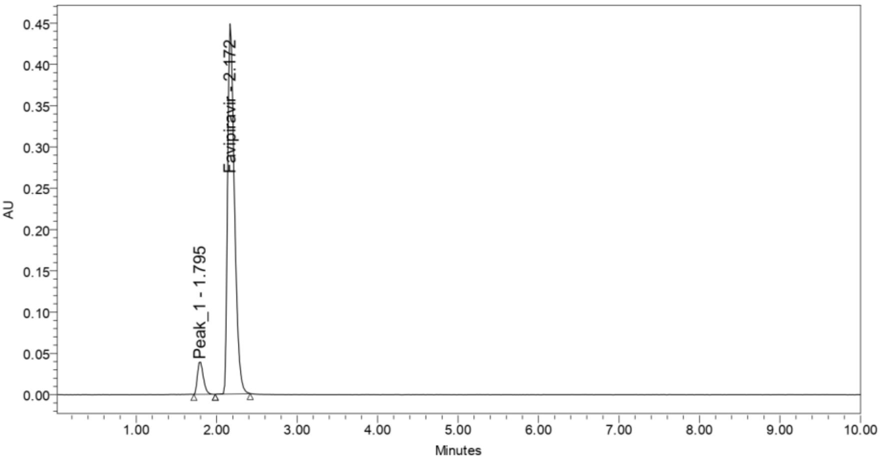

Figure 5: Oxidative Chromatogram

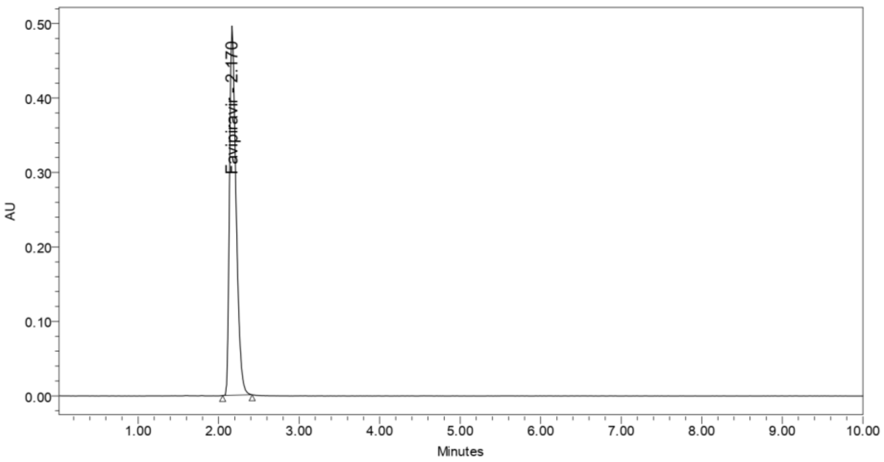

Figure 5: Thermal Chromatogram

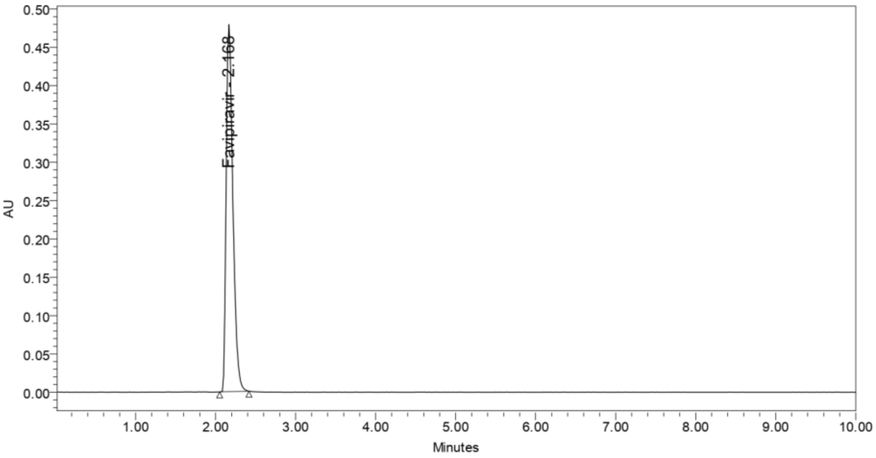

Figure 5: Photolytic Chromatogram

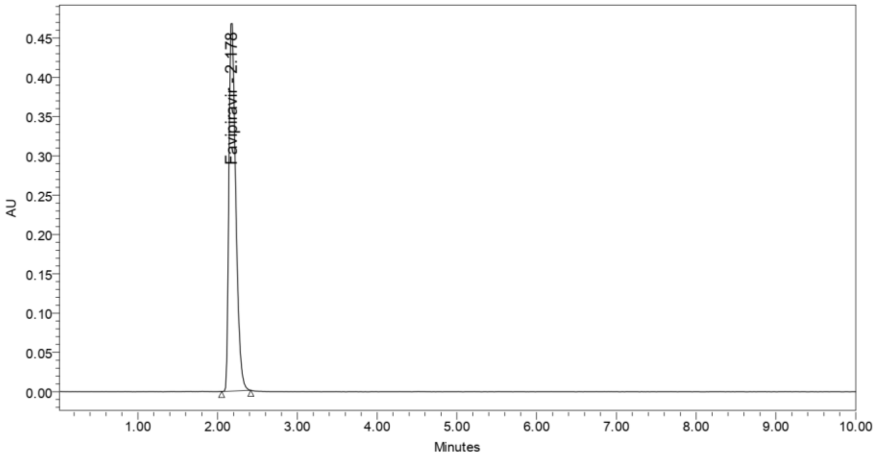

Figure 5: Water Chromatogram

## CONCLUSION:

In summary, the Favipiravir HPLC analysis findings demonstrate that this method can accurately ascertain the drug's concentration and purity. The technique is perfect for routine quality control and pharmacokinetic research because of its strong repeatability, clear peak resolutions, and consistent retention times. HPLC is a crucial method for evaluating the analytical qualities of favipiravir in order to guarantee its effectiveness and safety for therapeutic usage.

## ACKNOWLEDGEMENT:

The authors are thankful to, Department of Pharmaceutical Analysis, Malla reddy college of Pharmacy, Affiliated to Osmania University, India and Spectrum Pharma Research Solutions, Hyderabad, Telangana, India.

## REFERENCES:

1. *Minesh D. Patel et al., Review Article: Favipiravir as an Antiviral Drug, WJPMR, 2025, 11(2), 146-156.*
2. *Shashank Joshi et al. Role of favipiravir in the treatment of COVID-19, International Journal of Infectious Diseases 102 (2021) 501–508.*
3. *Umang Agrawal et al., Favipiravir: A new and emerging antiviral option in COVID-19, Med J Armed Forces India. 2020 Sep 2;76(4):370–376.*
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.*
5. *Madelain V, Nguyen TH, Olivo A, de Lamballerie X, Guedj J, Taburet AM, Mentre F: Ebola Virus Infection: Review of the Pharmacokinetic and Pharmacodynamic Properties of Drugs Considered for Testing in Human Efficacy Trials. Clin Pharmacokinet. 2016 Aug;55(8):907-23.*
6. *Venkataraman S, Prasad BVLS, Selvarajan R: RNA Dependent RNA Polymerases: Insights from Structure, Function and Evolution. Viruses. 2018 Feb 10;10(2).*
7. *DrugBank: Favipiravir*
8. *Yousuke Furuta et al., Favipiravir (T-705), a broad spectrum inhibitor of viral RNA polymerase, Proc Jpn Acad Ser B Phys Biol Sci, 2017;93(7):449-463.*
9. *Yousuke Furuta et al., Favipiravir (T-705), a novel viral RNA polymerase inhibitor, Antiviral Res. 2013 Nov;100(2):446-54.*
10. *Kimiyasu Shiraki, Tohru Daikoku, Favipiravir, an anti-influenza drug against life-threatening RNA virus infections, Pharmacol Ther, 2020 May:209:107512.*
11. *Ryta Łagocka et al., Favipiravir in Therapy of Viral Infections, J Clin Med, 2021 Jan 13;10(2):273.*
12. *Nandeesha Itigimatha et al, New Analytical Methods for the Determination of New Anti-Viral Drug Favipiravir: A Potential Therapeutic Drug Against Covid-19 Virus, in Bulk and Dosage Forms, Pharm Chem J. 2023*
13. *Shinde, J et al, Development and Validation of RP-HPLC Method for Estimation of Favipiravir API and It's Tablet Dosage Form Using Quality by Design Approach, Journal of Pharmaceutical Negative Results ; 2022.*

14. *İbrahim Bulduk, HPLC-UV Method For Quantification Of Favipiravir In Pharmaceutical Formulations, AK Journal, 2022*
15. *N S Yamani et al, Stability Indicating RP-HPLC Method for the estimation of Favipiravir in API and Pharmaceutical Dosage Forms (Tablets),RJPT, 2022*
16. *Dr. Amit Suryakant Tapkir, Method development and validation of Favipiravir by RP-HPLC, Innovative Journal of Medical Sciences, 2022*
17. *M. S. Kalshetti et al, Development and Validation of HPLC Method for Quantification of Favipiravir in Tablet, RJPT,2022*
18. *P. Ravisankar et al, RP-HPLC Method for Determination of Favipiravir (RdRp of RNA Viruses) in Pharmaceutical Dosage Form, Current Trends in Biotechnology and Pharmacy,2022*
19. *Sayyed Nazifa Sabir Ali et al, Analytical Method Development and Validation and Forced Degradation Stability-Indicating Studies of Favipiravir by RP-HPLC and UV in Bulk and Pharmaceutical Dosage Form, JPRI, 2021*
20. *A Bheema Nayak Et Al, A New Analytical Method Development And Validation For The Quantitative Determination Of Favipiravir In Bulk And Pharmaceutical Dosage Form By Rp-Hplc Method, Research Gate, 2023.*