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STABILITY INDICATING METHOD DEVELOPMENT AND METHOD VALIDATION FOR THE ESTIMATION OF FAVIPIRAVIR IN BULK AND PHARMACEUTICAL DOSAGE PREPARATIONS BY RP-UPLC

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ABSTRACT

A Precise, Specific, Accurate, Robust and Rugged stability indicating RP-UPLC method has been developed and validated for the estimation of Favipiravir in bulk and pharmaceutical dosage form (Tablets) was carried out by UPLC Instrument with Waters Acquity C18 (100mmx2.7mm ID) 1.7µm column as stationary phase by using mobile phase in Isocratic mode with a mixture of 20mM Phosphate Buffer of pH 2.5: Acetonitrile: Methanol (50:30:20 v/v/v)at a flow rate of 0.5mL/min and detection was carried out at 254nm. The Retention time of Favipiravir was 1.62 min. System precision results obtained within the acceptance criteria i.e., %RSD < 2.0. Correlation coefficient value obtained to be more than 0.999 and % Recovery for Favipiravir was obtained in between 98.0 to 102.0 in this method. In method precision, mean %Assay obtained between 95.0 to 105.0%. In forced degradation study, main analyte peak purity was passed; degradation also obtained in the range of 5-30%. Hence method is concluded as stability indicating.

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INTRODUCTION

Favipiravir is an antiviral used to manage influenza, and that has the potential to target other viral infections. Discovered by Toyama Chemical Co., Ltd. in Japan, favipiravir is a modified pyrazine analog that was initially approved for therapeutic use in resistant cases of influenza.7,9 The antiviral targets RNA-dependent RNA polymerase enzymes, which are necessary for the transcription and replication of viral genomes[1].

Not only does favipiravir inhibit replication of influenza A and B, but the drug has shown promise in the treatment of avian influenza, and may be an alternative option for influenza strains that are resistant to neuramidase inhibitors. Favipiravir has been investigated for the treatment of life-threatening pathogens such as Ebola virus, Lassa virus, and now COVID-19[1].

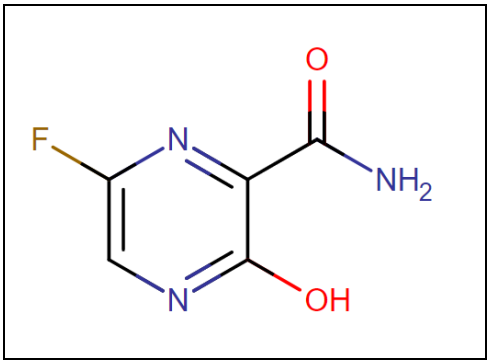

Fig No-01: Structure of Favipiravir[1]

The mechanism of action of Favipiravir is novel compared to existing influenza antivirals that primarily prevent entry and exit of the virus from cells. The active favipiravir-RTP selectively inhibits RNA polymerase and prevents replication of the viral genome. There are several hypotheses as to how favipiravir-RTP interacts with RNA dependent RNA polymerase (RdRp). Some studies have shown that when favipiravir-RTP is incorporated into a nascent RNA strand, it prevents RNA strand elongation and viral proliferation. Studies have also found that the presence of purine analogs can reduce favipiravir's antiviral activity, suggesting competition between favipiravir-RTP and purine nucleosides for RdRp binding[1].

Although favipiravir was originally developed to treat influenza, the RdRp catalytic domain (favipiravir's primary target), is expected to be similar for other RNA viruses. This conserved RdRp catalytic domain contributes to favipiravir's broad-spectrum coverage[1].

About Method:

Based on the previous works my UPLC method development work is novel, previous attempts were mostly on HPLC Developments but my method is developed on UPLC, with less elution time along with stability indicating. My method will reduce both Cost and time, easily we can use in industrial purpose to Analyse the Formulation under development samples and QC samples. Method also validated by using the ICH current guidelines.

Experimental Methodology:

Equipment:

Agilent UPLC with PDA Detector, Model Number:1290 with chemistation software, quaternary pump, auto sampler with thermostat, Analytical column:Waters AcquityC18 (100mmx2.7mm ID) 1.7µm, UV-Visible spectrophotometer, make:Shimadzu model: 1700UV with vision pro software, pH meter, make: Thermo Fisher and model: Orion star, Ultra sonic cleaner, Shimadzu balance (analytical)AY-0220.

Materials:

Favipiravir is obtained as gift sample from Chandra labs, Prashanthinagar, Kukatpally, Fabiflu Formulation was obtained in Local pharmacy, Acetonitrile, Methanol, Milli-Q water is used of HPLC-grade, Potassium di hydrogen phosphate purchased from Rankem and Merck and India.

Optimized Chromatographic conditions:

Analytical separation was carried out with column Waters AcquityC18 (100mmx2.7mm ID) 1.7µm by using Isocratic mode with a mixture of 20mM Potassium di hydrogen phosphate Buffer pH 2.5: Acetonitrile: Methanol (50:30:20 v/v/v). Column Oven Temperature: 30 ±0.2°C, flow rate was maintained at 0.5 mL/min and sample injection volume was maintained as 5µL with detection at 254nm with UV detector.

Preparation of standard solution

Weighed100mg of Favipiravir accurately, transferred into a 100 ml volumetric flask and dissolved in 70ml of mobile phase. Volume was made upto the mark with mobile phase. From above stock solution, 100µg/ml of Favipiravir was prepared by diluting 5ml to 50ml with mobile phase respectively.

Preparation of sample solution:

10tablets (each tablet contains 200mg of Favipiravir) were weighed and taken in a mortar and crushed into a fine powder and uniformly mixed. The crushed powder equivalent to 200 mg of Favipiravir was accurately weighed and transferred into a 200ml volumetric flask and dissolved in 150ml of mobile phase sonicating for 30min.The volume was made up to the mark with mobile phase. The sample was centrifuged at 5000rpm for 10min. Prepared 100µg/mL sample solution by further diluting 5mL of the above sample stock solution to 50mL with mobile phase. Then the sample was filtered with PVDF 0.45µm filter by discarding the 5mL of filtrate.

Selection of working wavelength (λmax):

UV spectrum of 5µg/mL solution of Favipiravir in methanol was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum, the wavelength maxima was identified as 254 nm. The spectrum was shown in Figure No: 2.

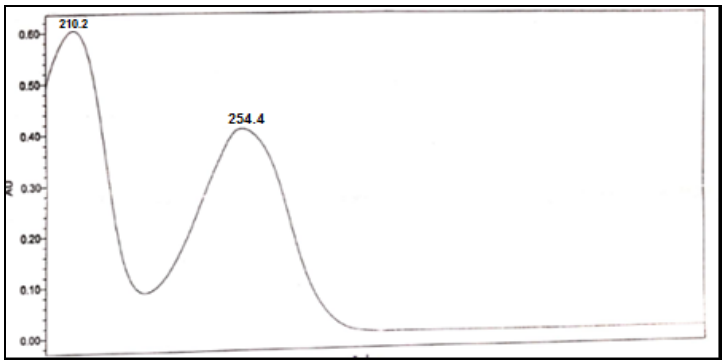

Fig No: 02 UV Spectrum of Favipiravir showingmaximum absorbanceat 254nm.

Analytical method validation[3]:

System suitability and System Precision[3]

Acceptance Criteria:

The % RSD of the area response of Standard peak obtained from the six injections of standard solution should not be more than 2.0. The Theoretical plates for 1st injection should be NLT 2000 for Favipiravir. The Tailing factor for 1st injection should be NMT 2.0 for Favipiravir peak.

Specificity[3]

Preparation of Placebo solution:

Weighed Placebo powder equivalent to 200 mg of Favipiravir in 200 ml of volumetric flask and dissolve in 350ml of mobile phase by 30min of sonication and make up the volume with mobile phase. Centrifuged sample at 5000rpm for 10min.
Prepared Placebo solution by further diluted 5mL above sample stock solution to 50mL with mobile phase and mixed well.

Acceptance Criteria:

No interference should be observed at the retention time of Favipiravir due to blank and Placebo.

Linearity[3]:

Linearity of the method was evaluated at the five equal-spaced concentration by diluting the standard stock solution to give solution over the range of 50-150% target of Favipiravir. Calibration curve was constructed at five linear concentrations of Favipiravir (50, 80, 100, 120&150µg/mL). The solutions were injected in to the chromatographic system, the results were plotted into a graph taking concentration versus area to evaluate correlation coefficient.
Acceptance criteria: Correlation coefficient Not less than 0.99.

Method Precision[3]:

Method precision Validation parameter investigated using the six individual sample preparations as reported above. Six samples were injected individually in to chromatographic system & calculated the % Assay of individual samples.
Acceptance Criteria: % Assay should be 95.0 to 105 & %RSD for six preparations assay should be ≤ 2.0

Accuracy & Recovery[3]:

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100% & 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100% & 150%.

Acceptance criteria of %Recovery should be 98.0 to 102.0 & Acceptance criteria of % RSD for nine preparations recover values should be ≤ 2.0.

Robustness[3]:

To determine the Robustness of the developed method, experimental conditions were deliberately changed and %RSD for Replicate injections of standard solution peak Areas, theoretical plates, tailing factor and resolution were evaluated. The mobile phase flow rate was changed to 0.4mL/min &0.6mL/min, the Column oven temperature was changed to 25°C&35°C. Acceptance Criteria: System suitability should be within the limit.

Intermediate precision (also called within-laboratory or within-device) is a measure of precision under a defined set of conditions: same measurement procedure, same measuring system, same location, and replicate measurements on the same or similar objects over an extended period of time. The Intermediate Precision (Ruggedness) was investigated using six individual sample preparations as reported above. Six preparations were injected individually in to chromatographic system & Calculated % Assay of individual samples. Acceptance criteria of %Assay should be 95.0 to 105.0, % RSD for six preparations assay values should be ≤ 2.0 & % RSD for Method precision and Intermediate Precision assay mean values should be ≤ 2.0.

RESULT AND DISCUSSION:

System suitability and System Precision[3]:

Table No.:01 Results for System suitability and System Precision.

Parameter	Results
Retention time(min)	1.622
Theoretical Plates	3629
Tailing factor	1.4
Resolution	NA
%RSD for Six replicated injection of Standard	0.3

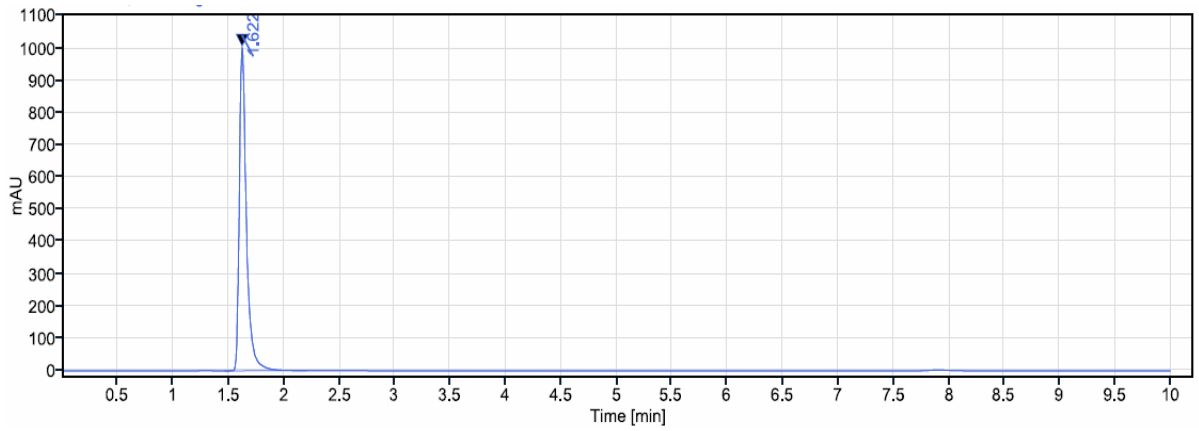

Fig No-03: Chromatogram of Standard.

Observation:

System suitability and System Precision results were met with acceptance criteria; hence system is precise.

Specificity[3]:

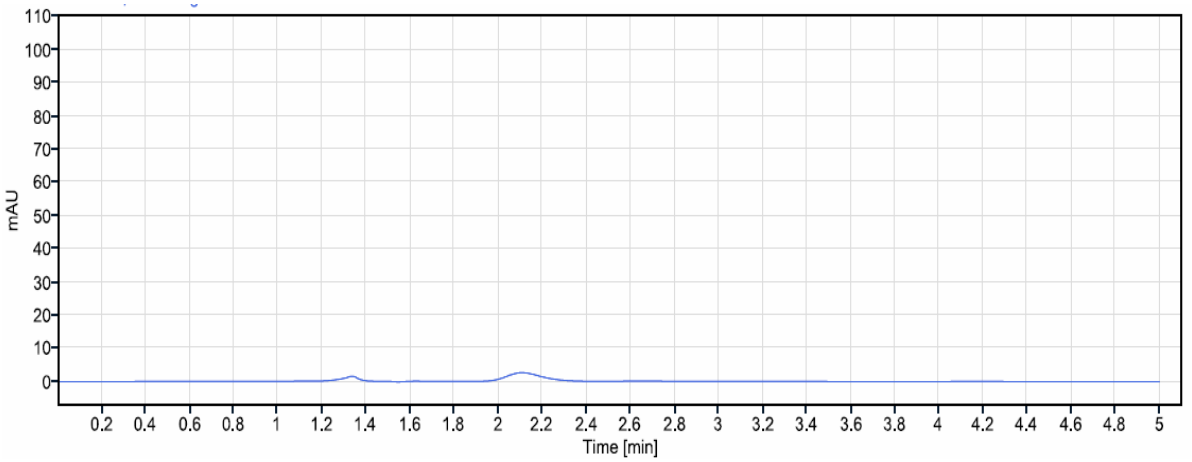

Fig No-04: Chromatogram of Blank.

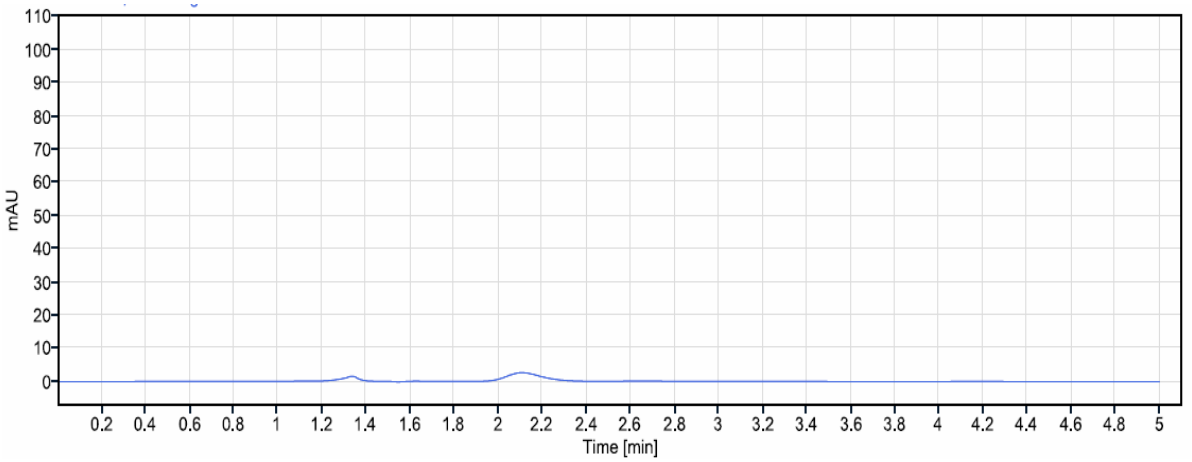

Fig No-05: Chromatogram of Placebo.

Observation:
There was no interference observed at the retention time of Favipiravir peak due to blank and Placebo. Hence System is specific

Forced Degradation studies[6]:
Acceptance Criteria:
Main analyte Peak purity should be Pass, peak purity value should be in positive.

Table No.:02 Forced Degradation results for Favipiravir.

Name of the Degradation	Condition	Peak Purity	Peak Purity Value	%Assay
Photolytic degradation	1.2mill/LUX Hours	PASS	+	99.7
Thermal Degradation	60°C/7Days	PASS	+	100.1
Acid Degradation	5mL of 0.1N HCl/4Hrs at 80°C	PASS	+	98.9
Base Degradation	5mL of 0.1N NaOH Solution/4Hrs at 80°C	PASS	+	98.4
Peroxide Degradation	5mL of 1% H_2O_2/4Hrs at Bench top	PASS	+	93.5
Control Sample	NA	PASS	+	99.8

Observation:
Peak purity was obtained Pass, Purity value obtained in Positive and %degradation 6.5% obtained in Peroxide degradation

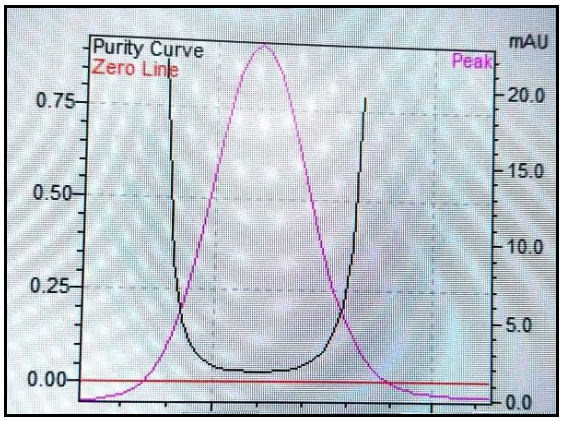

Fig No.:06Peak Purity Curve for Peroxide Degradation.

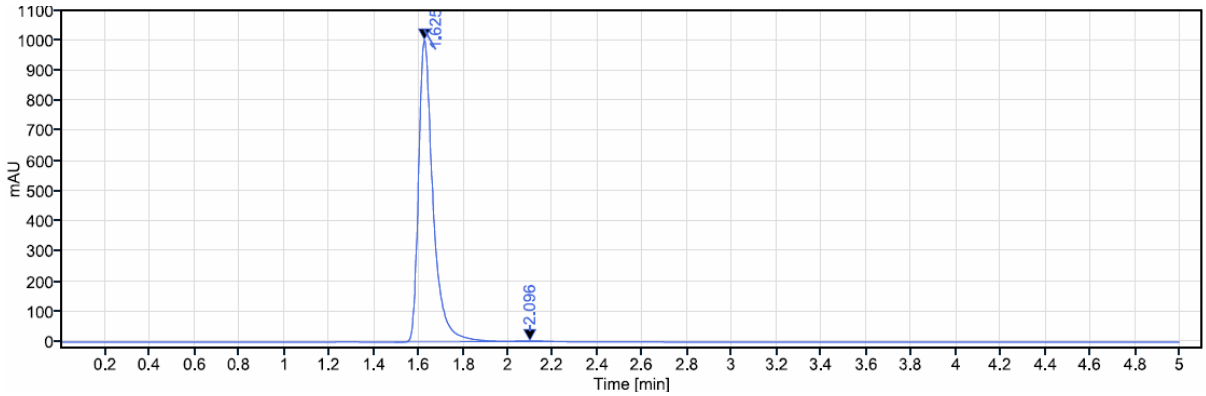

Fig No.:07 Chromatogram of Peroxide degradation.

Linearity[3]:

Table No.:03.

Parameter	Result
Concentration range in µg/mL	50µg/mL to 150µg/mL
Correlation coefficient	0.9993
Intercept	-45.616
Slope	69.249

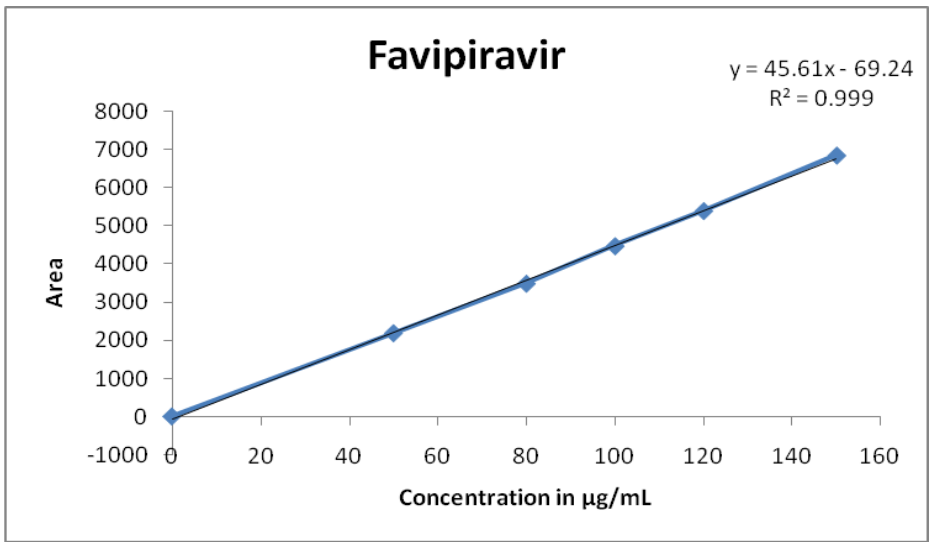

Fig No-08: Calibration curve for Favipiravir.

Observation: The correlation coefficient value obtained 0.9993 for Favipiravir.

Method Precision[3]:

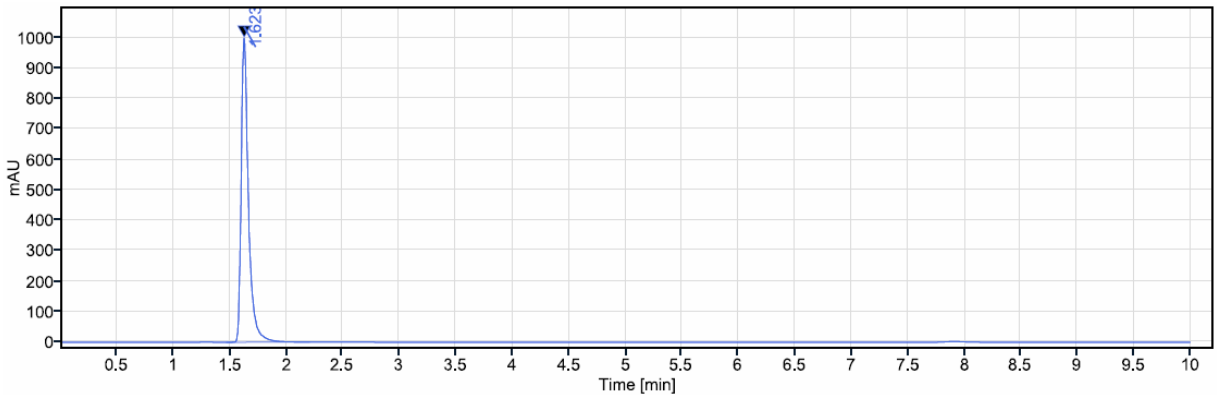

Fig No-09: Test solution Preparation-01 chromatogram.

Table No-04: Method Precision results of Favipiravir.

S.No.	Solution details	%Assay of Favipiravir
1	Test solution preparation-1	99.8
2	Test solution preparation-2	99.8
3	Test solution preparation-3	99.7
4	Test solution preparation-4	99.9
5	Test solution preparation-5	99.8
6	Test solution preparation-6	99.8
	Average	99.8
	Std Dev	0.13
	%RSD	0.1

Observation: Mean %Assay Obtained between 95.0 to 105.0% for Favipiravir and the % RSD of % Assay results obtained from Test solution was obtained less than 2.0% for Favipiravir

Accuracy and Recovery[3]:

Table No-05.

Parameter	Amount added (µg/m	Amount found (µg/mL)	% Recovery
50% Recover	**50**	**49.92**	**99.8**
100% Recov	**100**	**99.30**	**99.3**
150% Recov	**150**	**150.11**	**100.1**
Mean	-	-	**99.7**
%RSD	-	-	**0.9**

Observation: The % Recovery obtained between 98.0 to 102.0%, Mean % Recovery obtained between 98.0 to 102.0% for Favipiravir and %RSD obtained for All %recoveries less than 2.0%

Intermediate Precision[3]:

Table No-05 Anlyst-01 vs. Analyst-02.

S.No.	Solution details	%Assay
Analyst-01	Test solution preparation-1	99.8
	Test solution preparation-2	99.8
	Test solution preparation-3	99.7
	Test solution preparation-4	99.9
	Test solution preparation-5	99.8
	Test solution preparation-6	99.8
Analyst-02	Test solution preparation-1	100.1
	Test solution preparation-2	99.7
	Test solution preparation-3	98.6
	Test solution preparation-4	98.5
	Test solution preparation-5	98.2
	Test solution preparation-6	98.5
	Average	**99.3**
	Std Dev	**0.65**
	%RSD	**0.6**

Observation: Cumulative% RSD of % Assay results was obtained less than 2.0 for both analysts-01&02 of Favipiravir and Cumulative Mean of %Assay for both analysts-01&02 obtained to be 90.0 to 110.0% for Favipiravir

Robustness[3]:

Table No-06.

Name of the Parameter	%RSD	Theoretical Plates	Tailing factor
Low Column Oven Temperature (25°C)	0.67	3610	1.57
Low Column Oven Temperature (35°C)	0.91	3262	1.54
Lower Flow rate(0.4mL)	0.63	4137	1.65
Higher Flow rate(0.6mL)	0.81	3288	1.53

Observation: System suitability met the acceptance criteria in Robustness parameters hence method is Robust

CONCLUSION

Method was found to simple, accurate, specific, reliable and robust and method was described for the estimation of Pharmaceutical dosage form (Tablets) consisting of Favipiravir. Active ingredient was successfully resolved and quantified using UPLC with Waters Acquity C18(100mmx2.7mm ID) 1.7µm column in a relatively short run time of 10 minutes with 0.5mL/min flow in Isocratic mode of the chromatographic system. The proposed method provides a good resolution between active ingredients. The developed method was validated as per described in the ICH Q2B guidelines like system suitability, specificity, linearity, method precision, accuracy and recovery, robustness and ruggedness. The proposed method has the advantages of repeatability, sensitivity and requires less expensive reagents. In forced degradation studies all main peak purity values obtained in positive so this method is defined as stability indicating method.

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ABBREVIATIONS

UPLC : Ultra-performance liquid chromatography
Nm : Nanometer
ICH : International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
RSD : Relative Standard Deviation
mL/min : milliliter per minute
µg/Ml : microgram per milliliter
mM : millimolar
ID : internal diameter
ODS(C18): Octadecyl-silica
RNA : Ribonucleic acid
PVDF : Polyvinylidene fluoride
QC : Quality control
°C : Degrees centigrade**,** N:Normality

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