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DEVELOPMENT OF CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF FAVIPIRAVIR FROM BULK DRUG AND TABLET DOSAGE FORM

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ABSTRACT
The aim of study is to develop and validate a new, simple, rapid, precise, and accurate An Eco-friendly RP-HPLC and UV-Method Development and Validation for an estimation of Favipiravir in Bulk and pharmaceutical dosage form. To perform all the validation parameters according to ICH guidelines. To Optimize the validated parameters. FAV belongs to class II in the Biopharmaceutics Classification System (BCS). High permeability and low water solubility. To develop and validate a new, simple, rapid, precise, and accurate An Eco-friendly RP-HPLC and UV-Method Development and Validation for an estimation of Favipiravir in Bulk and pharmaceutical dosage form. To perform all the validation parameters according to ICH guidelines. To Optimize the validated parameters. Method development was done by using column (Cosmosil) C18 column (250 mm X 4.6 mm i.d.) 5µm, mobile phase Methanol: 0.2% Glacial acetic acid (pH 3.00) (20:80 % v/v), flow rate 0.5 ml/min, sample size 20 ml, run time 5 min. Validation parameters like accuracy, precision, repeatabilitystudies, specificity, solubility, LOD, LOQ and stability testing was performed on Favipiravir. From the calibration curve it was concluded that the Favipiravir shows linear response in the range of 2.0-30.0 µg /ml. All the parameters were within range hence above method is validated.

KEYWORDS: Favipiravir, HPLC, Accuracy, stability, specificity, linearity, filtration etc.

INTRODUCTION

HPLC

- High performance liquid chromatography (HPLC) is an important qualitative and quantitative technique, generally used for the estimation of pharmaceutical and biological samples.
- It is the most versatile, safest, dependable and fastest chromatographic technique for the quality control of drug components. This article was prepared with an aim to review different aspects of HPLC, such as principle, types, instrumentation and application.
- High performance liquid chromatography (HPLC) is an important qualitative and quantitative technique, generally used for the estimation of pharmaceutical and biological samples. It is the most versatile, safest, dependable and fastest chromatographic technique for the quality control of drug components.

There are 2 types of HPLC

1. **Reversed Phase Chromatography:-** Reversed phase HPLC (RP-HPLC or RPC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase.

RPC operates on the principle of hydrophobic interactions, which result from repulsive forces between a polar eluent, the relatively non-polar analyte, and the non-polar stationary phase. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand in the aqueous eluent.

2. **Normal phase chromatography:-** Also known Normal phase HPLC (NP-HPLC), this method separates analytes based on polarity.

NP-HPLC uses a polar stationary phase and a non-polar mobile phase.

The polar analyte interacted with and is retained by the polar stationary phase.

Adsorption strengths increase with increased analyte polarity, and the interaction between the polar analyte and the polar stationary phase increases the Elution time.

Analytical method validation Method validation is a documented evidence which provides a high degree of assurance for a specific method that the process used to confirm the analytical process is suitable for its intended use. The developed HPLC method for estimation ceftriaxone sodium was validated as per ICH Q2 (R1) guidelines.

- Faviparivir is antiviral drug against the SARS-CoV-2.

- One such drug is favipiravir, initially marketed as an antiinfluenza agent in Japan. This drug has just received emergency approval by the Drug Controller General of India (DCGI) and hence this comprehensive review of favipiravir comes at a timely juncture.
- As the pandemic spread to Europe, this drug received approval for emergency use in Italy, and currently has been in use in Japan, Russia, Ukraine, Uzbekistan, Moldova, and Kazakhstan. Approval has also recently been granted in Saudi Arabia and the UAE.

Mechanism of action

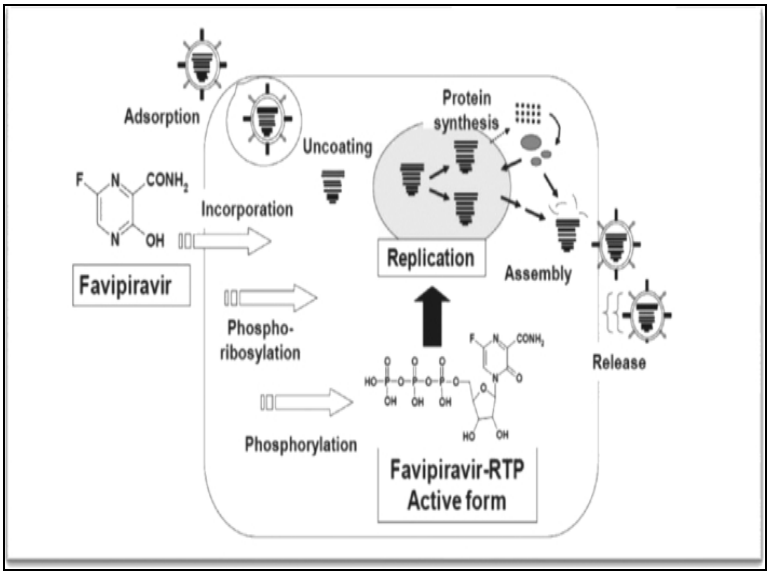


Fig. 1: Mechanism of action of Favipiravir.

a. This molecule acts as a substrate for the RNA-dependent RNA-polymerase (RdRp) enzyme, which is mistaken by the enzyme as a purine nucleotide, thus inhibiting its activity leading to termination of viral protein synthesis.
b. It gets incorporated in the viral RNA strand, preventing further extension.
c. It has recently been shown that favipiravir induces lethal mutagenesis in vitro during influenza virus infection, making it a virucidal drug.

Pharmacology

Favipiravir (T-705) is a synthetic prodrug, first discovered while assessing the antiviral activity of chemical agents active against the influenza virus in the chemical library.

Favipiravir is derived by chemical modification of the pyrazine moiety of T-1105.

Pharmacokinetics and pharmacodynamics

Favipiravir is administered as a prodrug.

It has an excellent bioavailability (~94%), 54% protein binding, and a low volume of distribution (10–20 L). It reaches Cmax within 2 h after a single dose. Both Tmax and half-life increase after multiple doses. Favipiravir has a short half-life (2.5–5 h) leading to rapid renal elimination in the hydroxylated form.Favipiravir exhibits both, dose-dependent and time-dependent pharmacokinetics. It is not metabolized by the cytochrome P450 system, but inhibits one of its components (CYP2C8).

Drug Profile

Drug name :- Favipiravir
Drug category :- Antiviral

Structure

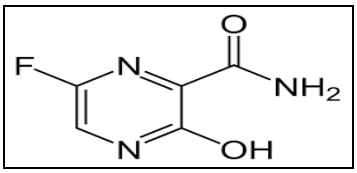


Fig.2: Structure of Favipiravir.

IUPAC Name:- 5-fluoro-2-oxo-1*H*-pyrazine-3-carboxamide
Molecular Formula :- C5H4FN3O2
Molecular weight :- 157.10
CAS NO :- 259793-96-9
Melting Point :- 187℃ to 193℃
Solubility :- slightly soluble in water
pKa :- 5.1
Physical State :- Lyophilized
Storage condition :-Short Term Storage: +4C. Long Term Storage: -20C. Handling Advice: Keep cool and dry. Use/Stability: Stable for at least 2 years after receipt when stored at -20C.

Methodology /Plan of Work

HPLC

Instruments

The analysis of the drug was carried out on Agilent Gradient System UV Detector. Equiped with Reverse

Phase (Cosmosil) C18 column (4.6 id x 250mm; 5µm), a SP930D pump, a 20µ l injection loop and UV630D Absorbance detector and running Open Lab EZ chrome software.

a. Selection of stationary phase: The column used in this method C_{18} Cosmosil. The configuration of the column is 4.6 x 250 mm, particle size 5 mm. C18 column gives high non polar retentivity, symmetric peak shape, highly reproducible and stable ideal for HPLC method.

b. Solubility Studies: This study was carried out to find an ideal solvent in which drugs are completely soluble. Various solvents were tried for checking solubility of Favipiravir. From solubility studies it was concluded that of Favipiravir is slightly soluble in water.

c. Chromatographic conditions: The following chromatographic conditions were established by trial and error and were kept constant throughout the experimentation.

d. UV-VIS Spectrophotometer: UV-VIS Spectrophotometer was selected as analytical technique for estimation of Favipiravir. UV absorbance range of 200-400nm.

e. Validation parameters

- Accuracy
- Precision
- Specificity
- Linearity
- Robustness
- Limit of detection
- Limit of Quantification
- Range
- Robustness

RESULTS AND DISCUSSION

PRELIMINARY CHARACTERIZATION AND IDENTIFICATION OF DRUG

1. Color, odour and appearance

Table no 1: Color, odour and appearance of Drug.

Sr. No	Name	Colour, odour and appearance of drug
1	Favipiravir	White to off white, odourless and Crystalline powder

2. Melting point determination

Table no. 2: Melting point of Drug.

Sr. No.	Name	Melting point std. value (°C)	Melting point observed (°C)
1	Favipiravir	183-192°C	189-192°C

3. Solubility study

Solubility study Favipiravir

DMSO - Drug was found soluble in DMSO

4. Selection of solvent

DMSO was selected as the solvent for dissolving Favipiravir.

5. Selection of analytical wavelength

Favipiravir STD solution: (20 PPM)

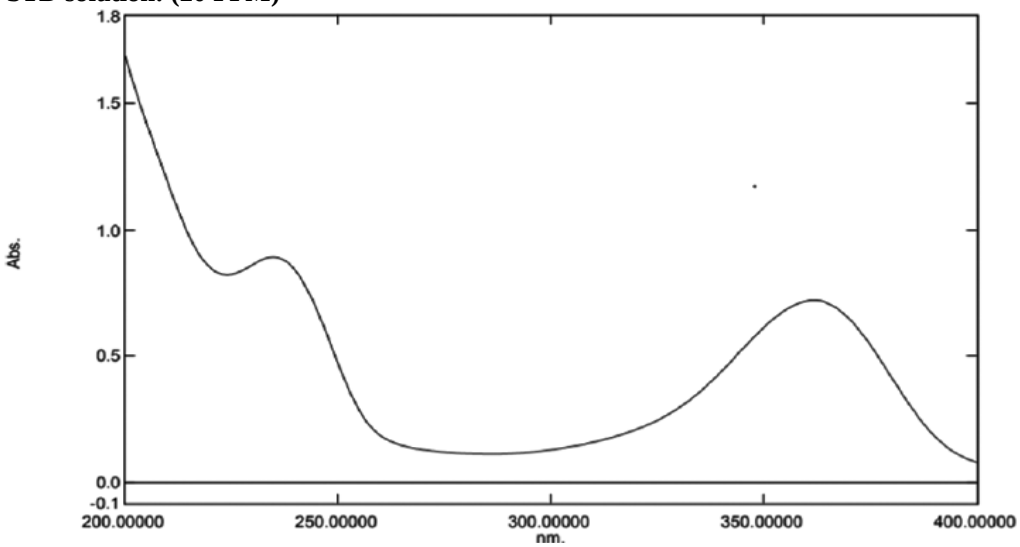


Fig. No 3. UVspectrum of Favipiravir.

Observation: The standard solution was scanned between 200 nm to 400nm. Wavelength of maximum absorption was determined for drug. Favipiravir showed maximum absorbance at 358 nm

6 Method Development by RP – HPLC
6.1 Optimization of HPLC method
Trial 5: Chromatogram

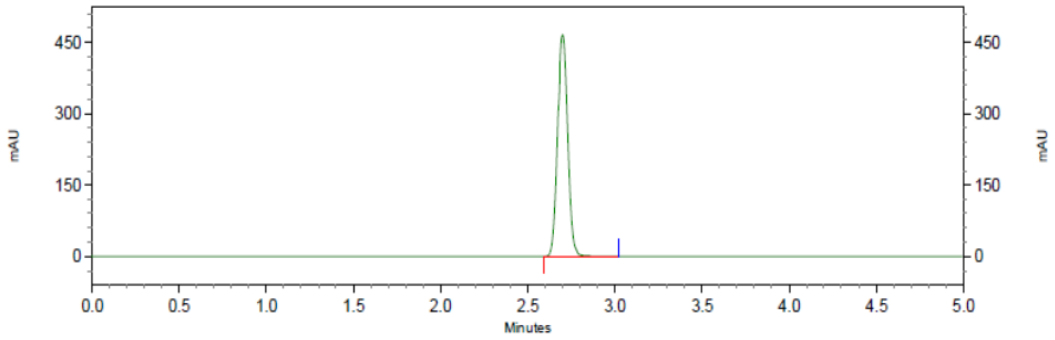


Fig. no.4: Chromatogram of optimized parameter.

Observation: Favipiravir eluted with acceptable Chromatography.

Conclusion: From the observations of trials first to five, it was concluded that chromatographic conditions in trial five gives better peak, good retention time and tailing factor therefore chromatographic conditions in trial five was used for method validation.

Optimized Chromatographic Conditions
Table no.3 Optimized Chromatographic Conditions.

Parameter	Description
Mode	Isocratic
Column Name	Cosmosil C18, 250 mm X 4.6mm ID, 5 µm
Detector	UV Detector
Injection Volume	20 µl
Wavelength	358 nm
Column Oven temp	25ºC
Mobile Phase	Methanol : 0.2% Glacial acetic acid (pH 3.00) (20:80 % v/v)
Flow Rate	0.5 ml/min
Run time	05 Minutes
Diluent	Mobile phase

6.2 Analysis of Marketed Test samples (Assay)
a) Fabiflu 400 Tablet
Weight of 20 tablets = 10.7080 gm
Average weight of tablet = 10.7080 / 20 = 0.5354 gm = 535.4 mg

- **Average weight of test sample** (Fabiflue 400 mg Tablet)**:**

Weighed the 20 tablets at a time and calculated average weight of tablet by following formula:

Average weight (mg) = Weight of 20 tablets (mg) / 20

- **Sample preparation of Marketed test sample:** 20 mcg of Favipiravir

Formula for % Assay calculation:

$$\%\ Assay = \frac{Favipiravir\ Spl\ area}{Favipiravir\ Std\ avg\ area} X \frac{Favipiravir\ STD\ wt\ (mg)}{20} X \frac{0.8}{20} X \frac{100}{Tablet\ sample\ weight\ (mg)} X \frac{20}{0.8} X \frac{Avg\ wt\ of\ tablet\ (mg)}{Label\ claim\ of\ Favipiravir\ (mg)} X\ 100$$

6.5**VALIDATION OF RP-HPLC METHOD :-**
The developed method for estimation of Favipiravir was validated as per ICH guidelines for following parameters.
1. **FILTRATION STUDY :-** Filtration study carried out with unfiltered and filtered test solution. During filtration activity 0.45 µm PVDF and 0.45 µm Nylon syringe filters used by discarding 5 mL of aliquot sample.

Assay results of Fabiflu 400 Tablet

Table no.4: Assay results of Fabiflu 400 Tablet.

Sample	Area	% Assay	Mean assay
Sample 1	5321506	97.46	97.62
Sample 2	5365125	97.82	

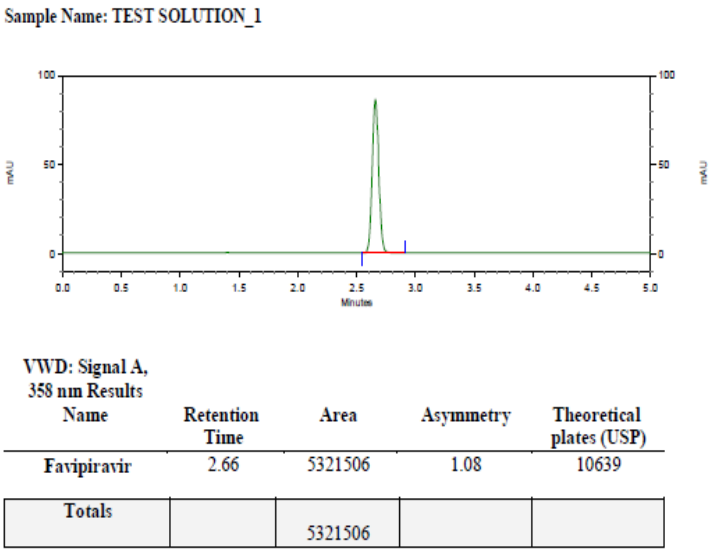


Fig. No. 5: Typical chromatogram of Fabiflu 400 Tablet sample.

Acceptance criteria

1) % Assay found should be in the range of 90-110%.

Data interpretation

From the above results, it can be concluded that the assay result is within the limit for selected marketed test sample and sample can be used for validation

Data interpretation: Both filters PVDF and Nylon passes the criteria for filter study, hence both filters can be used. We used PVDF filter because it showed less absolute difference as compare to Nylon filter.

7. VALIDATION OF RP-HPLC METHOD

1. Results of Filter study

Table no. 5: Results of Filter study.

Sample description	Area	% Absolute difference
Unfiltered	5368512	NA
0.45 µ PVDF filter	5325108	0.81
0.45 µ Nylon filter	5291527	1.43

1) **SOLUTION STABILITY:** Stability study was conducted for Standard as well as Test Sample. Stability study was performed at normal laboratory conditions. The solution was stored at normal illuminated laboratory conditions and analyzed at initial, after 12 hours and 24 hours.

Results of Solution stability.

Table no.6: Results of stability.

Sample solution			Standard solution		
Time point	**Area**	**% Absolute difference**	**Time point**	**Area**	**% Absolute difference**
Initial	5352106	NA	**Initial**	5462519	NA
12 Hours	5312519	0.74	**12 Hours**	5450210	0.23
24 Hours	5300025	0.97	**24 Hours**	5412517	0.92

Data interpretation: Standard and Test solution was found stable up to 24 Hrs. Hence both solutions can be used up to 24 Hrs.

3. SPECIFICITY: Specificity is the ability to access unequivocally the analyte in the presence of components which may be expected to be present.
Blank, placebo, standard and test solution prepared and injected to check peak purity.

RESULTS OF SPECIFICITY
Table no.7 Results of Specificity.

Description	Observation
Blank	No interference at R.T. of Favipiravir due to blank
Placebo	No interference at R.T. of Favipiravir due to placebo
Standard solution	Peak purity was 0.988
Test Solution	Peak purity was 0.976

Acceptance criteria
Blank: There should be no Interference at R.T. of Favipiravir

Placebo: There should be no Interference at R.T. of Favipiravir

Standard and Test sample solution: Peak purity: NLT 0.95.

Data interpretation: Blank and placebo were not having interference at R.T. of Favipiravir. Peak purity for Standard as well. Hence developed chromatographic method passes the criteria for specificity.

4. Linearity and Range
Linearity of an analytical method is its ability to elicit test results that are proportional to the concentration of analyte in samples within a given range.

Linearity Data for Favipiravir
Table no. 8: Linearity Data for Favipiravir.

Level	Conc (µg/mL)	Area	Mean	% RSD
10%	2.00	532015	532269	0.049
		532541		
		532251		
50%	10.00	2751942	2754535	0.085
		2756521		
		2755143		
100%	20.00	5468512	5467614	0.016
		5467528		
		5466802		
125%	25.00	6885211	6883220	0.028
		6881425		
		6883024		
150%	30.00	8252131	8252851	0.025
		8251206		
		8255216		

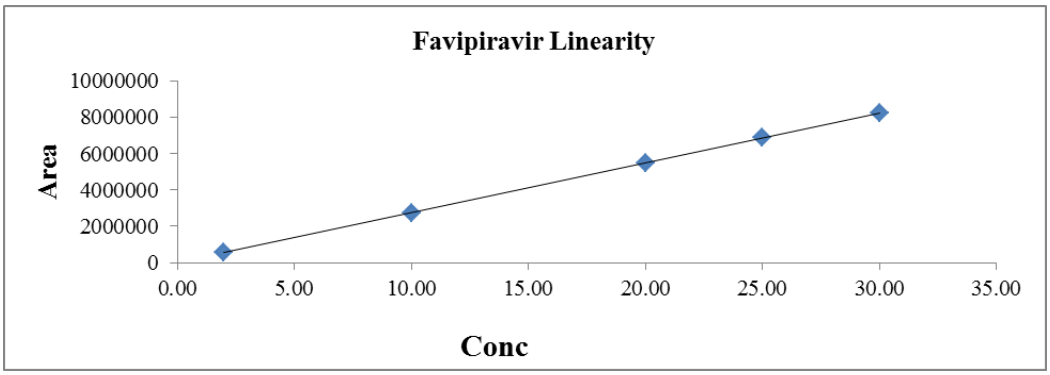
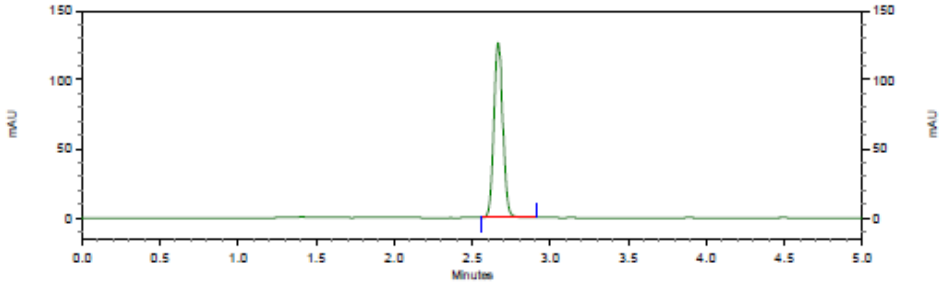


Fig. no. 6: Calibration curve of Favipiravir.

Sr no.	Parameter	Result value	Acceptance criteria
1	Beer's linearity range	2.00-30.00 µg/mL	NA
2	Correlation coefficient (R^2)	0.99999	NLT 0.98
3	Intercept	-15470.13	To be report
4	Slope	275492.41	To be report
5	% RSD for area at each level	NA	NMT 2.0

The respective linear equation for Favipiravir was
Y = M X + C
Y = 275492.41 x + -15470.13
Where, x = concentration of Analyte in µg/mL
y = is area of peak.
M = Slope
C= Intercept

Sample Name: LINEARITY 150%



VWD: Signal A, 358 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.67	8252131	1.09	10136
Totals		8252131		

Fig. No. 7 Typical chromatogram of Linearity 150%.

CONCLUSION

From the calibration curve it was concluded that the Favipiravir shows linear response in the range of 2.00-30.00 µg/ml. The Regression value was found well within the limit.

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

σ = 16555.84 (Residual standard deviation of a regression line)
s = 275492.41 (Slope)

Detection limit (LOD)

LOD = 3.3 σ / S
LOD = 3.3 x 16555.84 / 275492.41

LOD = 0.20 µg/mL

Quantitation limit (LOQ)

LOQ = 10 σ / S
LOQ = 10 x 16555.84 / 275492.41

LOQ = 0.60 µg/mL

6. ACCURACY (RECOVERY)

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value.

Acceptance criteria

% Recovery for each level and overall recovery: 98.0 to 102.0%
% RSD for each level and overall recovery: NMT 2.0

Data interpretation: Recovery of analytical procedure was found well within acceptance criteria at all 3 levels. % Recovery not get hampered by changed in analyte concentration.

7. PRECISION

Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample.

Result of Intra- day and Inter- Day Precision for Favipiravir test sample assay

Table no.9: Result of Intra- day and Inter- Day Precision for Favipiravir test sample assay.

	Sample	Test Sample (mg)	Area	% Assay
Repeatability	Sample 1	66.5	5421358	99.88
	Sample 2	66.8	5321651	97.61
	Sample 3	67.2	5295148	96.54
	Sample 4	66.4	5321064	98.18
	Sample 5	67.3	5421635	98.70
	Sample 6	66.9	5381524	98.56
	Mean			98.25
	STD DEV			1.122
	% RSD			1.142
Intermediate precision (Inter-Day)	Sample 1	66.8	5351694	98.16
	Sample 2	67.1	5403251	98.66

	Sample 3	66.2	5354136	99.09
	Sample 4	66.9	5302165	97.10
	Sample 5	67.4	5412899	98.40
	Sample 6	67.1	5341521	97.53
	Mean			98.16
	STD DEV			0.7339
	% RSD			0.748
Repeatability Plus Inter-day	Mean			98.201
	STD DEV			0.9051
	% RSD			0.922

Acceptance criteria

% Assay: % Assay value for each sample (Individual sample) and mean assay value for precision (6 sample), mean assay value intermediate precision (6 sample), and mean assay value for precision plus intermediate precision sample (12 sample): 90-110%

% RSD: % RSD for precision study samples(6 sample), Intermediate precision study samples (6 sample) and precision plus intermediate precision sample (12 sample): NMT 2.0

Data interpretation: % Assay and % RSD was found well within acceptance limit and hence method is precise (Reproducible).

8. ROBUSTNESS

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Following changes made under Robustness:

- Change in Wavelength
- Change in flow rate
- Change in column oven temperature

RESULT OF ROBUSTNESS STUDY

Table no.10: Result of Robustness study.

Change in Parameter	R.T.	Standard area	Asymmetry	Theoretical plates
Wavelengthby +3 NM (361 NM)	2.66	5402165	1.07	10924
Wavelength by -3 NM (355 NM)	2.62	5245628	1.09	10564
Flow rate by +10% (0.55 mL/min)	2.37	5026351	1.04	9751
Flow rate by -10% (0.45 mL/min)	2.89	6052149	1.08	11628
Column oven temp by +2ºC (27	2.64	5585362	1.02	11124
Column oven temp by -2ºC (23 ºC)	2.68	5346218	1.11	10251

Acceptance criteria

Chromatography (System suitability) acceptance criteria should not get failed.

Data interpretation: From the above results, it was concluded that the system suitability test result was found well within the limits and analytical method was robust.

8. CONCLUSION

Methods (RP-HPLC single - component mode of analysis) have been developed and validated for determination of **Favipiravir**

RP-HPLC methods are found to be accurate, precise, rugged and robust.

All these methods are adequately sensitive.

The only limitation of RPHPLC method is its cost.

UV-spectrophotometric methods are simple, accurate and economical and least calculations are involved for estimation of concentrations of both these drugs.

All the developed methods can be used for routine analysis of these drugs in their combined pharmaceutical formulation.

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