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Development And Validation of Stability –Indicating RP- HPLC Method For Analysis of Favipiravir Using Hydrotropic Solvent In Bulk And Pharmaceutical Formulation.

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ABSTRACT

The purpose of this work was to validate a stability-indicating HPLC method for the analysis of Favipiravir using c8 150x4.6 mm5µ HPLC Column with a mobile phase of 6% sodium acetate pH 7.0ACN (70:30 v/v) The ICH criteria were followed in the assessment of validation parameters. The method exhibited excellent linearity, accuracy, and precision over the specified concentration range. The method withstands several stress conditions like acid, alkali, peroxide, reduction, thermal, photolytic, and hydrolysis. Specificity studies confirmed that the developed method was stability-indicating in nature. Robustness testing demonstrated the developed method resilience to minor chromatographic condition. Hydrotropic solvent sodium acetate was used in the preparation of mobile phase. Which was economical then other solvents like methanol etc. hence the proposed method was said to be economical then reported methods.

Keywords: Favipiravir**,** HPLC, Green solvents, validation, stress studies,

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INTRODUCTION

Molecular Formula: C5H4FN3O2 Favipiravir 5-fluoro-2-oxo-1H-pyrazine-3-carboxamide 157.104 g•mol−1 in weight Favipiravir, which is marketed as A vigan. It's also being researched as a potential treatment for several other viral illnesses, such as SARS-CoV-2. It is a derivative of pyrazine carboxamide, just like the investigational antiviral medications T-1105 and T-1106. Two to five and a half hours. Classification An antiviral called favipiravir is used to treat influenza and may also be effective against other viral diseases. In Japan, favipiravir is authorized for the treatment of influenza. A review of the literature found that numerous analytical techniques have been documented alone or in conjunction with other medications. By using less organic solvent, the new approach demonstrated an outstanding green analytical procedure for the determination of Favipiravir and its degradation studies. An effort has been made [1-5].

MATERIALS AND METHODS

Drug Samples

Working standard Favipiravir (99.75%) was obtained as a gift sample from Shree icon laboratories, Vijayawada, India

Determination of Working Wavelength (λ_{max})

The PDA Detector was used to scan the wavelength region of 200–400 nm in 6% sodium acetate pH-7.0 as a blank to determine the wavelength of maximum absorption of the drug solution. At 228 nm, the absorption curve is visible. Thus, using the HPLC chromatographic procedure, a detector wavelength of 228 nm was used.

Preparation of standard solution

Weigh precisely and transfer 10 mg of the working standard favipiravir into a 10 ml dry volumetric flask. Use the same solvent to dilute, sonicate, and adjust volume to the desired level. (Stock A) Continue by pipetting 2 ml of the aforementioned stock solutions into a 10-ml volumetric flask, then dilute with diluent to the appropriate level. (Favipiravir200 ppm)

Preparation of Sodium acetate buffer solution

60gmof sodium acetate in1000mlHPLCgradewaterobservedpHis7.0adjustedwithOPA. Filtered through 0.45µ membrane filter.

Initial Chromatographic condition

Numerous trails were run in order to choose the ideal chromatographic conditions, and the best trail was chosen for the optimized procedure.

Use Waters Acquity HPLC.

Column	: Inert sustain c8150x4.6mm, 5µ
Mobile phase	: 6%sodiumacetatepH-7.0+CAN (70:30v/v)
Wavelength of detection	: 228 nm
Rate of flow	: 0.8 ml/min
Volume of injection	: 10µl
Run time	: 10 minutes

RESULTS AND DISCUSSION

System suitability

The peak caused by Favipiravir in Standard solution should have a tailing factor of not more than 2.0, and its theoretical plates should not be less than 2000.

Specificity

Three chromatograms—a blank, a standard, and a sample—were recorded for this purpose. The absence of any reaction in the blank chromatogram during the drug's retention durations indicates that the drug's response was specific.

Linearity

A series of aliquotes were prepared from 50 to 300 µg/ml from the stock solution. Plot the average peak area vs concentration (average peak area on the Y-axis and concentration on the X-axis) and the obtained correlation coefficient was in the acceptance limit.

Accuracy

Standard solution for accuracy studies 50%, 100%, 150% solutions were prepared using standard addition method. The prepared solution were injected to the HPLC and the results were recorded as shown below.

Precision

To make sure the analytical system is operating correctly, standard chemical substances are used to verify system precision. RSD should be computed in this peak location and the percentage of drug of the six determinations is assessed.

A homogeneous sample from a single batch should be examined six times for method precision. This shows if a procedure is producing consistent outcomes for a particular batch. This involves six analyses of the sample to determine the percentage RSD.

Robustness

Deliberate changes were made to the Flow rate and Temperature Variation as part of the Robustness to assess the effect on the procedure.

Standard solution of200ppm of Favipiravir was prepared and analyzed using the varied in method flow rate and column oven temperature ±2 ºC

Limit of detection (LOD) and limit of quantification (LOQ)

LOD for Favipiravir was found to be 0.6µg/mL and LOQ for Favipiravir was found to be2µg/ml.

Degradation Studies

Acid degradation

Pipette 1 ml of the aforementioned solution and 1 ml of 1N HCl were added to a 10 ml volumetric flask. After an hour at 60 degrees Celsius, the volumetric flask was neutralized with 1 N NaOH and made up to 10 milliliters with diluent.

Alkali degradation

1ml of the aforementioned Favipiravir solution was pipetted into a 10 ml volumetric flask, and 1 ml of 1N NaOH was added. After an hour at 60 degrees Celsius, the volumetric flask was neutralized with 1N HCl and made up to 10 milliliters with diluents

Peroxide degradation

A 10 ml volumetric flask was filled with 1 ml of the aforementioned stock solution and 1 ml of 3% w/v hydrogen peroxide, and the volume was adjusted to the necessary level with diluent. Following that,

the volumetric flask was held at 60°C for one hour. The volumetric flask was then allowed to stand at room temperature for 15 minutes.

Reduction degradation

Pipette 1mlof the above-stock solution was added to a 10ml volumetric flask, followed by 1ml of 10% sodium bisulfite. The volume was then increased with diluent to the required level. Following that, the volumetric flask was held at 60°C for one hour. The volumetric flask was left to stand at room temperature for 15 minutes.

Hydrolysis degradation

A 10 ml volumetric flask was filled with 1 ml of above-stock solution, 1 ml of HPLC grade water, and diluent was used to increase the volume to the necessary amount. After that, the volumetric flask was kept at 60°C for an hour. The volumetric flask was then allowed to set at room temperature for fifteen minutes.

Photolytic degradation

The photo stability chamber held the favipiravir sample for a duration of three hours. Subsequently, the material was extracted, diluted using diluents, and introduced into an HPLC for analysis.

Thermal degradation

A Petri dish containing a Favipiravir sample was placed in a hot air oven set at 105° C for three hours. Subsequently, the material was extracted, diluted using diluents, and introduced into an HPLC for analysis.

Specificity

All the stressed samples were Transfer into vials after filtering it via 0.22 micron syringe filters for analysis. Retention times of Favipiravir were 2.075 min. We did not found and interfering peaks in blank and placebo at retention times of this drug in this method. So this method was said to be specific.

Precision

System Precision

The % RSD obtained as 0.27 for Favipiravir. As the limit of Precision was less than “2” the system precision was within the acceptance limit.

The method precision %RSD obtained as 0.65.

Accuracy

Three levels of accuracy samples were created using the standard addition approach. Triplicate doses were given for each level of accuracy, and the mean % Recovery was 100.2% for Favipiravir.

Degradation studies

Favipiravir was more degraded in peroxide stress than other stress conditions. Purity angle was found to be less than purity threshold in all forced degradation studies without having signs of purity flags hence the proposed method was said to be stability indicating.

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Table 1: List of Apparatus

S.No	Name	Model	Manufacturer
1.	HPLC	Acquity	Waters
2.	pH meter	PICO+	Lab india
3.	Weighing balance	ELB300	Shimadzu
4.	UV/VIS spectrophotometer	UV-1800	Shimadzu
5.	Pipettes, beakers and Burettes	-	Borosil
6.	Ultra sonicator	UCA701	Unichrome

Table 2: List of chemicals and solvents

S. No	Name	Grade	Manufacturer
1.	Aceto nitrile	HPLC	Rankem
2.	Water (Milli Q)	HPLC	In house production
3.	Sodium acetate	Analytical reagents	Merc

Table 3: Optimized chromatographic conditions

S.NO		
1	Column	Inertsustainc8150*4.6mm5µ
2	Mobile phase	6%SodiumacetatepH-7.0 + ACN (70:30)
3	Flow Rate	0.8mL/min
4	Injection volume	10µl
5	Detection wave length	228 nm
6	Temperature	27±2^{0}c
7	Run time	10min

Table 4: System suitability

S.no	Parameter	Results(n=6)
1	Retention time(min)	2.075
2	Plate count	14327
3	Tailing factor	1.07
4	%RSD	0.27

Table 5: Assay of Favipiravir

Brand	Drug	Label amount (mg)	Estimated amount in mg(n=6)	%assay
Favivir	Favipiravir	200	199.98	99.9

Table 6: Results of linearity studies

S.NO	Favipiravir	
	Conc.(µg/ml)	Average Peak area (n=6)
1	50.00	542896
2	100.00	1086473
3	150.00	1633418
4	200.00	2158671
5	250.00	2695420
6	300.00	3161337
Regression equation	y=10615.18x+18896.04	
Slope	10615.18	
Intercept	18896.04	
R^2	0.99978	

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Table 7: Results of accuracy studies

Recovery level	Pre- Analyzed amount (mg)	Amount Added (mg)	Amount Found (mg)	%Recovery	Mean %Recovery
50%	20	10.0	30.05	100.6	100.1
		10.0	30.1	101.0	
		10.0	29.89	98.9	
100%	20	20.0	40.0	99.9	100.1
		20.0	39.93	99.6	
		20.0	40.15	100.7	
150%	20	30.0	50.06	100.2	100.3
		30.0	49.95	99.8	
		30.0	50.25	100.8	

Table 8: Results of precision studies

S.NO	Type Of Precision	Conc Of Favipiravir(ug/ml)	Mean Peak Area (n=6)	S.D	%RSD
1.	Repeatability	200	2163128	5871.705	0.27
2.	Method Precision	200	2165602	13986.926	0.65
3.	Inter day Precision	200	2166182	16066.268	16066.268

Table 9: Results of Robustness

Parameter	Favipiravir				
	Condition	Retention time(min)	Peak area	Tailing	Plate count
Flow rate Change (mL/min)	Less flow(0.72ml)	2.196	2026487	1.08	14543
	Actual flow(0.8ml)	2.075	2168971	1.07	14327
	More flow(0.88ml)	1.962	2319652	1.03	14255
Temperature condition	Less Org (25^0c)	2.354	1876763	1.11	14433
	Actual(27^0c)	2.078	2159032	1.08	14381
	More Org(29^0c)	1.827	2451679	1.04	14154

Table 10: Results of Forced Degradation

Stress conditions	Favipiravir			
	%Assay	%Degradation	Purity Angle	Purity Threshold
Control	100	0	1.625	4.461
Acid	84.1	15.9	1.608	4.486
Alkali	89.5	10.5	1.691	4.453
Peroxide	98.1	1.9	1.627	4.438
Reduction	96.8	3.2	1.633	4.494
Thermal	88.0	12.0	1.649	4.438
Photolytic	86.8	13.2	1.616	4.412
Hydrolysis	96.0	4.0	1.686	4.467

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Figure 1: Structure of Favipiravir

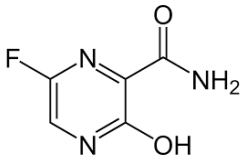

Figure 2: Absorption spectrum of Favipiravir

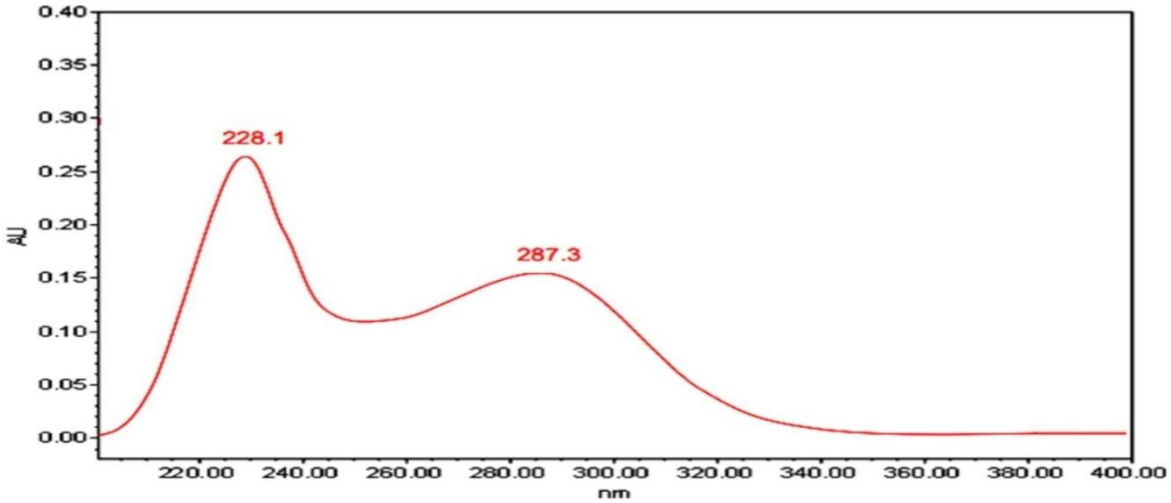

Figure 3: Optimized chromatogram

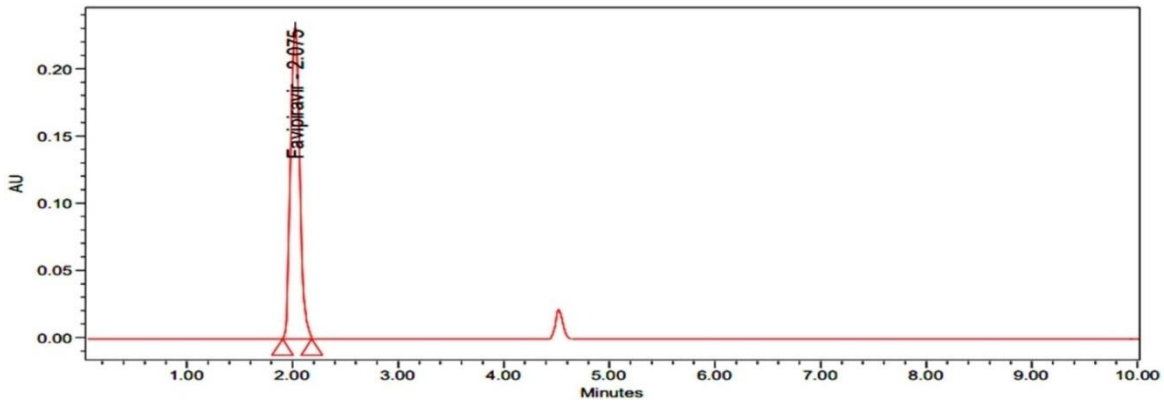

Figure 4: Chromatogram of Formulation

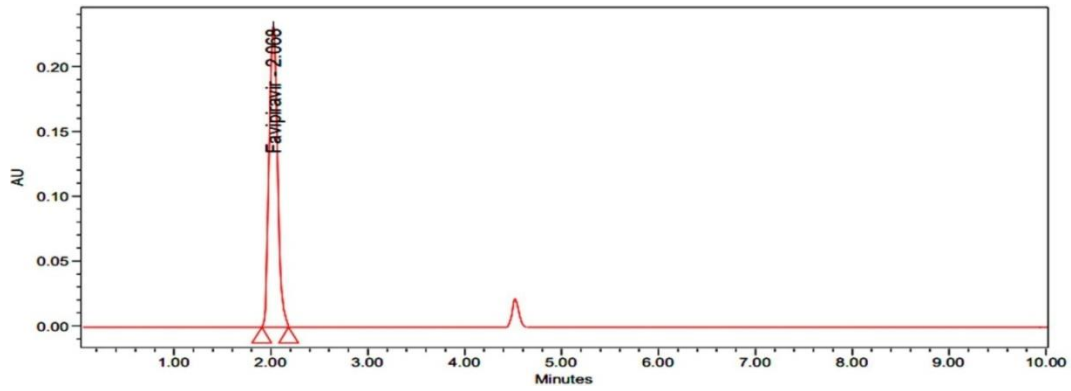

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Figure 5: Chromatogram of blank

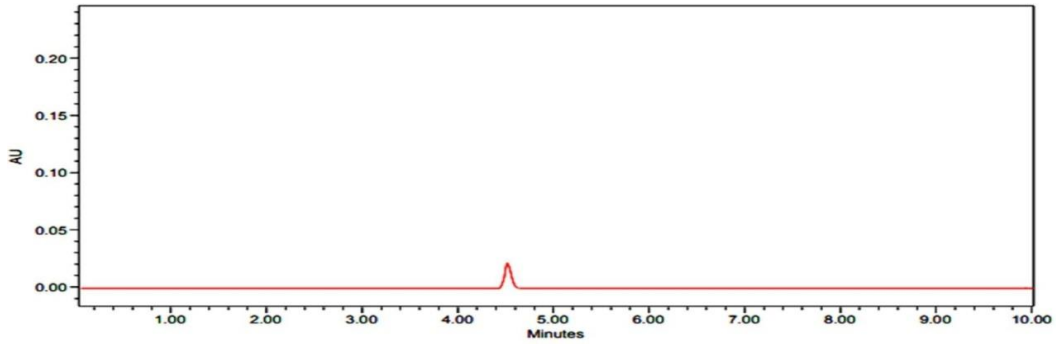

Figure 6: Chromatogram of placebo

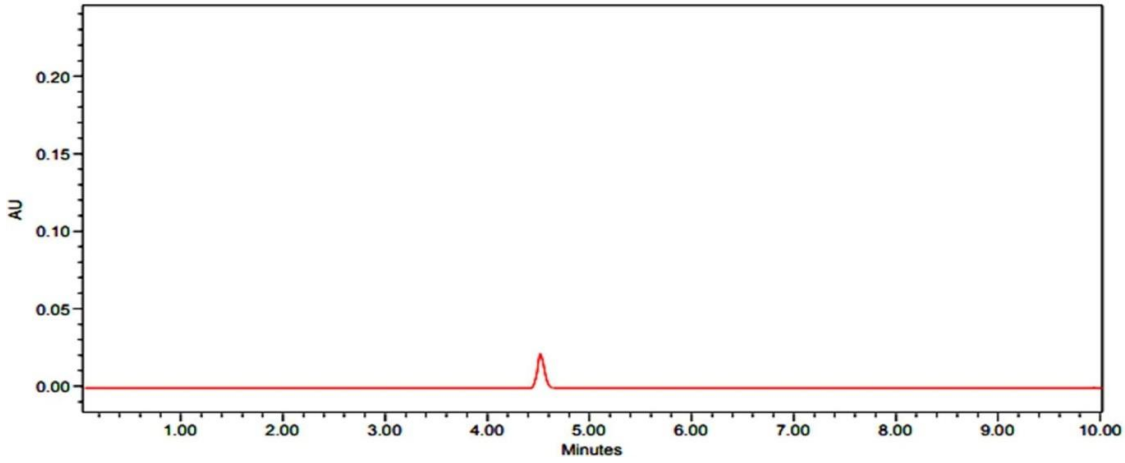

Figure 7: Chromatogram of Acid Degradation

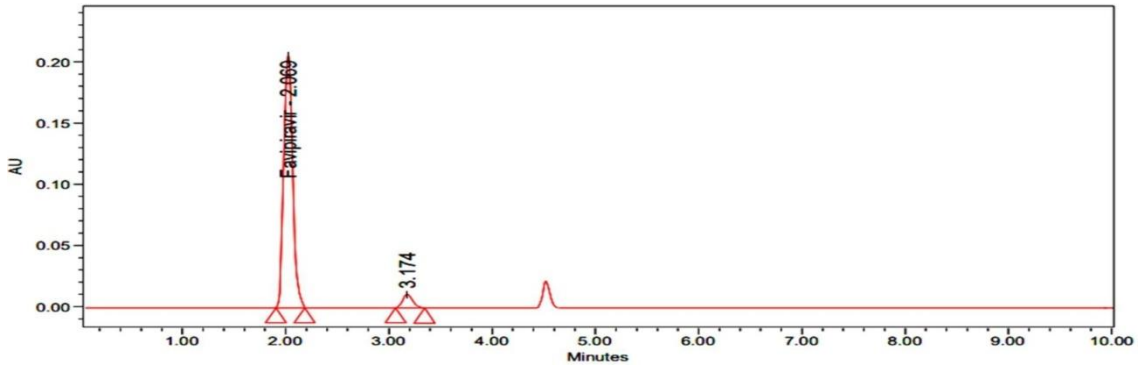

Figure 8: Purity Plot of Acid degradation

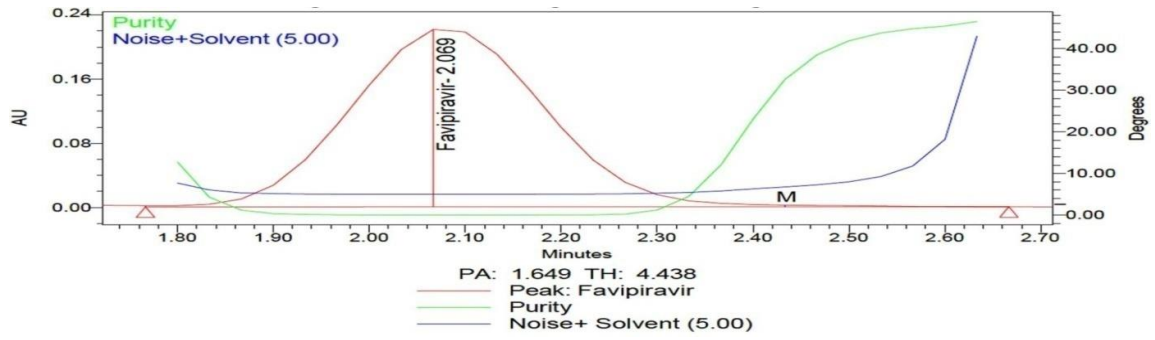

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Figure 9: Chromatogram of Alkali degradation

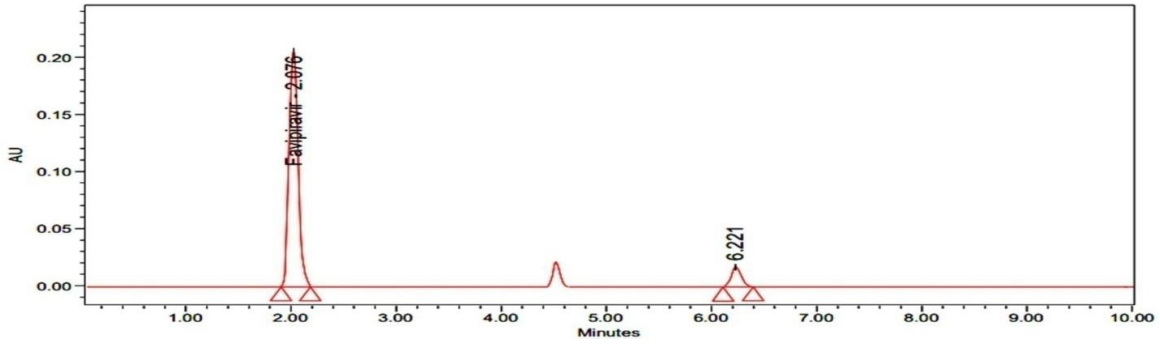

Figure 10: Purity Plot of Alkali degradation

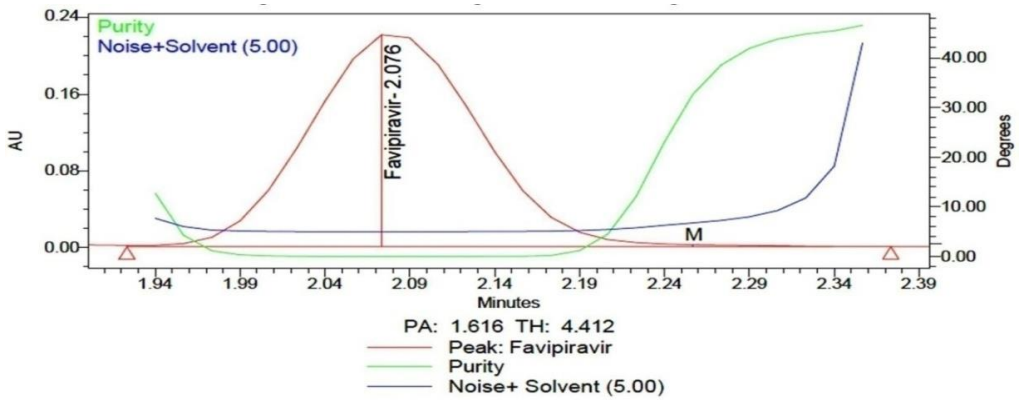

Figure 11: Chromatogram of Peroxide degradation

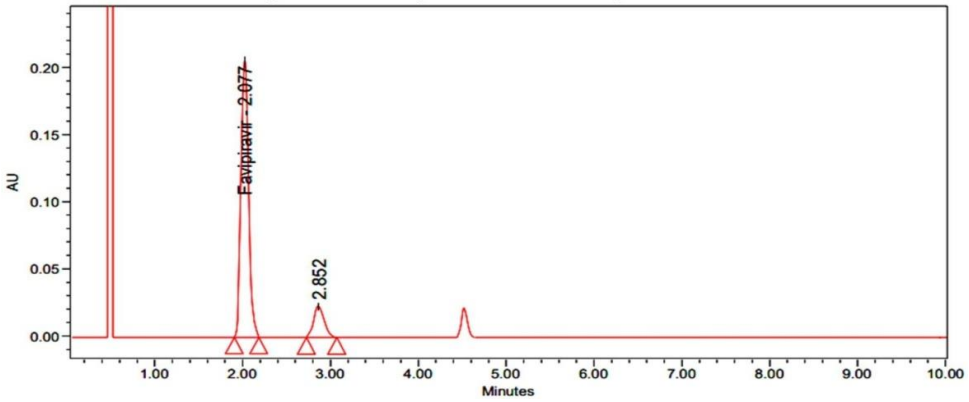

Figure 12: Purity Plot of Peroxide degradation

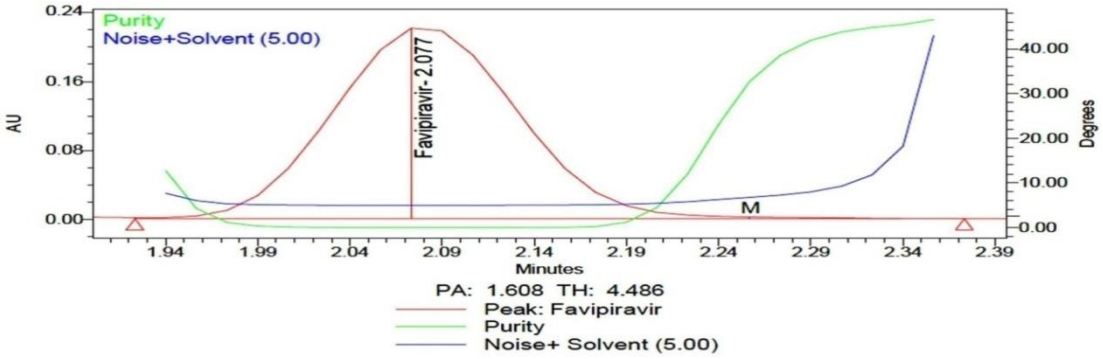

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Figure 13: Chromatogram of Reduction degradation

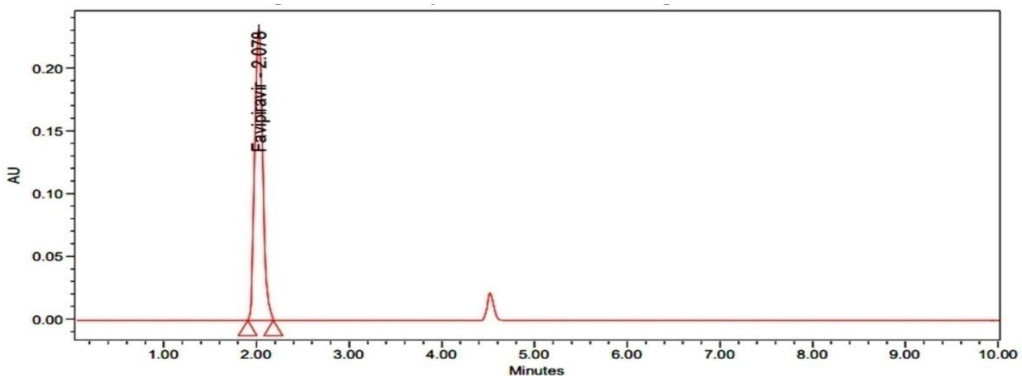

Figure 14: Purity Plot of Reduction degradation

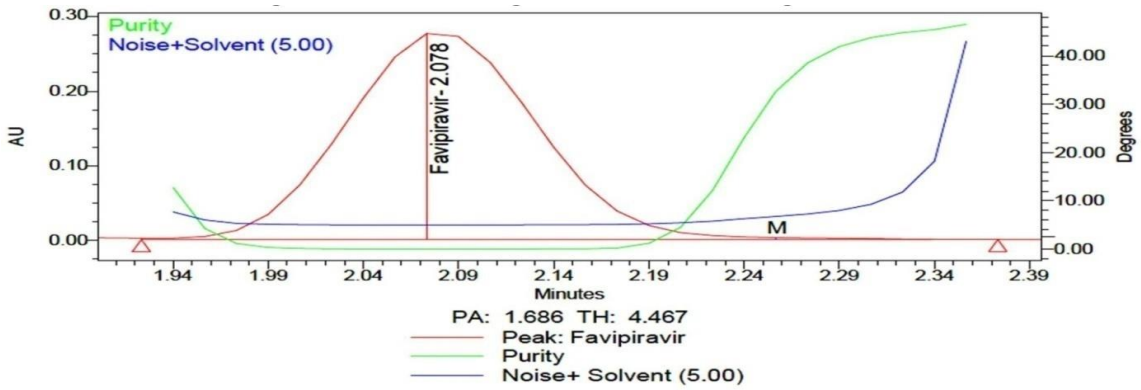

Figure 15: Chromatogram of Hydrolysis degradation

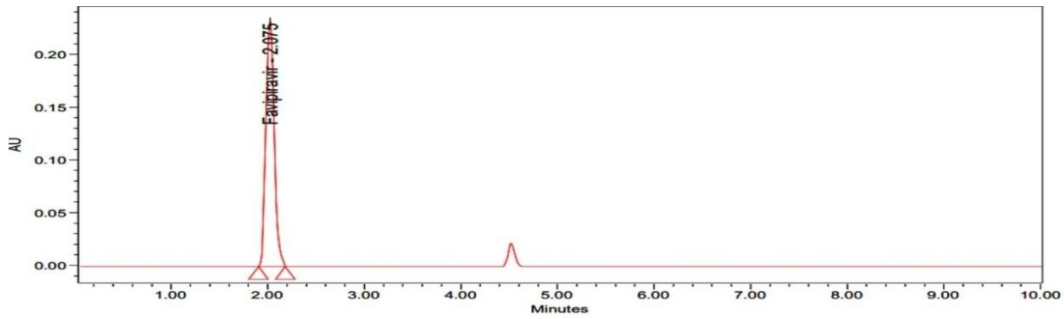

Figure 16: Purity Plot of Hydrolysis degradation

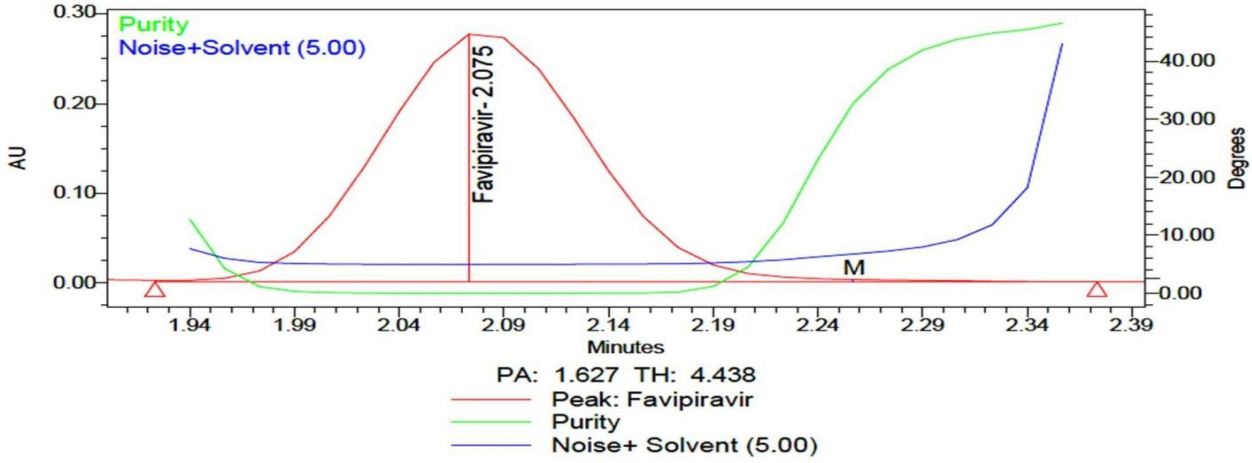

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Figure 17: Chromatogram of Photolytic degradation

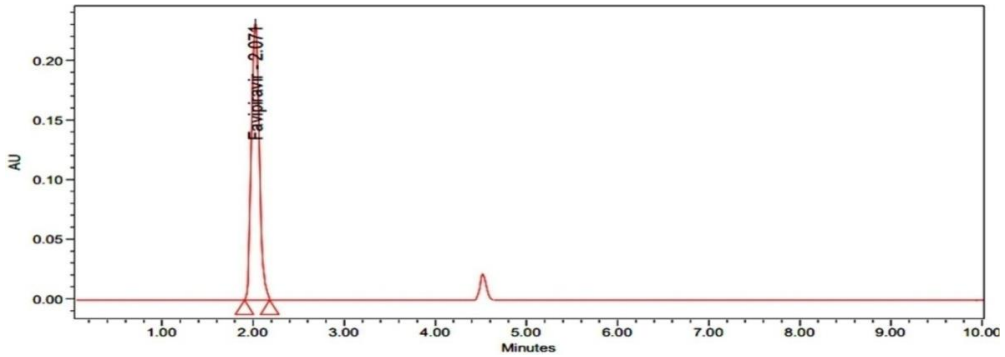


Figure 18: Purity Plot of Photolytic degradation

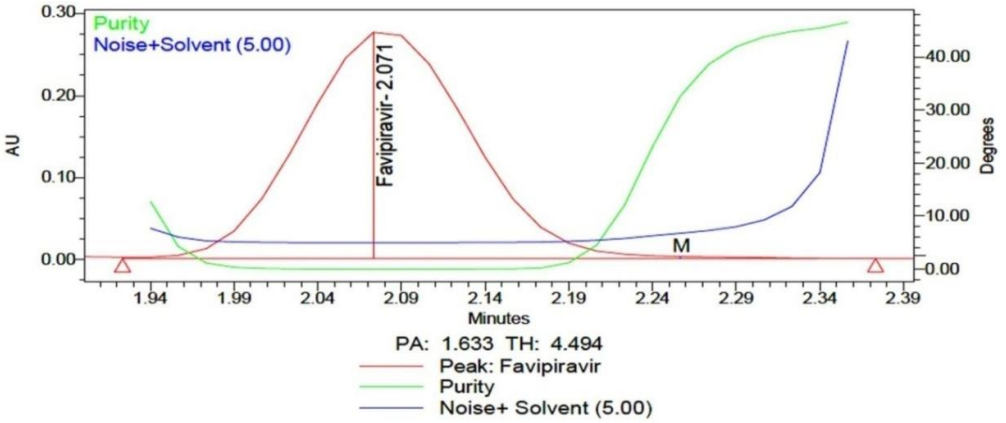


Figure 19: Chromatogram of Thermal degradation

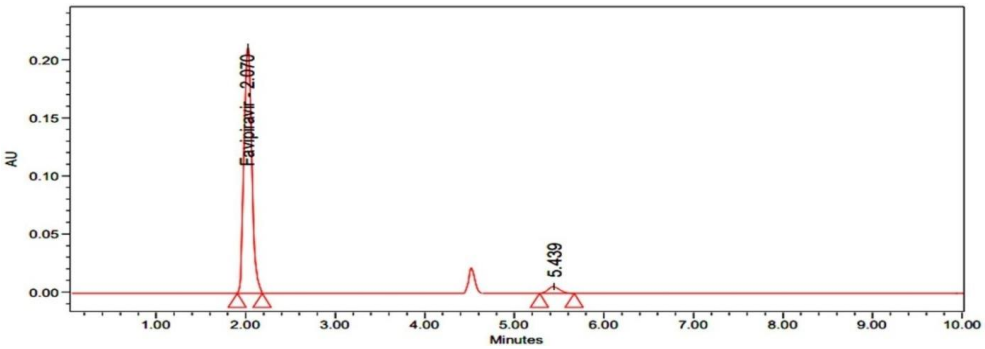
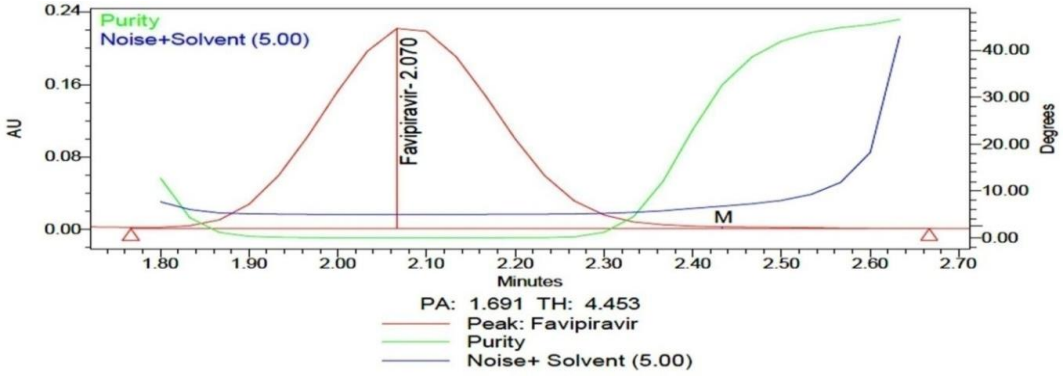


Figure 20: Purity Plot of Thermal degradation



CONCLUSION

The modern analyst is driven to create innovative, environmentally sustainable, and green analytical processes that decrease pretreatment stages, sample preparation, energy consumption, and solvent consumption, and replace toxic and hazardous solvents with safer alternatives.

The technique developed demonstrated a highly effective green analytical process for the estimation of Favipiravir and its degradation studies by reducing the usage of organic solvents.

For the measurement of Favipiravir, HPLC method using green solvents was devised that was easy to use, quick, accurate, precise, reliable, and affordable. The solvents and mobile phase are inexpensive, easy to prepare, dependable, sensitive, and require little time.

The sample recoveries indicated that formulation excipients did not interfere with the estimation, and they were in good agreement with the claims made on the labels. It can be concluded that the short and straightforward suggested methods are the most beneficial for analytical purposes.

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