An Environmentally Friendly Analytical Method for the Determination of Favipiravir Content in Drug Products

MS. P. S. SINGH[1*], DR. R.S. LOKHANDE[2], DR. D.A. KOCHREKAR[3]

[1,2] School of Basic Sciences, Department of Chemistry, Jaipur National University,
[3]FDC Limited, Mumbai

ABSTRACT:

A simple, cost-effective, stability-indicating, rapid method has been developed for the estimation of Favipiravir (FVR) in FVR drug products. The method developed is specific, precise, accurate, and linear. The analyte was quantified using an octadecylsilane HPLC column. The chromatographic conditions employed peak detection at 365 nm. The mobile phase used was Water: Methanol: Ethanol in the ratio of 60:20:20. The pH was adjusted to 3.0 to achieve better peak symmetry. The linear relationship between the peak area response and the concentration of the analyte was achieved from 20% to 150% of the concentration range with a correlation greater than 0.999. The method was found to be specific and stability-indicating. The analyte was subjected to stress conditions such as Acid hydrolysis, Base hydrolysis, Oxidation, UV light, and thermal degradation. The accuracy and precision of the method were established. The method was found to be accurate across the working range with recovery above 98.0%. The method precision and Robustness were demonstrated presenting a %RSD of NMT 2.0. The method stands validated as per the requirements of current ICH guidelines and can be used for routine analysis in Quality control laboratories. The method developed showed a greater greenness as compared to the official methods in use.

KEYWORDS:

Environmentally friendly method, Favipiravir (FVR), Reverse phase chromatography, Stability-indicating, Analytical Greenness measurement.

DOI: 10.48047/ecb/2022.11.10.31

INTRODUCTION:

Favipiravir drug (FVR) is an antiviral drug that is an analog of pyrazine (Fig. 1). This drug was developed initially for the treatment of Influenza by Toyama Chemical (Monika Sangani., Vol.4/Issue5/2022) [1]. It exhibits antiviral activity against several strains of viruses. It selectively inhibits the RNS polymerase of RNA viruses, thereby preventing the reproduction of the virus.

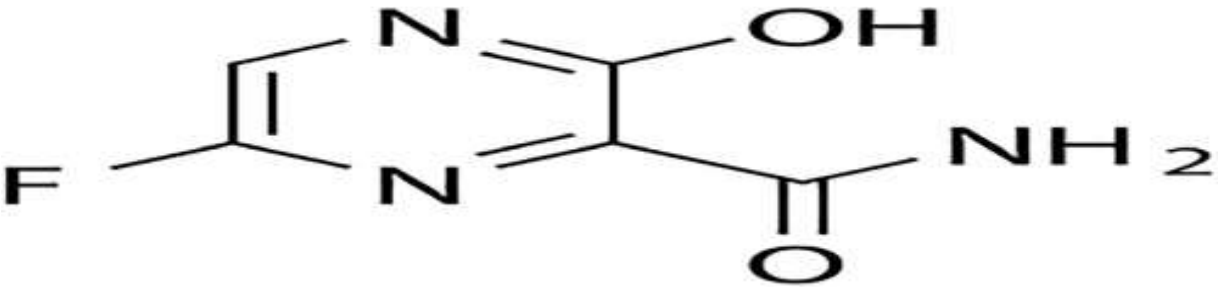

Figure 1: Structure of Favipiravir (FVR)

Favipiravir is an antiviral drug that was originally developed to treat influenza. It works by inhibiting the RNA-dependent RNA polymerase (RdRp) enzyme, which is essential for the replication of RNA viruses. Favipiravir has been shown to be effective against a variety of RNA viruses, including SARS-CoV-2, the virus that causes COVID-19. (A. Shannon, 2020) [2]

In clinical trials, favipiravir has been shown to be effective in reducing the severity of COVID-19 and shortening the duration of illness. It has also been shown to be safe and well-tolerated by most patients.

Favipiravir is currently approved for use in Japan, Russia, and India for the treatment of COVID-19. It is also being evaluated in clinical trials in other countries, including the United States.

Favipiravir is a promising repurposed drug for the treatment of COVID-19. It is an oral medication that is easy to administer, and it has been shown to be effective in reducing the severity of illness and shortening the duration of infection. More research is needed to confirm the long-term safety and efficacy of favipiravir, but it is a promising option for the treatment of COVID-19.

Here are some additional details about favipiravir:

- It is a prodrug, which means that it is converted into its active form in the body.
- It has a wide therapeutic index, which means that there is a large difference between the dose that is effective and the dose that is toxic.
- It has been shown to be effective against a variety of RNA viruses, including SARS-CoV-2, the virus that causes COVID-19.
- It is currently approved for use in Japan, Russia, and India for the treatment of COVID-19.
- It is also being evaluated in clinical trials in other countries, including the United States.

Several analytical methods have been developed for detecting and estimating Favipiravir in drug formulations. The official reported methods as well as the other methods developed make use of strong solvents and chemicals which have an impact on the environment. The current proposed method is greener and sustainable, with an acceptable analytical greenness factor. (Agnieszka Gałuszka, Piotr Konieczka, Zdzisław M. Migaszewski, Jacek Namies´nik, 2012) [3]. The application of Green analytical principles will render the method safe for use and superior to the available analytical methods for testing of the dosage form.

MATERIALS AND METHODS:

Chemicals

Analytical-grade chemicals were used for the method studies. Ortho-phosphoric acid (≥85%, Sigma–Aldrich), HPLC-grade Ethanol (≥99.9%, Sigma–Aldrich), and HPLC-grade Methanol (≥99.9%, Sigma–Aldrich) were used. Deionized water from the Milli-Q system (Millipore) with conductivity lower than 18.2 µs was used. FVR tablets (Fabiflu, 200 mg) from Glenmark Pharmaceuticals Ltd. were used for the study.

Stock solution

Weighed and transferred about 25 mg of the standard into a 50 ml volumetric flask, added enough mobile phase, and sonicated the solution to dissolve. Diluted to volume with the mobile phase. Further transferred the solution of the above flask to volume to achieve the solution of concentration of 200 µg/ml.

Sample solution

Transferred ten FVR tablets into a mortar pestle and crushed to a fine powder. Dispersed powdered tablets containing FVR equivalent to 200 mg into a 100 ml volumetric flask. Added enough diluent and sonicated for about 30 minutes with intermittent shaking. Dilute to volume with the mobile phase. Centrifuge the sample to obtain a clear supernatant solution. Further transferred 5 ml of the supernatant solution to a 50 ml volumetric flask and dilute to volume with the mobile phase.

Chromatographic conditions:

Chromatographic analysis was performed on a column of Inertsil C18 HPLC column (4.6 mm x 250 mm, 5 µm). The mobile phase consisted of Water (pH 3) and Ethanol and Methanol (60:20:20 v/v). The mobile phase was filtered and degassed through a 0.45 µm membrane filter before use. The mobile phase was pumped at a flow rate of 1.0 mL per minute. The column temperature was set at 40ºC. The run time was 8 min under these conditions. The detection wavelength was set at 365 nm for peak detection and quantitation.

METHOD DEVELOPMENT AND OPTIMIZATION:

HPLC method development can be iterative and requires several adjustments to successfully optimize the separation of analytes. Good record-keeping and systematic approaches are essential throughout the process to ensure the development of a robust and reproducible method.

High-Performance Liquid Chromatography (HPLC) method development is a systematic process that involves optimizing conditions to separate, detect, and quantify analytes in a sample. Below are the steps considered during the method development of the proposed method.

Favipiravir is a relatively new drug, and there is still a need to develop sensitive and specific analytical methods for its quantification. HPLC is a well-established technique for the analysis of drugs, and it is particularly well-suited for the analysis of favipiravir because it is a relatively polar molecule. Several methods are developed for the estimation of FVR using HPC methods using organic solvents. (Monika Sangani., Vol.4/Issue5/2022), (Bulduk, September 2020)[1,4]

Green analytical methods are becoming increasingly important as there is growing concern about the environmental impact of traditional analytical methods. HPLC is a relatively green technique, but it can be made even greener by using environmentally friendly solvents and mobile phases.

The development of a green HPLC method for favipiravir would be a valuable contribution to the field of analytical chemistry. It would provide a sensitive and specific method for the quantification of favipiravir, and it would also be a more environmentally friendly alternative to traditional HPLC methods. (Anastas, 1998) [5]

Here are some specific advantages of a green HPLC method for favipiravir:

It would use fewer toxic solvents and mobile phases, which would reduce the environmental impact of the analysis. (Armenta, 2008) [6]

It would use less energy, which would also reduce the environmental impact.

It would be more cost-effective, as the green solvents and mobile phases are often less expensive than traditional solvents.

The development of a green HPLC method for favipiravir is a worthwhile endeavor that would have several benefits. It would provide a sensitive and specific method for the quantification of favipiravir, it would be more environmentally friendly, and it would be more cost-effective.

RESULTS AND DISCUSSION

As opposed to treating the effects of toxic chemicals, the primary goal of the research is to apply the principles of green analytical chemistry by preventing the production of those substances. The biggest challenge is finding solvents that can replace the dangerous chemicals used in conventional analytical techniques while maintaining the proposed technique's effectiveness. It is advised to replace hazardous solvents with environmentally friendly substitutes since the selection of solvents is a crucial step in the development of an analytical method [6]. (Armenta et al. 2008).

The mobile phase's composition and various components were tested at various flow rates in the proposed HPLC method. Water, ethanol, and methanol were present in varying proportions (10

to 90%). Water (pH3): Methanol: Ethanol (60:20:20, v/v/v) at a flow rate of 1 mL/min produced the best separation. To achieve good sensitivity, the detection was carried out at 365 nm.

Method validation

The analytical method validation has been carried out in line with the ICH guidelines of Validation of Analytical Procedure: Q2 (R1) (ICH harmonized Tripartite guideline on Validation of Analytical Procedures:Text and Methodology Q2(R1), 1994) [7]. The parameters validated were addressed: specificity, system suitability, linearity, Limit of quantification (LOQ), accuracy, precision, and robustness (U.S. Food and Drug Administration, 2022) [8]. The stability indicating the nature of the method was assessed by performing the forced degradation studies.

Specificity/selectivity

The specificity of the analytical method was assessed by verifying for the interference from the matrix attributes such as blank, and placebo solutions. The selectivity was tested by comparing the chromatograms obtained with FVR standard to blank and placebo solutions at a specified wavelength of 365nm.

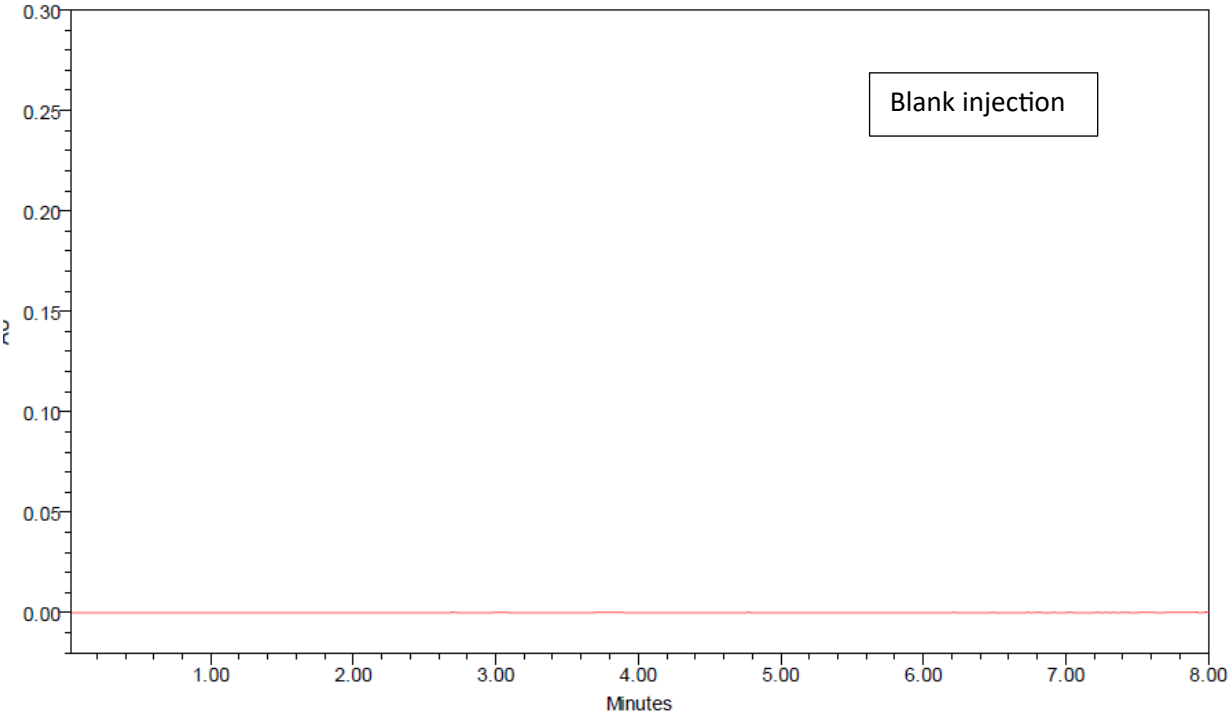

Figure 2: HPLC CHROMATOGRAM OF BLANK INJECTION

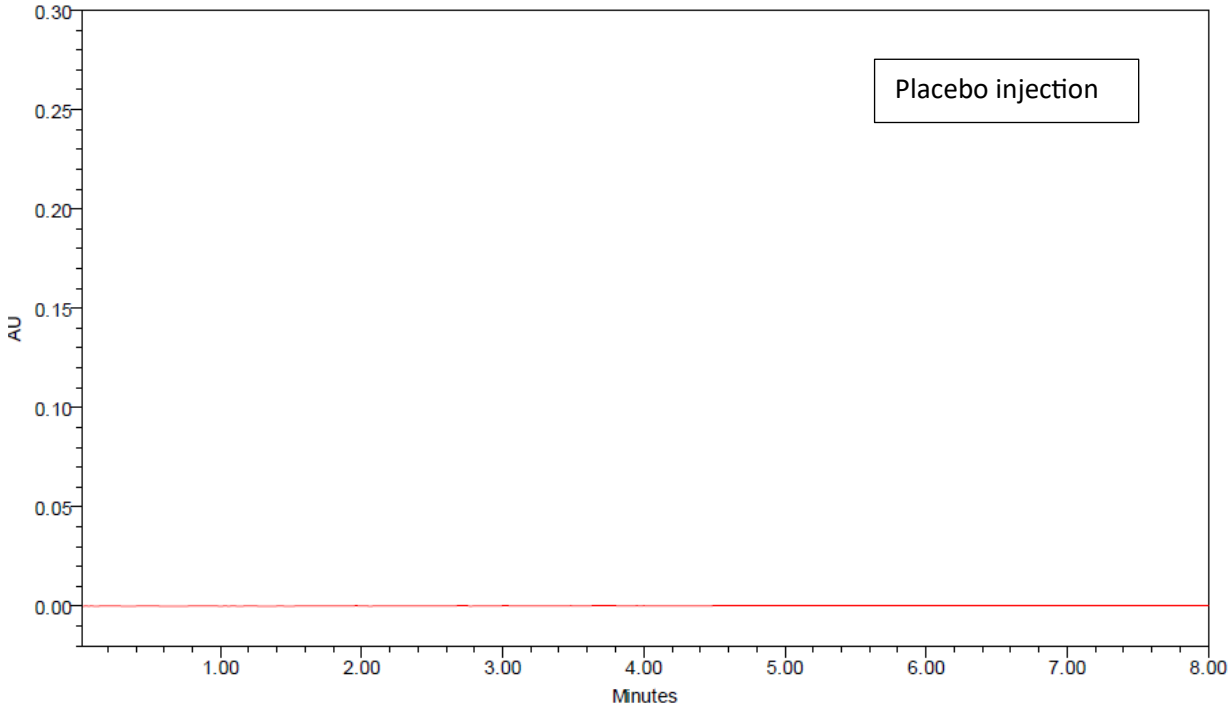

Figure 3: HPLC CHROMATOGRAM OF PLACEBO INJECTION

System suitability:

The suitability of the method was demonstrated by injecting five replicate standard solutions of FVR at a concentration of 200 µg/ml into the chromatograph. The peak area response and retention time were achieved acceptable to determine and quantitate the amount of FVR in the drug product formulations. The % RSD for peak area response was found to be 0.21% demonstrating good system precision. The theoretical plates were found to be greater than 2000 and the tailing factor was found to be less than 2.0.

Sr. No.	Standard Area
1	2259724
2	2254926
3	2260045
4	2267613
5	2264217
Mean	**2261305**
StdDev	**4823.11**
RSD	**0.21**

Table 1: SYSTEM SUITABILITY MEASUREMENT

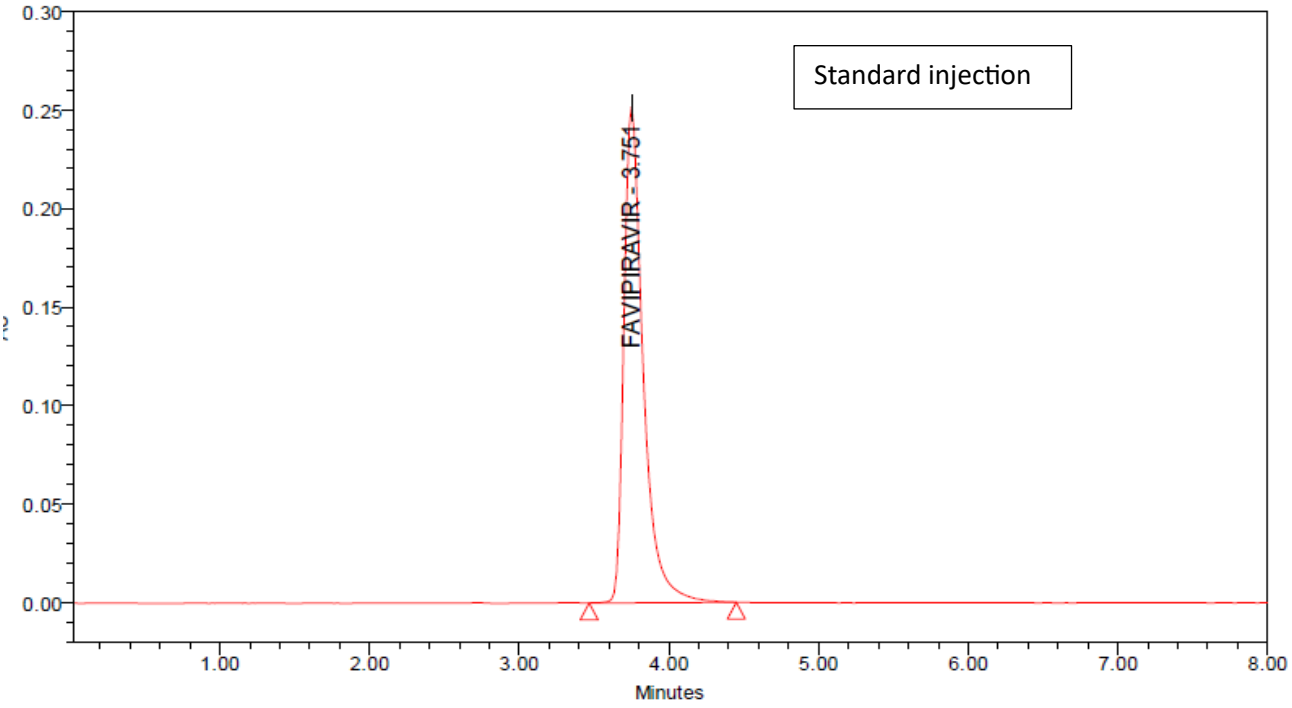

Figure 4: HPLC CHROMATOGRAM OF STANDARD INJECTION (FVR)

Linearity and Range

The linearity of the proposed method was established from 20% to 150% of the working range of 200µg/ml. The linearity solution was prepared at five different concentration levels. Each level was injected into the chromatographic system and the peak area response was recorded.

The correlation coefficient was determined by plotting the concentration versus the peak area response for the drug. The solution was prepared as below:

Sample details	Stock Taken (mL)	Final dilution	Conc (ppm)	Mean Conc (ppm)	Area	Mean	%RSD
Level-1 20%	1	10	50.14	50.14	551437	552721	0.23
	1.0	10	50.14		554027		
	1.0	10	50.14		552699		
Level- 2 50%	2.0	10	100.28	100.28	1119248	1121898	0.24
	2.0	10	100.28		1124593		
	2.0	10	100.28		1121854		
Level-3 80%	3.0	10	150.42	150.42	1666716	1667720	0.15
	3.0	10	150.42		1670644		
	3.0	10	150.42		1665799		
Level-4 100%	4.0	10	200.56	200.56	2271723	2266277	0.21
	4.0	10	200.56		2262542		
	4.0	10	200.56		2264567		
Level-5 120%	5.0	10	250.7	250.7	2813271	2807568	0.18

	5.0	10	250.7		2805692		
	5.0	10	250.7		2803741		
Level-6 150%	6.0	10	300.84	300.84	3375619	3363766	0.35
	6.0	10	300.84		3363600		
	6.0	10	300.84		3352078		

Table 2: LINEARITY LEVEL PREPARATIONS AND PEAK AREA OBTAINED.

The below plot represents the Linearity of FVR-peak area response versus concentration.

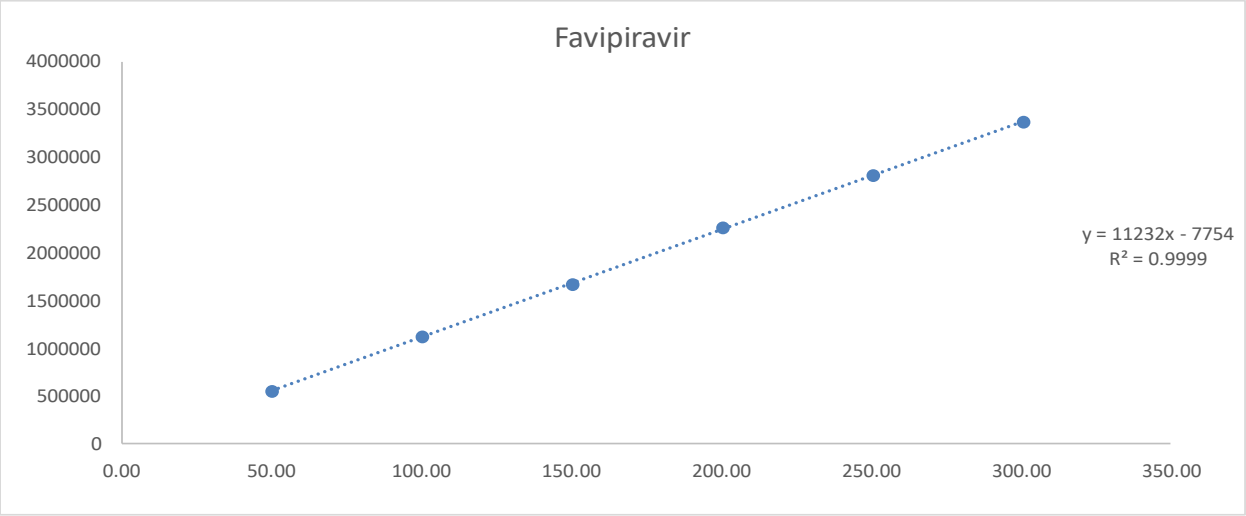

Figure 5: LINEARITY GRAPH REPRESENTING CORRELATION CO-EFFICIENT. THE X-AXIS REPRESENTS THE CONC. IN µG/ML AND THE Y-AXIS REPRESENTS THE PEAK AREA RESPONSE FOR FVR.

From the data used for demonstrating linearity, a good co-efficient correlation was achieved;

Correlation coefficient	0.9999
Regression coefficient	0.99987
Slope	11231.85937
Y Intercept	-7754.00
%Y Intercept (Bias)	-0.4649

Table 3: CORRELATION COEFFICIENT

Precision

The precision study was performed on the FVR tablets. Six sample preparations were prepared and injected into the chromatograph. The method was found to be precise with % RSD for the peak analyte obtained to be 0.2% which is within the acceptance criteria of not more than 2.0%.

Sample no.	Sample Wt. (mg)	Mean Area	% Assay
Sample 1	262.28	2217251	99.2
Sample 2	260.47	2199341	99.1
Sample 3	262.38	2224978	99.5
Sample 4	261.35	2213479	99.4
Sample 5	260.78	2206245	99.3
Sample 6	261.57	2205551	99
		StdDev	**0.2**
		RSD	**0.2**

Table 4: METHOD PRECISION

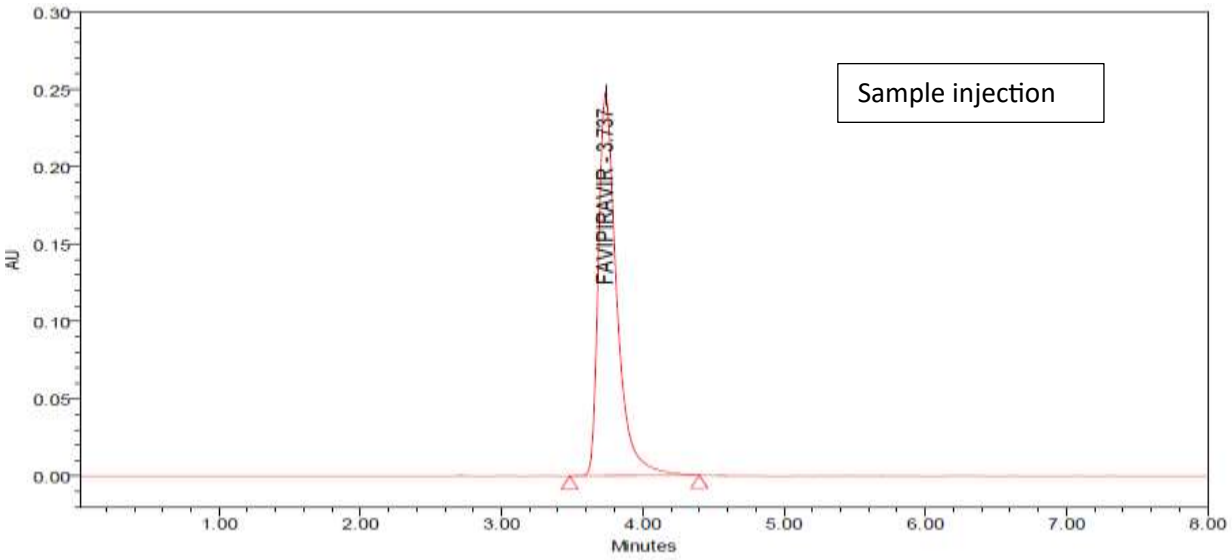

Figure 6: HPLC CHROMATOGRAM OF SAMPLE SOLUTION INJECTION- FVR TABLETS

Accuracy

The accuracy of the proposed method was conducted by the standard addition method. The standard solution across the range was spiked in the placebo solution and the accuracy of the method was assessed. Three levels of 50, 100, and 120% were studied. The % Recovery at each level was calculated at each level concentration.

Sample	Standard in ml	Final dilution	Amount added (µg/ml)	Area	Amount recovered (µg/ml)	% Recovery	Mean Recovery	SD	% RSD
50% / Prep – 1	2.0	10	100.28	1120053	99.42	99.14	99.01	0.28	0.28
50% / Prep – 2	2.0	10	100.28	1114939	98.97	98.69			
50% / Prep – 3	2.0	10	100.28	1120779	99.49	99.21			

Sample	Standard in ml	Final dilution	Amount added (µg/ml)	Area	Amount recovered (µg/ml)	% Recovery	Mean Recovery	SD	% RSD
100.0 % / Prep – 1	4.0	10	200.56	2257698	200.41	99.92	99.74	0.42	0.42
100.0 % / Prep – 2	4.0	10	200.56	2242832	199.09	99.26			
100.0 % / Prep – 3	4.0	10	200.56	2260269	200.63	100.04			
150.0 % / Prep – 1	6.0	10	300.84	3380812	300.1	99.75	99.53	0.19	0.19
150.0 % / Prep – 2	6.0	10	300.84	3369961	299.14	99.43			
150.0 % / Prep – 3	6.0	10	300.84	3368994	299.05	99.4			

Table 5: RECOVERY OF FVR FROM MATRIX

Robustness:

The robustness of the method provides confidence that the method will perform as intended for use under minor permitted variations and conditions. These variations analyze the impact due to minor variations in the chromatographic conditions and the preparations involved. The robustness of the proposed method was studied by making small deliberate changes to the flow rate (±0.2 ml/min), Change to the pH of the mobile phase (±0.2 units), and change in the column oven temperature (±0.2ºC). For each deliberate change, the system suitability runs were injected and the outcome of the Assay results for each condition was compared with the method proposed for the active ingredient, original method parameters.

Parameter	Variation	Mean Area	% Assay
Increase in Flow rate	1.2 ml/min	1857362	99.7%
Decrease in Flow rate	0.8 ml/min	2783474	99.8%
Increase in Column oven temperature	42ºC	2234402	99.2%
Decrease in Column oven temperature	38ºC	2216767	99.5
Increase in pH of mobile phase- 3.2	3.2	2244103	99.5
Decrease in pH of mobile phase- 2.8	2.8	2238583	99.3

Table 6: ASSAY OF ROBUSTNESS RESULTS- DELIBERATE CHANGES TO METHOD PARAMETERS

Flow rate 1.2 ml/min

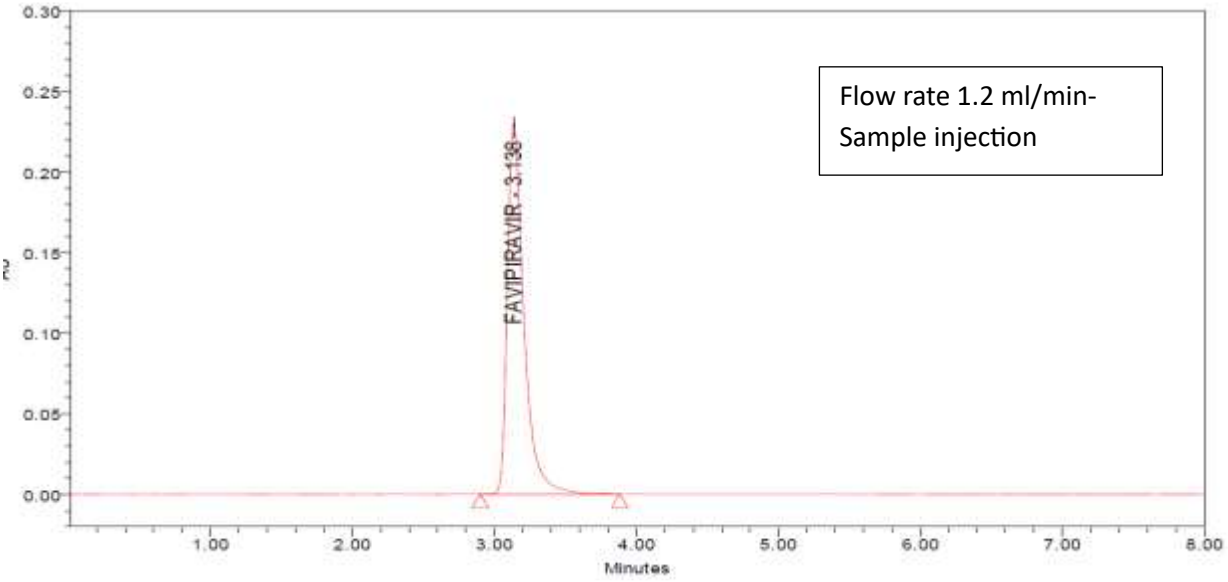

Figure 7: HPLC CHROMATOGRAM OF SAMPLE SOLUTION- FVR TABLETS AT ROBUSTNESS- 1.2 ML/MIN

Flow rate 0.8 ml/min

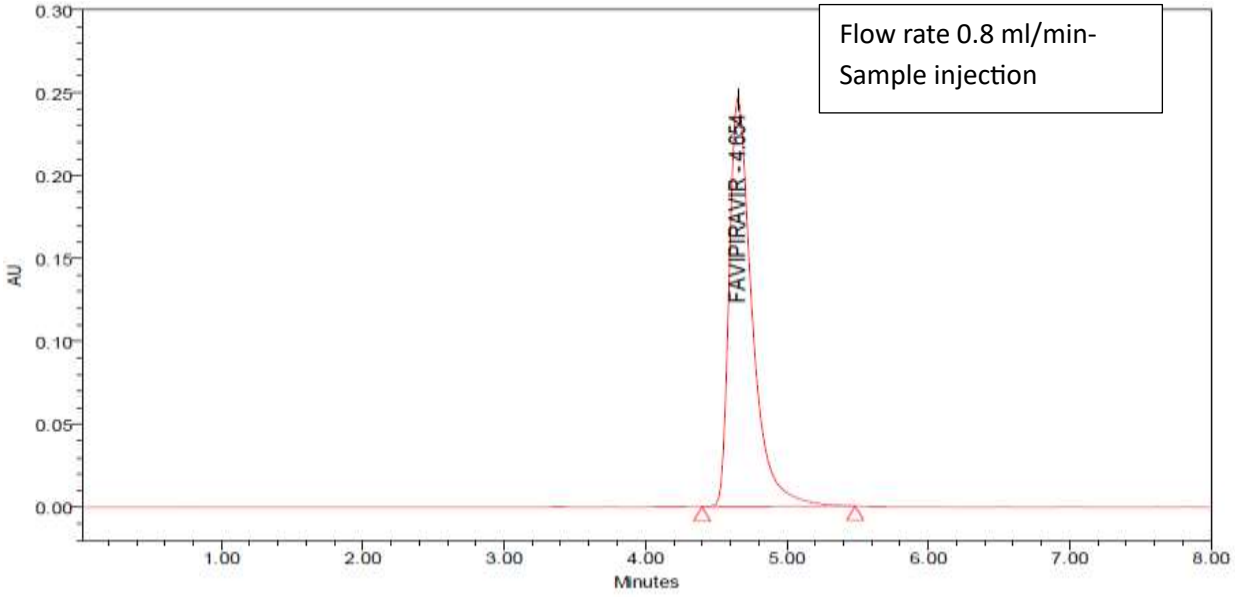

Figure 8: HPLC CHROMATOGRAM OF SAMPLE SOLUTION- FVR TABLETS AT ROBUSTNESS- 0.8 ML/MIN

Column oven temperature 42ºC

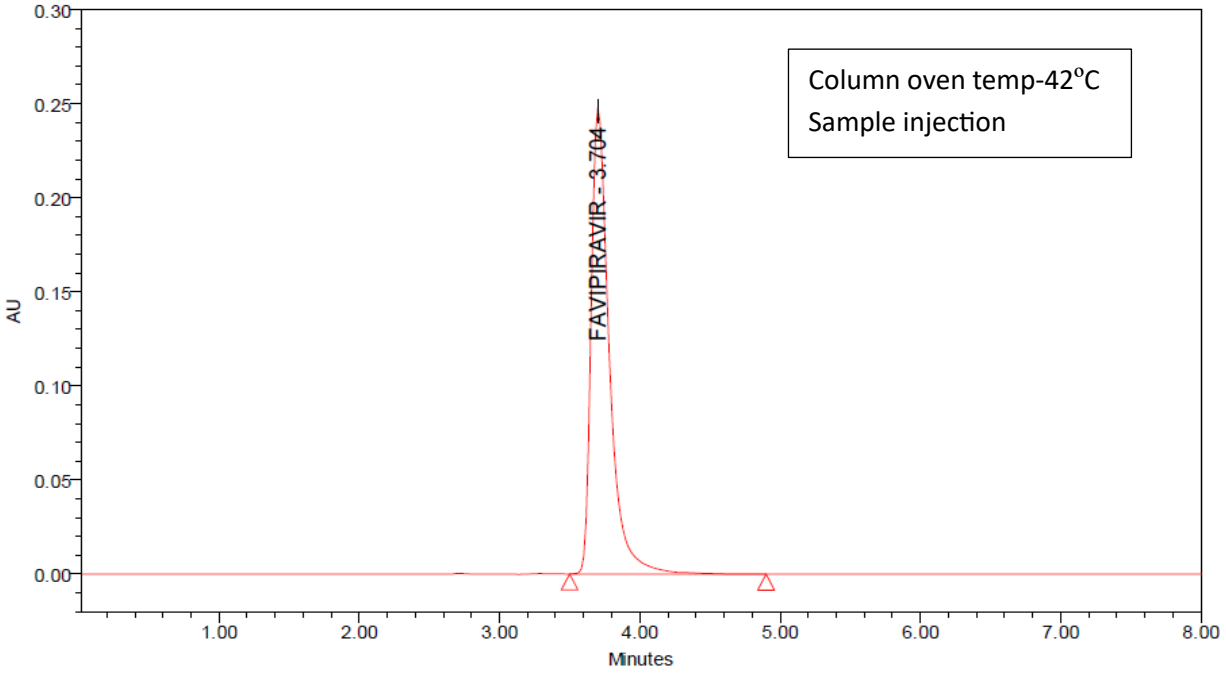

Figure 9: HPLC CHROMATOGRAM OF SAMPLE SOLUTION- FVR TABLETS AT ROBUSTNESS- 42ºC

Column oven temperature 38ºC

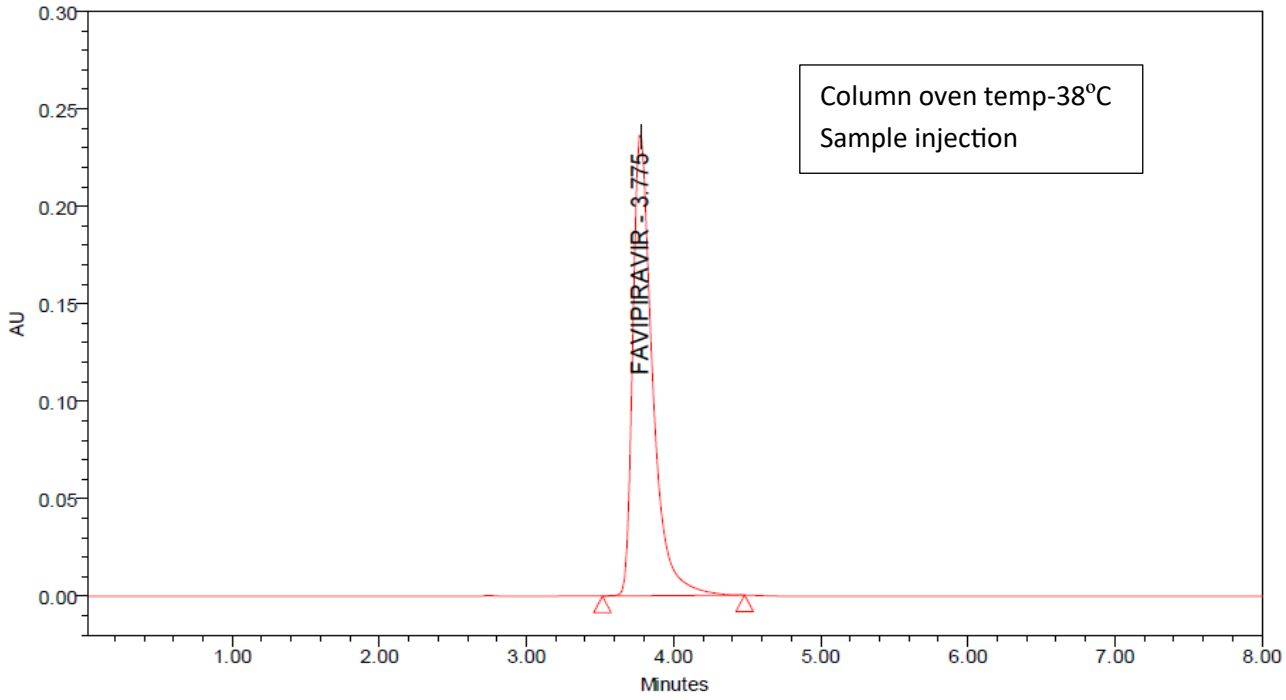

Figure 10: HPLC CHROMATOGRAM OF SAMPLE SOLUTION- FVR TABLETS AT ROBUSTNESS- 38ºC

pH of mobile phase-3.2

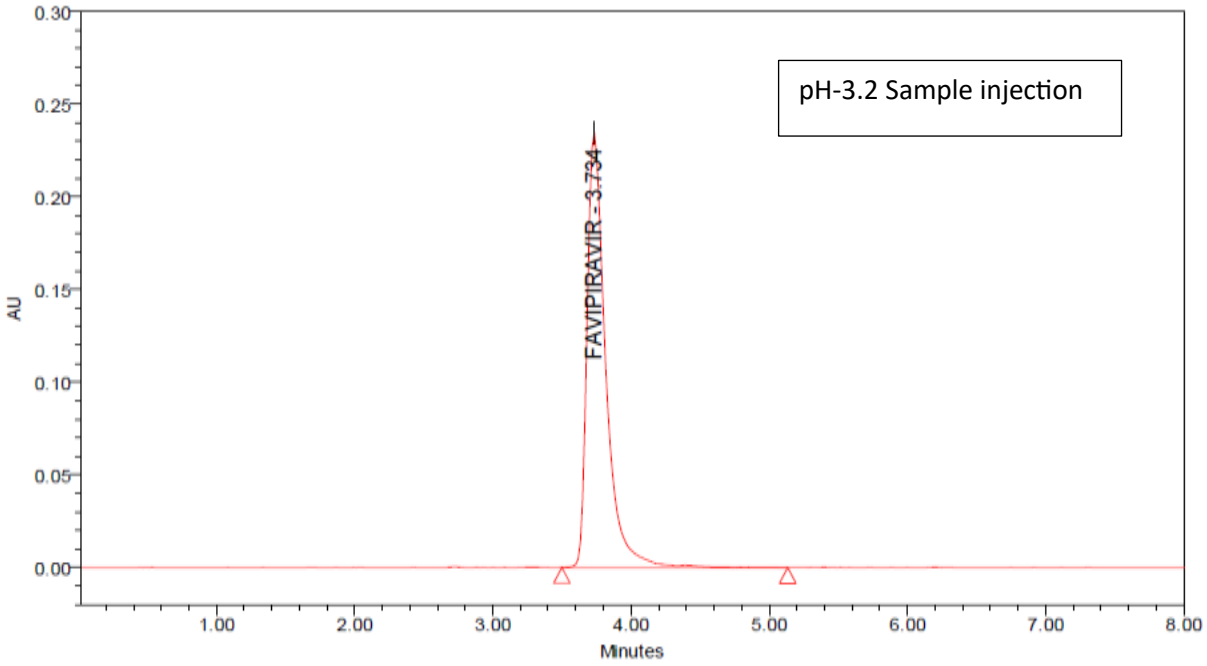

Figure 11: HPLC CHROMATOGRAM OF SAMPLE SOLUTION- FVR TABLETS AT ROBUSTNESS- PH 3.2

pH of mobile phase 2.8

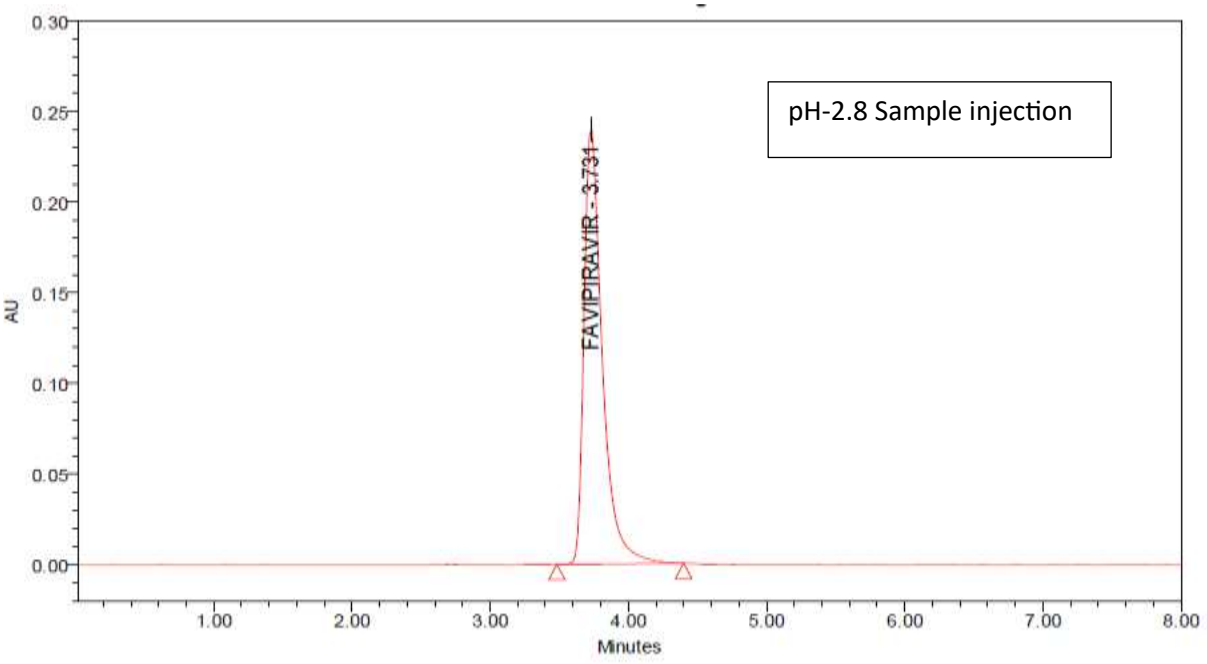

Figure 12: HPLC CHROMATOGRAM OF SAMPLE SOLUTION- FVR TABLETS AT ROBUSTNESS- PH 2.8

Solution stability

The sample solution was prepared by the proposed method of analysis and injected into the HPLC system at pre-determined time intervals to check the solution stability at ambient conditions. The sample solution stability achieved at room temperature is as reported below:

Test: Solution stability of FVR			
Sample	**Mean Area**	**% Assay**	**% change w.r.t initial**
Initial	2247491	99.3	-
6hrs	2230932	98.6	**0.7**
12hrs	2237490	98.9	**0.4**
24hrs	2195196	97.0	**2.3**

Table 7: SOLUTION STABILITY – ASSAY RESULTS

Stability indicating the nature of the method:

Forced degradation or stress study was conducted to demonstrate the stability indicating the nature of the method under development. Forced degradation study enables the identification of the compounds that could be detected as possible degradants in the drug formulation. The sample was subjected to stress conditions like Acid hydrolysis, Base hydrolysis, Oxidation, UV light, and Dry heat. (U.S. Food and Drug Administration, 1996) [9]. The exposed samples were injected into the HPLC system, and the degradation was calculated. There was no interference observed at the retention time of the principal peak in the chromatogram to any of the exposed conditions.

Forced degradation studies					
Sample	Area – 1	Area – 2	Mean Area	% Assay	% Degradation
Control sample	2213418	2221083	2217251	99.2	-
UV light	2245819	2277203	2261511	99.4	Nil
Acid hydrolysis	2202995	2096638	2149817	95.4	3.8
Base Hydrolysis	2294348	2278289	2286319	99.8	Nil
Oxidation	2238405	2240995	2239700	99.6	Nil
Thermal degradation	2320196	2240820	2280508	98.6	0.6

Table 8: FORCED DEGRADATION STUDIES

MEASUREMENT OF GREENNESS FACTOR:

The analytical greenness metric approach is a tool to measure the greenness factor an analytical method. The Analytical Greenness calculator is a thorough, adaptable, and simple assessment method that yields an understandable and instructive result. (Francisco Pena-Pereira, 2020) [10] The evaluation criteria are converted into a single 0–1 scale and taken from the 12 principles of green analytical chemistry. The principles are used to determine the final score. The outcome is a pictogram showing the final score, the analytical technique performed for each criterion, and the user-assigned weights. The method developed was calculated using the Analytical Greenness calculator and the report generated is as below:

Sr. no.	Criteria	Score	Weight
1.	Direct analytical techniques should be applied to avoid sample treatment.	0.3	1
2.	Minimal sample size and minimal number of samples are goals.	1.0	4
3.	If possible, measurements should be performed in situ.	0.66	1
4.	Integration of analytical processes and operations saves energy and reduces the use of reagents.	1.0	4
5.	Automated and miniaturized methods should be selected.	1.0	2
6.	Derivatization should be avoided by analyst	1.0	4
7.	The generation of a large volume of analytical waste should be avoided, and proper management of analytical waste should be provided.	0.29	1
8.	Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time.	0.38	1
9.	The use of energy should be minimized.	1.0	4
10.	Reagents obtained from renewable sources should be preferred.	0.5	2
11.	Toxic reagents should be eliminated or replaced.	1.0	4
12.	The operator's safety should be increased.	1.0	4

Table 9: ANALYTICAL GREENNESS REPORT

The overall score of 0.89 is displayed in the middle of the pictogram. The dark green values close to 1 denote that the technique being evaluated is more environmentally friendly. The color in the segment with the number corresponding to each criterion reflects the performance of the method in each of the assessment criteria. The results for the Green Analytical Chemistry principles 1, 3, 7, 8, and 10 depicted in Figure 13 are fair. However, the performance for principles 2, 4, 5, 6, 9, 11, and 12 is on the higher side of the scale.

Figure 13: Overall score

Table 10: ANALYTICAL GREENNESS SCORE

CONCLUSION:

The analytical method following the green analytical principles was developed and validated for the determination of favipiravir which was reported for activity against SARS-CoV-2 viral infection. The developed method is highly efficient and sensitive; additionally, it uses non-hazardous solvents. In order to demonstrate their negligible environmental impact, the greenness of AGREE was estimated. The techniques were successfully used to assay the dosage forms of marketed pharmaceuticals. The suggested approach is straightforward and reasonably priced, making it appropriate for use in all quality control and/or regulatory laboratories without the need for specialized handling or pricey equipment.

CONFLICTS OF INTEREST:

The authors have no conflicts of interest to declare that are relevant to the content of this article.

REFERENCES:

1. Monika Sangani., e. a. (Vol.4/Issue5/2022). An Application of Green Analytical Chemistry for RP-HPLC Method Development and Validation for Determination of Favipiravir in Bulk and Tablet Dosage Form. International Journal of PharmaO2. , 104-111.

2. A. Shannon, B. S. (2020). Favipiravir strikes the SARS-CoV-2 at its Achilles heel, the RNA polymerase. bioRxiv.

3. Agnieszka Gałuszka, Piotr Konieczka, Zdzisław M. Migaszewski, Jacek Namies´nik. (2012). .Analytical Eco-Scale for assessing the greenness of analytical procedures. Trends in Analytical Chemistry, 37, 61-72.

4. Bulduk, I. (September 2020). HPLC-UV method for quantification of favipiravir in pharmaceutical formulations. Acta Chromatographica.

5. Anastas, P. W. (1998). J.C green chemistry; theory and practice, oxford university press; New York.

6. Armenta, S. G. (2008). Green analytical chemistryTrends Anal. Chem., 27, 497-511.

7. ICH harmonized Tripartite guideline on Validation of Analytical Procedures: Text and Methodology Q2(R1). (1994). ICH guideline.

8. U.S. Food and Drug Administration. (2022). Reviewer Guidance, Validation of Chromatographic Methods.Available at: <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/reviewer-guidance-validation-chromatographic-methods> [Accessed 14.

9. U.S. Food and Drug Administration. (1996). FDA Guidance for Industry: Forced Degradation Studies for Drug Substances and Drug Products.

10. Francisco Pena-Pereira, W. W. (2020). AGREE Analytical GREEnness Metric Approach and Software. Anal. Chem. , 10076−10082.