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Research Article

An Application of Green Analytical Chemistry for RP-HPLC Method Development and Validation for Determination of Favipiravir in Bulk and Tablet Dosage Form

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ABSTRACT

An application of green analytical chemistry for RP-HPLC Method Development and Validation for determination of Favipiravir in Bulk and Tablet Dosage Form. The proposed method was validated to obtain official requirements including stability, accuracy, precision, linearity and selectivity as per ICH Guidelines. The estimation was developed on C (18) column reversed-phase using the mobile phase composition as Methanol: Water (10:90 v/v). The flow rate was set as 1mlmin^{-1}, and the maximum absorption was observed at 323 nm using Shimadzu PDA detector. The Favipiravir, drug showed a precise and good linearity at the concentration ranges of 10-50 µg/mL. The RP-HPLC assay showed the highest purity ranging from 99.90 % to 100.02 % for Favipiravir, Tablet dosage form. The Favipiravir retention time was found to be 5.00 minutes. A very quick, cost-effective, precise and accurate HPLC method for the determination of Favipiravir has been developed and validated in compliance with ICH guidelines Q2 (R1).

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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 IUPAC name 6-fluoro-3-hydroxypyrazine-2-carboxamide, Fig.1. The antiviral targets RNA-dependent RNA polymerase (RdRp) enzymes, which are necessary for the transcription and replication of viral genomes. (https://go.drugbank.com/drugs/DB12466)With over 16 million cases reported from across the globe, the SARS-CoV-2, a mere 125 microns in diameter, has left an indelible impact on our world. With the scarcity of new drugs to combat this disease, the medical community is

in a race to identify repurposed drugs that may be effective against this novel corona virus. One of the drugs which have recently garnered much attention, especially in India, is an anti-viral drug originally designed for influenza, called favipiravir (Agrawal et al, 2020).Several ongoing clinical trials will further substantiate favipiravir role. (Joshi *et al*, 2020). According to the literature search, only few high-performance liquid chromatography (HPLC) methods for determining favipiravir assay and impurities in active pharmaceutical ingredients were published (China patent (CN104914185B) 2016, China patent (CN104914185A) 2015, Buldukibrahim *et al*, 2021). Possibilities toward green LC include reducing solvent use, switching to more benign solvents and/or eliminating organic solvents (Armenta *et al*, 2008). In all of these methods, a gradient HPLC mode was used for chromatographic separation and the run time was more than 10 minutes which is not economic for routine analysis. Favipiravir is not officially available in any pharmacopoeia and there no single eco- friendly methods were developed for testing of Favipiravir in its dosage form. There is still a need for validated HPLC methods to determine Favipiravir in pharmaceutical formulations. Aim of current method development is to use eco-friendly and less hazardous solvents as a mobile phase. The proposed method was very fast, quick and accurate in terms of the chromatographic retention time and run time compared with other reported methods.

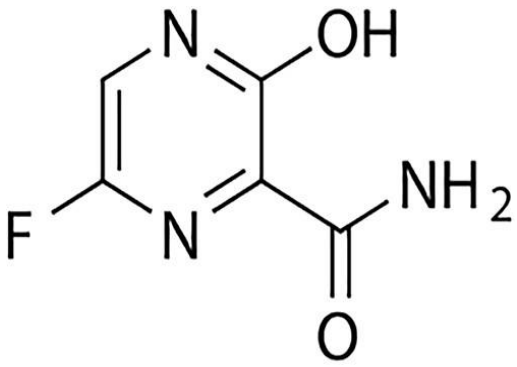

Fig. 1: Chemical structure (Favipiravir)

MATERIALS AND METHODS

Chemicals

Analytical grade chemicals were used without further purification in this study. Water (spectro chem India) and HPLC-grade methanol (≥99.9%, Molychem, India) were used. Favipiravir bulk powder was gifted by Sun Pharma Industries and a tablet (fabiflu (200 mg) Glenmark) was purchased from local market.

Stock standard solution

10 mg pure drug was accurately weighed, dissolved in about 3 mL of methanol and transferred to a 10 mL volumetric flask. Then the volume was completed to 10 mL with methanol to obtain 1 mg mL^{-1} of stock solution. The resulting stock solution was sonicated and filtered through a 0.45 mm filter. The stock solution was further diluted with Mobile phase to obtain the required concentration of standard solutions (10–50µgmL^{-1}) before being injected into the system for analysis.

Sample solution

Ten Favipiravir tablets were accurately weighed and transferred to a dry and clean mortar, then ground into a fine powder.

Next, tablet powder equal to 10 mg Favipiravir was transferred to a volumetric flask of 10 mL. About 3 mL methanol was added and this flask was attached to a rotary shaker for 10 min to completely disperse the ingredients. The mixture was sonicated for 30 min, diluted to volume with methanol to give a solution containing 1 mg $mL^{-1}$and then filtered through a 0.45 mm filter.

Determination of λmax

Standard solution (40 µgmL^{-1}) was subjected to scanning between 200 and 800 nm on an ultraviolet (UV) spectrophotometer (Shimadzu UV-1800 spectrophotometer). λmax was obtained from the UV spectrum of standard solution, Figure II.

Chromatographic conditions

Chromatographic analysis was performed on a column of Phenomenax C18 (4.6 mm 3 250 mm, 5.0 mm). The mobile phase consisted of methanol and water (10:90, v/v). The mobile phase was filtered and degassed through a 0.45 mm membrane filter before use and then pumped at a flow rate of 1 mL min^{-1}. The column has been thermostated at 30 °C. The run time was 10 min under these conditions.

Method validation

The analytical method validation had performed as per ICH guidelines of validation of Analytical Procedure: Q2(R1) [https://www.ich.org/page/quality-guidelines, [https://www.fda.gov/regulatory-information/search-fda-guidance-documents/reviewer-guidance-validation-chromatographic-methods>]. The validation parameters such as system suitability, linearity, the limit of detection (LOD), the limit of quantification (LOQ), accuracy, specificity, precision, and robustness were addressed.

Linearity

Standard calibration has been prepared using six standard solutions within the concentration range of 10–50 µgmL^{-1} in developed chromatographic conditions, each standard solution was chromatographed for 10 min three times.

Specificity/selectivity

Selectivity is the ability of the analytical method to produce a response for the analyte in the presence of other interference.

The selectivity of the method was tested by comparing the chromatograms obtained for Favipiravir standard, tablet, and blank solutions. The parameters retention time and tailing factor were calculated in order to prove that the method chosen was specific.

Limit of detection and limit of quantification

These values were determined using the standard error (s) and slope of the regression line (m) as shown in following equations:

LOD ¼ 3:3*s=m

LOQ ¼ 10*s=m

Precision

Precision was analyzed by calculating variations of the method in intraday (repeatability performed by analyzing standard solution on the same day) and inter-day (repeatability carried out by analyzing standard solution on three different days). Precision study was performed by injecting six times of standard solution at three different concentrations, 20, 40, and 60 μgmL^{-1}on the same day and three consecutive days.

Accuracy

Recovery studies were conducted by the standard addition technique to confirm the accuracy of the proposed method. In this method, 80, 100 and 120% of three different levels of pure drug were added to the previously analyzed sample solutions, and favipiravir recovery was calculated for each concentration.

Robustness

A robustness analysis was performed to determine the impact of minor yet systematic differences in chromatographic conditions. The modifications include different flow rates of the mobile phase (±0.1 $mLmin^{-1}$), methanol ratio in the mobile phase (±1%) and column temperatures (±2 ºC). After each change, system suitability parameters were checked by injecting the sample solution into the chromatographic system and the results were compared with those under the original chromatographic conditions.

Analysis of marketed formulations

Accurately weighed tablet powder equivalent to 10 mg Favipiravir has been transferred into a volumetric flask of 10 mL and filled the mark with deionized water than again dilute it to prepare at the concentration of 40 μgmL^{-1}sample solution. This sample solution was filtered using 0.45 mm filter and then analyzed.

RESULTS AND DISCUSSION

One of the main objectives of our work is to apply the principles of green chemistry by preventing the generation of toxic chemicals, rather than treating their effects. The main challenge is to replace hazardous chemicals used in traditional analytical methods with solvents more benign to the environment, without affecting the efficiency of the proposed method. The choice of solvents is a critical step during the development of an analytical method, so it is recommended to substitute harmful solvents with greener alternatives (Armenta *et al*, 2008).

In the proposed HPLC method, different ratios of the mobile phase were attempted at different flow rates. The percentage of water and methanol varied from 10 to 90%. The best separation was achieved using methanol: water (10: 90, v/v) at a flow rate of 1 $mLmin^{-1}$. Detection was done at 323 nm to provide good sensitivity (Fig.2).

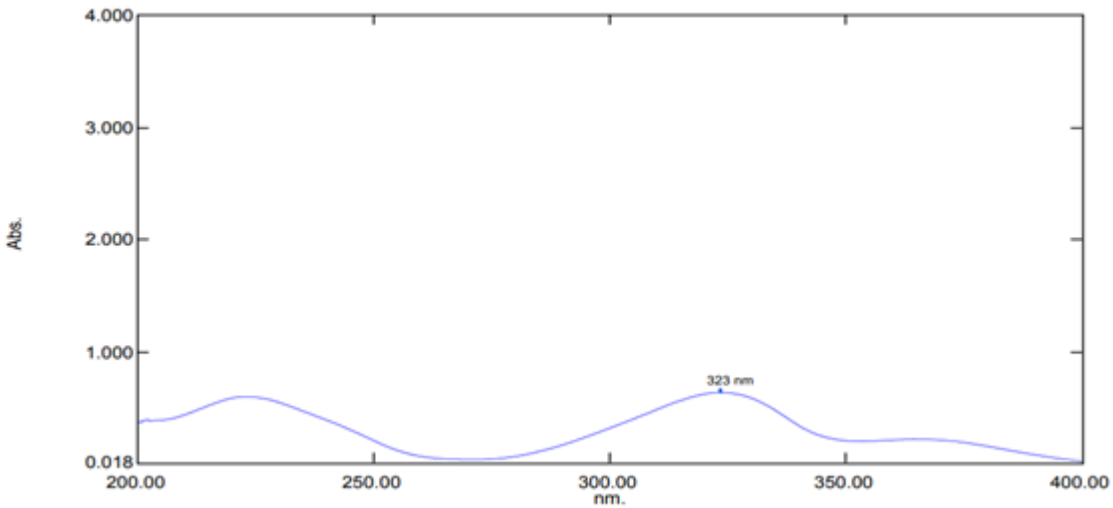

Fig.2: UV spectrum (standard solution, 40 µg/mL)

Separation was tried initially without using acetonitrile in place of, methanol but poor symmetric peaks was found. Retention time was 5.00 min, as shown in the HPLC chromatogram of the standard (Fig.3).

In this method short separation time was achieved (5.00 min), so the amount of waste generated was decreased (3–7 mL/run). Producing low amounts of waste is an essential measure to be considered, according to green chemistry principles.

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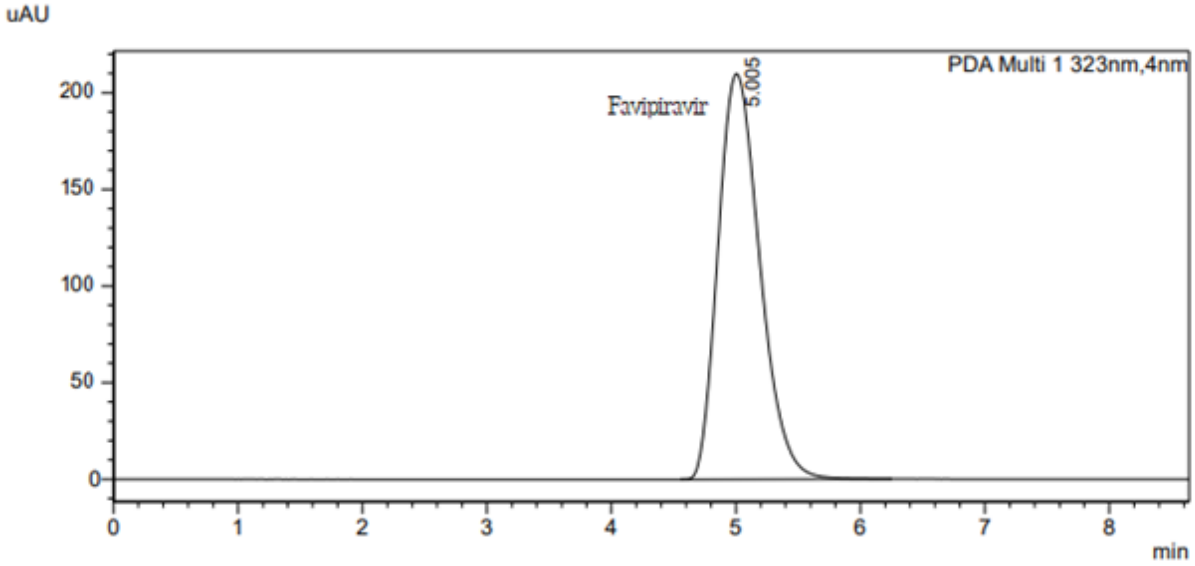

Fig. 3: HPLC chromatogram (standard solution, 40 µg mL-1)

METHOD VALIDATION

Linearity

The stock standard solution of favipiravir was diluted appropriately with deionized water to obtain standard solutions within the concentration range of 10–50µgmL^{-1}. Each standard solution was injected three times into the HPLC system under the above-mentioned chromatographic working conditions. Linearity of the proposed method has been estimated at six concentration levels in the range of 10– 50 µgmL^{-1} by regression analysis. The calibration curve was developed by plotting average peak area versus standard concentration (Fig. 4). The correlation coefficient, slope, and intercept of the regression line were determined using theleast squares method. Values of slope, intercept and correlation coefficient (r^2) were 11562, 85327 and 0.9998, respectively as shown in Table 1.

Table 1: Linearity Data of Favipiravir

Data	Conc. (ppm)	Avg. Area
	10	30032
	20	145145
	30	264758
	40	374027
	50	493685
Slope (a)	11562	
Intercept (b)	85327	
Correlation coefficient (R^2)	0.9998	

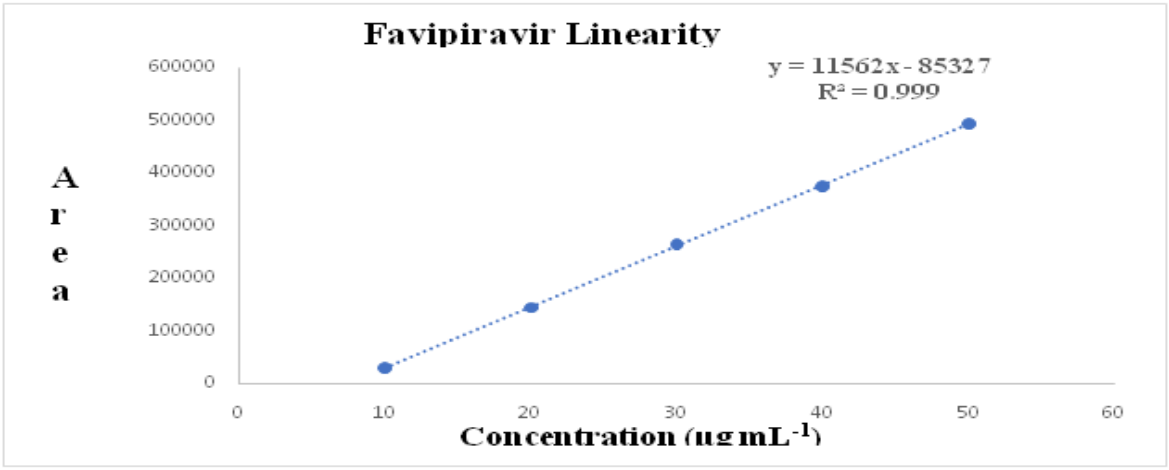

Fig. 4: Linearity Graph of Favipiravir

Specificity/selectivity

The chromatogram of favipiravir standard solution has been given in Fig. III. There was only one peak at the retention time of 5.00 min. The chromatogram of the tablet solution has been given in Fig. V. There was only one peak at the retention time of 5.004 min in this chromatogram. There were no other peaks caused by excipients and additives in this chromatogram. The chromatogram of the mobile phase has also given in Fig. VI. There are no other peaks caused by contents of the mobile phase in this chromatogram. This indicates that the analytical method is specific. The parameters retention time and tailing factor were calculated in order to prove that the method chosen was specific. Retention time, theoretical plate number, and peak tailing factor values were 5.00, 2754, and 1.02, respectively. All of the values were within the accepted level.

Precision

Precision study was performed by injecting six times of standard solution at three different concentrations, 20, 40and 60 µgmL^{-1} on the same day and three consecutive days. The precision data were given in Table 2. All RSD values for retention time and peak area for selected concentrations were less than 0.5 and 2.0%, respectively. In this case, the method is precise and can be used for our intended purpose.

Table 2: Precision Data

Std. conc. µg mL-1	Intraday Precision			Interday Precision		
	Found conc. µg mL-1	Peak area RSD (%)	Retention time RSD (%)	Found conc. µg mL-1	Peak area RSD (%)	Retention time RSD (%)
20	20.04	0.184	0.008	19.31	0.195	0.015
40	40.21	0.165	0.003	40.01	0.178	0.008
60	60.14	0.192	0.005	60.33	0.199	0.011

Accuracy study

A known quantity of standard solution has been added to the sample solutions previously analyzed at three different levels (80%, 100% and 120%). The amount recovered for favipiravir has been calculated for three concentrations. The recovery data were summarized in Table 3. Percent RSD values for all analyses were less than 2% indicating that excipients found in pharmaceutical formulations do not interfere and analytical method is very accurate.

Table 3: Recovery Data

Spiked level (%)	Amount added (µg mL-1)	Amount recovered(µg mL-1)	Recovery (%)	Average (%)	SD	RSD (%)
80	32	31.28	97.75	98.68	1.193161	1.193161
	32	32.01	100.03			
	32	31.45	98.28			
100	40	39.98	99.95	100.01	1.169672	1.169672
	40	39.62	99.05			
	40	40.55	101.37			
120	48	47.30	98.54	99.47	0.864812	0.864812
	48	47.82	99.62			
	48	48.12	100.25			

Robustness

The results showed that the change in flow rate and mobile phase concentration had little effect on the chromatographic behavior of favipiravir. The small change in the mobile phase flow rate and acetonitrile content have a small impact on the retention time of favipiravir. The change in the column temperature did not have a significant effect on the method. The results of this study, expressed as % RSD, were presented in Table 4.

Table 4: Robustness Data

Conditions	Variation	Assay %	SD	%RSD
Mobile Phase Flow Rate	0.90 mL min-1	99.32	0.58	0.58
	1.10 mL min-1	98.94	0.65	0.65
Methanol Ratio in Mobile	9 %	98.78	0.48	0.48
	11%	100.02	0.60	0.60
Column Temperature	28 ºC	100.12	0.39	0.39
	32 ºC	99.79	0.41	0.41

Limit of detection and limit of Quantitation (LOD and LOQ):

From the linearity plot the LOD and LOQ were calculated: LOD of favipiravir was found to be 1.41 µg $mL^{-1}$and LOQ of favipiravir was found to be 3.48 µg mL^{-1}**.**

System Suitability

A set of optimized conditions, i.e., combination of Water and Methanol90:10 % v/v as mobile phase at a flow rate of 1.0 mL min^{-1} over a Phenomenax C8 150 mm × 4.6 cm, 5 µm, was selected and system suitability was assessed according to BP guidelines. Statistical data of different parameters like retention time (Rt), theoretical plates (N), capacity factor (k') and Tailing Factor (Tf) of favipiravir was calculated for peak response by LC Solution software. The results showed that all the performance parameters of the analytical method comply with BP requirements for system suitability. The RSD for Rt of both analytes was less than 2.0 %, Theoretical plates more than 2000. Tailing factor (Tf) was between 0.8 to 1.5, capacity factor (k') was 0.5–10 Table 5.

Table 5: System Suitability using Proposed HPLC Method

	Favipiravir	Recommended Values
Retention time (Rt, min)	5.00	-
Theoretical Plates (N)	2754	The more theoretical plates better separation
Capacity Factor (k')	6.1	0.5<k'<10
Tailing Factor (Tf)	1.02	0.8<Tf< 1.5

Application of the method to the marketed tablets

The developed and validated method has been applied successfully for determination of favipiravir in pharmaceutical formulations. The result of assay of the marketed tablet of favipiravir is shown in Table VI**.** The results obtained are closely related to the amount indicated on the labels of the tablets. This shows that the method for content evaluation is useful. Developed method was validated in compliance with ICH guidance Q2. Besides the short run time, retention time (5.00) and flow rate of mobile phase (1 mL min^{-1}) made the method attractive because these features save analysis time and cost(Fig. V). Here we use simple methanol and water so simple method easy availability, low toxicity and cost, and greatly improved separation ability without column degradation. In short, this method is sensitive, selective, reproducible and rapid for favipiravir in bulk and tablets. The accuracy and precision are within reasonable limits, the maximum of quantification is as small as 3.48 µg $mL^{-1}$and finally analytical method is reliable and robust.

Table 6: Assay of Favipiravir Tablet Using Proposed HPLC Method

Formulation	Label Claim (mg)	Amount of drug (mg)	% Assay ± SD
Fabiflu Tablet	200	200.26	100.13 ± 0.29

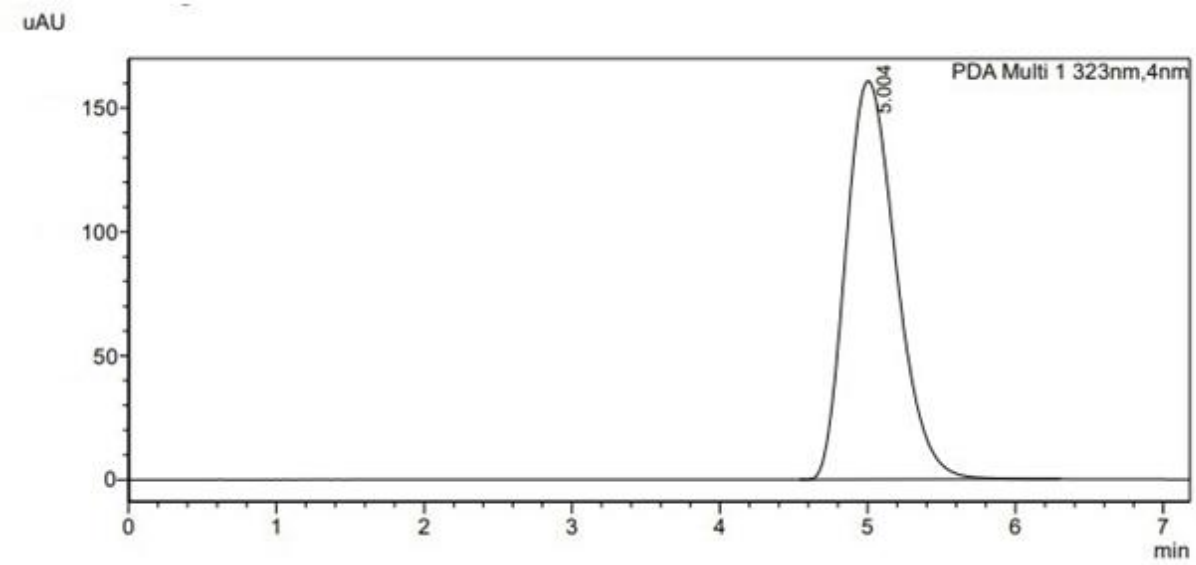

Fig. 5: HPLC chromatogram (Sample, 40 µg/mL)

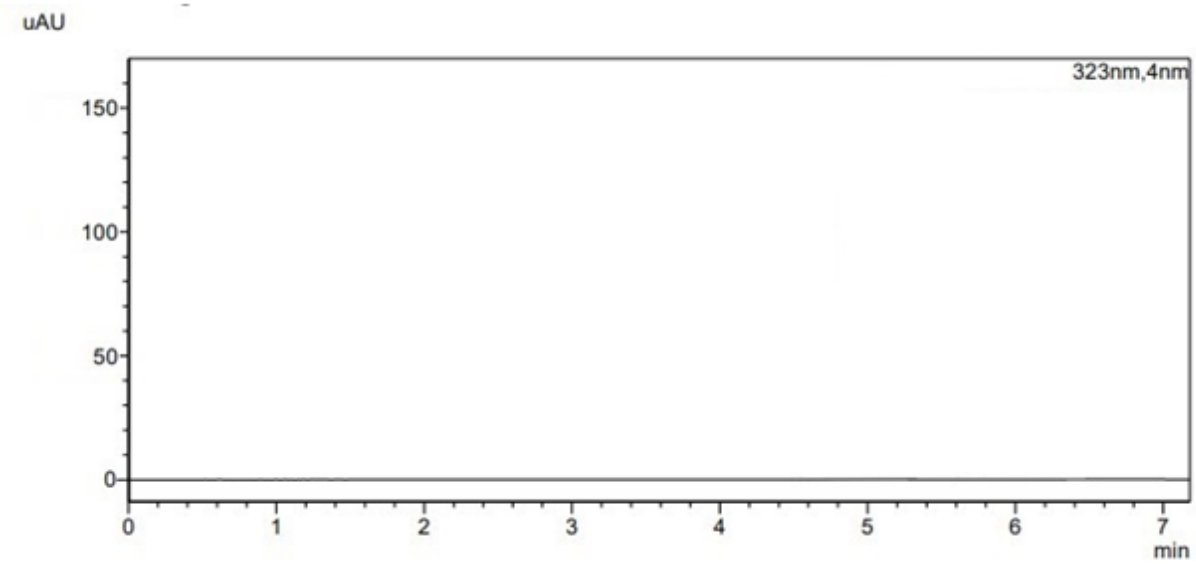

Fig. 6: HPLC chromatogram (Blank)

CONCLUSIONS

A very quick, cost-effective, precise and accurate HPLC method for the determination of Favipiravir has been developed and validated in compliance with ICH guidance Q2. Besides the short run time (7 min), retention time (5.00) and flow rate of mobile phase (1 mL min^{-1}) made the method attractive because these features save analysis time and cost. The most important of these features of this method is solvents selected for mobile phase are green analytical chemistry, easy availability, low toxicity and cost. In short, this method is sensitive, selective, reproducible and rapid for favipiravir in bulk and tablets. The accuracy and precision are within reasonable limits, the maximum of quantification is as small as 3.48 mg mL^{-1} and finally analytical method is reliable and robust.

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CONFLICTS OF INTEREST

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

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