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Development and Validation of a Rp-Hplc Method for the Quantification of Favipiravir and its Impurities in Pharmaceutical Dosage Forms

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An effective reversed phase high performance liquid chromatographic approach has been developed and validated for the determined for Favipiravir and its impurities, which may coexist in bulk drugs and solid pharmaceutical dosage forms. Using a mobile phase of pH 5.8 phosphate buffer and methanol (Gradient Program) at a flow rate of 1 ml/min at a wavelength of 325 nm, the separation was accomplished by an Inspire column (3.0*150mm, 5μ). Favipiravir recovery percentages at the three target concentrations were 99.67, 99.87, and 99.925, with a mean recovery of 99.59. The method's linearity varied from 0.1 to 0.5 PPM. This met the requirements for approval. The %RSD of 1.01, 0.63, and 0.95 for favipiravir, IMP-A, and IMP-B, respectively, showed how accurate the method was. The method's sensitivity is demonstrated by the LOD and LOQ values of 2.81 and 8.78, respectively. The method was validated as per ICH guidelines to prove its worth in order to adopt by pharmaceutical industries as a part of their routine quality control analysis.

Keywords: Favipiravir; ICH guidelines; Method development; RP-HPLC; Validation.

Favipiravir an anti-viral agent ($C_5H_4FN_3O_2$, 6-fluoro-3-hydroxypyrazine-2-carboxamide, its molecular weight is 157.104g/mol, available as tablet dosage form useful in the management of SARs infections. Triphosphorylated favipiravir stops the viral genome from replicating by specifically inhibiting RNA polymerase. Favipiravir inhibits viral growth and elongation when it gets integrated into a developing RNA strand. A few studies on the competitiveness between purine nucleosides and favipiravir for RdRp binding have been published. The catalytic domain is favipiravir's principal target, and it is anticipated to be comparable for different RNA viruses, contributing to its wide range of activity, despite it was first introduced to the market as an anti-influenza medication.[1-5]

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Favipiravir –impurity A is 6-Fluoro-3,5-dihydroxypyrazine-2-carboxamide with molecular formula C5H4FN3O3 and molecular weight 173.1g/mol which is stored at 2-8°C.

Favipiravir –impurity B is 6-Fluoro-3-oxo-3,4-dihydropyrazine-4,5-d2 carboxamide-N,N-d2with molecular formula $C_5D_4FN_3O_2$and molecular weight 171.13g/mol which is stored at 2-8°C.

A thorough review of the literature revealed that several analytical techniques were approved for the estimation of favipiravir and its related compounds using RP-HPLC and other analytical techniques [6-12] This study's main objective is to create and validate a liquid chromatographic approach that could separate and measure favipiravir and its related compounds in a single run. The approach will be straightforward, precise, cost-effective, and need minimal run time for regular quality control testing. Using ICH principles as a guide, the established approach was transferred for validation and applied to routine quantitative analysis.

MATERIALS AND METHODS

For this investigation, a Waters HPLC with a PDA detector was used. The columns employed were Phenomenex, YMC, the Inertsil-C18 ODS Plastil etc. Every glassware used in this investigation is borosil. A gift sample of Favipirair was obtained from Glenmark. MERCK chemicals are where we acquire HPLC quality water, methanol, acetonitrile, and potassium dihydrogen phosphate.

Method development

Standard solution

Precisely weighed 25 mg of the standard favipiravir, together with 1 mg of each of the impurities A and B, were taken into a 25 ml volumetric flask. 10 mL of diluent was added sonicated to fully dissolve the contents, and additional solvent was added to bring the volume to required level. 0.3 ml of the above stock solutions was taken into a 10-ml standard flask, diluted with diluent to the level.

Preparation of Impurity solution

The equivalent weight of 1 milligram of impurity A and 1 mg of impurity B was precisely weighed, transferred, and added to a 10 ml clean and a dry flask. Next, around 7 mL of diluent was added, and volume was then adjusted using the same. Additionally, 1 ml of the aforementioned stock solutions was pipetted into a 10 ml standard flask, diluted it to level with diluent.

Table 1. Gradient programme for optimized method

Time	Mobile Phase-A %	Mobile Phase-B %
0	70	30
3	60	40
7	55	45
12	50	50
16	45	55

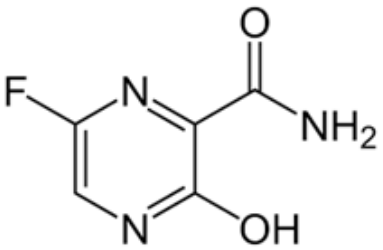

Fig. 1. Structure of Favipiravir

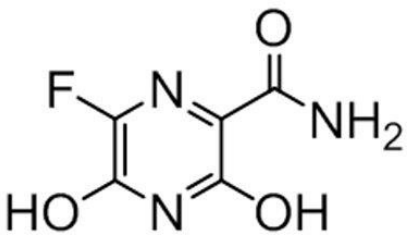

Fig. 2. Structure of Favipiravir impurity A

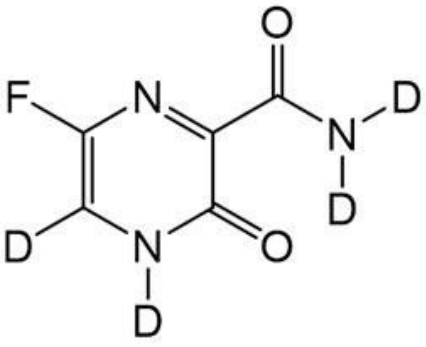

Fig. 3. Structure of Favipiravir-impurity B.

Method Optimization

At first, varied ratios of methanol, orthophosphoric acid buffer, phosphate buffer, and acetonitrile to methanol were explored as the mobile phase. Ultimately, the gradient program's mobile phase had been modified to comprise 0.1% ortho phosphoric acid buffer and acetonitrile.

The process of developing the approach was started utilizing a variety of columns, including the C18, Phenomenex, YMC, and Inertsil ODS columns. At last, the method was refined using Platsil (4.6*250mm, 5µ) at a flow rate of 1.0 ml/min. The chromatogram is shown in 4.

Table 2. Results of Optimized chromatogram

Peak Name	Retention time	Area	USP Plate count	USP Tailing
Impurity-A	4.366	67829	5219	1.2
Favipiravir	5.234	194265	2899	1.7
Impurity-B	8.448	45771	2766	1.5

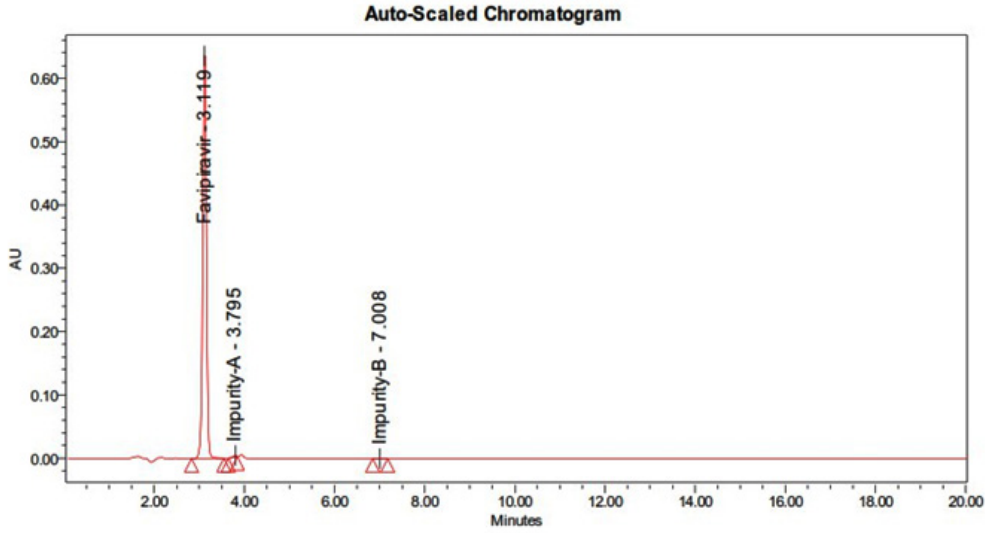

Fig. 4. Optimized chromatogram

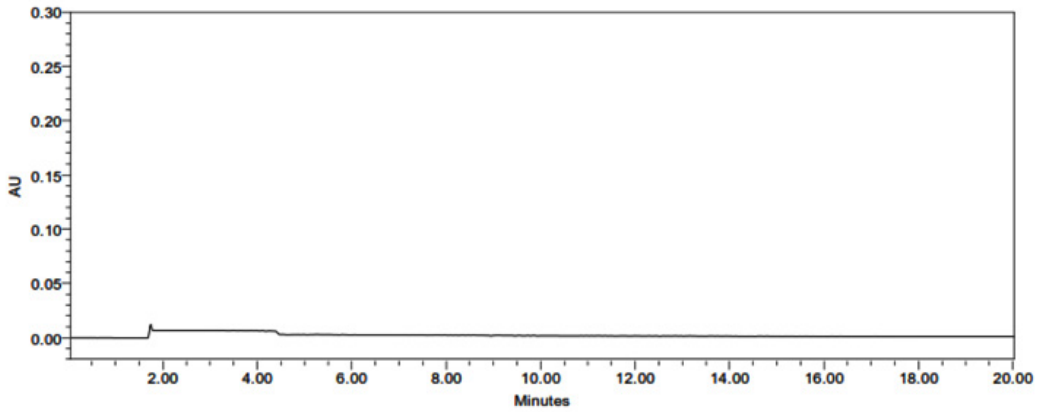

Fig. 5. Chromatogram of blank

Method validation

Specificity

A Standard solution was prepared by using Favipiravir spiked with impurities as per test method and Injected into the chromatographic system. The chromatograms were shown in figures 5 and 6

Assay

The following formula was used.

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity

Using favipiravir spiked with impurities as a working standard, a series of solutions were created. Peak areas were measured for replicate samples. The r2 value was calculated from a graph Table 3, Figures 7, 8, and 9 present the results of favipiravir, IMP-A, and IMP-B, respectively.

Accuracy

The newly created method's accuracy was assessed using recovery studies at three distinct levels, in triplicates which correspond to 50, 100, and 150%. The % recovery for favipiravir is given in Table 4.

Precision

Repeatability

Six duplicate injections were made, and each injection's peak area was recorded. The results showed that the percentage RSD for the six replicate injections fell between the acceptable limits. The table 5 displayed the outcomes for favipiravir, IMP-A, and IMP-B.

Intermediate Precision

A study was conducted on different days maintaining the same parameters. The results showed that the percentage RSD complied with the acceptable limits. Table 6 presented the findings for favipiravir, IMP-A, and IMP-B.

Table 3. Table for linearity of favipiravir and its impurities

Level	Concentration of Imp. A (ppm)	Peak Areas of Favipiravir	Peak Areas of Imp. A	Peak Areas of Imp. B
Level-1	0.1	1168012	3850	937
Level-2	0.2	2308831	8699	1659
Level-3	0.3	3458390	12695	2542
Level-4	0.4	4580110	16991	3279
Level-5	0.5	5567203	20999	4179
Correlation Coefficient		0.999	0.999	0.999

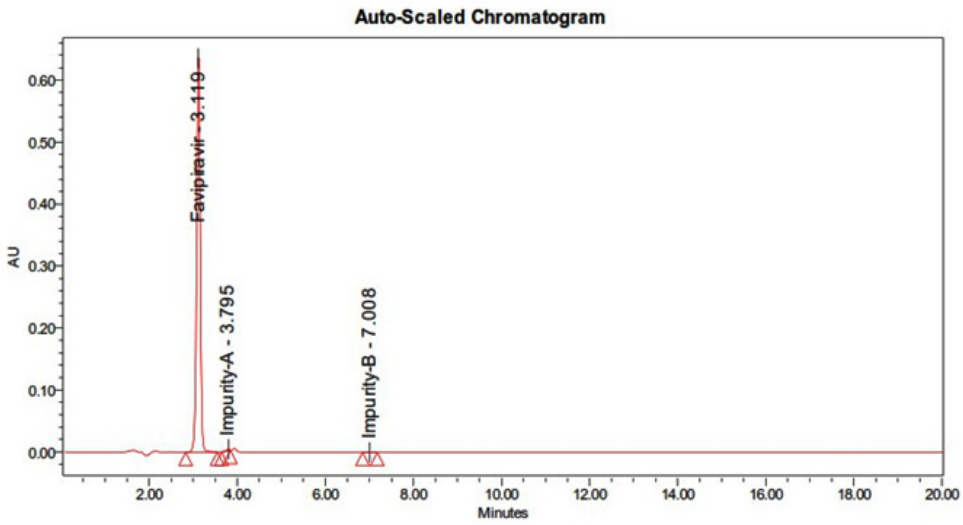

Fig. 6. Chromatogram of STD

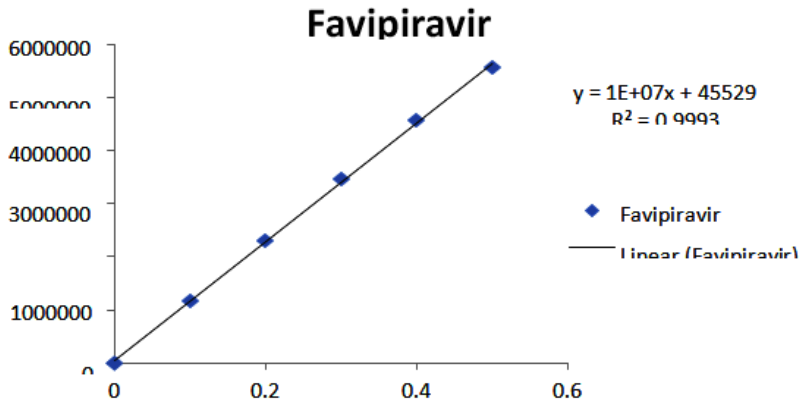

Fig. 7. Linearity chromatogram for favipiravir

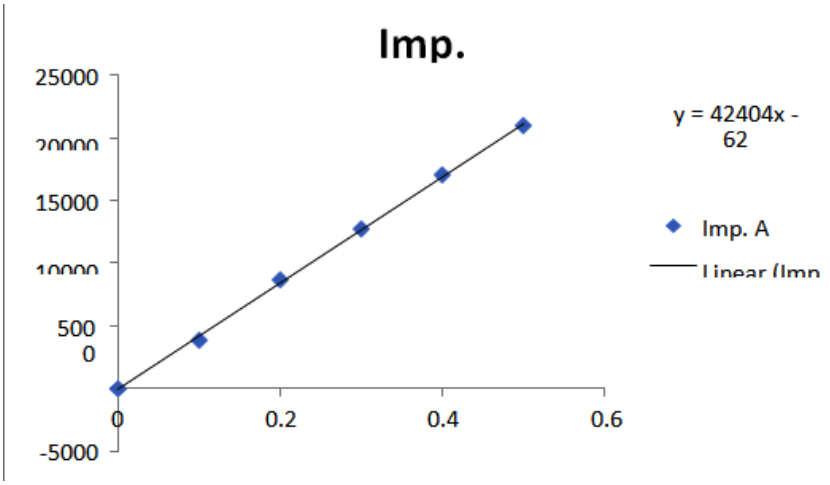

Fig. 8. Linearity chromatogram for impurity A

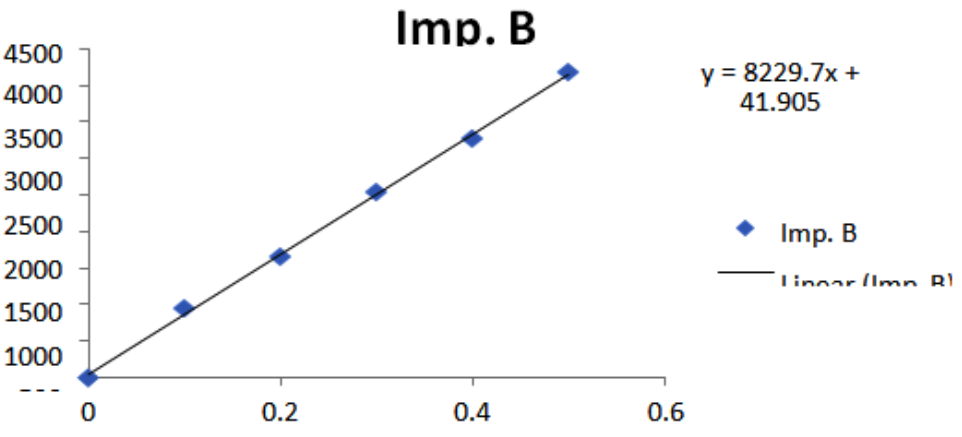

Fig. 9. Linearity chromatogram for impurity

Limit of Detection and Limit of Quantification

Determined by using the following formulae

$$LOD = 3.3\ \sigma / S$$

$$LOQ = 10\ \sigma / S$$

Robustness

A study was carried out to ascertain the impact of changes in the mobile phase and flow rate . Using flow rates of 0.9 ml/min and 1.1 ml/min, standard solution prepared and introduced into the HPLC system. The mobile phase composition was varied by 10% in the same investigations. The results were reported in fig 10 to 13 and in tables 7 and 8.

RESULTS

Optimized conditions

Column	: Platsil(3.0*150mm, 5µ)
Mobile phase	: pH 5.8
Phosphate buffer	: Methanol (Gradient)
Flow rate	: 1.0 ml per min
Injection volume	: 10 µl
Run time	: 20 min.

The aforementioned trail was deemed optimized since it had a plate count of above 2000 and a USP tailing of less than 2.

Method validation

Specificity

It is studied to assess ability of the method to measure the analyte of interest without any interference.

Table 4. Recovery Studies of favipiravir

Target level	Spiked (mg)	Recovered (mg)	% Recovery	Mean Recovery
50%	12.5	12.35	99.67	99.59
100%	25	24.91	99.87	
150%	37.5	37.45	99.25	

Table 5. Precision results of favipiravir and Impurity A & B

Injection	Areas of Favipiravir	Areas for IMP_A	Areas For IMP_B
Injection-1	3215992	26695	5392
Injection-2	3216832	26725	5321
Injection-3	3263239	26321	5319
Injection-4	3255759	26462	5420
Injection-5	3289296	26923	5324
Injection-6	3227621	26751	5364
Average	3244790	26646.17	5356.667
Standard Deviation	29486.3	217.2	42.6
%RSD	0.91	0.82	0.80

Table 6. Table for ID precision results of Favipiravir and Its impurities A & B

Injection	Areas of Favipiravir	Areas for IMP_A	Areas for IMP_B
Injection-1	3215986	26692	5381
Injection-2	3216843	26825	5461
Injection-3	3283392	26521	5329
Injection-4	3245729	26682	5420
Injection-5	3289127	26523	5338
Injection-6	3225621	26952	5355
Average	3246116	26699.17	5380.667
Standard Deviation	32937.4	169.0	51.3
%RSD	1.01	0.63	0.95

Table 7. Impact of variation in flowrate

S. No	Flow Rate	USP Plate Count	USP Tailing
1	0.9	3928.16	1.26
2	1.0	4417.64	1.12
3	1.3	4326.12	1.14

Assay results

194317/194265 x 25/25 x 0.3/10 x 5/20 x 10/0.3 x 2/0.5 x 99.8/ 100 x 100 = 99.8%

The percentage purity of favipiravir was found to be 99.8% which fells in acceptable range of 98.0% - 102.0%.

Linearity

It was determined at five different levels for favipiravir and its impurities by replicate injections.

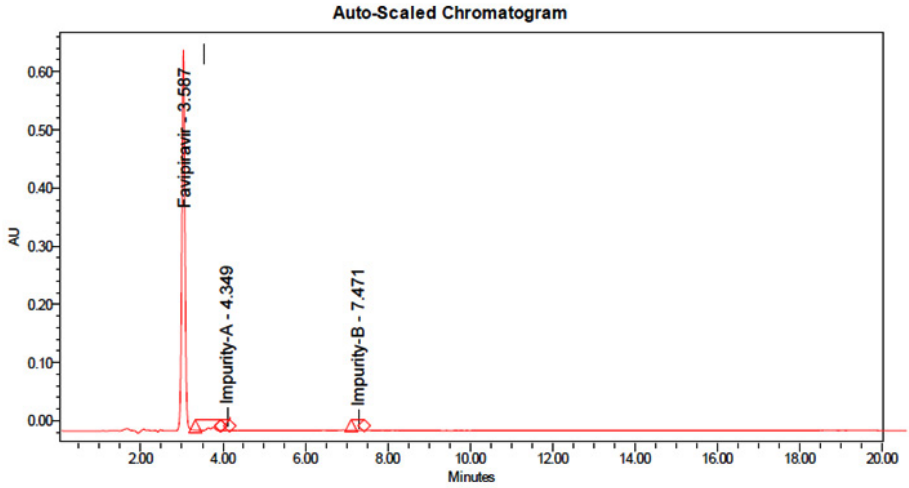

Fig. 10. Chromatogram at low flow rate

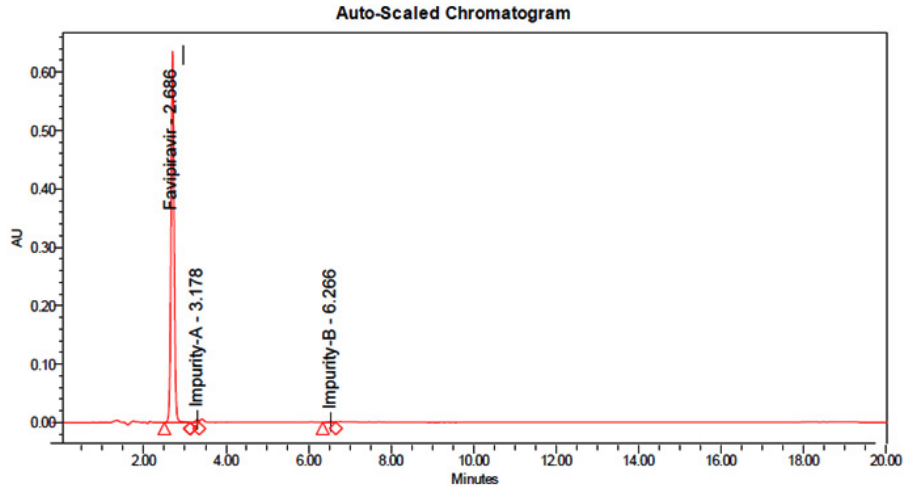

Fig. 11. Chromatogram at more flow rate

Table 8. Impact of variation in organic phase composition

Variation in organic phase	USP Plate Count	USP Tailing
<10%	3920.12	1.24
*Actual	4425.61	1.16
>10%	4325.11	1.32

The acceptance criteria were met by the correlation coefficient of favipiravir, Imp-A, and Imp-B, which was found to be 0.999

Accuracy

Assessed by recovery studies at three different concentrations with respect to target concentrations.

With a mean recovery of 99.59, the percentage recovery of favipiravir at 50%, 100%, and 150% was determined to be 99.67, 99.87, and

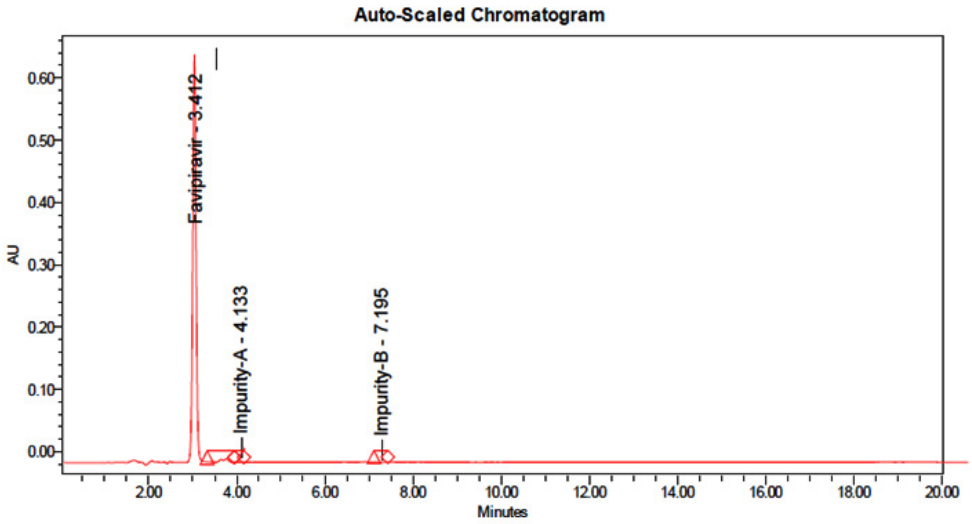

Fig. 12. Chromatogram at less organic phase

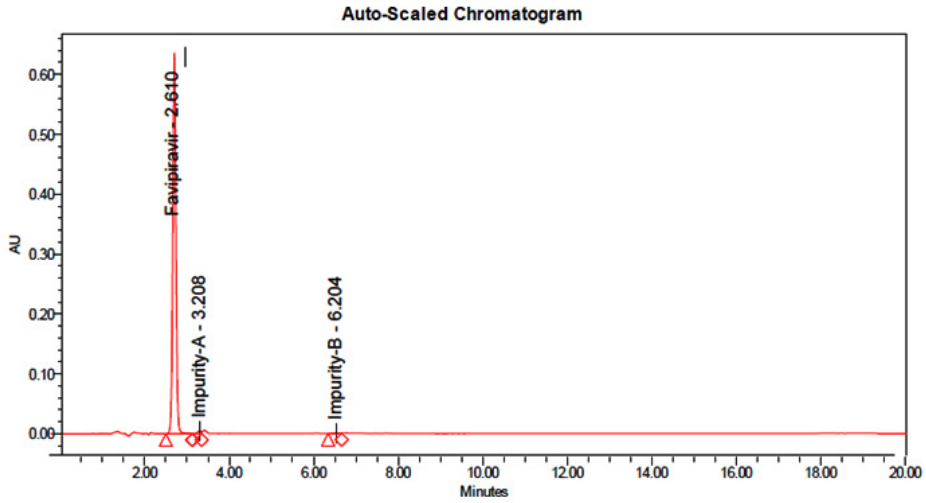

Fig. 13. Chromatogram at more organic phase

999.25, respectively, and it was in compliance with the acceptance criteria.

Precision

Precision was determined by injecting six replicate injections and peak areas were measured.

The results showed that the six injections of favipiravir, IMP-A, and IMP-B had %RSDs of 0.91, 0.82, and 0.80%, respectively, which met acceptance criteria.

Intermediate precision

The %RSD for ID precision of favipiravir, IMP-A and IMP-B was found to be 1.01, 0.63 and 0.95 respectively which was in compliance with acceptance criteria.

Limit of detection

S/N = 146/52 = 2.81

Limit of quantifictaion (LOQ)

S/N = 562/64 = 8.78

With the LOD & LOQ values 2.81 and 8.78 respectively the method was proven to be sensitive.

Robustness

It was dedicated to know how the variations in rate of flow and mobile phase affects the credibility of the method.

DISCUSSION

Favipiravir and its related substances were estimated using a novel, adaptable technique. Using a Platsil column (4.6*250mm, 5µ) with acetonitrile and 0.1% orthophosphoric acid buffer in a gradient program, the procedure was optimized at a flowrate of 1.0 ml/min. This particular mobile phase is suitable to achieve high resolution separations for ionizable compounds The gradient program was selected to reduce the analysis time, enhance sensitivity, resolution and most importantly in the present study it was selected to elute the strongly retained impurities. The method has been validated in accordance with ICH guidelines

At the three target concentrations, the percentage recovery of favipiravir was 99.67, 99.87, and 99.925, with a mean recovery of 99.59. The linearity of the approach ranged between 0.1 and 0.5 PPM This complied with the standards for acceptance. The precision of the approach was demonstrated by the %RSD of 1.01, 0.63, and 0.95 for favipiravir, IMP-A, and IMP-B, respectively. The method was found to be sensitive than the existing methods as the LOD and LOQ values were 2.81 and 8.78, respectively.

CONCLUSION

The development of an RP-HPLC method for favipiravir quantification in the presence of its contaminants has been considered because of its therapeutic significance. A Waters HPLC with a PDA detector was used in this investigation. The technique was optimized using Platsil (4.6*250mm, 5µ) at a flow rate of 1.0 ml/min. Lastly, the gradient program's mobile phase was optimized using acetonitrile and 0.1% ortho phosphoric acid buffer. According to ICH rules, the procedure was validated, and all of the results were deemed to be in compliance with the acceptance requirements. In light of the above findings, we recommend the proposed method is adoptable in regular quality control analysis to identify and estimate favipiravir and its impurities as it is more suitable than existing methods. The scope of the method can be elaborated by performing forced degradation studies which ensures the stability of the method.

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Conflict of interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials**.**

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Not Applicable

Authors Contribution

Sunil Kumar Chaitanya Padavala: Conceptualization- Methodology , Writing -Original draft; Murugan Nithya : Conceptualization-Methodology, Data collection, Analysis; Naga Haritha Pamujula : Visualization, Supervision, Project Administration; Sareesh Kankanala: Visualization, Supervision, Project Administration; Rajini Kolure: Writing- Review and editing.

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