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Stability-indicating Reverse Phase-HPLC Method Development and Method Validation for Quantitative Determination of Degradation Products in Favipiravir API and Drug Product

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: Favipiravir is an antiviral medication shown to be broad spectrum activity against RNA viruses, and potentially treating the COVID-19.
Methodology: In this study, the HPLC method for the quantification of degradation impurities (Favipiravir Acid Impurities) were developed and validated for Favipiravir in Tablet dosage form. The specificity of the method was achieved in analytical column Agilent HPLC-C18, 5µm, (4.6 x250) mm. using a suitable mobile phase was 10 mM Phosphate buffer (pH 3.5 with orthophosphoric acid) and Acetonitrile in the Isocratic more of 70:30 v/v. The flow rate is 1.0 mL/min. the injection

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volume is 10 µL, detection at 320 nm in UV and total run time is 8.0 minutes. The samples were made for forced degradation under hydrolysis, oxidation, photolytic and thermal conditions. The method was validated for specific, selective, linear, robust and accurate as per the ICH guidelines.
Results and Conclusion: The linearity of the method for Impurities and the analytes was found from 25 to 150 % concentration level with the correlation coefficient (r^2) > 0.999. The accuracy for impurity and the analytes was performed from 50 to 150% level concentration, and mean recovery was found from 98-102%. The analytical degradation and validated study results indicate its unstable nature in acidic, basic and thermal conditions. Therefore, this method is simple, selective and sensitive, this method can be used in pharmaceutical research and development and quality control departments.

Keywords: Favipiravir; HPLC; degradation impurities; ICH guidelines; forced degradation; stability indicating method.

1. INTRODUCTION

"Favipiravir is a member of the class of pyrazines that is pyrazine substituted by aminocarbonyl, hydroxy and fluoro groups at positions 2, 3 and 6, respectively. It is an anti-viral agent that inhibits RNA-dependent RNA polymerase of several RNA viruses and is approved for the treatment of influenza in Japan. It has a role as an antiviral drug, an anticoronaviral agent and an RNA-directed RNA polymerase inhibitor. It is a primary carboxamide, a hydroxypyrazine and an organofluorine compound" [1,2].

"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. The antiviral targets RNA-dependent RNA polymerase (RdRp) enzymes, which are necessary for the transcription and replication of viral genomes. Not only does favipiravir inhibit replication of influenza A and B, but the drug has shown promise in the treatment of avian influenza and may be an alternative option for influenza strains that are resistant to neuramidase inhibitors. Favipiravir has been investigated for the treatment of life-threatening pathogens such as Ebola virus, Lassa virus, and now COVID-19" [3–6].

"Favipiravir is a pyrazine carboxamide derivative with activity against RNA viruses. Favipiravir is converted to the ribofuranosyltriphosphate derivative by host enzymes and selectively inhibits the influenza viral RNA-dependent RNA polymerase. Favipiravir physicochemical properties includes, Molecular formula is $C_5H_4FN_3O$, its IUPAC name is 5-fluoro-2-oxo-1H-pyrazine-3-carboxamide, Molecular weight is 157.1 g/mol, Melting point is 159-160°C, Solubility is Sparingly soluble in water, Log P (octanol/water partition coefficient) is 0.83, pKa is 2.88 (acidic) and its Stability is stable under normal conditions" [7,8].

Favipiravir has been the subject of numerous pharmacokinetic and pharmacodynamic studies to understand its behaviour in the body and its effectiveness in treating viral infections. Here are some key findings from these studies:

Pharmacokinetics:

Absorption: Favipiravir is rapidly absorbed after oral administration, with peak plasma concentrations reached within 2-3 hours.

Distribution: It has a large volume of distribution, indicating extensive tissue distribution.

Metabolism: Favipiravir is primarily metabolized in the liver to its active form, favipiravir ribofuranosyl-5'-triphosphate.

Elimination: The drug is primarily eliminated unchanged in the urine, with a half-life of around 5-7 hours.

Pharmacodynamics:

Antiviral activity: Favipiravir exhibits broad-spectrum antiviral activity against RNA viruses, including influenza viruses, Ebola virus, and coronaviruses.

Mechanism of action: Favipiravir acts as a viral RNA-dependent RNA polymerase inhibitor, disrupting viral replication.

Resistance: Some studies have reported the emergence of resistance mutations to favipiravir, highlighting the importance of proper dosing and combination therapy.

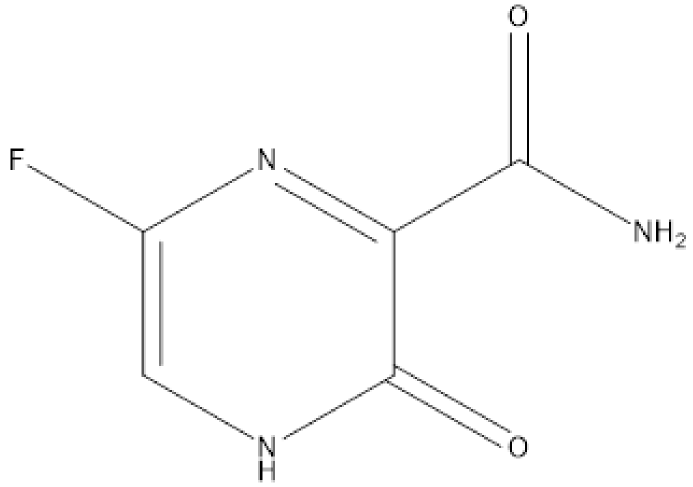

Fig. 1. Representative structure of favipiravir

Overall, pharmacokinetic and pharmacodynamic studies have provided valuable insights into the efficacy, safety, and dosing regimens of favipiravir in the treatment of viral infections.

Favipiravir is available in tablet form for oral administration. The strength of the tablets can vary depending on the brand and manufacturer, but common strengths include 200 mg and 400 mg per tablet [9,10].

literature survey indicated no chromatographic methods for determining degradation impurities (Favipiravir acid Impurities) presented in the Favipiravir. There are few reported literatures on the quantification of Favipiravir in chemical and biological matric. There are few reported literatures on the quantification of metabolite generated from the biological matrices of Favipiravir[11–17].

The objective of the current study was to create a simple, precise, linear, accurate, robust and stability-indicating assay method for identifying degrading impurities that are present in Favipiravir. A proven quantitative analytical process called the stability-indicating assay and related impurity method typically involves forced degradation and validation experiments[18–21].

2. MATERIALS AND METHODS

2.1 Instrumentation

The experiment was performed on a Waters HPLC 2695 with PDA detector integrated with Empower 2 Software equipped with Binary pumps, a PDA detector, an auto injector, a sample cooler, column heater. A pH meter from Digisun Electronics Hyderabad, India, to measure the buffer pH. Electronics Balance from Denver, India, was used. Vacuum microfiltration unit was used with 0.22µm PVDF filters from Millipore. Ultrasonicator from Labman India, was used for sonication of sample and standard. UV-VIS spectrophotometer integrated with UV win 6 Software from PG Instruments T60, India. Hot Air Oven from Servewell Instrument PVT LTD, Bangalore to study the stability of samples.

2.2 Chemicals, Reagents, and Standards

AR grade Potassium dihydrogen ortho phosphate, Ortho-phosphoric acid, was procured from Rankem, India. HPLC grade Acetonitrile and Water was procured from Merck, Mumbai, India. Favipiravir Tablets purchased from the local Pharmacy store. The Drug Substance (Favipiravir) and the impurities were obtained as a gratis sample from Hetero Laboratories.

2.3 Chromatographic Conditions

The buffer was prepared for 10 mM Potassium hydrogen phosphate buffer adjusted pH to 3.5 with Ortho-phosphoric acid in HPLC grade water and filtered through a 0.22µm membrane filter. HPLC mobile phase was composed of 10 mM Potassium hydrogen phosphate buffer is in A channel and Acetonitrile is in B channel in Isocratic mode in the ratio of 70:30 v/v. The selectivity was achieved using Agilent HPLC-C18, 5µm, (4.6 x 250) mm. the flow rate of 1.0 mL/min was employed. The HPLC column temperature and sample temperature were set at 30°C and 25°C respectively. The analytes were detected at 320 nm. The injection volume is 10µL and the total run time is 8.0 minutes. Water and Acetonitrile (50:50) v/v is used as diluent.

2.4 Preparation of the Favipiravir Standard Stock Solution

Weighed 1 mg of Favipiravir working Standard accurately and transferred into 50 ml clean dry volumetric flasks, added about 30ml of diluent, sonicated for 10 minutes, dissolved, and made up to the volume with diluent and mixed well. (20µg/ml Favipiravir).

2.5 Preparation of the Favipiravir Standard Solution

Transferred 1 ml of Favipiravir standard stock solutions into a 10 ml volumetric flask and made up to the volume with diluent and mixed well. (2 µg/ml Favipiravir).

2.6 Preparation of the Impurity Stock Solution

Weighed accurately 5 mg each of Favipiravir – Impurity A (Favipiravir acid Impurity) transferred into 50 ml clean dry volumetric flasks, added about 30ml of diluent, sonicated for 5 minutes and made up to the volume with diluent and mixed well. (100µg/ml impurity).

2.7 Preparation of the Favipiravir Stock Solution

Weighed accurately 10 mg each of Favipiravir standard and transferred into 50 ml clean dry volumetric flasks, added about 30 ml of diluent, sonicated for 5 minutes and made up to the volume with diluent and mixed well. (200µg/ml impurity).

2.8 Preparation of the Linearity Stock Solution

Transferred 1.0ml from the impurity stock solution and 1.0 ml of Favipiravir stock solutions into a 10 ml volumetric flask and made up to with diluent and mixed well. For Linearity solution preparation refer Table 1.

2.9 Preparation of the Sample solution (Drug Substance)

Weighed accurately and transferred about 20 mg of Favipiravir drug substance into 100ml clean and dry volumetric flask and added about 60 ml of diluent, sonicated for 10 minutes, and made up to the volume with diluent and mixed well. (200µg/ml Favipiravir).

2.10 Preparation of the Sample solution (Drug Product)

Calculate the average weight of 10 tablets and crush to fine powder. Weigh accurately powder equivalent to 20 mg of Favipiravir into a 100 mL volumetric flask and about 50 mL of diluent, sonicate for 10 minutes with intermittent shaking. Attain to room temperature. Dilute up to the volume with diluent and mix well.

2.11 Preparation of Spiked Solution for Precision

Pipette 1 ml of Impurity stock solution into a 100ml of volumetric flask, dilute to volume with diluent, mix well.

2.12 Preparation of the Spiked Sample Solution for Precision

Calculate the average weight of 10 tablets and crush to fine powder. Weigh accurately powder equivalent to 20 mg of Favipiravir into a 100 mL volumetric flask. Add 1 ml of Spiking solution for Precision and about 60 mL of diluent, sonicate for 10 minutes with intermittent shaking. Attain to room temperature. Dilute up to the volume with diluent and mix well.

Table 1. Linearity solution preparation from 25 to 150% level

S. No	Level	Dilution (From Linearity Stock Solution)	Con. of Favipiravir Acid (ppm)	Con. of Favipiravir API (ppm)
1	25%	0.5 ml to 20ml	0.25	0.50
2	50%	1 ml to 20 ml	0.50	1.00
3	75%	1.5 ml to 20ml	0.75	1.50
4	100%	1.0 ml to 10ml	1.00	2.00
5	125%	1.25 ml to 10ml	1.25	2.50
6	150%	1.50 ml to 10ml	1.50	3.00

2.13 Preparation of Spiked Solution for Accuracy

Pipette 1 ml of Impurity stock solution into a 100 ml of volumetric flask, dilute to volume with diluent, mix well.

2.14 Preparation of the 50% Spiked Sample Solution for Accuracy

Calculate the average weight of 10 tablets and crush to fine powder. Weigh accurately powder equivalent to 20 mg of Favipiravir into a 100 mL volumetric flask. Add 0.5 ml of Spiking solution for Accuracy and about 60 mL of diluent, sonicate for 10 minutes with intermittent shaking. Attain to room temperature. Dilute up to the volume with diluent and mix well.

2.15 Preparation of the 100% Spiked Sample solution for Accuracy

Calculate the average weight of 10 tablets and crush to fine powder. Weigh accurately powder equivalent to 20 mg of Favipiravir into a 100 mL volumetric flask. Add 1.0 ml of Spiking solution for Accuracy and about 60 mL of diluent, sonicate for 10 minutes with intermittent shaking. Attain to room temperature. Dilute up to the volume with diluent and mix well.

2.16 Preparation of the 150% Spiked Sample Solution for Accuracy

Calculate the average weight of 10 tablets and crush to fine powder. Weigh accurately powder equivalent to 20 mg of Favipiravir into a 100 mL volumetric flask. Add 1.5 ml of Spiking solution for Accuracy and about 60 mL of diluent, sonicate for 10 minutes with intermittent shaking. Attain to room temperature. Dilute up to the volume with diluent and mix well.

2.17 Preparation of Oxidative Degradation Sample Solution

Calculate the average weight of 10 tablets and crush to fine powder. Weighed accurately powder equivalent to 20 mg of Favipiravir into a 100 mL volumetric flask and added about 60 ml of diluent and sonicated for 10 minutes to dissolve and added 5 mL of 3% Hydrogen peroxide (H_2O_2) solution to the sample containing solution. The resultant solution was kept for 30 minutes at 60°C on a hot water bath. Finally, made up to the volume with diluent and mixed well. (200µg/ml Favipiravir). Injected 1.0 µl of the solution into HPLC and recorded the stability of the sample.

2.18 Preparation of Acid Degradation Sample Solution

Calculate the average weight of 10 tablets and crush to fine powder. Weighed accurately powder equivalent to 200 mg of Favipiravir into a 100 mL volumetric flask and added about 60 ml of diluent and sonicated for 10 minutes to dissolve and added 5 mL of 0.1N Hydrochloric acid (HCl) solution to the sample containing solution. The resultant solution was kept for 24 hours at 60°C on a hot water bath. Finally, made up to the volume with diluent and mixed well. (200µg/ml Favipiravir). Injected 1.0 µl of the solution into HPLC and recorded the stability of the sample.

2.19 Preparation of Alkali Degradation Sample Solution

Calculate the average weight of 10 tablets and crush to fine powder. Weighed accurately powder equivalent to 20 mg of Favipiravir into a 100 mL volumetric flask and added about 60 ml of diluent and sonicated for 10 minutes to dissolve and added 5 mL of 0.1N sodium hydroxide (NaOH) solution to the sample containing solution. The resultant solution was kept for 24 hours at 60°C on a hot water bath. Finally, made up to the volume with diluent and mixed well. (200µg/ml Favipiravir). Injected 1.0 µl of the solution into HPLC and recorded the stability of the sample.

2.20 Preparation of Thermal Degradation Sample Solution

Favipiravir tablets placed on the Petri dish and kept in an hot air oven at 105°C for 6h. After 6 hrs calculate the average weight of 10 tablets and crush to fine powder. Weighed accurately powder equivalent to 20 mg of Favipiravir into a 100 mL volumetric flask and added about 10 ml of diluent and sonicated for 10 minutes to dissolve, and finally made up to the volume with diluent and mixed well. (200µg/ml Favipiravir). Injected 1.0 µl of the solution into HPLC and recorded the stability of the sample.

2.21 Preparation of Photo Stability Degradation Sample Solution

The Favipiravir Tablets was placed in the Photo stability chamber exposing UV light and Visible light at 1.2 million Lux hours and 200-watt hours/minutes respectively. After exposed, calculate the average weight of 10 tablets and crush to fine powder. Weighed accurately powder equivalent to 20 mg of Favipiravir into a 100 mL volumetric flask and added about 60 ml of diluent and sonicated for 10 minutes to dissolve, and finally made up to the volume with diluent and mixed well. (200µg/ml Favipiravir). Injected 1.0 µl of the solution into HPLC and recorded the stability of the sample.

2.22 Preparation of Neutral Degradation Sample Solution

calculate the average weight of 10 tablets and crush to fine powder. Weighed accurately powder equivalent to 20 mg of Favipiravir into a 100 mL volumetric flask and added about 60 ml of diluent and sonicated for 10 minutes to dissolve, and finally made up to the volume with diluent and mixed well. (200µg/ml Favipiravir). Injected 1.0 µl of the solution into HPLC and recorded the stability of the sample.

3. RESULTS AND DISCUSSION

3.1 Method Development

This study aimed to develop a quantification of degradation products and Favipiravir in pharmaceutical dosage form (Tablets). Waters Alliance HPLC system equipped with DAD (Liquid Chromatography equipped with a Diode array detector) and UV as detector, the method was developed to provide the suitability of routine stability studies and QC analysis. The method was optimized to improve the resolution between DPs, symmetrical peak shape, Isocratic mode. To achieve the criteria many experiments were performed to optimize the column, diluent, and mobile phases. **Trail 1**: The initial HPLC method development was initiated using an Isocratic mode using mobile phase with 0.1% orthophosphoric acid and Acetonitrile (50:50) using the Sunfire C18 Column (250 mm x 4.6mm, 10µm) with a flow rate of 1.0 mL/min. The impurity Favipiravir acid peak shape got distorted. **Trail 2**: For the second trial a Isocratic mode using mobile phase with 10 mM Potassium phosphate buffer pH adjusted to 5.5 and Acetonitrile (60:40) using the Agilent C18 (4.6 x 250mm, 5.0µm) with a flow rate of 1.0 mL/min. The impurity Favipiravir acid peak shape merged with the Favipiravir. **Trail 3**: For the third trial a Isocratic mode using mobile phase with 10 mM Potassium phosphate buffer pH adjusted to 5.5 and Acetonitrile (80:20) using the Agilent C18 (4.6 x 250mm, 5.0µm) with a flow rate of 1.0 mL/min. The impurity Favipiravir acid peak shape got distorted. **Trail 4**: For the fourth trial a Isocratic mode using mobile phase with 10 mM Potassium phosphate buffer pH adjusted to 3.5 and Acetonitrile (70:30) using the Agilent C18 (4.6 x 250mm, 5.0µm) with a flow rate of 1.0 mL/min. The impurity Favipiravir acid peak shape good and well resolved from the Favipiravir peak. The analyte and DP peak shape was improved, and the plate count was above 5000. Hence the method was optimized, and above conditions are considered as final method. The final optimised chromatograms of standard solution are shown in the Fig. 3.

3.2 Method Validation

“The analytical method validation on HPLC method was performed (in terms of System suitability, Specificity, Sensitivity, Accuracy, Precision, Linearity, Range, Robustness, and Solution stability) in accordance with ICH guidelines”[22–24].

3.3 System Suitability

It is evaluated by injecting 6 replicate injections of Favipiravir standard solution according to the United States Pharmacopeia (USP) recommendations. The peak asymmetry, theoretical plates, and %RSD for main peak areas were calculated. The results are shown in Table 2.

3.4 Specificity and Forced Degradation Studies

“The analytical method was evaluated for the specificity by injecting the blank and as such sample prepared at the specified concentration (1000µg/mL) and Standard solution (1µg/mL)”. [25] The method was found specific as there is no interference observed in blank and placebo chromatograms at the main peak and DPs retention time, The representative chromatogram of blank, placebo, unspiked and spiked sample were shown in Fig. 4. The Mass balance, % degradation and peak purity at various degradation conditions are given for Favipiravir in the Table 3. The specificity of the method is also evaluated using forced degradation studies following ICH Q1A and Q1B guideline. The sample degradation was performed as per the experimental conditions in Table 3.

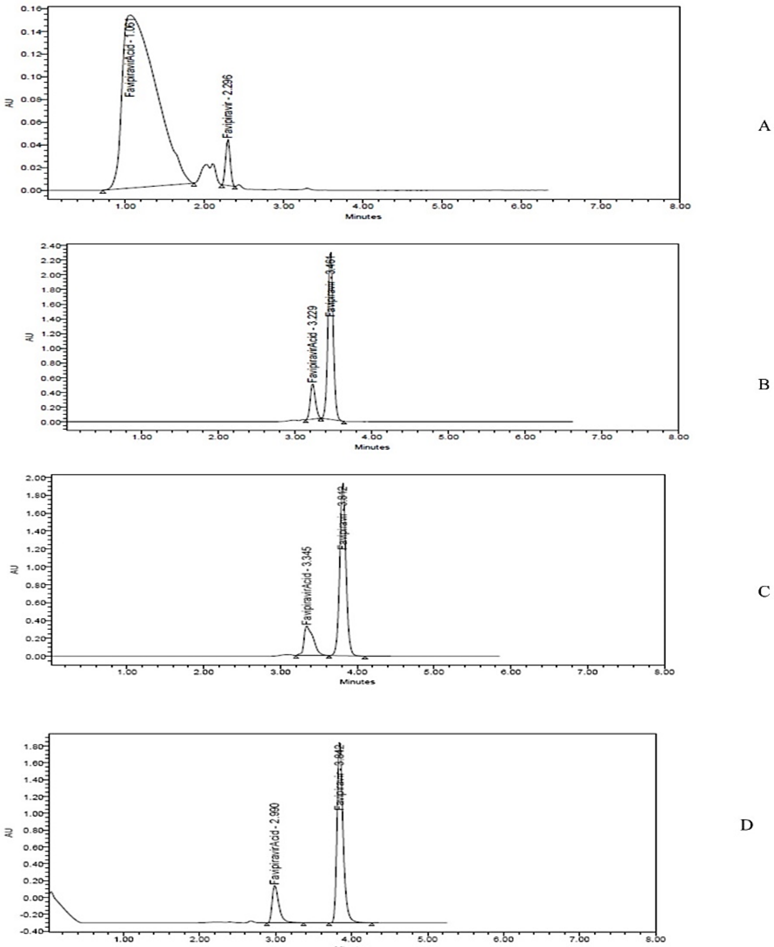

Fig. 2. Representative chromatograms of Trail 1 (A), Trail 2 (B), Trail 3 (c), Trail 4 (D)

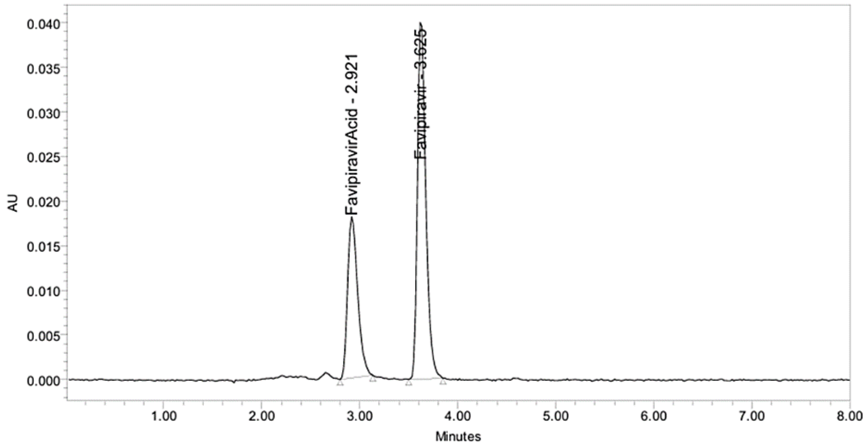

Fig. 3. Representative chromatograms of standard solution (Optimized condition)

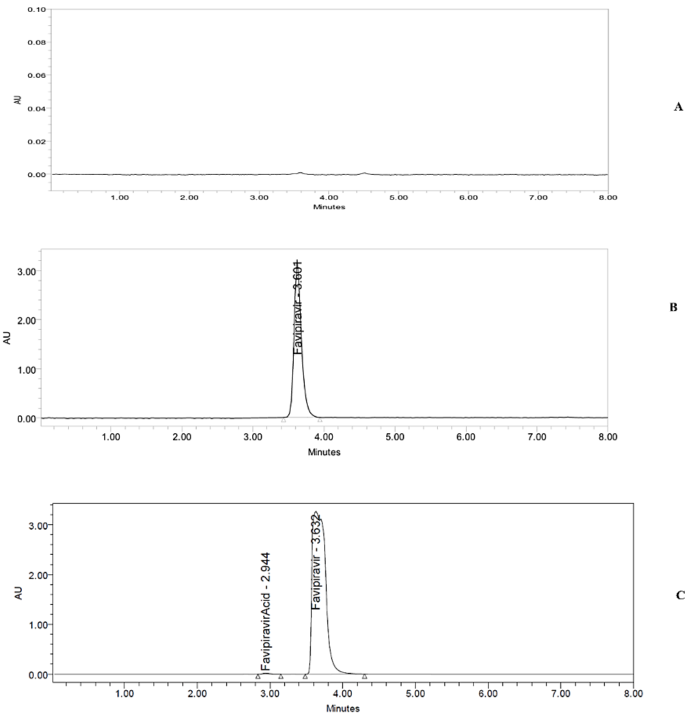

Fig. 4. Representative chromatograms of Blank (A), Un-spiked Sample (B) and Spiked Sample (C).

3.4.1 Acidic degradation

The obtained chromatogram shows significant degradation under the basic condition. The representative chromatogram shown in Fig. 5. The results of the percentage assay, percentage degradation, mass balance and peak purity of Favipiravir are in Table 3.

3.4.2 Base degradation

The obtained chromatogram shows significant degradation under the basic condition. The representative chromatogram shown in Fig. 5. The results of the percentage assay, percentage degradation, mass balance and peak purity of Favipiravir are in Table 3.

3.4.3 Hydrolysis (Neutral)

The obtained chromatogram shows significant degradation under the hydrolytic condition. The representative chromatogram shown in Fig. 5. The results of the percentage assay, percentage degradation, mass balance and peak purity of Favipiravir are in Table 3.

3.4.4 Peroxide degradation

The obtained chromatogram shows significant degradation under the oxidative degradation condition. The representative chromatogram shown in Fig. 5. The results of the percentage assay, percentage degradation, mass balance and peak purity of Favipiravir are in Table 3.

3.4.5 Thermal degradation

The obtained chromatogram shows no significant degradation under the thermal condition. The representative chromatogram shown in Fig. 5. The results of the percentage assay, percentage degradation, mass balance and peak purity of Favipiravir are in Table 3.

3.4.6 Photo degradation

The obtained chromatogram shows significant degradation under the Photo degradation condition. The representative chromatogram shown in Fig. 5. The results of the percentage assay, percentage degradation, mass balance and peak purity of Favipiravir are in Table 3.

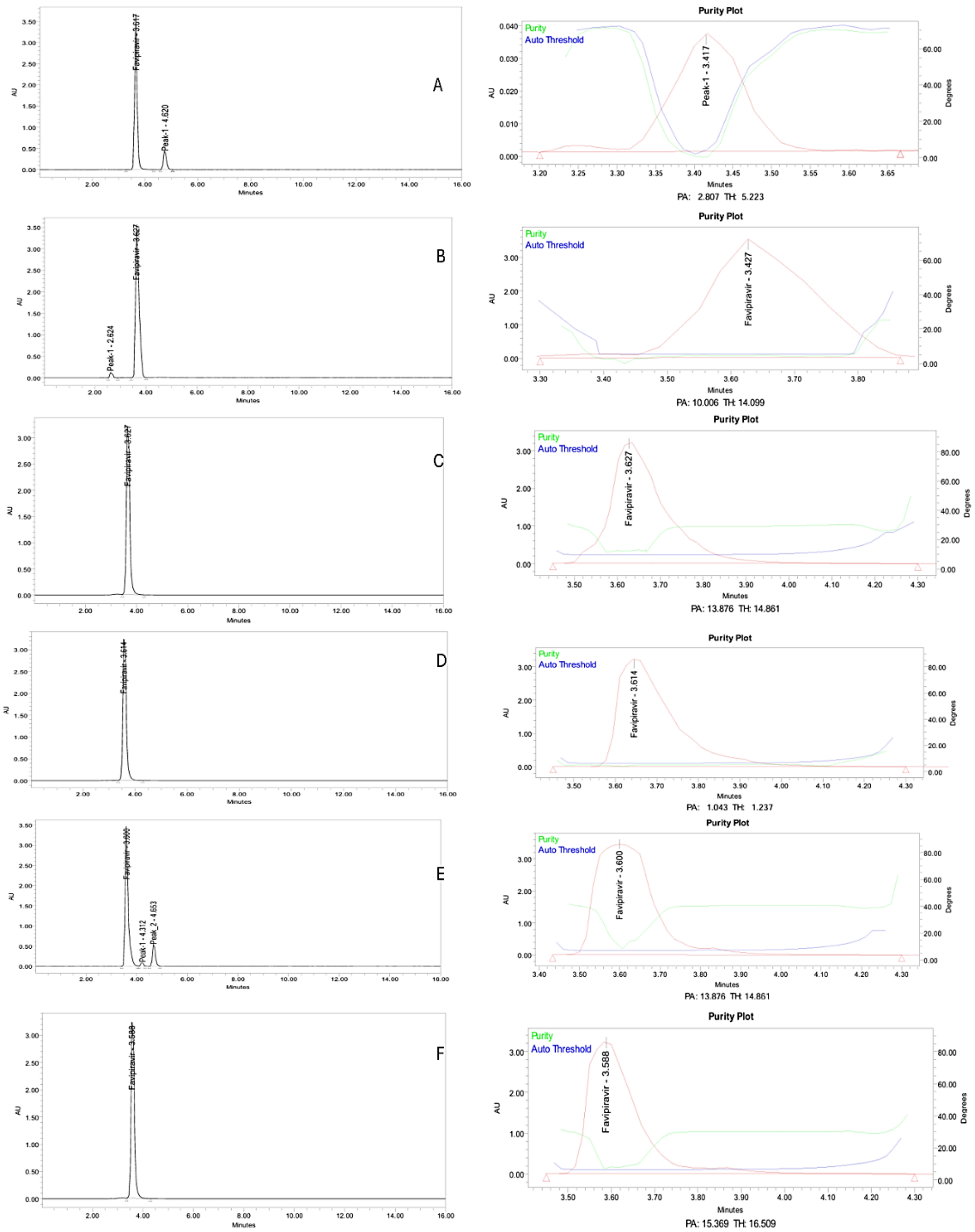

Fig. 5. Representative chromatogram and Purity plots for Acidic (A), Basic (B), Peroxide (C), Neutral (D), Thermal (E) and Photolytic (F) degradation conditions

3.5 Linearity

The analytical method was evaluated for the linearity by injecting the spiked standard solutions of Favipiravir and Favipiravir acid at concentrations ranging from 25 to 150% for more than 6 levels and 3 sets were prepared individually. The calibration curve was obtained by plotting a graph between the average peak areas of 3 sets and the concentrations of Favipiravir and Favipiravir acid. The obtained calibration curve showed a correlation coefficient greater than 0.9999 for both Favipiravir and Favipiravir acid, the method is found to be linear**.** The results are tabulated shown in Table 4. The Linearity plots and chromatograms are represented in Fig. 6 and Fig. 7.

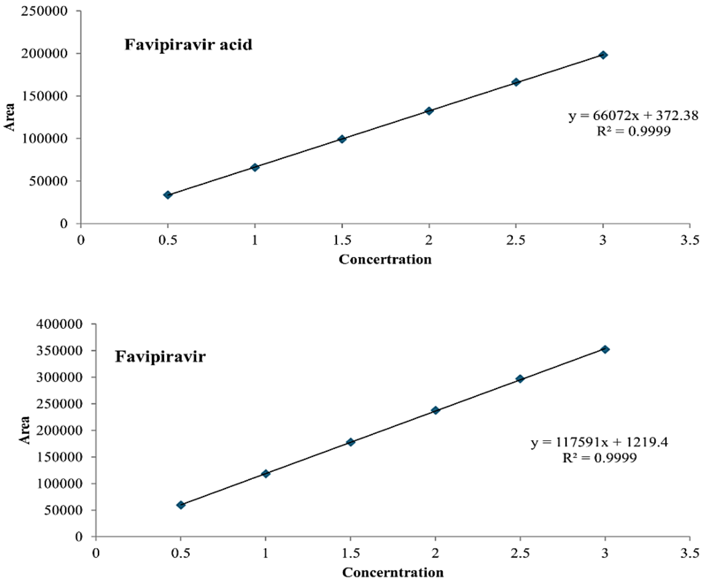

Fig. 6. Representative plots of favipiravir acid and favipiravir

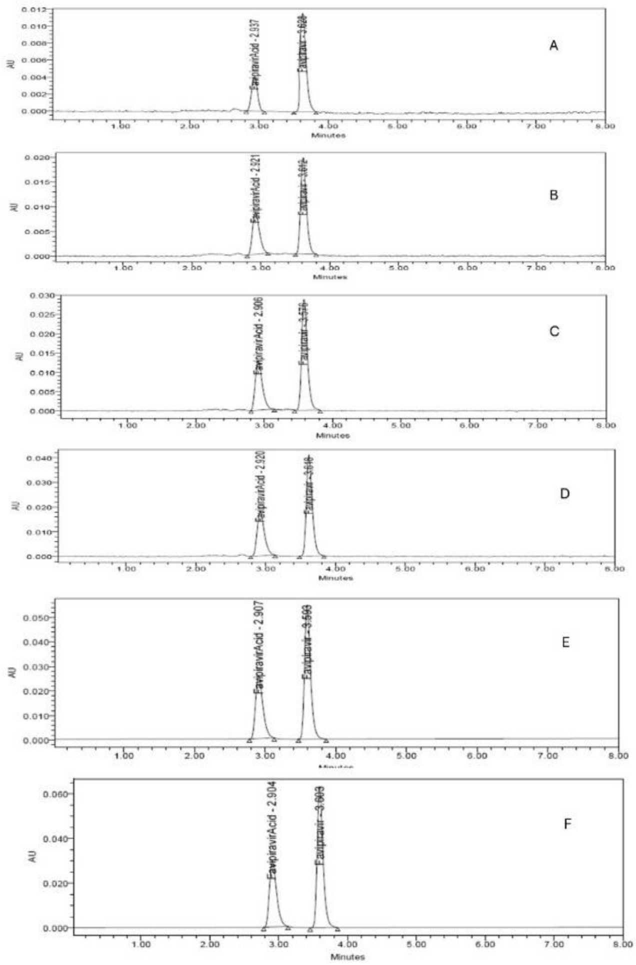

Fig. 7. Representative chromatogram for linearity levels at 25% (A), 50% (B), 75% (C), 100% (D), 125% (E) and 150% (F)

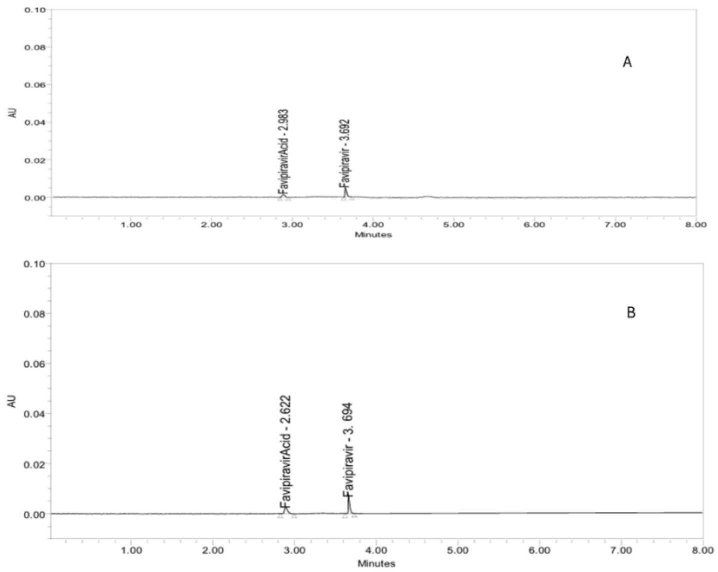

Fig. 8. Representative chromatograms of DL (A) and QL (B) of favipiravir acid and favipiravir

3.6 DL and QL

The DL and QL are defined as the lowest concentration of the analyte, where DL stands for Detection Limit, and QL stands for Quantification Limit. These was evaluated by using the Calibration plot. The calculated DL and QL for Favipiravir are 0.38 ppm and 0.13 ppm respectively, the calculated DL and QL for Favipiravir acid are 0.06 ppm and 0.10 ppm respectively injected into the HPLC. The QL and DL chromatograms are represented in Fig. 8. The precision results of QL are shown in Table 5. The % RSD of the peak areas of each analyte is not more than 10.0%.

3.7 Method Precision

The analytical method was evaluated for method precision by analysing 6 different preparations of Favipiravir spiked with the Favipiravir impurity at the specification level, the %RSD for impurities was calculated and the results are presented in Table 6. The results confirm that the method is precise for determining Favipiravir by HPLC.

3.8 Accuracy

The analytical method was evaluated to determine the accuracy of the method by using the standard addition method. The experiment was performed in triplicate at 50%, 100%, and 150% levels and the % recoveries were calculated. The % recovery values were in the range of 98.66 to 100.86 for Favipiravir acid, which is within the acceptance criteria. The %RSD values of the recoveries obtained for all impurities were less than 1.0. The results are shown in Table 7.

3.9 Solution Stability

The analytical method was evaluated for the solution stability of Favipiravir, Favipiravir acid Impurity was determined by storing the samples in tightly capped volumetric flasks at 25°C and 2-8°C for 48 hrs. The % recovery of samples was calculated against freshly prepared sample solution. The results were found that Favipiravir were stable at 2-8°C and 25°C after 48 hrs.

3.10 Robustness

The analytical method was evaluated for the robustness by deliberate change in the experimental conditions and the system suitability data were recorded. The variables evaluated in the study were column temperature from 25°C to 35°C as Temperature Minus (TM) and Temperature Plus (TP) respectively, the Flow rate from 0.8 to 1.2 mL/min as Flow Minus (FM) and Flow Plus (FP) respectively and mobile phase organic phase change with ±10% as Mobile phase Plus (MP) and Mobile phase Minus (MM). The results met the acceptance criteria, and the results are shown in Tables 8,9 and 10.

Table 2. System suitability parameters and retention time results

	Favipiravir Acid	Favipiravir
	132507	239359
	134239	239606
	131406	240597
	132653	241147
	134038	237427
	132185	238246
Average	132838	239397
STD.DEV	1097.6	1396.7
%RSD	0.8	0.6
Favipiravir USP Theoretical Plate Count	9745	
Favipiravir USP Tailing Factor	1.3	
Sample and impurity ID	**Retention time (min)**	
Favipiravir Acid Impurity	2.921 min	
Favipiravir	3.625 min	

Table 3. Forced degradation conditions for favipiravir and peak purity data

Sample	% Active remaining	% Total Impurities	% Total found (% w/w)	% Mass balance	Peak Purity
Unstressed sample	99.69	0.0	99.66	-	Pass
Light, solution, exposed, (1.2X10^6 lux hours and 200.25-watt hours/square meter of UV energy)	99.35	0.00	99.61	99.61	Pass
Hydrolytic at 60°C for 6 hrs	99.77	0.0	99.16	99.16	Pass
0.1N HCl at 60°C for 24 hrs min.	94.43	4.56	98.99	99.12	Pass
0.1N NaOH at 60°C for 24 hrs	95.02	3.69	98.71	99.06	Pass
3% Hydrogen Peroxide at 60°C for 24 hrs	99.32	0.0	99.24	99.42	Pass
Heat 105°C for 6 Hours.	96.90	3.2	99.14	99.14	Pass

Table 4. Linearity dilutions ranging from 25 to 150%

%Level	Conc (ppm)	Favipiravir Acid	Conc (ppm)	Favipiravir
25	0.25	33747	0.5	59453
50	0.50	66140	1.0	118320
75	0.75	99136	1.5	178005
100	1.00	132390	2.0	237410
125	1.25	166368	2.5	296753
150	1.50	198212	3.0	352081
R^2		**0.9999**		**0.9999**

Table 5. Precision at QL level

Precision at QL		
	Favipiravir	**Favipiravir Acid Impurity**
	10267	8556
	10029	8583
	10409	8436
	10125	8513
	10295	8514
	10193	8591
Average	**10220**	**512**
Standard Dev.	**134.0**	**4.8**
%RSD	**1.3**	**0.9**

Table 6. Method precision at specification level

Sample No.	Sample-1	Sample-2	Sample-3	Sample-4	Sample-5	Sample -6	% RSD
Impurity name	**% w/w**	**% w/w**	**% w/w**	**% w/w**	**% w/w**	**% w/w**	
Favipiravir Acid Impurity	0.098	0.099	0.10	0.099	0.099	0.099	**0.18**

Table 7. Accuracy results for favipiravir acid impurity

% Spike level	Amount found (%w/w)	Amount recovered (%w/w)	Amount added (%w/w)	% Recovery	Mean % Recovery	% RSD
50	0.052	0.048	0.0484	99.42	**99.30**	**0.2**
	0.052	0.048	0.0484	99.38		
	0.052	0.048	0.0484	99.09		
100	0.101	0.097	0.0968	99.98	**100.24**	**0.5**
	0.102	0.098	0.0968	100.86		
	0.101	0.097	0.0968	99.88		
150	0.147	0.143	0.1452	98.66	**98.91**	**0.4**
	0.147	0.143	0.1452	98.71		
	0.148	0.144	0.1452	99.36		

Table 8. Robustness study for flow variations

	FM	FP	FM	FP
	Favipiravir Acid		**Favipiravir**	
	144072	91208	263758	177226
	143723	92855	265211	176622
	144278	93366	263729	179820
	141780	93770	264962	178946
	144608	93767	261795	178761
Average	**143692**	**92993**	**263891**	**178275**
STD.DEV	**1116.2**	**1066.2**	**1353.4**	**1314.0**
%RSD	**0.8**	**1.1**	**0.5**	**0.7**

Table 9. Robustness study for column temperature variations

	TM	TP	TM	TP
	Favipiravir Acid		**Favipiravir**	
	99638	99951	197055	198591
	99020	99191	198872	202647
	98512	102072	198302	200697
	96576	100610	197962	199007
	97803	98875	197873	197647
Average	**98310**	**100140**	**198013**	**199718**
STD.DEV	**1180.3**	**1273.8**	**663.6**	**1975.0**
%RSD	**1.2**	**1.3**	**0.3**	**1.0**

Table 10. Robustness study for mobile phase organic variations

	MM	MP	MM	MP
	Favipiravir Acid		**Favipiravir**	
	127393	125494	236001	235005
	128987	126098	232966	234371
	127705	126946	235097	233514
	129962	128042	234253	234183
	131700	127175	236361	236930
Average	**129149**	**126751**	**234936**	**234801**
STD.DEV	**1758.1**	**986.3**	**1372.8**	**1303.6**
%RSD	**1.4**	**0.8**	**0.6**	**0.6**

4. CONCLUSION

The optimized experimental and validated results confirm that the analytical method on HPLC can quantify degradation impurities, known impurity (Favipiravir acid Impurity) and Favipiravir using suitable stationary and mobile phases. The proposed analytical method was validated according to ICH Q2 guidelines. Favipiravir is found to be susceptible to acidic, basic and thermal degradation conditions but remained stable under peroxide, photolytic and neutral forced degradation conditions. The methodology appears to be a specific, linear, accurate, precise robust, and stability-indicating method, according to the degradation and analytical validation. By employing HPLC, it is possible to quantify impurities and Favipiravir. This method is shown to be specific and with lesser run time quantify both impurities and drug in drug product. This method is useful for the Quality Control Laboratories and the Stability Studies.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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