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Original Article

A Stability Indicating RP-HPLC Method for Estimation of Favipiravir in Tablets

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ABSTRACT

Objectives: The focus of this research work was to develop an accurate and validated RP-HPLC technique for analysing Favipiravir (FAVI) drug and interpreting related degradants in drug and its formulation. **Materials and Methods:** The separation was performed on C_{18} column (Phenomenex Luna BDS Hypersil) with dimension of 4.6x150 mm and 5 µm of particle size at 40ºC in gradient mode. Two solvents mixture was used for analysis. Solvent A (water and 0.01% 10 mM ammonium acetate) and solvent B (acetonitrile) were used as a mobile phase at a rate of 0.5 mL/min and 323 nm wavelength used for detection. **Results:** The validated method was developed as per ICH parameters including LOD, accuracy, linearity, range, LOQ, precision and the observations were found to be satisfactory. The method was then processed for different degradation conditions. The FAVI was treated with various circumstances like hydrolysis, oxidation and photolytic stages for determination of degradation products. Retention time (t_R) was observed at 3.337±0.01 min for FAVI. After stress testing three degradants were found. The first degradant D1 found at 4.170 min (t_R), second D2 at 4.260 min (t_R) and third D3 at 3.881 min (t_R) were found in basic (1 N NaOH), acidic (1 N HCl) and oxidation (30% H_2O_2) conditions. All the degradants were interpreted by mass spectra. The molecular ion [M+1] m/z 156.8 was found in positive mode mass spectra. The various ion peaks were found at m/z 153.1, 130.0, 112.0 and 101.0. FAVI was observed to be degraded in all the conditions used. **Conclusion:** There FAVI drug, its formulations and degradants were identified and interpreted by quadrapole MS/MS system.

Keywords: Favipiravir, RP-HPLC, Stability indicating method, Tablets, Validation.

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INTRODUCTION

A new antiviral drug called Favipiravir (FAVI) is effective against viral infectious disorders including the Ebola virus, Lassa virus and now COVID19.[1,2] It hinders the replication of the viral genome by potently. An intracellular enzyme converts FAVI into FAVI Ribosyl Triphosphate (RTP), which selectively inhibits the influenza virus's RNA polymerase (RNA-dependent RNA polymerase) and stops the virus from replicating.[3] FAVI is used to treat influenza and may also work against other viral diseases (an antiviral medication). Chemically, FAVI (Figure 1) is 6-fluoro-3-hydroxypyrazine-2-carboxamide, molecular weight is 157.10 g mol^{-1} and molecular formula is $C_5H_4FN_3O_2$. The FAVI is a powdery white to pale yellow color. In addition to being less soluble in 99.5% ethanol, it is barely soluble in acetonitrile, methanol and water.[4]

Bulduk published work for comparison of HPLC and UV spectrophotometric methods as well as a separate validated RP-HPLC method for quantification of FAVI.[5,6] He was not reported the stability study of FAVI. In the literature survey, different methods of analysis have been reported for FAVI in drug and drug products.[7,8] Inas *et al.* reported menthol-assisted HPLC/UV method of FAVI determination in blood plasma from human by liquid micro-extraction.[9] Mohammad *et al.* described RP-HPLC method validation of FAVI in spiked blood plasma. FAVI is quantified with spiking human plasma using internal standard acyclovir at wavelength 242 nm with isocrate mode.[10] Duse *et al.* reported FAVI determination by using internal standard as carbamazepine in spiked human plasma.[11] Ibrahim *et al.* reported the Process Related Impurities (PRIs) profiling of FAVI by five multilevel design models. They developed and validated analytical methods for FAVI and two PRIs simultaneously.[12] Literature survey reveals that the UPLC-MS/MS method was developed for FAVI degradation study. In this method no degradation path was reported, and method was identified at 222 nm by UPLC.[13] Other

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LC-MS/MS in human serum were reported for FAVI by Vemuri, *et al.*[14] In this research study, FAVI degradation process related impurities impurity A, B and C were studied at 210 nm as detection wavelength along with root of degradation path. But they used all conditions such as acidic (0.01N HCl), basic (0.1 NaOH) and oxidation (0.05% H_2O_2) in FAVI on use of bench top for 5 days in degradation study of FAVI, hence less than 10% degradation of FAVI was observed. Whereas the present study included the mild as well as strong conditions for degradation of FAVI along with heating the drug. Other methods (LC-MS/MS) were found for determination of FAVI in biological sample by Onmaz *et al.*[15] Two stability indicating HPLC methods were reported in the literature survey.[16,17] But both methods did not identify the degradant for in the research study. Patel *et al.* were reported structure identification of only alkali degradant impurities for FAVI oral suspension.[18] Morzouk *et al.* published research work on degradation kinetic investigations and dissolution (*in vitro*) profiles of FAVI using stability-indicating HPLC-DAD approach.[19] They were reported development and optimizaton of FAVI drug formulation in the dissolution kinetic studies to maintain batch-to-batch homogeneity and the formation of an *in vitro/in vivo* dissolution profile. Ravisankar *et al.* published research work on FAVI with HPLC method development and validation study.[20] But degradants were not identified in FAVI.

According to existing research, the main issues with many published systems include high sample analysis costs, time-consuming processes, expensive initial investments, differing stability conditions and heavy equipment. The present study was focused on RP-HPLC method development and stability-indicating method with all validation parameters for determining FAVI. The method created is very helpful for finding and classifying degradants. We were capable to predict the probable degradant utilising the fragmentation pattern pathway and MS/MS spectra. This information will be helpful to decide the shelf life of FAVI under various circumstances as well as in the development of synthetic production procedures. The chromatographic separation with mass spectrometry was demonstrated to be an extraordinarily straightforward, quick (t_R=5 min). This is useful technology for qualitative and quantitative analysis of degradants in FAVI. The concept was validated using ICH criteria, proving its accuracy, precision, ruggedness and stability. Therefore, the routine investigation of FAVI stability under varied conditions can be conducted by this method.

MATERIALS AND METHODS

Chemicals and Reagents

Glenmark Life Sciences Ltd., Daund, Pune, Maharashtra supplied FAVI API drug. All the HPLC grade solvents such as water, ammonium acetate and acetonitrile were used and procured from Fisher Scientific, India. Formulation of FAVI in tablet dosage form was purchased from local market. The brand names are "Flumyc 400" (FAVI 400 mg) from Ipca Laboratories Ltd., Mumbai, India and "Fabiflu 800 co-pack" (FAVI 800 mg and 200 mg tablet) from Glenmark Pharmaceutical Ltd., Solan, Himachal Pradesh was used.

Instruments

All the analysis were performed on HPLC instrument (Shimadzu, Japan) with diode array detector (PDA). The column oven (CTO-10 ASVP), binary pump (LC 20AD) and autosampler (SIL-HTC) were used for analysis. The degasser (Prominence DGU-20A3) was used to remove the gas from the solutions. The software used for processing and collect the data was LC Postrun. It was chosen to use an octadecyl silane-filled, 4.6x250 mm stainless steel C_{18} column (Phenomenex Luna) with ligands bonded to the silica surface. The mass spectrometer was a Shimadzu HPLC system with an electrospray ionisation technique-equipped MDS SCIEX 4000 QTrap analyzer. Analyst software was used for collection and processing the data for the mass spectra. Photodegradation was examined by photostability chamber and (Vilber Lourmat, 6w, VLC, France) UV lamp with 1.2 million lux hr intensity and 200 Wh/m^2 output.

Throughout the analytical process, Shimadzu Japan Company, AX200 weighing balance with a minimum 0.1 mg capacity was used.

Chromatographic conditions

The C_{18} column for chromatography was BDS Hypersil used. The dimension (4.6x150 mm), particle size (5 µm) and the column temperature (40°C) used for stationary phase. Two solvents mixture was used for analysis in a gradient mode. Solvent A (water and 0.01% 10 mM ammonium acetate) and solvent B (acetonitrile) was used as a mobile phase at rate of 0.5 mL/min. For the first 5 min of the chromatographic separation in HPLC gradient mode, a ratio of solvent A (80%) to solvent B (20%) was used and for the next 3 min, 10% solvent A to 90% solvent B. This was increased up to solvent A (80%) and solvent B (20%) for the following 2 min. The gradient program for mobile phase was shown in Table 1. During the separation analysis, the total run time was 10 min. A membrane filter paper of size 0.45 µm was used to purify solvents and it was degassed using an ultrasonic sonicator for 10 min before being used in the analysis. The sample amount was 10 µL and the wavelength (323 nm) was used. A PDA detector was used for the detection. The identification of ions was performed in positive ionization mode. There were 10 Entrance Potentials (EP) and 40 Declustering Potentials (DP). At a temperature of 500°C and a pressure of 35 psi, nitrogen was being dried. The ion spray voltage in the positive mode was set to 5500 V.

Preparation of Analytical Solutions for FAVI

The FAVI standard (10 mg) was measured accurately and then transferred into a volumetric flask (10 mL), small amount of

Dimethyl Sulfoxide (DMSO) solvent was added, which was then thoroughly shaken and made up the volume with DMSO to form the solution of 1 mg/mL. The various diluted solutions were prepared from stock solutions with other solvents (methanol and water 80:20 v/v). This solutions were utilized to perform validation parameters for FAVI.

Preparation of Tablet Solutions for FAVI

After weighing twenty FAVI tablets, the average weight of FAVI was reported. The fine powder is prepared by triturate it in clean, dry mortar. The fine powder tablet FAVI 10 mg was weighed, mixed in a small amount of DMSO solvent thoroughly in a volumetric flask (10 mL) for conc. 1000 µg/mL. The further sample solutions were diluted with different solvents (methanol and water 80:20 v/v) from above solutions. These all-diluted solutions then pass through the membrane filter paper (0.45 µm) for filtration.

Validation

Linearity and range

In order to conduct the linearity test, different concentrations of FAVI solutions, i.e. 5, 10, 20, 50, 100, 200 µg/mL were prepared from 1000 µg/mL and diluted with other solvent (methanol and water 80:20 v/v). A linearity range of 5-200 µg/mL conc. was selected after trials and 6 distinct concentration levels as above were selected. For calibration curve, peak area was used with respect to conc. The equation reported for slope and Y-intercepts. correlation coefficient (r^2) was calculated to assess the linearity.

Accuracy

Recovery tests for accuracy use a standard addition technique in triplicate using a synthetic mixture process in a laboratory. Three different conc. levels of known amounts of FAVI were introduced to a synthetic mixture with a constant amount of 100 µg/mL. The volume of diluted samples was prepared by diluents, the same as above. The recovery and amount of analyte were estimated using the area of HPLC chromatogram.

Precision

Six replicates of the FAVI standard mixing solution were tested to determine the repeatability of the suggested method. The sample process was assessed on multiple days and by various analyzers inside the laboratory.

Sensitivity

The LOD and LOQ for FAVI were established using diluted solutions of FAVI. The concentrations of known amount for signal-to-noise ratios were reported at 3:1 (LOD). Similarly signal-to-noise ratios were reported 10:1 (LOQ).

Robustness

It was investigated what impact minute but intentional changes to the ideal chromatographic changes imposed for FAVI would have. The various parameters, i. e. change in flow rate of solvent used (10%), wavelength change (±2 nm) and change in column temp. (±10%) have been used for the robustness analysis. All the changes in the above parameters were studied for the three samples (conc. 100 µg/mL). In the observations of robustness, conditional changes were compared with the system suitability parameters of known FAVI.

Solution stability

For reliable and innovative substance stability testing, stress degradation studies are essential for determining the intrinsic stability parameters of the API. When dealing with content of drug, it requires accurate and exact dosing. At the initial stages of preformulation research for generic products, a validated stability-indicating analytical approach is necessary. It guarantees the stability of the API throughout the processes of formulation, manufacturing and storage. Well-designed analysis procedures can help prevent the entrance of inadequate medications into large markets.

Stress degradation study

ICH recommendations were followed when conducting the degradation testing. Several degradation scenarios, including basic, acidic, neutral (hydrolytic), oxidative and photolytic degradation, were applied to FAVI and its tablet formulation. The length of exposure to degradation conditions at a given temperature with various conditions were assessed by the study of FAVI degradation in RP-HPLC. FAVI heated with 1 N HCl (reflux at 80°C for 6 hr) to check degradation in acidic condition. Similarly, FAVI heated with 1 N NaOH (reflux at 80°C for 6 hr) as well as with H_2O (reflux at 80°C for 6 hr). Inject the 100 µg/mL solution to HPLC in same chromatographic conditions were used for hydrolytic degradation study. To check the oxidative

Table 1: Flow of mobile phase for solvent A and solvent B.

Time (min)	Solvent A (%) Pump A	Solvent B (%) Pump B
0.01 min	80	20
3 min	80	20
5 min	10	90
7 min	10	90
8 min	80	20
10 min	80	20

behavior of FAVI, it is heated with 30% H_2O_2 at 80°C (reflux) for 6 hr. To perform photolytic testing, a thin film of FAVI dry solid powder was left in the sun for 15 days. The FAVI drug layer is spread on petridish dish for exposure to light. UV light (200 Wh/m2) and visible light (1.2 million lux hr) were employed for the degradation experiment for 15 days. Up until a significant degradation was attained, all of these stress conditions were applied. The samples for degradation study were diluted and analyzed in the same instrumental conditions. The solution was injected in HPLC for the analysis. For stress study, the untreated samples were compared with the treated samples.

RESULTS

The objective of current research was to develop a validated RP-HPLC technique and stress degradation study for FAVI. Using mass spectrometry data FAVI degradant structure was elucidated. The RP-HPLC chromatographic technique was developed to examine significant impurities in FAVI. FAVI was introduced in the instrument along with all stress samples for the study as mention in chromatographic conditions. FAVI is very soluble in DMSO. The diluent DMSO for preparation of standard stock solution was chosen for FAVI drug because of the nature of solubility and its structure. Further dilutions were prepared using methanol and water (80:20 v/v) for final concentrations. For the separation of the FAVI and its degradants, different solvents with variations in combinations (ratios) were tried as a mobile phase. Based on the trials, gradient method of separation and mobile phase was selected. The sample injection, flow rate and investigation wavelength were used as described in chromatographic conditions throughout the analysis. The study employed a positive ionization-mode-operating Turbo ion Spray interface (TIS) under mass spectrometric settings. The potential was 40 and 10 was used as declustering and entry potential for analysis. A pressure of 35 psi, temperature of 500°C, voltage for ion spray 5500 V for positive mode and nitrogen gas were maintained for analysis of FAVI mass spectra. Retention time (t_R) was observed at 3.337±0.01 min. HPLC peak for FAVI was depicted in Figure 2.

Method Validation

Linearity and Range

Plotting peak area versus concentration used for the determination of the slope, Y-intercepts and correlation coefficient (r^2) for each FAVI concentration (1-200 µg/mL). The linear regression equations were found to be y=24590x-12344. The concentration levels for the FAVI method were linear, with regression coefficients of 0.9999. Graph of FAVI linearity is shown (Figure 3).

Accuracy

The concentrations of 25, 50 and 75 µg/mL were chosen from the FAVI sample curve ranges to evaluate the accuracy. In triplicate, the recovery of FAVI was evaluated at 50 µg/mL and level of 100% and 150% therapeutic component was selected. The sample's FAVI recovery demonstrated a high degree of quantitative proficiency. Table 2 displays the accuracy results.

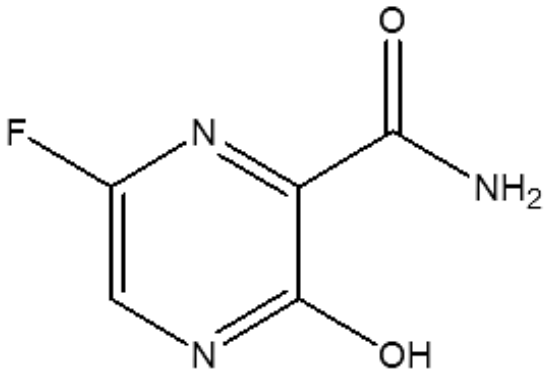

Figure 1: Structure of favipiravir.

Table 2: System suitability parameters and accuracy data for FAVI by HPLC.

Parameter	Results
No. of theoretical plate (N)	14138
Retention time (t_R)	3.337±0.01
Symmetry factor (A_s)	1.258
Linearity and range	1-200 µg/mL
Regression equation (y=mx+c)	y = 24590x-12344
Correlation coefficient (r^2)	0.9999
Accuracy (50%)* (mean±SD)	100.71±1.9538
Accuracy (100%)* (mean±SD)	101.80±0.3601
Accuracy (150%)* (mean±SD)	100.89±1.0047
Limit of Detection	0.5 µg/mL
Limit of Quantification	1 µg/mL

*Three determinations average.

Table 3: Results of intra-day and inter-day precision study for FAVI by RP-HPLC.

Level of quality control	Concentration of sample (µg/mL)	Area of chromatogram*	Amount found (µg/mL)*	% Assay	SD	% RSD
Intra-day precision						
LQC	2	52629	2.01	101.14	1.82	1.83
MQC	40	1247332	39.89	99.72	0.92	0.92
HQC	150	3321954	149.44	99.62	1.07	1.08
Inter-day precision						
LQC	2	51396	1.98	99.51	0.90	0.90
MQC	40	1223999	38.14	97.86	0.64	0.65
HQC	150	3358620	150.86	99.68	1.25	1.25

Mean of three replicants.

Table 4: Evaluation data for robustness study of FAVI.

Robustness parameters	t_R min	Peak area*	% Assay ± SD	RSD
Flow rate (0.4 mL/min)[a]	3.310	2438750	98.9644±0.7137	0.7211
Flow rate (0.5 mL/min)[a]	3.274	2517749	102.1703±1.2565	1.2298
Flow rate (0.6 mL/min)[a]	3.337	2412081	97.8823±0.5399	0.5516
Wavelength (321 nm)[a]	3.352	2458750	99.7761±1.7653	1.7693
Wavelength (323 nm)[a]	3.326	2447748	99.3297±0.7022	0.7070
Wavelength (326 nm)[a]	3.338	2462083	99.9113±1.5516	1.5529
Column oven temperature (30°C)	3.342	2492082	101.1287±0.1344	0.1329
Column oven temperature (40°C)	3.331	2462060	99.9104±0.9448	0.9456
Column oven temperature (50°C)	3.329	2424413	98.3827±0.4297	0.4368

[a] Mean of three replicants.

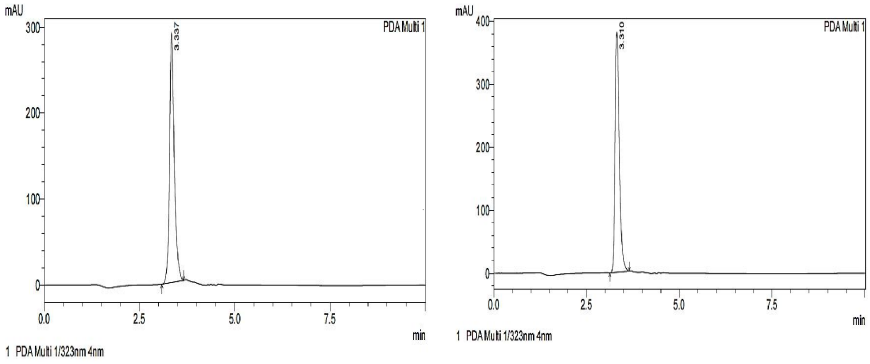

Figure 2: HPLC chromatogram of (A) FAVI drug and (B) FAVI tablet (100 µg/mL).

Precision

As per the ICH's requirements, the developed HPLC technology was assessed for intermediate and intraday precision research study. The percentage RSD was determined by observing the 6 replicates of FAVI samples. Intra-day precision (same day) was observed with FAVI samples. The inter-day precision (different days) for FAVI was examined on three consecutive days. Table 3 displays the precision results.

Robustness

When the chromatographic parameters, such as mobile phase flow rate, wavelength and column oven temperature were purposefully modified in small variations. All the analytes were

found to resolve at the same t_R. The flow rate change in ±0.1 mL/min, wavelength change in ±2 nm and column temp. Change in ±10% were studied for robustness. The claimed t_R, peak area and RSD all fall within acceptable parameters. Table 4 displays the robust results.

Quantitative aspects

To assess LOD and LOQ signal-to-noise ratios 3 and 10 were used for analysis of FAVI. After injection, FAVI exhibits enhanced sensitivity for detection LOD of 0.5 µg/mL and quantification LOQ of 1 µg/mL.

Degradation behavior of the FAVI

FAVI substantially deteriorates in hydrolytic and oxidative environments but is stable in photolytic and neutral treatments. Table 5 shows the degradation study of FAVI. Mass Spectrometry (MS/MS) was used to fragment the FAVI mass spectra at Entry Potential (EP) 10 and Declustering Potential (DP) 40. First, mild conditions such as NaOH (0.1 N), HCl (0.1 N) and hydrogen peroxide (3%) were used to test the degradability of FAVI. The sample was heated for 6 hr at 80°C. All these conditions had no impact on the chromatogram. The degradation of FAVI was then investigated using NaOH (1 N), HCl (1 N) and H_2O_2 (30%). Certain contaminants or derivatives of FAVI were analysed as derivative 1 (D1), Derivative 2 (D2) and Derivative 3 (D3) under various conditions given by HPLC. All HPLC chromatograms and mass spectra shown in Figure 4. Ion fragmentation peaks, based on data from MS/MS mass spectra and HPLC chromatograms were interpreted under various stress situations. All the fragmentation patterns of FAVI structure were interpreted in the scheme as depicted in Figure 5. For the comparison in the analysis of FAVI, blank samples run first followed by sample to avoid any excipients interference. Chromatograms and mass spectra from commercial tablets were compared with those from blank samples. It was found that the m/z 156.8 peak of FAVI in MS/MS spectra observed. Other fragments of FAVI were seen as m/z 114.07, 123.11 and 139.03 in MS/MS spectra by [M+H]+ESI mode. It is hypothesised that m/z 114.07 corresponds to the 5-fluoropyrazin-2-ol ion ($C_4H_3FN_2O$), m/z 123.11 corresponds to the pyrazin-2-carboxamide ion ($C_5H_5N_3O$) and m/z 139.03 relates to the 6-fluoro-3-hydroxypyrazine-2-carbonitrile ion produced.

Alkaline condition

For the basic condition (AK), FAVI was heated with NaOH (1 N) for 6 hr (1 mg/mL). This conc. (100 µg/mL) was then injected to the HPLC instrument while keeping the same instrumental and experimental parameters. It was discovered that the FAVI Retention Time (t_R) was 3.341 min. At t_R 4.170 min, another peak (D1) was found. In the mass spectrometer the identical solution was employed by $[M+H]^+$ ESI-MS/MS mode. In the mass spectrum shown in Figure 4, D1 fragments that were not visible

Table 5: Results of Degradation products of FAVI.

Stress condition	degradant Product	t_R (min)	Exact mass	Mass of fragments (m/z)	Degradation (%)
1N NaOH	D1	4.170	158.1	79.1, 106, 139.1	64%
1 N HCl	D2	4.260	130.1	127.0, 117.1, 83, 76	81%
30% H_2O_2	D3	3.881	105.1	64.1, 79.1, 158.1	79%

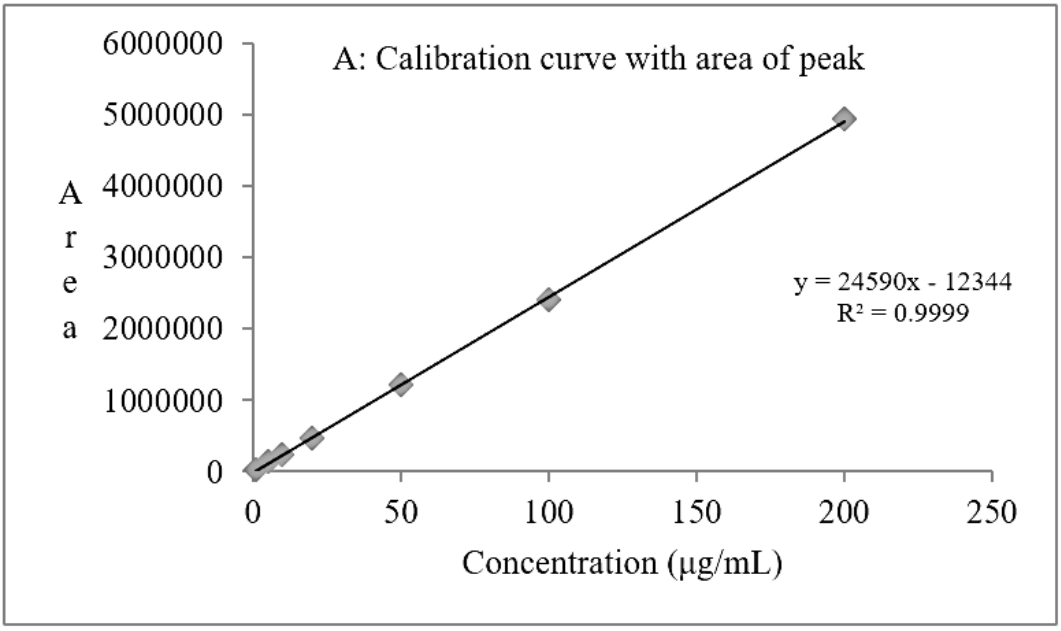

Figure 3: Calibration curve for linearity of FAVI with area of peak.

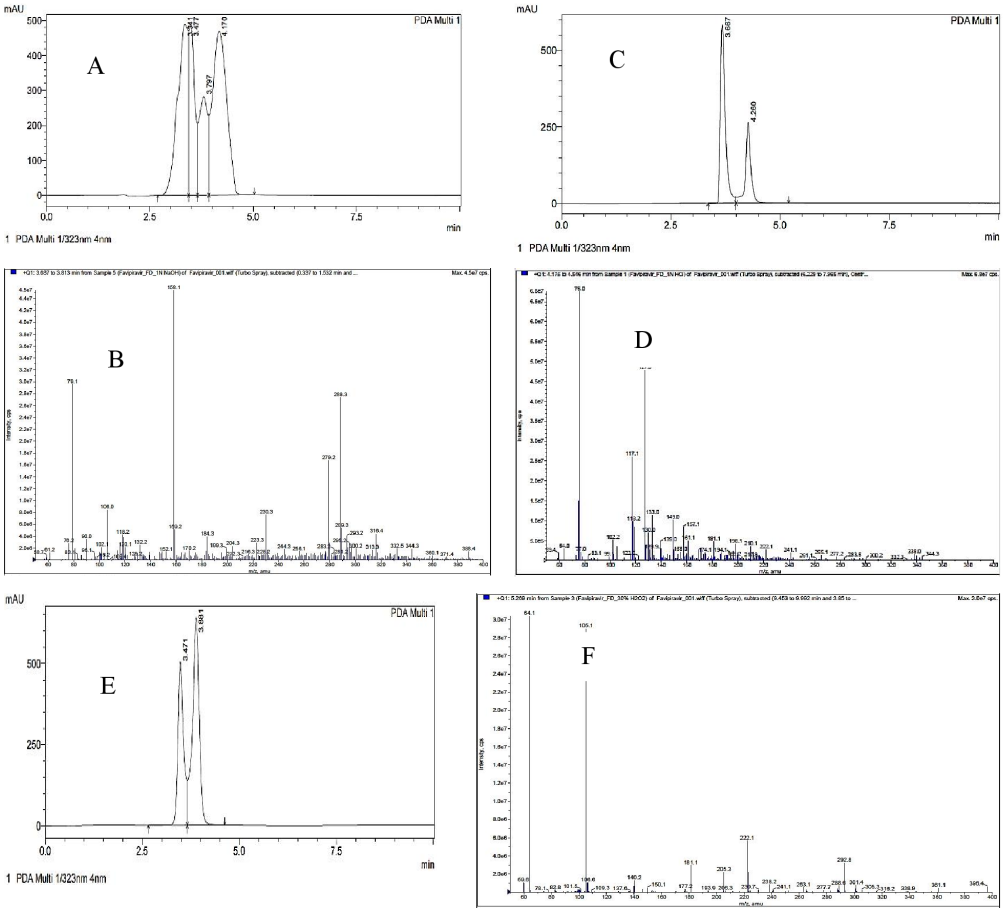

Figure 4: HPLC peaks and MS/MS spectra of FAVI in different stressed conditions. FAVI exposed to base, tR at 4.170 (D1) and MS/MS spectra with m/z 158.1, 139.1, 106, 79.1 fragment ion observed (A and B). FAVI exposed to acid, tR 4.260 min (D2) and MS/MS spectra with m/z 130.1, 127, 76 fragment ions observed (C and D). FAVI exposed to oxidation, tR 3.881 min (D3) and MS/MS spectra with m/z 105.1, 64.1, 79.1, 158.1 fragment ions observed (E and F).

in pure FAVI were seen as m/z 158.1, 139.1, 106.0 and 79.1. This m/z 158.1 was observed to have the chemical formula ($C_4H_4N_2$) because of oxidation. This shows that an alkaline media is where the degradant D3 was found.

Acid condition

FAVI was heated with HCl (1 N) for four hr at 80°C (1 mg/mL). The solution was introduced to the HPLC instrument under the same chromatographic conditions. The FAVI showed 3.341 min Retention Time (t_R) value. While at t_R 4.260 min, a Different peak (D2) was found. Fragmented (D2) as m/z 130, 127.0, 117.1, 83 and 76 (base peak) was found in mass spectrum (Figure 4). In acidic solution the extra peaks observed which are not seen in pure FAVI.

Oxidation condition

In the oxidation (30% hydrogen peroxide) condition (OX), FAVI was heated at 80°C for 8 hr (1 mg/mL). A diluted (100 µg/mL) solution was prepared from this oxidised solution and put into the HPLC instrument with the same chromatographic settings. Figure 5 shows that the FAVI Retention Time (t_R) was 3.471 min. While at t_R 3.881 min, another peak (D3) was discovered. The mass spectrum observed in ESI-MS/MS positive mode shown in Figure 4, D3 fragmented featured m/z 105.1, m/z 64.1, m/z 79.0 and m/z 158.1, which were not seen in pure FAVI. This m/z 105.1 was observed to have the molecular formula ($C_5H_3N_3$) as a result of oxidation. The result after oxidation state of FAVI showed that degradant D3 was detected.

Photochemical degradation

FAVI was exposed to sunlight for fifteen days. After exposure, degradation of drug not observed. Another source for photochemical behaviour used is ultraviolet chamber with 1.2 million lux h of visible light and 200 Wh/m^2 of Ultraviolet (UV) radiation used. In this condition FAVI exposed to 15 days. FAVI showed no change due to light. It means that there is no degradation in this condition observed.

DISCUSSION

In the current work, for routine quality control of tablet formulations containing FAVI was developed. The effectiveness of the method to determine the quantity accurately FAVI was not assessed in the many reported techniques. The approach is time-consuming and generally used for impurity quantitation, even though RP-HPLC techniques have reported for measurement of FAVI in the presence of impurities. The analysis did not take too long because the analyte FAVI was separted with an appropriate t_R=3.337±0.01 min. The approach is appropriate for FAVI formulation because of its linearity in the 1-200 µg/mL range. According to ICH criteria, the approach was validated. The standard addition method revealed that the percent recovery fell within the range of 102.72-98.82%, demonstrating the accuracy of the method and its ability to quantify FAVI just when excipients are present in the formulation. The degradation of the FAVI was seen after treatment under diverse circumstances. The data from studies on precision, robustness and ruggedness demonstrated that the suggested method's test results were not affected by operational factors. Forced degradation tests under various conditions were carried out to determine the capability of the presented technique to determine the quantity of FAVI in presence of its degradants. The suggested procedure was used to analyse the treated samples. FAVI was found to be stable under photochemical stress and neutral hydrolysis conditions, while it degraded under acid, base and oxidative stress conditions.

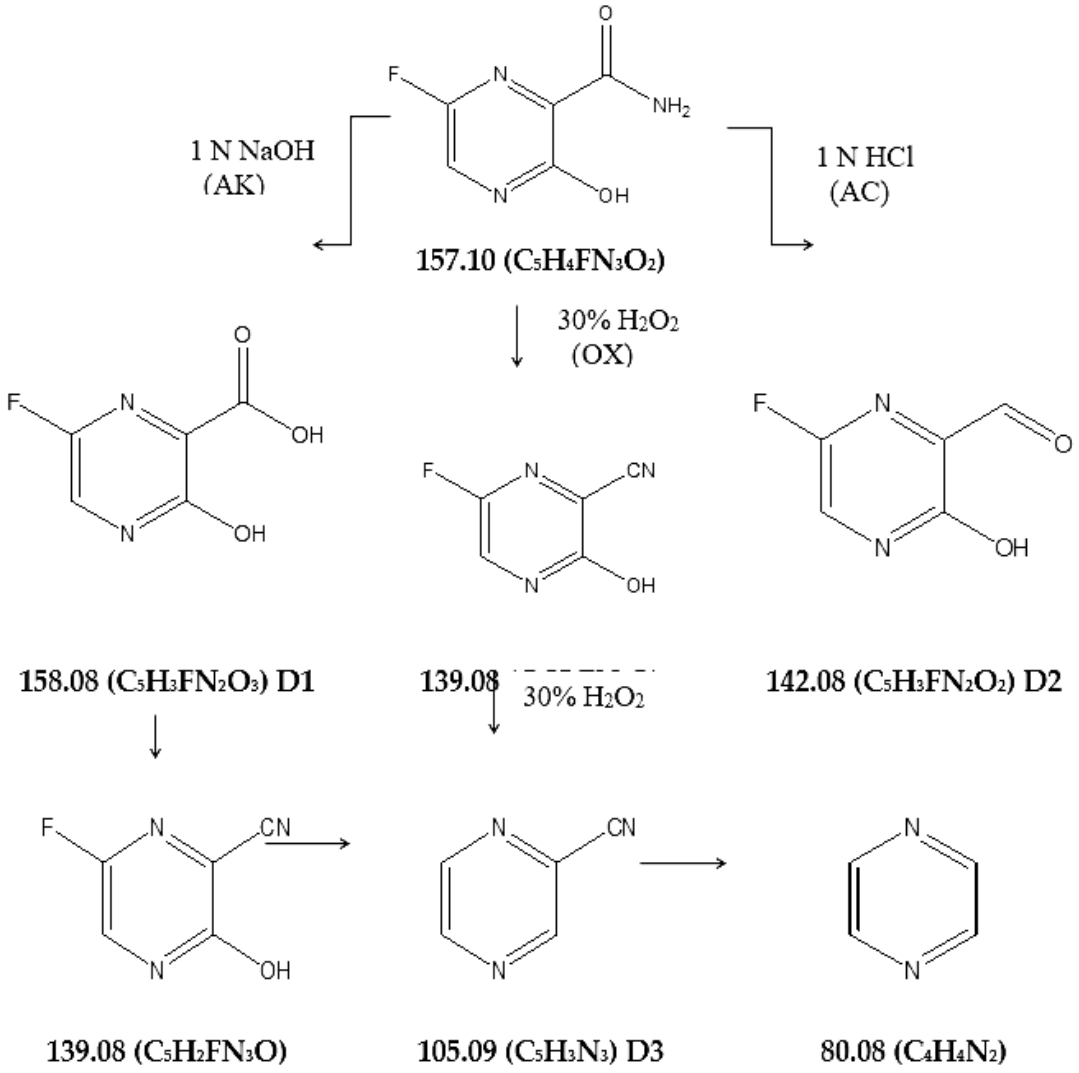

Figure 5: Elucidation of structure for the degradation products formed during FAVI exposal to various stress conditions.

The specificity of the approach was demonstrated by the ability to selectively quantify FAVI and isolate the FAVI peak from its degradation product peak. High-resolution ESI-MS/MS techniques were used to confirm the degradation of products identification.

CONCLUSION

According to ICH guidelines, the current analysis clearly demonstrated the purpose for developing and validating stability-indicating FAVI RP-HPLC technology under various stress conditions. High-resolution ESI-MS/MS studies revealed that the FAVI drug degradation products were found in three distinct stress states. The RP-HPLC procedure proved to be a very effective instrument for detecting and separating these degradants from FAVI. The different techniques such as linearity, accuracy, LOQ, LOD, precision and ruggedness were determined and tested for validation. FAVI tablets were analysed by these techniques. The proposed analytical method was simple and speedy HPLC technique. It avoids using more chemical also more economical and affordable techniques. The results of the FAVI degradation study bring up new pathways for understanding and researching drug stability, leading to the development of more tools for quality control and safer procedures.

ABBREVIATIONS

FAVI: Favipiravir; **HPLC:** High Performance Liquid Chromatography; **ICH:** International Conference on Harmonization; **LOD:** Limit of Detection; **LOQ:** Limit of Quantitation; **SD:** Standard Deviation; **RSD:** Relative Standard Deviation; $\mathbf{t_R}$**:** Retention Time.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

The aim of research work was to develop and validate stability-indicating FAVI RP-HPLC method under various stress conditions. All the validation parameters were tested as per ICH guideline for FAVI tablets. The proposed analytical method was simple, fast and accurate HPLC technique. The results of the FAVI degradation study were used in quality control test.

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