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A novel stability-indicating HPLC-DAD method for determination of favipiravir, a potential antiviral drug for COVID-19 treatment; application to degradation kinetic studies and in-vitro dissolution profiling

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ABSTRACT

Modern pharmaceutical analysis is paying a lot of attention to the stability of novel drug formulations as well as establishment of suitable stability-indicating approaches. In the current work, a comprehensive stability-indicating HPLC-DAD method has been developed and validated for determination of favipiravir (FAV) which is a novel and emerging antiviral option in COVID-19 treatment. The stability of FAV was examined under different stress conditions. FAV was found to be susceptible to acid, base hydrolysis and oxidative degradation. Structure elucidation of the forced degradation products was carried out using mass spectrometry (MS) operated in electrospray ionization mode. Effective separation of FAV and its induced degradation products was achieved using isocratic elution mode on Zorbax C₁₈ column maintained at 30 °C. The mobile phase used was comprised of 25.0 mM phosphate buffer (pH 3.5 ± 0.05) containing 0.1% (w/v) heptane sulphonic acid sodium salt-methanol-acetonitrile (62:28:10, by volume), delivered at flow rate of 1.0 mL/min. The diode array detector signal for FAV was monitored at 321.0 nm over a concentration range of 6.25–250.00 µg/mL. The potential mechanisms for generation of degradation products were postulated through comparison of MS¹ fragmentation pattern of FAV and its degradation products. Moreover, the proposed method was also extended to study the degradation kinetics. Additionally, dissolution profiling of FAV in different media was monitored. Clearly, the suggested approach is accurate, reliable, time-saving, and cost-effective. As a result, it may be utilized for regular quality control and stability assessment of FAV in its tablet dosage form.

1. Introduction

The coronavirus disease (COVID-19) outbreak across the world encouraged most researchers to exert effort to develop treatment strategies to overcome the fast progression of this respiratory disease. Favipiravir (FAV) is one among potential drugs that may possibly be used in the management of COVID-19 infection [1]. FAV is a pyrazine carboxamide derivative, that showed effective antiviral activity against a variety of RNA viruses including influenza A virus, adenovirus, and SARS Corona virus [2].

Innovation of FAV was reported firstly by Fujifilm Toyama Chemical Company. Later it was approved in Japan to be used for treatment of influenza. FAV undergoes an intracellular phosphoribosylation to be converted into an active form, FAV-RTP (favipiravir ribofuranosyl-5'-

triphosphate), which is recognized as a substrate by the RNA-dependent RNA polymerase (RdRp), suppress the RNA polymerase activity, acts as a competitor with purine nucleosides and can interfere with viral replication [3]. FAV is chemically recognized as 5-fluoro-2-oxo-1H-pyrazine-3-carboxamide, Fig. S1 [3]. Currently, FAV is being used for mild and moderate SARS-CoV-2 infections caused by coronavirus (COVID-19) and approved in many countries, including Japan, Italy, Bangladesh, Turkey, Egypt, India, Russia, KSA and UAE [4].

Chemical stability of new drug molecules has great importance in the study of safety and efficacy of the drug product, it aids in the selection of appropriate formulation and package, moreover providing proper storage conditions and shelf life. Forced degradation of drug products at drastic conditions is used to identify the potential degradation products and further helps in establishing the degradation pathways and

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validation of stability indicating methods [5].

Upon literature survey, few methods were reported for determination of FAV in biological fluids and/or pharmaceutical formulations using different analytical techniques; UV–VIS spectroscopy methods [6,7] and chromatographic methods [8–17]. Although, few HPLC methods were previously reported but up to our knowledge no stability-indicating HPLC method has been published yet for determination of FAV.

Accordingly, the proposed work aims to develop and validate for the first time a stability-indicating HPLC-DAD for determination of FAV in its pharmaceutical dosage form. The chemical stability of FAV was evaluated under different stress conditions such as hydrolysis, oxidation, photolytic, and thermal stress conditions. All potential degradation products were characterized via mass spectrometry and the degradation pathways were postulated. The proposed method was validated and optimized for quantification of intact drug in presence of possible degradation products and enable studying the kinetic order of the degradation reactions. Finally, in-vitro dissolution method is investigated for FAV as there is no official dissolution medium available in the literature.

2. Materials and methods

2.1. Experimental

Analysis was performed on Alliance HPLC system (Waters, USA) equipped with quaternary pump (M00SM4493M), autosampler (M17VSM029N), column oven (B18HLP981G) and photodiode array detector (2998PDA).

An Exion LCTM series UHPLC system (AB Sciex, USA) operated with AB Sciex QTRAP® 5500 mass detector (AB Sciex instruments, USA) worked in negative electrospray ionization (ESI) using Multiple Reaction Monitoring (MRM) mode was used for structure characterization of the forced degradation products. Mass spectrometric operating conditions were curtain gas, 20 psi; ion source gas 1, 30 psi; and ion source gas 2 pressure, 30 psi; source temperature, 600 °C; ion-spray capillary voltage, 5500 V and collision gas, 20 psi. Data acquisitions and LC-MS/MS parameters were controlled using Analyst version 1.6.3 software (Applied Biosystems/MDS Sciex, Concord, Canada).

Dissolution monitoring was achieved using eight-station Distek model 2500I, USP dissolution test system (Distek Incorporation Technologies, USA) in accordance with USP general dissolution method [18]. The dissolution media were carefully deaerated using vacuum filtration and the temperature was thermostatically maintained at 37.0 ± 0.5 °C throughout the study.

2.2. Chemicals and reagents

Favipiravir standard (FAV) was provided by BDR Pharmaceuticals International Pvt. Ltd. (Mumbai, India) with purity of 99.98% (according to supplier certificate of analysis). Avigan® tablets, produced by Fujifilm Toyama Chemical Co., Ltd, Japan, Batch No. FG1881, labelled to contain 200 mg FAV per tablet, was purchased from the local market.

Methanol, acetonitrile, monobasic potassium phosphate, heptane sulphonic acid sodium salt, orthophosphoric acid (HPLC grade) were purchased from Sigma-Aldrich (Milwaukee, USA). Hydrochloric acid (36%), sodium hydroxide, hydrogen peroxide (30%), analytical grade, were obtained from Merck (Mumbai, India). Ultra-pure deionized water was obtained from MilliQ UF-Plus water purification system (Millipore, Bedford, MA, USA).

2.3. Liquid chromatographic conditions

HPLC-DAD analysis and chromatographic separation was carried out on a reversed-phase Zorbax C₁₈ column (5 µm, 4.6 × 250 mm; Agilent technologies Inc, USA) using isocratic elution with a mobile phase

consisted of 25.0 mM phosphate buffer (pH 3.5 ± 0.05) containing 0.1% (w/v) heptane sulphonic acid sodium salt- methanol–acetonitrile (62:28:10, by volume) pumped at flow rate 1.0 mL/min. The column temperature was kept at 30 °C. For each sample, 5 µL was injected in triplicates. DAD detection was performed at 321.0 nm.

2.4. Standard solutions

Stock standard solution of FAV (500.0 µg/mL) was prepared in methanol. Working standard solutions were then prepared by diluting appropriate aliquots of the stock solution in deionized water. While, the stock solutions of the acid, base and oxidative degradation products were prepared in a concentration of 500.0 µg/mL in deionized water.

Different laboratory prepared mixtures containing different ratios of FAV and its corresponding degradation products were prepared by accurately transferring suitable aliquots from their respective working standard solutions (100.0 µg/mL) into a series of 10-mL volumetric flasks.

2.5. Linearity and construction of calibration curve

Linearity of FAV was checked over a concentration range of 6.25–250.00 µg/mL. The prepared solutions were injected, in triplicates, into the HPLC-DAD system using the previously stated chromatographic conditions. In order to construct the calibration graph, peak area values were plotted against the corresponding concentrations of FAV and the regression equation was computed.

Analytical method validation was consequently evaluated for accuracy, precision, linearity, specificity, limit of quantitation and robustness in accordance to ICH guideline (ICH (Q2 R1), 2005) [19].

2.6. Stress stability studies

Stress studies were carried out as per ICH guidelines [20] under different acid, base, oxidative, photolytic and thermal conditions.

Acid and basic hydrolysis were investigated by dissolving a mass of 50 mg FAV in different aqueous hydrochloric acid or sodium hydroxide solutions (0.1, 0.5, 1 N), separately, into screw cap tubes and left for 24 h protected from light at room temperature. Samples were withdrawn then neutralized with pre-calculated volume of sodium hydroxide or hydrochloric acid.

For oxidative studies, a mass of 50 mg FAV was separately incubated with 3, 10, 30% hydrogen peroxide aqueous solutions and protected from light at room temperature for 24 h. Samples were withdrawn and evaporated to dryness then re-dissolved in deionized water.

Photo-stability was investigated by exposing FAV stock solution (500.0 µg/mL) to direct sun light for 6 h.

Lastly, thermal stress testing of FAV in solid state was carried out by keeping the drug in a thermostatically controlled oven maintained at 105 °C for 6 h to study the effect of dry heat. Samples were withdrawn at regular intervals, to monitor the degradation process, with appropriate dilutions using deionized water and analyzed by the previously described LC method.

2.7. Analysis of FAV in its pharmaceutical formulation

Ten Avigan® tablets (each tablet labelled to contain 200 mg FAV) were separately weighed, grinded and finely powdered. An amount equivalent to average weight of one tablet was accurately transferred into 100-mL volumetric flask and dissolved in 60 mL methanol, sonicated for 10 min. The volume was then completed to mark with methanol and filtered through filters of 0.22 µm pore size (Millipore, Milford, MA). Appropriate dilutions were made with deionized water. The constructed calibration graph was used to estimate the concentration of FAV in tablets via the corresponding regression equation. For standard addition technique, different known concentrations of pure FAV

standard were added to known concentrations of the pharmaceutical dosage form and processed as described before. The recovered concentration of added FAV was obtained from corresponding regression equation and then the mean percentage recovery and the standard deviation were calculated.

2.8. Degradation kinetic studies

To monitor degradation kinetics, aliquots of FAV stock solution (500.0 µg/mL) were equally transferred into a series of 100-mL volumetric flasks then subjected to the aforementioned acid (20 mL 0.1 N HCl), alkali (0.5 N NaOH), and oxidative (10% H₂O₂) stress conditions, separately, at room temperature. At different time intervals, samples were transferred, neutralized or evaporated (if required), diluted with deionized water and analyzed sequentially by the proposed HPLC method and the remaining FAV concentration was estimated from its regression equation.

2.9. Application to in-vitro dissolution studies

The dissolution testing of FAV from Avigan® tablets was conducted at 37 ± 0.5 °C with stirring speed of 50, 75 or 100 rpm using USP apparatus I and II. Dissolution media including deionized water, 0.1 N HCl pH 1.2, acetate buffer pH 4.5, and phosphate buffer pH 6.8 with 500, 900 or 1000 mL were tested. Aliquots of 5 mL were withdrawn at the specified time intervals (5, 10, 15, 20, 30, 45 and 60 min) from each dissolution vessel, substituted with an equivalent volume of the corresponding fresh medium maintained at the same temperature and filtered through 0.45 µm membrane filter (Millipore, Milford, MA). Samples were diluted with respective dissolution medium and then analyzed by the proposed HPLC method.

3. Results and discussion

3.1. Degradation behavior of FAV under various stress conditions

As per ICH recommendations [20], FAV was exposed to various stress conditions including acid, base hydrolysis, oxidative, thermal, and photo-degradation. Degradation behavior was monitored periodically by the proposed HPLC-DAD method.

FAV was completely degraded upon standing for 24 h at room

temperature with 0.1 N HCl or with 0.5 N NaOH, as demonstrated by the disappearance of FAV peak (R_t 3.5 min) and appearance of new degradation peaks, Fig. S2. The postulated pathway proceeds through the cleavage of the amide group under acid and alkali hydrolysis giving rise to the free amine and the corresponding acid. The latter undergoes further decomposition in the acidic medium, where the acidic conditions catalyze the carbonyl carbon to perform radical carbo-cation, which then forms a resonance stabilized acylium cation via electron pairing as illustrated in Fig. 1.

FAV oxidation was achieved by standing with 10% H₂O₂ at room temperature for 24 h, confirmed by disappearance of FAV intact peak at R_t 3.5 min, Fig. S2. The reaction takes place through oxidation of the primary amine by hydrogen peroxide that functions to donate oxygen to nitrogen, thus oxidation of amines to the azanol, nitroso-, or nitro compound, depending on the amount of oxidizing agent, as illustrated in Fig. 1. On the other hand, FAV exhibits relative stability under the studied photolytic (direct sun light for 6 h) and thermal stress conditions (at 105 °C for 6 h), where no other peaks than the intact FAV peak appeared in their respective chromatograms, Fig. S2.

All induced degradation products were subjected to mass spectral analysis for subsequent identification. The assignment was based on comparison of mass spectral data for the degradation products with that of the intact drug using negative electrospray ionization through multiple reaction monitoring (MRM) mode. Mass spectra for the acid and alkali induced degradation products show molecular ion peak at *m/z* 140.9 and 156.9, in order. On the other hand, FAV oxidative degradation product has a mass spectrum characterized by its molecular ion peak at *m/z* 172.2, Fig. 2.

3.2. Method development and optimization

Optimization of chromatographic conditions was performed to achieve high resolution of the analyzed components with sharp symmetric peaks in short run time. As a result, it was necessary to study the impacts of various factors influencing sensitivity, selectivity, and separation efficiency.

Different mobile phase compositions were tried. Several aqueous solutions such as 0.1% (v/v) phosphoric acid (pH 2.5 ± 0.05), 0.1% (v/v) acetic acid (pH 3.0 ± 0.05), 25.0 mM ammonium formate solution (pH 4.5 ± 0.05), 25.0 mM phosphate buffer (pH 3.5 ± 0.05), 25.0 mM ammonium acetate solution (pH 5.5 ± 0.05), phosphate buffer (pH 6.0

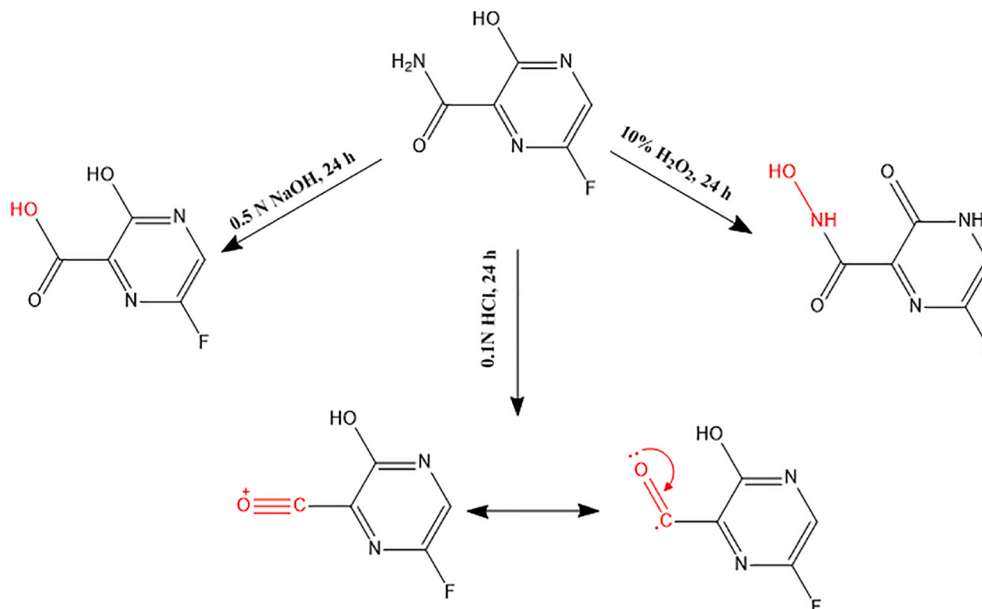


Fig. 1. The suggested pathways for FAV-induced degradation.

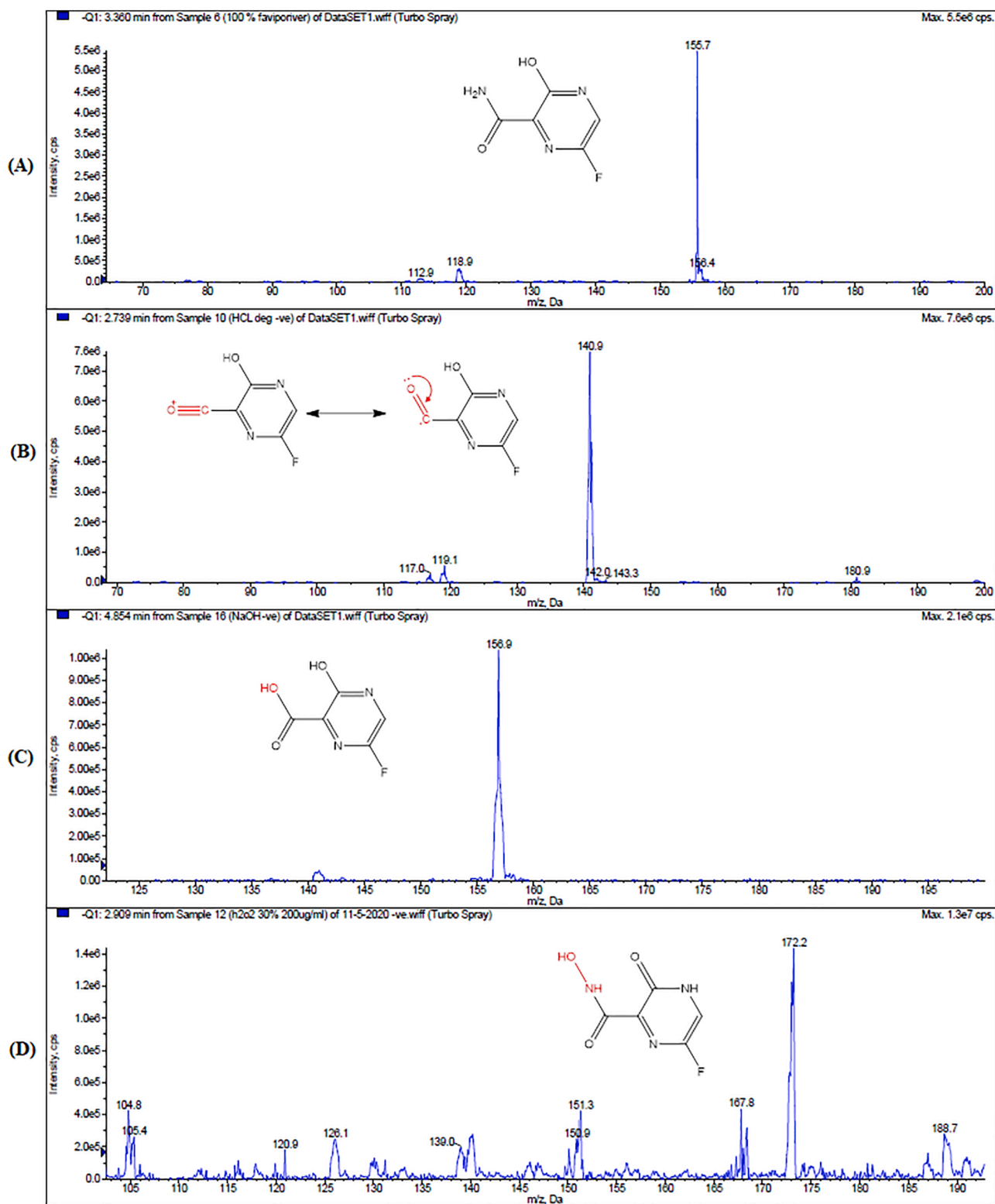


Fig. 2. LC-MS spectra, at negative mode ($[M-H]^-$) of (A) intact FAV, m/z 155.7 (B) acid-induced degradation product, m/z 140.9 (C) alkali-induced degradation product, m/z 156.9 (D) oxidative degradation product, m/z 172.2.

± 0.05) and deionized water, were tested. Because of the structure similarity between FAV and its stress induced degradation products, simultaneous separation was challenging. Therefore, ion pair method could successfully enhance the selectivity because of hydrogen bonding capacity and in the same time, it offers an electrostatic interaction with analytes of opposite charges. Sulfonate ions with negative charge (SO_3^-)

and different lengths of alkyl chain, such as hexane sulfonic acid and heptane sulfonic acid, were examined as proper ion pairing reagents. Using lower pH values, solvents offered better separation and enhanced peak resolution; therefore 0.1% (w/v) heptane sulphonic acid sodium salt solution was used as an ion pairing reagent. Concentration of the mixture was evaluated from 0.025 to 0.5% (w/v), higher number of

baseline-resolved peaks was detected when a mixture of 0.1% (w/v) heptane sulphonic acid sodium salt solution in presence of 25.0 mM phosphate buffer (pH 3.5 ± 0.05) was used. Additionally, the use of methanol or acetonitrile alone was investigated as an organic solvent, nevertheless, no progress regarding peak shape was obtained and even co-eluted peaks were detected under both conditions. Therefore, a mixture of acetonitrile and methanol was selected as organic solvent. Hence, optimum resolution of FAV and its stress degradation products was obtained by isocratic elution using 25.0 mM phosphate buffer (pH 3.5 ± 0.05) containing 0.1% (w/v) solution of heptane sulphonic acid sodium salt-methanol-acetonitrile in a ratio of 62:28:10, by volume with a flow rate of 1 mL/min, Fig. 3.

Different analytical columns were checked like Zorbax Eclipse XDB-C₁₈ (4.6 × 150 mm, 5 µm; Agilent technologies, USA), Kinetex C₈ (4.6 × 100 mm, 5 µm; Phenomenex, USA), XSelect HSS C₁₈ (4.6 × 50 mm, 2.5 µm; Waters, USA) and Xterra MS C₁₈ (4.6 × 50 mm 5 µm; Waters, USA). Using C₁₈ column rather than C₈ enhanced the separation and gave the optimum resolution for the analytes. Zorbax, C₁₈ (4.6 × 250 mm, 5 µm; Agilent technologies, USA) gave the best resolution with outstanding sharp peaks. Moreover, the effect of column temperatures was also investigated at 25 to 40 °C. However, no improvement was obtained in terms of shorter analysis time when higher column temperature is used owing to decreased mobile phase viscosity. Accordingly, no significant differences in the retention times were detected in the studied temperature range. Finally, optimum temperature was selected to be 30 °C, since all peaks were properly resolved under this condition, Fig. 3.

Different detection wavelengths 230.0, 254.0, 260.0 and 321.0 nm were tested to determine the best one for the studied analyte in term of sensitivity and peak shape. The optimum wavelength for FAV determination was 321.0 nm, Fig. 3.

System suitability parameters were calculated to verify the performance of the chromatographic system for the intended separation. Results are displayed in Table 1, for a set of parameters; including resolution, tailing factor, selectivity factor, column efficiency and theoretical plates. All are within the acceptance criteria [21], revealing complete baseline separation of the separated peaks and high selectivity of the proposed method.

3.3. Method validation

The proposed method was validated in accordance to ICH guidelines [19]. Table 2 summarizes the assay regression equation parameters over FAV concentration range of 6.25–250.00 µg/mL. The regression equation was computed using the least-squares regression analysis at the specified wavelength.

Accuracy is established across the specified range of the analytical

procedure. Three concentration levels, in triplicates, (60.0, 80.0 and 120.0 µg/mL) of pure samples of FAV were analyzed by the proposed method. Three concentrations (60.0, 80.0 and 120.0 µg/mL) were analyzed either within the same day or on three successive days for investigating intra-day and inter-day precision, respectively. Satisfactory results for accuracy and precision were obtained, Table 2. The limit of detection (LOD) and limit of quantification (LOQ) were determined based on standard deviation of intercept and slope of the calibration curve, Table 2.

Robustness of the analytical procedure was evaluated by deliberately varying in method parameters including scanning wavelength (± 1 nm), flow rate (± 0.1 mL/min) and column temperature (± 1 °C). It was found that minor variations of these variables didn't significantly affect the performance of the proposed method or the system suitability parameters. Good %RSD values were obtained providing an indication of proposed method reliability during normal usage, Table 2.

Specificity of the method was confirmed by good resolution of studied components by analyzing chromatographic blank/degradation products and examined for interference with accurate quantitation of FAV peak, as demonstrated in Figs. 3 and S2. Moreover, analysis of laboratory-prepared mixtures comprising different ratios of the four components was carried out. Results reveal accurate and specific determination of the intact drug in presence of up to 90% of the degradation products, Table S1. In addition, FAV peak purity was assessed by the DAD, and no signs of co-elution from any of the degradation products were detected.

3.4. Analysis of Avigan® tablets

The developed method was effectively applied for the analysis of FAV in its pharmaceutical dosage form (Avigan® tablets) without interference from the excipients, Table 3.

Furthermore, the validity of the procedure was successfully assured by applying standard addition technique, Table 3.

The obtained results for quantification of FAV in Avigan® tablets by the developed HPLC-DAD method were statistically compared to those attained by reported HPLC one [17], Table S2. Both student's *t* and *F*-test values are less than the tabulated ones, indicating no significant difference between the two methods regarding accuracy and precision.

3.5. Degradation kinetic studies

The proposed HPL-DAD method was utilized to investigate FAV degradation kinetics. This was accomplished by estimating the remaining FAV concentration (C_t) after specified time (t) intervals. Kinetics of acid, alkali hydrolysis and oxidative degradation were studied

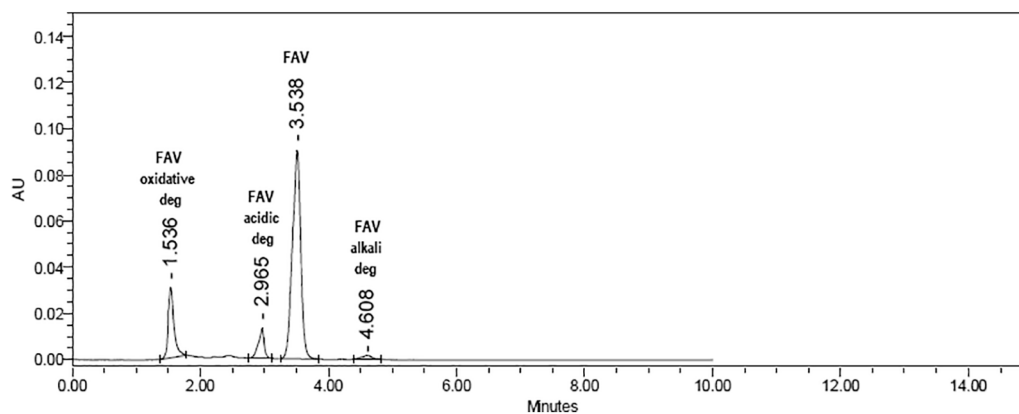


Fig 3. HPL Chromatogram of resolved mixture of FAV (100.0 µg/mL), FAV oxidative degradation (100.0 µg/mL), FAV acid degradation (100 µg/mL) and FAV alkali degradation (100.0 µg/mL) using 25.0 mM phosphate buffer (pH 3.5 ± 0.05) containing 0.1% (w/v) solution of heptane sulphonic acid sodium salt-methanol-acetonitrile (62:28:10, by volume) as mobile phase at 321.0 nm.

Table 1
System suitability parameters of the proposed HPLC-DAD method.

Parameter	Oxidative Degradate	Acid Degradate	FAV	Alkali Degradate	Reference value [20]
Selectivity (α) ^a	2.3	1.2	1.4		>1.0
Resolution (R_s) ^b	9.6	2.8	4.1		$R_s > 1.5$
Tailing factor (T)	1	1	1	1	$T \leq 2$
Column efficiency (N)	2003	4994	2698	3337	$N > 2000$
Height equivalent to theoretical plate HETP (cm/plate)	0.012	0.005	0.009	0.007	
Retention time (min \pm 0.2)	1.54	2.96	3.54	4.61	

^a Selectivity (α) = k'_2/k'_1 calculated for each of two successive peaks.

^b Resolution (R_s) = $2(t_{RB} - t_{RA}) / (W_{B+}W_A)$, where t_R is the retention time and w is the peak width calculated for each of two successive peaks.

Table 2
Validation parameters of the proposed HPLC-DAD method for determination of pure FAV.

HPLC-DAD method	Method Parameter
Range ($\mu\text{g/mL}$)	6.25 – 250.00
Regression equations parameters	
Slope (b) ^a	3172
Intercept (a) ^a	8898
Standard deviation of slope	± 6.94
Standard deviation of intercept	± 982.60
SD of residuals ($S_{y,x}$)	1948.82
Correlation Coefficient (r)	0.9999
Accuracy (Mean \pm SD) ^b	99.87 \pm 0.38
Precision	
(\pm %RSD) ^c	± 0.31
(\pm %RSD) ^d	± 0.35
LOD ($\mu\text{g/mL}$) ^e	1.02
LOQ ($\mu\text{g/mL}$) ^e	3.10
Robustness (Mean \pm SD) ^f	99.00 \pm 0.27

^a Regression equation: $A = a + bc$, where 'A' is the average peak area, 'c' is the concentration ($\mu\text{g/mL}$), 'b' is the slope and 'a' is the intercept.

^b Accuracy [average of three different concentrations of three replicates each ($n = 9$)].

^c Intra-day precision [average of three different concentrations of three replicates each ($n = 9$) within the same day].

^d Inter-day precision [average of three different concentration of three replicates each ($n = 9$) repeated on three successive days].

^e LOD and LOQ are calculated according to ICH, $3.3 \times \text{SD of } y\text{-intercept/slope}$ and $10 \times \text{SD of } y\text{-intercept /slope}$, respectively.

^f Average for the change in scanning wavelength (± 1 nm), flow rate (± 0.1 mL/min) and column temperature (± 1 °C).

for 12 h. Linear relationship was obtained by plotting the log % of FAV remaining concentration *versus* time, Fig. 4, indicating first-order degradation. However, under all investigated degradation conditions, the existence of two reactants in the reaction medium, one of which presents in a large excess, gives the reaction an overall term of pseudo first-order kinetics according to the following equation:

$$\log(C_t) = \log(C_0) - kt/2.303, t_{1/2} = \frac{0.693}{k}$$

where, C_t is FAV remaining concentration, C_0 is the initial concentration

Table 3
Determination of FAV in Avigan® film coated tablets and spiked dosage form samples by the proposed HPLC-DAD method.

Pharmaceutical formulation	HPLC-DAD method					
Avigan® Film Coated Tablets (Each Tablet is labelled to contain 200 mg FAV)	%Found \pm SD ^a		Standard Addition Technique			
			Claimed ($\mu\text{g/mL}$)	Pure added ($\mu\text{g/mL}$)	Pure Found ($\mu\text{g/mL}$)	%Recovery of the pure added ^b
	FAV	99.03 \pm 0.81	50	25.00	25.26	101.06
				50.00	50.69	101.39
				100.00	101.54	101.55
				Mean \pm SD		101.33 \pm 0.25

^a Average of five determinations.

^b Average of three determinations.

of FAV, k is the apparent first order rate constant with a negative sign and t is the time. For FAV acid and alkali hydrolysis, the observed reaction rate constants (k_{obs}) were 0.225 h^{-1} and 0.158 h^{-1} , with respective half life time ($t_{1/2}$) values of 3.08 h and 4.39 h. Whereas, FAV kinetic rate constant was 0.197 h^{-1} with $t_{1/2}$ value of 3.52 h for its oxidative degradation, Table S3.

3.6. Application of the proposed HPLC-DAD method for dissolution testing of Avigan® tablets

Establishment of dissolution profile is strongly recommended during development and optimization of drug formulations to ensure batch-to-batch uniformity as well as the construction of in-vitro/in-vivo correlation. As there is no available official monograph and the dissolution test is not defined for FAV tablets, dissolution testing under a variety of test conditions is recommended including different dissolution medium (pH 1 to 6.8) using basket or paddle with varying rotation speeds [22]. The sink conditions are determined and expressed as a percentage of drug released. In this study, deionized water, 0.1 N HCl pH 1.2, acetate buffer pH 4.5 and phosphate buffer pH 6.8 were tried as dissolution media and selected based on drug solubility. The drug release profiles are shown in Fig. 5. Phosphate buffer pH 6.8 dissolution medium delivered the highest drug release profile, with excellent sink conditions. Based on the dissolution study, the most discriminative release profile of FAV tablets was achieved by using phosphate buffer pH 6.8, USP type II, at 75 rpm and maintained at 37 ± 0.5 °C. Acceptance criteria for drug release in these conditions, were in the range of 65–83%. In the present study, the percentage of drug released in all media were $> 80\%$ in 45 min, Fig. 5. Accordingly, the suggested acceptance criteria in terms of quantity (Q) of active substance dissolved in specified time can be at least 80% of active substance is released in 45 min.

4. Conclusion

A rapid, accurate and specific HPLC-DAD method was established to determine FAV in presence of its forced degradation products for the first time. A comprehensive stress stability study revealed the susceptibility of FAV to acid, alkali hydrolysis and oxidative induced degradation. Moreover, degradation rates were studied and found to follow pseudo first-order kinetics. The suggested HPLC-DAD method makes a great use of DAD in determining peak purity and choosing the

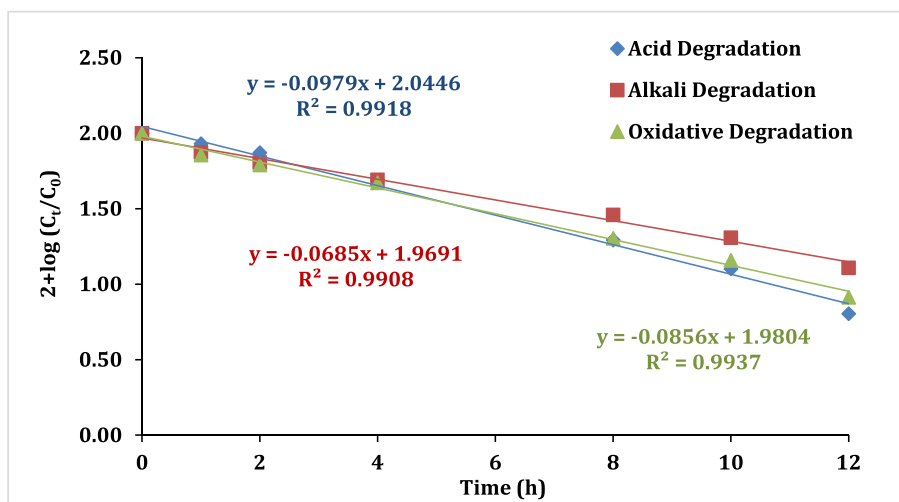


Fig. 4. Pseudo first-order kinetic plots for 100.0 µg/mL of FAV under acid, alkali and oxidative stress conditions.

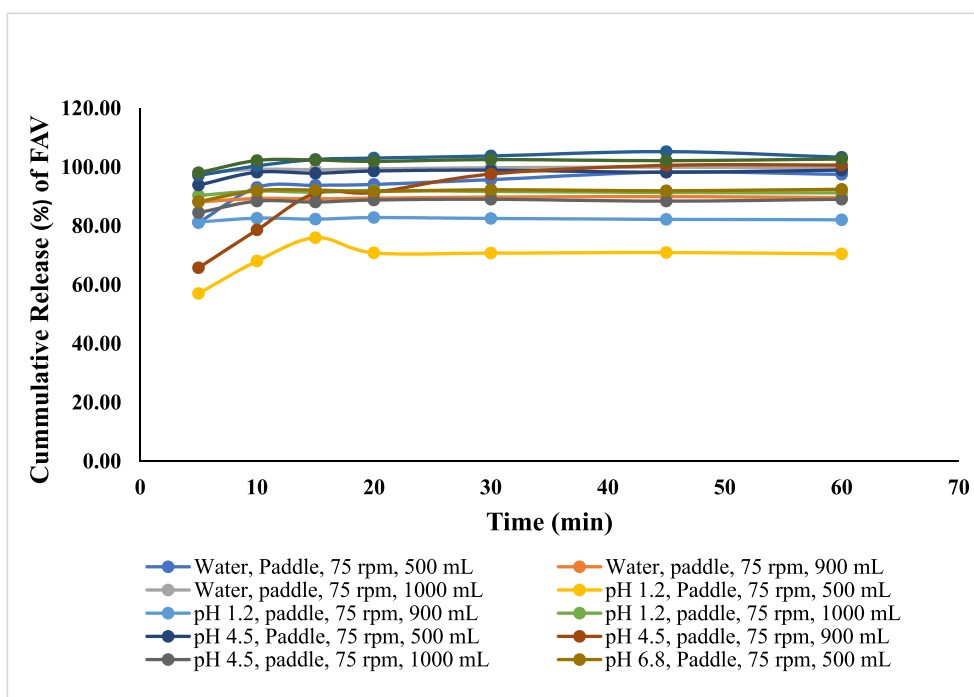


Fig. 5. Dissolution profiles of Avigan® tablets in tested dissolution media using the proposed HPLC-DAD method.

appropriate wavelength for analysis. The developed HPLC-DAD method was further applied for in-vitro dissolution monitoring to characterize FAV release rate from tablet dosage form. Successful assay of FAV in marketed pharmaceutical formulation without interference from common tablets' excipients or possible degradation products reveals method's suitability as stability-indicating method in routine quality control laboratories.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.microc.2021.106917>.

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