İstanbul Journal of Pharmacy
Istanbul J Pharm2024, 54 (2): 223–232
DOI: 10.26650/IstanbulJPharm.2024.1368223

Original Article

Development and validation of stability indicating HPLC method for favipiravir used in the treatment of the Covid-19 disease

Büşra Gülşen[1], Sıdıka Ertürk Toker[2]

[1]İstanbul University, Graduate School of Health Sciences, İstanbul, Türkiye
[2]İstanbul University, Faculty of Pharmacy, Department of Analytical Chemistry, İstanbul, Türkiye

ABSTRACT

Background and Aims: Favipiravir (FAV) is one of the active pharmaceutical ingredients used in the treatment of patients suffering from Covid-19. The epidemic started in 2019 and is still continuing all over the world. In this study, an analysis method was developed and validated for the simultaneous analysis of FAV and its degradation impurities.

Methods: The stationary phase of the developed method was determined using Kinetex® EVO C18 column and the mobile phase was pH 3.0 phosphate buffer:acetonitrile (90:10; v/v). Chromatographic separations were carried out at 30 °C column temperature and samples were monitored by a UV-Visible detector with a wavelength of 222 nm at 0.5 mL/min flow rate.

Results: Total analysis time was 25 minutes; FAV retention time was approximately 9 minutes. The retention times of major impurities formed under alkaline, acidic, oxidative conditions were observed at about 4, 5, 7 and,12 minutes (RRT 0.51, 0.54, 0.76, 1.31), respectively. According to the validation data, the linearity range was obtained as 0.030 – 0.750 µg/mL, the limit of quantitation and the limit of detection were 0.030 µg/mL and 0.010 µg/mL, respectively. Percentage relative standard deviation values obtained in intra-day and between day repeatability studies were determined as 0.17% and 0.28%, respectively, and the average recovery value was found to be 99.46%.

Conclusion: This validated method has been successfully applied to the determination of all possible degradation impurities of FAV that increase under stress conditions such as high temperature, humidity and photodegradation from tablet form. The developed HPLC method is extremely suitable for the routine analysis of this drug used in the treatment of the Covid-19 disease, especially in terms of speed and convenience.

Keywords: Covid-19, favipiravir, stability indicating method, HPLC, validation

INTRODUCTION

Favipiravir (FAV) is an antiviral drug whose chemical name is 6-fluoro-3-hydroxypyrazine-2-carboxamide (Figure 1). It was first approved in Japan in 2014 to treat pandemic influenza infections (Delong, Abdelnabi, & Neyts, 2018). This active ingredient has also been used in the treatment of Covid-19, a virus that first appeared in the Wuhan Province of China in December 2019 with respiratory symptoms such as fever, cough, and shortness of breath (Turkish Republic Ministry of Health, 2020).

FAV is an RNA-dependent RNA polymerase (RdRp) enzyme inhibitor, in addition to its anti-influenza virus activity, it has the ability to block the replication of flavi-, alpha-, filo-, bunya-, arena-, noro- and other RNA viruses (Drugbank, 2021; Baranovich et al., 2013). With these features, it was thought to have has fewer side effects than the combined drug group (Kiso et al., 2010; Watanabe et al., 2013).

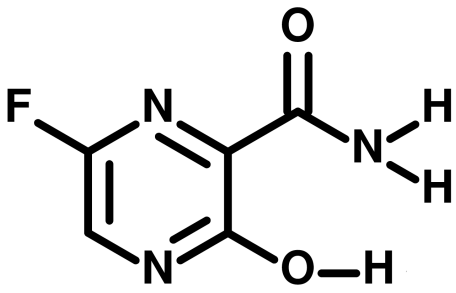

Figure 1. Chemical structure of FAV.

The first pharmaceutical preparation containing FAV is a

Corresponding Author: Sıdıka Ertürk Toker **E-mail:** serturk@istanbul.edu.tr
Submitted: 30.09.2023 • **Revision Requested:** 14.11.2023 • **Last Revision Received:** 19.01.2024 • **Accepted:** 22.01.2024
This article is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

medicine in tablet form (Avigan® 200 mg Tablet) originating in Japan. This preparation is typically used only in new influenza virus outbreaks where other anti-influenza virus agents are not effective enough (Pharmaceuticals and Medical Devices Agency, 2014). FAV became one of the preferred options in the treatment of COVID-19 symptoms during the pandemic period, where fast and effective treatment was essential. It was used in our country as well as in some other countries. During the manufacturing of the drug products of FAV, well-developed and validated analytical methods are required to enable quicker and easier analysis to be made.

To date, HPLC methods (Cuiyan, Yuanyuan, Lichao, Yangjin, & Lei, 2015; Yegorova, Scrypynets, Leonenko, Umetskaya, & Voitiuk, 2020; Bulduk, 2020), – as well as spectrophotometric methods (Yegorova et al., 2020; Megahed, Habib, Hammad, & Kamal, 2020) and a Luminescence method (Yegorova et al., 2020) have been developed for the quantification of FAV in its tablet forms. Moreover, the Chinese patents (CN104914185A (Guangling et al., 2015) and CN104914185B (Guangling et al., 2016)) and the stability-indicating HPLC method (Ali et al., 2021) are available on FAV impurity determination in studies conducted so far. In the stability-indicating HPLC method published by Ali et al. (Ali et al., 2021), impurities were not identified one by one and the degradation results were not published in detail.

In this study, a new HPLC method was developed and validated for the simultaneous analysis of FAV and its degradation impurities. Furthermore, its applicability in pharmaceutical preparations has been proven with detailed degradation studies.

MATERIAL AND METHODS

Chemicals

Analytical grade Potassium Dihydrogen Phosphate, 85% Phosphoric Acid, 37% Hydrochloric Acid and Sodium Hydroxide were purchased from Merck (Germany). HPLC grade Acetonitrile and Methanol were purchased from J.T.Baker (United States); distilled water was used from Merck Millipore Milli-Q Advantage A10 Ultrapure water system (United States). FAV working standard was obtained from Zhejiang Hengkang Pharmaceutical Co., Ltd. (China). FAV 200 mg film-coated tablet (FAAR1-V-01, ARIS) and adjuvant (placebo) mixture were supplied from Ali Raif Pharmaceutical Company (Türkiye); Avigan 200 mg film-coated tablet was purchased from Toyama Chemical Co. Ltd. (Japan) as a reference product.

Solutions

10 mM phosphate buffer was prepared by dissolving 1.36 g of potassium dihydrogen phosphate in approximately 950 mL of distilled water and adjusting the pH to 3.0±0.05 with 85% phosphoric acid. The solution was then made up to 1 L of volume.

A mobile phase solution was prepared by combining 10 mM phosphate buffer and acetonitrile at a ratio of 90:10 (v/v). The mobile phase solution was also used as the dilution solution.

FAV stock solution was prepared by dissolving 25 mg FAV working standard in approximately 25 mL of dilution solution and then kept in an ultrasonic bath for 15 minutes. After dissolution was achieved, it was made up to 50 mL volume with the dilution solution (C_{FAV} : 0.5 mg/mL).

For preparing the standard solution 1/100 dilutions of the FAV stock solution and 1/10 dilutions of the 1/100 solution were made and the volumes were completed with the dilution solution each time (C_{FAV} : 0.0005 mg/mL).

For the sample solution 5 FAV 200 mg film-coated tablets were crushed in a mortar and powdered. Tablet powder (32.58 mg) equivalent to 25 mg FAV was weighed and transferred to a 50 mL volumetric flask. 25 mL of dilution solution was added to it and left in an ultrasonic bath for 15 minutes and made up to volume with dilution solution (C_{FAV} : 0.5 mg/mL). The placebo solution consisted of excipients was also prepared similarly to the sample solution.

All prepared solutions were filtered through a 0.45 µm membrane filter (Millipore®, Germany).

HPLC system

Two different types of HPLC columns and mobile phases were examined during the selection of the column and mobile phase. C18 (Kinetex EVO C18 ; 100Å, 250 x 4.6 mm, 5 µm) and C8 (Waters® XBridge C8; 250 x 4.6 mm, 5 µm) columns were used and the mobile phases were tested according to the physicochemical properties of FAV. The HPLC system, showing the best achieved results and the conditions in which trials were carried out, is shown in Table 1. The run time of the method was 25 min.

Validation

The validation of the analytical method developed with this study was carried out in accordance with the parameters and explanations specified in the ICH Q2 (R1) – Validation of Analytical Procedures Guidelines (ICH Q2 (R1), 2005).

In the specificity studies, the system received one injection each of mobile phase solution, placebo solution, standard solution, sample solution, and FAV + placebo solution. A DAD detector was used throughout this analysis.

In the system suitability studies, 6 consecutive injections were applied from each of the prepared standard and sample solutions.

For the limit of quantitative determination (LOQ), 6 consecutive injections of the 0.030 µg/mL LOQ solution were applied.

Table 1. Chromatographic conditions of the developed method

HPLC System	HPLC LC-20A (Shimadzu Corporation, Japan)
Dedector	SPD-M20A Diode Array Dedector (DAD)
Column	Kinetex® EVO C18
Column Dimensions	100Å, 250 x 4.6 mm, 5 µm
Mobile Phase/Dilution Solvent	Phosphate Buffer (10 mM KH_2PO_4 (pH 3.0)):Acetonitrile, (90:10, *v/v*)
Mode	Isocratic
Flow Rate	0.5 mL/min
Detection Wavelenght	222 nm
Column Temperature	30 ºC
Injection Volume	10 µL

These studies were performed such that the FAV peak height to noise to signal to noise ratio (S/N) was in the 10:1 range and the RSD% between FAV peak areas from 6 consecutive injections was less than 10.0. For the lower limit of detection (LOD), an injection of the 0.010 µg/mL LOD solution was performed. It was found that the signal to noise (S/N) ratio of the FAV peak height to noise, which was acquired from the LOD injection, varied between 2:1 and 3:1.

Three solutions were made at the accuracy parameter, one for each of the percent LOQ, 100 percent, and 150 percent recovery levels, and three injections were made from each solution. The precision parameter was analyzed in two steps. In the first step, for reproducibility, two standard solutions were prepared. 6 consecutive injections of the first standard solution and two consecutive injections of the second standard solution were performed. The RSD% between the FAV peak areas obtained from both standard solutions, the tailing factor, and the theoretical plate number were evaluated, and the recovery calculation was made. During the second phase, the intermediate precision study, all solutions were prepared and administered on a different day by a different analyst as described in the repeatability study.

For the robustness parameter, three injections of each of the standard and sample solutions were made for the normal analysis conditions of the method and modified conditions given in Table 2. During the robustness study, results from flow rate, column temperature, and wavelength were evaluated.

For the solution stability parameter, prepared standard and sample solutions were divided into two and stored under HPLC conditions and at refrigerator temperature (2-8°C). From the solutions in HPLC conditions, at the start, 6, 21, 27 and 45 hours 3 injections were given. Similarly, of the solutions kept at refrigerator temperature (2-8 °C), 3 injections were given at the beginning, and thereafter in the 4^{th}, 19^{th}, 27^{th}, and 45^{th} hours.

Degradation and Stress Studies

A FAV 200 mg film-coated tablet was exposed to the conditions given in Table 3. The data from the deterioration studies were compared with the percent area findings and FAV peak purity values of the normal condition sample that was first injected into the HPLC system in the degradation and stress studies.

Checking the suitability of the determined total impurity limit was evaluated by Arrhenius Equation (lnk = lnA - Ea / R x T). 6-month analysis results of the most degraded impurity of FAV (RRT 0.51) under 30 °C and 40 °C heat stress conditions and 7-day analysis results under 70 °C heat stress conditions were used in this equation.

RESULTS AND DISCUSSION

Method Development

Initially, the λ_{max} values of the FAV molecule were obtained from the UV-Visible spectra of the FAV solution (C_{FAV} : 0.01 mg/mL, methanol) and the solvent was 222 nm and 322 nm, respectively. By injecting the sample solution into the HPLC system, an extra impurity peak - RRT 0.51 was seen in the chromatogram of 222 nm compared to the chromatogram of 322 nm, thus 222 nm was chosen as the method wavelength in order to monitor this impurity.

Due to the polar and basic character of FAV (Pharmaceuticals and Medical Devices Agency, 2014) and the capacity of its hydroxyl group to make intramolecular hydrogen bonds, it was decided to test the C8 and C18 columns for chromatographic separation. In addition, due to the pKa value of the molecule being 5.1, attention was paid to ensure that the pH ranges of the preferred C8 and C18 columns were wide so that the pH range to which the column would be resistant corresponded to $pK_{aFAV} \pm 2$. As a result of the column trial studies, it was decided to choose the Kinetex® EVO C18 100Å 250 x 4.6 mm, 5 µm column as the stationary phase that would provide separation in this analytical method. Upon examination of the findings produced by this column, it was observed that the

Table 2. Robustness parameters and their initial & changed conditions

Parameter	Initial	Lower Limit	Upper Limit
Flow Rate	0.5 mL/min	0.45 mL/min	0.55 mL/min
Column Temperature	30 °C	28 °C	32 °C
Dedection Wavelength	222 nm	215 nm	230 nm
Different Column*	Kinetex® EVO C18 250 x 4.6 mm, 5 gm, 100Â		

**Only serial number/part number differs from the column that was used initially.*

Table 3. Stress conditions that FAV tablet was exposed*

Stress Condition	Stressor	Exposure Condition	Exposure Duration
Acidic	0.1 N HCI	60 ₀C	4 hours
Alkali	0.1 N NaOH	60 °C	4 hours
Oxidative	3% H_2O_2	60 °C	4 hours
Photolysis	UV light	In a petri dish under 500 W/m^2 UV light	7 days
Thermal	-	70°C ± 2°C, 100% RH ± 5% RH	7 days
Humidity	Distilled Water	-	8 hours

**In order to compare the decomposition and stress studies, the reference sample and placebo solution without any treatment were prepared. For all conditions, placebo solutions were prepared exposed to the same conditions. For alkaline, acidic, oxidative decomposition and humidity studies, empty solutions exposed to these conditions were prepared.*

injection repeatability was guaranteed and that the theoretical plate number values and tailing factor satisfied the acceptance standards.

During the determination of the mobile phase, the type, concentration and pH value of the buffer solution and the determination of the organic solvent were sequentially carried out. Phosphate buffer and acetonitrile were chosen as the first materials to be used because of their extremely low absorbance at wavelengths near 222 nm. Buffer concentration trials were started at 10 mM, since in most applications the buffer concentration in the mobile phase is sufficient at 5 - 10 mM (Dolan, 2011). 1/10 of the total volume was chosen for the acetonitrile ratio to be used as the other component of the mobile phase. While determining the pH value of 10 mM buffer, considering the pH value at which FAV is not ionized, pH 3.0 value was chosen from the three pH ranges (1.1-3.1, 6.2-8.2, 11.3-13.3) where the phosphate buffer is effective.

After determining the column and mobile phase, it was observed that the retention time of the last impurity (RRT 1.99) was approximately 17.1 minutes in injections made under these conditions. Considering this value, 25 minutes, which is close to 1.5 times the retention time of the last impurity, was determined as the analysis time.

Determination of Sample and Standard Solutions Concentrations and Impurity Specifications

The maximum recommended daily dose for a pharmaceutical form containing FAV molecule is 3200 mg/day (Pharmaceuticals and Medical Devices Agency, 2014). According to ICH Q3B (R2) (European Medicines Agency, 2006), for drugs with a maximum daily dose of more than 1.0 g, the reporting limit, the limit of unknown impurities, and the limit of known impurities were determined as 0.05%, 0.10%, and 0.15%, respectively.

Based on an unknown impurity limit (0.10%) and reporting limit (0.05%) determined according to the ICH Q3B (R2) guideline, the FAV concentration for the sample solution was determined as 0.5 mg/mL since, the LOQ% of FAV was below or equal to the reporting limit. The FAV concentration for the limit level solution was also determined as 0.5 µg/mL. FAV peak purity was achieved at both concentrations. The S/N value obtained from the chromatograms of the solution at the limit level concentration was determined as 108.17. Based on this value, the LOQ level was calculated as 0.030 µg/mL, the S/N value as 10.52, and the LOQ as 0.006 (below reporting limit - 0.05%). These obtained values and the peak purity of FAV proved that the determined sample solution concentration was appropriate according to the ICH Q3B (R2) guideline.

The total impurity limit for the FAAR1-V-01 tablet was determined as 0.5% which was the result of both the mathematical calculation of the stress/stability data of the tablet and the stress/stability studies using the reference product Avigan®

Tablet (Serial No: PC1021, Japan). Evaluations in these studies were made on the RRT 0.51 impurity with the highest % result showing a regular increase in the chromatograms. 'Guidance for Industry ANDAS: Impurities in New Drug Products' was used to determine the total impurity limit by comparing it with the original product to be evaluated in terms of quality (Food and Drug Administration, 2010). The accelerated period 40 °C and 70 °C analysis results of the FAV tablet and Avigan® tablet are shown in Table 4. While checking the compliance of the determined total impurity limit, regardless of the relevant compound specifications of the reference product, 6-month analysis results at 30 °C and 40 °C stress conditions and 7-day analysis results at 70 °C stress conditions over RRT 0.51 impurity in FAV Tablet were evaluated by Arrhenius Equation (lnk = lnA - Ea / R x T). In this way, the behavior of the RRT 0.51 impurity against temperature change was determined. According to the Arrhenius Graph in Figure 2 obtained with the stress stability results of the RRT 0.51 impurity with the highest increase in 3 different temperature conditions for the product FAAR1-V-01, the shelf life of this product was approximately 72 months. The determined shelf life of the reference product of Avigan® Tablet was 60 months (Pharmaceuticals and Medical Devices Agency, 2014). The mathematical result obtained from the graph with the total impurity limit determined, since it corresponds to the shelf life of the reference product, showed that the determined total impurity limit is suitable for this product during shelf life.

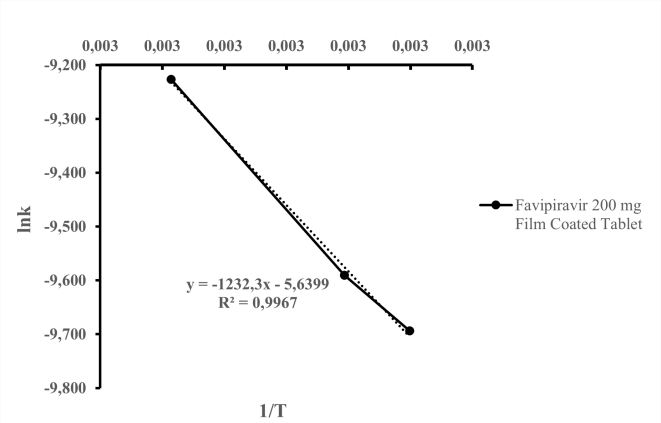

Figure 2. Arrhenius Graph for FAV 200 mg film-coated tablet.

Method Validation

It was observed that there was no interference between the FAV peak and the other peaks obtained in the chromatograms of the sample solution and placebo solution (Figure 3). FAV Peak purity index was also found between 0.99 and 1.00 as seen in Figure 3. The retention time, peak area, peak purity index, and single point threshold values of the FAV peak obtained from the injected solutions are reported in Table 5.

In the system suitability studies, the areas of FAV peak in all

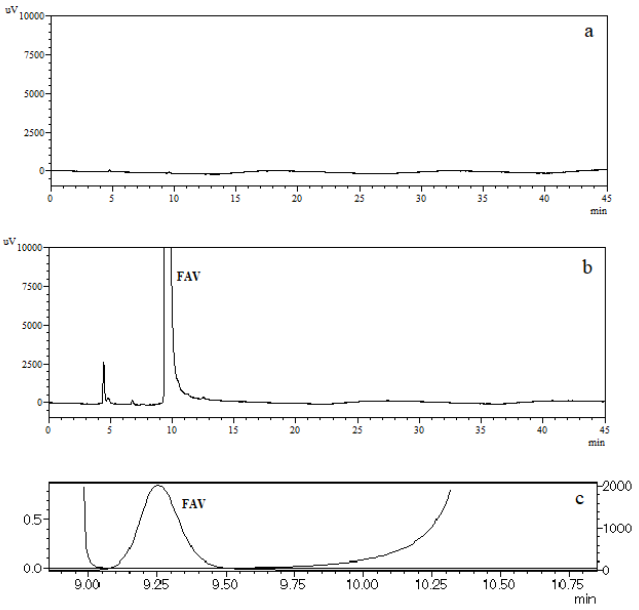

Figure 3. Representative chromatograms of placebo (a) and sample (b), and the figure of FAV peak purity (c).

chromatograms given separately from the standard and sample solutions, the standard deviation between the retention times and the peak areas, and the RSD% values were found to be less than 0.055.

In the LOD studies, the calculated S/N values of the LOD solution injection were found to be close to the ratio of 3:1 and these values provided the requested acceptance criterion. Similarly, the S/N ratios calculated from the LOQ solution injections were close to 10:1 and the RSD% value between the FAV peak areas in consecutive injections was below 10.0.

In the linearity graph obtained according to the least squares method from linearity studies, the peak areas corresponding to the concentrations were located directly above the line in the graph. The data of the obtained linearity equation are given in Table 6. From the reported values, the determination coefficient was 0.99980, which satisfies the acceptance criterion ($R^2 \geq 0.999$). At 2.54% the y-intercept criterion was obtained in accordance with the acceptance criterion (range ± 20.0% of the signal output by the standard solution).

Table 4. Temperature stress/stability results of FAV 200 mg film coated tablet

RRT 0.51 Impurity	FAV 200 mg Film Coated Tablet				
	Initial	3rd day	7th day	3rd month	6rd month
30°C ± 2°C, %65 RH[a] ± %5 RH[a]	0.019	-	-	0.025	0.056
40°C ± 2°C, %75 RH[a] ± %5 RH[a]	0.019	-	-	0.027	0.060
70°C ± 2°C, %100 RH[a] ± %5 RH[a]	0.019	0.039	0.078	-	-

[a] Relative Humidity

Table 5. Specificity results of FAV peak (222 nm) from different solutions

Solutions	Retention Time (minutes)	Peak Area	Peak Purity Index	Single Point Threshold
Mobile Phase/Dilution Solvent	-	-	-	-
Placebo Solution	-	-	-	-
Standard Solution	9.257	23187	0.999949	0.993503
Sample Solution	9.248	21328856	0.999999	0.999964
FAV + Placebo Spike Solution	9.261	23592	0.999962	0.988149

Table 6. Linearty parameters of the developed method

Parameter	**Result**
Linearity Range (p.g/mL)	0.030-0.750
Regression Equation	y=43971.286 x+582.978
Slope	43971.286
Intercept	582.978
Mean Correlation Coefficient, R	0.9999
Determination Coefficient, R^2	0.9998
y-Intercept Criterion (%)	2.54
LOD[a] (p.g/mL)	0.010
LOQb (p.g/mL)	0.030

[a]Limit of Detection; [b]Limit of Quantification

In the accuracy studies, the average recovery value at the LOQ%, 100% and 150% levels, were determined within the 90.0% - 110.0% limit. The results obtained are given in Table 7.

In the repeatability and intermediate precision studies, the results of 2 different standard solutions were all within the system suitability acceptance criteria. In both studies, the mean recovery values were found to be over 98% and the RSD% values were found to be below 0.28 (Table 8).

In the robustness studies, for both standard and sample solutions, the % change of FAV peak areas compared to the initial conditions was found to be less than 5.0 in both the column change and column temperature changes. The % change obtained in terms of flow rate and wavelength changes was found to be more than 5.0%. When the applied changes were evaluated in terms of sample solutions impurity results (%), it was seen that the results did not exceed the specification limits and similar results were obtained with the impurity results (%) obtained in the initial conditions.

The percent change between the standard and sample solutions held in refrigerated and HPLC settings at the beginning and injection times of the solution stability investigation was determined to be less than 5.0 for 45 hours in both scenarios. When the sample solution is evaluated in terms of impurities, no results exceeding specification limits for each unknown impurity and total impurity were observed for 19 hours under refrigeration conditions. However, the RRT 0.51 impurity showed a regular increase. The same impurity caused the sample solution

Table 7. Recovery results for FAV tablet samples

Concentration (p.g/mL)		Recovery (%)	RSD [b] (%)	Mean Recovery (%)
Added	Found (mean ± SD [a])			
0.030	0.032 ± 1.11	106.30	1.05	
0.500	0.480 ± 0.67	96.22	0.69	99.46
0.750	0.720 ± 0.80	95.85	0.84	

[a] Standard Deviation; [b] Relative Standard Deviation

Table 8. Intra-day & inter-day precision data for FAV tablet samples (n = 6)

Concentration (µg/mL)		Recovery (%)	RSD [b] (%)
Added	Found (mean ± SD [a])		
Intra-day			
0.501	0.492 ± 0.17	98.10	0.17
Inter-day			
0.504	0.496 ± 0.27	98.35	0.28

[a] Standart Deviation; [b] Relative Standart Deviation

results to exceed the limit from the 6th hour under HPLC conditions. Therefore, the stability time of the sample solution was determined by the 6-hour solution stability study performed in addition to both conditions. When all studies were evaluated, it was observed that the standard solution was stable for 45 hours under HPLC conditions and refrigerator conditions, but the sample solution was stable for impurities only for 2 hours under HPLC conditions and refrigerator conditions. According to this result, it was determined that the sample solutions should be injected into the system fresh after preparation.

Degradation and stress studies

The numerical data of all degradation studies obtained under alkaline, acidic, oxidative, thermal, humidity, and photolytic stress conditions are shown in Table 9.

In the degradation experiments carried out under alkaline conditions, only the RRT 0.54 impurity was detected in addition to the impurities in the normal condition sample. Results for this impurity were found to exceed each unknown impurity limit (0.10%). RRT 0.54 impurity and RRT 0.76 impurity, which were also detected in the normal condition sample, were observed as major impurities belonging to this condition. RRT 0.51, another impurity detected in the normal condition sample, increased with alkaline conditions, but the result was below the limit for each unknown impurity (Figure 4b).

No additional impurities were detected in the normal condition sample for degradation study under acidic conditions. RRT 0.51 impurity was observed as the major impurity for this condition. RRT 0.76 and RRT 1.18, the impurities in the normal condition sample, were the impurities that increased in acidic conditions; however, the results of both impurities under this condition were below each unknown impurity limit (Figure 4c).

In the oxidative conditions degradation study RRT 0.57, RRT 0.63, RRT 0.84, and RRT 1.31 impurities were detected in addition to the impurities in the normal condition sample. The results for impurities RRT 0.57, RRT 0.63, and RRT 0.84 remained below each unknown impurity limit (0.10%), while impurities RRT 0.51, RRT 0.76, and RRT 1.31 were observed as major impurities for this condition. RRT 1.99, one of the impurities in the normal condition sample, also increased with this condition, but each gave results below the unknown impurity limit (Figure 4d).

In the temperature effect study, RRT 0.54, RRT 1.31, and RRT 2.07 impurities were detected in addition to the impurities in the normal condition sample. Although RRT 0.51, RRT 0.76, and RRT 1.99 are the normal condition impurities, they were increased with the effect of temperature and all impurity results obtained as a result of this study were below each unknown impurity limit (0.10%). However, the RRT 0.51 impurity was increased under this condition, giving results above the reporting limit (0.05%) (Figure 5b).

In the humidity effect study, in addition to the impurities in the normal condition sample, RRT 0.54 and RRT 1.31 impurities were detected. The impurity RRT 0.51 in the normal condition sample increased with the effect of humidity and was close to each unknown impurity limit (0.10%) but still below each unknown impurity limit (Figure 5c).

In the photodegradation study, in addition to the impurities in the normal condition sample, RRT 0.54 and RRT 1.31 impurities were detected. The impurity RRT 0.51 increased with photodegradation and was detected close to each unknown im-

Table 9. Favipiravir 200 mg film coated tablet degradation results

	Reference	Alkali	Acidic	Oxidative	Thermal	Humidity	Photolysis
Favipiravir	99.867	95.699	88.261	94.120	99.830	99.833	99.864
RRT[a] 0.51	0.018	0.051	11.617	4.789	0.070	0.090	0.080
RRT[a] 0.54	-	0.128	-	-	0.005	0.029	0.027
RRT[a] 0.57	-	-	-	0.074	-	-	-
RRT[a] 0.63	-	-	-	0.007	-	-	-
RRT[a] 0.76	0.013	4.082	0.082	0.206	0.017	0.012	0.016
RRT[a] 0.84	-	-	-	0.007	-	-	-
RRT[a] 1.11	0.001	0.001	0.001	0.001	0.001	0.001	0.001
RRT[a] 1.18	0.005	0.005	0.006	0.004	0.005	0.005	0.003
RRT[a] 1.31	-	-	-	0.570	0.012	0.010	0.003
RRT[a] 1.99	0.003	0.003	0.003	0.019	0.006	0.003	0.007
RRT[a] 2.07	-	-	-	-	0.030	-	-
Total	99.907	99.969	99.970	99.797	99.976	99.983	100.001

[a] Relative Retention Time

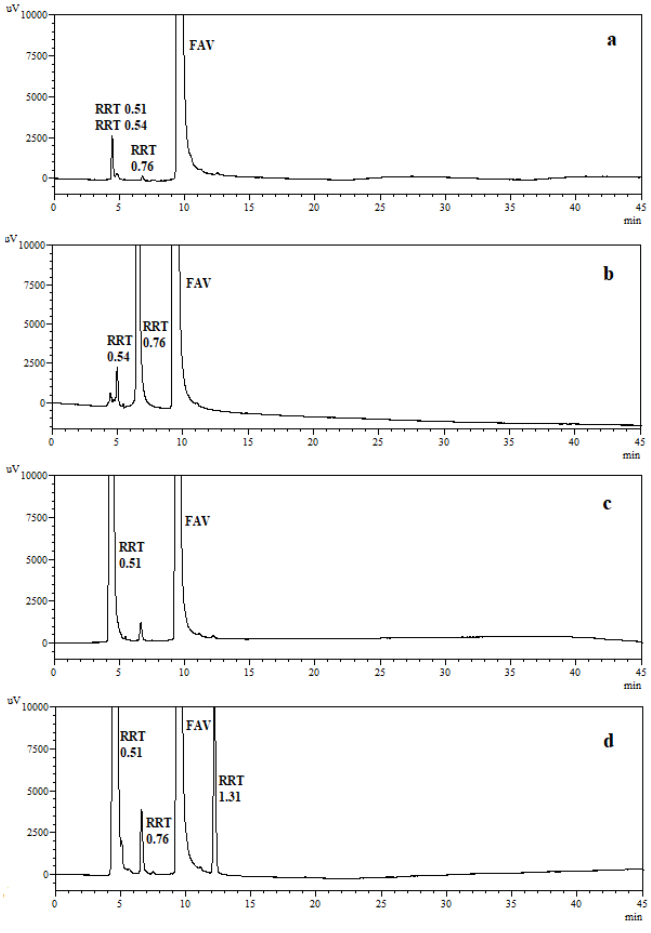

Figure 4. Chromatograms obtained under normal conditions (a) alkaline (b), acidic (c) and oxidative (d) stress conditions.

purity limit (0.10%). The other impurities, RRT 0.76 and RRT 1.99, which were found common in both conditions, were observed as impurities increasing with photodegradation. However, all impurity results obtained as a result of this study were below each unknown impurity limit (Figure 5d).

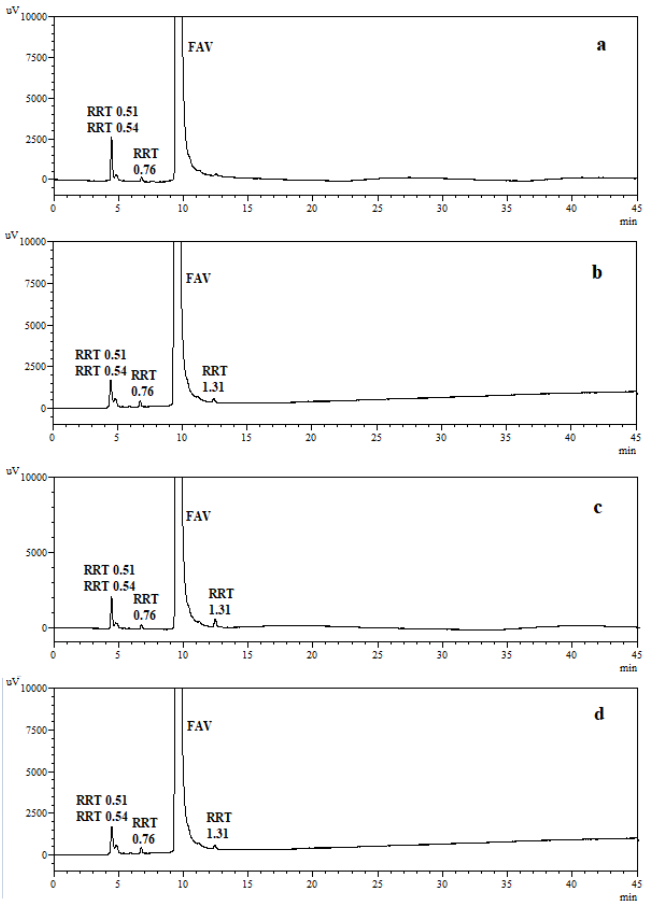

Figure 5. Chromatograms obtained under normal conditions (a), thermal (b), humidity (c) and photolytic (d) stress conditions.

CONCLUSION

Different medications were required by countries to treat the Covid-19 virus sickness, which can have a rapid and serious impact on public health. Drugs containing the FAV active ingredient were also used in this period. Currently, it is imperative for nations to manufacture these medications, and analytical techniques capable of guaranteeing the drug's quality are required. The method outlined in this study was developed to respond to this requirement. The developed method has some advantages including separating more than one impurity that does not interfere with the active ingredient of FAV, contributing to green chemistry with the use of a small number of organic solvents, and having a shorter analysis time compared to the analytical methods that determine impurities. Moreover, the developed method contributed to the analysis efficiency with low buffer solution concentration.

When the developed method is compared with the method given in Chinese patents, (Guangling et al., 2015 and Guangking et al., 2016) the method of the patent with an analysis time of 60 minutes is longer than the developed method. The LOD value of the patent method was reported as 0.1%. In the developed method, the LOD: 0.002%; R^2 was obtained as 0.9998. These values show that the linearity of the method and the detection limit are better than the Chinese patent methods. In the stability-indicating method published by Ali et al. (Ali et al., 2021), impurities were not identified one by one, and the degradation results were not published in detail. With the developed method, the impurities that occur in FAV and pharmaceutical preparations containing this active ingredient can be determined together and the conditions under which these impurities are formed can be explained supported by the results obtained from detailed degradation and stress studies.

In the developed method, the retention time of FAV was 9 minutes and the retention times of major impurities formed in alkaline, acidic, and oxidative conditions were approximately 4, 5, 7, and 12 minutes (RRT 0.51, 0.54, 0.76, 1.31). The total run time was completed in 25 minutes. The RSD% values of the method were obtained as 0.25 and the consistency between the results was supported by statistical tests. An average recovery of the active substance was 99.46% achieved with 5.03% RSD. The optimum time for which the sample solutions remained stable under HPLC conditions was found to be 2 hours, and for this reason, the sample solutions were given to the HPLC system immediately after preparation.

To determine the applicability of the validated method, pharmaceutical preparations containing FAV were kept under alkaline, acidic, oxidative conditions and exposed to high temperature, humidity, and photodegradation. Then, the impurity analyses of the pharmaceutical solid dosage form were successfully carried out with the developed method. Major impurities were detected in alkaline, acidic, and oxidative conditions. Although the impurities that increased when the same pharmaceutical preparations were kept under high temperature, humidity and light could be detected separately and the impurities formed remained below each unknown impurity limit (0.10%). This result showed that the preparations were resistant to these stress conditions. Since the analytical methods in which the impurity determination is given in detail with the degradation studies are not included in the literature, the developed method could not be compared from this perspective.

In conclusion, an HPLC method with high efficiency, reproducibility, and reliability was developed that can determine FAV and possible degradation impurities in solid dosage form. The developed method can be preferred in the pharmaceutical industry for its ease of use in the impurity analysis of pharmaceutical preparations containing FAV-active ingredients produced for antiviral treatment.

Peer-review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- S.E.T., B.G.; Data Acquisition- B.G.; Data Analysis/Interpretation- B.G.; Drafting Manuscript- S.E.T., B.G..; Critical Revision of Manuscript- S.E.T.; Final Approval and Accountability- S.E.T., B.G..

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared no financial support.

Note: Section Editor was not involved in the evaluation and peer-review processes of the article. These processes were carried out by the Editor-in-Chief and the member editors of the editorial management board.

ORCID IDs of the authors

Büşra Gülşen 0000 0002 7029 8004
Sıdıka Ertürk Toker 0000 0002 6827 8362

REFERENCES

Ali, S.N.S., Mobina, L., Mehfuza, M., Seema, P., Ahmed, A., & Khan, G.J. (2021). Analytical method development and validation and forced degradation stability-indicating studies of favipiravir by RP-HPLC and UV in bulk and pharmaceutical dosage form. *Journal of Pharmaceutical Research International, 33*, 254-271. doi: 10.9734/JPRI/2021/v33i48B33283

Baranovich, T., Wong, S. S., Armstrong, J., Marjuki, H., Webby, R. J., Webster, R. G., & Govorkova, E. A. (2013). T-705 (favipiravir) induces lethal mutagenesis in influenza A H1N1 viruses in vitro. *Journal of Virology, 87*(7), 3741-3751. doi:http://dx.doi.org/10.1128/JVI.02346-12T

Bulduk, İ. (2020). HPLC-UV method for quantification of Favipiravir in pharmaceutical formulations. *Acta Chromatographica*, 33, 209-215. doi:10.1556/1326.2020.00828

Cuiyan, L., Yuanyuan, Z., Lichao, B., Yangjin, L., & Lei, L. (2015).

Content determination of favipiravir tablets by HPLC. *China Pharmacist*, 7, 1231-1233.

Delong, L., Abdelnabi, R., & Neyts, J. (2018). Favipiravir as a potential countermeasure against neglected and emerging RNA viruses. *Antiviral Research*, 153, 85-94. doi:10.1016/j.antiviral.2018.03.003

Dolan, J. (2011) (Accessed 2 March 2021) A Guide to HPLC and LC-MS Buffer Selection, https://www.hplc.eu/Downloads/ACE_Guide_BufferSelection.pdf.

Drugbank, Favipiravir (2021, March 1), https://go.drugbank.com/drugs/DB12466

Guangling, F., Wenjuan, D., Yuxiao, D., Ren-Yang, Z., Yan, G., Chong-Gang, D., & Jinrui, S. (2015). HPLC method for measuring related substances in Favipiravir (Chinese Patent No CN104914185A). Shandong Academy of Pharmaceutical Sciences, https://patents.google.com/patent/CN104914185A/en

Guangling, F., Wenjuan, D., Yuxiao, D., Ren-Yang, Z., Yan, G., Chong-Gang, D., & Jinrui, S. (2016). A kind of Favipiravir has the HPLC assay method of related substance (Chinese Patent No. CN104914185B) Shandong Academy of Pharmaceutical Sciences, https://patents.google.com/patent/CN104914185B/en

Guidance for Industry ANDAs: Impurities in Drug Products, Food and Drug Administration, (2010) (Accessed 23 March 2021) https://www.fda.gov/files/drugs/published/ANDAs–Impurities-in-Drug-Products.pdf

ICH Q2 (R1):Validation of Analytical Procedures: Text and Methodology, 2005, European Medicines Agency, (Accessed 12 March 2021) https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-2-r1-validation-analytical-procedures-text-methodology-step-5en.pdf

Kiso, M., Takahashi, K., Sakai-Tagawa, Y., Shinya, K., Sakabe, S., Le, M., Ozawa, M., Furuta, Y., Kawaoka, Y. (2010). T-705 (Favipiravir) Activity against Lethal H5N1 Influenza A Viruses. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 882-887. doi: 10.1073/pnas.0909603107

Megahed, S. M., Habib, A. A., Hammad, S. F., & Kamal, A. H. (2020). Experimental design approach for development of spectrofluorimetric method for determination of favipiravir; a potential therapeutic agent against COVID-19 virus: Application to spiked human plasma. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 249, 1386-1425. doi:10.1016/j.saa.2020.119241

Japan Pharmaceuticals and Medical Devices Agency, 2014 Report on the deliberation results, Avigan 200 mg Tablet, (Accessed 31 December 2020) https://www.pmda.go.jp/files/000210319.pdf

Turkish Republic Ministry of Health, Covid-19 Information Platform, What is Covid-19? (December 2020) https://covid19.saglik.gov.tr/TR-66300/covid-19-nedir-.html

Yegorova, A. V., Scrypynets, Y. V., Leonenko, I. I., Umetskaya, I. V., & Voitiuk, O. D. (2020). Determination of favipiravir residues on the surfaces of pharmaceutical manufacturing equipment by HPLC, luminescence and spectrophotometry. *Odesa National University Herald Chemistry*, 25, 45-55. doi:10.18524/2304-0947.2020.4(76).216925

Watanabe, T., Kiso, M., Fukuyama, S., Nakajima, N., Imai, M., Yamada, S., . . . Kawaoka, Y. (2013). Characterization of H7N9 influenza A viruses isolated from humans. *Nature*, 501, 551-555. doi:10.1038/nature12392

How cite this article

Gülşen, B., & Ertürk Toker, S. (2024). Development and validation of stability indicating HPLC method for favipiravir used in the treatment of the Covid-19 disease. *İstanbul Journal of Pharmacy, 54*(2), 223–232. DOI: 10.26650/IstanbulJPharm.2024.1368223