Quantitative Determination of Favipiravir Tablets by Ultraviolet-Visible Spectrophotometry

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Abstract

The objective of this study was to develop and validate a simple UV-visible spectrophotometric method for determination of favipiravir in tablet formulation. Method: Ethanol : water (1:1, v/v) was used as a solvent for the preparation of standard and sample solutions. The results showed that favipiravir exhibited the wavelength of maximum absorbance (λ_{max}) at 227 nm. This analytical method also showed acceptable specificity, linearity, accuracy and precision. The linearity of favipiravir was noticed over the concentration range of 2 – 10 µg/mL. Similarly, the accuracy and precision results were also satisfied with average %recovery of 100.2 and %RSD of 1.28, respectively. Therefore, UV-visible spectrophotometry was suggested to be a simple, specific, accurate, and precise method for the quantification of favipiravir tablets.

Keywords : Favipiravir, tablets, UV-visible spectrophotometry, method validation

1. INTRODUCTION

Favipiravir (5-fluoro-2-oxo-1*H*-pyrazine-3-carboxamide) has been approved in Japan since 2014 for the treatment of new or recurrent influenza. As a prodrug, favipiravir is converted to an active form as favipiravir ribofuranosyl-5′-triphosphate (favipiravir-RTP) which shows antiviral activities by interacting with viral RNA-dependent RNA polymerase (RdRp), resulting to the termination of viral transcription and replication (Pavlova *et al.* 2021, pp. 2-7). Nowadays, due to the worldwide outbreak of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection or generally known as the coronavirus disease-2019 (COVID-19), favipiravir has been repurposed and also indicated as one of first-line therapy in some countries, including Thailand. Based on the clinical practice guideline, favipiravir is recommended in case of symptomatic COVID-19 without pneumonia and no risk factors for severe disease. The recommended dose for adult is 1,800 mg twice daily on day 1, followed by 800 mg twice daily for at least 4 days (Department of Medical Services, 2022). Favipiravir is available in oral tablet formulations (200 mg per tablet) which must be formerly imported from another country. Eventually, in 2021, the Government Pharmaceutical Organization (GPO) in collaboration with the National Science and Technology Development Agency (NSTDA) have announced a successful development and production of favipiravir film-coated tablets, starting from the synthesis of active pharmaceutical ingredient (API), with only half the prices of the imported equivalent.

According to the quality control process, determination of favipiravir in bulk materials and dosage forms was concerned. Japanese Pharmacopoeia (JP) with support from other pharmacopoeias has developed an official method for assaying favipiravir and favipiravir tablets,

using high-performance liquid chromatography (HPLC) (World Health Organization, 2021). In addition, validated HPLC method was also developed for the analysis of favipiravir in pharmaceutical products and human plasma (Bulduk, 2021, pp. 57-65; Mikhail, 2021). However, although HPLC was one of the most widely used technique for drug analysis with the ability of separation, qualification and quantification of single or multicomponent system, it was realized to be a costly and time-consuming method. A simple, economical, and easy-to-operate method, such as UV-visible spectrophotometry was reconsidered.

Recently, using of UV-visible spectrophotometric method for the determination of favipiravir in pharmaceutical formulations has been reported in previous studies (Bulduk, 2021, pp. 57-65; Bulduk, 2021, pp. 209-215; Rajan & Prathamesh, 2021, pp. 321-323; Jyothi & Kavya, 2021, pp. 67-69). Ethanol, ethanol and water (5 in 100 mL for standard stock solution), and deionized water only were used as solvent. Since favipiravir is slightly soluble in water and ethanol, selection of suitable solvent was a key step for further preparation and dilution of standard and sample solutions. To ensure that favipiravir was dissolved completely in a consistent component of the solvent, in this study, a validated UV-visible spectrophotometric method using a cosolvent system was developed for quantification of favipiravir in tablet dosage form.

2. Objectives

To develop and validate a UV-visible spectrophotometric method for the estimation of favipiravir in tablet formulation.

3. Materials and methods

3.1 Materials

Favipiravir reference standard (99.48% purity) was purchased from the Bureau of Drug and Narcotic, Department of Medical Sciences, Ministry of Public Health (Nonthaburi, Thailand). Ethanol (absolute, for analysis) was purchased from Merck (Darmstadt, Germany) for further dilution with deionized water. Favipiravir tablets (COVIVEL^{TM} 200, Strides Pharma Science Ltd., Bangalore, India) were kindly obtained from a hospital in Nakhon Pathom, Thailand. Each film-coated tablet contains 200 mg of favipiravir.

UV spectra and absorbance measurements were carried out using a double-beam UV-visible spectrophotometer (JASCO V-630, Tokyo, Japan) with 2 identical 1-cm quartz cuvettes. All spectral data were collected and analyzed using Spectra Manager 2.5 Software.

3.2 Methods

3.2.1 Method development

Preparation of stock standard solution

Favipiravir standard was weighed accurately about 10 mg into a 100-mL volumetric flask and 40 mL of ethanol : water (1:1, v/v) was then added. The flask was swirled vigorously for 10 min or until the standard was completely dissolved. Finally, adjusted to the volume with the same solvent to obtain a favipiravir stock standard solution at concentration of 100 µg/mL.

Preparation of standard solution

A series of favipiravir standard solution at 5 concentrations: 2, 4, 6, 8, and 10 µg/mL, was prepared by diluting the stock standard solution with ethanol : water (1:1, v/v). These solutions were scanned to determine the wavelength of maximum absorbance (λ_{max}), following by the measurement of absorbances.

Preparation of sample solution

Weighed and powdered 20 favipiravir tablets. The tablet powder was weighed accurately equivalent to 250 mg of favipiravir into a 50-mL volumetric flask. Dissolved with 20 mL of ethanol : water (1:1, v/v), swirled vigorously for 10 min, and adjusted to the volume with the same solvent. Mixed, filtered through an 11-µm filter paper, and discarded the first 10 mL of filtrate. Pipetted 1.0 mL of the previous solution into a 50-mL volumetric flask and adjusted to the volume with ethanol : water (1:1, v/v) to finally obtain a sample solution containing 100 µg/mL of favipiravir.

3.2.2 Method validation

The developed spectrophotometric method was validated for specificity, linearity, accuracy and precision according to the ICH and AOAC guideline.

Specificity

UV absorption spectra of the standard and sample solution were recorded in range of 200 – 400 nm. Characteristic and the λ_{max} obtained from the spectra were compared to evaluate the specificity of developed method.

Linearity and range

Linear regression analysis was established to verify the linearity, using the standard solution in 5 different concentrations (2 – 10 µg/mL). Calibration curve of favipiravir standard was constructed by plotting the measured absorbances at the λ_{max} against the concentrations. The regression equation and correlation coefficient (r) were also determined. The linearity was accepted when $r \geq 0.995$.

Accuracy and precision

Standard addition method was conducted to evaluate the accuracy. Briefly, 3.0 mL of the stock standard solution (as 100% level) was transferred into 6 of 50-mL volumetric flasks containing 1.0 mL of sample solution. Ethanol : water (1:1, v/v) was then added and adjusted to the volume. The standard addition mixtures in 6 replicates were measured at the λ_{max} to obtain a total absorbance from each flask, compared with un-spiked sample solution. The concentrations of spiked favipiravir standard, including percentage recoveries (%recovery) were calculated. The accuracy was accepted when %recovery were in range of 98 – 102.

For precision, percentage of relative standard deviation (%RSD) from average %recovery was consequently calculated. The precision was accepted when %RSD values were not more than 1.3.

3.2.3 Assay of favipiravir tablets

Referred to the preparation of sample solution (3.2.1), 100 µg/mL of favipiravir was prepared in triplicate. Firstly, the tablet powder was weighed accurately equivalent to 50 mg of

favipiravir into a 50-mL volumetric flask. Then, 20 mL of ethanol : water (1:1, v/v) was added, swirled vigorously for 10 min, and adjusted to the volume with the same solvent. After quantitative filtration, 5.0 mL of the filtrate was diluted to 50.0 mL with ethanol : water (1:1, v/v). Finally, diluted 3.0 mL of this solution to 50.0 mL with ethanol : water (1:1, v/v) and measured the absorbance at λ_{max}. Calculated the content of favipiravir per tablet by the absorptivity (a) obtained from linear regression analysis. For the acceptance criteria, favipiravir tablets contain not less than 95.0% and not more than 105.0% of the labeled amount.

4. Results

In this study, the analytical method was developed for further analysis of favipiravir tablets. Standard and sample solutions were prepared in a fixed ratio of ethanol : water (1:1, v/v) throughout the experiment. For method validation, the parameters such as specificity, linearity, accuracy and precision were examined.

4.1 Method validation

Specificity

UV absorption spectra of favipiravir standard and sample were illustrated in Figure 1. The λ_{max} of favipiravir standard was detected at 227 nm. Similarly, the λ_{max} of sample solution was also indicated at the same wavelength, as well as overall spectral characteristic was resemble. These findings demonstrated good specificity of the method for the detection of favipiravir with no inference from excipients in tablet formulation.

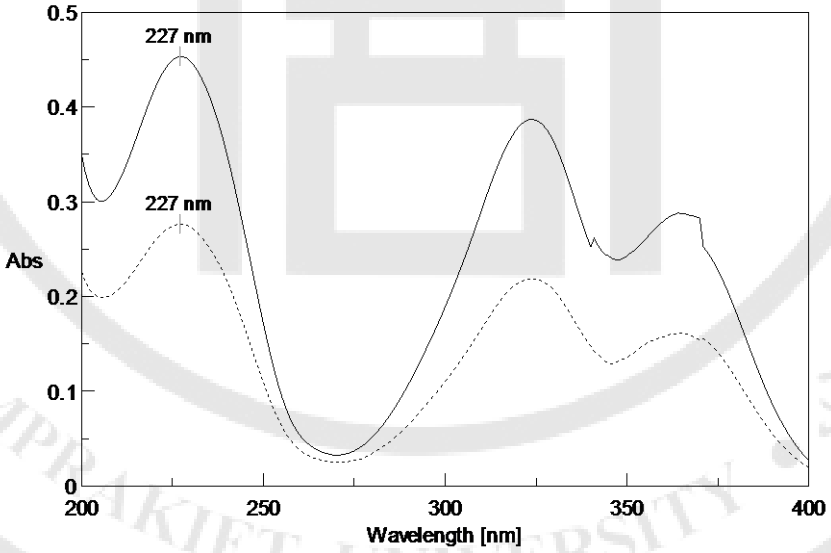

Figure 1 Overlay UV absorption spectra of standard favipiravir at 10 µg/mL (solid line), and sample solution at 6 µg/mL (dash line).

Linearity and range

The calibration curve of favipiravir obtained from the regression analysis was linear over the concentration range of 2 – 10 µg/mL, with the regression equation of y = 0.042x + 0.0152 and r-value of 0.9998 when measured at 227 nm. The absorptivity (a) of favipiravir was known from the slope of regression equation as 42 L/g·cm, resulting to favipiravir specific absorbance or A (1%, 1 cm) of 420 dL/g·cm.

Accuracy and precision

The standard addition mixtures of favipiravir standard (6 µg/mL) combined with sample (2 µg/mL) were prepared in 6 replicates. The absorbances were measured from all solution at 227 nm, and the concentration of spiked standard were calculated. The results were displayed in Table 1. For accuracy, calculated %recoveries were in range of 98.94 – 101.8% (average 100.2%). For precision, %RSD calculated from average %recovery were 1.28%. Thus, the developed method was considered to be accurate and precise for the estimation of favipiravir in tablets.

Table 1 Accuracy and precision results for favipiravir (n = 6)

No.	Amount added (µg/mL)	Amount found (µg/mL)	%Recovery
1	6.300	6.2595	99.36
2	6.300	6.4119	101.8
3	6.300	6.4000	101.6
4	6.300	6.2330	99.06
5	6.300	6.3452	98.94
6	6.300	6.2405	100.7
	Average		100.2
	SD		1.29
	%RSD		1.28

4.2 Assay of favipiravir tablets

Sample solution of 200-mg favipiravir tablets at 6 µg/mL was prepared in triplicate and determined for the absorbances at 227 nm. Based on the calculation using regression equation, the amount of favipiravir (mg/tablet) and percent labeled amount (%L.A.) were displayed in Table 2. Average %L.A. of favipiravir was 95.82, which met the acceptance criteria of favipiravir tablets (95.0 – 105.0 %L.A.).

Table 2 Assay result of favipiravir tablets (n = 3)

No.	Label claim (mg)	Amount found (mg)	%L.A.
1	200	190.5	95.27
2	200	191.2	95.60
3	200	193.2	96.59
	Average		95.82

5. Discussion

In this study, UV-visible spectrophotometric method which used to quantify the content of favipiravir in tablet formulation was modified from recent studies. Jyothi, *et al.* (2021) had introduced a simple, precise, and accurate UV spectrophotometric method for the estimation of favipiravir. Unfortunately, using of this validated method to analyze the marketed products was not applicable. As mentioned earlier, ethanol : water (1:1, v/v) was a suitable solvent in terms of cosolvent property with consistent ratio for the preparation of favipiravir standard and sample solutions. For method validation results, referred to ICH guideline, all parameters including specificity, linearity and range, accuracy, and precision were evaluated and also met the acceptance criteria. Interestingly, our study showed good linearity of the method with $r \geq 0.995$ over the concentration range of 2 – 10 µg/mL, lower than a previous study result which was reported in the range of 10 – 60 µg/mL for both UV and HPLC technique (Bulduk, 2021, pp. 57-65). This finding suggested higher sensitivity of developed method for favipiravir detection.

For favipiravir assay, although the %L.A. of faviravir (in triplicate) were in range of 95.0 – 105.0%, all values as well as the average %L.A. were near the lower limit criteria. Thus, more brands of commercially available favipiravir tablets should be tested to confirm the performance of this analytical method.

6. Conclusion

The developed UV-visible spectrophotometric method in this study was considered to be simple, specific, linear, accurate, precise and sensitive for the determination of favipiravir. Moreover, this analytical method was fully validated as per ICH guideline and successfully applied for the estimation of favipiravir in tablet formulation. Hence, this method can be used in routine quantitative analysis for the quality control of favipiravir tablets.

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