Bioequivalence Profile Prediction of Favipiravir Using Everted Gut Sac

Isra Muzaqiyah*, Itsna Fadlilatul Arifah, Lina Afriyani, Radika Ayu Prahesthi, Tri Ahadiat

Kimia Farma - Research and Development Division, Bandung - Indonesia
Email: isra.muzaqiyah@kimiafarma.co.id

Abstract. Favipiravir is one of broad-spectrum antiviral drugs approved for the treatment of the influenza virus and SARS-CoV-2, it is known to have inhibitory activity towards RNA dependent RNA polymerase (RdRp). The permeability of Favipiravir in tablet form was conducted using an everted gut sac model for evaluating ex vivo drug absorption as an approach before starting the Pivotal Bioequivalence Study. One formulated Favipiravir tablet 200 mg by Kimia Farma was selected. Avigan® 200 mg manufactured by Fujifilm Toyama Chemical Co. Ltd was used as reference. In vitro comparative dissolution testing and absorption experiments were conducted. Ex Vivo absorption experiment was developed by using an everted gut sac from male rats intestinal. Absorption rate was used to demonstrate drug molecule intestinal permeability. The rate between test and reference drug was evaluated using a paired student T-test. The resulting data is used to make the bioequivalence study decision. Open-label, randomized, single-dose, two-period, two-sequences, crossover bioequivalence study was carried out under fasting conditions which included 30 healthy adult male and female subjects. Bioequivalence pharmacokinetics parameters assessed in this study were C_{max} and AUC_{0-t}. Similarity factor (f2) of comparative dissolution testing between test and reference drug was more than 50 in three mediums (pH 1.2; pH 4.5; pH 6.8). Absorption rates of test and reference drugs were not significantly different (t =0.6683 (< t critical 2.571). Favipiravir tablets produced by Kimia Farma have similar absorption rates compared to the reference drugs. The Bioequivalence study result was satisfactory. The geometric mean ratios of the test drug to the reference drug (90% CI) were 101.27% (96.89-105.86) for AUC_{0-t} and 101.22% (96.80-105.84) for C_{max}. All values were within the accepted bioequivalence range of 80.00-125.00% for both AUC_{0-t} and C_{max}

Keywords: *Bioequivalence, Dissolution, Everted gut sac, Favipiravir, Pharmacokinetics.*

1 Introduction

Favipiravir is one of broad-spectrum antiviral drugs approved for the treatment of the influenza virus and SARS-CoV-2, it is known to have inhibitory activity towards RNA dependent RNA polymerase (RdRp). Favipiravir is a prodrug that is metabolized by the human hypoxanthine-guanine phosphoribosyltransferase (HGPRT) into its active metabolite favipiravir-ribofuranosyl-5'-triphosphate (favipiravir-FTP) [1,2]. The mechanism of action of favipiravir is its active

Received _____, Revised _____, Accepted for publication _____

metabolite favipiravir-FTP formed and acts as nucleoside analog that simulates Guanosine Triphosphate (GTP) or Adenosine Triphosphate (ATP), then integrates into the RNA of nascent viruses, causing the termination of viral RNA synthesis. The other mechanism is that the integration of favipiravir-FTP into viral RNA induces a high mutation rate in the genome, leading to the loss of infectivity in virion production and inhibiting viral replication and reproduction [2].

After oral administration, the level of favipiravir in plasma reaches a peak in about 2 hours, then undergoes an elimination phase with a plasma half-life of 2-5 hours. About 54% of favipiravir in plasma is bound to proteins, mainly albumin. This drug is metabolized in the liver by the aldehyde oxidase and xanthine oxidase enzymes, producing an inactive metabolite that is excreted through the kidneys [3]. The presence of a hydroxypyridne group and a primary amide makes favipiravir a weak acid, resulting in low solubility at pH 1, which is 0.526 mg/ml. With a potency of 200 mg, more than 250 ml of solvent is required to dissolve it, making this drug problematic in its dissolution phase. In preclinical tests with rats, oral bioavailability of 97.6% was observed. Based on these two data points, favipiravir is classified as a Class 2 drug in the Biopharmaceutics Classification System (BCS-2)[4].

The characteristic of the drug and the systemic conditions of the body are factors that can influence the systemic availability of drug molecules through oral route. In the last two to three decades, research on drug interactions, metabolic pathways, and drug absorption mechanisms have been extensively investigated using in vivo and in vitro models [5]. Several techniques have been used to investigate the drug transport in the intestine including using chambers, isolated epithelial cells, brush–border and basolateral membranes isolated from enterocytes, and the everted gut sac technique [6]. The everted gut sac model has been widely used as a research technique to investigate the pharmacokinetics of drugs, such as drug absorption, drug metabolism or pro-drug conversion in gastrointestinal, efflux transport, multidrug resistance, drug interaction and the impact of efflux transport modulators on drug absorption. A relatively large surface area for drug absorption and the presence of a mucus layer are advantages of using this model [7].

Based on the National Agency of Drug and Food Control (BPOM), through the Head of BPOM-RI Regulation on May 30, 2022, regarding Procedures for Conducting Bioequivalence Testing, it mandates Bioavailability/Bioequivalence (BA/BE) testing for 'copy' drugs circulating in the market [8]. Simultaneous in vitro dissolution and absorption tests through the small intestine or everted gut sac method are conducted to provide an overview of the drug absorption capability before the pivotal bioequivalence test. The present study assesses the

permeability of Favipiravir in tablet form using everted gut sac model for evaluating ex vivo drug absorption mechanism versus reference drug, before conducting pivotal bioequivalence study.

2 Material & Method

1.1. Study Product

The sample preparation (Favipiravir 200 mg film coated tablets, batch number G10110N) was manufactured by PT Kimia Farma (Bandung, Indonesia). The reference preparation (Avigan® 200 mg tablets, batch number HG2101A) was manufactured by Fujifilm Toyama Chemical Co., Ltd.). Comparative dissolution test was conducted between the test product and the reference product prior to the bioequivalence study. The comparative dissolution test was performed in three different media with pH levels representing the gastrointestinal tract pH where drug absorption may occur (ie, pH 1.2, pH 4.5, pH 6.8). The dissolution profiles between the test and reference drug in each media showed similarity factor results greater than 50 (ie, the similarity factors were 52.25, 54.19, 51.53, respectively).

2.2 Ex Vivo Test Using Everted Gut Sac and Bioequivalence Test

2.2.1 Experimental animals

Six three-week-old male Wistar rats (weight 150–200 g) supplied by Integrated Research and Testing Laboratory Gadjah Mada University were used for animal models. Animals were fed with pellets AD2, usually used for chickens and rats. The rats had *ad libitum* access with reverse osmosis (RO) water.

2.2.2 Design study

Experimental animals were fasted for 20-24 hours, but boiled water was given to drink. The rats were anesthetized with CO_2 gas, then their stomachs were opened along the median line and the intestines were removed. The pylorus and colon were determined as a benchmark for intestinal collection. The intestine was taken, i.e. 14 cm above and below the center mark. The intestines were cut into 4 parts of 7 cm for each. Each intestinal bag is filled with 1.0 ml of serosal fluid (phosphate buffer pH 7.4), then the anal part is tied to the oxygenation tube. The intestinal bag and equipment were placed in a dissolution device (Electrolab dissolution tester serial No. 1502048) which contained 900 ml of acetate buffer pH 4.5. Stirring using a paddle at a speed of 75 RPM. Favipiravir 200 mg tablets were inserted into the dissolution test medium. Sampling was carried out by taking 0.5 mL of serosal fluid through a cannula and immediately filling it again with 0.5 mL of new pH 7.4 phosphate buffer solution. Sampling was carried out

at 15, 30, 45, 60, 90, 120, 150 and 180 minutes. The same method is used for the control intestinal bag but using serosal fluid without medication.

2.2.3 Statistical Analysis

After the data of absorbed drug during the interval was obtained, the rate of absorption was calculated based on their slope. The paired Student's t-test was conducted to evaluate the ex vivo profile similarity between test drug and reference. Data analyses were performed using Microsoft Excel.

2.2.4 Bioequivalence Test

A randomized, single-dose, open-label, crossover bioequivalence study was conducted to evaluate in vivo profile in humans. The study was conducted by a contract research organization including ethical clearance submission, Bioequivalence Study Approval, subject screening, and organizing the main study. 30 subjects were involved in this study (19 males and 11 females). Several pharmacokinetics parameters (C_{max}, AUC_{0-t}, AUC_{0-inf}) were evaluated and ANOVA test was conducted to compare the in vivo profile of drugs tested.

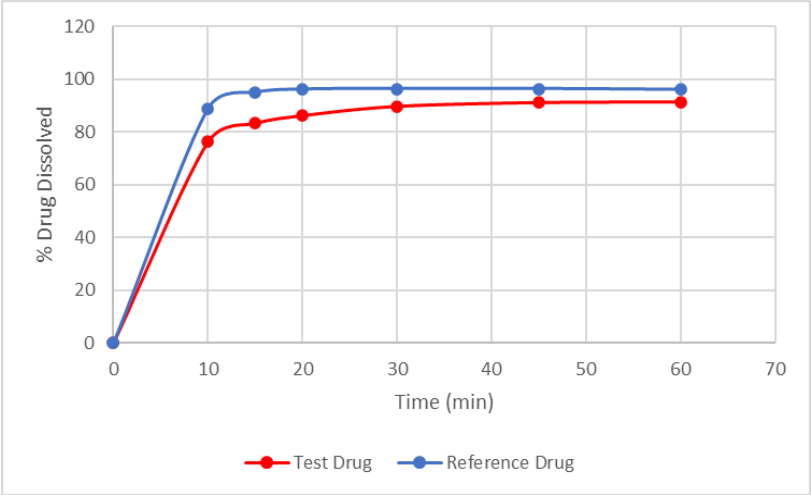
3 Result

3.1 Comparative Dissolution Test

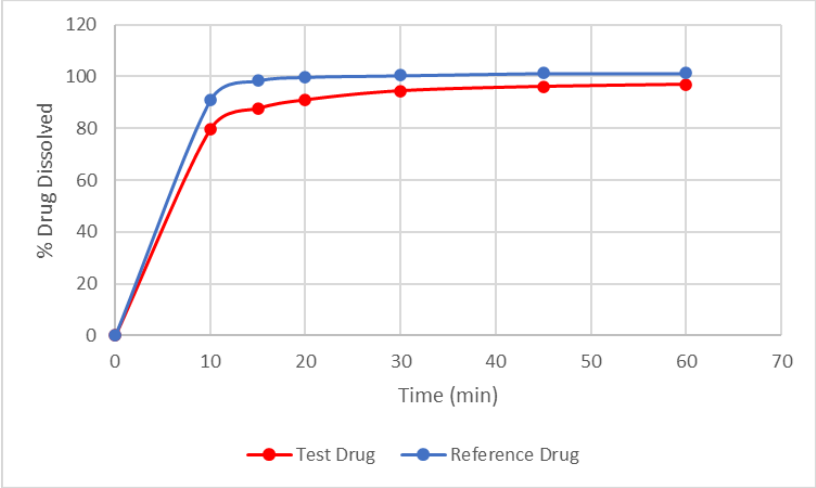
The comparative dissolution test of Favipiravir was conducted at three different media of certain pH levels (ie, pH 1.2, pH 4.5, and pH 6.8). Sampling was performed at 6 points for each dissolution medium (10, 15, 20, 30, 45 and 60 minutes). The relative standard deviation (%RSD) at the first sampling point (10 minutes) was less than 20%, while the %RSD at the subsequent points (15, 20, 30, 45, and 60 minutes) was less than 10%. The dissolution results of both the test drug and the reference drug showed values equal to or greater than 80% of the label claim at sampling times greater than 15 minutes but less than or equal to 30 minutes. The three comparative dissolution test results across the three different pH levels must adhere to the established criteria for difference factor (f1) with acceptance criteria of 1-15 and similarity factor (f2) with acceptance criteria of 50-100.

This study indicates that the comparative dissolution test results between the test drug and the reference drug meet the required criteria for each pH. The comparative dissolution profile of three different media of certain pH levels (ie, pH 1.2, pH 4.5, and pH 6.8) are shown in **Figure 1** and **Table 1.**

(a)


120
100
80
60
40
20
0
% Drug Dissolved
0
10
20
30
40
50
60
70
Time (min)
Test Drug
Reference Drug

(b)


120
100
80
60
40
20
0
% Drug Dissolved
0
10
20
30
40
50
60
70
Time (min)
Test Drug
Reference Drug

(c)

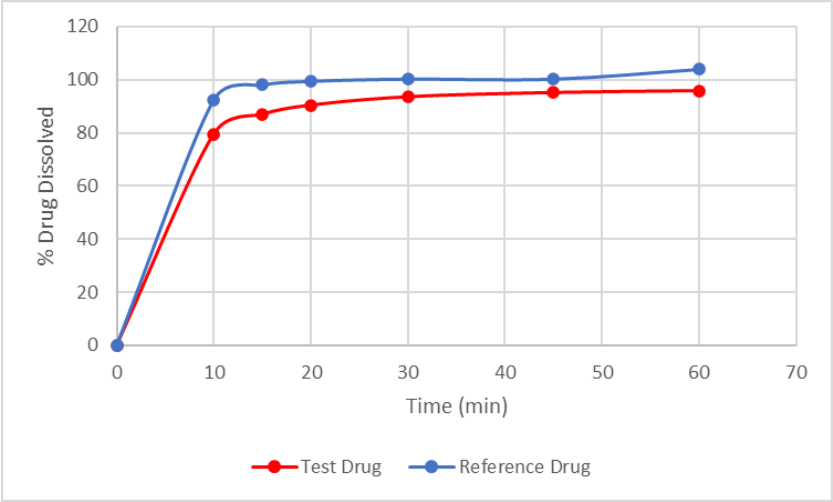


Figure 1. (a) The comparative dissolution test of test drug and reference drug in pH 1.2, (b) the comparative dissolution test of test drug and reference drug in pH 4.5, and (c) the comparative dissolution test of test drug and reference drug in pH 6.8.

Table I. The results of difference factor (f1) and similarity factor (f2) values from the comparative dissolution test at the three different media of certain pH levels (ie, pH 1.2, pH 4.5, and pH 6.8).

pH level	The Results	
	Difference Factor (f1) (Acceptance Criteria: 1-15)	**Similarity Factor (f2) (Acceptance Criteria: 50-100)**
pH 1.2	8.88	52.25
pH 4.5	7.81	54.19
pH 6.8	8.97	51.53

3.2 Ex Vivo Test Using Everted Gut Sac

The internal permeability testing of the Favipiravir 200 mg film-coated tablet was conducted on six Wistar strain rats, aged three weeks and weighing 150-200 grams, which had been fasted for 20-24 hours while still being provided with boiled water. The cellulose solution sampled at predetermined time points for both test drug and reference drug was analyzed using the HPLC system. The mass

of Favipiravir transported, along with the penetration profile graph of Favipiravir from both the test drug and the reference drug, is presented in **Table II** and **Figure 2.**

Table II. Amount of Transported Favipiravir

Time (minutes)	Amount of Transported Favipiravir (mg)	
	Test Drug	Reference Drug
15	0.0226	0.0216
30	0.0693	0.0646
45	0.1253	0.1211
60	0.1855	0.1726
90	0.2808	0.2360
120	0.3758	0.3470
150	0.4238	0.4134
180	0.5138	0.4942

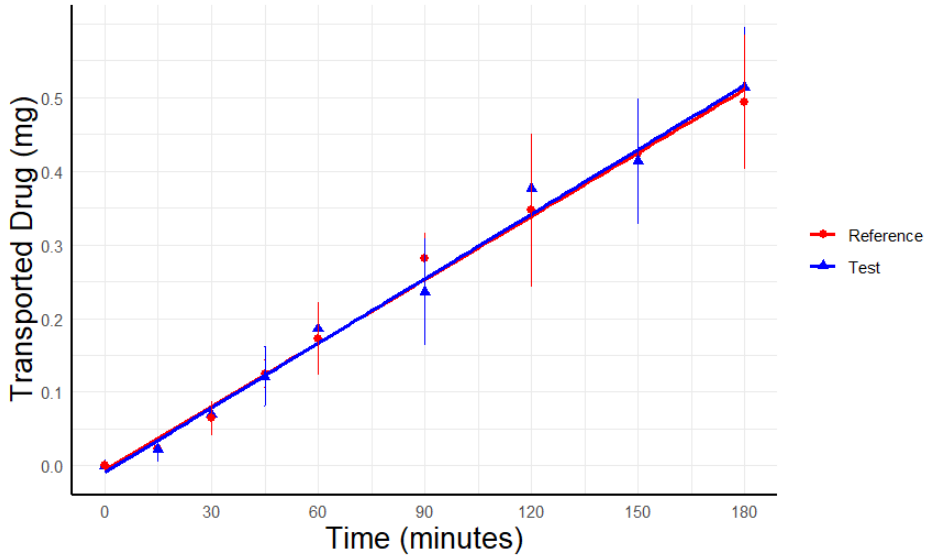


Figure 2. Average amount of transported drug into the sac versus time during dissolution of 200 mg favipiravir test and reference drug.

The absorption rate of Favipiravir in the test drug is expressed as the rate of absorption, derived from the slope of the time versus amount absorbed curve. The absorption rate data for the test drug are provided in **Table III**.

Table III. Absorption Rate of Favipiravir

Rats (i)	Absorption Rate (ng/min)	
	Test Drug	**Reference Drug**
A	2838.36	3181.97
B	2820.28	3336.93
C	3083.56	3467.99
D	3478.67	3012.69
E	1965.86	2232.13
F	3001.24	2640.34
Average	2864.66	2978.68
SD	500.82	465.20
RSD	17.48	15.62

The statistical analysis of both test drug and reference drug using a paired t-test yielded a calculated t-value of 0.6683, which is less than the critical t-value of 2.571. This result indicates that there is no statistically significant difference in the absorption rates between the two drugs.

3.3 Bioequivalence Study

The study involved a total of 30 healthy Indonesian subjects (19 males and 11 females) who met the inclusion and exclusion criteria. All participants had normal clinical laboratory parameters and were in good health. The subjects were aged between 22 and 51 years old, had body weights ranging from 47 to 72 kg, height between 143 and 172 cm, and body mass index (BMI) ranged between 18.47 kg/m^2 and 24.94 kg/m^2. Blood samples from these subjects after a single dose oral administration were analyzed to evaluate the pharmacokinetics of Favipiravir.

Figure 3 presents the area under the curve (AUC) profile of mean plasma concentration versus time for 30 subjects following oral administration of a Favipiravir 200 mg film-coated tablet, for both test and reference formulations. The geometric mean ratios of the test drug to the reference drug (90% CI) were 101.27% (96.89-105.86) for AUC_{0-t} and 101.22% (96.80-105.84) for C_{max}. All values were within the accepted bioequivalence range of 80.00-125.00% for both AUC_{0-t} and C_{max}. The detailed results of the statistical analysis for the bioequivalence test of Favipiravir 200 mg film-coated tablets are shown in **Table IV.**

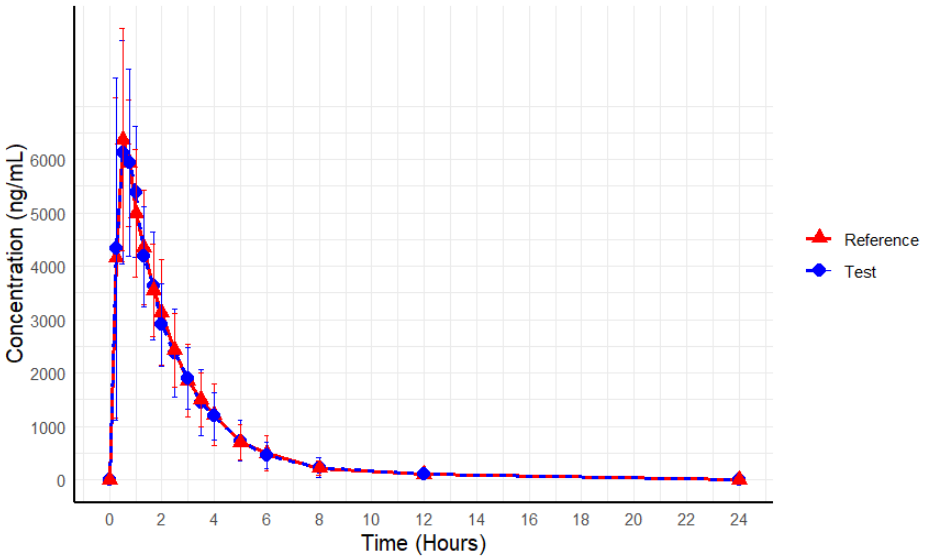


Figure 3. Mean Plasma Concentration of Favipiravir versus time profiles on human subjects (n = 30) after single-dose administration of 200 mg Favipiravir of the test and reference drug.

Table IV. Summary of bioequivalence data for Favipiravir

Parameter	Arithmetic Mean ± Standard Deviation	
	Test Drug	**Reference Drug**
AUC_{0-t} ($ng.h.mL^{-1}$)	1,5756.77 ± 4,773.47	1,5491.62 ± 4,288.43
AUC_{0-inf} ($ng.h.mL^{-1}$)	1,606.22 ± 4,900.70	1,5790.31 ± 4,364.66
C_{max} ($ng.mL^{-1}$)	7,237.49 ± 1,441.07	7,218.51 ± 1,896.11
$t_{1/2}$ (h)	1.43 ± 0.28	1.41 ± 0.25
t_{max} (h)	0.56 ± 0.26	0.61 ± 0.46

4 Discussion

The study proposes an in vitro approach using the everted gut sac method to predict bioequivalence. This method offers a valuable tool for conducting bioequivalence studies in a more sustainable manner. The simultaneous dissolution and ex vivo absorption test through the small intestine was carried out to provide an overview of the drug's absorption capacity, before commencing the actual bioequivalence test. Everted gut sac method has been conducted previously

to study intestinal P-glycoprotein [7]. However, this study apparently did not evaluate in vitro and in vivo for drug formulation.

Comparative dissolution testing of test and reference drugs was conducted at three different pH levels (pH 1.2, 4.5, and 6.8). Sampling was performed at 6 points for each pH (10, 15, 20, 30, 45 and 60 minutes). The result showed the F1 in pH 1.2; 4.5; 6.8 was at 8.88; 7.81; 8.97 respectively. The similarity factor (F2) at pH 1.2, 4.5, and 6.8 was at 52.24, 54.19, and 51.53 correspondingly. It means the test and reference drug had similar dissolution profiles in which the values met the acceptance range ($\leq$15 for F1 and $\geq$ 50 for F2).

The diffusion examinations were performed by utilizing everted gut sac methods which exhibit the average absorption rate of test and reference drug at 2978.68 ng/min and 2864.66 ng/min respectively. The everted gut sac study involved six male rats aged three weeks and 150 - 200 gram body weight range. There was not a significant difference between test drug and reference in the absorption rate shown by the student's paired t-test at 0.6683 ($<t_{critical}$ 2.57).

Later on, a bioequivalence study was conducted involving 30 healthy subjects. Crossover bioequivalence study, randomized, two-period, open-label, under fasting condition, two-sequence, was carried out to compare the bioavailability of favipiravir between in house formulation of favipiravir and the reference product (ie, the innovator or originator of Favipiravir), Avigan® (Fujifilm Toyama Chemical Japan).

In this study, the main parameters to evaluate bioequivalence between each preparation were defined by the value of AUC_{0-t}, AUC_{0-inf}, and C_{max} of favipiravir. Refer to the bioequivalence guidelines, the criteria for bioequivalence are that the value of AUC and Cmax should be within the range of 80%-125% for 90% CI of the geometric mean ratio of the test/reference. The results of the present study showed that mean ratio (90% CIs) of the AUC_{0-t}, AUC_{0-inf}, and C_{max} of favipiravir were 101.27% (96.89%-105.86%), 101.22% (96.80%-105.84%), and 101.8% (94.49%-109.67%).

Based on the provided data, it appears that the in vitro and in vivo studies align, which indicates a potential correlation. Favipiravir belongs to class II in the Biopharmaceutics Classification System (BCS) [9]. High permeability and low water solubility are two major characteristics of favipiravir. Thus, this study suggests that the everted gut sac method is beneficial for predicting the in vivo profile of favipiravir.

While an in vitro-in vivo correlation between the everted gut sac and in-vivo models has not been established, findings from the everted intestinal sac model

often align with in-vivo results, supporting its utility in early absorption assessment [6]. Drug absorption from gastrointestinal (GI) tract or any other extravascular site, depends on several factors: (1) the physicochemical properties of the drug and condition within small intestine, (2) the formulation of the dosage form, and (3) the anatomy and physiology of the absorption site [10]. Moreover, various factors such as animal mode, intestinal segment used, and experimental factor might influence the outcomes of the everted gut sac method [6].

5 Conclusion

The data obtained from the study suggest that there is a good correlation between ex vivo everted gut sac method and in vivo bioequivalence assessment on Favipiravir as a model. However, further research is required to find more general relationships and explore the limitation and potential application in developing another active ingredients' formulation.

6 References

[1] Faheem, Kumar B.K, Sekhar K.V.G.C, Kunjiappan S, Jamalis J, Balaña-Fouce R, Tekwani B.L, & Sankaranarayanan M., *Druggable Targets of SARS-CoV-2 and Treatment Opportunities for Covid-19.*, Bioorg Chem, **104**, pp. 15, Sep. 2020.

[2] Zhao L, Zhong W., *Mechanism of Action of Favipiravir against SARS-CoV-2: Mutagenesis or Chain Termination?.*,Innovation (Camb), **2**(4), pp. 1-2, Sep. 2021.

[3] Du Y-X & Chen X-P., *Favipiravir: Pharmacokinetics and Concerns About Clinical Trials for 2019-nCoV Infection*., Clinical Pharmacology & Therapy, **108**(2), pp. 242-247, Apr. 2020.

[4] Göktuğ Ö, Altaş E, Kayar G, Gökalp M., *The Development and the Validation of a Novel Dissolution Method of Favipiravir Film-Coated Tablets*. Scientia Pharmaceutica, **90**(1), pp. 1-14, Mar. 2022.

[5] Song, N, Zhang, S, & Liu, C., *Overview of factors affecting oral drug absorption.*, Asian Journal of Drug Metabolism and Pharmacokinetics, **4**(3), pp. 167-176, Aug. 2004.

[6] Alam, M. A., Al-Jenoobi, F. I., & Al-mohizea, A. M. *Everted gut sac model as a tool in pharmaceutical research: Limitations and application*s. Journal of Pharmacy and Pharmacology, **64**(3), 326–336. Sept. 2011.

[7] Barthe, L, Woodley JF, Kenworthy S, Houin G. *An improved everted gut sac as a simple and accurate technique to measure paracellular transport across the small intestine*. European Journal Drug Metabolism & Pharmacokinetics, **23**(2), pp. 313-323, 1998.

[8] Badan Pengawas Obat dan Makanan. Peraturan Kepala Badan Pengawas Obat dan Makanan Republik Indonesia Nomor 11 Tahun 2022 tentang *Tata Laksana Uji Bioekivalensi*. 2022.

[9] Seyed Ali Sajadian, Nedasadat S. A, Nadia E, Mahshid A, Abolghasem J., *Solubility of favipiravir (as an anti-COVID-19) in supercritical carbon dioxide: An experimental analysis and thermodynamic modeling*. The Journal of Supercritical Fluids. **183**. Feb. 2022.

[10] Leon Shargel, Andrew B.C. Yu., *Applied Biopharmaceutics & Pharmacokinetics*, ed. 7, McGraw-Hill Education, pp 177, 2012.

[11] Arellano, C., Philibert, C., Vachoux, C., Woodley, J., & Houin, G. *The metabolism of midazolam and comparison with other CYP enzyme substrates during intestinal absorption: In vitro studies with rat everted gut sacs.* Journal of Pharmacy & Pharmaceutical Sciences, **10**(1), 26-36, March. 2007.