# **Application News**

LC-MS

### **Analysis of Favipiravir in Human Plasma**

## No. **C229**

#### Introduction

Favipiravir (brand name: Avigan®), which was developed by FUJIFILM Toyama Chemical Co., Ltd, is one of the RNA polymerase inhibitors used to treat influenza. This report introduces the results of research into a system for analyzing favipiravir spiked with plasma using LC/MS/MS, a method that demonstrates outstanding selectivity (Fig. 1).

E. Imoto, D. Kawakami



Fig. 1 External Appearance of LCMS™-8060

#### Analytical Conditions and Pretreatment of Samples

Favipiravir (PN: C8720\*1), as the target compound, and [13C,15N]-favipiravir (PN: C8853\*1), as its stable isotope, were purchased from Alsachim, one of the companies of the Shimadzu Group. [13C,15N]-favipiravir was used as the internal standard (ISTD). The structural formulas of favipiravir and [13C,15N]-favipiravir are shown in Fig. 2. Favipiravir was spiked with commercially available human plasma treated with EDTA 2K to prepare a calibration curve. For analysis, the LC and MS analytical conditions shown in Table 1 and MRM transitions shown in Table 2 were used. Shim-pack Scepter C18-120 (50 mm×2.1 mm I.D., 1.9  $\mu$ m, P/N: 227-31012-03) was used as the analytical column. Fig. 3 shows the MS chromatograms.

A calibration curve was prepared using calibration points at plasma concentrations of 1, 2, 5, 10, 20, 50 and 100  $\mu$ g/mL for favipiravir (n = 5 for each calibration point). [ $^{13}$ C, $^{15}$ N]- favipiravir (20  $\mu$ g/mL) solution was prepared using acetonitrile and used as ISTD.

The pretreatment flow is shown in Fig. 4. Samples were prepared as follows ; 20 uL of 75% isopropyl alcohol(IPA), 50  $\mu L$  of plasma, 10  $\mu L$  of ISTD and 200  $\mu L$  of acetonitrile were mixed and shaken sufficiently, and then centrifuged. The obtained supernatant was transferred to an LC vial and analyzed.

\*1 Shimadzu GLC and Alsachim Product numbers

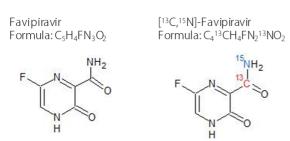


Fig. 2 Structural Formulas of Favipiravir and [13C,15N]-Favipiravir

Table 1 LC and MS Analytical Conditions

<lc analytical="" con<="" th=""><th>ditions&gt;</th><th colspan="4"><ms analytical="" conditions=""></ms></th></lc>	ditions>	<ms analytical="" conditions=""></ms>			
UHPLC	Nexera <sup>™</sup> X2	LC/MS/MS system	LCMS-8060		
Analysis column	Shim-pack Scepter C18-120 (50 mm×2.1 mm l.D., 1.9 μm)	Interface	Heated ESI		
Mobile phase	A: 0.05 % Formic acid – water B: 0.05 % Formic acid – acetonitrile	MS analysis mode	MRM (+)		
Gradient program (%B)	5 % (0 – 0.30 min) → 30 % (0.35 min) → 90 % (1.50 – 2.50 min) →	Heat block temperature DL temperature	400 °C 250 °C		
Flow rate	5 % (2.60 – 4.00 min) 0.4 mL/min	Interface temperature	300 °C		
Column oven temperature	40 °C	Nebulizing gas flow rate	3 L/min		
Injection volume	1.0 μL	Drying gas flow rate	10 L/min		
Rinse solution (for external rinse only)	MeOH	Heating gas flow rate	10 L/min		

Table 2 MRM Transitions of Favipiravir and [13C,15N]-Favipiravir

Compound	lon	Precursor ion (m/z)	Product ion (m/z)
Favipiravir	Quantifier ion	157.70	85.10
	Qualifier ion	157.70	113.20
[ <sup>13</sup> C, <sup>15</sup> N]- Favipiravir	Quantifier ion	159.70	85.10
	Qualifier ion	159.70	113.20

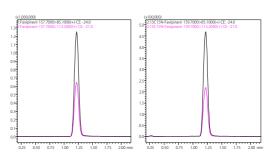


Fig. 3 MS Chromatograms of Favipiravir (Left) and [13C,15N]-Favipiravir (Right)



Fig. 4 Pretreatment Flow of Favipiravir in Plasma

#### Preparation of Calibration Curve

The calibration curve prepared using the samples spiked in plasma is shown in Table 3. Good linearity with  $R^2$  of 0.9998 was obtained over the set concentration range. The precision of favipiravir was %RSD 1.7 % – 6.5 %, accuracy ranged between 96 % – 103 %, within acceptance limits of 100  $\pm$  15 %.

Table 3 Linearity, Accuracy and Precision of Favipiravir in Plasma Obtained from Analysis

Favipiravir								
ID	Spiked Conc. (µg/mL)	Average Conc. (μg/mL)	Precision %RSD	Accuracy %	Calibration Curve			
Blank					Area ratio 3 Favipiravir			
Level 1	1	0.96	2.3	96	25.0 2 = 5.089523 × + 0.02217562 R1 = 0.9996239 R = 0.9999120 22.5			
Level 2	2	2.00	6.5	100	200 Weighting, Default (1 (C) 2002 Zero, Default (10 G) Feed (10 G) 2003 Zero, Default (10 G) Feed (10 G) 2003 Zero, Default (10 G) Feed (10 G) 2003 Zero, Default (10 G) 2003			
Level 3	5	4.98	2.3	100	15.0 9/RSD: 2.094884			
Level 4	10	10.3	1.9	103	12.5			
Level 5	20	20.5	1.7	103	$R^2 = 0.9998$			
Level 6	50	49.8	1.9	100	25			
Level 7	100	99.5	1.9	100	0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 Concentration ratio (jug/int.)			

#### Validation of Analytical System Using QC Samples

Favipiravir was prepared at the following plasma concentrations as QC samples: 3, 50, 90 µg/mL to evaluate its repeatability (Table 4) and between-days reproducibility comparing results obtained over three days (Table 5). Based on the repeatability test result, the precision of favipiravir (%RSD) was 1.7 % – 4.5 %. The accuracy ranged between 98 % – 104 % with acceptance limit of 100  $\pm$  15 %. Based on the test results for between-days reproducibility, the precision of favipiravir (%RSD) was 0.1 % – 5.2 %. The accuracy ranged between 89 % – 102 %, within acceptance limit of 100  $\pm$  15 % during QC sample analyses on each of the three days.

Table 4 Repeatability of Favipiravir in Plasma

		Spiked	Intra-Assay (n=6)				
Compound	QC Sample	Conc. (µg/mL)	Average Conc. (μg/mL)	Precision %RSD	Accuracy %		
Favipiravir	Low	3	3.12	2.6	104		
	Medium	50	49.2	4.5	98		
	High	90	89.8	1.7	100		

Table 5 Between-Days Reproducibility of Favipiravir in Plasma

Compound	Sample	Chilead	Day 1st (n=3)			Day 2nd ( <i>n</i> =3)			Day 3rd ( <i>n</i> =3)		
		Conc. (µg/mL)	Average Conc. (μg/mL)	Precision %RSD	Accuracy %	Average Conc. (μg/mL)	Precision %RSD	Accuracy %	Average Conc. (μg/mL)	Precision %RSD	Accuracy %
Favipiravir	Low	3	3.05	0.1	102	2.89	4.6	96	2.67	5.2	89
	Medium	50	49.0	1.1	98	47.5	1.0	95	46.4	2.2	93
	High	90	90.2	0.4	100	88.3	4.2	98	87.6	2.4	97

#### Stability Test of Analytical System

To evaluate the robustness and reproducibility of the developed analytical system, the sample prepared at a plasma concentration of 10  $\mu g/mL$  was injected consecutively 100 times. Fig. 5 shows the plotted results obtained by normalizing the areas from individual injections by the area from the first injection. As a result of consecutive injections 100 times, the %RSD of favipiravir was 2.9 %, while the %RSD of [ $^{13}C,^{15}N$ ]-favipiravir was 3.2 %, indicating that excellent injection stability was achieved. This result demonstrates that this analytical system can maintain outstanding sensitivity over a long period thanks to its exceptional robustness.

#### Conclusion

Using favipiravir spiked with plasma, an LC/MS/MS analytical system has been developed. The prepared calibration curve showed good linearity. Additionally, the samples were consecutively injected 100 times to evaluate the robustness of the analytical system. The findings demonstrated that the analytical system can maintain excellent sensitivity over a long period thanks to its exceptional robustness.

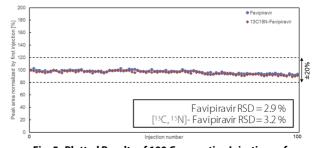


Fig. 5 Plotted Results of 100 Consecutive Injections of Favipiravir and [13C,15N]-Favipiravir Obtained by Normalizing the Areas Calculated from Individual Injections by the Area from the First Injection

The product described in this document has not been approved or certified as a medical device under the Pharmaceutical and Medical Device Act of Japan. It cannot be used for the purpose of medical examination and treatment or related procedures.

LCMS, Shim-pack Scepter and Nexera are trademarks of Shimadzu Corporation in Japan and/or other countries.

 $\ensuremath{\mathsf{AVIGAN}}$  is a registered trademark of Global Response Aid Inc. in the United States.

Third-party trademarks and trade names may be used in this publication to refer to either the entities or their products/services, whether or not they are used with the trademark symbol "TM" or "®".

First Edition: Oct. 2020



Shimadzu Corporation

www.shimadzu.com/an/

#### For Research Use Only. Not for use in diagnostic procedures

This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.

The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu. Shimadzu disclaims any proprietary interest in trademarks and trade names used in this publication other than its own. See <a href="http://www.shimadzu.com/about/trademarks/index.html">http://www.shimadzu.com/about/trademarks/index.html</a> for details.

The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.