SHIMADZU
Excellence in Science

Application News

No. C229

LC-MS

Analysis of Favipiravir in Human Plasma

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

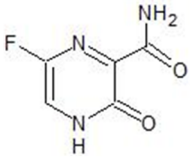
Favipiravir (PN: C8720*1), as the target compound, and [13C,15N]-favipiravir (PN: C8853*1), as its stable isotope, were purchased from Alsachim, one of the companies of the Shimadzu Group. [^{13}C,^{15}N]-favipiravir was used as the internal standard (ISTD). The structural formulas of favipiravir and [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 µm, 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 µg/mL for favipiravir (n = 5 for each calibration point). [^{13}C,^{15}N]- favipiravir (20 µg/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 µL of plasma, 10 µL of ISTD and 200 µL 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

Favipiravir
Formula: $C_5H_4FN_3O_2$



[^{13}C,^{15}N]-Favipiravir
Formula: $C_4{}^{13}CH_4FN_2{}^{13}NO_2$

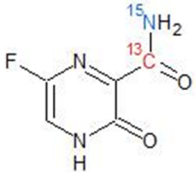


Fig. 2 Structural Formulas of Favipiravir and [13C,15N]-Favipiravir

Table 1 LC and MS Analytical Conditions

<LC analytical conditions>		<MS analytical conditions>	
UHPLC	Nexera™ X2	LC/MS/MS system	LCMS-8060
Analysis column	Shim-pack Scepter C18-120 (50 mm×2.1 mm I.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) ➡ 5 % (2.60 – 4.00 min)	Heat block temperature	400 °C
		DL temperature	250 °C
Flow rate	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 [^{13}C,^{15}N]-Favipiravir

Compound	Ion	Precursor ion (*m/z*)	Product ion (*m/z*)
Favipiravir	Quantifier ion	157.70	85.10
	Qualifier ion	157.70	113.20
[^{13}C,^{15}N]-Favipiravir	Quantifier ion	159.70	85.10
	Qualifier ion	159.70	113.20

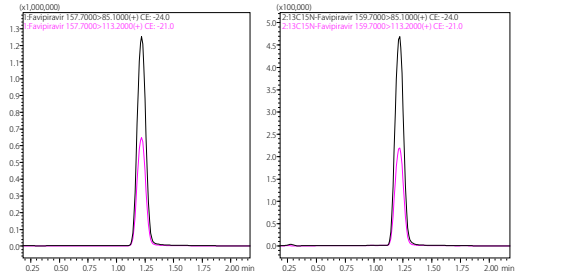


Fig. 3 MS Chromatograms of Favipiravir (Left) and [^{13}C,^{15}N]-Favipiravir (Right)

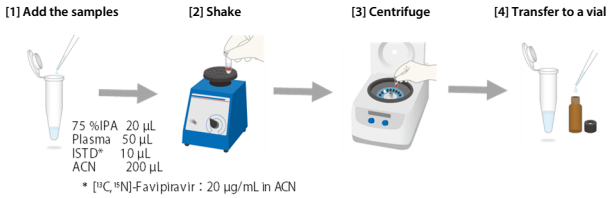
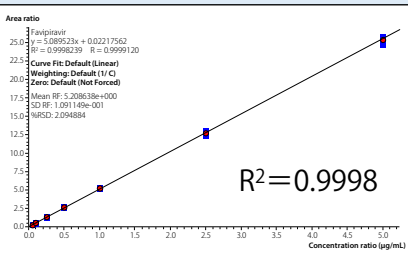


Fig. 4 Pretreatment Flow of Favipiravir in Plasma

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 ± 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	---	---	---	---	
Level 1	1	0.96	2.3	96	
Level 2	2	2.00	6.5	100	
Level 3	5	4.98	2.3	100	
Level 4	10	10.3	1.9	103	
Level 5	20	20.5	1.7	103	
Level 6	50	49.8	1.9	100	
Level 7	100	99.5	1.9	100	

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 ± 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 ± 15 % during QC sample analyses on each of the three days.

Table 4 Repeatability of Favipiravir in Plasma

Compound	QC Sample	Spiked Conc. (µg/mL)	Intra-Assay (*n*=6)		
			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	QC Sample	Spiked Conc. (µg/mL)	Day 1st (*n*=3)			Day 2nd (*n*=3)			Day 3rd (*n*=3)		
			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 µg/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.

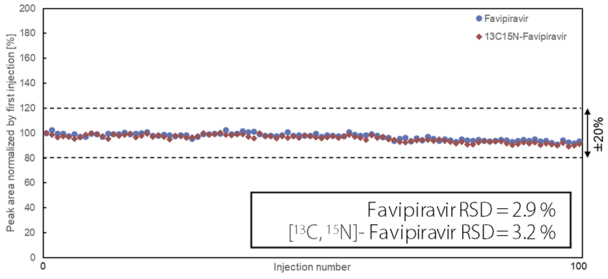


Fig. 5 Plotted Results of 100 Consecutive Injections of Favipiravir and [^{13}C,^{15}N]-Favipiravir Obtained by Normalizing the Areas Calculated from Individual Injections by the Area from the First Injection

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.

First Edition: Oct. 2020

SHIMADZU

Shimadzu Corporation

www.shimadzu.com/an/