SHIMADZU
Excellence in Science

Application News

No. C230

LC-MS

Analysis of Favipiravir in Human Plasma Using Fully Automated Sample Preparation LC/MS/MS System

Introduction

Favipiravir (brand name: Avigan®), which was developed by FUJIFILM Toyama Chemical Co., Ltd, is one of the RNA polymerase inhibitors used for treating influenza. In Application News C229, we introduced a robust, highly-sensitive analysis using LC/MS/MS with manual pretreatment. However, manual pretreatment of plasma samples entails a certain level of workload. This report introduces a method of analyzing favipiravir using a fully automated sample preparation LC/MS/MS system that can reduce variation between procedures, sample mix-ups, and risk of exposure to the samples (Fig. 1).

E. Imoto, D. Kawakami

Fig. 1 Fully Automated Sample Preparation LC/MS/MS System (CLAM™+LC/MS/MS)

Fully Automated Sample Preparation of Favipiravir in Plasma

For analysis of low-molecular weight compounds in plasma using a LCMS™, it is common to use supernatant collected following deproteinization by adding an organic solvent. With the fully automated sample preparation LC/MS/MS system, these preparatory steps are done automatically just by placing a blood collection tube in the system after plasma separation (Fig. 2). Pretreatment of the next sample can also be performed in parallel with LC/MS/MS analysis, which can greatly reduce the time required to analyze each sample.

This analysis was performed in a per-sample cycle time of 6.5 minutes from plasma pretreatment to the analysis of favipiravir using LC/MS/MS.

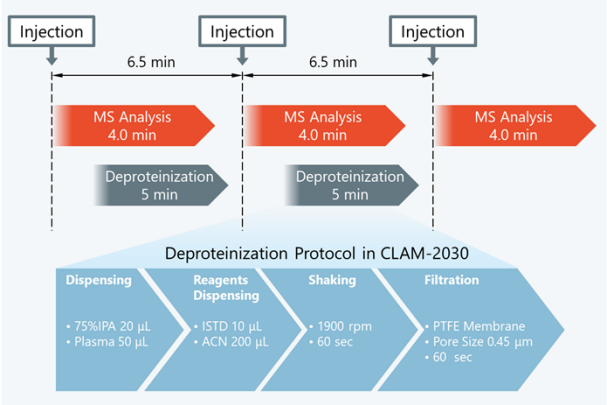


Fig. 2 Workflow of Fully Automated Sample Pretreatment

Analytical Conditions and Pretreatment of Samples

Favipiravir (PN: C8720*1), as the target compound, and [^{13}C,^{15}N]-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). Favipiravir was spiked with commercially available human plasma treated with EDTA 2K to prepare calibration curves and QC samples. Analysis was performed using the LC and MS analytical conditions shown in Table 1 and the MRM transition in Table 2. 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 and structural formulas of the compounds.

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 steps for the plasma sample spiked with favipiravir are shown in Fig. 2. Samples were automatically prepared through the following series of steps: mixing 20 µL of 75% isopropyl alcohol (IPA), 50 µL of plasma, 10 µL of ISTD and 200 µL of acetonitrile, shaking the mixture, and then filtration of the mixture using a PTFE membrane filter. Finally, the prepared sample was used for analysis.

*1 Shimadzu GLC and Alsachim Product numbers

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

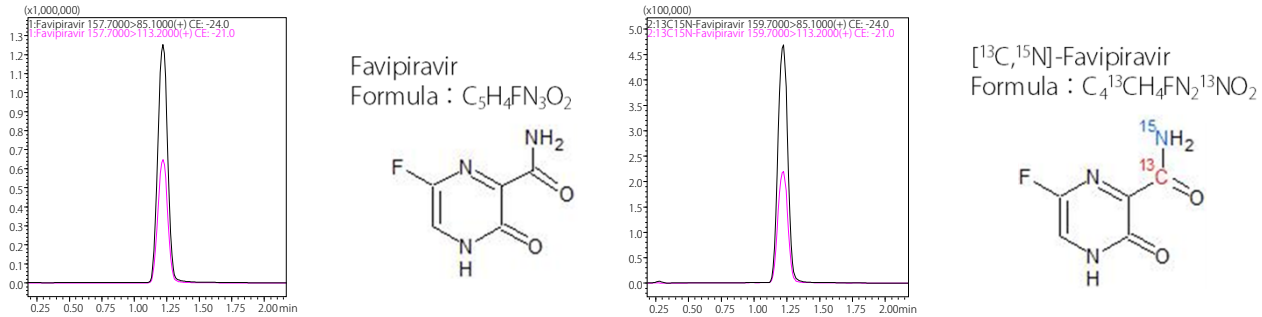
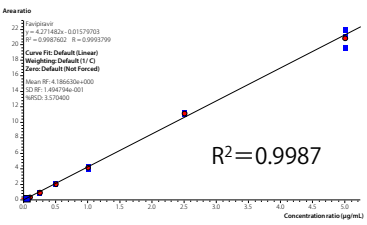


Fig. 3 MS Chromatograms and Structural Formulas of Favipiravir (Left) and [^{13}C,^{15}N]-Favipiravir (Right)

Preparation of Calibration Curve

The calibration curve prepared using the fully automated sample preparation LC/MS/MS are shown in Table 3. Good linearity with R^2 of 0.9987 was obtained in the set calibration range. The precision of favipiravir (%RSD) was 1.0 % – 5.6 % over the entire concentration range, including the quantitative lower limit. The accuracy of favipiravir ranged between 95 % – 105 %, within acceptance limits of 100 ± 15 %.

Table 3 Linearity, Precision and Accuracy of Favipiravir in Plasma Using Fully Automated LC/MS/MS Obtained from Analysis

Favipiravir					
ID	Spiked Conc. (µg/mL)	Average Conc. (µg/mL)	Precision %RSD	Accuracy %	Calibration Curve
Blank	---	---	---	---	
Level 1	1	1.04	4.3	104	
Level 2	2	2.00	1.9	100	
Level 3	5	4.77	2.4	95	
Level 4	10	9.87	1.9	99	
Level 5	20	19.7	3.5	99	
Level 6	50	52.7	1.0	105	
Level 7	100	97.9	5.6	98	

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 1.6 % – 3.0 %. The accuracy ranged between 94 % – 97 % with acceptance limit of 100 ± 15 %. Based on the test results for between-days reproducibility, the precision of favipiravir (%RSD) was 0.2 % – 7.6 %. The accuracy ranged between 88 % - 99 % 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	2.90	2.2	97
	Medium	50	48.3	3.0	97
	High	90	84.8	1.6	94

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	2.95	0.2	98	2.94	7.6	98	2.64	3.7	88
	Medium	50	49.3	1.0	99	47.1	2.8	94	46.0	3.6	92
	High	90	85.8	1.3	95	81.3	1.0	90	79.4	1.7	88

Conclusion

Using favipiravir spiked with plasma, a fully automated sample preparation LC/MS/MS analytical system has been developed. The prepared calibration curve showed good linearity. The repeatability and between-days reproducibility of favipiravir were evaluated using QC samples. Good accuracy and reproducibility were obtained.

First Edition: Oct. 2020

SHIMADZU

Shimadzu Corporation

www.shimadzu.com/an/