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Application News

No. L570

High Performance Liquid Chromatography

Quantitative Analysis of Favipiravir Spiked in Plasma Using by HPLC

Introduction

As of 2020, the development of both pharmaceuticals and vaccines remains urgent to overcome the global coronavirus disease 2019 (COVID-19) pandemic. Favipiravir (brand name: Avigan®), a promising drug candidate for COVID-19, is classified as an anti-influenza drug, evaluated and developed for both novel and re-emerging influenza viruses. [1], [2] Favipiravir undergoes renal excretion, eliminated in the urine mainly as a hydroxide. Notably, the plasma levels of this drug are difficult to control owing to its once daily dosing regimen. [3] Consequently, the accurate monitoring of drug levels is crucial.

In this report, we introduce a unique approach for the quantitative high sensitivity plasma analysis of favipiravir using only a standard high-pressure liquid chromatography (HPLC) setup without mass spectrometry.

R. Suzuki, Y. Osaka

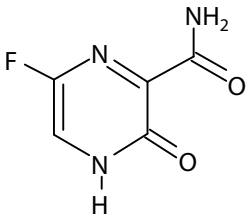

Fig. 1 Structural Formula of Favipiravir

Sample Preparation

Plasma and serum samples typically require deproteinization to prevent clogging and degradation of the analytical column. In this study, deproteinization was performed as follows. First, 25 µL of plasma and 100 µL of methanol were mixed well and centrifuged. Next, the obtained supernatant was recovered, and diluted by 15-fold with the mobile phase to be used in HPLC analysis. (Fig. 2).

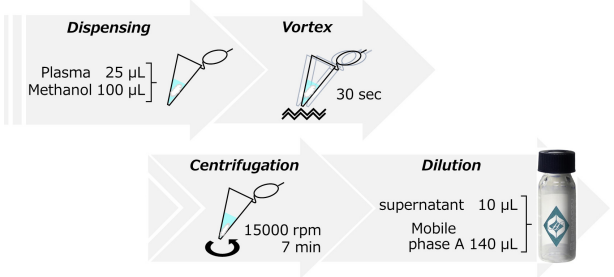

Fig. 2 Deproteinization Protocol

HPLC Analysis

Favipiravir *1 was purchased from Alsachim. The calibration curve and quality control (QC) samples were prepared by spiking healthy human plasma with favipiravir. The measurement was performed using HPLC analytical conditions shown in Table 1. The time program of gradient elution is shown in Table 2. Favipiravir was separated using an HPLC instrument fitted with Shim-pack Scepter™ C18-120 with a guard column. The chromatograms are shown in Fig. 3. A calibration curve was generated using favipiravir standard solutions of at 1, 10, 25, 50, and 100 µg/mL (n = 6) spiked in plasma.

*1: P/N C8720 (Alsachim's product number)

Table 1 Analytical Conditions

System	:	Nexera™ XR
Column	:	Shim-pack Scepter C18-120 *2 (150 mm × 4.6 mm I.D., 5.0 µm)
Guard Column	:	Shim-pack Scepter C18-120 (G) *3 (10 mm × 4.0 mm I.D., 5.0 µm)
Mobile Phase	:	A) 10 mmol/L (sodium) phosphate buffer pH 6.9 B) Methanol
Flow Rate	:	1.0 mL/min
Column Temp.	:	30 °C
Injection Volume	:	1.0 µL
Vial	:	TORAST-H Glass Vial (Shimadzu GLC) *4
Detection	:	Fluorescence detector (RF-20A) Ex. 360 nm [1], Em. 433 nm

*2: P/N 227-31020-05, *3: P/N 227-31126-01, *4: P/N 370-04301-01

Table 2 Time Program

Time (min)	A.conc	B.conc
0	100	0
2.5	100	0
7.5	30	70
9.5	30	70
9.51	100	0

Calibration Curve

In this quantitation range, good linearity was obtained ($R^2 = 0.999$, Weighting; (1/C)). Using accuracy and precision evaluations, the following results were obtained over the entire concentration range: favipiranvir precision (%RSD) was 0.21% – 0.31%, and accuracy ranged between 92.1% – 106%, within acceptance limits of 100±8.0%.

Validation Test with QC Samples

A validation test was performed using favipiravir at 2, 45, 90 µg/mL (n = 6) spiked in plasma like QC samples (Table 4). The validation test recorded favipiravir precision (%RSD) between 0.18% – 0.35%, with accuracy ranging between 96.5% – 100%, within acceptance limits of 100±4.0%.

Conclusion

We constructed a quantitative analysis method to assess plasma favipiravir levels using HPLC.

This system provided a high sensitivity quantitative analysis using a fluorescence detector.

In the validation test with QC samples, we obtained good accuracy and precision.

<References>
(1) Brian B. Gowen et. al., *"Alterations in favipiravir (T-705) pharmacokinetics and biodistribution in a hamster model of viral hemorrhagic fever"*, *Antiviral Res.*, 2015.
(2) E. Takashita et. al., *"Antiviral susceptibility of influenza viruses isolated from patients pre-and post-administration of favipiravir"*, *Antiviral Res.*, 2016.
(3) K. Shiraki et. al., *"Favipiravir, an anti-influenza drug against life-threatening RNA virus infections"*, *Pharmacol. Ther.*, 2020.

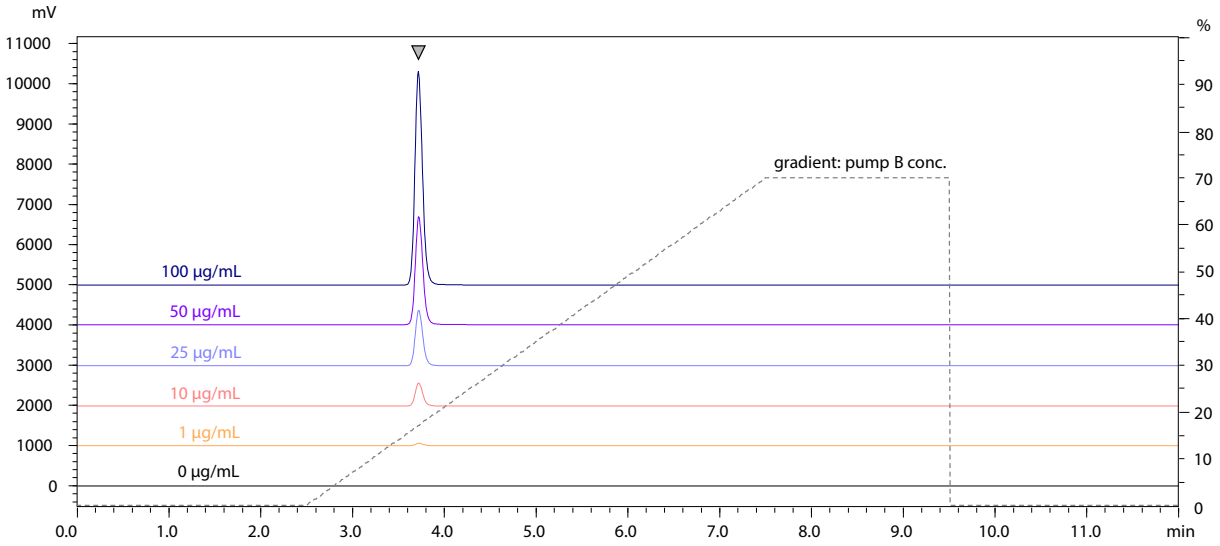

Fig. 3 Chromatograms of Favipiravir in Plasma and Gradient Profile

Table 3 Accuracy and Precision of Favipiravir in Plasma

ID	Spiked Conc. (µg/mL)	Intra-Assay (n=6)		
		Measured Conc. (µg/mL)	Precision %RSD	Accuracy %
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Level 1	1	0.921	0.25	92.1
Level 2	10	10.6	0.31	106
Level 3	25	25.8	0.26	103
Level 4	50	49.8	0.21	100
Level 5	100	98.8	0.24	98.8

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	2	1.93	0.18	96.5
	Medium	45	45.1	0.20	100
	High	90	88.7	0.35	98.5

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.

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