

# Application News

## No. C125

### Liquid Chromatography Mass Spectrometry

## High-Speed Analysis of Voriconazole in Plasma Using Triple Quadrupole LC/MS/MS (LCMS-8050)

Voriconazole is a triazole antifungal agent that is used widely for the treatment of infections by fungi of the genus *Aspergillus* and *Candida*.

This article introduces an example of high-speed analysis of voriconazole in plasma using the LCMS-8050 high-sensitivity triple quadrupole mass spectrometer. Although a simple method of sample pretreatment was used that involves only deproteinization, the quantitative results obtained were excellent in terms of accuracy and precision.

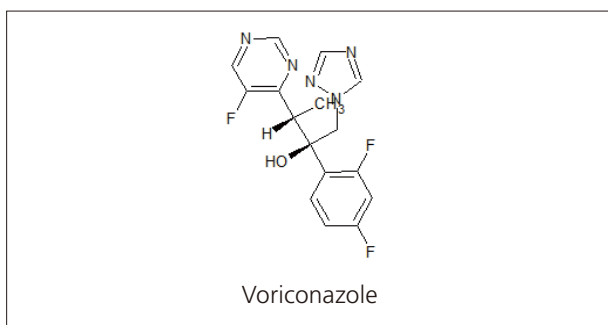


Fig. 1 Structural Formula of Voriconazole

### Sample Pretreatment

The pretreatment workflow is shown in Fig. 2. Standard (STD) samples with concentration of 0.1, 0.5, 1, 2.5, 5, and 10 µg/mL in plasma were prepared to create a calibration curve, and QC samples with concentration of 0.2, 4, and 8 µg/mL in plasma were prepared to validate the analysis results. An internal standard solution and acetonitrile were added to these standard samples and QC samples, and the deproteinized supernatant was then used for analysis. The stable isotope-labeled compound of voriconazole (voriconazole-d3) was used as the internal standard solution.

The pretreatment method used is simple and requires no labor-intensive steps such as solid phase extraction, which allows pretreatment to be performed at low cost and in a short period of time.

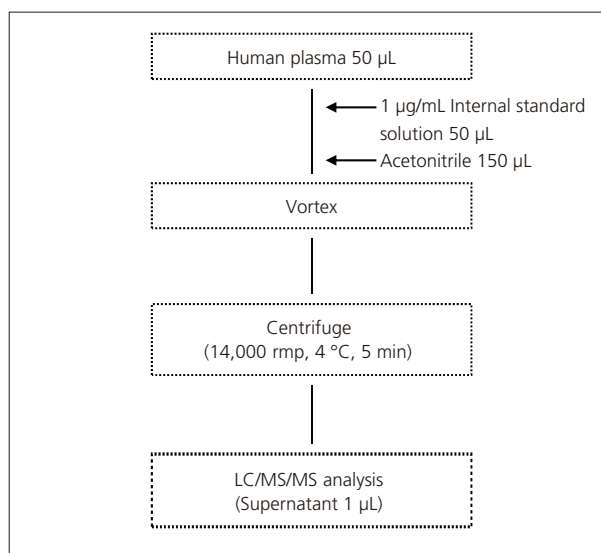


Fig. 2 Pretreatment Workflow

### LC/MS/MS Analytical Conditions

LC/MS/MS analytical conditions are shown in Table 1, and MRM transitions are shown in Table 2. A Shim-pack GIS column was used, and separation was performed in reverse-phase mode. Electrospray ionization (ESI) was used as the method of ionization, and MRM measurements were performed in positive ion mode.

Table 2 MRM Parameters

Compound Name	Polarity	MRM Transition
Voriconazole	+	350.20 > 281.20
Voriconazole-d3	+	353.20 > 284.20

Table 1 Analytical Conditions

Column	: Shim-pack GIS (75 mm L. × 2.1 mm I.D., 3 µm)
Mobile Phase	: A) 10 mmol/L Ammonium acetate in water B) Methanol
Time Program	: B. Conc. 30 % (0 - 0.50 min) → 100 % (1.50 - 3.00 min) → 30 % (3.01 - 5.00 min)
Flowrate	: 0.4 mL/min
Column Temperature	: 40 °C
Injection Volume	: 1 µL
Probe Voltage	: +4.0 kV (ESI-positive mode)
DL Temperature	: 250 °C
Block Heater Temperature	: 400 °C
Interface Temperature	: 300 °C
Nebulizing Gas Flow	: 3 L/min
Drying Gas Flow	: 10 L/min
Heating Gas Flow	: 10 L/min

■ Analysis Results

The calibration curve for voriconazole concentration in plasma is shown in Fig. 3, and the chromatograms for blank sample and each sample used for calibration are shown in Fig. 4.

Good linearity was obtained over the 0.1 to 10 µg/mL range of concentration in plasma, with a correlation coefficient of 0.999 or higher.

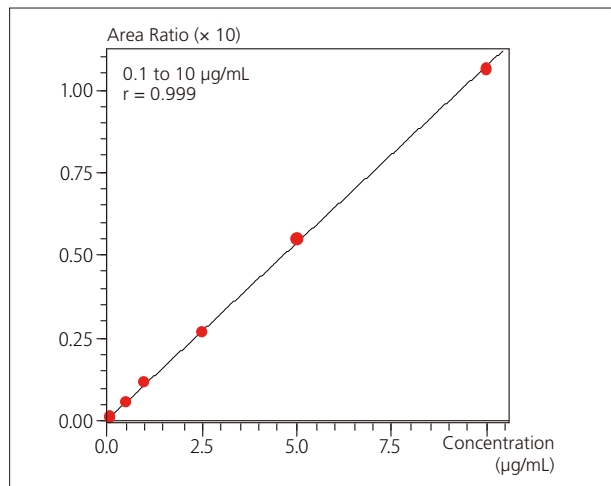


Fig. 3 Voriconazole Calibration Curve

The accuracy and repeatability of each point on the calibration curve for voriconazole concentration in plasma is shown in Table 3.

Good results were obtained for all points on the calibration curve, with repeatability of 5 % or below and accuracy within 100 ± 10 %.

Table 3 Measurement Results of Standard Sample in Plasma

	Concentration in Plasma (µg/mL)	Accuracy (%)	Concentration Repeatability (%)
STD1	0.1	93.1	3.04
STD2	0.5	101.2	1.58
STD3	1	105.8	2.61
STD4	2.5	99.6	1.50
STD5	5	101.7	1.22
STD6	10	98.7	1.16

QC sample measurement results are shown in Table 4. The results obtained were sufficiently precise and accurate, with repeatability of 2 % or lower and concentration accuracy of 95 to 105 %.

Table 4 Measurement Results of QC Samples

	Concentration in Plasma (µg/mL)	Accuracy (%)	Concentration Repeatability (%)
QC 1	0.2	98.6	1.74
QC 2	4	103.6	1.56
QC 3	8	99.0	1.70

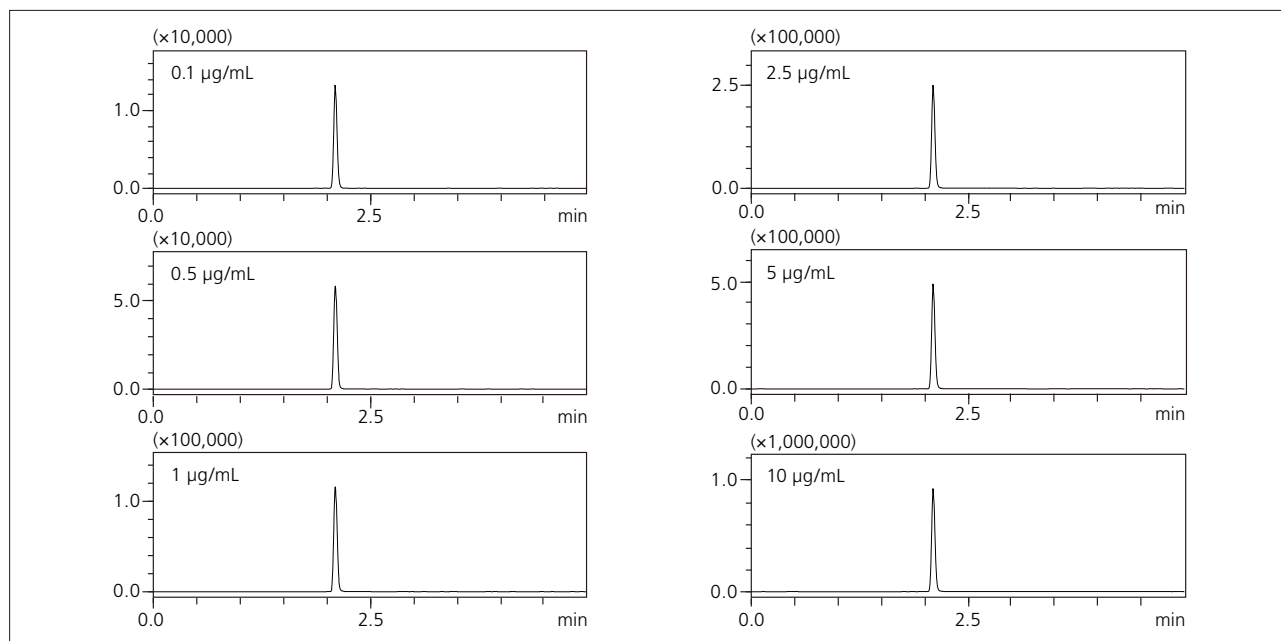


Fig. 4 Chromatograms for Samples with Voriconazole Spiked at Each Concentration in Plasma

This Application News was created with the cooperation of the Pharmaceutical Sciences Department of Tohoku University Hospital.

Notes: · The products mentioned in this article have not received approval for use as medical devices based on the Pharmaceutical and Medical Device Act.  
· The analytical methods mentioned in this article cannot be used for diagnostic purposes.

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