

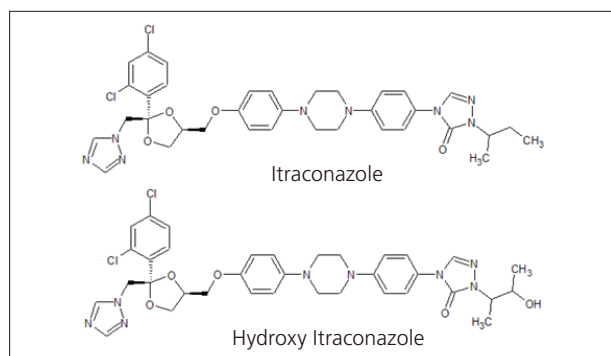
# Application News

## No. C127

### Liquid Chromatography Mass Spectrometry

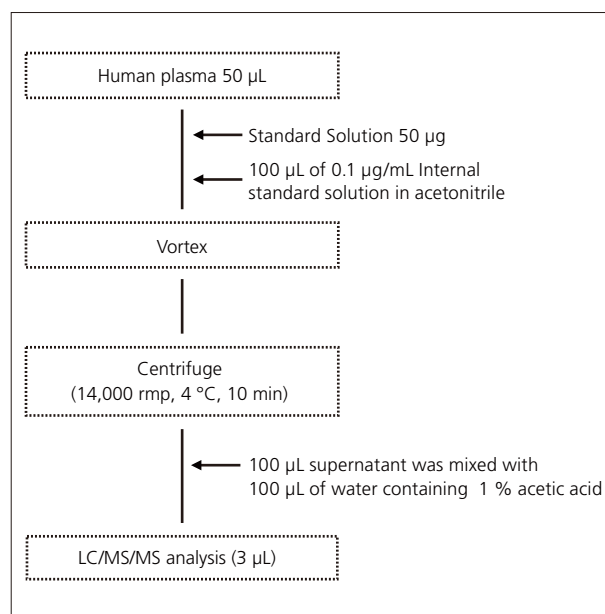
## High-Speed Analysis of Itraconazole in Plasma Using Triple Quadrupole LC/MS/MS (LCMS-8050)

Itraconazole is a triazole antifungal agent that is used widely for the treatment of infections by fungi including the genera *Trichophyton*, *Aspergillus* and *Candida*. This article introduces an example of high-speed analysis of itraconazole and its active metabolite hydroxy itraconazole 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 Itraconazole and Hydroxy Itraconazole**

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**

### Sample Pretreatment

The pretreatment workflow is shown in Fig. 2. Known concentrations of itraconazole and hydroxy itraconazole were solvated in a 1:1 water: methanol mixture, and used to prepare standard solutions for addition to plasma. These standard solutions for addition and an acetonitrile solution of an internal standard solution were added to human plasma, the deproteinized supernatant was diluted in an aqueous solution of 1 % acetic acid, and this mixture was used to prepare standard (STD) samples for a calibration curve and QC samples to validate the analysis results. The stable isotope-labeled compound of itraconazole (itraconazole-d3) was used as the internal standard solution.

### 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
Itraconazole	+	705.40 > 392.40
Hydroxy itraconazole	+	721.40 > 408.40
Itraconazole-d3	+	710.40 > 397.35

**Table 1 Analytical Conditions**

Column	: Shim-pack GIS (75 mm L. × 2.1 mm I.D., 3 µm)	Injection Volume	: 3 µL
Mobile Phase	: A) 10 mmol/L Ammonium acetate in water B) Acetonitrile	Probe Voltage	: +4.0 kV (ESI-positive mode)
Time Program	: B. Conc. 65 % (0 - 1.00 min) → 95 % (1.50 - 2.50 min) → 65 % (2.51 - 4.50 min)	DL Temperature	: 300 °C
Analysis Time	: 4.5 min	Block Heater Temperature	: 400 °C
Flowrate	: 0.4 mL/min	Interface Temperature	: 400 °C
Column Temperature	: 40 °C	Nebulizing Gas Flow	: 3 L/min
		Drying Gas Flow	: 5 L/min
		Heating Gas Flow	: 15 L/min

**Analysis Results**

The calibration curves for itraconazole and hydroxy itraconazole in plasma are shown in Fig. 3, and representative chromatograms are shown in Fig. 4. Good linearity was obtained over the 10 to 1000 µg/mL range of sample concentration in plasma, with a correlation coefficient of 0.999 or higher.

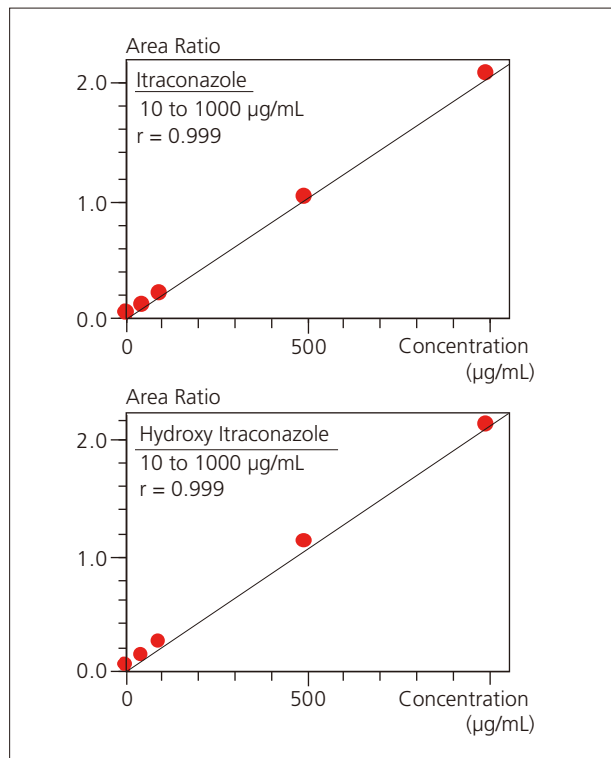


Fig. 3 Calibration Curves for Each Compound

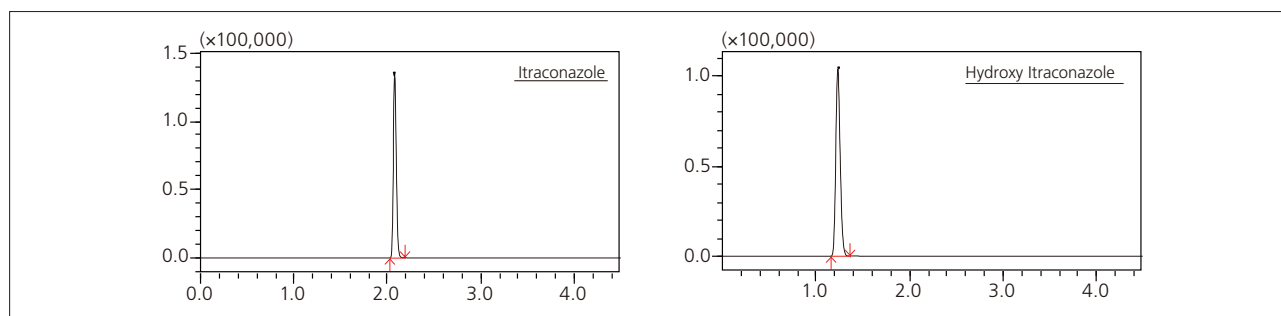


Fig. 4 Representative Chromatograms for Itraconazole and Hydroxy Itraconazole

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.

The accuracy and repeatability of each point on the calibration curves for itraconazole and hydroxy itraconazole concentration in plasma are shown in Table 3.

Good results were obtained for all points on the calibration curves, with repeatability of 15 % or below and accuracy within 100 ± 10 %.

Table 3 Measurement Results of Standard Samples in Plasma

		Concentration in Plasma (µg/mL)	Accuracy (%)	Concentration Repeatability (%)
Itraconazole	STD1	10	107.3	2.07
	STD2	50	98.9	1.52
	STD3	100	93.7	0.62
	STD4	500	99.1	0.30
	STD5	1000	101.1	0.59
Hydroxy Itraconazole	STD1	10	100.5	11.41
	STD2	50	98.3	4.47
	STD3	100	100.1	0.82
	STD4	500	102.1	1.15
	STD5	1000	99.0	5.55

QC sample measurement results are shown in Table 4. The results obtained were sufficiently precise and accurate, with repeatability of 5 % or lower and concentration accuracy of 80 to 105 %.

Table 4 Measurement Results of QC Samples

		Concentration in Plasma (µg/mL)	Accuracy (%)	Concentration Repeatability (%)
Itraconazole	QC1	25	98.7	0.65
	QC2	250	98.8	0.82
	QC3	750	103.3	0.38
Hydroxy Itraconazole	QC1	25	81.3	3.27
	QC2	250	90.4	4.36
	QC3	750	86.9	3.10