

Application News

Liquid Chromatography Mass Spectrometry

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

No.C127

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.

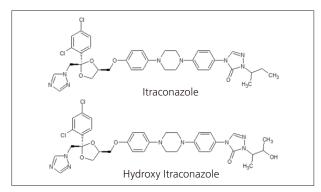


Fig. 1 Structural Formula of Itraconazole and Hydroxy Itraconazole

■ 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.

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.

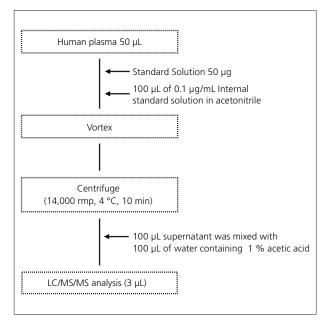


Fig. 2 Pretreatment Workflow

LC/MS/MS Analytical Conditions

LC/MS/MS analytical conditions are shown in Table 1, and MRM transitions are shown in Table 2. A Shimpack 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 : A) 10 mmol/L Ammonium acetate in water Mobile Phase Probe Voltage +4.0 kV (ESI-positive mode) B) Acetonitrile DL Temperature 300 °C : B.Conc. 65 % (0 - 1.00 min) → 95 % (1.50 - 2.50 min) Block Heater Temperature 400 °C Time Program → 65 % (2.51 - 4.50 min) Interface Temperature 400 °C Analysis Time : 4.5 min Nebulizing Gas Flow 3 L/min Flowrate : 0.4 mL/min Drying Gas Flow 5 L/min Column Temperature : 40 °C 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 μ g/mL range of sample concentration in plasma, with a correlation coefficient of 0.999 or higher.

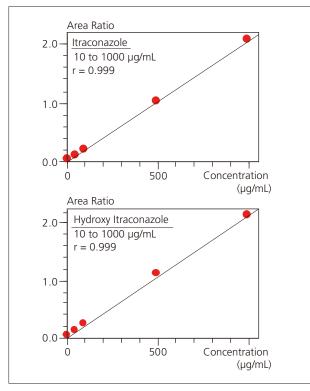


Fig. 3 Calibration Curves for Each Compound

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 %.

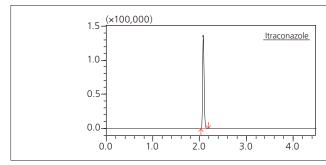
Table 3 Measurement Results of Standard Samples in Plasma

| | | 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 |



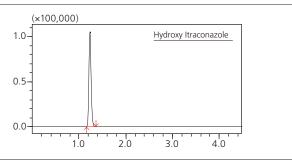


Fig. 4 Representative Chromatograms for Itraconazole and Hydroxy Itraconazole

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

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