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Application News

No. C196

Liquid Chromatography Mass Spectrometry

Simultaneous Analysis of Immunosuppressive and Antifungal Drugs in Human Blood Plasma Using LC/MS/MS

Mycophenolate mofetil, a prodrug for immunosuppressive drugs, is used to suppress rejection after organ transplantation. In addition, Voriconazole and Itraconazole are administered in combination as antifungal drugs to prevent infection after transplantation.

This article introduces a simultaneous analysis of an immunosuppressive drug (mycophenolic acid), antifungal drugs (Voriconazole and Itraconazole) and metabolites using the LCMS™-8050 triple quadrupole high-performance liquid chromatograph mass spectrometer.

T. Tsukamoto

Simultaneous Analysis of Mycophenolic Acid, Voriconazole and Itraconazole

Control human plasma samples spiked mycophenolic acid, Voriconazole, Itraconazole and its metabolite (hydroxyitraconazole) were deproteinized according to the process in Fig. 1. The resulting supernatants were subjected to analysis. MRM measurement with LC/MS/MS can selectively detect target drugs according to their molecular mass and structure (Fig. 2).

Table 1 Immunosuppressive Drug, Antifungal Drugs and Metabolites

Group	Compound	MRM transition *m/z*
Immunosuppressive drug	Mycophenolic acid (MPA)	321.1 > 207.1
Antifungal drug and metabolite	Voriconazole (VRC)	350.1 > 281.1
	Itraconazole (ITC)	705.2 > 392.2
	Hydroxyitraconazole (OH-ITC)	721.2 > 408.2
Internal Standard	$MPA\text{-}d_3$	324.1 > 210.1
	$VRC\text{-}d_3$	353.1 > 284.1
	$ITC\text{-}d_4$	709.2 > 396.2
	$OH\text{-}ITC\text{-}d_4$	725.2 > 396.2

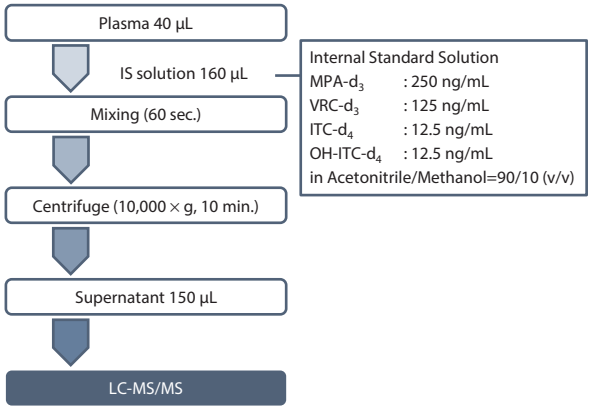

Fig. 1 Pretreatment Workflow of Blood Plasma Samples

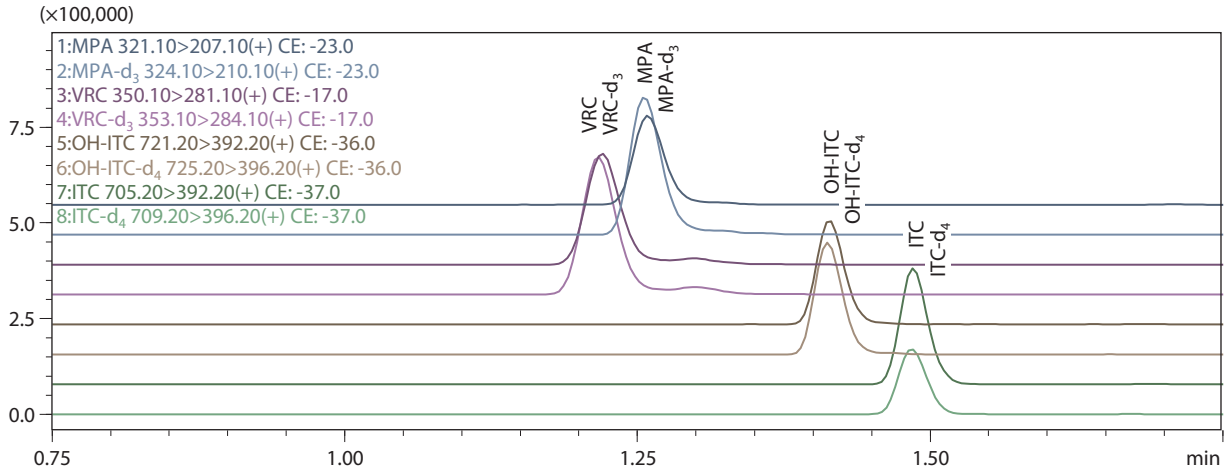

Fig. 2 Typical Chromatograms of Target Compounds and Internal Standards

Method Validation

Calibration curves were created using standard samples prepared with the pooled control plasma, and the validation of accuracy and precision based on the results of analysis of the QC samples (n = 6 for each level) was performed (Table 2).
Good linearity was seen in the calibration range for all compounds, and accuracy over the entire range including the quantitative lower limit was within 100 ± 15%. In the same manner, precision (%RSD) was within 15% and good repeatability was obtained [1), 2)].

The influence of matrix effect was evaluated with the results of six types of independent control plasma spiked the low QC level of standards. Accuracy was within 100 ± 15% and precision (%RSD) was within 15% for all the compounds, and no significant effect on the quantitative results due to difference in origin was observed.

Table 2 Results of Method Validation for Simultaneous Analysis of Immunosuppressive Drug, Antifungal Drugs and Metabolites

Compound	Cal. Range [ng/mL]	Correlation Coefficient R	Accuracy %				Precision %RSD, n=6				Matrix Effect at Low conc. n=6	
			LLOQ	Low	Med	High	LLOQ	Low	Med	High	Accuracy %	Precision %RSD
MPA *1	100-20,000	0.9983	94.5	97.8	100.2	101.7	4.0	3.7	1.7	0.3	95.5	1.6
VRC *2	50-10,000	0.9987	102.3	103.1	102.2	102.9	3.5	1.2	1.0	0.7	106.1	1.0
ITC *3	5-1000	0.9992	92.8	98.0	102.9	92.4	5.3	2.3	2.1	2.6	99.2	2.4
OH-ITC *3	5-1000	0.9987	101.6	102.5	103.9	100.7	8.9	6.4	1.6	1.7	107.9	2.8

*1: 100 ng/mL for LLOQ, 200 ng/mL for Low, 1000 ng/mL for Med, 15000 ng/mL for High
*2: 50 ng/mL for LLOQ, 100 ng/mL for Low, 500 ng/mL for Med, 7500 ng/mL for High
*3: 5 ng/mL for LLOQ, 10 ng/mL for Low, 50 ng/mL for Med, 750 ng/mL for High

Measurement of Plasma Samples

An analysis of plasma samples from patients taking mycophenolate mofetil and Itraconazole is shown in Fig. 3. With the actual blood plasma samples, as with the samples prepared with control blood plasma, no significant interference due to matrices in the blood plasma was observed, and selective detection of mycophenolic acid, Itraconazole and hydroxyitraconazole was possible.

This analysis method using LC/MS/MS is expected to be used as a simultaneous analysis method for immunosuppressive and antifungal drugs in plasma samples.

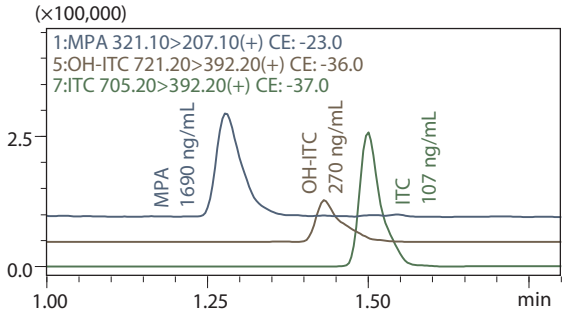

Fig. 3 Measurement Results of Plasma Samples of Patients Taking Mycophenolate Mofetil and Itraconazole

Table 3 Analysis Conditions (Method Validation)

System	: Nexera™ + LCMS-8050		
Column	: Shimadzu Shim-pack Scepter™ C18 Metal Free (50 mmL. × 2.1 mm I.D., 3 µm)		
Mobile Phase	: A: 10 mmol/L Formic acid + 10 mmol/L Ammonium formate - Water B: 10 mmol/L Formic acid + 10 mmol/L Ammonium formate - Methanol		
Flow Rate	: 0.45 mL/min		
Time program	: B Conc. 55% (0 – 0.55 min) – 100% (0.9 – 2.1 min) – 55% (2.11 – 3 min)		
Column Temp.	40 °C	Injection Volume	: 5 µL
Probe Voltage	: 4.0 kV (ESI-positive mode)		
Interface Temp.	: 300 °C	DL Temp.	: 250 °C
Block Heater Temp.	: 400 °C	Nebulizing Gas Flow	: 3 L/min
Heating Gas Flow	: 15 L/min	Drying Gas Flow	: 5 L/min

<Acknowledgments>
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<References>
1) Bioanalytical Method Validation: Guidance for Industry (2018, US FDA)
2) Guideline on Bioanalytical Method Validation in Pharmaceutical Development (2013, JP MHLW)

<Disclaimer>
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.
The samples described in this document were all sampled and measured at Tohoku University Hospital. Permission was obtained regarding the publication of measurement data.

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