C146-E464A

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Technical Report

Analysis for Voriconazole Using Fully Automated Sample Preparation LC-MS/MS System and Comparison of Measurement Methods

Daisuke Kawakami[1], Ai Tsutsui[1]

Abstract:

Shimadzu participated in the 2020 TDM (therapeutic drug monitoring) Control Survey for Anti-infective Drugs organized by the Japanese Society of TDM Quality Control. Shimadzu used a stable isotope-labeled reagent and LC-MS/MS system with a fully automated sample preparation module for its participation. The Japanese Society of TDM Quality Control measured the samples distributed to participating facilities beforehand to obtain reference values, and the measurements obtained by Shimadzu differed from these reference values by 0.0 to 2.9 %, indicating Shimadzu's results were highly accurate. This report presents the results of this control survey that were published by the Japanese Society of TDM Quality Control.

Keywords: Antifungal medication, infectious disease, voriconazole, LC-MS/MS, fully automated sample preparation module

1. Introduction

Voriconazole is a deep-seated mycosis azole therapeutic and the only antifungal medication that allows for TDM. According to the "Guidelines for TDM of Antimicrobial Agents" published in 2016, the recommended effective therapeutic range of voriconazole is a trough level in blood of 1.0 to 2.0 µg/mL and above, and risk of visual impairment and liver damage increases when trough levels in blood surpass 4.0 to 5.0 µg/mL.

The Act on Partial Revision of the Medical Care Act (Act No. 57 of 2017), implemented on December 1, 2018, specifies the TDM and requires that testing accuracy is assured. Blood drug concentration measurements often differ between testing facilities and testing methods, and third-party institutions play an important role in the external management of testing accuracy (via control surveys). The TDM control survey organized by the Japanese Society of TDM Quality Control covers 29 drugs in 4 disease groups, including anti-infective drugs. The TDM control survey sends identical samples to participating facilities throughout Japan, collects data on sample measurements performed by each facility, analyzes the data for differences between facilities and test methods, and shares the results of this analysis with the participating facilities.

Twenty-nine facilities (university hospitals, general hospitals, branch laboratories [clinical laboratories]) participated in the 2020 TDM control survey of anti-infective drugs that included seven drugs: vancomycin, teicoplanin, arbekacin, amikacin, gentamicin, tobramycin, and voriconazole.

Here are presented the results from Shimadzu's participation in the TDM control survey of voriconazole, in which Shimadzu measured voriconazole values using a mass spectrometer and fully automated LCMS sample preparation module.

Table 1 Drug name and Therapeutic Classification of Anti-Infective Drugs

Drug Name	Therapeutic Classification
Vancomycin	Glycopeptide antibiotic
Teicoplanin	Glycopeptide antibiotic
Arbekacin sulfate	Aminoglycoside antibiotic
Amikacin sulfate	Aminoglycoside antibiotic
Gentamicin sulfate	Aminoglycoside antibiotic
Tobramycin	Aminoglycoside antibiotic
Voriconazole	Deep-seated mycosis

2. Analysis Method

The sample preparation workflow of the CLAM™-2030 fully automated sample preparation module is shown in Fig. 1 and the analytical conditions and MRM transitions used by the LCMS-8050 system are shown in Table 2 and Table 3, respectively.

Measurements were taken using the column, mobile phases, and cleaning phase from the DOSIMMUNE™ immunosuppressant analysis kit for LC-MS/MS (Alsachim, a Shimadzu Group Company, France) a voriconazole standard (Cat#: C2674, Alsachim), and voriconazole labeled with a stable isotope ([$^{13}C_2$,$^{2}H_3$]-Voriconazole [Cat#: C4570, Alsachim]).

Calibration curve samples (0.1, 0.5, 1.0, 2.5, 5.0, and 10 µg/mL) and quality control (QC) samples (0.30, 0.75, and 1.75 µg/mL) were prepared by adding voriconazole standard to commercial blood plasma. A calibration curve was created from the calibration curve samples and the control survey samples were measured by an internal standard method.

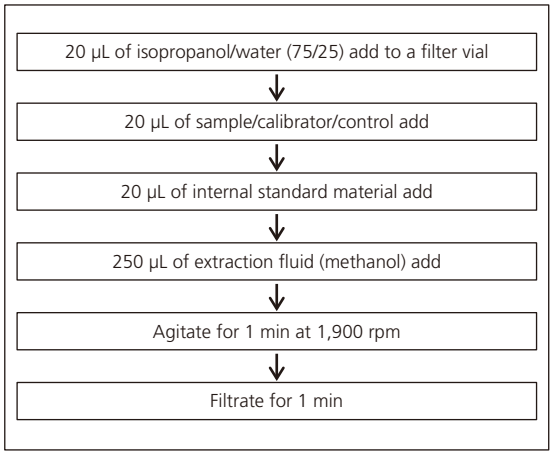

Fig. 1 CLAM-2030 Sample Preparation Workflow

1 Analytical & Measuring Instruments Division

Table 2 LC and MS Analytical Conditions

[HPLC conditions] (Nexera™)		
Column	:	DOSIMMUNE, Analytical Column*1
Mobile phase	:	DOSIMMUNE, Mobile phase A*2
	:	DOSIMMUNE, Mobile phase B*3
Column Temp.	:	40 ˚C
Flow rate	:	0.4 mL/min
Time program	:	30% (0 - 0.50 min) - 100% (1.50-3.00 min) - 30% (3.01- 4.00 min)
Cleaning phase	:	DOSIMMUNE, Cleaning Phase*4
Injection volume	:	0.5 µL
[MS conditions] (LCMS-8050)		
Ionization	:	ESI positive
DL temp.	:	250 ˚C
Heat block temp.	:	400 ˚C
Interface temp.	:	300 ˚C
Nebulizer gas flow	:	3 L/min
Drying gas flow	:	10 L/min
Heating gas flow	:	10 L/min

*1 P/N: 227-40130-58
*2 P/N: 227-40102-58
*3 P/N: 227-40103-58
*4 P/N: 227-40104-58

Table 3 MRM Parameters

Compound Name	Polarity	MRM Transition *m/z*
Voriconazole	+	350.20 > 281.20
[$^{13}C_2$,$^{2}H_3$]-Voriconazole	+	355.20 > 284.20

3. Measurement Results

The calibration curve of voriconazole-spiked plasma is shown in Fig. 2 and chromatograms for voriconazole at 1.686 µg/m and [$^{13}C_2$,$^{2}H_3$]-voriconazole at 1.000 µg/m are shown in Fig. 3. A correlation coefficient of above 0.999 was obtained in the range 0.1 to 10 µg/mL for voriconazole in plasma, showing good linearity.

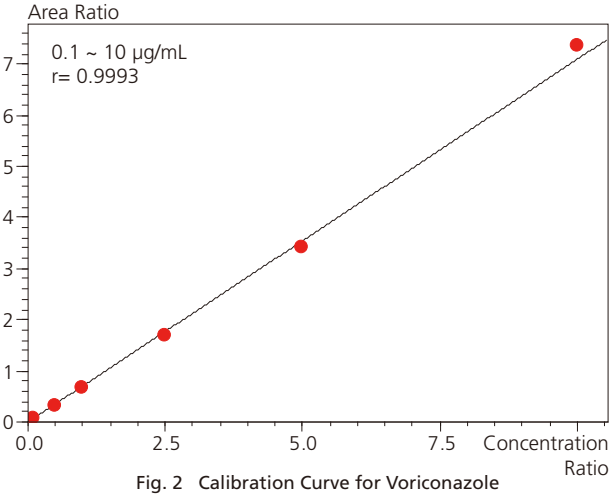

Fig. 2 Calibration Curve for Voriconazole

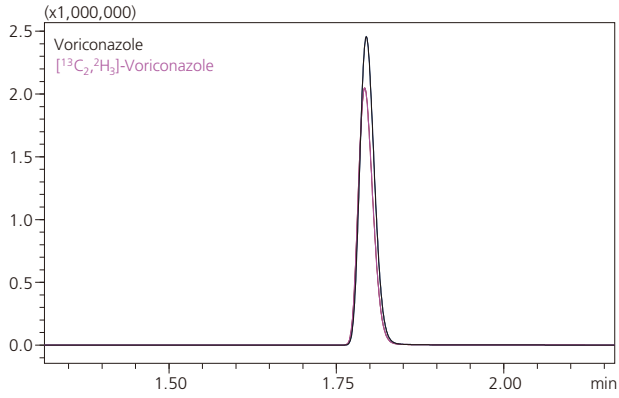

Fig. 3 Chromatograms of Voriconazole and [$^{13}C_2$,$^{2}H_3$]-Voriconazole

The measurements taken from the QC samples are shown in Table 4. In terms of repeatability, the relative standard deviation (%RSD) of the measurements was no more than 10 % and accuracy was within 90 to 110 %, confirming this measurement method was sufficiently precise and accurate.

Table 4 QC Sample Measurement Results

	Spiked conc. (µg/mL)	Repeatability (n=6)		
		Measured conc. (µg/mL)	Accuracy (%)	%RSD (%)
QC1	0.30	0.32	106.7	9.43
QC2	1.75	1.65	94.1	1.80
QC3	7.50	7.44	99.2	2.91

4. Control Survey Results

Fig. 4 shows the measurement values of voriconazole obtained by each participating facility and published at the 6th TMD-QC Workshop in "Report on the Results of the 2020 TDM Control Survey for Infectious Disease Therapeutics"*5. The vertical axis shows the concentration of voriconazole and the horizontal axis shows the measurement methods.

Reference values, which are the concentration of voriconazole in distributed samples measured in advance by the Japanese Society of TDM Quality Control, are shown above the graphs: Low 1.0 µg/mL, Middle 4.0 µg/mL, and High 7.0 µg/mL. The distributed samples were delivered to each facility without revealing their reference values. The red and blue circles indicate values measured by participating facilities using either measurement method. The empty circles indicate mean values and the bars indicate standard deviation. The red circle on the right side of each graph encircled by a gray circle shows the voriconazole value measured by Shimadzu using LC-MS/MS.

Table 5 shows the reference values for voriconazole, the voriconazole values measured by Shimadzu, and the difference between them. The difference (%) between voriconazole values measured by Shimadzu and reference values was calculated as (Shimadzu value - Reference value)/Reference value × 100. Table 5 shows the voriconazole values measured using the LC-MS/MS system with a fully automated sample preparation module and a stable isotope-labeled reagent and the reference values differed by 0 to 2.9 %, indicating the results obtained by Shimadzu were highly accurate.

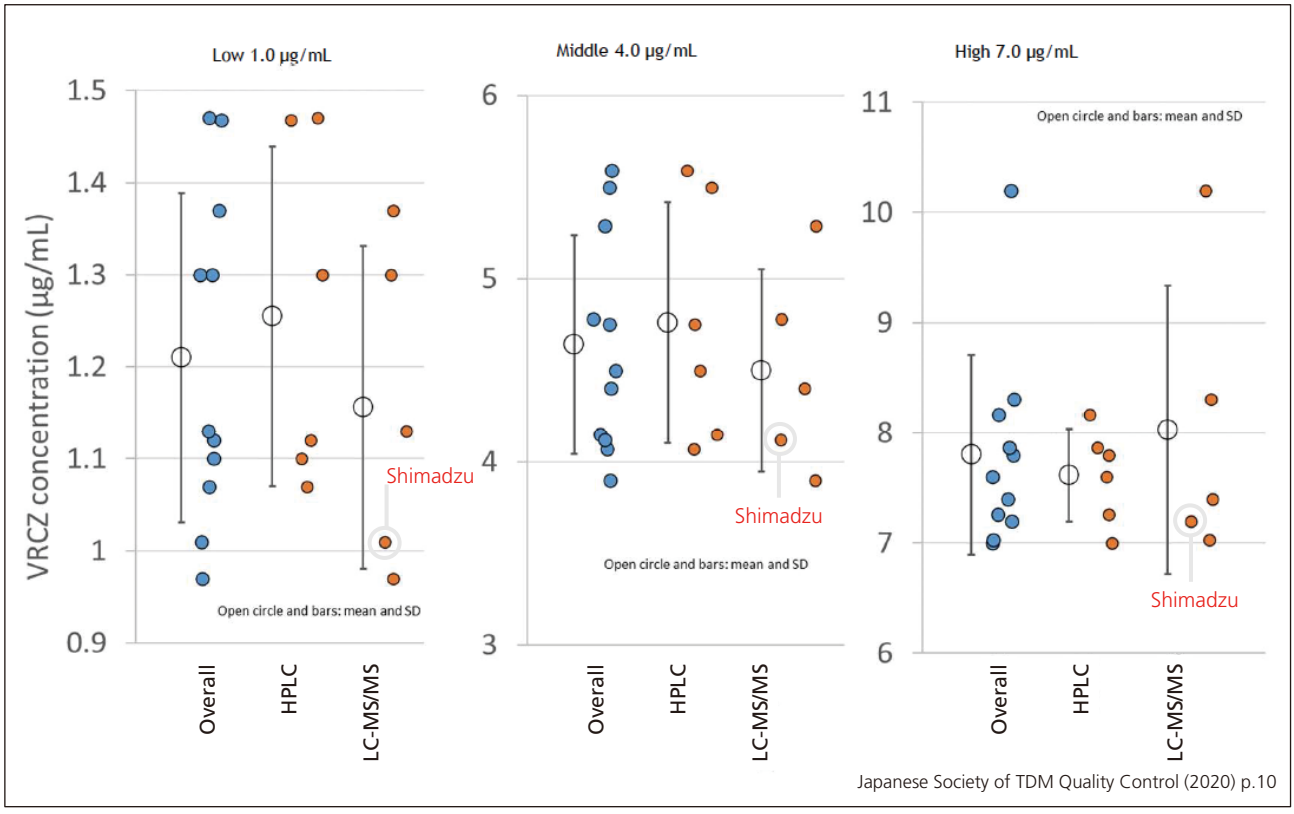

Fig. 4 Concentration of Voriconazole by Measurement Method

Table 5 Reference values and measurement value by Shimadzu

Distributed Sample	Reference value (μg/mL)	Shimadzu (μg/mL)	Difference from Reference value (%)
Low	1.0	1.0	0.0
Middle	4.0	4.1	2.5
High	7.0	7.2	2.9

5. Stable Isotope-Labeled Reagent

One of the matters to consider when assaying a biological sample by LC-MS/MS is the reduction in quantitative performance caused by matrix effects. Matrix effects refer to the change in ionization efficiency of target components caused by impurities present in biological samples (matrix components) and the resulting change in quantitative measurements.[*6] One approach to eliminating these matrix effects is to use a stable isotope-labeled reagent as an internal standard.

This internal standard method quantifies the concentration of a target component by adding a fixed amount of internal standard material to standard samples when creating a calibration curve, preparing a calibration curve based on the concentration ratio and peak area ratio of the standard material and internal standard material, then using this curve to quantify the concentration of the target component. Analysis must be able to separate the internal standard material from almost all other components in the sample and the internal standard material must elute close to the target component. A stable isotope-labeled reagent will have the same chemical properties as a given target component but be labeled with a stable isotope, which is an atom that possesses a different mass number to that of the corresponding atom on the target component. Because the stable isotope-labeled reagent and target component have identical chemical and physical characteristics and only differ in mass, under the same separation conditions, the target component and the stable isotope-labeled reagent (internal standard) have almost the same retention time and experience the same matrix effects. These characteristics make stable isotope-labeled reagents a suitable internal standard material for LC-MS/MS analysis.

The stable isotope-labeled voriconazole used in this study (Fig. 5) was labeled with stable isotopes of hydrogen (^{2}H) and carbon (^{13}C). Stable isotope-labeled reagents labeled with deuterium (^{2}H) are in widespread use due to their low cost. By contrast, labeling with a stable carbon isotope offers the advantage of labeling sites where deuterium cannot be used and of chemical stability. When an analyte is labeled with deuterium, detachment can occur due to solvent-related effects such as pH and acid-base reactions, but detachment does not occur with ^{13}C. The isotopic purity of ^{13}C is also higher than that of deuterium, which produces labeled reagents of higher purity and more accurate data.

Alsachim is a producer of reagents that possesses the advanced technology needed to synthesize and manufacture unique stable isotope-labeled reagents which only a few companies are capable of producing. Alsachim mainly specializes in labeling with the stable isotope ^{13}C, and can produce more than 6000 stable isotope-labeled materials, including pharmaceuticals, drug metabolites, biologically active substances, and controlled drugs. Alsachim also offers products at quantities of 1 mg and higher that are verified for weight, thereby reducing variability arising from human error during weighing and facilitating the acquisition of consistent data.

Fig. 5 Voriconazole: Standard Product and Stable Isotope-Labeled Reagent

6. Fully Automated Sample Preparation Module

Some advantages of LC-MS/MS compared to immunoassay include better analytical accuracy without cross-reactions with metabolites and other sample components and the ability to simultaneously measure multiple drugs. However, LC-MS/MS analysis also involves more complicated procedures and requires sample preparation. When using an LC-MS/MS system with the CLAM-2030 fully automated sample preparation module (Fig. 6), the user simply sets reagents and samples in the CLAM-2030, and subsequent analysis steps are performed fully automatically and seamlessly by the equipment, from sample preparation including sampling, dispensing of reagents, agitation, and filtration, to LCMS analysis. The combination of a stable isotope-labeled reagent and fully automated sample preparation module not only provides labor-saving benefits for the worker, but it also reduces the risk of omissions and variability associated with manual sample preparation and provides a rapid and highly accurate analysis workflow.

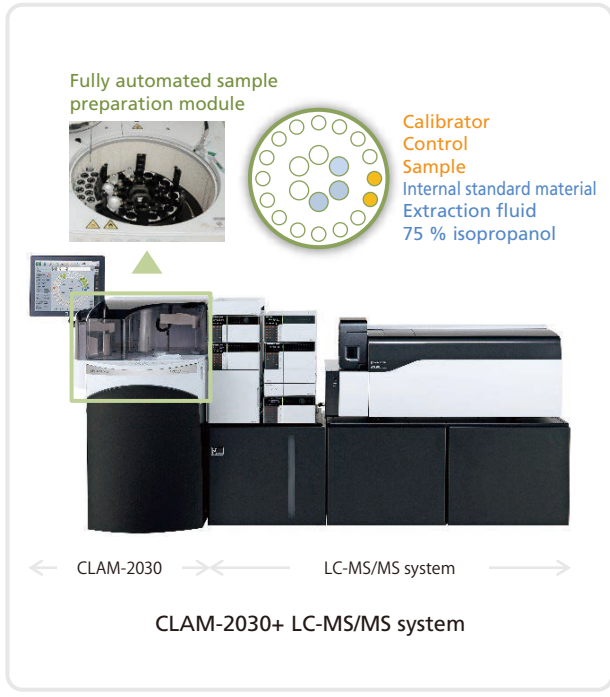

Fig. 6 Sample preparation for voriconazole using CLAM-2030

7. Summary

Shimadzu participated in the 2020 TDM Control Survey of Anti-infective Drugs organized by the Japanese Society of TDM Quality Control by measuring voriconazole values with an LC-MS/MS system with a fully automated sample preparation module and a stable isotope-labeled reagent. The voriconazole values measured by Shimadzu and the reference values measured by the Japanese Society of TDM Quality Control using the distributed samples differed by 0.0 to 2.9 %, indicating Shimadzu' s results were highly accurate.

Reference

*5: Japanese Society of TDM Quality Control, "Report on the Results of the 2020 TDM Control Survey for Infectious Disease Therapeutics"
*6: Y. Sano. (2016) .Mass Spectrometry on Drug Metabolism and Pharmacokinetics Studies" Intended for Beginners, J. Mass Spectrom Soc. Jpn. 64(3), 81-85

First Edition: July, 2022
(Second Edition: November, 2022)

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3655-11201-PDFIK, C146-E464A