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Sample Preparation Method for Determination of Voriconazole in Plasma Using ISOLUTE® SLE+

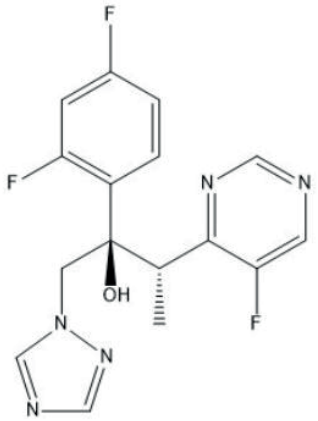

Figure 1. Molecular Structure of Voriconazole.

Introduction

Voriconazole (Figure 1) is a triazole-based antifungal drug that is widely used to treat fungal infections, including invasive aspergillosis, pulmonary aspergilloma, and candidemia. However, because side effects such as prolonged QT syndromes, toxic epidermal necrolysis, and anaphylaxis are known, it is included in Therapeutic Drug Monitoring (TDM) regimes. Especially for patients with severe or refractory fungal infections or hematopoietic stem cell transplantation, blood concentrations are measured for specific drug treatments.

Voriconazole is given in high doses. UV based methods are commonly used to measure voriconazole. This application note proposes a method that can be used for both UV and LC-MS/MS measurements. LC-MS/MS analyses have the advantage of being less susceptible to interference from concomitant drugs.

ISOLUTE® SLE+ Supported Liquid Extraction plates and columns offer an efficient alternative to traditional liquid-liquid extraction (LLE) for bioanalytical sample preparation, providing high analyte recoveries, no emulsion formation, and significantly reduced sample preparation.

Analytes

Voriconazole (CAS: 137234-62-9)

Internal Standards

6,7-Dimethyl-2,3-di-2 pyridyl-quinoxaline (6,7-dimethyl-2,3-di-(2-pyridyl)-chinoxaline, DDPC, CAS: 6627-38-9)

Sample Preparation Procedure

Format

ISOLUTE® SLE+ 400 µL Sample Volume Columns,
Part number: 820-0055-B

An equivalent 96-well plate format
(Part number 820-0400-P01 is also available)

Sample Pre-Treatment

To 200 µL of plasma, add 6,7-dimethyl-2,3-di-2-pyridyl-quinoxaline (internal standard) to give a concentration of 0.5 µg/mL. Add 200 µL of water (HPLC grade or higher) and vortex mix for 30 seconds.

Sample Loading

Load 400 µL of the sample solution onto the column and apply gentle pressure (3 psi) or vacuum (-0.2 bar) to initiate flow. Allow the sample to absorb for at least 5 minutes and wait for samples to stabilize. Ensure that all sample solution is absorbed onto the diatomaceous earth bed.

Sample Elution

Add 900 µL of hexane:diethyl ether (1:1, v/v) and allow to flow under gravity for 5 minutes. Then, add another 900 µL of the solvent, and allow to stand for 5 minutes or more. If required, the extraction can be completed with application of gentle pressure (3 psi) or vacuum (-0.2 bar) (up to 10 to 30 seconds).

Evaporation and Reconstitution

Evaporate the extract with a nitrogen gas evaporator and reconstitute the extract with water: methanol (1:1, v/v, 2 mL). In the case of UV measurement, the reconstituted solution should be measured as it is, or the reconstituted solution should be concentrated to a volume of less than 2 mL, depending on the sensitivity of UV measurement.

Dilution for LC/MS/MS

Additional dilution of the reconstituted assay solutions should be performed depending on the range of calibrated concentrations in the LC/MS/MS used*.

*In this application note, a 10-fold dilution was performed using water: methanol (1:1, (v/v)).

Biotage®

UHPLC Conditions

Instrument
Nexera LC-30AD (Shimadzu)

Column
ACQUITY UPLC® BEH C18 1. 7 µm (2.1 mm × 50 mm column; Waters)

Mobile Phase
A: 0.1% (v/v) Formic acid aqueous solution

B: Acetonitrile

Flow Rate
0.4 mL min

Column Temperature
40 °C

Injection Volume
1 µL

Mass Spectrometry Conditions

Equipment
LCMS-8060 (Shimadzu)

Ionization Mode
ESI positive

Nebulizer Gas Flow Rate
3 L/min

Flow Rate of Drying Gas
10 L/min

Heating Gas Flow
10 L/min

Interface Temperature
400 °C

DL Temperature
250 °C

Heat Block Temperature
400 °C

CID Gas
270 kPa

LC/MS/MS Conditions

Table 1 shows the gradient conditions for the LC/MS/MS analysis. The initial acetonitrile concentration in the gradient is initiated at a low level to accommodate a wide range of drug analyses.

In contrast, UV-measurement requires complete isolation of Voriconazole from the internal standard DDPC and this can be done by increasing the initial acetonitrile level in the gradient. Table 2 shows the LC/MS/MS SRMs transition.

Table 1. Gradient Condition.

Time (min)	%A	%B
0.0→3.0	95→5	5→95
3.0→4.0	5	95
4.0→4.1	5→95	9→5
4.1→6.5	95	5

Table 2. SRM Transitions.

Compound	SRM Transition	Collision Energy	Retention Time
Voriconazole	**Positive**		
Quant Ion	350.15>127.10	-34	2.21
Qual Ion	350.15>281.10	-18	2.21
DDPC	**Positive**		
Quant Ion	313.30>284.15	-45	2.24
Qual Ion	350.15>281.10	-32	2.24

Results

Figure 2 shows the quantitative SRM (Selected Reaction Monitoring) chromatograms for voriconazole and DDPC (used as an internal standard). Figure 3 shows the calibration curve for voriconazole. The quantitation range was 0.1–250 ng/mL with a wide dynamic range, and a good linearity was obtained with a multiple correlation coefficient (r^2) of 0.999. The required blood voriconazole concentration range for TDM is 0.1 to 10 µg/mL. This concentration is analyzed using UV. The purpose of this application note is to be applicable to a wide variety of antimicrobial agents, and to utilize the high selectivity and sensitivity of LC-MS/MS. The ISOLUTE® SLE+ pretreatment column effectively removes phospholipids, which can interfere with measurements by LC/MS analysis.

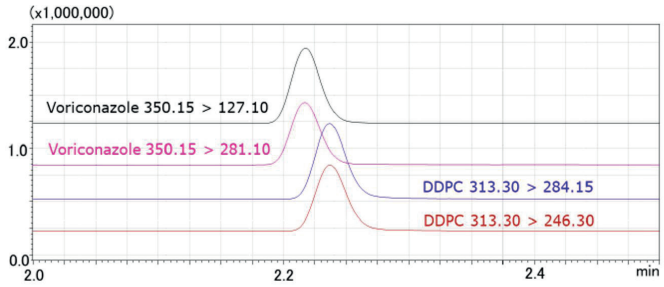

Figure 2. SRM chromatograms of Voriconazole and DDPC.

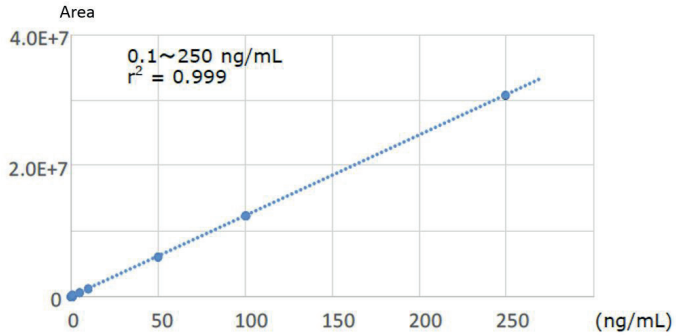

Figure 3. Calibration curve for Voriconazole.

Control Plasma Evaluation

Figure 4 shows the SRM chromatogram obtained for voriconazole spiked into control plasma at a concentration of 0.1 µg/mL and extracted using ISOLUTE® SLE + prior to diluting the reconstitution solution 100-fold. There was no interference from contaminants in the plasma.

Matrix effects were also evaluated. Three different concentrations of voriconazole (0.1, 1, and 10 µg/mL) were added to the control plasma, and the recovery rates and matrix factors are shown in the Table 3. Recovery was calculated by comparing the area of voriconazole in sample (A) after pre-treating the plasma spiked with voriconazole, and (B) adding voriconazole to the solution after pre-treating the plasma only. Matrix factors were calculated by comparing the area values of (B) with the standard solution (S). As a result, a recovery rate of more than 90% was obtained at each concentration. Matrix factor values were sufficiently small, and it was quantitatively confirmed that ISOLUTE SLE + pretreatment effectively eliminates matrix effects.

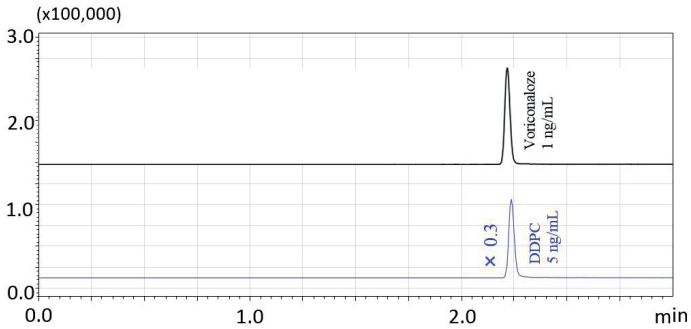

Figure 4. SRM chromatograms after ISOLUTE® SLE + treatment of Voriconazole at 0. 1 µg/mL in plasma.

Table 3. Recovery and Matrix Factor of each concentration of Voriconazole in plasma (n=3).

Blood Concentration (µg/mL)	Recovery Rate* (%)	Matrix Factors* (%)
0.1	97.2	8.6
1.0	98.0	1.0
10.0	103.1	8.6

* Recovery rate = [A]/[B] x 100

Matrix factor = 1-[B]/[S]

Ordering Information

Part Number	Description	Quantity
820-0055-B	ISOLUTE® SLE+ 400 µL Sample Volume Columns	50
820-0400-P01	ISOLUTE® SLE+ 400 µL 96-Well Plate	1
PPM-48	Biotage® PRESSURE+ 48 Positive Pressure Manifold	1
121-2016	Biotage® VacMaster™ 20 Sample Processing Manifold	1

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