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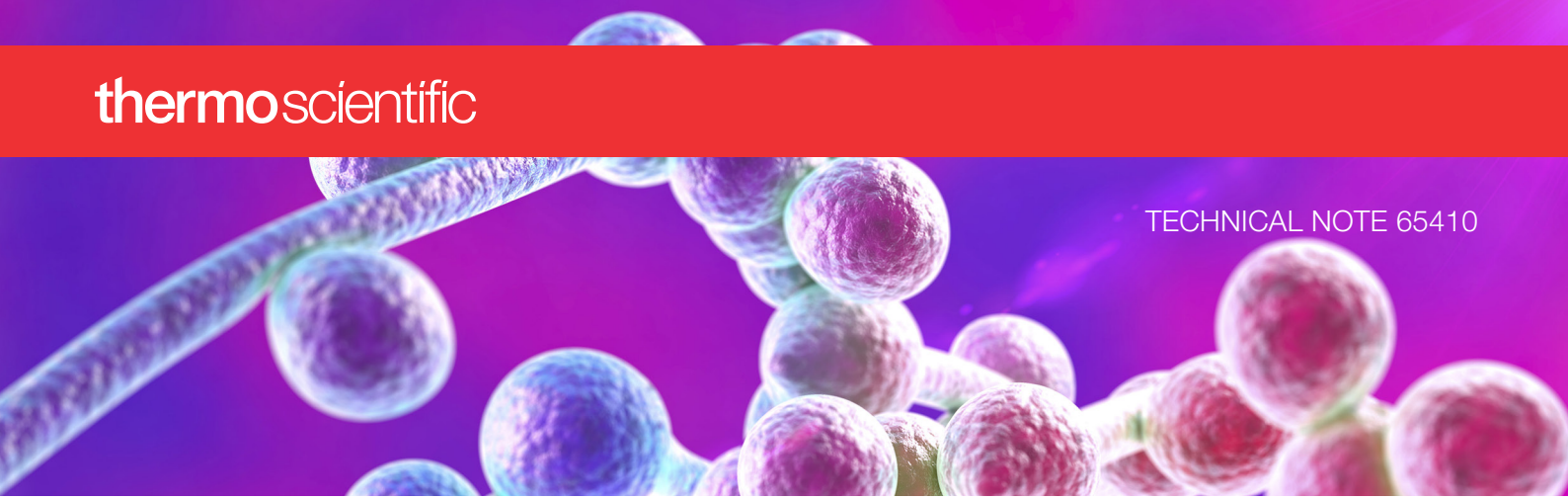

TECHNICAL NOTE 65410

Quantitative analysis of antifungal drugs using PaperSpray tandem mass spectrometry for clinical research

Authors

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Keywords

Antifungal drugs, PS-MS/MS, voriconazole, itraconazole, posaconazole, TSQ Altis, VeriSpray, TraceFinder, clinical research

Application benefits

- Simple, no sample preparation and fast analysis
- Three antifungal drugs in a single quantitative method

Goal

To develop a reliable and reproducible PaperSpray-mass spectrometry workflow for quantitative analysis of antifungal drugs in serum for clinical research using a Thermo Scientific™ TSQ Altis™ mass spectrometer with the Thermo Scientific™ VeriSpray™ PaperSpray ion source

Introduction

Voriconazole, itraconazole, and posaconazole (Figure 1) are drugs used to treat serious, invasive fungal infections. Invasive fungal infections are highly prevalent in individuals with seriously compromised immune defenses, including those on immunosuppressive drugs following organ or bone marrow transplant, or those undergoing chemotherapy for cancer treatment. Using antifungal drugs in pediatric populations is especially challenging because of the highly variable pharmacokinetics among individuals and across different ages. Adjustment of dose based on rapid and accurate drug monitoring results helps to achieve control of infection in clinical research.

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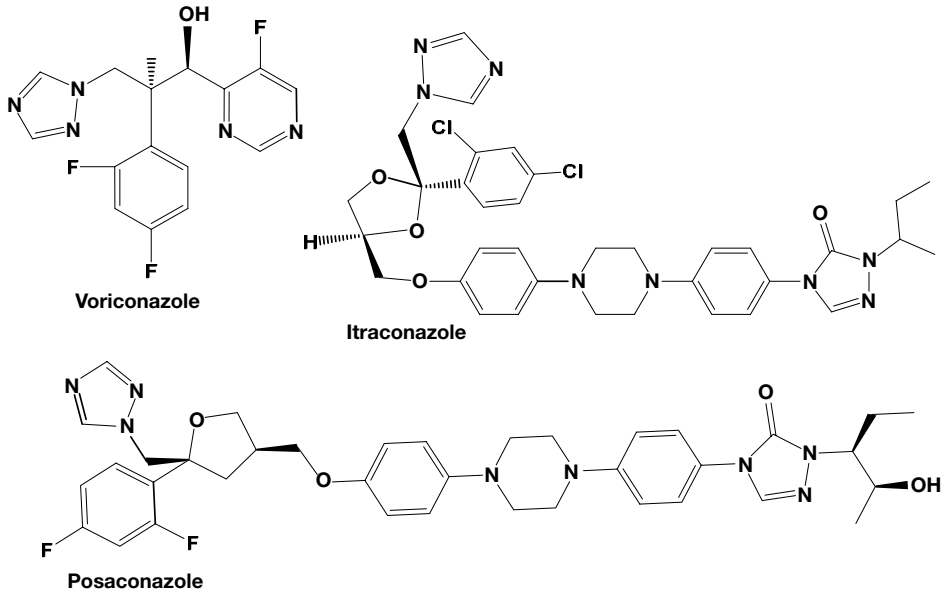

Figure 1. Antifungal drug structures

PaperSpray mass spectrometry, first described in 2010,[1] is a method for performing rapid, direct analysis of biological fluids spotted on paper or another porous substrate. In the PaperSpray technique, extraction and ionization of one or more analytes occur from a porous substrate containing the sample. Several reports in literature have demonstrated quantitative analysis of small molecules, including therapeutic and illicit drugs, directly from biological fluids.[2] PaperSpray MS has garnered significant interest because of its lack of sample preparation and shorter turn-around time while still maintaining good quantitative performance and sensitivity.

In this study, we describe the quantitation of three antifungal drugs (posaconazole, itraconazole, and voriconazole) using the VeriSpray PaperSpray ion source on a TSQ Altis triple-stage quadrupole mass spectrometer. The VeriSpray system enables robust, rapid, and automated PaperSpray analysis. Sample storage, extraction, and ionization all take place on VeriSpray sampling plates containing 24 individual paper spray tips, each of which analyzes a separate sample (Figure 2A). Analysis of the plate is carried out automatically via the VeriSpray ion source (Figure 2B) directly from the dried biofluid spots within minutes.

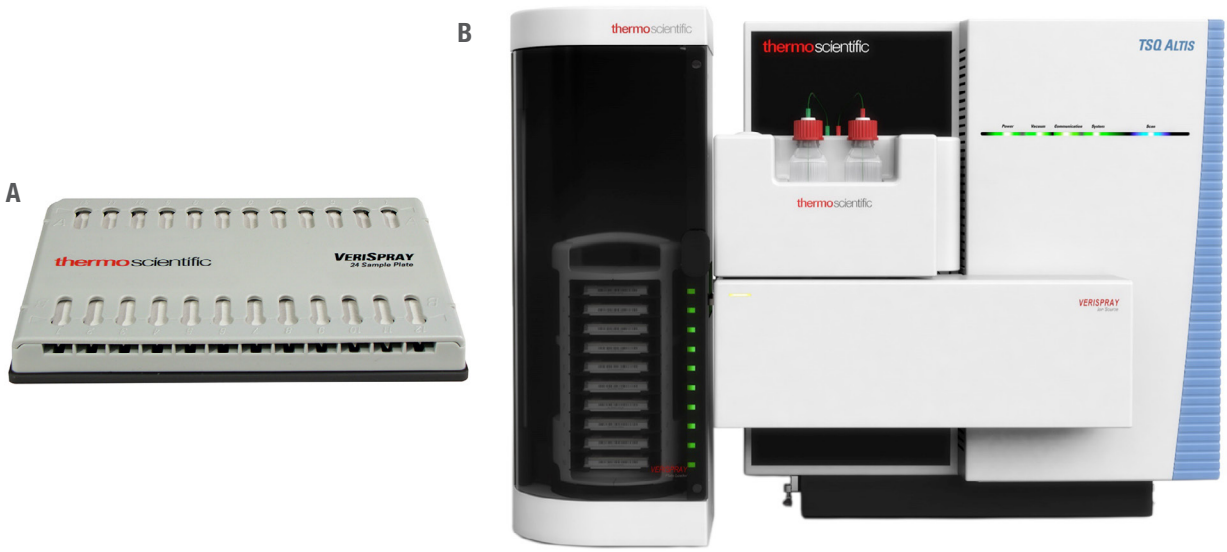

Figure 2. (A) VeriSpray sample plate and (B) VeriSpray PaperSpray system mounted to TSQ Altis triple quadrupole mass spectrometer

Experimental

Sample preparation

Calibration curve standards in the range of 0.1 to 10 µg/mL were prepared in pooled human serum. Working solutions at 20× concentration were prepared in methanol by serial dilution and spiked into serum on the day of analysis. Serum samples were mixed with an internal standard solution containing isotopically labeled voriconazole and itraconazole. A suitable isotopically labeled analog of posaconazole was not available; isotopically labeled itraconazole was used as its internal standard instead. Serum samples (4 µL) were then spotted on the Thermo Scientific™ VeriSpray™ cartridge and allowed to dry at ambient temperature for 30 minutes.

PaperSpray and MS conditions

For PaperSpray ionization, the VeriSpray system was used. The PaperSpray solvents (both sample rewet and spray solvents) were acetonitrile/acetone/water 85/10/5 with 0.01% acetic acid, applied according to the settings in Table 1. The TSQ Altis triple-stage quadrupole mass spectrometer was used for the analyses. The experimental conditions were optimized with a spray voltage of 4.2 kV, a cycle time of 0.8 s, and both Q1 and Q3 resolution of 0.7 Da FWHM. The source parameters and SRM table along with the critical MS features for all target analytes are listed in Tables 2 and 3, respectively. The optimum RF lens settings and collision energies for the product ions were determined by infusion of the individual standards into the mass spectrometer.

Data acquisition and analysis

Data acquisition and processing were conducted using Thermo Scientific™ TraceFinder™ software version 4.1. Limits of detection were calculated by the formula $3*s_b/m$, where s_b is the standard error of the intercept and m is the slope of the calibration line.

Table 1. VeriSpray solvent application parameters. Each rewetting and solvent dispense is 10 µL.

Rewetting dispense delay	
Dispense	Delay (s)
1	1
Solvent dispense delay	
Dispense	Delay (s)
1	1
2	1
3	1
4	1
5	3
6	3
7	5
8	5
9	5
10	5
11	7
12	7
13	7
14	7
15	7

Table 2. (A) MS conditions

Ion Source Parameter	Value
Spray Voltage	Time Dependent
Positive Ion	4200 V
Sweep Gas	0 Arb
Ion Transfer Tube Temperature	350 °C
CID Gas	2 mTorr

Table 2. (B) Time dependent spray voltage

Time (min)	Voltage (V)
0	0
0.1	4200
1.1	0

Table 3. Optimized SRM transitions for the antifungal drugs in serum with acquisition time of 1.2 min and positive polarity for each sample

Compound	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	RF Lens (V)
Voriconazole	350.1	224.1	19	59
Voriconazole	350.1	263.1	25	59
Voriconazole	350.1	281.2	16	59
Posaconazole	701.3	344.1	43	117
Posaconazole	701.3	370.2	43	117
Posaconazole	701.3	614.3	35	117
Itraconazole	705.2	335.1	42	115
Itraconazole	705.2	348.1	40	115
Itraconazole	705.2	392.2	36	115
Voriconazole D3	353.0	224.2	20	54
Voriconazole D3	353.0	266.2	25	54
Voriconazole D3	353.0	284.1	18	54
Itraconazole D4	709.2	339.3	43	119
Itraconazole D4	709.2	352.0	40	119
Itraconazole D4	709.2	396.4	36	119

Results and discussion

The three antifungal drugs were successfully quantitated simultaneously (Figure 3). The correlation coefficient (R^2) for each calibration curve was greater than 0.99, indicating good linearity. The detection limits (Table 4) are well below the concentrations normally found in research of fungal infections, and the assay measurement range is comparable to that offered by reference lab HPLC-MS/MS analysis. Total analysis time for the dried serum spots was about 2 minutes. This included the extraction step as well as the mass spectrometry analysis, which both take place automatically using the VeriSpray sample plate.

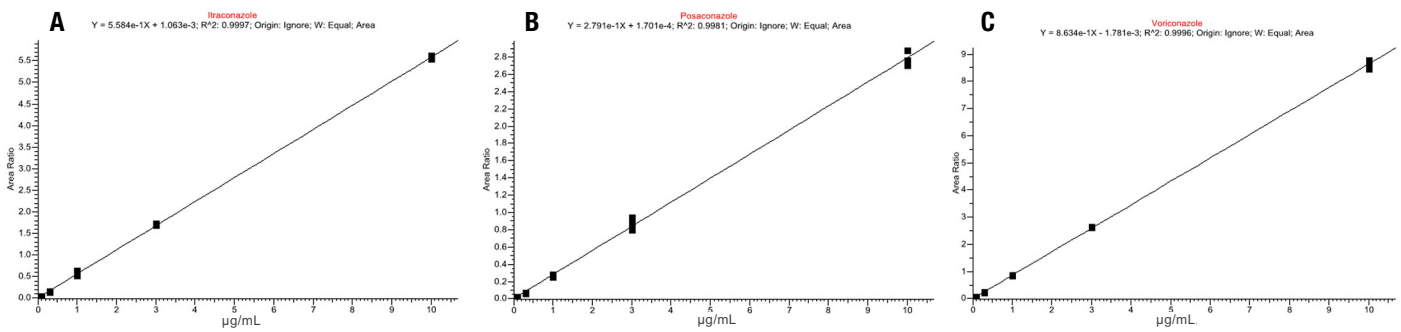

Figure 3. Calibration curves of (A) itraconazole, (B) posaconazole, and (C) voriconazole obtained from pooled human serum

Table 4. PaperSpray quantitative results in serum

Target	R^2	Limits of Detection in Serum (ng/mL)	Target Range
Itraconazole	0.9990	26	>500 ng/mL (local infection) >1000 ng/mL (systemic infection)
Posaconazole	0.9973	43	~1000 ng/mL (trough)
Voriconazole	0.9996	16	~1000 ng/mL (steady-state)

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Conclusions

PaperSpray mass spectrometry with the VeriSpray ion source was capable of accurate quantitation of antifungal drugs in human serum. In addition to providing fast analysis, this easy-to-implement workflow requires no additional sample pretreatment or separations. The linear dynamic range of the assay encompassed the range normally measured for clinical research.

References

1. Wang, H.; Liu, J.; Cooks, R. G.; Ouyang, Z., Paper Spray for Direct Analysis of Complex Mixtures Using Mass Spectrometry. *Angewandte Chemie International Edition* **2010,** *49* (5), 877–880.
2. Manicke, N. E.; Bills, B. J.; Zhang, C., Analysis of biofluids by paper spray MS: advances and challenges. *Bioanalysis* **2016,** *8* (6), 589–606.

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