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Determination of voriconazole in human plasma by liquid chromatography-tandem mass spectrometry and its application in therapeutic drug monitoring in Chinese patients

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Abstract

Objective: To develop a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantification of voriconazole in human plasma, and to evaluate its application in clinical therapeutic drug monitoring.

Method: Plasma samples were obtained from Chinese patients receiving voriconazole, precipitated with methanol (using fluconazole as an internal standard), and then subjected to LC-MS/MS using an SB C_{18} column with a methanol and water mobile phase at a flow rate of 0.4 mL/minute. Quantification was performed by multiple-reaction monitoring using the precursor and product ion pair m/z 350–280.9 for voriconazole and m/z 307–219.9 for fluconazole.

Results: The calibration curve was linear over a range of 0.1–10.0 μg/mL ($R^2 = 0.9995$). The inter-day and intra-day relative standard deviations were <7.68% and <8.97%, respectively. Extraction recovery, matrix effect, and stability were also validated. Sixty-eight plasma samples from 42 patients were analyzed, and the voriconazole concentrations in 25 samples (36.8%) were outside the optimal range of 1.5–4.5 μg/mL.

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Conclusions: We developed a simple and accurate method of drug monitoring, which could improve the efficacy and prevent adverse reactions of voriconazole.

Keywords
Voriconazole, therapeutic drug monitoring, plasma concentration, LC-MS/MS, antifungal agent, adverse drug reaction

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Introduction

The incidence of invasive fungal infections has recently been increasing due to the widespread clinical use of high-dose chemotherapy drugs and immunosuppressive agents.[1] Fungal infections are associated with higher mortality than other infections because of their rapid progression. The pathogens responsible for fungal infections are mainly conditional ones, such as *Candida* and *Aspergillus*. Antifungal drugs, especially propylenes, polyenes, azoles, and echinocandins, have thus become increasingly important for controlling these fungal infections in clinical practice. Terbinafine is a commonly used propylene-type antifungal agent mainly used to treat dermatophyte infections, but with poor efficacy for deep fungal infections. Its usual adverse reactions are mild gastrointestinal and skin reactions. Amphotericin B is the main polyene antifungal agent. As a broad-spectrum antifungal agent, amphotericin B has good activity against most fungi, except *Candida lusitaniae*, *Aspergillus terreus, Scedosporium apiospermum,* and *Scedosporium prolificans*. However, nephrotoxicity is an important adverse reaction of amphotericin B. The representative echinocalcin is the broad-spectrum antifungal agent caspofungin, which is active against both *Candida* and *Aspergillus*, but not against *Cryptococcus neoformans*, *Trichosporon*, or dimorphic fungi. Its main adverse reactions are fever, nausea, vomiting, and elevated transaminase activity. Voriconazole is a second-generation triazole antifungal drug developed by Pfizer that was approved in the US in 2002.[2] Voriconazole is known for its broad-spectrum antifungal activity and high bioavailability, and has become the first-choice drug for the treatment of invasive aspergillosis, esophageal candidiasis, and serious fungal infections caused by *S. apiospermum* and *Fusarium spp*.[3,4] Its adverse reactions include raised aminotransferases and visual disorders.

Previous studies have indicated that voriconazole shows the best curative effect and the least neurotoxicity within a concentration range of 1.5–4.5 μg/mL,[5] while others reported an optimal concentration of 1.0–5.5 μg/mL.[2,6] Matsumoto et al.[7] found that the trough concentration for hepatotoxicity was 5.55 ± 2.73 μg/mL, while 2.36 ± 1.67 μg/mL was non-hepatotoxic ($P<0.01$). They also reported that 90% of patients with trough concentrations >5 μg/mL were likely to develop hepatotoxicity, compared with only 31.6% of patients with trough concentrations <5 μg/mL.[7] Increasing attention has thus been paid to monitoring voriconazole concentrations in human plasma, highlighting the need for therapeutic drug monitoring of voriconazole in Chinese patients to guide clinical practice in China.

In the present study, we aimed to establish a rapid, accurate, and reliable analytical method for therapeutic drug monitoring of voriconazole. The findings will provide valuable reference data to support individualized treatment, improve treatment effects, and reduce adverse reactions in Chinese patients receiving voriconazole.

Materials and methods

Chemicals and reagents

Voriconazole and fluconazole were purchased from the National Institutes for Food and Drug Control (Beijing, China). High-performance liquid chromatography-grade acetonitrile, methanol, and water were from Thermo Fisher Scientific (Geel, Belgium). Formic acid was obtained from Thermo Fisher Scientific (Shanghai, China). Blank human plasma samples were provided by the Department of Blood Transfusion and stored at −20°C prior to further analysis.

Instruments and liquid chromatography-tandem mass spectrometry (LC-MS/MS) conditions

An Agilent 1260 system (Agilent Technologies Inc., Santa Clara, CA, USA) consisting of a vacuum degasser, binary pump, and auto-sampler was used for solvent and sample delivery. Compounds were separated on an Agilent SB C_{18} column (100 mm × 2.1 mm × 3.5 μm). The column was eluted with a gradient elution program using a mobile phase composed of 0.1% formic acid in water (mobile phase A) and methanol and ramped with a constant slope to 70% mobile phase B at 1.5 minutes, with a run time of 9 minutes. The injection volume was 2 μL.

An Agilent 6460A (Agilent Technologies) mass spectrometer equipped with an electrospray ionization source was used as a detector in positive ion mode. Quantification was performed by multiple-reaction-monitoring of the transitions at m/z 350–280.9 for voriconazole and m/z 307–219.9 for fluconazole.

Calibration standards and quality control (QC) samples

Primary stock solutions of voriconazole standards and QC samples were prepared as described previously.[8] The primary stock solutions (1.0 mg/mL) of voriconazole and fluconazole were prepared in a water/methanol mixture (1:1, V/V) and stored at −20°C. The stock solution was stable for at least 28 days. Working solutions of voriconazole were prepared by diluting the stock solution in 50% methanol to the desired concentration (0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 8.0, and 10.0 μg/mL for the calibration samples and 0.1, 0.2, 1.0, and 8.0 μg/mL for the QC samples).

Sample preparation

Briefly, 50 μL samples were dispensed into 5-mL polypropylene tubes, and 50 μL fluconazole solution (1 μg/mL) and 500 μL methanol were added into the tubes, followed by vortexing and centrifugation at 17,968 × *g* for 10 minutes. Finally, 2 μL of supernatant was injected into the chromatographic system.

Method validation

Method validation was carried out according to the Chinese Pharmacopoeia Bioanalytical Method Validation, including specificity, linearity of the calibration curves, lower limit of qualification (LLOQ), precision and accuracy, matrix effect (ME), and stability. Specificity was assessed by analyzing six blank plasma samples from different volunteers recruited via advertisements to donate blank plasma for routine drug monitoring in our hospital.

The plasma was stored at −80°C until use. Voriconazole concentrations ranging from 0.1–10 μg/mL were used to establish a calibration curve and six LLOQ samples were used to determine the lowest quantification concentration. The precision and accuracy were assessed by analyzing six replicates of voriconazole at low, medium, and high QC and LLOQ levels in three runs. The MEs were determined at three concentrations of voriconazole, with six samples for each concentration. Voriconazole stability was investigated by analyzing three concentrations of QC samples under four conditions (24 hour (in injector); 4 hour (bench top); three freeze–thaw cycles; and 90 days at −20°C), with three samples for each concentration. Each sample was analyzed once.

Patients and ethical approval

Patients who received at least four doses of voriconazole (Pfizer Ltd., United Kingdom) tablets or injection were included. All blood samples were obtained from patients before the following administration. The study was part of the routine therapeutic drug monitoring scheme in the hospital, and was thus exempt from the need for informed consent.

Results

Specificity

Chromatograms were obtained from six blank plasma samples from different volunteers and human plasma spiked with voriconazole and fluconazole. The retention times of voriconazole and fluconazole were 4.38 and 3.29 minutes, respectively. The results showed that endogenous substances did not interfere with the determination of voriconazole (Figure 1).

Calibration curve

A plasma calibration curve was prepared using voriconazole concentrations of 0.1–10.0 μg/mL. Chromatograms were recorded, and the ratio of voriconazole peak area to internal standard peak area was calculated as Ai ($f = As/Ai$). Using plasma voriconazole concentration (X) as the abscissa and the area ratio f (Y) as the ordinate, we calculated the following regression equation using the weighted least-squares method ($1/X^2$): $Y = 2.115*x + 0.013$, $R^2 = 0.9995$ (Figure 2). The average regression was 0.997. The accuracy for three linearity curves was within 90.08%–112.23%. The precision (%relative standard deviations (RSD)) ranged from 1.45%–6.68%. The LLOQ was 0.1 μg/mL and the accuracy ranged from 94.75%–107.88% with a %RSD ≤4.74% at LLOQ (n = 6).

Precision and accuracy

A standard curve was prepared with six replicate QC samples for each concentration (0.1, 0.2, 1.0, and 8.0 μg/mL) on 3 different days (Table 1). The intra-day accuracy and precision were 88.5%–108.99% and 1.35%–7.68%, respectively, and the inter-day accuracy and precision were 83.3%–111.0% and 3.38%–8.97%, respectively.

Extraction recovery and ME

ME was measured at three concentrations (0.2, 1, and 8 μg/mL) of voriconazole in six individual matrices. The peak areas of voriconazole and the internal standard dissolved in the pretreated blank plasma (A) were compared with those of pure standard solutions containing the same amount of voriconazole and internal standard (B). The ratio (A/B×100%) was then used to evaluate the ME. The extraction recovery was determined at three concentrations (0.2, 1, and 8 μg/mL) of voriconazole by comparing the peak area of voriconazole

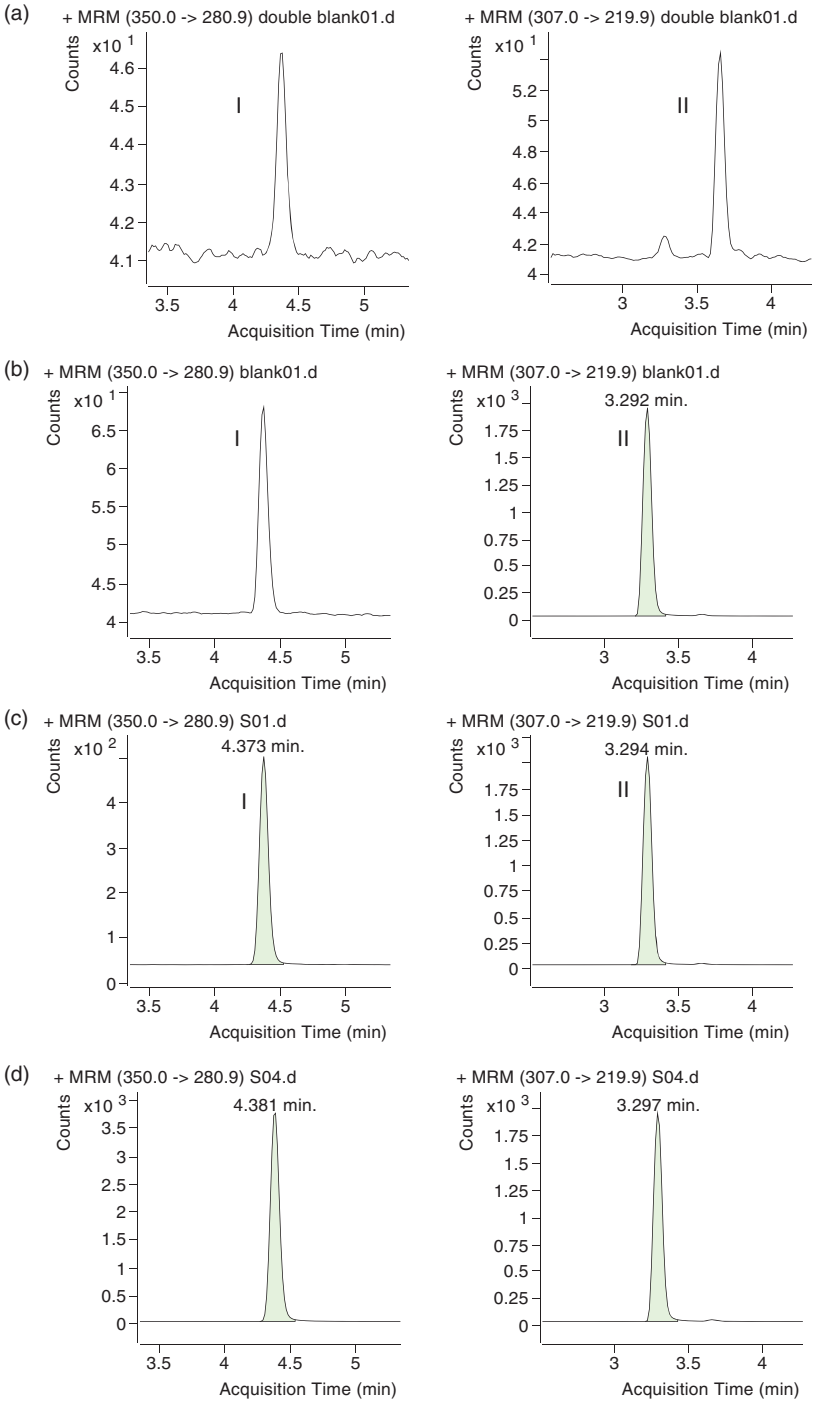

Figure 1. Typical extracted ion chromatograms of voriconazole and fluconazole (internal standard) in human plasma. (a) Blank human plasma; (b) blank human plasma with fluconazole (1.0 μg/mL); (c) blank human plasma with voriconazole (0.1 μg/mL) and fluconazole (1.0 μg/mL); (d) blank human plasma with voriconazole (1.0 μg/mL) and fluconazole (1.0 μg/mL).

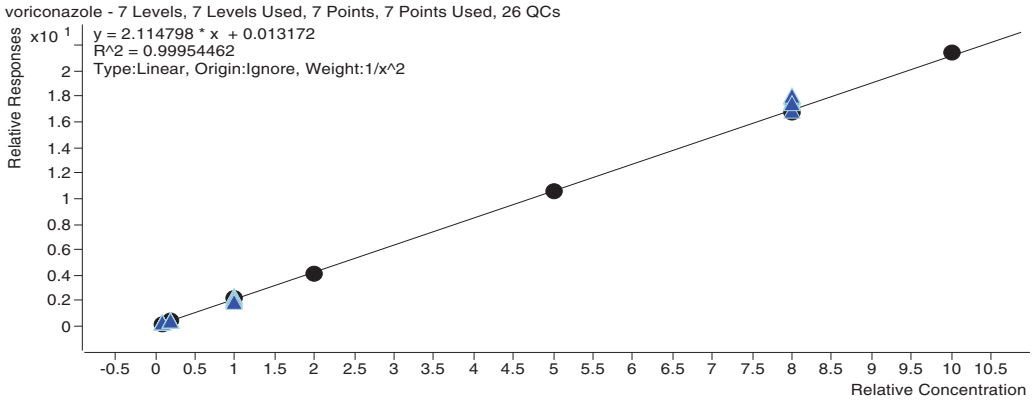

Figure 2. Calibration curve of voriconazole in human plasma ($R^2 = 0.995$).

Table 1. Intra-day and inter-day precision of voriconazole measurement in human plasma.

Batch \ Concentration	0.1 μg/mL	0.2 μg/mL	1.0 μg/mL	8.0 μg/mL
N	18	18	18	18
Mean	0.10	0.20	1.02	8.18
SD	0.006	0.018	0.066	0.276
RE (%)	0.92	−1.05	1.81	2.29
Intra-RSD (%)	1.35	6.46	7.68	2.37
Inter-RSD (%)	6.16	8.97	6.49	3.38

SD, standard deviation; RE, relative error; RSD, relative standard deviation.

dissolved in the supernatant of the extracted blank plasma (C) with that of (A). The ratio (A/C×100%) was used to evaluate the extraction recovery. The extraction recovery rates of voriconazole for low, medium, and high concentrations were $97.15 \pm 1.58\%$, $102.95 \pm 2.73\%$, and $107.11 \pm 6.41\%$, respectively, and the MEs were $102.42 \pm 2.47\%$, $95.16 \pm 7.28\%$, and $96.51 \pm 4.90\%$, respectively (Table 2).

Stability

QC samples were prepared at low, medium, and high concentrations (0.2, 1.0, and 8.0 μg/mL). Each sample was then divided into several portions: one portion was placed at ambient temperature for 4 hours; the second portion was subjected to three freeze–thaw cycles (at −20°C); and the third portion was frozen at −20°C for 3 months. The relative error (RE) and RSD for the low concentration were both <20%, and the RE and RSD for the medium and high concentrations were all <15%. These results indicated that plasma voriconazole was stable under all three storage conditions (Table 3).

Clinical application

In the current study, we monitored 68 plasma samples from 42 Chinese patients (22 male, 20 female). Forty-one patients had hematological system diseases, and only one patient was diagnosed with

Table 2. Recovery and matrix effects of voriconazole in human plasma (n = 5).

Nominal conc. (μg/mL)	Recovery/%	RSD/%	Matrix effect/%	RSD/%
0.2	97.15 ± 1.58	1.62	102.42 ± 2.47	2.41
1.0	102.95 ± 2.73	2.65	95.16 ± 7.28	7.66
8.0	107.11 ± 6.41	5.98	96.51 ± 4.90	5.08

RSD, relative standard deviation.

Table 3. Stability of voriconazole in human plasma (n = 3).

Nominal conc. (μg/mL)	Stability	Mean ± SD (μg/mL)	Accuracy/%	Precision/%
0.2	24 hour (in injector)	0.21 ± 0.01	103.77	3.24
	4 hour (bench top)	0.19 ± 0.01	97.04	2.55
	3rd freeze–thaw	0.21 ± 0.01	106.52	6.06
	90 day at −20°C	0.22 ± 0.02	110.04	7.12
1.0	24 hour (in injector)	1.02 ± 0.04	102.18	4.07
	4 hour (bench top)	1.11 ± 0.02	108.08	2.47
	3rd freeze–thaw	0.98 ± 0.14	98.17	13.96
	90 day at −20°C	1.04 ± 0.08	104.00	7.72
8.0	24 hour (in injector)	7.79 ± 0.11	97.41	1.40
	4 hour (bench top)	8.66 ± 0.42	108.26	4.86
	3rd freeze–thaw	8.25 ± 0.35	103.14	4.24
	90 day at −20°C	8.46 ± 0.95	105.72	11.24

SD, standard deviation.

respiratory failure complicated with pulmonary infection. All patients received voriconazole 200 mg every 12 hours. The voriconazole concentrations in 13 plasma samples from nine patients were >4.5 μg/mL, while 12 plasma samples from 10 patients were <1.5 μg/mL, indicating that plasma voriconazole levels were outside the range of 1.5–4.5 μg/mL in about 36.8% of samples and 45.2% of patients (Figure 3). Trough concentrations were monitored in each patient after continuous administration of voriconazole injection or voriconazole tablets. Most patients had pulmonary infections. The trough concentrations in patients 2 and 42 were 0.59 and 0.96 μg/mL after the dosage was changed from injection (200 mg every 12 hours) to oral form, respectively, and the dosing frequency remained at 200 mg every 12 hours. However, their plasma concentrations increased to 1.54 and 2.95 μg/mL when the dosages were changed to 250 and 300 mg every 12 hours, respectively. The trough concentration in patient 19 was 10.5 μg/mL after continuous administration of voriconazole injection at 200 mg every 12 hours for 7 days, and this decreased to 8.3 μg/mL at 5 days after switching to voriconazole tablets (200 mg every 12 hours). The dosage was subsequently adjusted to 150 mg every 12 hours in this patient for the following 11 days.

Discussion

Current methods for determining voriconazole plasma concentrations include high-performance liquid chromatography and LC-MS/MS.[7,9–18] Sample pretreatment for

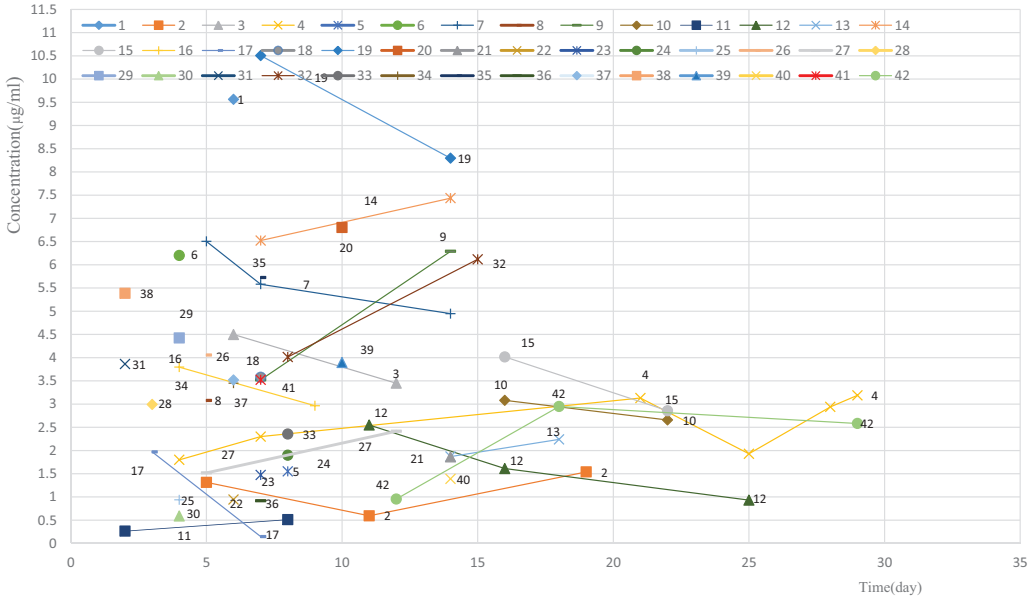

Figure 3. Voriconazole concentrations in plasma from 42 patients. Numbers represent different subjects. The X axis indicates the time of day when the plasma was collected

the existing methods includes liquid-liquid extraction and protein precipitation procedures, which are tedious and time-consuming due to multiple operation steps. In the present study, we adopted a direct protein precipitation method and simplified the pretreatment steps. Furthermore, this novel method only required 50 μL plasma. Gradient elution effectively reduced the ME due to its increased sensitivity, improved column efficiency, improved peak shape, smaller sample-injection volume (only 2 μL), voriconazole and internal standard peak times of 3.3–4.3 minutes, and the avoidance of endogenous substances and other drugs in plasma. This new method was thus considered more suitable for determining plasma voriconazole concentrations in patients.

The therapeutic concentrations of voriconazole range from 1.0–5.5 μg/mL, and the infection-cure rate was significantly increased at steady-state trough concentrations >1.0 μg/mL.[19] However, trough concentrations >6.0 μg/mL have been associated with adverse reactions, such as toxicity, visual impairment, and hepatotoxicity.[2,6] Pascual et al. performed a pharmacokinetic investigation in 55 patients with invasive fungal disease treated with conventional voriconazole therapy (intravenous: 4 mg/g every 12 hours; 200 mg every 12 hours orally).[5] They demonstrated that a voriconazole concentration of 1.5–4.5 μg/mL had the best curative effect and the least neurotoxicity. In the current study, we monitored 68 plasma samples from 42 Chinese patients, and found that voriconazole concentrations in 13 plasma samples from nine patients were >4.5 μg/mL, while 12 plasma samples from 10 patients were <1.5 μg/mL, indicating that plasma voriconazole levels were outside the range of 1.5-4.5 μg/mL in about 36.8% of samples and 45.2% of patients. A previous study in China suggested that the therapeutic concentration of voriconazole was 0.5–3.0 μg/mL in the Chinese population.[20] In this study, the concentrations in 32 samples

from 24 patients (47.1%) were >3.0 μg/mL, and none were <0.5 μg/mL. The lower threshold of voriconazole trough concentrations in Caucasians is significantly higher than in the Chinese population,[21] and it is therefore particularly important to pay attention to low voriconazole concentrations in Caucasians to ensure the optimal therapeutic effect. However, adverse reactions due to excessively high voriconazole concentrations need to be avoided in Chinese patients. The slower metabolism of voriconazole in the Chinese population is likely to be attributable to variations in cytochrome P450 enzymes, such as CYP2C19, which is present in 15%–20% of Asians compared with only 3%–5% of Caucasians.[22] The determination of voriconazole plasma concentrations provides real-time data to allow the clinical adjustment of dosing regimens, to improve the treatment efficacy and reduce adverse reactions.

Conclusion

We developed a simple and accurate method for the determination of voriconazole concentrations in human plasma. This method could be used for therapeutic drug monitoring and for pharmacokinetic studies of voriconazole. It is essential to monitor voriconazole plasma concentrations to improve treatment efficacy and prevent adverse reactions.

Declaration of Conflicting Interests

The authors declare that there is no conflict of interest.

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References

1. Editorial Board of Chinese J Internal Med. Diagnostic criteria and principles of treatment for invasive fungal infections in patients with hematologic disease/malignant tumors (draft). *Chin J Internal Med* 2009; 44: 555–556.
2. Cao Y, Zhang L, Wang Y, et al. Voriconazole and its clinical application. *Chin J New Drugs Clin Rem* 2005; 24: 330–332.
3. Caroline J. Jjingo. Voriconazole (VFEND®) oral tablets, IV for injection, and oral suspension. 2017; https://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/DevelopmentResources/UCM609569.pdf.
4. Limper AH, Knox KS, Sarosi GA, et al. An official American Thoracic Society statement: treatment of fungal infections in adult pulmonary and critical care patients. *Am J Respir Crit Care Med* 2011; 183: 96–128.
5. Andres P, Chantal C, Thierry B, et al. Challenging recommended oral and intravenous voriconazole doses for improved efficacy and safety: population pharmacokinetics-based analysis in adult patients with invasive fungal infections. *Clin Infect Dis* 2012; 55: 381–390.
6. Dolton MJ, Ray JE, Chen SC, et al. Multi-center study of voriconazole pharmaceutics and therapeutic drug monitoring. *Antimicrobial Agents Chemother* 2012; 56: 4973–4979.
7. Matsumoto K, Abematsu K, Shigemi A, et al. Therapeutic drug monitoring of voriconazole in Japanese patients: analysis based on clinical practice data. *J Chemother* 2016; 28: 198–202.
8. Mak J, Sujishi KK, French D. Development and validation of a liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay to quantify serum voriconazole. *J Chromatogr B Analyt Technol Biomed Life Sci* 2015; 986–987: 94–99.

9. Wu X, Dong X, Chen Y, et al. A HPLC method for voriconazole therapeutic drug monitoring. *Chin J Infect Chemother* 2009; 9: 118–122.
10. Feng S, Yong X, Du X, et al. Determination of voriconazole in human plasma and saliva with LC-MS/MS. *Chinese Journal of New Drugs* 2012; 21: 1908–1911.
11. Chen W, Liu Y, Wang X, et al. Pharmacokinetics of voriconazole in healthy volunteers. *Chin J Clin Pharmacol* 2013; 29: 433–436.
12. Fu S, Xiong X, Duan J, et al. Voriconazole plasma concentration monitoring in patients. *Chin J Clin Pharmacol* 2013; 29: 622–624.
13. Zhang Y, Lin X and Zhu J. Determination of serum voriconazole concentration by HPLC. *China Pharmaceuticals* 2014; 23: 6–8.
14. Xiao X, Meng D, Xie H, et al. Determination of voriconazole in human plasma by HPLC and methodology validation. *Anti Infect Pharm* 2014; 11: 292–294.
15. Wang J, Zhou Q, Yao Q, et al. A simple and accurate method for the determination of voriconazole concentration in human. *Chin Hosp Pharm J* 2014; 34: 1748–1751.
16. Yong X, Feng S, Du X, et al. Correlation between plasma and saliva concentration of voriconazole in healthy volunteers. *Chin J Clin Pharmacol* 2012; 28: 301–303.
17. Karthaus M, Lehrnbecher T, Lipp HP, et al. Therapeutic drug monitoring in the treatment of invasive aspergillosis with voriconazole in cancer patients-an evidence-based approach. *Ann Hematol* 2015; 94: 547–556.
18. Molinelli AR and Rose CH 4th. Quantification of the triazole antifungal compounds voriconazole and posaconazole in human serum or plasma using liquid chromatography electrospray tandem mass spectrometry (HPLC-ESI-MS/MS). *Methods Mol Biol* 2016; 1383: 39–47.
19. Pascual A, Csajka C, Buclin T, et al. Challenging recommended oral and intravenous voriconazole doses for improved efficacy and safety: population pharmacokinetics-based analysis of adult patients with invasive fungal infections. *Clin Infect Dis* 2012; 55: 381–390.
20. Chen K, Song Z, Liu W, et al. Chinese practice guideline for individualized medication of voriconazole: recommendations external review. *Chin J Pharmacoepidemiol* 2017; 26: 143–148.
21. Jin H, Wang T, Falcione BA, et al. Trough concentration of voriconazole and its relationship with efficacy and safety: a systematic review and meta-analysis. *J Antimicrob Chemother* 2016; 71: 1772–1785.
22. Bertilsson L. Metabolism of antidepressant and neuroleptic drugs by cytochrome p450s: clinical and interethnic aspects. *Clin Pharmacol Ther* 2007; 82: 606–609.