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A sensitive liquid chromatography–tandem mass spectrometry method for monitoring the caspofungin trough plasma concentration and its association with caspofungin efficacy in intensive-care-unit patients

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Abstract

Caspofungin is a common treatment for fungal infections in intensive care unit (ICU) patients, and in these patients their pharmacokinetics are highly variable. So a rapid and sensitive liquid chromatography–tandem mass spectrometry (LC-MS/MS) method was established for measuring C_{min} in 18 ICU patients, and the exposure–response characteristics of caspofungin were investigated. The calibration curve included clinically relevant caspofungin concentrations, ranging from 0.05 to 20 mg/L. The mean recovery rate ranged from 85.2% to 95.3%, while the intra- and interday precisions were <5.5% and their accuracies were within the range of 96.2–102.3%. The overall C_{min} was 2.13±0.99 mg/L (mean±SD; range, 0.51–3.79 mg/L). Patients were infected by either *Candida* spp. (*n* = 13) or *Aspergillus* spp. (*n* = 5), and caspofungin therapy was effective in 66.7% (*n* = 12) of them. 76.9% (10/13) patients (infected by *Candida* spp. and C_{min} > 1 mg/L) achieved clinical success while 23.1% (3/13) patients (C_{min} > 1 mg/L: n = 1; C_{min} < 1 mg/L: n = 2) failed to show a clinical response. All five patients infected by *Aspergillus* spp. had a mean plasma C_{min} above 0.5 mg/L, and only two achieved clinical success. Validated LC-MS/MS is a simple, rapid and accurate method that is suitable for monitoring the concentration of caspofungin. C_{min} exhibits a wide range in ICU patients, and relatively good treatment results are obtainable when C_{min} exceeds the 90% minimal inhibitory concentration (*Candida* spp: 1mg/L; *Aspergillus* spp: 0.5mg/L).

1 Introduction

Invasive fungal infections (IFIs) have high morbidity and mortality, and are the fourth most common cause of nosocomial infections in intensive care unit (ICU) patients, accounting for about one in five of all infections in critically ill patients [1, 2]. ICU patients are susceptible to fungal infections because they often suffer from multiple diseases and organ dysfunction after receiving major surgery that involves postoperative catheter indwelling [3-5]. Caspofungin was the first antifungal agent of the echinocandin family approved for the treatment of IFIs caused by *Candida* spp. and *Aspergillus* spp. in patients who are refractory to or intolerant of voriconazole [3, 6, 7]. Caspofungin works by inhibiting the synthesis of β-(1,3)-D-glucan, which is an essential component of *Candida* and *Aspergillus* cell walls. The recommended dosage regimen of caspofungin is a loading dose of 70 mg followed by 50 mg daily that is administered intravenously over a 1-h period. Caspofungin is highly protein-bound (~96%) and metabolizes slowly in the liver [8-10]. It is eliminated slowly from plasma, with a clearance rate of 10–12 ml/minute and a half-life of 9–11 h [8].

Therapeutic drug monitoring aims at optimizing the benefits and risks of pharmacotherapy specifically for drugs exhibiting significant pharmacokinetic (PK) variability. Clinical PK parameters and drug plasma concentrations in ICU patients are often different from those in healthy subjects [3]. Factors associated with alterations in PK include changes in organ function (e.g., renal and hepatic dysfunction), use of extracorporeal clearance techniques, and drug interactions [1, 4]. It has also been reported that caspofungin plasma concentrations are influenced by hypoalbuminemia

and hepatic impairment [1]. The caspofungin trough plasma concentration (C_{min}) exhibits relatively wide ranges in surgical intensive care unit (SICU) patients, and it is influenced by protein binding [3]. Thus, the recommended dosage regimen may not achieve the best curative result, and therapeutic drug monitoring might contribute to improvements in clinical management in these settings.

A rapid and sensitive method for analyzing caspofungin in human plasma is urgently needed for monitoring C_{min}. The methods used currently to determine the caspofungin concentration in human plasma include high-performance liquid chromatography (HPLC) and liquid chromatography–tandem mass spectrometry (LC-MS/MS) [11-17] HPLC has been used to estimate the caspofungin concentration in biological samples with a total run time of 10 min [17]. LC-MS/MS improves the sensitivity by employing a mass detector, and also provides better reliability, repeatability, and analysis time. But the studies that have utilized LC-MS/MS for determining the caspofungin concentration in human plasma have been subject to several limitations, including the use of complicated mobile phases [12, 14, 16, 17], time-consuming sample preparation and diluting steps [11-13]. Moreover, only brief descriptions have been provided of the methods used to measure caspofungin, without fully validation [15, 16], and using internal standards (IS) that are expensive [11-13] or no longer available [15]. Ambient mass spectrometric methods have recently been developed for drug analysis in order to reduce the complexity of LC-MS/MS [18-20]. However, no previously reported study has analyzed caspofungin using ambient mass spectrometry.

To resolve the above problems, we developed a LC-MS/MS method using simple and

economic analysis of mobile phases. The preanalytical plasma processing method using acetonitrile for protein precipitation in our approach was rather straightforward. What's more, our LC-MS/MS method had an excellent recovery rate and accuracy, and the IS (roxithromycin) was accessible. In a word, the present LC-MS/MS method was simple, accurate, precise and has been fully validated and specified. Furthermore, we used this method to analyze caspofungin plasma concentrations and evaluated the association between C_{min} and caspofungin efficacy in ICU patients.

2 Materials and methods

2.1 Chemicals, materials and equipment

All chemicals and reagents were of HPLC grade or analytical grade. Caspofungin was supplied by Merck Sharp & Dohme (Whitehouse, USA); Roxithromycin (IS) was supplied by Yangtze River Pharmaceutical Industry (Jiangsu, China); Acetonitrile and methanol were purchased from Merk (HPLC grade, Germany); Formic acid was purchased from Kemiou Chemicals (HPLC grade, Tianjin, China).

The LC-MS/MS system used consisted of a triple-stage quadruple (TSQ) Vantage triple quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA); The chromatographic analyses were conducted using a Dionex (Sunnyvale, USA) Ultimate 3000 HPLC system equipped by an Ultimate 3000 Pump and CTC Pal autosampler (CTC Analytics AG, Switzerland). Chromatographic separation of caspofungin and IS was achieved on a Hypersil GOLD C_{18} column (Thermo Fisher Scientific, 50 × 2.1 mm, 5 μm); The tri-distilled water was obtained by Millipore

using a water purification machine (Millipore, USA); High-speed centrifuge at low temperature (Allegra-22R, Beckman, USA); Vortex generator (Vortex-Genie2, Scientific Industries, USA); Thermo Finnpipette (France). The Xcalibure software (version 3.0.63) was used for instrument control and data collection.

2.2 Patients

The study was approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University. All subjects signed the informed consent before any screening item being performed. Forty-two blood samples were collected from a total of 18 ICU patients. Inclusion criteria: patients with proven invasive fungal infections caused by *Candida* spp. or *Aspergillus* spp. and being treated with caspofungin were enrolled in this study. Exclusion criteria: (1) patients < 18 years; (2) hypersusceptible or severe intolerance to caspofungin; and (3) concomitant with other antifungal agents. Acute physiology and chronic health evaluation (APACHE)-II score was used to measure the severity of disease of ICU patients. Clinical data (imaging tests, demographic data and underlying conditions) as well as laboratory data (liver and renal function) for each patient were recorded.

2.3 Caspofungin administration and blood sample collection

All patients received a loading dose of 70 mg on the first day, followed by 50 mg daily. Caspofungin was given as an intravenous infusion over 1 h. Blood samples for the determination of caspofungin C_{min} were taken directly before the next scheduled

dose at steady-state. The samples were centrifuged at 3,000 rpm for 10 minutes and the plasma samples were collected and stored at -80 ℃ for LC-MS/MS analysis.

2.4 Methodology of quantification of caspofungin in human plasma

2.4.1 LC-MS/MS system and conditions

Chromatography was performed on a Hypersil GOLD C_{18} column using two mobile phases-a mixture solution of 0.1% formicacid (A) and methanol (B). The flow rate was 0.4 mL/min and the run time was 6.5 min. The gradient elution was delivered as follows: at start of run 10/90% of A/B; from 5.5 to 6.5 min, the gradient starts at 10% A and ramps to 90% A in 60 s. 10 µl of sample was injected into the system by autosample, and the column temperature was maintained at 20 ℃. Besides, the tray temperature in the autosampler was kept at 4 ℃. The mass spectrometer was operated in an electronic spray ion positive mode. The selected reaction monitoring transitions which were used for quantification and qualification were performed at m/z 547.5 → 137.3 (collision energy: 26 eV) for caspofungin and m/z 837.7 → 679.3 (collision energy: 19 eV) for IS. Other ion source conditions were as follows: curtain gas was 25 psi, ion spary voltage was 3500 V, and source temperature was 350 ℃.

2.4.2 Stock solutions, calibration standard, quality control (QC) samples and sample preparation

Stock solutions of caspofungin (1.0 mg/mL) and IS (1.0 mg/mL) were prepared in deionized water and methanol, respectively, and stored at -80 ℃. Appropriate

amounts of caspofungin were added to achieve calibration concentrations of 0.05、0.1、0.5、1、5、10、20 mg/L and quality control (QC) concentrations of 0.1, 1, and 16 mg/L. Final concentration of the IS in calibration solutions was 4 mg/L. Plasma sample was prepared with protein precipitation using acetonitrile. After addition of 20 μl IS solution (40 mg/L), 600 μl acetonitrile was added into 200 μl plasma samples in tubes. After a thorough vortex mixing for 1 min, the mixture was centrifuged at 13,000 rpm for 10 min, and then 10 μl of the supernatant was injected into the LC-MS/MS system.

2.4.3 Method validation

The assay was fully validated according to the US Food and Drug Administration guidelines [21]. The specificity and selectivity of the method were performed by examining the presence or absence of interference, comparing chromatograms of six lots of blank human plasma samples from different sources, blank plasma spiked with standard, and human plasma sample after intravenously administration of caspofungin. The linearity was assessed by weighted ($1/X^2$) least-squares linear regression of calibration curves based on peak area ratios of caspofungin to IS versus actual concentrations. The limit of detection (LOD) was defined as the lowest concentration of an analyte that the bioanalytical procedure can reliably differentiate from bankground noise. It was calculated using the equation LOD = (3.3σ) /S', where σ is the standard deviation for the calibration curve and S' is its slope. The lower limit of quantification (LLOQ) was considered being the lowest concentration within the

calibration range with an acceptable accuracy and imprecision (⩽20%) and a signal-to-noise (>10:1). Carryover effect should be assessed by injecting blank samples following the calibration standard at the highest concentration, and the effect should not be >20% of LLOQ. Intra- and interday precisions were determined by analyzing QC plasma samples at low, medium, and high concentrations on the same day and on three different days. The matrix effects and extraction efficiency were determined at 3 concentrations (low, medium, and high) using 5 replicates of each. Matrix effects were performed by comparing extracted matrix samples against non-matrix samples and recovery compare against extracted matrix spiked with analyte. The stability of caspofungin in plasma was investigated by analyzing five QC samples at 3 concentrations (low, medium, and high) of caspofungin stored at room temperature for 8 h, -80 °C for 15 and 30 days, three freeze-thaw cycles (-80 °C to 25 °C) and the post preparative stability was examined after 8 h in the auto sampler maintained at 4 °C.

2.5 Clinical efficacy

Patients were assessed for clinical response by the investigators according to the following criteria: clinical efficacy was evaluated by assessing clinical and microbiological responses at the end of caspofungin treatment in patients with IFIs. Clinical success was defined as improvement in partial or resolution of clinically significant signs and symptoms (fever and inflammatory markers) associated with fungal infection, on proven or presumed eradication of the fungal pathogen (negative

198 culture results), and improvement or resolution of magnetic resonance imaging
199 findings or computed tomography. Lack of response or ineffective to caspofungin
200 therapy was defined by persistent IFI or by progressing IFI (clinical and radiological
201 progression, persistently positive culture results or death due to IFI) after 14 days of
202 caspofungin treatment [22].
203
204 **2.6 Statistical analysis**
205 The Xcalibure software (version 3.0.63) was used for instrument control and data
206 collection in process of sample analysis. Clinical data were analyzed and processed by
207 SPSS 19.0 and expressed as mean ± standard deviation (SD) or median ± SD. A
208 *P*-value of <0.05 was considered statistically significant.
209
210 # 3 Results
211 **3.1 Method validation and analytical methods**
212 Figure 1A shows that no interference peaks from endogenous substances were
213 observed at the retention times of caspofungin and IS in the chromatograms,
214 indicating the high specificity and selectivity of the method. Figure 1B shows the
215 chromatogram of a patient receiving caspofungin therapy. The calibration curve for
216 caspofungin in human plasma was highly linear over the concentration range of 0.05–
217 20 mg/L, with a correlation coefficient of R^2 = 0.9994. The LOD and LLOQ for
218 caspofungin were 0.001 and 0.05 mg/L, respectively. No peak in the chromatographic
219 region of the analyte of interest was observed by injecting blank plasma extract

immediately after the upper limit of quantification sample, indicating that any carryover effect from previous concentrated samples was negligible. Precision, accuracy, recovery, matrix effect, and stability data are listed in Table 1. Table S1 (in the supplemental material) provides an overview of several published methods [11-17] that have been used for quantifying caspofungin in human plasma.

3.2 Characteristics of 18 ICU patients with caspofungin therapy

Eighteen patients were enrolled and 42 plasma samples were monitored. Caspofungin therapy was administered to patients with proven fungal infections. Most of the yeast and mold species isolated from the samples were *Candida albicans* (*n* = 11), followed by *Aspergillus fumigatus* (*n* = 3), *Candida tropicalis* (*n* = 2), and *Aspergillus flavus* (*n* = 2). The APACHE II score was 26.0±6.1 (mean±SD; range, 15–37). C_{min} values were assessed in the 18 ICU patients, including 11 who were receiving continuous venovenous hemofiltration (CVVH). These patients had different kinds of diseases and underlying conditions, such as liver, renal, and multiple organ dysfunction. The characteristics of the patients, including their demographic and clinical data, are given in Table 2.

3.3 Characteristics of C_{min} in ICU patients

As indicated in Table 2, C_{min} as measured in 42 samples from 18 patients had a median of 2 per patient (range, 1–4). The overall C_{min} was 2.13±0.99 mg/L (range, 0.51–3.79 mg/L). Among the 18 patients, 5 liver dysfunction patients (patients 1, 2, 8,

9, and 10) had a C_{min} of 2.43±0.73 mg/L, and 7 renal dysfunction patients (patients1, 2, 3, 8, 11, 12, and 13) had a C_{min} of 2.37±0.69 mg/L. The overall C_{min} for the 11 CVVH patients was 2.02±0.45 mg/L. The C_{min} value of each patient is listed in Table 2; this ranged from ≤1 mg/L in 16.7% of cases to ≥2 mg/L in 50% of cases. As presented in Figure 2, the median C_{min} was maintained at a steady state from the second day after applying caspofungin therapy.

3.4 Relationship between C_{min} and efficacy of caspofungin therapy

As indicated in Table 2, 84.6% (11/13) patients infected by *Candida* spp. had a mean C_{min} above 1 mg/L, which was defined as the target concentration because this concentration exceeds the 90% minimal inhibitory concentration (MIC_{90}) for *Candida* spp. All five of the patients infected by *Aspergillus* spp. had a C_{min} above 0.5 mg/L, which was reported to be the MIC_{90} for *Aspergillus* spp. Caspofungin therapy was effective in 66.7% (12/18) of the patients in the present study. Ten of these patients with clinical success were infected by *Candida* spp., with a mean C_{min} above 1 mg/L, and the other two were patients infected by *Aspergillus* spp., with C_{min} above 0.5 mg/L. The other six patients who did not respond to the treatment were infected with *Candida albicans* (n = 2), *Candida tropicalis* (n = 1), *Aspergillus flavus* (n = 1), or *Aspergillus fumigatus* (n = 2). Patients 5 and 13, who showed failed responses, were infected by *Candida* spp. with a mean C_{min} below 1 mg/L.

4 Discussion

Caspofungin is widely used as an agent to prevent and treat IFIs in patients. Large interindividual variabilities of C_{min} have been described in ICU patients [3]. In order to monitor C_{min} and study its exposure–response characteristics, methods for measuring the plasma concentration of caspofungin are needed. We therefore established and validated a sensitive LC-MS/MS method for analyzing caspofungin plasma concentrations in ICU patients.

One of the advantages of our method over the currently available methods is the simple and economic analysis of mobile phases, which included a mixture solution of 0.1% formic acid in ultrapure water (mobile phase A) and methanol (mobile phase B). The preanalytical plasma processing method using acetonitrile for protein precipitation in our approach was rather straightforward. This simple, rapid, and inexpensive sample pretreatment step provided the best analytical sensitivity for the clinically relevant concentration ranges of caspofungin. In contrast, the sample preparation methods described by Rochat *et al.* [11] and Decosterd *et al.* [12] required an additional dilution step, which may increase the risk of errors and the assay variance. Farowski *et al.* [13] used diluted plasma as a matrix, which was obtained after centrifuging diluted blood layered onto a double discontinuous Ficoll-Hypaque density gradient and another dilution step, which represents a more complex sample preparation procedure. Egle *et al.* [15] used simple mobile-phase samples, but the run time was 30 min and they did not report the IS, which means that their approach is not suitable for further clinical research. The IS (e.g., caspofungin isotope) used by other methods [11-13] could be more expensive than ours.

Moreover, the present LC-MS/MS method has been fully validated based on the guidance from the US Food and Drug Administration for validating industrial bioanalytical methods [21]. The calibration curve included clinically relevant caspofungin concentrations ranging from 0.05 to 20 mg/L and exhibited excellent linearity. The LOD and LLOQ for caspofungin were 0.001 and 0.05 mg/L, respectively. The reason the LLOQ is 50 times compared to LOD was that the LLOQ was considered being the lowest concentration within the calibration range with an acceptable accuracy and imprecision (⩽20%) and a signal-to-noise (>10:1) while the equation LOD = (3.3σ) /S'. The mean recovery rate ranged from 85.2% to 95.3%, while the matrix effect ranged from 98.1% to 107.0%. The intra- and interday precisions were <5.5% and their accuracies were within the range of 96.2–102.3%. No carryover effect was observed on the column. Applying three freeze-thaw cycles or storing the plasma samples at –80 °C for 30 days did not result in significant changes of the caspofungin plasma concentrations in the QC samples—all values were within 90–110% of the initial values.

In short, compared with the previous literature, the LC-MS/MS method using a mixture solution of 0.1% formic acid in ultrapure water and methanol as mobile phases was simple and economic. The preanalytical plasma processing method using acetonitrile for protein precipitation was rather straightforward. The LC-MS/MS method was fully validated and with a mean recovery rate ranged from 85.2% to 95.3%, while the intra- and interday precisions were <5.5% and their accuracies were within the range of 96.2–102.3%. In addition, the IS (roxithromycin) which we used

was appropriate and affordable. In conclusion, the main advantages of this new LC-MS/MS method over the methods reported in the literature are (1) the simple and economic analysis of mobile phases, (2) rapid and inexpensive preanalysis processing, (3) accessible IS, (4) excellent recovery rate and accuracy; absence of a matrix and a carryover effect. All of the results already obtained indicate that this is a practical method for monitoring C_{min} in ICU patients with IFIs.

Forty-two plasma samples of C_{min} from 18 ICU patients were collected and analyzed. We found that the C_{min} values varied markedly between individuals. Hypoproteinemia and multiple organ dysfunctions may result in interindividual variations of plasma caspofungin concentrations in patients who are critically ill with life-threatening infections [23, 24]. Measuring the caspofungin concentrations in plasma might help to improve the clinical management in these settings as well as in patients treated with combinations of caspofungin and other antifungal agents [1, 3, 11].

Few studies have investigated the PK of caspofungin in ICU patients [1, 10]. Nguyen *et al.* [3] found that the mean C_{min} was 2.16 mg/L among 40 SICU patients, while Brüggeman *et al.* [1] found that it was 2.15 mg/L in 21 ICU patients. The mean C_{min} was 2.13 mg/L in our 18 ICU patients, with a large interindividual variability. The mean caspofungin concentrations in all of these ICU patients were slightly higher than that of 1.41 mg/L reported by Stone *et al.* [10] for healthy subjects. Additionally, we found that the C_{min} value was significantly lower in healthy subjects (1.41 mg/L) than in patients with liver dysfunction (2.43 mg/L, $P < 0.05$) and renal dysfunction (2.37 mg/L, $P < 0.05$). These higher C_{min} values in liver and renal dysfunction patients may

be due to physiological and physiopathological alterations caused by trauma, sepsis, septic shock, and surgery [1, 3]. Hemodynamic responses and vital support therapy are also known to influence PK parameters, such as clearance and the distribution volume. In addition, alterations in protein binding, lack of organ perfusion, and/or organ dysfunction are also common factors influencing the plasma concentration of caspofungin [2, 4]; for example, C_{min} was estimated to increase by 0.25 mg/L when the albumin concentration was >23.6 g/L [3]. However, we found no significant relationship between C_{min} and the albumin concentration (r = 0.197, P > 0.05). Extracorporeal devices exert barely detectable effects on drug disposition in ICU patients [2-4]. CVVH is the most common and important extracorporeal treatment method for patients with acute renal failure and systemic inflammatory response syndrome [25]. CVVH is associated with a higher glomerular filtration rate of 25–50 mL/min and can significantly reduce drug concentrations [25], but previous research had shown that caspofungin clearance by CVVH was very low [26]. Eleven CVVH patients were included in the present study and their mean C_{min} was 2.02 mg/L, which is lower than the overall average concentration but clearly higher than that in the healthy volunteers (P < 0.05).

Figure 2 shows that the median C_{min} was maintained at a steady state from the second day after caspofungin therapy, and that it varied between the individual patients. Similar to our findings, Nguyen *et al.* [3] found that C_{min} varied over a wide range (0.21–5.1 mg/L) among 40 SICU patients, with the median C_{min} also being maintained at a steady state after the second day. Caspofungin metabolizes in the liver

with a half-life of 9–11 h in healthy volunteers [8], resulting in steady-state concentrations being achieved on the second day after therapy involving a loading dose. However, Brüggeman *et al.* [1] found that the steady-state concentrations were not achieved on the second day after applying a loading dose of caspofungin therapy in 21 ICU patients, and they found that the half-life of caspofungin was 15.67 h on day 3 and 18.49 h on day 7. The half-life would be prolonged for ICU patients with liver dysfunction, which could explain the findings of Brüggeman *et al*.

Caspofungin is concentration-dependent antibacterial. Previous studies have often used the area under the concentration-time curve/minimum inhibitory concentration (AUC/MIC) as the caspofungin pharmacokinetic/pharmacodynamic parameter [27-29]. C_{min} is easy to measure clinically, and so we studied the relationship between C_{min} and the response to caspofungin therapy. Caspofungin was clinically effective in 66.7% (12/18) of our ICU patients. Most (84.6%, 11/13) of the patients who were infected by *Candida* spp. had a mean C_{min} above 1 mg/L (the target concentration exceeds the MIC_{90} for most clinically relevant *Candida* spp. [30-33]), and a successful clinical response occurred in 10 of the 11 patients. The mean C_{min} values of patients 5 and 13 were below 1 mg/L, and they exhibited clinical response failure. All five patients who were infected by *Aspergillus* spp. had plasma caspofungin concentrations above 0.5 mg/L (which is the MIC_{90} for *Aspergillus* spp. [34-36]), but only two patients achieved clinical success and one died during the treatment period. The clinical response is determined not only by drug factors but also underlying diseases, immune status, pathogenic species, and the susceptibility to antimicrobial agents. The five patients

374 who were infected by *Aspergillus* spp. were suffering from serious underlying
375 diseases, and these patients were treated with caspofungin when they failed to respond
376 to voriconazole, and they were mainly in an advanced disease stage.
377

378 5 Conclusion

379 Validated LC-MS/MS is a simple, rapid, sensitive, and reproducible method for
380 monitoring the concentration of caspofungin. C_{min} increased, and the caspofungin
381 concentration exhibited a wide range, suggesting the necessity of closely monitoring
382 the plasma concentrations of caspofungin in ICU patients when compared with
383 healthy subjects. Furthermore, monitoring C_{min} is necessary to ensure the efficacy of
384 clinical caspofungin treatment, and a successful response is obtainable when C_{min} >
385 MIC_{90}. Since this study involved a relatively small number of patients, future studies
386 should include larger samples and investigate pathogenic species in order to obtain
387 more reliable results.

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Competing interests

None to declare.

Ethical approval

The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University.

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Table 1. Inter- and intraday imprecision, accuracy, recovery, matrix effect and stability of caspofungin in human plasma

Concentration (mg/L)	Precision (RSD, %)		Accuracy (bias, %)		Recovery (%)	Matrix effect (%)	RSD (%) Stability analysis of caspofungin under various conditions (n=5)				
	Intraday (n=5)	Interday (n=3)	Intraday (n=5)	Interday (n=3)	Mean (n=5)	Mean (n=5)	8h, room temperature	8h, postpreparative	15 days -80°C	30 days -80°C	Three cylces, freeze-thaw, -80°C
0.1	2.5	5.5	99.3	102.2	85.2 ± 1.4	98.1 ± 1.8	2.9	2.9	4.9	4.3	5.9
1	5.3	5.2	100.6	96.3	91.9 ± 1.5	107.0 ± 1.5	4.9	6.3	5.7	2.3	6.1
16	3.2	3.0	98.0	96.2	95.3 ± 0.8	95.2 ± 1.4	2.1	1.1	3.1	3.5	1.5

Table 2. Characteristics of 18 patients with caspofungin treatment.

Patient	Age	Fungal organisms	Sex	Mean trough concentration (mg/L)	Albumin concentration (g/L)	Neutrophil concentration (%)	Days with therapy	No. of samples	Underlying conditions	CVVH	APACHE II score	Clinical response
1	30	*Candida albicans*	M	2.23	26.40	85.70	6	3	Liver dysfunction, lung dysfunction, MODS, respiratory failure	Yes	18	Clinical success
2	32	*Candida albicans*	M	1.70	27.01	80.98	8	2	Liver dysfunction, lung dysfunction, MODS	Yes	25	Clinical failure
3	33	*Candida tropicalis*	M	2.17	35.65	72.90	17	2	Respiratory failure, lung dysfunction, AKI, CPR	Yes	16	Clinical success
4	35	*Candida albicans*	F	1.57	32.32	75.56	14	3	Respiratory failure, AKI	Yes	15	Clinical success
5	36	*Candida tropicalis*	M	0.73	30.52	78.30	15	2	Respiratory failure, AKI	Yes	30	Clinical failure
6	40	*Aspergillus flavus*	M	1.01	20.86	91.90	21	2	Respiratory failure, MODS	No	26	Clinical success
7	45	*Aspergillus fumigatus*	M	0.51	28.49	90.53	18	2	Respiratory failure, MODS	No	20	Clinical success
8	61	*Aspergillus flavus*	F	1.85	22.93	82.30	13	4	Liver dysfunction, lung dysfunction, MODS, respiratory failure	Yes	27	Clinical failure
9	65	*Aspergillus fumigatus*	F	3.01	30.16	88.60	6	2	Liver dysfunction, respiratory failure, respiratory alkalosis	No	29	Clinical failure

									chronic cholecystitis			
10	71	*Aspergillus fumigatus*	F	3.38	28.45	88.35	8	2	Liver dysfunction, respiratory failure, chronic cholecystitis	No	31	Clinical failure
11	75	*Candida albicans*	F	3.53	26.62	93.80	4	3	Respiratory failure, lung dysfunction, hypertensive nephropathy	Yes	21	Clinical success
12	77	*Candida albicans*	F	1.99	25.83	90.22	7	2	Respiratory failure, lung dysfunction	Yes	26	Clinical success
13	79	*Candida albicans*	F	0.90	24.62	88.78	7	2	Respiratory failure, lung dysfunction, MODS	Yes	37	Clinical failure
14	80	*Candida albicans*	F	2.52	29.06	76.32	9	1	Respiratory failure, DN, heart disease	No	23	Clinical success
15	84	*Candida albicans*	M	2.72	30.04	78.55	8	4	Respiratory failure	No	22	Clinical success
16	85	*Candida albicans*	M	3.14	29.72	80.35	10	2	Respiratory failure, lung dysfunction	No	28	Clinical success
17	84	*Candida albicans*	F	3.79	28.45	82.63	11	2	Respiratory failure	Yes	35	Clinical success
18	87	*Candida albicans*	M	1.76	30.51	79.20	8	2	Respiratory failure, DN, heart disease	Yes	27	Clinical success

NOTE. F, female; M, male; CVVH, continuous vena-venous hemofiltration; MODS, multiple organ dysfunction syndrome; AKI, acute kidney injury ; CPR, cardiopulmonary resuscitation; DN, diabetic nephropathy.

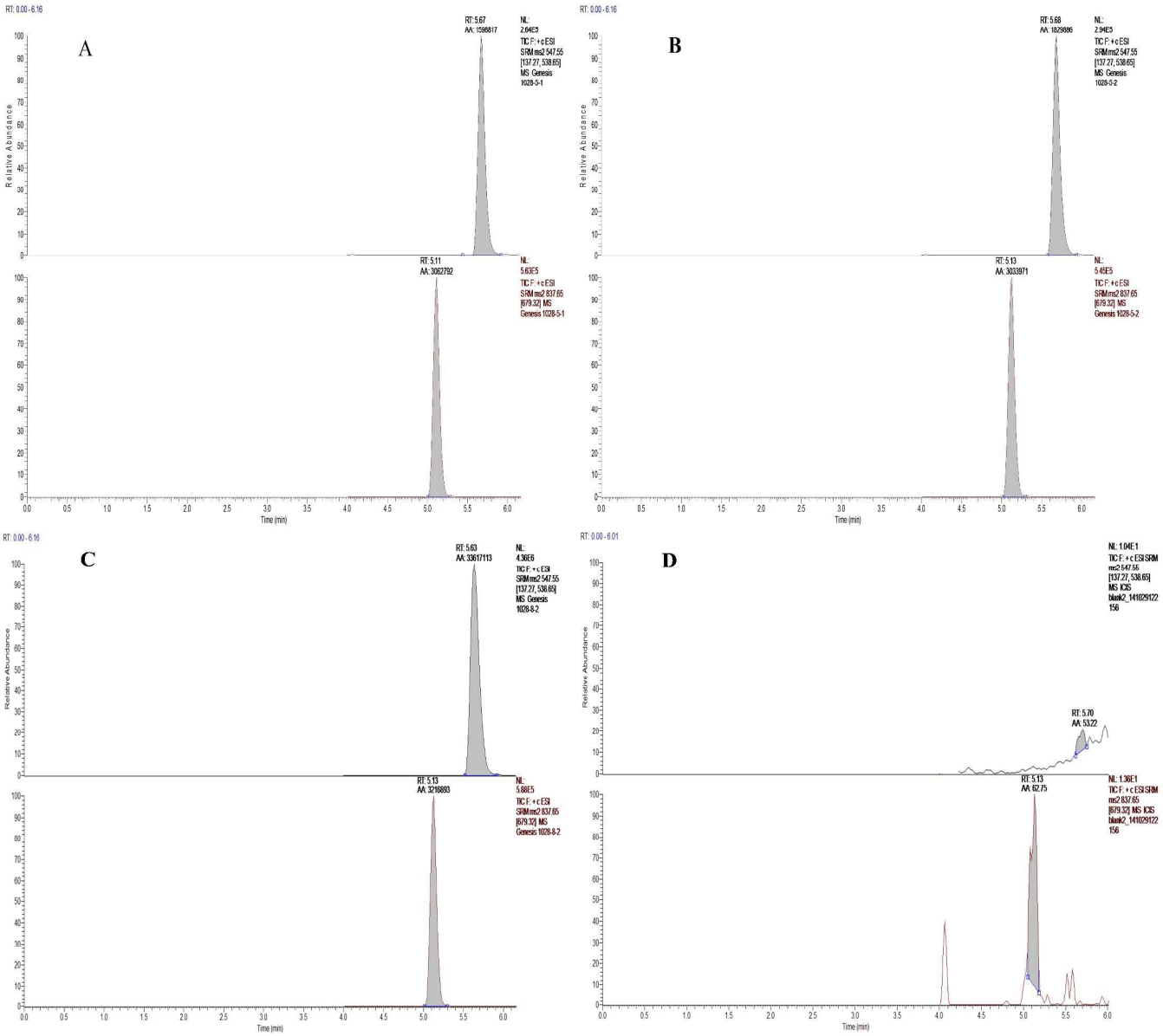


Figure 1. LC-MS/MS chromatograms of the ion transitions for caspofungin and the IS: (A) Injection (10 µl) of a QC sample (caspofungin and IS) spiked with 1 mg/L and 4 mg/L, respectively; (B) Injection of a plasma extract from a patient receiving caspofungin therapy; (C) Injection (10 µl) of a sample (caspofungin and IS) spiked with 20 mg/L (= ULOQ) and 4 mg/L, respectively; (D) A chromatogram of a plasma extract sample from a patient not received caspofungin therapy; RT is the retention time in min; AA is the peak area in arbitrary units.

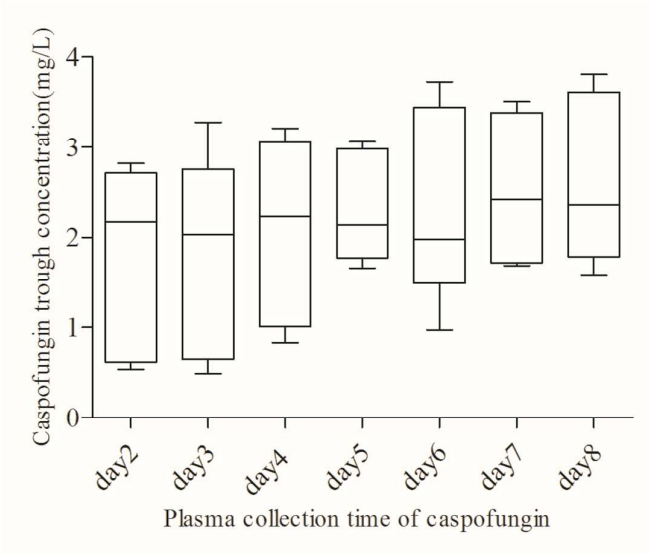


Figure 2. Distribution of caspofungin trough concentrations in 18 ICU patients.

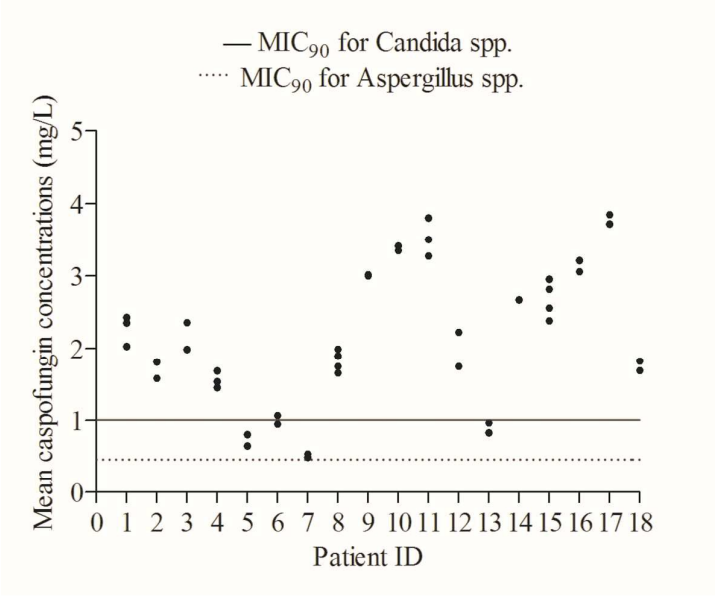
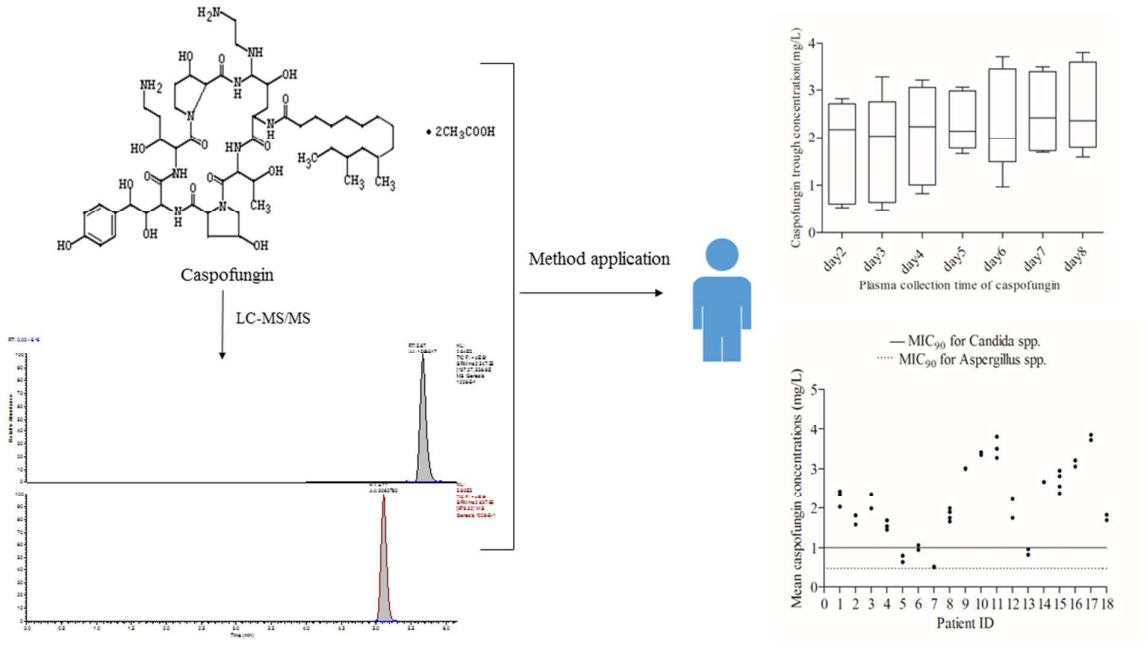


Figure 3. Caspofungin trough concentrations in plasma of the 18 ICU patients and MIC_{90} for *Candida* spp. and *Aspergillus* spp.



LC-MS/MS method for monitoring the caspofungin trough plasma concentration and its association with caspofungin efficacy in intensive-care-unit patients