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# Using sweeping-micellar electrokinetic chromatography to determine voriconazole in patient plasma

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ABSTRACT

Invasive fungal infection is a life-threatening condition; its occurrence has increased significantly over the past 20 years. We have developed a sensitive and efficient sweeping-micellar electrokinetic chromatography (sweeping-MEKC) method to quantify voriconazole, a potent triazole antifungal drug, in patient plasma. Solid phase extraction (SPE) conditions were first optimized to minimize plasma interference while maintaining a high recovery; the sweeping-MEKC conditions were then systematically optimized to obtain a high sweeping efficiency with good selectivity. Under the optimal analytical conditions, voriconazole was baseline-separated from endogenous materials within 10.5 min with a limit of detection of 0.075 μg mL$^{-1}$. The background electrolyte comprised 40 mM phosphoric acid, 110 mM sodium dodecyl sulfate, and 20% acetonitrile. In terms of method repeatability, the relative standard deviations (RSDs) of the migration time and the peak area (intra-day; $n = 6$) were both less than 5.5%; in terms of intermediate precision, and the RSDs of the peak area and the migration time (inter-day; $n = 3$) were both less than 6.3%. We successfully applied this developed method to the quantitative determination of plasma voriconazole levels in 16 patients; the results correlated well with those obtained through analyses using high-performance liquid chromatography. This sweeping-MEKC method is accurate and efficient and appears to be applicable to therapeutic drug monitoring and clinical research.



## 1. Introduction

The incidence of invasive fungal infections (IFIs) has significantly increased over the past 20 years. Fungal infection is a life-threatening condition; its rate of occurrence increases when the function of the immune system weakens. As a result, patients in cancer therapy, undergoing organ transplantation, infected with human immunodeficiency virus, or suffering from diabetes mellitus are at high risk of IFI [1]. From an examination of over 5000 hematopoietic stem cell transplant patients, Marr et al. found that the overall 1-year survival rate was less than 20% among patients with IFIs [2]. The high mortality of IFIs appears to be related to ineffective antifungal therapy [3–5].

Voriconazole (Fig. 1) is the first available second-generation triazole drug for the treatment of serious IFIs [6]. It is metabolized through N-oxidation mediated by the cytochrome P450 (CYP450) enzymes CYP2C19, CYP2C9, and CYP3A4 [7]. Genetic polymorphism of CYP2C19 results in approximately fourfold higher voriconazole exposure in poor metabolizers than in extensive metabolizers [8]. Other factors that influence voriconazole metabolism include age, liver disease and co-medication [9]. Therefore, the inter-individual variability of voriconazole pharmacokinetics is high. For poor metabolizers, long-term exposure to high concentrations of voriconazole may cause transient visual disturbances, rashes, and serious hepatic reactions [10]. On the other hand, concentrations of voriconazole in plasma lower than the minimal inhibitory concentration lead to poor treatment outcomes. In clinical practice, voriconazole therapeutic drug monitoring (TDM) is highly recommended for severely ill patients with IFIs to improve the efficacy and safety of the therapy [9].

Because therapeutic monitoring of voriconazole is important in clinical practice, efficient and accurate analytical methods are required. To date, there are no immunoassay-based methods available for voriconazole determination. Several chromatographic methods have been developed for quantitation of voriconazole in patient plasma. Among them, high-performance liquid chromatography with ultraviolet detection (HPLC-UV) has been the major tool for voriconazole analysis [11–14]. HPLC has also been coupled to mass detectors to improve the analytical sensitivity and speci-

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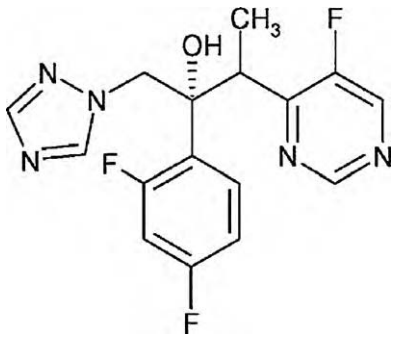


**Fig. 1.** Structure of voriconazole.

ficity [15–18]. Recently, Michael et al. used HPLC with fluorescence detection to determine voriconazole in human plasma and saliva [19].

Because of its high efficiency, short analysis times, and low sample and reagent consumption, capillary electrophoresis (CE) has become a powerful tool for pharmaceutical analysis, especially when considered from the point of view of green chemistry. Crego et al. used capillary zone electrophoresis/UV detection (CZE–UV) to separate a standard solution of several antifungal drugs and to study their *in vitro* metabolism [20,21]. Because of the small inner diameter of the capillary and the small amount of injected sample, the detection sensitivity of their method toward voriconazole was 3–5.2 $\mu g\,mL^{-1}$. Because the effective plasma concentration of voriconazole for IFIs is 2 $\mu g\,mL^{-1}$, this CZE–UV method is not suitable for clinical application. There are two major approaches toward improving the detection sensitivity of CE: using more-sensitive detectors (e.g., fluorescence, electrochemical, or mass spectrometric detectors) and applying on-line concentration strategies. Because highly sensitive detectors are expensive, they are not available in all laboratories; therefore, in this present study we used an on-line concentration strategy to improve the detection sensitivity. Stacking and sweeping are the two most commonly used on-line concentration techniques. Stacking is a phenomenon in which sample ions accumulate at the boundary separating the low-conductivity sample plug from the high-conductivity background electrolyte (BGE). Sweeping is defined as the selection and accumulation of analytes by a pseudostationary phase that fills the sample zone during the application of the voltage [22]. Relative to the stacking method, the matrix effect is much lower when using sweeping technique [23]. Because plasma samples contain complex endogenous components, we chose a sweeping approach to improve the limit of detection (LOD) of voriconazole in patient serum. The efficiency of the on-line concentration of the sweeping process depends on the types of micelles and the nature of the analytes; sensitivity enhancements of 80- to 5000-fold have been reported [24].

Our primary goal in this study was to develop a sensitive and accurate electrophoretic method for the determination of voriconazole in plasma. To achieve this goal, we employed solid phase extraction (SPE) for sample preparation and systematically optimized the factors affecting the sweeping-MEKC performance in our search for sensitive and selective conditions for voriconazole determination. We confirmed the applicability of our developed method by applying the optimal sweeping-MEKC conditions to quantify the levels of voriconazole in patient plasma. To the best of our knowledge, this paper is the first to report the use of an SPE/sweeping-MEKC method for the determination of voriconazole concentrations in patient plasma.

## 2. Materials and methods

### 2.1. Chemicals

Voriconazole (purity 99.9%) was obtained from Pfizer (UK). Urea, methanol (MeOH), and acetonitrile (ACN) were obtained from Mallinckrodt (Philipsburg, NJ, USA) in chromatography grade. Sodium tetraborate and phosphoric acid were obtained from Merck (Darmstadt, Germany). Sodium hydroxide and acetic acid were obtained from Fluka Chemicals (Buchs, Switzerland). Sodium dodecyl sulfate (SDS) was purchased from Sigma–Aldrich (St. Louis, MO, USA). The SPE cartridges were purchased from Waters (Milford, MA, USA). The SPE procedure was performed using a Waters extraction manifold system. Blank plasma was obtained from healthy volunteers.

### 2.2. Instrumentation

A fused-silica capillary from Polymicro Technologies (Phoenix, AZ, USA) was used for separation. The sweeping-MEKC experiments with UV detection were performed using a system comprising a Prince programmable injector from Lauer Labs (Emmen, The Netherlands) and a 30-kV high-voltage supply connected to a UV-C absorbance detector from Dynamax (Rainin, Emeryville, CA, USA). The electropherograms were recorded using an EZChrom (Scientific Software, San Ramon, CA, USA) chromatographic data system.

### 2.3. Separation conditions

Sweeping-MEKC was preformed on a 75 cm (60 cm effective length) × 50 $\mu$m I.D. fused-silica capillary. The BGE solution comprised 40 mM phosphoric acid, 110 mM SDS, and 20% ACN. The sample solution was injected hydrodynamically into the capillary at 50 mbar for 3 min. The applied voltage was −23 kV; the detection wavelength was 254 nm. The separation was performed at room temperature (28 °C). The new fused-silica capillary was conditioned by flushing sequentially with 1.0 M NaOH for 10 min, 0.2 M NaOH for 10 min, and water for 10 min. At the beginning of each experiment, the capillary was washed with 0.2 M NaOH for 3 min, followed by MeOH for 3 min, water for 3 min, and then BGE solution for 3 min to ensure reproducibility. Because the sample solution was different from the BGE, the inlet end of the capillary was immersed in DI water for 0.04 min to wash out the BGE adhesive on the outer capillary surface prior to each injection. Solutions containing phosphoric acid were filtered through a GHP Acrodisc® 0.45-$\mu$m filter (GelmanSciences, US); other solutions were filtered through a 0.45-$\mu$m filter from Millipore (Bedford, MA, USA) prior to use.

### 2.4. Preparation of stock and working solutions

Voriconazole stock solution was prepared in MeOH at a concentration of 1 $mg\,mL^{-1}$. An aliquot of this stock solution was diluted with DI water to obtain the working solution. For optimization of the SPE and sweeping-MEKC methods, a suitable amount of the working solution was spiked into blank plasma to obtain a final concentration of voriconazole of 2.5 $\mu g\,mL^{-1}$.

### 2.5. Preparation of plasma samples

Blood samples collected from healthy volunteers or patients were centrifuged (3000 × *g*, 15 min) to obtain plasma samples. Oasis HLB cartridges (1 mL; 30 mg) were used for sample extraction. The cartridges were conditioned with MeOH (1 mL) and DI water (1 mL) prior to use. 10 M Urea (300 $\mu$L) of was added to the plasma sample (300 $\mu$L) and vortex-mixed for 1 min to ensure protein

denaturation. The resulting solution (600 μL) was loaded into the pre-conditioned SPE cartridge. The cartridge was then washed with 0.1 M sodium tetraborate (1 mL) and 50% MeOH (1 mL). Voriconazole was then eluted with of 1% acetic acid (1 mL) in MeOH. The extraction cartridge was placed on the vacuum manifold and the vacuum pressure adjusted to 2 Torr to control the flow rate during the loading and eluting steps. The vacuum pressure was maintained at 10 Torr during the conditioning and washing steps. The eluent was evaporated to dryness under $N_2$ gas and then reconstituted in 75 mM phosphoric acid. The reconstituted solution was subjected to sweeping-MEKC analysis. All solutions were filtered through 0.22-μm PVDF filters prior to use (Millipore, Bedford, MA, USA).

### 2.6. Drug administration and sample collection

Voriconazole was orally administered to 16 patients in the National Taiwan University Hospital. This study was approved by the local ethical committee. Signed inform consents were received from all patients who participated in this study. The blood samples were collected in EDTA-containing tubes. Blood samples were collected 2 h after oral administration or 1 h after intravenous (i.v.) infusion of voriconazole to obtain the peak concentration. The trough concentration was obtained just prior to the administration of the next dosage. The blood samples were centrifuged and the plasma samples then stored at −80 °C until required for analysis using sweeping-MEKC.

### 2.7. HPLC analysis

The HPLC experiments were performed using a Hitachi HPLC system. The system comprised a programmable injector (Hitachi autosampler L-2200 with a cooling unit), a Hitachi binary solvent manager pump, and a UV absorbance detector (Hitachi UV–vis detector L-2420). The mobile phase comprised 50 mM phosphate buffer (adjusted to pH 7.5 using NaOH) and ACN (55:45, v/v). The flow rate was set at 1 mL $min^{-1}$. A C18 column (100 mm × 4.6 mm, 5.0 μm; Kanto Chemical Co., Inc.) was used for the separation. The sample injection volume was 20 μL. The detection wavelength was 255 nm.

ACN (500 μL) was added to a plasma sample (500 μL) and then vortex-mixed for 10 s. After centrifugation (17,900 × *g*, 5 min), the supernatant (400 μL) was collected and evaporated to dryness under $N_2$ gas. The residue was reconstituted in MeOH (200 μL) and subjected to HPLC analysis.

## 3. Results and discussion

### 3.1. Sample preparation method development

The degree of protein binding of voriconazole is 58% [25]; therefore, we examined the use of a protein denaturation reagent to increase the recovery of voriconazole. Because voriconazole is unstable under extreme pH conditions [26], we examined the efficiency of using ACN and urea for protein denaturation. The recoveries were calculated by comparing the peak area of voriconazole in the protein-denatured sample to standard solution. The recoveries of voriconazole obtained after denaturation with ACN and urea followed by SPE extraction were 95 ± 7 and 99 ± 3%, respectively. Because the urea-treated samples were of lower elution strength, they could be loaded directly into the SPE cartridge without the need for any further dilution steps. Therefore, we chose urea as our protein denaturant in this study.

The SPE procedures were performed according to the method proposed by Pennick et al., with slight modification [12]. Voriconazole is a basic drug, with values of p$K_a$ of 1.76, 4.98, and 11.54 [27,28]. In this study, we used 0.1 M sodium tetraborate (pH 9.12, 1 mL) and 50% MeOH (1 mL) as washing solutions to eliminate acidic and neutral interference. Plasma interference was greatly reduced when applying these proposed washing procedures.

### 3.2. Analytical method development

Our goal in this study was to develop sensitive and effective sweeping-MEKC conditions for the determination of voriconazole in plasma. Plasma samples spiked with voriconazole at 2.5 μg $mL^{-1}$ were used as working solutions for optimization of the sweeping-MEKC conditions. To achieve high sensitivity and selectivity, the parameters affecting the sweeping efficiency and MEKC selectivity were optimized in sequence.

#### 3.2.1. Optimization of the sweeping efficiency

Sweeping occurs when a pseudostationary phase penetrates the sample zone that entraps and accumulates the analytes. In theory, significantly enhancements in sweeping can be obtained when oppositely charged analytes interact with the pseudostationary phase under suppressed electroosmotic flow (EOF) conditions caused by strong interactions between the analytes and the pseudostationary phase [29]. In this study, we used 40 mM phosphoric acid as the BGE. Under such conditions, voriconazole carries a net positive charge and could, therefore, interact strongly with anionic micelles of SDS. Under the application of a voltage of reversed polarity, positively charged voriconazole migrated toward the incoming SDS micelles and then became swept into a narrower zone. According to sweeping theory [22], the length of the swept zone ($l_{sweep}$) is described using the

$$l_{sweep} = l_{ing}\left(\frac{1}{1+k}\right) \quad (1)$$

where $k$ is the retention factor of the analyte and $l_{ing}$ is the injection length. One would expect narrower bands of the swept zone to be obtained for larger values of $k$, which in turn can be obtained at higher concentrations of SDS. We investigated the effects of SDS in the background solution at concentrations ranging from 60 to 110 mM. Fig. 2 reveals that when the SDS concentration was less than 70 mM, it could not effectively sweep voriconazole molecules, resulting in a split signal. When we increased SDS concentration from 70 to 110 mM, the peak intensity increased because of the improved sweeping efficiency. On the other hand, the retention time of voriconazole decreased upon increasing the SDS concentration because increasingly more analyte molecules

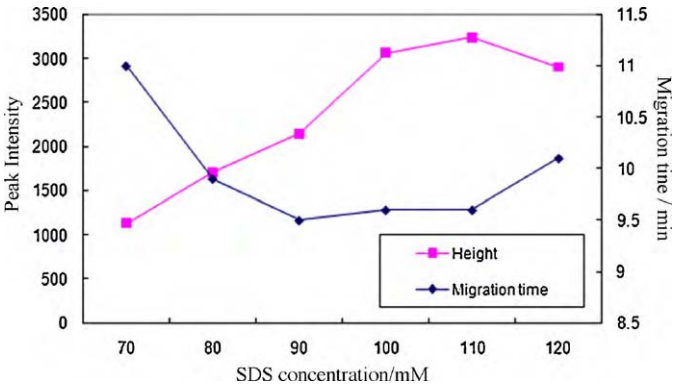


**Fig. 2.** Effect of SDS concentration on the peak intensity and migration time of voriconazole during sweeping-MEKC separation. Sweeping-MEKC conditions: BGE, 40 mM phosphoric acid, SDS (concentration indicated in the figure), and 20% ACN; sample, 2.5 μg $mL^{-1}$ voriconazole dissolved in 75 mM phosphoric acid; fused-silica capillary, 75 cm × 50 μm I.D., 60 cm effective length; injection, 50 mbar for 3 min; separation voltage, −23 kV.

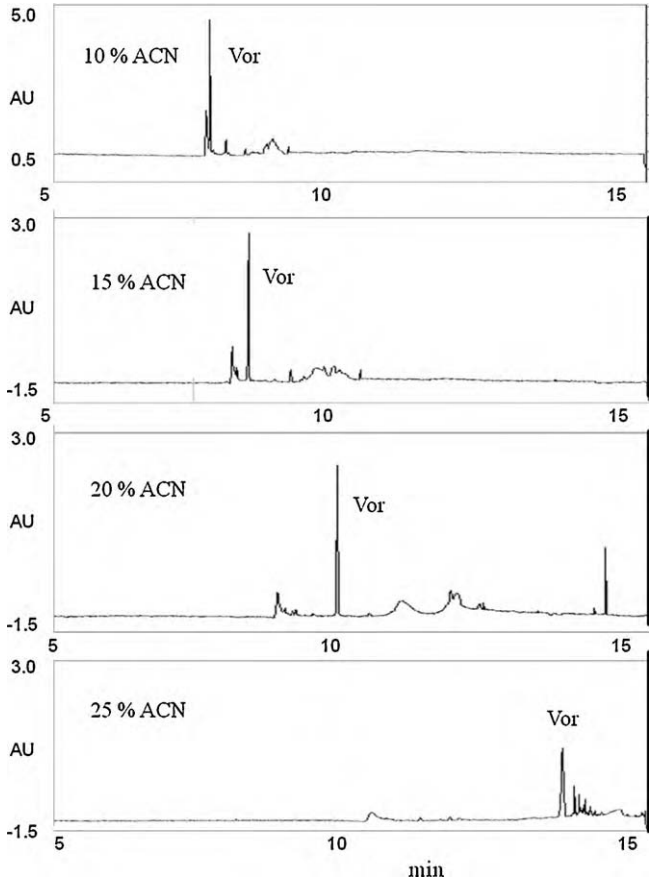


**Fig. 3.** Influence of ACN content on the separation of human plasma spiked with 2.5 μg mL$^{-1}$ of voriconazole (Vor). Sweeping-MEKC conditions were the same as those described in Fig. 2, with an SDS concentration of 110 mM.

were incorporated into the micellar phase and migrated with SDS toward the positive electrode. Because the peak intensity did not increase further at SDS concentrations of greater than 110 mM, we selected 110 mM as the optimal concentration to minimize Joule heating. Quirino et al. noted that the sample matrix can have a lower, similar, or higher conductivity than that of the separation solution in a sweeping system [30]. In this study, we used 75 mM phosphoric acid buffer as the sample solution to provide an acidic environment in which voriconazole would be protonated.

In general, a greater number of analyte molecules injected will result in a higher peak intensity. Nevertheless, overloading may cause peak broadening, without improved sensitivity, for analytes having limited values of *k*. When we increased the sample injection time (length) from 0.5 to 5 min, we found that the voriconazole peak intensity increased consistently over this range. Because we obtained the lowest quantitation limit (0.25 μg mL$^{-1}$) required for clinical application when the sample injection time was 3 min, this value was considered to be optimal. Although better detection sensitivity was obtained for increased injection times, the total analytical time also increased accordingly.

### 3.2.2. Optimization of the MEKC separation selectivity

An organic modifier changes the polarity of the original water phase, thereby affecting the retention of voriconazole in micelles and changing the separation selectivities. Voriconazole co-migrated with endogenous interference compounds when the BGE solution contained only SDS and phosphoric acid. Therefore, we tested the effects of various organic modifiers (MeOH, isopropanol, *n*-propanol, and ACN) added into the background solution on the separation selectivities. ACN provided the best selectivity improvement; therefore, we selected it to be the optimal organic modifier. We added different amounts of ACN (10–25%) into the BGE solution to determine the optimal percentage for voriconazole analysis. Fig. 3 reveals that when the ACN was present at 10%, the signal for voriconazole overlapped with that of interference compounds. Increasing the ACN content greatly improved the resolution of the signals for voriconazole and endogenous interference. Although voriconazole was baseline-separated from the endogenous interference when 15% ACN was added into the BGE solution, we further increased the ACN content to 20% to ensure good separation when applying this method to larger population. Therefore, we selected an ACN content of 20% as the optimal value.

The applied voltage plays a minor role on the MEKC selectivity. To determine the optimal voltage that provided good selectivity within the shortest analytical time, we tested applied voltages in the range from −20 to −25 kV. Because the ACN content was relatively high to facilitate the applicability of the developed method over a wider range, all of the tested voltages provided good selectivity. We obtained poor reproducibility of the analytical results, however, when the applied voltage was greater (i.e., more negative) than −24 kV, due to the high electric current. Therefore, we chose an applied voltage of −23 kV to obtain reproducible and rapid separations.

Fig. 4 presents the electropherogram obtained under the optimal separation conditions: a BGE solution comprising 40 mM phosphoric acid, 110 mM SDS, and 20% ACN; an applied voltage of −23 kV; and hydrodynamic sample injection at 50 mbar for 3 min. Under these conditions, voriconazole separated well from the endogenous matter in the plasma within 10.5 min. Relative to the results obtained using the conventional MEKC mode, the sweeping enhancement factor obtained under the optimal sweeping-MEKC conditions was 32.

### 3.3. Analytical method validation

Because of their structural similarity to voriconazole, fluconazole and itraconazole have been investigated for their suitability to be used as an internal standard. Under optimal sweeping-MEKC conditions, the peak shape of fluconazole was not satisfactory, and the peak of itraconazole overlapped with endogenous components. Although adding higher amounts of organic modifier to the background electrolyte will improve the peak shape and selectivity, the analytical time as well as system robustness will be sacrificed. Due

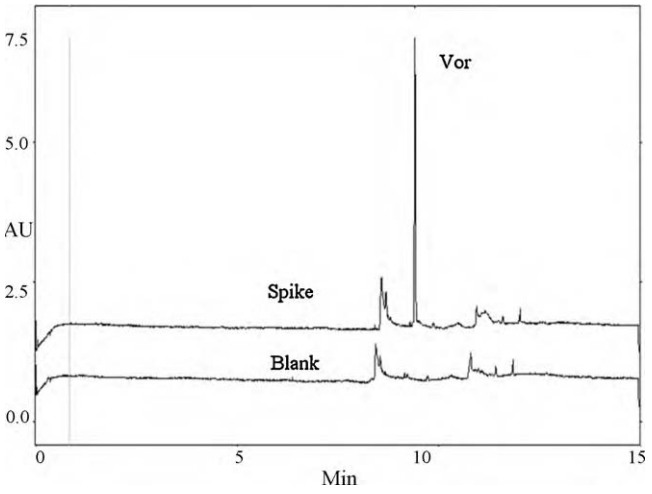


**Fig. 4.** Electropherograms of (A) blank and (B) voriconazole (Vor)-spiked plasma samples, recorded under the optimal sweeping-MEKC conditions: BGE, 40 mM phosphoric acid, 110 mM SDS, and 20% ACN. Other conditions were the same as those described in Fig. 2.

**Table 1**
Precision and accuracy of voriconazole determination in human plasma.

| | Voriconazole | | |
|---|---|---|---|
| | 0.25 ($\mu g\,mL^{-1}$) | 5 ($\mu g\,mL^{-1}$) | 15 ($\mu g\,mL^{-1}$) |
| Repeatability ($n=6$) % | | | |
| MT[a] | 1.5 | 0.7 | 2.8 |
| PA[b] | 5.5 | 2.9 | 3.9 |
| Reproducibility ($n=3$) % | | | |
| MT[a] | 2.6 | 2.3 | 4.1 |
| PA[b] | 5.5 | 6.3 | 4.5 |
| Accuracy (%) | 104 ± 2 | 107 ± 4 | 97 ± 4 |

[a] Migration time.
[b] Peak area.

to the large injection volume in the sweeping mode, the injection error frequently encountered in CE is very small in our study. Satisfactory precision could be obtained without the addition of the internal standard in the quantification process. Beside, the recovery of the SPE method was high. Therefore, we did not use internal standard in this study.

Koki et al. found that patients having a voriconazole plasma concentration of greater than 2 $\mu g\,mL^{-1}$ experienced better treatment outcomes; nevertheless, elevated levels of hepatic enzyme were frequently observed when the voriconazole concentration was greater than 6 $\mu g\,mL^{-1}$. Because elevation of hepatic enzyme might result in hepatic toxicity, it is recommended that the therapeutic range of voriconazole be executed and targeted within 2–6 $\mu g\,mL^{-1}$ to ensure treatment efficacy and avoid side effects [31]. Therefore, we performed our method validation within this concentration range.

### 3.3.1. Linearity

We tested the linearity of our method in the range from 0.25 to 15 $\mu g\,mL^{-1}$ necessary to satisfy clinical needs. Aliquots of the voriconazole working solution were added into blank human plasma to obtain samples spiked with 0.25, 1, 2.5, 5, 10, and 15 $\mu g\,mL^{-1}$ of voriconazole. The spiked plasma samples were subjected to SPE pretreatment and sweeping-MEKC analysis. We obtained a calibration curve by plotting the peak area of voriconazole ($y$) against the voriconazole concentration ($x$); the regression equation was

$$y = 2509.4x - 88.557$$

with a correlation coefficient of 0.999.

### 3.3.2. Precision and accuracy

To study the precision and accuracy, we added aliquots of voriconazole stock solution into blank human plasma to obtain plasma samples spiked with 0.25, 5, and 15 $\mu g\,mL^{-1}$ of voriconazole. The samples were analyzed six times consecutively on 1 day. The procedure was also repeated on three different days. The precision at each concentration level is expressed herein as the relative standard deviation (RSD) of the measurement results; the accuracy is expressed as the recovery of voriconazole in the tested samples.

Both the repeatability and the intermediate precision (inter-day reproducibility) of the determination of voriconazole, in terms of migration time, were less than 4.1% RSD. The repeatability and reproducibility of the peak area were both less than 6.3% RSD. The accuracy of our method was within 97 ± 4 and 107 ± 4%; Table 1 lists detailed data.

### 3.3.3. Limit of detection (LOD) and limit of quantification (LOQ)

We determined the LOD – the measured concentration at which the signal-to-noise (S/N) ratio was equal to 3 – to be 0.075 $\mu g\,mL^{-1}$, and the LOQ – the measured concentration at which the S/N ratio was equal to 10 – to be 0.25 $\mu g\,mL^{-1}$.

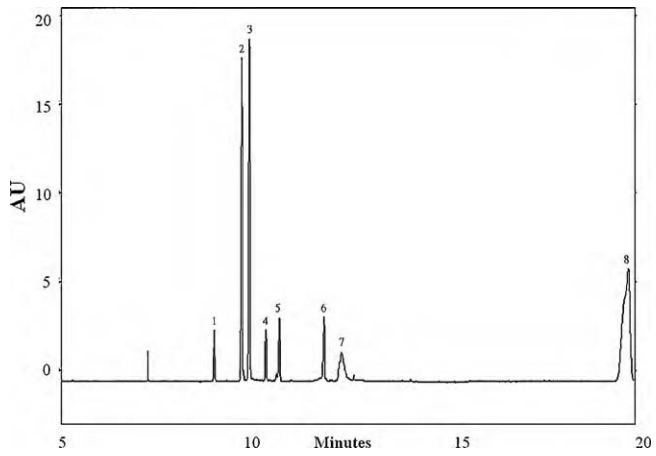


**Fig. 5.** Electropherogram of human plasma spiked with voriconazole and other co-medications. Sweeping-MEKC conditions were the same as those described in Fig. 4. Peaks: (1) ambroxol, (2) voriconazole, (3) levofloxacin, (4) methylprednisolon, (5, 8) co-trimoxazole (6) acetaminophen, and (7) vancomycin.

### 3.3.4. Selectivity and specificity

We tested the selectivity of the developed method by analyzing the plasma obtained from six healthy volunteers. We observed no overlapping of endogenous interference signals with the voriconazole peak. An investigation of the patients' drug list collected from our hospital revealed that the top ten drugs commonly used with voriconazole were acetaminophen, metoclopramide, furosemide, methylprednisolon, co-trimoxazole, imipenem and cilastin sodium, levofloxacin, vancomycin, ambroxol, and allopurinol. Fig. 5 displays the electropherogram obtained after spiking voriconazole with these co-medications in blank plasma. Notably, the co-medications did not interfere with voriconazole under the optimized conditions.

## 3.4. Performance of sweeping-MEKC method

Several bioanalytical methods have been developed to analyze voriconazole [11–14,16–19,32,33]. Table 2 listed the performance of these methods. LOD of the sweeping-MEKC method is 0.075 $\mu g\,mL^{-1}$. The sensitivity of the sweeping-MEKC method is superior to some LC–UV methods, and comparable to the LC–MS and LC–fluorescence methods. The precision of the sweeping-MEKC method in terms of the peak area is less than 6.3% RSD. It is superior to most of the LC methods. Compared with the LC-based method, sweeping-MEKC consumes greatly reduced amounts of organic solvent—a major advantage when performing routine TDM studies. Also, LC–MS instrument is much more expensive than CE. Therefore, the developed method is suitable to be used as TDM method for voriconazole.

## 3.5. Determination of voriconazole in patient plasma

A total of 16 plasma samples collected from patients receiving voriconazole treatment were analyzed using the validated sweeping-MEKC method and an HPLC-based method, which we performed according to the method proposed by Pennick et al., with slight modification [12]. Our developed sweeping-MEKC method was successfully applied to analyze these samples without encountering any problems from plasma interference. Fig. 6A and B display representative sweeping-MEKC electropherograms of a patient's plasma samples collected at the peak and trough

**Table 2**
Comparison of analytical performance of voriconazole bioanalytical methods.

| Method | Calibration range ($\mu g\,mL^{-1}$) | LOD ($\mu g\,mL^{-1}$) | LOQ ($\mu g\,mL^{-1}$) | Precision (% RSD) | References |
|---|---|---|---|---|---|
| CE | 0.25–15 | 0.075 | 0.25 | Intra-day: <5.5% Inter-day: <6.3% | Current method |
| LC–UV | 0.1–8 | 0.03 | 0.1 | Intra-day: <9.3% Inter-day: <6.6% | [11] |
| LC–UV | 0.2–10 | NA[a] | 0.2 | Intra-day: <15.7%Inter-day: <16.0% | [12] |
| LC–UV | 0.2–10 | NA[a] | 0.2 | Intra-day: <10.4%Inter-day: <10.1% | [13] |
| LC–UV | 0.05–40 | 0.02 | 0.05 | Intra-day: <8.4% Inter-day: <10.8% | [14] |
| LC–UV | 0.4–10 | 0.3 | 0.4 | Intra-day: 2.8% Inter-day: NA | [32] |
| LC–UV | 0.2–10 | NA[a] | 0.2 | Intra-day: <2.5% Inter-day: <4% | [33] |
| LC–MS | 0.38–20 | 0.01 | 0.1 | Intra-day: <4.4% Inter-day: <7.2% | [16] |
| LC–MS | 0.05–5.0 | 0.03 | 0.05 | Intra-day: <9.9% Inter-day: NA | [17] |
| LC–MS | 0.1–10 | NA[a] | 0.1 | Intra-day: <7.8% Inter-day: <3.1% | [18] |
| LC–fluorescence | 0.1–10 | 0.044 | 0.1 | Intra-day: <9.08%Inter-day: <4.41% | [19] |

[a] Not available.

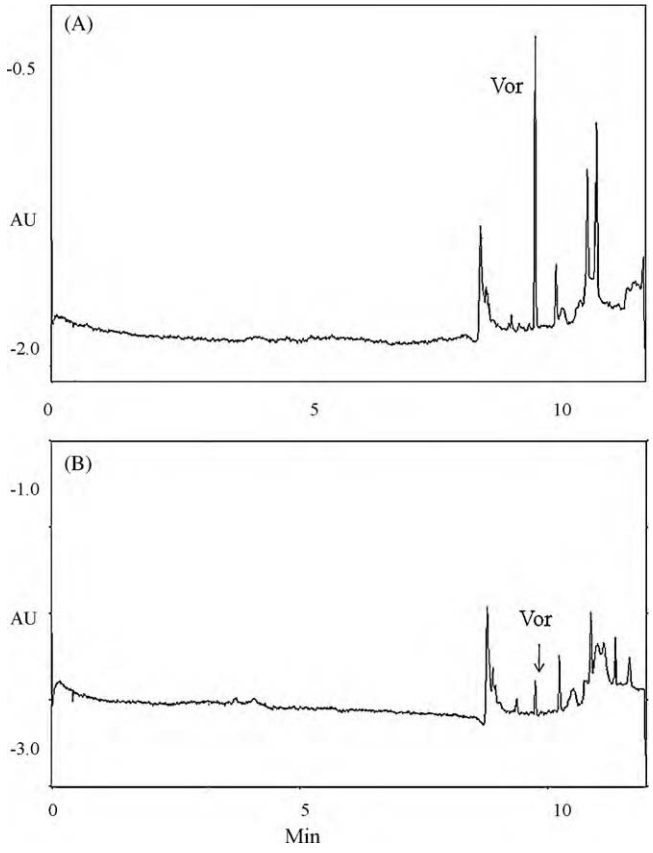


**Fig. 6.** Electropherograms of plasma samples obtained from patients undergoing voriconazole (Vor) treatment. Plasma samples were collected (A) 2 h after oral administration and (B) just before the administration of the next dosage. Sweeping-MEKC conditions were the same as those described in Fig. 4.

time points, respectively. They reveal that voriconazole was well separated from the endogenous interference, with satisfactory peak intensity, at concentrations of clinical interest. The correlation between the sweeping-MEKC and HPLC-based analytical results was 0.97; i.e., good correlation existed between these two methods.

## 4. Conclusion

IFIs are a significant cause of morbidity and mortality in hospitalized patients. Over the past two decades, the incidence of fungal infections has increased dramatically. Voriconazole is a potent antifungal agent used in the treatment of serious IFIs. In this study, we developed an efficient and accurate sweeping-MEKC method for the determination of voriconazole in patient plasma. Under the optimal analytical conditions, voriconazole was determined within 10.5 min with an LOD of 0.075 $\mu g\,mL^{-1}$. Compared with the traditional MEKC method, our proposed sweeping-MEKC method provides a sensitivity improvement of more than 30-fold; it could also be applied successfully to clinical samples. The quantification results obtained using the sweeping-MEKC and HPLC-based analytical methods were in good agreement; notably, however, sweeping-MEKC consumes much lower amounts of organic solvent. We anticipate that our developed sweeping-MEKC method could be applied to routine TDM studies of voriconazole to improve the treatment outcomes of IFIs.

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