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Rapid and sensitive determination of posaconazole in patient plasma by capillary electrophoresis with field-amplified sample stacking

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ABSTRACT

The high morbidity and mortality associated with invasive fungal infections have increased the importance of improving treatment efficacy. Posaconazole is a novel agent with strong antifungal activity and low toxicity. The success of posaconazole pharmacotherapy strongly depends on precise controlling of concentration of the drug in the blood. In the present study, a solid phase extraction-capillary electrophoresis (SPE-CE) method was developed for rapid and accurate determination of posaconazole in the plasma of patients. We used the field-amplified sample stacking (FASS) technique to improve the sensitivity of CE, and applied the SPE procedures to reduce the matrix effect that was frequently encountered by biological samples in FASS system. Effect of filter types on the recovery rate of compounds with high lipophilicity was carefully investigated. Parameters affecting FASS performance were all optimized to obtain the best sensitivity with the highest speed. When using 1.25 M formic acid as the background electrolyte and 0.2 M formic acid in 95% (v/v) methanol as the sample solution, the limit of detection (LOD) for posaconazole was 10 ng mL^{-1}, with an analytical run time of less than 5 min. The relative standard deviation (RSD) of the peak area ratios for repeatability (intra-day, $n = 6$) and intermediate precision (inter-day, $n = 3$) were lower than 7.2% and 7.5%, respectively. The accuracy was tested by recovery, and the recovery rates were within 95.1% and 106.4%, respectively. The successful application of the developed method demonstrated its feasibility as an effective method for clinical use.

1. Introduction

The incidence of invasive fungal infections (IFIs) has significantly increased over the past 20 years. IFIs are associated with significant patient mortality and health care costs. It was estimated that one-year survival rate for hematopoietic stem cell transplant patients (HSCT) was less than 20% among patients with IFIs [1]. The high mortality of IFIs is associated with ineffective antifungal therapy.

Posaconazole (chemical structure in Fig. 1) is a novel second-generation triazole agent, which has shown the strongest activity and the lowest toxicities among triazole antifungal agents [2–4]. It is a broad-spectrum antifungal drug that inhibits fungi, including *Fusarium*, *Candida*, *Aspergillus*, and *Zygomycetes* [5]. In addition, it can be used as a salvage therapy against difficult-to-treat fungal infections, such as chronic granulomatous disease (CGD), or used as prophylaxis for patients with prolonged neutropenia and HSCT recipients with graft-versus-host disease [6–9].

Several studies on the concentration of posaconazole in patient plasma revealed relatively high intra- and inter-individual differences in this drug [10]. The posaconazole concentration in different individuals ranges from 50 ng mL^{-1} to higher than 5000 ng mL^{-1} [8,11,12]. In order to ensure its treatment efficacy, the Food and Drug Administration (FDA) recommends that the posaconazole concentration in patient plasma should be above 700 ng mL^{-1} to work effectively [13]. Bioavailability of posaconazole is strongly associated with food intake [14–16]. In addition, drug–drug interactions have been demonstrated to decrease the concentration of posaconazole in plasma [10,17]. Therefore, it is highly recommended to monitor posaconazole plasma concentrations to optimize pharmacotherapy [18–20]. The high mortality rate of IFIs, and the importance of controlling posaconazole concentration in IFIs show the necessity of reliable and efficient bioanalytical methods to measure posoconazole concentration in patients.

Several methods have been developed to determinate posaconazole concentration [21]. High-performance liquid chromatography with UV detection (HPLC-UV) is widely used for measurements of posaconazole plasma concentrations [22–24].

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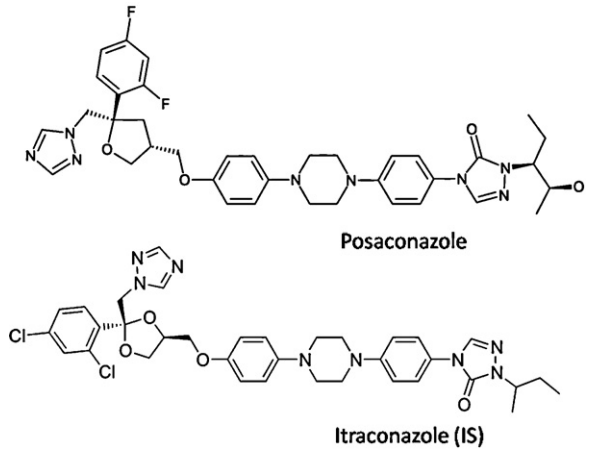

Fig. 1. Structure of posaconazole and itraconazole (internal standard).

One study applied HPLC with a laser-induced fluorescence (HPLC-LIF) detector to analyze posaconazole [25]. Another study applied HPLC with a single MS (HPLC–MS) detector to analyze posaconazole [26]. Compared to HPLC-UV and HPLC-LIF, liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) provides better sensitivity and specificity. In recent years, several studies have used LC–MS/MS for posaconazole quantification [27–30]. Capillary electrophoresis (CE) has been considered as an efficient and cost-effective method for the analysis of small molecules and biopolymers because of minimal sample and reagent consumption, fast separation speed, and high theoretical plate numbers. Its low organic solvent consumption also makes it a green chemistry technique. Until now, there has been no CE method developed for measuring posaconazole concentrations.

Due to the small internal diameter of the capillary and the small injection amount, detection sensitivity is the major drawback of CE, especially when coupled with UV detector. Two general approaches have been adopted to improve sensitivity. One method uses more sensitive detectors, such as fluorescence, electrochemical or mass spectrometry detectors, and the other employs on-line concentration strategies, such as stacking and sweeping. Stacking is a phenomenon through which sample ions accumulate at the boundary, which separates the low conductivity sample plug and the high conductivity background electrolytes (BGEs). Field-amplified sample stacking (FASS) has been applied to the analysis of DNA fragments, pharmaceuticals and drugs of abuse with up to a 1000-fold sensitivity enhancement [31].

The aim of this study was to develop a fast, accurate and sensitive CE method for the quantification of posaconazole in patient plasma. We used FASS technique to improve detection sensitivity. Our previous study revealed that the matrix effect was severe when using FASS technique to analyze complicated samples [32]. To reduce the matrix effect and improve the quantification accuracy for biological samples in FASS system, a solid phase extraction (SPE) method was applied to sample clean up. Parameters affecting stacking and SPE performance were optimized to achieve the best sensitivity with the highest speed. The developed FASS method was validated and then applied to analyze posaconazole concentrations in a patient who received posaconazole treatment.

2. Experimental

2.1. Chemicals and materials

The posaconazole standard was provided by Schering-Plough (Kenilworth, NJ, USA). The itraconazole standard and formic acid were purchased from Sigma (St. Louis, MO, USA). Acetonitrile (ACN) was purchased from Merck (Darmstadt, Germany). Methanol (MeOH) was purchased from Mallinckrodt (Paris, KY, USA). Urea was purchased from J.T. Bakers, Inc. (Phillipsburg, NJ, USA). All reagents and solvents used were of analytical or chromatographic grade. Plasma samples obtained from healthy volunteers were used as blanks. The plasma samples were frozen and stored at −80 °C before use. Oasis HLB SPE cartridges were purchased from Waters (Milford, MA, USA). The SPE procedure was performed on a Waters extraction manifold system.

2.2. Instrumentation

The capillary electrophoresis experiments were performed on a Beckman P/ACE MDQ capillary electrophoresis system equipped with a photodiode array detector (Beckman Coulter, Fullerton, CA, USA). The separations were performed using a 40-cm (30-cm effective length) × 50 μm ID fused-silica capillary from Polymicro Technologies (Phoenix, AZ, USA). The electropherograms were recorded using an EZChrom (Scientific Software, San Ramon, CA, USA) chromatographic data system.

2.3. Preparation of stock and working solutions

Posaconazole and itraconazole stock solutions, both with concentrations of 100 μg mL^{-1}, were prepared in MeOH. Blood samples collected from healthy volunteers were centrifuged (3000 rcf, 15 min) to obtain blank plasma samples. The blank samples were spiked with the posaconazole stock solution to obtain 1 μg mL^{-1} posaconazole-spiked plasma samples. Itraconazole (1 μg mL^{-1} in the plasma) was used as an internal standard. All of the solutions were filtered through 0.45-μm cellulose filters (Minisart RC 4, Sartorius, Germany) before use.

2.4. Sample preparation

Oasis HLB cartridges (1 mL; 30 mg) were used for sample extractions. Cartridges were conditioned with 1 mL MeOH and 1 mL water prior to use. Urea (10 M, 0.6 mL) was added to 0.6 mL serum sample and vortex-mixed for 1 min to ensure protein denaturation. The resulting solution (1.2 mL) was loaded into the pre-conditioned SPE cartridge, and the flow rate was at 1.2 mL/min. After the sample solution was thoroughly drained, the cartridge was washed with 1 mL DI water and 1 mL 80% MeOH. Posaconazole was then eluted with 1 mL acetonitrile. The eluent was evaporated to dryness under nitrogen gas and then reconstituted in 0.3 mL of 0.2 M formic acid in 95% MeOH. The reconstituted solution was filtered by a cellulose filter and subjected to CE analysis.

2.5. Separation conditions

The new silica capillaries (50-μm ID) were first flushed with 1.0 M NaOH for 10 min and then with DI water for 10 min. Before each run, the capillary was washed with MeOH for 2 min, 0.2 M NaOH for 2 min, DI water for 2 min and running buffer for 3 min in sequence to ensure reproducibility.

2.5.1. FASS

The FASS was performed on a 40-cm (30-cm effective length) × 50-μm ID fused-silica capillary. The BGE solution was composed of 1.25 M formic acid. The posaconazole sample solution was electrokinetically injected into the capillary at 8 kV for 48 s. The separation voltage was set at 25 kV to prevent joule heating. The detection wavelength was set to 195 nm, and the capillary was thermostatically controlled by a coolant at 25 °C.

2.5.2. Conventional capillary zone electrophoresis (CZE)

The posaconazole solution (10 μg mL^{-1}) was prepared in a buffer composed of 1.25 M formic acid and 20% MeOH (provide posaconazole solubility). The BGE solution was composed of 1.25 M formic acid. The sample was hydrodynamically injected at 0.7 psi for 5 s, and the separation voltage was set at 25 kV. Before each run, the capillary was flushed in sequence with 0.2 M NaOH for 2 min, DI water for 2 min and running buffer for 3 min.

2.6. Validation

2.6.1. Linearity

Aliquots of the posaconazole stock solution were added to blank human plasma to obtain 30, 50, 100, 500, 800, 1000, 3000, 5000 and 10,000 ng mL^{-1} posaconazole-spiked solutions. The five posaconazole-spiked solutions with lower concentrations, 30, 50, 100, 500 and 800 ng mL^{-1}, were used to establish a calibration curve to quantify posaconazole plasma concentrations lower than 800 ng mL^{-1}. The other five posaconazole-spiked solutions, 800, 1000, 3000, 5000 and 10,000 ng mL^{-1}, were utilized to establish a calibration curve to quantify posaconazole plasma concentrations higher than 800 ng mL^{-1}. Linear regression lines were obtained by plotting peak area ratios against posaconazole concentrations.

2.6.2. Precision, accuracy and extraction recovery

Precision and accuracy were tested at 30, 100, 800, 3000 and 10,000 ng mL^{-1} three times a day for 3 days. The accuracy of the method was studied by spiking posaconazole at 30, 100, 800, 3000 and 10,000 ng mL^{-1} in blank plasma, and the recoveries of posaconazole were calculated by the two calibration curves constructed in Section 2.6.1.

The extraction recovery was tested by comparing the peak area of samples pre-spiked with posaconazole (posaconazole was spiked before SPE extraction) and samples post-spiked with posaconazole (posaconazole was spiked after SPE extraction of blank plasma).

2.7. Drug administration and sample collection

Posaconazole was orally administered to patients in the National Taiwan University Hospital. The local ethics committee approved this study, and signed inform consents were received from the patients that participated. Blood samples were collected in EDTA-containing tubes 5 h after the oral administration of 400 mg posaconazole to obtain the trough concentration. The blood samples were centrifuged, and the plasma samples were stored at −80 °C until use.

3. Results and discussion

3.1. Optimization of sample preparation method

3.1.1. Deproteinization method

Posaconazole is a highly hydrophobic drug with a protein-binding rate greater than 98%. The most frequently used additive to denature serum protein when measuring posaconazole is acetonitrile (ACN) [25,28]. Although ACN provided a satisfactory extraction recovery (higher than 90%), the resulting solution showed high elution strength and could not be directly loaded into SPE cartridges. Compared to the ACN denaturation method, the urea denaturation method provided a similar recovery rate but with low elution strength. The urea-deproteinized solution could be directly loaded into SPE cartridges without any dilution steps; therefore, it was chosen as the protein-denaturing agent in this study.

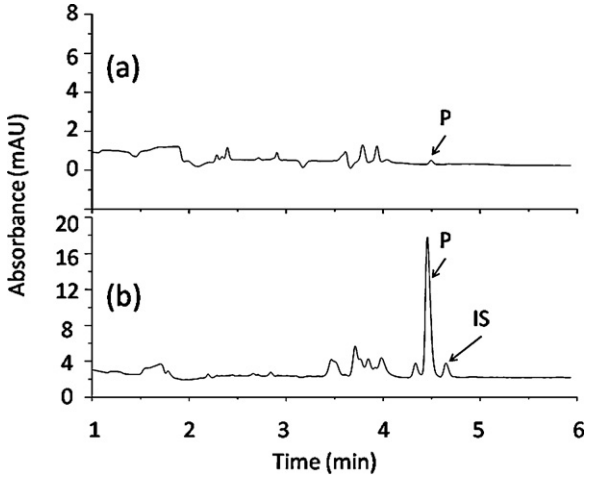

Fig. 2. Electropherograms of plasma samples spiked with 10 μg mL^{-1} posaconazole and 1 μg mL^{-1} itraconazole recorded under the optimal FASS conditions (a) without SPE pretreatment and (b) with SPE pretreatment. FASS separation conditions: background electrolyte, 1.25 M formic acid; sample solution, 0.2 M formic acid in 95% MeOH; separation voltage, +25 kV; separation temperature, 25 °C; sample injection, +8 kV for 0.8 min; fused-silica capillary, 40 cm × 50 μm ID, 30 cm effective length. P: posaconazole; IS: internal standard (itraconazole).

3.1.2. Solid phase extraction (SPE) procedures

We applied the SPE procedures to reduce the matrix effect that was frequently encountered by biological samples in FASS system. In the stacking mode of the online preconcentration method, the amount of endogenous materials in the sample matrix significantly affected the stacking efficiency. In addition, ions in the plasma samples will introduce an injection bias during electrokinetic injection in the FASS analysis and hamper the quantification accuracy in the clinical assay. As displayed in Fig. 2a, the posaconazole peak was very small if SPE procedure was not applied to clean up the plasma sample. The OASIS HLB cartridge was chosen as the extraction cartridge in this study. Two wash steps were employed in the SPE procedures. After sample loading, the salts were washed out with DI water followed by a MeOH solution to eliminate plasma interferences. Posaconazole is a highly hydrophobic compound ($\log P > 3$), and it can be well retained by the reversed phase extraction sorbent. We optimized the MeOH percentage of the wash solution, and an 80% MeOH solution was selected as the optimum wash solution. The extraction recovery of the SPE method was 96.75 ± 3.02% ($n = 3$). Fig. 2b shows the performance of the optimum SPE method. Although the concentration factor for plasma samples in Fig. 2a and b in sample preprocessing step is the same, the signal intensity of posaconazole significantly increased after SPE pretreatment.

3.1.3. Sample filtration

It should be noted that filter types for sample filtration affected posaconazole recovery to a great extent. The filtration step is conducted after the redissolving step to ensure no particles were injected into the capillary. Although very few studies discussed the effect of filter types on sample recovery, we found that this parameter showed a significant effect on sample recovery for highly hydrophobic compounds, such as posaconazole, in this study. Typical materials for syringe filters are nylon, polyvinylidene difluoride (PVDF), Teflon (PTFE), polypropylene (PP) and cellulose derivative membranes; these materials were tested for posaconazole recovery. We found that the posaconazole and itraconazole (internal standard) recovery was lower than 40% if we use hydrophobic filters or unmodified filters, such as nylon and PTFE. It is probably due to sample adsorption. Conversely, hydrophilic cellulose derivative material provided a much better recovery (>90%). In addition, the percentage of organic solvent in the sample solution during

sample filtration also influenced the posaconazole recovery to a great extent in that a higher percentage of organic solvent in the sample solution led to a higher sample recovery. In this study, 0.2 M formic acid in 95% MeOH was chosen as the sample solvent. The high percentage of MeOH in the sample solvent improved the recovery of posaconazole during sample filtration and also increased the difference in conductivity between the sample zone and the BGEs.

3.2. Analytical method development

Previous studies have revealed that the posaconazole concentrations in different patients could vary from 50 ng mL^{-1} to higher than 5000 ng mL^{-1}. In this study, conventional capillary zone electrophoresis (CZE) was tested for posaconazole analysis at first, but the sensitivity could not satisfy clinical need. FASS technique was therefore adapted to improve the detection sensitivity for plasma posaconazole. Posaconazole and itraconazole standard solution and drugs-spiked plasma sample were used to optimize the FASS method. Key parameters that affected the stacking efficiency included the composition of the BGEs and the sample matrix, the sample injection time, and the applied voltage. These parameters were optimized to achieve the highest sensitivity in the FASS system. The limits of detection (LOD) at a signal-to-noise ratio equal to three and the enhancement factor (EF) were determined to show the concentration efficiencies of FASS.

3.2.1. Effect of the sample matrix and the separation buffer

To ensure that the posaconazole was in its protonated form (pKa 3.6 and 4.6) [16,33], an acidic buffer was used as the sample solution and BGE. The buffer ranges of formic acid, phosphoric acid and citric acid are all lower than the pKa value of posaconazole, and these acids were evaluated for their suitability as the buffer system for FASS. Citric acid showed a relatively high UV cut-off value, which would sacrifice detection sensitivity, and phosphoric acid generated a higher current, which would generate joule heat and lead to an unstable system at high concentration. Considering these issues, formic acid was chosen as the analytical buffer in this study.

Theoretically, the stacking efficiency is proportional to the conductivity difference between the sample solution and BGE because of the much higher electric field being distributed in the sample zone. Formic acid at concentrations of 0.75 M, 1.0 M, 1.25 M and 1.5 M was tested as the BGE solution, and the results are displayed in Fig. 3a. When the concentration of formic acid was higher than 1.0 M, the posaconazole peak intensity reached its maximum. The resolution between posaconazole and the internal standard, itraconazole, continuously increased with increasing formic acid concentrations in all tested ranges. When formic acid was at concentration of 1.5 M, the electric current was higher than 100 μA, and the system became unstable. The method's precision was also sacrificed under high current. Therefore, 1.25 M formic acid was chosen as the optimum buffer concentration in the BGE.

For basic compound analysis in stacking mode, a small amount of acid is recommended to be added into the sample matrix to enhance compound protonation and to improve reproducibility of the method [32]. When the formic acid concentration in the sample matrix was varied from 0.05 M to 0.3 M, the increase in formic acid concentration resulted in an increase of the peak intensity. When the concentration of formic acid in the sample matrix was lower than 0.2 M, the results were irreproducible because of the incomplete protonation of posaconazole especially when analyzing posaconazole spiked plasma samples. As the formic acid concentration increased, number of theoretical plates decreased because of the decreasing conductivity differences between the sample solution and BGE (Fig. 3b). Considering peak intensity, reproducibility and peak efficiency, 0.2 M of formic acid was chosen as the optimum concentration in the sample matrix.

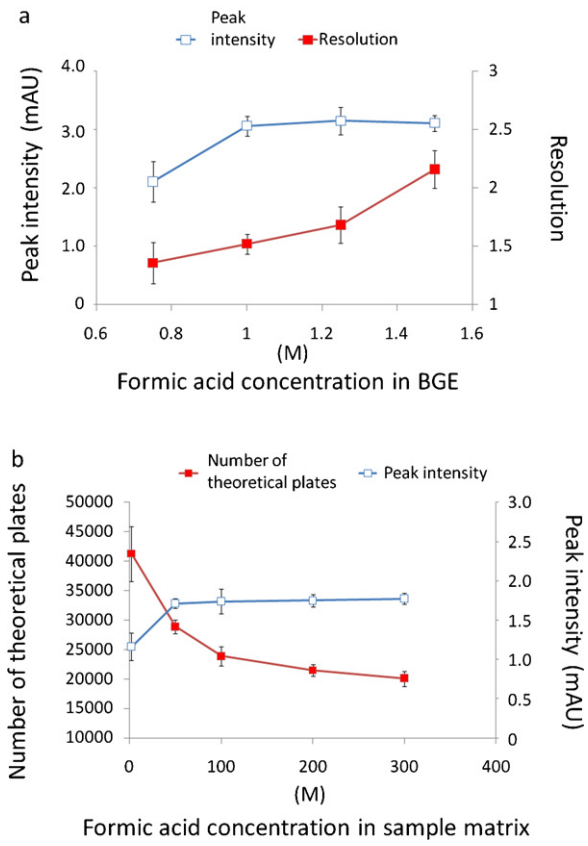

Fig. 3. (a) Effect of the formic acid concentration in the BGE on peak intensity and resolution of posaconazole and itraconazole (n = 3). Separation conditions are the same as indicated in Fig. 2, except formic acid concentration in BGE. (b) Influence of formic acid concentration in the sample matrix on peak intensity and theoretical plate number of posaconazole (n = 3). Separation conditions are the same as indicated in Fig. 2, except formic acid concentration in sample matrix.

The stacking efficiency has been shown to increase by adding water-miscible organic solvents into the sample matrix to enlarge the conductivity difference between the BGE and the sample plug [32]. Different concentrations (50–98%) of MeOH were added to the sample matrix to test its effect on peak intensity. As shown in Fig. 4, adding MeOH to the sample matrix significantly increased the peak intensity and peak area. This is because larger amount of posaconazole ions were injected into the capillary. This phenomenon is

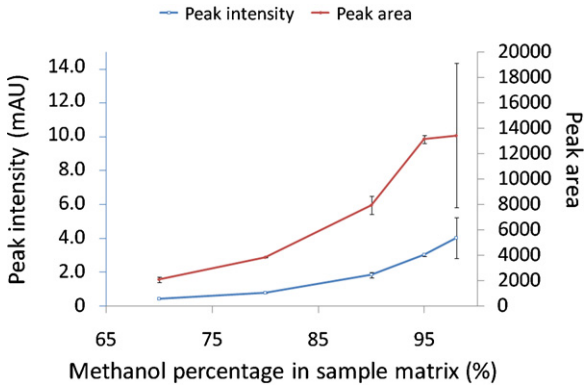

Fig. 4. The effect of MeOH percentage in the sample matrix on peak intensity and peak area of posaconazole (n = 3). Separation conditions are the same as indicated in Fig. 2, except MeOH percentage in sample matrix.

assumed to be caused by larger electric field being distributed in the sample matrix with increasing methanol percentage. Since the total length and the applied voltage did not change, the total electric field of the FASS system remained unchanged when changing the methanol percentage. Therefore, the electric field difference between the sample matrix and the background electrolyte is increased when adding higher percentage of methanol into the sample matrix. As posaconazole ions moved faster under the high electric field, more posaconazole ions were electrokinetically injected into the capillary. As a result, the peak area along with the peak intensity increased when the methanol percentage in the sample matrix was increased. When the percentage of MeOH was higher than 95%, the analytical results were irreproducible because of the decrease in protonation of posaconazole. Therefore, 95% MeOH was selected as the optimum percentage in the sample matrix.

3.2.2. Effect of the injection voltage and injection time

Theoretically, the injection voltage should be increased to reduce the injection time, assuming similar amount of sample is injected. The injection voltage was tested within 2–10 kV. Due to the high conductivity difference between the sample matrix and the separation buffer, excessive joule heat in the narrow part of the injection end could embrittle the capillary when the applied voltage was higher than 8 kV. Therefore, 8 kV was selected as the injection voltage. When a sample was injected into the capillary, the electric current remained constant for a while then gradually decreased. After the current reached 70% of the maximum current (approximately 48 s), the injection voltage was shut down. A longer injection time would lead to irreproducible results and even current disruption.

The separation voltage played a minor role on the separation result. To avoid too much joule heat, which can cause peak broadening and an irreproducible separation, the separation voltage was set at 25 kV. The electropherogram obtained under optimum separation conditions is displayed in Fig. 6. When using 1.25 M formic acid as the BGE and 0.2 M formic acid in 95% (v/v) MeOH as the sample solution, the limit of detection (LOD) for posaconazole was 10 ng mL^{-1}, with an analytical run time less than 5 min. Compared to the conventional CZE method, the FASS method improved the sensitivity by over 170-fold for the posaconazole analysis.

3.3. Method validation

To satisfy the clinical requirement, the developed method was validated within concentrations ranging from 30 to 10,000 ng mL^{-1}. Itraconazole was used as the internal standard to improve the precision and accuracy of the method.

3.3.1. Linearity

The linearity of the method was tested within 30–10,000 ng mL^{-1}. To obtain a better quantification accuracy at low concentrations, two calibration curves were generated (Fig. 5). The calibration curves were $y = 0.9108x + 0.1515$ ($r^2 = 0.9998$) for posaconazole concentrations in the range of 30–800 ng mL^{-1}, and $y = 1.0090x + 0.0106$ ($r^2 = 0.9998$) for posaconazole concentrations in the range of 800–10,000 ng mL^{-1}.

3.3.2. Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) was determined as the concentration when the signal-to-noise ratio (S/N) equaled 3. The limit of quantification (LOQ) was determined as the concentration when the signal-to-noise ratio (S/N) equaled 10. Under the optimized conditions, the LOD and LOQ of the posaconazole concentrations were 10 ng mL^{-1} and 30 ng mL^{-1}, respectively.

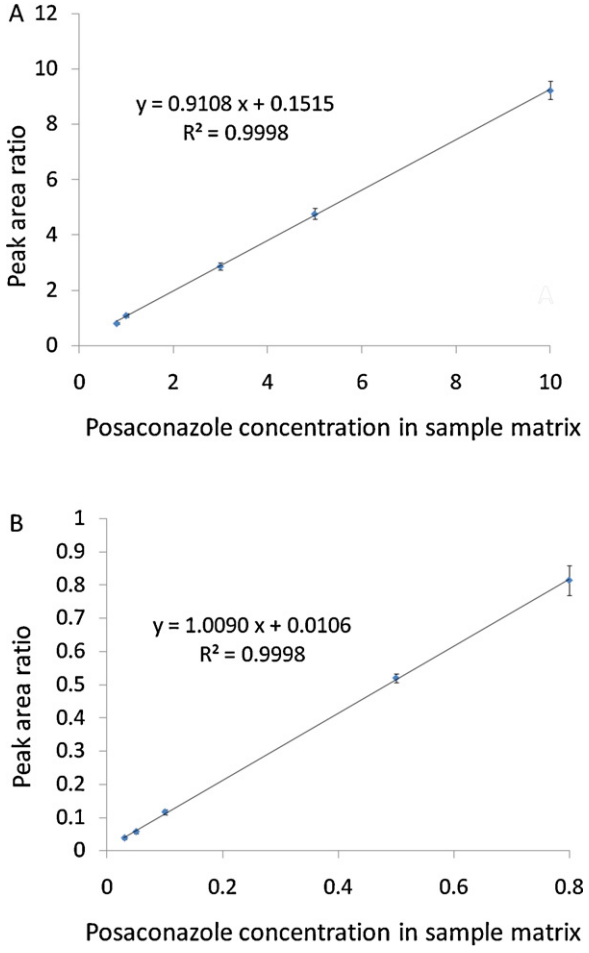

Fig. 5. Calibration curves of posaconazole ($n = 5$). Separation conditions are the same as indicated in Fig. 2.

3.3.3. Precision and accuracy

Run-to-run repeatability (intra-day, $n = 6$) and intermediate precision (inter-day, $n = 3$) of the migration time and peak area ratios of posaconazole to itraconazole were tested. In terms of migration time, both repeatability and intermediate precision of

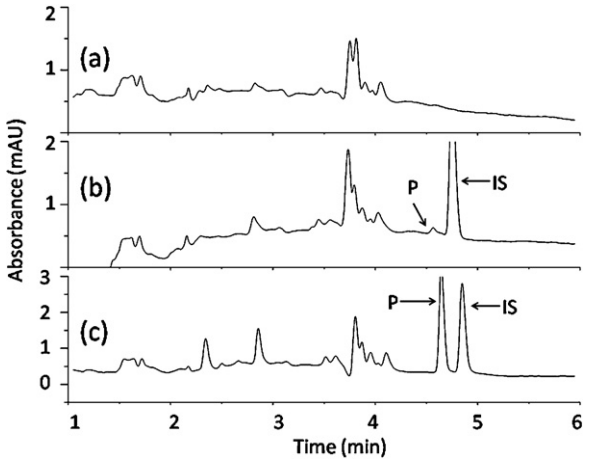

Fig. 6. CE electropherograms of (A) blank human plasma, (B) blank human plasma spiked with 30 ng mL^{-1} posaconazole (LOQ), and (C) blank human plasma spiked with 1000 ng mL^{-1} posaconazole, obtained under optimal SPE-FASS conditions. Separation conditions are the same as indicated in Fig. 2. P: posaconazole; IS: internal standard (itraconazole).

Table 1
Precision and accuracy of the SPE-FASS method.

	Posaconazole spiked concentration (ng mL^{-1})				
	10,000	3000	800	100	30[a]
Intra-day precision (n = 6) (RSD (%))	2.4	5.3	4.5	7.2	15.6
Inter-day precision (n = 3) (RSD (%))	6.7	4.6	5.7	7.5	17.5
Accuracy (n = 6) (mean recovery (%))	106.4	100.7	95.1	98.2	93.8

[a] Limit of quantification.

posaconazole were within a 3.2% relative standard deviation (RSD). The repeatability (intra-day, n = 6) and intermediate precision (inter-day, n = 3) of peak area ratios were tested at 30, 100, 800, 3000 and 10,000 ng mL^{-1}. The repeatability (n = 6) and intermediate precision (n = 3) at the tested ranges except LOQ, were within 7.2% and 7.5% RSD, respectively. The accuracy of the method was studied by spiking posaconazole into blank plasma samples. The recoveries, except LOQ, were within 95.1% and 106.4% (n = 6). The method precision and accuracy were also tested at LOQ (30 ng mL^{-1}). Both intraday precision and intermediate precision at the LOQ were lower than 17.5% RSD. The recovery of posaconazole at LOQ was 93.8% (n = 6) (Table 1).

3.4. Selectivity

The selectivity of the method was tested by analyzing blank plasma obtained from six healthy volunteers. There was no endogenous material found at the migration time of posaconazole and the internal standard for six tested samples.

3.5. Determination of posaconazole in patient plasma

Plasma samples collected from one patient receiving posaconazole treatment was analyzed using the validated FASS method. Fig. 7 displays the representative electropherogram, and the drug concentration was calculated to be 2.7 μg mL^{-1}. As displayed in Fig. 7, no endogenous interferences or co-medications overlapped peaks of posaconazole or the internal standard. The results revealed that the developed method could be used for therapeutic drug monitoring (TDM) for patients undergoing posaconazole treatment.

The high morbidity and mortality associated with invasive fungal infections have increased the importance of improving treatment efficacy. Therapeutic drug monitoring of posaconazole has been proven to improve the outcome of treatment. Several methods have been developed for the determination of posaconazole concentrations in patient plasma. HPLC-UV is the most frequently used method to measure posaconazole concentrations. The LOQs of the HPLC-UV method were in the range of 50–620 ng mL^{-1} [22–24]. One study applied the HPLC-LIF technique to improve the sensitivity of the method, and the LOQ was 100 ng mL^{-1} [25]. Another study applied the HPLC–MS technique for posaconazole quantification, and the LOQ was 31 ng mL^{-1} [26]. Several recent studies used LC–MS/MS method for posaconazole quantification. The LOQs of the LC–MS/MS method were in the range of 5–20 ng mL^{-1} [27–30]. As demonstrated in this study, the linear range of our developed FASS method was 30–10,000 ng mL^{-1}. The sensitivity of the current FASS method was superior to HPLC-UV or HPLC-LIF and comparable to HPLC–MS/MS method. The high sensitivity of the FASS method made it possible to analyze other noninvasive biofluids, such as urine and saliva, where posaconazole was found in low concentrations.

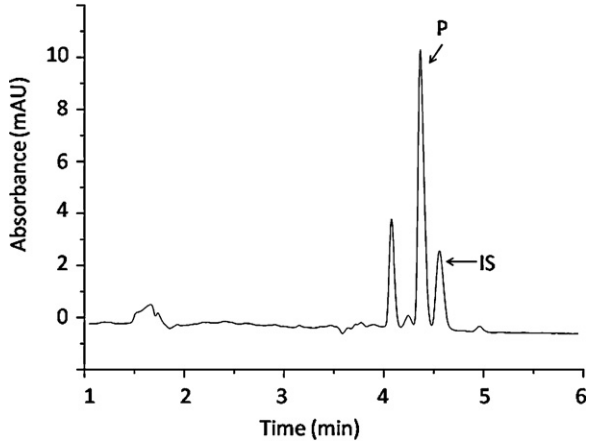

Fig. 7. Electropherogram of a plasma sample obtained from a patient undergoing posaconazole treatment. The posaconazole concentration in this patient was calculated to be 2.7 μg mL^{-1}. Separation conditions are the same as indicated in Fig. 2. P: posaconazole; IS: internal standard (itraconazole).

4. Conclusions

SPE-FASS analysis is an efficient, accurate and cost effective method to quantify posaconazole in human plasma. With the application of the optimized SPE procedures, the endogenous interferences and matrix effect in FASS system were greatly reduced. Due to the highly hydrophobic nature of posaconazole ($\log P > 3$), its recovery was found to be lower than 40% if hydrophobic filters or unmodified filters, such as nylon and PTFE, were used. The recovery was much higher with the use of a cellulose derivative filter (>90%).

To ensure a large injection amount during electrokinetic injection, 0.2 M formic acid was added to the sample matrix to maintain the posaconazole in the protonated state. A solution consisting of 95% MeOH was added to the sample matrix to increase the conductivity difference between the sample zone and the BGE. Compared to phosphoric acid, formic acid is relatively rarely used as the BGE in FASS, especially when CE is coupled with a UV detector instead of a mass spectrometer. In this study, formic acid was found to provide better system stability under high buffer concentrations, which is a big advantage when using the FASS method. With the use of 1.25 M formic acid as the BGE and 0.2 M formic acid in 95% (v/v) MeOH as the sample solution, the limit of detection (LOD) for posaconazole was 10 ng mL^{-1}, and the analytical run time was less than 5 min. The successful application of the developed method to determine posaconazole concentrations in patient sample has demonstrated its feasibility as an effective method for clinical use.

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