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A sensitive liquid chromatography and mass spectrometry method for the determination of posaconazole in human plasma

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Abstract

Posaconazole is a novel extended-spectrum triazole that has favorable in vitro, in vivo and clinical activity against a number of yeasts and moulds. Posaconazole is available as an oral suspension. The dosage found to result in monitored plasma levels that correlate with clinical evidence of good antifungal activity is 800 mg/day in divided doses. A liquid chromatographic/mass spectrometric method (LC-MS/MS) that can be used by clinicians wishing to quantitate, and thereby monitor, plasma levels of posaconazole in certain patients was validated. The method utilized semi-automated 96-well protein precipitation with gradient chromatographic separation of analytes using a Varian Polaris C-18A (2.0 mm × 50 mm, 5-μm particle size) column. The approximate retention time of posaconazole was 2.0 min. Analytes were detected by using tandem mass spectrometry. Sample introduction and ionization was performed by atmospheric pressure chemical ionization in the positive-ion mode. This method has been proven suitable for routine quantitation of posaconazole over the concentration range of 5.00–5000 ng/mL. Inter-run precision based on percent relative deviation for replicate quality controls was ≤6.2%. Inter-run accuracy expressed as %DIFF was ±4.0%. Posaconazole quality controls were stable in human plasma for up to five freeze-thaw cycles, when frozen at −20 °C for at least 105 days and when kept at room temperature for 24 h. The lower limit of quantitation was 5.00 ng/mL for a 100-μL sample aliquot. These data indicate that the LC-MS/MS method described is suitable for the rapid measurement of posaconazole over the concentration range of 5.00–5000 ng/mL.

Keywords: Posaconazole; Plasma analysis; Mass spectrometry; Liquid chromatography

1. Introduction

The azoles are part of a large group of synthetic compounds that includes the imidazoles and triazoles. Azoles are potent, selective inhibitors of 14α-demethylase (CYP51), an enzyme important in the biosynthesis of yeast and moulds. The spectrum of antifungal activity varies among the different azoles.

Posaconazole is a novel extended-spectrum triazole that has favorable in vitro, in vivo and clinical activity against a number of yeasts and moulds, including, among others, *Aspergillus*, *Candida*, *Cryptococcus*, *Coccidioides*, *Fusarium*, agents of chromoblastomycosis and mycetoma, and Zygomycetes [1–6]. Posaconazole has been shown to be safe and well tolerated in healthy volunteers and patients with refractory invasive fungal infections or febrile neutropenia [7,8]. Posaconazole is orally bioavailable, and administration with food or an oral nutritional supplement, such as Boost Plus® further enhances exposure of posaconazole [9–11]. The oral dosage of posaconazole found to maximize exposure and produce clinically adequate plasma levels that are associated with clinical evidence of good antifungal activity is 800 mg/day in divided doses [7]. Importantly, concentrations obtained with this dosage in both healthy volunteers and patients have been well above the minimum inhibitory concentrations for most organisms of interest [12–16].

Based on earlier toxicokinetic data and allometric scaling, the analytical method needed to be refined to measure concentrations as low as 5.00 ng/mL in order to accurately characterize the human plasma concentration–time profile. In this article, we present a 96-well sample preparation and LC-MS/MS method that has been utilized in clinical studies and

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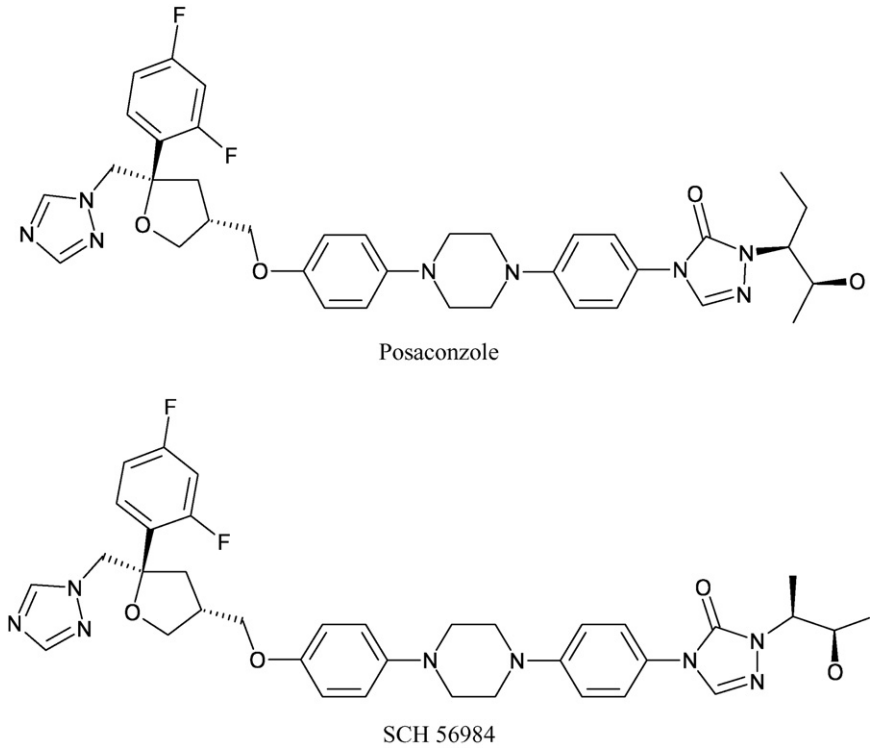

Fig. 1. Chemical structure of posaconazole and its internal standard (IS), SCH 56984.

is suitable for quantifying posaconazole over a dynamic range of 5.00–5000 ng/mL.

2. Materials and methods

2.1. Reference materials

Posaconazole and its internal standard (IS) SCH 56984 (Fig. 1) were synthesized at Schering-Plough Research Institute (Kenilworth, NJ) with a purity of 99.7% and 98.6%, respectively. An IV dosing solution (6 mg/mL in dosing solution) of posaconazole was provided by Schering-Plough Research Institute.

2.2. Biological matrix

Human plasma with ethylenediaminetetraacetic acid (EDTA) as the anticoagulant was obtained from Bioreclamation Inc. (Hicksville, NY). The blank matrix was used as received for the preparation of calibration standards (STD) and quality control (QC) samples without further treatment.

2.3. Reagents

HPLC-grade methanol, acetonitrile and American Chemical Society-grade formic acid were obtained from Fisher Scientific (Fair Lawn, NJ). Ultra-pure type 1 water was produced using a Millipore Milli-Q® water system (Bedford, MA). All other chemical reagents used were reagent grade or higher and used without further purification.

2.4. Liquid chromatography-tandem mass spectrometry

The liquid chromatographic system consisted of an integrated Shimadzu HPLC system consisting of two Shimadzu LC-10AD*VP* pumps (Shimadzu Corporation, Columbia, MD, USA) controlled by a Shimadzu SCL-10A system controller. A Shimadzu DGU-14A degasser was incorporated into this system. A LEAP CTC PAL (LEAP Technologies, Carrboro, NC) autosampler was used to perform sample injections. The CTC PAL autosampler had a Peltier-cooled sample storage compartment that refrigerated sample extracts to 4 °C. The detector was an Applied Biosystems/MDS Sciex (Concord, Ont.) API 3000™ triple quadrupole mass spectrometer. The HPLC column was a Varian (Varian Corp., Walnut Creek, CA) Polaris C-18A (2.0 mm × 50 mm, 5-μm particle size). A gradient elution program (Table 1) was used to separate posaconazole and its IS from the bulk of the endogenous matrix components. Mobile phase A consisted of water:methanol:formic acid (90:10:0.1, v:v:v) solution and mobile phase B consisted of ace-

Table 1
HPLC gradient program

Time (min)	% Mobile phase B	% Mobile phase A	Flow rate (μL/min)
0.3	10	90	250
1.3	75	25	250
2.5	75	25	250
2.6	100	0	500
3.5	100	0	500
3.6	10	90	400
4	10	90	250

tonitrile:methanol:formic acid (90:10:0.1, v:v:v). The retention times for posaconazole and the IS were 2.0 and 2.1 min, respectively. The sample injection volume was 10-μL. The cycle time of the method was four minutes per injection.

The mass spectrometer was operated in the multiple reaction monitoring (MRM) mode. Sample introduction and ionization was by atmospheric pressure chemical ionization (APCI) in the positive-ion mode. The corona discharge needle was set to 4.5 μA. The declustering potential was set to 80 V, and the collision energy was set to 47 eV. The probe temperature was set to 425 °C. The collision gas was set to maintain a pressure of approximately 3.1×10^{-5} Torr. To assay all analytes, the mass spectrometer was operated at unit mass resolution and set to select the MRM transitions for the $[M+H]^+$ ions of *m/z* 701 → *m/z* 683 for posaconazole and *m/z* 687 → *m/z* 669 for the IS. The dwell time for the posaconazole transition was 650 and 250 ms for the IS transition.

MRM data were acquired and integrated by using the Sciex Analyst™ software package (Version 1.3.1). Data regression was accomplished by using the Watson™ (Thermo LabSystems, Philadelphia, PA) drug metabolism laboratory information management system (Version 6.4.0.03).

2.5. Sample preparation

Fresh stock solutions containing posaconazole or SCH 56984 were prepared in methanol at a concentration of 1.0 mg/mL. Intermediate solutions for posaconazole and SCH 56984 were prepared in water:methanol (50:50, v:v) at concentrations of 50 and 1.0 μg/mL, respectively. The posaconazole calibration standard/quality control spiking solutions were prepared from the intermediate solution in water:methanol (50:50, v:v) at the nominal concentrations of 50.0, 100, 500, 2500, 5000, 12,500, 25,000 and 50,000 ng/mL for calibration standards and at 50.0, 150, 20,000 and 40,000 ng/mL for quality controls. Eight posaconazole calibration standards spanning concentrations from 5.00 to 5000 ng/mL were prepared in the appropriate matrix blanks using the corresponding spiking solution. Four levels of posaconazole quality controls were prepared in the appropriate matrix blanks at the nominal concentrations of 5.00 ng/mL (lower limit of quantitation, LLOQ), 15 ng/mL (QC of low concentration, QCL), 2000 ng/mL (QC of medium concentration, QCM) and 4000 ng/mL (QC of high concentration, QCH) by using the corresponding spiking solution. All samples were stored frozen at −20 °C until use.

On the day of analysis, a 100-μL aliquot of the appropriate sample was pipetted into a 96-well format dilution tube. A 20-μL aliquot of intermediate IS solution was added to all samples except matrix blanks, to which a 20-μL aliquot of water:methanol (50:50, v:v) was added. Samples were capped and vortexed for 60 s on a multitube vortexer. A 300-μL aliquot of acetonitrile was then added to each tube to precipitate the plasma proteins. After mixing, the samples were centrifuged at 4000 rpm on an Eppendorf 5810 R centrifuge (Brinkman Instruments, Westbury, NY) for 10 min. Using a Tomtec (Tomtec Corp., Hamden, CT) Quadra96® liquid handler, 300 μL of the supernatant was removed and transferred into a clean 96-well format collection plate. The extracts were then evaporated to near dryness under a stream of nitrogen at a setting of 50 °C for 15 min. The resulting residue was reconstituted in 250 μL of mobile phase A:mobile phase B (50:50, v:v). A 10-μL aliquot was injected into the LC-MS/MS system for analysis.

3. Study design

Bioanalytical validation was assessed with three core analytical runs. Each analytical run included eight calibration standards ($n = 2$ at each concentration) and quality control samples prepared at four concentrations ($n = 6$ at each concentration). Each run also contained two zero standards (blank human matrix samples with IS) and two control blanks (blank human matrix samples without IS). For each core analytical run, at least two thirds of the calibration standards must have individual accuracy within ±15% of the nominal value (±20% at the LLOQ). For each of the three core analytical runs, at least one of the two calibration standards at both the LLOQ and upper level of quantitation (ULOQ) must meet this criterion. If a calibration standard does not have accuracy within ±15% of the nominal value (±20% at the LLOQ), it is omitted from the calibration curve regression. For each validation run, the coefficient of determination (r^2) of the calibration curve must be greater than 0.98. For the three core validation runs, the intra-run (within-run) and inter-run (between-run) mean accuracy at each QC level should be within ±15% (±20% at the LLOQ). For each run, at least two thirds of the QC samples at each concentration level must have individual accuracy within ±15% (±20% at the LLOQ). The intra-run and inter-run precision should be ≤15% (≤20% at the LLOQ).

Selectivity of the method was assessed by screening six sources of blank plasma for interference at the retention times of posaconazole and SCH 56984. The selectivity of the method was also assessed to ensure that there was no interference between posaconazole and SCH 56984. To be acceptable, the response at the expected retention time of posaconazole must be less than 20% of the mean peak response calculated from the analysis of the LLOQ QC samples. The response at the expected retention time of SCH 56984 must be less then 5% of the mean peak response of the IS in the LLOQ QC samples.

Integrity of dilution was assessed to determine whether a sample with a concentration at the ULOQ could be diluted with matrix for accurate quantitation within the range of the calibration curve. QC samples at the concentration of 5000 ng/mL were prepared and diluted 10-fold and analyzed in six replicates. To be acceptable, the mean accuracy of the six replicates should be within ±15%, and two thirds of the dilution QC samples should have individual accuracy within ±15%.

The stability of posaconazole was assessed by analyzing QC samples at low and high concentrations ($n = 6$ at each concentration) that were stored under various conditions. To be acceptable, two thirds of all stability QC samples at each level should have individual accuracy within ±15% of the nominal value, and the mean accuracy at each level should be within ±15% of the nominal value. The stability of posaconazole in stock solutions during long-term storage at 4 °C was assessed by comparing stored

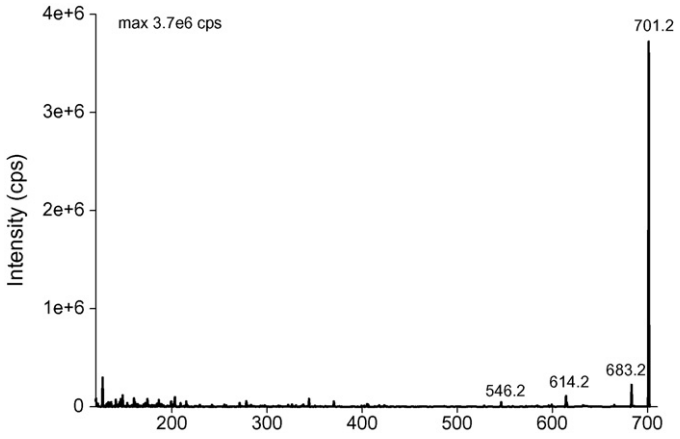

Fig. 2. Product ion mass scan for posaconazole.

stock solutions to fresh stock solutions. In addition, the stability of posaconazole in stock solutions at room temperature for at least 6 h was assessed. For stock solution stability, the mean difference of the peak response of the evaluated stock solution should be ≤5% from the mean peak response of a freshly prepared stock solution.

The recovery for posaconazole and SCH 56984 were assessed by comparing the mean peak responses from extracted QC samples to the mean peak responses from neat samples.

4. Results and discussion

4.1. Mass spectrometry

The positive-ion APCI mass spectrum of posaconazole is dominated by the protonated molecular ion of *m/z* 701. Under collisional activation, posaconazole fragments poorly, with a conversion efficiency of less then 20%. The predominant product ions of *m/z* 701 are *m/z* 683 (dehydration) and, to a lesser extent, *m/z* 614 (Fig. 2). While the loss of water is not normally a desirable choice for a selective MRM transition, the difference in signal to noise ratio between this transition and the latter was sufficient to warrant its selection for quantitation of posaconazole. The internal standard, SCH 56984, displayed similar fragmentation behavior and the MRM transition *m/z* 687 → *m/z* 669 was selected for detection.

4.2. Sample extraction optimization

Of the three commonly used extraction techniques, "dilute and shoot" or protein precipitation is typically the least suited for clinical studies because of its susceptibility to ion suppression and matrix effects [17]. Liquid–liquid extraction and solid-phase extraction (SPE) are techniques that offer much cleaner sample extracts that in turn make the method more robust and scalable. However, during the course of method development, we found that the use of SPE or liquid–liquid extraction techniques resulted in incomplete recovery for the posaconazole IV formulation. Fig. 3a describes a comparison of two liquid–liquid extraction experiments utilizing QC samples of the same nominal concentration, one prepared from the posaconazole IV dosing solution and the other from reference standard. For these experiments, 100 μL of human plasma was extracted with 900 μL of hexane:ethyl acetate (50:50, v:v). After thorough mixing and centrifugation at 3000 rpm for 10 min, the organic layer was removed and evaporated under a stream of nitrogen. The residue was reconstituted with 250 μL of mobile phase A:mobile phase B (50:50, v:v). A 10-μL aliquot was injected into the LC-MS/MS system for analysis. As shown in Fig. 3, the response for QC samples prepared using reference standard was almost twice that for QCs prepared with the posaconazole IV formulation. This pronounced difference in response was most likely because of nonspecific binding between the IV formulation additives, plasma protein and posaconazole. To minimize the discrimination between IV dosing solution and reference material, the use of protein precipitation afforded the best compromise to ensure uniform recovery irrespective of dose formulation. As shown in Fig. 3b, when QC samples prepared

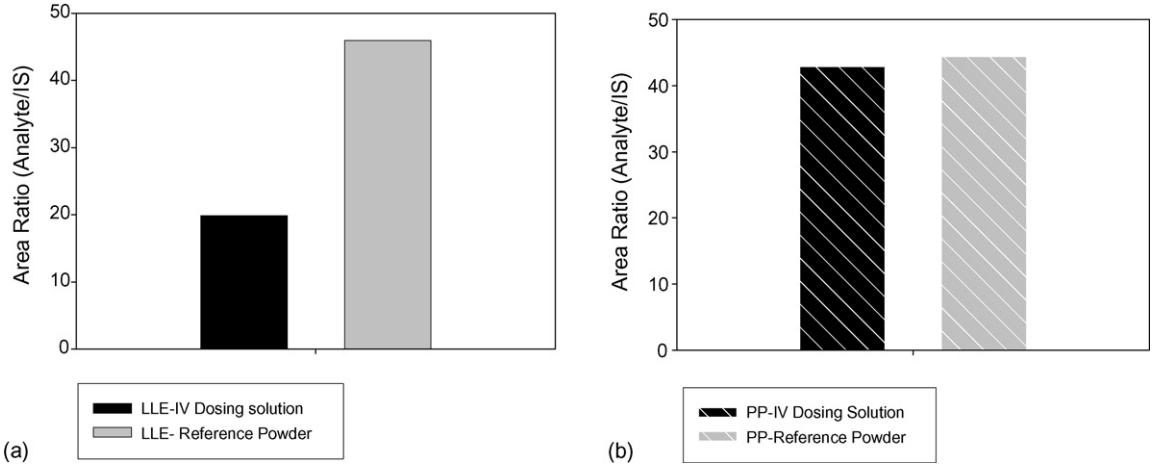

Fig. 3. (a) Comparison of two liquid–liquid extraction experiments of the same concentration quality control (QC) samples prepared from posaconazole IV dosing solution and posaconazole reference powder. Each data point is calculated based on average of three duplicate extractions. (b) Comparison of two protein precipitation extraction experiments of the same concentration QC samples prepared from posaconazole IV dosing solution and posaconazole reference powder. Each data point is calculated based on average of three duplicate extractions.

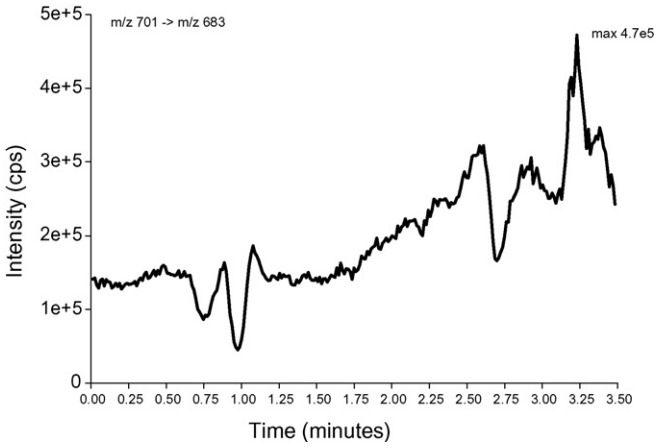

Fig. 4. Multiple reaction monitoring (MRM) ion chromatograms for posaconazole during post-column infusion and subsequent injection of an extracted control blank plasma sample.

with posaconazole IV dosing solution and reference material were subjected to a simple protein precipitation procedure using the extraction routine described in Section 2, response was equivalent.

4.3. Chromatography

Because of the relatively coarse sample preparation procedure, it was important to optimize the chromatography system to ensure that residual endogenous interferences did not interfere with the selectivity of the assay. Our first choice in using the APCI interface rather than electrospray ionization was based on the knowledge that APCI is less susceptible to ion suppression [18]. Additionally, a high-speed organic wash was incorporated into the gradient program to ensure that nonpolar endogenous material was removed from the column prior to the next injection. Following elution of posaconazole and the IS at approximately 2 min, the mobile phase flow rate and composition was increased from 250 μL/min at 75% mobile phase B to 500 μL/min at 100% mobile phase B. This one minute wash at 100% organic was sufficient to remove the endogenous lipids. Fig. 4 illustrates an ion suppression experiment similar to one previously described by King et al. [19]. In this experiment, posaconazole was infused post-column via a mixing tee at a concentration of 5 μg/mL. Ion suppression resulting from matrix components in the eluent was monitored during the gradient elution by continuously acquiring MRM spectra for posaconazole for a 3.5-min period. Control plasma blanks extracted using the protein precipitation procedure described above were injected into the LC-MS/MS system. A dip in the monitored mass trace was considered an effect of ion suppression. It is evident from the experiment that at least two major ion suppression regions were present in each gradient cycle. The first major ion suppression region, most likely caused by the elution of polar proteins and carbohydrates, occurred near the void volume of the column; i.e., at approximately 0.7 and 1.0 min. The second major ion suppression region, presumably caused by late-eluting phospholipids, occurred at approximately 2.7 min post-injection [20].

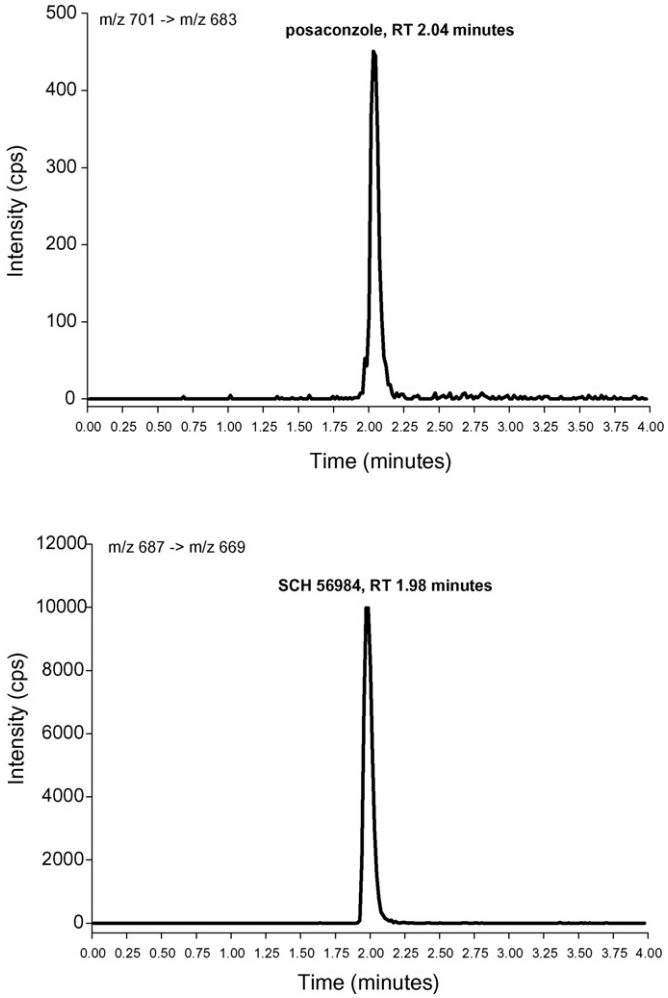

Fig. 5. Typical multiple reaction monitoring (MRM) chromatograms showing elution of 10-μL injection of an extracted lower limit of quantitation (LLOQ; 5.00 ng/mL) human plasma sample of posaconazole (top trace) and its internal standard (IS), SCH 56984 (bottom trace), under described HPLC gradient condition.

Given that the elution times of posaconazole and IS were approximately 2.0 min, it is likely the analytes are well resolved from the two major ion suppression regions.

The signal to noise ratio is excellent at the LLOQ level. Fig. 5 represents the typical response observed following an injection of a 10-μL aliquot of the reconstituted posaconazole LLOQ sample extract at 5.00 ng/mL. The injection represents a total column load of 20 pg of posaconazole.

4.4. Linearity

The peak area ratios of calibration standards were proportional to the concentration of analytes in each assay over the nominal concentration range of 5–5000 ng/mL. The calibration curves appeared quadratic and were well described by least-squares regression lines. A weighting factor of 1/concentration^2 was chosen to achieve homogeneity of variance. The slopes, intercepts and coefficients of determination (r^2) from the validation analyses appear in Table 2.

Table 2
Back-calculated posaconazole concentrations in human plasma calibration standards and calibration curve parameters[a]

Concentration (ng/mL)	STD 1 5.00 ng/mL	STD 2 10.0 ng/mL	STD 3 50.0 ng/mL	STD 4 250 ng/mL	STD 5 500 ng/mL	STD 6 1250 ng/mL	STD 7 2500 ng/mL	STD 8 5000 ng/mL	*A*	*B*	*C*	Coefficient of determination (r^2)
Core run 1	5.46	10.2	50.9	246	513	1280	3150[b]	4700	0.0000000700	0.00807	0.00563	0.9955
	4.89	8.50	47.7	243	507	1360	2570	5090				
Core run 2	4.54	9.71	47.6	256	530	1290	2640	4950	0.000000231	0.00762	0.0104	0.9969
	5.62	9.78	48.5	247	487	1240	2530	4870				
Core run 3	4.73	9.10	49.0	249	521	1270	2560	4900	0.0000000879	0.00832	0.00694	0.9976
	5.47	10.2	49.0	256	481	1240	2620	4910				
n	6	6	6	6	6	6	5	6				
Overall mean	5.12	9.58	48.8	250	507	1280	2580	4900				
S.D.	0.454	0.667	1.20	5.39	19.2	44.3	45.1	126				
%CV	8.9	7.0	2.5	2.2	3.8	3.5	1.7	2.6				
%DIFF	2.4	−4.2	−2.4	0.0	1.4	2.4	3.2	−2.0				

[a] $y = Ax^2 + Bx + C$, weighted 1/concentration^2, where y is the peak area ratio of posaconazole to IS, x the concentration of posaconazole and A–C are curve parameters of the calibration curve.
[b] Beyond ±15% limit from nominal concentration thus not included in calculation.

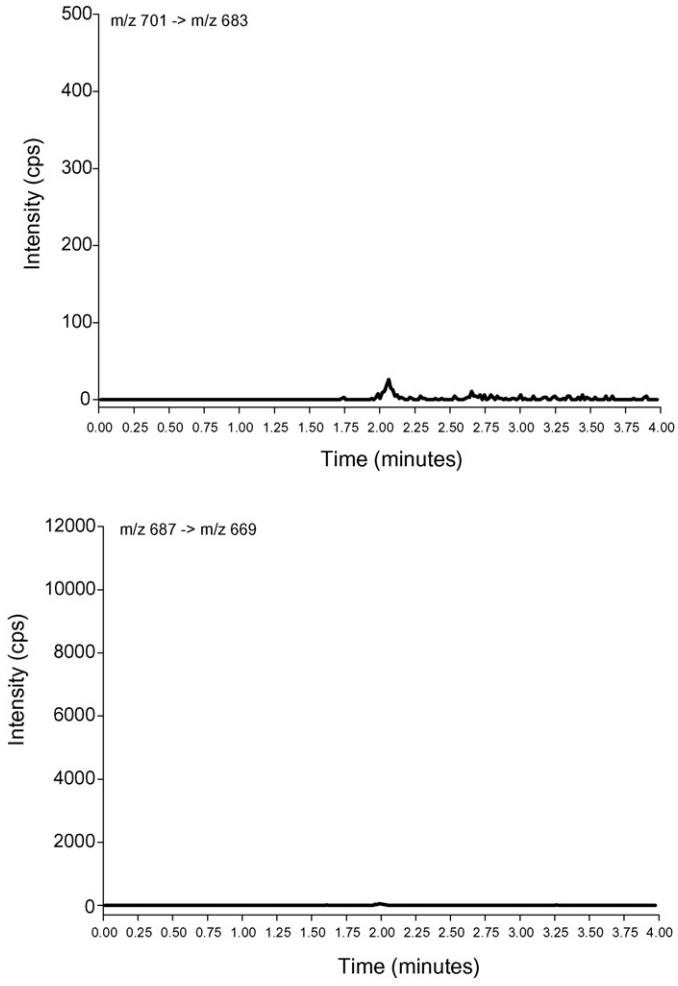

Fig. 6. Typical multiple reaction monitoring (MRM) chromatograms showing elution of 10-μL injection of an extracted blank human plasma sample of posaconazole (top trace) and its internal standard (IS), SCH 56984 (bottom trace), under described HPLC gradient condition.

4.5. Accuracy and precision

For calibration standards, the inter-run precision and accuracy results from the three analytical core runs are listed in Table 2. The inter-run accuracy (%DIFF) ranged from −4.2% to 3.2%. The inter-run precision (%CV) ranged from 1.7% to 8.9%.

For QC samples, the intra-run precision and accuracy from each of the three analytical core runs as well as the inter-run precision and accuracy results from three analytical core runs are listed in Table 3. To summarize, the inter-run accuracy ranged from −1.0% at the LLOQ to 4.0% at QCM. The inter-run precision (%CV) ranged from 6.2% at the LLOQ to 2.5% at the QCH.

4.6. Selectivity

Blank plasma samples from six sources were screened and were found to be free of interference from endogenous components or other sources at the same mass transitions and retention times as posaconazole and IS. Fig. 6 shows typical MRM chromatograms for an extracted blank human plasma sample of

Table 3
Analytical performance of posaconazole quality control samples in human plasma

Run parameter	Core run	LLOQ 5.00 ng/mL	%DIFF	QCL 15.0 ng/mL	%DIFF	QCM 2000 ng/mL	%DIFF	QCH 4000 ng/mL	%DIFF
	1	5.19	3.8	14.8	−1.3	2120	6.0	4200	5.0
		4.92	−1.6	14.3	−4.7	2140	7.0	4310	7.8
		4.70	−6.0	14.9	−0.7	2110	5.5	4280	7.0
		5.52	10.4	118[a]		2260	13.0	4210	5.3
		5.42	8.4	15.2	1.3	1960	−2.0	4280	7.0
		4.82	−3.6	14.9	−0.7	2150	7.5	4240	6.0
Intra-run mean		5.10		14.8		2120		4250	
Intra-run SD		0.334		0.327		96.5		43.7	
Intra-run %CV		6.5		2.2		4.6		1.0	
Intra-run %DIFF		2.0		−1.3		6.0		6.3	
n		6		5		6		6	
	2	4.62	−7.6	15.2	1.3	2040	2.0	3950	−1.3
		4.42	−11.6	15.8	5.3	2000	0.0	4070	1.8
		186[a]		14.9	−0.7	2060	3.0	4090	2.3
		5.32	6.4	15.3	2.0	2020	1.0	4030	0.8
		4.72	−5.6	17.2	14.7	2030	1.5	4130	3.3
		4.74	−5.2	14.9	−0.7	2100	5.0	4150	3.8
Intra-run mean		4.76		15.6		2040		4070	
Intra-run S.D.		0.336		0.873		34.9		72.7	
Intra-run %CV		7.1		5.6		1.7		1.8	
Intra-run %DIFF		−4.8		4.0		2.0		1.8	
n		5		6		6		6	
	3	5.27	5.4	15.0	0.0	2040	2.0	4170	4.3
		4.91	−1.8	15.4	2.7	2050	2.5	4010	0.3
		4.72	−5.6	15.6	4.0	2120	6.0	4130	3.3
		5.06	−1.2	15.6	4.0	2070	3.5	4090	2.3
		4.85	−3.0	16.6	10.7	2100	5.0	4070	1.8
		4.94	−1.2	15.6	4.0	2030	1.5	4040	1.0
Intra-run mean		4.96		15.6		2070		4090	
Intra-run S.D.		0.189		0.528		35.4		58.6	
Intra-run %CV		3.8		3.4		1.7		1.4	
Intra-run %DIFF		−0.8		4.0		3.5		2.3	
n		6		6		6		6	
Mean concentration found (ng/mL)		4.95		15.4		2080		4140	
Inter-run S.D.		0.305		0.696		68.5		102	
Inter-run %CV		6.2		4.5		3.3		2.5	
Inter-run %DIFF		−1.0		2.7		4.0		3.5	
n		17		17		18		18	

[a] Beyond ±15% limit from nominal concentration thus not included in calculation; LLOQ indicates lower limit of quantitation; %CV indicates % coefficient of variation (inter-run precision); %DIFF indicates %difference (inter-run accuracy); QCL indicates quality control of low concentration; QCM indicates quality control of medium concentration; QCH indicates quality control of high concentration; S.D. indicates standard deviation.

posaconazole (top trace) and the IS, SCH 56984 (bottom trace). The method is also sufficiently selective between posaconazole and the IS.

4.7. Integrity of dilution

Integrity of dilution was assessed using dilution quality control (QCD) samples prepared at a concentration of 5000 ng/mL and diluted 10-fold with $n = 6$. Accuracy data for posaconazole integrity of dilution are presented in Table 4.

4.8. Stability

The stability of posaconazole in human plasma was demonstrated under various storage conditions. A summary of these stability experiments is reported in Table 5. Posaconazole in methanolic stock solution was determined to be stable when refrigerated at 4 °C for at least 135 days. The methanolic stock solution was also stable for at least 6 h at room temperature.

4.9. Recovery

Recovery of posaconazole and IS were determined by comparing the peak area responses of extracted QC samples ($n = 3$) to the peak area responses of neat solutions at the same concentrations. Mean absolute recovery was 82.5%, 67.5% and 68.5%, respectively, for QCL, QCM and QCH samples.

Table 4
Accuracy data for 10× dilution integrity for posaconazole in human plasma

Sample	Posaconazole 5000 ng/mL
1	5320
2	5020
3	5310
4	5060
5	5240
6	5320
Mean	5210
S.D.	137
%CV	2.6
%Theoretical	4.2
%DIFF	6
n	5210

%CV indicates % coefficient of variation (inter-run precision); %DIFF indicates %difference (inter-run accuracy); S.D. indicates standard deviation.

Table 5
Stability data for posaconazole in human plasma

Conditions	Minimum stability
Short-term room temperature stability in human plasma	24 h
Frozen stability in human plasma stored in a −20 °C freezer	105 days
Freeze-thaw stability in human plasma	5 cycles
Autosampler stability at room temperature	5 days
Stock solution storage stability (at 4 °C)	135 days
Stock solution room temperature stability in methanol	6 h

5. Applicability of method

The method described herein and a number of unpublished LC-MS/MS bioanalytical methods have been used to support posaconazole studies conducted by Schering-Plough Research Institute (SPRI).

Systemic exposure to posaconazole has been found to be similar in healthy male and female and as well as in pediatric (8–17 years) and adult (18–64 years) patients with invasive fungal infections Older adults (≥65 years) have a slightly higher (∼27%) systemic exposure to posaconazole than do younger adults (18–45 years) and black volunteers, respectively, but these difference are not clinically relevant. In addition, posaconazole oral suspension is generally well tolerated at 800 mg/day in divided doses, and gastrointestinal adverse events, usually of mild to moderate intensity, are the most commonly reported adverse events [7,8]. No relationship between increasing posaconazole plasma levels and adverse events has been noted in electrocardiographic or laboratory evaluations (including alanine aminotransferase, aspartate aminotransferase and creatinine values) of healthy volunteers or patients [7]. Although it inhibits CYP3A4, posaconazole is primarily known to be metabolized by glucuronidation rather than CYP450 enzymes [21]. Consequently, coadministration of drugs that interact with the CYP450 enzyme system is unlikely to alter plasma concentrations of posaconazole. These findings suggest that routine monitoring of posaconazole levels may not be required.

6. Conclusion

We have validated a precise, accurate LC-MS/MS method for quantitating posaconazole in human plasma. This rapid method is suitable for the measurement of posaconazole over the concentration range of 5.00–5000 ng/mL.

Although the routine monitoring of posaconazole plasma levels may not be necessary, this rapid, accurate assay should be valuable in the context of clinical trials. The method outlined in this paper has been used in a number of studies conducted at Schering-Plough Research Institute to characterize the pharmacokinetics of posaconazole.

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