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ORIGINAL RESEARCH

A stereospecific high-performance liquid chromatographic assay for the determination of ketoconazole enantiomers in rat plasma

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ABSTRACT: A stereospecific high-performance liquid chromatographic assay was developed for the quantitation of ketoconazole enantiomers (KTZ) in rat plasma. After protein precipitation of 100 μL plasma using acetonitrile, a wash step was performed using hexane. The supernatant was removed and KTZ enantiomers and amiodarone, the internal standard, were extracted using liquid–liquid extraction with tert-butyl methyl ether. After transfer and evaporation of the organic layer, the residue was reconstituted in mobile phase and injected into the HPLC through a chiral column. The mobile phase consisted of hexane:ethanol:2-propanol with diethyl amine, pumped at 1.5 mL/min. All components eluted within 18 min. KTZ enantiomers were baseline resolved and peaks were symmetrical in appearance with no interferences. Calibration curves were linear over the range 62.5–5000 ng/mL of enantiomer. The intraday and interday CV% assessments were ≤19 and <13%, respectively, and mean error was <4% for both enantiomers. The lower limit of quantitation was 62.5 ng/mL for each enantiomer based on 100 μL rat plasma. In rats, plasma concentrations of (+)-KTZ were higher than those of antipode after single oral doses. The assay was shown to be sensitive and appropriate for use in pharmacokinetics study of KTZ in rat. Copyright © 2008 John Wiley & Sons, Ltd.

KEYWORDS: enantiospecific; HPLC; antifungals; pharmacokinetics

INTRODUCTION

Over the last two decades, there has been a dramatic increase in the incidence of serious mucosal and systemic fungal infections. This is mainly due to immunosuppression associated with human immunodeficiency virus, organ transplantation and anticancer chemotherapy (Thienpont *et al.*, 1999; Dilmaghanian *et al.*, 2004; Castro-Puyana *et al.*, 2005). Amongst the different antifungal agents, the azoles (imidazole- or triazole-based drugs) constitute an important class due to their broad spectrum of antifungal activity and reasonably good oral bioavailability (Como and Dismukes, 1994). Ketoconazole (KTZ) was the first introduced orally administered azole. However, due to the risk of KTZ-induced hepatotoxicity and serious drug–drug interactions, its use tends to be restricted to serious infections resistant to other safer azoles (Velikinac *et al.*, 2004; Korashy *et al.*, 2007).

KTZ, *cis*-1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3dioxolan-4-yl]methoxy]phenyl]-piperazine, is a chiral drug administered clinically as a racemic (1:1) mixture of the enantiomers of the *cis* configuration (Fig. 1). The *cis* configuration indicates that the hydrogen and the 2,4-dichlorophenyl group at the two chiral centers are on the same side of the five-membered (dioxolane) ring. Their absolute configuration has been reported via synthesis as (+)-(2*R*,4*S*) *dextro*rotatory enantiomer and (–)-(2*S*,4*R*) *levo*rotatory enantiomer (Rotstein *et al.*, 1992).

Since stereochemistry is an important modulator of drug biological effects, for new drug candidates the US Food and Drug administration (FDA) has recommended the development of quantitative assays for individual enantiomers in *in vivo* samples early in drug development (Anonymous, 1992). For many enantiomeric pairs of drugs, the pharmacological activity of one enantiomer may differ from that of its antipode (Jamali *et al.*, 1989; Brocks and Jamali, 1995). In many cases as well, the pharmacokinetic behaviour of one enantiomer may differ from the other (Brocks, 2006; Lu, 2007). Thus, it is worth examining the potential of stereoselectivity in KTZ pharmacokinetic and pharmacodynamic properties. To date, however, there has been no published report of the stereoselective pharmacokinetics of KTZ.

The enantiomeric separation of KTZ has been successfully achieved in solutions or pharmaceutical formulations using supercritical fluid chromatography (SFC) with a ChiralPak AD column (Thienpont

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Abbreviations used: AM, amiodarone; KTZ, ketoconazole; SFC, supercritical fluid chromatography; TBME, tert-butyl methyl ether.

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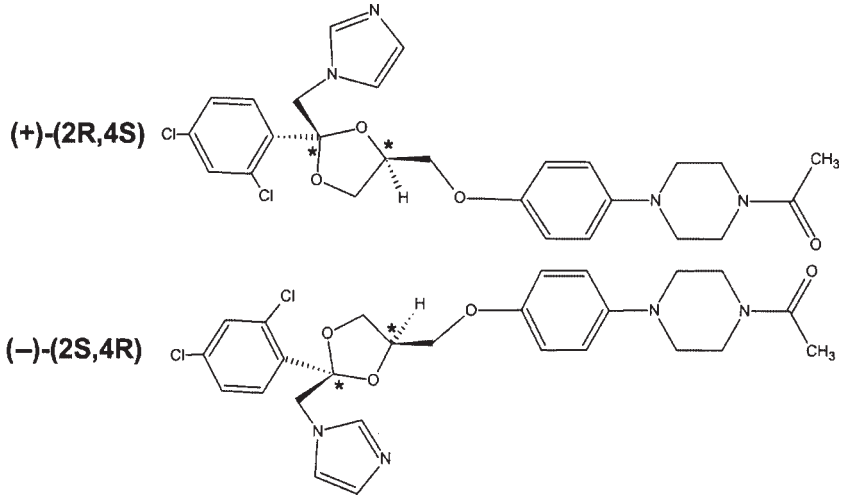

Figure 1. Ketoconazole enantiomer chemical structures.

et al., 1999; Bernal *et al.*, 2000) and electrokinetic capillary electrophoresis (Castro-Puyana *et al.*, 2005). Some HPLC separations have been reported using chiral columns (Thienpont *et al.*, 1999; Bernal *et al.*, 2002). Although some success in separating the enantiomers of KTZ has been reported, to our knowledge a method for quantifying the enantiomers in biological specimens has to date not been published. Therefore, in this report we describe a sensitive and specific HPLC method for the determination of KTZ enantiomers in rat plasma.

EXPERIMENTAL

Materials and reagents. Ketoconazole and amiodarone HCl were obtained from Sigma (St Louis, MO, USA). Hexane, isopropyl alcohol, absolute ethanol, tert-butyl methyl ether (TBME) (all HPLC grade), and diethylamine were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

Chromatographic conditions. The chromatography system consisted of a Waters 710B WISP auto-injector, Waters 510 pump (Waters, Milford, MA, USA) and an HP 1050 UV detector (Hewlett Packard, Palo Alto, CA, USA). The UV wavelength was set at 240 nm. The chromatographic separation of amiodarone (IS) and ketoconazole enantiomers was accomplished using a 250 × 4.6 mm ChiralPak AD column (Diacel Chemical Industries Ltd, NJ, USA) attached to a pre-column holder for PVDF-Cardridges 4-4 containing Lichrospher 100 Diol 5 µm guard column (Merck, KGaA, Darmstadt, Germany). Chromatographic data were collected and compiled by use of EZChrom software (Scientific Software, Pleasanton, CA, USA). The mobile phase used was a 70:20:10 (v/v) mixture of hexane, absolute ethanol and 2-propanol to which 0.1% diethylamine. The mobile phase was pumped at a flow rate of 1.5 mL/min.

Standard and stock solutions. A 100 mg/L stock solution of racemic KTZ was prepared by dissolving 10 mg of (±)-KTZ in 100 mL methanol. A 100 mg/L stock solution of AM (amiodarone, internal standard) was prepared by dissolving 10.6 mg of AM HCl in 100 mL methanol. To prepare samples for the calibration curve and validation assessment, three working solutions of 0.1, 1 and 10 mg/L of (±)-KTZ were prepared by successive 1:10 dilutions of the stock solution with methanol. The AM stock solution was stored at –30°C while KTZ stock and working solutions were stored at 2°C between uses.

Extraction procedure. IS (0.02 mL) was added to each 0.1 mL plasma sample in a 2 mL polypropylene microcentrifuge tube. To this, 0.3 mL of acetonitrile was added to precipitate the plasma proteins. Because methanol was present in the standard curve samples, an equivalent amount was added to the test samples as well (0.125 mL). The tubes were briefly vortex mixed (4 s) at high speed then subsequently centrifuged for 3 min at ~2500***g***. The supernatants were carefully transferred to new glass tubes using Pasteur pipettes. Then 0.05 mL of 0.1 M HCl and 1 mL of hexane were subsequently added to each tube as a washing step. The tubes were then vortex mixed for 30 s and centrifuged at ~2500***g*** for 3 min. The organic hexane supernatant was carefully aspirated and then 0.075 mL of 0.1 M NaOH and 5 mL of TBME were added. The tubes were then vortex mixed again for 30 s and centrifuged at ~2500***g*** for 3 min. The organic layer was transferred to new glass tubes and evaporated to dryness *in vacuo*. The residues were reconstituted in 150 µL mobile phase, of which 75–125 µL volumes were injected into the HPLC.

Recovery. The plasma recoveries were determined at KTZ enantiomer concentrations of 250, 1000 and 5000 ng/mL, using four replicates for each concentration. The extraction efficiency was determined by comparing the peak areas of analyte to the peak areas of the same amounts directly injected to the instrument, without extraction. Recovery of IS was performed using the concentrations used in the assay (20 µg/mL).

Calibration, accuracy and validation. Calibration curves were constructed using samples of 0.1 mL rat plasma containing KTZ and IS. The curve ranged from 62.5 to 5000 ng/mL of each KTZ enantiomer. The ratio of KTZ enantiomers to IS

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peak height was calculated and plotted vs nominal KTZ enantiomer concentration. Owing to the wide range of concentrations, the calibration curve data were weighed by a factor of 1/KTZ enantiomer concentration.

Intraday accuracy and precision of the assay were determined using five sample replicates of 62.5, 125, 500 and 2500 ng/mL of each KTZ enantiomer in rat plasma. To permit the assessment of interday accuracy and precision, the assay was repeated on three separate days. For each daily run, concentrations were determined by comparison with a calibration curve prepared on the day of the analysis. Precision was determined using percentage coefficient of variation (CV %) and bias was assessed using mean intra- or interday percentage error of the mean.

Assignment of KTZ enantiomer optical rotation. The order of elution of (+) and (–) enantiomers was determined by collecting eluent fractions from repeated injections. There were ~25 injections made into the HPLC, with ~180 µg racemate per injection. After compiling the eluent fractions corresponding to each enantiomer, they were dried *in vacuo*, reconstituted in methanol and the optical rotation determined using a Perkin Elmer 241 polarimeter (PerkinElmer Life and Analytical Sciences, Waltham, MA, USA).

Test for racemization. Ketoconazole enantiomer fractions of 10,000 ng each were collected. Each enantiomer fraction was divided into three aliquots, and then dried *in vacuo*. The contents of the tubes were extracted according to the procedure described above. The samples were reconstituted in 150 µL of mobile phase, and 125 µL was injected into the HPLC.

Assessment of chromatographic separation. The capacity factor (K') was calculated using the equation $K' = (t_r - t_m)/t_m$, where t_r and t_m are the retention times of the peak of interest and the non-retained peak (solvent front). The separation factor (α) was calculated as $\alpha = K'_J/K'_I$, where component J is the more strongly retained compound. The resolution factor (R_s) was calculated as $R_s = (t_{R,J} - t_{R,I})/0.5(W_{t,J} - W_{t,I})$, where W_t is the width at the base of the peak. The symmetry index was determined by first determining the peak width at 10% of peak height. The latter part of the width from time of peak height onwards was divided by the first part of the width up to the time of peak height.

Animal study. In order to assess the applicability of this method *in vivo*, two rats (280–300 g) were administered 10 mg/kg (±)-KTZ orally. The protocol was approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee. The day before the pharmacokinetic study, the right jugular vein of each rat was cannulated with Micro-Renathane tubing (Braintree Scientific, Braintree, MA, USA) under isoflurane anesthesia. The cannula was filled with 100 U/mL heparin in 0.9% saline. After cannula implantation, the rats were transferred to regular holding cages and allowed free access to water, but food was withheld overnight. The next morning, animals were transferred to metabolic cages for conduct of the pharmacokinetic experiments.

Serial blood samples were collected at 0.5, 1, 2, 3, 4, 6, 8, 10 and 24 h postdose into heparinized 2 mL polypropylene microcentrifuge tubes. Plasma was separated by centrifugation of the blood at 2500***g*** for 3 min. The samples were kept at –30°C until assayed using the developed HPLC method. The Cmax and tmax were examined from visual examination of the data, and the area under the plasma concentrations vs time curve from time of dosing to last measured concentration was calculated using log–linear trapezoidal rule.

RESULTS

The IS and KTZ enantiomers eluted chromatographically at approximately 3.8, 11.9 and 15.6 min, respectively (Fig. 2). From the polarimetry assessment, the *dextro* (+) enantiomer was found to be eluting first. KTZ enantiomer peaks showed baseline resolution and were symmetrical in appearance with no interferences from endogenous substances in plasma. The assay showed no observable racemization. The total analytical run time was 18 min.

The column capacity factors (K') for IS, (+)- and (–)-KTZ were calculated to be 0.3, 3.1 and 4.4 respectively. The column separation factor (α) and resolution factor for the KTZ enantiomers were calculated to be 1.4 and 2.3, respectively. The symmetry index were 1.1 and 1.2 for the (+) and (–) enantiomers, respectively.

The average extraction recoveries in plasma of (+)-KTZ were 89.0, 81.3 and 82.6% for the 250, 1000 and 5000 ng/mL enantiomer concentrations, respectively. For the (–) enantiomer, extraction efficiencies were 87.9, 79.8 and 82.0%, respectively, at concentrations of 250, 1000 and 5000 ng/mL enantiomer. The recovery of IS was 81.8%. Highly linear relationships were noted between the analyte/IS peak height or area ratios and rat plasma enantiomer concentrations ranging from 62.5 to 5000 ng/mL. The mean r^2 for the three standard curves were 0.999 for each enantiomer. The standard curve in plasma plotting concentration (ng/mL) vs peak height ratio yielded slopes of $1.17 \times 10^{-5} \pm 8.15 \times 10^{-7}$ and $9.31 \times 10^{-6} \pm 7.33 \times 10^{-7}$ for (+)- and (–)-KTZ, respectively. The corresponding intercepts were $2.87 \times 10^{-4} \pm 1.65 \times 10^{-4}$ and $3.02 \times 10^{-4} \pm 1.69 \times 10^{-4}$, respectively for the (+) and (–) enantiomers.

The validation data showed the assay to be sensitive, accurate and precise, with the intraday and interday assessment CV% less than or equal to 19 and 13%, respectively, for both enantiomers (Table 1). The mean interday error in rat plasma was less than 4% for both enantiomers. Since both CV% of interday and intraday assessment and interday mean error yielded values less that 20% at the lowest concentration tested, the lower limit of quantitation (LLQ) based on 100 µL of rat plasma was found to be 62.5 ng/mL for each enantiomer

In the rats dosed with (±)-KTZ orally, the concentrations of (+) enantiomer were much higher than those of (–)-KTZ (Fig. 3). The C_{max} of the (+) enantiomer was

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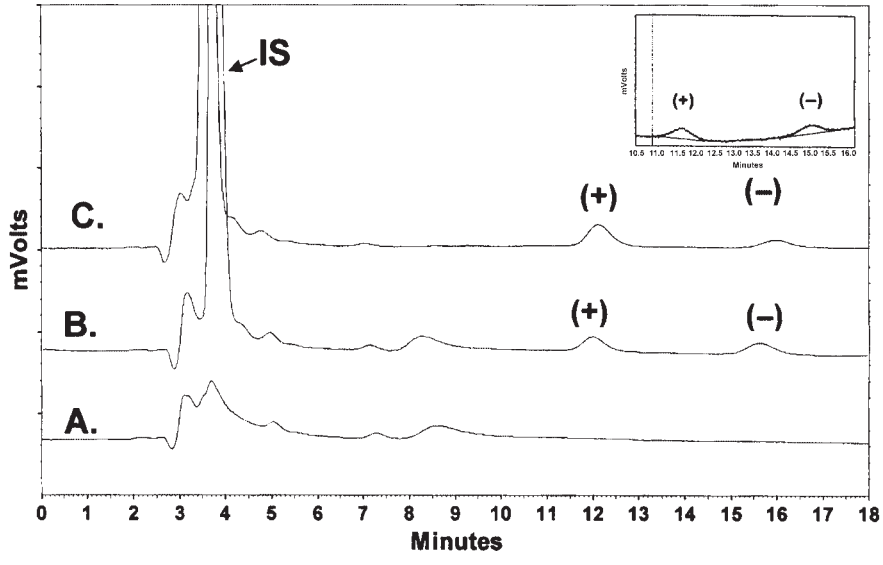

Figure 2. Chromatograms of (A) blank rat plasma sample, (B) rat plasma spiked with 2500 ng/mL of (±)-KTZ extracted from rat plasma samples, (C) rat plasma obtained after 2 h of 10 mg (±)-KTZ/kg. The inset shows a section of a chromatogram of a plasma sample spiked with 125 ng/mL of (±)-KTZ (the validated LLQ of the assay). IS denotes internal standard (amiodarone).

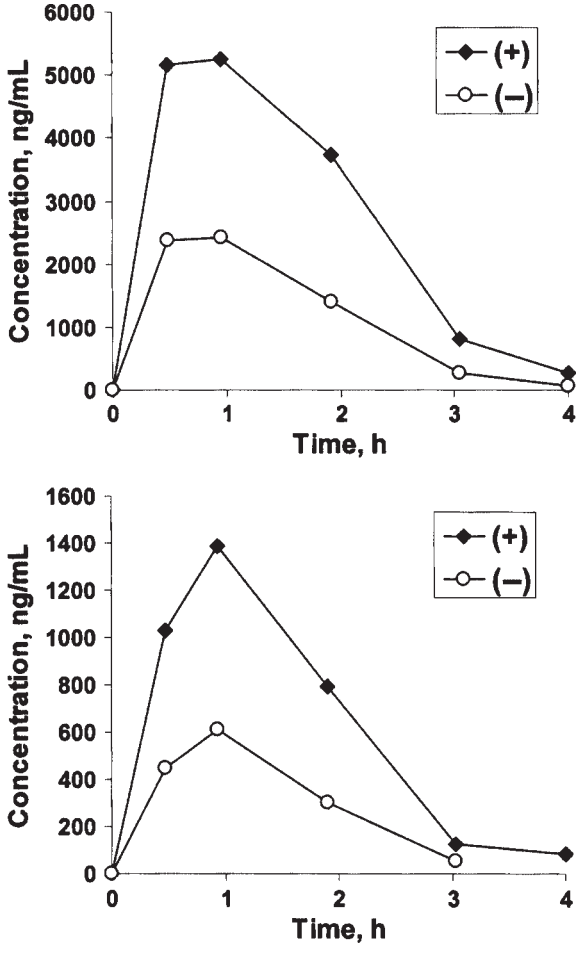

Figure 3. Plasma KTZ enantiomer concentrations vs time curves from two rats given an oral dose of 10 mg/kg KTZ racemate.

5246 and 1389 ng/mL for the two rats while those of the (–) enantiomer were 2424 and 614 ng/mL, respectively. The t_{max} for both enantiomers were similar, occurring at ~1 h post-dose in both rats. The KTZ enantiomer concentrations occurring after 4 h post-dosing below the LLQ of 62.5 ng/mL, thus limiting the AUC calculation from 0 to 4 h post-dose. The AUC for KTZ enantiomer concentrations in the two rats studied was 10596 and 2346 ng h/mL for the (+)-KTZ and 4404 and 945 ng h/mL for the (–)-KTZ.

DISCUSSION

The described assay was capable of quantifying the enantiomers of KTZ in a biological specimen, rat plasma. The assay was sensitive and specific, affording baseline resolution of the enantiomers. For an analytical run of 20 samples, it took approximately 90 min to prepare the samples for injection into the HPLC. To our knowledge, this is the first stereospecific assay for KTZ in plasma. However, it is not the first method reported for separation of the KTZ enantiomers. The first HPLC separation utilized ChiralPak AS and ChiralCel OD columns with combinations of hexanes and alcohols with octanoic acid. (Thienpont *et al.*, 1999) Although a chromatogram was not presented, the authors reported a baseline separation of KTZ enantiomers, albeit with a very long analysis time (~50 min). Similarly, Bernal *et al.* reported that KTZ enantiomers were strongly retained (over 60 min) on both ChiralPak AD and Chiracel OD columns, using either hexane:ethanol

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Table 1. Assay validation data based on 100 µL rat plasma

Expected enantiomer concentration ng/mL	KTZ enantiomer	Intraday mean ± SD (intraday CV %)			Interday, mean ± SD, ng/mL	Interday CV%	Interday mean error (%)
62.5	(+)	63.9 ± 4.36 (6.8)	65.3 ± 8.2 (12.5)	59.7 ± 9.7 (16.2)	63.0 ± 2.9	11.8	0.8
	(–)	65.3 ± 6.6 (10.0)	65.3 ± 7.1 (10.8)	55.9 ± 10.1 (18.0)	62.2 ± 5.4	12.9	–0.5
125	(+)	111 ± 11.6 (10.4)	130 ± 8.1 (6.2)	120.5 ± 9.7 (8.0)	121 ± 9.5	8.2	–3.4
	(–)	111 ± 21.3 (19.0)	130 ± 13.7 (10.6)	111 ± 3.5 (3.1)	118 ± 11	10.9	–6.0
500	(+)	476 ± 17.9 (3.8)	530 ± 15.3 (2.9)	517 ± 24.1 (4.7)	508 ± 28	3.8	1.6
	(–)	460 ± 14.3 (3.1)	529 ± 18.4 (3.5)	524 ± 29.0 (5.5)	505 ± 38	4.0	0.9
2500	(+)	2433 ± 82.5 (3.4)	2522 ± 44.1 (1.8)	2582 ± 164 (6.4)	2512 ± 72	3.9	0.5
	(–)	2464 ± 85.0 (3.5)	2515 ± 36.3 (1.4)	2549 ± 140 (5.5)	2510 ± 43	3.5	0.4

or hexane:2-propanol as mobile phase (Bernal *et al.*, 2002). Both authors reported better or similar resolution and shorter analytical run (~7 min) upon using SFC (Thienpont *et al.*, 1999; Bernal *et al.*, 2002). It was concluded by one group that SFC was the only technique that can provide appropriate enantiomeric separation for KTZ (Bernal *et al.*, 2002). However, our HPLC method provided sensitive and precise means of measuring KTZ enantiomers with sufficient resolution and sensitivity for application in biological specimens. Although the analytical run time was somewhat longer than determined by SFC (Bernal *et al.*, 2000, 2002), it was still entirely acceptable for an HPLC method (~18 min; Fig. 2).

The current method provided resolution parameters that were quite comparable and in some ways superior to those calculated from or reported in previously for SFC using similar chiral columns. Thienpont *et al.* reported an α of 1.3 and a resolution factor of 0.83. From the chromatograms presented by Bernal *et al.*, an α of 1.6 and a resolution factor of >2 were calculated (Thienpont *et al.*, 1999; Bernal *et al.*, 2000). In both cases our values of α and resolution factor were equivalent or superior. Both of the SFC methods were separation technique optimizations and were not tested for quantification; neither standard curves nor validation data were reported.

A capillary electrophoresis technique has been reported which was capable of measuring KTZ enantiomers in pharmaceutical formulations, and had superior separation qualities of 3 min elution time and resolution factor >2. However, the stated lower limit of detection was 250 ng/mL for each enantiomer and there was no mention of extraction from biological fluids, limiting its application to drug solutions only (Castro-Puyana *et al.*, 2005).

Several non-stereospecific analytical methods for the determination of KTZ in biological specimens have been reported. Using HPLC the non-stereospecific LLQ of KTZ ranged from 62.5 ng/mL using fluorescence detection, to 100 ng/mL using UV detection, based on 100 µL of human plasma (Alton, 1980; Yuen and Peh, 1998). In canine plasma, a LLQ of 15 ng/mL was reported using HPLC and UV detection based on 100 µL plasma (Vertzoni *et al.*, 2006). In rat plasma the LLQ ranged from 2 ng/mL using LC/MS to more than 200 ng/mL using UV detection based on 100 µL and 1 mL plasma, respectively (Riley and James, 1986; Huang *et al.*, 2007). Thus our reported LLQ is within the ranges reported using HPLC and UV detection in biological specimens.

In working up the method, we found that there was a need to incorporate a hexane wash step to remove an interfering peak from plasma which eluted at the initial part of the (+)-KTZ peak. Hexane was an excellent solvent for this purpose, as it caused virtually no

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extraction of KTZ enantiomers from the acetonitrile–aqueous phase. There was some loss of IS, but it did not have any adverse effects on linearity or validation parameters. We noticed that there was some noise in the baseline underlying the amiodarone peak, but due to the excess of amiodarone added, it similarly did not adversely affect KTZ enantiomer validation parameters. The optical rotation testing showed that (+)-KTZ is eluted first. This is in accordance with a reported preparative method for separation of KTZ enantiomers using the same HPLC column but different mobile phase (Gal *et al.*, 1993; Dilmaghanian *et al.*, 2004).

In vitro studies applying KTZ enantiomers to cytochrome P4503A4 using testosterone and methadone as substrates suggested stereoselective inhibition, wherein the (–)-KTZ enantiomer showed ~2 fold more inhibitory potency (Dilmaghanian *et al.*, 2004). In our report based on *C*max and AUC, we found that the plasma concentrations of (+)-KTZ were 2.1-2.4 fold higher than those of the (–)-KTZ, indicating a high level of stereoselectivity in KTZ pharmacokinetics *in vivo* after single oral doses. The t_{max} values of the two enantiomers were similar, indicating similar rates of absorption (Fig. 3).

(±)-KTZ plasma concentrations are reported to be much lower than in tissues (Riley and James, 1986). A previous report could not determine any measurable KTZ racemate plasma levels after 6 h of an i.v. administration of 5 mg/kg (±)-KTZ using nonstereospecific HPLC assay with 100 ng/mL LLQ based on 200 μL plasma (Remmel *et al.*, 1987). Our method could quantify KTZ enantiomer concentrations for 4 h post-dose using an oral dose of 10 mg/kg (±)-KTZ (Fig. 3). It is of note that (±)-KTZ is associated with nonlinear clearance (Sjoberg *et al.*, 1988; Matthew *et al.*, 1993), and therefore with repeated dosing it is likely that the time duration over which our assay could measure the drug would be longer than 4 h.

In conclusion, our method is the first to report separation and determination of KTZ enantiomers in plasma biological specimen. The method was sensitive and specific, with a lower limit of quantitation of 62.5 ng/mL, which is sufficient to permit use for pharmacokinetic studies involving rats.

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REFERENCES

Alton KB. Determination of the antifungal agent, ketoconazole, in human plasma by high-performance liquid chromatography. *Journal of Chromatography* 1980; **221**: 337.

Anonymous. FDA's policy statement for the development of new stereoisomeric drugs. *Chirality* 1992; **4**: 338.

Bernal JL, del Nozal MJ, Toribio L, Montequi MI and Nieto EM. Separation of ketoconazole enantiomers by chiral subcritical-fluid chromatography. *Journal of Biochemistry and Biophysics Methods* 2000; **43**: 241.

Bernal JL, Toribio L, del Nozal MJ, Nieto EM and Montequi MI. Separation of antifungal chiral drugs by SFC and HPLC: a comparative study. *Journal of Biochemistry and Biophysics Methods* 2002; **54**: 245.

Brocks DR. Drug disposition in three dimensions: an update on stereoselectivity in pharmacokinetics. *Biopharmaceutical Drug Disposition* 2006; **27**: 387.

Brocks DR and Jamali F. Stereochemical aspects of pharmacotherapy. *Pharmacotherapy* 1995; **15**: 551.

Castro-Puyana M, Crego AL and Marina ML. Enantiomeric separation of ketoconazole and terconazole antifungals by electrokinetic chromatography: rapid quantitative analysis of ketoconazole in pharmaceutical formulations. *Electrophoresis* 2005; **26**: 3960.

Como JA and Dismukes WE. Oral azole drugs as systemic antifungal therapy. *New England Journal of Medicine* 1994; **330**: 263.

Dilmaghanian S, Gerber JG, Filler SG, Sanchez A and Gal J. Enantioselectivity of inhibition of cytochrome P450 3A4 (CYP3A4) by ketoconazole: testosterone and methadone as substrates. *Chirality* 2004; **16**: 79.

Gal J, Aeschlimann C and Felix G. HPLC seperation of stereoisomersof ketoconazole, miconazole and itraconazole on the ChiralPak AD chiral stationary phase. *Fourth International Symposium on Chiral Discrimination*, Montreal, Canada, 1993; Abstract 130.

Huang Q, Wang GJ, Sun JG, Hu XL, Lu YH and Zhang Q. Simultaneous determination of docetaxel and ketoconazole in rat plasma by liquid chromatography/electrospray ionization tandem mass spectrometry. *Rapid Communications in Mass Spectrometry* 2007; **21**: 1009.

Jamali F, Mehvar R and Pasutto FM. Enantioselective aspects of drug action and disposition: therapeutic pitfalls. *Journal of Pharmaceutical Science* 1989; **78**: 695.

Korashy HM, Shayeganpour A, Brocks DR and El-Kadi AO. Induction of cytochrome P450 1A1 by ketoconazole and itraconazole but not fluconazole in murine and human hepatoma cell lines. *Toxicological Science* 2007; **97**: 32.

Lu H. Stereoselectivity in drug metabolism. *Expert Opinion on Drug Metabolism and Toxicology* 2007; **3**: 149.

Matthew D, Brennan B, Zomorodi K and Houston JB. Disposition of azole antifungal agents. I. Nonlinearities in ketoconazole clearance and binding in rat liver. *Pharmaceutical Research* 1993; **10**: 418.

Remmel RP, Amoh K and Abdel-Monem MM. The disposition and pharmacokinetics of ketoconazole in the rat. *Drug Metabolism and Disposition* 1987; **15**: 735.

Riley CM and James MO. Determination of ketoconazole in the plasma, liver, lung and adrenal of the rat by high-performance liquid chromatography. *Journal of Chromatography* 1986; **377**: 287.

Rotstein DM, Kertesz DJ, Walker KA and Swinney DC. Stereoisomers of ketoconazole: preparation and biological activity. *Journal of Medical Chemistry* 1992; **35**: 2818.

Sjoberg P, Ekman L and Lundqvist T. Dose and sex-dependent disposition of ketoconazole in rats. *Archives of Toxicology* 1988; **62**: 177.

Thienpont A, Gal J, Aeschlimann C and Felix G. Studies on stereoselective seperations of the azole antifungal drugs ketoconazole and itraconazole using HPLC and SFC on silica-based polysaccharides. *Analusis* 1999; **27**: 713.

Velikinac I, Cudina O, Jankovic I, Agbaba D and Vladimirov S. Comparison of capillary zone electrophoresis and high performance liquid chromatography methods for quantitative determination of ketoconazole in drug formulations. *Farmaco* 2004; **59**: 419.

Vertzoni MV, Reppas C and Archontaki HA. Optimization and validation of a high-performance liquid chromatographic method with UV detection for the determination of ketoconazole in canine plasma. *Journal of Chromatography B: Analytical Technology and Biomedical Life Sciences* 2006; **839**: 62.

Yuen KH and Peh KK. Simple high-performance liquid chromatographic method for determination of ketoconazole in human plasma. *Journal of Chromatography B: Biomedical Science Applications* 1998; **715**: 436.