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High performance liquid chromatographic assay for the simultaneous determination of midazolam and ketoconazole in plasma

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ABSTRACT

A high performance liquid chromatographic (HPLC) assay was developed for the simultaneous quantitation of midazolam (MDZ) and ketoconazole (KTZ) in plasma. MDZ, KTZ and diazepam (internal standard) were extracted from 100 μL or 500 μL plasma from rat or human, respectively, using liquid–liquid extraction with diethyl ether in the presence of 0.1N NaOH. After vortexing, centrifugation and freezing, the organic layer was transferred to clean tubes and evaporated. The dried residue was reconstituted in mobile phase and injected into the HPLC through a C18 column. The mobile phase consisted of acetonitrile:15 mM potassium dihydrogen orthophosphate (45:55, v/v), pumped at 1 mL/min and measured at λ = 220 nm. The method was tested in a pharmacokinetic study involving orally dosed KTZ 40 mg/kg in 1% methylcellulose followed by intravenous dosing of 5 mg/kg MDZ to rats 1.5 h latter. The components eluted within 10 min and were baseline resolved with no interferences from endogenous substances in plasma. The calibration curves were linear (r^2 = 0.999) over the range of 25–25,000 and 5–10,000 ng/mL of KTZ and MDZ in rat and human plasma, respectively. The intraday and interday CV% were <15% and <6% for KTZ and <7% and <4% for MDZ and the mean error was <13% for both drugs in rat plasma. In human plasma the intraday CV% and % error of the mean were <11% and <10% for KTZ, respectively; both values were <13% for MDZ. The validated lower limit of quantitation was 25 and 5 ng/mL for both drugs based on 100 μL rat plasma and 500 μL human plasma, respectively. In rats, plasma concentrations of MDZ and KTZ were simultaneously measured up to 8 and 9.5 h, respectively. In conclusion, the assay was shown to be rapid, sensitive and appropriate for use in drug–drug interaction studies involving MDZ and KTZ in rat, and potentially in humans.

1. Introduction

Cytochrome P450 3A (CYP3A) constitutes the most abundant subfamily in human liver and intestine. It facilitates the biotransformation of approximately half of all currently used therapeutic agents [1]. Since most pharmacokinetic drug interactions occur when two drugs share a common clearance pathway involving a drug metabolizing enzyme or transporter, the CYP3A family is frequently implicated in drug–drug interactions [2]. CYP3A-mediated inhibition of drug metabolism has been associated with some severe adverse effects prompting the withdrawal of some drugs from the market [3]. Consequently as part of safety assessment in drug development it is necessary to perform clinical drug interaction studies with CYP3A4 in mind [4]. Ketoconazole (KTZ) is a broad spectrum azole antifungal used for systemic and local infections, is widely used as a prototypical inhibitor of CYP3A for such studies [4].

The inhibitory effect of KTZ on CYP3A is thought to be attributed to two binding events, binding of the N-3 imidazole nitrogen to the heme iron, and the lipophilic portion of KTZ to a hydrophobic region of the enzyme protein [5]. KTZ is a chiral drug that is administered as a racemic (1:1) mixture of enantiomers of the cis configuration. Stereoselectivity has been shown between its enantiomers in pharmacokinetics and their CYP inhibitory potency [5–7]. Where the (−)-KTZ enantiomer showed lower plasma concentrations after i.v. and oral dosing of racemate [6], and displayed ~2-fold more inhibitory potency upon applying the enantiomers to human CYP3A4 supersomes using testosterone and methadone as substrates [5]. Although it is often believed to be a specific inhibitor of CYP3A isoforms, evidence is available that it can also inhibit some other CYP at concentrations expected in vivo [8].

Midazolam (MDZ) is a short acting benzodiazepine which undergoes extensive hepatic and gastrointestinal presystemic extraction [9]. Its clearance and oral bioavailability are primarily governed by CYP3A, and consequently, it is often used as a probe for measuring CYP3A activity [10]. The hydroxylation of MDZ has been

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used as a valuable tool for the measurement of metabolic activity of the CYP3A subfamily in both rats (CYP3A1/2) [2,11], and humans (CYP3A4/5) [12].

KTZ–MDZ drug–drug interaction assessments have been often reported in the literature [2,13]. The focus of these studies have involved comparisons of the inhibitory potency of KTZ to other azoles or CYP3A inhibitors [14], assessments of the ability of KTZ to affect MDZ pharmacokinetics/pharmacodynamics [9,15], and attempts to develop in vivo models for prediction of interactions for CYP3A4 substrates [2]. Most such studies have focused on the measurement of MDZ and/or its metabolites, and have ignored the kinetics of the inhibitor (e.g. KTZ). Thus most of the analytical methods used and developed for such studies were designed to measure either MDZ alone [16], MDZ hydroxylated metabolites alone [17], or MDZ and its hydroxy metabolites [18]. In the few cases where both analytes have been quantified, separate assays for the determination of MDZ and KTZ (and other inhibitors) have been used [14].

Measuring the blood/plasma concentrations of the probe substrate is valid in many cases where the only variable introduced in the study design is that of the probe and the inhibitor. The simultaneous measurement of both substrate and inhibitor, however, is advantageous when studying the drug interaction in presence of a third factor, such as a disease state. In such a situation, the disease state could conceivably alter the disposition and hence the inhibitory potency of the inhibitor. An analytical method capable of simultaneously measuring both probe substrate and inhibitor would be a valuable tool in such cases, facilitating the measurement of pharmacokinetics using a single blood draw for each time point with no need to split the sample for processing through two assay methods. In serial blood collection studies involving rats, in particular, this is an important consideration due to the need to keep cumulative blood volume withdrawal to a minimum.

There are some reported methods for the simultaneous determination of both MDZ and KTZ, but these utilize LC–MS or LC–MS/MS instrumentation [2,13]. Although these methods are more specific and sensitive than HPLC-UV assays, and provide very low limits of detection [2], the essential equipment may not be available in many laboratories. Therefore, in this report we describe a simple and rapid reverse phase HPLC-UV method for the simultaneous determination of MDZ and (±)-KTZ in plasma using a commercially available internal standard.

2. Experimental

2.1. Materials and reagents

(±)-Ketoconazole was obtained from Sigma (St. Louis, MO, USA). Diethyl ether, acetonitrile (all HPLC grade), and potassium dihydrogen orthophosphate (KH_2PO_4) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Midazolam (5 mg/mL) and diazepam (5 mg/mL) as sterile injectable solutions were manufactured by Sandoz (Boucherville, QC, Canada) and purchased from the University of Alberta Hospitals (Edmonton, Alberta, Canada).

2.2. Chromatographic conditions

The chromatography system consisted of a Waters 710B WISP auto-injector, Waters 510 pump (Waters, Milford, MA, USA), and a HP 1050 UV detector (Hewlett Packard, Palo Alto, CA, USA). The UV wavelength was set at 220 nm. The chromatographic separation of midazolam, ketoconazole and diazepam (IS) was accomplished using a Symmetry C18 analytical column, 3.5 μm, 150 mm × 4.6 mm (Waters, MA, USA) attached to a C18 2 cm × 4.0 mm, 5 μm guard column (Supelco, PA, USA). Chromatographic data were collected and compiled by use of EZChrom software (Scientific Software, Pleasanton, CA, USA). The mobile phase used was a 45:55 (v/v) mixture of acetonitrile:15 mM KH_2PO_4. The mobile phase was pumped isocratically at 1 mL/min.

2.3. Standard and stock solutions

A 100 mg/L stock solution of KTZ was prepared by dissolving 10 mg of KTZ in 100 mL methanol. A 100 mg/L stock solution of MDZ was prepared by dilution of 2 mL of (5 mg/mL) MDZ vial to 100 mL of methanol. A 40 mg/L stock solution of diazepam was prepared by dilution of 2 mL of (5 mg/mL) diazepam vial to 250 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 and MDZ were prepared by successive 1/10 dilutions of the stock solution with methanol. The diazepam, KTZ and MDZ stocks and working solutions were stored at 2 °C between uses.

2.4. Extraction procedure

The IS (0.05 mL) was added to each 0.1 mL rat plasma or 0.5 mL human plasma sample in a glass test tube. To rat plasma, 0.2 mL of 0.1N NaOH and 4 mL of diethyl ether were added whereas, to the human plasma 0.2 mL 0.1N NaOH and 5 mL diethyl ether were added. Because methanol was present in the standard curve samples, an equivalent amount was added to the test samples as well (0.2 mL). The tubes were vortex mixed (1 min) at high speed then subsequently centrifuged for 5 min at ~2500 × *g*. The tubes were then covered and placed in a −30 °C freezer for 5–7 min. The organic layer was transferred to new glass tubes and evaporated to dryness *in vacuo*. The residues were reconstituted in 200 μL mobile phase of which 75–125 μL volumes were injected into the HPLC.

2.5. Recovery

The plasma recoveries were determined at MDZ and KTZ concentrations of 250 and 5000 ng/mL in rat plasma, and 100 ng/mL in human plasma, using four replicates for each concentration. Recovery of the IS was conducted at 20 μg/mL. 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.

2.6. Calibration, accuracy and validation

Calibration curves were constructed using samples of 0.1 mL rat and human plasma containing KTZ, MDZ and IS. The curve ranged from 25 to 25,000 ng/mL of each drug in rat plasma, and 5 to 10,000 ng/mL in human plasma. The ratios of KTZ and MDZ peaks to IS peak height were calculated and plotted vs. the expected KTZ or MDZ concentrations. Owing to the wide range of concentrations, the calibration curve data were weighed by a factor of 1/KTZ and $1/MDZ^2$ concentration.

Intraday accuracy and precision of the assay were determined using five sample replicates of 25, 100, 1000 and 10,000 ng/mL in rat plasma; to permit for a measure of interday accuracy and precision, the assay was likewise repeated on two other days. In human plasma the assay was validated for intraday accuracy and precision as for rat but using concentrations of 5, 10, 100, 200 and 10,000 ng/mL of both analytes. 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. The stability of MDZ and KTZ in working solutions and plasma has been previously described extensively [19–21].

2.7. Assessment of chromatographic separation

The capacity factor (K') was calculated by use of the equation $K' = (\mathrm{tr} - \mathrm{tm})/\mathrm{tm}$, where tr and tm 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.

2.8. Demonstration of assay utility

To assess the applicability of this method in vivo, two rats (250–300 g) were administered 40 mg/kg KTZ orally followed by 5 mg/kg MDZ i.v. after 1.5 h of oral dosing. 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 Silastic® Laboratory Tubing (Dow Corning Corporation, Midland, MI, 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 0.083, 0.33, 0.67, 1, 1.5, 2, 3, 4, and 8 h post i.v. MDZ dose. Plasma was separated by centrifugation of the blood at 2500 × *g* for 3 min. The samples were kept at −30 °C until assayed.

2.9. Data and statistical analysis

Non-compartmental methods were used to calculate the pharmacokinetic parameters. The elimination rate constant (λz) was calculated by subjecting the plasma concentrations in the terminal phase to linear regression analysis. The $t½$ was calculated by dividing 0.693 by λz. The $AUC_{0-\infty}$ was calculated using the combined log-linear trapezoidal rule from time 0 h postdose to the time of the last measured concentration, plus the quotient of the last measured concentration divided by λz. The concentration at time 0 h after i.v. dosing was estimated by back extrapolation of the log-linear regression line using the first three measured plasma concentrations to time 0. The CL was calculated as the quotient of dose to $AUC_{0-\infty}$. All compiled data were reported as mean ± SD, unless otherwise indicated.

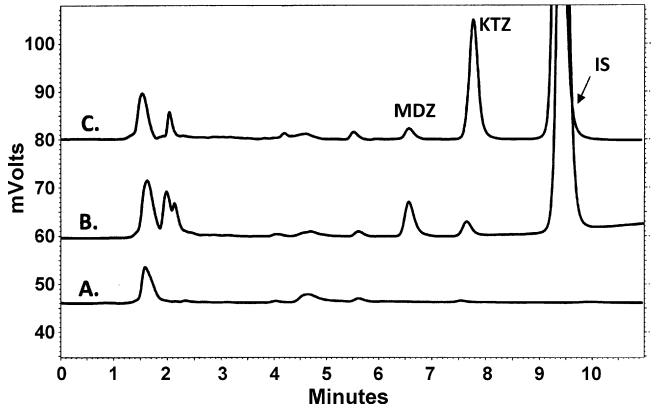

Fig. 1. Chromatograms of (A) blank rat plasma sample, (B) rat plasma spiked with 1000 ng/mL of KTZ and MDZ, and (C) rat plasma obtained after 1 h of i.v. MDZ administration (1.5 h of KTZ administration).

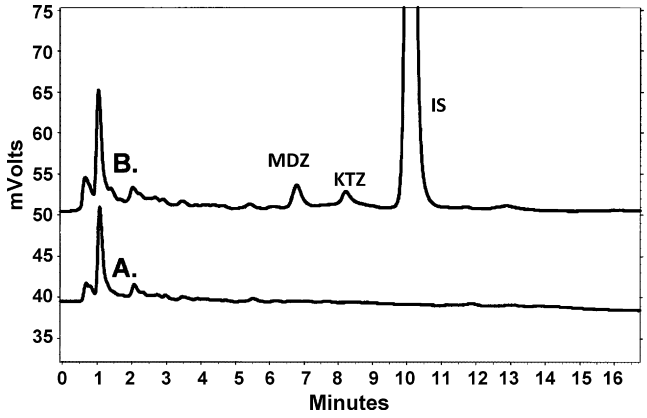

Fig. 2. Chromatograms of (A) blank human plasma sample and (B) human plasma spiked with 100 ng/mL of KTZ and MDZ.

3. Results

The MDZ, KTZ and IS eluted chromatographically at approximately 6.4, 7.5 and 9.1 min, respectively, in rat plasma and at approximately 7.2, 8.5 and 10.2 min, respectively, in human plasma (Figs. 1 and 2). All analyte peaks were symmetrical in appearance with baseline resolution with no interferences from endogenous substances in plasma. The total analytical run time was ~10 min for rat plasma and 19 min for human plasma. The column separation factor (α) and resolution factor for MDZ and KTZ were calculated to be 1.2 and 2.1, respectively. The column capacity factors (K') for MDZ, KTZ and IS were calculated to be 3.6, 4.3 and 5.5, respectively. The symmetry indexes were 1.2, 1.1 and 1.0 for the MDZ, KTZ and diazepam, respectively.

The recoveries of KTZ and MDZ and diazepam from plasma were complete (~100%) in both human and rat plasma. Highly linear relationships were noted between the peak height or area ratios of analyte:IS ranging from 25 to 25,000 and 5 to 10,000 ng/mL in rat and human plasma, respectively. The r^2 for human plasma was 0.999 and 0.9984 for MDZ and KTZ, respectively. The mean r^2 for the three standard curves in rat plasma were ≥0.999 for each drug.

The validation data showed the assay to be sensitive, accurate and precise, with the intraday and interday CV% less than or equal to 5.8% and 3.4%, respectively, for MDZ and KTZ (Tables 1 and 2). The mean interday error in rat plasma was less than 13% for both drugs. The intraday CV% and % error of the mean were less than 13% for both drugs in human plasma (Tables 1 and 2). Since both CV% of interday and intraday assessment and interday mean error yielded values less than 20% at the lowest concentration tested, the lower limit of quantitation (LLQ) based on 100 μL of rat plasma was 25 ng/mL, and based on 500 μL of human plasma, 5 ng/mL for both analytes.

In rats dosed with 40 mg/kg KTZ orally followed 1.5 h latter by 5 mg/kg MDZ i.v., KTZ plasma concentrations were higher than those of MDZ at all time points (Fig. 3). The assay managed to measure both KTZ and MDZ concentrations up to 8 h post MDZ administration. Table 3 shows the pharmacokinetic parameters for both drugs.

Table 1
Validation data for midazolam in 100 μL rat plasma and 500 μL human plasma.

Expected concentration (ng/mL)	Intraday mean ± SD (intraday CV%)			Interday mean ± SD (ng/mL)	Interday CV%	Interday mean error %
Rat plasma						
25	24.8 ± 1.32 (5.32)	23.2 ± 2.17 (2.18)	24.4 ± 1.50 (6.13)	24.1 ± 0.8	3.4	−3.5
100	109 ± 2.94 (2.70)	111 ± 1.58 (1.42)	116 ± 4.62 (3.98)	112 ± 3.6	3.2	12.3
1000	1028 ± 10.86 (1.06)	1034 ± 15.2 (1.47)	1009 ± 6.10 (0.61)	1023 ± 13	1.3	2.4
10,000	9665 ± 49.7 (0.51)	9592 ± 210 (2.19)	9499 ± 70.5 (0.74)	9585 ± 83	0.8	−4.1
Human plasma						
5	5.62 ± 0.70 (12.3)					
10	10.5 ± 1.24 (11.8)					
100	99.5 ± 5.32 (5.3)					
200	207 ± 3.09 (1.49)					
10,000	9358 ± 212 (2.26)					

Table 2
Validation data for ketoconazole in 100 μL rat plasma and 500 μL human plasma.

Expected concentration (ng/ml)	Intraday mean ± SD (intraday CV%)			Interday mean ± SD (ng/mL)	Interday CV%	Interday mean error %
Rat plasma						
25	25.2 ± 1.52 (6.02)	27.2 ± 3.86 (14.2)	27.4 ± 2.07 (7.54)	26.6 ± 1.2	4.6	6.5
100	103 ± 2.40 (2.34)	96.6 ± 3.95 (4.09)	109 ± 2.32 (2.13)	103 ± 6.0	5.8	2.6
1000	967 ± 29.5 (3.06)	1025 ± 18.0 (1.75)	1010 ± 24.1 (6.99)	1001 ± 30	3.0	0.08
10,000	9900 ± 425 (4.30)	10149 ± 138 (1.35)	9854 ± 78.6 (0.80)	9968 ± 158	1.6	−0.3
Human plasma						
5	4.53 ± 0.50 (10.9)					
10	10.8 ± 0.92 (11.0)					
100	110 ± 7.83 (7.14)					
200	196 ± 12.3 (5.3)					
10,000	10456 ± 194 (1.86)					

4. Discussion

The described assay was capable of the simultaneous quantitation of KTZ and MDZ in plasma. It is simple, rapid, sensitive and efficient for pharmacokinetics studies involving both drugs with less numbers of blood sampling taken from rats. The extraction solvents used by most of the HPLC reports for MDZ include using either diethyl ether [18,22], ethyl acetate [23], n-hexane [14] or diethyl ether/methylene chloride [24], while those used for KTZ extraction include the addition of just acetonitrile for protein precipitation [15], tertiary-butyl methyl ether [6] and diethyl ether [25]. The combination of acetonitrile and phosphate in different concentrations and proportions were previously used for the determination of each drug alone [14,26]. For the current method we found diethyl ether to be an ideal extraction solvent, and acetonitrile:15 mM KH_2PO_4 (45:55, v/v) to be a suitable mobile phase.

For the extraction procedure, this report used diazepam as an internal standard, and was based on the procedures described by Jurica et al. and Carrillo et al. for MDZ, with minor modifications [18,22]. The rat and human plasma volumes required in our assay, however, were only 100 and 500 μL compared to 450 μL by Jurica et al. and 1 mL by Carrillo et al., respectively. This lower volume permits the use of serial blood sampling in studies involving a small animal species, the rat. The total sample preparation time was decreased by about 40 min. Additionally, an improved lower limit of quantitation for MDZ (25 and 5 ng/mL based on 100 and 500 μL rat and human plasma, respectively) was achieved compared to 50 ng/mL based on 450 μL rat plasma [18] and 40 ng/mL based on 1 mL human plasma [22]. We must recognize, however,

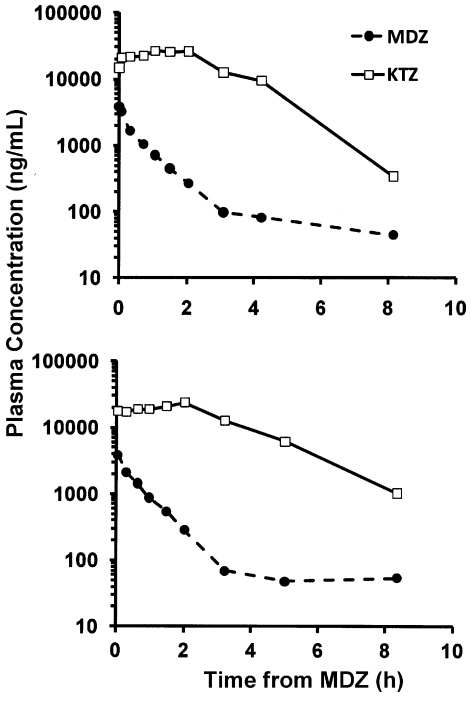

Fig. 3. Plasma KTZ and MDZ concentration vs. time curve taken from two rats given 40 mg/kg KTZ orally followed 1.5 h later by 5 mg/kg MDZ i.v.

Table 3
Plasma pharmacokinetic parameters of midazolam and ketoconazole after co-administration in individual rats.

	Rat 1		Rat 2	
	KTZ	MDZ	KTZ	MDZ
$AUC_{0-\infty}$ (mg h/L)	97.7	2.96	105	2.89
t½ (h)	1.40	3.43	0.83	4.46
CL (L/h/kg)	–	1.69	–	1.73
Vdss (L/kg)	–	2.66	–	4.53
Cmax (mg/L)	18.8	–	26.5	–
Tmax (h)	2.1	–	2.6	–

that the other two assays were not focused on quantitation of MDZ and KTZ, but rather determination of the MDZ and its hydroxylated metabolites.

The chromatographic run times reported for MDZ determination for other comparable assay methods range from 7.5 to 25 min [22,27]. For KTZ, run times have ranged from 10 to over 20 min [6,28]. Thus our analytical run time compares well to the reported ranges of the other methods. The sensitivity of our method represented with LLQ of 25 and 5 ng/mL in rat and human plasma, respectively, compares favorably to that reported for both medications using HPLC systems [9,29]. It was demonstrated to be of use in the study of MDZ and KTZ disposition in rat for up to 8 h after i.v. MDZ; this is similar to the available LC–MS and LC–MS/MS methods [2,13]. The published LC–MS/MS method for the simultaneous determination of MDZ and KTZ, although more sensitive with lower LLQ, did not state the volume of plasma assayed nor the internal standard used in the assay [2]. The LC–MS method described by Ogasawara et al., on the other hand, used a similar volume of 100 μL plasma (from monkeys), and a commercially available IS (reserpine) [13].

One limitation of the described method is that it is non-stereoselective and measures the sum of the (+) and (−)-KTZ enantiomer concentrations. This is potentially relevant because stereoselectivity in its pharmacokinetics and pharmacodynamics has been demonstrated [5,7]. In rats, the (+)-KTZ plasma concentrations were 2.5-fold higher than (−)-KTZ. In addition that (−)-KTZ displayed ∼2-fold more inhibitory potency upon applying the enantiomers to human CYP3A4 supersomes using testosterone and methadone as substrates [5]. Despite this limitation, the assay is still of use because of the very low number of assays that can simultaneously measure both KTZ and MDZ. In cases where it is desired to determine knowledge of plasma concentrations of KTZ enantiomers, use of a separate stereoselective assay is needed. To date there is only one HPLC assay reported for stereospecific measurement of KTZ enantiomers [6]. The assay was well suited for a pharmacokinetic study involving the interaction of (±)-KTZ and MDZ. The MDZ clearance in the presence of KTZ was found to be 28.5 and 28.9 mL/min/kg for the two rats, which is quite similar to a previously reported value of 29.8 mL/min/kg in presence of 20 mg/kg KTZ [2]. The $AUC_{0-\infty}$ of KTZ after 40 mg/kg oral dosing for the two rats was quite close to the values obtained (∼80 mg h/L) after the summation of the two KTZ enantiomers and in the absence of midazolam (Table 3) [6].

Because dose levels of the drugs are typically lower in human than animal studies, in human subjects there is a need for a greater assay sensitivity [9,30]. Furthermore, larger blood volumes can be drawn from humans than rats. Consequently a larger plasma volume of 500 μL plasma was used for human samples. As in rat, the extraction efficiency remained high (∼100%) in the human plasma. Because in the human plasma there were some later eluting peaks at 12 and 13 min, an extended run time of 19 min was needed (Fig. 2). As the validation data showed (Tables 1 and 2) the described method is potentially useful in examining human pharmacokinetic interactions involving MDZ and KTZ.

In conclusion, we have described a reverse phase HPLC-UV method for the simultaneous determination of KTZ and MDZ in plasma. It is simple, sensitive, and suitable for the quantitation of both drugs in drug–drug interaction pharmacokinetic studies in rats, and possibly in humans.

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References

[1] S. Rendic, Summary of information on human CYP enzymes: human P450 metabolism data, Drug Metab. Rev. 34 (2002) 83–448.
[2] S.V. Mandlekar, A.V. Rose, G. Cornelius, B. Sleczka, C. Caporuscio, J. Wang, P.H. Marathe, Development of an in vivo rat screen model to predict pharmacokinetic interactions of CYP3A4 substrates, Xenobiotica 37 (2007) 923–942.
[3] C.L. Alfaro, Emerging role of drug interaction studies in drug development: the good, the bad, and the unknown, Psychopharmacol. Bull. 35 (2001) 80–93.
[4] United States Food and Drug Adminitration, d.a., Draft guidance for industry: drug interaction studies—study design, and implications for dosing and labeling, http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM072101.pdf, in 2006 (accessed 5 January 2010).
[5] S. Dilmaghanian, J.G. Gerber, S.G. Filler, A. Sanchez, J. Gal, Enantioselectivity of inhibition of cytochrome P450 3A4 (CYP3A4) by ketoconazole: testosterone and methadone as substrates, Chirality 16 (2004) 79–85.
[6] D.A. Hamdy, D.R. Brocks, A stereospecific high-performance liquid chromatographic assay for the determination of ketoconazole enantiomers in rat plasma, Biomed. Chromatogr. 22 (2008) 542–547.
[7] D.A. Hamdy, D.R. Brocks, Nonlinear stereoselective pharmacokinetics of ketoconazole in rat after administration of racemate, Chirality 21 (2009) 704–712.
[8] M.E. Elsherbiny, A.O. El-Kadi, D.R. Brocks, The metabolism of amiodarone by various CYP isoenzymes of human and rat, and the inhibitory influence of ketoconazole, J. Pharm. Pharm. Sci. 11 (2008) 147–159.
[9] S.M Tsunoda, R.L. Velez, L.L. von Moltke, D.J. Greenblatt, Differentiation of intestinal and hepatic cytochrome P450 3A activity with use of midazolam as an in vivo probe: effect of ketoconazole, Clin. Pharmacol. Ther. 66 (1999) 461–471.
[10] K.E. Thummel, D.D. Shen, T.D. Podoll, K.L. Kunze, W.F. Trager, C.E. Bacchi, C.L. Marsh, J.P. McVicar, D.M. Barr, J.D. Perkins, et al., Use of midazolam as a human cytochrome P450 3A probe. II. Characterization of inter- and intraindividual hepatic CYP3A variability after liver transplantation, J. Pharmacol. Exp. Ther. 271 (1994) 557–566.
[11] M.M. Cotreau, L.L. von Moltke, M.C. Beinfeld, D.J. Greenblatt, Methodologies to study the induction of rat hepatic and intestinal cytochrome P450 3A at the mRNA, protein, and catalytic activity level, J. Pharmacol. Toxicol. Methods 43 (2000) 41–54.
[12] J.F Rogers, M.L. Rocci Jr., D.B. Haughey, J.S. Bertino Jr., An evaluation of the suitability of intravenous midazolam as an in vivo marker for hepatic cytochrome P4503A activity, Clin. Pharmacol. Ther. 73 (2003) 153–158.
[13] A. Ogasawara, T. Kume, E. Kazama, Effect of oral ketoconazole on intestinal first-pass effect of midazolam and fexofenadine in cynomolgus monkeys, Drug Metab. Dispos. 35 (2007) 410–418.
[14] K. Yamano, K. Yamamoto, H. Kotaki, Y. Sawada, T. Iga, Quantitative prediction of metabolic inhibition of midazolam by itraconazole and ketoconazole in rats: implication of concentrative uptake of inhibitors into liver, Drug Metab. Dispos. 27 (1999) 395–402.
[15] M. Kuroha, A. Azumano, Y. Kuze, M. Shimoda, E. Kokue, Effect of multiple dosing of ketoconazole on pharmacokinetics of midazolam, a cytochrome P-450 3A substrate in beagle dogs, Drug Metab. Dispos. 30 (2002) 63–68.
[16] N Van Brandt, P. Hantson, P. Mahieu, R.K. Verbeeck, A rapid high-performance liquid chromatographic method for the measurement of midazolam plasma concentrations during long-term infusion in ICU patients, Ther. Drug Monit. 19 (1997) 352–357.
[17] R.M. Arendt, D.J. Greenblatt, W.A. Garland, Quantitation by gas chromatography of the 1- and 4-hydroxy metabolites of midazolam in human plasma, Pharmacology 29 (1984) 158–164.
[18] J. Jurica, M. Dostalek, J. Konecny, Z. Glatz, E. Hadasova, J. Tomandl, HPLC determination of midazolam and its three hydroxy metabolites in perfusion medium and plasma from rats, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 852 (2007) 571–577.
[19] M.J. Ahsman, B.C. van der Nagel, R.A. Mathot, Quantification of midazolam, morphine and metabolites in plasma using 96-well solid-phase extraction and ultra-performance liquid chromatography–tandem mass spectrometry, Biomed, Chromatogr (Jan 14th 2010).
[20] B. Link, M. Haschke, M. Wenk, S. Krahenbuhl, Determination of midazolam and its hydroxy metabolites in human plasma and oral fluid by liquid chromatography/electrospray ionization ion trap tandem mass spectrometry, Rapid Commun. Mass Spectrom. 21 (2007) 1531–1540.
[21] Q. Huang, G.J. Wang, J.G. Sun, X.L. Hu, Y.H. Lu, Q. Zhang, Simultaneous determination of docetaxel and ketoconazole in rat plasma by liquid chromatography/electrospray ionization tandem mass spectrometry, Rapid Commun. Mass Spectrom. 21 (2007) 1009–1018.
[22] J.A. Carrillo, S.I. Ramos, J.A. Agundez, C. Martinez, J. Benitez, Analysis of midazolam and metabolites in plasma by high-performance liquid chromatography: probe of CYP3A, Ther. Drug Monit. 20 (1998) 319–324.
[23] R. Kato, S. Yamashita, J. Moriguchi, M. Nakagawa, Y. Tsukura, K. Uchida, F. Amano, Y. Hirotani, Y. Ijiri, K. Tanaka, Changes of midazolam pharmacokinetics in Wistar rats treated with lipopolysaccharide: relationship between total CYP and CYP3A2, Innate Immun. 14 (2008) 291–297.
[24] S. Kurosawa, S. Uchida, Y. Ito, S. Yamada, Effect of ursodeoxycholic acid on the pharmacokinetics of midazolam and CYP3A in the liver and intestine of rats, Xenobiotica 39 (2009) 162–170.

[25] R.J. Rodriguez, D. Acosta Jr., Metabolism of ketoconazole and deacetylated ketoconazole by rat hepatic microsomes and flavin-containing monooxygenases, Drug Metab. Dispos. 25 (1997) 772–777.
[26] M. de Diego, G. Godoy, S. Mennickent, Chemical stability of midazolam injection by high performance liquid chromatography, J. Sep. Sci. 30 (2007) 1833–1838.
[27] N. Yasui-Furukori, Y. Inoue, T. Tateishi, Sensitive determination of midazolam and 1′-hydroxymidazolam in plasma by liquid–liquid extraction and column-switching liquid chromatography with ultraviolet absorbance detection and its application for measuring CYP3A activity, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 811 (2004) 153–157.
[28] M.V. Vertzoni, C. Reppas, H.A. Archontaki, Optimization and validation of a high-performance liquid chromatographic method with UV detection for the determination of ketoconazole in canine plasma, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 839 (2006) 62–67.
[29] E.J. Portier, K. de Blok, J.J. Butter, C.J. van Boxtel, Simultaneous determination of fentanyl and midazolam using high-performance liquid chromatography with ultraviolet detection, J. Chromatogr. B: Biomed. Sci. Appl. 723 (1999) 313–318.
[30] V. Andersen, N. Pedersen, N.E. Larsen, J. Sonne, S. Larsen, Intestinal first pass metabolism of midazolam in liver cirrhosis—effect of grapefruit juice, Br. J. Clin. Pharmacol. 54 (2002) 120–124.