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A developed determination of midazolam and 1′-hydroxymidazolam in plasma by liquid chromatography–mass spectrometry: Application of human pharmacokinetic study for measurement of CYP3A activity

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Abstract

This paper describes sensitive and reliable determination of midazolam (MDZ) and its major metabolite 1′-hydroxymidazolam (1-OHMDZ) in human plasma by liquid chromatography–mass spectrometry (LC–MS) with a sonic spray ionization (SSI) interface. MDZ, 1-OHMDZ and diazepam as an internal standard were extracted from 1 ml of alkalinized plasma using *n*-hexane–chloroform (70:30, v/v). The extract was injected into an analytical column (YMC-Pak Pro C_{18}, 50 mm × 2.0 mm i.d.). The mobile phase for separation consisted of 10 mM ammonium acetate and methanol (50:50, v/v) and was delivered at a flow-rate of 0.2 ml/min. The drift voltage was 100 V. The sampling aperture was heated at 120 °C and the shield temperature was 260 °C. The total time for chromatographic separation was less than 16 min. The validated concentration ranges of this method were 0.25–50 ng/ml for both MDZ and 1-OHMDZ. Mean recoveries were 93.6% for MDZ and 86.6% for 1-OHMDZ. Intra- and inter-day coefficient variations were less than 6.5 and 5.5% for MDZ, and 6.1 and 5.7% for 1-OHMDZ at 0.3, 4, 20 and 40 ng/ml. The limits of quantification were 0.25 ng/ml for both MDZ and 1-OHMDZ. This method was sensitive and reliable enough for pharmacokinetic studies on healthy volunteers, and was applied for the measurement of CYP3A activity in humans after an intravenous (1 mg) and a single-oral administration (2 mg) of subtherapeutic MDZ dose.

Keywords: Midazolam; 1′-Hydroxymidazolam; CYP3A; LC–MS

1. Introduction

Cytochrome P450 (CYP) 3A subfamily, including CYP3A4, CYP3A5 and CYP3A7, is responsible for the metabolism of more one-third of drugs [1]. Wide inter-individual variations in intestinal and hepatic CYP3A activity are seen in the human population. Furthermore, the relationship between their pharmacokinetics and pharmacodynamics depends on the CYP3A activity [2]. Therefore, an in vivo marker to predict the CYP3A activity would be needed to determine its clinical significance and perform drug interaction studies in relation to CYP3A [3,4].

Midazolam (MDZ) is a short-acting benzodiazepine that is used for conscious sedation and induction of general anesthesia [5]. In humans, MDZ is extensively metabolized by CYP3A isozymes (CYP3A4/CYP3A5) to one major metabolite, 1′-hydroxymidazolam (1-OHMDZ) and to a lesser extent, 4-hydroxymidazolam [6]. The plasma clearance of MDZ [7] and 1-OHMDZ/MDZ plasma concentration ratio [8] have been used as an in vivo marker of CYP3A activity since 4-hydroxymidazolam is generally not detectable in human plasma [9]. The administration of intravenous MDZ is available to selectively assess the hepatic CYP3A activity, whereas orally administered MDZ is subject to both intestinal and hepatic CYP3A metabolism. Therefore, simultaneous intravenous and oral administration of MDZ and 1-OHMDZ could be used to examine the contribution of both intestinal and hepatic CYP3A [4,10,11]. The United

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States Food and Drug Administration advocates the use of both intravenous and oral administration of MDZ for the purpose of measuring CYP3A activity [12].

For measuring CYP3A activity in humans, the analytical method needs to be sensitive and reliable enough to evaluate the pharmacokinetics of MDZ and 1-OHMDZ. Previous analytical methods have been reported for simultaneous determination of MDZ and 1-OHMDZ in human plasma with different detection systems [7,13–29]. Among them, the limits of quantifications (LOQ) in high-performance liquid chromatography with UV detection (LC/UV) [7,13–17] and gas chromatography (GC) [18–20] methods were over 2 ng/ml for MDZ and 1-OHMDZ, and this was not adequate to characterize the pharmacokinetics of both compounds. A previous LC/UV method in our laboratory [21] had LOQ of 0.5 ng/ml for both compounds that was sensitive enough to evaluate the pharmacokinetics of MDZ and 1-OHMDZ, but it required 2 ml plasma. In addition, GC–mass spectrometry (MS) [22,23] appeared to be more sensitive than LC/UV and GC, but required lengthy extraction and derivation procedures before chromatographic analysis. Although LC–MS has replaced other detection systems, these methods also have various drawbacks, including large sample volume (2 ml) [24], uneconomical fast gradient LC procedure [24], use of expensive solid phase extraction cartridges [25], column switching procedure requiring an additional system [25], and inadequate sensitivity (LOQ; more than 1 ng/ml) [26,27]. The analytical method described by Shiran et al. has LOQ of 0.65 and 0.68 ng/ml for MDZ and 1-OHMDZ, but calibration curves for both compounds were constructed over the 6.5–208 ng/ml for application to human plasma [28]. A current LC–MS/MS assay had LOQ of 0.1 ng/ml using liquid–liquid extraction procedure from 1 ml plasma sample [29]. However, this method was applied to determine the plasma concentration of MDZ and 1-OHMDZ after a large oral dose of MDZ (15 mg) to human, but not a low dose. Therefore, an analytical method to measure CYP3A activity in patients receiving the lower dose of MDZ may be needed.

This paper describes a sensitive and reliable determination of MDZ and 1-OHMDZ in human plasma by LC–MS with a sonic spray ionization (SSI) interface, which was validated according to FDA Guidelines [30]. Additionally, this assay was suitable for measuring CYP3A activity in human volunteers following an intravenous (1 mg) and an oral administration (2 mg) of MDZ.

2. Experiment

2.1. Chemicals

MDZ and 1-OHMDZ were purchased from Diaich Chemical (Tokyo, Japan). Diazepam, the internal standard (I.S.) was purchased from Wako Pure Chemical Industries (Osaka, Japan) (Fig. 1). The purity of these materials was more than 98%. Ammonium acetate, methanol, *n*-hexane, and chloroform were also from Wako Pure Chemical Industries. Water was deionized and purified using a Milli-Q system (MP-650, IWAKI Millipore, Tokyo, Japan).

2.2. LC–MS conditions

The assay was developed using a LaChrom separation module (Hitachi, Tokyo, Japan) including a L-7100 low-pressure gradient pump, L-7360 column oven and a D-7000 interface module. The module was controlled by LC/3DQ-MS system Manager Software under Windows NT 4.0 and connected to an M-8000 ion-trap based mass spectrometer equipped with a SSI source (Hitachi, Tokyo, Japan). The analytes were separated on a YMC-Pack Pro C_{18} column (50 mm × 2.0 mm i.d., particle size 3 μm, YMC, Tokyo, Japan), preceded by a guard column packed with the same material (10 mm × 2.0 mm i.d.). The temperature of column was maintained at 25 °C. Samples were eluted iso-

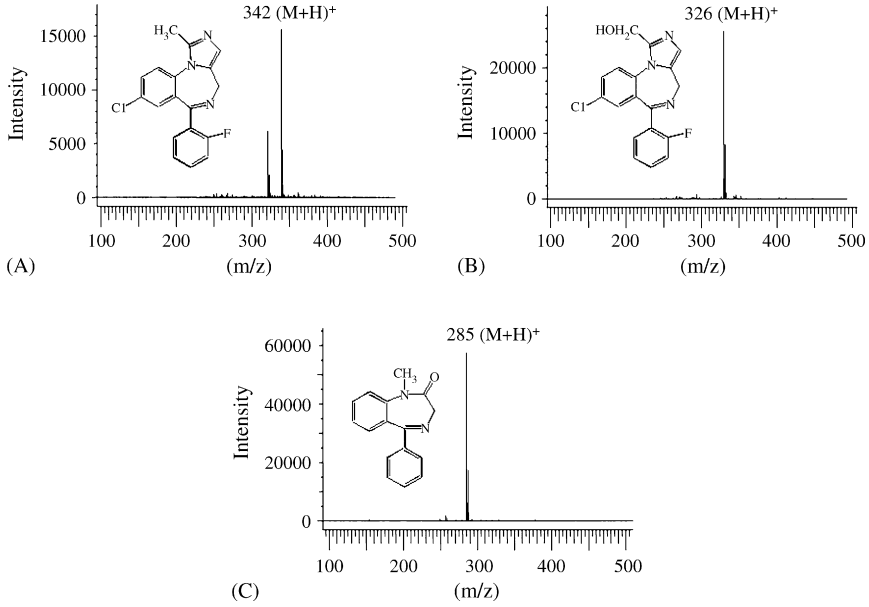

Fig. 1. Mass spectra of (A) midazolam, (B) 1′-hydroxymidazolam and (C) diazepam (I.S.).

cratically using a mobile phase composed of 10 mM ammonium acetate and methanol (50:50, v/v) at a flow-rate of 0.2 ml/min. The MS conditions were as follows: focus, 30 V; drift, 100 V; multiplier, 450 V; shield temperature, 260 °C; aperture 1 temperature, 160 °C; aperture 2 temperature (sampling aperture), 120 °C. Nitrogen gas was used as a carrier gas with output pressure of 0.5 MPa and ion source inlet pressure at 0.39 MPa. Positive ion mode was used, and selected-ion monitoring was accomplished at *m/z* 326 for MDZ, *m/z* 342 for 1-OHMDZ and *m/z* 285 for diazepam (I.S.).

2.3. Preparation of stock and working solutions

Stock solutions of MDZ, 1-OHMDZ and I.S. were prepared by dissolving an appropriate amount of each compound in methanol to yield concentrations of 0.2 mg/ml for generating standard curves. Working standard solutions of MDZ and 1-OHMDZ (1000, 100, 10 ng/ml) were prepared by serial dilution with 10 mM ammonium acetate–methanol (50:50, v/v). The working standard solution of I.S. (2 μg/ml) was obtained by 100 times diluting the stock solution (0.2 mg/ml) with 10 mM ammonium acetate–methanol (50:50, v/v). Stock solutions were stable at −20 °C for at least 10 months for the purpose of analysis.

2.4. Extraction procedure

I.S. (diazepam) 20 μl of 2 μg/ml and 0.5 ml NaOH (0.5N) were added to 1 ml of human plasma. The tubes were vortex-mixed for 10 s and 4 ml of *n*-hexan–chloroform (70:30, v/v) was added as extraction solution. After 10 min of shaking, the mixture was centrifuged at 2500 × *g* for 10 min at 4 °C, and the organic phase (3.5 ml) was evaporated in vacuo at 45 °C to dryness (TAITEC VC-960, Shimadzu, Kyoto, Japan). The residue was dissolved in 20 μl of 10 mM ammonium acetate–methanol (50:50, v/v) and vortex-mixed. 5 μl aliquot from each sample was injected into the LC–MS system.

2.5. Preparation of calibration standards and quality control samples

The human drug-free (blank) plasma was used for the validation studies of the analytical method. Calibration curves were prepared by spiking 10–50 μl of the respective working solutions in 1 ml of blank plasma to yield final concentrations of 0.25, 0.5, 1, 5, 20 and 50 ng/ml for MDZ and 1-OHMDZ. Standard curves were prepared daily and constructed by linear-regression analysis of compound to I.S. peak–area ratio versus the respective concentrations of MDZ and 1-OHMDZ, respectively. Stock solutions of MDZ and 1-OHMDZ were separately prepared for quality control samples in the same manner as for the standard curves. Quality control samples were obtained by spiking 20–40 μl of respective working solution in 1 ml of blank plasma to yield final concentrations of 0.3, 4, 20 and 40 ng/ml for MDZ and 1-OHMDZ, respectively. Quality control samples were kept at −20 °C until analysis.

2.6. Precision and accuracy

Intra- and inter-day precision and accuracy were evaluated by assaying quality controls with four different concentrations of MDZ and 1-OHMDZ. Intra- and inter-day precisions were assessed by analyzing six quality control samples at each concentration on the same day and mean values of a quality control for 6 days, respectively. The precision determined at each concentration level should not exceed 15% of the CV except for the lower limit of quantification (LLOQ), where it should not exceed 20% of the CV [30]. Accuracy was expressed as percent error (relative error) [(measured concentration − spiked concentration)/spiked concentration] × 100 (%), precision of which was quantitated by calculating intra- and inter-CV values.

2.7. Pharmacokinetic study design and sample collections

Fourteen healthy Japanese volunteers (7 men and 7 women) were enrolled in this study after giving written informed consent. The mean (±standard deviation) values of age and body weight of volunteers were 23.4 (±3.4) years (range 20–32 years) and 54.6 (±8.2) kg (range 40–68 kg), respectively. This study was approved by the Ethics Committee of Hirosaki University School of Medicine. A crossover-randomized study design was used at intervals of 2 weeks. They took either 1 mg MDZ by an intravenous injection for 5 min or 2 mg MDZ by an oral dose turbid in apple juice after overnight fast. Blood sampling (10 ml) was performed before and 5, 15, 30, 60, 120, 180, 240, 300 and 360 min after the intravenous administration and before and 15, 30, 60, 90, 120, 180, 240, 300, 360 min after the oral administration. Plasma samples were frozen and kept at −20 °C until analysis.

2.8. Pharmacokinetic data analysis and statistical analyses

The maximum plasma concentration (C_{max}) of MDZ and 1-OHMDZ and the time to reach C_{max} (t_{max}) were determined directly from the individual concentration–time data. The elimination rate constant (ke) was obtained by linear-regression analysis by use of at least three sampling points of the terminal log-linear declining phase to the last measurable concentration. The elimination half-life ($t_{1/2}$) was determined from the equation: $t_{1/2} = \ln 2/\text{ke}$. The area under the plasma concentration–time curve from 0 h to the last sampling time (tn) of plasma level (AUC_{0-tn}) was calculated by the trapezoidal rule. AUC from 0 to infinity ($AUC_{0-\infty}$) was calculated according to the equation: $AUC_{0-\infty} = AUC_{0-t} + \text{Cpt}/\text{ke}$. Cpt was the observed plasma concentration at tn. After intravenous administration of MDZ, total clearance (CL) was obtained from the equation: $CL = Dose_{iv}/AUC_{0-\infty,\,iv}$ and steady-state volume of distribution (Vd_{ss}) were calculated by use of non-compartmental methods based on statistical moment theory. The oral bioavailability (F) was calculated as follows: $F = (AUC_{0-\infty,\,po} \times Dose_{iv})/(AUC_{0-\infty,\,iv} \times Dose_{po})$. The CYP3A activity was calculated according to the equation: the CYP3A activity = $AUC_{0-\infty}$ (1-OHMDZ)/$AUC_{0-\infty}$ (MDZ).

3. Results and discussion

3.1. Chromatographic optimization

This paper describes a new method for the simultaneous determination of MDZ and 1-OHMDZ in human plasma by LC–MS with a SSI interface. Our goal was to develop a more sensitive and reliable method to evaluate precisely the human pharmacokinetics of MDZ and 1-OHMDZ after the low dose administration of MDZ for safety. A previous study by Kanazawa et al. has reported a simultaneous assay of MDZ and 1-OHMDZ by using LC–MS with SSI interface [31]. However, the method could not be applied to our human study because the calibration curves for MDZ and 1-OHMDZ validated were constructed over the 100–5000 ng/ml for monkey plasma. In this study with similar SSI interface, since we selected different MS conditions described in Section 2.2, calibration curve ranging from 0.25 to 50 ng/ml for MDZ and 1-OHMDZ in human plasma was achieved because it might be more effective to obtain a simultaneous ionization response for MDZ, 1-OHMDZ and diazepam. In addition to this result, a simple extraction procedure using *n*-hexane–chloroform (70:30, v/v) might lead to concentrate the sample volume, and then it could yield a lower LOQ. Although chloroform has certainly toxicity, the mixture of hexane–chloroform among several solvents we examined had the best extraction efficiency of MDZ and 1-OHMDZ from biological matrix. Consequently, since there were no interfering peaks of endogenous substances at the retention times of MDZ and 1-OHMDZ (Fig. 2), this method was applied to human pharmacokinetic study to 14 healthy volunteers. Therefore, our result was sensitive enough to obtain human pharmacokinetic parameters (Table 3), and various drawbacks of the previous published assay [7,13–29] were eliminated. To our knowledge, this is the first report that enables simultaneous determination of MDZ and 1-OHMDZ with LC–MS with SSI in human study after intravenous and oral administration of the low dose, which was validated according to FDA Guidelines [30].

3.2. Linearity

Calibration curves were linear over the concentration range from 0.25 to 50 ng/ml for MDZ ($r^2 = 0.9999$ and

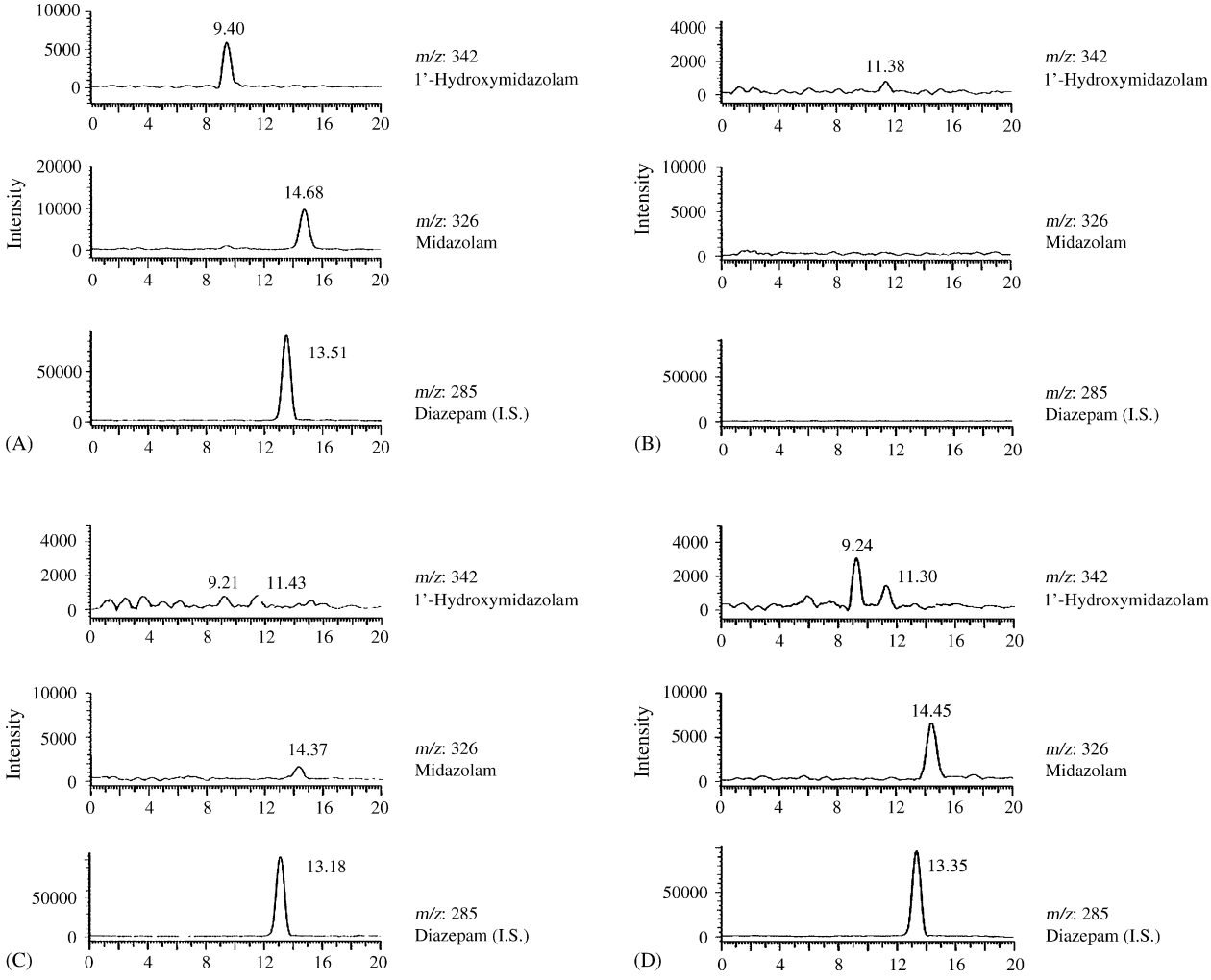

Fig. 2. Reconstructed mass chromatograms following the analysis of (A) working solution, (B) blank plasma, (C) plasma spiked with 0.25 ng/ml of midazolam and 1′-hydroxymidazolam and 2 ng/ml of I.S., and (D) plasma obtained from one volunteer at 180 min after oral administration of 2 mg midazolam. The concentration was 1.5 and 1.9 ng/ml for midazolam and 1′-hydroxymidazolam, respectively.

Table 1
Individual and mean values for slope, intercepts, correlation coefficients and F-values of five calibration curves for analytes

Analyte	Curve	Slope	Intercepts	r^2	F-test for lack of linearity		Concentration added (ng/ml)	Found (mean ± S.D.)	Accuracy (%)	CV (%)	n
					F	p-Value					
Midazolam	1	0.0415	0.0097	0.9999	34056.1	<0.001	0.25	0.24 ± 0.01	94.70	4.34	5
	2	0.0424	0.0065	0.9998	21787.8	<0.001	0.5	0.49 ± 0.03	98.83	6.98	5
	3	0.0413	0.0019	0.9999	29947.0	<0.001	1	1.07 ± 0.06	106.89	5.89	5
	4	0.0387	0.0057	0.9998	12731.4	<0.001	5	5.00 ± 0.17	99.99	3.44	5
	5	0.0454	0.0004	1.0000	71221.1	<0.001	20	20.19 ± 0.38	100.94	1.88	5
							50	50.64 ± 0.47	101.28	0.93	5
	Mean	0.0419	0.0048	0.9999	33948.7						
	S.D.	0.0024	0.0037								
	S.E.	0.0011	0.0017								
1′-Hydroxymidazolam	1	0.0174	−0.0005	1.0000	95011.1	<0.001	0.25	0.24 ± 0.02	94.49	6.96	5
	2	0.0184	−0.0031	0.9999	29643.5	<0.001	0.5	0.51 ± 0.02	102.90	4.21	5
	3	0.0169	0.0070	0.9999	21363.1	<0.001	1	1.02 ± 0.04	101.70	4.28	5
	4	0.0168	0.0035	0.9999	13940.1	<0.001	5	5.00 ± 0.02	99.95	0.33	5
	5	0.0166	0.0035	1.0000	56168.8	<0.001	20	20.36 ± 0.72	101.81	3.55	5
							50	49.75 ± 0.20	99.51	0.40	5
	Mean	0.0172	0.0021	0.9999	43225.3						
	S.D.	0.0007	0.0039								
	S.E.	0.0003	0.0018								

$F = 33{,}948.7$, $p < 0.001$, $n = 5$), and for 1-OHMDZ ($r^2 = 0.9999$ and $F = 43{,}225.3$, $p < 0.001$, $n = 5$), respectively (Table 1).

3.3. Specificity and sensitivity

Mass spectra of MDZ, 1-OHMDZ and diazepam (I.S.) are shown in Fig. 1, and ions at 326, 342 and 285, respectively, were selected for subsequently quantitative analysis. A typical chromatogram of working solution is shown in Fig. 2A; the retention times of 1-OHMDZ, I.S. and MDZ were 9.40, 13.51 and 14.68 min, respectively. A typical chromatogram of an extracted blank plasma sample is shown in Fig. 2B, while the chromatogram of an extracted sample spiked with 0.25 ng/ml of MDZ and 1-OHMDZ and 2 ng/ml of I.S. is shown in Fig. 2C. The chromatogram of extracted plasma sample obtained from one volunteer at 180 min after oral administration of MDZ (2 mg) did not show interference peaks (Fig. 2D).

The lower limit of quantification (LLOQ) defined as analytical responses was at least five times greater than the response as compared to blank response (signal-to-noise ratio = 5, and 0.1 ng/ml of MDZ and n1-OHMDZ). The lowest standard on the calibration curve was defined as the limit of quantification by which the analyte peaks for two compounds were identifiable, discrete and reproducible with a precision of 20% and accuracy of 80–120%. The limits of quantification were 0.25 ng/ml for MDZ and 1-OHMDZ.

Additionally, the plasma samples of healthy volunteers orally receiving CYP3A inhibitors (clarithromycin 400 mg/day, itraconazole 200 mg/day) and the blank plasma spiked with CYP3A inducers (rifampicin 8 μg/ml, hyperforin 0.5 μg/ml) were checked for interferences with the MDZ and 1-OHMDZ signals. Neither chromatogram revealed any limitations for the assay.

3.4. Recovery (extraction efficiency) from biological matrix

Recovery from plasma was calculated by comparing the peak areas of pure standards prepared in mobile phase, and injected directly into the analytical column with those of extracted plasma samples containing the same amount of the test compounds ($n = 6$). Mean absolute recoveries were 85.6–101.5% for MDZ, and 80.4–94.6% for 1-OHMDZ at 0.3, 4, 20 and 40 ng/ml, respectively, and their mean CV values were 3.6–6.5 and 3.6–5.2%, respectively. The mean recovery and their mean CV values for I.S. was 97.8 and 4.9%, respectively.

3.5. Precision and accuracy

Precision and accuracy evaluated over the method's linear range at 0.3, 4, 20 and 40 ng/ml are presented in Table 2. Intra- and inter-day relative standard deviations were less than 6.5 and 5.5% for MDZ, and 6.1 and 5.7% for 1-OHMDZ, respectively. All results for precision were within the acceptable limits. The mean accuracy for both the intra- and inter-day evaluations was between 96 and 104% over 6 days on which quality control samples were tested.

3.6. Stability

The stock solutions (methanol solution) of MDZ and 1-OHMDZ were stable at −20 °C for at least 10 months, and spiked MDZ and 1-OHMDZ in the blank plasma were stable at −20 °C for at least 10 months. Additionally, MDZ, 1-OHMDZ and I.S. in extracts from plasma samples reconstituted in the mixture of 10 mM ammonium acetate–methanol (50:50, v/v) were stable at room temperature for 72 h.

Three freeze–thaw cycles at −20 °C did not affect the stability of MDZ and 1-OHMDZ in plasma, as shown by deviation from

Table 2
Presicion and accuracy for determination of analytes in spiked plasma ($n = 6$)

Analyte	Concentration added (ng/ml)	Found (mean ± S.D.) (ng/ml)	Accuracy (%)	Intra-day		Found (mean ± S.D.) (ng/ml)	Accuracy (%)	Inter-day	
				CV (%)	Relative error (%)			CV (%)	Relative error (%)
Midazolam	0.3	0.31 ± 0.02	104.11	6.53	4.11	0.29 ± 0.02	96.27	5.53	−3.73
	4	3.95 ± 0.6	98.65	3.97	−1.35	3.99 ± 0.12	99.77	3.13	−0.23
	20	20.21 ± 0.60	101.07	2.95	1.07	20.16 ± 0.35	100.81	1.72	0.81
	40	39.90 ± 2.16	99.76	5.40	−0.24	40.41 ± 0.42	101.03	1.04	1.03
1′-Hydroxymidazolam	0.3	0.30 ± 0.02	100.71	6.12	0.71	0.30 ± 0.02	98.56	5.71	−1.44
	4	3.93 ± 0.10	98.32	2.59	−1.68	3.93 ± 0.08	98.29	2.13	−1.71
	20	20.03 ± 0.24	100.13	1.20	0.13	20.18 ± 0.37	100.91	1.83	0.91
	40	39.93 ± 0.90	99.82	2.24	−0.18	39.92 ± 0.12	99.80	0.31	−0.20

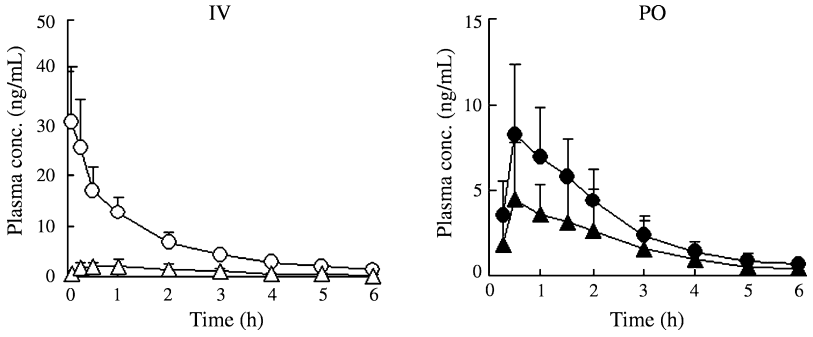

Fig. 3. Plasma concentration–time curves (mean ± S.D.) of midazolam (circles) and 1′-hydroxymidazolam (triangle) after intravenous administration (1 mg, left) and oral administration (2 mg, right).

nominal concentrations, which were within the acceptable limits of ±10% at all concentration levels.

3.7. Drug concentration in human plasma

Fig. 3 shows mean plasma concentration–time curves of MDZ and 1-OHMDZ after intravenous administration (1 mg) or oral administration (2 mg) of MDZ to 14 healthy volunteers. The mean pharmacokinetic parameters of MDZ and 1-OHMDZ are summarized in Table 3. Many sampling points in this study showed less than 1 ng/ml in both compounds, which were LOQ and/or the lowest concentration of calibration curve in previous methods [25–29]. LOQ in this developed analytical method was 0.25 ng/ml in both compounds. Plasma concentrations of MDZ were not shown <0.25 ng/ml up to 6 h in all volunteers after intravenous administration, and only one volunteer showed <0.25 ng/ml at 6 h after oral administration. Plasma concentrations of 1-OHMDZ were shown <0.25 ng/ml in 1, 2 and 5 volunteers at 3, 5 and 6 h after intravenous administration, respectively. After oral administration, they were shown <0.25 ng/ml in 1, 3 and 5 volunteers at 4, 5 and 6 h. Therefore, this analytical method appeared to be a more sensitive and practical means for monitoring of plasma concentrations of MDZ and 1-OHMDZ up to 6 h after intravenous dosing (1 mg) and oral dosing (2 mg) of MDZ in all volunteers.

Pharmacokinetic parameters of MDZ and 1-OHMDZ have wide inter-individual variation. AUC of MDZ and 1-OHMDZ/MDZ plasma concentration ratio, which were used as

Table 3
Pharmacokinetics of midazolam and 1′-hydroxymidazolam after 1 mg intravenous administration and 2 mg oral administration of midazolam in 14 healthy volunteers

	Parameters	Mean (range)	CV (%)
1 mg intravenous injection			
Midazolam	$AUC_{0-\infty}$ (ng h/ml)	46.7 (27.3–63.2)	21
	CL (l/h)	22.4 (15.8–36.6)	24
	Vd_{ss} (l)	47.4 (26.8–75.0)	28
	$t_{1/2}$ (h)	1.8 (1.3–3.0)	26
1′-Hydroxymidazolam	C_{max} (ng/ml)	2.4 (0.6–5.3)	58
	t_{max} (h)	0.5 (0.25–1)	53
	$AUC_{0-\infty}$ (ng h/ml)	7.6 (1.8–16.6)	54
	$t_{1/2}$ (h)	1.8 (1.1–4.0)	38
	CYP3A activity	0.16 (0.07–0.35)	54
2 mg oral administration			
Midazolam	C_{max} (ng/ml)	8.7 (3.8–17.4)	46
	t_{max} (h)	0.5 (0.25–1.5)	49
	$AUC_{0-\infty}$ (ng h/ml)	19.7 (7.9–32.3)	38
	$t_{1/2}$ (h)	1.3 (1.1–1.9)	15
	F	0.2 (0.09–0.37)	41
1′-Hydroxymidazolam	C_{max} (ng/ml)	5.0 (1.1–12.0)	64
	t_{max} (h)	0.5 (0.25–2.0)	58
	$AUC_{0-\infty}$ (ng h/ml)	10.9 (2.2–35.6)	75
	$t_{1/2}$ (h)	1.3 (1.0–2.0)	24
	CYP3A activity	0.64 (0.18–2.22)	87

Data are presented as geometric mean and range, except for t_{max} data, which are presented as median and range.

an in vivo marker of CYP3A activity, varied as much as 2.3-fold and 5.3-fold after intravenous administration and 4.1-fold and 12.4-fold after oral administration. The results of the pharmacokinetic parameters of MDZ determined in this study were in consistence with previously obtained values [21,32,33]. As long as we know, there is only one report from our laboratory for the pharmacokinetic parameters of 1-OHMDZ after intravenous dosing (1 mg) and oral dosing (2 mg) [21]. These values were similar to the results in the present study. This validated method was applied to determine plasma concentration of MDZ and 1-OHMDZ for CYP3A phenotypes in humans following the low intravenous and oral dose of MDZ.

4. Conclusion

This LC/MS procedure for the simultaneous determination of MDZ and 1-OHMDZ is simple, economic, well validated. The limit of quantification obtained justifies application to evaluating CYP3A activity after low intravenous and oral administration of MDZ in humans.

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