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Simultaneous determination of itraconazole and hydroxyitraconazole in human plasma by liquid chromatography–isotope dilution tandem mass spectrometry method

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ABSTRACT: A rapid and sensitive liquid chromatography–isotope dilution tandem mass spectrometry method was developed and validated for quantification of itraconazole (ITZ) and its active metabolite hydroxyitraconazole (OH-ITZ) in human plasma. The plasma samples were extracted with tert-butyl methyl ether and two isotope-labeled internal standards (D5-itraconazole and D5-hydroxyitraconazole) were used. The chromatographic separation was performed on a Capcell Pak C_{18} MG III (100 × 2 mm, 5 μm, Shiseido). The protonated ions of analytes were detected in positive ionization in multiple reaction monitoring mode. The plasma method has a lower limit of quantification of 1 ng/mL with a linearity range of 1–500 ng/mL for ITZ and OH-ITZ using 100 μL of plasma. The recoveries of the method were found to be 69.47–71.98% for ITZ and 75.68–82.52% for OH-ITZ. The intra- and inter-batch precision was less than 11% for all quality control samples at concentrations of 2.5, 200 and 400 ng/mL. These results indicate that the method was efficient with a short run time (4.5 min) and acceptable accuracy, precision and sensitivity.The validated method was successfully applied to analysis of human plasma samples in pharmacokinetics study. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: itraconazole; hydroxyitraconazole; liquid chromatography–isotope dilution tandem mass spectrometry; pharmacokinetics

Introduction

Itraconazole (ITZ, Fig. 1a) is an oral triazole antifungal agent with a broad spectrum of activity against most fungal pathogens. It is metabolized by the cytochrome P450 system to over 30 metabolites, and hydroitraconazole (OH-ITZ, Fig. 1b) is the main biologically active metabolite with its plasma concentration two-fold higher than ITZ at steady state (Haria *et al*., 1996).

Several analytical methods have been published for determination of the concentration of ITZ and OH-ITZ in human plasma. Methods of detection include ultraviolet detection (Abdel-Rahman *et al*., 2007; Uno *et al*., 2006; Ohkubo and Osanai, 2005), fluorometric detection (Redmann and Charles, 2006; Wong *et al*., 2003; Koks *et al*., 2002; Srivatsan *et al*., 2004) and LC-MS/MS (Bharathi *et al*., 2008; Kousoulos *et al*., 2006; Vogeser *et al*., 2003; Yao *et al*., 2001; Carrier and Parent, 2000). Mass spectrometry is considered to greatly improve the sensitivity and selectivity of ITZ and OH-ITZ. The UV detection method reported by Uno *et al*. (2006) and Abdel-Rahman et al (2007) utilized liquid–liquid extraction (LLE) when extracting from 1 mL of plasma. These two methods achieved lower limits of quantitation (LLOQs) of 2 and 3 ng/mL for ITZ, and 5 and 3 ng/mL for OH-ITZ, respectively. The fluorometric detection method reported by Srivatsan *et al*. (2004) and Wong *et al*. (2003) utilized LLE and achieved a sensitivity of LLOQ 5 and 2.8 ng/mL for ITZ, and 5 and 5.6 ng/mL for OH-ITZ, by extracting from 0.5–1 mL of plasma. A plasma method that uses solid-phase extraction (SPE) and positive ionization LC-MS/MS for detection has also been reported (Bharathi *et al*., 2008). The LC- MS/MS method reached high sensitivity sufficient for analyzing lower dosage clinical pharmaceutical samples when extracting from 0.5 mL of plasma (LLOQ at 0.5 ng/mL for ITZ and OH-ITZ). However, the multi-step LLE or SPE and large sample volume needed to achieve this sensitivity limits the application of the above methods.

Therefore, in this paper, a new method, utilizing simple one-step LLE for sample preparation and positive ionization LC-MS/MS for detection, is described for the simultaneous determination of ITZ and OH-ITZ in human plasma with a 0.1 mL of sample volume. Also, this method simultaneously determines ITZ and OH-ITZ in human plasma samples utilizing two isotope-labeled internal standards. The method has been successfully applied to

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Abbreviations used: ITZ, itraconazole; OH-ITZ, hydroxyitraconazole.

a pharmacokinetics study in Chinese healthy volunteers following oral administration of itrsaconazole tablet.

Experimental

Chemicals and Reagents

ITZ (99.3% purity) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). OH-ITZ (98% purity), D5-itraconazole (D5-ITZ, internal standard, 95.06 % purity, Fig. 1c) and D5-hydroxyitraconazole (D5-OH-ITZ, internal standard, 94.05% purity, Fig. 1d) were purchased from Toronto Research Chemicals, Inc. (North York, Canada). HPLC-grade acetonitrile, methanol, tert-butyl methyl ether (MTBE) and formic acid were from Tedia Company Inc. (Fairfield, OH, USA). All other reagents were of analytical grade. Double-distilled water was used throughout the work.

LC–MS/MS Equipment and Conditions

The liquid chromatography system (Shimadzu, Kyoto, Japan) was equipped with two LC-10ADvp pumps, a DGU-14AM vacuum degasser, a SIL-HTC autosampler and a controller module. Mass spectrometric detection was performed on an API 3000 triple quadrupole instrument (ABI-SCIEX, Toronto, Canada). The data was acquired and processed using Analyst 1.4.2 software.

The chromatographic separation was achieved on a Capcell Pak C_{18} MG III (100 × 2.0 mm, 5 µm, Shiseido, Japan) and a C_{18} guard column (4 × 3 mm, 5 µm, Phenomenex, USA) at room temperature. The mobile phase was consisted of 0.02% formic acid in acetonitrile and 0.02% formic acid in water (65 : 35, v:v) and run at a flow rate of 0.3 mL/min. The autosampler was kept at 4°C. Each run time was 4.5 min.

Meanwhile, the multiple reaction monitoring (MRM) analysis was applied in a positive ionization mode to detect precursor to product ion transitions at *m/z* 705.5 → 392.4 (ITZ), 710.5 → 397.4 (D5-ITZ), 721.5 → 408.4 (OH-ITZ) and 726.5 → 413.4 (D5-OH-ITZ). The source/gas conditions were set at 10 for nebulizer gas, 10 for curtain gas, 12 for collision gas, 7 for auxillary gas, 3000 V for ion spray voltage and 450°C for the source temperature. In the software of Analyst1.4.2, the parameters of source/gas conditions have no unit; therefore, these parameters were the internal data of the equipment with no arbitrary unit. The optimized compound dependent parameters are summarized in Table 1.

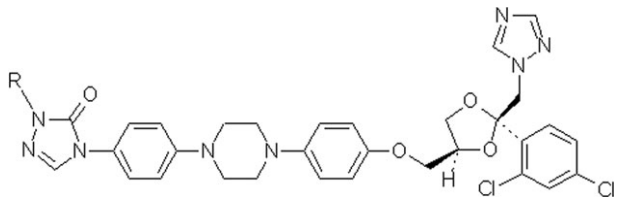

Figure 1. Chemical structure of ITZ, D5-ITZ, OH-ITZ and D5-OH-ITZ. (a) ITZ, R = CHCH3CH2CH3; (b) OH-ITZ, R = CHCH3CHOHCH3; (c) D5-ITZ, R = CHCH3CD2CD3; (d) D5-OH-ITZ, R = CDCH3CDOHCD3.

Sample Preparation

To a 2 mL polypropylene test tube, 100 µL plasma sample, 10 µL IS solution and 20 µL NaOH solution (0.1 M) were added, and the mixture was extracted with 1 mL MTBE and vortexed thoroughly for 3 min. Following centrifugation at 18,000 rpm for 3 min, the supernatant was transferred to a clean polypropylene tube and dried with a stream of nitrogen gas at 60°C. The residue was reconstituted with 100 µL acetonitrile–water (50 : 50, v/v) and a 10 µL volume was injected into LC–MS/MS system.

Standard, QC and IS Preparation

Primary stock solutions of ITZ and OH-ITZ for preparation of standard and quality control (QC) samples were prepared by weighing separately. The primary stock solutions of ITZ, OH-ITZ and D5-OH-ITZ (both 100 µg/mL) were prepared in acetone–water (70 : 30, v/v), and D5-ITZ (38 µg/mL) was prepared in acetonitrile–0.1 M HCl (70 : 30, v/v). The IS working solution was prepared by diluting its stock solution with acetonitrile–water (50 : 50, v/v) to 500 ng/mL for both D5-ITZ and D5-OH-ITZ. Appropriate dilutions were made in acetone–water (40 : 60, v/v) for ITZ and OH-ITZ to produce working solutions. All stock solutions and working solutions were stored at 4°C.

Calibration standards (1, 2, 10, 50, 100, 250 and 500 ng/mL) were prepared by spiking the working standard solutions of ITZ and OH-ITZ into human plasma. QCs were prepared in the same way at concentrations of 1, 2.5, 200 and 400 ng/mL. The standard samples and QCs were prepared for each analytical batch along with the unknown samples.

Method Validation

The method was validated by verifying specificity, linearity, LLOQ, intra- and inter-assay precision and accuracy, matrix effect, recovery and stability. The method specificity was evaluated by screening six different batches of blank human plasma prior to the main validation batches. These batches were spiked with known concentrations of ITZ and OH-ITZ at 0.00, 2.5, 200 and 400 ng/mL (six replicates for each of the six batches), extracted and analyzed along with a calibration curve prepared in one of the six batches to demonstrate the lack of chromatographic interference from endogenous plasma components and batch-to-batch variation.

The linearity for ITZ and OH-ITZ were evaluated over the range 1–500 ng/mL. A linear regression model with 1/*x* weighted factor was constructed based on the measured peak area ratio of ITZ to the D5-ITZ, and OH-ITZ to the D5-OH-ITZ vs the nominal concentration. The LLOQ was the lowest concentration of analytes measured with acceptable accuracy and precision (RSD less than 20%).

QCs at four concentration levels (1, 2.5, 200 and 400 ng/mL) were analyzed to evaluate intra- and inter-assay precision and accuracy of the method with six replicates for each of the three randomized batches. Precision was expressed as RSD% for replicate measurements and accuracy (%) by the percentage of deviation between nominal and calculated concentrations.

The matrix effect was assessed by comparing the peak areas of analytes from the standards spiked after extraction and the neat QC

Table 1. Parameters of the LC-MS/MS analysis for ITZ, D5-ITZ, OH-ITZ and D5-OH-ITZ

Compound	Q1 (amu)	Q3 (amu)	Declustering potential (V)	Focusing potential (V)	Entrance potential (V)	Collision energy (V)	Collision cell exit potential (V)
ITZ	705.5	392.4	70	350	14	52	22
D5-ITZ (IS)	710.5	397.4	70	350	14	52	22
OH-ITZ	721.5	408.4	79	350	14	52	22
D5-OH-ITZ (IS)	726.5	413.4	79	350	14	52	22

standards at three concentration levels. The recovery was assessed by comparing the standards spiked before and after extraction at three concentration levels.

The autosampler stability was assessed by keeping the processed QCs at 4°C for 18 h. The freeze–thaw stability was evaluated by analyzing the QCs after three cycles from −20°C to room temperature. The plasma samples were analyzed for bench-top stability after storage at room temperature for 24 h. Furthermore, stock solution stability of ITZ and OH-ITZ was evaluated by storing the stock solutions at 4°C for 40 day, then comparing the peak areas with those of freshly prepared solutions.

Clinical Application

The developed method was applied to determine the plasma concentrations of ITZ and OH-ITZ. The study was approved by the Ethics Committee of Shanghai Xuhui Central Hospital. Each subject was informed of the purpose of the study, and written informed consents were obtained. A total of 24 healthy male Chinese volunteers [mean (SD) age, 21.3 (2.7) years (range, 19–27 years); weight, 60.8 (5.1) kg (range, 55.0–74.0 kg); and height, 171.1 (4.2) cm (range, 164.0–176.0 cm)] were included in the study. After an overnight fast, the volunteers received a 200 mg ITZ tablet, taken with 200 mL water. Additional water intake was permitted after 2 h, and food intake was allowed 4 h after administration. The volunteers were under continuous medical supervision throughout the study. Blood samples of ~3 mL were drawn through a heparin-locked catheter (B. Braun Co., Penang, Malaysia) containing 0.5 mL 0.4% heparin sodium.

Results and Discussion

Optimization of Dilution Solvent

Most reports utilized methanol, and some with 0.1 M HCl–methanol (3 : 7, v/v) to prepare ITZ and OH-ITZ stock solution. However, we found that standards cannot completely resolve in such solvents except in acetone. Thus, 70% acetone was selected as the dilution solvent for ITZ and OH-ITZ in the study.

Optimization of Mass Spectrometric and Chromatographic Conditions

In order to achieve the quantitative determination of ITZ and OH-ITZ in plasma, the electrospray ionization interface parameters were optimized for maximum abundance of the molecular ions of the compounds. Acquisition parameters were determined by direct infusion into the mass spectrometer of 1 μg/mL solution of ITZ, OH-ITZ and IS, at a flow rate of 10 μL/min. Variable mass spectrometric conditions (source temperature, ion spray voltage, collision energy, etc.) were investigated. Figure 2 shows the positive ion electrospray mass spectrum of ITZ, D5-ITZ, OH-ITZ and D5-OH-ITZ presenting molecular ions ($[M + H]^+$) at *m/z* 705.6, 710.6, 721.0, 726.3 and product ions at *m/z* 392.9, 397.6, 408.4 and 413.4, respectively. These values were obtained by Q1 full-scan and Q2 product ion scan. These procedures were to determine the itiner-

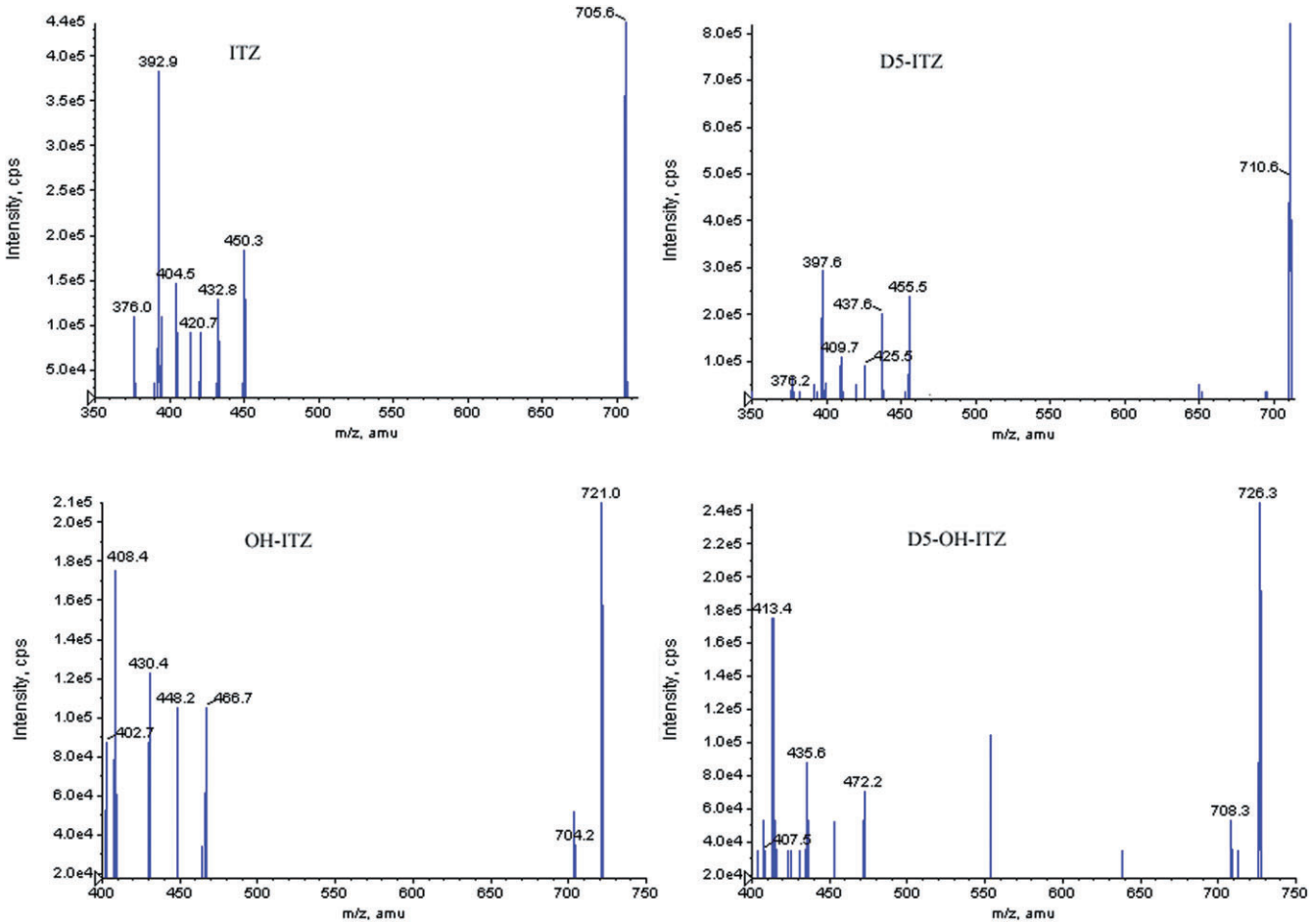

Figure 2. Product ion mass spectra of protonated ions obtained from ITZ, D5-ITZ, OH-ITZ and D5-OH-ITZ.

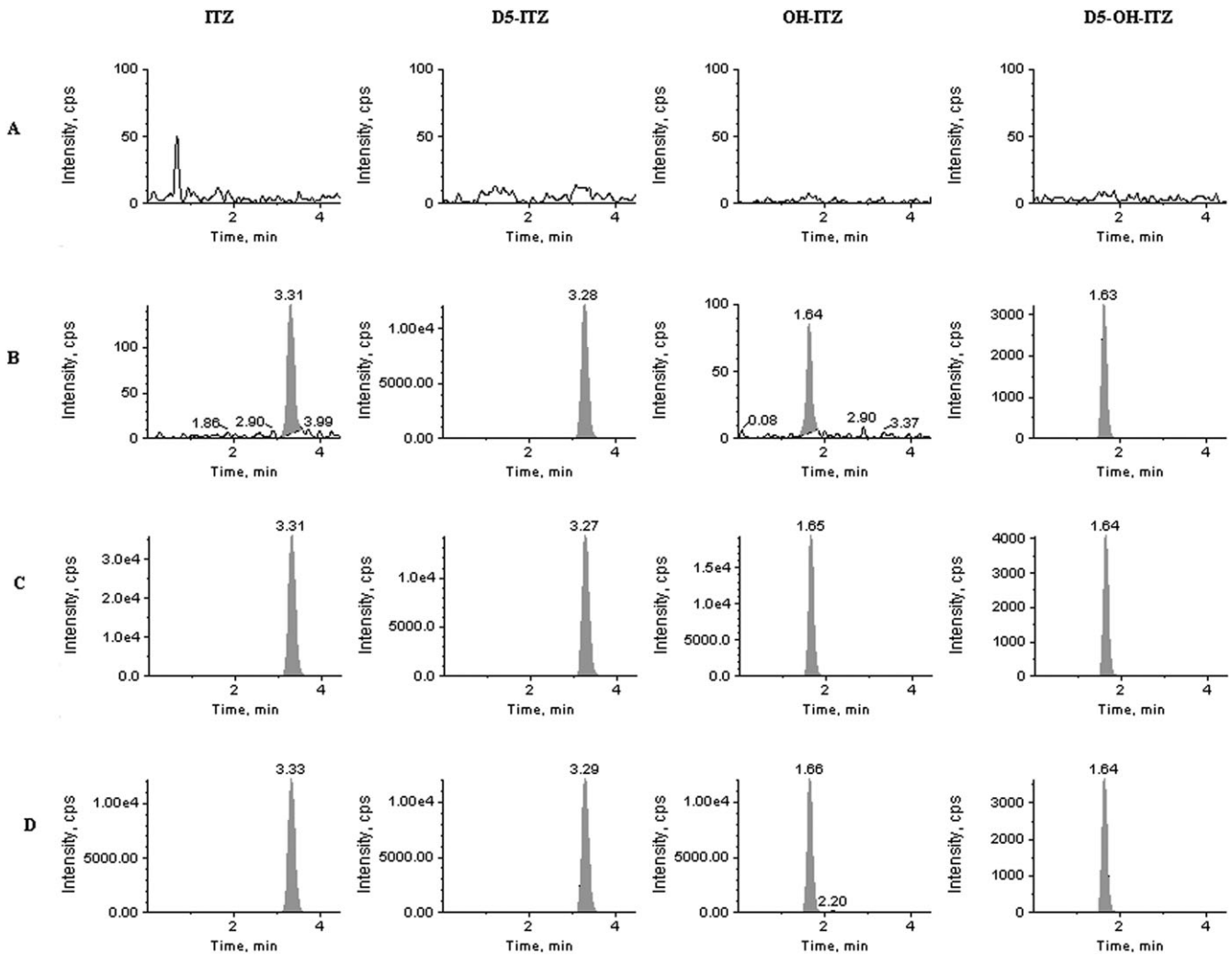

Figure 3. Representative LC-MS/MS chromatograms for ITZ, D5-ITZ, OH-ITZ and D5-OH-ITZ in human plasma. (a) Blank human plasma; (b) LLOQ plasma sample with 1 ng/mL ITZ and OH-ITZ; (c) QC plasma sample with 200 ng/mL ITZ and OH-ITZ; (d) human plasma sample collected 4.0 h after an oral dose of 200 mg ITZ.

ary condition of precursor ion and product ion. However, in the practical realm, the exact mass value of molecular ions and product ions should be optimized at MRM scan. In a range of about 1 amu, precursor ion and product ion would be optimized, comparing their intensities. Taking ITZ product ion, for example: Q1 full-scan has set 705.6 as its precursor ion, but in the MRM mode, we can select 705.5, 705.6, 705.7 and 705.8 as the precursor ions. This action can finally determine the specific precursor ion that has the best sensitivity among the selected conditions. Therefore, combined with liquid chromatographic separation, an excellent specificity and sensitivity were obtained from the precursor to the production *m/z*: 705.5/392.4, 710.5/397.4, 721.5/408.4 and 726.5/413.4amu for ITZ, D5-ITZ, OH-ITZ and D5-OH-ITZ. The retention time were about 3.35, 3.31, 1.69 and 1.67 min for ITZ, D5-ITZ, OH-ITZ and D5-OH-ITZ; therefore a total run time of 4.5 min was achieved. Figure 3 shows representative LC-MS/MS chromatograms for ITZ and OH-ITZ in plasma samples.

Optimization of the Extraction Method

In order to quantify the extracted amounts, the variables involved in the procedure such as extraction methods and extraction solvent were investigated. We tested a protein precipitation method with acetonitrile, but the sensitivity was not high enough for the pharmacokinetic studies as a result of sample dilution with the procedure. Although a method using a simple procedure of protein precipitation was reported by Redmann and Charles (2006), the LLOQ were set at 40 ng/mL for ITZ and 8 ng/mL for OH-ITZ.

Subsequently we adopted liquid–liquid extraction with different organic solvents. Ethyl ether and *tert*-butyl methyl ether were compared for sample extraction, and the extract recovery of the LLE method was much more than that of protein precipitation. Therefore, MTBE was selected as the extraction solvent for ITZ and its metabolite OH-ITZ in human plasma.

Method Validation

Specificity. Blank plasma from six batches was tested for endogenous interferences. Chromatograms representative of a blank matrix sample are shown in Fig. 3. The ITZ and OH-ITZ retention-time region was free from interferences. The back-calculated values from all six batches, spiked at concentration of 2.5, 200 and 400 ng/mL for both ITZ and OH-ITZ, were less than 6% of RSD

Table 2. Results of specificity analysis for ITZ and OH-ITZ ($n = 3$ for each matrix batch)

Spiked (ng/mL)	Matrix batch	ITZ Accuracy (%)	ITZ RSD (%)	ITZ Mean	ITZ RSD (%)	OH-ITZ Accuracy (%)	OH-ITZ RSD (%)	OH-ITZ Mean	OH-ITZ RSD (%)
2.5	1	105.1	2.16	104.4	2.13	99.95	3.82	103.3	5.40
	2	104.6	0.57			106.4	3.57		
	3	103.6	0.68			101.8	4.43		
	4	103.6	2.08			105.2	1.44		
	5	105.2	3.20			99.80	7.19		
	6	104.3	2.20			106.5	4.95		
200	1	104.6	0.25	103.0	1.29	101.4	1.02	100.7	0.91
	2	102.5	1.11			101.3	0.33		
	3	103.3	0.64			100.1	0.52		
	4	103.5	1.21			100.6	0.92		
	5	101.8	0.25			100.0	0.95		
	6	102.3	1.19			101.0	0.22		
400	1	99.73	1.16	100.7	1.38	97.32	0.57	99.07	2.03
	2	99.88	0.31			97.34	0.68		
	3	101.3	0.19			101.1	1.21		
	4	99.74	1.04			98.46	1.23		
	5	101.6	0.71			99.18	0.52		
	6	102.2	1.55			101.0	2.53		

Table 3. Calibration analysis of ITZ and OH-ITZ

Compound	Linearity range (ng/mL)	Calibration equation[a]	LLOQ (ng/mL)[b]	Correlation factor (r)
ITZ	1–500	$0.0169C + 0.001853$	1	0.9999
OH-ITZ	1–500	$0.0222C + 0.003450$	1	0.9998

C = the concentration of ITZ and OH-ITZ (ng/mL).
[a] Seven data points ($n = 6$).
[b] LLOQ = lower limit of quantification.

and the accuracies were 100.7–104.4% for ITZ and 99.07–103.3% for OH-ITZ, respectively, as shown in Table 2, indicating no significant batch-to-batch variation in matrix effects.

Linearity and LLOQ. Linear regression analyses for ITZ and OH-ITZ were performed by using internal standard method, ITZ/D5-ITZ and OH-ITZ/D5-OH-ITZ. The results of calibration curves of analytes in human plasma are summarized in Table 3. ITZ and OH-ITZ showed good linearity in a relatively wide concentration range, and the mean correlation coefficients (r) were 0.9999 and 0.9998, respectively. Based on the standard data presented here, it was concluded that the calibration curves used in this method were accurate for the determination of ITZ and OH-ITZ. The LLOQ were both 1 ng/mL for ITZ and OH-ITZ with a signal-to-noise (S/N) of more than 6. Although LLOQ is 1 ng/mL, the sensitivity is achieved using a limited sample volume of 0.1 mL plasma. The extracts were not condensed in the reconstitution step and 10% of final volume was typically injected onto LC-MS/MS for analysis. This indicates that a more sensitive linear range could be achieved if needed in future studies.

Accuracy and precision. Inter- and intra-batch accuracy and precision for assays were characterized by the four levels of QCs run on three sequential batches in six replicates. All QC samples were randomized daily, processed and analyzed together with calibration samples. The precision was expressed as a relative standard deviation (RSD) by calculating the standard deviation as the percentage of the mean calculated concentration, while the accuracy of the assay was determined as the percentage of the mean with reference to the true value.

Table 4 shows a summary of the individual QC data obtained in the three runs for the validation. As can be seen, the assays for ITZ and OH-ITZ were both accurate and precise inter-batch and intra batch for each level.

The intra- and inter-batch RSDs were 0.77–7.26 and 1.98–5.19% for ITZ and 0.35–9.34 and 2.31–10.56% for OH-ITZ, respectively. The inter- and intra-batch accuracies of the assay were 93.75–102.9 and 95.15–101.8% for ITZ, and 97.78–108.6 and 97.34–102.6% for OH-ITZ, respectively.

Recovery and matrix effect. Recovery was determined by comparing the peak areas of extracted QC samples with the peak areas of recovery standards (post-extraction-spiked plasma blanks at corresponding QC concentrations). Matrix effect was assessed by comparing the peak areas of post-extraction-spiked blanks with those of neat solutions to insure the robustness of the assay. Table 5 shows the summary of recovery and matrix effect results obtained from three QC levels. The mean recoveries of ITZ at 2.5, 200 and 400 ng/mL were 69.47, 71.98 and 71.77%. The mean recoveries of OH-ITZ at 2.5, 200 and 400 ng/mL were

Table 4. Results of precision analysis for ITZ and OH-ITZ ($n = 6$, for three batches)

Compound	Spiked (ng/mL)	Intra batch ($n = 6$) RSD (%)	Intra batch ($n = 6$) Accuracy (%)	Inter batch ($n = 18$) RSD (%)	Inter batch ($n = 18$) Accuracy (%)
ITZ	1	7.26	93.75	5.19	95.15
	2.5	2.93	102.9	2.83	101.8
	200	1.30	99.15	2.15	100.8
	400	0.77	101.5	1.98	101.8
OH-ITZ	1	9.34	103.4	10.56	97.34
	2.5	4.65	108.6	6.78	102.6
	200	0.35	97.78	2.31	98.98
	400	1.96	100.1	2.14	100.2

Table 5. Recovery and matrix effect of ITZ, OH-ITZ and IS in human plasma ($n = 6$)

Compound	Spiked (ng/mL)	Extraction recovery RSD (%)	Extraction recovery Accuracy (%)	Matrix effect RSD (%)	Matrix effect Accuracy (%)
ITZ	2.5	9.57	69.47	2.90	120.3
	200	8.60	71.98	3.47	95.32
	400	7.35	71.77	4.13	117.3
OH-ITZ	2.5	9.10	75.68	6.95	129.5
	200	8.76	78.23	3.23	109.0
	400	7.40	82.52	3.85	117.6
D5-ITZ(IS)	500	9.35	72.54	5.02	106.1
D5-OH-ITZ(IS)	500	8.69	81.84	9.42	114.3

75.68, 78.23 and 82.52%. The mean recoveries of D5-ITZ and D5-OH-ITZ were 72.54 and 81.84%. Both these replicates presented with an RSD of less than 10%.

The method demonstrates that the ionization efficiencies of analytes from plasma were somewhat enhanced, with a mean peak area ratio of 95.32–120.3, 109.0–129.5%, 106.1–114.3% for ITZ, OH-ITZ, D5-ITZ and D5-OH-ITZ obtained from the post-extracted samples; however plasma samples from different sources produced matrix effects at similar levels. No interferences from other compounds present in plasma were observed in the analytes assay. However, the accurate quantification could still be achieved even with the presence of the minor matrix effect under our experimental conditions, mainly owing to the using of isotope dilution mass spectrometry. To date, no significant interferences from endogenous substances have been observed during the process of method validation and sample assay.

Stability. The ITZ and OH-ITZ stabilities were investigated in autosampler, after three freeze–thaw cycles and on the bench-top. The results are listed in Table 6, indicating that the analytes remained considerably stable under the above conditions. The good stability of ITZ and OH-ITZ simplified the precautions needed for laboratory manipulations during the assay procedures. In addition, stock solutions were shown to be stable at 4°C for 40 days.

Table 6. Stability results of quality control samples for ITZ and OH-ITZ in human plasma

Compound	Spiked (ng/mL)	Calculated concentration (ng/mL)	RSD (%)	Accuracy (%)
Auto-sampler stability (4°C for 18 h after processing)				
ITZ	2.5	2.613	3.26	104.5
	200	194.6	0.79	97.29
	400	407.0	0.89	101.8
OH-ITZ	2.5	2.687	2.25	107.5
	200	195.0	1.91	97.51
	400	406.4	1.26	101.6
Freeze–thaw stability (three cycles)				
ITZ	2.5	2.513	3.89	100.5
	200	202.3	1.16	101.2
	400	397.5	1.81	99.36
OH-ITZ	2.5	2.470	5.46	98.81
	200	201.5	2.60	100.7
	400	397.6	1.54	99.40
Bench-top stability (room temperature for 24 h)				
ITZ	2.5	2.643	3.51	105.7
	200	202.5	2.20	101.3
	400	409.0	1.03	102.2
OH-ITZ	2.5	2.661	6.62	106.4
	200	198.0	1.67	99.02
	400	400.5	1.65	100.1

Application Study

The described liquid chromatography–isotope dilution tandem mass spectrometry (LC-ID/MS) method was successfully applied to determine the human plasma samples of ITZ up to 72 h after

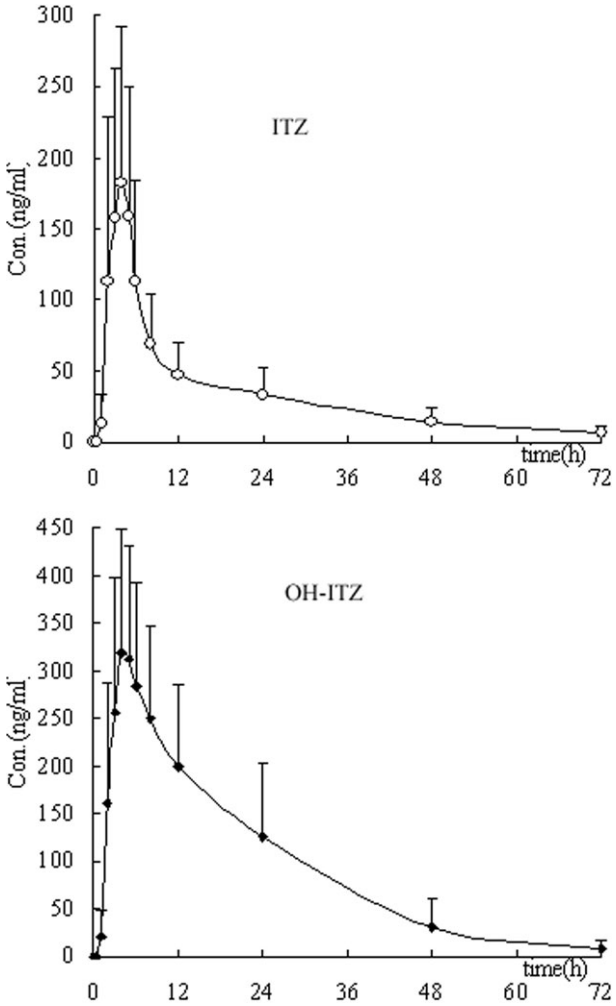

Figure 4. Mean plasma concentration–time curve of ITZ and OH-ITZ after an oral administration of 200 mg ITZ tablet to 24 healthy male volunteers.

a single oral dose administration of a 200 mg ITZ tablet to 24 healthy male Chinese volunteers. The sampling time was 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24, 36, 48, 72 h after administration. Figure 4 shows the mean plasma concentration vs time profile of ITZ and OH-ITZ in the 24 subjects.

Conclusions

A liquid chromatography–isotope dilution tandem mass spectrometry method was developed for the quantification of plasma itraconazole and hydroxyitraconazole. D5-itraconazole and D5-hydroxyitraconazole were added as internal standard. A simple one-step liquid–liquid extraction from 0.1 mL plasma was adopted for sample preparation and a turnaround of 4.5 min for sample assay was achieved. No interference with the LC–ID/MS method from endogenous substances was observed. The validated method has been successfully applied to the determination of itraconazole and hydroxyitraconazole in the plasma samples collected from pharmacokinetics studies.

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