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Original Research Article

Determination of fenticonazole in human plasma by HPLC–MS/MS and its application to pharmacokinetic studies

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ABSTRACT

Two simple and sensitive high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) methods were developed and validated for the determination of fenticonazole in human plasma after percutaneous and intravaginal administration. Mifepristone was used as an internal standard (IS), and simple protein precipitation by acetonitrile containing 2% acetic acid was utilized for extracting the analytes from the plasma samples. Chromatographic separation was performed on a Kinetex XB-C_{18} column. The quantitation was performed by a mass spectrometer equipped with an electrospray ionization source in multiple reactions monitoring (MRM) positive ion mode using precursor-to-product ion transitions of *m/z* 455.2–199.1 for fenticonazole and *m/z* 430.2–372.3 for mifepristone. The validated linear ranges of fenticonazole were 5–1000 pg/mL and 0.1–20 ng/mL in plasma for the methods A and B, respectively. For the two methods, the accuracy data ranged from 85% to 115%, the intra- and inter-batch precision data were less than 15%, the recovery data were more than 90%, and no matrix interference was observed. The methods A and B were successfully validated and applied to the pharmacokinetic studies of fenticonazole gel in Chinese healthy volunteers after percutaneous and intravaginal administration, respectively.

1. Introduction

Fenticonazole [1] is an imidazole derivative synthesized by the Recordati Research Division (Milan, Italy) [2–4]. It possesses a broad spectrum of antimycotic activity against superficial mycoses (Mycosporum, Tricophyton, and Epidermophyton) [5], as well as deep mycose (candidiasis, trichomoniasis, mixed infection and bacterial vaginosis) [6,7]. Fenticonazole exerts its antimycotic activity with damaging the fungal cell membrane via inhibition of the fungal cytochrome P450-dependent ergosterol synthesis [1]. It is also reported that there are two other antimycotic mechanisms for fenticonazole: inhibiting the synthesis of secretory aspartate protease acid of Candida albicans [8–11] and blocking yeast cytochrome oxidases and peroxidise [12]. Like other imidazole derivatives, fenticonazole is widely used for the therapy in dermatology and gynecology, for it is well tolerated in a variety of skin and vaginal infections and provides rapid control [7]. Some studies about fenticonazole have been reported mainly about clinical application and efficacy evaluation [13–18]. However, few studies focused on the pharmacokinetic characteristics of fenticonazole after single percutaneous or intravaginal administration in healthy subjects.

Several methods for the determination of fenticonazole in biological fluids have been reported, including high performance liquid chromatography with ultraviolet detection (HPLC–UV) [19] and high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) [19,20]. However, these reported methods are not appropriate for the pharmacokinetic study of 2% fenticonazole nitrate gel in human after single percutaneous or intravaginal administration because of their disadvantages such as large plasma volume, tedious sample extraction procedures, large injection volume and long analytical time. A new method with simple sample treatment procedure, short analytical run time and excellent reproducibility is needed for the high-throughput analysis.

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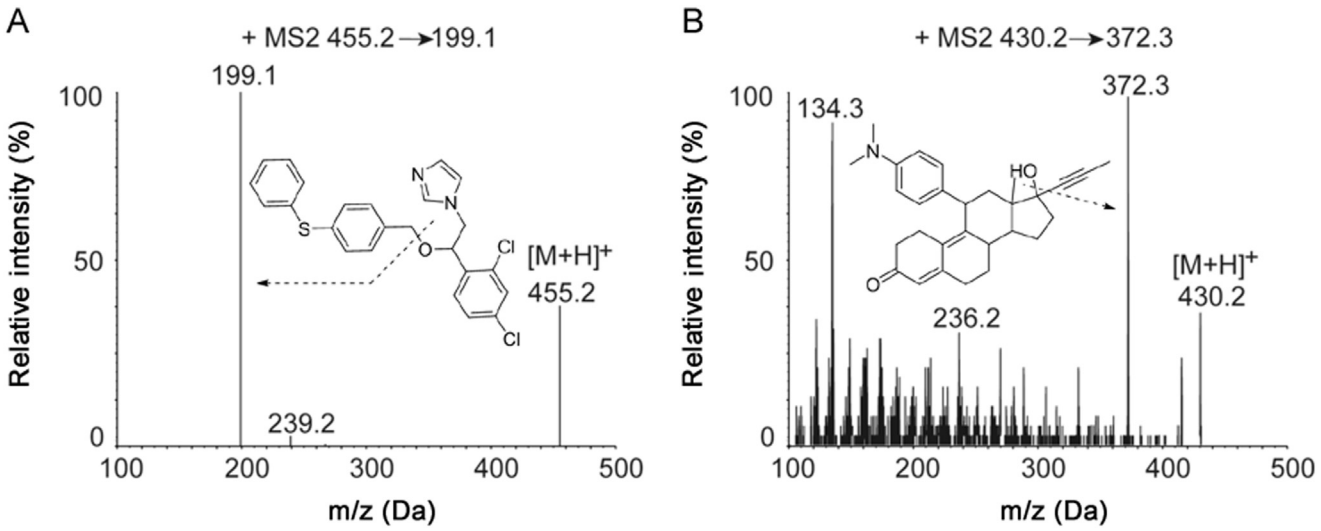

Fig. 1. MS/MS spectra of (A) fenticonazole and (B) mifepristone (IS).

In this paper, two studies, A and B, were conducted in healthy volunteers. Study A was a percutaneous administration pharmacokinetic study, and study B was an intravaginal administration pharmacokinetic study. Furthermore, the difference in plasma concentration levels of fenticonazole in plasma obtained from the above administration routes is very significant. The plasma concentration of fenticonazole in study A was at the level of pictogram per milliliter, while for study B it was at the level of nanogram per milliliter. The aim was to develop one method which could be applied to both studies, but unfortunately we could not achieve it. Therefore, two methods have been conceived for the studies A and B, respectively. In this paper, a sensitive method with the lower limit of quantification (LLOQ) of 5 pg/mL (method A) and a relative simple method with the LLOQ of 0.1 ng/mL (method B) were established for the determination of fenticonazole in human plasma obtained from the two studies, respectively. The two HPLC–MS/MS methods have been fully validated for the determination of fenticonazole in human plasma and successfully applied to the two different pharmacokinetic studies A and B of 2% fenticonazole nitrate gel in healthy Chinese volunteers. This is the first report on the pharmacokinetics of 2% fenticonazole nitrate gel in human. The information obtained from this study would be helpful in providing some reference to clinical application and clinical medication safety.

2. Experimental

2.1. Chemicals and reagents

The reference standard of fenticonazole nitrate (100% purity) was purchased from Salubris Pharmaceutical Co., Ltd. (Shenzhen, Guangdong, China). Mifepristone (99.5% purity), the internal standard (IS), was obtained from National Institutes for Food and Drug Control (Beijing, China). Fenticonazole nitrate gels (2%, 5 g: 0.1 g) were supplied by Nanjing Haina Pharmaceutical Technology Co., Ltd. Formic acid, acetic acid and ammonium acetate of analytical grade were purchased from Sigma-Aldrich, Co. (USA). Methanol and acetonitrile of HPLC grade were purchased from Merck KGaA (Darmstadt, Germany). Purified water obtained from a Milli Q (Millipore, Bedford, MA, USA) system was used throughout the experiment.

2.2. Instruments and conditions

2.2.1. Instruments and conditions for method A

HPLC–MS/MS method was performed with a Shimadzu HPLC system and a Triple Quad 5500 (Applied Biosystems/Sciex, USA) equipped with an electrospray ionization (ESI) interface. The Shimadzu HPLC system included a system controller (CBM-20A Lite), a binary pump (LC-30CE), a column oven (CTO-20AC), an online degasser (DGU-20A5R) and an autosampler (SIL-30ACMP). Chromatographic separation was performed on a Kinetex XB-C_{18} column (2.1 mm×50 mm, 2.6 μm, Phenomenex) with a C_{18} Security Guard Cartridges (2.0 mm×4.0 mm, Phenomenex). The column temperature was maintained at 40 °C with methanol (mobile phase B) and water containing 5 mM ammonium acetate and 0.2% formic acid (mobile phase A) as the mobile phase at a flow rate of 0.3 mL/min. The following gradient program was used for sample separation: 0–3.0 min, 68% B; 3.0–3.1 min, 68%–100% B; 3.1–4.6 min, 100% B; 4.6–4.8 min, 100%–68% B; 4.8–5.8 min, 68% B. Mass spectrometer was operated in a positive ion mode and the analytes were detected by multiple reaction monitoring (MRM) with the transitions of *m/z* 455.2–199.1 for fenticonazole (Fig. 1A), and *m/z* 430.2–372.3 for mifepristone (Fig. 1B), respectively. The Triple Quad 5500 instrument parameters were set as follows: ionspray voltage: 3500 V; curtain gas (CUR): 40 psi; nebulizer gas (GS1): 40 psi; heater gas (GS2): 50 psi; temperature (TEM): 600 °C; Collision-Activated Dissociation (CAD): 7 unit. The optimized compound-dependent parameters including declustering potential (DP), collision energy (CE), entrance potential (EP) and collision exit cell potential (CXP) were set at 80 V, 40 V, 8 V and 12 V for fenticonazole and 95 V, 30 V, 9 V and 10 V for the IS. System control and data acquisition were performed with the Analyst software version 1.6.2 (Applied Biosystems/Sciex, USA).

2.2.2. Instruments and conditions for method B

The liquid chromatography was achieved on a Shimadzu HPLC system (Kyoto, Japan) consisting of a system controller (CBM-20A

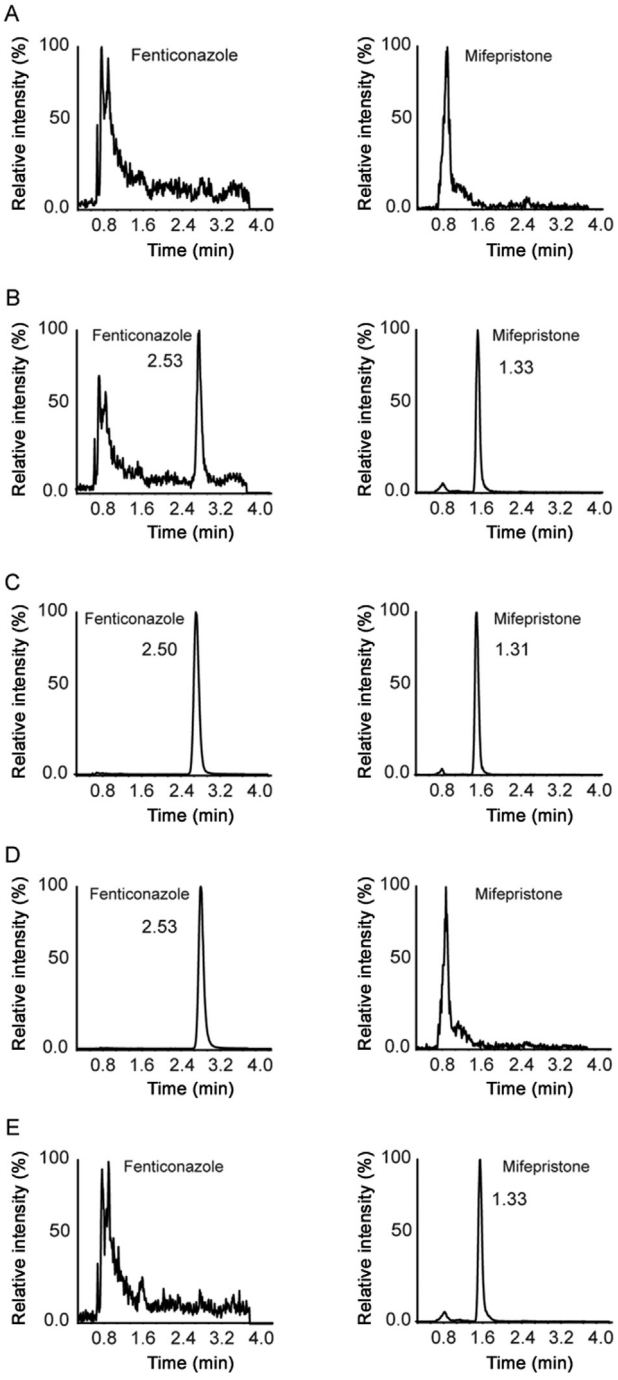

Fig. 2. The typical MRM chromatograms of (A) double blank sample; (B) plasma sample spiked with fenticonazole at LLOQ and IS; (C) plasma sample obtained from a subject after percutaneous application; (D) ULOQ without IS; and (E) control blank for method A.

Lite), a pump (LC-20ADXR), a column heater (CTO-20AC), an online degasser (DGU-20A5R) and an autosampler (SIL-30AC). The separation was carried out on a Kinetex XB-C_{18} column with a C_{18} Security Guard Cartridges at constant temperature of 40 °C. The mobile phase was composed of methanol (mobile phase B) and water containing 5 mM ammonium acetate and 0.1% formic acid (mobile phase A) with gradient elution at a flow rate of 0.3 mL/min. The gradient elution program was as follows: 0–2.40 min, 72% B; 2.40–2.45 min, 72%–100% B; 2.45–3.50 min, 100% B; 3.50–3.60 min, 100%–72% B; 3.60–5.50 min, 72% B. A 10-port switching valve (VICI, Houston, TX, USA) was used to direct column effluent to the mass spectrometer at the time interval of 0–3 min; otherwise the eluent was diverted to the waste. Autosampler was maintained at 8 °C. The mass spectrometric detection was carried out on an API 4000 tandem mass spectrometer (Applied Biosystems/Sciex, USA) equipped with a Turbo-V® ionspray source operating in the positive ESI mode and quantitation was performed with MRM mode. The ionization source conditions were optimized as follows: ionspray voltage: 4000 V; TEM: 400 °C; CAD: 10 unit; CUR, GS1 and GS2: 30, 50 and 50 psi, respectively. All gases were nitrogen. The optimized compound-dependent parameters including DP, CE, EP and CXP were set at 78 V, 42 V, 8 V and 14 V for fenticonazole and 95 V, 30 V, 9 V and 10 V for the IS. The system control and data analysis were performed by AB Sciex Analyst software (version 1.6.2).

2.3. Preparation of calibration curve and quality control samples

The stock solutions of fenticonazole (1.0 mg/mL) and the IS (1.0 mg/mL) were prepared in methanol. The working solutions for the calibration standards, quality control (QC) samples and IS were prepared by serially diluting the stock solution with methanol/water (1:1, v/v). All the stock and working solutions were stored at −20 °C for long-term use.

Calibration standards were prepared by 50-fold spiking the working solutions with blank plasma at the concentrations of 5, 10, 30, 100, 400, 800 and 1000 pg/mL for method A, and 20-fold spiking the working solutions with blank plasma at the concentrations of 0.1, 0.2, 0.6, 2.0, 8.0, 16 and 20 ng/mL for method B. The lower limit of quantification QC (LLOQ QC), low QC (LQC), medium QC (MQC), high QC (HQC) and dilution QC (DQC) were prepared with the same spiking procedure at concentrations of 5, 15, 150, 750, and 2000 pg/mL for method A, and 0.1, 0.25, 4.0, 15 and 50 ng/mL for method B.

2.4. Sample preparation

2.4.1. Sample preparation for method A

An aliquot of 50 μL of the IS working solution (10 ng/mL) and 450 μL acetonitrile containing 2% acetic acid was added to 150 μL of plasma sample in a 1.5 mL centrifuge tube and thoroughly vortex-mixed for 10 min. After centrifuging (14000 rpm, 10 min at 10 °C), 300 μL of the supernatant was transferred to a clean 96-well plate and evaporated to dryness under a gentle stream of nitrogen at 40 °C. The residue was reconstituted with 150 μL methanol/water (7:3, v/v) and 10 μL of the well-mixed extracts was injected to the HPLC–MS/MS system for analysis.

2.4.2. Sample preparation for method B

A volume of 50 μL plasma sample and an aliquot of 50 μL of the IS solution (667 ng/mL) were added to a 96-well plate and mixed well. Then the mixture was precipitated with 150 μL acetonitrile containing 2% acetic acid, vortex-mixed for 10 min, and then centrifuged at 4000 rpm for 10 min at 10 °C. A 70 μL aliquot of the supernatant was transferred into another clean 96-well plate which was added 210 μL methanol/water (7:3, v/v) in advance. After vortexing for 10 min, 10 μL of the mixture was injected to the LC–MS/MS system for analysis.

Table 1
The stability of fenticonazole in human plasma under different conditions in method A (n=3).

Storage conditions	Nominal conc. (pg/mL)	Mean conc. measured (mean ± SD, pg/mL)	RE (%)	RSD (%)
Room temperature stability (25 °C, 17.7 h)	15.0	15.60 ± 0.66	4.00	4.23
	750	857.67 ± 15.14	14.36	1.77
Freeze-thaw stability (−20 °C, five cycles)	15.0	16.13 ± 0.32	7.53	1.98
	750	861.00 ± 7.55	14.80	0.88
Long-term stability (−20 °C, 54 days)	15.0	17.07 ± 0.76	13.80	4.45
	750	857.00 ± 21.52	14.27	2.51
Autosampler stability (8 °C, 71.8 h)	15.0	15.67 ± 0.60	4.47	3.83
	750	799.33 ± 28.56	6.58	3.57

2.5. Method validation

The two methods were fully validated in terms of selectivity, carryover, linearity, inter-batch and intra-batch accuracy and precision, recovery, matrix effect and stability according to the guidelines for bioanalytical method validation published by the United States Food and Drug Administration (USFDA) [21].

Selectivity was evaluated by comparing the chromatograms of blank human plasma from six different single lots with those of plasma samples spiked with fenticonazole and IS. Moreover, the interference between fenticonazole and IS was evaluated with control blank sample (only IS working solution spiked into blank plasma) and upper limit of quantification (ULOQ) without IS (fenticonazole only spiked at ULOQ concentration). Carryover was carried out by immediately injecting blank plasma samples after the injection of ULOQ samples.

Calibration curves for the two methods were individually constructed by least-squares linear regression analysis of a seven-point calibration curve by plotting fenticonazole-to-IS peak area ratio versus its nominal concentration, with $1/x^2$ as a weighting factor. The LLOQ, defined as the lowest concentration on the calibration curve with acceptable accuracy, precision and satisfactory signal-to-noise ratio over 5, represents the sensitivity of the method. The back-calculated concentrations of each calibration standard have to be within ± 15% deviation (± 20% for LLOQ) of the nominal values.

Intra-batch and inter-batch precision and accuracy were assessed by analyzing QC samples at four concentration levels (LLOQ QC, LQC, MQC and HQC) for both methods in six replicates in three consecutive validation runs. The accuracy was expressed as the relative error (RE) which was required to be within ± 15% (± 20% for LLOQ). Inter-batch precision was assessed by One-Way analysis of variance (ANOVA), and intra- as well as inter-batch precision was expressed as relative standard deviation (RSD) and did not exceed 15% (20% for LLOQ). Moreover, the precision and accuracy of dilution were validated by analyzing six replicates of five-fold diluted QC samples at two concentration levels (HQC and DQC) for both methods.

Recovery of each analyte was determined by analyzing three concentration levels of QC samples (LQC, MQC and HQC) (n=6) and was expressed as the ratio of peak areas obtained from extracted spiked samples to that of samples spiked post-extraction at corresponding concentrations.

Blank plasma from six different single lots, and three replicates of each lot at three concentration levels (LQC, MQC and HQC) were investigated to assess the matrix effect. The matrix effect was calculated as the ratio of peak areas of extracted blank plasma samples spiked with the neat solutions to the peak areas of the neat solutions at corresponding concentrations. The IS normalized matrix factors calculated as the ratio of the matrix factor of fenticonazole to the matrix factor of the IS were used to assess the effects of matrix on ionization.

The stability of fenticonazole in human plasma was investigated at low and high concentration levels (n=3) under the conditions of room temperature, five freeze-thaw (−20 °C) cycles, long-term (−20 °C) storage, and autosampler (8 °C).

2.6. Pharmacokinetic study

The pharmacokinetics of fenticonazole was conducted in healthy Chinese volunteers after percutaneous and intravaginal administration. A total of 22 healthy Chinese volunteers (10 married non-pregnant females for intravaginal administration study, study B; and the rest of 12 consisted of half males and half females for percutaneous application study, study A) were recruited after strict physical examinations, medical history and laboratory examination and were given detailed information about the study before signing an informed consent approved by the Ethics Committee of National Medicine Clinical Trial Organization of Nanjing First Hospital (Nanjing, China) according to the principles of the Declaration of Helsinki.

All the volunteers were fasted overnight for at least 10 h before administration. Study A: each participant with prone position received 5 g of 2% fenticonazole nitrate gel on the back healthy skin areas of 14 cm×14 cm after cleaning and maintained prone position for 1 h, then covered the dosing areas with gauze, and the residual was not removed until 12 h after drug application. Study B: 10 married females with supine position were treated with 5 g of 2% fenticonazole nitrate gel via vagina by professionals. To ensure dosing, volunteers should keep supine position for 1 h.

No food was permitted until a standardized meal was served 4 h after administration. However, water was allowed as desired except for 1 h before and after drug administration. Blood samples were collected pre-dose and post-dose in a heparinized and labeled centrifuge tubes at 0, 2, 4, 6, 8, 10, 12, 15, 24, 30, 36, 48, 72 and 96 h for study A and 0, 1, 2, 4, 6, 8, 10, 12, 15, 24, 36, 48, 72 and 96 h for study B. Then the blood samples were well mixed and centrifuged immediately at 3500 rpm for 8 min under low temperature condition (5 °C), and the plasma was separated and stored at −20 °C until analysis.

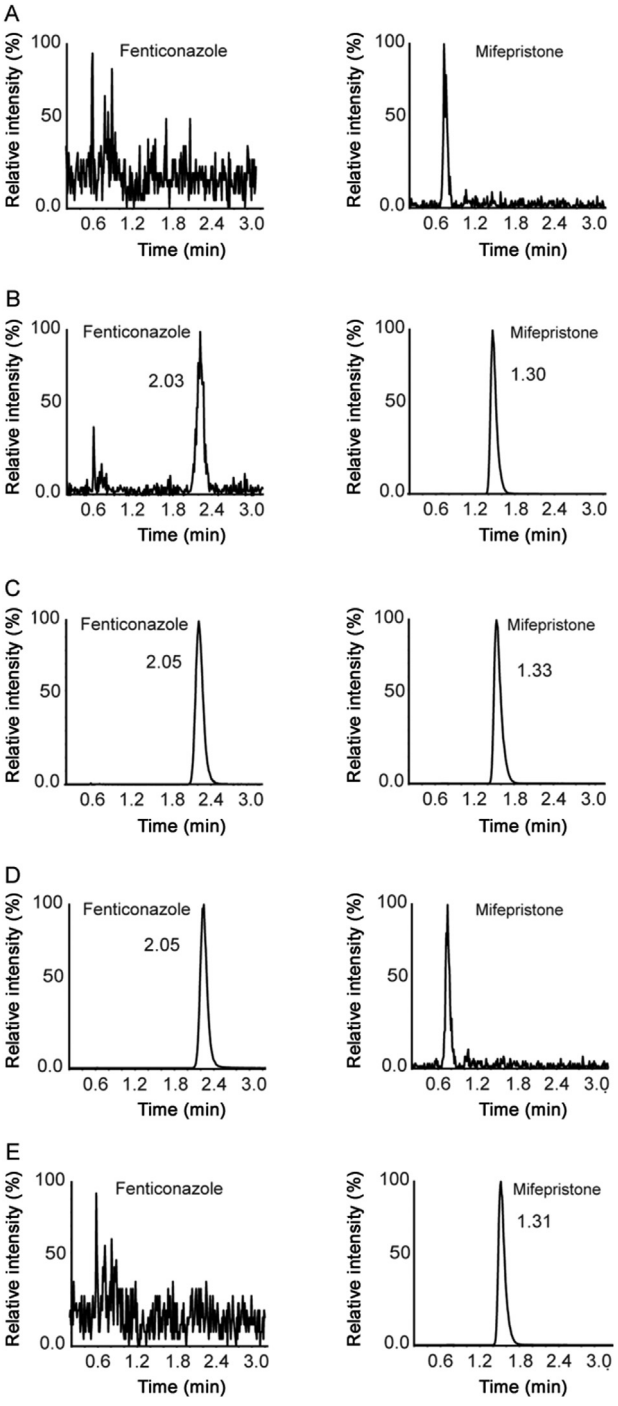

Fig. 3. The typical MRM chromatograms of (A) double blank sample; (B) plasma sample spiked with fenticonazole at LLOQ and IS; (C) plasma sample obtained from a subject after intravaginal administration; (D) ULOQ without IS; and (E) control blank for method B.

The concentration of fenticonazole in plasma was determined by the fully validated HPLC–MS/MS methods. The maximum plasma concentration (C_{max}) and the time to reach the C_{max} (T_{max}) were obtained directly from the concentration-time data and the pharmacokinetic parameters, such as area under the plasma concentration-time curve (*AUC*), elimination half-life ($t_{1/2}$), mean residence time (*MRT*) and plasma clearance (*CLz/F*) were obtained according to non-compartment model with DAS 3.2.7 (DAS®; professional edition version 3.2.7, Drug and Statistics, Shanghai, China).

3. Results and discussions

3.1. Method development

3.1.1. Sample preparation

Both protein precipitation (PPT) and liquid-liquid extraction (LLE) were taken into account as sample pretreatment. Initially, LLE with organic solvents (ethyl acetate, tert-butyl methyl ether (TBME), dichloroethane and the mixture of different proportions of the extraction solvents) and PPT with acetonitrile and methanol were investigated. The results indicated that the recovery values of PPT for fenticonazole and IS were higher than those of LLE, and the evaluated matrix effect result of PPT was acceptable. Therefore, PPT was selected for the sample pretreatment. The addition of acetic acid in the precipitant could greatly improve the test results of extraction recovery and matrix effect, and acetonitrile containing 2% acetic acid was selected as the precipitant for its high recovery rates for the both analytes without matrix interference. To meet the requirements of the detection sensitivity for the pharmacokinetic study of fenticonazole in human after percutaneous administration, centrifuging at 14000 rpm for 10 min for the precipitant treated plasma samples could provide more cleaner supernatants and the supernatants were separated and evaporated to dryness under a stream of nitrogen, and then the residues were reconstituted with moderate amounts of solvent.

3.1.2. Mass spectrometry

To optimize the mass spectrometric conditions, the standard solutions of fenticonazole and IS were respectively infused into the mass spectrometer by a syringe pump and the optimal parameters were obtained by manual tuning. Both fenticonazole and the IS showed strong intensity under positive ionization mode due to their tendency to easily capture a proton. In Q1 full-scan mass spectra, the intense molecular ions were *m/z* 455.2 for fenticonazole and *m/z* 430.2 for IS. Fig. 1 shows the product ion spectra of fenticonazole and the IS at *m/z* 199.1 and 372.3, respectively. A sensitive detector to quantify fenticonazole in plasma samples is crucial for accurate pharmacokinetic study of fenticonazole, especially at its low plasma concentration levels. Considering sensitivity requirement, Triple Quad 5500 was applied in method A to determinate fenticonazole in percutaneous pharmacokinetic study rather than API 4000. Moreover, the mass spectrometer parameters including compound-dependent parameters (DP, EP, CE and CXP) and ionization source parameters (ionspray, CUR, GS1, GS2, TEM and CAD) were optimized to achieve higher intensity of the mass response to the analytes.

3.1.3. Chromatography

Chromatographic elution conditions were optimized to achieve symmetrical peak shapes and short chromatographic retention times. Methanol, rather than acetonitrile, was chosen as the organic portion of the mobile phase, because it provided higher mass spectrometer response for the analytes. To separate the analytes from endogenous interfering substances and avoid the matrix effect, ammonium acetate and formic acid were added into the aqueous portion of the mobile phase, and different concentration levels of ammonium

Table 2
The stability of fenticonazole in human plasma under different conditions in method B (n=3).

Storage conditions	Nominal conc. (ng/mL)	Mean conc. measured (mean ± SD, ng/mL)	RE (%)	RSD (%)
Room temperature stability (25 °C, 17.0 h)	0.250	0.254 ± 0.005	1.60	1.97
	15.0	15.467 ± 0.404	3.11	2.61
Freeze-thaw stability (−20 °C, five cycles)	0.250	0.271 ± 0.009	8.40	3.32
	15.0	15.267 ± 0.153	1.78	1.00
Long-term stability (−20 °C, 77 days)	0.250	0.250 ± 0.018	0.00	7.20
	15.0	14.467 ± 0.115	−3.55	0.79
Autosampler stability (8 °C, 71.8 h)	0.250	0.257 ± 0.008	2.80	3.11
	15.0	15.217 ± 0.183	1.45	1.20

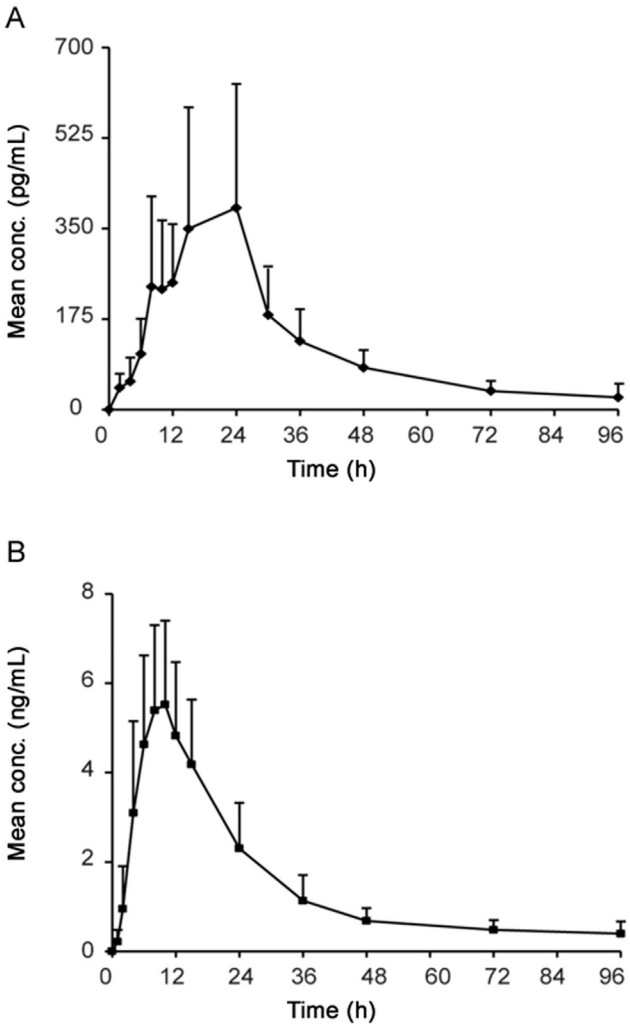

Fig. 4. Mean plasma concentration-time profiles of fenticonazole in healthy Chinese volunteers after (A) percutaneous administration (n=12) and (B) intravaginal administration (n=10) of 2% fenticonazole nitrate gel.

acetate (0, 2, 3, 5 and 10 mM) and formic acid (0, 0.05%, 0.1%, 0.2% and 0.5%) were investigated. Eventually, methanol (mobile phase B) and water containing 5 mM ammonium acetate and 0.1% formic acid

Table 3
The pharmacokinetic parameters of fenticonazole (mean ± SD) in healthy Chinese volunteers after percutaneous and intravaginal administration of 2% fenticonazole nitrate gel.

PK parameters	Percutaneous administration (n=12)	Intravaginal administration (n=10)
T_{max} (h)	16.917 ± 6.694	9.400 ± 1.647
C_{max} (ng/mL)	0.499 ± 0.235	5.957 ± 2.044
$t_{1/2}$ (h)	27.883 ± 16.317	26.510 ± 4.778
AUC_{0-t} (ng/mL·h)	11.876 ± 4.558	151.020 ± 46.806
$AUC_{0-\infty}$ (ng/mL·h)	12.914 ± 4.449	159.220 ± 48.004
MRT_{0-t} (h)	30.975 ± 5.391	26.460 ± 4.773
CL_z/F (L/h)	8890.833 ± 3858.688	559.724 ± 233.551

T_{max}: The time to reach the C_{max}; C_{max}: The maximum plasma concentration; $t_{1/2}$: Elimination half-life; AUC_{0-t}: Area under the plasma concentration-time curve from zero hour to 96 h; $AUC_{0-\infty}$: The AUC_{0-t} extrapolated to infinity; MRT_{0-t}: Mean residence time; CL_z/F: The plasma clearance.

(mobile phase A) were selected as the mobile phase for the method B. However, the same mobile phase system in the method B is not suitable to be applied in the method A for the determination of the concentrated samples; otherwise an interference peak would appear in the retention time of fenticonazole when the Triple Quad 5500 was used. Methanol (mobile phase B) and water containing 5 mM ammonium acetate and 0.2% formic acid (mobile phase A) with the initial proportion of 68% organic phase could avoid the interference substances and provide higher resolution and greater baseline stability.

In order to avoid later-eluted endogenous interference substances from the former injections stayed in column which may interfere its later injection, the proportion of organic phase of the mobile phase was increased to 100% after fenticonazole and the IS were eluted, and the system was equilibrated to initial sampling column pressure with initial proportion of organic phase before the next injection.

3.2. Method validation

3.2.1. Method A

The typical chromatograms of double blank sample, plasma sample spiked with fenticonazole at LLOQ and IS, plasma sample obtained from a subject after percutaneous application, ULOQ without IS and control blank are shown in Fig. 2. No significant interfering peaks were observed at the retention time of fenticonazole and IS, respectively. No

significant carryover peaks were observed for both fenticonazole and IS.

The calibration curve over the concentration range of 5–1000 pg/mL showed good linearity with weighted correlation coefficient $r > 0.9984$. The LLOQ of 5 pg/mL was the lowest reported so far [19,20].

The results suggested that the established method had a satisfactory reproducibility with accuracy ranging from −2.0% to 11.0%, and precision values were less than 10.6%. The precision and accuracy of the DQCs (RSD < 3.1%, RE < 4.7%) showed that the human plasma samples whose concentration exceeded ULOQ could be analyzed by five-fold dilution with blank human plasma.

The mean recovery of fenticonazole at three QC levels and the recovery of the IS were (92.0 ± 0.9)% and (94.1 ± 5.8)%, respectively. The IS normalized matrix factors at three concentration levels for fenticonazole were between 108.7% and 114.1% with RSD below 2.5%.

The stability results summarized in Table 1 indicate that fenticonazole is stable under routine processing and storage conditions.

3.2.2. Method B

The typical chromatograms of double blank sample, plasma sample spiked with fenticonazole at LLOQ and IS, plasma sample obtained from a subject after intravaginal administration, ULOQ without IS and control blank are shown in Fig. 3. No significant interference from endogenous peaks and carryover peaks was observed at the retention time of fenticonazole and IS.

Seven concentration levels were tested and the method exhibited good linearity over the range of 0.1–20 ng/mL with correlation coefficient $r > 0.9979$.

The intra- and inter-precision were below 7.2% and 6.8%, respectively and the accuracy ranged from 0.5% to 8.8%. For the precision and accuracy of the DQCs, the RE and RSD were below 3.1%, and the accuracy for both QC levels ranged from −4.7% to 4.0%.

The recoveries for fenticonazole and the IS were (101.0 ± 1.9)% and (100.1 ± 2.8)%, respectively. The matrix effect of fenticonazole by normalization with the IS was within the range of 105.3%–109.0%, and the matrix effect of the IS was (92.5 ± 1.3)%.

The stability results summarized in Table 2 indicate that fenticonazole is stable under routine processing and storage conditions.

3.3. Pharmacokinetic study

The validated HPLC–MS/MS methods were successfully applied to evaluate the pharmacokinetic profiles of fenticonazole in Chinese healthy volunteers after single percutaneous and intravaginal administration with 5 g of 2% fenticonazole nitrate gel. The mean plasma concentrations versus time profiles of fenticonazole are shown in Fig. 4. The main pharmacokinetic parameters are summarized in Table 3. The maximum plasma concentration (C_{max}) were 0.499 ± 0.235 ng/mL and 5.957 ± 2.044 ng/mL for percutaneous administration and intravaginal administration, respectively. The elimination half-life ($t_{1/2}$) were 27.883 ± 16.317 h and 26.510 ± 4.778 h for percutaneous administration and intravaginal administration, respectively. The results showed that after single percutaneous and intravaginal administration with 5g of 2% fenticonazole nitrategel, the Cmax of intravaginal administration was about 12 folds than that of percutaneous administration, and there was no significant difference between the two different administration routes in terms of $t_{1/2}$. The mean plasma concentrations versus time profiles of fenticonazole are shown in Fig. 4.

4. Conclusion

Two simple and sensitive HPLC–MS/MS methods for the determination of fenticonazole in human plasma were developed and fully validated. The good sensitivity made the method suitable for the determination of those low concentration plasma samples in the percutaneous pharmacokinetic study. This is the first report on the pharmacokinetics of fenticonazole in Chinese healthy volunteers and the pharmacokinetic profiles of fenticonazole under different administration routes would be helpful in providing some reference for clinical application and clinical medication safety.

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