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Comparative pharmacokinetic studies of peimine and peiminine in rat plasma by LC-MS-MS after oral administration of *Fritillaria thunbergii* Miq. and *Fritillaria thunbergii* Miq. - *Glycyrrhiza uralensis* Fisch. couple extract

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A sensitive LC-MS-MS method has been successfully applied to pharmacokinetic study of peimine and peiminine in rat plasma after oral administration of *Fritillaria thunbergii* Miq. exact and *Fritillaria thunbergii* Miq. - *Glycyrrhiza uralensis* Fisch. couple extract. The results indicated that plasma profiles of peimine and peiminine confirmed to two-compartment open model with weighting function of $1/C^2$ for data fitting and parameter estimation and the utilization with *Glycyrrhiza uralensis* Fisch. could decrease C_{max} and prolong $MRT_{0\text{-}\infty}$ and $t_{1/2}$ of peimine remarkly with the bioavailability of peimine remained practically unchanged. Meanwhile, the concentration of peimine in rat plasma was more stable. Nevertheless, there were no significant differences among all calculated parameters of peiminine.

1. Introduction

Modern pharmacological studies have elucidated that *Fritillaria thunbergii* Miq. has many activities such as preventing cough, eliminating phlegm, relieving pain and anti-inflammatory effect (Qian et al. 1985; Xiao et al. 1992; Zhou et al. 2003). In recent years, it has attracted more attention for its reversal effects on multidrug resistance (Li et al. 2001). According to the statistics of some ancient medical books, such as Shengji Zonglu, Taiping Shenghui Fang, Puji Fang, we found that there are 79 prescriptions which had been chosen *Fritillaria thunbergii* Miq. as Jun herb, and 80% of them containing *Glycyrrhiza uralensis* Fisch. as Shi hurb. Most herbal medicines are prescribed in combination based on the theory of Traditional Chinese medicine (TCM) to obtain synergistic effects or diminish the possible adverse reactions. As we know, Jun herb often provides the main therapeutic action and Shi herb serves to guide the bioactive components of the herbs to the proper organs to exert a harmonizing effect (Li et al. 2009). To a large extent, the combination of the two could represent the complex prescription to cure diseases and provide therapeutic action.

Given the multi-ingredient character of herbal medicines, the likelihood of herb–drug interactions is theoretically higher than that of drug–drug interactions. Moreover, multiple case reports of herb–drug interactions have been published in recent years (Lu et al. 2007). Nevertheless, pharmacokinetics is not only a valid way to investigate the synergistic interaction of medicines (Higashi et al. 2005; Koitabashi et al. 2006; Baldan et al. 2006; Bergamaschi et al. 2006; Shin et al. 2006), but also can provide information for the slection of dosage form.

It is well documented that the major constituents in *Fritillaria thunbergii Miq.* are isosteroidal alkaloids, of which peimine and peimine are the main types (Li et al. 1992), and are selected as the marker components for the quality control in China according to the Chinese pharmacopoeia (State Pharmacopoeia Committee 2010). Most of the published reports selected one or several representative compounds as the targets to perform pharmacokinetic study (Xu et al. 2008), and in this paper we chose peimine and peiminine as the targets. Several methods have been described for the identification and determination of peimine and peiminine in herbal extract. Such as GC with pre-column derivatization, RHPLC with pre-column derivatization, GC, HPLC-ELSD, LC/ESI-TOF-MS (Li et al. 1999; Kan et al. 1996; Li et al. 2000; Li et al. 2001; Zhou et al. 2008). On the contrary, few methods have been reported for the detection of peimine and peiminine in biological matrices. To our knowledge, few studies about LC-MS-MS method for the simultaneous detection of peimine and peiminine in plasma have been reported (Wu et al. 2010; Chen et al. 2010). And there is no report about its application in pharmacokinetic study after oral administration of compatible extract. The aim of this study was to explore the pharmacokinetic difference of peimine and peiminine in rats after oral administration of *Fritillaria thunbergii Miq.* extract and *Fritillaria thunbergii Miq.–Glycyrrhiza uralensis* Fisch. couple extract. Results of pharmacokinetic studies are valuable for evaluating the rationality and compatibility of herbs or prescriptions (Wu et al. 2009). It is expected that the results of this study would be useful for improving the clinical therapeutic efficacy of *Fritillaria* preparations and indicating the selection of dosage forms. Furthermore, we hope that the results of our study will provide information for the application of toxic chinese medicinal materials.

2. Investigations and results

2.1. Method validation

2.1.1. Selectivity

The representative MRM chromatograms of blank plasma, spiked plasma and real plasma samples after oral administration of *Fritillaria thunbergii* Miq. exact and *Fritillaria thunbergii*

Table 1: Precision and accuracy of the determination of peimine and peiminine in rat plasma (inter-day n = 6; intra-day n = 6 × 3)

Analytes	Spiked (ng mL^{-1})	Intra-day			Inter-day		
		Measured (ng mL^{-1})	RE (%)	RSD (%)	Measured (ng mL^{-1})	RE (%)	RSD (%)
Peimine	2.0	2.04 ± 0.06	1.5	3.2	2.14 ± 0.14	5.9	6.5
	80.0	80.2 ± 2.3	0.22	2.9	80.2 ± 1.4	0.30	1.7
	200.0	205.4 ± 5.1	2.7	2.5	203.8 ± 5.5	1.9	2.7
Peiminine	2.0	2.08 ± 0.04	1.9	2.2	2.05 ± 0.10	0.70	4.8
	80.0	78.8 ± 0.4	−1.5	0.54	80.1 ± 3.0	0.15	3.8
	200.0	203.0 ± 3.2	1.5	1.6	206.7 ± 5.0	3.3	2.4

Miq. - *Glycyrrhiza uralensis* Fisch. couple extract are shown in Fig. 1. The retention times of peimine, peiminine and IS were 2.20 min, 2.65 min and 5.40 min, respectively. No endogenous source of interference and no interference from other components of the extract or the couple extract was observed at the retention times of peimine, peiminine and IS in MRM mode. This indicated that the method was suitable for the selective detection of peimine and peiminine in rat plasma.

2.1.2. *Linearity and LLOQ*

The regression equation of the curves and the correlation coefficients (r) were calculated as $y = 0.008181x + 0.003920$ ($r = 0.9958$) for peimine and $y = 0.007691x + 0.007109$ ($r = 0.9942$) for peiminine using weighted least squares linear regression (weighting factor was $1/c$). The calibration curves were linear over the concentration range of 1.0−200.0 ng mL^{-1} for both of peimine and peiminine. The LLOQ for both of them was 1.0 ng mL^{-1} with precision not exceeding 20% and accuracy within ± 20%.

2.1.3. *Precision and accuracy*

The intra- and inter-day precision and accuracy values are summarized in Table 1. The intra- and inter-day precision values were not more than 6.5%, and the deviations were within ± 5.9%. The results demonstrated that the precision and accuracy of this assay were acceptable.

2.1.4. *Matrix effect and extraction recovery*

The extraction recoveries of peimine and peiminine from rat plasma are shown in Table 2. In addition, the extraction recovery of IS was 86.4 ± 3.1%. The extraction recoveries of analytes were shown to be consistent and reproducible. All the ratios (A/B × 100) % were between 85% and 115%, which excludes matrix effects for peimine, peiminine and IS in this method.

Table 2: Extraction recovery results for peimine and peiminine in rat plasma (n = 6)

Sample	Spiked concentration (ng mL−1)	Recovery (%) (mean ± SD)	RSD (%)
Peimine	2.0	96.4 ± 3.2	3.3
	80.0	97.3 ± 2.7	2.8
	200.0	100.4 ± 4.6	4.6
Peiminine	2.0	89.4 ± 4.5	5.0
	80.0	92.5 ± 2.2	2.4
	200.0	95.6 ± 3.1	3.2

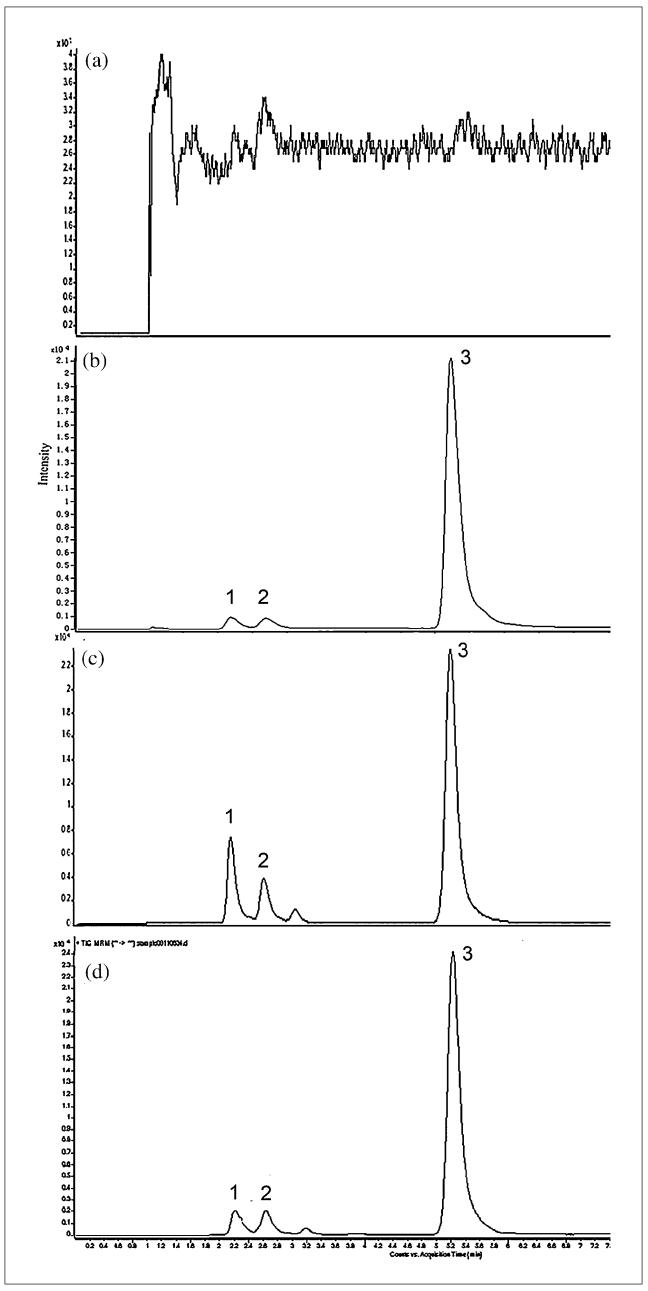


Fig. 1: Representative chromatograms of plasma samples. (a) rat blank plasma, (b) blank plasma spiked with peimine (1 ng mL^{-1}), peiminine (1 ng mL^{-1}) and carbamazepine (IS, 500 ng mL^{-1}), (c) rat plasma collected 2 min after oral administration of *Fritillaria thunbergii* Miq. extract, (d) rat plasma collected at 2 min after oral administration of *Fritillaria thunbergii* Miq. - *Glycyrrhiza uralensis* Fisch. couple extract. The retention times of peimine, peiminine and IS were 2.20 min, 2.65 min and 5.40 min, respectively. 1 = peimine; 2 = peiminine; 3 = carbamazepine.

Table 3: Stability of peimine and peiminine in rat plasma (n = 6)

Stability	Accuracy (mean ± RSD) (%)					
	Peimine			Peiminine		
	2.0 (ng mL^{-1})	80.0 (ng mL^{-1})	200.0 (ng mL^{-1})	2.0 (ng mL^{-1})	80.0 (ng mL^{-1})	200.0 (ng mL^{-1})
Freeze-thaw	1.98 ± 7.8	84.3 ± 5.2	201.8 ± 2.5	2.04 ± 2.2	80.6 ± 5.5	199.6 ± 1.1
Long-term	1.95 ± 11.3	78.9 ± 2.4	208.5 ± 3.6	1.98 ± 5.2	80.5 ± 3.1	205.3 ± 4.7
Short-term	1.97 ± 6.5	81.0 ± 1.9	200.9 ± 4.3	2.13 ± 8.6	80.4 ± 2.2	202.7 ± 5.0
Post-preparation	1.96 ± 6.8	80.4 ± 3.2	202.4 ± 3.0	2.18 ± 6.4	80.8 ± 3.7	202.5 ± 3.3

2.1.5. Stability

The results of stability studies are shown in Table 3. These results from short-term stability test, long-term stability test, freeze-thaw stability test and post-preparation stability demonstrate that peimine and peiminine were stable over all steps of determination.

2.2. Pharmacokinetics in rats

Plasma concentrations of peimine and peiminine were simultaneously determined after oral administration of *Fritillaria thunbergii* Miq. exact and *Fritillaria thunbergii* Miq.- *Glycyrrhiza uralensis* Fisch. couple extract and the plasma profiles of the two compounds demonstrated to two-compartment open model with weighting function of $1/C^2$ for data fitting and parameter estimation. Statistically significant differences ($p < 0.05$) in pharmacokinetic parameters of peimine including C_{max}, $t_{1/2}$, $MRT_{0\text{-}\infty}$ were obtained among the rats orally administered *Fritillaria thunbergii* Miq. and *Fritillaria thunbergii* Miq.- *Glycyrrhiza uralensis* Fisch. couple extract, the pharmacokinetic parameters of $AUC_{0\text{-}\infty}$, CL/F, V/F, and T_{max} have no significant changes. Acorrding to the increased values of $t_{1/2}$, $MRT_{0\text{-}\infty}$ and the decreased value of C_{max}, we surmise that glycyrrhizic acid reacts with peimine forming a complex salt which has a poor solubility in water, but could decompose into free peimine gradually in gastrointestinal fluids. Meanwhile, there were no significant differences among all calculated parameters for peiminine.

In short, the results indicated a potential pharmacokinetic interaction between *Fritillaria thunbergii* Miq. and *Glycyrrhiza uralensis* Fisch. (Fig. 2; Table 4). From this point of view, the combination is rather unsuitable. However, different from Western medicine, TCM concentrates on the overall functional state of the patient, which is becoming a trend in modern medicine for treating complicated diseases (Jiang 2005). Therefore, the multiherb remedy might be a reasonable prescription in spite of the existence of chemical and pharmacokinetic interactions between *Fritillaria thunbergii* Miq. and *Glycyrrhiza uralensis* Fisch.

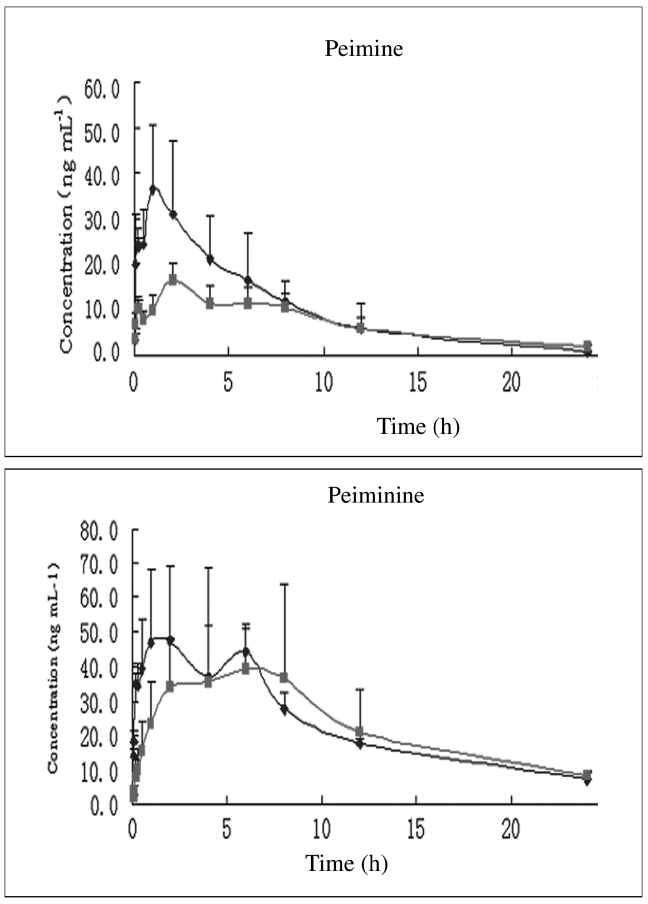


Fig. 2: Mean plasma concentration-time profile of peimine and peiminine after oral administration of *Fritillaria thunbergii* Miq. extract (4.25 g kg^{-1}) (♦) and *Fritillaria thunbergii* Miq.- *Glycyrrhiza uralensis* Fisch. couple extract (4.25 g kg^{-1} *Fritillaria thunbergii* Miq. and 1.46 g kg^{-1} *Glycyrrhiza uralensis* Fisch. extract) (■).

3. Discussion

In this paper, a sensitive and rapid LC-MS-MS method has been developed for the simultaneous determination of peimine and peiminine in rat plasma. Our findings suggest that the plasma profile of peimine and peiminine confirmed to two-compartment open model. In terms of mean plasma concentration-time profile of peimine, comparing with the previous one (orally administered *Fritillaria thunbergii* Miq. exact), the latter one (orally administered *Fritillaria thunbergii* Miq.- *Glycyrrhiza uralensis* Fisch. couple extract) was similar to the curve of sustained-release or controlled release preparations. While $AUC_{0\text{-}\infty}$ did not change significantly, the values of $t_{1/2}$ and $MRT_{0\text{-}\infty}$ increased remarkably ($p < 0.05$) and C_{max} decreased remarkably ($p < 0.05$). We surmise that glycyrrhizic acid reacts with peimine. Of course, it should be pointed out that further profound research is required. There were no significant differences among all calculated parameters for peiminine.

Compared with oral administration of *Fritillaria thunbergii* Miq. exact, the bioavailability of peimine and peiminine remained practically unchanged, but the concentration of peimine in rat plasma was more stable and the maximum plasma concentration (C_{max}) decreased remarkably after oral administration of *Fritillaria thunbergii* Miq.- *Glycyrrhiza uralensis* Fisch. couple extract. The information might be useful for improving the clinical therapeutic efficacy of *Fritillaria thunbergii* Miq. and indicating the selection of dosage forms. Furthermore, we hope that the results of our study could provide information for the application of toxic chinese medicinal materials.

Table 4: Pharmacokinetic parameters of peimine and peiminine after oral administration of *Fritillaria thunbergii* Miq.exact(4.25 g kg^{-1}) and *Fritillaria thunbergii* Miq.–*Glycyrrhiza uralensis* Fisch. couple extract (4.25 g kg^{-1} *Fritillaria thunbergii* Miq. exact and 1.46 g kg^{-1} *Glycyrrhiza uralensis* Fisch. exact), each value represents the mean ± SD (n = 6)

Parameters	Peimine (mean ± SD)		Peiminine (mean ± SD)	
	Fritillaria thunbergii Miq.exact	*Fritillaria thunbergii* Miq.– *Glycyrrhiza uralensis* Fisch. couple extract	*Fritillaria thunbergii* Miq. exact	*Fritillaria thunbergii* Miq.– *Glycyrrhiza uralensis* Fisch. couple extract
$t_{1/2}$ (h)	4.8 ± 0.8	8.5 ± 1.4	6.6 ± 3.2	9.2 ± 4.4
T_{max} (h)	1.5 ± 0.6	2.5 ± 1.0	4.5 ± 1.9	5.0 ± 2.6
CL/F (L h^{-1} kg^{-1})	119.6 ± 40.1	139.3 ± 23.6	34.1 ± 4.8	33.4 ± 9.1
V/F (L kg^{-1})	854.8 ± 363.9	1722.0 ± 504.3	321.1 ± 155.4	455.0 ± 237.0
C_{max} (ug L^{-1})	43.2 ± 5.4	17.5 ± 1.8	57.6 ± 23.0	51.2 ± 18.1
$MRT_{(0-\infty)}$ (h)	6.4 ± 0.3	11.9 ± 1.0	10.5 ± 3.6	14.4 ± 4.7
$AUC_{(0-\infty)}$ (ug h L^{-1})	260.5 ± 119.8	203.0 ± 36.8	618.3 ± 94.8	665.4 ± 224.2

4. Experimental

4.1. Chemicals and reagents

Standard of peimine (purity > 99%), peiminine (purity > 99%) and carbamazepine (IS, 99%) (Fig. 3) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). *Glycyrrhiza uralensis* Fisch. and decoction pieces of *Fritillaria thunbergii* Miq. were purchased from Huangqingren Huashi Pharmacy in Jiangxi province. Both of the two were identified by professor Cuisheng Fan (Department of Pharmacy, Jiangxi University of TCM, Nanchang, China). Acetonitrile, ethyl acetate, ammonia water and ammonium formate, were of HPLC grade, and other reagents used were of analytical grade.

4.2. Instruments and conditions

Liquid chromatographic separation and mass spectrometric detection were achieved by employing Agilent 6410 LCQQQ (Agilent Corporation, MA, USA) consisted of a G1311A quaternary pump, a G1316A column oven, a G1329A autosampler, a G1322A vacuum degasser, an Agilent 6410A triple quadrupole mass spectrometer, and MassHunter software was used for data acquisition and processing.

Chromatographic separation was on a luna $C_{18(2)}$ column, (3 μm, 150 × 2 mm phenomenex) equipped with a precolumn (3 μm, 4 × 2 mm phenomenex). Details of the LC-MS-MS method were previously published (Chen et al. 2010).

4.3. Extraction of the herbs

The coarse powder of *Fritillaria thunbergii* Miq. was soaked for 48 h before percolating with sevenfold ethanol-water (70:30, *v/v*) at the speed of 1.0 mL min^{-1} kg^{-1}. Followed by concentrating and vacuum drying, we got the extract equivalent to 0.05 g g^{-1} of crude powder. The concentrations of peimine and peiminine in the extract were determined as 6.50 mg g^{-1}, 4.88 mg g^{-1} by HPLC-ELSD, respectively.

Glycyrrhiza uralensis Fisch. was immersed in eightfold ethanol-water (10:90, *v/v*) and refluxed twice with 1 h endurance each time. After filtration, the liquid phase was retrieved until the concentration equivalent to 0.5 g mL^{-1} of crude powder. After holding for 12 h, adjusted pH to 2.0 to precipitate the resulting solution. Finally we got the extract powder which equivalent to 0.04 g g^{-1} of crude powder. The concentrations of glycyrrhizic acid in the extract were determined as 580.0 mg g^{-1} by HPLC.

The couple extract was prepared by extracting *Fritillaria thunbergii* Miq. and *Glycyrrhiza uralensis* Fisch. in the ratio of 2:1, respectively.

4.4. Preparation of standards and quality control samples

The stock solutions of peimine (200 μg mL^{-1}), peiminine (200 μg mL^{-1}) and IS (500 μg mL^{-1}) were prepared respectively by dissolving the accurately weighed reference compounds in methanol, and stored at 4 °C. On the day of analysis, standard working solutions of peimine and peiminine with concentrations of 2000, 800, 400, 200, 20 and 10 ng mL^{-1} were obtained by further dilution of the standard stock solution with methanol. By the same method, standard working solutions of IS with a concentration of 500 ng mL^{-1} was prepared. Calibration standards of 200.0, 80.0, 40.0, 20.0, 2.0 and 1.0 ng mL^{-1} for peimine and peiminine were acquired by diluting the standard working solution with rat plasma, respectively. For each calibration series, a blank sample and a zero sample were also prepared. Quality control (QC) samples were utilized in rat plasma at concentrations of 2.0, 80.0, 200.0 ng mL^{-1} for both of peimine and peiminine, each of the Qc sample contained 50.0 ng mL^{-1} IS. All the solutions were stored at 4 °C. Before processing each analytical batch, the spiked samples were brought to room temperature and processed together with the biological samples.

4.5. Sample preparation

An aliquot of 100 μL rat plasma was transferred into heparinized Eppendorf tube in the presence of 10 μL methanol and 10 μL IS working solution. After vortexing for 1 min, 20 μL ammonia water was added and the mixture was vortex-mixed for 1 min. Then, 0.8 mL ethyl acetate was added to extract by vortex-mixing for 5 min. The sample was centrifuged at 4,000 rpm for 10 min. The supernatant was aspirated and then evaporated to dryness at 40 °C under an N_2 stream. Finally, the residue was reconstituted with 100 μL mobile phase and centrifugated at 18000 rpm for 10 min. The reconstituted sample was filtered with Φ 0.22 μm polytetrafluoroethylene membrane prior to analysis by LC-MS-MS and the injection volume was 10 μL.

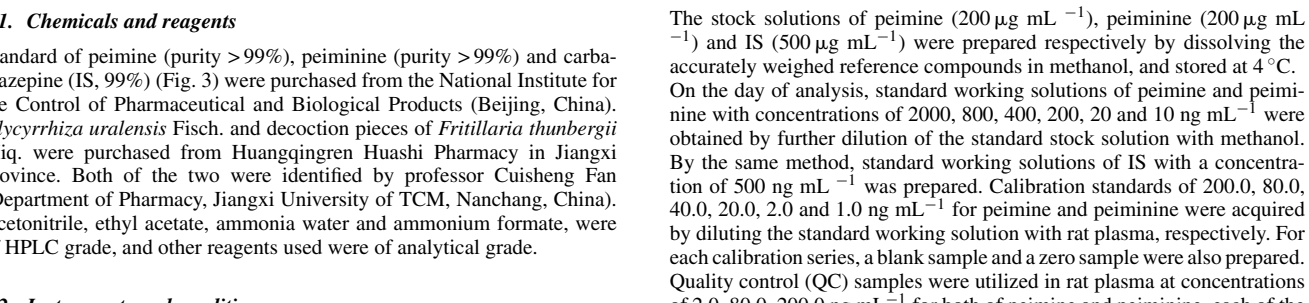
4.6. Method validation

4.6.1. Selectivity

Selectivity was studied by comparing chromatograms of six different batches of blank plasma with the corresponding spiked plasma and the plasma after oral dose of the extracts.

4.6.2. Linearity and LLOQ

Six calibration curves containing a blank sample, a zero sample and seven non-zero sample were analyzed for peimine and peiminine, respectively.



(a) (b) (c)

Fig. 3: Chemical structures of (a) peimine, (b) peiminine, (c) carbamazepine (IS).

The linear regression analysis was constructed by plotting peak-area ratio of analyte-to- IS (y) versus concentration of corresponding analyte in spiked plasma samples (x, ng mL^{-1}). The regression equation of the curves and the correlation coefficients were calculated by weighted least squares linear regression and the weighting factor was $1/c$. The lower limit of quantitation (LLOQ) of the assay was defined as the lowest concentration on the calibration curve that can be quantitated with accuracy within ± 20% bias of nominal concentration and precision not exceed 20% CV, according to the currently accepted Guidance for Industry Bioanalytical Method Validation of USFDA (Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research 2001).

4.6.3. Precision and accuracy

Accuracy and precision of the assay were evaluated by replicate analysis (n = 6) of QC samples on the same day (intra-day) and also on three consecutive days (inter-day). The concentration of each sample was calculated using a calibration curve constructed on the same testing day. According to Guidance for Industry Bioanalytical Method Validation of USFDA: In terms of accuracy, the mean value should be within 15% of the actual value except at LLOQ; The precision determined at each concentration level should not exceeding 15% of the coefficient of variation (CV) except for the LLOQ. Accuracy was described as relative error (RE), and precision was described as relative standard deviation (RSD).

4.6.4. Matrix effect and extraction recovery

A series of standard working solutions with concentrations of 20.0, 800.0, 2000.0 ng mL^{-1} for peimine and peiminine, and working solutions (500 ng mL^{-1}) for IS were evaluated.
In order to evaluate the matrix effect on the ionization of the analyte, such as the potential ion suppression or enhancement due to the matrix components, five replicates of 100 µL blank plasma were extracted and then spiked with above standard working solutions (final concentrations were 2.0, 80.0, 200.0 ng mL^{-1} for peimine and peiminine, 50.0 ng mL^{-1} for IS). The corresponding peak areas (A) were compared with those of standard solutions dissolved in 100 µL mobile phase mobile phase (B). The ratio (A/B × 100) % was used to evaluate the matrix effect. Endogenous matrix effect is implied if the ratio is less than 85% or more than 115% (Shah et al. 2000; Karnes and March 1993).
The extraction recovery of analytes were determined by comparing the peak areas obtained from blank plasma samples which were spiked with analytes before extraction with those obtained from blank plasma samples which were spiked with analytes after extraction. Six replicates of QC samples at each QC level were evaluated.

4.6.5. Stability

Stability of peimine and peiminine in rat plasma was assessed by analyzing six replicates of QC samples at each QC level.
The freeze and thaw stability was determined by subjecting unextracted QC samples to three freeze-thaw cycles. In each freeze-thaw cycle, the samples were frozen and stored at -20 °C for 24 h, then thawed at room temperature. After completion of every cycle, the samples were processed and analyzed. For long-term stability evaluation, unextracted QC samples were stored at -20 °C for two weeks. Short-term stability was evaluated by keeping unextracted QC samples at ambient temperature for 6 h. The stability of the post–preparation samples was evaluated by keeping processed QC samples in an autosampler maintain at room temperature for 12 h. These analysis results were compared with the nominal values. The analytes were considered to be stable in the biological matrix when 85-115% of the initial concentrations were found.

4.7. Animals

Female Sprague-Dawley rats, weighing 200-250 g, were obtained from the Experimental Animal Center of Jiangxi University of TCM. All the rats were kept in standard animal holding room at a temperature of 23 ± 1 °C and relative humidity of 55 ± 10%. Water and food were allowed ad libitum. The animals were acclimatized to the facilities for five days and then fasted with free access to water for 12 h prior to each experiment. All animal studies were carried out according to the Guidelines for the Care and Use of Laboratory Animals, and were approved by the Committee of Ethics of Animal Experimentation of Jiangxi University of TCM (Nanchang, China).

4.8. Biological specimen collection

Rats were divided into two groups randomly (n = 6). The rats of one group were administered with 4.25 g kg^{-1} extract powder of *Fritillaria thunbergii* Miq. For the other group, 4.25 g kg^{-1} extract powder of *Fritillaria thunbergii* Miq. and 1.46 g kg^{-1} extract powder of *Glycyrrhiza uralensis* Fisch. were administered. Both of the extracts were dissolved in distilled water before oral administration. 0.25 mL blood samples were collected in heparinized Eppendorf tubes via the posterior orbital venous plexus before dosing and subsequently at 0.033, 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 h after oral administration. After centrifuging at 3,000 rpm for 10 min, the plasma samples were obtained and frozen at −20 °C until analysis.

4.9. Pharmacokinetic analysis

DAS 2.0 package (Chinese Pharmacological Society) was used to calculate the pharmacokinetic parameters, such as T_{max}, C_{max}, $t_{1/2}$, $AUC_{0-\infty}$, $MRT_{0-\infty}$, CL/F, V/F, etc. Statistical analysis of the biological data was performed by the student′ s t test. All results were expressed as arithmetic mean ± standard deviation (SD).

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