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Turbulent-flow chromatography coupled on-line to fast high-performance liquid chromatography and mass spectrometry for simultaneous determination of verticine, verticinone and isoverticine in rat plasma

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ABSTRACT

A method based on the on-line turbulent-flow chromatography and fast high-performance liquid chromatography/mass spectrometry (TFC–LC/MS) was developed for sensitive and high throughput pharmacokinetic study of traditional Chinese medicines (TCMs). In this method, an on-line extraction column (Waters Oasis HLB) and a fast HPLC column with sub-2 μm particle size (Agilent Zorbax StableBond-C_{18}, 4.6 mm × 50 mm, 1.8 μm) in a column-switching set-up were utilized. HLB is a reversed-phase extraction column with hydrophilic–lipophilic balanced copolymer (2.1 mm × 20 mm, 25 μm particle size), which will exhibit some turbulent-flow properties at a high-flow rate. The method combines the speed and robustness of turbulent-flow extraction and the sensitivity and separation efficiency of fast HPLC–MS to analyze multiple and trace constituents of TCMs in plasma matrix. This method was successfully applied for pharmacokinetic study of verticine, verticinone and isoverticine, the chemical markers of *Fritillaria thunbergii*, after oral administration of total steroidal alkaloids extract of *F. thunbergii* to rats. Each plasma sample was analyzed within 7 min. The method demonstrated good linearity ($R > 0.999$) ranged from 0.505 to 96.0 ng/mL with satisfactory accuracy and precision, and the lower limit of quantifications of verticine, verticinone and isoverticine were estimated to be 0.120, 0.595 and 0.505 ng/mL, respectively. These results indicate that the proposed method is fast, sensitive, and feasible for pharmacokinetic study of TCMs.

1. Introduction

Traditional Chinese medicines (TCMs) have played an important role in human history for preventing and treating diseases in China and other Asian countries. Recently, the efficacy of TCMs has been gradually documented by pharmacological and clinical study [1], and the active constituents of TCMs have been revealed endlessly during these assays. Besides the pharmacological evaluation, the *in vivo* metabolism and pharmacokinetic data of the active constituents have become an important issue because these data can help us for better understanding their pharmacologic activities and clinical effects and guiding the device of clinical administration dose [2]. Therefore, the pharmacokinetic study of TCMs has been followed with interest more recently.

Due to the complex composition in TCMs, the qualitative and quantitative analyses of the active constituents *in vivo* are constant challenge for analytical techniques, especially in the aspects of sensitivity and throughput. There are, at present, many analytical methods used for pharmacokinetic study of bioactive compounds in TCMs, such as LC/UV [3], immunoassay [4], LC/ELSD [5], LC/MS [6,7] and LC/MS/MS [8]. Among these methods, LC/UV, immunoassay and LC/ELSD methods show lack of sensitivity, low selectivity and tend to suffer from cross-interference. LC/MS and LC/MS/MS methods were and still are the useful tools for analyzing multiple and trace constituents of TCMs in complex biological fluids because of the high selectivity and sensitivity provided by MS and their non-derivatization detection. Due to the existence of plasma proteins in the matrix, however, biological samples are not directly compatible with LC–MS or LC/MS/MS analysis since they could block LC columns and contaminate the ion source [9]. Therefore, sample pretreatment is always required before LC–MS and LC/MS/MS analyses. Several extraction methods have been reported to pretreat the biological samples, such as liquid–liquid extraction, protein precipitation, and traditional solid-phase extraction (off-line SPE), in which the samples are processed manually in a serial fashion

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[10]. However, the above-mentioned sample clean-up methods are time-consuming and labor intensive. As a result, higher throughput and simpler sample purification systems are continuously pursued.

Recently, turbulent-flow chromatography (TFC) technique has been demonstrated to be a rugged and time-efficient pretreatment method for biological samples [9,11]. By using large packing materials in TFC (e.g., HLB) [9], biological samples could be directly injected into a high-flow rate aqueous mobile-phase stream in which high-molecular-weight analytes (e.g., plasma proteins) are rapidly washed off, while the low-molecular-weight analytes (targeted compounds for analysis) are retained on the stationary phase. The extracted analytes were then eluted via column-switching for MS or LC–MS analysis. The use of turbulent flow resulted in a faster and more rugged extraction with reduced carryover compared with results obtained under laminar-flow conditions. However, using large packing materials considerably decreases the separation efficiency. It is thus necessary to tandem use a TFC column and an analytical column to maintain sufficient resolving power to avoid potential interference among the compounds and signal suppression in MS detection [9]. When on-line coupling the TFC to fast HPLC with sub-2 μm particle size, the analysis throughput of biological samples could be significantly improved because fast HPLC can dramatically increase the analysis speed without losing the resolution and sensitivity [12].

Therefore, the aim of this study was to show the potential of TFC on-line coupled to fast HPLC and mass spectrometry for high throughput and sensitive analysis of multiple and trace constituents of TCMs in complex biological fluids in regard to quantification of target analytes. As an illustrative case study, *Fritillaria thunbergii* Miq. (Liliaceae), an important medicinal plant used as antitussive and expectorant remedies in China [13,14], has been investigated. Three steroidal alkaloids, verticine, verticinone and isoverticine, are chosen as the chemical markers for pharmacokinetics study because these constituents posses various bioactivities, such as antitussive, expectorant, antibacterial and antihypertensive activities [15–17], and to some extent, they are responsible for the clinical curative effects of *F. thunbergii*.

2. Experiment

2.1. Chemicals and reagents

Methanol and acetonitrile (HPLC grade) were obtained from Merck (Darmstadt, Germany). Formic acid and ammonium formate of analytical grade were purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). Distilled deionized water was provided by a Millipore water purification system (Millipore, Bedford, MA, USA). Other reagents were of analytical purity.

The steroidal alkaloid standards verticine, verticinone, isoverticine and puqienine A (internal standard, IS) were extracted and purified from *Fritillaria* species in our laboratory. Their identities were confirmed by IR, ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR), MS analyses [18,19]. The purity of all chemicals determined to be more than 98% by normalization of the peak areas detected by HPLC with ESI/MS and their chemical structures are shown in Fig. 1.

2.2. Preparation of total steroidal alkaloids extract (TSAE) of *F. thunbergii*

The dried bulbs of *F. thunbergii* were powdered to a homogeneous size, and sieved through a No. 60 mesh, followed by drying at 60 °C in the oven for 2 h. The dried bulb powders (500 g) were pre-alkalized with 100 mL ammonium hydroxide for 1 h, and immersed in 4 L trichlormethane:methanol (4:1, v/v) mixture, then refluxed at 80 °C for 2 times and 2 h per time. After being filtered, the combined filtrate was concentrated to dryness under vacuum. The residue was dissolved in 0.1 M HCl and filtered, the filtrate was alkalized with saturate NaOH solution to make the pH value of filtrate more than 10.0, then the mixture was extracted for 2 times by trichlormethane, the extract was concentrated to dryness

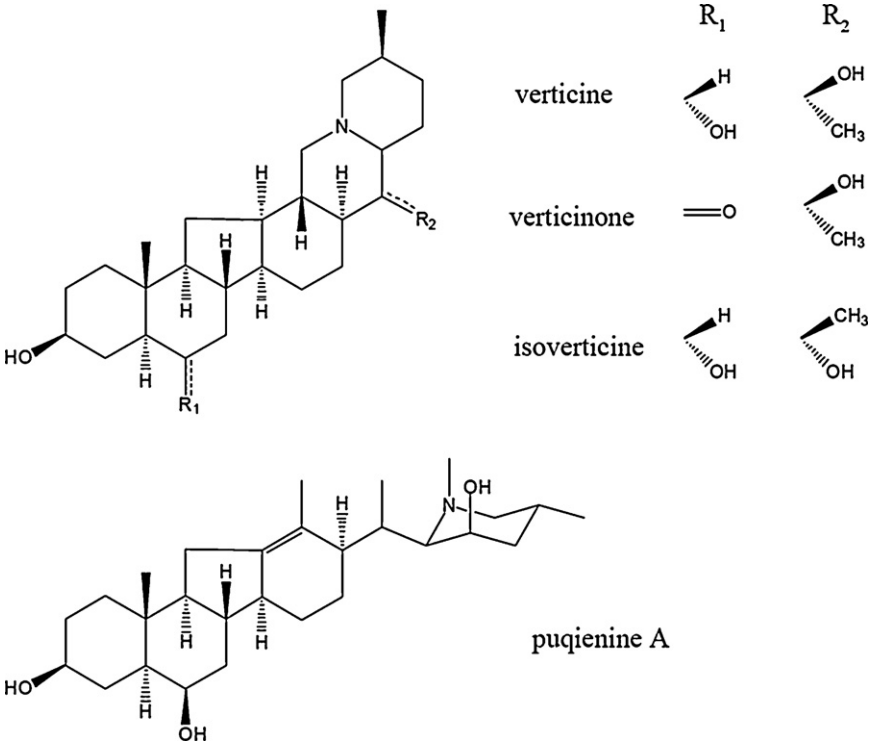

Fig. 1. Chemical structures of verticine, verticinone and puqienine A (IS).

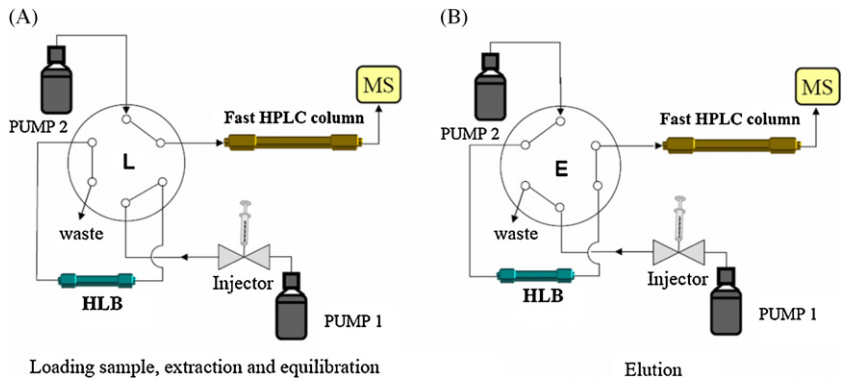

Fig. 2. Schematic representation of on-line turbulent-flow column-switching system.

under vacuum to obtain the TSAE, in which the contents of verticine, verticinone and isoverticine were 31.04%, 14.74% and 10.17%, respectively.

2.3. Preparation of standard solutions, calibration samples and quality control samples

The mixture of stock standard solution containing verticine (0.120 mg/mL), verticinone (0.119 mg/mL) and isoverticine (0.101 mg/mL) was prepared in methanol. The IS stock solution of 67.0 ng/mL was also prepared in methanol, and kept at 33.5 ng/mL in each working solution and samples. Calibration samples in plasma were prepared by spiking aliquots of the stock standard solutions into drug free plasma samples to obtain final concentrations in the ranges of 0.600–96.0 ng/mL for verticine, 0.595–95.2 ng/mL for verticinone and 0.505–80.8 ng/mL for isoverticine. Quality control (QC) samples were also prepared in the same way (2.40, 24.0, 96.0 ng/mL for verticine; 2.38, 23.8, 95.2 ng/mL for verticinone; 2.02, 20.2, 80.8 ng/mL for isoverticine). All solutions were stored at 4 °C before use. For a standard curve, the ratio of the chromatographic peaks area (analytes/IS) as ordinate variables were plotted versus the concentration of these drugs as abscissa.

2.4. Apparatus, sample pretreatment and chromatographic conditions

A schematic diagram of the on-line TFC-LC/MS instrument set-up based on column-switching and fast HPLC is shown in Fig. 2. A Shimadzu 10A pump (Shimadzu, Tokyo) was used to deliver a high flow through a hydrophilic–lipophilic balanced (HLB) reversed-phase column (Oasis HLB, 25 μm, 2.1 mm × 20 mm; Waters, Milford, USA), to load and wash the sample, and subsequently to flush and equilibrate the extraction column. Water (solvent A) was used as the solvent for this pump. An Agilent 1100 HPLC system (equipped with a binary pump, an on-line degasser, an auto-plate-sampler, and a thermostatically controlled column compartment) was used to deliver a gradient flow to elute the analytes from the extraction column and to perform the separation on a fast HPLC column (Agilent Zorbax StableBond-C_{18}, 4.6 mm × 50 mm, 1.8 μm). Water (10 mM ammonium formate) with 0.1% formic acid (solvents B) and acetonitrile (solvents C) were used as the mobile phase for this pump. Two Rheodyne six-port switching valves were used for the column-switching purposes. The L and E in the center of the each six-port valve designate "load" or "elute" positions for the flow path.

Aliquots of 100 μL of rat plasma samples (blank plasma, calibration standards, QC samples and pharmacokinetic plasma samples) were mixed with 100 μL of water with 2% formic acid and 200 μL of methanol containing 67.0 ng/mL of puqienine A (IS). The mixture was vortexed for 30 s and then centrifuged at 13,000 rpm for 10 min at 4 °C. A volume of 50 μL of the supernatant was injected for TFC-LC/MS analysis.

After injection of supernatant, the switching valve was switched to the L position, and the Shimadzu 10A pump started to deliver solvent A at 4.0 mL/min to load the sample onto the HLB extraction column and subsequently to clean the sample. The proteins and salts in the plasma sample were removed while the analytes were retained on the extraction column (see Fig. 2A). This loading and washing step was completed after 1 min when the switching valve was switched to the E position (see Fig. 2B). The extraction column was then in the flow path of the HP 1100 pump. The HP 1100 pump started a gradient using solvents B and C (0–6 min, 28–30% C; 6–8 min, 30–100% C) to elute the analytes from the extraction column to the RRHT column using a flow rate of 0.5 mL/min. This elution step was completed within 7 min when the switching valve was switched back to the L position. Solvent A delivered by Shimadzu 10A pump at 4.0 mL/min was then used to equilibrate the HLB column for the next sample.

2.5. Mass spectrometric conditions

Agilent SL G1946D single quadrupole mass spectrometer with an ESI source in positive mode was used as the detector and conditions of MS analysis were as follows: drying gas (N_2) flow rate, 9 L/min; drying gas temperature, 320 °C; nebulizing gas (N_2) pressure, 16 psi; capillary voltage, 3500 V; quad temperature, 100 °C; fragmentor, 100 V. The samples from the fast HPLC column were analyzed in selective ion monitoring (SIM) mode by monitoring the molecular ions $[M+H]^+$. SIM for each compound were restricted to specific retention time windows: 0–4.5 min, *m/z* 432.0; 4.5–6 min, *m/z* 446.5 by channel 1, and 0–5 min, *m/z* 430.5; 5–8 min, *m/z* 432.0 by channel 2.

2.6. Method validation

The method was fully validated for its specificity, linearity, lower limit of quantification (LLOQ), accuracy and precision. The assay precision was determined from inter- and intra-batch relative standard deviation (R.S.D.%), using five determinations per concentration (2.40, 24.0, 96.0 ng/mL for verticine; 2.38, 23.8, 95.2 ng/mL for verticinone; 2.02, 20.2, 80.8 ng/mL for isoverticine). The accuracy was determined by comparing the calculated concentration (obtained from the calibration curve) to the theoretical concentration of each sample and expressed as Bias%. The concentration-dependent matrix effect of analytes was examined by evaluation of the recovery which by comparing the peak areas obtained from five extracted samples spiked with known amounts of standards with those obtained from the pure compounds of the

same concentrations in the solvent. The LLOQ was considered as the concentration that produced a signal-to-noise (S/N) ratio of 10. The stability of analytes was evaluated by analyzing QC samples at three concentrations exposed to different time and temperature conditions: modeling three freeze/thaw cycles (−70 °C to ambient temperature), 12 h storage at room temperature and frozen at −70 °C for 1 month.

2.7. Application to pharmacokinetics study

Animal experiments were carried out in accordance with the Guidelines for Animal Experimentation of China Pharmaceutical University (Nanjing, China) and protocol was approved by the Animal Ethics Committee of this institution. Sprague-Dawley rats (n = 5, 240–270 g) were purchased from Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, China). The rats were fasted overnight before administration of drug with free access to water. For the oral route, dosing solutions were prepared by dissolving TSAE powder in isotonic saline containing 0.5% carboxymethyl cellulose sodium salt (CMC-Na) and mixed well. The preparation was accomplished immediately before drug administration. Blood samples (0.3 mL) were collected in 1.0 mL sodium heparinized tubes before and at 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 4.0, 8.0, 12.0, 24.0 h after oral administration of TSAE (30 mg/kg) and then centrifuged at 3000 rpm for 10 min to separate 100 μL plasma, which was kept at −70 °C until analysis.

Pharmacokinetic calculations were performed on each individual set of data using the pharmacokinetic program, Drug and Statistics 2.0 (DAS 2.0) (Mathematical Pharmacology Professional Committee of China, Shanghai, China) by the noncompartmental method. The area under the concentration-time curve (AUC) was calculated according to the log linear trapezoidal method. The half-life ($t_{1/2}$) was calculated as follows: $t_{1/2} = 0.693/k_e$ (k_e is the elimination rate constant).

3. Results and discussion

3.1. TFC-LC/MS method establishment

The TFC technique has been applied in pharmacokinetics study for many years with its advantages of rapidness and robustness. *Fritillaria* alkaloids are basic compounds ($pK_a > 7$), which make them particularly suitable for extraction using mixed-mode cation-exchange/reversed-phase column. In on-line system, these compounds should be eluted with basic or high-salt mobile phases from mixed-mode column. However, basic or high-salt mobile phases are not beneficial for the ionization of basic compounds, which decrease the ESI + sensitivity compared with acidic mobile phases [20]. Therefore, as an alternative, we use hydrophilic–lipophilic balanced reversed-phase column to extract the *Fritillaria* alkaloids from plasma samples in which the alkaloids could be eluted by acidic mobile phases. The plasma samples were firstly acidified with 2% formic acid to reduce protein binding because acidic condition is beneficial for the small molecules to dissociate from protein [21,22]. Meanwhile, a simple protein precipitation with methanol was applied prior to injection to extend the life of the solid-phase extraction column. With a fast flow rate of 4.0 mL/min for the extraction column, the proteins and salts in the plasma sample were removed while the analytes were retained on the extraction column. Then, the compounds were eluted by a gradient using solvents B and C and flowed onto the fast HPLC column. The whole analysis of each sample containing these three alkaloids and an internal standard (puqienine A) was achieved less than 7 min. Compared with the traditional off-line sample preparation methods, this on-line sample preparation technique eliminates labor and time involved in sample extraction, of which the turbulent-flow column-switching technique successfully combines the speed and robustness of turbulent-flow extraction and the sensitivity and separation efficiency of fast HPLC–MS.

3.2. Selectivity

Fig. 3 shows the chromatographic profiles of blank plasma, blank plasma spiked with three analytes and IS, and plasma obtained 0.25 h after oral administration of TSAE. No interfering peak from endogenous compounds was observed at the retention times of the analytes or IS in blank rat plasma, therefore an acceptable selectivity was obtained by this method.

3.3. Linearity and sensitivity

The calibration model was selected based on the analysis of the data by linear regression with intercepts and $1/x^2$ weight-

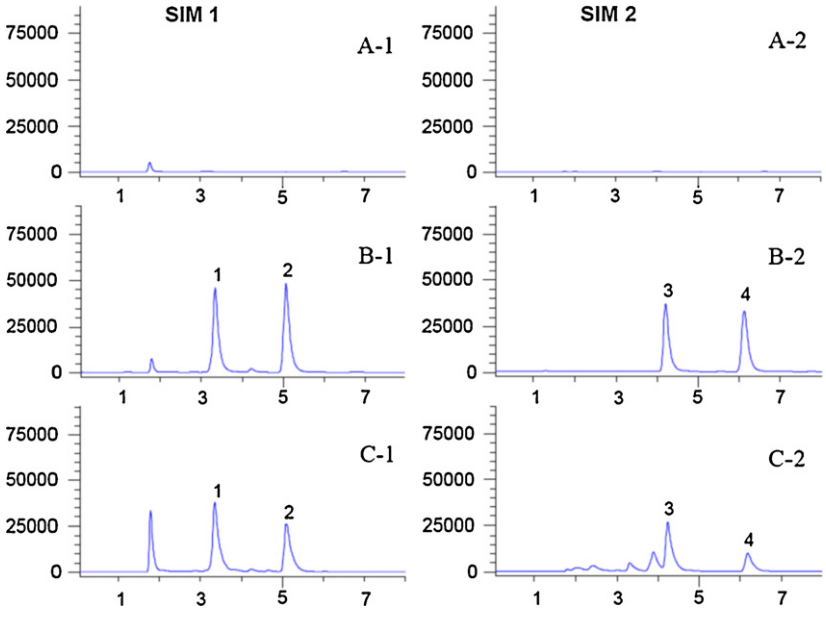

Fig. 3. Selected ion monitoring chromatograms from (A) blank plasma, (B) blank plasma spiked with verticine (1), IS (2), verticinone (3) and isoverticine (4), (C) samples 0.5 h after oral administration of TSAE (30 mg/kg) A-1, B-1 and C-1 represent SIM channel 1; A-2, B-2 and C-2 represent SIM channel 2.

Table 1
Calibration curve, linear range and LLOQ for verticine, verticinone and isoverticine in plasma.

Compounds	Calibration curves	R	Linear range (ng/mL)	LLOQ (ng/mL)
Verticine	$y = 0.0291x + 0.1677$	0.9994	0.600–96.0	0.120
Verticinone	$y = 0.0269x + 0.028$	0.9996	0.595–95.2	0.595
Isoverticine	$y = 0.0329x + 0.0356$	0.9993	0.505–80.8	0.505

Table 2
Precision and accuracy of verticine, verticinone and isoverticine in rat plasma ($n = 5$).

Compound	Spiked concentration (ng/mL)	Measured concentration (ng/mL) (mean ± S.D.)	R.S.D. (%)	Accuracy (%)	Bias (%)
Verticine					
Inter-day	2.40	2.10 ± 0.325	15.0	87.5	−12.3
	24.0	25.7 ± 1.70	6.62	107	7.05
	96.0	98.2 ± 3.91	3.98	102	2.27
Intra-day	2.40	2.03 ± 0.195	9.59	84.6	−15.4
	24.0	24.3 ± 0.625	2.58	101	1.25
	96.0	91.8 ± 0.853	0.929	95.6	−4.38
Verticinone					
Inter-day	2.38	2.23 ± 0.220	9.83	93.7	−6.01
	23.8	25.3 ± 1.43	5.66	106	7.95
	95.2	98.0 ± 3.81	3.88	103	2.94
Intra-day	2.38	2.19 ± 0.076	3.47	92.0	−7.98
	23.8	24.5 ± 0.404	1.65	103	2.94
	95.2	92.5 ± 0.881	0.952	97.2	−2.84
Isoverticine					
Inter-day	2.02	1.72 ± 0.141	8.24	85.1	−15.0
	20.2	21.5 ± 1.19	5.52	106	6.52
	80.8	83.4 ± 2.87	3.44	103	3.26
Intra-day	2.02	1.65 ± 0.0605	2.76	81.7	−18.3
	20.2	21.1 ± 0.307	1.46	104	4.46
	80.8	79.3 ± 0.742	0.935	98.1	−1.86

Table 3
Matrix effect evaluation of verticine, verticinone, isoverticine and IS in rat plasma ($n = 5$).

Compound	Spiked concentration (ng/mL)	Set1 (mean ± S.D.)	Set2 (mean ± S.D.)	Absolute matrix (%)
Verticine	2.40	$9.23 \times 10^4 \pm 182$	$8.13 \times 10^4 \pm 4.80 \times 10^3$	88.1
	24.0	$3.62 \times 10^5 \pm 1.28 \times 10^3$	$3.55 \times 10^5 \pm 1.85 \times 10^4$	98.1
	96.0	$1.09 \times 10^6 \pm 6.57 \times 10^4$	$1.17 \times 10^6 \pm 4.91 \times 10^4$	107
Verticinone	2.38	$4.04 \times 10^4 \pm 291$	$3.65 \times 10^4 \pm 2.59 \times 10^3$	90.3
	23.8	$2.97 \times 10^5 \pm 1.58 \times 10^3$	$2.88 \times 10^5 \pm 1.72 \times 10^4$	97.0
	95.2	$9.98 \times 10^5 \pm 6.05 \times 10^4$	$1.06 \times 10^6 \pm 4.68 \times 10^4$	106
Isoverticine	2.02	$4.35 \times 10^4 \pm 631$	$3.72 \times 10^4 \pm 2.87 \times 10^3$	85.5
	20.2	$3.32 \times 10^5 \pm 1.96 \times 10^3$	$3.08 \times 10^5 \pm 2.01 \times 10^4$	92.8
	80.8	$1.13 \times 10^6 \pm 7.15 \times 10^4$	$1.12 \times 10^6 \pm 6.01 \times 10^4$	99.1
IS	33.5	$3.61 \times 10^5 \pm 2.21 \times 10^4$	$3.17 \times 10^5 \pm 1.70 \times 10^4$	87.8

ing factor. Linear equation, calibration range, coefficient (R) and LLOQ were summarized in Table 1. The curves showed good linear response ($R > 0.999$) over the range from 0.505 to 96.0 ng/mL. The LLOQ of verticine, verticinone and isoverticine were 0.120, 0.595 and 0.505 ng/mL, respectively. These values of LLOQs obtained by on-line TFC-LC/MS were much improved, about 100-fold more sensitive over those reported previously by other methods, such as pre-column derivatization LC/UV [3] and LC/ELSD [5].

3.4. Precision and accuracy

Table 2 shows the intra- and inter-batch precision and accuracy at three concentrations. The intra-batch accuracy for verticine, verticinone and isoverticine samples were 84.6–95.6%, 92.0–103% and 81.7–98.1%, and the inter-batch accuracy ranged from 87.5 to 107%, 93.7 to 106% and 85.1 to 106%, respectively. The precisions (R.S.D.%) were less than 15% for the three analytes. These results indicated that the present method had a satisfactory accuracy and precision.

3.5. Recovery and matrix effect

In on-line TFC-LC/MS assays, it is challenging to directly determine the matrix effect. We monitored the concentration-dependent matrix effect for this assay. The recovery of verticine, verticinone and isoverticine were calculated by comparing the peak area ratios of the three analytes and internal standard in the extracted plasma samples (set1) with that of plasma-free standards prepared in pure solvent (set2). As shown in Table 3, no endogenous substances significantly affect the ionization of verticine, verticinone and isoverticine, suggesting that through the rigorous washing procedure no significant matrix effect was observed.

3.6. Stability

Stability data are summarized in Table 4 and indicated that the three analytes were all stable in plasma for three freeze/thaw cycles, 24 h at room temperature. Moreover, the results of the stability showed that all the analytes were stable for at least 1 month when kept frozen at −70 °C.

Table 4
Stability of verticine, verticinone and isoverticine in plasma ($n = 5$).

Compound	Spiked concentration (ng/mL)	Measured concentration (ng/mL) (mean ± S.D.)		
		After three freeze/thaw cycles in plasma	At room temperature for 12 h in plasma	At −70 °C for 1 month in plasma
Verticine	2.40	1.73 ± 0.070	2.25 ± 0.370	2.13 ± 0.330
	24.0	23.4 ± 0.630	25.0 ± 1.01	25.5 ± 1.58
	96.0	91.6 ± 2.11	93.1 ± 1.17	98.9 ± 2.54
Verticinone	2.38	2.04 ± 0.050	2.28 ± 0.130	2.28 ± 0.240
	23.8	24.0 ± 0.170	24.8 ± 0.950	25.1 ± 1.49
	95.2	91.6 ± 1.45	93.8 ± 1.04	98.6 ± 2.77
Isoverticine	2.02	1.56 ± 0.040	1.73 ± 0.10	1.74 ± 0.160
	20.2	20.7 ± 0.320	21.2 ± 0.72	21.6 ± 1.29
	80.8	78.3 ± 1.03	80.5 ± 0.48	83.5 ± 2.69

Table 5
Pharmacokinetic parameters of verticine, verticinone and isoverticine after oral administration of TSAE (30 mg/kg), each value represents the mean ± S.D. ($n = 5$).

Compounds	Parameter					
	$AUC_{(0-t)}$ (μg/L h)	$MRT_{(0-t)}$ (h)	$t_{1/2}$ (h)	CLz/F (L/h kg)	T_{max} (h)	C_{max} (μg/L)
Verticine	134	2.36	1.71	66.4	0.500	46.7
Verticinone	179	3.04	1.96	24.5	0.500	44.8
Isoverticine	48.1	2.83	2.40	55.5	0.500	12.8

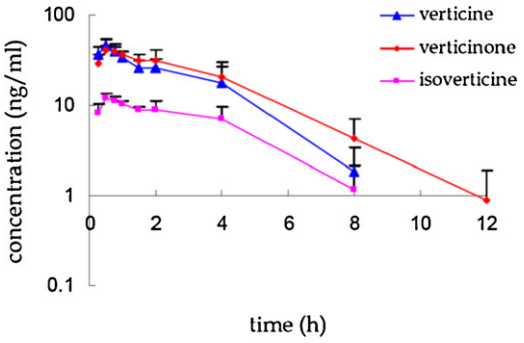

Fig. 4. Mean drug plasma concentration–time curve (mean ± SD, $n = 5$) of verticine, verticinone and isoverticine in rats after oral administration of TSAE (30 mg/kg).

3.7. Results of pharmacokinetic study

After oral administration of TSAE (30 mg/kg) to five rats, plasma concentrations of verticine, verticinone and isoverticine were simultaneously determined by the described on-line TFC-LC/MS method. The mean plasma concentration–time profiles ($n = 5$) are represented in Fig. 4 and the main pharmacokinetic parameters in rats are presented in Table 5. The results demonstrated that verticine, verticinone and isoverticine were absorbed rapidly from the gastrointestinal tract. The blood concentration reached their maximums (C_{max} 46.7 ng/mL for verticine, 44.8 ng/mL for verticinone, 12.8 ng/mL for isoverticine) at T_{max} of 0.500 h, and then quickly decreased monoexponentially with an elimination half-life of 1.71 h for verticine, 1.96 h for verticinone, 2.40 h for isoverticine. Our study demonstrated that verticine, verticinone and isoverticine are very rapidly absorbed orally and have a quick onset and a short duration of action in rats, which is consistent with some *Fritillaria* alkaloids studied ever [3,5].

4. Conclusion

The on-line TFC-LC/MS method described in this paper is a rapid, sensitive and reliable method for simultaneous quantification of verticine, verticinone and isoverticine in rat plasma. The use of on-line sample preparation technique could eliminate labor and time involved in sample extraction, and the use of fast HPLC with sub-2 μm particle size could rapidly separate 4 structurally similar steroidal alkaloids without sacrificing resolution and sensitivity. Our results demonstrated that the on-line TFC-LC/MS method was suitable for TCMs pharmacokinetic study at a low dose level. The improved method could also be used for high throughput screening of active compounds from TCMs. Further studies are underway in our laboratory to characterize the metabolites of *Fritillaria* alkaloids and the pharmacokinetics of TCMs which contain other active constituents.

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