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Simultaneous quantitation of aconitine, mesaconitine, hypaconitine, benzoylaconine, benzoylmesaconine and benzoylhypaconine in human plasma by liquid chromatography–tandem mass spectrometry and pharmacokinetics evaluation of "SHEN-FU" injectable powder

Fan Zhang[a,1], Ming-hai Tang[a,1], Li-juan Chen[a,*,1], Rui Li[a], Xian-huo Wang[a], Jun-guo Duan[b], Xia Zhao[c], Yu-quan Wei[a]

[a] State Key Laboratory of Biotherapy, West China Hospital, West China Medical School, Sichuan University, Keyuan Road 4, Gaopeng Street, Chengdu 610041, China
[b] The Affiliated Hospital of Chengdu University of Traditional Chinese Medicine, China
[c] Department of Gynecology and Obstetrics, Second West China Hospital, West China Medical School, Sichuan University, Chengdu, China

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ABSTRACT

A rapid, specific and sensitive liquid chromatography–tandem mass spectrometry (LC/MS/MS) method was developed for simultaneous quantitation of six *Aconitum* alkaloids, i.e. aconitine (AC), mesaconitine (MA), hypaconitine (HA), benzoylaconine (BAC), benzoylmesaconine (BMA) and benzoylhypaconine (BHA) in human plasma collected from 18 healthy volunteers after intravenous drop infusion of "SHEN-FU" injectable powder in three different dosages. Lappaconitine was selected as the internal standard (IS). LC/MS/MS system coupled with an electrospray ionization (ESI) source was performed in multiple-reaction monitoring (MRM) mode. The transitions of the *Aconitum* alkaloids executed as following: m/z 646.3 → 586.0 for AC; m/z 632.4 → 573.1 for MA; m/z 616.2 → 556.1 for HA; m/z 604.2 → 104.8 for BAC; m/z 590.1 → 104.8 for BMA; m/z 574.1 → 104.8 for BHA; m/z 585.2 → 161.8 for IS. Sample preparation was performed with solid-phase extraction (SPE) on a 1 mL HLB cartridge prior to analysis. The separation was applied on a Waters C_{18} column (1.7 μm, 2.1 mm × 100 mm) and a gradient elution of methanol and 0.1% formic acid–water was used as mobile phase. The retention time was less than 4.5 min. The concentrations ranged from 0.1 to 1000 ng/mL for all six *Aconitum* alkaloids and showed a good linearity with the correlation coefficient (r^2) >0.995. The validated method was employed to simultaneous quantitation and successfully used for the first time for the pharmacokinetic evaluation of the six *Aconitum* alkaloids after intravenous drop administration of "SHEN-FU" injectable powder in phase I clinical trial.

1. Introduction

"SHEN-FU", derived from the traditional Chinese medicine and mainly composed of red ginseng and aconite, had been commonly used in China for 1000 years. As a typical multiple-constituent traditional Chinese medicine, "SHEN-FU" injection had been clinically used in China for the protection from ischemia reperfusion injury after acute myocardial infarction, ameliorated the degree of injury, improved heart function and prevented myocardial fibrosis [1,2]. In order to increase the stability in storage and make it easy for quality evaluation, "SHEN-FU" injection was manufactured into "SHEN-FU" injectable powder by SAN-JIU Pharmaceutical Company of Ya-An in China. The "SHEN-FU" injectable powder was approved by State Food and Drug Administration of China (No. 2004L02334). At present, it was under clinical trial in phase I in National Clinical Trial Center of Traditional Chinese Medicine of China.

The main active components of "SHEN-FU" were ginsenosides and *Aconitum* alkaloids. *Aconitum* alkaloids constituted of three highly toxic diester-diterpence called aconitine (AC), measaconitine (MA) and hypaconitine (HA). Due to their high toxicity [3–5] this herb must be properly processed to decrease their toxicities by hydrolyzing AC, MA and HA to respective benzoylaconine (BAC), benzoylmesaconine (BMA) and benoylhypaconine (BHA) (see Fig. 1 for their structures). Therefore, the development of a rapid, valid and sensitive method to simultaneous determination of the six *Aconitum* alkaloids in biofluid and for pharmacokinetics study is of great importance.

* Corresponding author. Tel.: +86 28 85164063.
E-mail address: lijuan17@hotmail.com (L.-j. Chen).
[1] These authors equally contributed to this paper.

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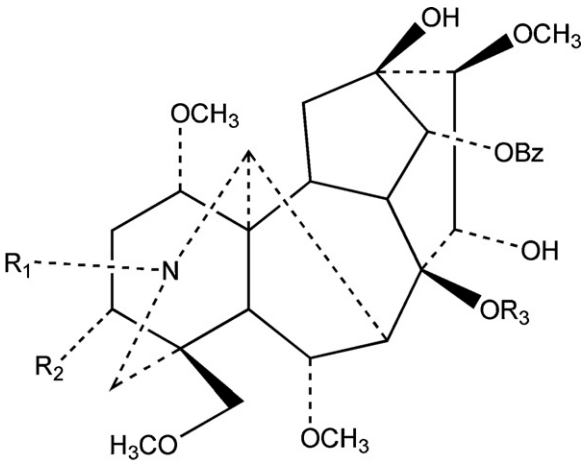

Name	R_1	R_2	R_3
AC	C_2H_5	OH	acetyl
MA	CH_3	OH	acetyl
HA	CH_3	H	acetyl
BAC	C_2H_5	OH	H
BMA	CH_3	OH	H
BHA	CH_3	H	H

Fig. 1. The chemical structures of AC, MA, HA, BAC, BMA and BHA.

Many methods had been established for the determination of AC, MA and HA, such as high-performance liquid chromatography (HPLC) [6–10], capillary electrophoresis [11], capillary liquid chromatographic-fric fast atom bombardment mass spectrometric (LC-frit-FAB-MS) [12], gas chromatography–tandem mass spectrometry (GC–MS) [13] and LC/MS [14–19]. Nevertheless, the HPLC methods often required long-time separation and complete resolution of all constituents. The reported GC–MS [13] method used trimethyl-silyl derivatization and the extraction procedure was tedious. Ohta et al. used LC-FAB-MS method to achieve the limit of detection (LOD) of 1 ng/mL in plasma for the six *Aconitum* alkaloids, nevertheless, the elution time was between 25 and 30 min, and 2 mL plasma was needed [12]. Beyer et al. used LC/MS/MS to achieve high sensitivity for quantitative determination of aconitine, however, this method required 1 mL human plasma and the elution time was 10 min [16] and the large volume of plasma was needed. Hayashida et al. introduced a column-switching LC/MS/MS method to get much lower LOD (0.2–1 ng/mL for AC, MA, HA and 2–50 ng/mL for BAC, BMA and BHA), needless of serum pretreatment, but 1 mL serum was still required [19] to be spiked to create a sample.

The objective of the present work was to develop a rapid and sensitive LC/MS/MS method for the simultaneous quantitation of the six *Aconitum* alkaloids of "SHEN-FU" injectable powder in 18 healthy volunteers' plasma in phase I. Samples and internal standard were detected in MRM mode to enhance specificity and sensitivity. This method was sensitive with a LOD at 0.033 ng/mL, and only 100 μL plasma was needed, which can satisfy the requirement of pharmacokinetics studies.

2. Experimental

2.1. Chemicals and reagents

AC, MA, HA and lappaconitine were obtained from State Food and Drug Administration of China. BAC, BMA and BHA were hydrolyzed in deionized water at 90 °C for 8 h using AC, MA and HA separately and purified by semi-preparitive HPLC. Their purity (≥99.0%) and structural identification were identified using HPLC, NMR and mass spectrometry. HPLC grade methanol was purchased from Fisher Chemicals (Fairlawn, NJ, USA). Formic acid also was HPLC grade and obtained from Sigma–Aldrich (Germany). Deionized water was prepared by the laboratory which was purified by means of a Milli-Q Plus water purification system (Millipore Ltd.). "SHEN-FU" injectable powder was supplied by a Chinese pharmaceutical company (SAN-JIU Pharmaceutical Company of Ya-An, China).

2.2. Equipments

A Waters Acquity ultra performance liquid chromatography (UPLC) consisted of a binary pump and a sample manager was applied as the LC system. Gradient elution was performed on an ACQUITY UPLC™ BEH C_{18} column (1.7 μm, 2.1 mm × 100 mm, Waters) and protected by a guard filter. The column temperature was maintained at 40 °C. The experiment was carried out at a flow rate of 0.25 mL/min with the mobile phases A (0.1% formic acid) and B (methanol). The gradient elution started at 40% B, ramped linearly to 65% in 5 min, and maintained at 60% for 0.5 min, then directly increased to 90% for 0.5 min and returned to the initial percentage. The backpressure was between 8500 and 9000 psi. Waters Quattro Premier XE Mass Spectrometer coupled with ESI source was employed in the MS/MS analysis and the Masslynx V 4.1 software was used to control the LC/MS/MS system. NaCsI was consecutively injected into MS system at 10 μL/min for the calibration and resolution adjustment of both quadrupoles before the experiment. The ESI source was performed on positive mode with a spray voltage at 2.8 kV. The source temperature was set at 110 °C and the desolvation temperature was set at 400 °C. Nitrogen was used as the vacuum gas and desolvation gas. The cone gas flow and the desolvation gas flow were performed at 40 and 800 L/h, respectively. Argon was used as collision gas and the flow rate was maintained at 0.2 L/h. MRM was applied as the method to measure the samples and internal standard. The cone voltage and collision energy were optimized to maximize the intensity of the transitions ions of $[M+H]^+$ precursor ions for AC, MA, HA, BAC, BMA, BHA and IS, respectively.

2.3. Sample preparation

Sample preparation was performed on SPE method using Waters HLB (30 mg sorbent). The Waters HLB cartridge was first conditioned with 1 mL methanol followed by 1 mL deionized water before use. 100 μL plasma and 10 μL (200 ng/mL) IS solution were loaded on the cartridge. The elution was carried out with 1 mL 5% methanol–water (v/v) and 1 mL methanol. The methanol fraction was collected and evaporated to dryness under a stream of nitrogen at 40 °C. The residue was reconstituted in 100 μL mobile phase, 5 μL sample was injected into the LC/MS/MS system for the analysis.

2.4. Preparation of calibration curves and quality control (QC) samples

Stock solutions of AC, MA, HA, BAC, BMA, BHA and IS were prepared in methanol at 200 μg/mL, respectively. The working solution of IS was prepared by diluting appropriate stock solution in methanol to get the concentration of 200 ng/mL. Standard spiking solutions were prepared in methanol at the concentrations of 1, 5, 10, 50, 100, 500, 1000, 5000 and 10,000 ng/mL, which were mixed with AC, MA, HA, BAC, BMA and BHA together. 10 μL of standard

spiking solution and 10 μL IS (200 ng/mL) were added into 100 μL blank plasma, mixed well, loaded on a HLB cartridge and treated with the procedure mentioned in the sample preparation section. The residue was reconstituted in 100 μL mobile phase to obtain the calibrators at the concentrations of 0.1, 0.5, 1, 5, 10, 50, 100, 500 and 1000 ng/mL.

QC samples for the validation were prepared daily by adding appropriate volume of standard spiking solutions into blank plasma, then processed in the same way as the calibrators to obtain three different concentration levels of low, medium and high at 1, 100 and 800 ng/mL, respectively.

2.5. Method validation

The method validation was performed in plasma using QC samples spiked with six *Aconitum* alkaloids at three different concentrations, 1, 100 and 800 ng/mL.

2.5.1. Linearity

The linearity was investigated by constructing calibration curves using analysis data from blank plasma sample at nine concentration levels from 0.1 to 1000 ng/mL. Three replicate analyses were performed for each calibration to evaluate the repeatability. The calibration was processed with MassLynx V 4.1 software. The correlation coefficient (r^2) must be >0.99.

2.5.2. Accuracy and precision

For measurement of intra-day precision (specified as R.S.D.) and accuracy (calculated by the ratio of the determined concentration and nominal concentration), five samples at each concentration levels (1, 100 and 800 ng/mL) were prepared to analyze three times in 1 day. The inter-day precision and accuracy was determined with the same QC samples in 3 consecutive days. The variation under 15% for the precision and accuracy was acceptable.

2.5.3. Recovery and ion suppression

Recovery experiments were performed by comparing the analytical results of QC samples using SPE method in blank plasma with the results in pure mobile phase at different concentrations of 1, 100 and 800 ng/mL. Five samples at each concentration level were evaluated.

Any reduction in MS/MS responses relative to reference solutions prepared by adding the same amounts of the analytes to mobile phase solution would attribute to ionization suppression from sample matrix. Ion suppression was calculated by comparing analyte peak areas obtained from supplemented plasma samples after SPE extraction with those obtained from pure mobile phase preparations ($n = 5$) at three level concentrations.

2.5.4. Stability

Five QC samples of each concentration at low, medium and high levels were prepared in the blank plasma and stored at −20 °C for the freeze–thaw experiments. Three freeze–thaw cycles were carried out between 36 h to evaluate the freeze and thaw stability.

Long-term stability was performed at the same QC samples which also stored at −20 °C for 30 days. The analytic results compared to the expected concentrations less than 15% were considered to be stable.

2.5.5. Lower limit of quantification (LLOQ) and limit of determination

The LLOQ of the method was determined by spiking the lowest point of calibrator in the blank plasma with a precision ≤20% which resulted in a signal-to-noise ratio ≥10:1. The LOD was defined as the signal of the components adding to the blank plasma can reliably distinguish from the background noise: signal/noise ≥3.

2.5.6. Specificity

Specificity was evaluated by comparing the chromatogram of blank plasma, which was processed by SPE with the chromatogram

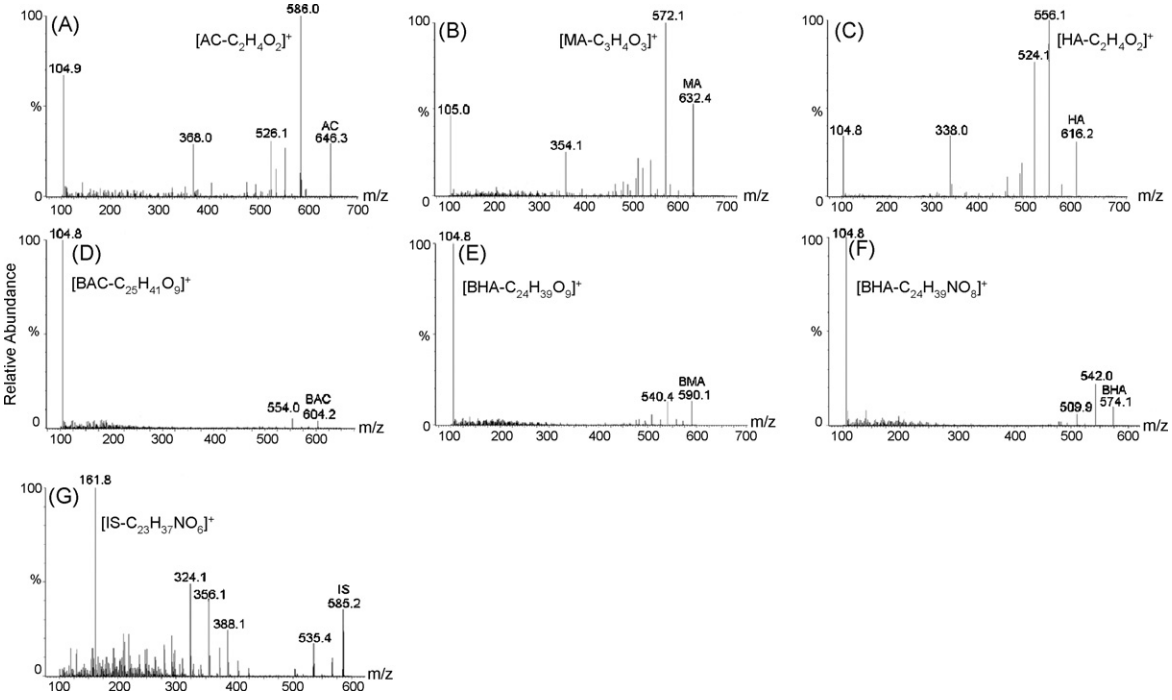

Fig. 2. MS/MS spectra of the product ions of AC (A), MA (B), HA (C), BAC (D), BMA (E), BHA (F) and IS (G), their product ions are *m/z* 586.0, *m/z* 572.1, *m/z* 556.1, *m/z* 104.8, *m/z* 104.8, *m/z* 104.8 and *m/z* 161.8, respectively.

spiked with respective standards to detect any peaks interfering the target compounds. Six different specimens of blank plasma were applied for the specificity evaluation.

2.6. Pharmacokinetics study

Three dosages at low (10.0 mg/kg), medium (13.3 mg/kg) and high (16.7 mg/kg) levels of "SHEN-FU" injectable powder were applied on 18 healthy volunteers (nine males and nine females) by intravenous drop infusion. Every 10 mg "SHEN-FU" injectable powder contained 0.65 ng AC, 16.8 ng MA, 11.45 ng HA, 1103.5 ng BAC, 24249.5 ng BMA and 7268.0 ng BHA. Six volunteers were involved in each experiment. 500 μL blood samples were collected into the heparin containing polypropylene tubes at 18 time-points: 0, 5, 15, 30, 45, 60, 75, 90, 105, 135, 165, 285, 405, 525, 645, 765, 1485 and 2205 min after intravenous drop infusion began. The plasma samples were harvested by centrifugating the blood samples at the speed of 3000 rpm for 10 min at 4 °C and stored at −20 °C until analysis.

The pharmacokinetic parameters were calculated by the results of the analysis through the DAS 2.0 software (Drug and Statistics for Windows). Apparent elimination half-life ($t_{1/2}$) was calculated as $t_{1/2} = 0.693/k_e$. The area under the curve (AUC_{0-t}) was calculated using the linear-trapezoidal rule, with extrapolation to infinity ($AUC_{0-\infty}$) from the last detectable concentration using the terminal elimination rate constant (k_e) calculated by linear regression of the final log-linear part of the drug concentration–time curve. Maximum plasma concentration (C_{max}) and the time-to-maximum concentration (T_{max}) were estimated by visual inspection of semi-logarithmic plots of the concentration–time curves. The study was approved by the Independent Ethics Committee (IEC), Affiliated Hospital of Chengdu University of Traditional Chinese Medicine (2006ZL-013-1). All 18 volunteers gave written informed consent.

3. Results and discussion

3.1. Optimization of the extraction method

Early in this experiment, SPE, protein precipitation process and liquid–liquid extraction using ethyl acetate, chloroform and methyl *tert*-butyl ether were intended to apply on the six *Aconitum* alkaloids extraction. SPE and ethyl acetate demonstrated good recovery efficiency. Considering the much cleaner chromatogram, better repeatability, less contamination of the column and convenient processed procedure, SPE was finally selected to process the biological samples.

3.2. Optimization of LC/MS/MS method

All of the compounds were investigated in the positive mode and negative mode. The signal intensities for all these compounds showed stronger intensities in positive mode than in the negative mode. The MS/MS method was optimized to obtain the highest response using the MRM pairs comprising the precursor and product ions. Unlike the reported selected ion monitoring (SIM) methods [12,19], quantitation by MRM mode provided high sensitivity. After optimization, the protonated precursor molecular ions $[M+H]^+$ (see in Fig. 2) were chosen to be monitored. The ions of AC at m/z 646.3 → 586.0, MA at m/z 632.4 → 572.1, HA at m/z 616.2 → 556.1, BAC at m/z 604.2 → 104.8, BMA at m/z 590.1 → 104.8, BHA at m/z 574.1 → 104.8 and IS at m/z 585.2 → 161.8 were selected for quantification, respectively. The MS/MS spectrums were shown in Fig. 2. The acquisition parameter cone voltage was set at 45 V for all the compounds, collision energy was optimized to be 40 eV for AC, MA, HA and 52 eV for BAC, 47 eV for BMA, 45 eV for BHA and 40 eV for IS, respectively.

Comparing the common HPLC, UPLC system utilizes pressure-tolerant 1.7 μm hybrid particles containing a bridged ethylsiloxane/silica structure allowing the Acquity to run routinely at pressures up to 15,000 psi [20], which can greatly reduce the analytic time, improve the resolution and enhance the sensitivity. Also, the injection volume was only 5 μL and the left volume provided sufficient volume for reinjection.

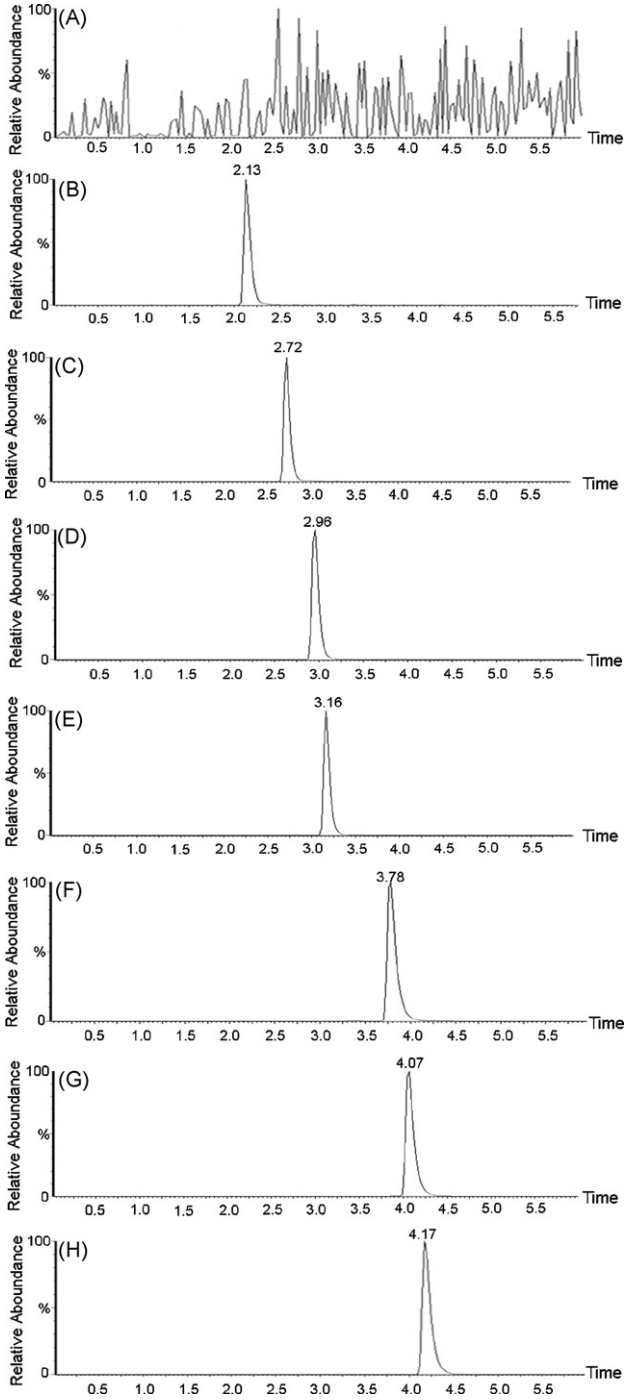

Fig. 3. Chromatograms of blank plasma (A) and blank plasma spiked with 50 ng/mL AC, MA, HA, BAC, BMA, BHA and 20 ng/mL IS, the retention times of AC (H), MA (F), HA (G), BAC (D), BMA (C), BHA (E) and IS (B) are 4.17, 3.78, 4.07, 2.96, 2.72, 3.16 and 2.13 min, respectively.

Table 1
Regression equations and respective correlation coefficients (r^2) of AC, MA, HA, BAC, BMA and BHA in plasma

Compounds	Regression equation	Correlation coefficient (r^2)
AC	$Y = 0.1304(\pm0.0011)X + 0.01379(\pm0.0015)$	0.997
MA	$Y = 0.07255(\pm0.0008)X + 0.02170(\pm0.0010)$	0.997
HA	$Y = 0.05743(\pm0.0009)X + 0.003762(\pm0.0011)$	0.998
BAC	$Y = 0.06470(\pm0.0009)X + 0.002381(\pm0.0012)$	0.996
BMA	$Y = 0.0620(\pm0.0012)X - 0.003750(\pm0.0016)$	0.996
BHA	$Y = 0.004396(\pm0.0008)X - 0.0003089(\pm0.0009)$	0.996

The numbers in parentheses were standard errors of slope and intercept, respectively obtained in the linear regression.

In order to get appropriate retention time and better resolution, methanol, acetonitrile, water, 5 mM/10 mM ammonium acetate and formic acid were tested as mobile phase. 0.1% formic acid was found to enhance efficiency of ionization, the use of methanol led to better peak shape rather than acetonitrile.

After optimization, methanol and 0.1% formic acid were selected as the mobile phase. The retention times of AC, MA, HA, BAC, BMA, BHA and IS were 4.17, 3.78, 4.07, 2.96, 2.72, 3.16 and 2.13 min, respectively. The chromatogram, which spiked with 50 ng/mL of six *Aconitum* alkaloids and 20 ng/mL of IS in blank plasma was shown in Fig. 3(B–H). The chromatographic peaks were symmetrical with good resolution and the retention time was less than 4.5 min, which was much shorter than the previous reports [12,19].

3.3. Method validation

There was no interference from endogenous substance observed in the blank plasma at the respective retention times (see in Fig. 3A). The standard calibration curves fitted with weights of 1/concentration and showed satisfactory linearity over the concentration range (0.1–1000 ng/mL), which provided wider linear range than that of Hayashida et al. (0.1–100 ng/mL for AC, MA and HA, 0.1–500 ng/mL for BAC, BMA and BHA) [19]. The correlation coefficient (r^2) was >0.995 for all six compounds (shown in Table 1).

For all six *Aconitum* alkaloids, the intra-day, inter-day precision with R.S.D. is less than 8.61% and accuracy between 96.5% and 110.2% in human plasma were shown in Table 2. The results suggested that the analytical method is acceptable with the CV ≤15%. The recoveries of the six compounds in plasma were specified in Table 3, which showed no relevant difference in extraction recovery at different concentrations. The ion suppression was demonstrated in Table 3, the results indicated that the endogenous substances suppressed ionization slightly.

Table 3
The recovery and ion suppression of AC, MA, HA, BAC, BMA and BHA in human plasma ($n = 5$)

Analytes	Spiked concentration (ng/mL)	Recovery (%) (mean ± S.D.)	Ion suppression (%) (mean ± S.D.)
AC	1	87.7 ± 4.5	90.7 ± 6.7
	100	97.4 ± 5.2	93.2 ± 3.2
	800	93.5 ± 4.5	92.6 ± 3.5
MA	1	88.4 ± 5.2	101.5 ± 6.3
	100	96.4 ± 0.4	97.3 ± 3.7
	800	96.7 ± 4.8	93.6 ± 2.5
HA	1	88.7 ± 11.1	95.1 ± 5.1
	100	92.4 ± 5.1	96.0 ± 2.8
	800	91.6 ± 2.4	93.2 ± 3.7
BAC	1	101.4 ± 3.1	103.7 ± 4.3
	100	96.4 ± 2.0	95.1 ± 1.1
	800	88.8 ± 1.3	90.2 ± 1.2
BMA	1	91.9 ± 12.9	93.0 ± 8.0
	100	99.5 ± 0.5	98.0 ± 1.9
	800	90.0 ± 1.5	90.3 ± 2.4
BHA	1	86.8 ± 7.5	91.3 ± 2.4
	100	84.6 ± 2.4	89.3 ± 2.0
	800	83.4 ± 1.7	90.7 ± 2.6

Freeze–thaw stability of the compounds was assessed after three freeze–thaw cycles in 36 h. No significant variability in drug concentration was observed in all freeze–thaw stability tests. After three freeze–thaw cycle, the variation were <4.3% for AC, MA and HA, and <7.3% for BAC, BMA and BHA. Comparing with the nominal concentrations, the variation of 1 month stability was less than 12.5%. Long-term stability demonstrated that the six *Aconitum* alkaloids were stable in 1 month with the variation less than 15%.

The LLOQ were 0.1 ng/mL for all six *Aconitum* alkaloids. The LOD was 0.033 ng/mL for all target compounds, comparing to Ohta et

Table 2
Intra-day and inter-day precision and accuracy of AC, MA, HA, BAC, BMA and BHA in human plasma

Compounds	Concentration (ng/mL)	Intra-day			Inter-day		
		Mean (ng/mL)	Accuracy (%)	Precision (R.S.D., %)	Mean (ng/mL)	Accuracy (%)	Precision (R.S.D., %)
AC	1	1.027	102.7	8.61	1.030	103.0	5.99
	100	101.57	101.6	5.27	102.6	102.6	4.73
	800	813.46	101.7	2.95	809.1	101.1	1.77
MA	1	1.081	108.1	3.17	1.03	103.0	5.99
	100	103.24	103.2	4.04	102.6	102.6	4.73
	800	814.00	101.8	2.53	809.1	101.1	1.77
HA	1	1.04	103.8	3.22	1.04	103.9	4.31
	100	101.4	101.4	2.22	103.6	103.6	3.71
	800	810.9	101.4	2.33	816.4	102.1	3.09
BAC	1	1.03	103.4	6.86	1.02	102.3	6.42
	100	108.3	108.3	3.59	111.5	111.5	1.84
	800	880.4	110.1	3.20	772.1	96.5	4.56
BMA	1	1.05	104.9	4.27	1.09	108.8	4.38
	100	108.7	108.7	3.11	109.5	109.5	3.12
	800	881.2	110.2	3.12	820.5	102.6	1.52
BHA	1	0.98	98.0	6.59	0.99	99.16	8.00
	100	96.5	96.5	6.70	110.2	110.2	3.49
	800	879.7	110.0	2.97	820.1	102.5	4.43

Table 4
The parameters of the pharmacokinetics for benzoyl-type *Aconitum* alkaloids in 18 healthy volunteers ($n = 6$, mean ± S.D.)

	Dosage	$t_{1/2}$ (min)	$AUC_{(0-t)}$ (μg/L min)	T_{max} (min)	C_{max} (μg/L)
BAC	Low	44.35 ± 1.86	847.3 ± 198.6	45 ± 0	9.120 ± 2.02
	Medium	62.17 ± 2.81	1.312E3 ± 78.60	30 ± 0	11.80 ± 0.290
	High	48.00 ± 14.0	1.528E3 ± 32.40	45 ± 0	17.58 ± 1.76
BMA	Low	23.97 ± 0.33	7.695E3 ± 1249	45 ± 0	115.5 ± 12.9
	Medium	36.17 ± 1.72	1.233E4 ± 1415	30 ± 0	162.6 ± 10.0
	High	18.01 ± 0.66	1.169E4 ± 731.0	45 ± 0	207.5 ± 19.2
BHA	Low	20.33 ± 6.49	1.527E3 ± 363.4	45 ± 0	26.47 ± 4.29
	Medium	22.26 ± 1.63	2.003E3 ± 40.40	30 ± 0	37.17 ± 2.37
	High	14.60 ± 7.23	2.167E3 ± 280.5	45 ± 0	46.08 ± 9.35

al. (1 ng/mL) [12] and Hayashida et al. (0.2–1 ng/mL for AC, MA, HA and 2–50 ng/mL for BAC, BMA and BHA) [19]. The high sensitivity could be attributed to the LC/MS/MS technique, especially in MRM mode, it was useful to provide an excellent procedure to quantitate target compounds at very low concentration level.

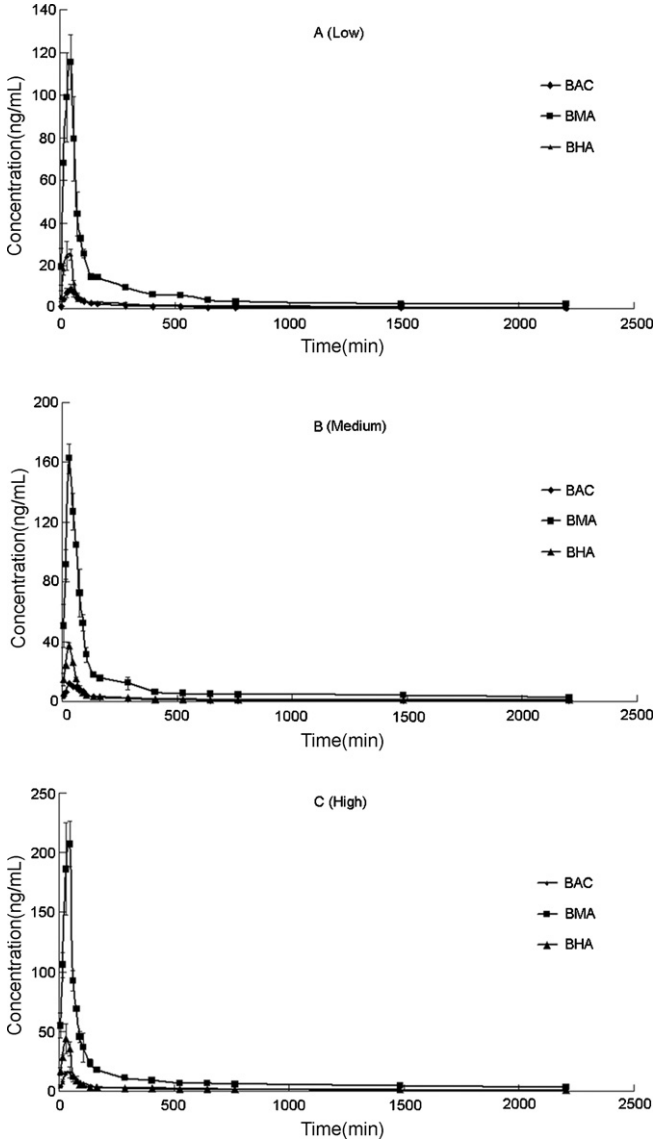

Fig. 4. The plasma concentration–time profiles of BAC, BMA and BHA in low (A), medium (B) and high (C) dosages. Each point was given as the form of mean ± S.D.

3.4. Pharmacokinetics

The results of pharmacokinetics demonstrated that the concentrations of AC, MA and HA were at very low levels (AC and MA were less than 0.2 ng/mL, HA was less than 0.7 ng/mL or under detection limit for all) and the content of BMA was highest, which was in accordance to literature [7]. Fig. 4 demonstrates the concentration–time curves of BAC, BMA and BHA in plasma after the administration of three dosages of "SHEN-FU" injectable powder. The fitting of the concentration–time profiles were based on pharmacokinetics one-compartment model. The parameters of pharmacokinetics were demonstrated in Table 4.

The pharmacokinetics showed a short half-life for all three benzoyl-type *Aconitum* alkaloids, which were almost less than 1 h. They all achieved the maximum concentration at 30 min in medium dosage, however, 45 min in low and high dosages. These results could be helpful for the rational use of this multiple-constituent traditional Chinese medicine "SHEN-FU" injectable powder.

4. Conclusion

In present study, a rapid, specific and sensitive LC/MS/MS was established to simultaneous quantitation of AC, MA, HA, BAC, BMA and BHA in human plasma samples of 18 volunteers in the phase I. The small volume plasma (100 μL) and high sensitivity with LOD low to 0.033 ng/mL were achieved. Up to 150 samples could be processed in a working day. It is for the first time for the LC/MS/MS method applied on human plasma to evaluate the pharmacokinetics of benzoyl-type *Aconitum* alkaloids after intravenous drop infusion of "SHEN-FU" injectable powder. Furthermore, this method is suitable for the forensic and clinical study in phase II of *Aconitum* alkaloids.

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