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LC–MS determination and pharmacokinetic study of six phenolic components in rat plasma after taking traditional Chinese medicinal-preparation: Guanxinning lyophilized powder for injection

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ABSTRACT

A traditional Chinese medicinal preparation (TCMP) named Guanxinning lyophilized powder for injection composed of *Salvia miltiorrhiza* Bge. (SMB) and *Ligusticum chuanxiong* Hort. (LCH) was studied. In order to learn the kinetic behaviors of the lyophilized powder and provide proofs for rational administration, we have developed a sensitive and reproducible method for determination and pharmacokinetic study of six main phenolic components {danshensu (DSS), protocatechuic acid (PAC), protocatechuic aldehyde (PAL), chlorogenic acid (CHA), caffeic acid (CAA) and salvianolic acid B (SAB)} of Guanxinning in rat plasma using liquid chromatography–mass spectrometric (LC–MS) method. Sample preparations were carried out by protein precipitation with the addition of methanol followed by liquid–liquid extraction with ethyl acetate–ethyl ether (3:1, *v*/*v*) after internal standard (IS, galic acid) spiked. After evaporation to dryness, the resultant residue was reconstituted in methanol and injected onto a Kromasil C_{18} column (150 mm × 4.6 mm i.d. with 5 μm particle size). The analytes were analyzed by using negative electrospray ionization (ESI) mass spectrometry in selected ion monitoring (SIM) mode. The method was with good linearity in the range 0.342–85.0 μg mL^{-1} for DSS, 0.0647–12.9 μg mL^{-1} for PAC, 0.0933–18.7 μg mL^{-1} for PAL, 0.0085–3.40 μg mL^{-1} for CHA, 0.0138–2.75 μg mL^{-1} for CAA and 0.0272–810 μg mL^{-1} for SAB ($r > 0.99$). The average extract recoveries of the six analytes from rat plasma were all no less than 75%, the precision and accuracy determined were all within the required limits. This LC–MS method was successfully applied to pharmacokinetic study of the six phenolic components of Guanxinning lyophilized powder for injection in rats.

1. Introduction

Traditional Chinese medicines (TCMs) are natural therapeutic agents used in accordance with the guiding theory of traditional Chinese medical science. TCMs are used mostly in combinations in China and made into preparations for easy and efficient use. Guanxinning injection, which was recorded in a ministerial standard [1], is a clinical medicine commonly used in China for the treatment of coronary artery disease and angina pectoris [2–4]. It consists of two well-known Chinese TCMs *Salvia miltiorrhiza* Bge.(SMB) and *Ligusticum chuanxiong* Hort. (LCH). In order to improve the stability of Guanxinning liquid injection, the injection was made into lyophilized powder. It was reported that the phenolic acids (DSS, PAC, PAL, CHA, CAA, SAB, and so on) in SMB and LCH were the main effective components for the treatment of coronary heart disease, angina pectoris, heart-stroke and cardiovascular diseases [5–15].

Pharmacokinetic studies of active ingredients in TCMs and TCMPs will greatly help in illustrating their action mechanisms and in promoting the development of TCM and TCMP. It is well known that there are a lot of complicated chemical constituents contained in TCMs and TCMPs, and there are synergistic actions among the components, the cooperation may affect the therapeutical effects or action mechanisms, in this case pharmacokinetic study of one component or two is not enough to explain the kinetic behaviors of TCMs and TCMPs *in vivo*, it is necessary to find out how the total effective components metabolized. Currently, there are a limited number of reports on DSS, PAC, PAL, CHA, CAA and SAB in crude drugs and TCMPs [16–24], HPLC, MEKC and LC–MS^n methods were used in those papers, LC–MS exceeds other meth-

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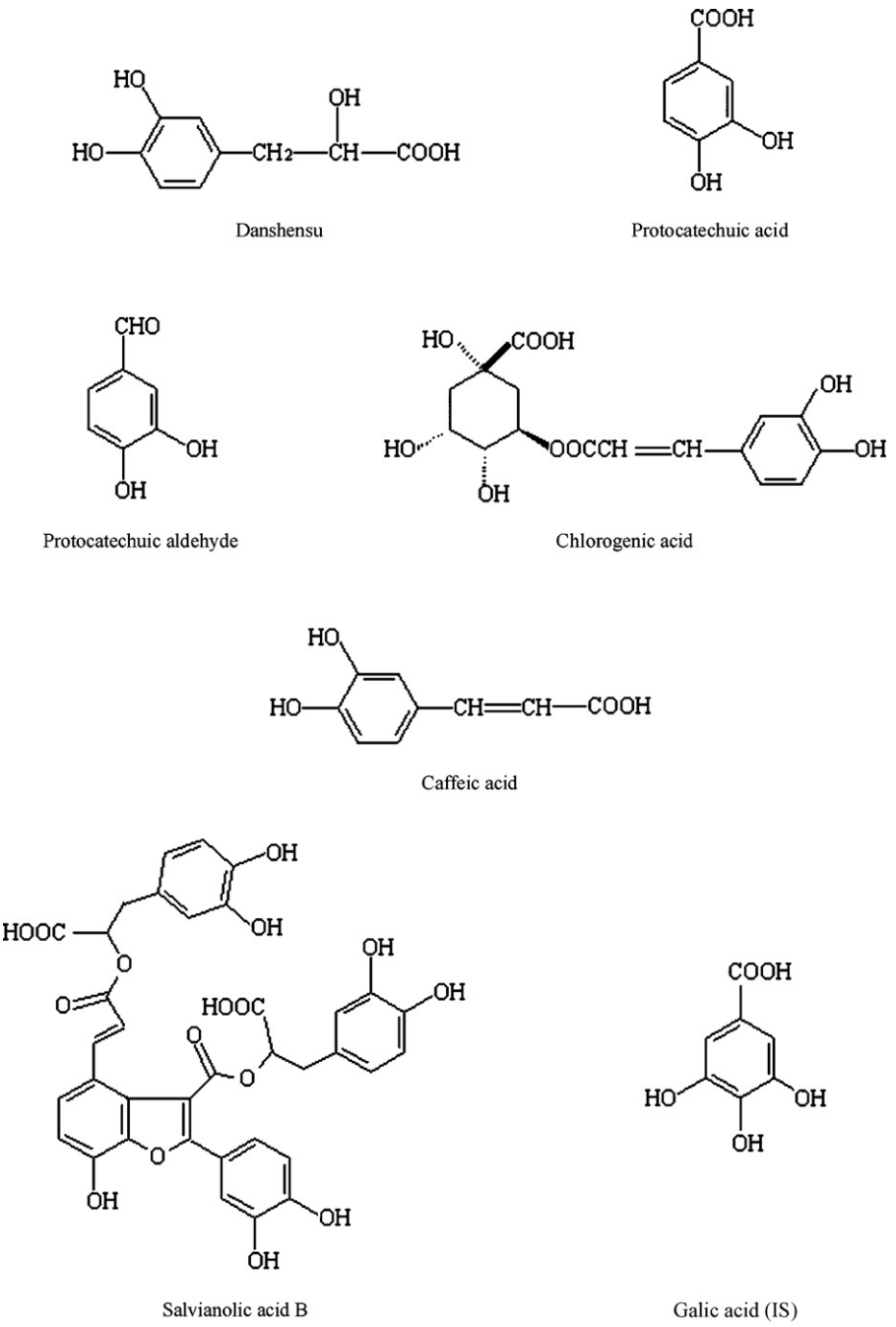

Fig. 1. The chemical structures of the investigated six main components and IS.

ods because of the higher selectivity. And we know that there was no report on pharmacokinetic studies of those six components together. Due to the different polarity of the components, they are quite different in retention behaviors, which made it difficult to analyze them at the same time. As for pharmacokinetic studies, how to extract all of these components from the blood is even more difficult. As far as we are aware, no method has been reported on pharmacokinetic studies of Guanxinning injection. In order to support preclinical pharmacokinetic studies requiring quantitation of DSS, PAC, PAL, CHA, CAA, SAB, a sensitive and accurate method for the determination and pharmacokinetic study of six main components in rat plasma after intravenous administration of Guanxinning lyophilized powder for injection was developed.

2. Experimental

2.1. Chemical

Six standards, danshensu, protocatechuic acid, protocatechuic aldehyde, chlorogenic acid, caffeic acid and salvianolic acid B (Fig. 1), were purchased from the National Institute for Control of Biological and Pharmaceutical Products (Beijing, China). Galic acid (purity >98%, Fig. 1) used as IS was isolated and purified in our laboratory. Methanol (Caledon, Georgetown, Canada), acetonitrile (Caledon, Georgetow, Canada) and formic acid (Kermel, Tianjin, China) were of HPLC grade. All the other reagents were of analytic grade. Distilled water, prepared from demineralized water, was used throughout the experiments.

2.2. Instrumentation

A Shimadzu 2010 liquid chromatograph-mass spectrometer (Shimadzu, Japan) equipped with an electrospray ionization (ESI) interface was used in the study. The HPLC system consisted of a DGU-14 AM degasser, two LC-10AD*vp* pumps, a CTO-10A*vp* column oven and a SIL-HTC auto sampler (Shimadzu, Japan). The data acquisition and peak integration were performed using LC–MS solution Version 3.0.

2.3. Chromatographic and mass spectrometric condition

The separation was performed on a Kromasil C_{18} column (150 mm × 4.6 mm i.d. with 5 μm particle size) preceded by a C_{18} guard column (12.5 mm × 4.6 mm i.d.) from Zhonghuida Co. (Dalian, China).

The mobile phase, which was mixed with solvent A (methanol–acetonitrile, 3:1, *v*/*v*) and solvent B (0.2% formic acid), was gradient elution. The gradient program was 0–15 min from A-B (25:75, *v*/*v*) to A-B (70:30, *v*/*v*). The flow rate was 0.8 mL min^{-1} with 25% of the eluent being splitted into the inlet of the mass spectrometer and the column temperature was maintained at 35 °C.

The electrospray ionization (ESI) was performed using nitrogen to assist nebulisation (the flow rate was set at 1.5 L min^{-1}). Selected ion monitoring (SIM) with negative ion mode, capillary voltage at 1.6 KV and temperature of Curved Desolvation Line (CDL) and Heat block both set at 200 °C were used. Target ions were monitored at m/z 197.05 for DSS, 153.10 for PAC, 137.10 for PAL, 353.05 for CHA, 179.05 for CAA, 717.05 for SAB and 169.00 for IS.

2.4. Preparation of stock and working solutions

By dissolving different accurately weighted amounts of standards in solution of methanol the stock solutions were gained as follows: DSS 510 μg mL^{-1}, PAC 77.6 μg mL^{-1}, PAL 112 μg mL^{-1}, CHA 10.2 μg mL^{-1}, CAA 16.5 μg mL^{-1}, SAB 1.62 mg mL^{-1} and IS 19.9 μg mL^{-1}.

Different volumes of each stock solution were transferred into volumetric flasks and then diluted to volume to make working standard solutions with methanol. Then assay standard samples were prepared by spiking 200 μL blank rat plasma with appropriate amounts of the standard working solutions, the concentration ranges were 0.342–85.0 μg mL^{-1} for DSS, 0.0647–12.9 μg mL^{-1} for PAC, 0.0933–18.7 μg mL^{-1} for PAL, 0.0085–3.40 μg mL^{-1} for CHA, 0.0138–2.75 μg mL^{-1} for CAA and 0.0272–810 μg mL^{-1} for SAB. Quality control (QC) samples (DSS 0.684, 17.1, 68.0 μg mL^{-1}, PAC 0.129, 2.59, 10.3 μg mL^{-1}, PAL 0.187, 3.73, 14.9 μg mL^{-1}, CHA 0.0170, 0.680, 2.72 μg mL^{-1}, CAA 0.0275, 0.550, 2.20 μg mL^{-1}, SAB 0.0544, 5.44, 648 μg mL^{-1}) were independently prepared in the same manner. All the working solutions were kept at 4 °C. The working and assay standard solutions were prepared everyday.

2.5. Sample preparation

To a 200 μL aliquot of plasma sample, 10 μL of 0.2% (mL/mL) HCl, 20 μL of the IS working solution and 500 μL methanol were added and vortexed for 3 min to mix. The mixed sample was then extracted with 2 mL ethyl acetate–ethyl ether (3:1, *v*/*v*) by vortex mixing for 5 min, after centrifugation at 12000 rpm for 5 min, the upper organic layer was transferred to a clean test tube and evaporated to dryness at 30 °C under a stream of nitrogen. The residuals were reconstituted in 120 μL methanol with vortex mixing for 1 min. After centrifuged at 12000 rpm for 5 min, a 20 μL aliquot was injected onto the LC–MS system.

2.6. Appplication of the analytical method

Male Wistar rats, weighing approximately 250–280 g, were provided by the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). They were kept in an environmentally controlled breeding room for three days before starting the experiments. Before the test they were fed with food and water *ad libitum* and fasted overnight. Animal experiments were carried out in accordance with the Guidelines for Animal Experimentation of Shenyang Pharmaceutical University (Shenyang, China) and the protocol was approved by the Animal Ethics Committee of this institution. Six rats received an intravenous injection of Guanxinning powder at a dose of 0.8 g kg^{-1}. Blood samples (0.5 mL) were collected from the ophthalmic vein before (0 min) administration and at different time points (2, 4, 6, 10, 15, 20, 30, 45, 60, 120, 240 and 480 min) after administration. Then the plasma was separated from heparinized blood by centrifugation and stored frozen at −20 °C until analysis.

The plasma concentrations of the analytes at different times were expressed as mean ± S.D. and the mean concentration–time curves were plotted, respectively. All data were processed by use of drug and statistics (DAS) software (version 2.0) for Windows (China's State Drug Administration). The maximum plasma concentration (C_{max}) and the time to reach this concentration (t_{max}) were obtained directly from the observed values. The apparent elimination rate constant (K_e) was calculated by fitting mean data at four terminal points of the plasma concentration profile with a log-linear regression equation using the least-squares method. The plasma half-life, $t_{1/2}$, was calculated as $0.693/K_e$. The area under the plasma concentration–time curve from zero to the time of the final measurable sample ($AUC_{0\text{-}t}$) was calculated using the linear-trapezoidal rule up to the last sampling point with a detectable level (C). The area under the plasma concentration–time curve from zero to infinity ($AUC_{0\text{-}\infty}$) was calculated using the trapezoidal rule with extrapolation to infinity with K_e.

3. Results and discussion

3.1. LC–MS optimization

The purpose of this study was to develop a sensitive and accurate LC–MS method for the determination of DSS, PAC, PAL, CHA, CAA and SAB in rat plasma. Because of the complexity of TCMs and TCMPs, it is very difficult to separate the analytes completely. Due to the carboxyl group and phenolic hydroxyls existed in the analytes, we added a bit of acid to decrease the pH value of the mobile phase to get better separation and less interference from other components in the plasma or in the drugs. The optimal mobile phase consisted of a mixture of methanol–acetonitrile–0.2% formic acid. And because of the different polarity of those analytes, we found that gradient elution made better separation than isocratic elution. The degree of interference by endogenous plasma constituents was assessed by inspection of chromatograms derived from processed blank plasma and spiked plasma samples. As regards to column temperature, it suggested that the retention time was shorter and the separation was better when column temperature was kept at 35 °C.

The six components and IS were analyzed by MS in ESI negative ion mode. Positive mode was also test, but the sensitivity obtained was not satisfactory for all of the six analytes. The full scan mass spectrum of six analytes and IS after direct injection in mobile phase are shown in Fig. 2. The most sensitive ions were $[M-H]^-$, so the quantitative analysis was carried out by SIM for DSS 197.05, PAC 153.10, PAL 137.10, CHA 353.05, CAA 179.05, SAB 717.05 and IS

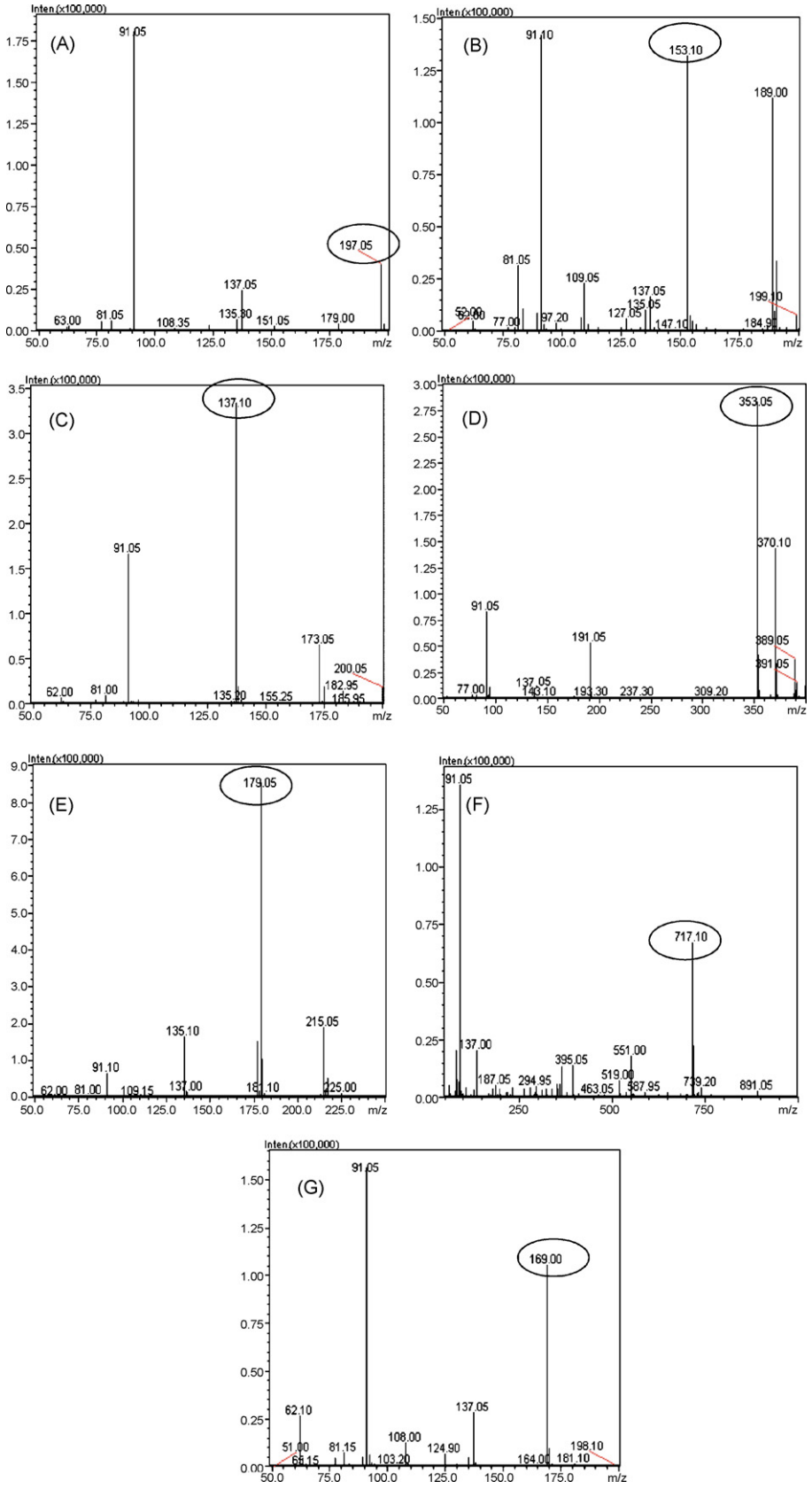

Fig. 2. Full-scan mass spectra of DSS (A), PAC (B), PAL (C), CHA (D), CAA (E), SAB (F) and IS (G).

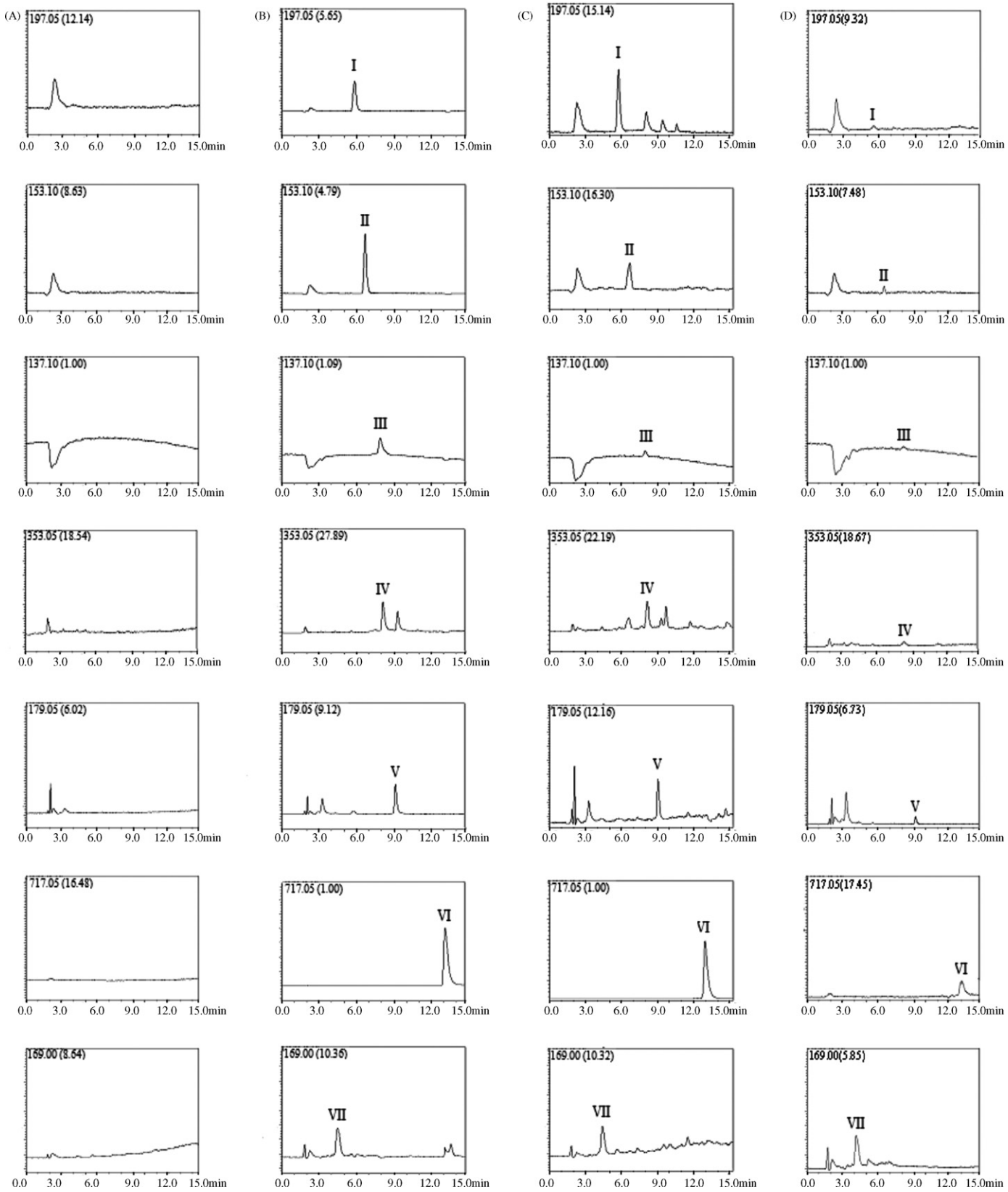

Fig. 3. SIM chromatograms of blank rat plasma (A), blank rat plasma spiked with the six components and IS (B), a rat plasma sample 0.17 h after intravenous administration of Guanxinning Lyophilized Powder for Injection (C), and LLOQ sample (D). Peak I: DSS; II: PAC; III: PAL; IV: CHA; V: CAA; VI: SAB; VII: IS.

Table 1
Intra- and inter-day precision and accuracy of DSS, PAC, PAL, CHA, CAA and SAB assay in rat plasma ($n = 6$)

	Concentration added ($\mu g\,mL^{-1}$)	Intra-day concentration measured ($\mu g\,mL^{-1}$)	RE(%)	RSD(%)	Inter-day concentration measured ($\mu g\,mL^{-1}$)	RE(%)	RSD(%)
DSS	0.684	0.658 ± 0.060	−3.7	8.7	0.681 ± 0.053	−0.4	7.7
	17.1	17.6 ± 1.77	3.3	10.0	17.6 ± 0.92	3.2	5.2
	68.0	67.6 ± 4.76	−0.7	7.2	66.1 ± 4.65	−2.8	7.0
PAC	0.129	0.136 ± 0.008	5.0	6.3	0.133 ± 0.007	2.5	5.4
	2.59	2.54 ± 0.140	−1.7	5.4	2.61 ± 0.143	0.9	5.5
	10.3	10.2 ± 0.828	−1.2	8.0	10.3 ± 0.235	−0.3	2.3
PAL	0.187	0.182 ± 0.011	−2.4	6.2	0.181 ± 0.006	−2.8	3.1
	3.73	3.74 ± 0.291	0.3	1.8	3.80 ± 0.219	1.8	5.8
	14.9	14.9 ± 1.22	−0.1	8.4	14.6 ± 0.759	−2.4	5.2
CHA	0.0170	0.0171 ± 0.001	0.8	4.1	0.0175 ± 0.001	3.3	5.1
	0.680	0.683 ± 0.037	0.4	5.5	0.672 ± 0.024	−1.2	3.5
	2.72	2.79 ± 0.104	2.7	3.7	2.81 ± 0.109	3.4	3.9
CAA	0.0275	0.0272 ± 0.0019	−0.9	7.0	0.0275 ± 0.0018	0.0	6.5
	0.550	0.552 ± 0.040	−0.4	7.4	0.540 ± 0.031	−1.8	5.7
	2.20	2.26 ± 0.093	2.8	4.2	2.19 ± 0.193	−0.6	8.8
SAB	0.0544	0.0563 ± 0.0048	3.5	8.5	0.0564 ± 0.0041	3.6	7.2
	5.44	5.57 ± 0.468	2.4	8.6	5.42 ± 0.450	−0.4	8.3
	648	.650 ± 57.2	0.3	8.7	.657 ± 48.3	1.3	7.3

169.00. The analytes were identified by comparing their retention time and SIM chromatograms with standard solutions containing the corresponding compound.

3.2. Sample preparation

During development of the extraction method, protein precipitation and liquid–liquid extraction were explored. But due to the different polarity of the six analytes, neither precipitation nor liquid–liquid extraction could be used alone to extract all the six analytes completely enough. For precipitation, the extraction recovery of SAB was only around 20%, and for liquid–liquid extraction, recoveries of DSS, PAC and PAL were no more than 40%. When these two methods were combined together after HCl being added to modify the pH value of blood sample, results showed that the combined method was better than each one alone.

In order to improve the recoveries of the six analytes and IS, several organic solvents were tested. For precipitation, methanol, acetonitrile and 10% trichloroacetic acid were compared. As for acetonitrile, PAL could not be detected, when 10% trichloroacetic acid was used, extract recovery of SAB was no more than 10%. Results showed that methanol was better. For liquid–liquid extraction, ethyl ether, ethyl acetate, dichloromethane and their mixture in different combinations and ratios were tested. Finally, ethyl acetate–ethyl ether (3:1, v/v) appeared to be optimal, especially for CAL, CAA and SAB, content of which were at least doubled.

3.3. Selection of internal standard

An internal standard should be used when performing pharmacokinetic study. An appropriate internal standard will control for extraction, HPLC injection and ionization variability. In this method galic acid was chosen as IS for its phenolic structure, extraction recovery, ionization response in ESI mass spectrometry and chromatographic retention time with the analytes. It was also stable during the period of pretreatment and assaying of the plasma samples. Moreover, galic acid is not a component of SMB or LCH.

Table 2
Stability of DSS, PAC, PAL, CHA, CAA and SAB assay in rat plasma ($n = 6$)

	Concentration ($\mu g\,mL^{-1}$)	Long-term stability storage at −20 °C for 14 days (RE%)	Post-preparative stability at room temperature for 24 h (RE%)	Freeze and thaw stability (RE%)
DSS	0.684	−3.7	−0.1	−6.7
	17.1	4.2	5.3	2.4
	68.0	2.3	−1.8	−3.6
PAC	0.129	5.8	0.7	2.6
	2.59	−0.3	2.2	−2.6
	10.3	4.9	0.6	−9.9
PAL	0.187	−8.7	−1.8	3.9
	3.73	4.5	0.5	−3.9
	14.9	0.4	−3.4	−0.6
CHA	0.0170	−4.7	4.8	−0.9
	0.680	1.1	−1.7	−3.6
	2.72	−1.2	2.2	0.9
CAA	0.0275	−0.2	−1.4	−4.5
	0.550	−1.2	−4.2	−2.1
	2.20	2.8	−4.3	2.8
SAB	0.0544	8.1	0.8	−1.1
	5.44	3.4	−4.1	1.4
	648	−1.0	−1.1	1.7

3.4. Method validation [25,26]

3.4.1. Assay selectivity and matrix effect

LC–MS method is with high selectivity because only selected ions are monitored. In this report, six lots of blank plasma were extracted to assess assay selectivity. Comparison of the chromatograms of the blank and the spiked rat plasma (see Fig. 3) indicated no significant interference at the retention times of the analytes and the IS.

As for matrix effect experiment, stock solution of each analyte was diluted with water into the chosen concentration as the working standard solution in plasma in six replicates. After corresponding diluted samples and working standard samples were analyzed, concentrations determined were compared. Results, which were described as percentage in concentration of diluted samples to working standard solutions, were no less than 98.7% for all the six analytes, and showed that there was no significant difference between the peak areas of samples prepared from rat plasma and from water which indicated that no co-eluting unseen compounds significantly influenced the ionization of analytes and IS.

3.4.2. Linearity of calibration curves and lower limit of quantification

Calibration curve for each analyte in spiked rat plasma was linear with a correlation coefficient (r) >0.99. The LLOQ for determination of each analyte in plasma, defined as the lowest concentration analyzed with accuracy within ±20% and precision ≤20%, were 0.342 μg mL^{-1} for DSS, 0.0647 μg mL^{-1} for PAC, 0.0933 μg mL^{-1} for PAL, 0.0085 μg mL^{-1} for CHA, 0.0138 μg mL^{-1} for CAA and 0.0272 μg mL^{-1} for SAB. These limits are sufficient for the pharmacolinetic study of the six analytes following an intravenous injection of Guanxinning lyophilized powder for injection to rats.

3.4.3. Assay precision and accuracy

Intra- and inter-day precision was assessed from the results of QCs by using a one-way analysis of variance (ANOVA). The mean values and relative standard deviation (RSD) for QC samples at three concentration levels (DSS 0.684, 17.1, 68.0 μg mL^{-1}, PAC 0.129, 2.59, 10.3 μg mL^{-1}, PAL 0.187, 3.73, 14.9 μg mL^{-1}, CHA 0.0170, 0.680, 2.72 μg mL^{-1}, CAA 0.0275, 0.550, 2.20 μg mL^{-1}, SAB 0.0544, 5.44, 648 μg mL^{-1}) were calculated over three validation runs. Six replicates of each QC level were determined in each

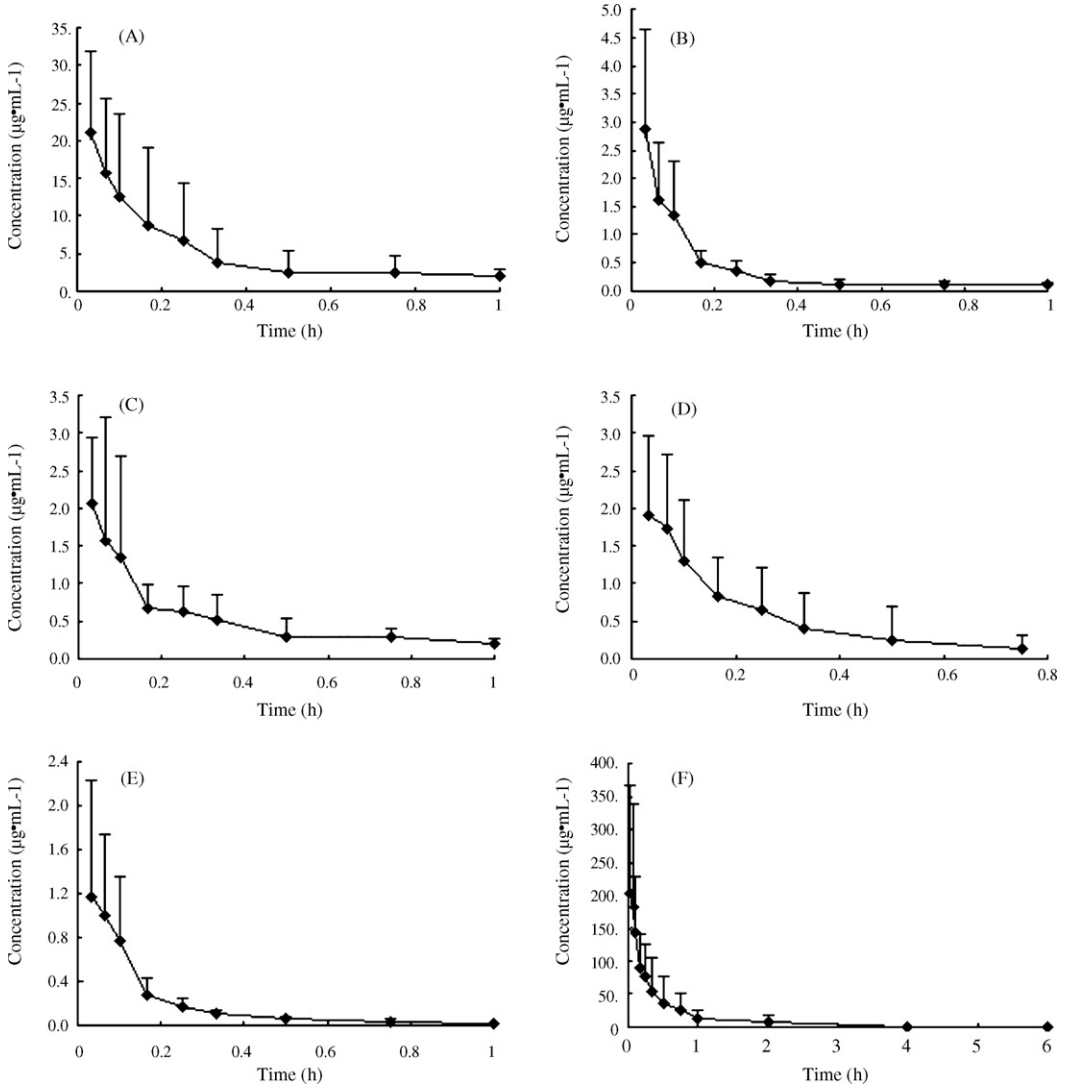

Fig. 4. Plasma concentration–time curves of DSS (A), PAC (B), PAL (C), CHA (D), CAA (E), and SAB (F) after intravenous injection of Guanxinning Lyophilizer to rats at a dose of 0.8 g kg^{-1}. Each value is expressed as mean ± S.D.

Table 3
Mean pharmacokinetic parameters of DSS, PAC, PAL, CHA, CAA and SAB after administration of Guanxinning Lyophilizer at a dose ($n = 6$)

Parameter	Estimate (mean ± S.D.)					
	DSS	PAC	PAL	CHA	CAA	SAB
$t_{1/2}$ (h)	0.390 ± 0.408	0.384 ± 0.230	0.419 ± 0.385	0.114 ± 0.069	0.270 ± 0.122	1.18 ± 0.502
C_{max} (μg mL^{-1})	21.2 ± 10.7	2.90 ± 1.73	2.45 ± 1.45	2.01 ± 1.04	1.29 ± 1.00	205 ± 163
t_{max} (h)	0.033 ± 0	0.033 ± 0	0.039 ± 0.014	0.050 ± 0.019	0.044 ± 0.018	0.039 ± 0.014

C_{max}: maximum plasma concentration; t_{max}: time to reach peak concentration; $t_{1/2}$: apparent elimination half-life.

run. The accuracy of the method was determined by calculating the percentage deviation observed in the analysis of QCs and expressed as the relative error (RE). For inter-day validation, six sets of QC samples at three different concentrations were evaluated for three different days. For intra-day validation, six sets of QC samples at three different concentrations were assayed on the same day.

Table 1 summarizes the intra- and inter-day precision and accuracy for the six analytes from the QC samples. The precision of this method was no more than 10.0% RSD and the accuracy ranged from −2.8% to +5.0%. All results were satisfactory.

3.4.4. Extraction recovery

The mean recoveries were 86.8% for DSS, 85.6% for PAC, 78.1% for PAL, 76.5% for CHA, 84.7% for CAA, 82.3% for SAB and 70.5% for IS.

3.4.5. Analyte stability

Six replicates of QC samples (0.684, 17.1, 68.0 μg mL^{-1} for DSS, 0.129, 2.59, 10.3 μg mL^{-1} for PAC, 0.187, 3.73, 14.9 μg mL^{-1} for PAL, 0.0170, 0.680, 2.72 μg mL^{-1} for CHA, 0.0275, 0.550, 2.20 μg mL^{-1} for CAA, 0.0544, 5.44, 648 μg mL^{-1} for SAB) were used to evaluate the stabilities of the six analytes in rat plasma under a variety of storage conditions: long-term stability storage at −20 °C for 14 days, post-preparative stability at room temperature for 24 h and three cycles of freeze (−20 °C) and thaw (room temperature) stability. Samples were to be concluded stable if the average deviations of them were within ±15% of the actual value. Results were shown in Table 2.

3.5. Application of the analytical method to pharmacokinetic studies

The assay was applied to a preliminary pharmacokinetic study of DSS, PAC, PAL, CHA, CAA and SAB in rats after intravenous injection of Guanxinning lyophilized powder for injection. The pharmacokinetic parameters were estimated by use of DAS software (version 2.0). The mean plasma concentration–time profiles for the six analytes are illustrated in Fig. 4. The corresponding pharmacokinetic parameters obtained were listed in Table 3.

Among the six main active components of Guanxinning powder, the AUC values for DSS and SAB were high while those for the mark compounds of SMB. DSS, PAC, PAL, CHA and CAA eliminated quickly *in vivo* after intravenous injection, which were undetectable 1 h after administration. Compared to them, SAB eliminated slowly after administration, it was detectable at least for 6 h.

DSS, PAC, PAL, CAA and SAB are main active ingredients of water-soluble components in SMB, but there is still great difference in the chromatographic behaviors among these components, and up to know, although many researchers have done pharmacokinetic studies of them respectively, little paper has been reported about the simultaneous determination and pharmacokinetic study of these components together. So we developed a sensitive and accurate LC–MS method for determination and pharmacokinetic study of these main water-soluble components, which could be used not only for Guanxinning lyophilized powder for injection, but also for SMB or TCMPs contained SMB.

4. Conclusion

An LC–MS method was developed for the simultaneous determination of danshensu, protocatechuic acid, protocatechuic aldehyde, chlorogenic acid, caffeic acid and salvianolic acid B in rat plasma. This method was sensitive, with high accuracy and met all requirements in bioanalytical method. The analytical method has been successfully applied to assay plasma concentration of six phenolic acids in rat plasma. If appropriately improved it may also be applied to other routes of administration, or to other animal species and humans, or to the TCMs included in Guanxinning lyophilized powder for injection and TCMPs including them. In this paper, protein precipitation and liquid–liquid extraction were combined together to extract all the target analytes as completely as possible. This is the first report of pharmacokinetic studies of danshensu, protocatechuic acid, protocatechuic aldehyde, chlorogenic acid, caffeic acid and salvianolic acid B together *in vivo*. The pharmacokinetic results provide a firm basis for evaluating the clinical efficacy of Guanxinning lyophilized powder for injection and Guanxinning injection.

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