Journal of Chromatography B, 878 (2010) 2184–2190

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Comparative pharmacokinetics of baicalin and wogonoside by liquid chromatography–mass spectrometry after oral administration of Xiaochaihu Tang and Radix scutellariae extract to rats

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ARTICLE INFO

Article history:
Received 18 November 2009
Accepted 18 June 2010
Available online 25 June 2010

Keywords:
Baicalin
Wogonoside
Pharmacokinetics
Liquid chromatography
Mass spectrometry

ABSTRACT

The aim of this study was to compare the pharmacokinetics of baicalin and wogonoside in rats following oral administration of Xiaochaihu Tang (Minor Radix Bupleuri Decoction) and Radix scutellariae extract. Thus, a specific LC–MS method was developed and validated for the determination of these flavonoids in the plasma of rats after oral administration Xiaochaihu Tang and Radix scutellariae extract. Chromatographic separation was performed on a Zorbax SB C_{18} column (150 mm × 4.6 mm, i.d.: 5 μm) with 0.1% formic acid in water and acetonitrile by linear gradient elution. Baicalin, wogonoside and carbamazepine (internal standard, I.S.) were detected in select-ion-monitoring (SIM) mode with a positive electrospray ionization (ESI) interface. The following ions: *m/z* 447 for baicalin, *m/z* 461 for wogonoside and *m/z* 237 for the I.S. were used for quantitative determination. The calibration curves were linear over the concentration ranges from 0.1231 to 6.156 μg mL^{-1} for baicalin and 0.08832 to 4.416 μg mL^{-1} for wogonoside. The lower limit of detection (LLOD) based on a signal-to-noise ratio of 2 was 0.06155 μg mL^{-1} for baicalin and 0.04416 μg mL^{-1} for wogonoside. Intra-day and inter-day precisions (RSD%) were within 10% and accuracy (RE%) ranged from −6.4 to 4.4%. The extraction recovery at three QC concentrations ranged from 74.7 to 86.0% for baicalin and from 71.3 to 83.7% for wogonoside. The plasma concentrations of baicalin and wogonoside in rats at designated time periods after oral administration were successfully determined using the validated method, pharmacokinetic parameters were estimated by a non-compartment model. Following oral administration of Xiaochaihu Tang and Radix scutellariae extract, the $t_{1/2}$ of baicalin was 3.60 ± 0.90 and 5.64 ± 1.67, the C_{max1} was 1.64 ± 0.99 and 5.66 ± 2.02, the t_{max1} was 0.13 ± 0.05 and 0.20 ± 0.07, the C_{max2} was 2.43 ± 0.46 and 3.18 ± 1.66, and the t_{max2} were 6.40 ± 1.67 and 5.66 ± 2.02, respectively. Following oral administration of Xiaochaihu Tang and Radix scutellariae extract, the $t_{1/2}$ of wogonoside was 4.97 ± 1.68 and 7.71 ± 1.55, the C_{max1} was 1.39 ± 0.83 and 1.45 ± 0.37, the t_{max1} was 0.21 ± 0.20 and 0.17 ± 0.01, the C_{max2} was 1.90 ± 0.55 and 1.42 ± 0.70, and the t_{max2} was 5.60 ± 1.67 and 5.20 ± 1.79, respectively. A significant difference ($p < 0.05$) was observed for $t_{1/2}$, and the elimination of baicalin and wogonoside in Xiaochaihu Tang was increased.

1. Introduction

Xiaochaihu Tang (Minor Radix Bupleuri Decoction, or Sho-saiko-to in Japanese) is an important multiherbal medicine in Traditional Chinese Medicine (TCM) and was first described in Shang Han Lun, a treatise on exogenous febrile diseases written by the famous Chinese physician Zhang Zhongjing (150 to 219 A.D. in the Chinese Eastern Han Dynasty). It is composed of seven herbs including Radix bupleuri, Radix scutellariae, Radix glycyrrhizae, Radix ginseng, Rhizoma pinelliae, Rhizoma zingiberis recens, and Fructus jujubae. Xiaochaihu Tang is widely used to treat chronic hepatitis [1–3], it also has cytoprotective effects in experimental liver injuries [4,5], preventive and therapeutic effects in experimental hepatic fibrosis *via* inhibition of hepatic stellate cells and lipid peroxidation in hepatocytes [6–8].

Radix scutellariae is a well-known TCM used as a ministerial ingredient in Xiaochaihu Tang to treat inflammation, fever, hepatitis, allergic diseases and hypertension. Baicalin and wogonoside are characteristic flavonoids isolated from the roots of Radix scutellariae. Baicalin is used as a phytochemical marker for the quality control of Radix scutellariae in the Chinese pharmacopoeia, and wogonoside is also a major flavonoid [9,10]. It has been reported that baicalin and wogonoside have anti-

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doi:10.1016/j.jchromb.2010.06.021

inflammatory, anti-allergic, anti-oxidant and hepatoprotective properties [11,12].

Several methods for the separation and quantification of flavonoids in Radix scutellariae have been described using high performance liquid chromatography (HPLC) with ultra-violet detection [13–19]. The pharmacokinetic profiles of baicalin in blood, brain and eyes of rats and rabbits have been reported using liquid chromatography–mass spectrometry (LC–MS) [20–22]. Deng et al. [23] investigated the pharmacokinetic profiles of baicalin and wogonoside in diabetic rats following oral administration of Huanglian-Jiedu Decoction using HPLC. Wang et al. [24] evaluated baicalin and wogonoside in rat plasma following administration of GegenQinlin Decoction using ultra-performance liquid chromatography–mass spectrometry (UPLC–MS). However, there are few data on the pharmacokinetics of baicalin and wogonoside following administration of oral Xiaochaihu Tang. A selective and sensitive analytical method for the simultaneous determination of baicalin and wogonoside in plasma is therefore needed to characterize the pharmacokinetics of baicalin and wogonoside following oral administration of Xiaochaihu Tang. The aim of this study was to develop a simple, rapid and reliable LC–MS assay for the simultaneous determination of baicalin and wogonoside in rat plasma and to compare the pharmacokinetic profiles of baicalin and wogonoside after oral administration of Xiaochaihu Tang and Radix scutellariae extract.

2. Experimentation

2.1. Materials and reagents

Radix bupleuri, Radix scutellariae, Radix glycyrrhizae, Radix ginseng, Rhizoma pinelliae, Rhizoma zingiberis recens, and Fructus jujubae were purchased from Shanghai Huayu Pharmaceutical Limited Company (Shanghai, China) and authenticated by Prof. Sun from the Department of Pharmacognosy, the Second Military Medical University (Shanghai, China). Standards of baicalin (purity > 98%) and wogonoside (purity > 98%) and carbamazepine (purity > 98%) as the internal standard were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile and formic acid of HPLC grade were from Burdick & Jackson (Ulsan, Korea). Purified water was used in the experiments. All the other reagents were of analytical grade.

2.2. Instrumentation and conditions

A 1100 HPLC system (Agilent, Santa Clara, CA, USA) used consisted of a G1322A degasser, a G1311A quaternary pump, a G1367A well-plate autosampler, a G1316A thermostated column compartment and a G1315B DAD detector. A G1946D mass spectrometer (Agilent, Santa Clara, CA, USA) equipped with an electrospray source was connected to the LC system. Data acquisition and analysis were performed using Agilent ChemStation for LC/MSD version B.02.01. The sample was separated on an Agilent Zorbax SB C_{18} column (150 mm × 4.6 mm, i.d.: 5 μm) and eluted with a gradient of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). The gradient program increased B from 28 to 45% in 0–10 min, B returned to 28% at 10.1 min and was maintained for 20 min to analyze the next sample. The column temperature was 25 °C at a flow rate of 1 mL min^{-1} and an injection volume of 10 μL. The flow outlet was introduced into the mass spectrometer by a post-column split ratio of 1:1 with a three-way joint. The mass conditions of electrospray ionization were optimized as follows: capillary 4000 V, nebulizer 40 psi, drying gas 10 L min^{-1}, gas temperature 350 °C and fragmentor 100 V. Select-ion-monitoring (SIM) in the positive ion mode was used. The baicalin, wogonoside and carbamazepine ions were recorded as *m*/*z* 447, *m*/*z* 461 and *m*/*z* 237 for quantification respectively.

2.3. Preparation of Xiaochaihu Tang and Radix scutellariae extract

Pieces of Radix bupleuri 125 g, Radix scutellariae 46.9 g, Radix glycyrrhizae 46.9 g, Radix ginseng 46.9 g, Rhizoma pinelliae 50 g, Rhizoma Zingiberis Recens 46.9 g and Fructus jujubae 12 g were mixed and decocted twice with water (2400 mL and then 1200 mL) for 1 h. The extracted solution was concentrated to 200 mL to obtain Xiaochaihu Tang. Radix scutellariae (250 g) was decocted with water (2500 mL and then 1250 mL) for 1 h and the extracted solution was concentrated to 1000 mL to obtain Radix scutellariae extract.

To calculate the administration dose, the contents of baicalin and wogonoside in Xiaochaihu Tang and Radix scutellariae extract were analyzed quantitatively by HPLC-DAD. Baicalin and wogonoside were separated using a 5 μm Agilent Zorbax XDB C_{18} column (150 mm × 4.6 mm i.d.). The LC mobile phase was 0.2% H_3PO_4 (v/v) for solvent A and CH_3CN for solvent B. The binary gradient program consisted of an initial 20 min linear gradient segment of B increasing from 15 to 23%, followed by B increasing to 35% from 20 to 35 min. The linear gradient was changed progressively by increasing B to 90% from 35 to 45 min, and then maintaining B at 90% from 45 to 50 min. Finally, B was returned back to 15% at 50.1 min and maintained from 50.1 to 65 min for analysis of the next sample. The effluent was delivered at 1 mL min^{-1} during the gradient program. The UV detection wavelength was set at 275 nm and the column temperature was 25 °C. Baicalin and wogonoside were separated at baseline within 30 min, baicalin was linear ($y = 44.16x - 36.22$, $r = 0.9999$) over the concentration range 6.156–307.8 μg mL^{-1} and wogonoside was linear ($y = 52.08x - 28.69$, $r = 0.9999$) over the concentration range 2.208–110.4 μg mL^{-1}. The solutions of Xiaochaihu Tang and Radix scutellariae extract were diluted 1000 times with water, respectively. A 15 μL sample was injected into the HPLC system after filtration using a 0.45 μm millipore filter.

2.4. Preparation of calibration standards, quality control and internal standard

Stock solutions were prepared by dissolving various accurate amounts of standards in methanol solution: 1.026 mg mL^{-1} of baicalin and 0.736 mg mL^{-1} of wogonoside. The internal standard stock solution was prepared by dissolving carbamazepine in methanol to 0.0993 mg mL^{-1}. Working standard solutions of baicalin and wogonoside were prepared by combining the aliquots of each primary stock solution and diluting to the scale of a volumetric flask with methanol. The internal standard was diluted to 198.6 ng mL^{-1} before use. The assay standard samples containing baicalin (6.156, 3.078, 1.231, 0.6156, 0.3078, and 0.12312 μg mL^{-1}) and wogonoside (4.416, 2.208, 0.8832, 0.4416, 0.2208, and 0.08832 μg mL^{-1}) were prepared by spiking 30 μL of the working standard solution into 100 μL of blank rat plasma. Quality control samples (QCs) at 3.078, 1.231 and 0.3078 μg mL^{-1} of baicalin and 2.208, 0.8832 and 0.2208 μg mL^{-1} of wogonoside were independently prepared in the same manner. All the solutions were kept at 4 °C. The assay standard samples and quality control samples were prepared during each analysis batch.

2.5. Sample preparation

To a 100 μL aliquot of plasma sample, 30 μL of the carbamazepine internal standard solution (198.6 ng mL^{-1}), 30 μL

methanol and 140 μL acetonitrile were added and vortexed for 1 min in a 1.5 mL polypropylene tube, and then centrifuged at 10,400 × *g* for 10 min before injection into the LC–MS system.

2.6. Validation method

2.6.1. Selectivity

The selectivity of the method was investigated by analyzing six individual blank rat plasma samples. The chromatographic findings of each blank plasma sample were compared with the spiked rat plasma containing baicalin (1.231 μg mL^{-1}), wogonoside (0.8832 μg mL^{-1}) or I.S.(19.86 ng mL^{-1}) to check interference.

2.6.2. Linearity of calibration curves, LLOD and LLOQ

The calibration curves for baicalin and wogonoside were constructed by plotting peak area ratios of the analyte to I.S. against plasma concentrations using a $1/C^2$ weighted linear least-squares regression model. The linearity of baicalin and wogonoside determined in spiked rat plasma was obtained using six calibration standards in five independent runs.

The lower limit of detection (LLOD) was defined as the lowest concentration with a signal-to-noise ratio of at least 2-fold, and the lower limit of quantification (LLOQ) was the concentration giving a signal-to-noise ratio at least 5-fold with acceptable accuracy within 20% deviation and precision between 80 and 120%.

2.6.3. Precision and accuracy

Three concentrations (high, medium and low) of baicalin and wogonoside standard stock solutions were added to plasma to obtain control samples respectively, and were determined in five separate runs on the same day for intra-day and on 5 consecutive days for the inter-day accuracy variation. The accuracy of the method was determined by calculating the percentage deviation observed during the analysis of quality controls and expressed as the relative error (RE).

2.6.4. Recovery and matrix effect

The extraction recovery of baicalin, wogonoside and I.S. were determined at three QC concentrations by comparing the peak area from plasma samples with those obtained by injecting the diluted standard solution at the same concentration using five replicates.

The matrix effect was determined at three different QC concentrations using three replicates. The peak area of the post-extraction blank plasma spiked with standard solutions of baicalin and wogonoside was known as set 1, and the peak area of the diluted standard solutions at the same concentration was known as set 2. When the peak area ratio of the analytes in set 1 compared to that in set 2 was between 85 and 115%, the matrix effect was considered to be negligible.

2.6.5. Stability

QCs were used to evaluate the stability of the analytes in rat plasma under different storage conditions: long-term stability at −40 °C for 14 days, post-preparative stability at room temperature for 8 h, and three freeze–thaw cycles.

2.7. Study in vivo

The experimental protocol was approved by the Animal Ethics Committee of the Second Military Medical University. Male Sprague–Dawley (SD) rats weighing 250–280 g were supplied by Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, China). The rats were maintained in air-conditioned animal quarters at 22 ± 2 °C and 50 ± 10% relative humidity. Water and food (laboratory rodent chow, Shanghai, China) were allowed ad libitum. The animals were acclimatized to the facilities for 5 days, and then fasted with free access to water for 12 h prior to each experiment. The rats were randomized into two groups, 5 in each group: animals in group A were administered an oral dose of 2 mL/100 g Xianchaihu Tang (265.4 mg/kg baicalin and 58.78 mg/kg wogonoside), and animals in group B were administered an oral dose of 2 mL/100 g Radix scutellariae extract (249.6 mg/kg baicalin, and 52.00 mg/kg wogonoside). Blood samples (0.5 mL) were collected into a heparinized tube *via* the oculi chorioideae vein before drug administration and at 0.083, 0.167, 0.333, 0.5, 1, 2, 4, 6, 8, 10, 12, 14, 16 and 24 h after drug administration. Following centrifugation at 2200 × *g* for 10 min, plasma samples were obtained and frozen at −40 °C until analysis.

The area under the concentration–time curve from 0 h to the last experimental point at 24 h (AUC_{0-24}) was estimated by the linear trapezoidal rule. Terminal half-life ($t_{1/2}$) was calculated as $t_{1/2} = 0.693/k_e$, and k_e was determined by linear regression of the logarithmic plasma concentration versus time for the last 3 or 4 (criteria was linearity with coefficients of correlation not less than 0.9) data points on the concentration–time curve. C_{max} and t_{max} values were obtained directly from the observed concentration–time curves. An unpaired Student's *t*-test was used to compare the differences in pharmacokinetic parameters between the two groups. All results were expressed as arithmetic mean ± standard deviation (S.D.).

3. Results and discussion

3.1. LC–MS optimization

Due to the complexity of Traditional Chinese Medicines (TCMs) and Traditional Chinese Medicinal preparations (TCMPs), many analogues may be co-eluted during analyses. In order to develop a sensitive and accurate LC–MS method for the determination of baicalin and wogonoside in rat plasma, the qualification analysis was performed using SIM owing to its high selectivity and sensitivity. We investigated the response of analytes after the addition of different concentrations (0.01, 0.05, 0.1 and 0.15%) of formic acid into the mobile phase (water and acetonitrile), and found that when 0.1% formic acid was used in the mobile phase, better separation was obtained as well as the highest response with protonated molecular ions of baicalin and wogonoside. The mass spectra of baicalin, wogonoside and carbamazepine are shown in Fig. 1. Abundant protonated molecular ions after electrospray ionization of baicalin (*m*/*z* 447), wogonoside (*m*/*z* 461) and carbamazepine (*m*/*z* 237) were selected for MS detection. The main parameters of electrospray ionization including pressure of the nebulizer, the flow rate of drying gas and voltage of the fragmentor in SIM were optimized by flow injection analysis (FIA) using the baicalin standard, The time segment in one record channel of SIM was set as follows: 0–4.8 min for baicalin at *m*/*z* 447, 4.8–7 min for wogonoside at *m*/*z* 461 and 7–10 min for carbamazepine at *m*/*z* 237. The analytes in rat plasma were identified by comparing the retention time and the molecular weight.

3.2. Optimization of the extraction procedure

In generally, numerous samples need to be analyzed in a pharmacokinetic study. Thus, simple, rapid and economic sample preparation is necessary. It is not advisable that plasma samples containing of baicalin and wogonoside undergo pretreatment with liquid–liquid extraction using organic solvents, because the flavonoid glycoside is a hydrophilic, poor lipophilic compound, which makes it difficult to extract from an aqueous biological medium. Nevertheless, we compared liquid–liquid extraction and protein precipitation for sample preparation, and

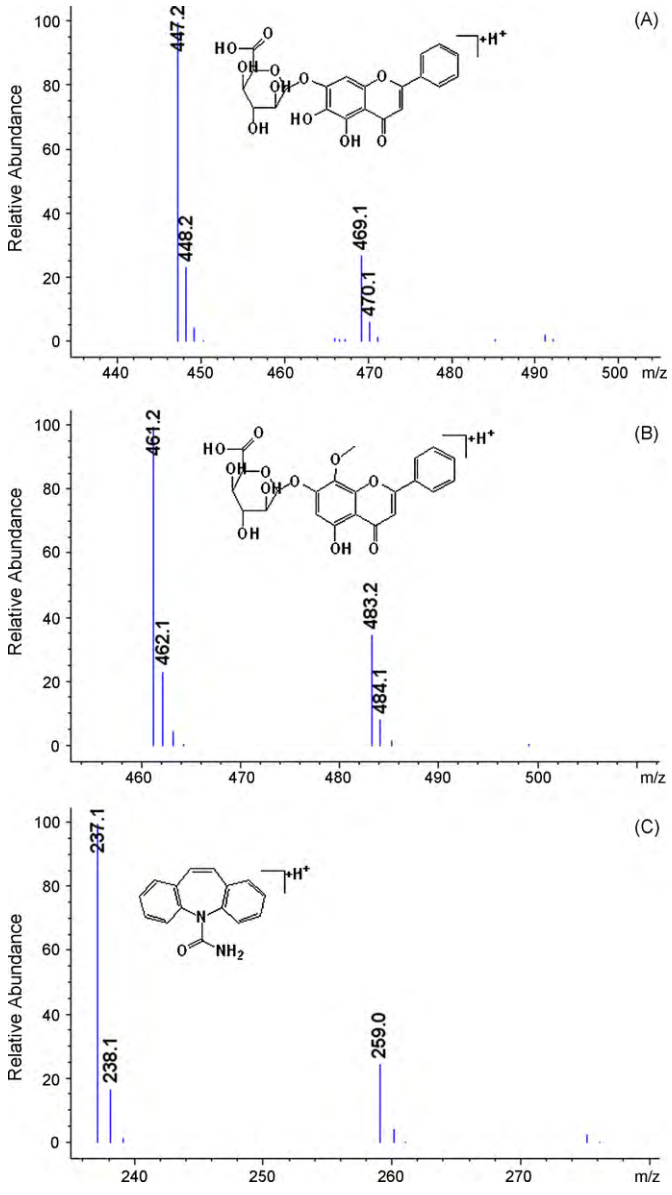

Fig. 1. Protonated molecular ions in mass spectra for baicalin (A), wogonoside (B) and internal standard carbamazepine (C).

protein precipitation was subsequently used for sample preparation. Methanol, acetonitrile and their mixture in different ratios were investigated. We found that interference in blank plasma cannot be eliminated when methanol is used for precipitation, and the sensitivity of detection was limited by a 3-fold volume of acetonitrile due to a dilution effect, therefore, a mixture of organic solvents including 140 μL acetonitrile and 60 μL methanol (sample to precipitant ratio of 1:2) was chosen as the precipitation agent. Protein precipitation was advantageous in this study, because it could not only ensure less endogenous interference, adequate recovery and high sensitivity, but also ensure easy performance.

3.3. Method validation

3.3.1. Selectivity

The representative chromatograms in SIM of baicalin, wogonoside and carbamazepine are shown in Fig. 2. The retention time of baicalin, wogonoside and carbamazepine was found to be approximately 3.8, 5.8 and 8.0 min, respectively, indicating good resolution of the two flavonoids and the I.S. The analysis of blank rat plasma samples did not show any interference on the retention time of baicalin, wogonoside or I.S.

3.3.2. Linearity of calibration curves, LLOD and LLOQ

The calibration curve of baicalin was $y = 3.069x - 0.2925$ ($r = 0.9996$) and was $y = 4.816x + 0.1450$ ($r = 0.9998$) for wogonoside. LLOQ was set at 0.1231 μg mL^{-1} for baicalin and 0.08832 μg mL^{-1} for wogonoside in rat plasma. The signal-to-noise ratio was higher than 5-fold and the deviation was no more than 15% ($n = 5$). LLOD of baicalin and wogonoside was obtained when the solution was diluted to 0.06155 μg mL^{-1} and 0.04416 μg mL^{-1}, respectively, and the signal-to-noise ratio was 2-fold.

3.3.3. Precision and accuracy

The intra-day and inter-day precisions of this method were not more than 10%, and the accuracy ranged from −6.4 to 4.4%. All results are shown in Table 1.

3.3.4. Extraction recovery and matrix effect

The results showed that the mean recovery of baicalin was 86.0 ± 3.1, 74.7 ± 6.1 and 81.8 ± 4.9% ($n = 5$) at the concentration of 0.3078, 1.2312 and 3.078 μg mL^{-1}, and that of wogonoside was 83.7 ± 3.1, 71.3 ± 5.0 and 80.9 ± 4.7% ($n = 5$) at the concentration of 0.2208, 0.8832 and 2.208 μg mL^{-1}. The mean recovery of the internal standard was 93.6 ± 4.4% ($n = 5$) at the concentration used in the assay procedure (Table 2).

The matrix effect was described as percentage in the response of set 1 to set 2, the results were 90.7 ± 1.3, 87.3 ± 1.8 and 95.2 ± 2.8% for the three QCs ($n = 3$). The relative matrix effect of QCs in three concentrations was 3.6% showed that the effect of plasma matrix was equal to different concentrations. It is suggested that the method to be considered reliable and free from the matrix effect.

3.3.5. Sample stability

The analytes remained stable and the concentrations were still within 15% deviation of the initial values. These results are shown in Table 3.

3.3.6. Application of the method to pharmacokinetic study

The contents of baicalin and wogonoside were calculated as 13.27 ± 0.06 and 2.94 ± 0.02 mg mL^{-1} in Xiaochaihu Tang, and 12.48 ± 0.06 and 2.60 ± 0.02 mg mL^{-1} in Radix scutellariae extract, respectively.

The mean concentration–time curve of baicalin and wogonoside in the two treatments is shown in Fig. 3. We attempted to estimate the pharmacokinetic parameters using 3P97 (Practical Pharmacokinetics Program Version 1.0), however the degree of fit with the compartment model was unfavorable, which is consistent with the findings of Wang et al. [24]. A non-compartment model was used to calculate the pharmacokinetic parameters. The pharmacokinetic parameters of baicalin and wogonoside in male SD rats following oral administration of Xiaochaihu Tang and Radix scutellariae extract are shown in Table 4.

The concentration–time curves of baicalin and wogonoside displayed double peaks as shown in Fig. 3. This phenomenon was similar to that in other reports [25–27], and was probably due to enterohepatic circulation. The main pharmacokinetic parameters after oral administration of Radix scutellariae extract have been reported by Shi et al. [28]. However, as shown in Table 4, $t_{1/2}$ of baicalin and wogonoside after oral administration of Xiaochaihu Tang was less than that after oral administration of Radix scutellariae extract, and C_{max1} of baicalin after oral administration of Xiaochaihu Tang was smaller than that after oral administration of Radix scutellariae extract. The difference in the two treatment groups was significant ($p < 0.05$) by unpaired Student's t-test,

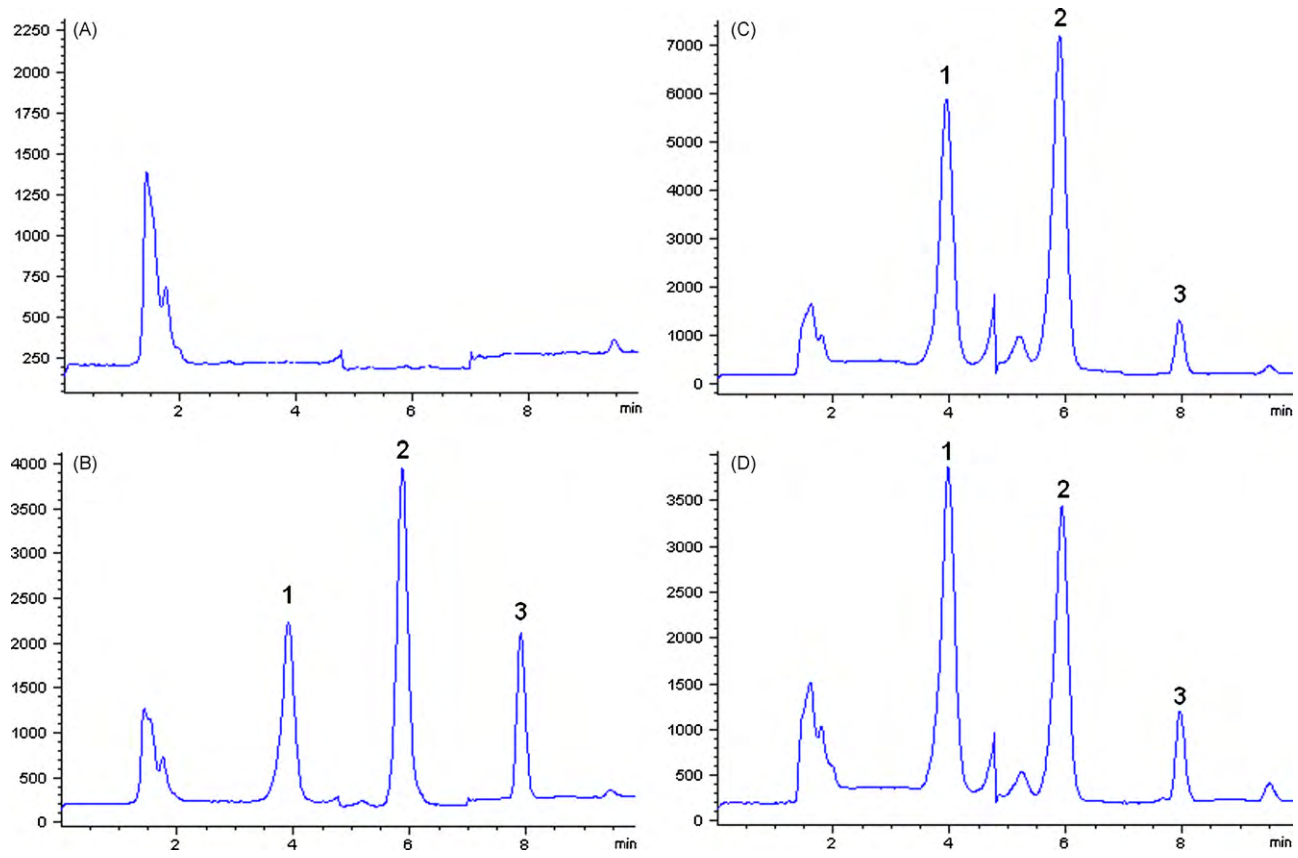

Fig. 2. Representative chromatograms of the two analytes in blank plasma (A), spiked standard solution in blank plasma (B), rat plasma sample after oral administration of Xiaochaihu Tang (C) and rat plasma sample after oral administration of Radix scutellariae extract (D). (1) Baicalin; (2) wogonoside; and (3) carbamazepine (I.S.).

suggesting that drug interactions occurred in this compound prescription formula, which possibly prohibited the absorption of baicalin and accelerated the elimination of baicalin and wogonoside in rats. These results were similar to those observed in the study of Huang-Lian-Jie-Du-Tang in rats by Lu et al. [13]. Many case reports have shown [29–33] that Xiaochaihu Tang can produce adverse drug reactions, and in our previous study [34] we reported that Radix scutellariae was the main toxic component. The pharmacokinetic parameters of baicalin and wogonoside determined in the present study may imply that compound TCM prescriptions may be safer than a single Chinese medicine.

Many publications have reported that baicalin cannot enter the blood directly because of its large polarity, but can be partly transformed into baicalein by glucuronidase (GUS). Baicalein absorbed

Table 1
Precision and accuracy of analytes in rat plasma.

Analytes	Spiked concentration ($\mu g\,mL^{-1}$)	Intra-day			Inter-day		
		Measured ($\mu g\,mL^{-1}$)	RSD (%)	RE (%)	Measured ($\mu g\,mL^{-1}$)	RSD (%)	RE (%)
Baicalin	0.3078	0.3144	4.0	+2.1	0.2999	3.5	−2.6
	1.231	1.245	1.8	+1.1	1.153	8.5	−6.4
	3.078	3.176	3.1	+3.2	3.195	4.0	+3.8
Wogonoside	0.2208	0.2212	7.6	+0.2	0.2084	5.1	−5.6
	0.8832	0.8835	0.6	+0.0	0.8420	4.0	−4.7
	2.208	2.294	1.1	+3.9	2.306	3.4	+4.4

Table 2
Recovery of baicalin, wogonoside and I.S. from spiked rat plasma.

Analytes	Spiked concentration ($\mu g\,mL^{-1}$)	Plasma sample		Standard solution		Recovery (%)
		Peak of area	RSD (%)	Peak of area	RSD (%)	
Baicalin	0.3078	17154.3	3.8	19975.9	5.5	86.0 ± 3.1
	1.231	95947.7	2.3	128827.1	6.9	74.7 ± 6.1
	3.078	290219.0	4.0	355416.5	6.1	81.8 ± 4.9
Wogonoside	0.2208	29137.0	3.6	34834.8	3.7	83.7 ± 3.1
	0.8832	112253.1	3.8	157991.7	6.2	71.3 ± 5.0
	2.208	311067.4	4.8	397449.0	5.6	80.9 ± 4.7
I.S.	0.1986	30502.2	2.2	32631.9	4.5	93.6 ± 4.4

Table 3
Stability of baicalin and wogonoside in rat plasma.

Analytes	Theoretical concentration ($\mu g\,mL^{-1}$)	Long-term stability storage		Room temperature		Freeze–thaw cycles	
		Measured ($\mu g\,mL^{-1}$)	RE (%)	Measured ($\mu g\,mL^{-1}$)	RE (%)	Measured ($\mu g\,mL^{-1}$)	RE (%)
Baicalin	0.3078	0.2967	−3.6	0.2867	−6.9	0.2998	−2.6
	1.231	1.188	−3.5	1.157	−6.0	1.191	−3.3
	3.078	3.264	+6.0	3.332	+8.3	3.273	+6.3
Wogonoside	0.2208	0.2225	+0.8	0.1993	−9.7	0.2173	−1.6
	0.8832	0.8368	−5.3	0.8387	−5.0	0.8317	−5.8
	2.208	2.305	+4.4	2.448	+10.9	2.311	+4.7

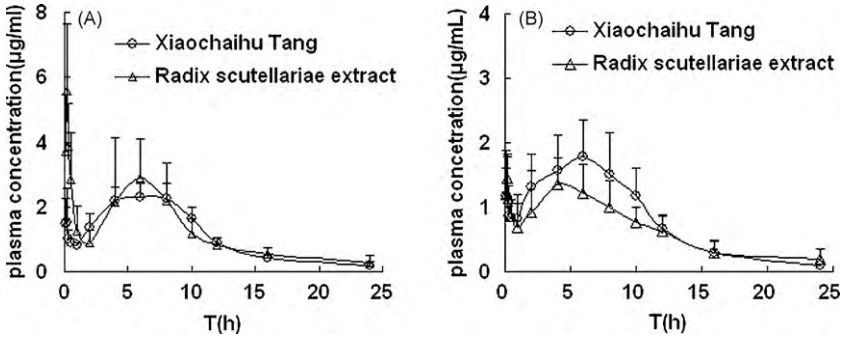

Fig. 3. Mean concentration–time curves in rat plasma after oral administration of Xiaochaihu Tang and Radix scutellariae extract. (A) Baicalin and (B) wogonoside.

Table 4
Pharmacokinetic parameters of baicalin and wogonoside in male SD rats following oral administration of Xiaochaihu Tang and Radix scutellariae extract.

Parameter	Baicalin		Wogonoside	
	Xiaochaihu Tang	Radix scutellariae extract	Xiaochaihu Tang	Radix scutellariae extract
C_{max1} ($\mu g\,mL^{-1}$)	1.64 ± 0.99*	5.66 ± 2.02	1.39 ± 0.83	1.45 ± 0.37
t_{max1} (h)	0.13 ± 0.046	0.20 ± 0.07	0.21 ± 0.20	0.17 ± 0.01
C_{max2} ($\mu g\,mL^{-1}$)	2.43 ± 0.46	3.18 ± 1.66	1.90 ± 0.55	1.42 ± 0.70
t_{max2} (h)	6.40 ± 1.67	5.60 ± 0.89	5.60 ± 1.67	5.20 ± 1.80
$AUC_{(0-24)}$ ($\mu g\,h\,mL^{-1}$)	26.14 ± 3.95	28.48 ± 12.21	19.56 ± 4.82	15.66 ± 7.75
$AUC_{(0-\infty)}$ ($\mu g\,h\,mL^{-1}$)	27.22 ± 4.16	30.62 ± 12.74	20.27 ± 4.65	17.64 ± 9.33
$t_{1/2}$ (h)	3.60 ± 0.90*	5.64 ± 1.67	4.97 ± 1.68*	7.71 ± 1.55

Mean ± S.D., $n = 5$.
* $p < 0.05$

by the small intestinal mucosa was partly transformed into baicalin by UDP-glucuronosyltransferase(UGT), which was then partly absorbed into the blood [27,35,36]. Baicalein was partly absorbed into the blood directly and transformed into baicalin or metabolized by hepatic microsomal enzymes [18,26,37], and baicalein was the main metabolite of baicalin *in vivo*. In our study, we found a peak (named M) with a retention time of about 5 min at *m*/*z* 461 was similar to wogonoside (*m*/*z* 461) after oral administration of Xiaochaihu Tang and Radix scutellariae extract (Fig. 2C and D), which was possibly a methylated product of baicalin. It is known that methylation generally exists during the drug metabolism *in vivo*, and due to the multiple hydroxyl groups on the baicalin benzene ring, the combination of a methyl group with a hydroxyl group is easy [38]. Therefore, it was tentatively concluded that compound M was the methylated metabolite of baicalin, and the presumed metabolic pathway of baicalin is shown in Fig. 4.

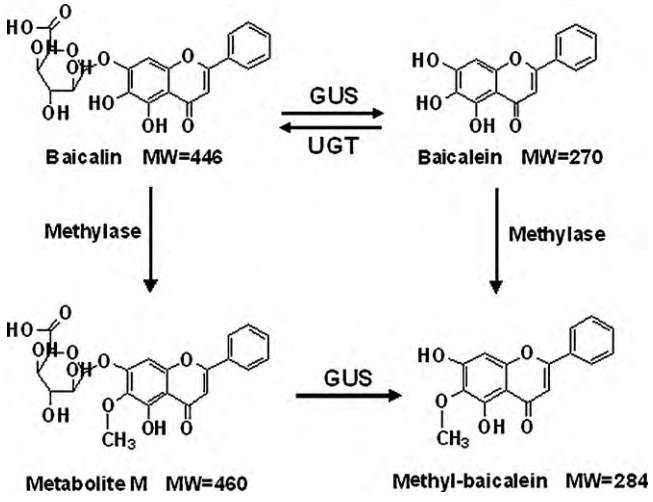

Fig. 4. Presumed metabolic pathway of baicalin *in vivo*.

4. Conclusion

A LC–MS method was developed for the simultaneous pharmacokinetic determination of baicalin and wogonoside in rat plasma. This method is sensitive, highly accurate, simple and appropriate to the bioanalytical requirements. A potential methylated metabolite of baicalin was found, which will be identified in a subsequent study.

The results of the present study showed that there were significant differences between the pharmacokinetic parameters of baicalin and wogonoside after oral administration of Xiaochaihu Tang and Radix scutellariae extract, indicating that competition

or inhibition between the chemical constituents in Xiaochaihu Tang could lead to a decrease in the absorption of baicalin and an increase in the elimination of baicalin and wogonoside. The change in baicalin and wogonoside during metabolism was possibly related to the toxicity of Radix scutellariae in Xiaochaihu Tang, and seemed to suggest that Xiaochaihu Tang is safer than Radix scutellariae. These results might be helpful in explaining the action mechanism of action of traditional Chinese compound prescriptions.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (No. 30873196) and Medical Scientific Research Foundation of Army (06MB205). The authors would like to thank Prof. Lian-na Sun for identifying the medicinal herbs purchased for the study.

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