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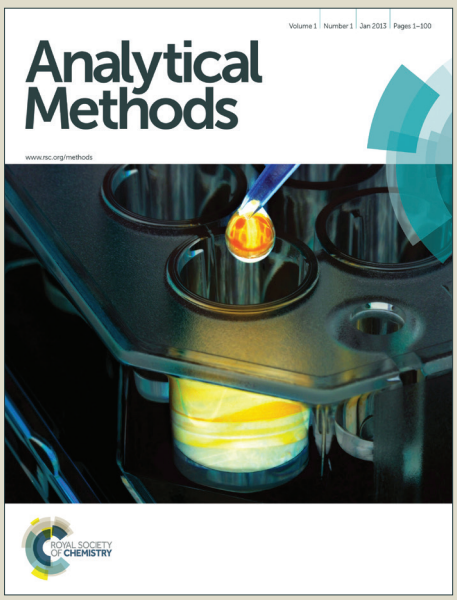

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Comparative Pharmacokinetic and Tissue Distribution Study of Baicalin, Baicalein, Wogonoside, Wogonin and Oroxylin-A after Oral Administration of Component Compatibility of Sanwu-Huangqin-Tang and Total Flavonoids Fractions of Radix Scutellariae to Rats

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Abstract

Sanwu-Huangqin-Tang (SHT) is a classical prescription used for treatment of gynecological disease, and its key ingredient is Radix scutellariae (*Scutellaria baicalensis* Georgi, Labiatae). Baicalin, wogonoside, baicalein, wogonin and oroxylin-A are five main effective ingredients enriched in Radix scutellariae. In the present study, pharmacokinetic and tissue distribution difference of the five compounds following oral administration of Component Compatibility of Sanwu-Huangqin-Tang (CCSHT) and total flavonoids fractions of Radix scutellariae (FSR) were investigated in male Sprague-Dawley rats with approximately the same dose. The amount of flavonoids in plasma and tissues were measured by a rapid and sensitive HPLC-MS/MS method. Unpaired student's-test was used for statistical comparison. The bimodal phenomenon was observed in plasma profile after oral administration of FSR and CCSHT. Statistical significant increase (P<0.05) in pharmacokinetic parameters (including C_{max} and $AUC_{0\to t}$) and tissue distribution of the target compounds were observed after oral administration CCSHT comparing with FSR, but there were no significant differences between the two groups in parameters of T_{max}. The results indicated that compared with FSR, the bioavailability and distribution amount of flavonoids could be greatly improved by co-administrating alkaloids in *Radix sophora flavescentis* and polysaccharide in *Radix Scutellaria.*

Key words Pharmacokinetic; Tissue distribution; Sanwu-Huangqin-Tang; Flavonoids

1 Introduction

Chinese herb preparations, the organic combinations of various crude drugs under the guidance of the theory of traditional Chinese medical (TCM) science, have played an indispensable role in the prevention and treatment of disease in China for centuries. Because the TCM prescriptions contain the scientific principles and rules of drug combination, which are important to guide the clinical treatment and new drug development, the drug-drug interaction of TCM compatibility has long been focused on, and pharmacokinetics are the most commonly used techniques for assessing the rationality of TCM prescriptions.

Sanwu-Huangqin-Tang, a classical TCM prescription recorded for treatment of gynecological disease, originates from Qian Jin Yao Fang and Jin Kui Yao Lve. The prescription contains king herb of Radix scutellariae (6 g), ministerial herb of Radix sophorae flavescentis (6 g), adjuvant and envoy herb of Rehmanniae Rhizoma (12 g). It is famous for the funcation of clearing heat and drying dampness, cooling blood and detoxification, and Ziyin yangxue. In the modern clinical practice of TCM science, SHT has been widely used as a remedy for treatment of fever , hepatitis B, autoimmune liver disease, nephritis, diabetes, tetter, cancer and so on (Zhang *et al.*, 2008 a; Shi *et al.*, 2002; Lv, 2001). The phytochemistry study has revealed that the main active fractions of Radix scutellariae, Radix sophorae flavescentis and Rehmanniae Rhizoma are flavonoids, alkaloids and polysaccharide, respectively. These results suggested that the function of SHT own much to the synergistic interaction of these active fractions.

Radix scutellariae is a well-known TCM used as a king ingredient in SHT. Baicalin(BL),

wogonoside (WL), baicalein (B), wogonin (W) and oroxylin-A (OA) (structures shown in Fig.1) are the main characteristic flavonoids in Radix scutellariae. BL is used as a phytochemical marker for the quality control of Radix scutellariae in the Chinese pharmacopoeia, WL, B, W and OA are also major flavonoids. It have been reported that they have anti-inflammatory (Kim *et al.*, 2009; Yoon *et al., 2009*), antioxidative (Salini *et al.*, 2013; Huang *et al.*, 2006) , antiallergic (Li *et al.*, 2011), antiviral (Tseng *et al*., 2010) antigenotoxic (Chu *et al.*, 2007), anxiolytic (Liao *et al.*, 2003) and anti-carcinogenic activities (Ikemoto *et al.*, 2000; Kumagai *et al.*, 2007), and have become the subjects of intense research interest. Kim *et al.* have investigated the pharmacokinetic profiles of BL, B, W and OA in rats after intravenous administration of Scutellariae radix extract using LC–MS/MS (Kim *et al.*, 2006). Hou *et al.* investigated the pharmacokinetic and tissue distribution profiles of BL, B, WL and W in rats after multiple dosing of Scutellaria baicalensis using HPLC (Hou *et al.*, 2011). Tong *et al*. evaluated the pharmacokinetic behavior of BL, B, WL, W and OA in rats plasma following administration of Radix scutellariae extract using LC–MS/MS (Tong *et al.*, 2010 a). However, up to now, few studies on the comparative pharmacokinetic and tissue distribution behaviors of the five flavonoids were reported when they were included as a part of component compatibility of SHT. Given the therapeutic effect of herbal might be caused by multi-ingredient synergism rather than the independent actions of individual ingredients, the likelihood of herb–drug interactions is exsit. To improve our understanding of those complex processes, it is necessary to study the differences of the pharmacokinetics and tissue distribution of flavonoids in FSR and CCSHT. Therefore, in this study, an analytical method

was established, validated, and employed to compare the pharmacokinetics and tissue distribution of BL, WL, B, W and OA in rats after oral administration of FSR and CCSHT. It was expected to explore whether there are some herbal ingredients in Radix sophorae flavescentis and Rehmanniae Rhizoma combined with Radix scutellariae effecting the pharmacokinetic and tissue distribution behavior of total flavonoids in Radix scutellariae. So as to the results of this study would be helpful for improving clinical therapeutic efficacy and further pharmacological studies of total flavonoids.

Fig.1 The structure of baicalin, wogonoside, baicalein, wogonin, oroxylin-A and daidzein

2 Materials and methods

2.1 Chemicals and reagents

Standard substances including baicalin, baicalein, wogonin, oroxyline-A and daidzein (Da) as the internal standard (IS) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Wogonoside was obtained from Xi'an Rongsheng Biological Technology Co., Ltd (Xi'an, China). HPLC grade methanol and acetonitrile were applied by Merk (Darmstadt, Germany). Hydrochloric acid and formic acid of analytical grade were from Tianjin Fuchen Chemical Reagent Factory (Tianjin, China). Deionized water used throughout the experiments was generated by a Millipore water purification system (Milford, MA, USA). Radix scutellariae, Radix sophorae flavescentis and Rehmanniae Rhizoma were obtained from a local drug store in Guangzhou and authenticated by professor Jin-song Zhou from Guangzhou University of Traditional Chinese Medicine.

2.2 Instruments and LC-MS/MS conditions

The LC-MS/MS system was composed of a Surveyor ^{TM} HPLC system and a TSQ quantum triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source (Thermo Finnigan, USA). Data acquisition was performed with Xcalibur 1.3 software. Chromatographic separation was achieved on a ZORBAX SB-C_{18} (150 mm × 2.1 mm, i.d. 5 μm, Agilent, USA) protected by a Luna C_{18} guard column at 10 ºC. The mobile phase consisted of 0.3% (*v/v*) aqueous formic acid (A) and acetonitrile (B) using a gradient elution of 36% B at 0-3.5 min, 36-70% B at 3.5-13 min, 70-36% B at 13-15 min. The flow rate was 250 μL/min, 100 μL/min and 250 μL/min correspondingly. The mass spectrometer was operated in the positive detection mode with spray voltage set at 3500 V. The heated capillary temperature was 350 °C. The nitrogen sheath gas and the auxiliary gas were set at 35 and 10 psi, respectively. Quantification was performed with multiple selected reaction monitoring (MRM) mode with argon at a pressure of 1.5 m Torr for collision induced dissociation (CID) of the following *m/z* transitions (with collision energy): 447→271 for BL (21 eV), 461→285 for WL (15 eV), 271→123 for B (30 eV), 285→270 for W and OA (23 eV) and 255→181 for Da (29 eV) respectively.

2.3 Preparation of the total FSR, ASF and PRR

Radix scutellariae, Radix sophorae flavescentis. and Rehmanniae Rhizoma were decocted twice by refluxing with water (1: 10, g/mL) for 1 h respectively. The extracted solutions were concentrated to 0.5 g/mL calculated according to crude drug quantity. The total flavonoids of Radix scutellariae (FSR), total alkaloids of Radix sophorae flavescentis. (ASF) and total polysaccharide of Rehmanniae Rhizoma (PRR) were purified and enriched by acid precipitation, macroporous resin and alcohol precipitation methods reported by literature (Yao

et al., 2006; Zhang *et al.*, 2008 b; Zhao and Liu, 2010), respectively. The final there effective parts were obtained after spray drying or lyophilization.

To calculate the administration dose, the content total flavonoids，total alkaloids and total polysaccharide in corresponding crude herbs and in FSR, ASF, PRR fractions were analyzed quantitatively by Mg-HCl colorimetric method, acid fuel colorimetric method and sulphoacid-anthranone colorimetric method based on literature (Gong *et al.*, 2010; Liu *et al.*, 2004; Ding *et al.*, 2008), respectively. Furthermore, the content of baicalin, wogonoside, baicalein, wogonin and oroxylin-A in FSR were accurately quantified by HPLC-DAD using our previous method (Feng *et al.*, 2012), and the results indicated that 50.1% of BL, 1.06% of WL, 12.08% of B, 6.93% of W and 0.083% of OA were contained in FSR.

2.4 Preparation of standard solutions

The stock solutions of BL, B, WL, W, OA and Da were prepared by dissolving a proper amount of them in methanol to furnish a nominal concentration of 100 μg/mL each. Several series of standard mixture working solutions for plasma and different tissue homogenates were obtained by diluting the mixture of the stock standard solutions with methanol. Da was diluted to 4 μg/mL. All solutions were stored at 4 ºC.

2.5 Animals, drug administration and sampling

Male Sprague–Dawley rats (180–220 g) were obtained from the Center Animal Laboratory of Guangzhou University of Traditional ChineseMedicine, and maintained in a normally controlled breeding room (temperature 25 ± 2 ^{o}C, relative humidity 60 ± 5%) on a 12-h light–dark cycle with standard laboratory food and water for a week before experiments, and

fasted for 12 h with free access to water prior to the experiments. All animal experiments were carried out according to the Guidelines for the Care and Use of Laboratory Animals, and were approved by the Animal Ethics Committee of Guangzhou University of Traditional Chinese -Medicine. Rats were randomly divided into FSR group and CCSHT group for each study (n=6). A single dose equivalent to 100 mg/kg FSR and (100: 100: 200) mg/kg of CCSHT (FSR, ASF and PRR were mixed in 0.5% sodium carboxymethyl cellulose aqueous solution at the ratio of 1: 1: 2) were orally administered by gastric intubation to the two groups over-night fasted rats, respectively.

For pharmacokinetic study, the blood samples (approximately 300 μL each) were collected into heparinized eppendorf tube via jugular vein according to the specific schedule at 0, 5, 10, 20, 40 min, 1, 2, 3, 4, 6, 8, 10, 12, 24 h after dosing (six rats were used for blood collection at six time points ahead and the other six rats were used at the remained eight time points to make up the blood loss). The blood samples were followed by centrifuging at approximately 12000×g at 4 ℃ for 5 min. The resulting plasma layers were separated, and then immediately acidified with con. HCl (Per milliliter rat plasma added with 20 μL con. HCl), and stored at -20 ℃ until analysis performed with the procedure described below.

In the tissue distribution study, after oral administration FSR and CCSHT at the same dosage as above, various organs including the liver, kidney, stomach and small intestine were collected at 0.3, 3, 8 and 24 h, respectively. Tissue samples were following washed with ice-saline solution, blotted dry on filter paper and accurately weighed. Twice of cold methanol was added into tissue samples (g/mL), which were thenhomogenized for 5 min under the

condition of ice-water bath. The obtained tissue homogenates were centrifuged at approximately 12000×g at 4 ºC for 5 min and stored at -20 ºC.

2.6 Sample Preparation

To 100 μL of the above plasma or tissue homogenates, 50 μL of daidzein (4 μg/mL) as internal standard was added as well as 350 μL of methanol. The resulting solution was thoroughly vortex-mixed for 3 min. After centrifugation at 12000 ×g at 4 ºC for 5 min, (1) the supernatant of plasma samples was collected and evaporated to dryness under a gentle stream of nitrogen. The residue was reconstituted with 100 μL mobile phase before injected into the HPLC-MS system; (2) the supernatant of tissue samples was directly injected into the HPLC-MS system.

3 Results and discussion

3.1 Detection condition

In this paper, LC-MS/MS method for analysis of plasma and tissue homogenous samples was developed based on literature (Tong *et al.*, 2012 b), and some identical experiment parameters were applied including the condition of positive scan mode, selecting $[M+H]^+$ at *m/z* 447, 461, 271, 285, 285 and 255 as parent ions, selecting SRM transitions at *m/z* 447→271 (BL), 461→285 (WL), 271→123 (B), 285→270 (W, OA) and 255→181 (Da) for quantification. To obtain maximum sensitivity of SRM, some other parameters such as spray voltage, capillary temperature, source CID, sheath gas pressure, auxiliary gas pressure, collision gas pressure and collision energy were optimized.

3.2 Our thought about the component compatibility of SHT

Previous similar research on TCM mainly focused on performing comparative study between single herb and TCM prescriptions. As far as SHT is concerned, our previous study has revealed that the content of target compounds in SHT varied greatly compared with those in crude drugs (Zhang *et al.*, 2007). The phenomenon is because the extraction efficiency could be affected, and the interchange of compounds could occur when Radix scutellaria, Radix sophorae flavescentis and Rehmanniae Rhizomac were co-decocted together. Thus, it is difficult to guarantee the equivalent administration dose for the comparative study. So we adopted the idea of "component compatibility of TCM". After a serious procedure of extraction, purification and enrichment, total flavonoids, total alkaloids and total polysaccharide were obtained respectively. Then according to their content in medicinal materials and compatibility proportion in SHT, the physical mixing ratio and dosage could be easily calculated. Although the "component compatibility of TCM" was not fully consistent with TCM prescriptions, it could more clearly clarifies the interaction rules between components, and ensures the accuracy and comparability of the experiment results under the condition of equal dosage.

3.3 Method Validation

The method was validated with reference to Guidance for Industry Bioanalytical Method Validation of Food and Drug -Administration (FDA 2001). Specificity, matrix effect, linearity, precision, accuracy, extraction recovery and stability multiple stabilities were evaluated in the method validation.

3.3.1 Specificity

The degree of interference by endogenous substances was assessed by inspection of chromatograms derived from processed blank and rat samples (liver was chosen as representative tissue). Fig. 2 illustrated representative chromatograms of blank bio-samples (liver homogeneous), blank bio-samples spiked with target compounds and IS, and bio-samples after administration of FSR and CCSHT spiked with IS. Five flavonoids and IS were eluted at approximately 2.4, 3.8, 8.5, 11.2, 12.3 and 4.0 min, respectively. No detectable interference was found.

Fig. 2. The typical chromatograms of the blank liver tissue homogenate sample (A), the blank liver tissue homogenate sample spiked with analytes at LLOQ with IS (B), and the liver tissue homogenate sample at 3 h after administration of CCSHT to the rats (C)

3.3.2 Linearity of calibration curve and lower limit of quantification

Calibration curves of seven concentration levels were constructed by plotting the peak area radio of the analytes to internal standard versus their respective concentrations using weighted ($1/x^2$) least regression method. The results were listed in Table 1. The calibrations were linear over a certain range in all bio-samples with a correlation coefficient larger than 0.9934. LLOQs were defined as the lowest concentration points of calibration curves at which both precision and accuracy were less than or equal to 20%. The limits were sufficient for investigation of pharmacokinetics and tissue distribution.

Table 1. Regression equations, correlation coefficients and linear ranges of baicalin, wogonoside, (baicalein), wogonin and oroxylin-A in rat plasma and tissue samples (n =7)

3.3.3 Precision and accuracy

Accuracy and precision were evaluated by determining QC samples at three concentration levels in six replicates during a single day and by duplicating the experiments on three consecutive days. The concentration of each sample was calculated using a calibration curve constructed on the same testing day. Accuracy expressed as relative error (RE) was determined by calculating the percentage deviation observed in QC samples. The intra- and inter-day precisions were expressed by relative standard deviation (RSD). The results (Table 2) suggested that the RSD of intra- and inter-day precision were both less than 10.8%. Method accuracy varied from -11.0% ~10.3%.

Table 2 Intra-day and inter-day variability and extraction recovery of baicalin, wogonoside, (baicalein), wogonin and oroxylin-A in rat plasma and liver homogenate (n =6)

3.3.4 Extraction recovery and matrix effect

Extraction recoveries were performed at three QC levels by comparing the peak area obtained from biological sample spiked before extraction with those spiked after extraction. The results (Table 2) showed that the extraction recoveries were acceptable in different biological samples.

The matrix effect was evaluated by comparing the absolute peak area of blank biological sample and then spiked with a known amount of analytes to that of neat standard samples at equivalent concentrations. The same procedure was performed for IS. The results indicated a neglectable matrix effect on the ionization of the analytes.

3.3.5 Stability

Due in part to the presence of 6, 7-dihydroxyl and 5, 6, 7-trihydoxyl groups in the benzene ring of BL and B accordingly, BL and B call for the assessment of the stability during biological sample collection, processing and storage. Referencing to literature, we comparatively evaluated the stability of baicalin and baicalein in their monomer and in FSR form (have not been published). The results showed that baicalin and baicalein were relatively stable in organic solvents but rather unstable in biological fluids without chemical protection, and their degradation was pH-, concentration and temperature-dependent, and baicalein has more unstable than baicalin. Interestingly, baicalin and baicalein exhibited the better stability in FSR or CCSHT than in their monomer forms. We also found that baicalein monomer was the worst stability in plasma, and could hardly be stabilized enough to meet the requirement for quantification in plasma whatever protection was performed. But in FSR and CCSHT at above microgram concentration level, they could be leisurely analyzed if some suitable protection measures for pretreatment and storage of biological samples were performed, such as adjusting medium pH to acidic range, adding antioxidant, operation at low temperature and so on. However, in the present study, we found baicalin could be accurately quantified in plasma and tissue samples, while baicalein could only be detected in tissue samples (homogenized by cold-methanol). Further research suggested that baicalein, owning to its worst stability and biotransformation effect of endogenous substances in plasma, could hardly be stabilized enough in plasma at low concentration level. So in our present study, baicalein was not determined in plasma sample.

The stability of BL, B, WL, W and OA in plasma after three freeze-thaw cycles and stored at -20 ºC for 1 month have been reported by literature (Tong *et al.*, 2012 c). Here post-preparative stability, which could more directly reflects the stability of analytes in the matrix post-extracted, was evaluated extracted samples stored at 10 ºC for 12 h. The results showed that the stability of QC_10.0 ng/mL for baicalin (low concentration) in plasma and liver homogenates were tested to be RE of -13.3% and -12.7%, respectively. The other analytes were rather stable (RE < 8.4%) in all bio-samples within the tested periods.

3.4 Sample collection and preparation

Blood was collected with the ice-cold test tubes or on ice. Plasma should be immediately acidified with hydrochloric acid after centrifuging at low temperature. Tissues removed from the animals should be placed in ice-cold water at once. Proper aliquots of tissue slices were asked to immediately homogenize with acidified methanol after weighing and cutting. The biological samples pretreated according to above measures remain stable at 10 ºC for at least 12 h and stable for 1 month at -20 ºC. When preparing the samples for LC-MS/MS analysis, in order to inhibit the biotransformation of baicalin and baicalein, the chemical reagents and apparatus were advised to be pre-cooled, and the prepared samples were proposed to store at 4 ºC ~10 ºC before injection.

3.5 Pharmacokinetic study

The mean plasma concentration-time profiles of these compounds following oral administration of FSR and CCSHT are shown in Fig. 3, and the estimated pharmacokinetic parameters are listed in Table 3. Baicalin, wogonoside, wogonin and oroxylin-A

concentration-time profiles conformed to a two-compartment pharmacokinetic model. Model analyses of the parameters of flavonoids in rat plasma in the two experimental groups reached the same conclusion: it showed that the four flavonoids all exhibited rapid absorption and bimodal phenomenon in plasma concentration-time profiles. The first peak occurred at about 10 ~ 20 min and the second peak occurred between 2 ~ 10 h after oral administration of FSR and CCSHT. This phenomenon was similar to that in other reports (Tong *et al.*, 2012 c; Xing *et al.*, 2005), and may be contributed to enteric circulation and enterohepatic circulation. That may be because baicalin is poorly absorbed from the gastrointestinal tract in its native form and must be hydrolyzed by microflora enzymes (bacterial β - glucuronidase) in gut to its aglycone (baicalein) (Yim *et al*., 2004; Zuo *et al*., 2002). A large portion of the aglycone is subsequently conjugated to balcalin in the gut mucosal cell by UDP-glucuronosyltransferase and approximately half of the conjugate is excreted back into the gut lumen, mainly through MRP2 (Akao *et al.*, 2000) .

As shown in Table 4, $t_{1/2}$ of baicalin after oral administration of CCSHT was less than that after oral administration of FSR, and $AUC_{0-\infty}$ of baicalin after oral administration of CCSHT was greater than that after oral administration of FSR. The difference in the two treatment groups was significant (P<0.05) by unpaired Student's t-test, T_{max} for all target compounds and $t_{1/2}$ for WL, W and OA has no significant differences between FSR group and CCSHT group. But the differences among the parameters of AUC_{0-t} and C_{max} of the four flavonoids were considered to be significant (P<0.05). It was suggested that drug interactions occurred in this compound prescription formula, which possibly promote the absorption and enhance the

bioavailability of BL, WL, W and OA, and accelerate the elimination of baicalin.

Fig.3 Mean ± SD plasma concentration-time profile for baicalin, wogonoside, wogonin and oroxylin-A in rat plasma after oral administration of FSR and CCSHT

Table 3 Estimated pharmacokinetic parameters for baicalin, wogonoside, wogonin and oroxylin-A in rat plasma (*n*=6) after oral administration of FSR and CCSHT

3.6 Tissues distribution study

In regard to tissue analysis, rats were sacrificed near the plasma peak time of the five flavonoids based on our pharmacokinetic data. After a single-dose administration of FSR and CCSHT, the results (Table 4 and Fig. 4) indicated that total flavonoids could be detected in all assayed tissues and the amount of them underwent a rapid and wide distribution in the tissues throughout the body. Except WL in stomach and small intestine, the concentration of most target compounds in almost all investigated tissues increase to the maximum at 0.17 h and few or undetected at 24 h after rats were administrated of FSR or CCSHT. The change trend is consistent with that of concentration of the flavonoids in plasma, which implied that the distribution of them depended on the blood flow or perfusion rate of the organ and there was no long-term accumulation of them in the tissues. Among various tissues, the distribution of the flavonoids except WL gained the highest level in stomach, followed by small intestine, kidney and liver. We found that with the distribution amount of BL, B, WL, W and OA at 0.17 h in various tissues as reference, the amount of the five flavonoids maintained a relative higher level at 3 h and 8 h in small intestine than in other tissues, and could form a

concentration platform between 3 h and 8 h. Pharmacokinetic study have revealed that the second gentle absorption peaks of target compounds appeared in the positions of 2 h ~ 10 h, and predicted it exist the enterohepatic circulation. So we speculated that the phenomenon of relative higher concentration level of target compounds at 3 h and 8 h in small intestine was probably caused by enterohepatic circulation too. Compared with oral administration of FSR, the concentration of the target compounds in tissues at different time were greatly increased when co-administrated with ASF and PRR, which is conformed with the traditional Chinese medicine theory of mutual promotion action.

Table 4. Tissue distribution of of baicalin, wogonoside, baicalein, wogonin and oroxylin-A in rats after oral administration of FSR and CCSHT at different time (n = 6, mean ± SD)

Fig. 4 Tissue distribution of baicalin, wogonoside, baicalein, wogonin and oroxylin-A in liver, stomach, kidney and small intestine at 0.17, 3, 8 and 24 h after a single dosage administration of FSR and CCSHT

Conclusions

In this report, we first delivered comparative pharmacokinetic and tissue distribution study of BL, WL, B, W and OA in rat plasma or tissue sample after oral administration of component compatibility of Sanwu-Huangqin-Tang and total flavonoids fractions of Radix scutellariae. A sensitive and reliable LC-MS/MS method was established, validated, and successfully applied in the comparison study of pharmacokinetic and tissue distribution of FSR and CCSHT. The groups had similar pharmacokinetic profiles but showed different C_{max}

and *AUC*, and different concentration level for the target compounds in tissues. Improvement of absorption and distribution effects were found when different effective fractions of herb were used in combination.

Acknowledgement

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1. Zhang L, Liu WT, Zhang RW, Wang ZW, Shen ZD, Chen XH, Bi KS. Pharmacokinetic study of matrine, oxymatrine and oxysophocarpine in rat plasma after oral administration of Sophora flavescens Ait. extract by liquid chromatography tandem mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 2008; 47: 892 - 898.
2. Shi WL, Tang SL, Zhang W, Zhao N. The new clinical application of Sanwu-Huangqin-tang. Journal of Beijing University of TCM 2002; 25: 70 - 71.
3. Lv K. Sanwu-Huangqin-Tang treatment of 34 fanre disease cases. Journal of Practical Traditional Chinese Internal Medicine 2001; 15: 33.
4. Kim EH, Shim B, Kang S, Jeong G, Lee JS, Yu Y B, Chun M. Anti-inflammatory effects of Scutellar ia baicalensis extract via suppression of immune modulators and MAP kinase signaling molecules. Journal of Ethnopharmacol 2009; 126: 320 - 331.
5. Yoon SB, Lee YJ, Park SK, Kim HC, Bae H, Kim HM, Ko SG, Choi HY, Oh MS, Park W. Anti-inflammatory effects of Scutellaria baicalensis water-extraction LPS-activated RAW 264.7 macrophages. Journal of Ethnopharmacol 2009; 125: 286 - 290.

6. Salini S, Chubicka T, Sasidharan N, Sindhu ER, Babu TD. Cytotoxic and antioxidant properties of selected Scutellaria species from the Western Ghats of Peninsular India. Pharmaceutical Biology 2013; 51: 152-159.

7. Huang WH, Lee AR, Yang CH. Antioxidative and anti-inflammatory activities of polyhydroxyflavonoids of Scutellaria baicalensis Georgi. Biosci Biotechnol Biochem 2006; 70: 2371- 2380.

8. Li CR, Lin G, Zuo Z. Pharmacological effects and pharmacokinetics properties of Radix Scutellariae and its bioactive flavones. Biopharmaceutics & drug disposition 2011; 32: 427 - 445.

9. Tseng YP, Wu YC, Leu YL, Yeh SF, Chou CK. Scutellariae Radix suppresses hepatitis B virus product ion in human hepatoma cells. Front Biosci(Elite Ed) 2010; 2: 1538 - 1547.

10. Chu M, Chu ZY, Wang DD. The extract of compound Radix Scutellariae on mRNA replication and IFN expression of influenza virus in mice. Zhong Yao Cai 2007; 30: 63-65.

11. Liao JF, Hung WY, Chen CF. Anxiolytic-like effects of baicalein and baicalin in the Vogel conflict test in mice. European journal of pharmacology 2003; 464: 141-146.

12. Ikemoto S, Sugimura K, Yoshida N, Yasumoto R, Wada S, Yamamoto K, Kishimoto T. Antitumor effects of Scutellariae Radix and its components baicalein, baicalin, and wogonin on bladder cancer cell lines. Urology 2000; 55: 951- 955.

13. Kumagai T, Müller CI, Desmond JC, Imai Y, Heber D, Koeffler HP. Scutellaria baicalensis, a herbal medicine: anti-proliferative and apoptotic activity against acute

lymphocytic leukemia, lymphoma and myeloma cell lines. Leukemia Research 2007; 31: 523 - 530.

14. Kim YH, Jeong DW, Paek IB, Ji HY, Kim YC, Sohn DH, Lee HS. Liquid chromatography with tandem mass spectrometry for the simultaneous determination of baicalein, baicalin, oroxylin A and wogonin in rat plasma. Journal of Chromatography B 2006; 844: 261- 267.

15. Hou YC, Lin SP, Tsai SY, Ko MH, Chang YC, Lee PD. Flavonoid pharmacokinetics and tissue distribution after repeated dosing of the roots of Scutellaria baicalensis in rats. Planta Medica 2011; 77: 455 - 460.

17. 16. Tong L, Wan MX, Zhang LH, Zhu YH, Sun H, Bi KS. Simultaneous determination of baicalin, wogonoside, baicalein, wogonin, oroxylin A and chrysin of Radix scutellariae extract in rat plasma by liquid chromatography tandem mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 2012; 70: 6 - 12Yao G, He ZY, Fang JN. Extraction and purifiction process of total flavonoid compounds in Scutellaria baicalensis. Chinese Traditional Patent Medicine 2006; 28: 1568 - 1571.

18. Zhao PG, Liu X. Study on Extraction and Purification of Rehmannia glutinosa Polysaccharide. China Pharmacy 2010; 21: 2146-2148.

19. Gong Y, Li YM, Chen M. Determination of total flavones in polygonam capitarum D.Don based on hydrochloric acid-Mg reaction colormietry method. Journal of Anhui Agriculture Science 2010; 38: 5636-5637.

20. Liu B, Shi RB, Zhou SR. Research on the Method for Quantitatively Determining Total Alkaloid in the Effective Fraction of the Extract of Kushen Decoction. Journal of Beijing

University of TCM 2004; 27: 76-79.

21. Ding LQ, Liu L, Xu DS. An throne-sulfuric acid colorimetric methods for determination of total sugar content in the extract of Rehmannia glutinosa Libosch. Shanghai Pharmaceutica 2008; 29: 368-370.

22. Feng ZQ, Han J, Xie ZY, Liao QF, Zhang L. Determination of plasma protein binding rate of multicomponent in scutellaria baicalensis Georgi. Chinese Pharmacological Bulletin 2012; 28: 289-293.

23. Zhang L, Xu L, Tan XJ, Liao QF, Guo W, Chen XH, Bi KS. Simultaneous Determination of Baicalin, Baicalein, Wogonin, Oxysophocarpine, Oxymatrine and Matrine in the Chinese Herbal Preparation of Sanwu-Huangqin-Tang by Ion-Paired HPLC. Chromatographia 2007; 66: 115-120.

24. Yim JS, Kim YS, Moon SK, Cho KH, Bae HS, Kim JJ, Park EK, Kim DH. Metabolic activities of ginsenoside Rb1, baicalin, glycyrrhizin and geniposide to their bioactive compounds by human intestinal microflora. Biological and Pharmaceutical Bulletin 2004; 27: 1580-1583.

25. Zuo F, Zhou ZM, Yan MZ, Liu ML, Xiong YL, Zhang Q, Song HY, Ye WH. Metabolism of constituents in Huangqin-Tang, a prescription in traditional Chinese medicine, by human intestinal flora. Biological and Pharmaceutical Bulletin 2002; 25: 558-563.

26. Akao T, Kawabata K, Yanagisawa E. Baicalin, the predominant flavone glucuronide of scutellariae radix, is absorbed from the rat gastrointestinal tract as the aglycone and restored to its original form. The Journal of Pharmacy and Pharmacology 2000; 52:

1563-1568.

27. Xing J, Chen XY, Zhong DF. Absorption and enterohepatic circulation of baicalin in rats. Life Sciences 2005; 78: 140-146.

Tables:

Table 1. Regression equations, correlation coefficients and linear ranges of baicalin, wogonoside, (baicalein), wogonin and oroxylin A in rat plasma and tissue samples (*n* =7)

Sample	constitues	linery equation($\times 10^{-3}$)	*r*	Range (ng/mL)
plasma	BL	y =0.30 x + 1.67	0.9944	5~2000
	WL	y =0.88 x - 0.91	0.9958	5~2000
	W	y =10.3 x - 4.23	0.9971	1~400
	OA	y =5.30 x – 3.24	0.9988	0.5~200
liver	BL	y = 0.56 x - 0.26	0.9984	5~5000
	WL	y = 1.51 x +1.46	0.9989	5~5000
	B	y = 0.95 x + 1.59	0.9962	5~5000
	W	y = 5.76 x + 3.82	0.9986	5~5000
	OA	y = 6.00 x + 3.0	0.9980	5~5000
stomatch	BL	y =0.63 x + 0.58	0.9990	5~5000
	WL	y =1.65 x + 3.37	0.9934	2.5~2500
	B	y = 1.32 x +5.32	0.9997	10~10000
	W	y = 5.58 x + 3.84	0.9954	5~5000
	OA	y =5.59 x + 15.3	0.9953	2.5~2500
kidney	BL	y = 0.61 x + 0.37	0.9981	5~5000
	WL	y = 1.51 x + 3.28	0.9982	5~5000
	B	y = 0.75 x +1.33	0.9975	2.5~2500
	W	y = 5.50 x +7.9	0.9948	2.5~2500
	OA	y =5.53 x + 8.65	0.9970	2.5~2500
Small intestine	BL	y =0.65 x + 0.36	0.9979	20~10000
	WL	y = 1.54 x +2.42	0.9937	10~10000
	B	y = 1.14 x +3.84	0.9965	5~2500
	W	y = 6.01 x + 6.8	0.9984	5~2500
	OA	y =5.93 x + 8.1	0.9961	5~2500

Table 2. Intra-day and inter-day variability and extraction recovery of baicalin, wogonoside, (baicalein), wogonin and oroxylin A in rat plasma and liver homogenate (*n* = *6*)

Compound	Sample	Spiked (ng/mL)	Intra-day		Inter-day		Recovery (%)
			RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)	
BL	Plasma	10	4.5	8.61	9.7	10.3	61.3 ± 6.6
		200	6.8	-0.3	4.5	-3.4	65.5± 5.2
		1600	0.4	1.65	9.1	1.9	60.1 ± 7.0
	Liver	10	5.2	6.00	6.9	-1.52	83.7 ± 2.4
		400	6.5	0.68	3.6	-3.46	81.2 ± 4.1
		4000	2.9	2.50	2.3	0.41	76.5 ± 2.3
WL	Plasma	10	2.7	9.4	5.4	3.87	89.6 ± 5.2
		200	6.5	2.72	3.8	-5.40	91.5 ± 6.6
		1600	3.9	2.06	10.8	0.81	81.6 ± 1.4
	Liver	10	7.6	-4.74	7.8	9.5	90.1 ± 6.0
		400	6.9	-3.84	6.6	-4.62	86.8 ± 5.1
		4000	5.9	6.9	2.3	1.61	94.6 ± 3.8
B	Liver	10	7.2	-11.0	9.5	-5.59	87.3 ± 7.1
		400	5.4	-6.04	7.6	7.08	83.9 ± 4.6
		4000	3.1	3.01	3.9	-0.80	98.3 ± 4.2
W	plasma	2	7.5	8.50	9.3	6.73	76.5 ± 3.3
		40	2.8	-4.21	1.5	4.45	81.5 ± 1.8
		320	3.4	4.72	6.4	-5.33	82.8 ± 6.8
	Liver	10	5.4	-6.51	7.9	2.50	81.3 ± 6.4
		400	5.6	8.06	7.3	-2.04	89.7 ± 5,3
		4000	2.8	3.75	3.2	2.50	88.4 ± 3.7
OA	Plasma	1	5.1	-9.00	4.2	8.72	80.65 ± 7.5
		20	9.4	2.57	3.3	5.50	87.21 ± 4.2
		160	7.5	3.98	5.2	4.03	74.53 ± 4.1
	Liver	10	7.2	5.50	9.2	-6.55	88.6 ± 8.1
		400	4.8	9.96	7.1	8.02	93.6 ± 5.3
		4000	3.2	4.38	4.4	1.31	90.2 ± 2.7

Table 3. Estimated pharmacokinetic parameters for baicalin, wogonoside, wogonin and oroxylin A in rat plasma (*n*=6) after oral admisistration of FSR and CCSHT

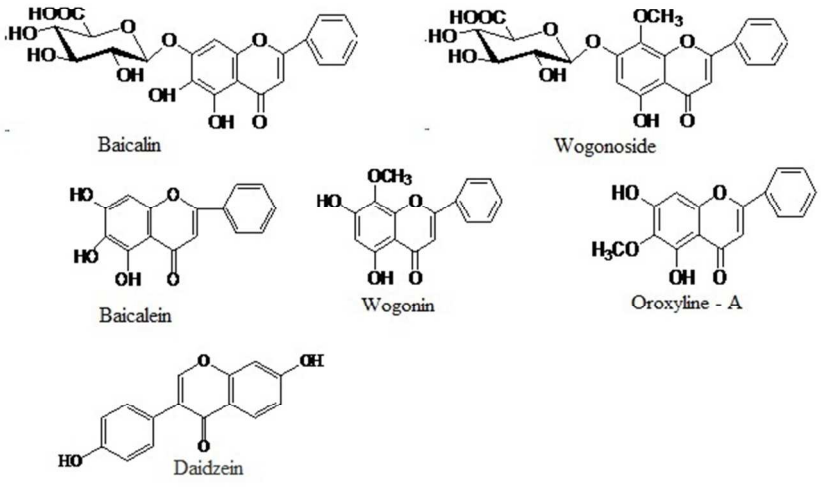
Pharmacokinetic parameters	BL		WL		W		OA	
	FSR	CCSHT	FSR	CCSHT	FSR	CCSHT	FSR	CCSHT
T_{max}(h)	0.18 ± 0.10	0.23 ± 0.05	0.23 ± 0.10	0.18 ± 0.05	0.33 ± 0.26	0.23 ± 0.10	0.25 ± 0.06	0.25 ± 0.05
C_{max}(ng/mL)	504 ± 50.3	709 ± 281*	228 ± 97	353 ± 161*	53.12± 34.73	74.48±19.15*	31.66 ± 5.83	57.52 ± 4.8*
$t_{1/2}$(h)	6.39 ± 3.09	4.44 ± 1.96*	4.75 ± 3.45	4.93 ± 2.98	7.84 ± 5.76	8.72 ± 4.75	7.85 ± 0.52	6.16 ± 2.38
$K_e(\times 10^2\ h^{-1})$	10.85±0.78	15.61±2.51*	14.59±3.65	14.06±4.33	8.84±3.22	7.95±2.15	8.83±0.33	11.25±2.15
$AUC_{0-\infty}$(ng/h/mL)	2650 ± 1870	3420± 1750*	1750 ± 203	2290 ± 1200*	309 ± 75	486 ± 349*	98 ± 35	317 ± 179**

**P<0.05; **P<0.01*

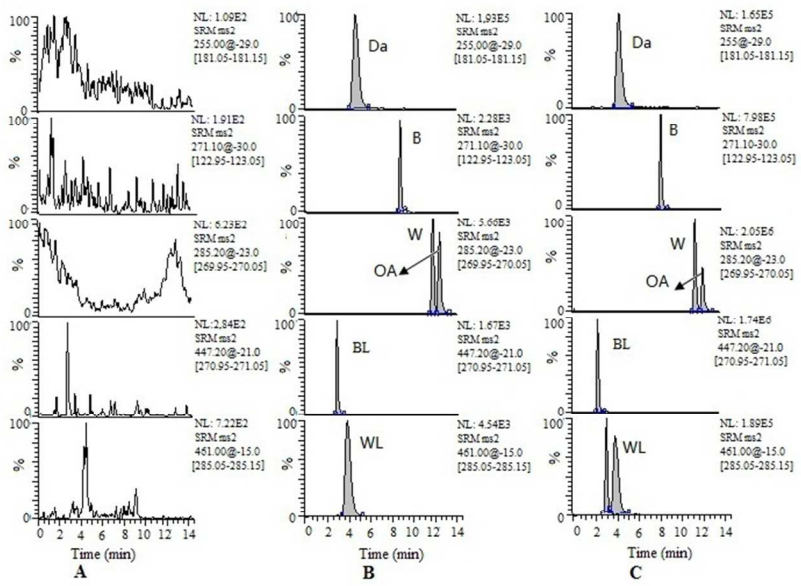
Table 4. Tissue distribution of of baicalin, wogonoside, baicalein, wogonin and oroxylin A in rats after oral administration of FSR and CCSHT at different time (*n* = 6, *mean* ± *SD*)

Tissues	Time (h)	BL（μg/g)		WL(μg/g)		B(μg/g)		W(μg/g)		OA(μg/g)	
		FSR	CCSHT	FSR	CCSHT	FSR	CCSHT	FSR	CCSHT	FSR	CCSHT
Liver	0.17	1.02±0.42	2.68±1.21	0.88 ±0.35	3.60±1.00	0.24±0.11	0.60±0.17	0.57±0.25	1.18±0.65	0.52±0.14	1.25±0.50
	3.0	0.29±0.08	1.74±0.41	0.49±0.17	1.12±0.36	0.06±0.03	0.22±0.05	0.21±0.03	0.41±0.16	0.19±0.04	0.35±0.13
	8.0	0.59±0.25	0.13±0.04	0.73±0.37	0.39±0.11	0.20±0.17	0.02±0.01	0.32±0.11	0.18±0.07	0.22±0.09	0.13±0.07
	24.0	0.08±0.03	0.29±0.10	0.02±0.01	0.34±0.08	0.02±0.01	0.03±0.02	0.03±0.02	0.14±0.04	0.03±0.01	0.31±0.33
Stomach	0.17	93.6±11.3	86.1±18.0	5.09±1.26	4.96±1.17	45.4±15.8	44.8±7.70	18.1±4.9	18.0±3.38	11.7±5.61	8.78±1.78
	3.0	23.9±4.67	47.7±15.4	8.43±1.37	2.66±0.89	12.2±1.94	19.6±3.6	4.62±1.20	8.20±3.20	1.81±0.18	3.03±1.04
	8.0	7.64±2.32	11.6±0.74	2.58±1.77	0.76±0.21	2.53±0.03	11.5±1.53	1.02±0.20	3.33±0.46	1.34±0.82	1.31±0.05
	24.0	0.21±0.04	0.58±0.13	0.04±0.03	0.46±0.17	0.53±0.13	0.38±0.12	0.04±0.00	0.08±0.02	0.36±0.11	0.44±0.17
Kidney	0.17	2.18±0.41	13.2 ± 5.7	1.30±0.36	4.06±1.13	0.10±0.00	1.63±0.30	0.21±0.06	1.64±0.36	0.12±0.04	1.17±0.47
	3.0	0.91±0.21	0.85±0.18	0.95±0.63	0.87±0.45	0.07±0.02	0.05±0.02	0.10±0.03	0.09±0.03	0.10±0.15	0.09±0.04
	8.0	1.73±0.42	0.25±0.06	1.19±0.20	0.46±0.17	0.04±0.02	0.02±0.01	0.05±0.01	0.03±0.01	0.06±0.02	0.03±0.01
	24.0	0.09±0.03	0.24±0.04	0.02±0.01	0.30±0.06	0.01±0.00	0.01±0.00	—	0.01±0.00	—	0.02±0.00
Small intestine	0.17	21.3±5.24	4.25±0.10	3.21±0.96	3.25±0.92	8.00±1.69	10.65±2.66	2.57±0.75	2.03±0.43	1.47±0.41	1.04±0.21
	3.0	7.21±1.65	12.0±2.53	5.79±1.41	12.2±4.70	2.21±0.58	2.81±0.74	0.59±0.17	1.73±0.34	0.48±0.12	0.75±0.15
	8.0	6.92±2.43	10.7±6.33	4.88±1.80	12.1±1.83	3.48±0.72	1.74±0.31	0.90±0.39	1.63±0.37	0.56±0.16	1.00±0.17
	24.0	0.31±0.09	2.91±1.26	0.10±0.04	1.07±0.44	0.11±0.04	0.58±0.17	0.03±0.01	0.36±0.08	0.06±0.19	0.47±0.11

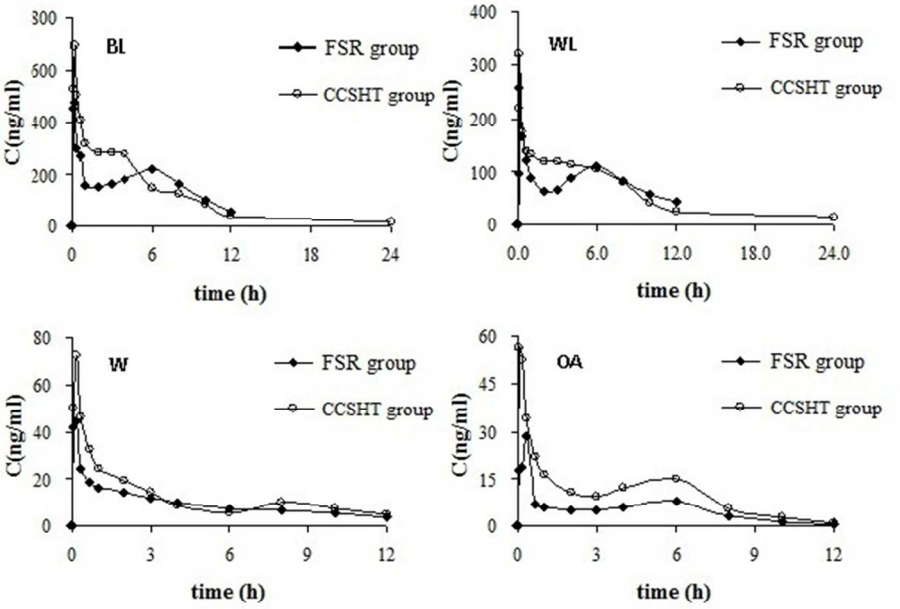
—not detectable.



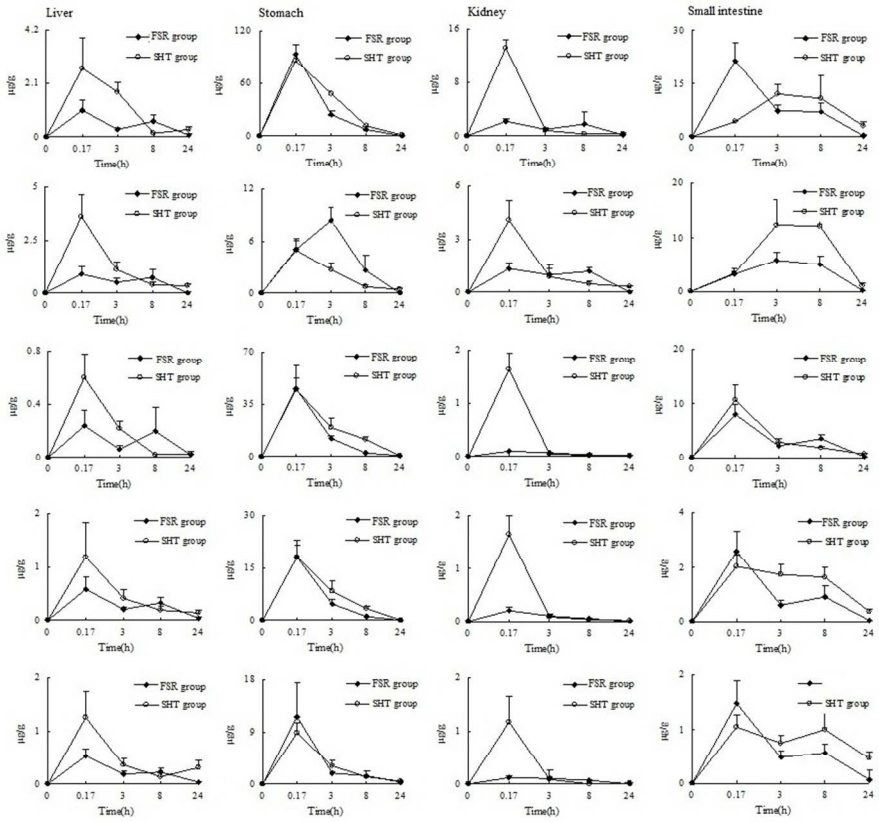
The structure of baicalin, wogonoside, baicalein, wogonin, oroxylin A and daidzein
199x137mm (96 x 96 DPI)



The typical chromatograms of the blank liver tissue homogenate sample (A), the blank liver tissue homogenate sample spiked with analytes at LLOQ with IS (B), and the liver tissue homogenate sample at 3 h after administration of CCSHT to the rats (C)
228x154mm (96 x 96 DPI)



Mean ± SD plasma concentration-time profile for baicalin, wogonoside, wogonin and oroxylin A in rat plasma after oral administration of FSR and CCSHT
177x135mm (96 x 96 DPI)



Tissue distribution of baicalin, wogonoside, baicalein, wogonin and oroxylin A in liver, stomach, kidney and small intestine at 0.17, 3, 8 and 24 h after a single dosage administration of FSR and CCSHT
272x239mm (96 x 96 DPI)