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Full Length Research Paper

Comparison of the tissue distributions of flavonoids after oral administration of pure baicalin, *Radix scutellariae* and *Scutellariae-Paeoniae* couple extracts to rats

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Baicalin, baicalein, wogonoside and wogonin are the major flavones of *Radix scutellariae* with various beneficial biological activities. The purpose of this study was to investigate the tissue distribution and compare the differences of these flavones after orally administrating the pure baicalin, *R. scutellariae* and *Scutellariae-Paeoniae* couple extracts with the same dose of 200 mg/kg baicalin. Tissue concentrations of baicalin, baicalein, wogonoside and wogonin were determined by high performance liquid chromatography (HPLC). Organ samples including the heart, liver, spleen, lung, kidney, large intestine, small intestine and stomach were collected at three specific times (15 min, 8 h, 24 h). Unpaired Student's t-test was used for statistical comparison. The results indicated that the baicalin, wogonoside, baicalein and wogonin all occurred in the investigated tissues after orally administrating pure or compound to the rats. Considerable concentrations of baicalein and wogonin could be detected in the investigated tissues, but were not found in rat serum. The contents of glycosides were higher than that of aglycones in small intestine, stomach, lung, heart and kidney; conversely in liver, large intestine and spleen. Pure baicalin was absorpted faster than *R. scutellariae* and *Scutellariae-Paeoniae* couple extracts. The concentrations of baicalin, baicalein, wogonoside and wogonin in the investigated tissues after orally administrating *Scutellariae-Paeoniae* couple extract (24 h) were higher ($P < 0.05$) than after orally administrating the pure baicalin and *R. scutellariae* extract.

Key words: Baicalin, wogonoside, baicalein, wogoin, distribution, *Scutellariae-Paeoniae.*

INTRODUCTION

Herb couples have special clinical significance in traditional Chinese medicine (TCM) and are much simpler than other complex formulas without altering their basic therapeutic features (Feng et al., 2010). The combination of *Radix scutellariae* and *Radix paeoniae Alba*, as a herb couple, is now commonly used clinically to cure viral diarrhea, bacillary dysentery, general fever, and so on, in which *R. scutellariae* (RS), has exhibited various bioactivities such as antiinflammation (Kim et al., 2009; Yoon et al., 2009), antihepatitis (Tseng et al., 2010), antitumor (Ikemoto et al., 2000; Kumagai et al., 2007) and antivirus effects (Wu et al., 2001; Ma et al., 2002). More recently, RS has been reported to inhibit H1N1 virus replication in mice (Chu et al., 2007). The major flavones in RS include baicalin, baicalein, wogonoside, and wogonin (structures shown in Figure 1), which presented anti-inflammatory (Yang et al., 2009), antioxidative (Hsieh et al., 2007), antiallergic (Shao et al., 2002), antiviral (Huang et al., 2006), and anticarcinogenic activities

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Abbreviations: HPLC, High performance liquid chromatography; **TCM,** traditional Chinese medicine; **RS,** *Radix scutellariae*; **IS,** internal standard; **HCl,** hydrochloric acid; **FDA,** food and drug administration; **QC,** quality control; **RE,** relative error; **RSD,** relative standard deviation; **LLOQ,** lower limit of quantification; **GUS,** glucuronidase; **LC-MS-MS**, liquid chromatography-mass spectrometry.

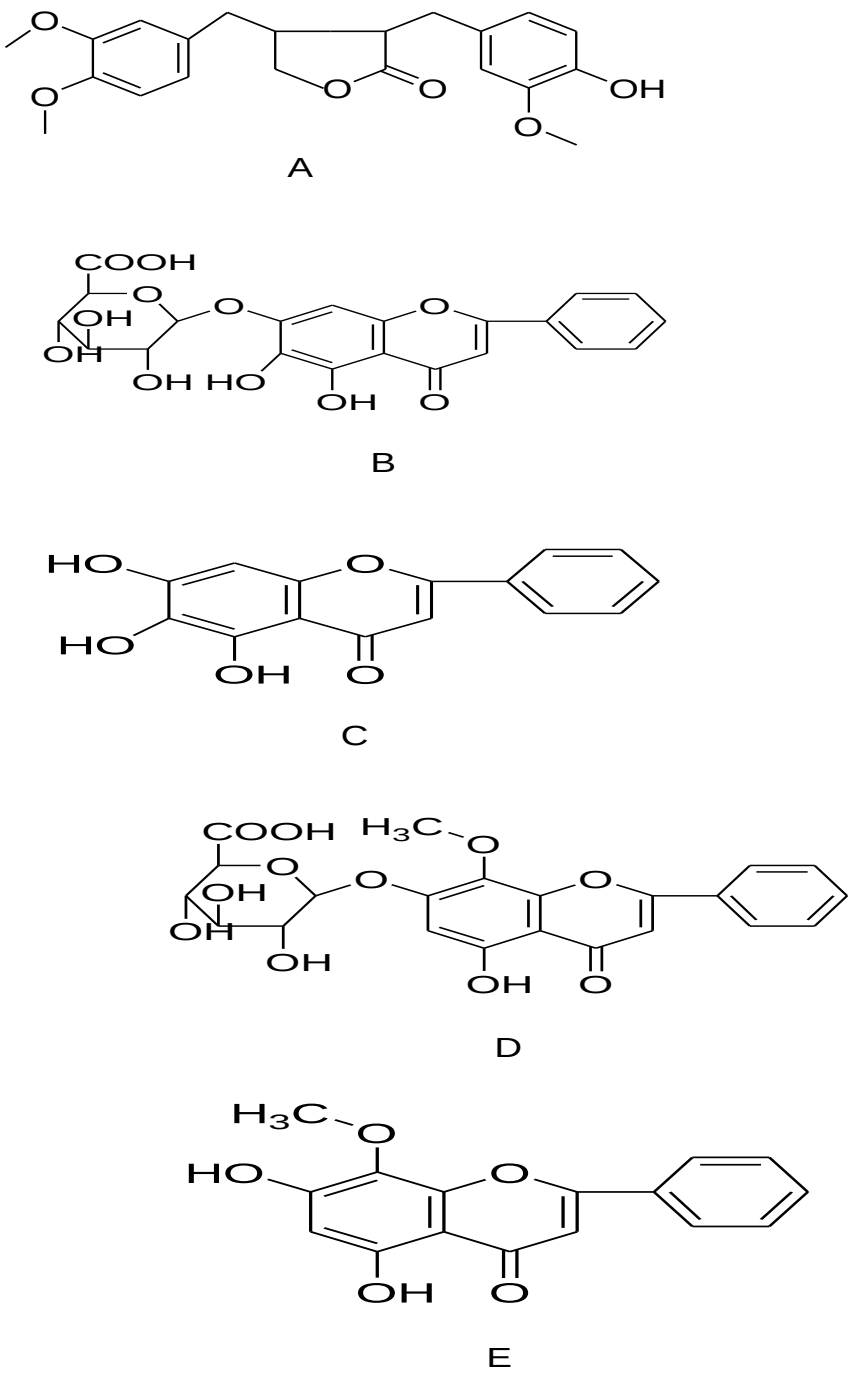


Figure 1. Chemical structures of arctigenin (A), baicalin (B), baicalein (C), wogonoside (D) and wogonin (E).

(Kimata et al., 2000; Huang et al., 2000).

Pharmacokinetic studies of herb-herb interactions had proved useful in elucidating the rationale of TCM compatibility and in guiding the clinical application of TCM prescriptions. Many researches have been done to investigate the pharmacokinetic profiles of baicalin and wogonoside in blood, brain, and eyes of rats and rabbits using high performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC–MS–MS), ultra-performance liquid chromatography mass spectrometry (UPLC–MS–MS) or ultra high performance liquid chromatography (UHPLC-ed) (Lu et al., 2007; Kim et al., 2007; Ju et al., 2007; Kotani et al., 2006; Ye et al., 2010; Wang et al., 2009; Young et al., 2006; Zhu et al.,

2010; Zeng et al., 2010). The investigations of flavones in RS decoction tissue distribution have been reported recently (Hou et al., 2010). Wen et al. have studied the tissue distribution of wogonin and its metabolite in tumor-bearing nude mice (Llu et al., 2010). However, there is few data on the tissue distribution of baicalin, wogonoside, baicalein and wogonin following oral administration of pure baicalin, RS and *Scutellariae-Paeoniae* couple extracts to rats. The knowledge of the difference in tissue distribution of baicalin, wogonoside, baicalein and wogonin among pure baicalin, RS and *Scutellariae-Paeoniae* couple extracts could be helpful in designing rational dosage regimens. The aim of this study was to develop a simple, rapid and reliable HPLC assay for the simultaneous determination of baicalin, baicalein, wogonoside and wogonin in rat tissues and compare the tissue distribution profiles of these flavones after orally administrating the pure baicalin, RS and *Scutellariae Paeoniae* couple extracts.

MATERIALS AND METHODS

Animals and tissue collection

Male Sprague–Dawley rats, weighing 250 to 280 g, were supplied by the Experimental Animal Department of Dalian University (Dalian, China). Animal welfare and experimental procedures were strictly in accordance with the guide for the care and use of laboratory animals (US National Research Council, 1996) and the related ethics regulations of Liaoning University of TCM. The rats were maintained in an air-conditioned animal quarter at a temperature of 22 ± 2°C and a relative humidity of 50 ± 10%. Water and food (laboratory rodent chow, Dalian, China) were allowed ad libitum. The animals were acclimatized to the facilities for 5 days, and then fasted with free access to water for 24 h prior to each experiment.

Male Sprague–Dawley rats were randomly divided into three groups: the pure baicalin group, RS group and *Scutellariae-Paeoniae* couple group. Three groups of rats were, respectively administrated an oral dose of 200 mg/kg baicalin (in the form of pure compound or co-administrated mixture), 0.21 g/kg pure baicalin, 0.46 g/kg RS extract and 1.16 g/kg *Scutellariae-Paeoniae* couple extract, all of which were suspended in water and homogenized by utilizing ultrasonic technology just before each experiment. At predefined times (15 min, 8 h and 24 h post-dose; n = 5 each time) they were sacrificed. The heart, liver, spleen, lung, kidney, large intestine, small intestine and stomach were immediately harvested, rinsed in saline solution to remove blood or other contents, blotted on filter paper and then weighed. After homogenization in four times the volume of 0.9% NaCl, tissue samples were stored at −20°C until analysis.

Chemicals and reagents

RS and *R. paeoniae* Alba were purchased from Bozhou medicine Company (Anhui, China) and authenticated by Prof. Li Feng from the College of Pharmacy, University of TCM (Liaoning, China). The voucher specimen has been deposited in Liaoning University of TCM (No. 20081205, No. 20081206). The pure baicalin (95.5%) was provided by Prof. Zhang Zhen-Qiu and Prof. Li feng (Department of Chemistry in our institute).

Baicalin, wogonin (purity > 98%, respectively) reference standards were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Internal standard (IS) arctigenin was supported by Prof. Dou De-Qiang (Department of Phytochemistry in our institute). Baicalein (purity >98%) was provided by Tianjin Yifang Science and Technology Co. Ltd and wogonoside (purity > 98%) was obtained from Shanghai Ronghe Science and Techmology Co. Ltd.

Acetonitrile used for HPLC was chromatographic grade (Fisher Company, Inc., USA) and filtered through 0.45 µm organic membrane prior to use. Deionized water was prepared in a Mill-Q academic water purification system (Millipore, Bedford, MA, USA) and used throughout the study. All the other reagents were analytical grade and were provided by Kermel Chemical Co., (Tianjin, China).

Apparatus and chromatographic conditions

Chromatography was performed on an Agilent 1100 series HPLC system equipped with pump (Agilent model G1314A VWD). Chromatographic separation was performed on an Agilent reverse-phase Eclipsel XDB-C_{18} column (250 mm × 4.6 mm, 5 µm particle size). The mobile phase was composed of A (acetonitrile):B (water–phosphoric acid 100:0.1, v/v) with gradient elution (0 min 16:84; 10 min 22:78; 15 min 25:75; 25 min 34:64; 30 min 42:58) at a flow rate of 1.0 ml/min with linear isocratic elution. The detector operated at 278 nm and the operating temperature was maintained at 30°C.

Preparation of RS and *Scutellariae-Paeoniae* couple extracts

RS and *R. paeoniae* Alba were mixed in the ratio of 1.5:1 and the total weight was 200 g. The mixture was decocted twice by refluxing with 70% ethanol (1:10 and then 1:8 w/v) for 1 h, and the solution obtained was concentrated to give an extract of 60.5 g. RS 200 g was treated as well as *Scutellariae-Paeoniae* couple, the extract was 43.3 g. The dried powder was stored at 4°C before use. To calculate the administration dose, the concentrations of baicalin, wogonoside, baicalein and wogonin in the extracts and pure baicalin were quantitatively determined. The extract powder (0.10 g) was ultrasonicated with 100 ml 60% methanol for 30 min. The solution was filtered through 0.45 µm organic membrane before HPLC analysis. The contents of baicalin, baicalein, wogonoside and wogonin in *Scutellariae-Paeoniae* couple extract were determined to be 17.3, 7.6, 2.4 and 1.7% and 43.6, 19.1, 6.2 and 4.5% in RS extract, respectively. The content of baicalin was 95.5% in pure baicalin.

Biosample preparation

Tissue homogenate (100 µl) was mixed with 15 µl internal standard arctigenin (32.0 µg/ml in methanol) and 10 µl hydrochloric acid (HCl) solution (0.1 mol/L), and then spiked with methanol 75 µl and acetonitrile 100 µl by vortex mixing for 5 min. The mixture was centrifuged at 10000 rpm for 15 min. An aliquot of 50 µl of the supernatant was injected into the HPLC system.

Preparation of standard solutions and quality control samples

The method was validated according to the currently accepted US food and drug administration (FDA) bioanalytical method validation guidance.

For the calibrator preparation, 800 µl of blank tissue homogenate was mixed with various concentrations of standard solutions of

baicalin, wogonoside, baicalein and wogonin. The baicalin, wogonoside, baicalein and wogonin reference standards and IS were accurately weighed and dissolved in methanol, and then diluted to appropriate concentration ranges for the establishment of calibration curves in rat tissues. The concentrations of stock solutions of reference substance and IS were baicalin (126.4 μg/ml), wogonoside (36.8 μg/ml), baicalein (68.0 μg/ml), wogonin (7.9 μg/ml)and IS 32.0 μg/ml, respectively. These solutions were stored at 4°C before analysis. The calibration graphs were plotted by linear regression of the peak area ratios (baicalin, wogonoside, baicalein and wogonin to internal standard) against concentrations of baicalin, wogonoside, baicalein and wogonin. Blank tissue samples, no matter with IS or without IS, were assayed to confirm the absence of interference.

The extraction recovery and matrix effect at three quality control (QC) concentrations were assayed in sets of six replicates. Extraction recovery was calculated by comparing the peak area of analytes added to tissue homogenate from untreated tissue homogenate and then extracted, with analytes added into preextracted tissue homogenate from untreated rats. The matrix effect was evaluated by comparing the peak area of analytes added into preextracted tissue homogenate from untreated rats, with analytes dissolved in matrix component-free reconstitution solvent.

QC samples were used for the study of intra-day and inter-day accuracy and precision. Five replicates analyzed in each of the three analytical runs. The accuracy was expressed by the relative error (RE), and the precision was evaluated by the relative standard deviation (RSD).

Stability was prepared in the same way as described previously and was prepared from blank tissue homogenate at three concentrations. The stabilities of baicalin, wogonoside, baicalein and wogonin were evaluated under a variety of storage conditions likely to be encountered during sample storage and the analytical process by analyzing five replicates of QC samples. The freeze–thaw stability was determined after one freeze and thaw cycle. The QC samples were stored at −20°C for 24 h and thawed at room temperature.

Data analysis

The distribution profiles of baicalin, wogonoside, baicalein and wogonin in the three groups of tissues were assessed after s15 min, 8 h and 24 h. The concentrations of baicalin, wogonoside, baicalein and wogonin in the specific tissue were calculated by the regression equations. The concentrations were used to evaluate the extent of tissue distribution. An unpaired Student's t test was used for comparison.

RESULTS

HPLC method validation

The selectivity of the method was evaluated by analyzing blank rat small intestine samples prior to administration. The chromatograms were free of interfering peaks at the retention times of IS (34.503 min), baicalin (20.527 min), wogonoside (25.840 min), baicalein (31.880 min) and wogonin (40.823 min). Figure 2 shows the representative chromatograms of blank rat small intestine sample (A), blank rat small intestine sample spiked with baicalin, wogonoside, baicalein and wogonin (B), blank rat small intestine sample spiked with IS (C), small intestine samples 15 min after oral administration of *Scutellariae-Paeoniae* couple extracts (D). No interfering peaks were observed at the elution times of baicalin, wogonoside, baicalein and wogonin and the IS.

The calibration ranges of baicalin (0.1215 to 126.4 μg/g), baicalein (0.2875 to 36.8 μg/g), wogonoside (0.1328 to 68.0 μg/g) and wogonin (0.1234 to 7.9 μg/g) in various tissue homogenates had good linearities ($r > 0.9920$). Based on a signal-to-noise ratio (S/N) = 6, the lower limit of quantification (LLOQ) for baicalin, wogonoside, baicalein and wogonin were 0.1215, 0.2875, 0.1328, 0.1234 μg/g, respectively.

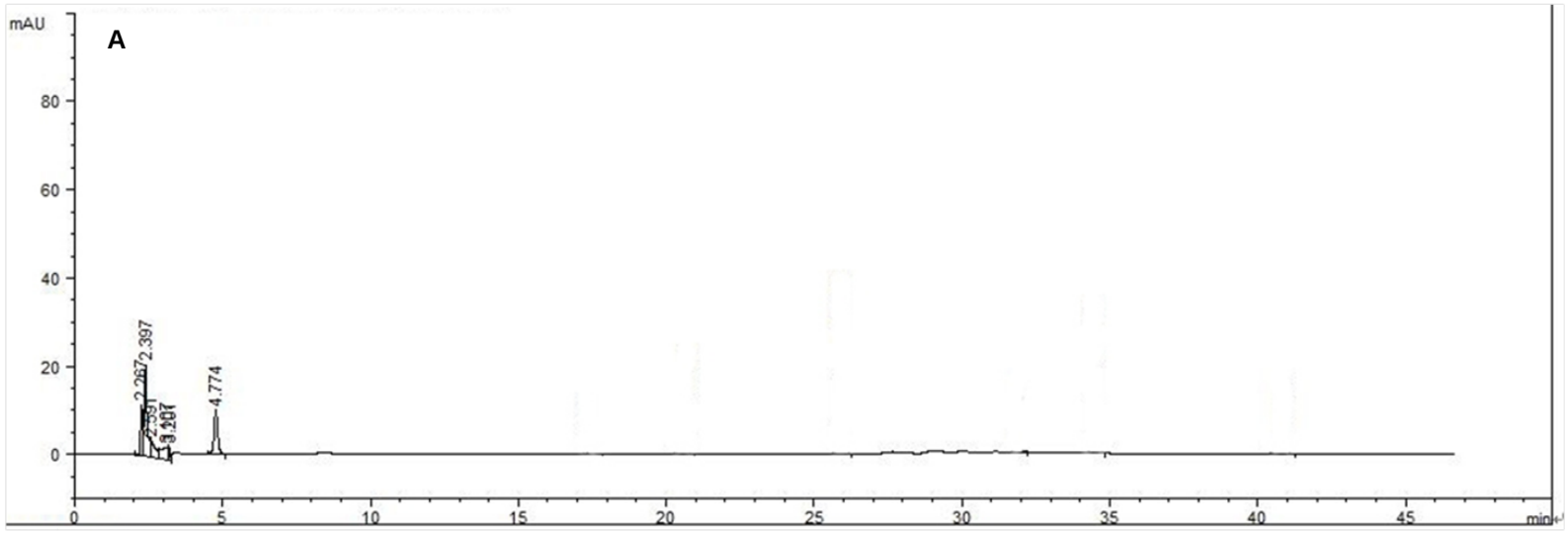
The average precisions, expressed as (%, RSD), were for baicalin 1.6 to 5.9%, wogonoside 0.8 to 5.2%, baicalein 2.8 to 6.1% and wogonin 1.6 to 6.3%, respectively. In this study, precisions were within acceptable limits at every QC concentration. The recoveries of the method were 81.4 to 91.0% for baicalin, 84.3 to 103.0% for wogonoside, 76.7 to 96.9% for baicalein and 79.7 to 99.3% for wogonin; the results obtained from analysis of the three QC solutions were within acceptable limits. Studies of the stability of the analytes in biological samples showed that under all the sample-analysis conditions investigated results were consistently within the given limits compared with those for freshly prepared QC samples.

Tissue distribution study

Concentrations and distribution profiles of baicalin wogonoside, baicalein and wogonin in rat main tissues after oral administration of pure baicalin, RS and *Scutellariae-Paeoniae* couple extracts with the same dose of 200 mg/kg baicalin were shown in Tables 1 to 4. It was observed that the liver, stomach, large intestine and small intestine were the main distribution tissues for baicalin, wogonoside, baicalein and wogonin. And these flavones could be detected in the investigated tissues at 15 min, but most tissue concentrations after orally administrating the pure baicalin were below the detection limit after 24 h. The relative concentrations of glycosides were higher than those of aglycones in small intestine, stomach, lung, heart and kidney; whereas the concentrations of aglycones were higher than glycosides ($P<0.05$) in liver, large intestine and spleen. After orally administrating *Scutellariae-Paeoniae* couple extract (24 h), the concentrations of baicalin, wogonoside, baicalein and wogonin were higher ($p<0.05$) than that after orally administrating the pure baicalin and RS extract; but were smaller than that after orally administrating the pure baicalin (15 min). The concentrations of these flavones in most investigated tissues were almost equal after orally administrating RS extract and *Scutellariae-Paeoniae* couple (8 h).

DISCUSSION

The quantitation methods of baicalin, wogonoside,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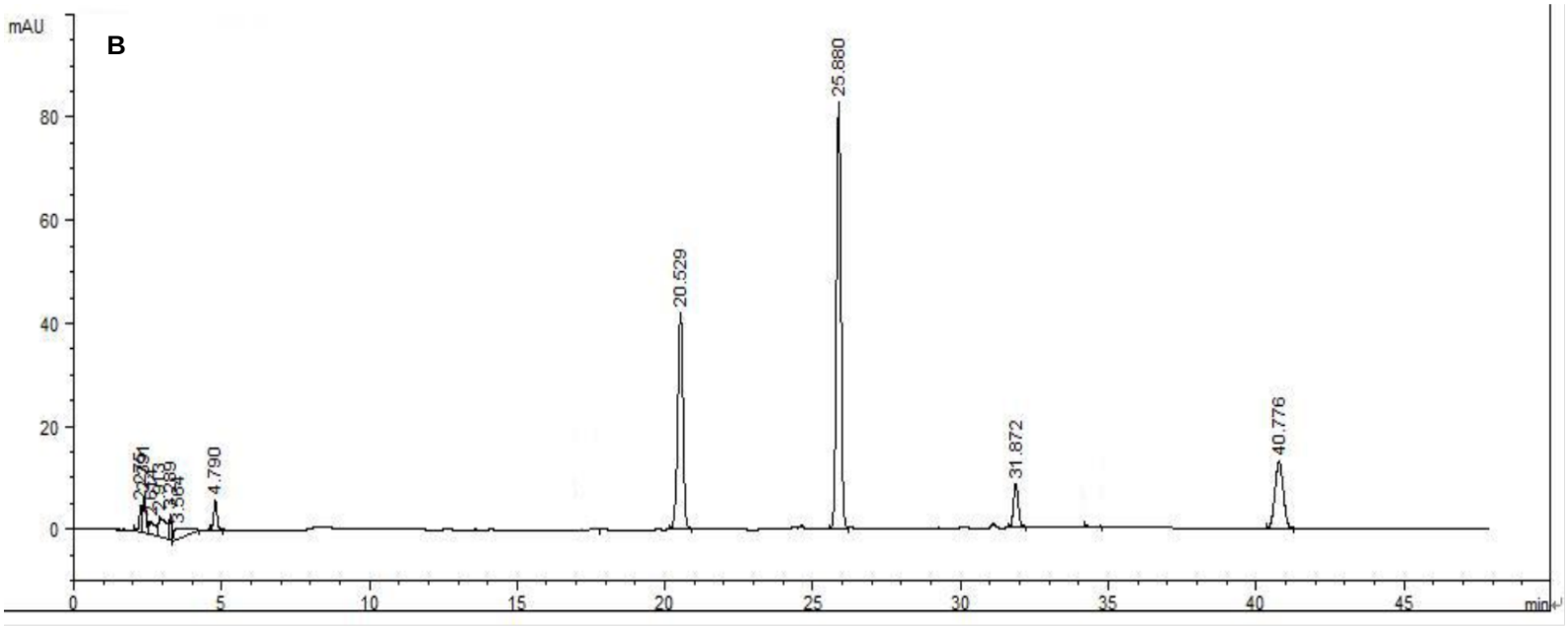
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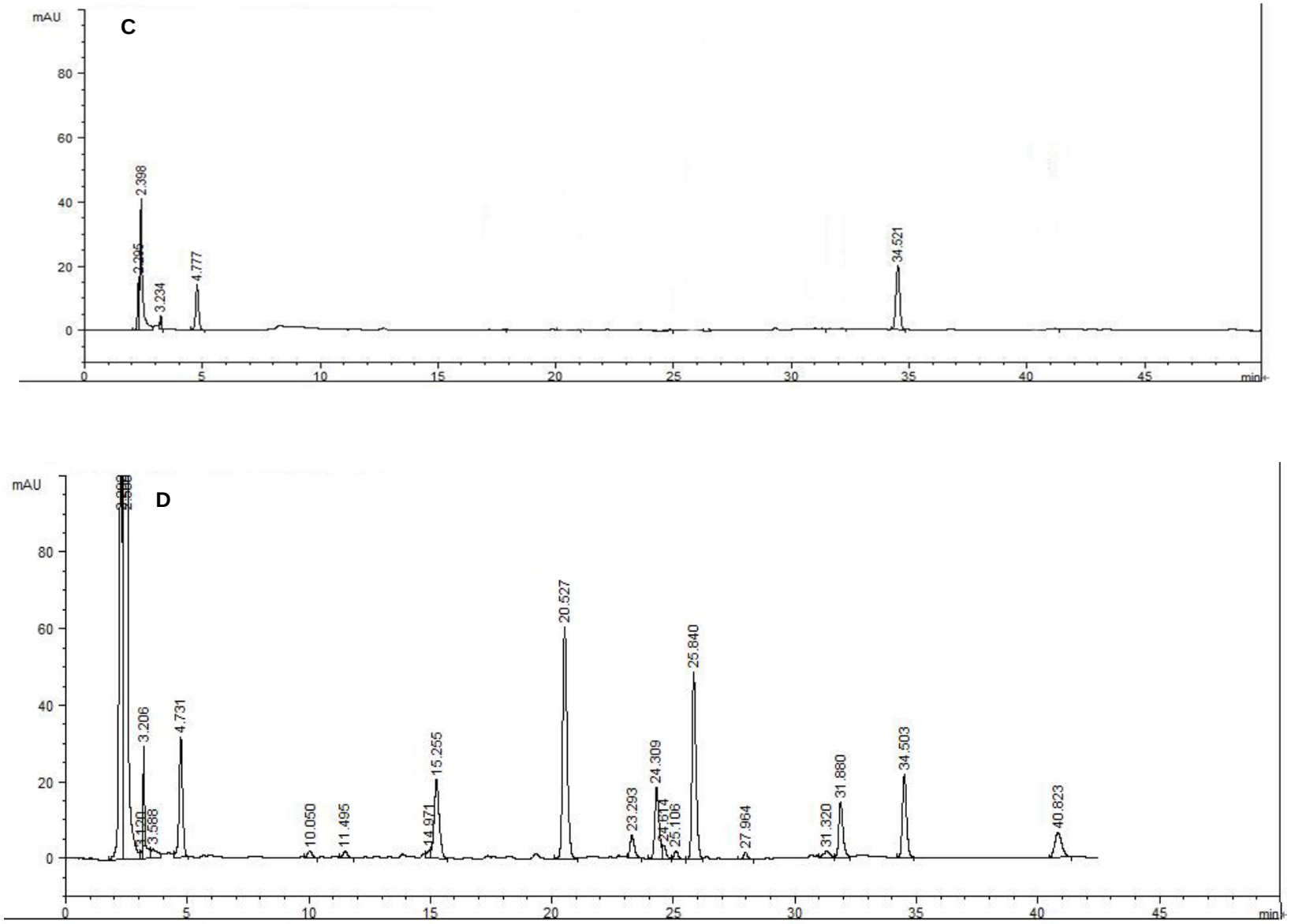

Figure 2. Chromatograms of rat plasma samples. (A) Blank rat small intestine sample; (B) blank rat small intestine sample spiked with baicalin (t = 20.527 min), wogonoside (t = 25.840 min), baicalein (t = 31.880 min) and wogonin (t = 40.823 min); (C) blank rat small intestine sample spiked with I.S., (t = 34.503); (D) small intestine samples 15 min after oral administration of *Scutellariae-Paeoniae* couple extracts to rat.

Table 1. Concentrations mean of baicalin in rat tissues at predefined times (15 min, 8 h and 24 h post-dose; n = 5 per time point, μg/g).

Tissue	Heart	Liver	Spleen	Lung	Kidney	Large intestine	Small intestine	Stomach
Pure baicalin (15 min)	15.52	2.48	1.22	15.15	5.71	3.25	12.07	11.28
Radix scutellariae (15 min)	0.98	0.71	0.28	2.39	1.83	0.35	5.36	4.95
Scutellariae-Paeoniae (15 min)	4.53	1.27	0.44	10.62	6.77	0.56	1.53	6.48
Pure baicalin (8 h)	0.22	ND	ND	5.19	ND	0.83	5.41	5.56
Radix scutellariae (8 h)	ND	ND	ND	0.56	0.87	2.76	15.57	11.65
Scutellariae-Paeoniae (8 h)	14.37	ND	ND	0.63	0.85	3.11	16.11	12.88
Pure baicalin (24 h)	ND	ND	ND	ND	ND	ND	ND	ND
Radix scutellariae (24 h)	ND	ND	ND	ND	ND	0.96	1.29	1.95
Scutellariae-Paeoniae (24 h)	1.08	ND	ND	0.25	0.56	1.22	8.71	3.52

Table 2. Mean concentrations of baicalein in rat tissues at predefined times (15 min, 8 h and 24 h post-dose; n = 5 per time point, μg/g).

Tissues	Heart	Liver	Spleen	Lung	Kidney	Large intestine	Small intestine	Stomach
Pure baicalin (15 min)	1.08	8.34	3.87	7.58	0.71	7.76	6.83	6.57
Radix scutellariae (15 min)	ND	1.45	0.96	2.41	0.52	0.86	1.67	9.63
Scutellariae-Paeoniae (15 min)	0.69	3.83	0.55	5.51	0.34	1.74	3.26	3.34
pure baicalin (8 h)	ND	ND	ND	0.29	ND	3.51	2.82	1.61
Radix scutellariae (8 h)	ND	ND	ND	0.57	ND	7.74	5.55	6.71
Scutellariae-Paeoniae (8 h)	1.51	8.73	0.93	ND	2.32	7.45	6.54	7.44
Pure baicalin (24 h)	ND	ND	ND	ND	ND	0.68	ND	ND
Radix scutellariae (24 h)	ND	ND	ND	ND	ND	2.36	0.86	1.67
Scutellariae-Paeoniae (24 h)	0.45	1.24	0.28	0.61	0.31	4.62	1.16	3.27

Table 3. Mean concentrations of wogonoside in rat tissues at predefined times (15 min, 8 h and 24 h post-dose; n = 5 per time point, μg/g).

Tissues	Heart	Liver	Spleen	Lung	Kidney	Large intestine	Small intestine	Stomach
Pure baicalin (15 min)	0.51	ND	ND	4.87	2.13	ND	1.26	0.52
Radix scutellariae (15 min)	0.59	ND	ND	0.54	1.95	0.62	3.51	ND
Scutellariae-Paeoniae (15 min)	0.44	0.34	ND	3.45	2.31	0.26	1.96	1.11
Pure baicalin (8 h)	ND	ND	ND	ND	ND	0.24	0.74	0.25
Radix scutellariae (8 h)	ND	0.44	ND	ND	2.91	ND	9.61	3.49
Scutellariae-Paeoniae (8 h)	1.52	ND	ND	0.56	3.71	0.95	10.61	3.82
pure baicalin (24 h)	ND	ND	ND	ND	ND	ND	ND	ND
Radix scutellariae (24 h)	ND	ND	ND	ND	ND	ND	1.78	ND
Scutellariae-Paeoniae (24 h)	0.84	ND	ND	0.24	0.52	0.53	2.55	1.84

baicalein and wogonin in various organ homogenates were established and validated. The results indicated that the considerable concentrations of baicalein and wogonin could be detected in the investigated tissues when the rats were sacrificed at 15 min. This time was the serum peak of baicalin, wogonoside based on our own pharmacokinetic experiments, but baicalein and wogonin were not found in serum (15 min). The emergence of considerable concentrations of the free forms of baicalein and wogonin in the heart, liver, spleen, lung, kidney, large intestine, small intestine and stomach may have been biotransformed from their glucuronides (baicalin, wogonoside) in the blood circulation. (O'Leary et al., 2001; Pasqualini and Chetrite, 2005; Reed et al., 2005).

Zhu et al. reported that after oral administration of *Xiaochaihu Tang* and RS extract, wogonoside, could be

Table 4. Mean concentrations of wogonin in rat tissues at predefined times (15 min, 8 h and 24 h post-dose; n = 5 per time point, μg/g).

Tissues	Heart	Liver	Spleen	Lung	Kidney	Large intestine	Small intestine	Stomach
Pure baicalin (15 min)	ND	0.29	ND	0.38	0.23	ND	0.22	0.31
Radix scutellariae (15 min)	ND	0.74	0.27	0.44	0.42	0.97	1.53	1.14
Scutellariae-Paeoniae (15 min)	0.24	0.26	0.23	0.76	0.27	0.34	0.86	0.81
Pure baicalin (8 h)	ND	ND	ND	ND	ND	0.84	ND	ND
Radix scutellariae (8 h)	ND	0.45	ND	ND	ND	1.74	1.05	1.07
Scutellariae-Paeoniae (8 h)	ND	0.99	ND	ND	0.76	1.42	1.48	1.34
Pure baicalin (24 h)	ND	ND	ND	ND	ND	ND	ND	ND
Radix scutellariae (24 h)	ND	ND	ND	ND	ND	0.68	0.38	ND
Scutellariae-Paeoniae (24 h)	0.26	0.39	0.14	0.27	0.22	0.87	0.71	0.32

ND, concentration below detection limit.

detected in serum, which was thought as a methylated product of baicalin. As it is known that wogonoside existed in *X. Tang* and RS extract, so we could not be sure that wogonoside was transformed from baiclain. In this paper, we found that wogonoside, baicalein and wogonin occured in the investigated tissues after oral administration of pure baicalin (purity >95.5%). This further proved methylation generally existed during the drug metabolism *in vivo* and wogonoside was a methylated product of baicalin. And then baicalin and wogonoside were partly transformed into baicalein and wogonin by glucuronidase (GUS) and distributed in the rat tissues.

After oral administration of pure baicalin, RS extract or *Scutellariae- Paeoniae* couple extract, the concentrations of baicalein and wogonin in the liver, large intestine and spleen were higher ($P<0.05$) than baicalin, wogonoside; but conversely in small intestine, stomach, lung, heart and kidney. This discrepancy may arise from the different catalytic capability of deconjugation enzymes in organs on the hydrolysis of various flavones glucuronides/ sulfates. The deconjugation reaction was higher and reconjugation was lower in the liver, large intestine and spleen when compared with the small intestine, stomach, lung, heart and kidney. All these findings led us to suggest that the metabolic fates of flavones in organs were substrate-dependent.

Tables 1 to 4 showed that the pure baicalin occurred and distributed more rapidly in the tissues than the RS extract and *Scutellariae-Paeoniae* couple extract. The concentrations of the flavones in the specific tissues after oral administration of *Scutellariae-Paeoniae* couple extract were higher than ($P<0.05$) those of pure baicalin, RS extract at 24 h, suggesting that pure baicalin could expedite the distribution of the flavones to the various tissues, and *Scutellariae-Paeoniae* couple extract could postpone the tissue distribution of the flavones. In a word, most constituents in the *Scutellariae-Paeoniae* couple extract delayed absorption and elimination, produced a longer residence time in the body than they did in the monomer and single herb extract. Therefore, the constituents in the compound prescription were more efficient and durable, making them more promising in exerting pharmacological effects *in vivo*.

Conclusion

The developed HPLC method was simple, sensitive, and appropriate for the simultaneous distribution determination of baicalin, wogonoside, baicalein and wogonin in rat tissues. The results indicated that wogonoside, a potential methylated metabolite of baicalin after orally administrating the pure baicalin, was found in the rat tissues, and there were significant differences among the tissue distribution profiles of baicalin, wogonoside, baicalein and wogonin after oral administration of pure baicalin, RS and *Scutellariae-Paeoniae* couple extracts, indicating that competition or inhibition between the chemical constituents in *Scutellariae-Paeoniae* couple extract could delay retention time of baicalin, wogonoside, baicalein and wogonin in the rat tissues.

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