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Simultaneous determination of baicalin, baicalein, wogonin, berberine, palmatine and jatrorrhizine in rat plasma by liquid chromatography–tandem mass spectrometry and application in pharmacokinetic studies after oral administration of traditional Chinese medicinal preparations containing scutellaria–coptis herb couple

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ABSTRACT

A sensitive, rapid and selective liquid chromatography–tandem mass spectrometry (LC–MS–MS) method was developed and validated for the simultaneous determination of baicalin, baicalein, wogonin, berberine, palmatine and jatrorrhizine in scutellaria–coptis herb couple in rat plasma. After protein precipitation with acetonitrile and 0.1% NaH_2PO_4, chromatography was performed using a C_{18} column, with gradient elution with 0.1% formic acid and acetonitrile at 0.25 ml/min. All analytes including internal standards were monitored under positive ionization conditions by selected reaction monitoring with an electrospray ionization source. The lower limit of quantification was 10 ng/ml for baicalin, baicalein and wogonin, and 0.6 ng/ml for berberine, palmatine and jatrorrhizine. The validated method was applied in pharmacokinetic studies after oral administration of Yiqing Capsule and Gegen-Qinlian Tablet to rats.

1. Introduction

Herb couples, as the basic composition units of Chinese herbal formulas, have special clinical significance in traditional Chinese medicine (TCM), and are much simpler than other complex formulas without altering their basic therapeutic features [1]. The combination of Radix Scutellariae and Rhizoma Coptidis, so-called scutellaria–coptis herb couple, is the main herb couple in many TCMs such as Yiqing Capsule and Gegen-Qinlian Tablet. Yiqing Capsule composed of Rhizoma Coptidis, Radix Scutellariae and Radix et Rhizoma Rhei has been used clinically in the treatment of intense heat in the body, inflammation and painful swelling of the eyes, sore throat, gingival bleeding and reddish urine. Gegen-Qinlian Tablet composed of Rhizoma Coptidis, Radix Scutellariae, Radix Puerariae Lobatae and Radix Glycyrrhizae is now commonly used clinically to cure viral diarrhea, bacillary dysentery, general fever and dipsesis. According to the theory of TCM and thousands of years of medicinal practice, both herbs of the scutellaria–coptis herb couple, as the core of Yiqing Capsule and Gegen-Qinlian Tablet, have characteristics of bitter and cold and common medicinal functions such as purging fire for removing toxins, eliminating the wetness evil from the upper warmer, clearing away the heat-evil and expelling superficial evils [2].

Flavonoids like baicalin, baicalein and wogonin are the main bioactive ingredients in Radix Scutellariae [3], while the quaternary protoberberine-type alkaloids berberine, palmatine and jatrorrhizine are the main bioactive components in Rhizome Coptidis [4,5] (Fig. 1). The development of a sensitive and rapid method to simultaneously determine as many active components as possible in herb couple is quite necessary to predict interactions between the various compounds. Several recent studies have reported on the detection of active components in Radix Scutellariae or Rhizome Coptidis, separately, however methods regarding the simultaneous determination of baicalin, baicalein, wogonin, berberine, palmatine and jatrorrhizine *in vivo* in a single run are not available in the literatures. Methods have been reported for the determination of the active components of *Scutellaria baicalensis* in rat plasma samples using high-performance liquid chromatography with UV detection (HPLC-UV), micro-HPLC with electrochemical detection (μHPLC-ED) [6] or liquid chromatography–tandem mass spectrom-

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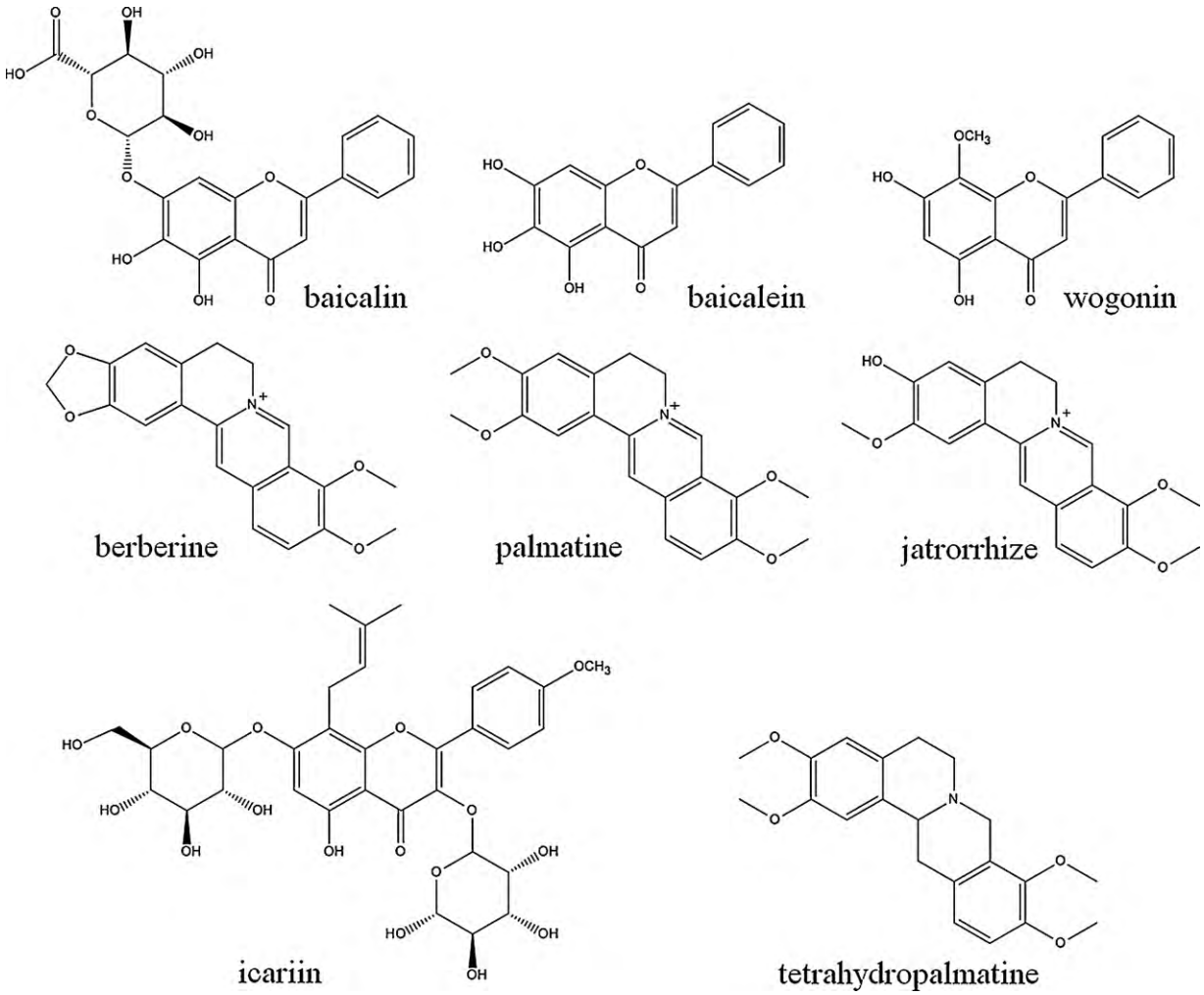

Fig. 1. Chemical structures of baicalin, baicalein, wogonin, berberine, palmatine, jatrorrhizine, icariin (IS) and tetrahydropalmatine (IS).

etry (LC–MS–MS) [7,8]. The liquid chromatography–electrospray ionization-mass spectrometry (LC–ESI-MS) methods can simultaneously determine the active components of *Coptis chinensis Franch* in rat plasma [9–12] and LC–MS–MS methods have been developed to detect palmatine in canine plasma [13], and berberine in human plasma [14,15]. Although Li et al. [16] reported the simultaneous determination of baicalin, rhein and berberine in rat plasma with column-switching HPLC, the long chromatographic run time (60 min) and low berberine sensitivity (0.4 μg/ml of low limit of quantification, LLOQ) limit its use for the determination of large numbers of samples, or low clinical monitoring of berberine. It is therefore necessary to develop a rapid, sensitive and convenient method which can simultaneously determine the main components of scutellaria–coptis herb couple in biological samples. The distinguished physicochemical differences between flavonoids and alkaloids, the low absorption of protoberberine-type alkaloids following oral administration [9–12] and the low contents of the components in TCM may be reasons why a new method for simultaneous determination is difficult to establish.

In this study, we developed and validated an LC–MS–MS method for the simultaneous determination of baicalin, baicalein, wogonin, berberine, palmatine and jatrorrhizine in scutellaria–coptis. This method proved to be rapid, sensitive and convenient, and can be applied in pharmacokinetic studies of the scutellaria–coptis herb couple contained in Yiqing Capsule and Gegen-Qinlian Tablet.

2. Experimental and methods

2.1. Chemicals and reagents

Baicalin, baicalein, wogonin, berberine, palmatine, jatrorrhizine, icariin and tetralydropalmatine were obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Yiqing Capsule was from Kanghong Pharmaceutical Co., Ltd. (Chengdu, China). Gegen-Qinlian Tablet was from Lijun Modrem TCM Co., Ltd. (Shaanxi, China). MS-grade acetonitrile was purchased from Merck (Darmstadt, Germany). HPLC-grade formic acid was purchased from the Tedia Company Inc. (Tedia, Fairfield, OH, USA). All other reagents were of analytical grade and obtained commercially.

2.2. Equipment and LC–MS–MS conditions

The LC–MS–MS system was an Agilent 1200 liquid chromatograph and a 6410 triple quadrupole mass spectrometer with an electrospray ionization source. Data were analyzed by MassHunter software (Agilent Corporation, MA, USA).

Separation was on a Zorbax SB-C_{18} column (2.1 mm × 150 mm i.d., 3.5 μm, Agilent Corporation, MA, USA) by gradient elution with 0–1.6 min, 76% → 20% mobile phase A, 1.6–4.6 min, 20% → 76% A, and 4.6–10 min, 76% A, flowing at 0.25 ml/min. Eluent A was water with 0.1% formic acid, and B was acetonitrile. Mass spectrometry conditions were optimized for maximal sensitivity. All analytes,

including the internal standard (IS), were monitored under positive ionization conditions and quantified by the selected reaction monitoring transitions. High purity nitrogen served as both the nebulizing and drying gas. Other parameters of the mass spectrometer were set as follows: drying gas flow 10.0 l/min; drying gas temperature 350 °C; nebulizer pressure 40 psi; capillary voltage 4000 V.

2.3. Preparation of calibration standards and quality control (QC) samples

Stock solutions at 1 mg/ml for each compound were prepared in methanol and stored at −20 °C. For calibration standards, stock solutions were diluted with acetonitrile–water (50:50, v/v) to produce standard working solutions at concentrations of 100, 200, 400, 800, 1600, 3200, 6400, 12800 and 23600 ng/ml for baicalin, 100, 200, 400, 800, 1600, 3200, 6400 and 12800 ng/ml for baicalein and wogonin, 6, 30, 60, 300, 600, 3000 and 6000 ng/ml for berberine, and 6, 12, 24, 48, 96, 192 and 384 ng/ml for palmatine and jatrorrhizine. These solutions were then added to blank plasma (1:10) to make calibration samples of 10–2360 ng/ml for baicalin, 10–1280 ng/ml for baicalein and wogonin, 0.6–600 ng/ml for berberine, and 0.6–38.4 ng/ml for palmatine and jatrorrhizine. Solutions of 20, 160, and 1280 ng/ml for baicalin; 20, 160, and 640 ng/ml for baicalein and wogonin; 3, 30, and 300 ng/ml for berberine, and 1.2, 4.8, and 19.2 ng/ml for palmatine and jatrorrhizine were prepared as QC samples.

2.4. Sample preparation

Fifty microliters of rat plasma was mixed with 20 μl 0.1% NaH_2PO_4 and 100 μl acetonitrile containing the IS of 100 ng/ml icariin and 1 ng/ml tetrahydropalmatine. After vortexing and centrifugation, 50 μl supernatant was mixed with an equivalent volume of water, 20 μl of which was injected into the LC–MS–MS system.

2.5. Method validation

The method was validated according to the industrial guidelines for bioanalytical method validation from the U.S. Food and Drug Administration (FDA) [17].

For selectivity, blank plasma obtained from six rats was assayed. The response in each blank sample was compared with samples at the LLOQ to ensure no interference. Analyte response at the level of LLOQ should be at least five times the blank plasma.

Calibration curves of baicalin at nine concentrations, baicalein and wogonin at eight concentrations, and berberine, palmatine and jatrorrhizine at seven concentrations, all in the ranges listed above, were prepared and assayed. Blank samples (blank plasma processed without IS) and zero samples (blank plasma processed with IS) were also assayed to confirm the absence of interference.

Accuracy and precision were assessed using five batches per concentration of QC samples as described above and calculated using a calibration curve constructed on the same testing day, ensuring that the intra-batch and inter-batch precision did not exceed ±15% of the coefficient of variation (CV), and accuracy did not exceed ±15% of the relative error (RE).

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

Stability of the analytes was investigated at three QC concentrations. Freeze–thaw stability was determined after three freeze–thaw cycles; short-term temperature stability was evaluated using QC batches kept at room temperature for 6 h; long-term stability was measured using samples kept at −20 °C for 2 weeks; and post-preparative stability was determined by reanalyzing QC samples after keeping them under autosampler conditions (4 °C) for 12 h.

2.6. Applications in pharmacokinetic studies

The developed LC–MS–MS method was applied in the pharmacokinetic studies of baicalin, baicalein, wogonin, berberine, palmatine and jatrorrhizine after oral administration of Yiqing Capsule and Gegen-Qinlian Tablet. The animal studies were approved by the Animal Ethics Committee of the Second Military Medical University, according to the Guidelines for Animal Experimentation of the Second Military Medical University. Ten male Sprague–Dawley rats weighing 180–220 g were kept in air-conditioned animal quarters at 22 ± 2 °C and relative humidity of 50 ± 10%, with free access to food and water until 12 h prior to the experiment, and acclimatized for several days. The Yiqing Capsule at 300 mg/kg and Gegen-Qinlian Tablet at 600 mg/kg were administered by oral gavage, as suspensions in 0.5% carboxymethyl cellulose sodium aqueous solution. Blood samples (150 μl) were collected before dosing, and at 5, 10, 20, 30, 45 (min), 1, 1.5, 2, 3, 4, 6, 8, 12, 24 and 48 (h) after administration, and centrifuged at 13000 × *g* for 10 min at 4 °C. The plasma was stored at −20 °C until analysis.

Concentration–time data were analyzed by the non-compartmental method to obtain pharmacokinetic parameters. The maximum plasma drug concentration (C_{max}), and the time to reach maximum plasma drug concentration (t_{max}) were observed directly from the concentration–time data. The elimination rate constant (K_e) was calculated from the slope of the logarithm of the plasma concentration versus time using the final four points. The apparent elimination half-life ($t_{1/2}$) was calculated as $0.693/K_e$. The area under the plasma concentration–time curve (AUC) was calculated by the trapezoidal rule. The values were calculated by Microsoft Excel (Microsoft, Seattle, Washington, USA).

3. Results and discussion

3.1. Optimization of LC–MS–MS conditions

Liquid chromatography of baicalin in plasma samples was optimized. After assaying the biological samples following drug administration, an impurity peak, which may be attributed to the metabolites, was observed in the baicalin chromatography (Fig. 2). Therefore, a different gradient was applied with a higher water content of 76% at the beginning of the gradient cycle. This improved the LC separation and removed the interfering substance, and necessitated the 10 min stop time.

A scan of all flavonoids and alkaloids in negative and positive modes showed sensitivity for flavonoids, but because of the quaternary nitrogen, only the positive mode was suitable for protoberberine alkaloids ion monitoring. Therefore, the positive mode was applied, and other parameters were also optimized for maximum sensitivity (Table 1).

3.2. Method validation

The lowest concentration that could be quantified was 10 ng/ml for baicalin, baicalein and wogonin, and 0.6 ng/ml for berberine, palmatine, and jatrorrhizine. Each analyte response at this level was at least five times greater than the blank response. This was defined as the lower limit of quantification for this method (Fig. 2A

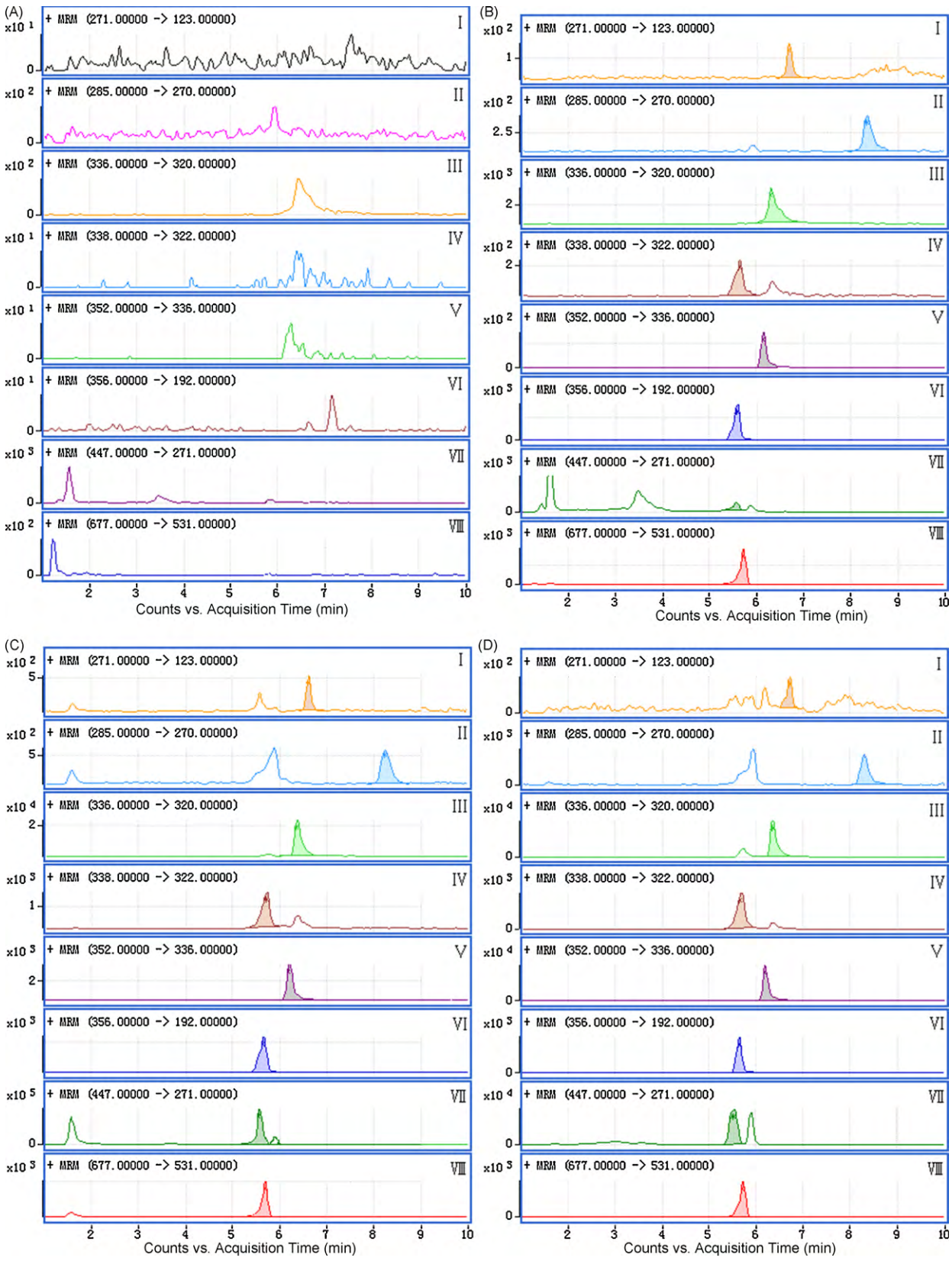

Fig. 2. Representative MRM chromatograms of baicalein (I), wogonin (II), berberine (III), jatrorrhizine (IV), palmatine (V), tetrahydropalmatine (VI, IS), baicalin (VII) and icariin (VIII, IS) in rat plasma. (A) Blank plasma sample; (B) plasma sample of LLOQ; (C) plasma sample 10 min after oral administration of Yiqing Capsule and (D) plasma sample 10 min after oral administration of Gegen-Qinlian Tablet.

Table 1
Optimized multiple reaction monitoring (MRM) parameters for the detection of analytes and internal standards.

Analyte	*m/z*	Fragmentor energy (V)	Collision energy (eV)
Baicalin	447 → 271	120	10
Baicalein	271 → 123	80	35
Wogonin	285 → 270	100	16
Berberine	336 → 320	125	30
Palmatine	352 → 336	100	26
Jatrorrhizine	338 → 322	150	35
Icariin (IS)	677 → 531	140	18
Tetrahydropalmatine (IS)	356 → 192	150	25

and B). At this level, CVs were 5.4, 7.8, 6.5, 12.1, −9.7, and 9.1 and RE values were 3.2, −4.6, 4.1, 7.6, 5.5, and −10.5 for baicalin, baicalein, wogonin, berberine, palmatine, and jatrorrhizine, respectively.

Calibration curves showed good linearity in the range of 10–2360 ng/ml for baicalin, 10–1280 ng/ml for baicalein and wogonin, 0.6–600 ng/ml for berberine, and 0.6–38.4 ng/ml for palmatine and jatrorrhizine. The correlation coefficients (r^2) were 0.9957, 0.9942, 0.9983, 0.9997, 0.9997 and 0.9917 for baicalin, baicalein, wogonin, berberine, palmatine and jatrorrhizine, respectively, and the calibration curves were $y = 1.3053x - 0.4052$, $y = 0.1346x + 0.0213$, $y = 1.8987x - 0.1687$, $y = 0.2636x - 0.1175$, $y = 0.2279x - 0.7833$ and $y = 0.1191x - 2.5136$, where y is the peak area ratio of the component to the IS, and x is the concentration of the component added to plasma.

Accuracy and precision of the method were validated for intra- and inter-day data, by comparing the mean of five replicates of each QC sample. Neither the CV, which evaluated precision, nor did the RE, which evaluated accuracy, exceeded ±14.6% (Table 2).

Table 3 shows the extraction recovery for the six components, indicating that the method was consistent, precise and reproducible. Assessment of the matrix effect showed no effect.

Freeze–thaw stability, short-term temperature stability, long-term stability and post-preparative stability were determined by comparing the mean of the five replicates per level of QC samples (Section 2.5) (Table 4). This new method for the simultaneous deter-

Table 2
Precision and accuracy in measurement of baicalin, baicalein, wogonin, berberine, palmatine and jatrorrhizine in rat plasma ($n = 5$).

Analyte	Theoretical concentration (ng/ml)	Intra-day			Inter-day		
		Mean measure concentration (ng/ml)	CV (%)	RE (%)	Mean measure concentration (ng/ml)	CV (%)	RE (%)
Baicalin	20.0	22.3	4.4	11.5	20.8	8.5	4.0
	160.0	153.2	8.2	−4.3	179.5	3.2	12.2
	1280.0	1220.8	1.8	−4.6	1217.2	7.5	−4.9
Baicalein	20.0	22.2	8.7	11.0	21.0	10.9	5.0
	160.0	168.4	5.2	5.3	156.3	7.8	−2.3
	640.0	702.7	2.7	9.8	686.0	6.6	7.2
Wogonin	20.0	21.2	8.2	6.0	21.9	10.3	9.5
	160.0	146.6	10.5	−8.4	138.5	7.0	−13.4
	640.0	717.8	5.9	12.2	697.0	2.9	8.9
Berberine	3.0	3.3	7.8	10.0	3.2	13.1	6.7
	30.0	33.1	3.4	10.3	32.5	10.5	8.3
	300.0	265.9	8.7	−11.4	287.3	3.8	−4.2
Palmatine	1.2	1.3	6.7	8.3	1.1	12.8	−8.3
	4.8	4.5	4.4	−6.3	5.3	9.6	10.4
	19.2	16.9	6.7	−12.0	17.1	2.3	−10.9
Jatrorrhizine	1.2	1.3	11.5	8.3	1.3	12.7	8.3
	4.8	4.2	3.4	−12.5	4.1	3.8	−14.6
	19.2	17.1	2.9	−10.9	18.2	2.4	−5.2

Table 3
Extraction recovery and matrix effect of baicalin, baicalein, wogonin, berberine, palmatine and jatrorrhizine in rat plasma ($n = 6$).

Analyte	Concentration (ng/ml)	Extraction recovery (%, mean ± S.D.)	Matrix effect (%, mean ± S.D.)
Baicalin	20	83.2 ± 2.1	97.7 ± 2.4
	160	82.7 ± 3.4	95.4 ± 4.0
	1280	91.5 ± 2.4	94.0 ± 1.2
Baicalein	20	84.6 ± 7.4	113.0 ± 2.6
	160	71.5 ± 4.9	106.5 ± 7.1
	640	75.2 ± 6.1	107.6 ± 5.6
Wogonin	20	62.6 ± 2.8	88.3 ± 6.6
	160	74.1 ± 2.4	89.4 ± 6.8
	640	65.8 ± 2.5	88.5 ± 9.9
Berberine	3	83.4 ± 3.6	94.0 ± 8.8
	30	91.2 ± 1.6	90.5 ± 6.9
	300	90.5 ± 5.1	93.4 ± 9.6
Palmatine	1.2	89.1 ± 1.4	87.7 ± 9.4
	4.8	80.5 ± 5.4	89.3 ± 5.2
	19.2	81.3 ± 2.6	89.7 ± 9.6
Jatrorrhizine	1.2	53.4 ± 4.1	86.2 ± 1.8
	4.8	59.0 ± 8.0	87.2 ± 8.3
	19.2	65.1 ± 6.0	87.6 ± 1.6

Table 4
Stability of baicalin, baicalein, wogonin, berberine, palmatine and jatrorrhizine in rat plasma ($n = 5$).

Analyte	Theoretical concentration (ng/ml)	Short-term stability		Long-term stability		Freeze and thaw stability		Post-preparative stability	
		Concentration (mean ± S.D., ng/ml)	RE (%)	Concentration (mean ± S.D., ng/ml)	RE (%)	Concentration (mean ± S.D., ng/ml)	RE (%)	Concentration (mean ± S.D., ng/ml)	RE (%)
Baicalin	20.0	20.6 ± 1.6	3.0	17.9 ± 2.9	−10.5	17.3 ± 1.4	−13.5	22.4 ± 1.8	12.0
	160.0	142.2 ± 2.4	−11.1	139.1 ± 3.2	−13.1	138.3 ± 2.4	−13.6	175.2 ± 2.1	9.5
	1280.0	1112.2 ± 7.8	−13.1	1159.6 ± 8.3	−9.4	1167.0 ± 4.2	−8.8	1331.8 ± 2.7	4.0
Baicalein	20.0	18.9 ± 1.5	−5.5	19.1 ± 2.5	−4.5	18.4 ± 0.3	−8.0	19.4 ± 1.6	−3.0
	160.0	148.1 ± 1.6	−7.4	138.9 ± 4.6	−13.2	146.1 ± 1.8	−8.7	155.5 ± 2.7	−2.8
	640.0	648.3 ± 4.6	1.3	572.2 ± 7.9	−10.6	558.9 ± 3.8	−12.7	699.5 ± 4.4	9.3
Wogonin	20.0	21.7 ± 1.2	8.5	19.3 ± 1.9	−3.5	17.2 ± 0.6	−14.0	18.5 ± 0.5	−7.5
	160.0	145.8 ± 1.5	−8.9	148.5 ± 3.1	−7.2	139.7 ± 0.8	−12.7	169.2 ± 0.9	5.7
	640.0	688.5 ± 2.5	7.6	562.6 ± 5.8	−12.1	571.7 ± 1.6	−10.7	697.8 ± 6.3	9.0
Berberine	3.0	2.6 ± 0.4	−13.3	3.4 ± 0.4	13.3	3.2 ± 0.3	6.7	2.8 ± 0.3	−6.7
	30.0	26.3 ± 1.2	−12.3	26.8 ± 1.3	−10.7	26.9 ± 1.0	−10.3	29.2 ± 2.2	−2.7
	300.0	307.6 ± 1.7	2.5	307.5 ± 2.7	2.5	307.6 ± 1.5	2.5	307.8 ± 3.0	2.6
Palmatine	1.2	1.3 ± 0.1	8.3	1.3 ± 0.2	8.3	1.3 ± 0.1	8.3	1.3 ± 0.1	8.3
	4.8	4.4 ± 0.6	−8.3	4.2 ± 0.5	−12.5	4.2 ± 0.6	−12.5	4.2 ± 0.3	−12.5
	19.2	18.6 ± 2.8	−3.1	16.7 ± 1.6	−13.0	17.1 ± 1.2	−10.9	19.9 ± 0.5	3.6
Jatrorrhizine	1.2	1.3 ± 0.2	8.3	1.1 ± 0.1	−8.3	1.3 ± 0.2	8.3	1.3 ± 0.1	8.3
	4.8	4.2 ± 0.6	−12.5	4.3 ± 0.5	−10.4	4.2 ± 0.7	−12.5	4.3 ± 0.4	−10.4
	19.2	20.6 ± 1.5	7.3	17.6 ± 1.2	−8.3	18.4 ± 1.4	−4.2	19.8 ± 0.9	3.1

mination of baicalin, baicalein, wogonin, berberine, palmatine and jatrorrhizine offered satisfactory stability.

3.3. Applications

Yiqing Capsule was administered orally to five rats at a dose of 300 mg/kg (42.9 mg/kg baicalin, 1.92 mg/kg baicalein, 1.47 mg/kg wogonin, 1.29 mg/kg berberine, 0.48 mg/kg palmatine, and 0.66 mg/kg jatrorrhizine), and Gegen-Qinlian Tablet to the remaining five rats at a dose of 600 mg/kg (6.87 mg/kg baicalin, 0.54 mg/kg baicalein, 1.44 mg/kg wogonin, 2.7 mg/kg berberine, 0.69 mg/kg palmatine, and 1.2 mg/kg jatrorrhizine). Plasma samples at the points indicated in Figs. 3–6 were analyzed to determine the pharmacokinetic parameters of baicalin, baicalein, wogonin, berberine, palmatine and jatrorrhizine in Yiqing Capsule and Gegen-Qinlian Tablet (Section 2.6).

As shown in Table 5, the plasma concentration of quaternary protoberberine-type alkaloids was low (AUC_{0-t} was 41.54 ± 9.40 ng h/ml for palmatine, 121.76 ± 48.30 ng h/ml for jatrorrhizine in Yiqing Capsule, and 39.96 ± 10.55 ng h/ml for

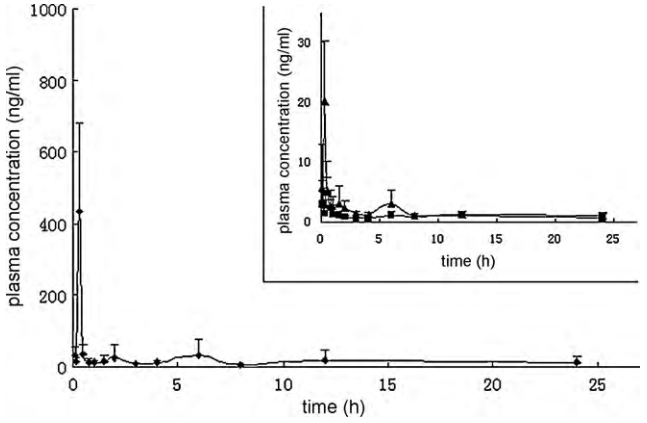

Fig. 4. Mean plasma concentration–time profiles of berberine (♦), palmatine (■) and jatrorrhizine (▲) after oral administration of Yiqing Capsule at a dose of 300 mg/kg. Each point represents mean ± S.D. ($n = 5$).

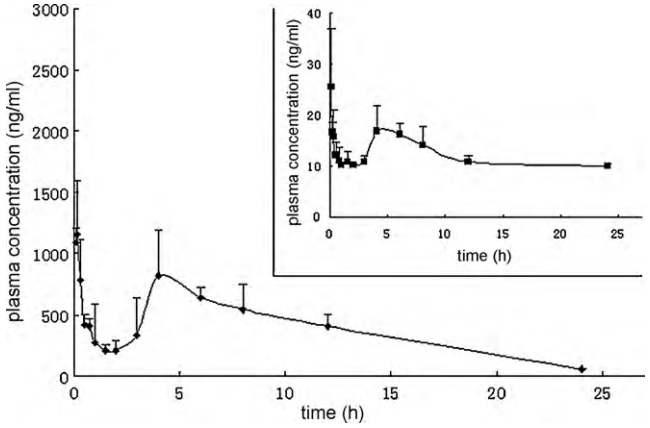

Fig. 3. Mean plasma concentration–time profiles of baicalin (♦) and wogonin (■) after oral administration of Yiqing Capsule at a dose of 300 mg/kg. Each point represents mean ± S.D. ($n = 5$).

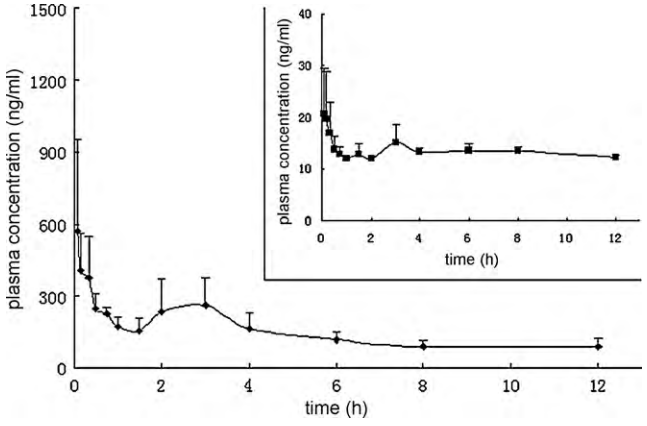

Fig. 5. Mean plasma concentration–time profiles of baicalin (♦) and wogonin (■) after oral administration of Gegen-Qinlian Tablet at a dose of 600 mg/kg. Each point represents mean ± S.D. ($n = 5$).

Table 5
Pharmacokinetic parameters (mean ± S.D.) of baicalin, wogonin, berberine, palmatine and jatrorrhizine after oral administration of 300 mg/kg Yiqing Capsule and 600 mg/kg Gegen-Qinlian Tablet, respectively (n = 10).

	Yiqing Capsule				Gegen-Qinlian Tablet			
	C_{max} (ng/ml)	t_{max} (min)	$t_{1/2}$ (h)	AUC_{0-t} (ng h/ml)	C_{max} (ng/ml)	t_{max} (min)	$t_{1/2}$ (h)	AUC_{0-t} (ng h/ml)
Baicalin	1319 ± 367.9	4.81 ± 2.88	4.81 ± 0.61	7082 ± 1975	529.5 ± 147.0	8.75 ± 2.50	3.73 ± 0.69	3256 ± 429.4
Wogonin	24.0 ± 7.13	6.25 ± 2.50	7.29 ± 0.18	335.3 ± 55.19	21.6 ± 8.86	6.25 ± 2.50	5.09 ± 0.62	419.4 ± 62.94
Berberine	465 ± 30.9	16.3 ± 7.64	13.9 ± 1.11	558.1 ± 205.7	206 ± 20.8	101 ± 39.4	13.5 ± 1.22	809.2 ± 190.2
Palmatine	6.68 ± 2.19	28.0 ± 11.6	16.8 ± 1.25	41.54 ± 9.400	16.9 ± 5.89	30.0 ± 8.66	14.2 ± 1.32	39.96 ± 10.55
Jatrorrhizine	23.3 ± 3.91	26.0 ± 9.57	13.9 ± 1.28	121.8 ± 48.30	19.4 ± 6.42	35.0 ± 8.66	13.8 ± 1.81	175.4 ± 12.72

palmatine, 175.40 ± 12.72 ng h/ml for jatrorrhizine in Gegen-Qinlian Tablet), which was consistent with previous reports [9–12], however the plasma concentration of berberine was not low (AUC_{0-t} was 558.08 ± 250.65 ng h/ml in Yiqing Capsule, and 809.24 ± 190.23 ng h/ml in Gegen-Qinlian Tablet). In addition, t_{max} of berberine was 16.25 min in Yiqing Capsule and 101.25 min in Gegen-Qinlian Tablet. Deng et al. [12] observed multiple blood concentration peaks in alkaloid pharmacokinetics, probably due to distribution re-absorption and enterohepatic circulation. Zhou et al. [18,19] reported that many herbs and natural compounds isolated from herbs have been identified as substrates, inhibitors, and/or inducers of various CYP enzymes, and herb-CYP interactions may occur and affect the pharmacokinetics. Moreover, Li et al. [16] showed that the pharmacokinetics may be relative to the administrated drugs which were complex prescriptions.

Flavonoids showed rapid absorption (t_{max} was 4.81–8.75 min in both Yiqing Capsule and Gegen-Qinlian Tablet) and a bimodal phenomenon in this study. Due to the low content of baicalein in Yiqing Capsule and Gegen-Qinlian Tablet, it was too low to detect in this study.

3.4. Method comparison with existing reports

There are several reports on assaying flavonoids in Radix Scutellariae or alkaloids in Rhizome Coptidis [6–15], but no method about simultaneous determination of baicalin, baicalein, wogonin, berberine, palmatine and jatrorrhizine *in vivo* is available in the literature. However, simultaneous determination of the different types of compounds in TCM in biological samples is essential in clinical practice. There is only one report on the simultaneous determination of baicalin, rhein and berberine in rat plasma [16]. The LLOQ was 0.4 μg/ml for baicalin and berberine. The chromatographic run time was 60 min. In comparison, the method described in this study is more suitable for clinical applications. The LLOQ in this study was 10 ng/ml for baicalin, baicalein and wogonin, and 0.6 ng/ml for berberine, palmatine and jatrorrhizine. The chromatographic run time was within 10 min for all the six compounds.

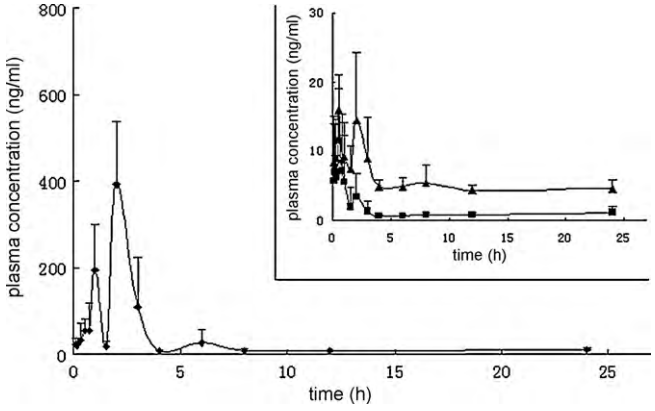

Fig. 6. Mean plasma concentration–time profiles of berberine (♦), palmatine (■) and jatrorrhizine (▲) after oral administration of Gegen-Qinlian Tablet at a dose of 600 mg/kg. Each point represents mean ± S.D. (n = 5).

Scutellaria–coptis is the main herb couple in many TCMs such as Yiqing Capsule, Gegen-Qinlian Tablet, Qinlian Pill and Lianqin-Zhuyu Pill. To improve the understanding of the relationship between pharmacokinetics, pharmacology and bioavailability of multiple components in scutellaria–coptis, a selective and sensitive analytical method for the simultaneous quantification of the main bioactive components in this herb couple and in pharmacokinetic studies after oral administration of TCM preparations containing scutellaria–coptis herb couple in plasma is required. To the best of our knowledge, this is the first time baicalin, baicalein, wogonin, berberine, palmatine and jatrorrhizine has been simultaneously determined *in vivo* using an LC–MS–MS method.

4. Conclusions

A rapid, sensitive and convenient LC–MS–MS method for the simultaneous determination of baicalin, baicalein, wogonin, berberine, palmatine and jatrorrhizine in scutellaria–coptis herb couple was developed, validated and successfully applied in pharmacokinetic studies of Yiqing Capsule and Gegen-Qinlian Tablet. The pharmacokinetic parameters obtained from this study and the simple method developed herein would prove useful in clinical applications and further research on herb interactions of the scutellaria–coptis herb couple.

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