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Journal of Chromatography B, 844 (2006) 261–267
JOURNAL OF CHROMATOGRAPHY B
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Liquid chromatography with tandem mass spectrometry for the simultaneous determination of baicalein, baicalin, oroxylin A and wogonin in rat plasma

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Received 5 April 2006; accepted 13 July 2006
Available online 8 August 2006

Abstract

A rapid, sensitive and selective liquid chromatography–tandem mass spectrometric (LC–MS/MS) method for the determination of baicalein, baicalin, oroxylin A and wogonin, *Scutellaria baicalensis* active components in rat plasma was developed. After liquid–liquid extraction with 2-(3,4-dimethoxy-phenyl)-5,7-dihydroxy-chromen-4-one as internal standard, baicalein, baicalin, oroxylin A and wogonin were eluted from an Atlantis C_{18} column within 7 min with isocratic mobile phase consisting of methanol and 0.1% formic acid (60:40, v/v). The analytes were detected using an electrospray ionization tandem mass spectrometry in the multiple reaction monitoring (MRM) mode. The standard curves were linear ($r = 1.000$) over the concentration ranges of 5–500 ng/ml for baicalein, wogonin and oroxylin A and 5–5000 ng/ml for baicalin. The coefficients of variation and relative errors of baicalein, wogonin, oroxylin A and baicalin for intra- and inter-assay at three or four quality control (QC) levels were 0.8–6.1% and −4.0 to 5.8%, respectively. The lower limits of quantification for baicalein, wogonin, oroxylin A and baicalin were 5 ng/ml using 50 μl of plasma sample. This method was successfully applied to the pharmacokinetic study of baicalein, baicalin, wogonin and oroxylin A after an intravenous administration of *Scutellariae radix* extract to male Sprague–Dawley rats.

Keywords: LC–MS/MS; Baicalein; Baicalin; Oroxylin A; Wogonin; Rat plasma

1. Introduction

Scutellaria baicalensis is a perennial herb cultivated in Korea. The roots of this plant have been used in traditional oriental medicine for the treatment of various ailments including fevers, ulcers, inflammation and cancers. *Scutellaria baicalensis* contains a variety of flavones, phenylethanoids, amino acids, sterols and essential oils. Baicalein, baicalin, oroxyiln A and wogonin (Fig. 1) are the main active components in *Scutellaria baicalensis* [1]. These flavonoids possess anti-allergic [2], antioxidant [3,4], anti-HIV [5,6], anti-inflammatory [7], anti-tumor [8–10], anxiolytic [11], anti-hepatitis B virus [12] and antigenotoxic activity [13].

A better understanding of the pharmacokinetics and bioavailability of herbal medicinal products (HMP) can link data from pharmacological assays to clinical effects and also help in designing rational dosage regimens [14]. The absorption, metabolism and excretion studies of pure baicalein and baicalin [15–18,29], or wogonin [19–21] in rats and humans were evaluated. A few pharmacokinetics data of baicalein, baicalin, wogonin and/or oroxylin A after oral administration of *Scutellariae radix* or medicinal preparations were available [22–26]. Selective and sensitive analytical method for the simultaneous determination of baicalein, baicalin, oroxylin A and wogonin in plasma is needed in order to characterize the pharmacokinetics of baicalein, baicalin, oroxyiln A and wogonin after administration of *Scutellariae radix* extracts. Baicalein, baicalin, wogonin and/or oroxylin A in plasma and urine were determined by high-performance liquid chromatography (HPLC) with UV [15–17,19,22–26] or electrochemical detection [27–29]. LC–MS/MS method was described for the

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doi:10.1016/j.jchromb.2006.07.021

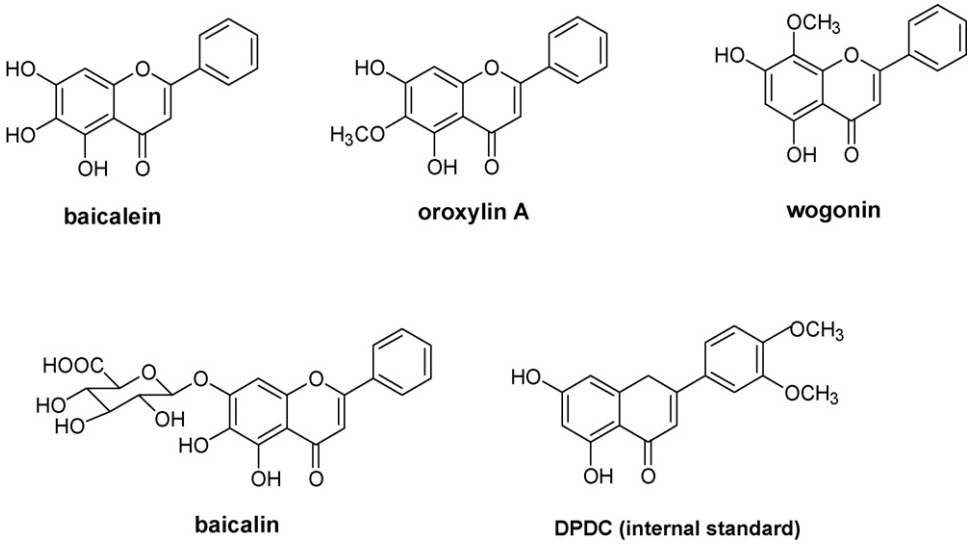

Fig. 1. Chemical structures of baicalein, oroxylin A, wogonin, baicalin and DPDC (internal standard).

determination of baicalin [18] or wogonin [20,21] in rat plasma. The analytical methods for the quantitative determination of the active components in *Scutellaria baicalensis* plants and medicinal preparations were reviewed by Li et al. [30]. To the best of our knowledge, the simultaneous determination of *Scutellaria baicalensis* active components, i.e., baicalein, baicalin, oroxylin A and wogonin in plasma by LC–MS/MS has not been described.

The purpose of this study was to develop the simple, rapid and reliable LC–MS/MS assay using one-step liquid–liquid extraction procedure for the simultaneous determination of baicalein, baicalin, oroxylin A and wogonin in rat plasma.

2. Experimental

2.1. Materials and reagent

Baicalein, wogonin and oroxylin A were separated by our previous method [13], and their purities were 99.0%. Baicalin was obtained from Sigma Chemical Co. (St. Louis, MO, USA). 2-(3,4-Dimethoxy-phenyl)-5,7-dihydroxy-chromen-4-one (DPDC, purity of 99.0%, internal standard) was supplied by Dong-A Pharmaceutical Co. (Yongin, Korea). Methanol and ethyl acetate (HPLC grade) were obtained from Burdick & Jackson Inc. (Muskegon, MI, USA) and the other chemicals were of the highest quality available.

2.2. Preparation of calibration standards and quality control samples

Primary stock solutions of baicalein, baicalin, oroxylin A, wogonin and DPDC (1 mg/ml) were prepared in DMSO (Fig. 1). Working standard solutions of baicalein, baicalin, oroxylin A and wogonin were prepared by combining the aliquots of each primary stock solution and diluting with acetonitrile. The DPDC solution (0.1 μg/ml) was prepared by diluting an aliquot of stock solution with acetonitrile. Baicalein, baicalin, oroxylin A, wogonin and DPDC solutions were stored at ca 4 °C in polypropylene tubes in the dark for 1 week.

Rat plasma calibration standards of baicalein, oroxylin A, wogonin (5, 10, 20, 50, 100, 200 and 500 ng/ml) and baicalin (5, 10, 20, 50, 100, 200, 500, 1000, 2000 and 5000 ng/ml) were prepared by spiking the working standard solutions into a pool of 11 lots of drug-free rat plasma. Quality control (QC) samples at 12.0, 120 and 400 ng/ml were prepared in bulk by adding 25 μl of the appropriate working standard solutions (0.24, 2.40, 8.00 μg/ml) to drug-free rat plasma (475 μl). The bulk samples were aliquoted (50 μl) into polypropylene tubes and stored at −20 °C until analysis.

2.3. Sample preparation

Fifty microliters of rat plasma standard and QC samples were mixed with 10 μl of 10% ascorbic acid as an antioxidant [31], 350 μl of 0.05 M hydrochloric acid, 5 μl of DPDC in acetonitrile solution and 1000 μl of ethyl acetate in 1.5 ml-polypropylene tubes. The mixtures were centrifuged at 13,000 × *g* for 5 min. The organic layer was pipette-transferred and evaporated to the dryness using a vaccum concentrator. The residues were dissolved in 40 μl of 50% methanol by sonicating for 3 min and centrifuged. The aliquots (10 μl) were injected into LC–MS/MS system.

2.4. LC–MS/MS analysis

The chromatographic system consisted of a Nanospace SI-2 pump, a SI-2 autosampler and a S-MC system controller (Shiseido, Tokyo, Japan). The separation was performed on an Atlantis C_{18} column (5 μm, 2.1 mm i.d. × 100 mm, Waters Co, Milford, MA, USA) using mixture of methanol and 0.1% formic acid (60:40, v/v) at a flow rate of 0.2 ml/min. The column and autosampler tray temperatures were 50 °C and 4 °C, respectively. The analytical run time was 7.0 min. The eluent was introduced directly into the positive ionization electrospray

source of a tandem quadrupole mass spectrometer (Quattro LC, Micromass UK Ltd., UK). The ion source and desolvation temperature were held at 120 °C and 350 °C, respectively. The optimum cone voltages were 35 V for baicalein, 33 V for oroxylin A and wogonin 28 V for baicalin and 55 V for DPDC. Multiple-reaction-monitoring (MRM) mode using specific precursor/product ion transitions was employed for the quantification. The molecular ions of baicalein, baicalin, oroxylin A, wogonin and DPDC were fragmented at collision energy of 31, 16, 25, 25 and 32 eV using argon as collision gas. Detection of the ions was performed by monitoring the transitions: *m/z* 271.2 → 123.3 for baicalein, *m/z* 447.3 → 271.1 for baicalin, *m/z* 285.3 → 270.1 for oroxylin A and wogonin and *m/z* 315.0 → 299.0 for DPDC. Peak areas for all components were automatically integrated using MassLynx version 3.5 software. (Micromass UK Ltd.)

2.5. Method validation

This method was validated to meet the acceptance criteria of industrial guidance for the bioanalytical method validation [32]. Batches, consisting of triplicate calibration standards at each concentration, were analyzed on three different days to complete the method validation. In each batch, QC samples at 12.0, 120, 400 and 4000 ng/ml (for baicalin) were assayed in sets of six replicates to evaluate the intra- and inter-day precision and accuracy. The percentage deviation of the mean from true values, expressed as relative error (RE) and the coefficient of variation (CV), serve as the measure of accuracy and precision, respectively.

The recoveries of baicalein, baicalin, oroxylin A and wogonin were determined by comparing the peak area of six extracted samples at the concentrations of 12.0, 120 and 400 ng/ml with the mean peak areas of five replicates of the recovery standards. Recovery standards were prepared by spiking baicalein, baicalin, oroxylin A, wogonin and internal standard solutions to blank rat plasma extracts.

The relative matrix effects for baicalein, baicalin, oroxylin A, wogonin and DPDC were assessed by analyzing standards spiked at three concentrations (15.0, 75.0 and 400 ng/ml) into different plasma extracts originating from five different lots of blank rat plasma and comparing the peak areas of the analytes [33]. The variability in the peak areas of the analytes, expressed as CV (%), was considered as a measure of the relative matrix effect for these analytes.

Analyte stability was tested by subjecting QC samples at the concentrations of 12.0 and 400 ng/ml through three freeze–thaw cycles and extended time (4 h) on bench at room temperature (short-term). Post-extraction batch integrity was determined by batch re-injection.

2.6. Application

The developed LC–MS/MS method was used in the pharmacokinetic study of baicalein, baicalin, oroxylin A and wogonin after an intravenous administration of *Scutellariae radix* extract to male Sprague–Dawley rats (7–8 weeks of age, body weight 210–230 g, Biogenomics, Seoul, Korea). Animals were kept in plastic cages with free access to standard rat diet (Biogenomics, Seoul, Korea) and water. The animals were maintained at a temperature of 22–24 °C with a 12 h light/dark cycle and relative humidity of 50 ± 10%. The rats were anesthetized by ketamin and cannulated with polyethylene tubing (0.58 mm i.d. and 0.96 mm o.d., Clay Adams Co., Parsippany, NJ, USA) in the left femoral vein and right jugular vein. After a 1-day recovery period, *Scutellariae radix* extract was administered intravenously at a dose of 10 mg/kg to the femoral vein in the rats ($n = 4$). Blood samples (150 μl) were collected at 0, 1, 5, 15, 30, 45 min, 1, 1.5, 2, 4, 6, 8, 10 and 24 h after intravenous administration. Following centrifugation (3000 × *g*, 10 min, 4 °C), plasma samples were transferred to polypropylene tubes and stored at −20 °C until analysis.

The plasma concentration versus time data were analyzed by a non-compartmental method using the non-linear least squares regression program WinNonlin (Scientific Consulting Inc., Cary, NC). The area under the plasma concentration–time curve (AUC) and the area under the first moment curve (AUMC) were calculated using the trapezoidal rule extrapolated to infinite time. The terminal elimination half-life ($t_{1/2}$), the systemic clearance (*Cl*), mean residence time (MRT) and volume of distribution at steady state (V_{ss}) were obtained. The peak plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) of baicalin, a major metabolite of baicalein after intravenous administration of *Scutellariae radix* extract, were obtained directly from the experimental data.

3. Results and discussion

3.1. LC–MS/MS

The electrospray ionization of baicalein, oroxylin A, wogonin and DPDC produced the abundant protonated molecular ions ($[MH]^+$) at *m/z* 271.2, 285.3, 285.3 and 315.0, respectively, under positive ionization conditions, without any evidence of fragmentation and adduct formation. The electrospray ionization of baicalin produced the abundant $[MH]^+$ ion at *m/z* 447.3 with a fragment ion at *m/z* 271.2 (baicalein) due to a loss of glucuronic acid. $[MH]^+$ ions from baicalein, baicalin, oroxylin A, wogonin and DPDC were selected as the precursor ion and subsequently fragmented in MS/MS mode to obtain the product ion spectra yielding useful structural information (Fig. 2). The fragment ions were produced as the prominent product ions at *m/z* 123 (trihydroxyphenyl moiety) for baicalein, *m/z* 271 (the loss of glucuronic acid moiety from $[MH]^+$ ion) for baicalin, *m/z* 270 (the loss of methyl group from $[MH]^+$ ion) for wogonin, *m/z* 270 (the loss of methyl group from $[MH]^+$ ion) for oroxylin A and *m/z* 299 (the loss of methyl group from MH^+) for DPDC. The quantification of the analytes was performed using the MRM mode due to the high selectivity and sensitivity of MRM data acquisitions. Four pairs of MRM transitions were selected: *m/z* 271.2 → 123.3 for baicalein, *m/z* 447.3 → 271.1 for baicalin, *m/z* 285.3 → 270.1 for oroxylin A and wogonin and *m/z* 315.0 → 299.0 for DPDC.

The two isomers, oroxylin A and wogonin have the same product ions (Fig. 2), and the electrospray ionization of baicalin

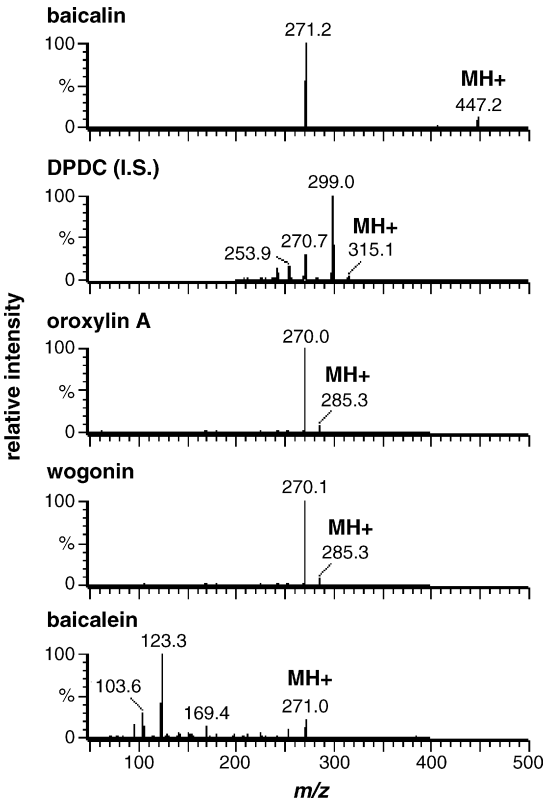

Fig. 2. Product ion mass spectra of baicalein, baicalin, oroxylin A, wogonin and DPDC. Collision energies were 31 eV for baicalein, 16 eV for baicalin, 25 eV for oroxylin A, 25 eV for wogonin and 32 eV for DPDC.

produced baicalein ion as well as [MH^+] ion, and therefore, the chromatographic separation was needed to avoid any potential isobaric interferences. The representative MRM chromatograms for the separation of baicalein, baicalin, oroxylin A and wogonin on an Atlantis C_{18} column with isocratic mobile phase are shown in Fig. 3. The retention times of baicalein, baicalin, oroxylin A and wogonin were found to be approximately 4.36, 2.59, 5.17 and 6.17 min, respectively, indicating the good resolution of these four flavonoids. The analysis of blank rat plasma samples from 30 different sources did not show any interference at the retention times of baicalein, baicalin, oroxylin A, wogonin and DPDC (Fig. 3a), confirming the selectivity of the present method. Sample carryover effect was not observed.

3.2. Method validation

Calibration curves were obtained over the concentration range of 5.0–500 ng/ml for baicalein, oroxylin A and wogonin and 5.0–5000 ng/ml for baicalin in rat plasma. Linear regression analysis with a weighting of 1/concentration^2 gave the optimum precision and accuracy of the corresponding calculated concentrations at each level (Table 1). The low CV values for the slopes of baicalein (3.6%), baicalin (5.7%), oroxylin A (2.6%) and wogonin (3.0%) indicated the repeatability of the method (Table 1).

Table 2 shows a summary of intra- and inter-assay precision and accuracy for QC samples containing baicalein, baicalin, oroxylin A and wogonin. Both intra- and inter-assay CV values were 0.8–6.1% for baicalein, 1.7–5.2% for oroxylin A and 1.4–5.9% for wogonin at three QC levels and 1.6–4.1% for baicalin at four QC levels. The intra- and inter-assay RE values were −4.0 to 4.2% for baicalein, −1.3 to 1.7% for oroxylin

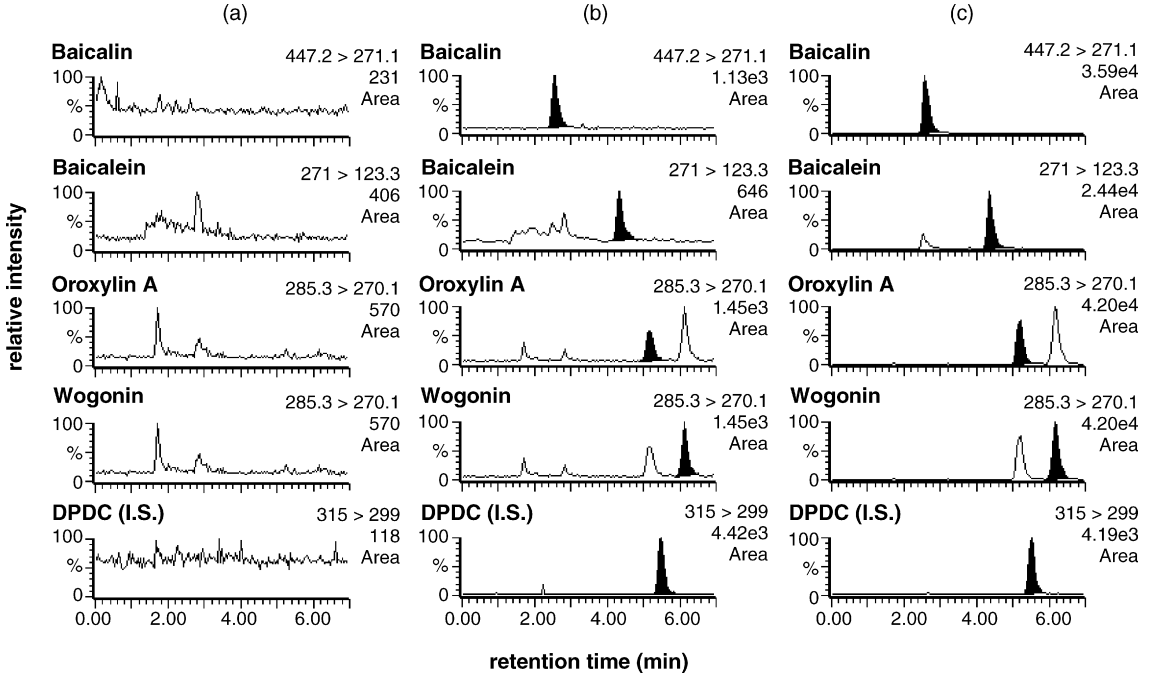

Fig. 3. MRM chromatograms of (a) a human blank plasma and blank plasma samples spiked with (b) 5.0 ng/ml and (c) 100 ng/ml of baicalein, baicalin, oroxylin A and wogonin.

Table 1
Calculated concentrations of baicalein, baicalin, oroxylin A and wogonin in calibration standards prepared in rat plasma

Analytes	Statistical variable	Theoretical concentration (ng/ml)										Slope	r
		5.0	10.0	20.0	50.0	100	200	500	1000	2000	5000		
Baicalein	Mean	4.8	9.7	19.1	49.7	102	201	499	–	–	–	0.0251	1.000
	CV (%)	2.8	4.4	6.3	5.2	6.6	3.6	3.1	–	–	–	3.6	
	RE (%)	−4.0	−3.0	−4.5	−0.6	2.0	0.5	−0.2	–	–	–		
Baicalin	Mean	5.1	9.8	19.6	50.8	99.1	193	512	1019	1960	5001	0.0236	1.000
	CV (%)	6.0	7.2	7.1	9.1	8.5	8.0	5.6	6.9	4.7	5.8	5.7	
	RE (%)	2.0	−2.0	−2.0	1.6	−0.9	−3.5	2.4	1.9	−2.0	0.0		
Oroxylin A	Mean	4.8	9.9	20.3	50.5	100	199	500	–	–	–	0.0374	1.000
	CV (%)	3.9	5.2	3.6	4.7	2.8	3.3	2.4	–	–	–	2.6	
	RE (%)	−4.0	−1.0	1.5	1.0	0.0	−0.5	0.0	–	–	–		
Wogonin	Mean	5.0	10.2	20.7	49.8	100	198	501	–	–	–	0.0571	1.000
	CV (%)	3.1	2.3	4.4	3.3	3.1	5.0	2.7	–	–	–	3.0	
	RE (%)	0.0	2.0	3.5	−0.4	0.0	−1.0	0.2	–	–	–		

Values are mean ± SD ($n = 9$). (–) Not assayed.

A and −3.8 to 5.8% for wogonin at three QC levels and −1.7 to 1.7% for baicalin at four QC levels. These results indicated that the present method has the acceptable accuracy and precision.

The lower limit of quantitation (LLOQ) was set at 5.0 ng/ml for baicalein, baicalin, oroxylin A and wogonin using 50 μl of rat plasma. Representative chromatograms of LLOQ are shown in Fig. 3b and the signal-to-noise ratios for baicalein, baicalin, oroxylin A and wogonin were higher than 5. At LLOQ level, CVs were 4.1–4.3% and RE values were −4.0 to 3.3% (Table 2).

The extraction recoveries of baicalein, baicalin, oroxylin A and wogonin from spiked rat plasma were determined using one-step liquid–liquid extraction with ethyl acetate at acidic pH at the concentrations of 15.0, 75.0 and 400 ng/ml in six replicates. The recoveries were 82.8–87.1% for baicalein, 71.1–76.9% for baicalin, 81.5–83.2% for oroxylin A and 81.4–84.7% for wogonin (Table 3). The recovery of DPDC was 76.3 ± 4.7%.

The assessment of the presence of a relative matrix effect was made on he basis of direct comparison of the peak areas of baicalin, baicalin, oroxylin A, wogonin and DPDC spiked post-extraction into extracts originating from five different sources of rat plasma. The CVs of the determination of peak areas of baicalin, baicalin, oroxylin A, wogonin and DPDC at three different concentrations (15.0, 75.0 and 400 ng/ml) varied from 6.5 to 7.4% for baicalin, 6.3 to 8.1% for baicalin, 4.0 to 8.5% for oroxylin A, 4.5 to 7.8% for wogonin and 4.3 to 5.5% for DPDC. The CVs of the ratio of analytes/I.S. for standards spiked post-extraction into extracts from five different lots of plasma were 6.4–7.0% for baicalein, 6.1–8.0% for baicalin, 5.7–7.4% for oroxylin A and 5.5–7.2% for wogonin. These data confirm

Table 3
Recoveries of baicalein, baicalin, oroxylin A and wogonin from spiked rat plasma

Concentration (ng/ml)	Recovery (%, mean ± SD, $n = 6$)			
	Baicalein	Baicalin	Oroxylin A	Wogonin
15.0	82.8 ± 2.8	73.0 ± 5.9	81.5 ± 3.4	84.5 ± 5.9
75.0	86.1 ± 8.7	76.9 ± 7.7	83.2 ± 2.9	84.7 ± 3.7
400.0	87.1 ± 7.5	71.1 ± 4.6	82.7 ± 3.2	81.4 ± 4.3

Table 2
Precision and accuracy of baicalein, baicalin, oroxylin A and wogonin in rat plasma quality control samples

Analytes	Statistical variable	Intra-batch (ng/ml, $n = 6$)					Inter-batch (ng/ml, $n = 18$)			
		5	12	120	400	4000	12	120	400	4000
Baicalein	Mean	5.1	11.6	125	393	–	11.6	117	384	–
	CV (%)	4.2	0.8	3.4	3.1	–	3.9	6.1	3.6	–
	RE (%)	2.0	−3.3	4.2	−1.8	–	−3.3	−2.5	−4.0	–
Baicalin	Mean	4.8	11.8	122	400	3965	11.9	122	400	3978
	CV (%)	4.2	3.7	2.7	4.1	1.6	4.0	2.6	3.6	2.0
	RE (%)	−4.0	−1.7	1.7	0.0	−0.9	−0.8	1.7	0.0	−0.6
Oroxylin A	Mean	4.9	12.2	122	401	–	11.9	118.5	396	–
	CV (%)	4.3	3.3	1.7	2.5	–	5.2	2.7	2.6	–
	RE (%)	−2.0	1.7	1.7	0.3	–	−0.8	−1.3	−1.0	–
Wogonin	Mean	5.2	12.7	123	385	–	12.4	120	402	–
	CV (%)	4.1	3.2	1.4	2.4	–	5.9	3.7	5.0	–
	RE (%)	3.3	5.8	2.5	−3.8	–	3.3	0.0	0.5	–

(–) Not assayed.

Table 4
Stability of samples ($n = 6$)

Statistical variable	Theoretical concentration (ng/ml)							
	Baicalein		Baicalin		Oroxylin A		Wogonin	
	12.0	400	12.0	400	12.0	400	12.0	400
Three freeze–thaw stability								
Mean	11.3	381	12.3	421	12.1	401	12.6	418
CV (%)	6.0	2.2	2.0	3.1	5.0	5.5	3.9	2.2
RE (%)	−5.8	−4.8	2.5	5.3	0.8	0.3	5.0	4.5
Short-term temperature stability (4 h at room temperature)								
Mean	11.5	385	11.4	384	11.9	399	12.4	401
CV (%)	2.2	3.1	2.4	3.1	5.9	2.4	1.9	4.2
RE (%)	−4.2	−3.8	−5.0	−4.0	−0.8	−0.3	3.3	0.3
Post-preparative stability								
Mean	12.2	412	11.2	369	11.9	409	11.7	396
CV (%)	3.6	0.6	2.1	2.6	3.4	2.3	2.6	3.9
RE (%)	1.7	3.0	−6.7	−7.8	−0.8	2.3	−2.5	−1.0

that the relative matrix effects for baicalein, baicalin, oroxylin A and wogonin have practically no effect on the determination of baicalein, baicalin, oroxylin A and wogonin spiked into five different lots of plasma.

Stabilities of processing (freeze–thaw and short-term) and chromatography (re-injection) were tested and shown to be of insignificant effect (Table 4). QC samples that went through three freeze–thaw cycles showed the acceptable accuracy (RE: −5.8 to 5.3%) and precision (CVs: ≤6.0%). QCs showed the acceptable accuracy (RE: −5.0 to 3.3%) and precision (CVs: ≤5.9%) when exposed to room temperature for 4 h under UV shielded lights. The reanalysis of the reconstituted extracts stored for 24 h at 4 °C showed the acceptable accuracy (RE: −7.8 to 3.0%) and precision (CVs: ≤3.9%) for QC samples.

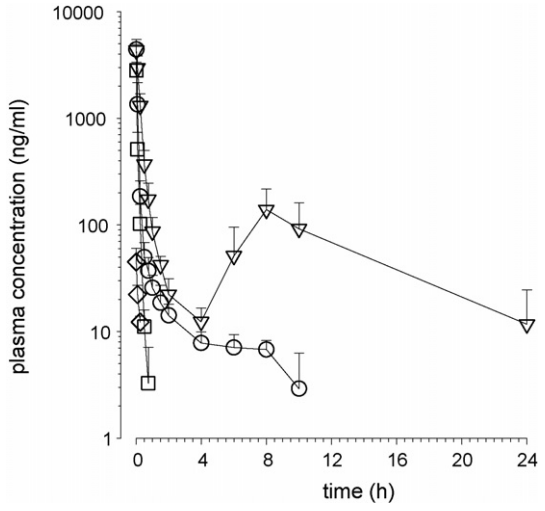

Fig. 4. Mean plasma concentration–time plots of baicalein (◯), baicalin (▽), oroxylin A (◇) and wogonin (□) after an intravenous administration of *Scutellariae radix* extract at a dose of 10 mg/kg (equivalent to 4.4 mg/kg of baicalein, 1.15 mg/kg of oroxylin A and 0.3 mg/kg of wogonin) to male Sprague–Dawley rats. Each point represents mean ± SD ($n = 4$).

3.3. Application

This method has been successfully used for the pharmacokinetic study of baicalein, baicalin, oroxylin A and wogonin after an intravenous administration of *Scutellariae radix* extract at a dose of 10 mg/kg (equivalent to 4.4 mg/kg of baicalein, 1.15 mg/kg of oroxylin A and 0.3 mg/kg of wogonin) to male Sprague–Dawley rats. Fig. 4 shows mean plasma concentration plots of baicalein, baicalin, oroxylin A and wogonin in male Sprague–Dawley rats. AUC, $t_{1/2}$, MRT, V_{ss} and Cl of baicalein were 32.1 ± 4.9 μg min/ml, 316 ± 77.1 min, 118 ± 11.2 min, 15.3 ± 3.59 l/kg and 129 ± 20.2 ml/min/kg, respectively. C_{max}, T_{max} and AUC of baiclain, a metabolite of baicalein, were 4508 ± 802 ng/ml, 2.0 ± 2.0 min and 132.7 ± 51.9 μg min/ml, respectively. AUC, $t_{1/2}$, and MRT of oroxylin A were 0.54 ± 0.10 μg min/ml, 9.8 ± 5.6 min and 5.2 ± 0.8 min, respectively. AUC, $t_{1/2}$ and MRT of wogonin were 14.3 ± 2.2 μg min/ml, 5.0 ± 1.8 min and 3.4 ± 0.9 min, respectively.

4. Conclusions

A sensitive and reliable LC–MS/MS method for the simultaneous determination of baicalein, baicalin, oroxylin A and wogonin in rat plasma has been successfully developed and validated using liquid–liquid extraction as sample clean-up procedure. This assay method demonstrated the acceptable sensitivity (LLOQ: 5.0 ng/ml), precision, accuracy, selectivity, recovery and stability. This method was successfully applied to the pharmacokinetic study of baicalein, baicalin, oroxylin A and wogonin after an intravenous administration of *Scutellariae radix* extract to male Sprague–Dawley rats.

Acknowledgements

This research was supported by a grant (PF 0320401-00) from Plant Diversity Research Center of 21st Century Frontier

Research Program funded by Ministry of Science and Technology of Korea Government and Spela Ltd.

References

[1] F. Ye, H. Wang, S. Jiang, J. Wu, J. Shao, X. Cheng, Y. Tu, D.Y. Zhang, Nutr. Cancer 49 (2004) 217.
[2] A. Koda, S. Watanabe, Y. Yanagihara, H. Nagai, K. Sakamoto, Jpn. J. Pharmacol. 27 (1977) 31.
[3] Z. Gao, K. Huang, X. Yang, H. Xu, Biochim. Biophys. Acta 1472 (1999) 643.
[4] D.E. Shieh, L.T. Liu, C.C. Lin, Cancer Res. 20 (2000) 2861.
[5] B.Q. Li, T. Fu, Y.D. Yan, N.W. Baylor, F.W. Ruscetti, H.F. Kung, Cell Mol. Biol. Res. 39 (1993) 119.
[6] J.A. Wu, A.S. Attele, L. Zhang, C.S. Yuan, Am. J. Chin. Med. 29 (2001) 69.
[7] B.Q. Li, T. Fu, W.H. Gong, N. Dunlop, H.F. Kung, Y.D. Yan, J. Kang, J.M. Wang, Immunpharmacology 49 (2000) 295.
[8] T. Konoshima, M. Kokumai, M. Kozuka, M. Iinuma, M. Mizuno, T. Tanaka, H. Tokuda, H. Nishino, A. Iwashima, Chem. Pharm. Bull. 40 (1992) 531.
[9] F.L. Chan, H.L. Choi, Z.Y. Chen, P.S.F. Chan, Y. Huang, Cancer Lett. 160 (2000) 219.
[10] F. Ye, L. Xue, J. Yi, W. Zhang, D.Y. Zhang, J. Aktern. Complement Med. 8 (2002) 351.
[11] K. Hui, M. Huen, H. Wang, H. Zheng, E. Sigel, R. Baur, H. Ren, Z.W. Li, J.T.F. Wong, H. Xue, Biochem. Pharmacol. 64 (2002) 1415.
[12] R.L. Huang, C.C. Chen, H.L. Huang, C.G. Chang, C.F. Chen, C.M. Chang, M.T. Hsieh, Planta Med. 66 (2000) 694.
[13] B.H. Lee, S.J. Lee, T.H. Kang, D.H. Kim, D.H. Sohn, G.I. Ko, Y.C. Kim, Planta Med. 66 (2000) 70.
[14] V.A. Bhattaram, U. Graefe, C. Kohlert, M. Veit, H. Derendorf, Phytomedicine 9 (Suppl. 3) (2002) 1.
[15] M.Y. Lai, S.L. Hsiu, S.Y. Tsai, Y.C. Hou, P.D.L. Chao, J. Pharm. Pharmacol. 55 (2003) 205.
[16] K. Abe, O. Inoue, E. Yumioka, Chem. Pharm. Bull. 38 (1990) 208.
[17] L. Zhang, G. Lin, Z. Zuo, J. Pharm. Biomed. Anal. 36 (2004) 637.
[18] J. Xing, X. Chen, D. Zhong, Life Sci. 78 (2005) 140.
[19] T.H. Tsai, C.J. Chou, T.R. Tsai, C.F. Chen, Planta Med. 62 (1996) 263.
[20] Y. Du, X.Y. Chen, H.Y. Yang, D.F. Zhong, Yaoxue Xuebao 37 (2002) 362.
[21] X.Y. Chen, H.Y. Wang, Y. Du, D.F. Zhong, J. Chromatogr. B 775 (2002) 169.
[22] M.Y. Lai, S.L. Hsiu, C.C. Chen, Y.C. Hou, P.D.L. Chao, Biol. Pharm. Bull. 26 (2003) 79.
[23] F. Zuo, Z.M. Zhou, Q. Zhang, D. Mao, Y.L. Xiong, Y.L. Wang, M.Z. Yan, M.L. Liu, Biol. Pharm. Bull. 26 (2003) 911.
[24] F. Zuo, Z.M. Zhou, M.Z. Yan, M.L. Liu, Y.L. Xiong, Q. Zhang, H.Y. Song, W.H. Ye, Biol. Pharm. Bull. 25 (2002) 558.
[25] C. Li, M. Homma, K. Oka, Biol. Pharm. Bull. 21 (1998) 1251.
[26] M. Homma, K. Oka, C. Taniguchi, T. Niitsuma, T. Hayashi, Biomed. Chromatogr. 11 (1997) 125.
[27] Y. Wakui, E. Yanasigawa, E. Ishibashi, Y. Matsuzaki, S. Takada, H. Sasaki, M. Aburada, T. Oyama, J. Chromatogr. 113 (1992) 131.
[28] T.H. Tsai, S.C. Liu, P.L. Tsai, L.K. Ho, A.Y.C. Shum, C.F. Chen, Br. J. Pharmacol. 137 (2002) 1314.
[29] A. Kotani, S. Kojima, H. Hakamata, F. Kusu, Anal. Biochem. 350 (2006) 99.
[30] H.-B. Li, Y. Jiang, F. Chen, J. Chromatogr. B 812 (2004) 277.
[31] M.V. Martinez-Ortega, M.C. Garcia-Parrilla, A.M. Troncoso, Anal. Chim. Acta 502 (2004) 49.
[32] Guidance for Industry-Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Veterinary Medicine, May 2001. http://www.fda.gov/cder/guidance/index.htm.
[33] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.