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# Simultaneous quantification of four active schisandra lignans from a traditional Chinese medicine *Schisandra chinensis*(*Wuweizi*) in rat plasma using liquid chromatography/mass spectrometry

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**Abstract**

A simple, rapid and sensitive method was developed for the simultaneous quantification of four active schisandra lignans (schisandrin, schisantherin A, deoxyshisandrin and γ-schisandrin) from a traditional Chinese medicine *Schisandra chinensis*(*Wuweizi*) in rat plasma using a high-performance liquid chromatography system coupled to a positive ion electrospray mass spectrometric analysis. The plasma sample preparation was a simple deproteinization by the addition of three volumes of methanol followed by centrifugation. The analytes and internal standard (IS) bicyclol were separated on a Zorbax SB-C18 column (3.5 μm, 2.1 mm × 100 mm) with mobile phase of methanol/water (70:30, v/v) containing 0.1% formic acid at a flow rate of 0.2 mL/min with an operating temperature of 25 °C. Detection was performed on a Trap XCT mass spectrometer equipped with an electrospray ionization (ESI) source operated in selected ion monitoring (SIM) mode. Positive ion ESI was used to form sodium adduct molecular ions at *m/z* 455 for schisandrin, *m/z* 559 for schisantherin A, *m/z* 439 for deoxyshisandrin, *m/z* 423 for γ-schisandrin, and *m/z* 413 for the internal standard bicyclol. Linear detection responses were obtained for the four test compounds ranging from 0.010 to 2.0 μg/mL and the lower limits of quantitation (LLOQs) for four lignans were 0.010 μg/mL. The intra- and inter-day precisions (R.S.D.%) were within 12.5% for all analytes, while the deviation of assay accuracies was within ±13.0%. The average recoveries of analytes were greater than 80.0%. All analytes were proved to be stable during all sample storage, preparation and analytic procedures. The method was successfully applied to the pharmacokinetic study of the four lignans after oral administration of *Schisandra chinensis* extraction to rats.


*Keywords:* *Schisandra chinensis*; *Wuweizi*; Schisandrin; Schisantherin A; Deoxyshisandrin; γ-Schisandrin; Schisandra lignans; HPLC–MS; Quantification; Pharmacokinetics

## 1. Introduction

*Schisandra chinensis*, officially listed as a sedative and tonic in the Chinese Pharmacopoeia, has been used as an important component in various prescriptions in traditional Chinese medicine (TCM) and more recently in western-based medicine for its antihepatotoxic effect [1]. *Schisandra chinensis* was also considered as a fortifying agent in cases of physical exhaustion for many years in Russian states [2]. Schisandra lignans are the major constituents of *Schisandra chinensis* and more than 40 of them have been isolated by now [3,4] in which the most abundant ingredients include schisandrin, schisantherin A, deoxyshisandrin and γ-schisandrin. Schisandra lignans have been shown to have various biological activities, such as hepatoprotective effect [5], potent antioxidative property [6], anticarcinogenic activity through the activation of key liver enzymes [7] and strong inhibiting effect on human immunodeficiency virus (HIV) [8] reported recently. Moreover, *Schisandra chinensis* has also been shown to increase the physical performance and recovery rates in race horses [9–11].

Up to now, several schisandra lignans have been separated by using TLC [12–14], RP–LC [12,15–21], LC–MS [22], GC–FID [23], GC–MS [24,25] and high-speed counter current chromatography [26,27]. However, these methods, which

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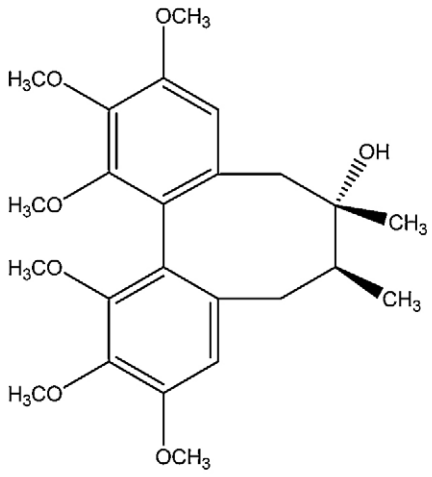
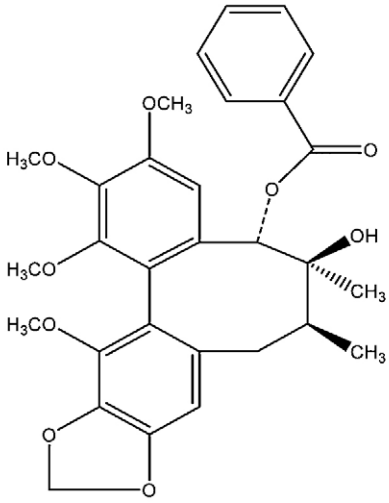
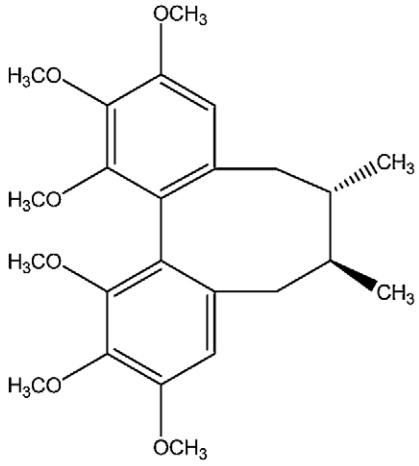


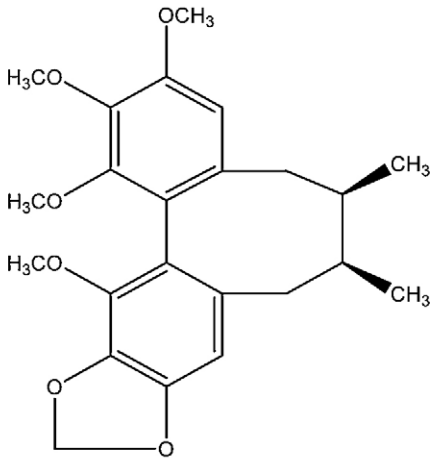

Schisandrin, MW：432



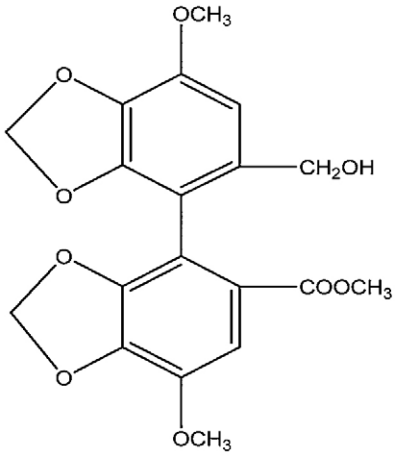

Schisantherin A, MW:536




Deoxyshisandrin, MW:416




γ-schisandrin, MW:400




Bicyclol, MW:390

Fig. 1. Chemical structures of schisandrin, schisantherin A, deoxyshisandrin and γ-schisandrin from *Schisandra chinensis*(*Wuweizi*). Bicyclol, internal standard for plasma analysis.

are commonly applied to analyze the lignan compounds in raw herb or extraction preparation of *Schisandra chinensis*, are time-consuming with too complex sample pretreatments and not sensitive enough for pharmacokinetic studies. To our knowledge there was no analytical method for simultaneous quantification of schisandrin, schisantherin A, deoxyshisandrin and γ-schisandrin, the four major active ingredients in biological samples.

We have developed a simple, sensitive and specific LC/MS method for the simultaneous determination of these four active schisandra lignans in plasma with bicyclol as the internal standard (see Fig. 1). The validation and application of this high precision assay is demonstrated for the quantitative analysis of schisandrin, schisantherin A, deoxyshisandrin and γ-schisandrin in plasma after oral administration of commercial dried extraction of *Schisandra chinensis* to rats.

## 2. Experimental

### 2.1. Materials

Schisandrin, schisantherin A, deoxyshisandrin and γ-schisandrin (>99% purity) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Bicyclol (>99% purity) was provided by Beijing Union Pharmaceutical Plant. Dried extraction of *Schisandra chinensis* (batch NO.: SC070428) which was predetermined containing schisandrin of 1.84%, schisantherin A of 1.54%, deoxyshisandrin of 2.43% and γ-schisandrin of 1.23% was purchased from Nantong Sihai Plant Extracts Co., Ltd. Methanol was of HPLC grade (Fisher, USA). All other chemicals were of analytical reagent grade. Ultrapure water, prepared

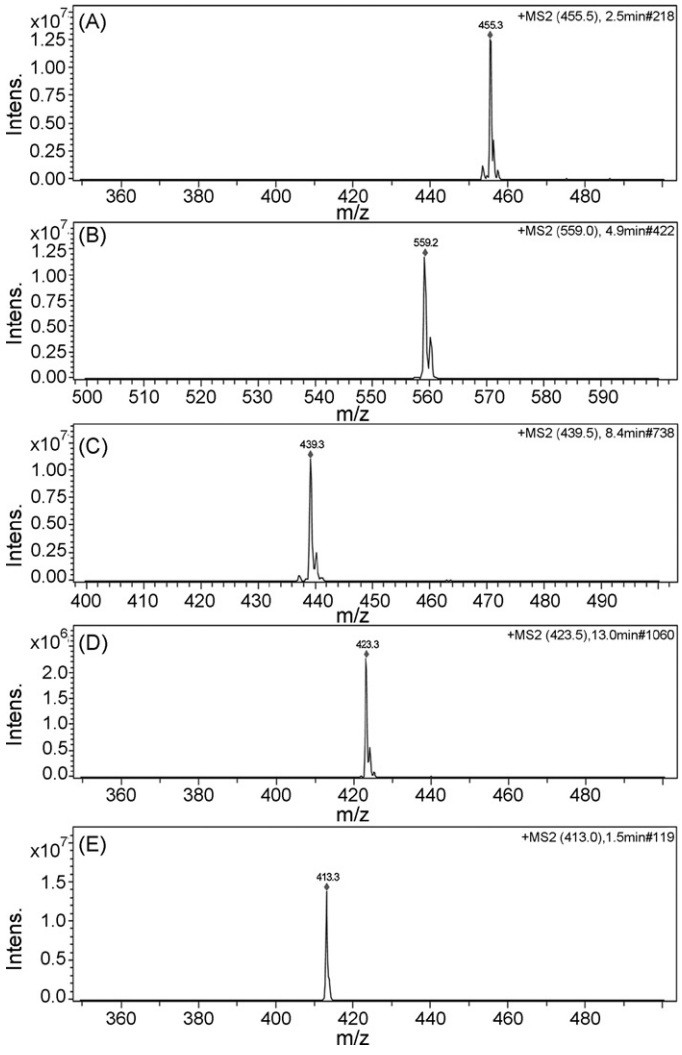



Fig. 2. Mass spectra of (A) schisandrin (*m/z* 455), (B) schisantherin A (*m/z* 559), (C) deoxyshisandrin (*m/z* 439), (D) γ-schisandrin (*m/z* 423) and (E) the internal standard (IS) bicyclol (*m/z* 413) in SIM mode.

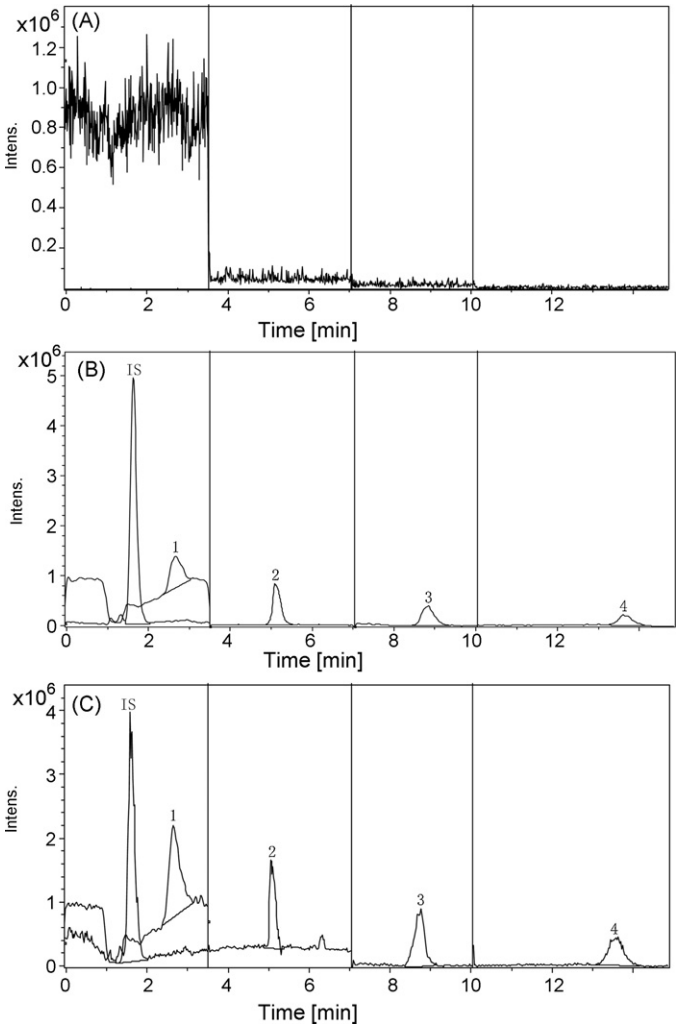



Fig. 3. Selected ion monitoring mass chromatograms obtained during LC/MS with positive ion ESI of the sodium adduct molecular ions of (1) schisandrin (*m/z* 455), (2) schisantherin A (*m/z* 559), (3) deoxyshisandrin(*m/z* 439), (4) γ-schisandrin(*m/z* 423) and the internal standard(IS) bicyclol (*m/z* 413) in (A) blank rat plasma and (B) a blank plasma spiked at 0.02 μg/mL of four schisandra lignans, and 0.2 μg/mL internal standard (bicyclol). (C) A rat plasma sample at 24 h after oral administration of commercial extraction of *Schisandra chinensis* at 1.5 g/kg.

using a Milli-Q Reagent water system (Millipore, MA, USA), was used throughout the study.

### 2.2. Preparation of stocks, calibration standards and quality control samples

The stock solution containing the four schisandra lignans was prepared in methanol at the same concentration of 1 mg/mL for each single ingredient and appropriate dilutions were made with methanol. A 0.5 mg/mL solution of bicyclol in methanol was prepared for use as the internal standard stock solution. All stock solutions were stored at −20 °C prior to use.

Calibrations standards samples were prepared by spiking diluted stock solutions into blank rat plasma, giving final concentrations of 2.0, 1.0, 0.50, 0.20, 0.10, 0.050, 0.020 and 0.010 μg/mL for each analyte.

High-, mid- and low-level quality control samples contained 1, 0.2 and 0.02 μg/mL of four analytes. These samples were prepared in a manner similar to that used for the preparation of the calibrator samples.

### 2.3. Sample processing

The internal standard bicyclol (8 μg/mL) 10 μL and 0.3mL of methanol were added to plasma sample (100 μL/sample). The mixture was vortexed for 30 s followed by centrifugation at 14,000 rpm for 5 min at room temperature. A 10 μL aliquot of each supernatant was injected into the LC/MS system for the analysis.

### 2.4. LC/MS

LC/MS was carried out using an Agilent (USA) LC/MSD Trap XCT mass spectrometer equipped with a series 1100 HPLC system consisting of a quaternary pump, automatic solvent degasser, autosampler, and an automatic thermostatic column compartment. The analytical column used was a Zorbax SB-C18(3.5 μm, 2.1 mm × 100 mm, Agilent, USA) with a 0.5 online filter(Upchurch Scientific Ltd.). The mobile phase, methanol/water containing 0.1% formic acid (70:30, v/v), was run at a flow rate of 0.2 mL/min with an operating temperature of 25 °C. Positive ion electrospray ionization (ESI) was used to form sodium adduct molecular ions in selected ion monitoring (SIM) mode at *m/z* 455 for schisandrin, *m/z* 559 for schisantherin A, *m/z* 439 for deoxyshisandrin, *m/z* 423 for γ-schisandrin, and *m/z* 413 for the internal standard bicyclol, respectively. The optimum ESI conditions for these compounds included a nitrogen nebulizer pressure of 40 psi, a nitrogen-drying gas temperature of 350 °C at 8 L/min, spray voltage of 4000 V, skimmer of 40 V, cap exit of 200 V, oct RF of 200 V and trap drive of 65 V.

### 2.5. Bioanalytical method validation

#### 2.5.1. Calibration curves, precision and accuracy

The linearity of the LC/MS method for the determination of four analytes was evaluated by a calibration curve in the range of 0.010–2.0 μg/mL. The calibration curves were obtained by plotting the peak-area ratio versus the analyte concentration prepared. Least squares linear regression analysis was used to determine the slope, intercept and correlation coefficient. The calibration curve requires a correlation coefficient ($r^2$) of 0.99 or better. To evaluate the precision, at least five QC samples of three different concentrations of the four substances were processed and injected in a single day (intra-day) and at different days (inter-day). The variability of four compounds determination was expressed as the coefficient of variation (%CV) which should be ≤15% at all tested concentrations. Accuracy is expressed as % bias which should be within the limits of ±15% at all concentrations of four analytes.

#### 2.5.2. Recovery

The recoveries of the four compounds from spiked rat plasma were determined by comparing peak-area ratios (analyte/internal standard) from those plasma samples with that obtained from the standard methanolic solutions. The recoveries at three QC concentration levels of four analytes in rat plasma were examined at least five times.

#### 2.5.3. Stability

The stabilities of four compounds in rat plasma were evaluated. The effect of three freeze/thaw cycles and the stability of compound in plasma at room temperature for 12 h were determined by repeated analysis ($n = 5$) of plasma samples. Long-term stability in plasma was also tested by assaying frozen plasma samples after storage at −80 °C for 1 month. The amount of the compounds in plasma samples was determined using a newly prepared calibration curve. Stability of analytes was expressed as a percentage of nominal concentration.

### 2.6. Pharmacokinetic experiments design

All animal protocols were approved by Institute Animal Care and Welfare Committee. Sprague–Dawley rats (adult male), weighing 180–220 g, were obtained from Beijing Vital River

Table 1
Standard curves of the four schisandra lignans in rat plasma

| Compound | Concentration ranges (μg/mL) | Regression equation | Correlation coefficient ($r^2$) |
|---|---|---|---|
| Schisandrin | 0.010–2.0 | $Y = 2.0332X - 0.01694$ | 0.994 |
| Schisantherin A | 0.010–2.0 | $Y = 6.1895X - 0.04829$ | 0.999 |
| Deoxyshisandrin | 0.010–2.0 | $Y = 3.4379X - 0.01159$ | 0.997 |
| γ-Schisandrin | 0.010–2.0 | $Y = 2.5303X - 0.14666$ | 0.993 |

*Y*, peak-area ratio (analyte/internal standard); *X*, concentration of compound in plasma (μg/mL).

Experimental Animal Co., Ltd. The animals were quarantined for 1 week prior to the study. The rats were maintained on a 12-h light/12-h dark cycle at 22 ± 1 °C and at 60% relative humidity. All animals were weighed daily and observed twice daily, in order to assess their general health. Diet was prohibited for 12 h before the experiment while water was taken freely.

The dosing solutions used for all animal studies were prepared by suspending the required amounts of dried extraction of *Schisandra chinensis* in 0.5% sodium carboxymethyl cellulose. The plasma pharmacokinetics of four lignans were studied in rats after a single oral administration at dose of 1.5 g/kg (equivalent to 27.6 mg/kg of schisandrin, 23.1 mg/kg of schisantherin A, 36.5 mg/kg of deoxyshisandrin and 18.5 mg/kg of γ-schisandrin). Blood samples were collected from rats at intervals of 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24 and 36 h after oral dosing. The blood samples of about 0.25 mL were collected in heparinized 1.5 mL polythene tubes by orbital bleeding, via heparinized capillary tubes, and centrifuged at 3000 × *g* for 10 min. A 0.1 mL plasma sample was subsequently collected and stored at −80 °C until analysis.

### 2.7. Pharmacokinetics analysis

Data fitting and pharmacokinetic parameter calculations were carried out using the DAS 2.0 pharmacokinetic program (Chinese Pharmacological Society). An appropriate pharmacokinetic model was chosen on the lowest Akaike's information criterion (AIC) value under equal weight scheme.

## 3. Results and discussion

### 3.1. Method development

The LC/MS method described in this report has high sensitivity and specificity that enables the simultaneous determination of four active schisandra lignans of *Schisandra chinensis* in rat plasma. The four lignans and the internal standard (IS) bicyclol in methanol formed predominantly sodium adduct molecular ions ($[M+Na]^+$) in full scan spectra, and sodium adduct ions ($[M+Na]^+$) were detected at *m/z* 455 for schisandrin, *m/z* 559 for schisantherin A, *m/z* 439 for deoxyshisandrin, *m/z* 423 for γ-schisandrin and *m/z* 413 for the IS (see Fig. 2). No potassium or other solvent adducts or dimers were observed. Under the described HPLC conditions, the retention times of schisandrin, schisantherin A, deoxyshisandrin, γ-schisandrin and the IS were 2.7, 5.1, 8.7, 13.5 and 1.6 min, respectively, as shown in Fig. 3. The resolution of method is sufficiently satisfactory through both chromatographic separation and SIM mode mass detection. No interfering peaks were detected at these retention times in blank plasma samples from rats (see example in Fig. 3). The overall

Table 2
Intra-day and inter-day accuracy and precision of LC/MS determination of the four schisandra lignans in rat plasma ($n = 5$)

| Sample | Spiked conc. (μg/mL) | Measured conc. (μg/mL) | Accuracy (%) | Precision (%) |
|---|---|---|---|---|
| Schisandrin | | | | |
| intra-day | 0.02 | 0.0175 ± 0.0014 | 87.5 | 8.0 |
| | 0.2 | 0.1986 ± 0.0104 | 99.3 | 5.2 |
| | 1 | 1.0206 ± 0. 1028 | 102.1 | 10.1 |
| inter-day | 0.02 | 0.0180 ± 0.0013 | 90.1 | 7.2 |
| | 0.2 | 0.2048 ± 0.0151 | 102.4 | 7.4 |
| | 1 | 1.0008 ± 0.0637 | 100.1 | 6.4 |
| Schisantherin A | | | | |
| intra-day | 0.02 | 0.0196 ± 0.0008 | 97.8 | 3.9 |
| | 0.2 | 0.2076 ± 0.0156 | 103.8 | 7.5 |
| | 1 | 0.9956 ± 0.0145 | 99.6 | 1.5 |
| inter-day | 0.02 | 0.0179 ± 0.0006 | 89.6 | 3.4 |
| | 0.2 | 0.1996 ± 0.0104 | 99.8 | 5.2 |
| | 1 | 0.9928 ± 0.0204 | 99.3 | 2. 1 |
| Deoxyshisandrin | | | | |
| intra-day | 0.02 | 0.0177 ± 0.0015 | 88.4 | 8.5 |
| | 0.2 | 0.2036 ± 0.0088 | 101.8 | 4.3 |
| | 1 | 1.0254 ± 0.0550 | 102.5 | 5.4 |
| inter-day | 0.02 | 0.0221 ± 0.0015 | 110.4 | 6.8 |
| | 0.2 | 0.2010 ± 0.0119 | 100.5 | 5.9 |
| | 1 | 1.0336 ± 0.0470 | 103.4 | 4.6 |
| γ-Schisandrin | | | | |
| intra-day | 0.02 | 0.0181 ± 0.0013 | 90.7 | 7.2 |
| | 0.2 | 0.2004 ± 0.0119 | 100.2 | 5.9 |
| | 1 | 1.0150 ± 0.0529 | 101.5 | 5.2 |
| inter-day | 0.02 | 0.0214 ± 0.0026 | 107.2 | 12.1 |
| | 0.2 | 0.2044 ± 0.0135 | 102.2 | 6.6 |
| | 1 | 1.0056 ± 0.0646 | 100.6 | 6.4 |

Table 3
Stability of Schisandrin, Schisantherin A, Deoxyshisandrin and γ-Schisandrin in rat plasma ($n = 5$)

| Compound | Nominal conc. (μg/mL) | Three freeze/thaw cycles | | Room temperature (12 h) | | Stored at −80 °C for 1 month | |
|---|---|---|---|---|---|---|---|
| | | Measured conc. (μg/mL) | Accuracy (%) | Measured conc. (μg/mL) | Accuracy (%) | Measured conc. (μg/mL) | Accuracy (%) |
| Schisandrin | 0.02 | 0.017 ± 0.002 | 87 | 0.019 ± 0.002 | 92.8 | 0.018 ± 0.003 | 91.5 |
| | 0.2 | 0.195 ± 0.009 | 97.6 | 0.190 ± 0.011 | 95.3 | 0.205 ± 0.018 | 102.5 |
| | 1 | 0.983 ± 0.034 | 98.3 | 0.983 ± 0.028 | 98.3 | 1.003 ± 0.036 | 100.3 |
| Schisantherin A | 0.02 | 0.017 ± 0.003 | 86.5 | 0.018 ± 0.001 | 90.5 | 0.018 ± 0.003 | 92.1 |
| | 0.2 | 0.192 ± 0.013 | 95.8 | 0.190 ± 0.016 | 94.9 | 0.192 ± 0.021 | 96.2 |
| | 1 | 0.953 ± 0.019 | 95.3 | 1.042 ± 0.032 | 104.2 | 0.922 ± 0.029 | 92.2 |
| Deoxyshisandrin | 0.02 | 0.019 ± 0.001 | 93.1 | 0.017 ± 0.002 | 86 | 0.018 ± 0.003 | 91.8 |
| | 0.2 | 0.185 ± 0.008 | 92.5 | 0.187 ± 0.019 | 93.5 | 0.192 ± 0.007 | 96.2 |
| | 1 | 1.005 ± 0.021 | 100.5 | 0.993 ± 0.051 | 99.3 | 1.038 ± 0.026 | 103.8 |
| γ-Schisandrin | 0.02 | 0.019 ± 0.003 | 93.4 | 0.019 ± 0.002 | 92.4 | 0.018 ± 0.002 | 92.3 |
| | 0.2 | 0.192 ± 0.012 | 95.8 | 0.187 ± 0.006 | 93.6 | 0.185 ± 0.010 | 92.7 |
| | 1 | 0.953 ± 0.033 | 95.3 | 1.033 ± 0.014 | 103.3 | 0.964 ± 0.023 | 96.4 |

Accuracy (%): Measured conc./nominal conc. ×100%.

chromatographic run time was finished within 15 min. The retention time is short, thus suitable for the high number of samples determined in the pharmacokinetic study.

### 3.2. Recovery

The recovery from each lignan compound in rat plasma was 80.2–90.3% for schisandrin, 84.1–95.1% for schisantherin A, 89.6–94.3% for deoxyshisandrin and 81.3–96.5% for γ-schisandrin at final concentration from 0.02 to 1 μg/mL ($n = 5$). Ion suppression was investigated and was not obvious in the assay.

### 3.3. Calibration curves

The calibration curves generated from detection of rat plasma containing known amounts of the four compounds were linear over the quantities ranges from 0.010 to 2.0 μg/mL as shown in Table 1. The correlation coefficient ($r^2$) for each of these calibration curves was over 0.99, indicating a good linear detector response over the 200-fold dynamic range that was investigated. The limit of detection (LOD) of four analytes were 0.005 μg/mL (50 pg on column) with a signal-to-noise (S/N) ratio of at least 3, while the lower limit of quantitation (LLOQ) were 0.010 μg/mL (100 pg on column) with a signal-to-noise (S/N) ratio of at least 10. This sensitivity has proven useful in the analysis of pharmacokinetic data of rats treated orally.

### 3.4. Precision and accuracy of the detection method

Analytical accuracy and precision data are shown in Table 2. The reproducibility of the method was defined by examining

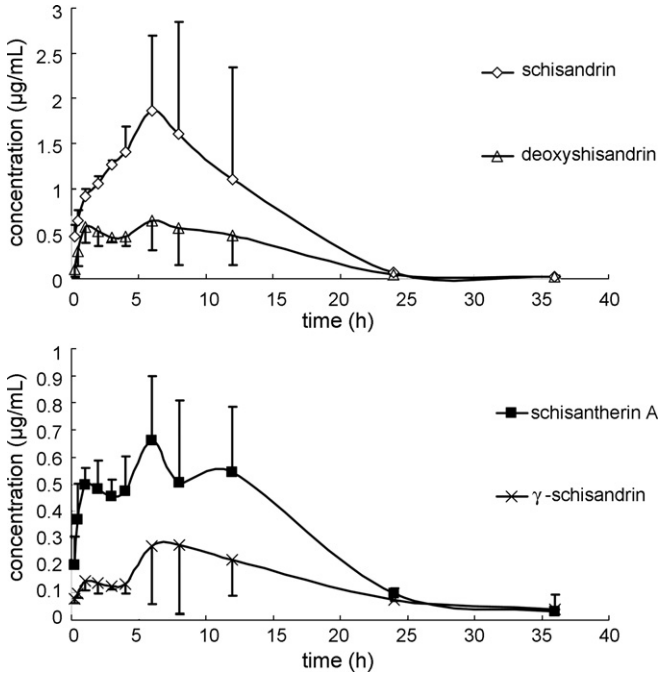



Fig. 4. Plasma concentration–time curves of the four lignans after oral administration of commercial extraction of *Schisandra chinensis* at 1.5 g/kg to rats. Each point represents the mean ± S.D. of 5 rats.

Table 4
Mean pharmacokinetic parameters of the four schisandra lignans after oral administration of commercial extraction of *Schisandra chinensis* at 1.5 g/kg to rats ($n = 5$)

| Parameter | Schisandrin | Schisantherin A | Deoxyshisandrin | γ-Schisandrin |
|---|---|---|---|---|
| $t_{1/2}$ (h) | 4.46 | 6.41 | 13.20 | 4.68 |
| $C_{max}$ (mg/L) | 1.865 | 0.662 | 0.650 | 0.276 |
| $T_{max}$ (h) | 6 | 6 | 6 | 8 |
| $AUC_{(0\text{-}t)}$ (mg/L*h) | 23.77 | 12.28 | 9.76 | 4.92 |
| $MRT_{(0\text{-}t)}$ (h) | 8.69 | 12.33 | 9.40 | 12.82 |
| Vz/F (L/kg) | 434.44 | 1486.20 | 1405.09 | 3897.36 |
| CLz/F (L/h/kg) | 62.64 | 114.70 | 149.77 | 273.22 |

both intra- and inter-day variance. The intra-day and inter-day coefficients of variance (CVs) of four analytes at low-to-high concentrations were less than 12.5%. Assay accuracy was found to range from 87.5 to 110.4%. These low CVs indicate that the LC/MS method was reliable and reproducible for the quantitative analysis of four lignan compounds in plasma samples.

### 3.5. Stability studies

All stability results are shown in Table 3. Four analytes were stable for at least 12 h at room temperature in plasma samples; the mean recoveries from the nominal concentration were more than 85% at the concentration range from 0.02 to 1 μg/mL. Three freeze–thaw cycles of the QC samples appeared to have no effect on quantification of the analytes. Test compounds remained stable when stored at −80 °C for a 1-month period with their mean recoveries from the nominal concentration over 90%.

### 3.6. Pharmacokinetic study of four lignans in rats

The utility of LC/MS assay for the quantitative analysis of four schisandra lignans in rat plasma in support of *in vivo* studies was demonstrated. The mean plasma concentration versus time profiles of four lignans are shown in Fig. 4 and the main pharmacokinetic parameters assessed using the noncompartmental method are summarized in Table 4. After a single oral dosage, the rat plasma concentrations of four lignans were relatively high at 0.25 h which suggested the entrance of four compounds into plasma was fast. Through comparing $C_{max}$ and AUC, the bioavailability of schisandrin was higher than other three analytes while γ-schisandrin was the poorest one. The results also revealed that the distribution and elimination processes of four lignans might be slow indicated by longer $T_{max}$ (6–8 h) and MRT (about 10 h). The above information should be useful for further studies on pharmacokinetics, pharmacy and toxicity of traditional Chinese medicine *Schisandra chinensis* and in support of studies to determine the efficacy of this TCM in clinical therapeutic researches.

In conclusion, a LC/MS method has been developed which showed excellent sensitivity, good linearity of response, and high precision for the simultaneous quantification of schisandrin, schisantherin A, deoxyshisandrin and γ-schisandrin in plasma and successfully applied to the preliminary pharmacokinetic study of four major active schisandra lignans following oral administration of commercial dried extraction of traditional Chinese medicine *Schisandra chinensis* to rats.

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