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Journal of Pharmaceutical and Biomedical Analysis 46 (2008) 121–126
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Determination of deoxyschizandrin in rat plasma by LC–MS

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Received 5 July 2007; received in revised form 26 September 2007; accepted 3 October 2007
Available online 11 October 2007

Abstract

A simple, rapid and sensitive LC–MS method was developed for quantification of deoxyschizandrin in rat plasma. A 50 μl plasma sample was extracted by ether and performed on Elite Hypersil C_{18} column (200 mm × 4.6 mm, 5 μm) with the mobile phase of methanol–water (84:16, v/v) in a run time of 6.5 min. The analyte was monitored with positive atmospheric pressure chemical ionization (APCI) by selected ion monitoring (SIM) mode. A good linear relationship was obtained over the range of 1.0–50.0 ng/ml and the validated method was successfully applied for the pharmacokinetic studies of deoxyschizandrin in rat. After oral administration of 4 mg/kg deoxyschizandrin and *Schisandra* extract which contained the same dose of deoxyschizandrin to male rats, the C_{max} of deoxyschizandrin were 15.8 ± 3.1 and 34.3 ± 16.8 ng/ml, T_{max} were 0.51 ± 0.13 and 3.83 ± 1.83 h, $T_{1/2}$ were 5.3 ± 2.2 and 6.5 ± 3.4 h.

Keywords: Deoxyschizandrin; *Schisandra*; Rat plasma; Pharmacokinetic; LC–MS

1. Introduction

Schisandra chinensis (Turcz.) Baill., a well-known traditional Chinese medicine, is a common ingredient in prescriptions and could also be used alone as a tonic or sedative. Lignans are major active compounds of *S. chinensis* [1–4], and among them deoxyschizandrin (Fig. 1) is the most important one which provides hepatoprotection [5,6], antioxidation [7–9] and antitumor [10] activities. The pharmacokinetic studies of deoxyschizandrin could be helpful for the reasonable usage of *Schisandra* and its preparations. However, there is little report on the pharmacokinetic studies of deoxyschizandrin because of its low concentration in plasma. While the high performance liquid chromatography coupled with mass spectrometry (LC–MS) is widely used recent years, the studies of deoxyschizandrin *in vivo* become possible [11]. The LC–MS method takes the advantages of good specification, short analytical time, low limit of detection and less biological samples used. In this paper, we applied the sensitive LC–MS method successfully for the pharmacokinetic studies of deoxyschizandrin *in vivo* [12].

2. Experimental

2.1. Materials

S. chinensis (Turcz.) Baill. was purchased from the TCM shop of Tianyitang (Shenyang, China). Deoxyschizandrin (purity >95%) was separated from *S. chinensis* (Turcz.) Baill. by the author, and the structure was validated by comparing the chemical and spectroscopic (UV, NMR and MS) data with those reported in literatures [13,14]. Dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylene dioybiphenyl-2,2′-diearboxylate (DDB, Fig. 1), which was used as the internal standard (IS), was purchased from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). It was a synthetic compound derived from schizandrin C, a component processing a similar structure to deoxyschizandrin in *S. chinensis*. Methanol (Dikma Company, USA) was of HPLC grade and water was double distilled. Ether of analytical grade was commercially available.

2.2. Animals

Male Sprague–Dawley rats weighing 180–220 g were provided by the Experimental Animal Center of Shenyang Pharmaceutical University. Animal experiments were conducted

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doi:10.1016/j.jpba.2007.10.006

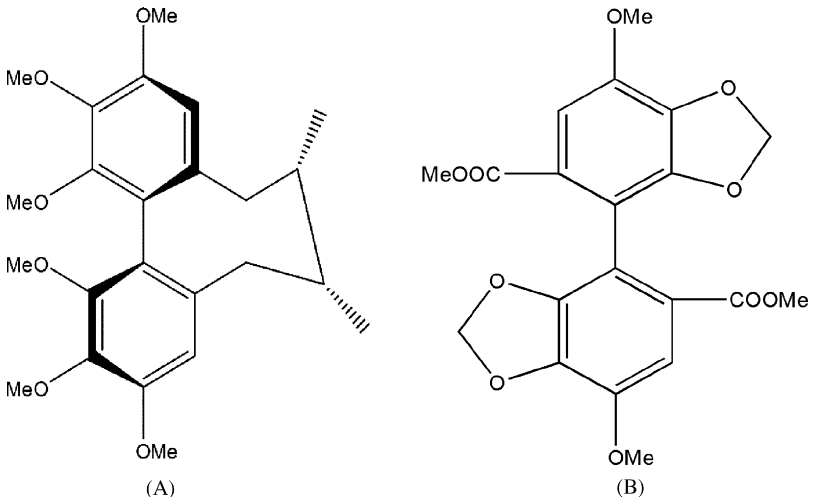

Fig. 1. Chemical structures of deoxyschizandrin (A) and IS (B).

in accordance with the Guidelines for Animal Experimentation of Shenyang Pharmaceutical University (Shenyang, China) and the procedure was approved by the Animal Ethics Committee of this institute.

2.3. LC–MS instruments and conditions

The assay was performed on Shimadzu (Japan) LC–MS 2010A system. Liquid chromatographic separations were achieved by Elite Hypersil C_{18} column (200 mm × 4.6 mm, 5 μm) at room temperature with the mobile phase of methanol–water (84:16, v/v) at a flow rate of 0.8 ml/min in a run time of 6.5 min. The injection volume was 10 μl. The analyte and IS were ionized by APCI source in positive ion mode under the following source conditions: nebulizing gas, 2.5 l/min; drying gas, 2.0 l/min; CDL temperature, 250 °C; heat block temperature, 200 °C; interface temperature, 400 °C; the other parameters were fixed as the tuning file. Analysis was carried out by SIM for deoxyschizandrin $[M+H]^+$ *m/z* 417.15 and IS $[M+H]^+$ *m/z* 419.0.

2.4. Sample preparation

Plasma samples (50 μl) were spiked with 50 μl IS (10 ng/ml, dissolved in methanol). After drying with nitrogen, 200 μl of water was added and then the samples were extracted with 1 ml double-distilled ether, respectively. The organic layer was transferred to another tube and evaporated to dryness at 40 °C under a stream of nitrogen. The residue was reconstituted in 100 μl of methanol and 10 μl was injected.

2.5. Bioanalytical method validation

The method validation was conducted by the Guidance for Industry Bioanalytical Method Validation.

2.5.1. Specificity

The specificity of this method was examined by analyzing blank rat plasma extract and an extract spiked with deoxyschizandrin and IS.

2.5.2. Calibration and sensitivity

The working solutions of deoxyschizandrin were prepared in methanol. Rat plasma calibration standards of deoxyschizandrin were prepared by adding 50 μl working solution into 50 μl free plasma. The concentrations of deoxyschizandrin in rat plasma were 1.0, 2.0, 5.0, 10.0, 25.0 and 50.0 ng/ml, and the lower limit of quantitation (LLOQ) was 1.0 ng/ml. The samples were dealt with the method "2.4 Sample preparations" and injected into HPLC. Linearity was assessed by a weighted $(1/x^2)$ least squares regression analysis. The calibration curve had to have a correlation coefficient $r > 0.99$. The acceptable criterion for each standard concentration was ±15%, except for LLOQ which was ±20%.

2.5.3. Accuracy and precision

The accuracy and precision of the assay were evaluated by quality control (QC) samples of three concentrations (2.0, 10.0 and 40.0 ng/ml) which were prepared in the same way as calibration standards. The within-batch precision and accuracy were determined by analyzing five sets of quality control samples of three concentrations in a batch. The between-batch precision and accuracy were determined by analyzing three sets of quality control samples in five different batches. The concentrations of these samples were determined by using a calibration curve prepared with each batch. The relative standard deviation (R.S.D.) was calculated by SPSS 11.5 (Statistical Package for the Social Science).

2.5.4. Recovery

Recovery of deoxyschizandrin in the extract procedure was determined by comparison of the peak area between samples spiked with deoxyschizandrin (2.0, 10.0 and 40.0 ng/ml) at the beginning and drug-free extract spiked with deoxyschizandrin at the step just prior to chromatography. Recovery of IS was determined in the same way.

2.5.5. Stability

Three replicates of QC samples at each of 2.0, 10.0 and 40.0 ng/ml concentrations were used to evaluate the stability

of the analyte in rat plasma after three freeze–thaw cycles and the 24 h post-preparative stability at room temperature, as well as the long-term stability when the QC samples were stored at −20 °C for 15 days. Samples were to be concluded stable if the average deviation was within ±15% of the actual value.

2.6. Application of the assay

The method was applied for the pharmacokinetic studies after oral administration of 4 mg/kg deoxyschizandrin and the extract of *S. chinensis* (equal to 4 mg/kg deoxyschizandrin) to rats. The deoxyschizandrin concentration of *Schisandra* extract had been evaluated by the HPLC method. Animals were fasted 12 h before dosing and 4 h afterwards with free access to water. Both of the solutions were prepared by 7% ethanol. Venous blood samples were withdrawn to the heparinized tubes by eye puncture at 0, 0.08, 0.16, 0.33, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 h, and centrifuged at 16,000 rpm for 5 min immediately. The plasma samples were stored at −20 °C.

The plasma concentrations of deoxyzchizandrin at different time were expressed as mean ± S.D. and the concentration versus time curve was plotted. All the data was calculated by DAS 2.0 statistical software (Pharmacology Institute of China).

3. Results and discussion

3.1. Mass spectra analysis

The full scan mass spectrum of deoxyschizandrin and IS after direct injection in mobile phase are shown in Fig. 2. The most sensitive ions were $[M+H]^+$, so the quantitative analysis was carried out by SIM for deoxyschizandrin *m/z* 417.15 and IS *m/z* 419.0.

The limit of detection in the analytical method studies of deoxyschizandrin, such as HPLC-UV and TLC, was higher than 100 ng/ml [15,16]. So even increasing the dose or more plasma used, the deoxyschizandrin in plasma could not be detected. However, deoxyschizandrin of 1 ng/ml had been easily quantitated by LC–MS with only 50 μl plasma used. Therefore, the method described here would facilitate the study of deoxyschizandrin *in vivo*.

3.2. Method validation

3.2.1. Specificity

The chromatograms for blank plasma, blank plasma spiked with deoxyschizandrin and IS, as well as the rat plasma samples

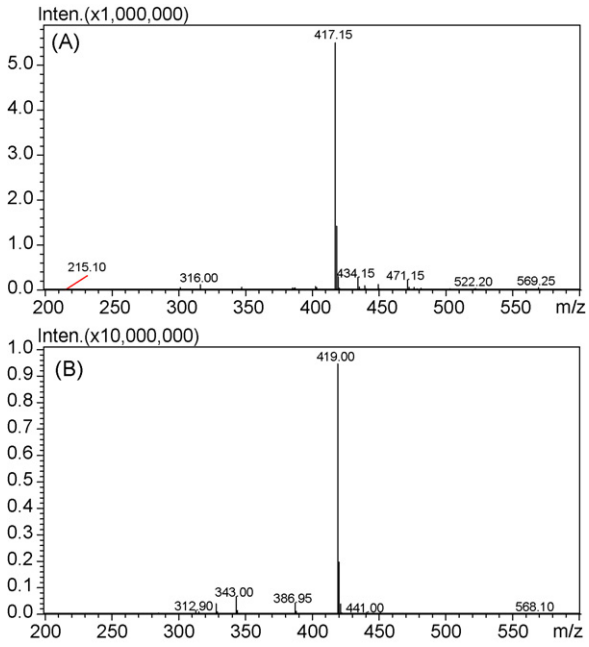

Fig. 2. Full scan mass spectrum of deoxyschizandrin (A) and IS (B).

are presented in Fig. 3, and no interference was observed at the retention time of the analyte 5.9 min and IS 3.2 min due to endogenous substances in drug-free rat plasma.

3.2.2. Linearity

The linear regression analysis of deoxyschizandrin was constructed by plotting the peak-area ratio of deoxyschizandrin to IS (y) versus analyte concentration in rat plasma (x). The linear range of the curve was 1.0–50.0 ng/ml, and the LLOQ was 1.0 ng/ml. Representative calibration curve was as follows: $y = 0.06680x + 0.00641$ ($r = 0.9975$, weighting factor: $1/x^2$). The $1/x^2$ weighting yielded better accuracy at lower standard levels [17].

3.2.3. Precision and recovery

The within-batch and between-batch precision and accuracy (presented as R.S.D.) are shown in Table 1. All the results of the tested samples were within the acceptable criteria of ±15%.

The absolute recoveries of deoxyschizandrin at three concentration levels (2.0, 10.0 and 40.0 ng/ml) were 78.3%, 75.9% and 75.9%, respectively, and the absolute recovery of IS was 81.3%.

Table 1
Precision and accuracy of deoxyschizandrin in rat plasma

Spiked concentration (ng/ml)	Within-batch precision ($n = 5$)			Between-batch precision ($n = 15$)		
	2.0	10.0	40.0	2.0	10.0	40.0
Measured concentration (ng/ml)	1.8 ± 0.1	10.1 ± 0.9	39.3 ± 1.4	2.0 ± 0.1	10.2 ± 0.6	41.2 ± 1.1
Accuracy (%)	97.1 ± 7.8	101.9 ± 10.0	99.2 ± 5.2	101.8 ± 8.5	101.0 ± 9.3	101.0 ± 4.2
R.S.D. (%)	5.5	8.6	3.6	6.3	5.9	2.7

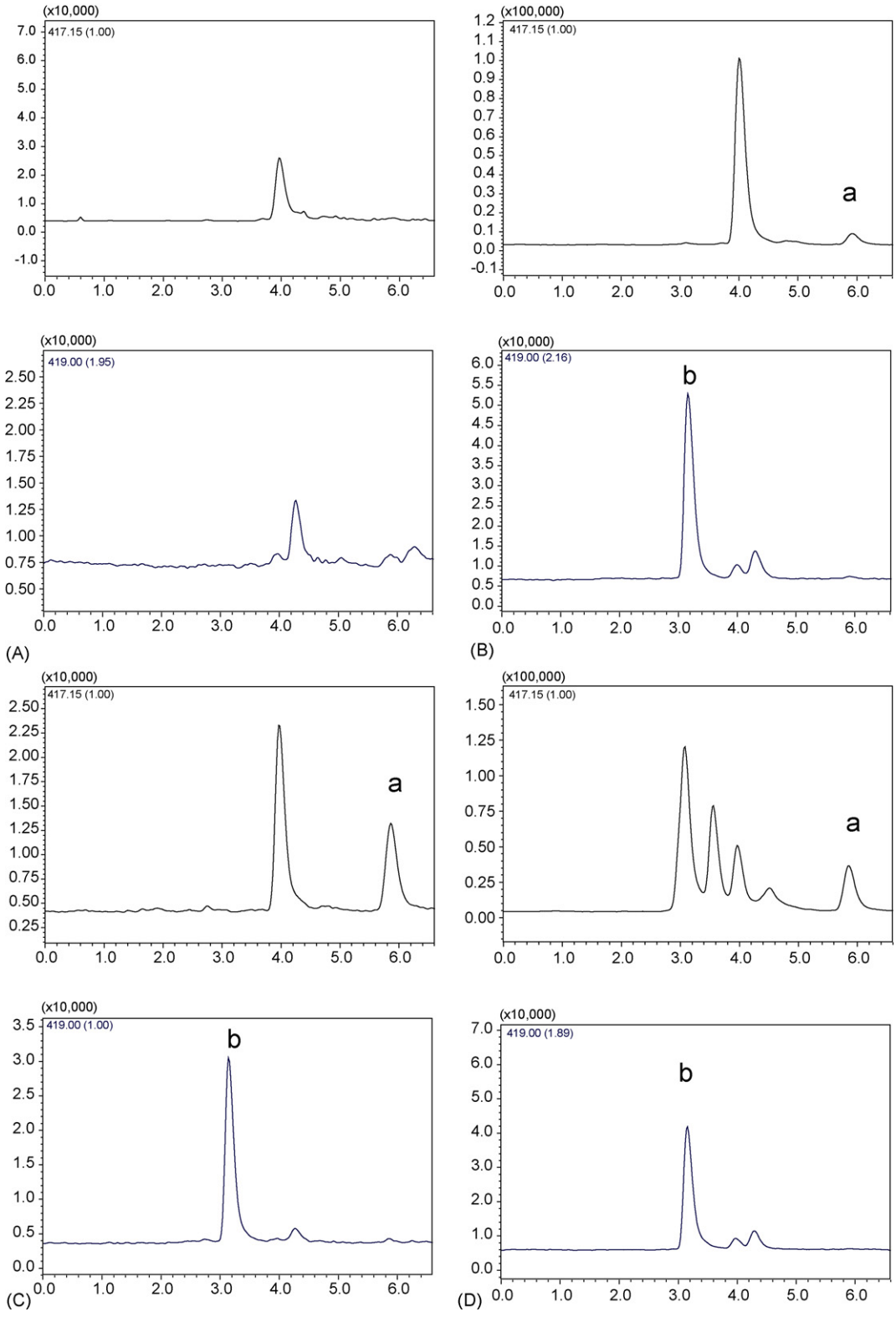

Fig. 3. SIM chromatograms of a blank rat plasma (A), a blank rat plasma spiked with deoxyschizandrin (2 ng/ml) and IS (B), a rat plasma sample 0.5 h after oral administration of deoxyschizandrin (C), and a rat plasma sample 0.5 h after oral administration of *Schisandra chinensis* extract (D)—a: deoxyschizandrin; b: IS.

3.2.4. Stability

It was shown that the rat plasma samples were stable after three freeze–thaw cycles and at the 24 h post-preparative condition. The study also indicated that the plasma samples could be stored at −20 °C for at least 15 days (Table 2).

3.2.5. Pharmacokinetic study of deoxyschizandrin in rats

In the study we compared the pharmacokinetic parameters of deoxyschizandrin in rats which were administered deoxyschizandrin and *S. chinensis* extract with the same dose of deoxyschizandrin. Figs. 4 and 5 show the mean

Table 2
Stability of deoxyschizandrin in rat plasma

Measured concentration (ng/ml)	Spiked concentration (ng/ml)		
	2.0	10.0	40.0
Freeze and thaw stability			
Mean ± S.D.	1.9 ± 0.1	10.4 ± 0.2	39.5 ± 1.8
R.S.D. (%)	5.8	1.7	4.5
Post-preparative stability (24 h at room temperature)			
Mean ± S.D.	2.2 ± 0.1	10.4 ± 0.4	38.4 ± 4.7
R.S.D. (%)	5.3	3.9	12.3
Stability for 15 days at −20 °C			
Mean ± S.D.	1.9 ± 0.2	9.6 ± 1.4	38.4 ± 1.9
R.S.D. (%)	10.7	14.4	4.8

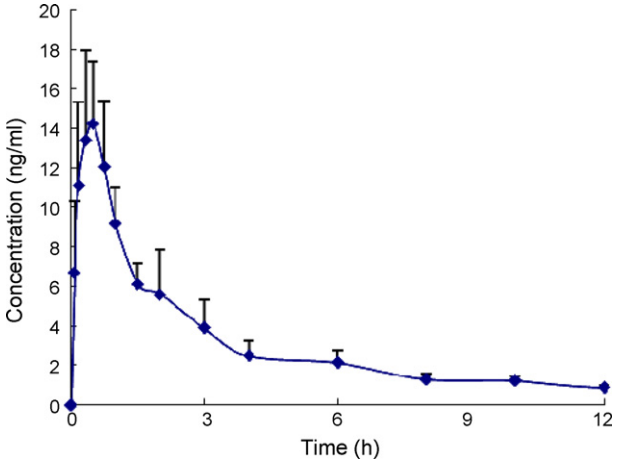

Fig. 4. Mean plasma deoxyschizandrin concentrations vs. time profile in six rats after oral administration of deoxyschizandrin (4 mg/kg).

deoxyschizandrin plasma concentrations versus time profile in rat with the LC–MS method. The pharmacokinetic parameters of deoxyschizandrin in rats are shown in Table 3.

After oral administration to rats with the same dose of deoxyschizandrin, the T_{max}, C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ had

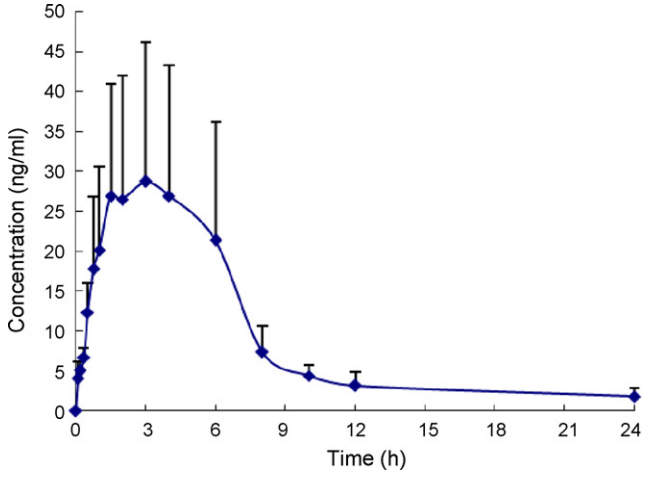

Fig. 5. Mean plasma deoxyschizandrin concentrations vs. time profile in six rats after oral administration of *S. chinensis* extract containing deoxyschizandrin (4 mg/kg).

Table 3
The pharmacokinetic parameters of deoxyschizandrin in rat plasma after orally administered deoxyschizandrin (4 mg/kg) and *Schisandra chinensis* extract containing the same dose of deoxyschizandrin

Parameter	Deoxyschizandrin (mean ± S.D., $n = 6$)	*Schisandra* extract (mean ± S.D., $n = 6$)
$T_{1/2}$ (h)	5.3 ± 2.2	6.5 ± 3.4
k_e (h^{-1})	0.2 ± 0.1	0.1 ± 0.1
T_{max} (h)	0.5 ± 0.1	3.8 ± 1.3
C_{max} (ng/ml)	15.8 ± 3.1	34.3 ± 18.6
AUC_{0-t} (ng h/ml)	37.0 ± 6.7	212.4 ± 106.2
$AUC_{0-\infty}$ (ng h/ml)	44.8 ± 7.2	230.3 ± 119.5

significant differences between deoxyschizandrin alone and deoxyschizandrin in *Schisandra* extract. These differences could be caused by the following reasons: (1) other ingredients extracted from *S. chinensis* facilitated and prolonged the absorption procedure of deoxyschizandrin, and thus increased the bioavailability of deoxyschizandrin; (2) recent study of my laboratory showed that deoxyschizandrin was mainly metabolized by the CYP3A (unpublished data), and many ingredients in *Schisandra* were the inhibitors of CYP3A as the report showed [18], so the metabolism procedure of deoxyschizandrin in *Schisandra* extract might be inhibited and thus its concentration was increased; (3) other ingredients were metabolized into compounds with the same *m/z* 417.15 as deoxyschizandrin. The possible reasons above should be confirmed by further studies. The $T_{1/2}$ of deoxyschizandrin in *Schisandra* extract was a little longer than deoxyschizandrin alone, but did not have significant difference, suggesting that other ingredients had little effect on the elimination of deoxyschizandrin.

4. Conclusion

A simple, rapid and sensitive LC–MS method has been developed and applied successfully for the pharmacokinetic studies of deoxyschizandrin in rat plasma. It offers significant advantages of lower sample volume requirement, higher sensitivity and selectivity. It is the first report of analytical method on the determination of deoxyschizandrin *in vivo* and also provides necessary information for the further studies of *Schisandra*.

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