Journal of Pharmaceutical and Biomedical Analysis 52 (2010) 741–746

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Simultaneous determination of five lignan constituents of *Wuzhi* capsule in rat plasma by LC–MS/MS: Application to pharmacokinetic study

Hua Wei [a], Wen Xu [a], Fei Cai [a], Gang Zhao [a], Jin Feng [a], Lianna Sun [b], Wansheng Chen [a,*]

[a] Department of Pharmacy, Changzheng Hospital, Second Military Medical University, No. 415 Fengyang Road, Shanghai 200003, PR China
[b] Department of Pharmacognosy, School of Pharmacy, Second Military Medical University, No. 325 Guohe Road, Shanghai 200433, PR China

ARTICLE INFO

Article history:
Received 8 October 2009
Received in revised form 29 January 2010
Accepted 3 February 2010
Available online 10 February 2010

Keywords:
Liquid chromatography–tandem mass spectrometry
Wuzhi capsule
Schisandra sphenanthera
Pharmacokinetic
Rat plasma

ABSTRACT

A rapid sensitive and selective liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was developed for simultaneous determination of multiple bioactive lignan constituents of *Wuzhi* capsule in rat plasma. The extraction, separation, and analytical conditions were optimized. Five constituents of the *Wuzhi* capsule (schisandrin, schisandrol B, schisantherin A, schisanhenol, and deoxyshisandrin) were determined by the LC–MS/MS method. Liquid–liquid extraction with methyl tert-butyl ether was carried out using bifendate as the internal standard. The five bioactive constituents were separated on a Zorbax SB-C18 reserved-phase column (100 mm × 2.1 mm i.d., 3.5 μm) by isocratic elution using a mobile phase consisting of acetonitrile, methanol, and 0.1% aqueous formic acid (72:18:10, v/v/v) at a flow rate of 0.3 mL/min. The total run time was only 3.5 min. All analytes showed good linearity over a wide concentration range ($r^2 > 0.99$) and their lower limit of quantification was 0.5 ng/mL. The average extraction recovery of the five analytes from rat plasma was more than 85%, and the intra-day and inter-day accuracy and precision of the assay were less than 15%. Our method was successfully used for pharmacokinetic study of the five components in the *Wuzhi* capsule.

1. Introduction

The ripe fruits of *Schisandra sphenanthera*, known as "*Nan-wuweizi*" in Chinese folk medicine, have long been used as antitussive agents and tonics [1]. Traditional Chinese medicines (TCMs) containing *Nan-wuweizi* are available in the form of tablets, pills, and syrup. Investigations have revealed that the schisandra lignans, including deoxyshisandrin, schisantherin A, and schisanhenol with a dibenzocyclooctadiene skeleton, are the principal active constituents of *Nan-wuweizi*, and they have liver-protective, anti-inflammatory, anti-oxidant, anti-tumor and anti-HIV activities [2]. The *Wuzhi* capsule (WZC), an ethanol extract from the ripe fruits of *S. sphenanthera*, is widely used as a hepar-protecting and enzyme-decreasing drug. Recently WZC has been increasingly used in combination with other drugs. Occasionally, WZC was found to markedly increase the blood concentration of other drugs or to influence their absorption [3–5]. A reliable pharmacokinetic study of WZC is therefore very important for optimizing the dosage of WZC and developing better combination therapies.

There have been increasing reports on pharmacokinetics of bioactive lignan constituents in *Nan-wuweizi*. However, the reported methods still have room for improvement. First, the protein precipitation extraction [9] or solid-phase extraction (SPE) [2] protocol described in the literatures could not achieve high extraction recoveries of the five lignans from rat plasma. Second, previous methods were based on thin-layer chromatography (TLC) [5,7,8], high-performance liquid chromatography (HPLC) [8–10], nuclear magnetic resonance (NMR) [11], micellar electrokinetic capillary chromatography (MEKC) [12], or gas chromatography coupled with mass spectrometry (GC/MS) [13,14]. Compared with them, the LC–MS is widely use for analyzing biological samples; it has the advantages of good specification, short analytical time, low limit of detection and requirement of less biological samples. Although there have been reports [2,6,15,16] on LC–MS/MS methods, but they mainly used the selected ion monitoring (SIM) mode rather than the multiple-reaction-monitoring (MRM) mode. During SIM, the MS analysis time is focused only on analytes of specific masses. MRM is one of the most sensitive approaches for quantification of known analytes. An essential feature of MRM is that both the parent ion and one or more transitional product masses are known. This approach is particularly powerful for analyzing complex drug metabolic pathways in which both the parent drug and multiple metabolites can be monitored simultaneously with high sensitivity and precision [17].

In the present study, we, for the first time, developed and validated a sensitive and rapid LC–MS/MS method in MRM mode for the simultaneous determination of the five bioactive

* Corresponding author. Tel.: +86 21 81886181; fax: +86 21 33100038.
E-mail addresses: chenws@vent.citiz.net, chenwanshengchzh@yahoo.com.cn (W. Chen).

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.02.003

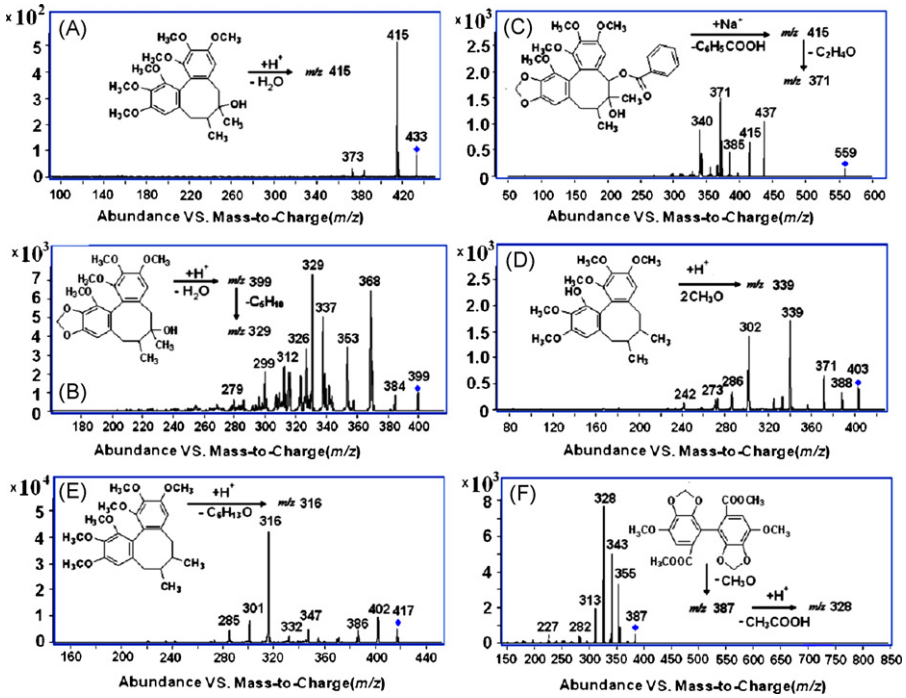

Fig. 1. Chemical structures and full scan product ion of precursor ions of schisandrin (A), schisandrol B (B), schisantherin A (C), schisanhenol (D), deoxyshisandrin (E) and bifendate (F; IS).

lignan constituents in WZC: schisandrin (A), schisandrol B (B), schisantherin A (C), schisanhenol (D), and deoxyshisandrin (E) in rat plasma using bifendate (DDB) as the internal standard (IS) (Fig. 1). The method was applied to pharmacokinetic study.

2. Experimental

2.1. Chemicals and reagents

WZC (batch no. 070601) were purchased from Hezheng Pharmaceutical Company, Chengdu, China. Reference compounds, including schisandrin, schisandrol B, schisantherin A, and deoxyshisandrin, were purchased from Shanghai R&D Center for Standardization of Traditional Chinese Medicines, Shanghai, China (purity >98%). Schisanhenol (purity 99%) was isolated and purified from the ripe fruits of *Schisandra chinensis* by Prof. Daofeng Chen from the Department of Pharmacognosy, Fudan University School of Pharmacy, Shanghai, China. The internal standard (IS) bifendate (DDB) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products of China (Beijing, China). HPLC-grade acetonitrile and methanol were purchased from Merck Company (Darmstadt, Germany). HPLC-grade formic acid was purchased from Tedia Company Inc. (Fairfield, USA). All other reagents were of analytical grade.

2.2. Chromatographic conditions

Separation was performed by a Zorbax SB-C18 reserved-phase column (100 mm × 2.1 mm i.d., 3.5 μm) with an mobile phase consisting of acetonitrile, methanol and water–0.1% formic acid (72:18:10, v/v/v) at a flow rate of 0.3 mL/min. The column temperature was maintained at 35 °C, and the injection volume was 10 μL.

2.3. Mass spectrometric condition

An Agilent 6410A triple quadrupole LC–MS system (Agilent Corporation, MA, USA) was used. Ionization was achieved using electrospray in the positive mode with the spray voltage set at 4000 V. Nitrogen was used as nebulizer gas and nebulizer pressure was set at 40 psi with a source temperature of 105 °C. Desolvation gas (nitrogen) was heated to 350 °C and delivered at a flow rate of 10 L/min. For collision-induced dissociation (CID), high purity nitrogen was used as collision gas at a pressure of about 0.1 MPa. The system was controlled by MassHunter software (Agilent Corporation, MA, USA). Table 1 shows the optimized MRM parameters for the detected drugs and IS. The peak widths of precursor and product ions were maintained at 0.7 amu at half-height in the MRM mode.

2.4. Preparation of standard and quality control (QC) samples

The standard stock solution (100 μg/mL) of the five constituents were prepared by dissolving appropriate amount of the chemical reference substance in methanol. The stock solutions of schisandrin and schisantherin A were further diluted with water/acetonitrile (90:10, v/v) to obtain standard working solutions at concentrations of 2.50, 5.00, 25.0, 50.0, 250.0, 500.0, and 1000 ng/mL. The stock solutions of schisandrol B, schisanhenol, and deoxyshisandrin were further diluted with water/acetonitrile (90:10, v/v) to obtain standard working solutions at concentrations of 2.50, 5.00, 10.0, 25.0, 50.0, 100, and 250 ng/mL. Quality control samples were prepared in the same way. Internal standard working solution (100 ng/mL) was prepared by diluting the stock solution of bifendate with water/acetonitrile (90:10, v/v).

2.5. Sample preparation

100 μL plasma sample was transferred to a 10 mL centrifuge tube together with 20 μL IS solution and 20 μL water/acetonitrile (90:10, v/v). After vortex shaking for 30 s, 3 mL methyl tert-butyl ether was added. The analyte and IS were extracted from plasma by

Table 1
Optimized multiple-reaction-monitoring (MRM) parameters for schisandrin, schisandrol B, schisantherin A, schisanhenol, deoxyshisandrin and bifendate(IS).

Components	Precursor ion	Fragmentor energy (V)	Collision energy (eV)	Product ion
Schisandrin	433	100	7	415
Schisandrol B	399	100	26	329
Schisantherin A	559	160	27	371
Schisanhenol	403	150	23	339
Deoxyshisandrin	417	150	25	316
Bifendate	387	145	16	328

vortexing for 2 min. Then the sample was centrifuged at 3000 × *g* for 10 min. The organic layer was quantitatively transferred to a 5 mL glass tube and evaporated to dryness in an evaporator at 35 °C. Then, the dried extract was reconstituted in 100 μL solvent (water–acetonitrile, 20:80, v/v), followed by injection of 10 μL aliquot into LC–MS/MS.

2.6. Method validation

2.6.1. Selectivity

Six pre-dose plasma samples from different rats were used to evaluate the specificity. Blank samples were extracted and analyzed by LC–MS/MS for potential interfering peaks within the range of the retention time of each analyte.

2.6.2. Linearity of calibration curves

Calibration curves ranging from 0.5 to 200 ng/mL for each analytes were run on three separate days, and constructed from the peak-area ratios of each analyte to IS versus plasma concentrations using a $1/x^2$ weighted linear least-squares regression model.

2.6.3. Extraction recovery and matrix effect

Extraction recovery of the five analytes was determined at three QC levels by comparing peak areas obtained from plasma samples to those found by direct injection of a standard solution of the same concentration. To develop a reliable and reproducible method, the matrix effect was evaluated by the following experiment: Triplicates of QC samples at three levels of the five analytes and IS were added into 0.1 mL pre-extracted blank rat plasma and water separately, and then the spiked samples were pretreated with exactly the same procedure as described in Section 2.5.

2.6.4. Precision and accuracy

Six replicates of QC samples of each analyte at three levels were included in each run to determine the intra- and inter-day precision of the assay. Accuracy was determined as the percentage difference between the mean concentrations detected and the nominal concentrations. The lower limit of quantification (LLOQ) is defined as the lowest concentration of standard that can be measured with an acceptable accuracy and precision (≤20% for both parameters).

2.6.5. Stability

Stability of the five analytes in plasma was assessed by analyzing triplicate QC samples stored for 24 h at ambient temperatures, three cycles of freezing at −20 °C and thawing and stored for one month at −20 °C, respectively. The stability of the five analytes and IS in reconstituted extract was also assessed at room temperature for 24 h. Concentrations following storage were compared with freshly prepared samples of the same concentrations.

2.7. Pharmacokinetic study

Male Sprague–Dawley rats (about nine weeks old, weighting 250 ± 20 g) were acclimated for 5 days under environmental conditions at 20–25 °C, 55 ± 15% relative humidity and a 12-h light/dark cycle, and deprived of food for 12 h with water *ad libitum* before the experiment. The experimental protocols were approved by the Animal Care and Use Committee of the Second Military Medical University. Six rats received an intragastric administration of 150 mg/kg WZC (equivalent to 21.51 μg/kg of schisandrin, 14.62 μg/kg of schisandrol B, 868.21 μg/kg of schisantherin A, 94.59 μg/kg of schisanhenol, and 895.45 μg/kg of deoxyshisandrin). The drug was suspended in 0.5% carboxymethyl cellulose sodium (CMC-Na) (w/v). Blood samples (about 250 μL) were collected in heparinized tubes via the postorbital venous plexus veins from each rat at 0, 2.5, 5.0, 10.0, 15.0 and 30.0 min, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0 and 12.0 h after administration, and were immediately centrifuged and stored at −20 °C until analysis. Plasmas collected from six vehicle-administrated rats served as blank control. To determine the pharmacokinetic parameters of the five analytes, the pivotal pharmacokinetic parameters were calculated using DAS software (Ver. 2.0, Medical College of Wannan, China).

3. Results and discussion

3.1. Sample extraction

Liquid–liquid extraction (LLE) of the five analytes from plasma samples was explored. The deproteinization step is very important. If deproteinization is not thorough, the impurity in sample liquid may block the LC–MS. Protein precipitation (PPT) was initially developed with methanol and acetonitrile, but which could not eliminate the interferences from the sample matrix. Compared with the more recent and popular technique of solid-phase extraction (SPE), separation with LLE resulted in less potential interfering compounds, and it was simple and much more economical.

Different types of solvents with different pH conditions were tested to extract the analytes and IS. Although the five analytes could be extracted with methyl tert-butyl ether, diethyl ether, dichloromethane, or ethyl acetate, the result showed that neutralized conditions with methyl tert-butyl ether offered the best recovery. Moreover, IS, which had a structure similar to the analytes, even had a better recovery under current condition.

3.2. Method validation

3.2.1. Selectivity

Selectivity was evaluated by extracting blank rat plasma from six different matrix and comparing the MS/MS response at the retention times of five analytes to the responses of the LLOQ (see Fig. 2). No significant peaks were observed in any of the blank plasma samples for the five analytes.

3.2.2. Linearity of calibration curves and lower limits of quantification

The standard calibration curves for spiked rat plasma containing schisandrin and schisantherin A were linear over the range 0.5–200 ng/mL, with a correlation coefficient (r^2) > 0.99, and those of schisandrol B, schisanhenol and deoxyshisandrin were linear over the range 0.50–50.0 ng/mL, with a correlation coefficient (r^2) > 0.99. Typical equations for the calibration curve are shown in Table 2. The lower limits of quantification of each analyte in plasma

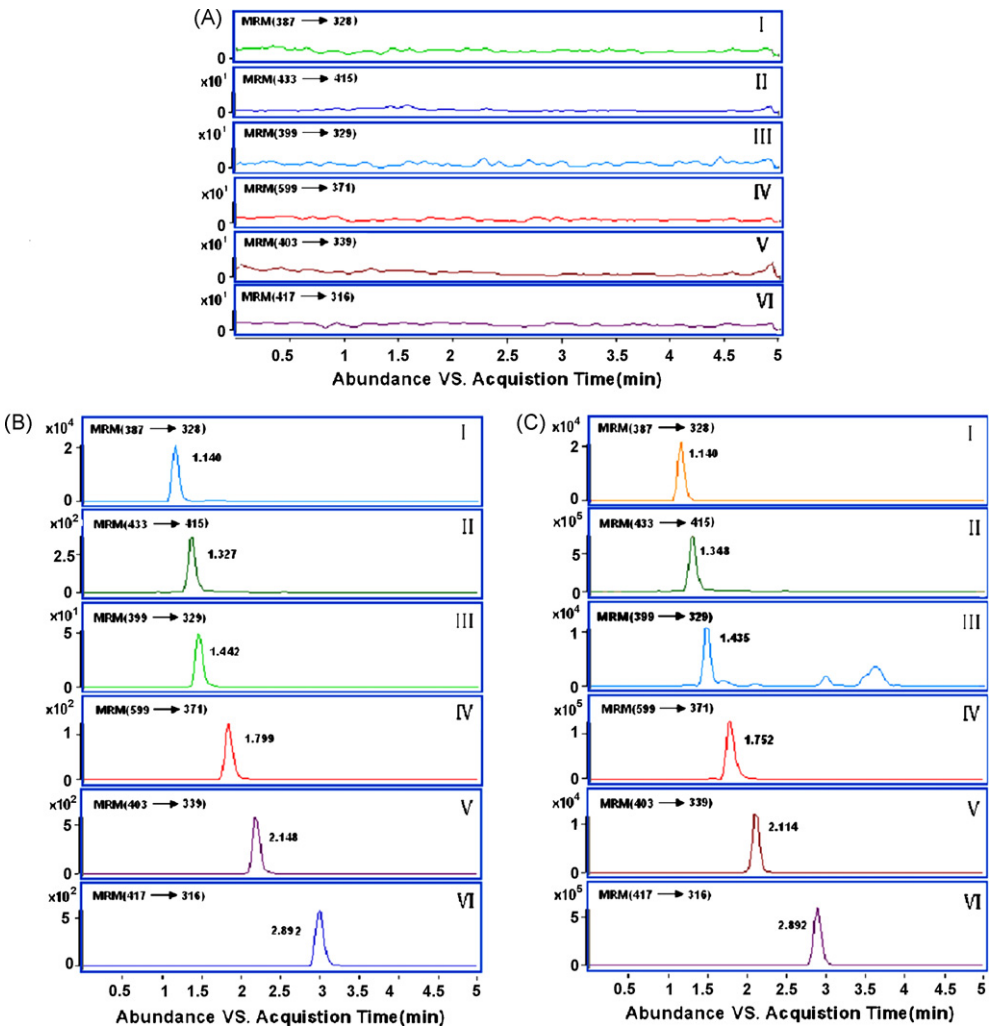

Fig. 2. Representative MRM chromatograms of schisandrin (II), schisandrol B (III), schisantherin A (IV), schisanhenol (V), deoxyshisandrin (VI) and bifendate (I; IS) in rat plasma. (A) A blank plasma sample, (B) a blank plasma sample spiked with schisandrin, schisandrol B, schisantherin A, schisanhenol, deoxyshisandrin at the lower limit of quantification and IS, and (C) plasma sample from a rat 2.0 h after intragastric administration of WZC at a dose of 150 mg/kg.

were all 0.50 ng/mL. These limits are sufficient for the pharmacokinetic study.

3.2.3. Extraction recovery and matrix effect

A single-step LLE with methyl tert-butyl ether proved to be simple, rapid and successful, with an mean recovery rate >85% at concentrations of QC samples, indicating that LLE efficiency was acceptable. The extraction recovery of schisandrin and schisantherin A at concentrations of 1.00, 10.0, and 100 ng/mL (QC samples) was $88.42 \pm 8.80/89.87 \pm 7.25/90.81 \pm 3.81$ and $88.74 \pm 6.72/90.48 \pm 8.84/93.56 \pm 5.82$, respectively. The extraction recovery of schisandrol B, schisanhenol and deoxyshisandrin at concentrations of 1.00, 5.00, and 20.0 ng/mL (QC samples) was $86.44 \pm 6.52/85.81 \pm 4.74/88.75 \pm 3.44$; $90.78 \pm 7.12/93.89 \pm 4.77/92.42 \pm 3.19$; and $91.04 \pm 8.99/91.55 \pm 5.96/92.13 \pm 4.78$, respectively. The recovery of the internal standard was $93.89 \pm 3.10\%$ in rat plasma ($n = 6$).

3.2.4. Precision and accuracy

The intra- and inter-day precision was analyzed by injecting replicates of QC samples. Table 3 summarizes the intra- and inter-day precision and accuracy of the method. The intra- and inter-day precision ranged 2.65–13.72% and 3.71–14.82%, respectively. The accuracy derived from QC samples was within ±4.29% for each QC level of the five analytes.

3.2.5. Stability

Stability of the five analytes during the sample storing and processing procedures was fully evaluated by analysis of QC samples. The concentration variation after one cycle of freezing and thawing was within ±15% of nominal concentrations, indicating that

Table 2
Regression data and LLOQs of the multi-components determined.

Components	Linear range (ng/mL)	Linear regression equation	Correlation coefficient (r)
Schisandrin	0.50–200	$Y = 0.0812X - 0.0068$	0.9988
Schisandrol B	0.50–50.0	$Y = 0.0213X - 0.0103$	0.9976
Schisantherin A	0.50–200	$Y = 0.1624X - 0.0440$	0.9992
Schisanhenol	0.50–50.0	$Y = 0.1231X - 0.0023$	0.9970
Deoxyshisandrin	0.50–50.0	$Y = 0.2031X - 0.0418$	0.9986

Table 3
Precision and accuracy from QC samples of rat plasma extracts (n = 3 days and six replicates per day).

Analyte	Added C (ng/mL)	Found C (ng/mL)	Intra-day R.S.D. (%)	Inter-day R.S.D. (%)	Relative error (%)
Schisandrin	1.00	1.09	10.95	14.82	1.34
	10.00	10.92	8.70	9.74	−2.80
	100.00	101.25	5.13	6.65	−3.12
Schisandrol B	1.00	0.89	6.87	7.78	−2.15
	5.00	4.91	2.65	5.62	−3.81
	20.00	20.97	4.50	3.71	4.29
Schisantherin A	1.00	0.87	13.72	6.20	2.35
	10.00	9.45	8.60	5.20	3.75
	100.00	99.10	4.44	4.85	−1.93
Schisanhenol	1.00	1.16	11.94	12.15	−2.94
	5.00	5.10	5.81	7.82	3.08
	20.00	21.12	2.80	6.51	3.22
Deoxyshisandrin	1.00	0.91	9.75	10.20	−1.22
	5.00	5.21	4.89	4.68	−1.17
	20.00	18.94	4.32	3.70	2.82

Table 4
Pharmacokinetic parameters of schisandrin, schisandrol B, schisantherin A, schisanhenol and deoxyshisandrin after intragastric administration of WZC to rats at a dose of 150 mg/kg (n = 6).

Parameter	Values				
	Schisandrin	Schisandrol B	Schisantherin A	Schisanhenol	Deoxyshisandrin
T_{max} (h)[a]	0.50 ± 0.02	0.25 ± 0.01	0.71 ± 0.06	0.46 ± 0.10	0.50 ± 0.01
C_{max} (ng/mL)[b]	79.21 ± 23.21	16.54 ± 8.58	125.70 ± 42.21	11.92 ± 5.29	32.89 ± 15.53
$t_{1/2}$ (h)[c]	0.66 ± 0.22	0.79 ± 0.22	1.17 ± 0.29	1.64 ± 0.56	3.71 ± 0.54
$AUC_{0\to t}$ (ng h/mL)[d]	98.74 ± 42.15	17.50 ± 4.81	453.62 ± 253.10	17.34 ± 7.85	44.31 ± 21.19
$AUC_{0\to\infty}$ (ng h/mL)[e]	99.01 ± 42.69	18.94 ± 4.69	454.56 ± 253.83	18.38 ± 8.46	46.57 ± 21.84
$AUMC_{0\to t}$ (ng h^2/mL)[f]	124.06 ± 57.67	28.05 ± 5.47	1264.08 ± 958.52	40 ± 13.79	189.31 ± 45.24
MRT (h)[g]	1.26 ± 0.11	1.74 ± 0.10	2.56 ± 0.51	2.23 ± 0.16	3.89 ± 0.21

[a] Time to reach maximum concentration.
[b] C_{max}: maximum plasma concentration.
[c] The apparent elimination half-life.
[d] The area under the plasma concentration–time curve from time zero to last sampling time.
[e] The area under the plasma concentration–time curve from time zero to infinity.
[f] The area under the first moment of the plasma concentration–time curve.
[g] The sum mean absorption and mean residence time.

there was no significant substance loss during thawing and freezing. When the processed samples were stored at room temperature, the five analytes and IS showed good stability as the responses varied no more than ±13% at QC concentrations during 24 h. After storage at −20 °C, the concentrations of the five analytes showed no obvious substance loss during 10 days (RE: −12.27% to 4.32%, RSD <10.80%). Meanwhile, storing at −20 °C for a whole month followed by three freeze–thaw cycles did not markedly affect its stability, only leading to a reduction of less than 15%. In addition, they were stable before being extracted and stored at room temperature for 6.0 h (RE: −8.51% to 3.92%, RSD < 9.60%).

3.3. Pharmacokinetic study

Our method was applied to a pharmacokinetic study after intragastric administration of 150 mg/kg WZC solution in six rats. Table 4 shows the main pharmacokinetic parameters of the five constituents. The method with 0.50 ng/mL LLOQ for the five lignans is more sensitive than previously reported ones (LLOQ = 10 ng/mL) [16]. So our method is more sensitive, and it can be used to study the pharmacokinetics, pharmacy and toxicity of *S. sphenanthera*.

4. Conclusion

We have developed a rapid, sensitive, and selective LC–MS/MS method for simultaneous determination of five bioactive constituents of WZC in rat plasma. Compared with previous methods, the present method has a lower limit of detection, high sensitivity, more satisfactory selectivity, and a short run time of 3.5 min, which makes it particularly suitable for routine assay. The validation parameter tests and pharmacokinetic sample analysis indicate that our method could be used to study the pharmacokinetics, pharmacology, and toxicity of *Nan-wuweizi* and other related preparations, and it could also be used for clinical drug monitoring as well.

Acknowledgements

The authors thank Shanghai R&D Center for Standardization of Traditional Chinese Medicines (Shanghai) and Prof. Daofeng Chen from the Department of Pharmacognosy of Fudan University School of Pharmacy for their excellent assistance.

References

[1] S.J. Jing, Y.H. Wang, D.F. Chen, Sphenanlignan, a new Lignan from the seeds of *Schisandra sphenanthera*, Chin. J. Nat. Med. 3 (2005) 78–82.
[2] M. Xu, G. Wang, H. Xie, Determination of schizandrin in rat plasma by high-performance liquid chromatography–mass spectrometry and its application in rat pharmacokinetic studies, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 828 (2005) 55–61.
[3] H.W. Xin, X.Ch. Wu, Q. Li, Ai-R. Yu, Effects of *Schisandra sphenanthera* extract on the pharmacokinetics of tacrolimus in healthy volunteers, Br. J. Clin. Pharmacol. 64 (2007) 469–475.
[4] L.J. Zhao, Sh.T. Fu, Ch.L. Zhang, Effects of Wuzhi capsule on pharmacokinetics of indinavir, Bull. Acad. Mil. Med. Sci. 32 (2008) 353–369.
[5] Q. Huang, Q. Pei, W.L. Zhou, Pharmacokinetic effect of Wuzhi capsules Oil lamivudine tablets in healthy Chinese subjects, Cent. South Pharmacy 5 (2007) 461–463.

[6] M.J. Xu, G.J. Wang, H.T. Xie, Pharmacokinetic comparisons of schizandrin after oral administration of schizandrin monomer, *Fructus Schisandrae* aqueous extract and Sheng-Mai-San to rats, J. Ethnopharmacol. 115 (2008) 483–488.
[7] X.Y. Niu, Z.J. Bian, Z.H. Ren, The metabolic fate of schizandrola and its distribution in brain in rats determined by TLC method, Acta Pharm. Sin. 18 (1983) 491–495.
[8] A. Chen, C. Li, W. Gao, Z. Hu, X. Chen, Separation and determination of active components in *Schisandra chinensis* Baill. and its medicinal preparations by non-aqueous capillary electrophoresis, Biomed. Chromatogr. 19 (2005) 481–487.
[9] Y.Y. Cui, M.Z. Wang, Aspects of schizandrin metabolism in vitro and in vivo, Eur. J. Drug Metab. Pharmacokinet. 18 (1993) 155–160.
[10] Y.Y. Cui, M.Z. Wang, Metabolic transformation of schizandrin, Acta Pharm. Sin. 27 (1992) 57–63.
[11] Y. Ikeya, K. Sugama, M. Tanaka, T. Wakamatsu, H. Ono, S. Takeda, T. Oyama, M. Maruno, Structure determination of biliary metabolites of schizandrin in rat and dog, Chem. Pharm. Bull. 43 (1995) 121–129.
[12] H. Sterbova, P. Sevcikova, L. Kvasnickova, Z. Glatz, J. Slanina, Determination of lignans in *Schisandra chinensis* using micellar electrokinetic capillary chromatography, Electrophoresis 23 (2002) 253–258.
[13] H. Ono, Y. Matsuzaki, Y. Wakui, S. Takeda, Y. Ikeya, S. Amagaya, M. Maruno, Determination of schizandrin in human plasma by gas chromatography–mass spectrometry, J. Chromatogr. B: Biomed. Appl. 674 (1995) 293–297.
[14] H.J. Sohn, J.Y. Bock, Identification of lignan compounds in fruits of *Schisandra chinensis* BAILLON by gas chromatography/mass spectrometry, J. Korean Chem. Soc. 32 (1989) 344–349.
[15] X.X. Deng, X.H. Chen, R. Yin, Determination of deoxyschizandrin in rat plasma by LC–MS, J. Pharm. Biomed. Anal. 46 (2008) 121–126.
[16] B.L. Wang, J.P. Hu, W. Tan, Simultaneous quantification of four active schisandra lignans from a traditional Chinese medicine *Schisandra chinensis* (*Wuweizi*) in rat plasma using liquid chromatography/mass spectrometry, J. Chromatogr. B 865 (2008) 114–120.
[17] N.R. Kitteringham, R.E. Jenkins, C.S. Lane, V.L. Elliott, B.K. Park, Multiple reaction monitoring for quantitative biomarker analysis in proteomics and metabolomics, J. Chromatogr. B 877 (2009) 1229–1239.