Journal of Pharmaceutical and Biomedical Analysis 51 (2010) 685–690

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Liquid chromatograph/tandem mass spectrometry assay for the simultaneous determination of chlorogenic acid and cinnamic acid in plasma and its application to a pharmacokinetic study

Jun Zhang*, Min Chen, Wenzheng Ju, Shijia Liu, Meijuan Xu, Jihong Chu, Ting Wu

Clinic Pharmacology Laboratory, The Affiliated Hospital of Nanjing University of Traditional Chinese Medicine, No. 155 Hanzhong Road, Nanjing, Jiangsu 210029, PR China

ARTICLE INFO

Article history:
Received 22 July 2009
Received in revised form
24 September 2009
Accepted 25 September 2009
Available online 2 October 2009

Keywords:
Mailuoning injection
Chlorogenic acid
Cinnamic acid
LC/MS/MS
Pharmacokinetic study

ABSTRACT

A rapid and high sensitive liquid chromatography/tandem mass spectrometry (LC/MS/MS) method for simultaneous determination of chlorogenic acid and cinnamic acid in human plasma was developed. The analytes and internal standard (IS), tinidazole, were extracted from human plasma via liquid/liquid extraction with ether–ethyl acetate (1:1, v/v) and separated on an Agilent Zorbax SB C18 column within 5 min. Quantitation was performed on a triple quadrupole mass spectrometer employing electrospray ionization technique, operating in multiple reaction monitoring (MRM) and negative ion mode. The precursor to product ion transitions monitored for chlorogenic acid, cinnamic acid and IS were m/z 352.9 → 191.1, 146.8 → 103.1, 245.6 → 126.0, respectively. The assay was validated with linear range of 1.00–800.00 ng/mL for chlorogenic acid and 0.50–400.00 ng/mL for cinnamic acid. The intra- and inter-day precisions (RSD%) were within 9.05% for each analyte. The absolution recoveries were greater than 74.62% for chlorogenic acid and 76.21% for cinnamic acid. Each analyte was proved to be stable during all sample storage, preparation and analytic procedures. The method was successfully applied to a pharmacokinetic study of Mailuoning injection in 10 healthy volunteers.

1. Introduction

Mailuoning injection [1–3], a well-known composite formulation of traditional Chinese medicine (TCM), is commonly used in clinical practice. It is made from the extracts of TCM including *Flos lonicerae*, *Artemisiae scopariae*, *Scrophularia ningpoensis* and *Achyranthes bientata* Bl. The pharmacological and clinical studies show that Mailuoning injection can expand the vessels, improve micro-circulation, increase the blood flow, suppress platelet congestion and thrombosis, dissolve the thrombus and lower the whole blood viscosity. Chlorogenic acid is one of the primary ingredients of *Flos lonicerae* and cinnamic acid is one of the primary ingredients of *Achyranthes bientata* Bl. Both are the active ingredients of Mailuoning injection and have relatively high concentrations. The concentrations of chlorogenic acid and cinnamic acid are the quality indication of Mailuoning injection. Few studies on the preclinic pharmacokinetics of Mailuoning injection [4,5], chlorogenic acid [6,7] and cinnamic acid [8,9] have been reported. No report about clinic pharmacokinetics of Mailuoning injection, chlorogenic acid and cinnamic acid has been published. Several methods for the determination of chlorogenic acid or cinnamic acid in plasma have been described in the literature. These methods include high performance liquid chromatography (HPLC) [4,7–16] and liquid chromatography/mass spectrometry (LC/MS) [5,6]. For chlorogenic acid, the LC/MS method reported by Wang et al. [5] presents lower limit of quantification (LLOQ) of 5 ng/mL, the highest LLOQ among others, is not adequately sensitive for the quantification due to low concentration in human plasma. For cinnamic acid, the HPLC–UV method reported by Zhang et al. [4] presents LLOQ of 11.2 ng/mL, is not adequately sensitive for the quantification due to low concentration in human plasma. Now a rapid and high sensitive LC/MS/MS method for the simultaneous determination of chlorogenic acid and cinnamic acid in human plasma was developed. The application of this method was demonstrated for the quantification of chlorogenic acid and cinnamic acid in plasma after intravenous administration of 20 mL Mailuoning injection in 10 healthy volunteers.

2. Experimental

2.1. Chemicals, materials and reagents

Chlorogenic acid, cinnamic acid, and tinidazole (IS) (Fig. 1) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products. Mailuoning injection, 10 mL/vial, lot number 200806262, containing 3.7×10^5 ng/mL chlorogenic acid and 3.3×10^5 ng/mL cinnamic acid, expired before June 2011, was

* Corresponding author. Tel.: +86 25 86617141 80518; fax: +86 25 86555033.
E-mail address: jennifer2886@126.com (J. Zhang).

0731-7085/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2009.09.039

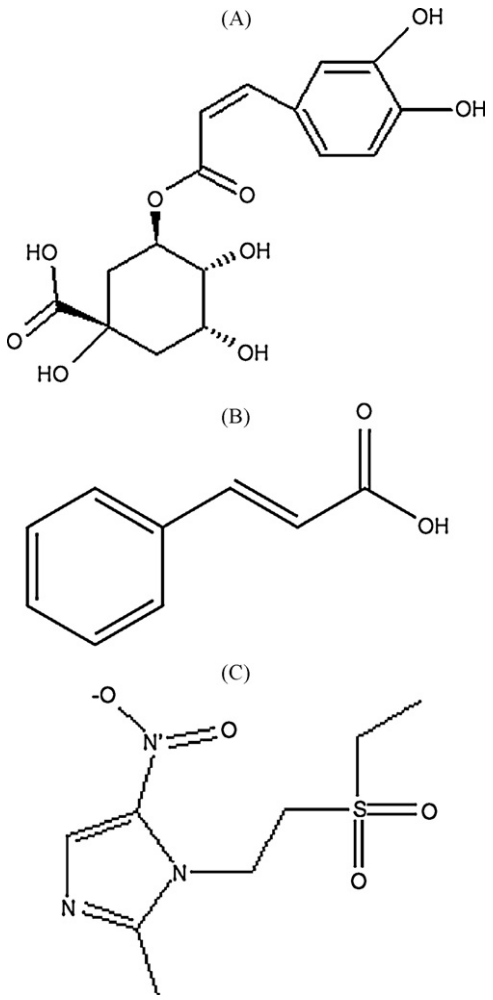

Fig. 1. Structures of two analytes and IS. (A) Chlorogenic acid; (B) cinnamic acid; (C) tinidazole.

manufactured by Jin Ling Pharmaceutical Co. Ltd. (Nanjing, China). Recommendation dosage is 10–20 mL/each with intravenous guttae. Methanol and acetonitrile was of HPLC grade (Merck, USA). Others were of AR grade. Ultrapure water used for the LC/MS/MS was from Milli-Q water purification system (Millipore, USA).

2.2. Preparation of standard solutions and plasma samples

The standard stock solutions were prepared by dissolving requisite amount of chlorogenic acid, cinnamic acid and IS in methanol, and were further diluted appropriately with methanol separately. Combined working solutions of two analytes, required for spiking plasma calibration and quality control samples, were subsequently prepared. The concentration of IS working solution was 108.7 ng/mL. All the solutions were stored at 4 °C until use. Drug free plasma, blank plasma, was stored in the freezer and allowed to completely thaw before use. The calibration standards and quality control (QC) samples were prepared by spiking blank plasma with combined working solutions. Calibration standards were made at 1.00, 2.00, 4.00, 20.0, 40.0, 200, 400, 800 ng/mL for chlorogenic acid, and 0.50, 1.00, 2.00, 10.0, 20.0, 100, 200, 400 ng/mL for cinnamic acid. Quality controls were prepared at 3.00, 60.0, 600 ng/mL for chlorogenic acid, and 1.50, 30.0, 300 ng/mL for cinnamic acid. The spiked plasma samples were stored at −20 °C for validation.

2.3. LC/MS/MS instrumentation and conditions

A series 1200 HPLC system (Agilent, USA) consisted of a quat pump, an autosampler and an online degasser. The analytes and IS were separated on an Agilent Zorbax SB C18 column (5 μm; 4.6 mm × 150 mm) with the mobile phase, methanol–2 mmol/L ammonium acetate solution (45/55, v/v), at a flow rate of 0.5 mL/min. The autosampler temperature was maintained at 4 °C and the injection volume was 5 μL. The total LC run time was 5 min. Detection of analytes and IS was performed on API4000 tandem quadrupole mass spectrometer (Applied Biosystems, USA). Negative ion electrospray ionization (ESI) was used to form deprotonated molecules. Multiple reaction monitoring (MRM) was used to monitor precursor to product ion transition of m/z 352.9 → 191.1 for chlorogenic acid, m/z 146.8 → 103.1 for cinnamic acid, and m/z 245.6 → 126.0 for IS. All the parameters of LC and MS were controlled by Analyst software version 1.4.1.

For analytes and IS the source parameters were ion spray voltage (ISV) 4000 V, turbo heater temperature (TEM) 400 °C, collision activation dissociation (CAD) 6 psi, curtain gas (CUR) 20 psi. The compound dependent parameters like declustering potential (DP) and collision energy (CE) were optimized at 52 and 25 for chlorogenic acid, 50 and 15 for cinnamic acid, and 25 and 15 for IS, respectively. Quadrupole 1 and quadrupole 3 were maintained at unit resolution. Dwell time set was 200 ms for both the analytes and IS.

2.4. Procedure for sample extraction

In a 10 mL centrifuge tube an aliquots of 500 μL plasma, mixed with 50 μL working solution of IS (108.7 ng/mL) and 50 μL 1 mol/L hydrochloric acid, was vortexed until thoroughly mixed. After 4 mL ethyl-ether–ethyl acetate (1:1, v/v) was added, the tube was vortexed vigorously for 3 min, followed by centrifugation for 10 min at 3000 × *g*. 3.2 mL of supernatant was transferred into another tube and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue, dissolved into 100 μL mobile phase, was vortexed for 1 min and then centrifuged for 5 min at 12,000 × *g*. 5 μL supernatant was injected into the LC/MS/MS system for analysis.

2.5. Method validation

The method was validated for selectivity, sensitivity (LLOQ), matrix effect (ME), linearity, precision and accuracy, recovery and stability.

Specificity was assessed by comparing the total ion chromatography (TIC) of 6 blank plasma samples with TIC of plasma samples spiked with chlorogenic acid, cinnamic acid and IS to check any possible interference at the retention time of analytes and IS. Further, 5 μL working solution was prepared in the mobile phase and was injected to check the interference.

Matrix effect was checked by comparing the area response of unextracted samples (Ai) to that of aqueous sample (Ar). Unextracted samples were prepared in three different lots of blank plasma. Matrix effect at three QC levels had been observed:

$$\mathrm{ME}\,(\%) = \frac{\mathrm{Ai}}{\mathrm{Ar}} \times 100$$

The lowest limit of quantification (LLOQ), defined as the minimum concentration at which the analyte could be quantified with acceptable accuracy and precision (RSD < 20%), was determined by the experimental analysis of different samples with known concentrations of the analyte.

Five linearity curves containing eight non-zero concentrations were analysed. Best-fit calibration curves of peak area ratio (chlorogenic acid versus IS, cinnamic acid versus IS) versus concentration

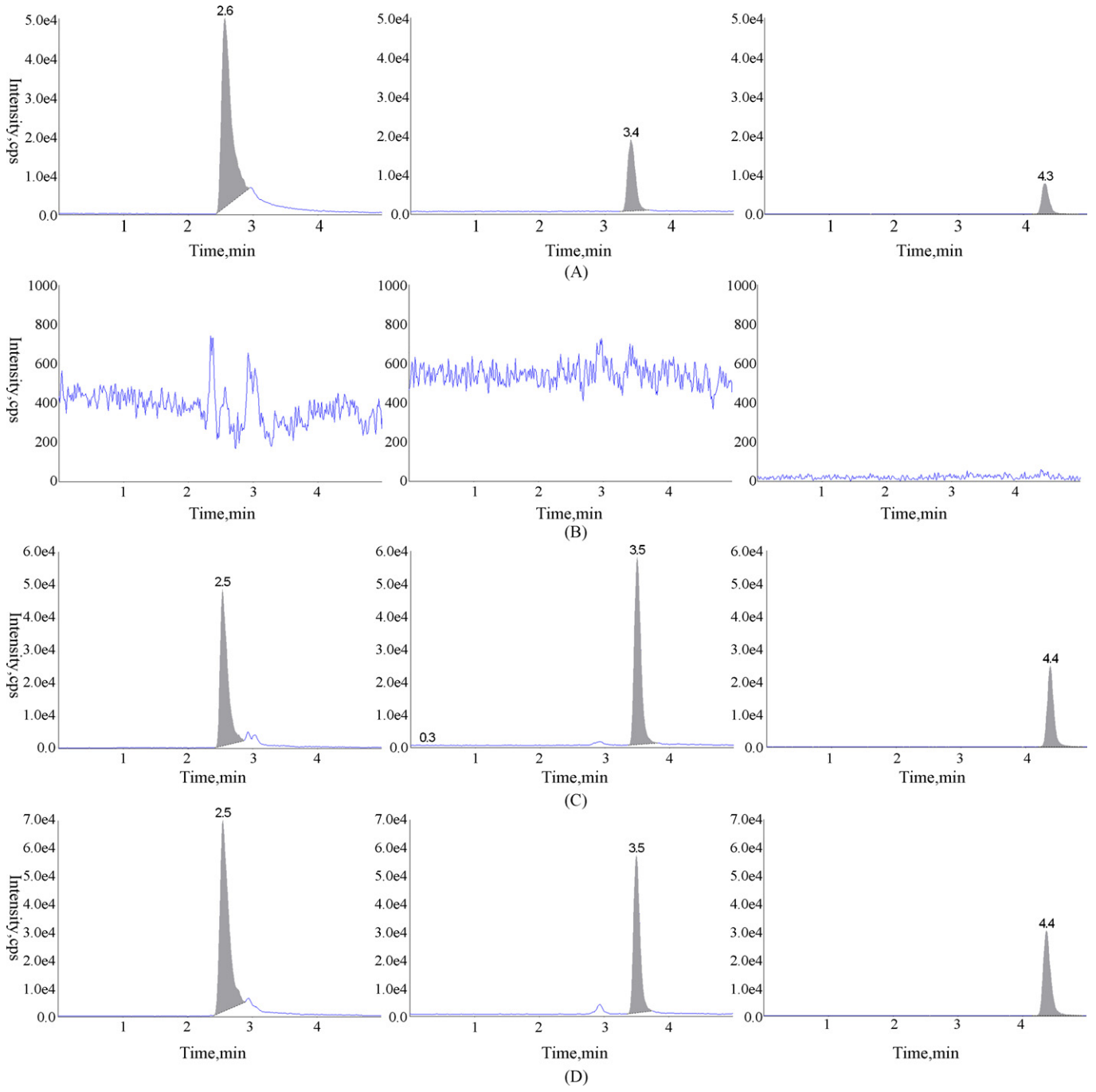

Fig. 2. TIC of chlorogenic acid, cinnamic acid, and IS in different conditions. (A) TIC of chlorogenic acid (0.05 μg/mL), cinnamic acid (0.025 μg/mL), and IS in methanol; (B) TIC of double blank sample; (C) TIC of spiked plasma with chlorogenic acid (1000 ng/mL), cinnamic acid (500 ng/mL) and IS (108.7 ng/mL); (D) TIC of plasma after an intravenous administration of Mailuoning injection (20 mL) at intervals of 30 min.

were drawn. The concentration of the analytes was calculated from calibration curve ($f = a \times C + b$, where f is the peak area ratio and C is the concentration) using least-squares linear regression analysis with reciprocate of the drug concentration as a weighing factor ($1/C^2$) for chlorogenic acid and cinnamic acid. The regression equation for the calibration standards was also used to back-calculate the measured concentration at each QC level. The peak area ratio values of calibration standards were proportional to the concentration of the drugs in plasma over the range tested.

The recovery of the analytes from the extraction procedure was performed at each QC level. It was evaluated by comparing peak area of extracted samples (spiked before extraction) to the peak area of unextracted samples (QC working solutions spiked in extracted plasma).

The intra-batch precision and accuracy were evaluated in five replicate analyses for chlorogenic acid and cinnamic acid at three QC levels on the same analytical run. Inter-batch precision and accuracy were calculated after repeated analysis in three different analytical runs. Concentrations were calculated from the calibration curve. The accuracy and precision was calculated and expressed in terms of %bias and coefficient of variation (%CV), respectively.

Stability experiments were performed to evaluate the analyte stability in stock solutions and in plasma samples under differ-

ent conditions. Room temperature stability, refrigerated stability of extracted sample, freeze–thaw stability and long term stability were performed using three replicates at each QC level.

2.6. Pharmacokinetic study

The study was conducted at the Clinic Pharmacology Laboratory, The Affiliated Hospital of Nanjing University of Traditional Chinese Medicine. Ethics committee (IRB of The Affiliated Hospital of Nanjing University of Traditional Chinese Medicine, Nanjing, China) approved the study protocol. All the volunteers were informed of the aim and risk involved in the study and written consent were obtained. Health check up was done by general physical examination, ECG and laboratory tests like hematology, biochemistry and urine examination. Ten healthy volunteers (half male, half female) were selected for the study. An 12-h overnight fasting was necessary before the intravenous administration. Lunch, served 4 h after the administration, should exclude vegetables containing chlorogenic acid and cinnamic acid.

Based on the clinical dose to patients, healthy volunteers were given Mailuoning injection via intravenous guttae with 20 mL/each. Blood samples were collected from forearm vein before the administration, 0.5 h during the administration, and after the administration at intervals of 0, 5, 10, 20, 30, 60, 90, 120, 180, 240, 360, and 480 min. Blood samples were in heparinized polythene tubes, and centrifuged at 3000 × *g* for 10 min. Plasma was separated and stored at −75 °C until use.

2.7. Pharmacokinetic analysis

Pharmacokinetic parameters were calculated by Drug And Statistic (DAS) 1.0 pharmacokinetic software (Chinese Pharmacological Association, Anhui, China). Pharmacokinetic parameters include compartment parameters and statistic parameters.

Maximum plasma concentrations (C_{max}) and the time point of maximum plasma concentration curve (t_{max}) were observed from the plasma concentration–time curve. Area under the plasma concentration–time curve (AUC) was calculated by the linear trapezoidal rule. The elimination rate constant (kel) was calculated by linear regression of the terminal points of the semi-log plot of plasma concentration against time. Half-life of drug elimination during the terminal phase ($t_{1/2}$) was calculated from the formula $t_{1/2} = 0.693/\text{kel}$. Mean residence time (MRT) was calculated as AUMC/AUC, where AUMC is the area under the first moment plasma concentration–time curve. Volume of distribution (Vd) of the central compartment was calculated as dose/C_0, where C_0 is the concentration measured just after the administration. Plasma clearance (CL) was calculated as dose/AUC.

3. Results and discussion

3.1. Selectivity and sensitivity (LLOQ)

The liquid–liquid extraction methodology in combination with mass spectrometry detection gave very good selectivity for the analytes and IS. Fig. 2(A) shows the total ion chromatography (TIC) of chlorogenic acid (0.05 μg/mL), cinnamic acid (0.025 μg/mL), and IS in methanol. The retention time was 2.5 min for chlorogenic acid, 3.5 min for cinnamic acid, and 4.4 min for IS, respectively. No endogenous interference was detected in blank plasma samples at the retention time of two analytes and IS, as shown in Fig. 2(B). Fig. 2(C) shows the TIC of spiked plasma with two analytes (1000 ng/mL for chlorogenic acid, 500 ng/mL for cinnamic acid) and IS (108.7 ng/mL). Fig. 2(D) shows the TIC of plasma after an intravenous administration of Mailuoning injection (20 mL) at intervals of 30 min.

Table 1
Matrix effect of chlorogenic acid, cinnamic acid and tinidazole (IS).

	Injecting concentration (ng/mL)	ME (%)
Chlorogenic acid ($n = 3$)	15	76.5 ± 8.11
	300	76.9 ± 7.24
	3,000	81.9 ± 6.01
Cinnamic acid ($n = 3$)	7.5	85.5 ± 5.63
	150	83.4 ± 2.09
	1,500	82.5 ± 3.38
Tinidazole ($n = 9$)	54,350	81.1 ± 2.76

The lower limit of quantitation (LLOQ) was defined as the lowest concentration that could be reliably quantitated from the background level. In this case, the LLOQs of chlorogenic acid and cinnamic acid were 1.00 and 0.50 ng/mL, respectively.

3.2. Linearity and matrix effect

For chlorogenic acid, the linear regression equation for mean of five calibration curves was $y = 0.0505x - 0.0232$, $r = 0.9994$. For cinnamic acid, the equation for mean of five calibration curves was $y = 0.0916x + 0.0759$, $r = 0.9986$ (Fig. 3).

Matrix effect represented as ion suppression for this method. ME was consistent in all the lots and did not affect the quantitative analysis of analytes and IS peak. The results are shown in Table 1.

3.3. Recovery

The recoveries of chlorogenic acid and cinnamic acid are shown in Table 2.

The recoveries of two analytes and IS could be promoted by acidifying the plasma samples with hydrochloric acid, so 1 mol/L hydrochloric acid 50 μL was added to plasma samples before extraction. The recoveries using different organic solvents as extractant were compared. Since the polarities of two analytes were varied, ethyl-ether/ethyl acetate (1:1) as the extractant was chosen to obtain suitable recoveries of each analyte.

3.4. Accuracy and precision

The accuracy between 85% and 115% and precision less than 15% are acceptable. Intra-batch and inter-batch precision and accuracy for the analysis of chlorogenic acid and cinnamic acid in human plasma are presented in Tables 3 and 4. These show that the LC/MS/MS assay is excellent for the simultaneous quantitative analysis of two analytes in human plasma.

3.5. Stability

Chlorogenic acid and cinnamic acid were found stable in extracted plasma samples for 24 h at room temperature. Both ana-

Table 2
Recoveries of chlorogenic acid, cinnamic acid and IS from human plasma using ethyl-ether–ethyl acetate (1:1, v/v) as extracting solvent.

	Injecting concentration (ng/mL)	Recoveries ($n = 5$)
Chlorogenic acid	15.0	74.6 ± 3.11
	300	75.9 ± 2.61
	3000	81.6 ± 2.85
Cinnamic acid	7.5	80.7 ± 3.62
	150	77.3 ± 2.36
	1500	76.2 ± 2.26
Tinidazole	54.35	76.0 ± 3.02

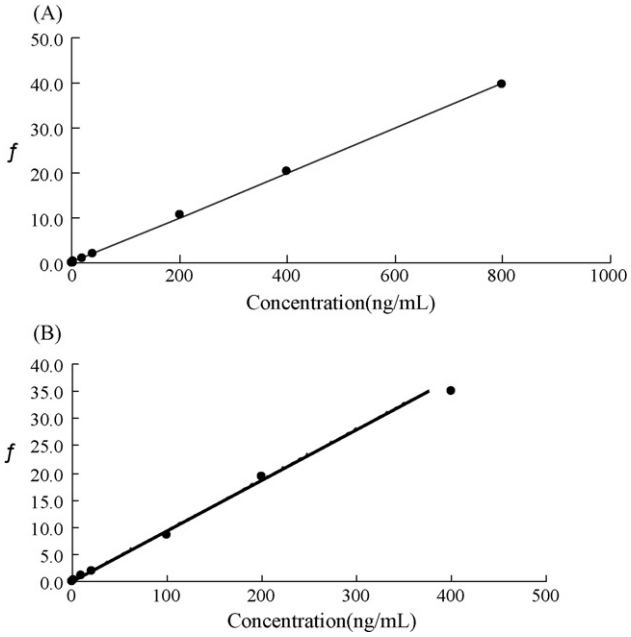

Fig. 3. Mean calibration curves of analytes in human plasma. (A) Chlorogenic acid and (B) cinnamic acid.

Table 3
Intra-batch accuracy and precision for the analysis of chlorogenic acid and cinnamic acid in human plasma.

	Nominal concentration (ng/mL)	Measured concentration (ng/mL)	Accuracy (%)	Precision (%)
Chlorogenic acid ($n=5$)	3.00	2.96 ± 0.119	98.8 ± 4.05	4.00
	60.0	57.0 ± 1.88	95.1 ± 3.36	3.30
	600	587 ± 31.1	97.7 ± 5.02	5.29
Cinnamic acid ($n=5$)	1.50	1.39 ± 0.064	92.5 ± 4.16	4.63
	30.0	27.4 ± 0.900	91.3 ± 3.02	3.28
	300	296 ± 6.19	98.8 ± 2.08	2.09

lytes were stable under three freeze/thaw cycles. The chlorogenic acid and cinnamic acid spiked plasma samples stored at −20 °C for long term stability were found stable for 20 days.

3.6. Application

The pharmacokinetic parameters like C_{max}, T_{max}, $AUC_{0-7.17}$, $t_{1/2}$, $MRT_{0-7.17}$, CL and Vd were calculated for chlorogenic acid and cinnamic acid. The pharmacokinetic parameters are presented in Table 5.

Table 4
Inter-batch accuracy and precision for the analysis of chlorogenic acid and cinnamic acid in human plasma.

	Nominal concentration (ng/mL)	Measured concentration (ng/mL)	Accuracy (%)	Precision (%)
Chlorogenic acid ($n=15$)	3.00	2.86 ± 0.257	95.2 ± 8.62	9.00
	60.0	60.3 ± 4.61	101 ± 7.76	7.65
	600	618 ± 45.2	103 ± 7.45	7.32
Cinnamic acid ($n=15$)	1.50	1.41 ± 0.119	94.2 ± 7.92	8.41
	30.0	28.4 ± 2.24	94.6 ± 7.47	7.89
	300	285 ± 11.8	95.0 ± 3.95	4.15

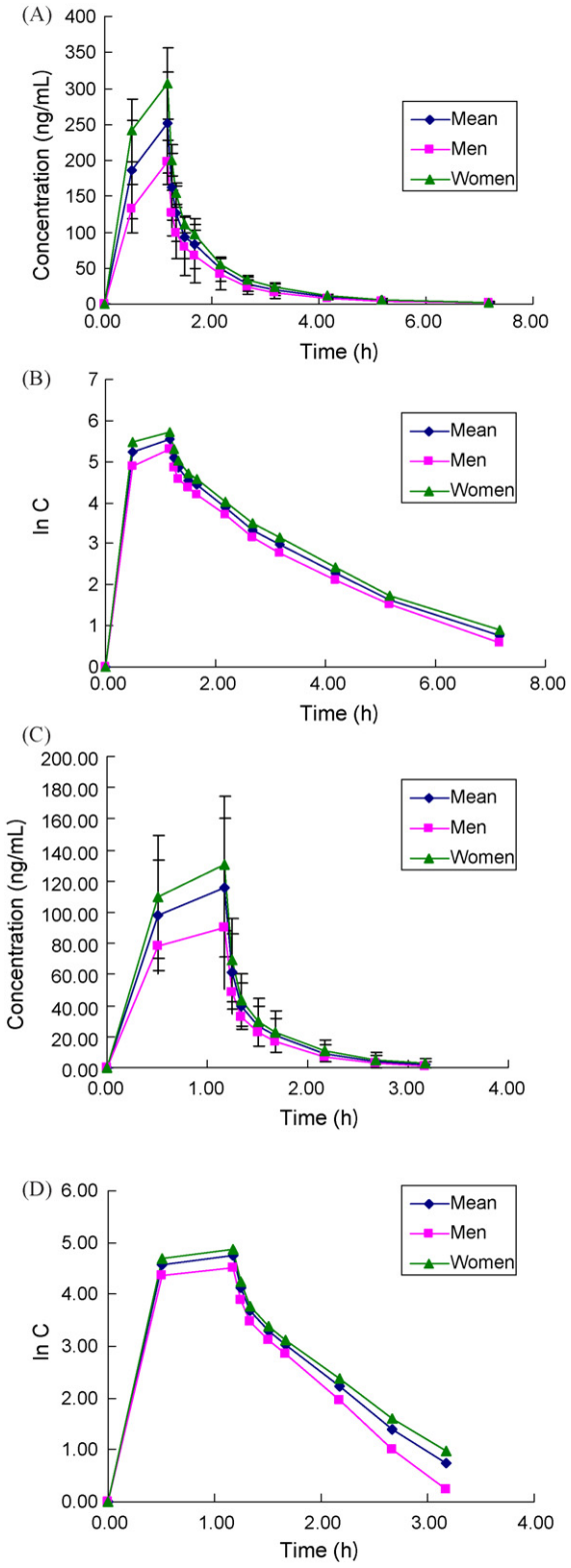

Fig. 4. Average plasma concentration–time curves and the corresponding semi-logarithmic plots after an intravenous administration of Mailuoning injection to healthy volunteers. (A) Average plasma concentration–time curves of chlorogenic acid; (B) the corresponding semi-logarithmic plots of chlorogenic acid; (C) average plasma concentration–time curves of cinnamic acid; (D) the corresponding semi-logarithmic plots of cinnamic acid.

Table 5
Pharmacokinetic parameters of chlorogenic acid and cinnamic acid in 10 healthy volunteers with an intravenous administration of Mailuoning injection.

	Chlorogenic acid	Cinnamic acid
C_{max} (μg/L)	255.70 ± 66.425	119.63 ± 40.303
T_{max} (h)	1.04 ± 0.282	1.00 ± 0.310
$AUC_{0-7.17}$ (μg/L h)	403.62 ± 109.296	158.64 ± 56.019
$MRT_{0-7.17}$ (h)	1.26 ± 0.116	0.80 ± 0.065
Vd (L/kg)	0.700 ± 0.244	0.519 ± 0.314
$t_{1/2\beta}$ (h)	1.35 ± 0.533	0.42 ± 0.087
CL (L/kg/h)	0.370 ± 0.105	0.899 ± 0.295

Table 6
Pharmacokinetic parameters for chlorogenic acid from different reports.

	Human (our results)	Rat [4]	Rat [5]
$MRT_{0-7.17}$	1.26 ± 0.116 h	No report	28.4 ± 4.3 min
Vd	0.700 ± 0.244 L/kg	0.0517 L/kg	0.66 ± 0.36 L/kg
$t_{1/2\beta}$	1.35 ± 0.533 h	18.4 min	27.9 ± 5.4 min
CL	0.370 ± 0.105 L/kg/h	0.0013 L/kg/min	1.60 ± 0.59 L/kg/h

Table 7
Pharmacokinetic parameters for cinnamic acid from different reports.

	Human (our results)	Rat [4]
$MRT_{0-7.17}$	0.80 ± 0.065 h	No report
Vd	0.519 ± 0.314 L/kg	1.5939 L/kg
$t_{1/2\beta}$	0.42 ± 0.087 h	213.5 min
CL	0.899 ± 0.295 L/kg/h	0.0035 L/kg/min

A two-compartment, first-order pharmacokinetic model appeared to fit the concentration–time curve after an intravenous administration of Mailuoning injection with 20 mL/each to the volunteers (equivalent to 7.4 mg of chlorogenic acid and 6.6 mg of cinnamic acid). Fig. 4 shows the average plasma concentration–time curves and the corresponding semi-logarithmic plots after an intravenous administration of Mailuoning injection to healthy volunteers.

The maximum concentrations of chlorogenic acid and cinnamic acid reached at 1 h after an intravenous administration of Mailuoning injection to healthy volunteers. C_{max} of chlorogenic acid was much higher than that of cinnamic acid. It showed that the velocities of absorption for the two analytes were similar, but the degree of absorption for chlorogenic acid was greater than that of cinnamic acid.

The pharmacokinetic parameters revealed the distribution volumes of two analytes were less than 1 L/kg, suggesting that two analytes mainly existed in peripheral blood in human. The $t_{1/2}$ of cinnamic acid was less than that of chlorogenic acid. It revealed that cinnamic acid eliminated faster than chlorogenic acid did. The plasma clearances of two analytes were less than 1 L/kg/h, showing that two analytes eliminated quickly in blood.

There was no gender differences ($P > 0.05$) for main pharmacokinetic parameters of two analytes. Some pharmacokinetic parameters for chlorogenic acid from different reports are shown in Table 6 and some pharmacokinetic parameters for cinnamic acid from different reports are shown in Table 7. It revealed that there was species difference in these pharmacokinetic parameters for two analytes.

4. Conclusions

A simple, specific, rapid and sensitive analytical method for simultaneous determination of chlorogenic acid and cinnamic acid in human plasma had been developed and validated. The proposed method is the first LC/MS/MS method for determination of two analytes. Simple liquid–liquid extraction procedure and short run time can increase sample throughput that is important for large sample batches. The method provided excellent specificity and linearity with a limit of quantification of 1.00 ng/mL for chlorogenic acid and 0.50 ng/mL for cinnamic acid. The application of this method was demonstrated for the quantitative analysis of two analytes in plasma after intravenous administration of Mailuoning injection to healthy volunteers. The pharmacokinetic parameters of chlorogenic acid and cinnamic acid in human were also reported for the first time. This information should be useful for further investigating in pharmacokinetics, pharmacy and toxicity of Mailuoning injection. It also provides the academic support to the clinic therapy study of this traditional Chinese medicine.

References

[1] K. Hou, Neimenggu Tradit. Chin. Med. 1 (2007) 26–27.
[2] Ch.N. Tan, Abstr. Clin. Pract. 17 (2002) 284–286.
[3] F. Yu, Y.T. Jin, Ch.Y. Zuan, W. Xie, Y.W. Shi, Chin. J. New Drugs 11 (2002) 920–924.
[4] J. Zhang, W.Zh. Ju, M. Chen, Sh.J. Liu, H.Sh. Tan, Chin. Pharmacol. Bull. 24 (2008) 558–559.
[5] S.J. Wang, Zh.Q. Zhang, Y.H. Zhao, J.X. Ruan, J.L. Li, Rapid Commun. Mass Spectrom. 20 (2006) 2303–2308.
[6] S.J. Wang, Zh.W. Zhang, Y.H. Zhao, Zh.Q. Zhang, H.T. Xie, J.X. Ruan, Chin. J. Clin. Pharmacol. Ther. 11 (2006) 1340–1344.
[7] X. He, T.M. Zhao, X.D. Jun, G.J. Jing, Sh.S. Gongqi, Chin. Tradit. Pat. Med. 21 (1999) 161–162.
[8] Ch.M. Yang, Sh.X. Hou, Y.Y. Sun, Ch.Y. Li, Chin. Tradit. Herbal Drugs 32 (2001) 616–618.
[9] R.H. Dai, Z.H. Song, T. Ju, K.Sh. Bi, J. Chin. Pharm. Sci. 13 (2004) 130–133.
[10] B. Yuan, M. Qiao, H. Xu, L. Wang, F. Li, Yakugaku Zasshi 126 (2006) 811–814.
[11] Q. Li, L.X. Sun, L. Xu, Y. Jia, Z.W. Wang, Z.D. Shen, K.S. Bi, Biomed. Chromatogr. 20 (2006) 1315–1320.
[12] R. Gao, Q. Zheng, T. Gong, Y. Fu, L. Deng, Z.R. Zhang, J. Pharm. Biomed. Anal. 43 (2007) 335–340.
[13] X.P. Li, J. Yu, J.Y. Luo, H.S. Li, F.J. Han, X.G. Chen, Z.D. Hu, Biomed. Chromatogr. 20 (2006) 206–210.
[14] H. Yang, B. Yuan, L. Li, H. Chen, F. Li, J. Chromatogr. Sci. 42 (2004) 173–176.
[15] R. Wang, T. Sun, D. Jing, X. Chen, Z. Yu, K. Bi, Se Pu 23 (2005) 273–275.
[16] Z. Song, K. Bi, X. Luo, J. Chromatogr. Sci. 40 (2002) 198–200.