Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 850 (2007) 1–6
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Review

Simultaneous enantiomer determination of 20 (*R*)- and 20 (*S*)-ginsenoside-Rg_2 in rat plasma after intravenous administration using HPLC method

Fang-Jin Gui [a], Xiu-Wei Yang [a,*], Long-Yun Li [b], Jian-Ming Tian [b]

[a] *The State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, and Medical and Healthy Analysis Center, Peking University, Beijing 100083, China*
[b] *Jilin Academy of Traditional Chinese Medicine, Changchun 130021, China*

Received 24 December 2005; accepted 6 November 2006
Available online 20 November 2006

Abstract

20 (*R*,*S*)-Ginsenoside-Rg_2, an anti-shock agent, is prescribed as a racemate. To analyze simultaneously the enantiomers of 20 (*R*)-ginsenoside-Rg_2 and 20 (*S*)-ginsenoside-Rg_2 in plasma, a simple and reproducible high-performance liquid chromatographic (HPLC) method has been developed. The enantiomeric separation and determination were successfully achieved using a Diamonsil^{TM} ODS C_{18} reversed-phase column (5 μm, 250 mm × 4.6 mm) with an RP18 (5 μm) guard column and a mobile phase of MeOH-*aq*. 4% H_3PO_4 (65:35, v/v, pH 5.1) with UV detection at 203 nm. Both enantiomers, 20 (*R*)-ginsenoside-Rg_2 and 20 (*S*)-ginsenoside-Rg_2, were well separated at 14.5 min and 13.6 min, respectively. The linear ranges of the standard curves were 2.0–250 μg/ml. The intra- and inter-day precision (R.S.D.) were ≤1.59% and ≤0.54% and the mean extraction recoveries were 95.8% and 96.5% for 20 (*R*)-ginsenoside-Rg_2 and 20 (*S*)-ginsenoside-Rg_2 in rat plasma, respectively. The limits of detection and quantification were 2.0 μg/ml and 7.8 μg/ml (S/N = 3:1) for 20 (*R*)-ginsenoside-Rg_2, and 2.0 μg/ml and 3.9 μg/ml (S/N = 3:1) for 20 (*S*)-ginsenoside-Rg_2, respectively. This validated method was applicable to pharmacokinetic studies in rat plasma after intravenous administration of 20 (*R*,*S*)-ginsenoside-Rg_2. A pharmacokinetic study in rat plasma indicated that the enantiomers were rapidly absorbed and eliminated. These assay results are necessary for the evaluation of the metabolism of ginsenoside-Rg_2 *in vivo*.

Keywords: 20 (*R*,*S*)-Ginsenoside-Rg_2; 20 (*R*)-Ginsenoside-Rg_2; 20 (*S*)-Ginsenoside-Rg_2; Enantiomeric separation; Pharmacokinetics

Contents

1. Introduction 2
2. Experimental 2
   2.1. Chemicals, regents, and animals 2
   2.2. Apparatus and chromatographic conditions 2
   2.3. Sample preparation 3
   2.4. Separation and extraction procedure 3
   2.5. Ginsenoside-Rg_2 administration and sample collection 3
   2.6. Validation of the assay 3
   2.7. Application of the method and pharmacokinetic study 3
3. Results and discussion 3
   3.1. The column and mobile phase 3
   3.2. Solid phase extraction 4
   3.3. Selectivity 4

* Corresponding author. Tel: +86 10 82805106; fax: +86 10 82802724.
E-mail address: xwyang@bjmu.edu.cn (X.-W. Yang).

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.11.008

3.4. Linearity, and LOD and LOQ . . . 4
3.5. Extraction recovery . . . 4
3.6. Precision and accuracy . . . 5
3.7. Application to pharmacokinetics study . . . 5
4. Conclusions . . . 6
References . . . 6

1. Introduction

In traditional Chinese medicine, ginseng (the root of *Panax ginseng* C. A. Mey.) is an important component in various prescriptions. Ginsenosides are glycosides with a dammarane skeleton that are classified as protopanaxadiol or protopanaxatriol compounds (except ginsenoside-Ro) and are isolated from the root of *P. ginseng*. They have been regarded as the principal ingredients responsible for the pharmacological activities of the drug [1–3]. Several researchers have reported that orally administered ginsenosides are detected in the blood not in the intact form, because of a low absorption rate from the intestines, but in forms metabolized by intestinal bacteria [4]. Since few studies have been performed on the pharmacokinetics of ginsenosides, further experiments are necessary for their evaluation. Ginsenoside-Rg_2 (Fig. 1) is protopanaxatriol-type compound that is one of the major active components in the root and stem-leaves of *P. ginseng*. In our previous reports, ginsenoside-Rg_2 had a therapeutic effect on acute cardiogenic shock in dogs, increased the heart rate of rabbits, and protected against apoptosis in ischemia and reperfusion of the rat myocardium [5–9].

Ginsenoside-Rg_2 is a racemic mixture of 20 (*R*)- and 20 (*S*)-enantiomers, which exhibit similar activity. The pharmacokinetic profile of ginsenoside-Rg_2 enantiomers remains unknown. Thus, analytical determination of the ginsenoside-Rg_2 enantiomers is important for insight into their pharmacokinetics and pharmacodynamics. Recently, enantiomeric separation of racemic drugs [10–15] on chiral stationary phases by high-performance liquid chromatography (HPLC) has been widely investigated, since the two enantiomers of some racemic drugs have been shown to have different pharmacokinetics in animals and humans.

In this study, the enantiomeric separation of racemic mixtures of ginsenoside-Rg_2 was examined by HPLC using a Diamonsil™ ODS-C-18 reversed-phase column with a RP18 guard column and a mobile phase of MeOH-*aq*. 4% H_3PO_4 with UV detection at 203 nm. An efficient analytical method for simultaneous enantiomeric determination of ginsenoside-Rg_2 was developed and validated. Furthermore, the method was used to determine the plasma concentrations of 20 (*R*)-ginsenoside-Rg_2 and 20 (*S*)-ginsenoside-Rg_2 in rats after intravenous administration of 20 (*R*,*S*)-ginsenoside-Rg_2.

2. Experimental

2.1. Chemicals, regents, and animals

20 (*R*,*S*)-Ginsenoside-Rg_2, 20 (*R*)-ginsenoside-Rg_2, and 20 (*S*)-ginsenoside-Rg_2 were prepared from ginseng, and the structures were determined by IR, 2D NMR (including GCOSY, GHMQC, GHMBC), a DEPT experiment, and FT-MS [16]. Methanol of HPLC grade was purchased from Biaoqi Ltd Co. (Tianjin, China). All other chemicals and solvents used were of analytical grade and water was milli-Q grade. Wister rats were obtained from the Laboratory Animal Center of Peking University Health Science Center (Beijing, China).

2.2. Apparatus and chromatographic conditions

A Thermo Separation Products liquid chromatograph system consisting of a SpectraSYSTEM P 2000 binary pump and SpectraSYSTEM UV3000 detector coupled with a PC1000 workstation was used. Separations were performed on a Diamonsil™ ODS C_{18} reversed-phase column (5 μm, 250 mm × 4.6 mm;

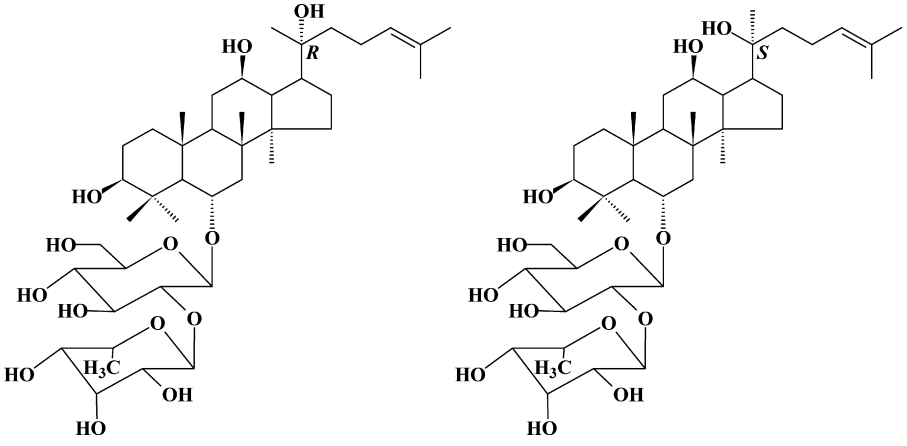

Fig. 1. Chemical structures of enantiomers of 20 (*R*)-ginsenoside-Rg_2 and 20 (*S*)-ginsenoside-Rg_2.

Dikma, China) with a C_{18} guard column (5 μm, 8 mm × 4 mm; Dikma, China). The isocratic mobile phase was *aq*. 4% orthophosphoric acid–methanol (35:65, v/v, pH 5.1). The pump flow rate was 1 ml/min. Peaks of 20 (*R*)-ginsenoside-Rg_2 and (*S*)-ginsenoside-Rg_2 were detected by UV absorbance at 203 nm. The injection volume was 20 μl. All measurements were performed at 25 °C.

2.3. Sample preparation

Stock solutions of 20 (*R*)-ginsenoside-Rg_2 and (*S*)-ginsenoside-Rg_2 were prepared with methanol as the solvent. Working solutions were diluted with methanol before experiments. Plasma standards were prepared using drug-free rat plasma. Standard samples were prepared in blank rat plasma at concentrations of 7.8–250 μg/ml for 20 (*R*)-ginsenoside-Rg_2 and 3.9–125 μg/ml for 20 (*S*)-ginsenoside-Rg_2. Calibration curves were obtained by plotting peak area versus ginsenoside-Rg_2 concentrations in the standard sample.

2.4. Separation and extraction procedure

Plasma samples (200 μl) were slowly added onto the solid phase cartridge SEP-PAK C_{18} (Millipore®, Waters, USA), which had been previously activated with 10 ml methanol and balanced with 5 ml water. After the sample had been absorbed by the cartridge, the cartridge was successively washed with 3 ml of water, and 20%, 55%, 65% methanol in water, respectively. The elutions of 55% and 65% methanol in water, which contained ginsenoside-Rg_2, were combined and then evaporated under an air stream at 50 °C in a water-bath. The residue was redissolved in 1 ml methanol with vortexing. After centrifugation at 6000 × *g* for 15 min, about 80 μl supernatant was drawn and 20 μl injected into the HPLC system.

2.5. Ginsenoside-Rg_2 administration and sample collection

Six Wister rats, weighing 270–290 g and including both sexes, were used in this study. They were kept in a controlled-environment breeding room for 3 days before the start of the experiments. The rats were fasted overnight before dosing, but with free access to water. For intravenous administration, ginsenoside-Rg_2 powder was dissolved in isotonic saline containing 25% of polyethylene glycol-400 and 25% of 1,2-propanediol, and the solution was delivered using a syringe into the tail vein at a dose of 25 mg/kg for 20 (*R*,*S*)-ginsenoside-Rg_2, corresponding to 2 mg/kg for 20 (*R*)-ginsenoside-Rg_2 and 23 mg/kg for 20 (*S*)-ginsenoside-Rg_2. The preparations were made immediately before drug administration.

The rats were then fixed on a wooden plate under pentobarbital sodium (40 mg/kg, i.p.) anesthesia, and blood samples (800 μl) were collected immediately before and at 1 min, 3 min, 5 min, 10 min, 20 min, 30 min, 45 min, and 60 min after administration of 20 (*R*,*S*)-ginsenoside-Rg_2. After each blood sampling, the cannula was flushed with physiologic saline containing heparin. Each blood sample was transferred into a heparinized Eppendorf tube and mixed gently, then centrifuged (1500 × *g*, 10 min) to obtain 300 μl plasma, which was kept at −20 °C until analysis.

2.6. Validation of the assay

Calibration curves for each analyte were obtained on a single day using 20 (*R*)-ginsenoside-Rg_2 and 20 (*S*)-ginsenoside-Rg_2 spiked rat plasma samples. Standard peak area versus concentration curves were fit by a least-squares linear regression method. The precision and accuracy of the method was evaluated by analyzing plasma samples spiked with three concentrations of 20 (*R*)-ginsenoside-Rg_2 and 20 (*S*)-ginsenoside-Rg_2. The intra-day variabilities were determined by assaying the three replicates on the same day and inter-day variabilities were assayed on three consecutive days. Precision was expressed as the percentage coefficient of variation. The accuracy for these samples was determined by comparing the calculated concentration obtained from the calibration curve with the known concentration. Extraction recoveries for 20 (*R*)-ginsenoside-Rg_2 and 20 (*S*)-ginsenoside-Rg_2 from rat plasma were assessed at low, medium, and high concentrations (7.8 μg/ml, 31.3 μg/ml, and 125 μg/ml for 20 (*R*)-ginsenoside-Rg_2 and 3.9 μg/ml, 15.6 μg/ml, and 62.5 μg/ml for 20 (*S*)-ginsenoside-Rg_2) by comparing the peak area of authentic samples of 20 (*R*)-ginsenoside-Rg_2 and 20 (*S*)-ginsenoside-Rg_2 added to blank rat plasma and solid phase extraction with those of the same quantities added in the mobile phase. Validation was carried out at three concentration levels.

2.7. Application of the method and pharmacokinetic study

The utility of the method was demonstrated in *in vivo* conditions. Samples were prepared as described in Section 2.5, and 20 μl of the samples was analyzed. The concentrations of 20 (*S*)-ginsenoside-Rg_2 and 20 (*R*)-ginsenoside-Rg_2 at different time points were evaluated by means of linear regression analysis. All statistical analysis was performed using Microsoft Excel 2000. The relevant pharmacokinetic parameters were calculated using the computer program 3p87 (Chinese Society of Mathematical Pharmacology).

3. Results and discussion

3.1. The column and mobile phase

Chiral compounds cannot be generally directly separated by conventional HPLC stationary phases without prior derivatization of the enantiomers into diastereomers. Over the last 15 years, increasingly powerful chiral stationary phases have become available (Pirkle-type, protein-base, cyclodextrins, cellulose/amylose, etc.) [17]. However, in this study a universal C_{18} column was chosen as the stationary phase. The C_{18} column is generally used for the separation of different ginsenosides such as Rg_1, Re, Rb_1 Rc, and Rd [18]. Now, separation of enantiomers has been successfully achieved using a universal C_{18} column. MeOH-*aq*. 4% H_3PO_4 was chosen as the mobile phase to obtain baseline separation of the enantiomers. As a component of the

mobile phase, *aq*. 4% H_3PO_4 had a strong effect on the separation of ginsenosides.

3.2. Solid phase extraction

To exclude interference from rat plasma samples, several extraction methods were tested, including solid phase extraction using an SEP-PAK C_{18} cartridge and the liquid–liquid extraction method by ether or ethyl acetate. It was shown that solid phase extraction could remove proteins and other interfering components in rat plasma with satisfactory drug recovery. Loading of the sample should be slow, and different concentrations of methanol were selected for the fine eluting procedure. It was found that removal of interfering substances was better on a loaded cartridge washed successively with 3 ml of water, and 20%, 55%, and 65% methanol in water, than with 3 ml of water, 20% methanol in water, and 6 ml of 65% methanol in water. The eluents of 55% and 65% methanol in water contained ginsenoside-Rg_2. This procedure provided sufficient sample clean-up, higher recovery rate, and little interference on the chromatograms.

3.3. Selectivity

Chromatograms obtained from a blank and from rat plasma are shown in Fig. 2 (B). The enantiomers 20 (*R*)-ginsenoside-Rg_2 and 20 (*S*)-ginsenoside-Rg_2 were well separated at 14.5 min and 13.6 min, respectively. Baseline separation of the two compounds was obtained under the specified chromatographic conditions. No interfering peaks were observed in extracts of the matrix. This indicated that the selectivity of the procedure was appropriate.

3.4. Linearity, and LOD and LOQ

20 (*R*)-Ginsenoside-Rg_2 and 20 (*S*)-ginsenoside-Rg_2 standards, spiked at 7.8 μg/ml, 15.6 μg/ml, 31.3 μg/ml, 62.5 μg/ml, 125 μg/ml, and 250 μg/ml and 3.9 μg/ml, 7.8 μg/ml, 15.6 μg/ml, 31.3 μg/ml, 62.5 μg/ml, and 125 μg/ml in plasma, were analyzed in each run, and the peak area for each was plotted against the concentration to generate a calibration curve. The equation of the line was calculated by a least-squares linear regression method as shown in Table 1. Standard curves generated acceptable data over the ranges 7.8–250 μg/ml in plasma for 20 (*R*)-ginsenoside-Rg_2 and 3.9–125 μg/ml in plasma for 20 (*S*)-ginsenoside-Rg_2. All curves had correlation coefficients >0.999. Limits of detection were 2.0 μg for 20 (*R*)-ginsenoside-Rg_2/ml and 2.0 μg for 20 (*S*)-ginsenoside-Rg_2/ml in plasma. Limits of quantitation were 7.8 μg for 20 (*R*)-ginsenoside-Rg_2/ml and 3.9 μg for 20 (*S*)-ginsenoside-Rg_2/ml in plasma.

Table 1
Standard curve for 20 (*R*)-ginsenoside-Rg_2 and 20 (*S*)-ginsenoside-Rg_2 in rat plasma

Compound	Regression equation	Coefficient r^2	Linear range (μg/ml)
20 (*R*)-Ginsenoside-Rg_2	$y = 6525x - 1870.9$	0.9997	7.8–250
20 (*S*)-Ginsenoside-Rg_2	$y = 6812.3x + 2075$	0.9999	3.9–125

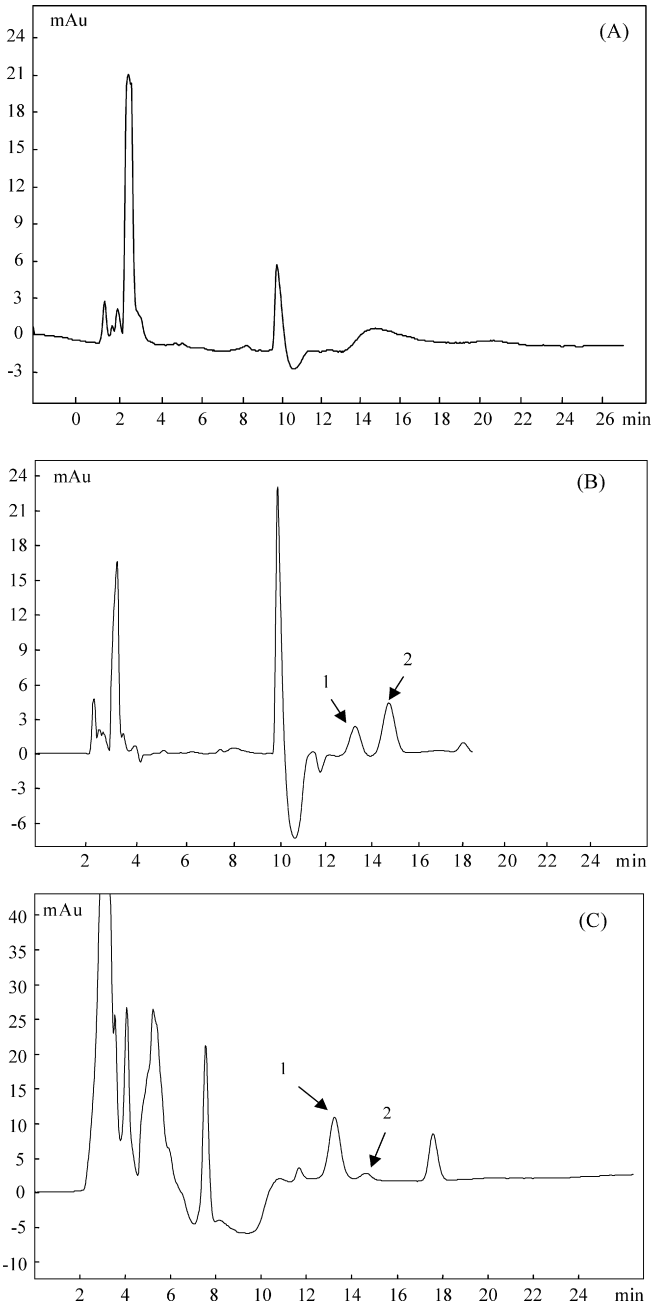

Fig. 2. Typical chromatograms of the determination of 20 (*S*)-ginsenoside-Rg_2 and 20 (*R*)-ginsenoside-Rg_2 in plasma samples: (A) chromatogram of a blank plasma; (B) chromatogram of a blank plasma sample spiked with 20 (*S*)-ginsenoside-Rg_2 (1) and 20 (*R*)-ginsenoside-Rg_2 (2); (C) chromatogram of the plasma sample from a rat after 3 min of intravenous administration of 20 (*R*,*S*)-ginsenoside-Rg_2.

3.5. Extraction recovery

The peak areas of unextracted analyte solutions were compared with those of extracted plasma to determine the recovery of 20 (*R*)-ginsenoside-Rg_2 and 20 (*S*)-ginsenoside-Rg_2. The average extraction recoveries from rat plasma were

Table 2
Extraction recovery of 20 (*R*)-ginsenoside-Rg_2 and 20 (*S*)-ginsenoside-Rg_2 from rat plasma ($n = 3$)

Compound	Spiked concentration (μg/ml)	Recovery (%)	CV (%)	Average (%)
20 (*R*)-Ginsenoside-Rg_2	7.8	96.4 ± 1.20	1.25	95.8 ± 1.0
	31.3	94.6 ± 0.47	0.50	
	125	96.4 ± 0.08	0.09	
20 (*S*)-Ginsenoside-Rg_2	3.9	96.0 ± 0.47	0.50	96.5 ± 0.5
	15.6	96.6 ± 5.18	5.26	
	62.5	96.9 ± 0.70	0.73	

Table 3
Validation of the intra- and the inter-day assay in rat plasma ($n = 3$)

Compound	Concentration (μg/ml)	The intra-day assay		The inter-day assay	
		Accuracy (%)	CV (%)	Accuracy (%)	CV (%)
20 (*R*)-Ginsenoside-Rg_2	15.6	88.52	1.59	88.52	0.09
	62.5	103.46	0.32	103.63	0.22
	250	99.68	0.19	99.48	0.54
20 (*S*)-Ginsenoside-Rg_2	7.8	91.67	0.78	92.05	0.53
	31.3	102.84	0.16	102.78	0.28
	125	99.70	0.10	99.80	0.12

95.8 ± 1.0% for 20 (*R*)-ginsenoside-Rg_2 and 96.5 ± 0.5% for 20 (*S*)-ginsenoside-Rg_2, as shown in Table 2.

3.6. Precision and accuracy

The data presented in Table 3 show the intra- and inter-day precision for plasma quality control samples. The intra- and inter-day precision and accuracy in rat plasma were evaluated at three concentration levels (15.6 μg/ml, 62.5 μg/ml, and 250 μg/ml for 20 (*R*)-ginsenoside-Rg_2 and 7.8 μg/ml, 31.3 μg/ml, and 125 μg/ml for 20 (*S*)-ginsenoside-Rg_2). As shown in Table 3, the intra-day accuracies for 20 (*R*)-ginsenoside-Rg_2 and 20 (*S*)-ginsenoside-Rg_2 were 88.52–103.46% and 91.67–102.84% with CV values of less than 1.59% and 0.78%, respectively. The inter-day accuracies for 20 (*R*)-ginsenoside-Rg_2 and 20 (*S*)-ginsenoside-Rg_2 were 88.52–103.63% and 92.05–102.78% with CV values of less than 0.54% and 0.53%, respectively. The overall reproducibility of the method was acceptable.

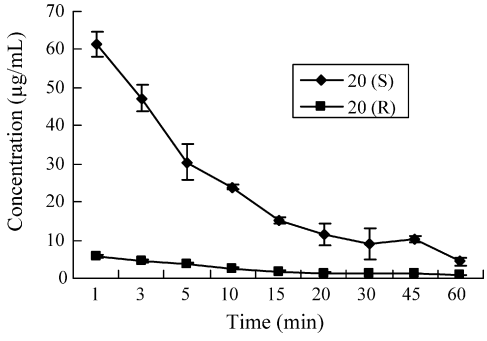

Fig. 3. The concentration–time profile of 20 (*S*)-ginsenoside-Rg_2 and 20 (*R*)-ginsenoside-Rg_2 ($n = 3$): after a rat was fixed on a wooden plate under pentobarbital sodium (40 mg/kg, i.p.) anesthesia, the ginsenoside-Rg_2 solution was delivered using a syringe into a rat tail vein at the dose of 25 mg/kg for 20 (*R*,*S*)-ginsenoside-Rg_2 corresponding to 2 mg/kg for 20 (*R*)-ginsenoside-Rg_2 and 23 mg/kg for 20 (*S*)-ginsenoside-Rg_2. Blood samples (800 μl) were collected immediately before and at 1 min, 3 min, 5 min, 10 min, 20 min, 30 min, 45 min, 60 min from the arteria carotis communis after 20 (*R*,*S*)-ginsenoside-Rg_2 administration.

3.7. Application to pharmacokinetics study

The method presented here was successfully used to quantify 20 (*R*)-ginsenoside-Rg_2 and 20 (*S*)-ginsenoside-Rg_2 in plasma samples after tail vein injection of ginsenoside-Rg_2 at a dose of 25 mg/kg for 20 (*R*,*S*)-ginsenoside-Rg_2, corresponding to 2 mg/kg for 20 (*R*)-ginsenoside-Rg_2 and 23 mg/kg for 20 (*S*)-ginsenoside-Rg_2. Representative chromatograms obtained from the study are shown in Fig. 2 (C). 20 (*R*)-Ginsenoside-Rg_2 and 20 (*S*)-ginsenoside-Rg_2 were measured in plasma up to 1.5 h post-dosing. The concentration–time profiles of the enantiomers are

Table 4
Pharmacokinetic parameter of 20 (*R*)- and 20 (*S*)-ginsenoside-Rg_2 [20 (*R*)-ginsenoside-Rg_2 for 2 mg/kg and 20 (*S*)-ginsenoside-Rg_2 for 23 mg/kg] in rat plasma ($n = 3$)

Parameter	Unit	Value	
		20 (*R*)-Ginsenoside-Rg_2	20 (*S*)-Ginsenoside-Rg_2
A	$\mu g\,ml^{-1}$	5.0005 ± 0.0648	55.3000 ± 3.7722
α	min^{-1}	0.1740 ± 0.0220	0.1963 ± 0.0633
B	$\mu g\,ml^{-1}$	1.6259 ± 0.1232	14.5104 ± 5.6855
β	min^{-1}	0.0097 ± 0.0004	0.0181 ± 0.0057
$t_{1/2}\alpha$	min	4.0246 ± 0.0087	3.7242 ± 0.0459
$t_{1/2}\beta$	min	71.1999 ± 3.1586	38.4414 ± 1.1134
K21	min^{-1}	0.0504 ± 0.0065	0.0562 ± 0.0274
K10	min^{-1}	0.0336 ± 0.0003	0.0640 ± 0.0100
K12	min^{-1}	0.0997 ± 0.0157	0.0942 ± 0.0358
V_c	$l\,kg^{-1}$	3.8115 ± 0.0988	0.3620 ± 0.0459
V_d	$l\,kg^{-1}$	7.5398 ± 0.5373	0.6068 ± 0.3821
AUC	$\mu g\,min\,ml^{-1}$	197.7176 ± 5.1766	1092.5109 ± 83.9747
CL_s	$l\,min^{-1}$	0.1264 ± 0.0003	0.0232 ± 0.0013

shown in Fig. 3, fitted with a two-compartment model, and were used to calculate the pharmacokinetic parameters from the raw data, as shown in Table 4.

4. Conclusions

A simple and reproducible HPLC method has been developed to determine simultaneously the enantiomers of 20 (*R*)-ginsenoside-Rg_2 and 20 (*S*)-ginsenoside-Rg_2 using a Diamonsil^{TM} ODS C_{18} reversed-phase column and a mobile phase of MeOH-*aq*. 4% H_3PO_4. The quantitative ranges of 20 (*R*)-ginsenoside-Rg_2 and 20 (*S*)-ginsenoside-Rg_2 were 7.8–250 μg/ml and 3.9–125 μg/ml, respectively. The intra- and inter-day coefficients of variation were satisfactory for pharmacokinetic studies of the two enantiomers. The method was used for simultaneous determination of the plasma concentrations of 20 (*R*)-ginsenoside-Rg_2 and 20 (*S*)-ginsenoside-Rg_2 in rats after tail vein administration of 20 (*R*,*S*)-ginsenoside-Rg_2. This is the first report of a pharmacokinetic study of 20 (*R*)-ginsenoside-Rg_2 and 20 (*S*)-ginsenoside-Rg_2 in rat plasma. The results provide a firm basis for evaluating the clinical efficacy of 20 (*R*,*S*)-ginsenoside-Rg_2.

References

[1] A.S. Attele, J.A. Wu, C.S. Yuan, Pharmacol. Biochem. Pharmacol. 58 (1999) 1685.
[2] B.H. Lee, S.M. Jeong, J.H. Lee, D.H. Kim, J.H. Kim, J.I. Kim, H.C. Shin, S.M. Lee, S.Y. Nah, Mol. Cells 17 (2004) 51.
[3] D. Kiefer, T. Pantuso, Am. Fam. Physician 68 (2003) 1539.
[4] X.W. Yang, M.R. Hao, M. Hattori, Metabolite Analysis for Chemical Constituents of Traditional Chinese Medicines, China Medical-Pharmaceutical Science and Technology Publishing House, Beijing, 2004, p. 346.
[5] W.J. Sun, J. Liu, S.C. Qu, Y. Jin, Z.W. Zhang, L.Y. Li, Chin. J. Mod. Appl. Pharm. 21 (2004) 447.
[6] W.W. Lu, J. Liu, L.J. Zhao, J.M. Tian, L.Y. Li, S.J. Yang, J. Jilin Univ. (Med. Ed.) 30 (2004) 534.
[7] X.M. Cui, Y.N. Cui, Y.R. Li, L. Cui, J. Chin. Electron Microsc. Soc. 23 (2004) 309.
[8] J.M. Tian, S.Q. Zheng, W.F. Guo, J.M. Ye, L.Y. Li, Chin. Pharmacol. Bull. 20 (2004) 480.
[9] J.M. Tian, H. Li, J.M. Ye, W.F. Guo, L.Y. Li, L.P. Wang, China J. Chin. Mater. Med. 28 (2003) 1191.
[10] A. Suzuki, S. Takagaki, H. Suzuki, K. Noda, J. Chromatogr. 617 (1993) 279.
[11] A. Doroudian, K. Yeleswaram, D.W. Rurak, F.S. Abbott, J.E. Axelson, J. Chromatogr. 619 (1993) 79.
[12] U. Becker-Scharfenkamp, G. Blaschke, J. Chromatogr. 621 (1993) 199.
[13] J.X. de Vries, E. Schmitz-Kummer, J. Chromatogr. 644 (1993) 315.
[14] L. Wallen, O. Ericsson, I. Wikstrom, U. Hellgren, J. Chromatogr. B 655 (1994) 153.
[15] D. Demetriou, K. Rustemeier, P. Voncken, G. Schepers, Chirality 5 (1993) 300.
[16] X.W. Yang, Chin. J. Magn. Reson. 17 (2000) 9.
[17] I.W. Wainer, Drug Stereochemistry: Analytical Methods and Pharmacology, 2nd Ed., Marcel Dekker, New York, 1993, p. 139.
[18] N. Fuzzati, J. Chromatogr. B 812 (2004) 119.