A Simple HPLC Assay for Ginsenoside-Rh2 in Plasma and Its Application for Pharmacokinetic Study in Rats

Haijun Li1,2, Ping Ya Li2 and Pollen Yeung*1

1Pharmacokinetics and Metabolism Laboratory, College of Pharmacy and Department of Medicine, Dalhousie University, Halifax, NS, Canada
2Institute of Frontier Medical Science, Jilin University, Changchun, China

Abstract

Ginsenoside-Rh2 (G-Rh2) is an important constituent in ginseng and has significant anti-tumor property. A simple HPLC assay was developed to study pharmacokinetics of G-Rh2 in rat plasma. The HPLC consisted of a C18 reversed phase analytical column, a variable wavelength ultraviolet (UV) spectrophotometric detector, and a mobile phase made up of 0.005 M KH2PO4 (pH 7.2); acetonitrile: methanol (23:7:70). The system was operated at ambient temperature isocratically at a flow rate of 0.5 mL/min and wavelength at 203 nm. Extraction of G-Rh2 from plasma was achieved by solid phase extraction (SPE) using 100 mg/mL C18 SPE columns. The results showed that standard curves using 50 µL of plasma sample were linear from 0.25 to 100 µg/mL, with regression coefficient (r2)>0.99. The intra- and inter-assay variations over a 3-month study period were <10% and <20%, respectively. After 10 mg/kg twice daily for 4 doses by subcutaneous (sc) injection, the mean maximum plasma concentration (Cmax) and time to Cmax (Tmax) of G-Rh2 was 0.79 ± 0.06 µg/mL and <0.5 hour, respectively. The described HPLC is readily performed in most laboratories and should have adequate sensitivity and specificity to study pharmacokinetics of G-Rh2 in rats following multiple doses.

Keywords: Ginsenoside-Rh2; Ginseng; HPLC; Pharmacokinetics; Rats

Introduction

Natural products such as traditional Chinese medicines (TCM) are increasingly used in our societies to enhance health and prevention of chronic diseases [1-9]. There are also evidences to suggest that these herbal products may enhance the therapeutic effects of conventional medicines and they should be incorporated in prescription drug therapies [8,10-12].

Ginseng is a well-known medicinal herb native to China and Korea, and has been used as a herbal remedy in eastern Asia for thousands of years [13]. In TCM, ginseng is used for a variety of illnesses such as anorexia, shortness of breath, palpitation, insomnia, impotence, hemorrhage and diabetes. In western medicines, however, it is recognized for its effect on physical and psychomotor performance, cognitive function, immunomodulation, diabetes mellitus, improving cardiovascular risk factors, quality of life, as well as for counteracting adverse effects [9]. It is known that ginseng contains close to 40 different ginsenosides some of which have potent and varying pharmacologic properties [14-16] including anti-inflammatory and anti-tumor effects [17,18]. 20(S)-Ginsenoside-Rh2 (G-Rh2) is a trace constituent in ginseng which was first isolated from red ginseng by Kitagawa et al. [19]. It has an aglycone skeleton of 20(S)-protopanaxadiol dammarane structure (Figure 1) and exhibits cytotoxic effects in various cancer cells in vitro [20,21]. G-Rh2 has been shown to reverse resistance developed for daunomycin or vinblastine by P388/Adr cancer cells [22], synergistically enhance the activities of paclitaxel and mitoxantrone in prostate cancer cells [23], and increase the antitumor activity of cyclophosphamide while decreasing its genotoxic effects [24,25]. There is also evidence to suggest that G-Rh2 could increase the absorption of drugs which are substrates of P-glycoprotein in rats [26]. Thus G-Rh2 has considerable therapeutic potential for oncology therapy, and understanding the pharmacokinetics of G-Rh2 could help to better design dosage and route of administration for its optimal use to aid cancer chemotherapy.

Materials and Methods

Chemicals

G-Rh2 was isolated and purified from Panax ginseng C.A. Meyer cv. Silvatica, purchased from the Institute of Frontier Medical Science of Jilin University (Changchun, China), and was >98% pure as determined by HPLC [38]. Imipramine (IMI) was purchased from Sigma-Aldrich Ltd. (St. Louis, MO, USA) (Figure 1). Other pharmaceutical products tested for interference with the assay were either received as gifts from their respective manufacturers, or purchased from Sigma-Aldrich Ltd. (St. Louis, MO, USA). Solid phase extraction (SPE) columns were 50 µL, materials (100 mg/mL) purchased commercially (Extra-Sep®, Chromatographic Specialties Inc., Brockville/ ON, Canada.). Solvents were HPLC grade and other chemicals were reagent grade (Fisher Scientific, Ont., Canada).

HPLC system

The HPLC system consisted of a Beckman 114 M solvent delivery...
module (Berkeley, CA, USA), a Rheodyne syringe loading injector (model 9725) with a 100 μL PEEK injection loop (Scientific Products & Equipment, Concord, ON, Canada), a Shimadzu ultraviolet (UV) spectrophotometric detector (UV VIS SPD-20A, Man-Tech Assoc. Inc., Guelph, ON, Canada), and a Hewlett-Packard HP3395 Integrator (Agilent Technologies, Santa Clara, CA, USA). Chromatographic separation was achieved on a 3 μm 110 A 150×3.0 mm i.d. C<sub>18</sub> reversed phase analytical column (Gemini<sup>®</sup>-NX, Phenomenex, Torrance, CA 90501, USA) coupled with a 5 μm 4.0× 3.0 mm i.d. C<sub>18</sub> reversed phase guard column (Security® Guard Cartridges, Phenomenex, Torrance, CA 90501, USA) using a mobile Phase of a mixture of 0.005 M potassium phosphate buffer at pH 7.2 (KH<sub>2</sub>PO<sub>4</sub>):acetonitrile:methanol (23:7:0).

The system was operated at room temperature isocratically with a flow rate of 0.5 mL/min at an operating pressure of 2.5 ksi (ca. 183 kgf/cm<sup>2</sup>). G-Rh<sub>2</sub> and IMI were detected and quantified at 203 nm. Detector output was recorded by an integrator (Hewlett-Packard HP3395 Integrator/Palo Alto, CA, USA), and digitalized using the Peak Simple<sup>®</sup> software (Chromatographic Specialties Inc., Brockville/ON, Canada).

Preparation of standard solutions

Stock solutions of G-Rh<sub>2</sub> and IMI were prepared in methanol at 1 mg/mL and 0.1 mg/mL, respectively. Serial dilution of the stock solutions using HPLC water was performed to prepare standard spiking solutions for preparing plasma standards of 100, 40, 10, 4, 2, 1, and 0.25 μg/mL. The working internal standard (IS) solution of 2 μg/mL was prepared by diluting the stock IS solution with the HPLC water. Intra-assay and inter-assay variations were assessed over a 3-month study period using quality control (QC) samples at 1 and 10 μg/mL. These spiking solutions should be prepared on the same day of the extraction.

Solid phase extraction (SPE)

50 μL of each of the spiking solution of G-Rh<sub>2</sub> or HPLC water was added to 50 μL of rat blank plasma or study plasma sample in a 1.5 mL polystyrene micro-centrifuge tube, respectively, followed by 50 μL of 0.01 M KH<sub>2</sub>PO<sub>4</sub> buffer solution (pH 6.5), and then 50 μL of the working IS solution containing 0.1 μg of IMI. Each tube was gently mixed, and add to it was 150 μL of 62% cold methanol followed by vortex mix for 5 min at 1200 rpm in room temperature (Eppendorf Model 5436 Thermomixer, Hamburg, Ger) to precipitate the plasma proteins. After centrifugation at 5000xg for 10 min at room temperature (Eppendorf Model 5415 Microcentrifuge, Hamburg, Ger), the supernatant fluid was collected and loaded on top of the C<sub>18</sub> 100 mg/mL SPE column, and allowed to equilibrate for 5 min before passing it through the column slowly (ca. 1 mL/5 min) at a pressure of 10 inches of Hg (Vac-Elut<sup>®</sup>, Varian, Harbor City, CA, USA). Each SPE column was washed with 2×1 mL of 62% methanol in water and then air dried at a vacuum of 30 inches of Hg for 30 min. G-Rh<sub>2</sub> and the IS were recovered from the column by eluting with 2×0.5 mL of 90% methanol in water. The filtrate was collected into a round bottom glass culture tube (Kimax<sup>®</sup>, Fisher Scientific Co., Ottawa, ON, Canada), and evaporated to dryness under a gentle stream of nitrogen at 55°C (Thermolyrn Dri-Bath<sup>®</sup>, Fisher Scientific Co., Ottawa, ON, Canada). The residues were stored at -20°C until analysis. Each sample was reconstituted in 200 μL of mobile phase immediately prior to injection, and an aliquot (10-50 μL) was injected into the HPLC.

Pharmacokinetics study

The study protocol was approved by the Dalhousie University Committee on Laboratory Animals (UCLA) using the Canadian Council of Animal Care (CCAC) guidelines. Male SD rats (300-350 g) with a carotid artery catheter were purchased from Charles River Laboratories (Wilmington, DE, USA). They were each (n=6) given twice daily G-Rh<sub>2</sub> (10 mg/kg) for 4 doses by subcutaneous (sc) injection. Blood samples (0.3 mL each) were obtained from the catheter serially before the last dose, and at 0.1, 0.25, 1, 1.2, 1.5, 2, 3, 4, 5 and 6 hours post dose. The plasma (>0.1 mL) was immediately separated by centrifugation (4°C, 1720xg, 5 min) and then stored at -80°C until analysis. An aliquot of the plasma samples (50 μL) was used for analysis of G-Rh<sub>2</sub> by the described HPLC. All the samples were analyzed within 3 months after collection.

Data analysis

Recoveries of G-Rh<sub>2</sub> and IMI from plasma samples were determined by measuring the amounts (expressed as peak heights) after the SPE extraction, and compared to the amounts added to the QC samples. Standard curves were plotted using known plasma concentrations of G-Rh<sub>2</sub> (x-axis) and the peak height ratios (PHR) of G-Rh<sub>2</sub> to the IS (y-axis) from 0.25 to 100 μg/mL, and the data analyzed by linear regression (Lotus 1-2-3, IBM Canada). The QC samples at each concentration were performed in 4 replicates. Intra- and inter-assay variations were assessed from the in study QC samples (1 and 10 μg/mL) for each batch analysis over a 3-month period. The intra-assay variation was the average coefficient of variation (%CV) assessed over the study period, whereas the inter-assay variation (%CV) was determined using the mean PHR of the QC samples obtained from each period. Sensitivity of the assay was assessed by determining the smallest amount of G-Rh<sub>2</sub> injected on-column which resulted in a signal to noise ratio of greater than 3. The lower limit of quantization (LLQ) of the assay was determined by the lowest concentrations of G-Rh<sub>2</sub> measurable with a CV of less than 15%. Accuracy was assessed by comparing the concentrations determined by the assay with the spiked concentrations of the QC samples. Pharmacokinetic variables such as area under the curve (AUC), maximum plasma concentration (C<sub>max</sub>), time to maximum concentration (T<sub>max</sub>) and half-life (T<sub>1/2</sub>) were calculated using Rstrip<sup>®</sup> (MicroMath Scientific Software, St. Louis, MO, USA) assuming one compartment model after first order input (sc injection). The mean plasma concentration-time data from the rats (n=6) were used for data fitting and calculation of the pharmacokinetic parameter estimates.

Results

Under the described chromatographic conditions, the average retention times of IMI, and G-Rh<sub>2</sub> were 7.6 and 24 minutes, respectively (Figure 2). The standard curves of the HPLC assay for G-Rh<sub>2</sub> constructed during method development were linear between 0.25 to 100 μg/mL with regression coefficients (r<sup>2</sup>)>0.99. A typical standard curve is shown in (Figure 3). The recoveries using the described SPE were >85% for G-Rh<sub>2</sub> and the IS (IMI). The sensitivity of the assay based on absolute on-column injection of G-Rh<sub>2</sub> was 5 ng which produced a signal to noise ratio >3 (Figure 2). The LLQ was <0.25 μg/mL using 50
µL of plasma sample with CV of <15%. The intra assay variations at 1 and 10 µg/mL determined from 4 study batches (n=4 in each batch) over a 3-month period were <10%; and inter-assay variations were <20%. Accuracy of the assay was 100% and 107% for the 10 and 1 µg/mL QC samples, respectively, over the same study period (Table 1). We have tested a series of natural products and therapeutic agents which may be encountered in concurrent pharmacotherapy, and shown that there was no interference from any of the agents tested (Table 2).

Plasma concentration of G-Rh-2 was still detectable in some of the rats at the 0 h sample before the last injection. On average the plasma concentrations of G-Rh2 over the 6 h study period was 0.50 ± 0.24 µg/mL, and it was barely measurable in the rats 6 h after the injection, and thus the 6 h sample was not included in the pharmacokinetic analysis. The mean plasma concentration-time data could be adequately described by a one compartment open model following first order kinetics.

Discussion

Despite many HPLC assays which have been reported for ginsenosides to-date [27-29,31,32,34-37,39,40], there is still need for specific HPLC assay that is simple, easily accessible and sensitive enough for pharmacokinetics study of G-Rh2. Most of the HPLC assays reported for ginsenosides were based on tandem HPLC/MS techniques which are not readily amenable for routine analyses in general laboratory setting. On the other hand, the HPLC assay described by Shangguan and co-workers did not require mass-spectrometry, but it needed chemical derivatization followed by detection with fluorescent detector [28]. The only other non-mass spectrometric method as described by Wang and co-workers used similar UV detection, but it was not specific for G-Rh2 [27]. The HPLC method described in the current communication employed a 3 µm 150x3 mm ID column, which minimized mobile phase consumption and kept the cost down for the analysis. The method is specific for G-Rh2 as it is separated from other ginsenosides such as ginsenoside-Rg1 (G-Rg1) and ginsenoside-Rg3 (G-Rg3) (Table 2). Thus the method is simple, specific, economical to run, and should be easily adoptable in most laboratory settings.

The method utilized SPE technique coupled with reversed phase HPLC separation and UV detection using 62% methanol in water as a cleanup step before desorbing the analytes with 90% methanol. The extraction method achieved over 85% recoveries for G-Rh2 and the IS. During method development we also explored G-Rg3 as internal standard as it is chemically similar to G-Rh2 and had a retention time of about 10 min which should make it more suitable for the HPLC assay. However, the recovery was poor (<20%) and highly variable using the described SPE, for that we chose to use IMI despite having a more distinct chemical structure and less retained in the column (retention time 7.6 min). We have found that separation of imipramine (IS) from endogenous plasma materials was sensitive to mobile phase composition. Using 60% methanol in water (vs. 62%) as a cleanup step resulted in much dirtier sample and more interference from plasma matrix. On the other hand, using higher concentration of methanol in the cleanup step led to significant reduction of recovery from the SPE. Both precision as well as accuracy of the assay were within acceptable limits (± 10%) for the high (10 µg/mL) and low (1 µg/mL) QC samples. The assay was also robust enough with an inter-assay variation of <20% over the 3-month period (Table 1). Further, none of the compounds we tested in (Table 2) interfered with the assay. These include commonly used therapeutic products that could be potentially taken together with G-Rh2 in other research study and/or clinical situations. These are desirable attributes for an analytical method particularly for pharmacokinetic and drug interaction studies.

Table 1: Intra- and inter-assay variations of the HPLC assay of G-Rh2 in plasma.

| Drug test | Retention time (min) | Drug test | Retention time (min) |
|-----------|----------------------|-----------|----------------------|
| Ginsenoside-Rg1 | 24 min | Imipramine | 7.6 min |
| Ginsenoside-Rg1 | 3.2 min | Digoxin | 2.5 min |
| Ginsenoside-Rg3 | 10 min | Clomipramine | 8.3 min |
| Hydroxysafflor Yellow A | 5.4 min | Irinotecan | 3.7 min |
| Neomycin | >60 min | Losartan | 2.4 min |
| Bosentan | >30 min | Diltiazem | 3.5 min |
| CoQ10 | >60 min | Dipyridamole | 3.6 min |

Table 2: Retention times of drugs tested for interferences.
The current HPLC assay is not as sensitive as the LC/MS assays previously reported for G-Rh2 [32,36,40]. However despite the limited sensitivity, we were still able to measure plasma concentrations of G-Rh2 up to 5 h for pharmacokinetic measurement in rats following 4 doses of 10 mg/kg given by sc injection. Based on the pilot study, it appeared that G-Rh2 was rapidly absorbed following sc injection with $T_{\text{max}}$ attained at <0.5 h after injection. After that plasma concentration of G-Rh2 followed a mono-exponential decline with $T_{1/2}$ about 3 h, which was adequately characterized by a one-compartment model with 1st order input. However, it should be noted that the 6 h sampling time was inadequate for an accurate estimate of $T_{1/2}$. In addition, since G-Rh2 was present in the plasma samples collected before the last injection in some rats, it is probable that G-Rh2 was extensively distributed into extra-vascular tissues, and released back to circulation from tissue compartment beyond the 6 h sampling period. Thus the $T_{1/2}$ reported in this study was only an approximate which may underestimate the true terminal $T_{1/2}$. Very little is known of the pharmacokinetics of G-Rh2 in humans or animal models. One brief report which used a LC/MS method to measure plasma G-Rh2 in rat (n=3) up to 1 h following a single intravenous injection of 5 mg/kg [36]. The duration was too short in this study to provide meaningful estimates of pharmacokinetic parameters. Another study using LC/MS which measured G-Rh2 up to 24 h in rats following a single intragastric (ig) or intravenous (iv) administration. It reported for G-Rh2 a $T_{1/2}$ of 4.5 h which is similar to the 3 h found in the current study. The absolute oral bioavailability was <10% in rat, but was considerably higher in dog (20-30%) [41]. Thus despite the limitation, the current HPLC can provide a reasonable estimate of the pharmacokinetic of G-Rh2 in rats following multiple injections (Figure 4). However, it remains to be tested if the assay has adequate sensitivity for study requiring a lower dose or oral route of administration.

While no interference was found from a wide variety of therapeutic agents (Table 2), it is still questionable if the method has enough sensitivity for clinical therapeutic study which employs a lower dose. The current HPLC uses 50 μL of plasma sample and has a LLQ of about 0.25 μg/mL. It may be possible measuring lower than 20 ng/mL if a larger plasma sample size is used (e.g. 1 mL or larger). However, it will need to be evaluated further for therapeutic monitoring in a clinical setting.

In summary, the HPLC assay as described is simple, economical to run, readily accessible in general laboratory setting and have adequate sensitivity and specificity to determine G-Rh2 concentrations in plasma for pharmacokinetics studies in rats after multiple doses given by subcutaneous injection. The suitability for clinical study awaits further investigation.

### References

1. Middleton E Jr, Kandaswami C, Theoharides TC (2000) The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev 52: 673-751.

2. Mozaffarian D, Rimm EB (2006) Fish intake, contaminants, and human health: evaluating the risks and the benefits. JAMA 296: 1885-1899.

3. Calder PC, Yaqoob P (2009) Omega-3 polyunsaturated fatty acids and human health outcomes. Biofactors 35: 266-272.

4. Xiang YZ, Shang HC, Gao XM, Zhang BL (2008) A comparison of the ancient and modern safety and efficacy of Chinese medicinal materials. Phytomedicine 15: 443-452.

5. Shiou WL, Liu L (2010) The role of traditional Chinese herbal medicines in cardiovascular prevention: Lessons from Studies on Endothelial Function. Cardiovasc Ther 28: 187-201.

6. Calder PC, Yaqoob P (2009) Omega-3 polyunsaturated fatty acids and human health outcomes. Biofactors 35: 266-272.

7. Zucchi C, Ambrosio G, Luscher TF, Landmesser U (2010) Nutraceuticals in Cardiovascular Prevention: Lessons from Studies on Endothelial Function. Cardiovasc Ther 28: 187-201.

8. Calder PC, Yaqoob P (2009) Omega-3 polyunsaturated fatty acids and human health outcomes. Biofactors 35: 266-272.

9. Weant KA, Smith KM (2005) The Role of Coenzyme Q10 in Heart Failure. Ann Intern Med 142: 1522-1526.

10. Ceylan-Isik AF, Flixtman RM, Wold LE, Ren J (2008) Herbal and traditional Chinese medicine for the treatment of cardiovascular complications in diabetes mellitus. Curr Diabetes Rev 4: 320-328.

11. Hsiao WL, Liu L (2010) The role of traditional Chinese herbal medicines in cancer therapy--from TCM theory to mechanistic insights. Planta Med 76: 1118-1131.

12. Chan E, Tan M, Xin J, Sudarsanam S, Johnson DE (2010) Interactions between traditional Chinese medicines and Western therapeutics. Curr Opin Drug Discov Devel 13: 50-65.

13. Xiang YZ, Shang HC, Gao XM, Zhang BL (2008) A comparison of the ancient use of ginseng in traditional Chinese medicine with modern pharmacological experiments and clinical trials. Phytother Res 22: 851-858.

14. Berman M, Erman A, Ben-Gal T, Dvir D, Georghiou GP, et al. (2004) Coenzyme Q10 in patients with end-stage heart failure awaiting cardiac transplantation: a randomized, placebo-controlled study. Clin Cardiol 27: 295-299.

15. Weant KA, Smith KM (2005) The Role of Coenzyme Q10 in Heart Failure. Ann Pharmacother 39: 1522-1526.

16. Sander S, Coleman CI, Patel AA, Kluger J, White CM (2006) The impact of coenzyme Q10 on systolic function in patients with chronic heart failure. J Card Fail 12: 464-472.

17. Shibata S, Fujita M, Itokawa H, Tanaka O, Ishii T (1963) Studies on the constituents of Japanese and Chinese crude drugs. XI. Panaxadiol, A sapogenin of ginseng roots. Chem Pharm Bull 11: 759-761.

18. Lu JM, Yao Q, Chen C (2009) Ginseng compounds: an update on their molecular mechanisms and medical applications. Curr Vasc Pharmacol 7: 293-302.

19. Deng J, Lv XT, Wu Q, Huang XN (2009) Ginsenoside Rg1 inhibits rat left ventricular hypertrophy induced by abdominal aorta coarctation: involvement of calcineurin and mitogen-activated protein kinase signalings. Eur J Pharmacol 608: 42-47.

20. Chan RY, Chen WF, Dong A, Guo D, Wong MS (2002) Estrogen-like activity of ginsenoside Rg1 derived from Panax notoginseng. J Clin Endocrinol Metab 87: 3691-3695.
21. Kim HS, Lee EH, Ko SR, Choi KJ, Park JH, et al. (2004) Effects of ginsenosides Rg3 and Rh2 on the proliferation of prostate cancer cells. Arch Pharm Res 27: 429-435.

22. Hasegawa H, Sung JH, Matsumiya S, Uchiyama M, Inouye Y, et al. (1995) Reversal of daunomycin and vinblastine resistance in multidrug-resistant P388 leukemia in vitro through enhanced cytotoxicity by triterpenoids. Planta Med 61: 409-413.

23. Jia WW, Bu X, Philips D, Yan H, Liu G, et al. (2004) Rh2, a compound extracted from ginseng, hypersensitizes multidrug-resistant tumor cells to chemotherapy. Can J Physiol Pharmacol 82: 431-437.

24. Xie X, Eberding A, Madera C, Fazi L, Jia W, et al. (2006) Rh2 synergistically enhances paclitaxel or mitoxantrone in prostate cancer models. J Urol 175: 1926-1931.

25. Wang Z, Zheng Q, Liu K, Li G, Zheng R (2006) Ginsenoside Rh(2) enhances antitumor activity and decreases genotoxic effect of cyclophosphamide. Basic Clin Pharmacol Toxicol 98: 411-415.

26. Zhang J, Zhou F, Wu X, Gu Y, Ai H, et al. (2010) 20(S)-ginsenoside Rh2 noncompetitively inhibits P-glycoprotein in vitro and in vivo: a case for herb-drug interactions. Drug Metab Dispos 38: 2179-2187.

27. Wang H, Zou H, Kong L, Zhang Y, Pang H, et al. (1999) Determination of ginsenoside Rg3 in plasma by solid-phase extraction and high-performance liquid chromatography for pharmacokinetic study. J Chromatogr B Biomed Sci Appl 731: 403-409.

28. Shangguan D, Han H, Zhao R, Zhao Y, Xiong S, et al. (2001) New method for high-performance liquid chromatographic separation and fluorescence detection of ginsenosides. J Chromatogr A 910: 367-372.

29. Kwon SW, Han SB, Park IH, Kim JM, Park MK, et al. (2001) Liquid chromatographic determination of less polar ginsenosides in processed ginseng. J Chromatogr A 921: 335-339.

30. Li L, Zhang JL, Sheng YX, Ye G, Guo HZ, et al. (2004) Liquid chromatographic method for determination of four active saponins from Panax notoginseng in rat urine using solid-phase extraction. J Chromatogr B Analyl Technol Biomed Life Sci 808: 177-183.

31. Yu K, Ma Y, Shao Q, Qu H, Cheng Y (2007) Simultaneously determination of five ginsenosides in rabbit plasma using solid-phase extraction and HPLC/MS technique after intravenous administration of ‘SHENMAI’ injection. J Pharm Biomed Anal 44: 532-539.

32. Li X, Sun J, Wang G, Hao H, Liang Y, et al. (2007) Simultaneous determination of panax notoginsenoside R1, ginsenoside Rg1, Rd, Re and Rs1 in rat plasma by HPLC/ESI/MS: platform for the pharmacokinetic evaluation of total panax notoginsenoside, a typical kind of multiple constituent traditional Chinese medicine. Biomed Chromatogr 21: 735-746.

33. Joo KM, Lee JH, Jeon HY, Park CW, Hong DK, et al. (2010) Pharmacokinetic study of ginsenoside Re with pure ginsenoside Re and ginseng berry extracts in mice using ultra performance liquid chromatography/mass spectrometric method. J Pharm Biomed Anal 51: 278-283.

34. Zhang X, Zhang D, Xu J, Gu J, Zhao Y (2007) Determination of 25-OH-PPD in rat plasma by high-performance liquid chromatography-mass spectrometry and its application in rat pharmacokinetic studies. J Chromatogr B Analyt Technol Biomed Life Sci 858: 65-70.

35. Yang L, Xu SJ, Zeng X, Liu YM, Deng SG, et al. (2006) Determination of ginsenoside Rg3 and its metabolites in rat urine by LC-MS. Yao Xue Xue Bao 41: 742-746.

36. Qian T, Cai Z, Wong RN, Jiang ZH (2005) Liquid chromatography/mass spectrometric analysis of rat samples for in vivo metabolism and pharmacokinetic studies of ginsenoside Rh2. Rapid Commun Mass Spectrom 19: 3549-3554.

37. Xie HT, Wang GJ, Sun JG, Tucker I, Zhao XC, et al. (2005) High performance liquid chromatographic-mass spectrometric determination of ginsenoside Rg3 and its metabolites in rat plasma using solid-phase extraction for pharmacokinetic studies. J Chromatogr B Analyt Technol Biomed Life Sci 818: 167-173.

38. Jiang L, Zhao S, Ya L, Li J (2004) Determination of Ginsenoside Rh2 in Enzyme Conversion by HPLC. Special Wild Economic Animal and Plant Research 28: 41-44.

39. Li XW, Gui MY, Zheng Y, Jin YR, Zhang HQ (2006) Determination of 20(S)-ginsenoside Rh2 in the alkali-hydration product of saponins from leaves of Panax quinquefolium by RP-HPLC. Zhongguo Zhong Yao Za Zhi 31: 386-389.

40. Xie HT, Wang GJ, Lv H, Sun RW, Jiang XL, et al. (2005) Development of a HPLC-MS assay for ginsenoside Rh2, a new anti-tumor substance from natural product and its pharmacokinetic study in dogs. Eur J Drug Metab Pharmacokinet 30: 63-67.

41. Gu Y, Wang GJ, Sun JG, Jia YY, Wang W, et al. (2009) Pharmacokinetic characterization of ginsenoside Rh2, an anticancer nutrient from ginseng, in rats and dogs. Food Chem Toxicol 47: 2257-2268.