Journal of Chromatography B, 879 (2011) 2011–2017

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Simultaneous determination of ginsenoside (G-Re, G-Rg1, G-Rg2, G-F1, G-Rh1) and protopanaxatriol in human plasma and urine by LC–MS/MS and its application in a pharmacokinetics study of G-Re in volunteers

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ARTICLE INFO

Article history:
Received 10 December 2010
Accepted 11 May 2011
Available online 23 May 2011

Keywords:
Ginsenoside Re
LC–MS/MS
Metabolite
Pharmacokinetics

ABSTRACT

Ginsenoside Re (G-Re) improved the memory function of experimental animals in a preclinical study. Several types of saponins including G-Rg1, G-Rg2, G-F1, G-Rh1, and protopanaxatriol (PPT) may be the metabolites of G-Re according to reports from preclinical trials. In order to support a study of the pharmacokinetics of G-Re, an analytical method for G-Re and the co-detection of its probable metabolites using liquid chromatography tandem mass spectrometry (LC–MS/MS) was developed and validated. Solid phase extraction was utilized in the sample preparation. Separation of the analytes was achieved using a gradient elution (0.05% formic acid–methanol–acetonitrile, each organic phase containing 0.05% formic acid) at a flow rate of 0.3 mL/min with a retention time of approximately 2.88 min for G-Re. Data were acquired in the multiple reaction mode (MRM) and the linear range of the standard curve of plasma and urine samples for G-Re was 0.05–20 ng/mL with $r^2 \geq 0.99$. In the analysis of probable metabolites, G-Re, G-Rg1, G-F1, G-Rh1 and PPT were all detected in samples; however, G-Rg2 was not detected.
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1. Introduction

The use of ethnobotanical drugs as complementary medicine is prevalent in Asia and is also gaining increasing popularity in the West. The most well-known herb, traditionally used as a drug, is the root of the ginseng species (Panax ginseng C.A. Meyer). It is widely used in traditional Chinese medicine and is taken orally to enhance stamina and capacity to cope with fatigue as well as physical stress. Ginsenosides are normally classified into two groups based on the type of aglycone present, namely the protopanaxadiol group (e.g., G-Rb1 and G-Rc) and the protopanaxatriol group (Fig. 1). G-Re, is an active component and major constituent of ginseng, and is widely known to have several physiological effects including antioxidant effects [1,2], immunomodulatory effects [3], it improves diabetes and its complications [4–6], enhances tissue regeneration [7], modulates lymphocyte proliferation [8], regulates cardiovascular function [9,10], reduces the side-effects of prednisone acetate [11] and ameliorates impaired performance [12]. A preclinical study indicated that G-Re improved memory function in experimental animals by promoting the formation of synapse long-term potentiation. The *in vivo* and *in vitro* metabolism of many ginsenosides has been investigated in detail [13–21]. According to preclinical trials and published reports, several types of saponins including G-Rg1, G-Rg2, G-F1, G-Rh1, and PPT may be the metabolites of G-Re [13–17]. Studies on the metabolism of G-Re in human intestinal microflora [13] and in rats [14–16] have been carried out.

There are several previously published reports on the determination of G-Re. A chromatographic immunostaining method [22] using a monoclonal antibody was developed for the detection of G-Re in ginseng samples, however, this method had a limited quantitative range and a low limit of quantitation (LLOQ). The use of micellar electrokinetic chromatography coupled to Ultraviolet–Visible detection [23] and LC–MS/MS [24] have also been reported in the determination of G-Re, however, similar quantitative problems were observed. Recently, bioanalytical methods for G-Re have been developed using LC–MS [13] and LC–MS/MS [15,17]. However, these methods either had a poor LLOQ or limited linear range. In addition, few reports have focused on co-detection of the five probable metabolites (G-Rg1, G-Rg2, G-F1, G-Rh1, and PPT) of G-Re in human samples.

Solid-phase extraction (SPE) is a convenient method for sample preparation. Specific advantages of SPE include faster sample processing, economical use of solvent, good purification of compounds from complex samples and procedural simplicity potentially reducing the risk of manipulation errors in routine analysis. LC–MS/MS is a rapid and sensitive analytical method. To support published

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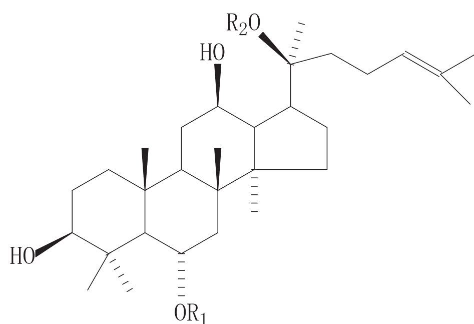
1570-0232/$ – see front matter © 2011 Published by Elsevier B.V.
doi:10.1016/j.jchromb.2011.05.018



Compound	M.F.	M.W.	R_1	R_2	Rt (min)	Channel	Cone Volt.	Col. Energy
G-Re	$C_{48}H_{82}O_{18}$	947	Glc$\overset{2}{-}$Rha	Glc	2.88	969.6→789.3	80	45
G-Rg1 *	$C_{42}H_{70}O_{14}$	801	Glc	Glc	2.90	823.5→643.2	90	40
G-F1 *	$C_{36}H_{58}O_{9}$	639	H	Glc	3.33	807.7→349.2	70	45
G-Rg2	$C_{42}H_{70}O_{13}$	785	Glc$\overset{2}{-}$Rha	H	3.38	661.4→481.3	70	32
G-Rh1 *	$C_{36}H_{58}O_{9}$	639	Glc	H	3.36	621.6→423.3	20	15
PPT *	$C_{30}H_{46}O_{4}$	475	H	H	3.77	441.4→423.3	20	18
Digoxin (IS)					3.07	781.3→651.3	20	10

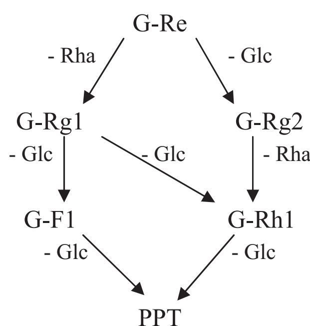


Fig. 1. Chemical structures and the main parameters of G-Re and the probable metabolites in addition to their major fragment ions observed in LC–MS/MS, and the probable metabolic pathway. Glc, β-D-glucopyranosyl; Rha, α-L-rhamnopyranosyl; *, the metabolites of G-Re detected in this study.

clinical studies and to evaluate the pharmacokinetics of G-Re in healthy Chinese subjects, SPE and LC–MS/MS were developed and validated for the direct quantification of G-Re, G-Rg1, G-Rg2, G-F1, G-Rh1, and PPT in human plasma and urine.

2. Experimental

2.1. Chemicals and reagents

Standards of G-Re and G-Rg1 with the purity of ≥98.0% were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). G-Rg2, G-F1, G-Rh1 and PPT with the purity of ≥98.0% were provided by Xinliheng Pharmaceutical Corporation (Beijing, China). The internal standard, digoxin, with the purity of ≥99.0% was purchased from Sigma Chemical Corporation (USA). Formic acid for mass spectroscopy was also purchased from Sigma and acetonitrile as well as methanol purchased from Tedia, USA were both of HPLC grade. Ultrapure water (18.2 MΩ cm, 25 °C) was prepared by passing through a Milli-Q System (Millipore Corporation, Germany). Solid-phase extraction cartridges (HLB 1 cc, OASIS) were purchased from the Waters Corporation (USA) and human blank plasma samples for the development, validation and quality control of the method were obtained from healthy, drug-free volunteer blood donors at the Blood Center of Tongzhou (Beijing, China). Blank urine was also supplied by the healthy, drug-free volunteers.

2.2. Working solution preparation

Stock solutions of G-Re, G-Rg1, G-Rg2, G-F1, G-Rh1, PPT and digoxin (IS) [14] were prepared in methanol at the concentration of 200 μg/mL. A mixed working solution containing G-Re, G-Rg1, G-Rg2, G-F1, G-Rh1 and PPT at eleven concentrations (Table 1) was prepared by appropriate dilution of the stock solution with 50% methanol. The stock solution of digoxin was further diluted with 50% methanol to obtain the working IS solution at the concentration of 50 ng/mL. All solutions were stored at 4 °C and were allowed to reach room temperature before use.

2.3. Liquid chromatographic conditions

LC–MS/MS analyses were performed using a Waters 2795 HPLC system (Waters Corporation, USA) coupled to a Quattro Premier electrospray ionization tandem mass spectrometer

Table 1
Concentration of the six compounds in the 11 levels of mixed working solution.[a]

Working solution (ng/mL)	Re	Rg1	Rg2	F1	Rh1	PPT
1	0.5	0.5	2	0.5	5	5
2/QC1	1	1	5	1	10	10
3	2	2	10	2	20	20
4	5	5	20	5	50	50
5	10	10	50	10	100	100
QC2	15	15	75	15	150	150
6	20	20	100	20	200	200
7	50	50	200	50	500	500
8	100	100	500	100	1000	1000
QC3	160	160	800	160	1600	1600
9	200	200	1000	200	2000	2000

[a] By diluting each working solution 10 times with blank plasma or urine, the calibration curve points (standard concentrations of each analyte) and QC were obtained.

(Micromass, Waters Corporation, USA). Chromatographic separation was obtained using a Waters Symmetry C_{18} Column (2.1 mm × 150 mm, 3 μm), fitted with a filter cartridge. An injection volume (10 μL) of sample was kept in an autosampler set at 4 °C. The column temperature was maintained at 22 °C. Separation of the analytes was achieved using a gradient elution (0.05% formic acid–methanol–acetonitrile, each organic phase containing 0.05% formic acid) at a flow rate of 300 μL/min with continuous 5% acetonitrile and under a linear gradient of 12–85% methanol over 2 min, 85% methanol hold for 2 min, then 12% methanol hold for 4 min. The retention time (Fig. 1) was approximately 2.88 min for G-Re and approximately 3.07 min for the IS.

2.4. Mass spectrometric conditions

The mass spectrometer was operated under the positive ionization mode in electrospray MS/MS conditions, and data were acquired under multiple reaction monitoring (MRM) incorporating a molecular weight scan from m/z 100 to m/z 1100. The following electrospray MS/MS parameter settings were applied: electrospray capillary voltage was 3.5 kV; ultrapure nitrogen was used as the nebulizer and cone gas at a rate of 500 L/h and 50 L/h, respectively; source and desolvation temperatures were set at 105 °C and 350 °C, respectively. With the collision energy off, the analytes yielded predominantly $[M+Na]^+$ for G-Re, G-Rg1, G-Rg2, and G-F1, $[M-H_2O+H]^+$ for G-Rh1, $[M-2H_2O+H]^+$ for PPT and protonated molecular ions for the IS. Each of the precursor ions was subjected to collision-induced dissociation (CID) to determine the resulting daughter ions. Argon was used as the collision gas at a flow rate of 0.24 mL/min. The acquired data were quantified using Masslynx 4.0 (Waters Corp., USA). The monitor ion and collision energy are shown in Fig. 1.

2.5. Biological samples

For the G-Re pharmacokinetics study, blood samples were obtained from healthy volunteers (five males and five females) enrolled in the study *via* an indwelling cannula inserted into a forearm vein immediately prior to dosing and 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 5, 6, 8, 10 and 12 h post-dosing. Urine samples were collected prior to dosing and at 2 h intervals up to 8 h, then at 4 h intervals up to 12 h and finally at 12 h intervals up to 24 h after G-Re administration. The samples were then separated by centrifugation, and each 1 mL was stored in a 1.5-mL Eppendorf tube at −70 °C until analysis. All subjects provided written informed consent prior to enrollment in the study, which was conducted in the Department of Pharmacy at Beijing Hospital (Beijing, China) following review by the Institutional Review Board. Study procedures were performed in accordance with the Declaration of Helsinki (concerning medical research in humans).

2.6. Sample preparation

Sample preparation involved solid phase extraction (SPE) with *OASIS* HLB cartridges. To the 1.5-mL Eppendorf tubes containing aliquots of human plasma or urine (1 mL), 50 μL of the IS working solution was added to the tubes which were then vortexed for 10 s before each sample was centrifuged (13,000 rpm) for 3 min and then loaded onto a HLB cartridge, which was preconditioned with 1 mL of methanol and then 1 mL of ultrapure water. Each cartridge was washed sequentially with 1 mL of ultrapure water, 1 mL of methanol/water (5/95, vol/vol), and then eluted with 0.5 mL of methanol/water (9/1, vol/vol). The eluate containing the analytes was transferred into an autosampler vial and 10 μL of the sample was injected into the LC–MS/MS system for analysis.

2.7. Standard solution preparation

Calibration curve points (standard solution) were prepared by spiking blank plasma or urine with the appropriate volume of one of the above-mentioned mixed working solutions to dilute each working solution 10 times with blank plasma or urine to obtain final standard concentrations of each analyte. Quality controls (QC) for the analytes were prepared using the same procedure. The subsequent assay procedures were the same as those described in Section 2.6.

2.8. Method validation

The specificity of the method was evaluated by comparing the baseline blank plasma or urine samples with samples spiked with each analyte standard or IS using the SPE and LC–MS/MS procedures outlined to certify the absence of interference from endogenous compounds.

The matrix effect on the ionization of analytes and IS was evaluated by the peak area ratio of the post-extraction blank plasma or urine sample then spiked with analytes/IS and the neat standard solutions at an equivalent concentration to the QC levels.

To assess the precision and accuracy of the procedure, reproducibility for both intra-day (6 samples at each QC level) and inter-day (3 consecutive days) variations was measured. The precision results are depicted using RSD. The recovery of SPE (5 samples at each QC level) was evaluated by comparing the peak area of analytes at the QC levels when adding the working solution to the blank plasma or urine before and after SPE.

Stability was studied at the QC levels under various conditions. Short-term stability was assessed by analyzing QC samples kept at ambient temperature for 8 h. Long-term stability was tested by assaying QC samples after storage at −70 °C for 12 weeks. Post-preparative stability was evaluated by analyzing the processed samples kept in the autosampler at the setting of 4 °C for 24 h. Freeze–thaw stability was estimated over three cycles.

Calibration curves were prepared by plotting the peak area ratios between the analytes and the IS against the concentration of analytes. Linear regression was then performed. The acceptance criterion for the correlation coefficient (r) was $r^2 \geq 0.99$. Linearity was determined by the correlation coefficients from the calibration curves of nine concentrations of each analyte (i.e. G-Re) which ranged from 0.05 to 20 ng/mL after extraction and assay. Blank plasma or urine samples were analyzed to confirm the absence of interference by endogenous compounds but were not used to construct the calibration function. The LLOQ ($<$0.05 ng/mL for G-Re) was determined as the concentrations with a signal-to-noise (S/N) ratio of 10:1. Each concentration standard needed to meet the fol-

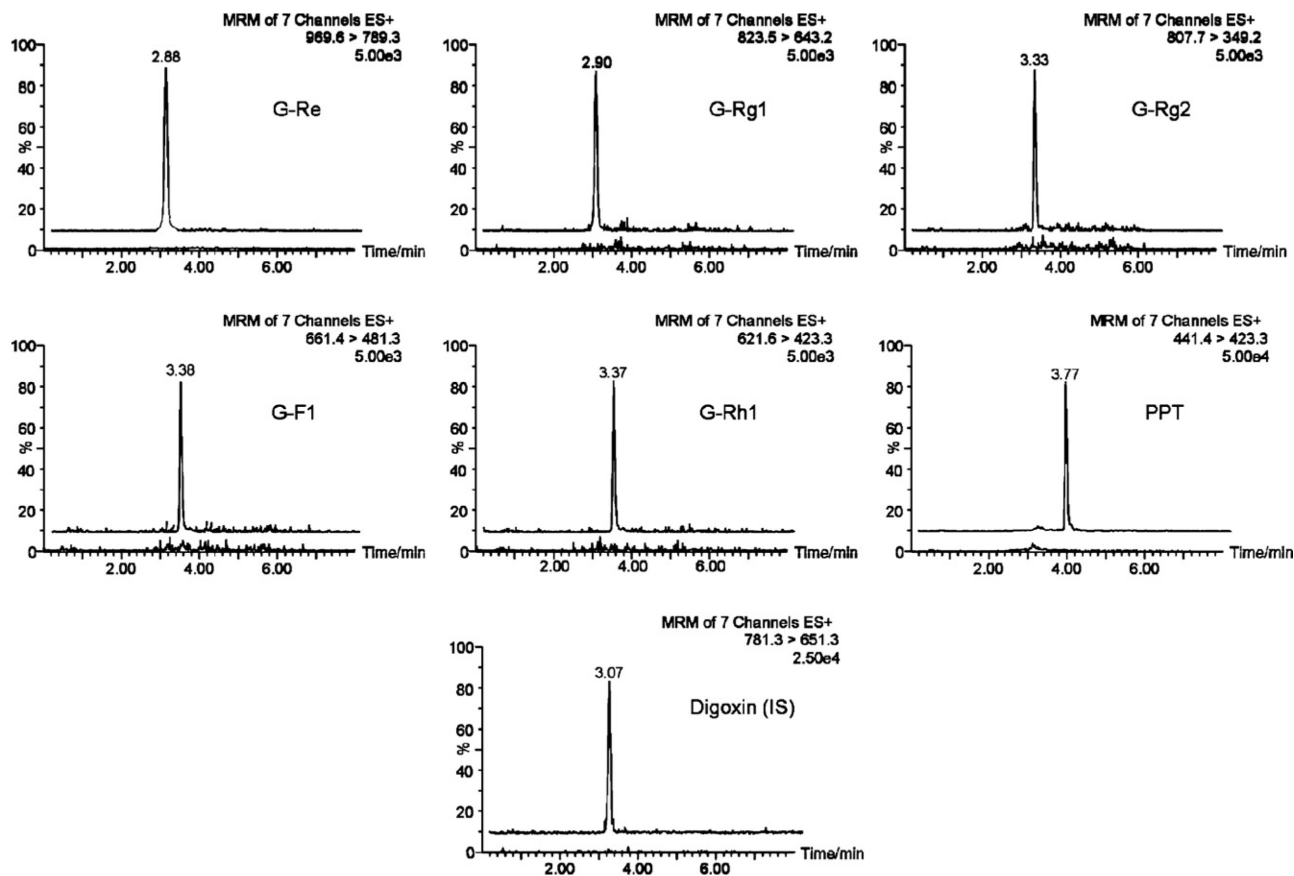


Fig. 2. Typical representative ion chromatograms of blank and standard samples (near LLOQ, the lowest standard) in plasma.

lowing acceptable criteria: no more than 20% deviation near the LLOQ and no more than 15% deviation for the standards above the LLOQ.

3. Results and discussion

3.1. Development of the analytical method

The mobile phase system was composed of 0.05% formic acid–methanol–acetonitrile (each organic phase containing 0.05% formic acid). A good peak shape but poor response of the analytes was seen with acetonitrile alone, while methanol alone induced peak broadening and tail formation of the analytes. The combination of acetonitrile and methanol was then chosen as the organic phase. When acetonitrile was controlled at the proportion of 5%, a good peak shape and response of the analytes were noted.

A satisfactory chromatographic peak shape and response were observed with the aqueous phase of ultrapure water, but the retention times of the analytes were unstable in different matrices. Ultrapure water with a modifier such as ammonium formate or formic acid, which usually causes suppression [25] of the response of the analytes, was used to fix the retention time. Ammonium formate is a common buffer which is selected as a modifier in the mobile phase in LC–MS/MS analysis, however, the response of ginsenosides was decreased following the introduction of $[M+NH_4]^+$. 0.05% formic acid which yielded a satisfactory chromatographic peak shape and response for the analytes in the samples was chosen as the aqueous phase. Thus, the mobile phase system (0.05% formic acid–methanol–acetonitrile, each organic phase containing 0.05% formic acid) was set up for gradient separation which provided good sensitivity and stable retention times for the analytes.

The mean matrix effects of the QC concentration levels of the analytes were (98.7 ± 5.5)%, (98.5 ± 4.4)%, (97.3 ± 5.4)%, (101.6 ± 5.3)%, (99.0 ± 8.2)% and (99.7 ± 7.1)% for G-Re, G-Rg1, G-Rg2, G-F1, G-Rh1 and PPT in plasma samples, and were (99.6 ± 5.5)%, (94.9 ± 6.0)%, (101.1 ± 4.1)%, (99.3 ± 8.2)%, (97.3 ± 7.0)% and (95.1 ± 6.0)% for G-Re, G-Rg1, G-Rg2, G-F1, G-Rh1 and PPT in urine samples, respectively. The matrix effects for the IS were (95.8 ± 4.1)% and (95.0 ± 5.2)% in plasma and urine samples, respectively.

3.2. SPE for plasma and urine extraction

In order to reduce HPLC run time during the analysis and lengthen the lifetime of the HPLC column for large samples, the SPE procedure was established for sample preparation.

The recovery of SPE was evaluated by comparing the peak areas of the QC concentration levels of the analytes when adding the working solution of analytes to plasma or urine before and after SPE. The extraction recovery of the 6 compounds was shown to be consistent, precise and reproducible. The mean recoveries of the QC concentration levels of the analytes were (84.5 ± 1.4)%, (78.2 ± 5.6)%, (75.0 ± 5.0)%, (76.9 ± 4.6)%, (75.1 ± 5.3)% and (81.1 ± 5.0)% for G-Re, G-Rg1, G-Rg2, G-F1, G-Rh1 and PPT in plasma samples, and were (82.3 ± 1.9)%, (77.5 ± 3.3)%, (76.9 ± 5.0)%, (77.1 ± 4.7)%, (76.0 ± 6.0)% and (82.7 ± 5.8)% for G-Re, G-Rg1, G-Rg2, G-F1, G-Rh1 and PPT in urine samples, respectively. The mean recoveries for the IS were (79.3 ± 3.1)% and (81.7 ± 4.5)% in plasma and urine samples, respectively.

3.3. Specificity

The described method proved to be specific with respect to interference by endogenous compounds using the same retention

Table 2
Calibration curves of the six compounds in plasma and urine samples ($n = 6$).

Compound	Calibration curves in plasma		Calibration curves in urine	
Ginsenoside Re	$R = 0.4981C + 0.0782$	$r^2 = 0.9988$	$R = 0.6062C + 0.1047$	$r^2 = 0.9973$
Ginsenoside Rg1	$R = 1.2627C + 0.1601$	$r^2 = 0.9981$	$R = 1.2035C + 0.1614$	$r^2 = 0.9981$
Ginsenoside Rg2	$R = 0.2029C + 0.1461$	$r^2 = 0.9996$	$R = 0.2028C + 0.1087$	$r^2 = 0.9998$
Ginsenoside F1	$R = 0.6119C + 0.0125$	$r^2 = 0.9998$	$R = 0.5778C + 0.0379$	$r^2 = 0.9996$
Ginsenoside Rh1	$R = 0.1922C + 0.0838$	$r^2 = 0.9998$	$R = 0.1785C + 0.2291$	$r^2 = 0.9994$
Protopanaxatriol	$R = 0.0499C + 0.1841$	$r^2 = 0.9962$	$R = 0.0495C + 0.0888$	$r^2 = 0.9990$

time and MRM channel (Fig. 2). The S/N ratio for the lowest standard concentration of G-Re and its probable metabolites in plasma and urine samples was more than 10, which was accepted as the minimum S/N ratio of the LLOQ.

3.4. Linearity

The method exhibited a good linear response over the selected concentration range using linear regression analysis. Calibration curves were constructed on 3 consecutive days. The correlation coefficient (r) and the goodness-of-fit coefficient (g) for the concentration range were >0.99 and <15%, respectively. The mean calibration curve was typically described by the least-square equation with the weight coefficient $1/C^2$, $R = 0.4981C + 0.0782$, and $r^2 = 0.9988$ ($n = 6$, G-Re in plasma sample), where R corresponds to the peak-area ratio of G-Re compared to the IS, and C refers to the concentration of G-Re added to plasma over a concentration range of 0.05–20 ng/mL. The results of six representative calibration curves of G-Re, G-Rg1, G-Rg2, G-F1, G-Rh1 and PPT in plasma or urine samples are listed in Table 2.

3.5. Stability

The mixed working solutions were found to be stable for 12 weeks at 4 °C, as were freeze and thaw stability, short-term stability, long-term stability and post-preparative stability of the samples spiked with G-Re, G-Rg1, G-Rg2, G-F1, G-Rh1 and PPT. These results (Table 3) showed that there were no stability-related problems during routine analysis in the pharmacokinetic study.

3.6. Precision and accuracy

To assess the precision and accuracy of the procedure, reproducibility for both inter-day and intra-day variations was determined. The RSD values for intra-day precision were in the range 0.1–7.1%, whereas the corresponding inter-day values were 0.8–11.1%. While the relative recoveries for G-Re, G-Rg1, G-Rg2, G-F1, G-Rh1 and PPT in the samples were 90.7–114.8%. These results revealed good precision and accuracy.

3.7. Results of the pharmacokinetics study

The developed method was used to analyze plasma and urine samples obtained from 10 healthy Chinese volunteers who each received a dose of 200 mg G-Re in the pharmacokinetics study. The procedure was sensitive enough to ensure the quantitative analysis

Table 3
The results of stability for G-Re ($n = 5$).

Conc. %	1.0 ng/mL	15 ng/mL	160 ng/mL
Working solution (4 °C)	100.5 ± 6.3	100.8 ± 6.2	102.6 ± 6.0
Conc. %	0.1 ng/mL	1.5 ng/mL	16 ng/mL
Short-term stability	102.6 ± 7.0	103.9 ± 6.7	101.0 ± 4.8
Long-term stability (−70 °C)	102.6 ± 7.0	109.3 ± 7.4	102.1 ± 3.5
Post-preparative stability	95.6 ± 5.5	106.1 ± 3.6	101.5 ± 2.6
Freeze–thaw stability	102.0 ± 6.7	103.9 ± 6.5	103.6 ± 5.6

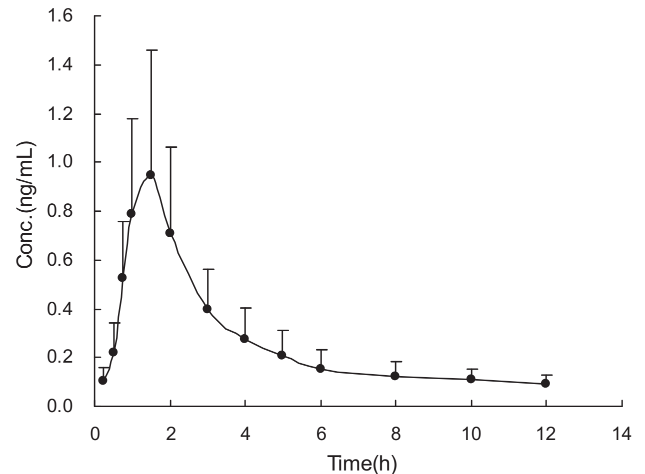


Fig. 3. The mean plasma concentration (Mean + SD)–time curve obtained from 10 volunteers after oral administration of 200 mg G-Re tablets.

of G-Re in plasma and urine with acceptable accuracy over a period of 12 h after administration. The mean plasma concentration–time curve obtained from the 10 volunteers after oral administration of G-Re tablets is shown in Fig. 3. The curve showing the amount of G-Re recovered from urine is shown in Fig. 4. Pharmacokinetic parameters are listed as follows: T_{max} and $t_{1/2}$ were (1.19 ± 0.44) h and (1.82 ± 0.75) h, respectively. AUC_{0-t} and $AUC_{0-\infty}$ were (2.476 ± 2.281) ug/L h and (2.699 ± 2.284) ug/L h, respectively. C_{max} was (0.939 ± 0.549) ug/L and CL/F was (124,054 ± 84,725) L/h.

With the exception of ginsenoside Rg2, which has been detected in a metabolic study of G-Re in rats [15,16], all the other probable metabolites were detected in the plasma and urine samples. The low concentration or fast degradation of G-Rg2 may be the

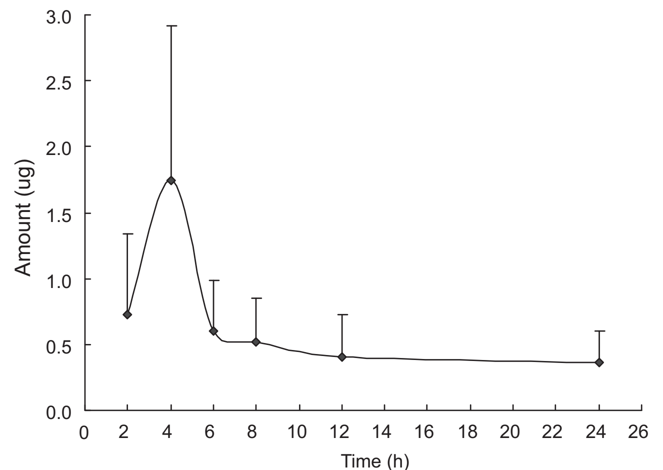


Fig. 4. The mean amount (Mean + SD) of G-Re recovered from the urine-time curve obtained from 10 volunteers after oral administration of 200 mg G-Re tablets.

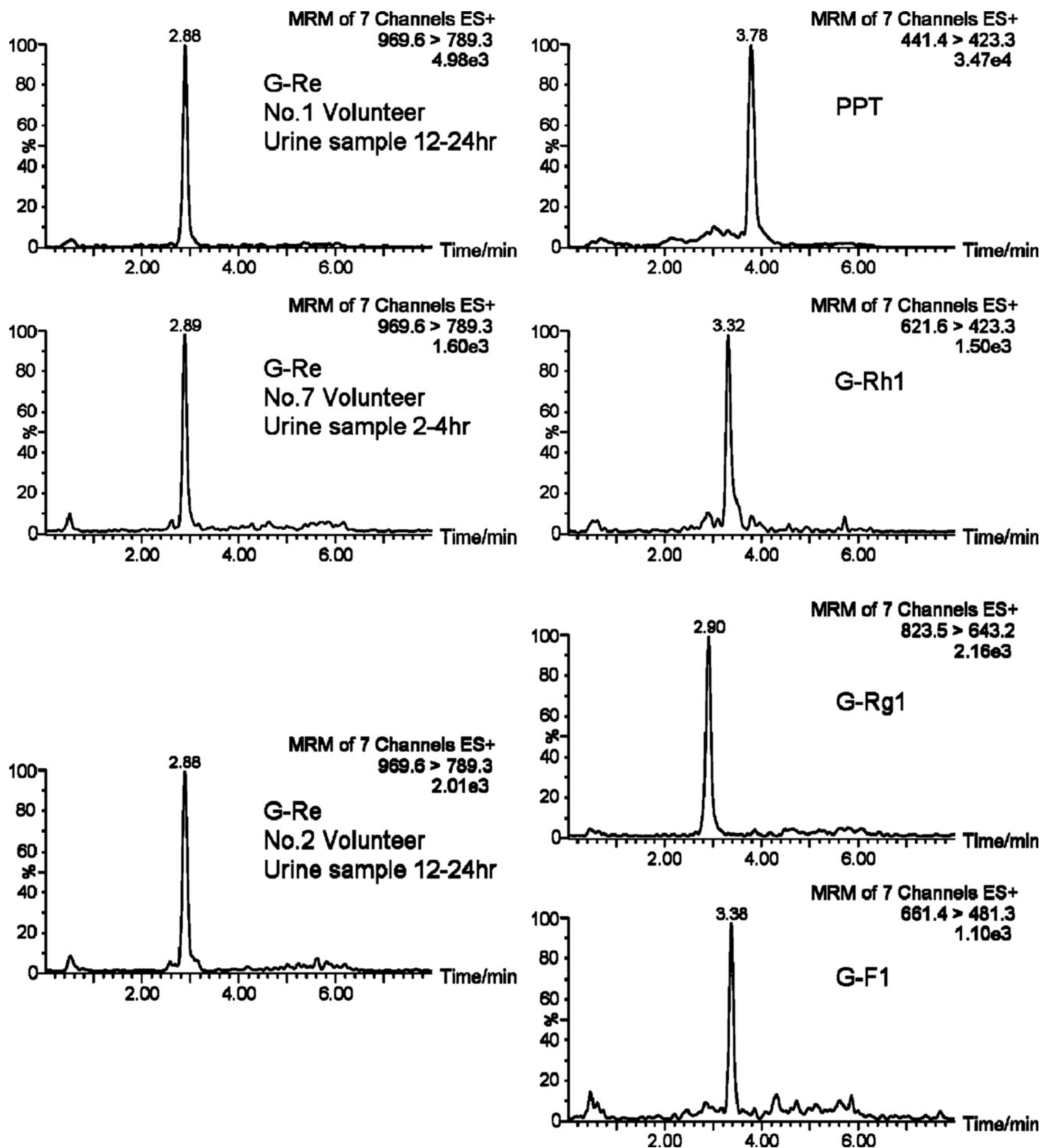


Fig. 5. Representative ion chromatograms of G-Re and the corresponding metabolites detected in samples from volunteers post-dosing.

reason why it was not detected in this study. G-Rh1 and G-F1 have also been detected by Tawab et al. [17], however, the drug used for oral administration in their study was a commercial ginseng preparation containing several types of ginsenosides. Fig. 1 shows the probable metabolic pathway, and chromatograms of the detected metabolites, G-Re, G-Rg1, G-F1, G-Rh1 and PPT are shown in Fig. 5. The results showed that the urine samples supplied more information on the metabolites than the plasma samples which is consistent with the results obtained in the study by Tawab et al. [17].

4. Conclusions

The described high performance liquid chromatography–electrospray ionization mass spectrometry (LC–ESI-MS/MS) method for the determination of G-Re, G-Rg1, G-Rg2, G-F1, G-Rh1 and PPT in plasma and urine was proved to be rapid, specific and sensitive. No endogenous compounds were co-eluted with the six compounds studied and the IS, and the analysis of these compounds was simplified by the solid phase extraction procedure. The addition of this procedure made the

method suitable for detection of the metabolites and the analysis of a large number of samples resulting from the pharmacokinetics study of G-Re in humans.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jchromb.2011.05.018.

References

[1] J.T. Xie, Z.H. Shao, T.L. Hoek Vanden, W.T. Chang, J. Li, S. Mehendale, C.Z. Wang, C.W. Hsu, L.B. Becker, J.J. Yin, C.S. Yuan, Eur. J. Pharmacol. 532 (2006) 201.
[2] S. Mehendale, H. Aung, A. Wang, J.J. Yin, C.Z. Wang, J.T. Xie, C.S. Yuan, Cancer Chemother. Pharmacol. 56 (2005) 63.
[3] J.L. Yu, D.Q. Dou, X.H. Chen, H.Z. Yang, N. Guo, G.F. Cheng, Planta Med. 71 (2005) 202.
[4] W.C. Cho, T.T. Yip, W.S. Chung, S.K. Lee, A.W. Leung, C.H. Cheng, K.K. Yue, J. Ethnopharmacol. 108 (2006) 272.
[5] J.T. Xie, S.R. Mehendale, X. Li, R. Quigg, X. Wang, C.Z. Wang, J.A. Wu, H.H. Aung, P.A. Rue, G.I. Bell, C.S. Yuan, Biochim. Biophys. Acta 1740 (2005) 319.
[6] A.S. Attele, Y.P. Zhou, J.T. Xie, J.A. Wu, L. Zhang, L. Dey, W. Pugh, P.A. Rue, K.S. Polonsky, C.S. Yuan, Diabetes 51 (2002) 1851.
[7] Y.C. Huang, C.T. Chen, S.C. Chen, P.H. Lai, H.C. Liang, Y. Chang, L.C. Yu, H.W. Sung, Pharm. Res. 22 (2005) 636.
[8] J.Y. Cho, A.R. Kim, E.S. Yoo, K.U. Baik, M.H. Park, Planta Med. 68 (2002) 497.
[9] G.I. Scott, P.B. Colligan, B.H. Ren, J. Ren, Br. J. Pharmacol. 134 (2001) 1159.
[10] C.X. Bai, K. Takahashi, H. Masumiya, T. Sawanobori, T. Furukawa, Br. J. Pharmacol. 142 (2004) 567.
[11] D. Dou, Y. Wen, Y. Pei, Y. Chen, Z. Ma, Zhongguo Zhong Yao Za Zhi 22 (174–176) (1997) 192.
[12] Y. Yamaguchi, M. Higashi, H. Kobayashi, Eur. J. Pharmacol. 312 (1996) 149.
[13] E.A. Bae, J.E. Shin, D.H. Kim, Biol. Pharm. Bull. 28 (2005) 1903.
[14] K.M. Joo, J.H. Lee, H.Y. Jeon, C.W. Park, D.K. Hong, H.J. Jeong, S.J. Lee, S.Y. Lee, K.M. Lim, J. Pharm. Biomed. Anal. 51 (2010) 278.
[15] L. Yang, S. Xu, C. Liu, Z. Su, Anal. Bioanal. Chem. 395 (2009) 1441.
[16] G. Chen, M. Yang, D. Guo, Zhongguo Zhong Yao Za Zhi 34 (2009) 1540.
[17] M.A. Tawab, U. Bahr, M. Karas, M. Wurglics, M. Schubert-Zsilavecz, Drug Metab. Dispos. 31 (2003) 1065.
[18] L. Yang, Y.H. Deng, S.J. Xu, X. Zeng, J. Chromatogr. B 854 (2007) 77.
[19] J. Lee, E. Lee, D. Kim, J. Lee, J. Yoo, B. Koh, J. Ethnopharmacol. 122 (2009) 143.
[20] E.A. Bae, M.J. Han, M.K. Choo, S.Y. Park, D.H. Kim, Biol. Pharm. Bull. 25 (2002) 58.
[21] Y. Wang, B.X. Wang, T.H. Liu, M. Minami, T. Nagata, T. Ikejima, Acta Pharmacol. Sin. 21 (2000) 792.
[22] O. Morinaga, H. Tanaka, Y. Shoyama, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 830 (2006) 100.
[23] W.F. Yang, W.L. Zhao, Zhongguo Zhong Yao Za Zhi 28 (2003) 135.
[24] X. Wang, T. Sakuma, E. Asafu-Adjaye, G.K. Shiu, Anal. Chem. 71 (1999) 1579.
[25] S.A. Gustavsson, J. Samskog, K.E. Markides, B. Langstrom, J. Chromatogr. A 937 (2001) 41.