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Liquid chromatographic method for determination of four active saponins from *Panax notoginseng* in rat urine using solid-phase extraction

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Abstract

Four major active saponins (ginsenosides Rg_1, Rb_1, Rd and notoginsenoside R_1) in *Panax notoginseng* were determined in rat urine after oral and intravenous administration of total saponins of *P. notoginseng* (PNS), and the urine samples were treated with solid-phase extraction (SPE) prior to liquid chromatography. A reversed-phase liquid chromatography system with ultraviolet detection and a Zorbax SB-C_{18} column was used. The within-day and between-day assay coefficients of variation for the four saponins in urine were less than 7% and the recovery of this method was higher than 85%. Using this method, the excretion profile of the drug in rat urine after administration of PNS was revealed for the first time.

Keywords: *Panax notoginseng*; Saponins; Ginsenosides

1. Introduction

The root of *Panax notoginseng* is an important component in various prescriptions in traditional Chinese medicine. It has been used for thousands of years as an important folk medicine in China. The total saponins of *P. notoginseng* (PNS), phytochemically obtained from the roots of *P. notoginseng*, have been regarded as the principal components manifesting the pharmacological activities of the drug [1]. Currently, PNS are used to treat coronary heart disease, cardiac angina, apoplexy and atherosclerosis in clinics [2–4]. PNS mainly contained four active saponins, which are ginsenosides Rg_1, Rb_1, Rd and notoginsenoside R_1, respectively (their total content ≥60% in PNS). Therefore, the pharmacokinetic studies of PNS were focused on the above-mentioned four saponins. A number of reports on the absorption, distribution, metabolism and excretion (ADME) of ginsenosides have been performed on animals with the administration of high doses of isolated individual ginsenosides. For example, Odani et al. [5–8] studied systematically the ADME of ginsenosides Rg_1 and Rb_1 in rat using TLC method, but no report was found on the ADME of notoginsenoside R_1 and ginsenoside Rd. Up to now, there has been little information on the degree of absorption and excretion of saponins after consumption of PNS or PNS preparations except that Xu et al. [9] studied the pharmacokinetics and bioavailability of ginsenosides Rb_1 and Rg_1 after administration of PNS in rat plasma by HPLC method.

Analysis of intact saponins in biological fluids is difficult since there are a variety of single saponins and other constituents in PNS or PNS preparations. Methods used for the detection of these saponins in animal biological fluids include thin layer chromatography [5–8], high-performance liquid chromatography [9,10] and gas chromatography [11,12], etc. TLC method was widely used to study the ADME of ginsenosides in Odani's reports. This method has a detection limit higher than 500 ng, which is insufficient for the determination of saponins in biological fluids, especially after oral administration of PNS but not the individual ginsenosides. GC method is more suitable for the determination of small molecular weight compounds with low boiling points. Therefore, we have developed and validated a HPLC method for the quantification of ginsenosides Rg_1, Rb_1, Rd and notoginsenoside R_1 in rat urine after oral and intravenous administration of PNS.

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In the present report, we described a simple method for the determination of four saponins in rat urine using a solid-phase extraction (SPE) technique and HPLC with UV detection, which proved to be simple, sensitive and specific, and could be applied in metabolic and pharmacokinetic studies of PNS.

2. Experimental

2.1. Chemicals and reagents

HPLC grade acetonitrile was purchased from Merck Company (Merck, Darmstadt, Germany); deionized water was purified by Milli-Q system (Millipore, Bedford, MA, USA); methanol was analytical grade from Beijing Reagent Co. Ltd. (Beijing, PR China) and Extract-clean^{TM} C_{18} (SPE) cartridge column was purchased from Alltech Company (Deerfield, IL, USA).

Ginsenosides Rg_1 and Rb_1 were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China); notoginsenoside R_1 and ginsenoside Rd were kindly provided by Professor Yinjie Chen (Shenyang Pharmaceutical University, Shenyang, PR China); β-estradiol was purchased from Fluka Chemical Company (Buchs, Swizerland, purity ≥97%). The total saponins of *P. notoginseng*, phytochemically obtained from the roots of *P. notoginseng*, were purchased from Wanfang Co. Ltd. (Wenshan, Yunnan, PR China). Injection prepared from PNS was manufactured by Kangyuan Pharmaceutical Co. Ltd. (Inner Mongolia, PR China).

2.2. Apparatus

An Agilent 1100 liquid chromatography system (Agilent Technologies, Deutschland, Waldbronn, Germany), equipped with a quaternary solvent delivery system, an autosampler and UV detector, was used. The column configuration consisted of an Agilent Zorbax SB-C_{18} reversed-phase column (5 μm, 250 × 4.6 mm) and an Aglient Zorbax SB-C_{18} guard column (5 μm, 20 × 4 mm). UV absorption was measured at 203 nm.

2.3. Animals

Experimental animals used were Sprague–Dawley rats, weighting 220–230 g, from Laboratory Animal Center of Peking University Health Science Center (Beijing, PR China).

2.4. Animal study

An 80 mg/ml PNS aqueous solution was administered orally with gavage at a dose of 80 mg/100 g to rats deprived of food but free access to water for 20 h before the experiment (800 mg PNS was easily dissolved in 10 ml distilled water after ultrasonication for 10 min). For intravenous experiments, an 8 mg/ml solution of PNS in 0.9% saline was given via the femoral vein at a dose of 50 mg/kg to fasted rats. Each rat was held in metabolic cage and the urine was collected periodically and stored at −10 °C prior to analysis. The aqueous and saline solutions of PNS could be kept stable for at least 1 week at 4 °C when not in use.

2.5. Preparation of stock, working and internal standard solutions

A concentrated stock solution of ginsenosides Rg_1, Rb_1, Rd and notoginsenoside R_1 was prepared by dissolving the reference substances in mixture of acetonitrile–water (1:1,v/v) to a final concentration of 1.0 mg/ml for ginsenoside Rb_1 and notoginsenoside R_1, 3.0 mg/ml for ginsenoside Rg_1 and 2.0 mg/ml for ginsenoside Rd. For the assay of urine samples, working solutions were prepared by appropriate dilution of the stock solution with the mixture of acetonitrile–water (1:1, v/v). The internal standard solution was prepared at a final concentration of 100 μg/ml in acetonitrile. All solutions were stored at −10 °C and were found to be stable for at least 1 month.

2.6. Preparation of standard and quality control (QC) samples for SPE

Standard samples for HPLC analysis and calibration were made by spiking control blank urine with working solutions to achieve analytical concentration ranges. The working solution (600 μl) and internal standard solution (100 μl) were placed in a glass tube, and the solvent was evaporated to dryness under nitrogen gas flow, then 2 ml of blank urine was added to the residue. The sample was vortex-mixed and applied to Extract-clean^{TM} C_{18} cartridge column. Quality control samples were similarly prepared at high, medium and low concentrations for the four saponins.

2.7. Sample processing

For the assay of urinary samples, 600 μl of acetonitrile-water (1:1,v/v) and 100 μl of internal standard solution were added to a glass tube, and the solvent was evaporated to dryness under the nitrogen gas flow. Urinary sample (2 ml) was added to the residue; the mixture was vortex-mixed for 5 min and applied to Extract-clean^{TM} C_{18} column.

2.8. Solid-phase extraction

The 2 ml standard urine sample was loaded and drew through by gravity on a SPE cartridge (5 ml, packed with 200 mg of 40 μm octadecyl silica), which was preconditioned by passing through 5 ml methanol followed by 5 ml water before loading. The solid-phase cartridge was washed with 10 ml water and 4 ml 20% (v/v) aqueous methanol solution in that order followed by elution with 5 ml 70% aque-

ous methanol solution. The eluate was evaporated at ambient temperature to dryness under the stream of nitrogen and reconstituted in 400 μl of 50% aqueous acetonitrile solution. A 5 μl aliquot was injected into the HPLC system for analysis.

2.9. Chromatographic conditions

Gradient elution of the analytes was performed using water (A) and acetonitrile (B). Initial condition was A–B (80:20,v/v), linearly changed to A–B (75:25,v/v) at 20 min. The mobile-phase composition was 69.0% A and 31.0% B at 25 min. Over the next 30 min, the percentage of mobile-phase B increased linearly to 40%. The total run time was 55 min. The column temperature was maintained at 35 °C and the flow rate was 0.8 ml/min.

2.10. Validation procedure

2.10.1. Calibration curve

The four calibration curves were constructed with standard samples in duplicate in two separate analytical runs. Every calibration curve consisted of at least seven concentration levels. The linear weighted regression was performed based on peak area ratio (analyte/internal standard (IS)). For every calibration curve, the calibration concentrations were back-calculated from the peak area of the analytes. The deviation from the nominal concentration was equal to or less than 9%.

2.10.2. Accuracy and precision

The within-day precision and accuracy were calculated by analyzing QC samples in six replicates. The between-day variability of the assay method was assessed by replicate analysis of QC samples on four separate days. QC samples were prepared from the stock solutions at three different concentrations – 78.75, 9.84 and 3.94 μg/ml for notoginsenoside R_1; 247.5, 30.9 and 12.38 μg/ml for ginsenoside Rg_1; 52.5, 6.56 and 2.63 μg/ml for ginsenoside Rb_1, and 172.5, 21.56 and 8.63 μg/ml for ginsenoside Rd. These validation criteria for precision and accuracy were used to assess the suitability of the method.

The precision was evaluated by within-day and between-day percent relative standard deviation (R.S.D., %): within ±15% for all concentrations. The accuracy is a measure of the systematic error or bias and is defined as the agreement between the measured concentration and nominal value. Accuracy of the method can be calculated from the expression: accuracy = measured concentration/nominal concentration, and should be in the range of 75–115% for all concentrations [13].

2.10.3. Limit of quantification

The limit of quantification (LOQ) was investigated in a series of different diluted urine standard samples. For the concentration to be accepted as LOQ, the percent deviation of the nominal concentration and the relative standard deviation has to be within the range of ±20% and less than 20%, respectively.

2.10.4. Recovery

The recovery of this method was determined by comparing peak-area ratios of the extracts of spiked urine (three different concentrations) to those obtained by direct injection of standards. Recoveries were calculated as the mean of four replicates.

2.10.5. Stability

To examine the stability in urine, pools of urine were spiked with the reference substances to obtain high, medium and low concentrations. These QC urinary samples were stored at room temperature for 48 h and analyzed. The long-term storage stability was checked by analyzing the QC samples that had been stored at −10 °C for a period of time. The freeze–thaw stability was determined after three freeze and thaw cycles. In each cycle, the QC samples were stored at −10 °C for 24 h and thawed unassisted at room temperature. When completely thawed, the sample was re-frozen within 24 h. This cycle was repeated two more times, and then the samples were analyzed after the third cycle.

3. Results and discussion

Ginsenosides Rg_1, Rb_1, Rd and notoginsenoside R_1 are the four major saponins contained in PNS (structures shown in Fig. 1). They all show poor UV absorption. To our knowledge, there is no report for derivatization of these saponins to improve their sensitivity. Therefore, it must be detected at low UV wavelengths. In this study, the detection wavelength was set at 203 nm. All the four saponins have sugar moieties attached, which cannot be readily extracted into organic solvents. High efficiency pretreatment technique was required to remove excessive interferences and achieve good selectivity for the four saponins. This problem could be overcome by the use of solid-phase extraction method, which could also ensure the efficient cleaning up of the urine samples. SPE method has been used for the pretreatment of ginsenoside Rb_1 [7,8], ginsenoside Rg_3 [10], ginsenoside Rb_2 [14–16] and PNS [9] in biological fluid. In these reports, 90% aqueous methanol and absolute methanol were used to elute the ginsenosides from the SPE, and 20% aqueous acetonitrile, 40 and 60% aqueous methanol solutions were utilized to wash the interferences in biological samples. The retention behaviors of these saponins in rat urine on C_{18} stationary phase with methanol–water system were also investigated. When the concentration of methanol was below 30%, the ginsenosides were strongly absorbed and difficult to elute from the column. However, many of the interferences could be removed at low methanol concentration. After eluting with 5 ml of 70% aqueous methanol, the column was continuously washed with 5 ml methanol and the eluent was

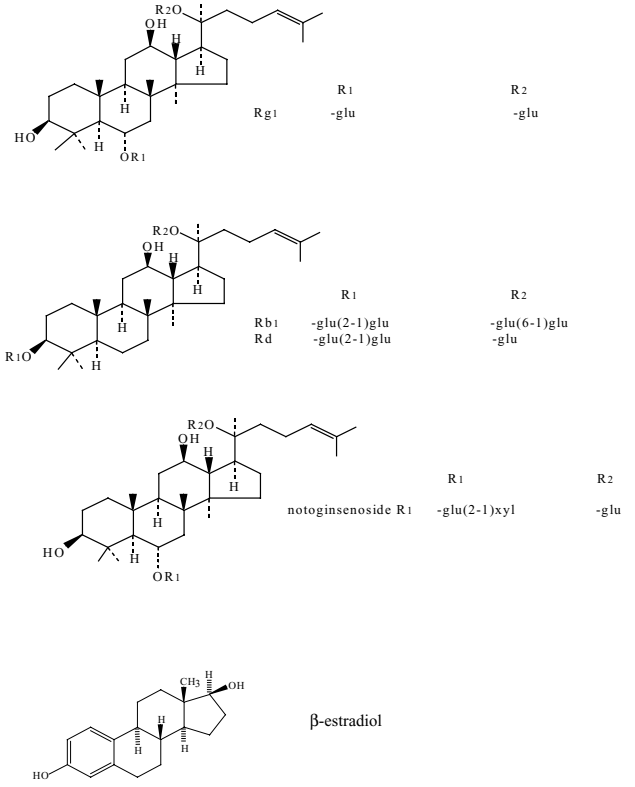

Fig. 1. The structures of the four saponins in *Panax notoginseng* and the internal standard.

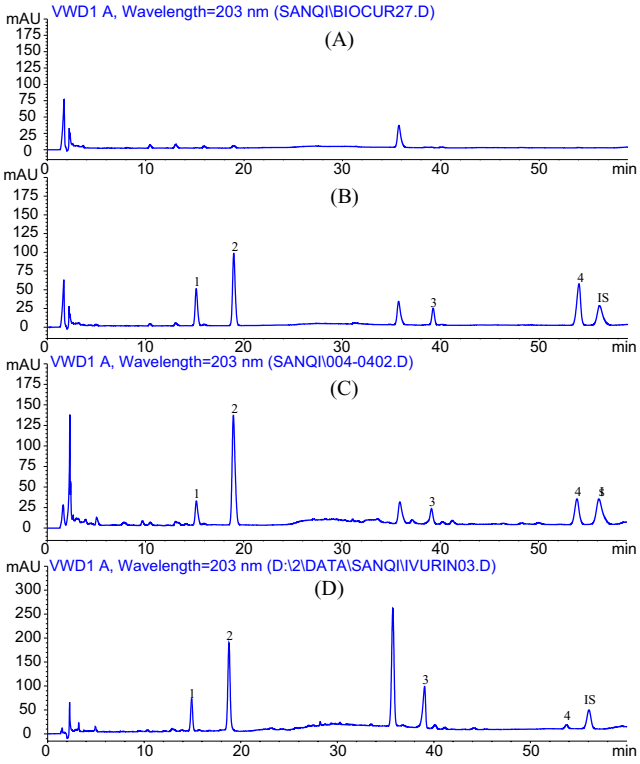

Fig. 2. Representative chromatograms of the four saponins in urine samples. (A) Blank urine. (B) Blank urine spiked with (1) notoginsenoside R_1 (78.75 μg/ml), (2) ginsenoside Rg_1 (247.5 μg/ml), (3) ginsenoside Rb_1 (52.5 μg/ml), (4) ginsenoside Rd (172.5 μg/ml) and internal standard (IS, 25 μg/ml). (C) Urine sample obtained 24 h after oral administration of saponins of *P. notoginseng*. (D) Urine sample abtained 7 h after intravenous administration of PNS.

detected by HPLC; no saponins and internal standard were found. It was also found that 5 ml of 70% aqueous methanol was optimal for the recovery of all the analytes and IS.

Internal standard is generally incorporated into an assay in order to improve precision, especially in the assays of biological samples. The primary requirement for an internal standard in a bioanalytical procedure is that it mimics the behavior of the target analytes. In this experiment, β-sitosterol, digtoxin and β-estradiol were compared. It was found that β-estradiol was the most suitable internal standard, which was structurally close to the four saponins and possessed analogous behavior with the analytes during both the sample preparation procedure and the detection in the HPLC system.

All the four saponins and internal standard in urine were completely separated within 55 min as shown in Fig. 2. The results presented here showed that we have developed a simple, efficient and precise HPLC method for the measurement of the four saponins in rat urine after oral and intravenous administration of PNS. The run time of this method is relatively long in the analysis of biological samples. In order to optimize this method, we tried to shorten the analysis time, including sharpening the gradient elution condition between ginsenosides Rg_1 and Rb_1. However, urinary samples collected in different time contained many compounds which would potentially interfere with the assay if the run time was considerably shorter.

In this method, standard curves were linear over the range of 3.9–78.8 μg/ml for notoginsenoside R_1, 12.4–495 μg/ml for ginsenoside Rg_1, 2.6–157.5 μg/ml for ginsenoside Rb_1 and 8.6–172.5 μg/ml for ginsenoside Rd, with the correlation coefficient $\geq$0.99. The limit of quantification was within the range of 0.66–1.55 μg/ml. The results of analysis are shown in Table 1.

In general, the assay precision and accuracy were derived from the QC samples. From these data, the mean standard derivation and coefficient of variation at each level were determined. The results are shown in Table 2. It was clear that the R.S.D. (%) was less than 6.8%.

Urinary samples containing three different concentrations of the analytes (low, medium and high levels) were used for the determination of the recoveries of the four saponins. Results are shown in Table 3. The percent recoveries for ginsenosides Rg_1, Rb_1, Rd and notoginsenoside R_1 were all over 85%.

The stability study showed that the four saponins were stable in urine at room temperature for 72 h and −10 °C for at least 7 days (shown in Table 4). The freeze–thaw stability results showed that the QC samples were stable after at least three freeze–thaw cycles. The results are summarized in Table 5.

Table 1
Calibration curves for the four saponins of *P. notoginseng* in urine

Analyte	Standard curves	r^2	Test range (μg/ml)	Limit of quantification (μg/ml)
Notoginsenoside R_1	$Y = 0.011X - 0.0010$	0.9997	3.94–78.75	0.79
Ginsenoside Rg_1	$Y = 0.0072X + 0.0543$	0.9997	12.38–495	2.47
Ginsenoside Rb_1	$Y = 0.0085X + 0.0213$	0.9999	2.62–157.5	0.66
Ginsenoside Rd	$Y = 0.0095X - 0.01993$	0.9995	8.63–172.5	1.15

Y, peak area ratio (analyte/internal standard); X, concentration of compound in urine (μg/ml).

Table 2
Within-day and between-day variability for the assay of the four saponins of *P. notogineng* in urine

Spiked concentration (μg/ml)	Within-day precision ($n = 6$)			Between-day precision ($n = 4$)		
	Measured concentration (μg/ml)	R.S.D.[a] (%)	Accuracy[b] (%)	Measured concentration (μg/ml)	R.S.D. (%)	Accuracy (%)
Notoginsenoside R_1						
78.75	78.89 ± 1.09	1.4	100.2	74.97 ± 4.5	6.0	95.2
9.84	9.75 ± 0.20	2.1	99.1	9.59 ± 0.21	2.1	97.5
3.94	4.30 ± 0.19	4.4	109.1	4.08 ± 0.28	6.9	103.6
Ginsenoside Rg_1						
247.5	250.19 ± 8.37	3.3	101.1	240.06 ± 12.26	5.1	97.0
30.94	29.65 ± 0.67	2.3	95.8	29.16 ± 0.71	2.4	94.2
12.38	11.17 ± 0.34	3.0	90.2	11.26 ± 0.70	6.2	91.0
Ginsenoside Rb_1						
52.5	51.74 ± 1.32	2.6	98.6	49.85 ± 2.29	4.6	95.0
6.56	6.89 ± 0.30	4.4	105.0	6.70 ± 0.35	5.2	102.1
2.63	2.70 ± 0.14	5.2	102.7	2.58 ± 0.16	6.2	98.1
Ginsenoside Rd						
172.5	170.63 ± 2.07	1.2	98.9	163.01 ± 8.85	5.4	94.5
21.56	21.79 ± 0.30	1.4	101.1	21.39 ± 0.50	2.3	99.2
8.63	8.85 ± 0.23	2.7	102.5	8.73 ± 0.26	3.0	101.2

[a] Relative standard deviation.
[b] Accuracy = [1 − (nominal concentration − mean of measured concentration)/nominal concentration] × 100.

The time variations of urinary excretions of ginsenosides Rg_1, Rb_1, Rd and notoginsenoside R_1 after oral administration of PNS are shown in Fig. 3. The cumulative urinary excretion of notoginsenoside R_1, ginsenoside Rg_1, Rb_1 and Rd within 48 h was 1.79, 5.14, 0.75 and 6.62% of the dose, respectively. Urine samples were collected periodically. The maximum urinary excretions (the greatest percentage dose of recovered in the urine collected in different time intervals) of all the four saponins after p.o. dosing of PNS were observed between 9 and 12 h and the results are shown in Fig. 4. To our knowledge, there are no reports on the excretion studies of notoginsenoside R_1, ginsenoside Rd and PNS. Odani et al. reported the excretion of ginsenosides Rg_1 and Rb_1 into urine after oral administration of the isolated individual ginsenosides in 1983 [5–8]. According to his reports, the cumulative urinary excretions of ginsenosides Rg_1 and Rb_1 within 12 h are lower than our results. The reasons of this inconsistence might result from the following facts. In our study, 800 mg/kg PNS (about 200 mg/kg for ginsenoside Rg_1, 250 mg/kg for ginsenoside Rb_1, 90 mg/kg for ginsenoside Rd and 70 mg/kg for notoginsenoside R_1) was given to rats via p.o. and the dose of oral administration in documental reports was 100 mg/kg for ginsenosides Rg_1 and Rb_1. Under the condition of our experiment, the enzymes and bacteria in gastric and intestinal tract of rats might be saturated and consequently the intact ginsenosides Rg_1 and Rb_1 entered into blood and urine. Additionally, the detection limits for ginsenosides Rg_1 and Rb_1 in our experiment were about 60 and 5.0 ng, respectively, which were

Table 3
Recovery of the four saponins in *P. notoginseng* from urine

Saponins	Spiked concentration (μg/ml)	Recovery[a,b] (%)	R.S.D. (%)
Notoginsenoside-R_1	65.25	91.8 ± 2.10	2.3
	26.25	91.5 ± 2.00	2.2
	6.56	87.8 ± 3.01	3.4
Ginsenoside-Rg_1	206.25	93.9 ± 1.97	2.1
	82.50	92.3 ± 3.11	3.4
	20.63	89.4 ± 4.01	4.5
Ginsenoside-Rb_1	43.75	90.0 ± 1.26	1.4
	17.50	90.0 ± 2.16	2.4
	4.38	85.0 ± 3.20	3.8
Ginsenoside-Rd	143.75	92.7 ± 1.39	1.5
	57.50	96.5 ± 3.09	3.2
	14.38	88.8 ± 3.73	4.2

[a] Recovery = [1 − (spiked concentration−measured concentration)/spiked concentration] × 100.
[b] All values quoted are the mean ± S.D. ($n = 4$).

Table 4
Stability of the four saponins in rat urine stored at −10 °C and at ambient temperature ($n = 4$)

QC concentration (μg/ml)	Stored at ambient temperature			Stored at −10 °C		
	Before storage	After storage	Recovered[a] (%)	Before storage	After storage	Recovered (%)
Notoginsenoside R_1						
78.75	78.48	79.30	101.0	78.03	78.48	100.6
9.84	9.82	9.34	95.1	9.99	9.68	96.9
3.94	4.14	3.84	92.7	4.23	4.45	105.2
Ginsenoside Rg_1						
247.5	254.73	230.23	90.4	252.77	254.73	100.6
30.94	29.48	28.16	95.5	29.75	29.17	98.1
12.38	11.16	11.34	101.6	11.45	11.18	97.6
Ginsenoside Rb_1						
52.5	52.50	47.24	90.0	53.04	48.17	90.8
6.56	6.56	6.25	95.3	6.86	6.25	91.1
2.63	2.63	2.73	103.8	2.55	2.36	92.5
Ginsenoside Rd						
172.5	174.5	155.25	89.0	173.3	171.78	99.1
21.56	20.56	20.75	100.9	21.34	21.71	101.7
8.63	8.93	8.45	94.6	8.71	8.64	99.2

[a] Recovered = (after treatment/before treatment) × 100.

lower than that in Odani's reports, 500 ng for ginsenosides Rg_1 and Rd by TLC. It is likely that at the low concentration, ginsenosides undetected by TLC could be determined by HPLC in this experiment. Furthermore, the parent components might also be transformed to their prosapogenins or other sapogenins by glycosidic hydrolysis as well as other reactions in rat digestive tract as suggested by the results from animal experiments [15,16]. After oral administration of PNS, there are many interactions and changes occurring in rat body, which might be the reason to make the amounts of ginsenoside Rg_1 and Rb_1 excreted into urine increased.

Table 5
Freeze–thaw stability of the four saponins in rat urine

QC concentration (μg/ml)	Measured concentration[a]		
	Before treatment	After treatment	Recovered[b] (%)
Notoginsenoside R_1			
78.75	79.45	78.62	99.0
9.84	9.96	9.42	94.6
3.94	4.19	4.48	106.9
Ginsenoside Rg_1			
247.5	252.63	234.7	92.9
30.94	29.55	28.89	97.8
12.38	11.15	10.51	94.3
Ginsenoside Rb_1			
52.5	52.66	49.28	93.6
6.56	6.86	7.01	102.2
2.63	2.69	2.56	95.2
Ginsenoside Rd			
172.5	173.3	167.6	96.7
21.56	22.15	21.34	96.3
8.63	8.05	8.64	107.3

[a] Values are reported as mean of four determinations.
[b] Recovered = (after treatment/before treatment) × 100.

The time variations of cumulative urinary excretions of the four saponinis after intravenous dosing of PNS to rats are shown in Fig. 5. Urine samples were collected periodically. The greatest urinary excretion of notoginsenoside R_1 and ginsenoside Rg_1 were observed between 0 and 4 h after intravenous administration of PNS, and the results are shown in Fig. 6. The cumulative urinary excretion of notoginsenoside R_1 and ginsenoside Rg_1 within 12 h was 37.3 and 73.5% of the dose, respectively. Most of the urinary excretion of ginsenoside Rb_1 occurred within 24 h after administration, but excretion of ginsenoside Rb_1 continued after that. The maximum excretion of Rd into urine was observed at 12 h. The cumulative urinary excretion of Rd within 12 h was 7.0%, but undetectable after 12 h.

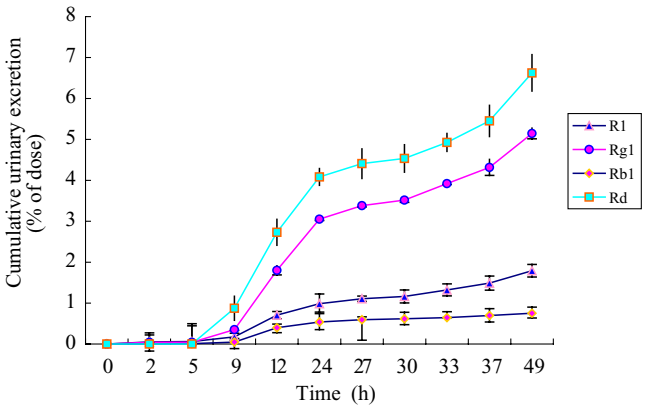

Fig. 3. Cumulative excretion of notoginsenoside R_1 (▲), ginsenoside Rg_1 (●), ginsenoside Rb_1 (◆), ginsenoside Rd (■) into urine after oral administration of saponins of *P. notoginseng* to rats. Each point with bar represents mean ± S.D. ($n = 3$) as the percentage of the administered dose of PNS.

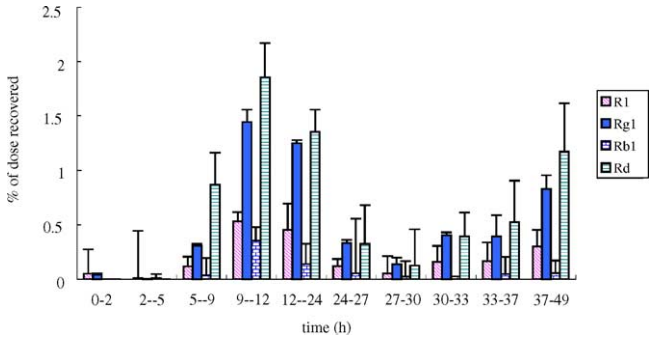

Fig. 4. Urinary excretion–time profile of notoginsenoside R_1, ginsenoside Rg_1, Rb_1 and Rd after oral administration of PNS to rats. urinary excretion (%dose of recovered) = amount of the drug excreted to urine/amount of the drug administered to rats.

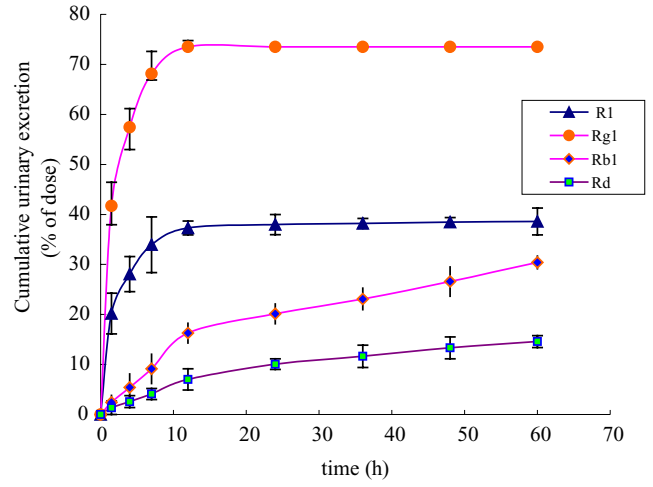

Fig. 5. Cumulative excretion of notoginsenoside R_1 (▲), ginsenoside Rg_1 (●), ginsenoside Rb_1 (◆), ginsenoside Rd (■) into urine after intravenous administration of saponins of *P. notoginseng* to rats. Each point with bar represents mean ± S.D. ($n = 3$) as the percentage of the administered dose of PNS.

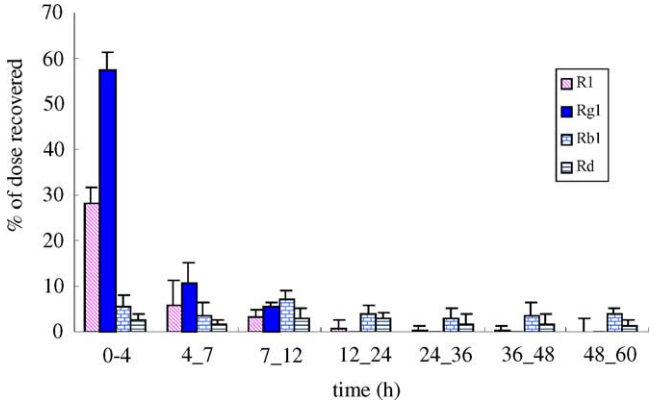

Fig. 6. Urinary excretion–time profile of notoginsenoside R_1, ginsenoside Rg_1, Rb_1 and Rd after intravenous administration of PNS to rats.

The method reported here was the first HPLC–UV assay with which the four major saponins in PNS could be quantitated simultaneously in rat urine after oral administration of PNS. Sample preparation is quite simple and without derivatization procedure. SPE preparation used in this study has improved recovery in particular for more polar compounds such as ginsenosides and notoginsenosides. This method possessed adequate sensitivity, selectivity, accuracy and precision for the quantitative analysis of the four saponins in PNS in rat urine. It can be used for the biopharmaceutical study of traditional Chinese formulations containing *P. notoginseng*.

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