Comparative pharmacokinetic analysis of raw and steamed *Panax notoginseng* roots in rats by UPLC-MS/MS for simultaneously quantifying seven saponins

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**ABSTRACT**

**Context:** After being steamed, the restorative effects of *Panax notoginseng* (Burk.) F. H. Chen (Araliaceae) will be strengthened. However, the underlying mechanism remains elusive.

**Objective:** To compare the pharmacokinetics of ginsenosides Rg1, Rb1, Rd, Re, Rg5, Rk1, notoginsenoside R1 (GRg1, GRb1, GRd, GRE, GRg5, GRk1, and NGR1) in the raw and steam-processed *P. notoginseng* (RPN and SPN).

**Materials and methods:** The pharmacokinetics of seven components after oral administration of SPN and RPN extracts (1.0 g/kg) were investigated, respectively, in SD rats (two groups, n = 6) using UPLC-MS/MS.

**Results:** The approach elicited good linear regression (r^2 > 0.991). The accuracy, precision and stability were all within ± 15%. The extraction recoveries and matrix effects were 75.0–100.8% and 85.1–110.3%, respectively. Compared with the RPN group, AUC_{0-24} of GRg1 (176.63 ± 42.94 ng/h/mL), GRb1 (509.06 ± 1453.14 ng/h/mL), GRd (1396.89 ± 595.14 ng/h/mL), and GRk1 (135.95 ± 54.32 ng/h/mL), along with C_{max} of GRg1 (17.41 ± 5.43 ng/mL), GRb1 (361.48 ± 165.57 ng/mL), GRd (62.47 ± 33.65 ng/mL) and NGR1 (23.97 ± 16.77 ng/mL) decreased remarkably with oral administration of the SPN extracts, while GRE showed no significantly difference. Of note, GRg5 and GRk1 could not be detected in the plasma.

**Conclusions:** Influence of the processing reduced the systemic exposure levels to GRg1, GRb1, GRd and NGR1. It is the first report of comparative pharmacokinetic study of multiple saponins analysis after oral administration of RPN and SPN extract, which might be helpful for further studies on its steam-processing mechanism.

**Introduction**

Processing of Chinese Materia Medica means special crafts of cooking, baking, steaming and other methods with solid or liquid excipients. After processing of specifications of Chinese Herbal Slices, the raw can be transformed into the cooked, thus strengthening the curative efficacy, reducing toxicity, increasing new effects and transforming the properties of medicines. In clinical application of traditional Chinese medicine (TCM), raw and cooked products are selected according to different diseases to achieve different therapeutic effects. The concept of ‘the different treatment for raw and cooked’ of TCM is a distinctive characteristic and major advantage of Chinese medicine (Sun et al. 2014).

*P. notoginseng* (Burk.) F. H. Chen (Araliaceae) is a precious herbal medicine in China. The root and rhizome are used as the famous TCM San-Qi. As first documented in *Compendium of Materia Medica* (Bencao Gangmu) in the Ming dynasty of China, *P. notoginseng* was regarded as ‘holy medicine of traumatology’ with the functions of haemostasis, promoting blood circulation to remove blood stasis, alleviating swelling and pain (Liu et al. 2014; Wang et al. 2016). Currently, *P. notoginseng* is listed as an herbal medicine in Chinese Pharmacopoeia (2015), European Pharmacopoeia (EP 8.0), British Pharmacopoeia (BP 2014), USA Herbal medicines Compendium (1.0), etc., and shows a huge development prospect and commercial value in medicine, functional food, dietary supplements and other industries. Processing of *P. notoginseng* has long history and the traditional processing methods include steaming, baking and frying, among which the steaming method is the most common processing method and still currently used (Wu et al. 2018). Pharmacological effects of *P. notoginseng* change during the steaming process and there is a description for *P. notoginseng* medicinal properties that ‘the raw products eliminate and the steamed forms tonify’ (Wu et al. 2018). That is to say, RPN can remove blood stasis and arrest bleeding, while SPN has a restorative effect for its nourishing function and blood cell-increasing (Zhou et al. 2020). Prevailing studies confirmed that the RPN displayed high potency for haemostasis, analgesic effects, anticoagulant, anti-inflammatory, while the SPN has better antitumor, antioxidation, antiplastic anaemia and enhances immune function activities (Lau et al. 2009; Li et al. 2011; Chen et al. 2013; Wu et al. 2014; Wei et al. 2018; Zhang et al. 2019). However, the underlying mechanism remains elusive.
Numerous studies have shown that most of the pharmacological activities of *P. notoginseng* are believed to be associated with saponins derived mainly from the tetracyclic dammarane. These triterpene saponins are classified according to their structures as 20(S)-protopanaxadiol type (ppd-type) saponins, such as ginsenosides R$_{a3}$, R$_b1$ and Rd and 20(S)-protopanaxatriol type (ppt-type) saponins, such as ginsenosides Re and R$_g1$ and notoginsenoside R$_1$. The change of chemical composition of *P. notoginseng* will occur during the steaming process. After steaming process, some saponins were decreased (ginsenosides R$_g1$, R$_b1$, Rd, Re and notoginsenoside R$_1$), and a large number of new effective compounds generated, such as ginsenosides R$_g5$, R$_g5$, R$_k1$, R$_k3$, R$_h4$, F$_2$ and Rh$_b$, which are unique saponins that only existed in SPN but not in RPN (Sun et al. 2010; Toh et al. 2016; Xiong et al. 2017; Zhu et al. 2018).

In this article, a simple, reliable and sensitive UPLC–MS/MS method was developed for the simultaneously determination of seven components in rat plasma, including five major saponins (ginsenosides R$_g1$, R$_b1$, Rd, Re and notoginsenoside R$_1$) and two unique saponins (ginsenosides R$_g5$ and R$_k1$), and applied to compare the pharmacokinetics of these components involving oral administration with the raw and steam-processed *P. notoginseng* aqueous extracts. Our study might be helpful for *P. notoginseng* clinical reasonable application and further studies on its steam-processing mechanism.

**Materials and methods**

**Chemicals and reagents**

The standard substances ginsenosides R$_g1$ (GRg1), R$_b1$ (GRb1), Rd (GRd), Re (GRe), R$_g5$ (GRg5), R$_k1$ (GRk1), notoginsenoside R$_1$ (NGR1) and theophylline (IS) (purity > 99%) were purchased from Shanghai Yuanye Biotechnology Co., Ltd, Shanghai, China. HPLC-grade methanol and formic acid were purchased from CNW Technologies Gmbh (Dusseldorf, Germany) and Sigma-Aldrich (St. Louis, MO), respectively. Ultra-pure water was obtained from a Mill-Q water purification system (Millipore, Burlington, MA). All other reagents were of LC-MS grade.

**Apparatus and operation conditions**

Separation was conducted on a Shimadzu UPLC instrument (Kyoto, Japan) with an Agilent Extend-C$_{18}$ column (i.d., 1.8 μm, 2.1 mm × 100 mm) at 35°C with a gradient mobile phase consisting of 0.005% HCOONH$_4$ and 3.0 mM formic acid aqueous solution (A) and methanol (B). The linear gradient elution programme was started at 20–50% B at 0–2.0 min, 50–87% B at 2.0–4.0 min, 87–90% B at 4.0–9.0 min, and the re-equilibration time of gradient elution was 2 min. The flow rate was 0.3 mL/min and the sample injection volume was 3 μL.

The MRM analysis was carried out on an AB Sciex QTRAP 5500 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) probe set in positive ionization mode. The optimized MS parameters were as follows: ion spray voltage, 5.5 kV; curtain gas, 35.0 psi; collision gas, 8.0 psi; ion source gas, 55.0 psi; entrance potential, 10 V; capillary temperature, 550°C. The data were acquired by PeakView software version 1.2 and instrumentation were controlled by Analyst software version 1.5.2 from AB Sciex (Framingham, MA).

**Preparation of standard and quality control (QC) samples**

The stock solutions of GRg1, GRe, GRb1, GRd, GRg5, GRk1 and NGR1 were separately prepared with 50% methanol. A series of working solutions were then obtained by diluting successively the stock solution with 50% methanol. Adding 5 μL of the above-mentioned series working solutions to 95 μL of blank rat plasma to obtain standard calibration curve in the concentration levels of 0.50–63.8 ng/mL for GRe, 1.00–127.5 ng/mL for GRg1 and NGR1, 3.98–255.0 ng/mL for GRd, 3.98–2040 ng/mL for GRb1, 7.97–255 ng/mL for GRg5 and GRk1. A quantity of theophylline was prepared in methanol to produce the IS solution with a concentration of 50 ng/mL.

Three concentration levels of 1.20, 23.9, 47.9 ng/mL for GRe, 23.9, 47.9, 95.6 ng/mL for GRd and NGR1, 9.56, 95.6, 191.3 ng/mL for GRb1, 7.65, 765.1, 150.2 ng/mL for GRg1, 19.1, 95.6, 191.3 ng/mL for GRg5 and GRk1 were prepared independently as QC samples. All solutions were stored at −40°C and were brought to room temperature before analysis.

**Procedure of steam-processing**

A single batch of dried *P. notoginseng* root was purchased from Jiangxi Jianhong Chinese Pharmaceutical Products Co., Ltd. (Jiangxi, China, lot #1906163) and was authenticated by Tulin Lu, a professor at Nanjing University of Traditional Chinese Medicine (Nanjing, China). Steaming the raw *P. notoginseng* in a small steamer for 2, 4 and 6 h at 100°C to obtain steamed *P. notoginseng*, respectively. The SPN was then dried at 50°C to constant weight using a heating-air drying oven, then powdered and sieved through a 40-mesh sieve.

**Preparation of raw and steam-processed Panax notoginseng aqueous extract**

Powdered RPN or SPN samples (100 g) were steeped in 600 mL water for 30 min before ultrasonicate for three times, 20 min each time, then filtrated. The residue was dissolved in 400 mL water and then repeated the above steps. The combined filtrate was concentrated under reduced pressure at 40°C to obtain a concentration of 0.4 g/mL. In order to calculate the administered dose, 1 mL aqueous extract of RPN or SPN sample was filtered through 0.22 μm filter membrane and diluted 5000 times with water before quantitatively analysis.

**Preparation of plasma samples**

A one-step protein precipitation method was adopted to prepare plasma samples. Aliquots (300 μL) of IS working solution (50 ng/mL in methanol) was added to 50 μL rat plasma in a 1.5 mL Eppendorf tube in order to precipitate protein. After vortexing for approximately 5 min and centrifugation at 12,000 rpm for 10 min, 3 μL of the supernatant was injected UPLC–MS-based for analysis.
**Method validation**

Validation procedures were fully accomplished according to Food and Drug Administration (US FDA) guidelines for validation of bioanalytical method, including selectivity, linearity, accuracy, precision, extraction recovery, matrix effect and stability (US FDA 2018).

**Selectivity and specificity**

The chromatograms of blank rat plasma were compared with those of corresponding spiked rat plasma containing seven target compounds and IS, and real plasma samples after oral administration of P. notoginseng aqueous extract. Specificity is the ability of the analytical method to distinguish the analytes from potential interfering substances and determine the analytes in the blank biological matrix.

**Linearity and lower limit of quantification (LLOQs)**

Spiking 5 μL corresponding working solutions into 45 μL rat blank plasma to prepare calibration standards solutions of 1.00–127.5 ng/mL for GRg1, NGR1, 0.5–63.8 ng/mL for GRe, 3.98–255.0 ng/mL for GRd, 3.98–2040 ng/mL for GRb1, 7.97–255.1 ng/mL for GRg5, GRk1. Plotting the peak-area ratio of seven analytes to IS versus the corresponding concentrations of calibration constituents to yield Linearity. Theophylline was chosen as the internal standard. The regression equations were evaluated by weighted (weighting factor, 1/x²) least squares analysis. Sensitivity was evaluated based on signal-to-noise (S/N) ratio of 10:1.

**Accuracy and precision**

To test the intra- and inter-day precision and accuracy, six replicates of seven compounds were analysed at three concentrations (LQC, MQC and HQC) over three verification days. Precision was expressed as relative standard deviation (RSD) and accuracy as relative error (RE). The intra- and inter-day precision and accuracy were required to be within the acceptable criteria (±15%) at LQC, MQC and HQC levels.

**Matrix effect and extraction recovery**

Five replicates at two QC levels (LQC and HQC) were used to determine recovery and matrix effect. The extraction recoveries of all seven analytes were determined based on the comparison between the peak areas of extracted QC samples and those obtained from reference standards spiked in post-extracted blank rat plasma. Meanwhile, the matrix effects were evaluated by the peak area ratio of the extracted QC sample dissolved in pre-extracted blank plasma and those of the pure standard solutions at an equivalent concentration to the QC levels.

**Stability**

The stability of GRg1, GRe, GRb1, GRd, GRg5 and GRk1 and NGR1 was assessed at low and high QC levels with three replicates at each level, including ambient temperature stability (storage at ambient temperature for two weeks), long-term stability (storage for 30 d at −40°C), freeze-thaw cycles (freeze-thaw) and post-preparative stability (the ready-to-inject samples in an autosampler for 12 h at 4°C).

**Application to PK study**

Male Sprague–Dawley rats (12 rats, 200–250 g) were purchased from the Nanjing Qinglong mountain animal breeding farm; licence approval number: SYXK (Su) 2018-0049. All rats were housed in rat cages under a controlled standard condition for 7 d prior to the experiment. All rats were fasted overnight with free access to water. After oral gavage of a single dose of RPN or SNG extract at 1 g/kg, serial heparinized blood samples were collected from orbital vein for 400 μL at pre-dosing and 5, 15, 30 min and 1, 2, 4, 6, 8, 10, 24, 32 and 48 h post-dosing. After centrifuging (12,000 rpm, 10 min, 4°C), the harvested supernatants were frozen at −40°C until analysis. The study protocol was reviewed and approved by the University Animals Care and Use Committee, Nanjing University of Chinese Medicine (ethical committee approval numbers: 201912A022).

**Data analysis**

The time to reach the maximum concentration (Tmax) and maximum plasma concentration (Cmax) of seven compounds in rats were observed directly from the concentration–time (CT) profile. Other compartmental parameters, such as area under the plasma CT curve (AUC0–t and AUC0–∞) and terminal elimination half-life (t1/2) were calculated using the Kinetta software version 5.0 (InnaPhase Corporation, Philadelphia, PA) by the non-compartmental model. Each value was expressed as mean ± SD.

**Results and discussion**

**Optimization of extraction procedure and mass spectrometry conditions**

We found in our experiments that glycosides and one-sugar-containing ginsenosides could be better extracted using ethyl acetate or other organic solvents. However, the extraction efficiency of ginsenosides with two or more sugar moieties attached was very low. Solid phase extraction (SPE) could extract all ginsenosides together, but strong eluting solvent required to be used due to large differences in polarity of the ginsenosides. This method could not reduce the matrix effect very well. In addition, it was complicated and expensive to use SPE column, which was not suitable for a large number of pharmaceutical biological samples. Protein precipitants such as methanol and acetonitrile could extract the ginsenosides together. Considering the mobile phase system was MeOH/H2O, we used methanol as precipitator in order to reduce the solvent effect.

On the positive ESI ion source, all ginsenosides can be well ionized. Among all these ions, [M + H]⁺ and [M + Na]⁺ have the highest signal and become our target parent ions. Previous research results show that adding a small amount of electrolyte in the mobile phase can enhance the ionization efficiency of target compounds and reduce the matrix effect of mass spectrometry and the ionization capacity of target compounds, which we called ‘LC-electrolyte effects’ (Wang et al. 2007). For this reason, we have studied the effect of electrolyte in the mobile phase on the ionization of [M + H]⁺ and [M + Na]⁺. We found that the ionization efficiency of [M + H]⁺ and [M + Na]⁺ can be increased by adding a very small amount of electrolyte (HCOONa) into the mobile phase. [M + Na]⁺ has the highest signal strength at 0.005% electrolyte concentration, while [M + H]⁺ has the highest signal strength at 0.02% electrolyte concentration.
Figure 1 shows the chemical structures and product ion mass spectra of the analytes and IS. The compounds detection and quantification were performed for acquiring higher sensitivity and selectivity by MRM mode. The MS parameters were optimized to achieve the higher signal for both precursor ions and product ions. After optimization, the transitions of 823.3 → 643.3, 969.4 → 789.6, 595.6 → 775.4, 969.6 → 789.4, 789.3 → 365.0, 789.3 → 365.1, 1131.4 → 365.0 and 180.9 → 124.0 were applied for analysis of GRg1, GRe, GRb1, GRd, GRg5, GRk1, NGR1 and theophylline (IS), respectively.

Method validation

Selectivity and specificity

The selectivity of the approach was interrogated by analysing five individual blank plasma samples. The chromatograms were compared including each blank plasma and spiked rat tissue homogenate containing the seven components and IS, along with real plasma samples collected from RPN and SPN aqueous extract treated groups (Figure 2). Absence of interfering peaks for GRg1, GRe, GRb1, GRd, GRg5, GRk1, NGR1 and IS were observed in plasma samples. Compounds 1–8 and IS were eluted at 4.83, 4.25, 5.30, 4.05, 4.20, 7.10, 7.31 and 2.05 min, respectively. The compounds could be separated from the endogenous interference.

Linearity and LLOQs

The validated method revealed a good linear regression over a wide concentration range of 1.00–127.5 ng/mL for GRg1 and NGR1, 0.5–63.8 ng/mL for GRe, 3.98–255.0 ng/mL for GRd, 3.98–2040 ng/mL for GRb1 and 7.97–255.1 ng/mL for GRg5 and GRk1, in rat plasma with correlation coefficient ($r^2$) of at least 0.991. The lower limit of quantification (LLOQ) of GRg1, GRb1, GRd, GRe, GRg5, GRk1 and NGR1 in plasma was defined as 1.00, 0.50, 3.98, 3.98, 7.97, 7.97 and 1.00 ng/mL, respectively.

Accuracy and precision

The intra- and inter-day precision and accuracy of the approach were assessed at three QC levels (2.39, 47.9, 95.6 ng/mL for GRg1 and NGR1, 1.20, 23.9, 47.9 ng/mL for GRe, 9.56, 95.6, 191.3 ng/mL for GRd, 7.56, 765.1, 1530.2 ng/mL for GRb1, 19.1, 95.6, 191.3 ng/mL for GRg5 and GRk1) on three different days. The accuracy was calculated by RE, ranging from −11.2 to 10.9%. RSD values of precision for all the seven analytes were within the acceptable criteria of ± 15%. The approach was considered reliable and reproducible. The results are summarized in Table 1.

Matrix effect and extraction recovery

As shown in Table 2, the matrix effect of the seven compounds at LQC and HQC levels ranged from 85.1 to 110.3%, which showed that no significant matrix effect was observed. The mean extraction recoveries of the seven compounds ranged from 75.0% to 100.8% at LQC and HQC levels, indicating the extraction recoveries were consistent and reproducible in rat plasma. Both assessments were carried out for five replicates.

Stability

The stabilities of the seven analytes and IS under four tested conditions were assessed by analysis of three different QC levels in the blank plasma. As shown in Table 3, the results demonstrated that the seven analytes and IS were stable under these conditions including 4 h at ambient temperature, 30 d at −40°C, three freeze-thaw cycles and in an autosampler at 4°C for 12 h, with accuracy in the range of −5.07–6.78%, −9.62–7.46%, −7.43–7.37% and −8.93–10.6%, respectively.

Saponin content in steaming Panax notoginseng

The dynamic alteration and content of GRg1, GRe, GRb1, GRd, GRg5, GRk1 and NGR1 in the RPN and SPN extracts were investigated and results are shown in Figure 3. The content of ginsenosides was changed during the steaming: decreased as steaming time increases, Rg5 and Rk1 significantly increased. High temperature induced the cleavage of C-20 glycosidic bond of GRb1 and GRd to form new saponins, the contents of five major saponins (GRg1, GRe, GRb1, GRd and NGR1) in the RPN were decreased gradually, whereas new converted saponins (GRg5 and GRk1) were formed (Wang et al. 2012). The content change of the seven saponins in P. notoginseng became steady after 6 h of steaming, which was coincident with previous report by Xiong et al. (2019). Based on the above analysis, P. notoginseng steamed

Figure 1. Chemical structures and product ion mass spectra of GRg1 (A), GRe (B), GRb1 (C), GRd (D), GRg5 (E), GRk1 (F) and NGR1 (G).
for 6 h was used for the subsequent pharmacokinetic study. The content of GRg1, GRe, GRb1, GRd, GRg5, GRk1 and NGR1 was 202, 32.9, 278, 99.9, 0.92, 0.28, 68.8 mg/g in the RPN, respectively, and 105, 17.3, 87.3, 35.7, 4.46, 7.40, 36.7 mg/g in the SPN, respectively. The results indicated a distinct difference in saponin composition between the SPN and RPN.

Application to PK study

The developed LC–MS/MS approach was successfully applied to compare the pharmacokinetics of the seven bioactive constituents following an oral administration of RPN and SPN aqueous extracts. The mean CT curves of the two treatments are displayed in Figure 4. The pharmacokinetic parameters of five triterpenoid saponins in male SD rats including time to reach the maximum concentrations (T_{max}), maximum plasma concentration (C_{max}), half-time (t_{1/2}), area under CT curve (AUC_{0–t} and AUC_{0–∞}) are illustrated in Table 4. As shown in Table 4, GRg1,
GRb1 and GRd achieved the maximum plasma concentration in 9.33 h after oral administration. GRe and NGR1 achieved the maximum plasma concentration in 1.63 h. Double absorption peaks occurred in the CT curve of GRg1 and GRe after an oral dose of raw and steamed *P. notoginseng* aqueous extracts. No significant difference was observed on Tmax and t1/2 between the two treatments. Compared to those of the raw group, AUC0–t values of GRg1 (176.63 ± 42.49 ng/h/mL, *p* < 0.01), GRb1 (5094.06 ± 1453.14 ng/h/mL, *p* < 0.001), GRd (1396.89 ± 595.14 ng/h/mL, *p* < 0.001) and NGR1 (135.95 ± 54.32 ng/h/mL, *p* < 0.01), along with Cmax values of GRg1 (17.41 ± 5.43 ng/mL, *p* < 0.01), GRb1 (361.48 ± 165.57 ng/mL, *p* < 0.001), GRd

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| Compounds | Spiked concentration (ng/mL) | Accuracy (RE%) | Precision (RSD%) | Accuracy (RE%) | Precision (RSD%) |
|-----------|-----------------------------|---------------|------------------|---------------|-----------------|
| Rg1       | 2.39                        | −11.2         | 7.62             | −3.26         | 5.24            |
|           | 47.9                        | 3.08          | 8.49             | −0.37         | 5.14            |
|           | 95.6                        | 1.29          | 2.39             | −1.53         | 3.35            |
| Re        | 1.20                        | −4.83         | 13.4             | −0.67         | 12.1            |
|           | 2.39                        | −0.59         | 15.1             | 10.0          | 7.56            |
|           | 47.9                        | 4.94          | 4.95             | −5.30         | 3.78            |
|           | 95.6                        | −7.70         | 1.75             | −2.28         | 2.37            |
| Rd        | 9.56                        | 8.33          | 11.5             | 2.64          | 10.5            |
|           | 95.6                        | 7.01          | 5.74             | 5.65          | 5.25            |
|           | 191.3                       | −2.96         | 3.28             | −1.53         | 4.06            |
| Rb1       | 7.65                        | 1.23          | 11.1             | −4.71         | 7.30            |
|           | 765.1                       | 7.87          | 5.88             | 1.72          | 5.08            |
|           | 1530.2                      | −3.91         | 6.40             | −6.03         | 8.05            |
| Rg5       | 19.1                        | 9.42          | 6.08             | 10.9          | 3.74            |
|           | 95.6                        | 0.31          | 2.76             | 4.02          | 2.02            |
|           | 191.3                       | −0.90         | 8.37             | −4.20         | 4.50            |
| Rk1       | 19.1                        | 12.0          | 16.4             | 4.23          | 10.8            |
|           | 191.3                       | 8.77          | 9.19             | 77.9          | 4.42            |
| IS        | 2.39                        | 6.78          | 1.18             | −9.62         | 2.17            |

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| Compounds | Spiked concentration (ng/mL) | Accuracy (RE%) | Precision (RSD%) | Accuracy (RE%) | Precision (RSD%) |
|-----------|-----------------------------|---------------|------------------|---------------|-----------------|
| Rg1       | 2.39                        | −5.07         | 6.9              | 3.52          | 9.6             |
|           | 95.6                        | −0.83         | 14.6             | 7.21          | 15.0            |
| Re        | 1.20                        | −0.53         | 5.40             | 5.83          | 8.00            |
|           | 47.9                        | 110.0         | 7.09             | 100.3         | 3.31            |
| NGR1      | 2.39                        | −4.35         | 3.4              | 2.05          | 5.93            |
|           | 95.6                        | −0.77         | 3.13             | −8.63         | 8.24            |
| Rd        | 9.56                        | 191.3         | 103.4            | 108.8         | 8.30            |
|           | 765.1                       | 94.7          | 10.3             | 101.2         | 5.72            |
|           | 1530.2                      | 100.7         | 8.33             | 103.2         | 6.58            |
| Rg5       | 19.1                        | 89.3          | 5.31             | 76.8          | 4.83            |
|           | 191.3                       | 85.1          | 7.52             | 75.0          | 3.47            |
| Rk1       | 19.1                        | 87.7          | 9.19             | 77.9          | 4.42            |
| IS        | 2.39                        | 6.78          | 1.18             | −9.62         | 2.17            |

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| Compounds | Spiked concentration (ng/mL) | Accuracy (RE%) | Precision (RSD%) | Accuracy (RE%) | Precision (RSD%) |
|-----------|-----------------------------|---------------|------------------|---------------|-----------------|
| Rg1       | 2.39                        | −5.07         | 6.9              | 3.52          | 9.6             |
|           | 95.6                        | −0.83         | 14.6             | 7.21          | 15.0            |
| Re        | 1.20                        | −0.53         | 5.40             | 5.83          | 8.00            |
|           | 47.9                        | 110.0         | 7.09             | 100.3         | 3.31            |
| NGR1      | 2.39                        | −4.35         | 3.4              | 2.05          | 5.93            |
|           | 95.6                        | −0.77         | 3.13             | −8.63         | 8.24            |
| Rd        | 9.56                        | 191.3         | 103.4            | 108.8         | 8.30            |
|           | 765.1                       | 94.7          | 10.3             | 101.2         | 5.72            |
|           | 1530.2                      | 100.7         | 8.33             | 103.2         | 6.58            |
| Rg5       | 19.1                        | 89.3          | 5.31             | 76.8          | 4.83            |
|           | 191.3                       | 85.1          | 7.52             | 75.0          | 3.47            |
| Rk1       | 19.1                        | 87.7          | 9.19             | 77.9          | 4.42            |
| IS        | 2.39                        | 6.78          | 1.18             | −9.62         | 2.17            |

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| Compounds | Spiked concentration (ng/mL) | Accuracy (RE%) | Precision (RSD%) | Accuracy (RE%) | Precision (RSD%) |
|-----------|-----------------------------|---------------|------------------|---------------|-----------------|
| Rg1       | 2.39                        | −5.07         | 6.9              | 3.52          | 9.6             |
|           | 95.6                        | −0.83         | 14.6             | 7.21          | 15.0            |
| Re        | 1.20                        | −0.53         | 5.40             | 5.83          | 8.00            |
|           | 47.9                        | 110.0         | 7.09             | 100.3         | 3.31            |
| NGR1      | 2.39                        | −4.35         | 3.4              | 2.05          | 5.93            |
|           | 95.6                        | −0.77         | 3.13             | −8.63         | 8.24            |
| Rd        | 9.56                        | 191.3         | 103.4            | 108.8         | 8.30            |
|           | 765.1                       | 94.7          | 10.3             | 101.2         | 5.72            |
|           | 1530.2                      | 100.7         | 8.33             | 103.2         | 6.58            |
| Rg5       | 19.1                        | 89.3          | 5.31             | 76.8          | 4.83            |
|           | 191.3                       | 85.1          | 7.52             | 75.0          | 3.47            |
| Rk1       | 19.1                        | 87.7          | 9.19             | 77.9          | 4.42            |
| IS        | 2.39                        | 6.78          | 1.18             | −9.62         | 2.17            |
(62.47 ± 33.65, \( p < 0.001 \)) and NGR1 (23.97 ± 16.77 ng/mL, \( p < 0.05 \)), decreased remarkably with oral administration of the SPN aqueous extracts. The main reason for the differences on \( C_{\text{max}} \) and AUC_{0–t} values seem to be the degradation of ingredients in preparation of processed \( P. \) notoginseng. The AUC_{0–t} and \( C_{\text{max}} \) values of GRe demonstrated no significantly difference after an oral dose of raw and steamed \( P. \) notoginseng aqueous extracts, indicating the systemic exposure of the compound was not changed.

Although the concentration of GRg5 and GRk1 increased due to steaming, the relative contents were still very low compared to the other five ginsenosides. On the other hand, the bioavailabilities of ginsenosides Rg5 and Rk1 were extremely low, 0.67 and 0.98%, respectively (Ma et al. 2021). For these reasons, ginsenoside GRg5 and GRk1 were not detected in real rat plasma. According to the Lipinski’s rule of five (Lipinski et al. 2001) and the molecular surface properties (number of rotatable bonds and, total hydrogen bond count, topological polar surface area [TPSA]) (Veber et al. 2002), ginsenosides Rg1, Rb1, Rd, Re, Rg5, Rk1 and notoginsenoside R1 all had unfavourable traits underlying poor membrane permeability, including the molecular weight ranging from 767 to 1109, high total hydrogen bond counts ranging from 20 to 38, high flexibility ranging from 9 to 16 and high TPSA ranging from 199 to 377 Å. In addition, the lipophilicity of the GRg5 and GRk1 was significantly higher (Log\( P \), 4.45 and 4.47, respectively) than that of the ginsenosides (Log\( P \), 0.28–2.77). Collectively, in silico calculations suggested that the

![Figure 3. Saponin content in steaming Panax notoginseng root. The label on the left was for GRb1, GRe, GRd, NGR1 and GRg1 and the label on the right was for GRg5 and GRk1.](image)

![Figure 4. Mean plasma concentration–time profiles of NGR1 (A), GRb1 (B), GRg1 (C), GRd (D) and GRe (E) in the Sprague-Dawley rat after oral administration the extracts of Panax notoginseng at 5 mL/kg. Each point represents the means ± SD.](image)

| Analyte | Groups     | \( C_{\text{max}} \) (ng/mL) | \( T_{\text{max}} \) (h) | AUC_{0–t} (ng h/mL) | AUC_{0–\infty} (ng h/mL) | \( t_{1/2} \) (h) |
|---------|------------|-------------------------------|--------------------------|---------------------|--------------------------|----------------------|
| Rg1     | Raw        | 66.67 ± 32.81                 | 9.33 ± 2.07              | 808.42 ± 382.14     | 860.90 ± 354.81          | 7.92 ± 6.73          |
|         | Steam-processed | 17.41 ± 5.43**              | 8.67 ± 1.63              | 176.63 ± 42.49**    | 194.85 ± 46.90**         | 7.46 ± 2.47          |
| NGR1    | Raw        | 94.31 ± 55.29                | 1.12 ± 0.33              | 578.05 ± 273.25     | 627.83 ± 303.61          | 7.63 ± 0.38          |
|         | Steam-processed | 23.97 ± 16.77*              | 1.25 ± 0.00              | 135.95 ± 54.32**    | 156.52 ± 57.94**         | 9.27 ± 2.01          |
| Re      | Raw        | 28.15 ± 13.10                | 1.24 ± 0.49              | 87.71 ± 20.68       | 91.69 ± 19.68            | 3.16 ± 1.73          |
|         | Steam-processed | 21.53 ± 10.02               | 1.63 ± 0.41              | 102.08 ± 24.69      | 106.50 ± 24.22           | 4.14 ± 1.98          |
| Rb1     | Raw        | 997.00 ± 143.69             | 8.67 ± 1.63              | 15110.08 ± 2383.47  | 15242.07 ± 2435.24       | 9.58 ± 1.11          |
|         | Steam-processed | 361.48 ± 165.57***         | 8.33 ± 1.97             | 5094.06 ± 1453.14*** | 5446.36 ± 1611.83***     | 9.91 ± 0.83          |
| Rd      | Raw        | 164.37 ± 28.80              | 8.67 ± 2.73              | 3633.64 ± 568.69    | 4172.87 ± 733.16         | 13.95 ± 1.71         |
|         | Steam-processed | 62.47 ± 33.65***         | 8.33 ± 1.97             | 1396.89 ± 595.15*** | 1538.71 ± 594.79***      | 12.01 ± 3.06         |

\( C_{\text{max}} \): maximum concentration; \( T_{\text{max}} \): time to maximum concentration; AUC_{0–t}: area under the concentration–time curve from zero to last sampling time; AUC_{0–\infty}: area under the concentration–time curve from zero to infinity; \( t_{1/2} \): elimination half-life.

*Indicated \( p < 0.05 \), **indicated \( p < 0.01 \) and ***indicated \( p < 0.001 \), versus oral administration of raw Panax notoginseng aqueous extract.
poor intestinal absorption of the ginsenosides could be attributed to poor membrane permeability. Although these traits were improved to some extent for GRG5 and GRK1, significant increases in intestinal absorption appeared to be limited by their lowered solubility. Further studies are in progress to find the possible explanation why GRG5 and GRK1 were not detected in plasma after orally administering *P. notoginseng*.

**Conclusions**

This study validated a UPLC–MS/MS method based on methanol precipitation for the simultaneously pharmacokinetic determination of ginsenosides Rg5, Re, Rb1, Rd, Rg5, Rk1 and notoginsenoside R1. The validated analytical procedure has been successfully applied to evaluate the influences of steaming process from *P. notoginseng* on comparative pharmacokinetic study of multiple components following oral administration of *P. notoginseng* root extracts in raw and steamed forms. The pharmacokinetic characters of ginsenosides Rg1, Re, Rb1, Rd and notoginsenoside R1 showed significant differences using SPN even though the overall kinetic profiles were still similar between RPN and SPN extract. It was also the first report of comparative pharmacokinetic study of multiple triterpenoid saponins analysis after oral administration of raw and steam-processed *P. notoginseng* extract. In conclusion, influence of the processing reduced the systemic exposure levels to ginsenosides Rg1, Rb1, Rd and notoginsenoside R1. Ginsenosides Re, Rg5 and Rk1 have a low likelihood to cause the different efficacy between SPN and RPN. Our study might be helpful for its steam-processing mechanism.

**Disclosure statement**

No potential conflict of interest was reported by the author(s). Jiajia Dong and Tulin Lu supervised the project and designed the experiments. Jiajia Dong performed the experiments and statistical analysis as well as the writing of the article. Zhenzhen Yin, Lianlin Su, Lin Li and Chunqin Mao collected samples and analysed the data. Mengting Yu and Meng Wang collected samples and modified the manuscript.

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