# Biosynthesis and pharmacokinetics of *Panax notoginseng* enteric-coated soft capsules

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**Background:** To investigate the biosynthesis and pharmacokinetic course of enteric-coated soft capsules of *Panax notoginseng* saponins (PNS) in a beagle dog model.

**Methods:** To satisfy the enteric properties of soft capsules, the PNS enteric soft gelatin capsules were prepared by formaldehyde impregnation and orthogonal experimental design. The fluidity of gelatin and the disintegration time were selected as evaluation indexes; the soft gelatin capsule content was self-emulsifying, and the Km value and the optimal prescription were determined by making three-phase diagrams; *in vivo* pharmacokinetics studies were performed on six beagle dogs with 3 dogs in each group. Beagle dogs were divided into two groups randomly. One group was given PNS self-emulsifying enteric capsule and the other was given market conventional capsules. Plasma samples were collected at different times. After 1 week, the crossover experiment was carried out. The plasma concentration was detected by HPLC-MS (high performance liquid chromatography-mass spectrometry). Then the pharmacokinetic parameters were calculated by non-compartment model analysis.

**Results:** The range and variance analysis of the orthogonal test determined that the best prescription of total saponins of *Panax notoginseng* enteric soft capsule was:gelatin:glycerol:water =1:1:2, Soak the films in 1% formaldehyde for 1 hour. The contents of the soft capsule self-microemulsion are prescribed as: IPM (isopropyl myristate):Cremophor RH40:PEG400 (polyethylene glycol 400) =1:4.5:4.5 (with suitable PNS); the pharmacokinetic parameters of PNS self-emulsified enteric capsules and conventional capsules in the market are as follows: $Rb_1$:$C_{max}$ is (18.05±0.26) and (15.50±0.51) ng/mL, $T_{max}$ is (2.00±0) and (3.00±0) h, $AUC_{0\rightarrow t}$ is 98.49±1.16 and 34.46±2.02 (ng/mL)·h, relative bioavailability is 196.2%; $Rg_1$: $C_{max}$ is 4.16±0.25 and 3.88±0.28 ng/mL, $T_{max}$ is 2.00±0 and 1.50±0 h, area under drug time curve $(AUC)_{0\rightarrow t}$ is 11.80±2.93 and 10.45±2.29 (ng/mL)·h, relative bioavailability is 77.2%; $R_1$:$C_{max}$ is 1.84±0.25 and 1.48±0.21 ng/mL, $T_{max}$ is 2.08±0.49 and 1.92±0.20 h, $AUC_{0\rightarrow t}$ is 7.06±2.07 and 7.16±2.59 (ng/mL)·h, relative bioavailability is 117.7%.

**Conclusions:** The experiment *in vivo* showed the higher relative bioavailability of PNS self-emulsifying enteric capsule compared with market conventional capsules. This will provide a potential application prospect for the clinical research and applications of PNS.

**Keywords:** *Panax notoginseng* saponins (PNS); enteric soft capsule; self-emulsifying; mass spectrum; pharmacokinetic

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## Introduction

*Panax notoginseng* saponins (PNS) has been used in the treatment of cardiovascular and cerebrovascular diseases, respiratory system diseases, urinary system diseases, and digestive system diseases (1). PNS preparations commonly used in clinical practice are available in orally administered form or injectable form. Oral preparations include Xuesaitong soft capsule, Xuesaitong dropping pill and Xuesaitong granules, Xuesaitong tablets, and Xueshuantong capsules. The main injectable preparations include Xuesaitong injection, Xuesaitong injection (freeze-dried), Xueshuantong injection, and Xueshuantong (freeze-dried) injection. The injection has definite curative effect, but it has high cost, inconvenient operation, poor patient compliance, and it is easy to cause adverse reactions such as allergy, so there are safety problems; However, conventional oral preparations may have low bioavailability, slow and uncertain efficacy, and limited clinical application.

Soft-gel capsule is a newly developed dosage form (2). Compared with other formulations, the soft capsule has the characteristics of high bioavailability, accurate content, good uniformity, and beautiful appearance (3). Substances such as oily drugs can be encapsulated to avoid its absorption and solidification. It can effectively prevent the oily drug from seeping from the absorption excipients (4). In addition, low melting point drugs, hydrophobic drugs with poor bioavailability, drugs with bad smell, micro active drugs, and drugs unstable to light, humidity, heat, and oxidation, are also suitable for soft capsules (5).

In recent years, research into soft capsules has developed rapidly in China, but only a few varieties are available. The main products include Maitong capsules, vitamin E capsules, and Duoxikang capsules, most of which are stomach soluble capsules (6-9). Except the merits of ordinary soft capsules, enteric soft capsules also have the following advantages: (I) enteric-coats protect the contained drug from degradation until it is released in the intestine (10); (II) avoids strong stimulation of the drugs on the gastric mucosa; (III) localized delivery of the drug; (IV) provides delayed release; and (V) maximizes the concentration of the drugs absorbed by the small intestine (11). Therefore, research and development of enteric coated soft capsules has positive clinical significance.

The research on saponins at home and abroad is mostly limited to chemical components, pharmacological effects and clinical indications, and the research on new preparations to improve their bioavailability has not been reported yet. Therefore, it is of great academic value and practical significance to select PNS as a model drug and develop new high bioavailability preparations for the research and development of saponin pharmaceutical preparations.

In this study, the enteric soft capsule rubber was prepared by formaldehyde impregnation, and the contents of the soft capsule were prepared into the self-microemulsion form of PNS. Compared with the commercially available soft capsules, this preparation has the characteristics of rapid onset and high bioavailability. We present the following article in accordance with the MDAR and ARRIVE reporting checklists (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5751/rc).

### Highlight box

**Key findings**

- PNS enteric-soluble soft capsule is not available in the market, it is a new formulation developed by us. The content of it is in the form of self-microemulsion, and the dosage form is novel and meaningful.
- In this paper, the pharmacokinetic behavior of *Panax notoginseng* enteric-coated soft capsules in beagle dogs was studied for the first time, and the pharmacokinetic parameters were obtained.

**What is known and what is new?**

- The commercial preparations of PNS are only soft capsules and tablets.
- PNS enteric-soluble soft capsule is not available in the market.

**What is the implication, and what should change now?**

- It provides a basis for the development of new models of *Panax notoginseng* saponins.
- This study is novel and meaningful, and can provide a theoretical basis for the pharmacodynamics and clinical research of *Panax notoginseng* saponins.

## Methods

### *Drugs and reagents*

Isopropyl myristic acid was purchased from Haiyan County Fine Chemical Co., Ltd. (Jiaxing, China). Soybean oil was purchased from Shanghai Fulinmen Group. Tween80 (chemically pure) was purchased from Tianjin Guangfu Fine Chemical Research Institute. Peanut oil was purchased from Shandong Luhua Group. Polyoxyethylene ether (40) hydrogenated castor Oil (Cremophor RH40) was purchased from BASF (Germany). PEG400 (Chemical pure) was

purchased from Tianjin Guangfu Fine Chemical Research Institute. Isopropyl alcohol (chromatography-pure) was purchased from Tianjin Concord Technology Co., Ltd. (Tianjin, China). Ethanol (analytical pure) was purchased from Tianjin Yongda Chemical Reagent Development Center (Tianjin, China). *Panax notoginseng* total saponin enteric self emulsified soft capsule (Batch Number: 20081215) was homemade. Xuesaitong soft capsule (Batch Number: 20081010) was purchased from Kunming Shenghuo Pharmaceutical Co., Ltd. (Kunming, China). Ginsenoside $Rg_1$ (ginsenoside $Rg_1$, $Rg_1$) Reference Product (Batch Number: 200415), Ginsenoside $Rb_1$(ginsenoside $Rb_1$, $Rb_1$) Reference Product (Batch Number: 200318) and Ginsenoside $R_1$ ($R_1$) reference (Batch Number: 200415) were gained from China Pharmaceutical and Biological Products Inspection Institute. Puerarin internal standard (Batch Number: 06082824) was purchased from Shanghai TAUTO Biotech. Co. Ltd. (Shanghai, China). Acetonitrile (chromatographic pure) was purchased from DIKMA Corporation. Heparin sodium was purchased from Tianjin Biochemical Pharmaceutical Factory (Tianjin, China).

### *Preparation of enteric soft capsule rubber*

In this experiment, formaldehyde impregnation method was used to prepare *Panax notoginseng* total saponin enteric-soluble soft capsule. The basic preparation process was determined through single factor investigation of the preparation process and prescription, and orthogonal experimental design optimization method was used to optimize the preparation. Three different levels were selected to consider the proportion of gelatin and glycerin, the proportion of gelatin and water, and the concentration of formaldehyde during immersion. Optimized by $L_9(3^4)$ orthogonal test. Using the fluidity of gelatin, the appearance and shape of gelatin and the disintegration time of gelatin as evaluation indexes, the optimal process conditions of enteric-coated soft capsule rubber were determined and the results were predicted.

### *Preparation of self microemulsion contents of PNS soft capsules*

#### Prescription screening of blank self microemulsion

##### *Oil phase selection*

The earliest oil used in self-microemulsifying drug delivery system (SEDDS) is peanut oil, soybean oil and other natural vegetable oils, but these oils are not strong in the ability to dissolve fat soluble drugs. On the basis of references and preliminary tests, this paper taked oleic acid and isopropyl mycopic acid as the oil phase, Cremophor RH40 as the surfactant, and various oils and surfactants were mixed in different ratios. Titrated with distilled water under constant temperature magnetic stirring at 37 ℃ at medium speed, if a bright white emulsion is formed and no oil droplets exist, it is an effective self-emulsification. Otherwise, it is ineffective self emulsification if the emulsion is slowly emulsified into dark gray or the degree of emulsification is very low and there is oil drop suspended on the surface.

##### *Selection of surfactant*

Using isopropyl myristic acid as oil phase and PEG400 as auxiliary surfactant, the surfactant (Tween 80, lecithin, Cremophor RH40) and the auxiliary surfactant were mixed in different proportions (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1). Gradually added water and saw if a microemulsion forms.

##### *Selection of auxiliary surfactants*

Using isopropyl myristic acid as oil phase, Cremophor RH40 as surfactant, the surfactant and auxiliary surfactant (PEG400, isopropyl alcohol, ethanol) mixed according to different ratios (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1), and then gradually added water, the formation of self - microemulsion was observed.

##### *Preparation of three-phase diagram*

According to the above experiments, isopropyl myristic acid was selected as oil phase, Twain 80 and Cremophor RH40 as surfactants, and PEG400 as auxiliary surfactants. Different ratios (1:9, 2:8, 3:7, 4:6, 5:5, 6:3, 6:4, 7:3, 8:2, 9:1) of surfactants and auxiliary surfactants were mixed evenly. The mixture and oil phase were respectively mapped as three phases, and the Km value was determined by taking the area of microemulsion formation as the evaluation index.

The oil phase, surfactant and auxiliary surfactant were accurately weighed in the test tube, stirred at medium speed on a 37 ℃ magnetometer, and mixed evenly. Water was gradually added until it was clear or slightly opalescent. The contents of each component during the formation of microemulsion were calculated and the three-phase diagram was drawn to compare the area of microemulsion area in the two three-phase diagrams.

#### Prescription of total saponins of Notoginseng self-microemulsion

According to the optimized prescription selected from the above blank self-emulsifying prescription screening

experiment, the total saponin self-microemulsion solution of *Panax notoginseng* was prepared. The prescription is *Table 1*.

According to the prescription amount, the isopropyl myristic acid, polyoxyethylene hydrogenated castor oil and PEG400 were put in the beakers, stirred and mixed at 37 ℃ with magnetic force, and then the total saponin of notoginseng was added, stirring to dissolve all of them, and the total saponin of notoginseng was obtained.

### Self-micro milk character investigation

#### *Particle size and its distribution*

The particle size of the self-micro emulsion was investigated and measured by 90 type particle size analyzer. The samples were diluted 30 times before test and tested three times consecutively. After one month, test again to see if the particle size changes.

#### *Viscosity*

Measure the viscosity of self-microemulsion (diluted 30 times) with a rotary viscometer.

**Table 1** The prescription of the total saponin self-microemulsion solution of *Panax notoginseng*

| Components | Dosage, g |
|---|---|
| Isopropyl myristic acid | 0.1 |
| Polyoxyethylene hydrogenated castor oil | 0.45 |
| PEG400 | 0.45 |
| Total PNS extract | 1.1 |

PEG400, polyethylene glycol 400; PNS, *Panax notoginseng* saponins.

#### *Study on pharmacokinetics of Panax notoginseng total saponins enteric soft capsules*

### Test animals

Six beagle dogs (about 9.5–11.5 kg), half male and half female, were purchased from Shanghai Xingang Laboratory Animal Farm, raised in Animal Center of Hebei Medical University, Animal Qualification Certificate No. SCXK(Shanghai)2002-0014. Experiments were performed under a project license granted by the Animal Ethics Committee of Hebei Medical University (IACUC-Hebmu-GLP2009006), and the animal study was conducted based on the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No. 85–23, revised in 1985). A protocol was prepared before the study without registration.

### Establishment of a method for determination of three main components $R_1$, $Rb_1$, $Rg_1$ in total saponins of *Panax notoginseng* in whole blood

#### *Conditions of liquid chromatography and mass spectrometry detection*

Liquid chromatography conditions: Chromatographic column: Kromasil C18 (5 μm, 250 mm × 4.6 mm); Protective column: YWG2 C18 10 mm × 4.6 mm; Detection wavelength: 203 nm; Sample size: 50 μL; Mobile phase—A: 2 mmol/L acetonitrile, B: 2 mmol/L ammonium acetate for gradient elution according to the *Table 2*.

Mass spectrometry detection conditions: ion source was electrospray ionization (ESI) source; The source injection voltage (IS) is 5,500 V; The source temperature is 650 ℃. Atomized gas (GS1, N2) pressure is 60 psi; Auxiliary gas (GS2, N2) pressure is 60 psi; Curtain gas (N2) pressure

**Table 2** Liquid chromatography conditions

| Step | Total time (min) | Flow rate (μL/min) | A (%) | B (%) |
|---|---|---|---|---|
| 0 | 8.00 | 800 | 10.0 | 90.0 |
| 1 | 5.00 | 800 | 50.0 | 50.0 |
| 2 | 5.10 | 800 | 90.0 | 10.0 |
| 3 | 10.00 | 800 | 90.0 | 10.0 |
| 4 | 10.20 | 800 | 10.0 | 90.0 |

A: 2 mmol/L acetonitrile, B: 2 mmol/L ammonium acetate.

is 25 psi; Positive ion detection; The ionic reactions used for quantitative analysis are m/z 799.4→m/z 637.3 ($Rg_1$), the unbunching voltage (DP) is –90 eV, and the collision energy (CE) is –35 eV. m/z 1,107.5→m/z 179.0 ($Rb_1$), the unbunching voltage (DP) is –130 eV, the collision energy (CE) is –72 eV; m/z 931.45→m/z 475.3 ($Rb_1$), the clustering voltage (DP) is –110 eV, the CE is –64 eV; m/z 418.1→m/z 267.1 (Puerarine, internal standard), cluster-breaking voltage (DP) is –50 eV and CE is –45 eV.

***Experimental design***

Six Beagle dogs were randomly divided into two groups with 3 dogs in each group. Fasting for 12 hours before taking the medicine, without water. The first group was given commercially available Xuesaitong soft capsule (equivalent to 1,200 mg of total saponin of *Panax notoginseng*), put the soft capsule on the back of the tongue of Beagle dogs, closed the mouth, and let them swallow naturally. The second group was given self-made enteric-soluble self-emulsifying soft capsule of total PNS (equivalent to 1,200 mg of total PNS). The soft capsule was placed on the tongue of Beagle dogs, and the mouth was closed for natural swallowing. Drank water freely after 2 hours and ate food after 4 hours. Three milliliters of venous blood from Beagle canine strands was taken into heparinized test tubes at 0.25, 0.5, 0.75, 1.5, 2, 4, 6, 8, 10, 12 and 24 h after drug administration,, centrifuged at 4,000 r/min for 10 min, and the upper plasma was taken and frozen in the refrigerator at –20 ℃ for use. After a one-week washout period, the experiment was crossed again.

***Treatment and determination of plasma samples***

The plasma sample of 0.5 mL was precisely measured and placed in a 5 mL centrifuge tube. Puerarin internal standard solution of 30 μL was added, followed by vortex for 30 seconds. Then anhydrous methanol of 1.5 mL was added and swirled for 3 min. After centrifugation at 15,000 r/min for 10 min, the supernatant was precisely taken into another centrifuge tube, and then dried with nitrogen in a water bath at 40 ℃. The residue was dissolved in a swirl of 20% acetonitrile water 100 μL, and then centrifuged at 15,000 r/min for 10 min. The supernatant was precisely absorbed 50 μL and then injected for analysis.

***Preparation of standard curve for content determination of $R_1$, $Rb_1$ and $Rg_1$, three main components of total saponins of notoginseng in plasma***

Preparation of standard solution: Precisely weighed 10 mg of the total saponins of *Panax notoginseng*, placed them in 10 mL measuring bottle, dissolved them with mobile phase and diluted them to the scale to obtain 1,000 μg/mL reserve solution. A series of standard solutions with concentrations of 2.5, 6.25, 25, 125, 250, 500 and 1,000 μg/mL were prepared by taking appropriate amount of the above-mentioned reserve solution and diluting it step by step with mobile phase.

Plasma standard curve: 0.5 mL blank plasma was taken into a 5 mL centrifuge tube, and 30 μL internal standard solution and 50 μL series standard solution were added, respectively. The plasma samples were treated in the same way as plasma sample treatment items. 50 μL was injected, and the chromatographic peak area was recorded. The ratio (Y) of the extracted ion peak area (As) of the sample to the extracted ion peak area (Ais) of the internal target was used for linear regression of the concentration (C) to obtain the standard curve.

***Method recovery rate test***

The total saponin reference solution of notoginseng was added into the blank plasma to prepare the samples with three concentrations (the concentrations were 6.25, 125, 500 μg/mL, respectively). The chromatograms were recorded as described above, and the peak area of the substance to be measured was substituted into the standard curve equation to obtain the corresponding determination values. The determination amount was compared with the added amount to obtain the recovery rate of the method.

***Precision test***

The total saponins of notoginseng were added into the blank plasma to prepare the samples of low, medium and high concentrations (the concentrations were 6.25, 125, 500 μg/mL, respectively). The operation was carried out according to the above description, and the chromatogram was recorded and the determination values were calculated. The relative standard deviation (RSD) was calculated after 5 consecutive injections of each concentration in one day, which was intraday variation. The RSD was calculated for 5 consecutive days, which was diurnal variation.

***Sensitivity test***

Simulated plasma samples with different concentrations were tested, and the minimum detection limit was calculated when the SNR was S/N ≥3. The lowest quantitative limit is S/N ≥10.

***Pharmacokinetic analysis***

The data of blood drug concentration and time in Beagle dogs were processed by non-atrioventricular model analysis. $T_{max}$ and $C_{max}$ were calculated by parabola method according to the maximum blood drug concentration and the sub-maximum values before and after, and the pharmacokinetic parameters were calculated. The trapezoidal method

**Table 3** IPM/Tween80/PEG400/Water Km =2:1 (37 ℃)

| Number | Oil (g) | S + Co-S (g) | Water (g) | Phenomenon |
|---|---|---|---|---|
| 1 | 0.10 | 0.90 | 0.98 | ME |
| 2 | 0.20 | 0.80 | 0.62 | ME |
| 3 | 0.30 | 0.70 | 0.58 | ME |
| 4 | 0.40 | 0.60 | – | – |
| 5 | 0.50 | 0.50 | – | – |
| 6 | 0.60 | 0.40 | – | – |
| 7 | 0.70 | 0.30 | – | – |
| 8 | 0.80 | 0.20 | – | – |
| 9 | 0.90 | 0.10 | – | – |

S + Co-S, surfactant + cosurfactant; ME, microemulsion

**Table 4** IPM/Tween80/Cremophor RH40/Water Km =1:1 (37 ℃)

| Number | Oil (g) | S + Co-S (g) | Water (g) | Phenomenon |
|---|---|---|---|---|
| 1 | 0.10 | 0.90 | 0.46 | ME |
| 2 | 0.20 | 0.80 | 0.48 | ME |
| 3 | 0.30 | 0.70 | 0.48 | ME |
| 4 | 0.40 | 0.60 | 0.51 | ME |
| 5 | 0.50 | 0.50 | – | – |
| 6 | 0.60 | 0.40 | – | – |
| 7 | 0.70 | 0.30 | – | – |
| 8 | 0.80 | 0.20 | – | – |
| 9 | 0.90 | 0.10 | – | – |

S + Co-S, surfactant + cosurfactant; ME, microemulsion.

was used to calculate the area under the drug-time curve ($AUC_{0\to\infty}$), and the relative bioavailability $F=AUC_{0\to\infty}(T)/AUC_{0\to\infty}(R)\times100\%$.

***Statistical method analysis***

The area $AUC_{0\to\infty}$ and $C_{max}$ under the curve of blood drug concentration time of test preparation (total saponin enteric self-emulsified soft capsule of notoginseng) and reference preparation (Xesaitong soft capsule) were logarithmic converted, and then the analysis of variance (ANOVA) with three factors (weekly, inter-preparation and inter-individual) was conducted.

## Results

### *Manufacture of enteric coated soft capsules and the self-microemulsion content*

The range and variance analysis of the orthogonal test determined that the best prescription of total saponins of *Panax notoginseng* enteric soft capsule was: gelatin:glycerol:water=1:1:2, the films were soaked in 1% formaldehyde in 1 hour. See *Table 3* and *Table 4* for the content of each component when microemulsion is formed, and see *Figure 1* and *Figure 2* for the three-phase diagram. It can

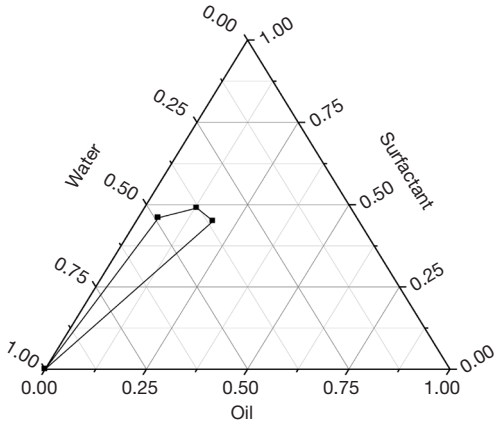


**Figure 1** The pseudo-ternary phase diagram of IPM/Tween80/PEG400/Water system Km =2:1 (37 ℃) (A: O/W Microemulsion phase). O/W, oil-in-water. A, microemulsion area in three phase diagram drawn by surfactant.

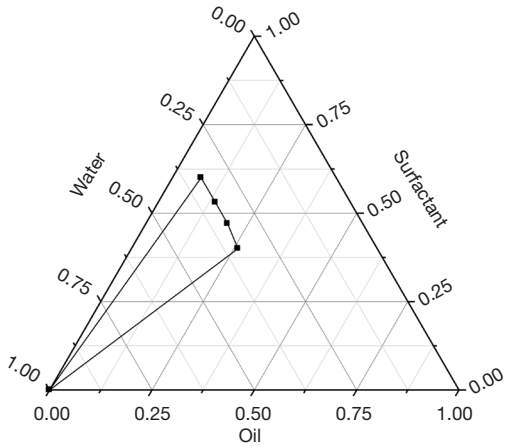


**Figure 2** The pseudo-ternary phase diagram of IPM/Cremophor RH40/PEG400/Water system Km =1:1 (37 ℃) (A: O/W Microemulsion phase). O/W, oil-in-water. A, microemulsion area in three phase diagram drawn by surfactant.

be seen from the diagram that the area of microemulsion area in the three-phase diagram with Cremophor RH40 as surfactant is larger than Tween 80. The contents of the soft capsule self-microemulsion are prescribed as:IPM:Cremophor RH40:PEG400 =1:4.5:4.5 (with suitable PNS).

### *Pharmacokinetic analysis*

#### Chromatographic behavior

Under the chromatographic conditions adopted, the retention times of the 3 main components $Rg_1$, $Rb_1$, and $R_1$, and the puerarin internal standard in PNS are 7.97 minutes, 8.50, 7.80, and 6.24 minutes, respectively. Blank plasma did not interfere with the determination after treatment, and all chromatographic peaks were completely separated. The results are shown in *Figure 3*.

#### Standard curve and linear range

The $Rg_1$ standard curve equation was y=0.00124×–0.0164, r=0.9956 (n=6). The $Rb_1$ standard curve equation was y=0.00168×–0.158, r=0.9968 (n=6). The standard curve equation of $R_1$ was y=0.00117×–0.119, r=0.9973 (n=6). The drug concentrations of $Rg_1$, $Rb_1$, and $R_1$ in the blood were linear and in the range of 2.5–1,000 μg/mL.

#### Method recovery rate test

The relative average recoveries of $Rg_1$, $Rb_1$, and $R_1$ were (105.51±7.93)%, (97.20±9.33)%, and (95.91±9.58)%, respectively (*Table 5*). The recovery rate of this method was stable and satisfied the range specified in the *Chinese Pharmacopoeia* (2020) (12).

#### Method precision

For the intra-day precision of $Rb_1$, the RSD of low, medium, and high concentrations were 13.58%, 4.87%, and 5.37%, respectively. For the daytime precision, the RSD of low, medium, and high concentrations were 14.89%, 3.98%, and 6.31%, respectively. For the intraday precision of Rg1, the RSD of low, medium, and high concentrations were 7.99%, 10.29%, and 1.63%, respectively. For the daytime precision, the RSD of low, medium, and high concentrations were 11.35%, 10.60%, and 1.63%, respectively. For the intra-day precision of R1, the RSD of low, medium, and high concentrations were 10.61%, 5.87%, and 3.42%, respectively. For the interday precision, the RSD of low, medium, and high concentrations were 10.61%, 9.21%, and 3.42%, respectively. The precision satisfied the requirement that the general RSD value should be less than 15% as stipulated in the Chinese Pharmacopoeia (2020) (12). See *Table 6* for the results.

#### Sensitivity test

The lowest detection concentration in plasma was 2.5 μg/mL, and the lowest limit of quantification was 7.5 μg/mL.

### *Pharmacokinetic test results*

#### Blood drug concentration data

Beagle dogs were given Xuesaitong soft capsule or self-

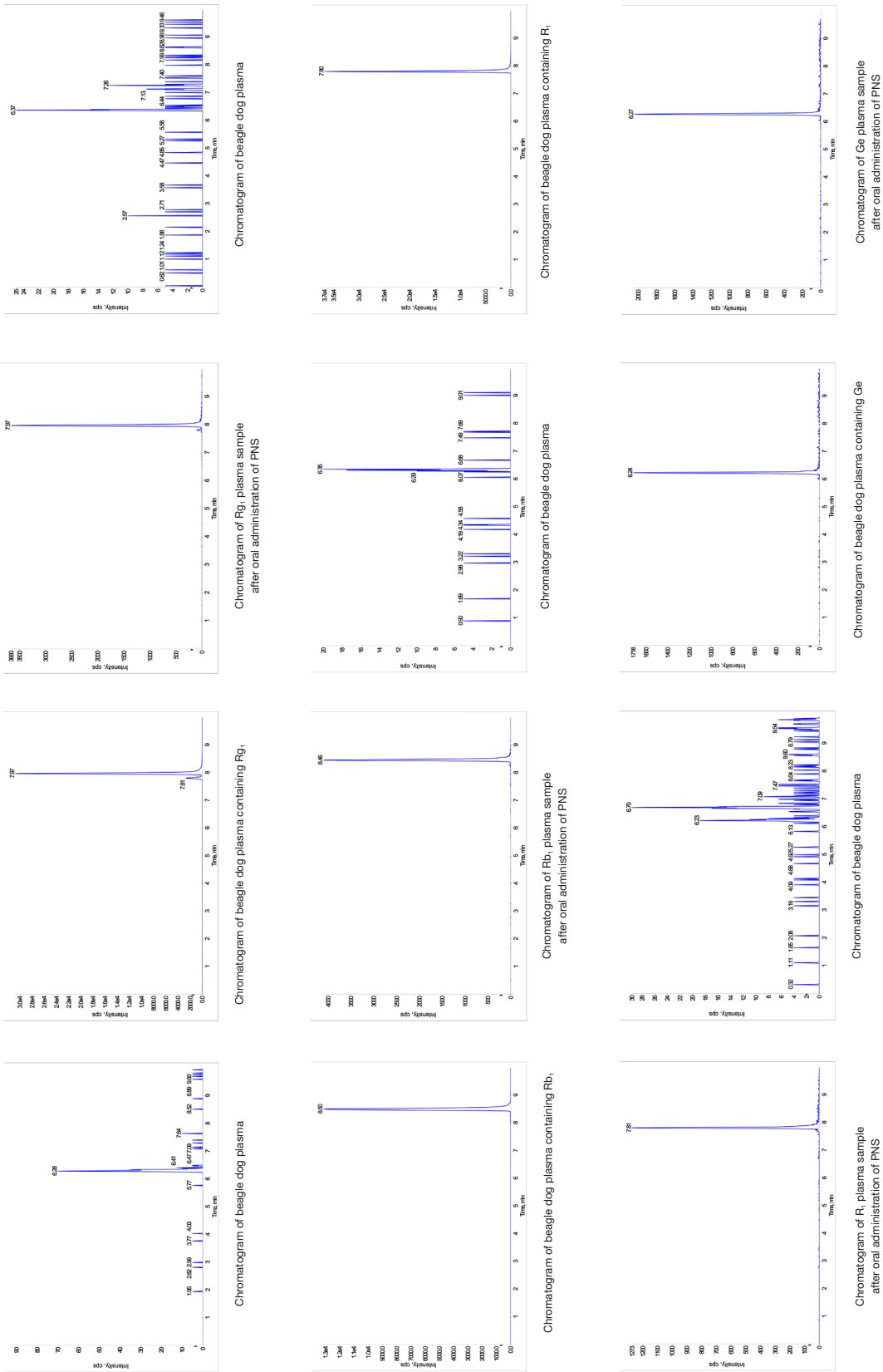


**Figure 3** Chromatograms showing the saponin contents after oral administration of *Panax notoginseng saponins*. PNS, *Panax notoginseng* saponins; Rb1, ginsenoside, Rg1, ginsenoside, $R_1$, notoginsenoside.

**Table 5** The recovery of *Panax notoginseng* saponins in plasma (n=3)

| PNS | Amount added (μg/mL) | Amount detected (μg/mL), mean ± SD | Recovery (%) | RSD (%) |
|---|---|---|---|---|
| $Rb_1$ | 5.5 | 6.20±0.23 | 113.01 | 4.12 |
| | 120.76 | 128.14±6.24 | 106.31 | 5.02 |
| | 509.12 | 494.76±8.03 | 97.22 | 1.98 |
| $Rg_1$ | 6.2 | 5.48±0.44 | 88.45 | 8.35 |
| | 124.23 | 118.89±12.24 | 96.14 | 10.41 |
| | 501.13 | 533.98±8.71 | 107.01 | 1.89 |
| $R_1$ | 5.78 | 4.99±0.09 | 86.09 | 2.46 |
| | 121.01 | 127.46±2.47 | 105.24 | 2.33 |
| | 498.7 | 477.14±8.46 | 96.41 | 1.99 |

$Rb_1$, ginsenoside; $Rg_1$, ginsenoside; $R_1$, notoginsenoside; SD, standard deviation; RSD, relative standard deviation.

**Table 6** The precision of *Panax notoginseng* saponins in plasma (n=3)

| PNS | Amount added (μg/mL) | Amount detected (μg/mL), mean ± SD | | RSD (%) | |
|---|---|---|---|---|---|
| | | Within-day | Between-day | Within-day | Between-day |
| $Rb_1$ | 5.45 | 5.63±0.76 | 5.73±0.85 | 13.58 | 14.89 |
| | 121.76 | 128.14±6.24 | 129.81±5.16 | 4.87 | 3.98 |
| | 505.12 | 483.43±25.94 | 486.76±30.72 | 5.37 | 6.31 |
| $Rg_1$ | 5.20 | 5.48±0.44 | 5.38±0.61 | 7.99 | 11.35 |
| | 122.23 | 118.89±12.24 | 115.56±12.24 | 10.29 | 10.60 |
| | 504.13 | 533.98±8.71 | 533.98±8.71 | 1.63 | 1.63 |
| $R_1$ | 6.78 | 4.66±0.49 | 4.66±0.48 | 10.61 | 10.61 |
| | 121.01 | 124.13±7.29 | 121.79±11.21 | 5.87 | 9.21 |
| | 498.70 | 463.80±15.85 | 463.80±15.80 | 3.42 | 3.42 |

$Rb_1$, ginsenoside; $Rg_1$, ginsenoside; $R_1$, notoginsenoside; SD, standard deviation; RSD, relative standard deviation.

made PNS enteric self-emulsifying soft capsule. The peak concentration ($C_{max}$) of $Rb_1$ was 15.50±0.51 and 18.05±0.26 ng/mL, and the peak time ($T_{max}$) was 3.00±0 and 2.00±0 hours, for Xuesaitong soft capsule and self-made PNS enteric self-emulsifying soft capsule, respectively. The $C_{max}$ and $T_{max}$ of $Rg_1$ were 3.88±0.28 ng/mL and 4.16±0.25 ng/mL, and 1.50±0 and 2.00±0 hours, respectively. The $C_{max}$ of $R_1$ was 1.48±0.21 and 1.84±0.25 ng/mL, respectively, and the $T_{max}$ was 1.92±0.20 and 2.08±0.49 hours, respectively. See *Tables 7-12* for blood drug concentration-time data. The relative bioavailability of $Rb_1$ was 196.2%; the relative bioavailability of $Rg_1$ was 77.2%; and the relative bioavailability of $R_1$ was 117.7%.

**Blood drug concentration-time curve**

The mean ± standard deviation (SD) of blood concentration after beagle dogs were administered oral Xuesaitong soft capsule and self-made self-microemulsifying enteric-coated capsule was plotted against time. The results are shown in and *Figure 4*.

### *Analysis of variance results*

The analysis of variance results of $\ln AUC_{0\to\infty}$ and $\ln C_{max}$ are shown in *Tables 13-18*, respectively.

The results of variance analysis of $Rg_1$, $Rb_1$ and $R_1$ showed that there was no significant difference in $\ln AUC_{0\to\infty}$

**Table 7** The estimated pharmacokinetic parameters of $Rb_1$ in market conventional capsule (μg/mL)

| Parameter | No. 1 | No. 2 | No. 3 | No. 4 | No. 5 | No. 6 | Mean | SD |
|---|---|---|---|---|---|---|---|---|
| Ka ($h^{-1}$) | 0.36 | 0.36 | 0.37 | 0.33 | 0.35 | 0.42 | 0.36 | 0.03 |
| Ke ($h^{-1}$) | 0.29 | 0.31 | 0.31 | 0.29 | 0.30 | 0.32 | 0.30 | 0.01 |
| $T_{1/2Ka}$ (h) | 1.94 | 1.93 | 1.88 | 2.09 | 2.01 | 1.65 | 1.92 | 0.15 |
| $T_{1/2Ke}$ (h) | 2.40 | 2.27 | 2.24 | 2.41 | 2.31 | 2.17 | 2.30 | 0.09 |
| $T_{max}$ (h) | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 0.00 |
| $C_{max}$ (ng/mL) | 15.13 | 15.99 | 14.99 | 14.98 | 16.02 | 15.87 | 15.50 | 0.51 |
| $AUC_{0\rightarrow t}$ (ng/mL)·h | 37.03 | 32.64 | 36.36 | 33.60 | 35.07 | 32.06 | 34.46 | 2.02 |
| $AUC_{0\rightarrow \infty}$ (ng/mL)·h | 40.79 | 35.27 | 40.25 | 37.91 | 39.18 | 34.75 | 38.03 | 2.54 |
| CLz/F (L/h/kg) | 1.03 | 1.06 | 1.01 | 0.99 | 0.97 | 1.01 | 1.01 | 0.03 |
| Vz/F (L/kg) | 3.36 | 2.94 | 3.91 | 3.87 | 3.70 | 3.67 | 3.58 | 0.37 |
| $MRT_{0\rightarrow t}$ (h) | 4.34 | 4.17 | 4.26 | 4.28 | 4.26 | 4.13 | 4.24 | 0.08 |
| $MRT_{0\rightarrow \infty}$ (h) | 4.76 | 4.41 | 4.96 | 5.04 | 4.96 | 4.77 | 4.82 | 0.23 |

$Rb_1$, ginsenoside; K, elimination rate constant; $T_{1/2}$, half-life; Tmax, time to reach the peak plasma concentration; $C_{max}$, peak plasma concentration; $AUC_{0\rightarrow t}$, area under the plasma concentration-time curve from time of dosing to the last sampling time; $AUC_{0\rightarrow \infty}$, area under the plasma concentration-time curve from time of dosing to infinity; CLz/F, apparent clearance; Vz/F, apparent volume of distribution; MRT, mean residence time; SD, standard deviation.

**Table 8** The estimated pharmacokinetics parameters of $Rb_1$ in self-emulsifying enteric capsule (μg/mL)

| Parameter | No. 1 | No. 2 | No. 3 | No. 4 | No. 5 | No. 6 | Mean | SD |
|---|---|---|---|---|---|---|---|---|
| Ka ($h^{-1}$) | 1.01 | 0.87 | 0.64 | 0.97 | 0.86 | 0.86 | 0.87 | 0.13 |
| Ke ($h^{-1}$) | 0.17 | 0.18 | 0.26 | 0.18 | 0.20 | 0.20 | 0.20 | 0.03 |
| $T_{1/2Ka}$ (h) | 0.69 | 0.80 | 1.09 | 0.72 | 0.80 | 0.81 | 0.82 | 0.14 |
| $T_{1/2Ke}$ (h) | 3.99 | 3.76 | 2.71 | 3.87 | 3.46 | 3.53 | 3.55 | 0.46 |
| $T_{max}$ (h) | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 0 |
| $C_{max}$ (ng/mL) | 18.00 | 17.98 | 18.53 | 17.93 | 17.78 | 18.03 | 18.05 | 0.26 |
| $AUC_{0\rightarrow t}$ (ng/mL)·h | 99.02 | 99.53 | 98.99 | 96.55 | 97.60 | 99.26 | 98.49 | 1.16 |
| $AUC_{0\rightarrow \infty}$ (ng/mL)·h | 116.88 | 120.09 | 110.39 | 113.11 | 112.71 | 114.00 | 114.53 | 3.44 |
| CLz/F (L/h/kg) | 0.51 | 0.48 | 0.52 | 0.52 | 0.53 | 0.52 | 0.52 | 0.02 |
| Vz/F (L/kg) | 2.94 | 3.33 | 2.78 | 2.96 | 3.02 | 2.78 | 2.97 | 0.21 |
| $MRT_{0\rightarrow t}$ (h) | 4.61 | 4.62 | 4.41 | 4.56 | 4.48 | 4.57 | 4.54 | 0.08 |
| $MRT_{0\rightarrow \infty}$ (h) | 6.57 | 7.30 | 6.04 | 6.43 | 6.24 | 6.13 | 6.45 | 0.46 |

K, elimination rate constant; $T_{1/2}$, half-life; $T_{max}$, time to reach the peak plasma concentration; $C_{max}$, peak plasma concentration; $AUC_{0\rightarrow t}$, area under the plasma concentration-time curve from time of dosing to the last sampling time; $AUC_{0\rightarrow \infty}$, area under the plasma concentration-time curve from time of dosing to infinity; CLz/F, apparent clearance; Vz/F, apparent volume of distribution; MRT, mean residence time; SD, standard deviation.

**Table 9** The estimated pharmacokinetics parameters of Rg1 in market conventional capsule (μg/mL)

| Parameter | No. 1 | No. 2 | No. 3 | No. 4 | No. 5 | No. 6 | Mean | SD |
|---|---|---|---|---|---|---|---|---|
| Ka ($h^{-1}$) | 1.01 | 0.87 | 0.64 | 0.97 | 0.86 | 0.86 | 0.87 | 0.13 |
| Ke ($h^{-1}$) | 0.25 | 0.06 | 0.12 | 0.32 | 0.05 | 0.08 | 0.15 | 0.11 |
| $T_{1/2Ka}$ (h) | 1.05 | 2.10 | 1.98 | 1.82 | 20.21 | 0.80 | 4.66 | 7.63 |
| $T_{1/2Ke}$ (h) | 0.25 | 0.06 | 0.12 | 0.32 | 0.05 | 0.08 | 0.15 | 0.11 |
| $T_{max}$ (h) | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 0.00 |
| $C_{max}$ (ng/mL) | 3.53 | 3.98 | 3.79 | 4.01 | 3.67 | 4.31 | 3.88 | 0.28 |
| $AUC_{0\rightarrow t}$ (ng/mL)·h | 10.37 | 14.10 | 11.45 | 7.43 | 10.56 | 8.80 | 10.45 | 2.29 |
| $AUC_{0\rightarrow \infty}$ (ng/mL)·h | 11.40 | 56.30 | 15.62 | 8.36 | 15.88 | 10.96 | 19.75 | 18.14 |
| CLz/F (L/h/kg) | 4.66 | 0.97 | 3.31 | 2.28 | 2.92 | 3.96 | 3.01 | 1.30 |
| Vz/F (L/kg) | 23.90 | 48.23 | 28.55 | 30.38 | 22.95 | 15.99 | 28.33 | 10.96 |
| $MRT_{0\rightarrow t}$ (h) | 3.82 | 5.00 | 4.55 | 4.74 | 5.18 | 4.36 | 4.61 | 0.49 |
| $MRT_{0\rightarrow \infty}$ (h) | 4.96 | 47.26 | 8.30 | 15.03 | 8.69 | 5.00 | 14.87 | 16.28 |

K, elimination rate constant; $T_{1/2}$, half-life; $T_{max}$, time to reach the peak plasma concentration; $C_{max}$, peak plasma concentration; $AUC_{0\rightarrow t}$, area under the plasma concentration-time curve from time of dosing to the last sampling time; $AUC_{0\rightarrow \infty}$, area under the plasma concentration-time curve from time of dosing to infinity; CLz/F, apparent clearance; Vz/F, apparent volume of distribution; MRT, mean residence time; SD, standard deviation.

**Table 10** The estimated pharmacokinetics parameters of $Rg_1$ in self-emulsifying enteric capsule (μg/mL)

| Parameter | No. 1 | No. 2 | No. 3 | No. 4 | No. 5 | No. 6 | Mean | SD |
|---|---|---|---|---|---|---|---|---|
| Ka ($h^{-1}$) | 0.67 | 0.67 | 0.71 | 16.28 | 1.04 | 1.47 | 3.47 | 6.28 |
| Ke ($h^{-1}$) | 0.60 | 0.57 | 0.68 | 0.44 | 0.33 | 0.38 | 0.50 | 0.14 |
| $T_{1/2Ka}$ (h) | 1.04 | 1.04 | 0.97 | 0.04 | 0.67 | 0.47 | 0.71 | 0.40 |
| $T_{1/2Ke}$ (h) | 0.60 | 0.57 | 0.68 | 0.44 | 0.33 | 0.38 | 0.50 | 0.14 |
| $T_{max}$ (h) | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 0 |
| $C_{max}$ (ng/mL) | 4.31 | 4.24 | 4.01 | 3.98 | 3.87 | 4.54 | 4.16 | 0.25 |
| $AUC_{0\rightarrow t}$ (ng/mL)·h | 12.58 | 11.97 | 8.97 | 7.72 | 14.56 | 14.98 | 11.80 | 2.93 |
| $AUC_{0\rightarrow \infty}$ (ng/mL)·h | 12.72 | 12.18 | 9.10 | 7.83 | 15.31 | 15.24 | 12.07 | 3.09 |
| CLz/F (L/h/kg) | 7.85 | 8.68 | 9.72 | 17.50 | 11.92 | 10.42 | 11.01 | 3.47 |
| Vz/F (L/kg) | 4.72 | 4.93 | 6.59 | 7.66 | 3.92 | 3.94 | 5.29 | 1.52 |
| $MRT_{0\rightarrow t}$ (h) | 3.20 | 3.45 | 3.82 | 4.04 | 3.95 | 3.49 | 3.66 | 0.33 |
| $MRT_{0\rightarrow \infty}$ (h) | 3.25 | 4.05 | 3.82 | 4.04 | 5.62 | 3.56 | 4.06 | 0.82 |

K, elimination rate constant; $T_{1/2}$, half-life; $T_{max}$, time to reach the peak plasma concentration; $C_{max}$, peak plasma concentration; $AUC_{0\rightarrow t}$, area under the plasma concentration-time curve from time of dosing to the last sampling time; $AUC_{0\rightarrow \infty}$, area under the plasma concentration-time curve from time of dosing to infinity; CLz/F, apparent clearance; Vz/F, apparent volume of distribution; MRT, mean residence time; SD, standard deviation.

**Table 11** The estimated pharmacokinetics parameters of $R_1$ in market conventional capsule (μg/mL)

| Parameter | No. 1 | No. 2 | No. 3 | No. 4 | No. 5 | No. 6 | Mean | SD |
|---|---|---|---|---|---|---|---|---|
| Ka ($h^{-1}$) | 1.67 | 1.96 | 0.57 | 1.36 | 1.16 | 1.04 | 1.29 | 0.49 |
| Ke ($h^{-1}$) | 0.23 | 0.25 | 0.14 | 0.09 | 0.18 | 0.52 | 0.23 | 0.15 |
| $T_{1/2Ka}$ (h) | 0.42 | 0.36 | 1.23 | 0.51 | 0.60 | 0.67 | 0.63 | 0.32 |
| $T_{1/2Ke}$ (h) | 3.03 | 2.78 | 4.95 | 8.02 | 3.95 | 1.34 | 4.01 | 2.31 |
| $T_{max}$ (h) | 1.50 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 1.92 | 0.20 |
| $C_{max}$ (ng/mL) | 1.40 | 1.72 | 1.31 | 1.69 | 1.54 | 1.19 | 1.48 | 0.21 |
| $AUC_{0\rightarrow t}$ (ng/mL)·h | 5.41 | 5.95 | 8.61 | 11.37 | 7.47 | 4.16 | 7.16 | 2.59 |
| $AUC_{0\rightarrow \infty}$ (ng/mL)·h | 5.96 | 6.33 | 12.25 | 22.22 | 8.85 | 4.19 | 9.97 | 6.62 |
| CLz/F (L/h/kg) | 9.68 | 8.67 | 4.73 | 2.53 | 6.56 | 12.56 | 7.46 | 3.60 |
| Vz/F (L/kg) | 50.25 | 31.05 | 44.12 | 47.47 | 39.68 | 18.47 | 38.51 | 11.90 |
| $MRT_{0\rightarrow t}$ (h) | 3.98 | 3.71 | 5.25 | 5.16 | 4.76 | 4.57 | 4.57 | 0.62 |
| $MRT_{0\rightarrow \infty}$ (h) | 5.33 | 4.32 | 10.24 | 18.46 | 6.59 | 4.58 | 8.25 | 5.45 |

K, elimination rate constant; $T_{1/2}$, half-life; $T_{max}$, time to reach the peak plasma concentration; $C_{max}$, peak plasma concentration; $AUC_{0\rightarrow t}$, area under the plasma concentration-time curve from time of dosing to the last sampling time; $AUC_{0\rightarrow \infty}$, area under the plasma concentration-time curve from time of dosing to infinity; CLz/F, apparent clearance; Vz/F, apparent volume of distribution; MRT, mean residence time; SD, standard deviation.

**Table 12** The estimated pharmacokinetics parameters of $R_1$ in self-emulsifying enteric capsule (μg/mL)

| Parameter | No. 1 | No. 2 | No. 3 | No. 4 | No. 5 | No. 6 | Mean | SD |
|---|---|---|---|---|---|---|---|---|
| Ka ($h^{-1}$) | 1.22 | 1.15 | 0.04 | 0.46 | 0.02 | 0.57 | 0.58 | 0.52 |
| Ke ($h^{-1}$) | 0.45 | 0.30 | 0.03 | 0.36 | 0.00 | 0.48 | 0.27 | 0.21 |
| $T_{1/2Ka}$ (h) | 0.57 | 0.61 | 17.91 | 1.49 | 42.10 | 1.23 | 10.65 | 16.84 |
| $T_{1/2Ke}$ (h) | 1.53 | 2.29 | 23.66 | 1.93 | 6931.47 | 1.46 | 1160.39 | 2827.26 |
| $T_{max}$ (h) | 2.00 | 2.00 | 3.00 | 2.00 | 2.00 | 1.50 | 2.08 | 0.49 |
| $C_{max}$ (ng/mL) | 1.86 | 2.09 | 1.98 | 1.65 | 1.43 | 2.01 | 1.84 | 0.25 |
| $AUC_{0\rightarrow t}$ (ng/mL)·h | 5.55 | 4.99 | 10.85 | 7.38 | 6.41 | 7.17 | 7.06 | 2.07 |
| $AUC_{0\rightarrow \infty}$ (ng/mL)·h | 5.64 | 5.19 | 12.53 | 7.83 | 6.66 | 7.34 | 7.53 | 2.65 |
| CLz/F (L/h/kg) | 9.57 | 8.17 | 5.89 | 6.96 | 10.39 | 6.54 | 7.92 | 1.78 |
| Vz/F (L/kg) | 19.63 | 14.57 | 16.49 | 24.39 | 12.47 | 15.96 | 17.25 | 4.22 |
| $MRT_{0\rightarrow t}$ (h) | 3.06 | 3.23 | 4.94 | 4.00 | 3.83 | 4.36 | 3.90 | 0.70 |
| $MRT_{0\rightarrow \infty}$ (h) | 3.10 | 3.27 | 5.15 | 4.57 | 3.83 | 4.46 | 4.06 | 0.80 |

K, elimination rate constant; $T_{1/2}$, half-life; $T_{max}$, time to reach the peak plasma concentration; $C_{max}$, peak plasma concentration; $AUC_{0\rightarrow t}$, area under the plasma concentration-time curve from time of dosing to the last sampling time; $AUC_{0\rightarrow \infty}$, area under the plasma concentration-time curve from time of dosing to infinity; CLz/F, apparent clearance; Vz/F, apparent volume of distribution; MRT, mean residence time; SD, standard deviation.

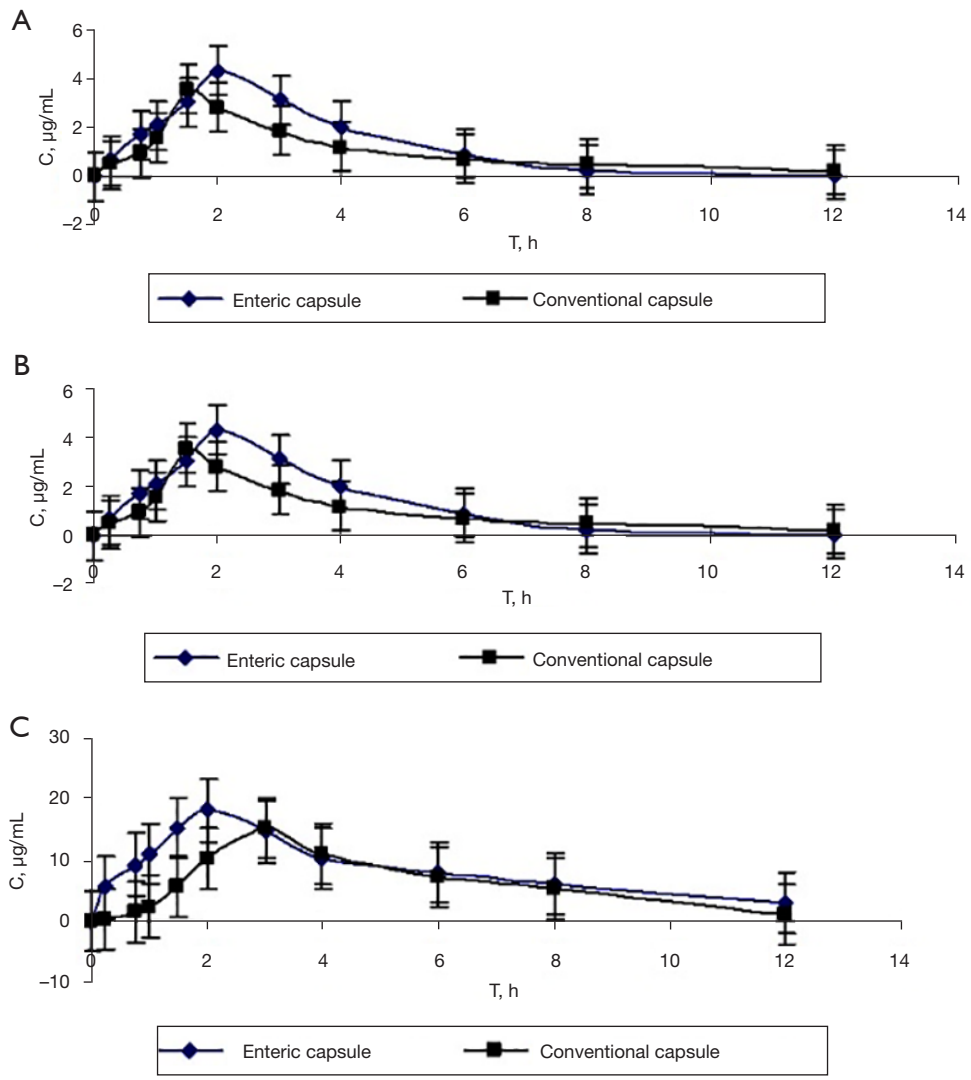


**Figure 4** The mean plasma concentration time curves of the self-emulsifying enteric capsule and the conventional capsule. (A) Rg1concentration, (B) $Rb_1$ concentration, and (C) $R_1$ concentration. $Rb_1$, ginsenoside, $Rg_1$, ginsenoside, $R_1$, notoginsenoside.

between the two preparations during the week and between individuals (P>0.05), but there was a significant difference between the two preparations (P<0.05). Therefore, the bioavailability of self-made preparations is higher than that of commercially available preparations.

There was significant difference in the $C_{max}$ between the test preparation and the reference preparation (P<0.05), and there was no difference during the test week (P>0.05). Therefore, the peak concentration of self-made preparations is higher than that of commercially available preparations.

## Discussion

(I) Accumulating clinical and laboratory evidence have indicated the significant effects of P. notoginseng in treating chronic human diseases, including cardiovascular diseases (39%), cancer (19%), liver diseases (10%), neurodegenerative diseases (9%), inflammatory diseases (6%) and diabetes (6%), as well as acute injury (4%), wound pair (3%), osteoporosis (2%) and hyperlipemia (2%). With its

**Table 13** Variance analysis for $lnAUC_{0\to\infty}$ of $Rb_1$

| Source | df | SS | MS | F | Sig. |
|---|---|---|---|---|---|
| Individual | 0.001 | 5 | 0.000 | 0.045 | P>0.05 |
| Period | 0.000 | 1 | 0.000 | 0.137 | P>0.05 |
| Preparation | 1.362 | 1 | 1.362 | 583.003 | P<0.05 |
| Error | 1.372 | 11 | 0.125 | | |
| Total | 0.009 | 4 | | | |

$AUC_{0\to\infty}$, area under the plasma concentration-time curve from time of dosing to infinity; $Rb_1$, ginsenoside; df, degree freedom; SS, sum of square; MS, mean square; F, bioavailability; Sig., significance.

**Table 14** Variance analysis for $lnC_{max}$ of $Rb_1$

| Source | df | SS | MS | F | Sig. |
|---|---|---|---|---|---|
| Individual | 0.002 | 5 | 0.000 | 0.370 | P>0.05 |
| Period | 0.000 | 1 | 0.000 | 0.166 | P>0.05 |
| Preparation | 1.362 | 1 | 1.362 | 583.003 | P<0.05 |
| Error | 0.076 | 11 | 0.007 | | |
| Total | 0.004 | 4 | 0.001 | | |

$C_{max}$, peak plasma concentration; $Rb_1$, ginsenoside; df, degree freedom; SS, sum of square; MS, mean square; F, bioavailability; Sig., significance.

**Table 15** Variance analysis for $lnAUC_{0\to\infty}$ of $Rg_1$

| Source | df | SS | MS | F | Sig. |
|---|---|---|---|---|---|
| Individual | 0.064 | 5 | 0.013 | 1.889 | P>0.05 |
| Period | 0.005 | 1 | 0.005 | 0.786 | P>0.05 |
| Preparation | 0.080 | 1 | 0.080 | 11.856 | P>0.05 |
| Error | 0.175 | 11 | 0.016 | | |
| Total | 0.027 | 4 | 0.007 | | |

$AUC_{0\to\infty}$, area under the plasma concentration-time curve from time of dosing to infinity; $Rg_1$, ginsenoside; df, degree freedom; SS, sum of square; MS, mean square; F, bioavailability; Sig., significance.

**Table 16** Variance analysis for $lnC_{max}$ of $Rg_1$

| Source | df | SS | MS | F | Sig. |
|---|---|---|---|---|---|
| Individual | 0.031 | 5 | 0.006 | 2.204 | P>0.05 |
| Period | 0.000 | 1 | 0.000 | 0.148 | P>0.05 |
| Preparation | 0.014 | 1 | 0.014 | 5.094 | P>0.05 |
| Error | 0.057 | 11 | 0.005 | | |
| Total | 0.011 | 4 | 0.003 | | |

$C_{max}$, peak plasma concentration; $Rg_1$, ginsenoside; df, degree freedom; SS, sum of square; MS, mean square; F, bioavailability; Sig., significance.

**Table 17** Variance analysis for $lnAUC_{0\to\infty}$ of $R_1$

| Source | df | SS | MS | F | Sig. |
|---|---|---|---|---|---|
| Individual | 0.000 | 5 | 0.000 | 0.115 | P>0.05 |
| Period | 0.000 | 1 | 0.000 | 0.008 | P>0.05 |
| Preparation | 0.976 | 1 | 0.976 | 2,033.140 | P<0.05 |
| Error | 0.978 | 11 | 0.089 | | |
| Total | 0.002 | 4 | | | |

$AUC_{0\to\infty}$, area under the plasma concentration-time curve from time of dosing to infinity; $R_1$, notoginsenoside; df, degree freedom; SS, sum of square; MS, mean square; F, bioavailability; Sig., significance.

**Table 18** Variance analysis for $lnC_{max}$ of $R_1$

| Source | df | SS | MS | F | Sig. |
|---|---|---|---|---|---|
| Individual | 0.001 | 5 | 0.000 | 0.045 | P>0.05 |
| Period | 0.000 | 1 | 0.000 | 0.137 | P>0.05 |
| Preparation | 1.362 | 1 | 1.362 | 583.003 | P<0.05 |
| Error | 1.372 | 11 | 0.125 | | |
| Total | 0.009 | 4 | 0.002 | | |

$C_{max}$, peak plasma concentration; $R_1$, notoginsenoside; df, degree freedom; SS, sum of square; MS, mean square; F, bioavailability; Sig., significance.

excellent medical property, patented health products and medicines containing PNS have been widely used in China (13). Therefore, it is of great academic value and practical significance to select PNS as a model drug and develop new high bioavailability preparations for the research and development of saponin drug preparations. PNS is a kind of chemical mixture containing different dammaranetype saponins where notoginsenoside $R_1$, ginsenosides $Rb_1$ and $Rg_1$ are the main saponins isolated and identified from notoginseng (14).

(II) PNS have UV terminal absorption, low response sensitivity, and require gradient elution, but there is baseline drift (15). The cost of analyzing PNS is high due to the mutiple components of *Panax notoginseng* that require large numbers of reference materials. Common methods for the multi-component determination of the reference substance include response factor correction method and control extract method (16). The conditions of gradient elution vary with different chromatographic columns and analytical instruments. Under different analytical conditions, different reference substances of PNS were used for calibration, and the best gradient conditions were determined. To ensure that the chromatographic column can reach a better equilibrium state at the next injection, it is necessary to ensure that there is enough equilibrium time to ensure the stability of the baseline, the repeatability of the chromatogram, and the extension of the column life.

(III) $Rg_1$ and $Rb_1$,, are not stable in artificial gastric juice and large intestine contents, but relatively stable in near neutral buffer, small intestine contents, and intestinal mucosa (17). Drug preparations of PNS enteric coated soft capsules are not available in the market. It is a new dosage form developed by us. They can reduce the irritation of drugs to the stomach. After entering the intestine, the change of PH will cause the rapid dissolution of the casing layer. Control the release of drugs in the small intestine or colon to achieve the purpose of targeted release and improve the bioavailability. As a stable, safe and efficient drug delivery system, PNS content self microemulsion has simple preparation process, no irritating odor of the excipients used, and is

 

convenient for oral administration, which can not only protect the stability of the drug, It can enhance the pharmacological effect of the drug and prolong the efficacy time.

(IV) The protein in the plasma can combine with certain components of PNS to affect the determination (18). Therefore, methanol is needed to precipitate the protein. Generally, 3–5 times of methanol is used to precipitate the protein. However, considering that the boiling point of methanol is high and it is difficult to evaporate with nitrogen, the least amount of methanol is used to achieve better protein precipitation. In this study, 3 times of methanol is used. The absolute bioavailability of PNS was very low, with the absolute bioavailability of $Rg_1$ and $Rb_1$ being 4.35% and 18.40%, respectively when PNS was administered directly (19,20). Therefore, it is difficult to detect the normal oral dose in beagle dogs using HPLC, and it can only be detected by increasing the dose of PNS and using liquid chromatography-mass spectrometry with higher sensitivity. We herein established a HPLC-MS method for the determination of PNS in plasma (21). The method had high sensitivity and specificity, and the accuracy and precision of the method satisfied the requirements. Compared with Xuesaitong soft capsule, PNS enteric coated self microemulsion soft capsule had a higher peak concentration and higher bioavailability, which suggested that the drug disintegrated in the small intestine in the form of self microemulsion, and this is beneficial for drug efficacy and improved bioavailability.

(V) During the treatment of plasma samples, after the supernatant was volatilized with nitrogen, the residue needs to be redissolved with appropriate solvents, namely, with acetonitrile, 20% acetonitrile water, and 50% ethanol. There was no peak after redissolving in pure acetonitrile. After redissolving in 50% ethanol water, the solution was relatively clear, but the peak type was not good. After redissolving in 20% acetonitrile water, the peak type was improved. Therefore, 20% acetonitrile water was selected as the complex solvent. The boiling point of methanol is 64.5 ℃ (22), and it is therefore difficult to volatilize with nitrogen. We found that volatilization was relatively faster in a 40 ℃ water bath. The pre-test also demonstrated that the total saponins of *Panax notoginseng* were relatively stable in a 40 ℃ water bath, and did not affect the determination of the drugs.

(VI) The relative bioavailability of $Rb_1$ and $Rg_1$ in self-made enteric coated soft capsules of PNS has been significantly improved, but the relative bioavailability of $R_1$ has decreased, which may be due to the fact that the ingredient is not easily absorbed or stable in this dosage form, which needs further research.

## Conclusions

In this paper, PNS was used as the model drug, while gelatin, glycerin (plasticizer), and water (softener and disintegrant) were used to prepare the capsule shell of the soft capsule. The formaldehyde impregnation method was used to prepare the soft capsule with enteric-coated property, and the contents of the soft capsule were prepared into the form of self-microemulsion to improve the stability and absorption rate of the drug. Finally, a pharmacokinetic study of the soft capsule in beagle dogs was conducted, and the following conclusions were obtained:

(I) The main components $Rg_1$ and $Rb_1$ in PNS were unstable in artificial gastric juice and large intestine contents, but relatively stable in buffer solution in near neutral environment, small intestine contents, and intestinal mucosa. Therefore, preparing enteric soft capsules of PNS to disintegrate and release in the small intestine can improve the bioavailability of the drug.

(II) The saponin content in the self-made preparation was higher than that of common soft capsules after a single dose administration, which achieved the expected purpose of this experiment. The results showed that the peak concentration of PNS enteric coated self-microemulsion soft capsule was increased, the blood concentration was stable and the relative bioavailability was improved compared with the commercial Xuesaitong soft capsule. The pharmacokinetic parameters of the two groups were significantly different.

(III) In this paper, a high-performance liquid chromatography-mass spectrometry method was established to analyze the total saponins of *Panax notoginseng in vivo*. This method is simple, rapid, sensitive and specific. In addition, the pharmacokinetic behavior of PNS in Beagle dogs was studied for the first time, and the pharmacokinetic parameters were obtained. This

study is novel and meaningful, which can provide a theoretical basis for the pharmacodynamics and clinical research of PNS.

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## Footnote

*Reporting Checklist:* The authors have completed the MDAR and ARRIVE reporting checklists. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-5751/rc

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*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5751/coif). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Animal experiments were performed under a project license granted by the Animal Ethics Committee of Hebei Medical University (IACUC-Hebmu-GLP2009006), and they were conducted based on the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No. 85–23, revised in 1985).



## References

1. National Clinical Medical Research Center for Cardiovascular Diseases of Traditional Chinese Medicine; Branch of Integrated Traditional and Western Medicine of Chinese Physicians Association; Professional Committee of Promoting Blood Circulation and Removing Blood Stasis of Chinese Integrated Traditional and Western Medicine Association; Compiling group of Chinese Expert Consensus on the Clinical Application of Panax notoginseng saponins Preparations. Chinese Expert Consensus on the Clinical Application of Panax notoginseng saponins Preparations. Chinese Journal of Integrated Traditional and Western Medicine 2021;41:1157-67.
2. Vita R, Fallahi P, Antonelli A, et al. The administration of L-thyroxine as soft gel capsule or liquid solution. Expert Opin Drug Deliv 2014;11:1103-11.
3. Wittwer F, Mayer JP. Foam soft gelatin capsules and their method of manufacture. Patent No. US4609403A. 1984.
4. Choi W, Park JH, Ha H, et al. Flow induced deformation of vulnerable stenosis under pulsatile flow condition. Phys Rev Fluids 2020;5:043101.
5. Wang J. Study on extraction technology, quality standard and pharmacodynamics of Xihuang soft capsule. Shandong University of Traditional Chinese Medicine; 2005:7-8.
6. Felton LA, Shah NH. Physical-mechanical properties of film-coated soft gelatin capsules. Int J Pharm 1996;127:203-11.
7. Pissinati R, Oliveira WP. Enteric coating of soft gelatin capsules by spouted bed: effect of operating conditions on coating efficiency and on product quality. Eur J Pharm Biopharm 2003;55:313-21.
8. Pearnchob N, Dashevsky A, Bodmeier R. Improvement in the disintegration of shellac-coated soft gelatin capsules in simulated intestinal fluid. J Control Release 2004;94:313-21.
9. Seibel J, Neumann A, Müller A, et al. Food Interactions Observed in a Pharmacokinetic Investigation Comparing Two Marketed Cold Preparations (BNO1016 and ELOM-080) after Administration to Beagle Dogs - A Pilot Study. Planta Med 2022. [Epub ahead of print]. doi: 10.1055/a-1821-8690.
10. Limmatvapirat S, Limmatvapirat C, Luangtana-Anan M, et al. Modification of physicochemical and mechanical properties of shellac by partial hydrolysis. Int J Pharm 2004;278:41-9.
11. Rao CB, Chakrabarti PK, Ravishankar H. Novel soft

 

gelatin capsule comprising S-adenosylmethionine and a method for producing the same. Patent No. US20020164369A1. 2001.
12. National Pharmacopoeia Commission. Chinese Pharmacopoeia. Beijing: China Medical Science and Technology Press; 2020:12-3.
13. Xu Y, Tan HY, Li S, et al. Panax notoginseng for Inflammation-Related Chronic Diseases: A Review on the Modulations of Multiple Pathways. Am J Chin Med 2018;46:971-96.
14. Xu C, Wang W, Wang B, et al. Analytical methods and biological activities of Panax notoginseng saponins: Recent trends. J Ethnopharmacol 2019;236:443-65.
15. Bai CC, Han SY, Chai XY, et al. Sensitive Determination of Saponins in Radix et Rhizoma Notoginseng by Charged Aerosol Detector Coupled with HPLC. J Liq Chromatogr Relat Technol 2009;32:242-60.
16. Liu S. Study on the application of ultra high pressure extraction method, one test multiple evaluation method and control extraction method in Phellodendron amurense. Suzhou University; 2014.
17. Han H. Study on oral absorption mechanism of Panax notoginseng total soaps (PNS) and W/O Microemulsion. Fudan University; 2006.
18. Guo YP, Chen MY, Shao L, et al. Quantification of Panax notoginseng saponins metabolites in rat plasma with in vivo gut microbiota-mediated biotransformation by HPLC-MS/MS. Chin J Nat Med 2019;17:231-40.
19. Xu QF, Fang XL, Chen DF. Pharmacokinetics and bioavailability of ginsenoside Rb1 and Rg1 from Panax notoginseng in rats. J Ethnopharmacol 2003;84:187-92.
20. Xu Q. Study on high bioavailability preparation of Panax notoginseng saponins. Fudan University; 2003.
21. Vestal ML. High-performance liquid chromatography-mass spectrometry. Science 1984;226:275-81.
22. Olah GA. Beyond oil and gas: the methanol economy. Angew Chem Int Ed Engl 2005;44:2636-9.

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