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Simultaneous determination of notoginsenoside R_1, ginsenoside Rg_1, ginsenoside Re and 20(S) protopanaxatriol in beagle dog plasma by ultra high performance liquid mass spectrometry after oral administration of a *Panax notoginseng* saponin preparation

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ABSTRACT

20(S) protopanaxatriol is the main metabolite of notoginsenoside R_1, ginsenoside Rg_1, ginsenoside Re in *Panax notoginseng* and has significant activities. A ultra high performance liquid mass spectrometry method has been developed and validated for the simultaneous determination of notoginsenoside R_1 (R1), ginsenoside Rg_1 (Rg1), ginsenoside Re (Re) and 20(S) protopanaxatriol (PPT) in beagle dog plasma after oral administration of a *Panax notoginseng* saponin preparation. After the addition of the internal standard (digoxin), plasma samples were subjected to liquid–liquid extraction with acetone and methanol and separated on a 100 × 2.1 mm ACQUITY 1.7 μm C_{18} column (Waters, USA), with acetonitrile and water as the mobile phase, within a runtime of 7.0 min. The analytes were detected without interference in Selected Reaction Monitoring mode with a change in the electrospray ionization from positive to negative. The detection limits were 0.01 to 0.04 mg/L and the calibration curves of the peak areas for the four ingredients were linear over four orders of magnitude with a correlation coefficient greater than 0.9957. The intra-day and inter-day precision values (relative standard deviation, RSD, %) were within 10.25% and 13.51%, respectively, and the accuracy (relative error, RE, %) was less than 7.81%. The validated method was successfully applied to a comparative pharmacokinetic study of four saponins in beagle dogs after oral administration of a *Panax Notoginseng* Saponins preparation. The pharmacokinetic parameters were calculated with DAS 3.20. The T_{max} and C_{max} values indicate a dose–dose relationship between the saponins (R1, Rg1, and Re) and their sapogenin (PPT).

1. Introduction

Panax notoginseng (Burk.) F.H. Chen, also known as Sanchi is well-known in China and other countries for its obvious therapeutic effects on the cardiovascular system [1,2], in including resistance against cerebral infarction and ischemia [3,4], antioxidant property [5], therapeutic effect on atherosclerosis [6]. Most of the bioactivities of Sanchi are believed to be associated with triterpene saponins derived mainly from the tetracyclic dammarane. These compounds, known as ginsenosides, can be classified according to their structures as 20(S)-protopanaxadiol-type (ppd-type) and 20(S)-protopanaxatriol-type (ppt-type). The ppd-type ginsenosides possess sugar moieties at the C-3 and/or C-20 positions, whereas the ppt-type ginsenosides have a hydroxyl group at C-3 and sugar moieties at C-6 and/or C-20. The major saponins present in Sanqi include the ppd-type ginsenosides Ra3, Rb1, and Rd and the ppt-type ginsenosides Re and Rg_1 and notoginsenoside R_1 [7]. 20(S) protopanaxatriol (PPT), a well-known end metabolite of protopanaxatriol-type saponins has recently been reported to have the same bioactivity as its prototype [8,9].

Numerous studies on the pharmacokinetic behavior, metabolism, and biotransformation of ginsenosides have been conducted. Metabolic studies of Re in animals has been reported. The structures of the metabolites were identified as the 20(S)-ginsenoside Rg2, 20(S)-ginsenoside Rh_1, 20(R)-ginsenoside Rh_1, ginsenoside F_1, 3-oxo-ginsenoside Rh_1 and PPT [10]. Rg_1 is easily biotransformed. After oral administration, two metabolites of ginsenosides, Rh_1 and protopanaxatriol, were found in the human intestine [11], but three metabolites, Rh_1, ginsenosides F_1 and PPT, were found in the rat intestine [12]. Notoginsenoside R_1 metabolism by human intestinal bacteria and liver subcellular fractions, and the permeability properties of notoginsenoside

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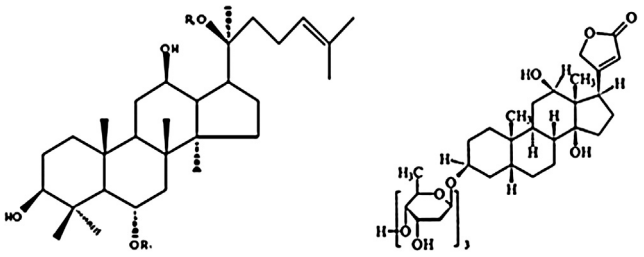

(1) (2)

	compound	R1	R2
(1)	PPT	H	H
	Re	Glc(2-1)Rha	Glc
	Rg1	Glc	Glc
	R1	Glc(2-1)xyl	Glc

Fig. 1. Chemical structure of ginsenoside Re, Rg1, notoginsenoside R1, PPT (1), and internal standard digoxin (2).

R_1 and resultant metabolites in a Caco-2 model [13], 20(S) protopanaxatriol was found to be the major metabolite with a formation rate close to that of notoginsenoside R_1 and a low elimination rate.

However, there are few reports describing the determination of 20(S) protopanaxatriol after oral administration of the saponins but not the sapogenin and the relationship between the saponins and sapogenin. Here, we describe a simple, selective and highly sensitive UPLC–MS/MS method for the determination of notoginsenoside R_1 (R1), ginsenoside Rg_1 (Rg1), ginsenoside Re (Re) and 20(S) protopanaxatriol (PPT) in beagle dog plasma after oral administration of a *Panax notoginseng* saponin preparation. The method was fully validated and applied to the pharmacokinetic analysis of R1, Rg1, Re and PPT. The results show a dose–dose relationship between the saponins and sapogenin.

2. Experimental

2.1. Chemical and reagents

The reference standards of notoginsenoside R_1 (R1), ginsenoside Rg_1 (Rg1), ginsenoside Re (Re), 20(S) protopanaxatriol (PPT) and digoxin (IS) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Chemical structures are shown in Fig. 1. Acetonitrile of LC/MS grade was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Ultrapure water was generated from the Synergy UV water purification system (Millipore Corp., USA). Other chemicals were of analytical grade.

XUESAITONG(LIXUWANG^{TM})soft capsule is an extract of *Panax notoginseng* (Burk.) F.H. Chen made by Kunming Shenghuo pharmaceutical holdings, INC. (Yunnan, China). One capsule contained 60 mg *Panax Notoginseng* Saponins [Batch: 121017]

The *Panax notoginseng* saponins preparation was analyzed by UPLC–MS/MS. The content levels of ginsenoside Re, Rg1, notoginsenoside R1are 1.83 mg/capsule, 18.91 mg/capsule, and 4.34 mg/capsule, respectively.

2.2. UPLC–MS/MS system and operating conditions

The UPLC–MS/MS system was composed of an ACQUITY UPLC system (Waters Corp., Milford, MA, USA) and a TQS triple quadrupole tandem mass spectrometer (Waters Corp., Milford, MA, USA) equipped with an electrospray ionization (ESI) source. Data were acquired and processed using MassLynx 4.1 software (Waters Corp., Milford, MA, USA).

Chromatographic separation was performed on an ACQUITY UPLC BEH C_{18} column (100 mm × 2.1 mm ID, 1.7 μm; Waters Corp., Milford, MA, USA). The column temperature was maintained at 40 °C and the auto sampler was conditioned at 4 °C.

The UPLC mobile phase consisted of acetonitrile (solution A) and Water (solution B) at a flow rate of 0.4 mL/min in only 7.0 min. Gradient condition of the mobile phase was as follows: 20% B at 0–1.0 min; 20% → 40% B at 1.0–2.5 min; 40% → 58% B at 2.5–3.0 min; 58% → 95% B at 3.0–6.0 min; 95% → 95% B at 6.0–6.5 min;95% → 20% B at 6.5–6.6 min; then the system was equilibrated using the initial condition (acetonitrile–water, 20:80, v/v) for 0.4 min. The injection volume was 3 μL and the partial loop with a needle overfill mode was used for sample injection.

Mass spectrometer was operated both in the positive ion mode (0–3.7 min) and negative ion mode (3.71–7 min) using a selected reaction monitoring (SRM) approach. The capillary voltage was set at 2.5 kV and cone voltage was set at 30 V, and source and desolvation temperatures were set at 150 °C and 400 °C, respectively. Nitrogen was used as the desolvation gas and cone gas with the flow rates of 800 L/h and 150 L/h, respectively. Argon was used as the collision gas at a pressure of approximately 3.4×10^{-3} mbar. The specific parameters for each analyze are shown in Table 1.

2.3. Animals

This study was in accordance with the regulations for animal experimentation issued by the State Committee of Science and Technology of the People's Republic of China. Six male beagle dogs weighing 11.5–12.5 kg were obtained from Beijing Tongli experimental animal farms (Beijing, China). Animals were housed in a well-lighted air-conditioned room (25 ± 2 °C) under standard environmental condition s (12 h light and 12 h dark cycle) and had free access to water prior to the study. Beagle dogs were fasted for 12 h and had free access to water prior to the experiments.

2.4. Preparation of stock and working solutions

Nine separate primary stock solutions for notoginsenoside R_1, ginsenoside Rg_1, ginsenoside Re, PPT were prepared by dissolving the accurately weighed reference compounds in methanol at the concentrations of 168, 250, 94.4, 59.5 μg/mL, respectively. The stock solutions of notoginsenoside R_1, ginsenoside Rg_1, ginsenoside Re, PPT were then mixed together and serially diluted with methanol to produce a series of standard or quality control (QC) working solutions at the desired concentrations.

Table 1
MS/MS condition of five compositions.

Composition	Formula (mass)	Pareat (*m*/*z*)	Doughters	Coae voltage	Collision energy	Ion mode
R1	933.13	955.29	775.26	94	40	ES+
Rg1	801.01	823.35	643.29	88	36	ES+
PPT	476.39	475.29	391.18	70	26	ES−
Re	946.55	969.39	789.3	98	48	ES+
Digoxin	780.43	803.27	283.09	100	40	ES+