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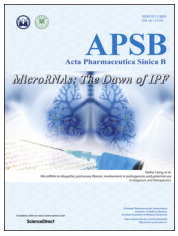

ORIGINAL ARTICLE

Simultaneous quantification of ginsenoside Rg1 and its metabolites by HPLC–MS/MS: Rg1 excretion in rat bile, urine and feces

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KEY WORDS

Ginsenoside Rg1;
Ginsenoside Rh1;
Protopanaxatriol;
Excretion;
LC–MS/MS

Abstract Ginsenoside Rg1 (Rg1), the major effective component of ginseng, has been shown to have multiple bioactivities, but low oral bioavailability. The aim of this study was to develop a simple, sensitive and rapid high performance liquid chromatography–tandem mass spectrometry (LC–MS/MS) method, which could be used to validate and quantify the concentrations of Rg1 and its metabolites in Sprague-Dawley rat bile, urine, and feces after oral administration (25 mg/kg). Calibration curves offered satisfactory linearity ($r > 0.995$) within the determined ranges. Both intra-day and inter-day variances were less than 15%, and the accuracy was within 80–120%. The excretion recoveries of Rg1, ginsenoside Rh1 (Rh1), and protopanaxatriol (Ppt) in bile, urine, and feces combined were all greater than 70%. The fecal excretion recoveries of Rg1, Rh1, and Ppt were 40.11%, 22.19%, and 22.88%, respectively, whereas 6.88% of Rg1 and 0.09% of Rh1 were excreted in bile. Urinary excretion accounted for only 0.04% of Rg1. In conclusion, the observed excretion profiles for Rg1 and its metabolites after oral administration are helpful for understanding the poor oral bioavailability of Rg1 and will aid further investigations of Rg1 as a pharmacologically active component.

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1. Introduction

Ginsenoside Rg1 (Rg1) is one of the major active saponins originating from *Panax ginseng* of the Araliaceae family and is classified as a panaxatriol saponin[1]. The chemical structure of Rg1 is shown in Fig. 1A. It has long been regarded as the main active component responsible for the pharmacological activities of *Panax* herbs. Multiple bioactivities of Rg1 have been studied, including stimulation of the central nervous system, improvement of memory, attenuation of fatigue, and formation of acetylcholine, proteins and lipids[2,3]. It has also been demonstrated that Rg1 can improve the hematopoietic function of bone marrow in a model of cyclophosphamide-induced myelosuppression[4]. Furthermore, Rg1 was reported to significantly improve survival of septic mice[5].

A few reports on the metabolism and pharmacokinetics of Rg1 have been published[6–8]. Other reports revealed that Rg1 could be biotransformed *via* deglycosylation in intestine. Ginsenoside Rh1 (Rh1) and protopanaxatriol (Ppt) are major metabolites in human intestine, whereas another ginsenoside F1 (F1), an isomer of Rh1, is also found in rat intestine[9]. In addition, metabolites of Rg1 have even greater biological effects than Rg1[10]. However, pharmacokinetic studies indicate that Rg1 has very poor oral bioavailability, Odani et al. and Xu et al. reported that the amount of ginsenoside Rg1 absorbed *via* oral administration was the range of 1.9%–20.0% of the dose[11,12]. Poor membrane permeability and active biliary excretion could be primary factors limiting the oral bioavailability of Rg1 and its metabolites[13]. Therefore, comprehensive studies of the excretion of Rg1 are required and a validated bioanalytical method is necessary to support any pharmacokinetic studies. Some methods for quantifying concentrations of Rg1 have been published, such as thin-layer chromatography (HPTLC)[14], which has low sensitivity and long analysis time, but such methods are not suitable for pharmacokinetic studies. Although high performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometer (LC–MS) are the most commonly employed analytical methods[15–17], such methods have not been applied to the comprehensive study of Rg1 and its metabolites in bile, urine and feces. Thus, the development of a much more sensitive, accurate and simple LC–MS/MS method was necessary to determine Rg1 in complex matrices. The successful method should also be capable of separating the analytes from interfering substances (including analyte degradation products) from biological samples.

The objective of this study was to develop and validate a reliable and rapid high performance liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for simultaneously quantifying the concentrations of Rg1 and its metabolites (Rh1 and Ppt) in bile, urine, and feces of Sprague–Dawley (SD) rats. Combining the advantages of HPLC and MS/MS, we examined the overall excretion of Rg1 and its metabolites in bile, urine, and feces, and provide an *in vivo* excretion profile for Rg1 after oral administration.

2. Material and methods

2.1. Chemicals and reagents

Rg1 (No. 110703-201027, purity > 98%) and androlin (No. 100008-200505, purity > 98%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Rh1 (No. 200612, purity > 95%) and Ppt (No. 200612, purity > 95%) were supplied by Jilin University (Jilin, China). HPLC grade acetonitrile was acquired from Fisher (Fairlawn, NJ, USA), and water was purified by a Milli-Q Plus water purification system (Millipore, Ltd., Billerica, MA, USA). Formic acid was of LC–MS grade (99% purity, Wako, Osaka, Japan). The other chemical reagents were of analytical grade and were purchased from Beijing Chemical Reagent Co., Ltd. (Beijing, China).

2.2. HPLC–MS/MS conditions

HPLC–MS/MS was carried out using the HPLC–MS/MS8040 (Shimadzu Corp., Kyoto, Japan). A Shim-Pack XR-ODS II (75 mm × 2 mm, 2.3 μm) column was used for separation. The mobile phase consisted of 0.05% formic acid (A) and 0.05% formic acid in acetonitrile (B) with a gradient elution. The linear

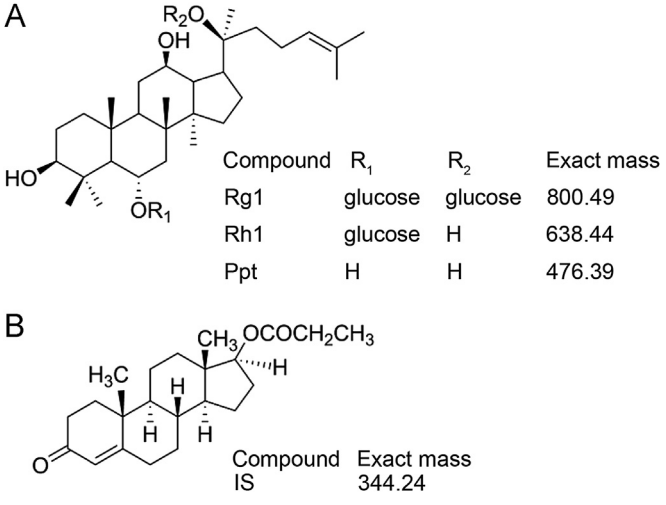

Figure 1 The chemical structures of (A) Rg1, Rh1 and Ppt, and (B) IS.