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Simultaneous determination of ginsenoside (G-Re, G-Rg1, G-Rg2, G-F1, G-Rh1) and protopanaxatriol in human plasma and urine by LC–MS/MS and its application in a pharmacokinetics study of G-Re in volunteers

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ABSTRACT

Ginsenoside Re (G-Re) improved the memory function of experimental animals in a preclinical study. Several types of saponins including G-Rg1, G-Rg2, G-F1, G-Rh1, and protopanaxatriol (PPT) may be the metabolites of G-Re according to reports from preclinical trials. In order to support a study of the pharmacokinetics of G-Re, an analytical method for G-Re and the co-detection of its probable metabolites using liquid chromatography tandem mass spectrometry (LC–MS/MS) was developed and validated. Solid phase extraction was utilized in the sample preparation. Separation of the analytes was achieved using a gradient elution (0.05% formic acid–methanol–acetonitrile, each organic phase containing 0.05% formic acid) at a flow rate of 0.3 mL/min with a retention time of approximately 2.88 min for G-Re. Data were acquired in the multiple reaction mode (MRM) and the linear range of the standard curve of plasma and urine samples for G-Re was 0.05–20 ng/mL with $r^2 \geq 0.99$. In the analysis of probable metabolites, G-Re, G-Rg1, G-F1, G-Rh1 and PPT were all detected in samples; however, G-Rg2 was not detected.

1. Introduction

The use of ethnobotanical drugs as complementary medicine is prevalent in Asia and is also gaining increasing popularity in the West. The most well-known herb, traditionally used as a drug, is the root of the ginseng species (Panax ginseng C.A. Meyer). It is widely used in traditional Chinese medicine and is taken orally to enhance stamina and capacity to cope with fatigue as well as physical stress. Ginsenosides are normally classified into two groups based on the type of aglycone present, namely the protopanaxadiol group (e.g., G-Rb1 and G-Rc) and the protopanaxatriol group (Fig. 1). G-Re, is an active component and major constituent of ginseng, and is widely known to have several physiological effects including antioxidant effects [1,2], immunomodulatory effects [3], it improves diabetes and its complications [4–6], enhances tissue regeneration [7], modulates lymphocyte proliferation [8], regulates cardiovascular function [9,10], reduces the side-effects of prednisone acetate [11] and ameliorates impaired performance [12]. A preclinical study indicated that G-Re improved memory function in experimental animals by promoting the formation of synapse long-term potentiation. The *in vivo* and *in vitro* metabolism of many ginsenosides has been investigated in detail [13–21]. According to preclinical trials and published reports, several types of saponins including G-Rg1, G-Rg2, G-F1, G-Rh1, and PPT may be the metabolites of G-Re [13–17]. Studies on the metabolism of G-Re in human intestinal microflora [13] and in rats [14–16] have been carried out.

There are several previously published reports on the determination of G-Re. A chromatographic immunostaining method [22] using a monoclonal antibody was developed for the detection of G-Re in ginseng samples, however, this method had a limited quantitative range and a low limit of quantitation (LLOQ). The use of micellar electrokinetic chromatography coupled to Ultraviolet–Visible detection [23] and LC–MS/MS [24] have also been reported in the determination of G-Re, however, similar quantitative problems were observed. Recently, bioanalytical methods for G-Re have been developed using LC–MS [13] and LC–MS/MS [15,17]. However, these methods either had a poor LLOQ or limited linear range. In addition, few reports have focused on co-detection of the five probable metabolites (G-Rg1, G-Rg2, G-F1, G-Rh1, and PPT) of G-Re in human samples.

Solid-phase extraction (SPE) is a convenient method for sample preparation. Specific advantages of SPE include faster sample processing, economical use of solvent, good purification of compounds from complex samples and procedural simplicity potentially reducing the risk of manipulation errors in routine analysis. LC–MS/MS is a rapid and sensitive analytical method. To support published

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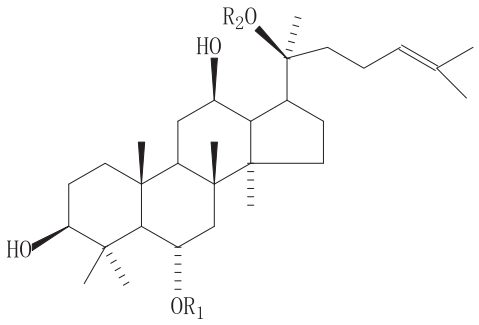

Protopanaxatriol type

Compound	M.F.	M.W.	R_1	R_2	Rt (min)	Channel	Cone Volt.	Col. Energy
G-Re	$C_{48}H_{82}O_{18}$	947	Glc-2-Rha	Glc	2.88	969.6→789.3	80	45
G-Rg1 *	$C_{42}H_{70}O_{14}$	801	Glc	Glc	2.90	823.5→643.2	90	40
G-F1 *	$C_{36}H_{58}O_9$	639	H	Glc	3.33	807.7→349.2	70	45
G-Rg2	$C_{42}H_{70}O_{13}$	785	Glc-2-Rha	H	3.38	661.4→481.3	70	32
G-Rh1 *	$C_{36}H_{58}O_9$	639	Glc	H	3.36	621.6→423.3	20	15
PPT *	$C_{30}H_{46}O_4$	475	H	H	3.77	441.4→423.3	20	18
Digoxin (IS)					3.07	781.3→651.3	20	10

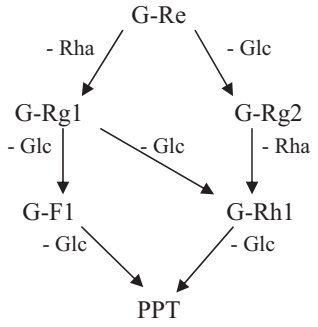

Fig. 1. Chemical structures and the main parameters of G-Re and the probable metabolites in addition to their major fragment ions observed in LC–MS/MS, and the probable metabolic pathway. Glc, β-D-glucopyranosyl; Rha, α-L-rhamnopyranosyl; *, the metabolites of G-Re detected in this study.

clinical studies and to evaluate the pharmacokinetics of G-Re in healthy Chinese subjects, SPE and LC–MS/MS were developed and validated for the direct quantification of G-Re, G-Rg1, G-Rg2, G-F1, G-Rh1, and PPT in human plasma and urine.

2. Experimental

2.1. Chemicals and reagents

Standards of G-Re and G-Rg1 with the purity of ≥98.0% were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). G-Rg2, G-F1, G-Rh1 and PPT with the purity of ≥98.0% were provided by Xinliheng Pharmaceutical Corporation (Beijing, China). The internal standard, digoxin, with the purity of ≥99.0% was purchased from Sigma Chemical Corporation (USA). Formic acid for mass spectroscopy was also purchased from Sigma and acetonitrile as well as methanol purchased from Tedia, USA were both of HPLC grade. Ultrapure water (18.2 MΩ cm, 25 °C) was prepared by passing through a Milli-Q System (Millipore Corporation, Germany). Solid-phase extraction cartridges (HLB 1 cc, OASIS) were purchased from the Waters Corporation (USA) and human blank plasma samples for the development, validation and quality control of the method were obtained from healthy, drug-free volunteer blood donors at the Blood Center of Tongzhou (Beijing, China). Blank urine was also supplied by the healthy, drug-free volunteers.

2.2. Working solution preparation

Stock solutions of G-Re, G-Rg1, G-Rg2, G-F1, G-Rh1, PPT and digoxin (IS) [14] were prepared in methanol at the concentration of 200 μg/mL. A mixed working solution containing G-Re, G-Rg1, G-Rg2, G-F1, G-Rh1 and PPT at eleven concentrations (Table 1) was prepared by appropriate dilution of the stock solution with 50% methanol. The stock solution of digoxin was further diluted with 50% methanol to obtain the working IS solution at the concentration of 50 ng/mL. All solutions were stored at 4 °C and were allowed to reach room temperature before use.

2.3. Liquid chromatographic conditions

LC–MS/MS analyses were performed using a Waters 2795 HPLC system (Waters Corporation, USA) coupled to a Quattro Premier electrospray ionization tandem mass spectrometer