Journal of Pharmaceutical and Biomedical Analysis 109 (2015) 91–104

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Identification of ginsenoside markers from dry purified extract of *Panax ginseng* by a dereplication approach and UPLC–QTOF/MS analysis

Heejung Yang[a], Dong Young Lee[b], Kyo Bin Kang[b], Jeom Yong Kim[c], Sun Ok Kim[c], Young Hyo Yoo[c], Sang Hyun Sung[b,*]

[a] *College of Pharmacy, Kangwon National University, Chuncheon 200-701, Republic of Korea*
[b] *College of Pharmacy and Research Institute of Pharmaceutical Science, Seoul National University, Seoul 151-742, Republic of Korea*
[c] *Green Cross Health Science, Suntechcity, Sangdaewon-Dong, Jungwon-Gu, Sungnam 513-15, Republic of Korea*

ARTICLE INFO

Article history:
Received 13 January 2015
Received in revised form 16 February 2015
Accepted 17 February 2015
Available online 25 February 2015

Keywords:
Panax ginseng
Ginsenoside
UPLC–QTOF
Dereplication

ABSTRACT

A dry purified extract of *Panax ginseng* (PEG) was prepared using a manufacturing process that includes column chromatography, acid hydrolysis, and an enzyme reaction. During the manufacturing process, the more polar ginsenosides were altered into less polar forms via cleavage of their sugar chains and structural modifications of the aglycones, such as hydroxylation and dehydroxylation. The structural changes of ginsenosides during the intermediate steps from dried ginseng extract (DGE) to PEG were monitored by ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectroscopy (UPLC–QTOF/MS). 22 ginsenosides isolated from PEG were used as the reference standards for determining of unknown ginsenosides and further suggesting of the metabolic markers. The elution order of 22 ginsenosides based on the type of aglycones, and the location and number of sugar chains can be used for the structural elucidation of unknown ginsenosides. This information could be used in a dereplication process for quick and efficient identification of ginsenoside derivatives in ginseng preparations. A dereplication approach helped the identification of the metabolic markers in the UPLC–QTOF/MS chromatograms during the conversion process with multivariate analyses, including principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) plots. These metabolic markers were identified by comparing with the dereplication information of the reference standards of 22 ginsenosides, or they were assigned using the pattern of the MS/MS fragmented ions. Consequently, the developed metabolic profiling approach using UPLC–QTOF/MS and multivariate analysis represents a new method for providing quality control as well as useful criteria for a similarity evaluation of the manufacturing process of ginseng preparations.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Ginseng (*Panax ginseng* C.A. Meyer) has been used for thousands of years in Korean traditional medicine as an herbal medication for a variety of disorders. Ginseng is believed to be an important medicinal resource and health supplement for enhancing bodily functions, maintaining human health, and balancing bodily conditions [1,2]. Recently, ginseng has become popular as a dietary health supplement and as an additive in foods and beverages. Ginsenosides, the major pharmacologically active ingredients in ginseng, are triterpenoidal saponins that are primarily attributed to a wide range of pharmacological and therapeutic properties, i.e., maintaining homeostasis of the body, improving brain function, preventing cancer, enhancing the immune system, and adjusting blood pressure. Ginsenosides also exhibit anti-aging, anti-obesity, and anti-diabetic effects [3,4]. Ginsenosides are classified into three groups according to the type of aglycones, i.e., dammarane, ocotillol and oleanane triterpenes. Furthermore, the dammarane type to which most

Abbreviations: DGE, dry ginseng extract; MeOH, methanol; MS, mass spectrometer; OPLS-DA, orthogonal partial least squared discriminant analysis; PCA, principle component analysis; PEG, the final dry purified extract of *P. ginseng*; PPD, protopanaxadiol; PPT, protopanaxatriol; QTOF, quadruple time-of-flight; UPLC, ultra performance liquid chromatography.

* Corresponding author at: College of Pharmacy and Research Institute of Pharmaceutical Science, Seoul National University, Seoul 151-742, Republic of Korea. Tel.: +82 2 880 7859; fax: +82 2 877 7859.
E-mail address: shsung@snu.ac.kr (S.H. Sung).

http://dx.doi.org/10.1016/j.jpba.2015.02.034
0731-7085/© 2015 Elsevier B.V. All rights reserved.

ginsenosides belong can be generally classified as protopanaxadiol (PPD; ginsenosides Rb1 (Rb1), Rc, Rd, Rg3, Rh2, etc.) or protopanaxatriol (PPT; Rg1, Re, Rg2, Rh1, etc.) [5,6]. Although the sugar chains in the PPD-type group are attached to C-3 or C-20, the sugar chains in the PPT-type group are linked to a hydroxyl moiety at C-6 or C-20 [7]. In addition, the two types can be further differentiated based on the types of sugar chains and the aliphatic chain at C-17. The less polar ginsenosides (F2, Rg2, Rg3, Rh1, Rh2, compound K, etc.), which are rarely present or even absent in wild ginseng, can be produced by transformation of the more polar ginsenosides (Rb1, Rb2, Rc, Rd, Re, etc.), which account for more than 80% of the total ginsenosides in wild ginseng [7]. Biotransformation methods, i.e., hydrolysis, steaming, heating, and enzymatic and microbial transformations, have been attempted to produce less polar ginsenosides with less than two sugar chains, which show more potent pharmacological activities than the more polar varieties [8,9]. In particular, Rh2 (one glucose at C-3) and Rg3 (two glucoses at C-3) have been well studied as anti-cancer agents in various cell lines [10–14].

Recently, ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectroscopy (UPLC–QTOF/MS) has been applied as a powerful analytical tool for rapid analysis of the complex components or metabolites and chemical transformations in ginseng-related products [15,16]. The development of the UPLC–QTOF/MS system offers much higher resolution for the separation of single components in ginseng extracts and also facilitates quantification with high sensitivity and selectivity for identification of the components in ginseng extract [17,18]. The value of accurate mass acquired from QTOF/MS can be the most important information and have been applied to predict and find the known components in natural products extracts as a dereplicaiton strategy.

The final dry purified extract of *P. ginseng* (PEG) product was produced from dry ginseng extract (DGE) via patented technologies [19,20]. It was designed for elevating the content of many less polar ginsenosides, especially Rh2 and Rg3, in the three steps of the manufacturing processes, i.e., column chromatography, acid and enzyme hydrolysis, and pressurized heating.

Recently, a dereplication process using LC/MS was demonstrated as a powerful technique for rapid isolation and identification of known compounds from natural product extracts including ginsenosides [21,22]. The chromatograms and spectroscopic data acquired from the LC/MS technique could be used to monitor changes in the chemical profile during the manufacturing processes in natural product preparations [23–26]. With this perspective, we attempted to discovery of the metabolic markers during the manufacturing steps from DGE to PEG. The metabolic markers could be identified via a dereplication process using the spectroscopic features of 22 ginsenosides isolated from PEG as the reference standards. The suggested metabolic markers and a dereplication approach might be used in quality control as well as for observation of the metabolic changes in ginsenosides during the manufacturing process of ginseng preparation.

2. Material and methods

2.1. Chemicals

The preparations of ginseng extracts and reference standards used in this study were gifted from Green Cross Health Science, Inc. (Sungnam, Korea) [19,20]. The manufacturer provided four dry ginseng extracts (DGE), seven of the first intermediate products (I-1), eight of the second intermediate products (I-2), and eleven of the final products (PEG) produced from different batches and prepared with patented technology. In brief, the harvested crude ginseng was ground and repeatedly extracted with aqueous ethanol followed by evaporation in vacuo to yield DGE. Next, DGE was suspended in distilled water and subjected to HP-20 resin column chromatography to yield I-1. The I-2 was obtained from I-1 by reaction with an enzyme containing ginsenoside-β-glucosidase. After mild acid hydrolysis (acetic acid) of the mixture of I-1 and I-2, the reactant was purified with HP-20 resin followed by washing with aqueous ethanol. Aqueous ethanol extract was concentrated and designated as PEG, also named *P. ginseng* dry purified extract.

2.2. Preparation of samples and reference standards

The powdered samples were resolved in 80% MeOH to obtain a concentration of 5 mg/ml and filtered through a 0.2-μm membrane filter prior to the analysis. The reference standards of Rb1, Rb2, Rc, Rd, Re, and compound K were provided by Green Cross Health Science, Inc., and 22 ginsenosides (Table 2) were isolated using a series of column chromatography techniques according to the procedure in the previous study [26]. The reference standards were dissolved in 80% MeOH and mixed at a concentration of 100 μg/ml for injection into the UPLC–QTOF/MS system. The volume injected to the column was 1 μl, and each sample was injected three times. The run sequence was randomly generated, and the blank (80% MeOH) was injected once every five runs.

2.3. UPLC–QTOF/MS analysis

The UPLC–QTOF/MS analyses were performed on a Waters Acquity UPLC system (Waters Co., Milford, MA, USA), which consists of a binary solvent delivery system and an auto sampler. The UPLC column was a Waters Acquity UPLC BEH C18 (150 mm × 2.1 mm, 1.7 μm). The mobile phases were 0.1% formic acid in H_2O (A) and acetonitrile (B), with the following gradient: 15% B (0–2 min), 15–50% B (2–10 min), 50–70% B (10–17 min), 70–90% B (17–20 min), 90% B (20–21 min), and 15% B (21–23 min). The flow rate was set at 400 μl/min. The temperatures in the auto sampler and in the column oven were set at 10 °C and 45 °C, respectively. The MS experiments were performed on a Waters Xevo G2 QTOF mass spectrometer (Waters MS Technologies, Manchester, UK) connected to the UPLC system through an electrospray ionization (ESI) interface. The ESI conditions were set as follows: negative ion mode, capillary voltage of 2.5 kV, cone voltage of 45 V, source temperature of 100 °C, desolvation temperature of 350 °C, cone gas flow of 50 l/h, and desolvation gas flow of 800 l/h. The ion acquisition rate was 0.2 s with resolution in excess of 20,000 FWHM. The energy for collision-induced dissociation (CID) was set to 4 V for the precursor ion, and the MS/MS fragment information was obtained using a collision energy ramp from 40 eV to 45 eV in MSe mode. The instrument was calibrated using sodium formate solution as the calibration standard suggested by the manufacturer allowing for mass accuracies of <5 ppm. To ensure the mass accuracy and reproducibility of the optimized MS condition, leucine encephalin (m/z 554.2615 in negative mode) was used as the reference lock mass at a concentration of 200 pg/μl and a flow rate of 5 μl/min and was sprayed into the MS instrument every 10 s.

2.4. Data processing and multivariate statistical analysis

For the chemical profiles of the samples (DGE, I-1, I-2 and PEG), accurate and reproducible MS data acquired from UPLC–QTOF/MS were processed using MassLynx^{TM} software (Ver. 4.1, Waters Co., Milford, MA, USA). For identification of the peaks, all possible molecular formulae (elements C, H, O, tolerance of 5 mDa, at least 2 carbons) were extracted with the Element Composition analysis software provided by the manufacturer.

For multivariate statistical analysis, the chromatographic data and MS spectral data for each peak were extracted in MakerLynx^{TM}

Download English Version:

https://daneshyari.com/en/article/7629590

Download Persian Version:

https://daneshyari.com/article/7629590

Daneshyari.com