Vol. 9, No. 2, 2018
ISSN 2233-4203/ e-ISSN 2093-8950
LETTER www.msletters.org | Mass Spectrometry Letters

Simultaneous Quantification of 13 Ginsenosides by LC-MS/MS and its Application in Diverse Ginseng Extracts

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Received December 29, 2017; Revised March 12, 2018; Accepted March 12, 2018
First published on the web June 30, 2018; DOI: 10.5478/MSL.2018.9.2.41

Abstract : Ginseng (*Panax ginseng* Meyer) has been used as traditional herbal drug in Asian countries. Ginsenosides are major components having pharmacological and biological efficacy like anti-inflammatory, anti-diabetic and anti-tumor effects. To control the quality of the components in diverse ginseng products, we developed a new quantitative method using LC-MS/MS for 13 ginsenosides; Rb1, Rb2, Rc, Rd, Re, Rf, 20(*S*)-Rh1, 20(*S*)-Rh2, Rg1, 20(*S*)-Rg3, F1, F2, and compound K. This method was successfully validated for linearity, precision, and accuracy. This quantification method applied in four representative ginseng products; fresh ginseng powder, white ginseng powder, red ginseng extract powder, and red ginseng extract. Here the amounts of the 13 ginsenosides in the various type of ginseng samples could be analyzed simultaneously and expected to be suitable for quality control of ginseng products.

Keywords : LC-MS/MS, ginsenoside, fresh ginseng, white ginseng, red ginseng

Introduction

Ginseng (*Panax ginseng* Meyer) has been used as traditional herbal medicine in Asian countries. Ginsenosides, which are ginseng saponins, are major components of ginseng and have diverse pharmacological and biological efficacy, including anti-inflammatory, anti-diabetic, and anti-tumor effects.[1,2] Based on the structure of aglycone, ginsenosides can be divided into a 20(*S*)-protopanaxadiol (PPD) group, such as ginsenoside Rb1, Rb2, Rc, Rd, 20(*S*)-Rh2, 20(*S*)-Rg3, F2, and compound K (CK), and a 20(*S*)-protopanaxatriol (PPT) group, such as ginsenoside Re, Rf, 20(*S*)-Rh1, 20(*S*)-Rg1, and F1 (Fig. 1).

For diverse medicinal usage, ginseng products are prepared by using different processing methods. For example, red ginseng is made by steaming and drying fresh ginseng, whereas white ginseng is made by simply drying fresh ginseng.[3,4] During processing, it is known that the amounts of the individual ginsenosides could change. To quantify the major ginsenosides in diverse ginseng products, many methods have been developed using diverse analytical instruments such as thin layer chromatography (TLC), gas chromatography (GC), high-performance liquid chromatography (HPLC), and liquid chromatography-mass spectrometry (LC-MS).[5-7] However, it is still difficult to quantify all ginsenosides in ginseng products because of the long analytical time, insufficient classification for ginsenosides having the same molecular weight, or an insufficient number of ginsenosides for simultaneous quantification of their chemical and physical properties.[5-7]

In this study, we developed a rapid and simple method using HPLC coupled with tandem mass spectrometry (MS/MS) for the simultaneous quantitative determination of 13 major ginsenosides. After method validation, the developed method was successfully applied to compare a concentration of ginsenosides in fresh ginseng, white ginseng, and red ginseng produced from Punggi area where is the oldest ginseng producing area in Korea since 1908. The content of ginsenosides is very diverse based on producing area or each company, this study showed the ginsenoside contents of ginseng produced special historic area.

Experimental Methods

Materials and Calibration

To apply absolute quantification of the 13 ginsenosides Rb1, Rb2, Rc, Rd, Re, Rf, 20(*S*)-Rh1, 20(*S*)-Rh2, Rg1, 20(*S*)-Rg3, F1, F2, and compound K, we obtained standard

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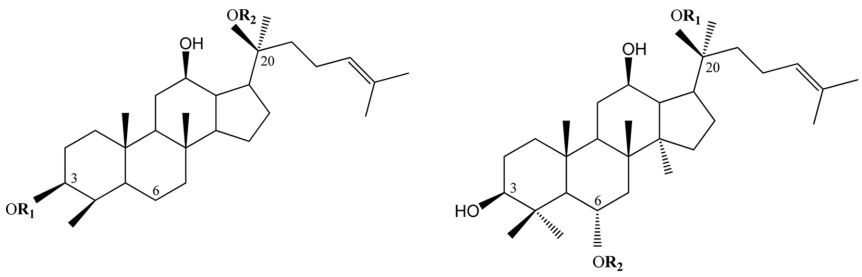

20(*S*)-protopanaxadiol **20(*S*)-protopanaxatriol**

Ginsenoside (PPD)	R_1 (C-3)	R_2 (C-20)
Rb1	Glc(2→1)Glc	Glc(6→1)Glc
Rb2	Glc(2→1)Glc	Glc(6→1)Arap
Rc	Glc(2→1)Glc	Glc(6→1)Araf
Rd	Glc(2→1)Glc	Glc
Rh2	Glc	H
Rg3	Glc(2→1)Glc	H
F2	Glc	Glc
Compound K	H	Glc
PPD	H	H

Ginsenoside (PPT)	R_1 (C-20)	R_2 (C-6)
Re	Glc	Glc(2→1)Rha
Rf	H	Glc(2→1)Glc
Rh1	H	Glc
Rg1	Glc	Glc(2→1)Xyl
F1	Glc	H
PPT	H	H

Fig. 1. Chemical structures of protopanaxadiol (PPD)-type ginsenosides and protopanaxatriol (PPT)-type ginsenosides. Glc: β-D-glucopyranosyl, Arap: α-L-arabinopyranosyl, Araf: α-L-arabinofuranosyl, Xyl: β-D-xylopyranosyl, and Rha: α-L-rhamnopyranosyl.

compounds from Ambo Institute (purity >98%, Daejeon, Korea). Stock solutions of each ginsenoside were prepared in water at concentrations of 5 mg/mL and diluted to appropriate concentrations for construction of calibration curves: 0.5, 1, 2.5, 5, 10, 25, and 50 μg/mL. Calibration curves were constructed by plotting the peak area ratios of the internal standard (IS) versus the concentration of each ginsenoside. Validation of the methodology was carried out by analyzing replicates of the ginsenoside standard mixtures.

To quantify the concentrations of the 13 ginsenosides in the ginseng products, we analyzed the three types of ginseng extracts with the newly developed quantification method. Each ginseng stock was diluted with water to 10 mg/mL and mixed with 90 μL of acetonitrile containing 0.1 μM reserpine (IS). After mixing, the samples were centrifuged at 13,000 rpm for 10 min at 4°C and injected into the LC-MS/MS system. Two different concentrations of the standard (5 and 50 μg/mL) were added into each sample in at least triplicate.

Sample preparation

The ginseng products were prepared from Punggi Ginseng Cooperative Association (Punggi, Korea) by a traditional routine process.[4,8-10] The roots are cleaned, dried by air and sun, and ground to fresh ginseng powder (FGP). After drying, the roots are baked under low temperature and then dried under the sun again. After repeating this process 2-3 times and grinding, white ginseng powder (WGP) is obtained. However, red ginseng is produced by steaming the roots in a closed steam chamber and then cooling the roots and removing the moisture in open air. Then, the ginseng roots are baked under low temperature to dry. Red ginseng extract (RGE) is produced after hot-water extracting and concentrating, and red ginseng extract powder (RGEP) is prepared by freeze-dried of RGE. All FGP, WGP, RGEP, and RGE powders were dissolved in water rather than in organic solvents because ginsenosides are water-soluble compounds. The final concentrations were 50 mg/mL.

Instruments

Samples were analyzed using an LC system coupled to a triple-stage quadrupole (TSQ) mass spectrometer (Thermo Scientific Inc., Waltham, MA, USA) with an electrospray ionization (ESI) source. The LC system consisted of a LC-20AD liquid chromatograph, an SIL-20A auto sampler, a CTO-20A column oven, and a CVM-20A communication bus module (Shimadzu, Kyoto, Japan). A Shim-pack GIS ODS column (3 μm, 150 mm × 3.0 mm, Shimadzu, Japan) and a guard C18 column (2 mm, 2.1 mm i.d., Phenomenex, USA) were used for LC separation. A gradient program was employed, with the mobile phase combining solvent A (acetonitrile) and solvent B (5 mM ammonium formate, pH 4) at a flow rate of 500 μL/min. The gradient was as follows: 20-20% A (0-1.0 min), 20-95% A (1.0-39.0 min), 95-95% A (39.0-40.0 min), 95-20% A (40.0-40.1 min), and 20-20% A (40.1-45.0 min). During the analysis, the column oven was maintained at 30°C. The mass spectrometer was operated in the positive ESI mode with nitrogen as the auxiliary gas pressure, sheath gas pressure, and vaporizer temperature, with

optimum values set at 10 psi (69 kPa), 35 psi (241 kPa), and 300°C, respectively. The ESI spray voltage was adjusted to 4000 V and the capillary temperature was set at 350°C. The selected reaction monitoring (SRM) mode is described in Supplemental Table S1. Data procurement was controlled by Xcalibur software (Version 3.0.63)

Result and Discussion

Then, we analyzed 13 ginsenosides using LC-MS/MS after determining the mass spectrometric conditions and fragmentation behaviors using MS/MS (Fig. 2). The representative SRM chromatograms for the ginsenoside standard mixture and reserpine as the IS are shown in Supplemental Fig. S1: Rb1(1132.1→365.2) at 13.5 min, Rb2 (1102.2→334.9) at 14.0 min, Rc (1102.2→334.9) at 14.4 min, Rd (969.6→789.6) at 15.6 min, Re (969.6→789.6) at 9.3 min, Rf (823.8→364.7) at 13.4 min, 20(*S*)-Rh1 (603.0→423.3) at 5.0 min, 20(*S*)-Rh2 (587.0→407.0) at 27.3 min, Rg1 (823.5→643.4) at 9.4 min, 20(*S*)-Rg3 (807.7→364.9) at 21.2

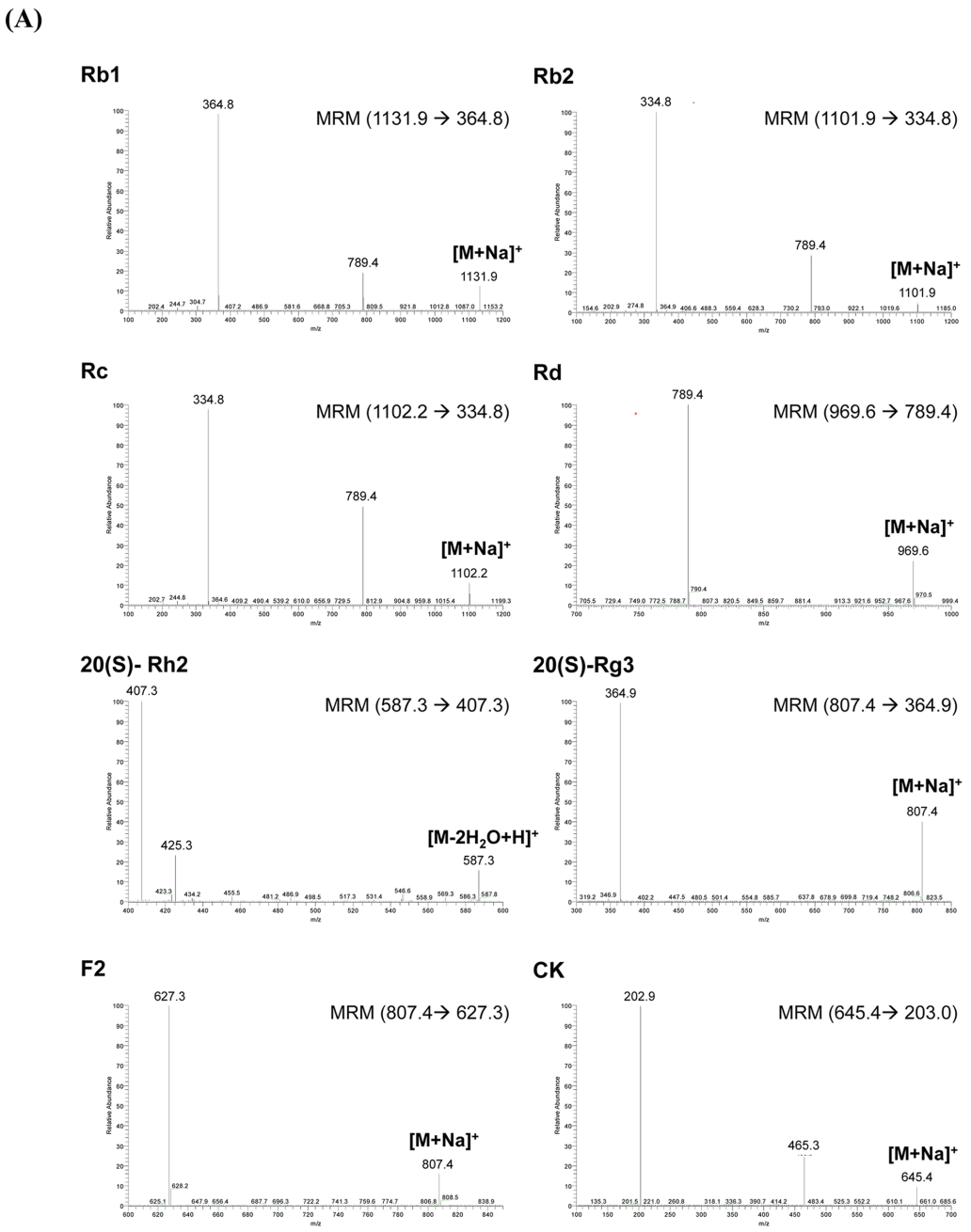

Fig. 2. MS/MS fragmentation spectrum of 20(S)-Protopanaxadiol (A) and 20(S)-Protopanaxatriol (B).

(B)

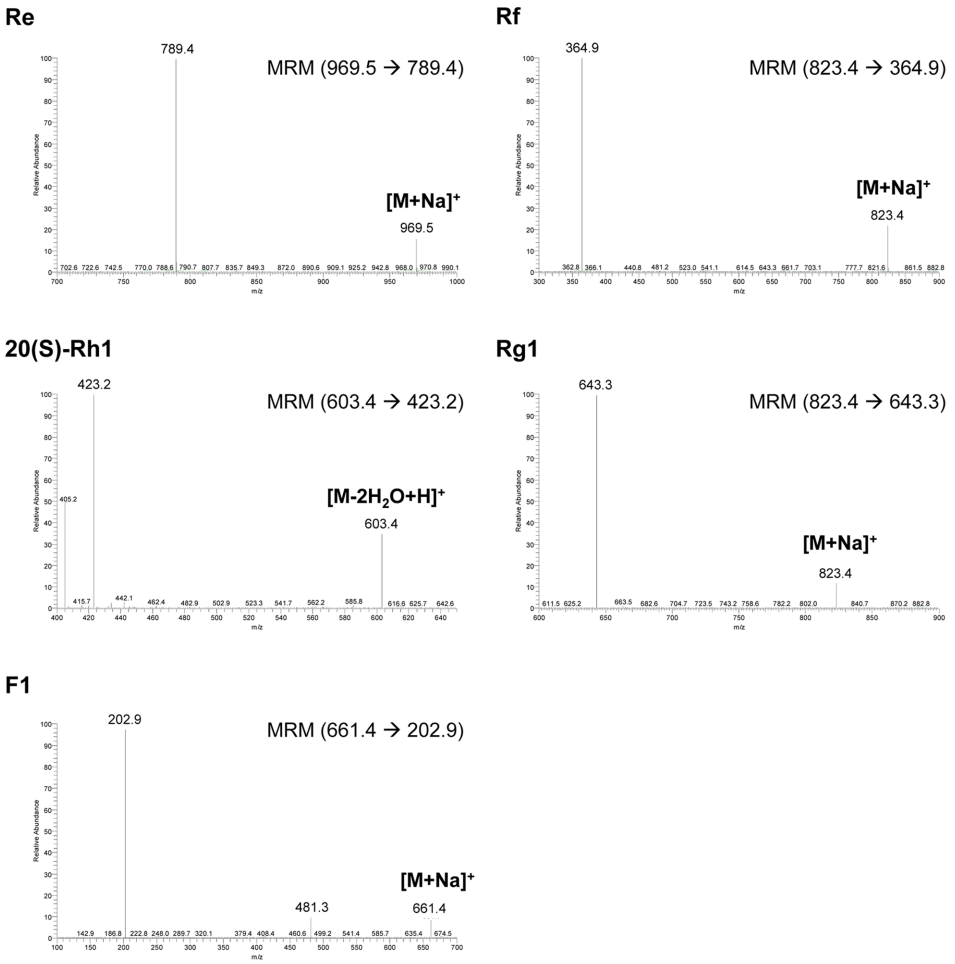

Fig. 2. Continued.

min, F1 (661.4→203.0) at 16.0 min, F2 (807.8→627.6) at 19.3 min, and compound K (645.7→203.0) at 26.3 min. Under these LC-MS/MS conditions, each ginsenoside and IS peak were clearly eluted and resolved in positive ion mode. The representative SRM for each ginsenoside in the four types of ginseng are shown in Supplemental Fig. S2. The final concentrations of the ginsenosides in each ginseng products are shown in Fig. 3.

Supplemental Table S2 provides the equation of the calibration curve, the limit of detection (LOD), and the limit of quantification (LOQ) for each ginsenoside. Linear calibration curves with correlation coefficients greater than 0.9953 were observed for analytes in the concentration range 0.5-50 μg/mL. LODs of 0.4-6.0 μg/mL and LOQs of 0.9-18.3 μg/mL were achieved. Validation of the analytical methods for the ginsenosides was performed to evaluate the 13 ginsenoside standard mixtures. As shown in Supplemental Table S3, the LC-MS/MS method showed good reproducibility for the quantification of the 13 ginsenosides and the IS. The overall recoveries were 85.5-103.1%, with the relative standard deviations of 1.6-37.2%. Therefore, the proposed method can be applied for determination of the ginsenoside content in ginseng products.

In FGP, the amounts of Rb1, Rb2, Rc, and Rd, which are 20(*S*)-PPD group ginsenosides, were calculated as 1353.2, 652.3, 480.5, and 126.0 μg/g ginseng, respectively. The amounts of Re, Rf, and Rg1, which are 20(*S*)-PPT group ginsenosides, were detected as 1492.1, 332.6, and 1737.1 μg/g ginseng, respectively. 20(*S*)-Rh1, F1, F2, 20(*S*)-Rg3, and compound K were detected as 20.5, 24.2, 26.0, and 1.5 μg/g ginseng, respectively, and 20(*S*)-Rh2 was not detected.

Because white ginseng and red ginseng are produced by simple drying or steaming, an increase in the ginsenoside content after processing was expected. Following this prediction, the amounts of Rb1, Rb2, Rc, and Rd in WGP were measured as 5724.5, 3696.9, 2661.1, and 936.8 μg/g powder, values that were higher than those of FGP. Also,

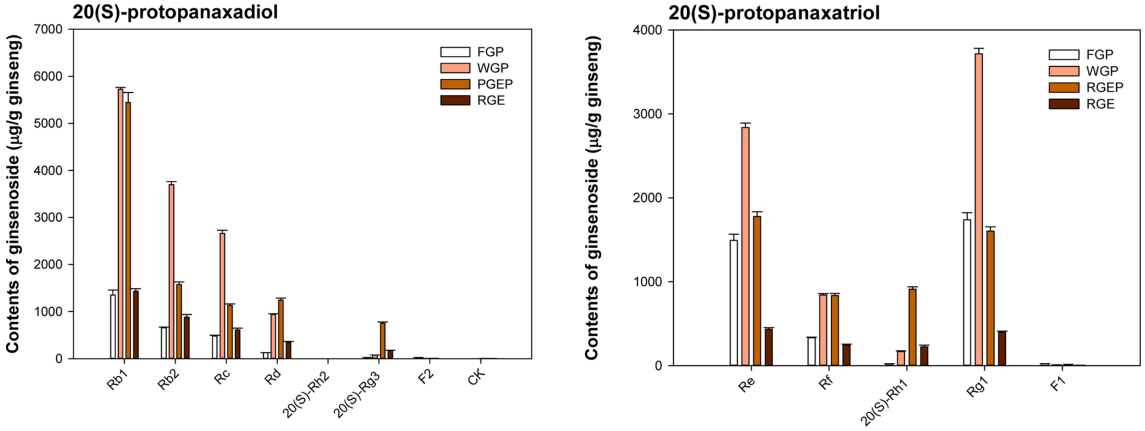

Fig. 3. The final concentrations of 13 ginsenosides in four types of extracts; fresh ginseng powder (FGP), white ginseng powder (WGP), red ginseng extract powder (RGEP) and grinding red ginseng extract (RGE). All FGP, WGP, RGEP, and RGE powders were dissolved in water at 50 mg/mL. The concentrations of the 13 ginsenosides in the ginseng products were quantified by the LC-MS/MS system.

RGEP contained 5443.8, 1750.8, 1126.6, and 1242.2 μg/g powder for Rb1, Rb2, Rc, and Rd, respectively, which were also higher than the FGP contents. In case of the 20(*S*)-PPT group, Re, Rf, and Rg1 were detected as 2838.2, 841.8, and 3716.6 μg/g in WGP, values that are greater than those of FGP. The amounts of 20(*S*)-Rh1, 20(*S*)-Rg3, and compound K also increased after the processing method for drying to WGP; however, ginsenosides F1 and F2 decreased compared to FGP. These results show a different pattern from those of a previous study.[10] In RGE, Rb1, Rb2, Rc, Rd, 20(*S*)-Rg3, and compound K in the 20(*S*)-PPD group were detected as 1431.8, 880.4, 609.2, 347.0, 76.0, and 2.6 μg/g ginseng, amounts that were greater than those of FGP but less than those of WGP and RGEP.[11]

Fresh ginseng can be used to produce ginseng products such as white and red ginseng using several processing methods. The processing of fresh ginseng into white or red ginseng generates changes in the ginsenoside content in the end product.[6,10] As a result, each ginseng product has different amounts of ginsenosides depending on the processing method.[8] We therefore need to know the ginsenoside amount for each manufacturing process. Especially, we need to pay attention to the amounts of Rb1, Rb2, Rc, Rd, Re, Rf, 20(*S*)-Rh1, Rg1, and 20(*S*)-Rg3 because they change significantly after processing. In this study, we developed an improved and rapid LC-MS/MS method to simultaneously determine the concentrations of 13 ginsenosides in FGP, WGP, RGEP, and RGE to maintain the quality of ginseng products.[12]

Supporting Information

Supplementary information is available at https://drive.google.com/file/d/1B6RBTjHzeRkcCUBLFRefkoXCSY4xWDGm/view?usp=sharing.

Acknowledgments

This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Export Promotion Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (grant number 316017-3)

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