

Short communication

Simultaneous quantification of 14 ginsenosides in *Panax ginseng* C.A. Meyer (Korean red ginseng) by HPLC-ELSD and its application to quality control

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Abstract

A new method of high-performance liquid chromatography coupled with evaporative light scattering detection (HPLC-ELSD) was developed for the simultaneous quantification of 14 major ginsenosides, which are the marker compounds of *Panax ginseng* C.A. Meyer (Korean red ginseng). Various types of ginseng samples were extracted, and the amounts of the 14 ginsenosides (Rg1, Re, Rf, Rh1, Rg2, Rb1, Rc, Rb2, Rb3, Rd, Rg3, Rk1, Rg5, and Rh2) were determined by reverse-phase HPLC-ELSD using digoxin as an internal standard. The mobile phase consisted of a programmed gradient of aqueous acetonitrile. Calibration curves for each ginsenoside were determined for the quantification. The method was validated for linearity, precision, accuracy, limit of detection, and limit of quantification. This quantification method was applied to several finished ginseng products including white ginseng, red ginseng powder, and red ginseng concentrate. The amounts of the 14 ginsenosides in the various ginseng samples could be analyzed simultaneously. This validated HPLC method is expected to provide a new basis for the quality assessment of ginseng products.

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1. Introduction

Ginseng, the root of *Panax ginseng* C.A. Meyer, has been used frequently in traditional Oriental medicine and is now widely used around the world. Ginsenosides, the ginseng saponins, are the major components having pharmacological and biological activities, including antidiabetic and antitumor activities [1–11]. More than 30 different ginsenosides have been isolated and characterized, and they have different pharmacological effects. Ginsenosides can be divided into 20(S)-protopanaxadiol (ginsenoside Rb1, Rb2, Rb3, Rc, Rd, and Rg3) and 20(S)-protopanaxatriol (ginsenoside Re, Rg1, Rg2, and Rh1) groups based on their aglycone moieties (Fig. 1).

Ginseng is one of the most famous herbal medicines used as a dietary supplement in recent years. There are various types of commercial ginseng products, the most popular being red ginseng. Korean red ginseng is made by steaming and drying fresh ginseng by a traditional method [12], whereas white ginseng is made by simply drying the fresh ginseng. The processed red ginseng contains some distinct constituents such as the ginsenosides Rg3, Rg5, and Rk1, which are not found in white ginseng [13–17]. There are several reports that these unique compounds have potent biological activities, such as anticancer and anti-inflammatory activities [1,18–20].

Many methods for quantifying ginsenosides in various types of ginseng samples have been developed, including thin layer chromatography [21,22], HPLC coupled with an ultraviolet (UV) detector [15,23–28], evaporative light scattering detector (ELSD) [13,14,29,30], and mass spectrometry (MS) [31–33]. However, ginsenosides could not be fully resolved due to their

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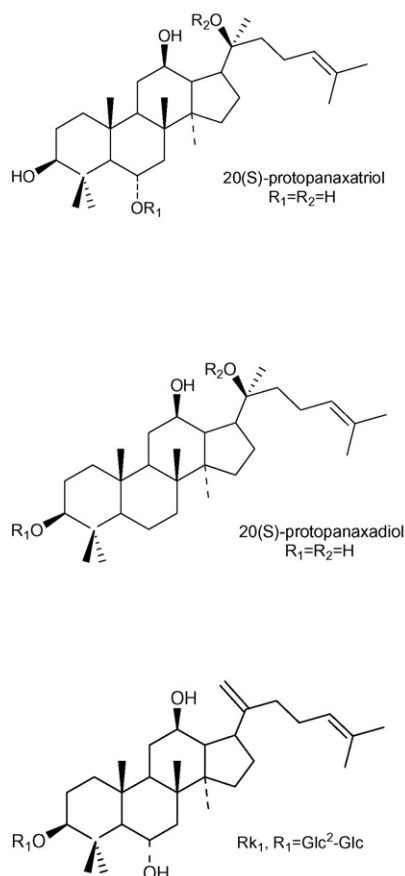


Fig. 1. Structure of the major ginsenosides in *Panax ginseng*. Glc: glucose, Rha: rhamnose, Ara: arabinose, Xyl: xylose.

| Ginsenoside | R ₁ | R ₂ |
|-------------|-----------------------|----------------|
| Rg1 | Glc | Glc |
| Re | Glc ² -Rha | Glc |
| Rf | Glc ² -Glc | H |
| Rh1 | Glc | H |
| Rg2 | Glc ² -Rha | H |

| Ginsenoside | R ₁ | R ₂ |
|-------------|-----------------------|--------------------------|
| Rb1 | Glc ² -Glc | Glc ⁶ -Glc |
| Rc | Glc ² -Glc | Glc ⁶ -Ara(f) |
| Rb2 | Glc ² -Glc | Glc ⁶ -Ara(p) |
| Rb3 | Glc ² -Glc | Glc ⁶ -Xyl |
| Rd | Glc ² -Glc | Glc |
| Rg3 | Glc ² -Glc | H |
| Rh2 | Glc | H |

very similar structures [14,24,32], or the number of determined ginsenosides was insufficient to identify all of the ginsenosides in red ginseng products [23,25–27,34]. Moreover, most reports concerning quality control of commercial ginseng products are limited to the root of ginseng or to fresh ginseng [28,30,35–37]. Also, the number of ginsenosides quantified was typically not enough to account for the total ginsenosides in the finished products [31,38]. Therefore, the amount of each ginsenoside could not be determined precisely [39].

In the present study, a new HPLC method coupled with ELSD was developed for the simultaneous quantitative determination of 14 major ginsenosides including ginsenosides Rg3, Rg5, and Rk1, which are the unique compounds in red ginseng. This method was applied to various types of finished ginseng products processed from *Panax ginseng* C.A. Meyer, such as white ginseng root, red ginseng powder, and red ginseng concentrate. This newly developed quantitative method could be applied for the quality control of several types of commercial ginseng products.

2. Experimental

2.1. Materials and equipment

Ginsenosides Rg1, Re, Rf, Rh1, Rg2, Rc, Rb3, Rd, and Rh2 were purchased from Chengdu Cogon Bio-tech Co., Ltd.

(China), and ginsenoside Rb2 was obtained from the National Institute for the Control of Pharmaceutical Products (China). Ginsenosides Rb1, Rg3, Rk1, and Rg5 were preparatively purified from steamed ginseng extract by countercurrent chromatography [40]. Digoxin, the internal standard, was obtained from Sigma–Aldrich (St. Louis, MO, USA), and HPLC-grade acetonitrile and methanol were purchased from J.T. Baker (Phillipsburg, NJ, USA). Mobile phases were degassed by sonication for 60 min before use. Solid phase extraction columns (Sep-Pak[®] Vac C₁₈) were obtained from Waters (Milford, MA, USA). White ginseng root, red ginseng powder, and red ginseng concentrate were purchased from the local herbal shop (Seoul, Korea).

2.2. Sample preparation

To test the extraction efficiency of four different solvent systems, 1 g red ginseng powder or red ginseng concentrate was extracted three times with 50 ml of each solvent system (water, 70% MeOH [35], 100% MeOH [15], or 100% MeOH after wetting the samples in 10 ml of water for 2 h [13]) by sonication for 60 min. After filtration using filter paper (ADVANTEC, Dublin, CA, USA), the solvent was removed using an evaporator, and the residue was dissolved in 10 ml water. One milliliter of the aqueous sample solution was applied to a Sep-Pak[®] Vac C₁₈ column to remove polar compounds. The sample

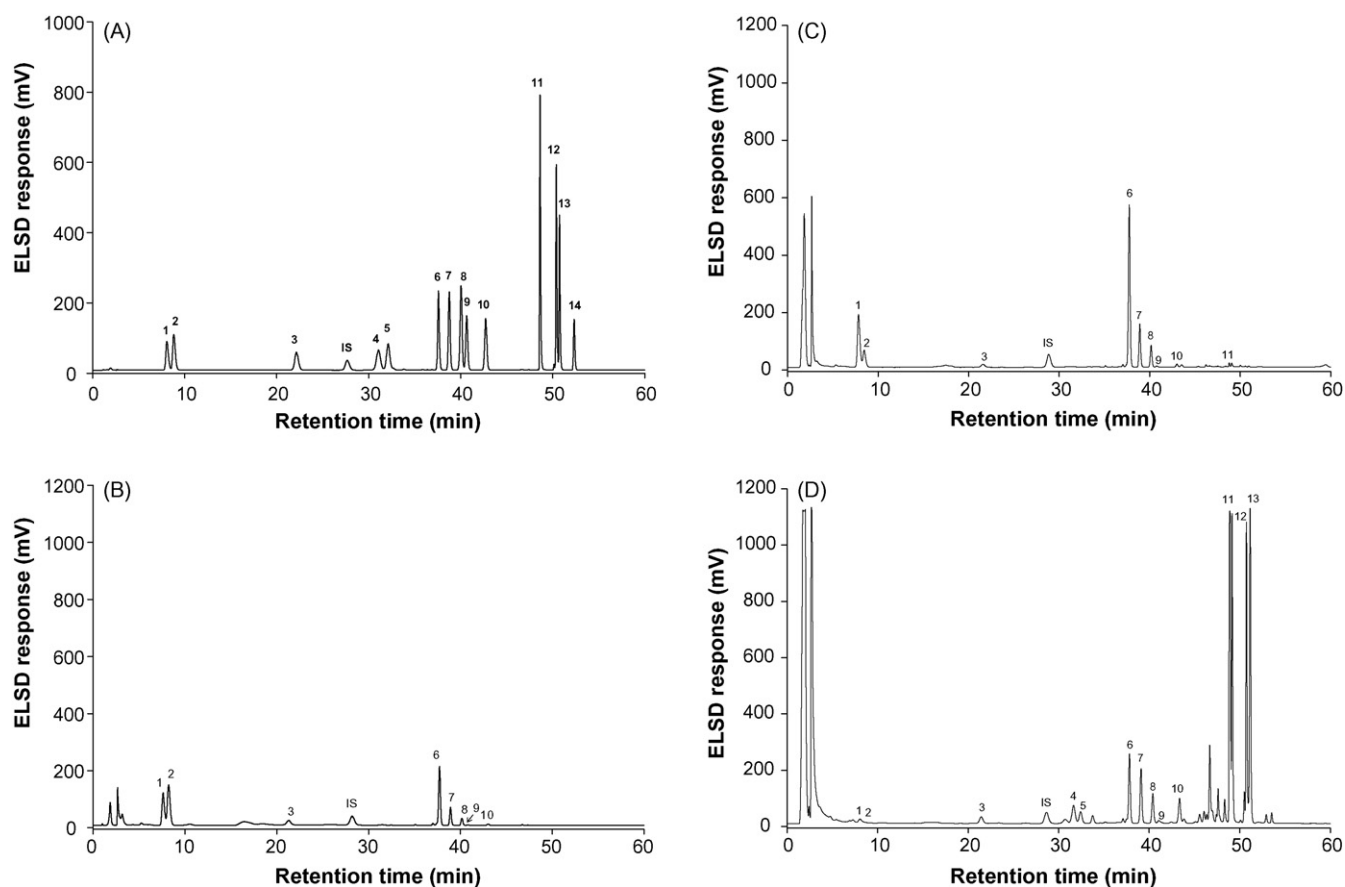


Fig. 2. Representative HPLC chromatograms of mixed standards and extracts of finished ginseng products. Column: Agilent Zorbax SD-Aq C18 column (4.6 mm × 150 mm, 5 μm), detector: ELSD. (A) Mixed standards with internal standard, (B) extract of white ginseng powder, (C) extract of red ginseng powder, (D) extract of red ginseng concentrate. 1, Rg1; 2, Re; 3, Rf; 4, Rh1; 5, Rg2; 6, Rb1; 7, Rc; 8, Rb2; 9, Rb3; 10, Rd; 11, Rg3; 12, Rk1; 13, Rg5; 14, Rh2; IS, internal standard (digoxin).

was eluted sequentially with water (3 ml) and 100% methanol (3 ml). The 100% methanol elution was filtered through a 0.45 μm syringe filter (Whatman, Brentford, Middlesex, UK), and 50 μl filtrate containing the internal standard (4 μg digoxin) was injected into the HPLC system. The content of each ginsenoside was determined from the corresponding calibration curves.

Table 1
Linearity of calibration curve for 14 ginsenosides

| Ginsenoside | Calibration curve ^a | R ² | LOD (μg) | LOQ (μg) |
|-------------|--------------------------------|----------------|----------|----------|
| Rg1 | $y = 0.6291x - 0.9457$ | 0.9938 | 0.20 | 0.40 |
| Re | $y = 0.7497x - 1.1074$ | 0.9937 | 0.25 | 0.45 |
| Rf | $y = 0.6655x - 1.1154$ | 0.9917 | 0.30 | 0.60 |
| Rh1 | $y = 0.3548x - 0.6021$ | 0.9886 | 0.30 | 0.80 |
| Rg2 | $y = 0.4466x - 0.7510$ | 0.9891 | 0.30 | 0.80 |
| Rb1 | $y = 0.4775x - 0.5953$ | 0.9965 | 0.15 | 0.30 |
| Rc | $y = 0.4550x - 0.6599$ | 0.9921 | 0.15 | 0.30 |
| Rb2 | $y = 0.3660x - 0.5540$ | 0.9906 | 0.15 | 0.30 |
| Rb3 | $y = 0.5285x - 0.7250$ | 0.9936 | 0.20 | 0.45 |
| Rd | $y = 0.4320x - 0.5941$ | 0.9943 | 0.20 | 0.45 |
| Rg3 | $y = 0.3332x - 0.4791$ | 0.9833 | 0.075 | 0.125 |
| Rk1 | $y = 0.3072x - 0.1202$ | 0.9874 | 0.075 | 0.125 |
| Rg5 | $y = 0.2543x - 0.3336$ | 0.9958 | 0.075 | 0.125 |
| Rh2 | $y = 0.1415x - 0.0325$ | 0.9998 | 0.10 | 0.15 |

^a y, peak area ratio (analyte/internal standard); x, amount of analyte (μg).

2.3. Analysis of ginsenosides by HPLC-ELSD

HPLC analysis was carried out by using a Hitachi L-6200 pump coupled with a Sedex 75 ELSD (Sedere, Virty-sur-Seine, France) and a SIL-9A auto injector (Shimadzu, Japan). A Zorbax SB-Aq C₁₈ column (4.6 mm × 150 mm, 5 μm particle size) from Agilent Technologies (Palo Alto, CA, USA) was used for all separations. HPLC conditions were as follows: solvent A, water; solvent B, acetonitrile; gradient, 0–6 min (21–22% B), 6–7 min (22–23% B), 7–25 min (23–24% B), 25–30 min (24–30% B), 30–40 min (30–32% B), 40–45 min (32–50% B), 45–60 min (50–65% B), 60–61 min (65–100% B). The column was then washed with 100% B for 10 min at a flow rate of 1 ml/min. ELSD was set to a probe temperature of 70 °C, and the nebulizer for nitrogen gas was adjusted to 2.5 bar [41]. Five micrograms of each standard ginsenoside were injected for the HPLC analysis, and peaks were assigned by comparing their retention times with that of each reference compound.

2.4. Calibration

The standard solutions containing 1–20 μg of each ginsenoside were injected into the HPLC with 4 μg digoxin. Calibration curves were plotted as the peak area ratio (ginsenoside/digoxin)

Table 2
Ginsenoside contents upon extraction of red ginseng powder (A) and red ginseng concentrate (B) with different solvent systems ($n = 3$)

| Compound | Solvent system | | | |
|------------|----------------|----------------|----------------|----------------|
| | 1 ^a | 2 ^b | 3 ^c | 4 ^d |
| (A) | | | | |
| Rg1 | 1.45 ± 0.04 | 3.29 ± 0.07 | 1.72 ± 0.01 | 3.44 ± 0.01 |
| Re | 0.96 ± 0.03 | 1.53 ± 0.04 | 1.12 ± 0.01 | 1.56 ± 0.04 |
| Rf | 0.93 ± 0.01 | 1.13 ± 0.01 | 1.07 ± 0.01 | 1.05 ± 0.01 |
| Rh1 | ND | ND | ND | ND |
| Rg2 | ND | ND | ND | ND |
| Rb1 | 2.30 ± 0.08 | 7.40 ± 0.19 | 2.70 ± 0.02 | 8.44 ± 0.09 |
| Rc | 1.17 ± 0.02 | 2.63 ± 0.06 | 1.45 ± 0.01 | 2.68 ± 0.07 |
| Rb2 | 1.04 ± 0.01 | 2.02 ± 0.01 | 1.24 ± 0.01 | 2.10 ± 0.05 |
| Rb3 | ND | 0.82 ± 0.00 | ND | 0.72 ± 0.01 |
| Rd | 0.77 ± 0.01 | 0.94 ± 0.01 | 0.87 ± 0.01 | 0.85 ± 0.01 |
| Rg3 | 1.02 ± 0.01 | 1.06 ± 0.01 | 0.93 ± 0.01 | 1.03 ± 0.01 |
| Rk1 | ND | ND | ND | ND |
| Rg5 | ND | ND | ND | ND |
| Rh2 | ND | ND | ND | ND |
| Total | 9.65 ± 0.17 | 20.82 ± 0.32 | 11.11 ± 0.05 | 20.87 ± 0.43 |
| (B) | | | | |
| Rg1 | 0.90 ± 0.03 | 0.94 ± 0.01 | 0.84 ± 0.02 | 1.10 ± 0.02 |
| Re | 0.91 ± 0.02 | 0.81 ± 0.01 | 0.74 ± 0.01 | 0.93 ± 0.01 |
| Rf | 1.14 ± 0.00 | 1.21 ± 0.01 | 1.17 ± 0.01 | 1.42 ± 0.00 |
| Rh1 | 2.38 ± 0.07 | 2.25 ± 0.01 | 2.28 ± 0.02 | 2.26 ± 0.03 |
| Rg2 | 1.69 ± 0.04 | 1.48 ± 0.01 | 1.35 ± 0.02 | 1.74 ± 0.07 |
| Rb1 | 2.69 ± 0.04 | 2.81 ± 0.06 | 2.59 ± 0.04 | 3.18 ± 0.09 |
| Rc | 2.40 ± 0.02 | 2.42 ± 0.04 | 2.26 ± 0.04 | 2.66 ± 0.07 |
| Rb2 | 1.97 ± 0.04 | 1.94 ± 0.02 | 1.71 ± 0.02 | 2.10 ± 0.04 |
| Rb3 | 0.96 ± 0.01 | 0.95 ± 0.01 | 0.74 ± 0.01 | 0.89 ± 0.01 |
| Rd | 1.60 ± 0.03 | 1.63 ± 0.01 | 1.58 ± 0.01 | 1.95 ± 0.05 |
| Rg3 | 23.27 ± 0.65 | 25.63 ± 0.32 | 22.51 ± 0.33 | 26.62 ± 0.34 |
| Rk1 | 10.44 ± 0.01 | 11.70 ± 0.04 | 12.15 ± 0.23 | 13.00 ± 0.20 |
| Rg5 | 15.99 ± 0.03 | 17.86 ± 0.42 | 16.31 ± 0.21 | 18.11 ± 0.20 |
| Rh2 | ND | ND | ND | ND |
| Total | 66.34 ± 0.52 | 71.61 ± 1.40 | 66.23 ± 1.08 | 75.96 ± 0.99 |

Content (mg/g). ND, not detected.

^a Solvent system 1: water.

^b Solvent system 2: 70% methanol.

^c Solvent system 3: 100% methanol.

^d Solvent system 4: 100% methanol after wetting the samples in water for 2 h.

versus the amount of each analyte. The linearity was evaluated by linear regression analysis calculated by the least squares regression method. The limit of detection (LOD) and limit of quantification (LOQ) under the present chromatographic conditions were determined on the basis of response at a signal-to-noise ratio (S/N) of 3 or 10, respectively.

2.5. Validation

The precision of the HPLC method was determined by intra- and inter-day variations. One gram of ginseng sample was extracted and analyzed as described in Sections 2.2 and 2.3. The intra-day precision was performed by triplicate extraction and analysis on a single day. The inter-day precision was carried out on 3 different days. Variations were expressed by the relative standard deviations (R.S.D.). The recovery test was used to evaluate the accuracy of this quan-

Table 3

Intra- and inter-day variations of the HPLC-ELSD method for the determination of 14 ginsenosides ($n = 3$)

| Compound | Intra-day precision | | Inter-day precision | |
|----------|---------------------|------------|---------------------|------------|
| | Content (mg/g) | R.S.D. (%) | Content (mg/g) | R.S.D. (%) |
| Rg1 | 3.22 ± 0.08 | 2.62 | 3.14 ± 0.07 | 2.19 |
| Re | 1.54 ± 0.02 | 1.45 | 1.52 ± 0.03 | 1.86 |
| Rf | 1.11 ± 0.02 | 1.51 | 1.12 ± 0.03 | 3.07 |
| Rh1 | 2.97 ± 0.08 | 2.84 | 2.83 ± 0.09 | 3.12 |
| Rg2 | 1.79 ± 0.07 | 4.02 | 1.70 ± 0.02 | 0.93 |
| Rb1 | 3.44 ± 0.14 | 3.94 | 3.36 ± 0.12 | 3.71 |
| Rc | 3.13 ± 0.10 | 3.18 | 3.22 ± 0.07 | 2.31 |
| Rb2 | 2.52 ± 0.06 | 2.47 | 2.46 ± 0.04 | 1.79 |
| Rb3 | 0.84 ± 0.04 | 4.65 | 0.83 ± 0.02 | 1.98 |
| Rd | 2.00 ± 0.09 | 4.37 | 2.06 ± 0.04 | 1.83 |
| Rg3 | 26.02 ± 1.07 | 4.12 | 25.05 ± 0.91 | 3.65 |
| Rk1 | 10.69 ± 0.21 | 1.94 | 10.89 ± 0.47 | 4.35 |
| Rg5 | 17.53 ± 0.53 | 3.04 | 17.68 ± 0.75 | 4.26 |
| Rh2 | ND | ND | ND | ND |

ND, not detected. Rh2 was not detected in ginseng samples; R.S.D. (%) = (S.D./mean) × 100%.

tification method. Accurate amounts of 14 ginsenosides were added to 1 g of ginseng sample and then extracted and analyzed as described in Sections 2.2 and 2.3. The average recoveries were determined by the following formula: recovery (%) = (observed amount – original amount)/spiked amount × 100%, with R.S.D. (%) = (S.D./mean) × 100%.

2.6. Analysis of finished ginseng products

White ginseng powder was prepared by grinding the purchased white ginseng root with a pulverizer (Hanil, Seoul, Korea). One gram of each prepared white ginseng powder and other finished ginseng products such as red ginseng powder and red ginseng concentrate was extracted and analyzed as described in Sections 2.2 and 2.3. The red ginseng powder and concentrate were purchased from two different companies, A and B. The amounts of the 14 ginsenosides in each ginseng sample were determined simultaneously by HPLC.

3. Results and discussion

3.1. Analysis of ginsenosides by HPLC-ELSD

To analyze the amounts of 14 ginsenosides, the HPLC conditions were optimized by changing the elution gradient. The chromatogram for the mixture of 14 major ginsenosides and the internal standard is shown in Fig. 2A. Under these HPLC conditions, each ginsenoside and the internal standard are clearly resolved, indicating that this method can be used for the quantitative determination of ginsenosides in ginseng samples. The differences in peak height are due to the solvent gradient system and differing interactions of ginsenosides with the column. However, the area of each peak corresponds to the calibration curve of each sample. Table 1 provides the equation of the calibration curve, the LOD, and the LOQ for each compound. All calibration curves showed good linearity ($R^2 > 0.9833$).

Table 4
Accuracy of HPLC-ELSD method for the determination of 14 ginsenosides

| Compound | Original (mg) | Spiked (mg) | Observed (mg) | Recovery (%) | Mean (%) | R.S.D. (%) |
|----------|---------------|-------------|---------------|--------------|----------|------------|
| Rg1 | 3.22 | 3.00 | 6.09 | 95.75 | 100.46 | 6.62 |
| | | 2.50 | 5.85 | 105.16 | | |
| Re | 1.53 | 1.50 | 3.02 | 99.23 | 102.99 | 5.16 |
| | | 1.00 | 2.60 | 106.75 | | |
| Rf | 1.12 | 1.10 | 2.21 | 99.22 | 100.23 | 1.42 |
| | | 0.80 | 1.93 | 101.23 | | |
| Rh1 | 2.91 | 3.00 | 6.08 | 105.51 | 102.62 | 3.99 |
| | | 2.50 | 5.40 | 99.72 | | |
| Rg2 | 1.75 | 1.70 | 3.48 | 101.89 | 107.79 | 7.74 |
| | | 1.20 | 3.11 | 113.69 | | |
| Rb1 | 3.41 | 3.40 | 6.87 | 101.69 | 104.88 | 4.29 |
| | | 2.50 | 6.11 | 108.06 | | |
| Rc | 3.16 | 3.00 | 6.45 | 109.50 | 106.68 | 3.75 |
| | | 2.50 | 5.76 | 103.85 | | |
| Rb2 | 2.50 | 2.50 | 5.07 | 102.69 | 105.63 | 3.93 |
| | | 2.00 | 4.67 | 108.56 | | |
| Rb3 | 0.84 | 1.00 | 1.79 | 94.74 | 98.34 | 5.17 |
| | | 0.70 | 1.55 | 101.93 | | |
| Rd | 2.02 | 2.00 | 4.04 | 100.91 | 98.14 | 3.99 |
| | | 1.50 | 3.45 | 95.37 | | |
| Rg3 | 25.62 | 26.00 | 51.98 | 101.40 | 104.32 | 3.96 |
| | | 20.00 | 47.07 | 107.24 | | |
| Rk1 | 10.86 | 10.00 | 21.68 | 108.18 | 105.05 | 4.22 |
| | | 8.00 | 19.01 | 101.91 | | |
| Rg5 | 17.74 | 10.00 | 27.34 | 95.99 | 101.08 | 7.11 |
| | | 6.00 | 24.11 | 106.16 | | |
| Rh2 | ND | 10.00 | 10.20 | 101.95 | 101.88 | 0.10 |
| | | 7.00 | 7.13 | 101.80 | | |

Recovery (%) = (observed amount – original amount)/spiked amount × 100%; R.S.D. (%) = (S.D./mean) × 100%; ND, not detected.

Table 5
The contents of 14 ginsenosides in three types of ginseng products ($n = 3$)

| Compound | White ginseng | Red ginseng powder | | Red ginseng concentrate | |
|----------|---------------|--------------------|----------------|-------------------------|----------------|
| | | A ^a | B ^b | A ^a | B ^b |
| Rg1 | 2.33 ± 0.02 | 2.75 ± 0.05 | 3.22 ± 0.08 | 1.01 ± 0.01 | 0.85 ± 0.01 |
| Re | 2.69 ± 0.03 | 2.00 ± 0.02 | 1.53 ± 0.03 | 0.84 ± 0.00 | 0.87 ± 0.03 |
| Rf | 1.17 ± 0.01 | 1.16 ± 0.01 | 1.12 ± 0.02 | 1.08 ± 0.01 | 1.19 ± 0.03 |
| Rh1 | ND | ND | ND | 1.58 ± 0.01 | 2.88 ± 0.08 |
| Rg2 | ND | ND | ND | 1.22 ± 0.01 | 1.71 ± 0.04 |
| Rb1 | 3.46 ± 0.04 | 8.09 ± 0.12 | 7.02 ± 0.13 | 2.46 ± 0.04 | 3.49 ± 0.08 |
| Rc | 1.67 ± 0.03 | 4.26 ± 0.06 | 2.71 ± 0.08 | 1.90 ± 0.04 | 3.28 ± 0.04 |
| Rb2 | 1.34 ± 0.01 | 2.94 ± 0.03 | 2.04 ± 0.01 | 1.59 ± 0.03 | 2.51 ± 0.06 |
| Rb3 | 0.80 ± 0.00 | 0.90 ± 0.00 | 0.86 ± 0.01 | 0.78 ± 0.01 | 0.82 ± 0.02 |
| Rd | 0.88 ± 0.01 | 1.03 ± 0.01 | 0.98 ± 0.01 | 1.14 ± 0.01 | 2.08 ± 0.04 |
| Rg3 | ND | 1.51 ± 0.05 | 1.07 ± 0.01 | 16.51 ± 0.24 | 25.62 ± 0.45 |
| Rk1 | ND | ND | ND | 4.15 ± 0.04 | 10.86 ± 0.21 |
| Rg5 | ND | ND | ND | 7.75 ± 0.16 | 17.74 ± 0.54 |
| Rh2 | ND | ND | ND | ND | ND |
| Total | 14.34 ± 0.08 | 24.64 ± 0.22 | 20.22 ± 0.44 | 42.03 ± 0.63 | 74.95 ± 0.90 |

Content (mg/g). ND, not detected.

^a A: produced by company A.

^b B: produced by company B.

LODs of 0.075–0.30 μg and LOQs of 0.125–0.80 μg were achieved.

This newly developed HPLC-ELSD method was applied to compare the extraction efficiency of four different solvent systems for the 14 ginsenosides in the red ginseng powder and concentrate (Table 2). The ginsenoside contents depended on the solvent system used for extraction. Ginsenosides in both types of ginseng samples were well extracted with 100% methanol after dissolving ginseng samples in water for 2 h (solvent system 4). Thus, ginseng samples were extracted by using solvent system 4.

A validation study was conducted using red ginseng powder and red ginseng concentrate manufactured by the company B, but the amounts of the ginsenosides Rg1, Re, and Rf were very small in red ginseng concentrate. For this reason, red ginseng powder was used for the validation of ginsenosides Rg1, Re, and Rf. As shown in Table 3, the HPLC-ELSD method showed good reproducibility for the quantification of the 14 ginsenosides, with intra- and inter-day variations of less than 4.65 and 4.35%, respectively. The overall recoveries ranged from 94.74 to 109.50%, with the R.S.D. ranging from 0.10 to 7.74% (Table 4). These results demonstrated that the HPLC-ELSD method is precise, accurate, and sensitive for the quantitative determination of 14 ginsenosides in ginseng samples.

3.2. Application to finished ginseng products

Three types of ginseng samples were analyzed with the newly developed quantification method. The HPLC chromatograms of the various ginseng extracts are shown in Fig. 2, and the contents of 14 ginsenosides in the ginseng products are presented in Table 5. There are various types of commercial red ginseng products such as red ginseng powder, concentrate, tablets, candy, and tea, and they are produced by several ginseng companies. White ginseng and two types of red ginseng products (red ginseng powder and concentrate) were analyzed because it was assumed that the different types of red ginseng products contain different types of ginsenosides and contents of total ginsenosides. In addition, the finished red ginseng products manufactured by two different companies were compared.

Fig. 2B and C show the HPLC chromatograms for white ginseng powder and red ginseng powder, respectively. Ginsenosides Rg1, Re, Rf, Rb1, Rc, Rb2, Rb3, and Rd were found in both white and red ginseng powder, but only the red ginseng powder contained ginsenoside Rg3. These results are consistent with previous reports of the generation of new types of ginsenosides, including Rg3, under conditions of high temperature and pressure [13,42,43]. Because antitumor activity of ginsenoside Rg3 has been reported, many studies have been performed recently using Korean red ginseng [20,44,45]. Red ginseng powder was found to contain more ginsenosides than white ginseng powder (Table 5).

Fig. 2C and D are the chromatograms from red ginseng powder and concentrate manufactured by the same company. Ginsenosides Rh1, Rg2, Rk1, and Rg5 were observed in red ginseng concentrate but were not detected in red ginseng powder. Furthermore, the red ginseng concentrate contained a sub-

stantially higher amount of ginsenoside Rg3 than red ginseng powder. Ginsenosides Rh1, Rg2, and Rg3 can be formed by deglycosylation of Rg1, Re, and many protopanaxadiol ginsenosides such as Rb1 and Rd, respectively [42,43,46]. Thus, Rh1, Rg2, and Rg3 were likely produced during the process of making the concentrate. To make the concentrate, red ginseng roots are boiled and condensed for a long period of time. The sugars attached to the R₂ position (Fig. 1) can be removed under conditions of high temperature and pressure, producing various transformed ginsenosides such as Rh1, Rg2, and Rg3. Ginsenosides Rk1 and Rg5, which have side chains that are quite different from other ginsenosides, have reduced polar properties (Fig. 1), but the glycosylation patterns are similar to Rg3. These observations indicate that Rk1 and Rg5 were formed from Rg3 under high temperature and pressure, but the pathway of formation has not been defined.

Table 5 shows the amounts of 14 ginsenosides in red ginseng powders and concentrates manufactured by two different companies. There were no differences in the numbers of ginsenosides between the two products, but the total quantity of ginsenosides differed. The determined amount of total ginsenosides in the red ginseng concentrate from company B fulfills the labeled amount of ginsenosides (total ginsenosides > 70 mg/g). However, the concentrate produced by company A does not contain an appropriate amount of ginsenosides. The labeled amount of total ginsenosides in the red ginseng concentrate from company A was also >70 mg/g, but the determined amount of total ginsenosides was only 42 mg/g.

From these results, it can be concluded that substantial differences exist between the different types of ginseng products such as white ginseng powder, red ginseng powder, and red ginseng concentrate. Furthermore, it was discovered that some commercial ginseng products contained less ginsenoside than the labeled amount. These results strongly suggest that guidelines and quality control for commercial ginseng products are required.

4. Conclusions

In this study, HPLC-ELSD conditions were optimized for the quantitative and qualitative determination of 14 major ginsenosides in white and red ginseng. This developed method can be used to analyze ginsenosides Rg3, Rg5, and Rk1, which are the unique compounds of steamed *Panax ginseng* (Korean red ginseng). The quantification method is rapid, accurate, and precise, and it can simultaneously determine the amounts of 14 ginsenosides in various ginseng samples. We identified differences in ginsenoside content among different types of commercial ginseng products. These results confirmed the need for quality control of ginseng products, and the validated HPLC method is expected to provide a new basis for the assessment of samples.

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