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Sensitive high-performance liquid chromatography method of non-polar ginsenosides by alkaline-enhanced pulsed amperometric detection

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ABSTRACT

We determined the minute amount of non-polar ginsenosides in red ginseng with a reversed-phase high-performance liquid chromatography-pulsed amperometric detection (RP-HPLC-PAD) method. Non-polar ginsenosides efficiently extracted by ethyl acetate were well separated in 40 min using a water–acetonitrile gradient eluent and detected by PAD under NaOH alkaline conditions. The ginsenoside detection limits (S/N = 3) were 0.03–0.10 ng. The coefficients of linear regression were 0.9972–0.9990. Intra- and inter-day precision (RSDs) was less than 8.34% and average recovery was 98.06–102.73%. The total amount of non-polar ginsenosides in hairy root of red ginseng was slightly higher than in the main root.

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1. Introduction

Ginseng, the root of *Panax ginseng*, has been widely used in Chinese traditional medicine, and contains ginsenosides as medicinal components. Red ginseng is made through additional process of steaming and drying from ginseng. During the steaming process, some of the polar ginsenosides are transformed into non-polar ginsenosides by hydrolysis of sugar moiety. Consequently, non-polar ginsenosides are produced in small quantities in red ginseng. Therefore, red ginseng contains both polar and non-polar ginsenosides, whereas ginseng contains only polar ginsenosides. In Korea Pharmacopoeia (VIII), the pharmacopoeia names for ginseng and red ginseng is differently described as Ginseng Radix Alba and Ginseng Radix Rubra, respectively. Typical red ginseng has non-polar ginsenosides, such as ginsenoside Rh_1 (G-Rh_1), ginsenoside Rh_2 (G-Rh_2), ginsenoside Rg_2 (G-Rg_2), ginsenoside Rg_3 (G-Rg_3), ginsenoside Rg_5 (G-Rg_5), ginsenoside Rk_1 (G-Rk_1) as well as polar ginsenosides, such as ginsenoside Rb_1(G-Rb_1), ginsenoside Rb_2 (G-Rb_2), ginsenoside Rb_3 (G-Rb_3), ginsenoside Rc (G-Rc), ginsenoside Rd (G-Rd), ginsenoside Rf (G-Rf), ginsenoside Rg_1 (G-Rg_1), and ginsenoside Re (G-Re) [1,2]. The chemical structures of ginsenosides are shown in Fig. 1. The non-polar ginsenosides in red ginseng have shown diverse biologically beneficial activities, such as anticancer effects (G-Rg_3, G-Rg_5, G-Rh_2) [3–6], antioxidant activities (G-Rg_3, G-Rg_3, G-Rh_1, G-Rh_2, G-Rk_1) [7–9], a neuroprotective effect (G-Rg_2, G-Rg_3) [10,11], a vasodilating effect (G-Rg_3) [12], and hepatoprotective effects (G-Rg_3, G-Rh_2) [13].

The various methods for the analysis of polar ginsenosides in ginseng have widely been used without any problem [14–17], while small amount of non-polar ginsenosides in red ginseng was not easily detected because of lack of detector sensitivity [18,19]. The HPLC-evaporate light scattering detection (ELSD) method has been a general method for the analysis of ginsenosides in red ginseng, but could not detect small amount of non-polar ginsenosides [18]. Therefore, it is desirable to develop sensitive detection methods for non-polar ginsenosides in red ginseng.

High-performance anion-exchange chromatography-pulsed amperometric detection (HPAEC-PAD) has been used for the quantitation of carbohydrates in plant resources [20–22], because of its strong anion-exchange properties, that efficiently separate carbohydrates. PAD, an electrochemical detector that measures the positive potential produced by carbohydrate oxidation on a gold electrode, allows the direct detection of carbohydrates at low pico-mole levels [23].

Although PAD has been used for the analysis of sugars, it has not been applied for the analysis of glycosides because of the difficulty in separating glycosides from sugars and glycosides on an anion-exchange column. We have developed a reversed-phase method

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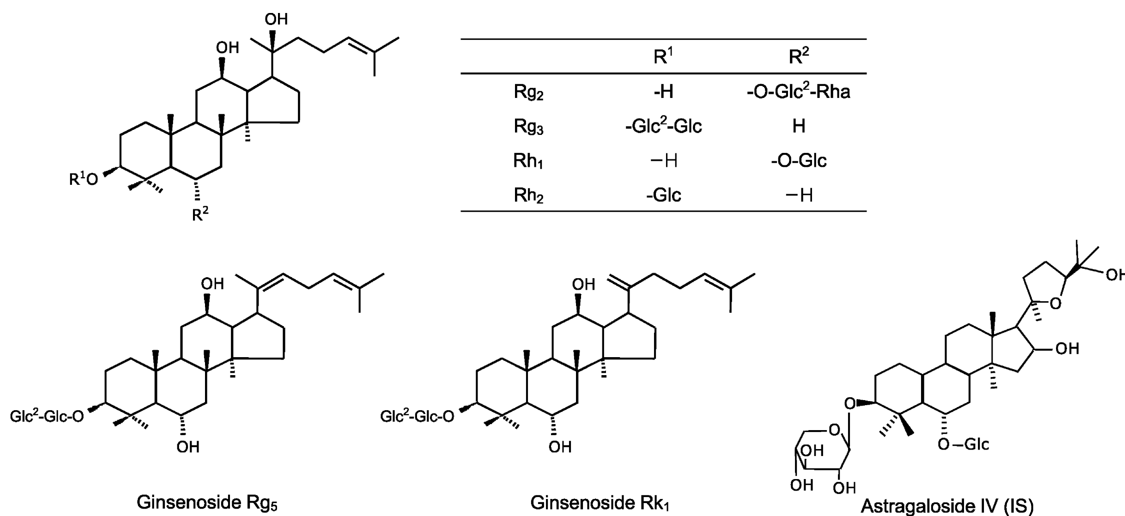

	R^1	R^2
Rg_2	-H	-O-Glc^2-Rha
Rg_3	-Glc^2-Glc	H
Rh_1	−H	-O-Glc
Rh_2	-Glc	−H

Fig. 1. Chemical structures of ginsenosides and astragaloside IV (I.S.).

of high-performance liquid chromatography-pulsed amperometric detection (RP-HPLC-PAD) for glycoside analysis. Previously, we demonstrated the analysis of several polar ginsenosides in ginseng [24]. We demonstrated highly efficient separation of polar ginsenosides from sugars on a reversed-phase column and their detection by PAD. Here, we established a noble method for determining non-polar ginsenosides in red ginseng by modified extraction protocol and optimized separation conditions. By this new method, we successfully analyzed non-polar ginsenosides in 6-year-old red ginseng (MR-6), 4-year-old red ginseng (MR-4), and hairy root of 4-year-old red ginseng (HR-4).

We also compared the limits of detection (LODs) and limits of quantitation (LOQs) by PAD to those reported for the ELSD method. The robustness of this method was also evaluated by intra- and inter-day validation. The six non-polar ginsenosides (G-Rh_1, G-Rh_2, G-Rg_2,G-Rg_3, G-Rg_5 and G-Rk_1) in red ginseng were successfully separated with high recovery.

2. Experimental

2.1. Materials

Crude drugs were purchased from the *Kyungdong* Market (Seoul, South Korea), in accordance with the standards stipulated in Korea Pharmacopoeia (VIII). The main roots of 6-year-old red ginseng (MR-6) and 4-year-old red ginseng (MR-4), as well as the hairy root of 4-year-old red ginseng (HR-4), were purchased from a local drug store. HPLC-grade acetonitrile and 50% sodium hydroxide were purchased from Fisher Scientific (Fairlawn, NJ, USA). All other reagents and solvents used were of guaranteed or analytical grade. G-Rg_3, G-Rh_2, G-Rg_2, G-Rh_1, G-Rb_2, G-Rb_3, G-Rf and astragaloside IV (AST IV) were purchased from ChromaDex (Santa Ana, CA, USA). G-Rk_1 (95.2%) and G-Rg_5 (96.1%) were working standards obtained from Seoul National University [25]. G-Rb_1, G-Rc, G-Rd, G-Re, and G-Rg_1 were purchased from Wako (Tokyo, Japan). A Millipore membrane filter (type HA, pore size 0.45 μm) was used for solvent filtration. All samples were filtered through disposable syringe filters (Hydrophobic PTFE, pore size 0.20 μm, Advantec MFS, Tokyo, Japan) before injection. To prepare the standard solutions, sample solutions, and mobile phase, we used 18 MΩ purified water produced by our laboratory's water purification system, Automatic Aquarius AW-1001 (Top Trading, Seoul, South Korea). The weight of each sample was measured on a Mettler Toledo AX 105 DeltaRange (Greifensee, Switzerland). Mass spectra were obtained with a JEOL AccuTOF TLC JMS-T100TD (Tokyo, Japan).

2.2. Apparatus and high-performance liquid chromatography

The PAD system from the ICS-3000 series Dionex (Sunnyvale, CA, USA) was equipped with an Au-Flow cell containing a gold working electrode and a solvent compatible cell containing an Ag/AgCl reference electrode. We cleaned up the gold electrode by rubbing the surface twice a week with the pink eraser (Dionex P/N. 049721) provided in the polishing kit. HPLC equipment, consisting of a Model Nanospace SI-2/3201 pump and a 3004 column oven, was purchased from Shiseido (Tokyo, Japan). Nanospace SI-2/3201 pump has metal-free head made of polyetheretherketone (PEEK) resin, which resist aggressive chemicals such as alkaline solutions.

Chromatographic separation was performed by using a Unison UK-C-18 column (150 mm × 2.0 mm I.D.; 3 μm, Imtakt, Kyoto, Japan). The potential waveform was as follows: E1 = −0.2 V (from 0.00 to 0.04 s); E2 = 0 V (from 0.05 to 0.21 s); E3 = +0.22 V (from 0.22 to 0.46 s); E4 = 0 V (from 0.47 to 0.56 s); E5 = −2 V (from 0.57 to 0.58 s); and E6 = +0.6 V (0.59 s). The mobile phase consisted of 30% (v/v) acetonitrile (solvent A) and 80% (v/v) acetonitrile (solvent B). The following procedure was employed: isocratic elution with A:B (92:8) for 8 min, linear gradient elution from A:B (92:8) to (68:32) from 8 to 10 min, and isocratic elution with A:B (68:32) from 10 to 45 min. The column was then washed with 100% B for 10 min. The flow rate was 0.2 mL/min, and the separation temperature was 30 or 40 °C. A post-column delivery system of 200 mM sodium hydroxide with a flow rate of 0.8 mL/min was added to the RP-HPLC-PAD system. The mobile phase was made through being degassed by vacuum filtration after the mixture of water with acetonitrile on a daily basis, followed by sonication for 20 min before use. A post-column delivery system was purged to remove carbonate from the water with helium throughout the experiment. The data were controlled on a computer running the Chromeleon client program supplied by Dionex. The injection volume was 10 μL.

2.3. Standard preparation

Stock solutions were prepared by dissolving 1 mg of each standard (G-Rg_2 G-Rg_3, G-Rh_1, G-Rh_2, G-Rg_5, and G-Rk_1) in 1 mL of 50% (v/v) acetonitrile/water in an Eppendorf tube. Each stock solution was diluted to create six calibration points (1, 2, 5, 10, 25, and

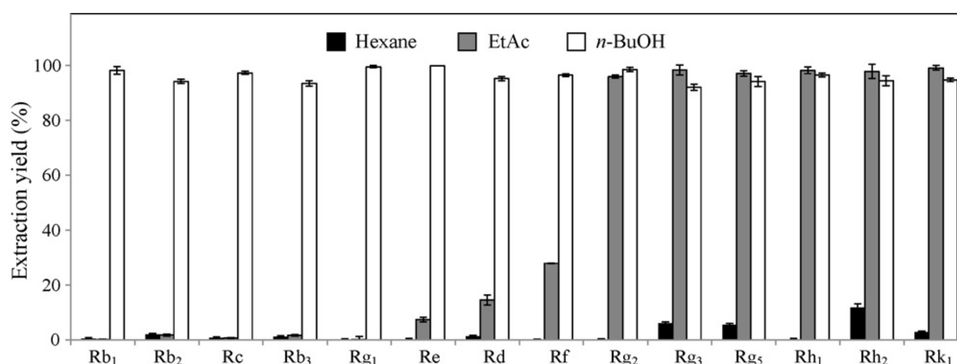

Fig. 2. Extraction yield (%) of 14 ginsenosides by hexane, ethyl acetate and *n*-butanol ($n = 3$).

50 μg/mL) for the preparation of the calibration curves. The concentration of the internal standard, AST IV, was 10 μg/mL for all analytes.

2.4. Sample preparation

Each 50 mg of red ginseng sample powder was added to 5 mL of ethanol and then extracted under sonication for 2 h before being filtered. The extract solution was evaporated to dryness. The residue was suspended in 1 mL of distilled water, and then extracted by vortexing three times with 1 mL of ethyl acetate. The ethyl acetate layer (top layer) was evaporated to dryness in a vacuum rotary evaporator. The residue was dissolved in 1 mL of 50% (v/v) acetonitrile/water and filtered through a MFS 13 disposable syringe before injection.

2.5. Validation of the method

The linear calibration curves were made at least eight times for each reference compound. The regression equation was $y = ax + b$, where y and x were the ratio of the peak area (analytes/internal standard) and sample concentration, respectively. Repeatability was evaluated by performing intra- and inter-day ($n = 5$) assays. Recovery tests of non-polar ginsenoside were done by adding known amounts of standards (50, 100, 250 ng) to samples from the main roots of 6-year-old red ginseng. Three injections of each sample were carried out to measure recovery.

3. Results and discussion

3.1. Use of liquid–liquid extraction for analyzing non-polar ginsenosides

A long time is required to simultaneously separate ginsenosides because red ginseng contains both polar and non-polar ginsenosides. Even if the running time is reduced, the resulting overlapping peaks make it difficult to analyze the non-polar ginsenoside content. Therefore, a selective liquid–liquid extraction method was employed to extract only non-polar ginsenosides while leaving polar ginsenosides behind. The transfer ratios into hexane, ethyl acetate, and *n*-butanol from water (the extraction yield) were examined for 14 polar and non-polar ginsenoside standards. Mixtures of 14 ginsenoside standards (each 0.1 mg) were suspended in 1 mL of distilled water and then 1 mL of hexane, water-saturated ethyl acetate, or water-saturated *n*-butanol was added. After vortexing the mixtures for 30 s, the mixtures were centrifuged at 12,000 rpm for 5 min and the organic solvent layer was transferred to a microtube. The extraction procedure was repeated three times. All organic solvent layers were collected and evaporated to dryness, and re-dissolved in 50% (v/v) acetonitrile.

The extraction yield was evaluated by the following formula [26].

$$\text{Extraction yield (\%)} = \frac{G_o}{G_o + G_w} \times 100$$

where G_o is the amount of ginsenoside in the organic solvent layer, and G_w is the amount of ginsenoside in the water layer. The amount of ginsenoside in each layer was determined by the RP-HPLC-PAD method (Fig. 2).

In hexane extraction, the extraction yields of non-polar ginsenosides were quite low (G-Rh_2, 11%; G-Rg_3, 6%; G-Rg_5, 5%). When using water-saturated *n*-butanol, all ginsenosides were equally extracted with high yield. In ethyl acetate, non-polar ginsenosides were extracted by more than 95%, while polar ginsenosides were only partially extracted (G-Rf, 28%; G-Rd, 15%; G-Re, 7%). The peaks of the polar ginsenosides extracted under this extraction condition (G-Rf, G-Rd, G-Re) did not overlap with six non-polar ginsenoside peaks under our chromatographic conditions. Consequently, ethyl acetate was the most suitable solvent for non-polar ginsenoside extraction.

3.2. RP-HPLC-PAD system

The RP-HPLC-PAD system was composed as follows. A mixture of acetonitrile and water was used in gradient eluent system. In this condition, non-polar ginsenosides were strongly retained and clearly separated on the C-18 column. The eluent after passing through the column was mixed with sodium hydroxide solution (NaOH) in a T-shaped mixer before entering PAD. The RP-HPLC-PAD method focuses on the detection of sugar moieties in non-polar ginsenosides.

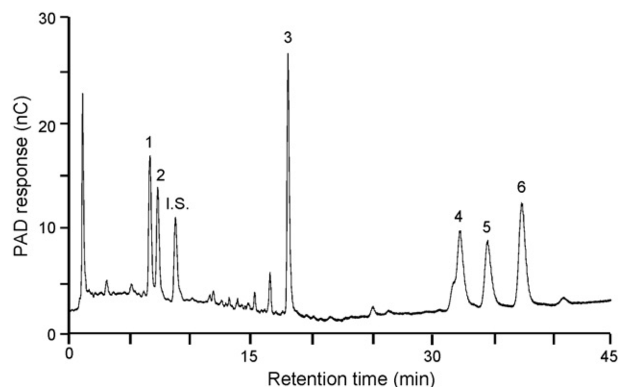

Fig. 3. Standard chromatogram of non-polar ginsenosides. Peaks: 1, G-Rg_2; 2, G-Rh_1; 3, G-Rg_3; 4, G-Rk_1; 5, G-Rg_5; 6, G-Rh_2; I.S., AST IV. Injected amount: 100 ng ginsenoside.

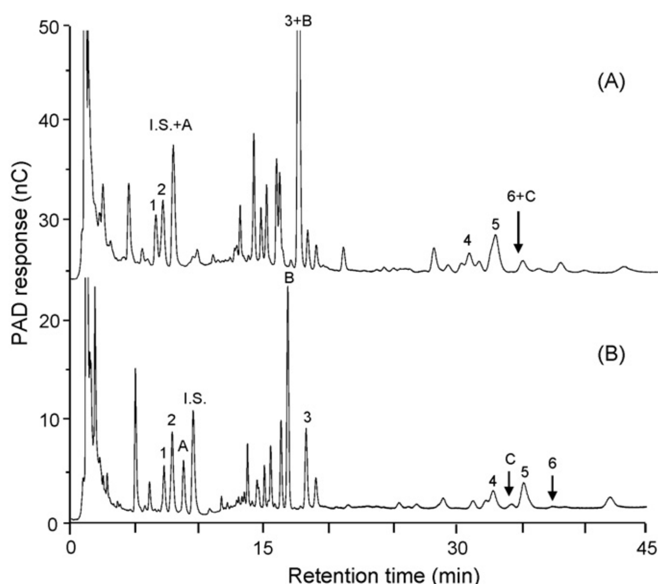

Fig. 4. Chromatograms of non-polar ginsenosides in 6-year-old red ginseng at two column temperatures. (A) Column temperature, 30 °C; (B) column temperature, 40 °C. Peaks: 1, G-Rg_2; 2, G-Rh_1; 3, G-Rg_3; 4, G-Rk_1; 5, G-Rg_5; 6, G-Rh_2; I.S., AST IV. Injected volume: 10 μL.

As PAD usually senses the redox of sugar residues, ginsenoside detection was highly responsive. The optimal column, post-column reagent, and PAD waveform conditions have been discussed in our previous paper [24]. As discussed, we used a 2.0 mm-diameter C-18 column (length, 150 mm) at a flow rate of 0.2 mL/min to reduce the volume of acetonitrile in the mobile phase because large volumes of acetonitrile reduce PAD sensitivity. To obtain high sensitivity, the concentration and flow rate of the post-column eluent were adjusted to 200 mM NaOH and 0.8 mL/min, respectively. Of the three different types of PAD waveform (triple-potential, quadruple-potential, and six-potential), we used the most sensitive six-potential waveform.

3.3. Separation of non-polar ginsenosides in red ginseng

Fig. 3 shows the chromatograms of non-polar ginsenoside standards and internal standards. Astragaloside IV (10 μg/mL) was used as an internal standard. Six non-polar ginsenosides were completely separated in 40 min using a water–acetonitrile gradient elution system.

Fig. 4 demonstrated the labile retentions of unknown peaks near non-polar ginsenosides depending on column temperatures of 30 °C (A) and 40 °C (B). At 30 °C, it was difficult to separate non-polar ginsenosides owing to the several overlapping peaks of internal standard with unknown A, G-Rg_3 with unknown B and G-Rh_2 with unknown C (Fig. 4A). Especially, the overlapping of G-Rg_3 with large unknown B leads to overestimate the content of G-Rg_3. At 40 °C, however, non-polar ginsenosides were completely separated (Fig. 4B). The $[M+H]^+$ molecular ion of the unknown B peak was measured at m/z 579.4 by electrospray ionization time-of-flight mass spectrometry (ESI-TOF-MS). The unknown B might not be a ginsenoside class because most of ginsenosides have molecular weights higher than 620 [27–29]. We could not determine the structures of unknown A and C because of insufficient amounts. Interestingly, the unknown A, B and C peaks exhibited variable retention times depending on column temperature, whereas the non-polar ginsenosides showed relatively stable retention times regardless of column temperature. For these reasons, unknown A, B and C peaks might have different structures from ginsenosides. We greatly improved the resolutions of non-polar ginsenoside peaks by changing column temperature.

Table 2
Intra-and inter-day validations for determination of non-polar ginsenosides in the main root of 6-year-old red ginseng (n = 5).

Ginsenoside	Intra-day precision		Inter-day precision	
	Content (mg/g)	RSD (%)	Content (mg/g)	RSD (%)
Rg_2	0.174 ± 0.009	5.043	0.174 ± 0.012	7.132
Rh_1	0.298 ± 0.012	3.915	0.287 ± 0.019	6.326
Rg_3	0.215 ± 0.017	7.887	0.220 ± 0.014	6.168
Rk_1	0.269 ± 0.007	2.788	0.269 ± 0.005	1.891
Rg_5	0.293 ± 0.024	8.345	0.294 ± 0.024	8.101
Rh_2	0.023 ± 0.001	4.969	0.024 ± 0.001	5.543

Table 3
Recovery test for determination of non-polar ginsenosides in the main root of 6-year-old red ginseng (n = 3).

Ginsenoside	Added (ng)	Recovery (%)	Mean (%)	RSD (%)
Rg_2	50	102.692 ± 8.112	98.061	6.002
	100	97.312 ± 3.424		
	250	94.179 ± 2.327		
Rh_1	50	99.869 ± 3.388	99.859	3.533
	100	103.340 ± 0.930		
	250	96.369 ± 1.002		
Rg_3	50	107.989 ± 4.113	102.726	5.959
	100	101.922 ± 6.410		
	250	98.266 ± 4.417		
Rk_1	50	105.027 ± 5.815	102.204	5.355
	100	103.748 ± 5.747		
	250	97.837 ± 2.972		
Rg_5	50	102.823 ± 6.504	99.171	5.904
	100	97.820 ± 6.656		
	250	96.868 ± 4.452		
Rh_2	50	96.874 ± 5.205	99.344	5.628
	100	97.794 ± 6.983		
	250	103.364 ± 3.490		

Recovery (%) = (observed amount – original amount)/spiked amount × 100%.

Table 1
Investigated linear range, linear equation, correlation coefficient, limits of detection (LOD) and limits of quantitation (LOQ) for non-polar ginsenosides.

Ginsenoside	Linear range (μg/mL)	Linear equation[a]	r^2	PAD		ELSD[b]	
				LOD (ng)	LOQ (ng)	LOD (ng)	LOQ (ng)
Rg_2	0.03–50	$y = 0.1358\,x + 0.1518$	0.9990	0.10	0.30	300	800
Rh_1	0.03–50	$y = 0.0884\,x + 0.2383$	0.9972	0.10	0.30	300	800
Rg_3	0.01–50	$y = 0.1636\,x + 0.2931$	0.9978	0.03	0.10	75	125
Rk_1	0.02–50	$y = 0.1466\,x + 0.0165$	0.9983	0.05	0.20	75	125
Rg_5	0.03–50	$y = 0.0967\,x + 0.0297$	0.9990	0.10	0.30	75	125
Rh_2	0.02–50	$y = 0.1623\,x + 0.1049$	0.9979	0.05	0.20	100	150

[a] y = peak area ratio (analyte/I.S.), x = theoretical concentration.
[b] Data from *J. Pharm. Biomed. Anal.* 2007, 45, 164–170.

Table 4
The contents of non-polar ginsenosides detected by ELSD or PAD in red ginseng.

Ginsenoside	ELSD[a]	RP-HPLC-PAD[b]					
	Red ginseng powder	Main root of 6-year-old		Main root of 4-year-old		Hairy root of 4-year-old	
	Content (mg/g)	Content (mg/g)	RSD (%)	Content (mg/g)	RSD (%)	Content (mg/g)	RSD (%)
Rg_2	ND[c]	0.174 ± 0.012	7.132	0.127 ± 0.009	7.345	0.494 ± 0.046	9.237
Rh_1	ND	0.287 ± 0.019	6.326	0.286 ± 0.009	3.121	0.283 ± 0.016	5.801
Rg_3	1.510 ± 0.050	0.220 ± 0.014	6.168	0.210 ± 0.012	5.895	0.139 ± 0.006	4.233
Rk_1	ND	0.269 ± 0.005	1.891	0.197 ± 0.017	8.819	0.145 ± 0.009	6.408
Rg_5	ND	0.294 ± 0.024	8.101	0.265 ± 0.022	8.353	0.245 ± 0.010	4.142
Rh_2	ND	0.024 ± 0.001	5.543	0.070 ± 0.007	9.654	0.025 ± 0.002	6.046
Total		1.268 ± 0.044	3.450	1.154 ± 0.053	4.596	1.332 ± 0.069	5.153

[a] Ginsenoside contents in red ginseng powder analyzed by ELSD method (Data from *J. Pharm. Biomed. Anal.* 2007, 45, 164–170).
[b] $n = 4$ determinations.
[c] ND, not detected.

3.4. Analysis of non-polar ginsenosides

The analytical method was validated with respect to parameters such as linearity, LODs, LOQs, precision, accuracy and recovery.

The linearity of detection for each analyte was examined using 6 different standard solutions (1, 2, 5, 10, 25, and 50 μg/mL). A calibration curve was constructed by performing linear regression of the peak area ratio (analyte/internal standard) *vs*. analyte concentration. The equations and linear ranges are listed in Table 1. The concentration range of non-polar ginsenosides in 50 mg of red ginseng powder was calculated as 1.2–24.7 μg. The red ginseng powder was dissolved in 1 mL of 50% (v/v) acetonitrile/water after pretreatment steps (final concentration: 1.2–24.7 μg/mL). The concentration of non-polar ginsenosides would be within the linearity ranges (0.01–50 μg/mL). The correlation coefficient was 0.9972–0.9990. The LODs (S/N = 3) and LOQs (S/N = 10) obtained for each standard ginsenoside were as follows: G-Rg_2, 0.10 and 0.30 ng; G-Rh_1, 0.10 and 0.30 ng; G-Rg_3, 0.03 and 0.10 ng; G-Rk_1, 0.05 and 0.20 ng; G-Rg_5, 0.10 and 0.30 ng; G-Rh_2, 0.05 and 0.200 ng. Significantly, PAD showed 750–3000 times higher sensitivity in detecting ginsenosides than the ELSD method [18].

3.5. Analysis of non-polar ginsenosides in crude drugs

To validate our method, we analyzed the reproducibility and recovery of non-polar ginsenosides in 6-year-old red ginseng. The results are summarized in Tables 2 and 3. The intra- and inter-day precisions (RSDs) were 2.79–8.34% and 1.89–8.10%, respectively. The recoveries of non-polar ginsenosides were evaluated by adding 500 μL of non-polar ginsenosides mixture (each 5, 10, and 25 μg) to 500 μL of each sample and then applying 10 μL of mixed solutions (each 50, 100, and 250 ng) into HPLC system. The results are summarized in Table 3. The mean recoveries were as follows: G-Rg_2, 98.06%; G-Rh_1, 99.86%; G-Rg_3, 102.73%; G-Rk_1, 102.20%; G-Rg_5, 99.17%; G-Rh_2, 99.34%. The mean recoveries and RSD ranges for non-polar ginsenosides were 98.06–102.73% and 3.53–6.00%, respectively. Therefore our method showed good precision and accuracy.

3.6. Applications

Previous ELSD methods showing universal detection failed to determine non-polar ginsenosides in red ginseng powder, whereas our sensitive method showed greatly enhanced detectability for the analysis of non-polar ginsenosides in 6-year-old red ginsengs (MR-6), 4-year-old red ginsengs (MR-4), and the hairy roots of 4-year-old (HR-4) red ginsengs. The results are summarized in Table 4. Distinctively, five among six non-polar ginsenosides were not detected (indicated as ND) in ELSD method, while all non-polar ginsenosides were well detected by our new method. Although these samples showed a little difference in total ginsenosides amount, HR-4 contained more non-polar ginsenosides (1.332 mg/g). Especially, G-Rg_2 was 3–4 times higher in HR-4 (0.494 mg/g) than in MR-6 (0.174 mg/g) or MR-4 (0.127 mg/g). G-Rh_1 and G-Rg_5 were present at similar levels in three samples (G-Rh_1, 0.283–0.287 mg/g; G-Rg_5, 0.245–0.294 mg/g). G-Rk_1 was 1.4–1.9 times higher in MR-6 (0.269 mg/g) than in MR-4 (0.197 mg/g) or HR-4 (0.145 mg/g). G-Rh_2 was almost 3 times higher in MR-4 (0.070 mg/g) than in MR-6 (0.024 mg/g) or HR-4 (0.025 mg/g). Our results demonstrated that individual red ginseng have similar non-polar ginsenoside contents.

4. Conclusion

In this report, we introduced a new analytical method for non-polar ginsenosides in red ginseng with a RP-HPLC-PAD method. Previous methods had low sensitivity for non-polar ginsenoside. In contrast, our PAD method could detect non-polar ginsenosides on a C-18 column with high selectivity, high sensitivity, and good reproducibility by combining with post-column NaOH solution. In contrast to polar ginsenosides, non-polar ginsenosides were efficiently extracted by ethyl acetate extraction. The extraction method gave excellent recoveries, and was successfully applied to the analyses of various red ginseng samples. Our method should make it possible to screen for non-polar ginsenosides that are difficult to micro-analyze. Additionally, the method is expected to provide a new basis for the assessment of the ginsenoside content of red ginseng.

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