

Simultaneous determination of 30 ginsenosides in *Panax ginseng* preparations using ultra performance liquid chromatography

Hee-Won Park¹, Gyo In^{1*}, Sung-Tai Han¹, Myoung-Woo Lee¹, So-Young Kim², Kyung-Tack Kim³,
Byung-Goo Cho¹, Gyeong-Ho Han¹, and Il-Moo Chang¹

¹Korea Ginseng Corporation Research Institute, Korea Ginseng Corporation, Daejeon 305-805, Korea

²Waters Korea Limited, KICOX Venture Center, Seoul 152-759, Korea

³Processing Technology Research Group, Korea Food Research Institute, Seongnam 463-746, Korea

A quick and simple method for simultaneous determination of the 30 ginsenosides (ginsenoside Ro, Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, 20(S)-Rg2, 20(R)-Rg2, 20(S)-Rg3, 20(R)-Rg3, 20(S)-Rh1, 20(S)-Rh2, 20(R)-Rh2, F1, F2, F4, Ra1, Rg6, Rh4, Rk3, Rg5, Rk1, Rb3, Rk2, Rh3, compound Y, compound K, and notoginsenoside R1) in *Panax ginseng* preparations was developed and validated by an ultra performance liquid chromatography photo diode array detector. The separation of the 30 ginsenosides was efficiently undertaken on the Acquity BEH C-18 column with gradient elution with phosphoric acids. Especially the chromatogram of the ginsenoside Ro was dramatically enhanced by adding phosphoric acid. Under optimized conditions, the detection limits were 0.4 to 1.7 mg/L and the calibration curves of the peak areas for the 30 ginsenosides were linear over three orders of magnitude with a correlation coefficients greater than 0.999. The accuracy of the method was tested by a recovery measurement of the spiked samples which yielded good results of 89% to 118%. From these overall results, the proposed method may be helpful in the development and quality of *P. ginseng* preparations because of its wide range of applications due to the simultaneous analysis of many kinds of ginsenosides.

Keywords: *Panax ginseng*, *Panax ginseng* preparations, Ginsenosides, UPLC-PDA, Simultaneous analysis

INTRODUCTION

Korean ginseng (*Panax ginseng*) has been considered one of the most valuable medicinal herbs in oriental countries for 2,000 years, and in the modern age it is widely used as a complementary and alternative medicine and health food [1]. In ginseng, the ginsenosides are triterpenes considered to be the main bioactive constituents of ginseng and show various pharmacological effects such as an anti-carcinogenic effect, immune-modulatory effect, and anti-inflammatory and anti-allergic effects [1,2].

Up to now more than 150 naturally occurring ginsen-

osides have been isolated from the roots, leaves, stem, fruits and flower heads of various *Panax* genera [2]. These ginsenosides can be classified into four types of aglycone moieties: protopanaxadiol, protopanaxatriol, ocotillol-type, and oleanolic acid [3]. Due to the fact that ginseng is a very popular phytomedicine used all around the world, a huge quantity of work has been carried out during the last 40 years in order to develop analytical methods for the identification, quantification and quality control of the ginsenosides in the raw plant material, extracts and marketed products [3-5].

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*Corresponding author

E-mail: 20109042@kgc.or.kr

Tel: +82-42-870-3051, Fax: +82-42-870-3182

The analysis of ginsenosides has been performed with various analytical methods such as TLC [6], GC [7,8], HPLC, capillary electrophoresis [9], near infra-red spectroscopy [10] and enzyme immunoassay [11]. Among these techniques, HPLC is by far the most employed analytical method. Because of its speediness, sensitivity and adaptability to non-volatile and polar compounds, HPLC is ideal for the analysis of ginsenosides [2-5]. Another advantage is versatility due to the possibility of using different detection techniques such as an ultraviolet detector (UVD), evaporative light scattering detector (ELSD), fluorescence detector (FLD), pulsed amperometric detector (PAD), and a mass spectrometry detector. Among the different detection techniques of ginsenoside analysis, the HPLC-UVD method is the most employed since it is by far the most common detector found in phytochemical laboratories. With the ease of this method, therefore, many research papers have been published for the simultaneous determination of ginsenosides [12-17]. However, the main issues encountered in performing HPLC-UVD analyses of ginseng are the high levels of baseline noise and the poor sensitivity due to the weak UV absorption of the ginsenosides [2,3]. Thus many other researchers developed the analytical method of ginsenosides using the HPLC-ELSD method [18-21]. This method is a universal, non-specific detector which can provide a stable baseline even with gradient elution. Other detection techniques of HPLC such as FLD and PAD have also been established for simultaneous ginsenosides determination [22,23].

In the conventional HPLC system, the choice of particle size must be a compromise. The smaller the particle size, the higher the column back-pressure that is occurring in the HPLC system. Recently, ultra performance liquid chromatography (UPLC) could be considered to be a new direction in liquid chromatography. UPLC, which utilize silica particles 1.7 μm , makes it possible to perform better separations in short periods of time. It also has the advantages of the fast analysis, high peak capacity, great resolution and good sensitivity [24-26].

In this paper, a new UPLC method for the simultaneous determination of 30 ginsenosides, namely ginsenoside Ro, Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, 20(S)-Rg2, 20(R)-Rg2, 20(S)-Rg3, 20(R)-Rg3, 20(S)-Rh1, 20(S)-Rh2, 20(R)-Rh2, F1, F2, F4, Ra1, Rg6, Rh4, Rk3, Rg5, Rk1, Rb3, Rk2, Rh3, compound Y, compound K, and notoginsenoside R1 in *P. ginseng* preparations was developed and validated.

MATERIALS AND METHODS

Chemicals and reagents

Ginsenoside Rg1, Re, Rf, 20(S)-Rh1, Rb1, Rc, Rb2, Rd, 20(S)-Rg3 and 20(R)-Rg3 standards were purchased from the Chromadex (Irvine, CA, USA) and ginsenoside Ro, 20(S)-Rg2, 20(R)-Rg2, 20(S)-Rh2, 20(R)-Rh2, F1, F2, F4, Ra1, Rg6, Rh4, Rk3, Rg5, Rk1, Rb3, Rk2, Rh3, compound Y, compound K, and notoginsenoside R1 standards were obtained from the Ambo Institute (Seoul, Korea). Ginsenoside Rg6 and F4, Rk3 and Rh4, Rg5 and Rk1, Rk2, and Rh3 are epimer compound mixtures. The concentrations of mixture solutions were calculated using the normalization area percentage in the UPLC chromatogram because the UV spectrum of the epimer molecules is the same.

Phosphoric acid was purchased from the Junsei Chemical (Tokyo, Japan), and HPLC-grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). For method validation, red ginseng powder (lot no. 1019008; Korea Ginseng Corporation, Daejeon, Korea) and red ginseng concentrate (lot no. 2019119, Korea Ginseng Corporation) were used. Other *P. ginseng* preparations were obtained from the Korea Ginseng Corporation Research Institute. All distilled water used in this experiment was purified by a Milli-Q gradient system (Millipore, Bedford, MA, USA) and the resistance value was measured as 18 M Ω prior to use.

Sample preparation

The sample preparations of the red ginseng powders and concentrates were performed in a similar manner as in our previous studies using the ultrasonic cleaner [27].

A half gram of red ginseng powder was weighed in a centrifugal tube (15 mL, PP-single use; BioLogix Group, Jinan, China) and shaken vigorously after the addition of 10 mL of 70% MeOH. Extraction was performed in an ultrasonic cleaner (60 Hz; Wiseclean, Seoul, Korea) for 30 min. After ultrasonic extraction, centrifugal separation (Legand Mach 1.6R; Thermo, Frankfurt, Germany) was performed for 10 min at 3,000 rpm. The resulting supernatant solution was filtered (0.2 μm ; Acrodisk, Gelman Sciences, Ann Arbor, MI, USA) and injected into the UPLC system.

In the case of the concentrate type samples, two grams of sample was weighed in a beaker, and 15 mL of deionized water was added. After being left to stand at room temperature for 1 h, the diluted sample was transferred into a 50 mL volumetric flask where the volume was brought up to 50 mL by adding MeOH. The extraction

was performed in an ultrasonic cleaner (60 Hz, Wise-clean) for 30 min. Then, the solution was filtered (0.2 μm , Acrodisk) and injected into the UPLC system.

Chromatographic conditions

The instrumental analysis was performed by a Waters ACQUITY UPLC system (Waters, Millford, MA, USA) composed of a binary solvent manager, sample manager and photo diode array detector (PDA). The chromatographic separation was accomplished on a ACQUITY BEH C18 column (100 mm \times 2.1 mm, 1.7 μm ; Waters). The column temperature was 40°C. The binary gradient elution system consisted of 0.001% phosphoric acid in water (A) and 0.001% phosphoric acid in acetonitrile (B). The separation was achieved using the following gradient program: 0-0.5 min (15% B), 14.5 min (30% B), 15.5 min (32% B), 18.5 min (38% B), 24.0 min (43% B), 27.0 min (55% B), 27.0-31.0 min (55% B), 35.0 min (70% B), 38.0 min (90% B), 38.1 min (15% B), and 38.1-43.0 min (15% B). The flow rate was set at 0.6 mL/min and the sample injection volume was 2.0 μL . The 30 ginsenosides were detected by PDA at 203 nm.

Validation of the developed method

The method validation was performed in accordance with International Conference of Harmonization guidelines [28,29]. The precision and accuracy of the analytical methods were determined by the comparison of results from 3 different concentrations using the same sample and conditions all done in triplicate.

Calibration curves, limits of detection (LOD) and limits of quantification (LOQ) were obtained as follows. Due to the distinct variation in contents of ginsenosides in red ginseng powders and red ginseng concentrates, individual ginsenoside standard solutions were prepared and diluted with methanol to appropriate concentrations for

the establishment of calibration curves. Five concentrations of 30 ginsenoside solutions were injected and then the calibration curves were constructed by plotting the peak areas against the concentration of each ginsenoside. The LOD and LOQ under present chromatographic conditions were determined on the basis of the responses at a signal-to-noise ratio of 3 or 10, respectively.

Precision and accuracy were obtained as follows. Intra- and inter-day variations were chosen to explain the precision of the UPLC method. Red ginseng powder and concentrate were extracted and analyzed as describe in the previous section of sample preparation. The intra-day precision was performed by extraction and analysis on a single day all done in triplicate. The inter-day precision was carried out on 3 different days. Variations were expressed by the relative standard deviations (RSD). The recovery test was performed in a similar manner as in our previous study [27]. Accurate amounts of the crude saponin fraction were added to approximately a half gram of red ginseng powder and two grams of red ginseng concentrate, then extracted and analyzed as described in the sample preparation sections. The average recoveries were calculated by the following formula: $\text{recovery (\%)} = 100 \times (\text{amount found} - \text{original amount}) / \text{amount spiked}$, with $\text{RSD (\%)} = (\text{standard deviations} / \text{mean}) \times 100\%$.

Optimization of extraction conditions

In order to obtain the quantitative extractions of the ginsenosides, variables involved in the procedure such as solvent and extraction times were optimized. Ultrasonic extraction was compared with refluxing. As a result of our previous studies, ultrasonic extraction was more simple and effective for the extraction of ginsenosides [27,30]. Thus, ultrasonic extraction was chosen as the extraction method. It involved the following experimental

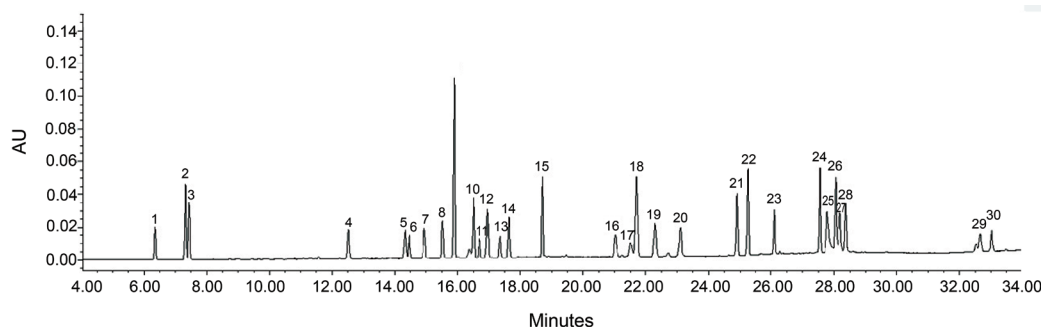


Fig. 1. Standard chromatogram of 30 ginsenosides by ultra performance liquid chromatography. NG-R1, 1; G-Rg1, 2; G-Re, 3; G-Rf, 4; 20(S)-G-Rh1, 5; 20(S)-G-Rg2, 6; 20(R)-G-Rg2, 7; G-Ro, 8; G-Rb1, 9; G-Rc, 10; G-Ra1, 11; G-F1, 12; G-Rb2, 13; G-Rb3, 14; G-Rd, 15; G-Rg6, 16; G-Rk3, 17; G-F4, 18; G-Rh4, 19; G-F2, 20; 20(S)-G-Rg3, 21; 20(R)-G-Rg3, 22; C-Y, 23; C-K, 24; G-Rk1, 25; G-Rg5, 26; 20(S)-G-Rh2, 27; 20(R)-G-Rh2, 28; G-Rk2, 29; G-Rh3, 30. NG, notoginsenoside; G, ginsenoside; C, compound.

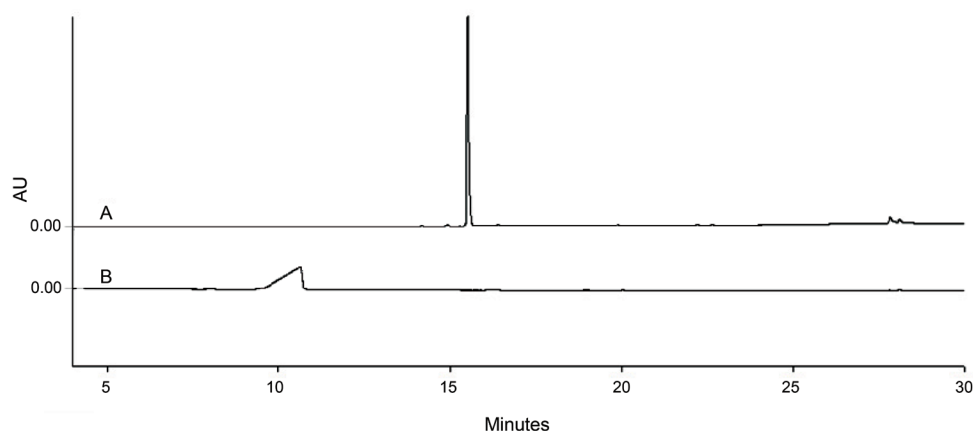


Fig. 2. Effect of acid on the chromatogram of ginsenosides Ro in ultra performance liquid chromatography system. In the presence of 0.001% phosphoric acid (A), in the absence of phosphoric acid (B).

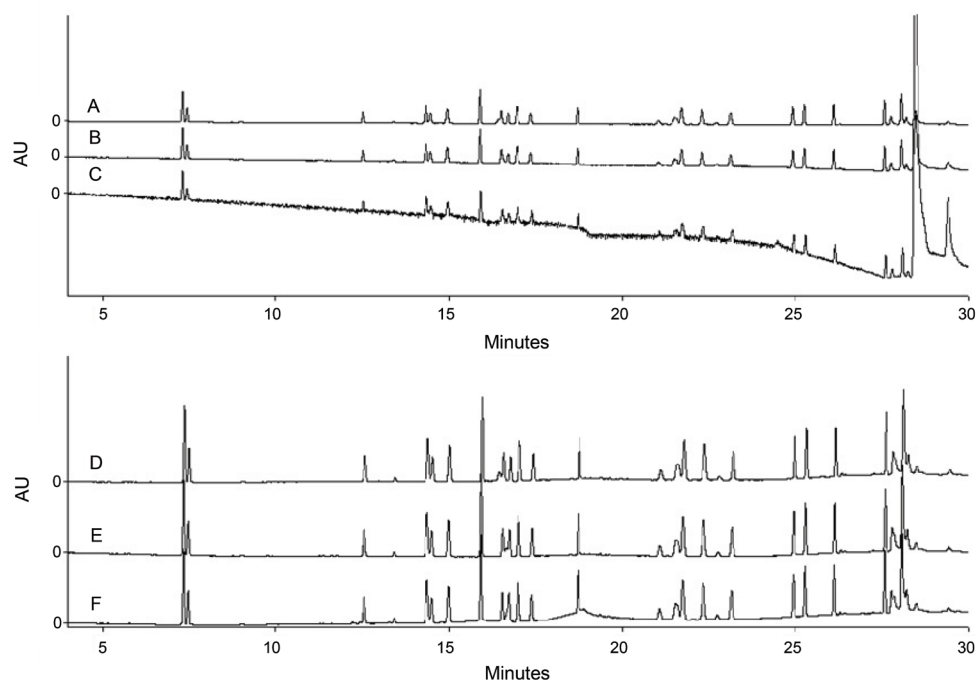


Fig. 3. Change of ginsenoside standard chromatogram depending on the type of acid and its concentration. 0.001% formic acid (A), 0.01% formic acid (B), 0.1% formic acid (C), 0.001% phosphoric acid (D), 0.01% phosphoric acid (E), and 0.1% phosphoric acid (F).

factors and corresponding levels: solvent volume (10, 20, or 40 times the material), methanol concentration (50, 70, or 100%, v/v), extraction repetitions (1, 2, or 3 times) and extraction time (10, 30, or 60 min). The optimal conditions for the extraction of ginseng powders and concentrates were selected and presented in detail in the sample preparation section. According to statistic analysis theory, methanol concentration was the most important factor in the extract conditions of red ginseng powders and concentrates and 70% was the best concentration for the extraction of the investigated ginsenosides.

RESULTS AND DISCUSSION

Optimization of UPLC-PDA conditions

For the separation of the 30 ginsenosides, it is key to obtain a good resolution between 20(*S*)-ginsenoside Rh1 (5) and 20(*S*)-Rg2 (6), ginsenoside Rk3 (17) and F4 (18), as well as ginsenoside Rg5 (26) and 20(*R*)-Rh2 (27), which are the main factors increasing the running time of the UPLC. With the great resolution of UPLC, the problem was resolved under appropriate gradient elution and flow rates. A higher column temperature was used so

as to decrease the pressure at the higher flow rate, which could improve the resolutions and peak shapes. Finally, under the optimized UPLC conditions, the resolutions between 20(*S*)-ginsenoside Rh1 (5) and 20(*S*)-Rg2 (6), ginsenoside Rk3 (17) and F4 (18), as well as ginsenoside Rg5 (26) and 20(*R*)-Rh2 (27) were 1.25, 1.18 and 1.43 respectively. As shown in Fig. 1, the investigated ginsenosides were well separated within 35 min for the standard solution. The peaks were identified by comparing the

retention times of the peaks with those of the individual standard solutions under the same conditions.

In the case of ginsenoside Ro, especially, the acidity of the mobile phase was the key factor for quantitative determination. As shown in Fig. 2, in the chromatogram of ginsenoside Ro, a huge difference was observed in the respective results when phosphoric acid was added or not added. It can be seen that by using the mobile phase without acid, the peak shape was broadened be-

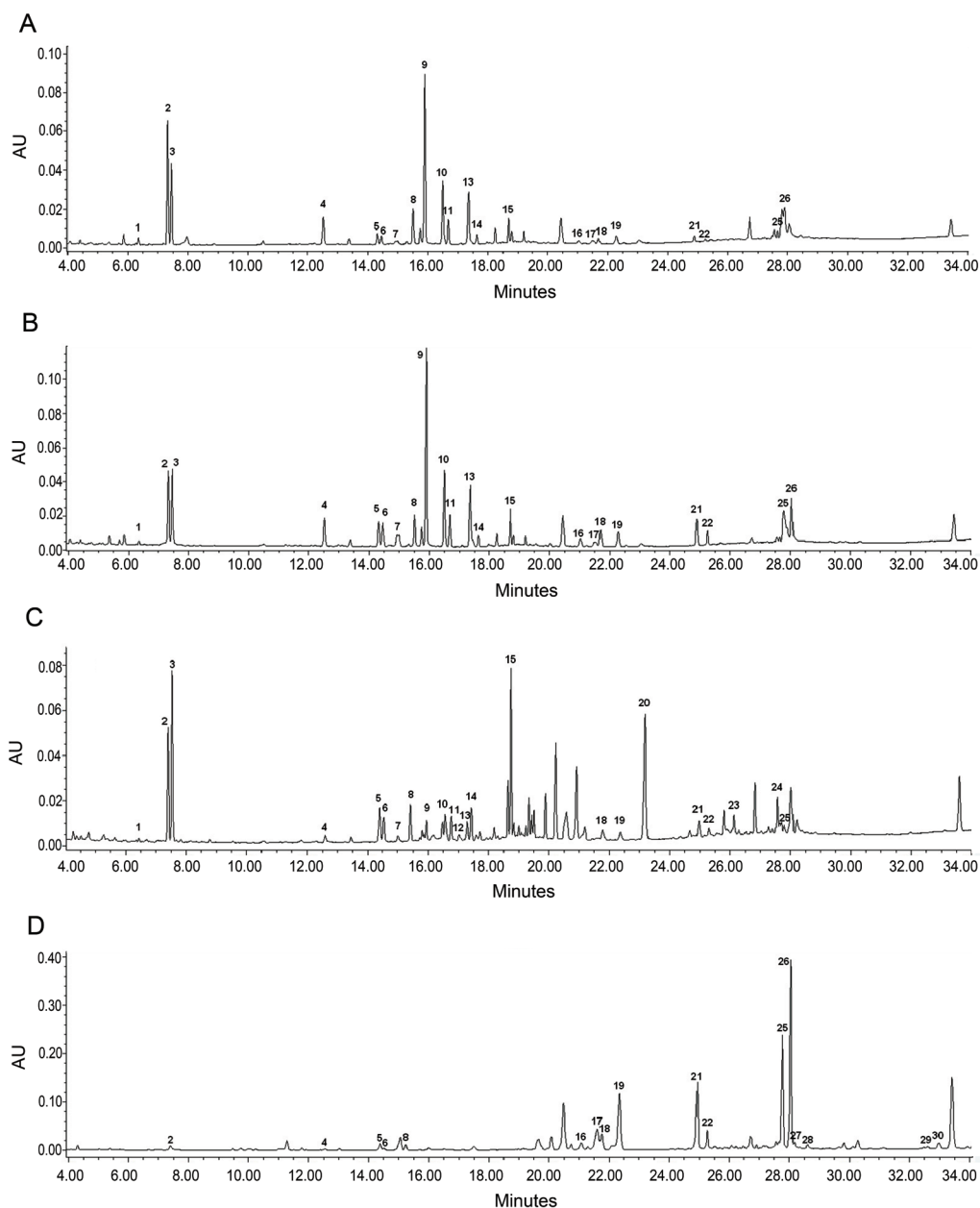


Fig. 4. Representative ultra performance liquid chromatography chromatogram of various *Panax ginseng* preparations. Red ginseng powder (A), red ginseng concentrate (B), fermented ginseng extract (C), and cosmetic raw materials (D). NG-R1, 1; G-Rg1, 2; G-Re, 3; G-Rf, 4; 20(*S*)-G-Rh1, 5; 20(*S*)-G-Rg2, 6; 20(*R*)-G-Rg2, 7; G-Ro, 8; G-Rb1, 9; G-Rc, 10; G-Ra1, 11; G-F1, 12; G-Rb2, 13; G-Rb3, 14; G-Rd, 15; G-Rg6, 16; G-Rk3, 17; G-F4, 18; G-Rh4, 19; G-F2, 20; 20(*S*)-G-Rg3, 21; 20(*R*)-G-Rg3, 22; C-Y, 23; C-K, 24; G-Rk1, 25; G-Rg5, 26; 20(*S*)-G-Rh2, 27; 20(*R*)-G-Rh2, 28; G-Rk2, 29; G-Rh3, 30. NG, notoginsenoside; G, ginsenoside; C, compound.

cause of insufficient interaction between analytes and the solid sorbent in the analytical column. However, when small amounts of phosphoric acid were added, the peak shape of ginsenoside Ro was dramatically sharpened and more retained. This is due to the fact that the solid sorbents were protonated by the added phosphoric acid and thus this phenomenon made better interactions between the ginsenoside Ro and solid sorbents in the analytical column. The importance of the acidity of the mobile phase was also emphasized by other previous studies and these results concurred with those of the previous studies [14-16].

We also investigated the following experimental factors including the type of acid and concentration of acid for the simultaneous analysis of 30 ginsenosides. As shown in Fig. 3, in the case of formic acid, the signal to noise ratio was higher and baseline drift was deeper with an increase in the concentration. Therefore, the optimum solvent system was selected as adding phosphoric acid to the mobile phases to a concentration of 0.001%.

Validation of the developed method

The specificity of individual ginsenosides was confirmed by demonstrating the sufficient separation of the

Table 1. Linearity of calibration curves for 30 ginsenosides

Analytes	Linear regression data			LOD (mg/L)	LOQ (mg/L)
	Calibration curve	r ²	Test range(mg/L)		
NG-R1	Y=1.03*10 ³ x+4.89*10 ³	0.999853	9.1-914	0.94	3.12
G-Rg1	Y=4.52*10 ³ x-4.25*10 ³	0.999933	11.2-1120	1.74	5.80
G-Re	Y=3.82*10 ³ x+8.88*10 ²	0.999961	3.1-306	1.72	5.70
G-Rf	Y=3.92*10 ³ x-9.62*10 ²	0.999958	4.5-448	1.08	3.59
20(S)-G-Rh1	Y=6.45*10 ³ x-7.48*10 ³	0.99997	5.0-504	0.85	2.82
20(S)-G-Rg2	Y=5.03*10 ³ x-1.68*10 ⁴	0.999932	3.9-387	0.36	1.21
20(R)-G-Rg2	Y=5.30*10 ³ x+1.16*10 ⁴	0.999978	4.0-398	0.48	1.58
G-Ro	Y=1.08*10 ³ x+2.37*10 ³	0.999928	10.1-1010	1.18	3.94
G-Rb1	Y=3.33*10 ³ x-4.70*10 ³	0.999937	11.0-1104	0.89	2.97
G-Rc	Y=3.69*10 ³ x-1.12*10 ⁴	0.999937	3.7-366	1.91	6.37
G-Ra1	Y=7.68*10 ³ x+3.83*10 ³	0.999841	8.2-824	1.33	4.43
G-F1	Y=1.44*10 ³ x+5.22*10 ³	0.999903	8.5-852	0.98	3.26
G-Rb2	Y=3.55*10 ³ x-5.54*10 ³	0.999937	4.0-400	1.76	5.86
G-Rb3	Y=7.64*10 ³ x+7.07*10 ³	0.999653	11.0-1100	1.67	5.56
G-Rd	Y=4.12*10 ³ x-5.72*10 ³	0.999924	3.4 -340	1.46	4.85
G-Rg6	Y=2.33*10 ³ x-5.34*10 ²	0.9999	1.9-191	0.80	2.67
G-Rk3	Y=3.48*10 ³ x+1.10*10 ⁴	0.999846	4.2-422	0.72	2.42
G-F4	Y=2.26*10 ³ x-9.96*10 ²	0.999939	6.6-655	0.75	2.51
G-Rh4	Y=3.47*10 ³ x+1.70*10 ⁴	0.999827	6.0-603	0.47	1.58
G-F2	Y=1.18*10 ³ x-1.05*10 ³	0.999978	6.2-624	1.26	4.21
20(S)-G-Rg3	Y=4.18*10 ³ x-3.41*10 ³	0.99991	3.3-385	0.34	1.12
20(R)-G-Rg3	Y=5.71*10 ³ x-6.03*10 ³	0.999919	3.9-386	0.20	0.66
C-Y	Y=1.25*10 ³ x+3.28*10 ³	0.999865	8.2-816	0.76	2.54
C-K	Y=1.46*10 ³ x+3.42*10 ³	0.999964	8.5-853	0.56	1.88
G-Rk1	Y=3.17*10 ³ X +2.56*10 ⁴	0.999706	4.3-427	0.31	1.04
G-Rg5	Y=2.77*10 ³ x-2.35*10 ⁴	0.996517	1.5-149	1.16	3.87
20(S)-G-Rh2	Y=2.62*10 ³ x+4.37*10 ³	0.999924	3.8-383	0.13	0.43
20(R)-G-Rh2	Y=8.51*10 ³ x+1.23*10 ²	0.999939	3.6-360	0.04	1.14
G-Rk2	Y=2.96*10 ³ x+4.48*10 ⁴	0.996686	6.6-492	0.36	1.19
G-Rh3	Y=2.94*10 ³ x+2.70*10 ⁴	0.996538	3.8-284	0.34	1.15

LOD, limit of detection; LOQ, limit of quantification; NG, notoginsenoside; G, ginsenoside; C, compound.

substance present in the sample matrix. As shown in Fig. 1 and Fig. 4, the sample chromatograms compared with that of the standard solution was sufficient to confirm the specificity of the ginsenosides. In other words, appropriate separation means that there is adequate resolution between the analyte peaks and the impurity and placebo peaks that need not be separated from each other [29].

A linear dependence of the signal and the analyte concentration is the most convenient indicator of sample quality or purity and is widely used in pharmaceutical

analysis [29]. The standard solutions of each ginsenoside were injected into the UPLC and the calibration curves were plotted as the peak area versus the amount of each ginsenoside. The linearity was evaluated by linear regression analysis, which is calculated by the least squares regression method. The LOD and LOQ under the present chromatographic conditions were determined on the basis of the response at signal to noise ratios of 3 and 10, respectively. These data are summarized in Table 1.

The precision of the developed UPLC method was de-

Table 2. Intra- and inter-day variations of the ultra performance liquid chromatography photo diode array detector method for the determination of 30 ginsenosides ($n=3$)

Analytes	Red ginseng powders				Red ginseng concentrates			
	Intra-day precision		Inter-day precision		Intra-day precision		Inter-day precision	
	Content (mg/g)	RSD (%)	Content (mg/g)	RSD (%)	Content (mg/g)	RSD (%)	Content (mg/g)	RSD (%)
NG-R1	0.16	2.30	0.16	2.28	0.13	2.40	0.13	3.69
G-Rg1	2.96	1.89	2.95	1.92	2.26	1.11	2.47	8.84
G-Re	1.96	1.94	1.95	1.95	2.40	2.82	2.58	7.50
G-Rf	0.76	1.79	0.76	2.04	1.04	1.27	1.11	6.78
20(S)-G-Rh1	0.25	1.96	0.25	1.99	0.81	0.80	0.88	7.26
20(S)-G-Rg2	0.27	2.82	0.27	2.62	0.90	0.89	0.96	7.02
20(R)-G-Rg2	0.08	4.63	0.08	4.00	0.44	2.96	0.45	4.01
G-Ro	0.91	2.38	0.92	3.92	1.23	3.34	1.25	4.68
G-Rb1	5.47	3.30	5.46	3.33	8.90	0.57	9.57	7.26
G-Rc	1.38	3.79	1.38	3.92	2.67	1.18	2.85	6.61
G-Ra1	1.63	2.63	1.63	2.67	3.19	1.39	3.24	2.17
G-F1	ND	ND	ND	ND	ND	ND	ND	ND
G-Rb2	2.33	2.51	2.32	2.74	3.81	0.58	3.77	7.73
G-Rb3	0.38	2.44	0.37	2.48	0.62	0.85	0.62	2.11
G-Rd	0.46	3.24	0.46	3.41	0.94	0.77	1.02	7.54
G-Rg6	0.06	2.51	0.06	2.71	0.26	0.96	0.26	1.55
G-Rk3	0.07	3.30	0.07	2.68	0.16	2.99	0.16	3.36
G-F4	0.11	2.71	0.11	3.16	0.53	1.31	0.52	1.77
G-Rh4	0.11	2.69	0.11	3.04	0.31	1.36	0.31	1.68
G-F2	ND	ND	ND	ND	ND	ND	ND	ND
20(S)-G-Rg3	0.15	5.53	0.15	6.04	0.88	1.92	0.95	7.64
20(R)-G-Rg3	0.05	8.81	0.05	10.19	0.43	8.29	0.46	9.99
C-Y	ND	ND	ND	ND	ND	ND	ND	ND
C-K	ND	ND	ND	ND	ND	ND	ND	ND
G-Rk1	0.05	2.78	0.05	2.88	0.31	24.98	0.33	32.17
G-Rg5	0.05	6.06	0.05	6.48	0.57	20.59	0.58	20.43
20(S)-G-Rh2	ND	ND	ND	ND	ND	ND	ND	ND
20(R)-G-Rh2	ND	ND	ND	ND	ND	ND	ND	ND
G-Rk2	ND	ND	ND	ND	ND	ND	ND	ND
G-Rh3	ND	ND	ND	ND	ND	ND	ND	ND

RSD, relative standard deviations; NG, notoginsenoside; G, ginsenoside; C, compound; ND: not detected.

Table 3. Accuracy of ultra performance liquid chromatography photo diode array detector method for the determination of 30 ginsenosides (*n*=3)

Analytes	Red ginseng powder					Red ginseng concentrate				
	Original (mg)	Spiked (mg)	Found (mg)	Recovery (%)	RSD (%)	Original (mg)	Spiked (mg)	Found (mg)	Recovery (%)	RSD (%)
NG-R1	ND	ND	ND	ND	ND	0.05	0.03	0.07	84.8	16.18
G-Rg1	1.33	1.24	2.56	99.1	1.74	1.03	1.48	2.47	97.4	0.67
G-Re	0.90	1.40	2.28	98.5	1.65	1.09	1.70	2.72	96.1	0.72
G-Rf	0.35	0.84	1.16	97.0	1.12	0.46	0.98	1.40	95.8	0.70
20(S)-G-Rh1	0.11	0.66	0.75	97.3	0.65	0.37	0.81	1.10	90.5	4.17
20(S)-G-Rg2	0.11	1.00	1.08	97.5	0.60	0.41	1.33	1.64	92.8	0.66
20(R)-G-Rg2	0.02	0.59	0.66	107.6	3.51	0.23	0.69	0.92	101.0	1.11
G-Ro	0.70	0.40	1.18	121.0	4.71	0.52	0.32	0.80	88.3	6.57
G-Rb1	2.69	5.11	7.63	96.5	0.89	4.02	6.12	9.74	93.4	0.95
G-Rc	0.79	1.52	2.30	99.2	0.80	1.22	1.93	3.05	94.7	0.28
G-Ra1	1.03	2.14	3.19	100.7	1.06	1.36	2.02	3.31	96.0	0.30
G-F1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G-Rb2	0.96	2.42	3.39	100.8	0.48	1.76	2.90	4.52	94.9	1.02
G-Rb3	0.20	0.45	0.66	101.5	0.69	0.26	0.44	0.68	93.9	0.44
G-Rd	0.22	1.02	1.20	95.5	0.05	0.43	1.24	1.62	96.6	0.49
G-Rg6	0.03	0.53	0.54	96.0	0.98	0.10	0.47	0.56	99.5	1.32
G-Rk3	0.04	0.28	0.31	95.5	0.35	0.06	0.26	0.32	98.8	1.68
G-F4	0.07	1.05	1.07	95.0	0.26	0.21	0.95	1.14	97.2	0.99
G-Rh4	0.07	0.51	0.56	95.1	1.65	0.12	0.46	0.57	96.7	1.37
G-F2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
20(S)-G-Rg3	0.08	2.92	2.86	95.1	0.13	0.42	3.56	3.89	97.4	1.26
20(R)-G-Rg3	0.05	1.00	0.95	90.9	0.62	0.25	1.21	1.40	95.1	1.10
C-Y	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C-K	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G-Rk1	0.04	1.64	1.55	92.0	0.32	0.13	1.63	1.70	96.0	1.37
G-Rg5	0.08	2.92	2.73	90.8	0.91	0.25	2.64	2.81	96.9	1.16
20(S)-G-Rh2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
20(R)-G-Rh2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G-Rk2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G-Rh3	ND	ND	ND	ND	ND	0.00	0.03	0.03	111.8	2.27

RSD, relative standard deviations; NG, notoginsenoside; G, ginsenoside; C, compound; ND, not detected.

terminated by intra-and inter-day variations. Red ginseng and red ginseng concentrate samples were extracted and analyzed as described in the materials and methods sections. Table 2 shows a summary of the intra-and inter-day precision. The intra-and inter-day precision (RSD) of the red ginseng powder ranged from 1.8 to 10.2 with the three different sample amounts. However, in the case of the red ginseng concentrate, the intra-and inter-day variations tended to be a little too wide for the ginsenoside Rk1 and ginsenoside Rg5.

The accuracy of the developed method was tested by

spiking experiments for recovery investigations. Thus, crude saponin fractions were spiked into the analytical samples for recovery tests of the 30 ginsenosides. As shown in Table 3, the recovery values of the red ginseng powders and concentrates ranged from 89% to 108% and from 98% to 118%, respectively. However, with regards to the precision and accuracy experiments, it should be confirmed that the developed method was suitable for simultaneous determination of ginsenosides in the red ginseng and concentrates.

Table 4. The contents of 30 ginsenosides in various *Panax ginseng* preparations (n=3)

Analytes	Red ginseng powder	Red ginseng concentrate	Fermented ginseng extract	Cosmetic raw materials
NG-R1	0.153±0.004	0.125±0.003	0.030±0.002	ND
G-Rg1	2.886±0.068	2.245±0.017	1.057±0.083	7.118±0.117
G-Re	1.920±0.045	2.361±0.026	1.577±0.126	ND
G-Rf	0.745±0.016	1.028±0.006	0.067±0.003	1.746±0.157
20(S)-G-Rh1	0.241±0.007	0.813±0.004	0.334±0.027	7.270±0.455
20(S)-G-Rg2	0.272±0.010	0.897±0.007	0.261±0.021	2.092±0.455
20(R)-G-Rg2	0.076±0.004	0.442±0.010	0.067±0.005	ND
G-Ro	0.914±0.052	1.248±0.013	0.384±0.030	5.432±0.282
G-Rb1	5.222±0.166	8.901±0.040	0.204±0.016	ND
G-Rc	1.346±0.038	2.682±0.020	0.267±0.022	ND
G-Ra1	1.593±0.049	3.214±0.026	0.633±0.055	ND
G-F1	ND	ND	0.043±0.003	ND
G-Rb2	2.260±0.068	3.816±0.023	0.458±0.037	ND
G-Rb3	0.368±0.009	0.619±0.004	0.112±0.022	ND
G-Rd	0.448±0.014	0.949±0.004	1.230±0.096	ND
G-Rg6	0.063±0.001	0.264±0.002	ND	6.940±0.716
G-Rk3	0.067±0.001	0.163±0.003	ND	15.643±0.208
G-F4	0.110±0.002	0.535±0.003	0.073±0.005	13.042±1.302
G-Rh4	0.109±0.004	0.313±0.003	0.046±0.003	37.570±0.763
G-F2	ND	ND	1.929±0.138	ND
20(S)-G-Rg3	0.144±0.006	0.884±0.005	0.189±0.011	80.291±2.569
20(R)-G-Rg3	0.045±0.003	0.442±0.005	0.061±0.003	17.957±0.837
C-Y	ND	ND	0.107±0.007	ND
C-K	ND	ND	0.169±0.009	ND
G-Rk1	0.051±0.004	0.296±0.002	0.023±0.001	43.184±0.815
G-Rg5	0.051±0.004	0.569±0.006	ND	61.347±0.773
20(S)-G-Rh2	ND	ND	ND	1.158±0.107
20(R)-G-Rh2	ND	ND	ND	0.332±0.005
G-Rk2	ND	ND	ND	3.923±0.195
G-Rh3	ND	ND	ND	0.969±0.027

NG, notoginsenoside; G, ginsenoside; C, compound; ND, not detected.

Analysis of 30 ginsenosides in *Panax ginseng* preparations

There are various types of commercial red ginseng products such as red ginseng powder, concentrates, tonic, tablets, tea, and candy. Among these, the most representative red ginseng products are red ginseng powder and red ginseng concentrates. Thus, these two types of samples were analyzed with the newly developed UPLC method because it was assumed that the different types of red ginseng products contain different types of ginsenoside patterns and different contents of total ginsenosides. To demonstrate the usefulness of the proposed method, a fermented ginseng extract and cosmetic raw

materials were analyzed. The UPLC chromatograms of the various *P. ginseng* preparations are shown in Fig. 4 and the contents of the 30 ginsenosides involved in the *P. ginseng* preparations are represented in Table 4.

Ginsenoside Ro, Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, 20(S)-Rg2, 20(R)-Rg2, 20(S)-Rg3, 20(R)-Rg3, 20(S)-Rh1, F4, Ra1, Rg6, Rh4, Rk3, Rg5, Rk1, Rb3, and Notoginsenoside R1 were found in both the red ginseng powder and red ginseng concentrates samples. Furthermore, the red ginseng concentrate contained a substantially higher amount of 20(S)-ginsenoside Rg2, 20(R)-Rg2, Rg6, Rk3, F4, Rh4, 20(S)-Rg3, 20(R)-Rg3, Rk1, Rg5 than the red ginseng powder. 20(S)-ginsenosides

Rg3, 20(R)-Rg3, Rk1 and Rg5 can be formed by heat processing through glucosyl elimination and the epimerization of Rb1 [2,31,32]. 20(S)-ginsenosides Rg2, 20(R)-Rg2, Rg6, Rk3, F4, and Rh4 can be formed by the deglycosylation process from Rg1 and Re [2,31,33]. Thus these ginsenosides were likely produced during the process of making the concentrates. To make the concentrates, red ginseng roots are boiled and condensed for a long period of time. In this period, the sugars attached to the aglycones can be removed under conditions of high temperature and pressure, producing various transformed ginsenosides such as 20(S)-Rg2, 20(R)-Rg2, Rg6, Rk3, F4, Rh4, 20(S)-Rg3, 20(R)-Rg3, Rk1, and Rg5. We know as a fact that compound Y and compound K were observed only in the fermented ginseng extract sample; in addition, the cosmetic raw materials contained high amounts of conversion products of ginsenoside such as ginsenoside Rk3, F4, Rh4, Rk3, 20(S)-Rg3, 20(R)-Rg3, Rk1, and Rg5. Because of this raw material was acid hydrolyzed product from ginsenosides enriched *P. ginseng* preparations.

In this study, UPLC-PDA conditions were optimized for the quantitative and qualitative determinations of 30 ginsenosides. Also, 30 ginsenosides were determined in various *P. ginseng* preparations. This developed method is rapid, accurate and precise, and it can simultaneously determine the 30 ginsenosides in various *P. ginseng* preparations. These results are definitely helpful in the process of quality control of ginseng products and provide a scientific basis for the research on the components that are responsible for the pharmacological effects of red ginseng and related products. In conclusion, this method may be helpful in the development of new functional materials for cosmetics and natural drugs using other parts of the ginseng plant such as the leaf, flower, seed and berry because of its wide range of applications due to the simultaneous analysis of many kinds of ginsenosides.

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