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# Simplified Extraction of Ginsenosides from American Ginseng (*Panax quinquefolius* L.) for High-Performance Liquid Chromatography−Ultraviolet Analysis

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Four methods were tested for extraction and recovery of six major ginsenosides (Rb1, Rb2, Rc, Rd, Re, and Rg1) found in roots of American ginseng (*Panax quinquefolius*): method A, sonication in 100% methanol (MeOH) at room temperature (rt); method B, sonication in 70% aqueous MeOH at rt; method C, water extraction (90 °C) with gentle agitation; and method D, refluxing (60 °C) in 100% MeOH. After 0.5−1 h, the samples were filtered and analyzed by high-performance liquid chromatography (HPLC)−UV. A second extraction by methods C and D was done, but 85−90% of ginsenosides were obtained during the first extraction. Lyophilization of extracts did not influence ginsenoside recovery. Method D resulted in the highest significant recoveries of all ginsenosides, except Rg1. Method C was the next most effective method, while method A resulted in the lowest ginsenoside recoveries. Method B led to similar recoveries as method C. All methods used one filtration step, omitted time-consuming cleanup, but maintained clear peak resolution by HPLC, and can be used for quantitative screening of ginsenosides from roots and commercial ginseng preparations.

**KEYWORDS: American ginseng; *Panax quinquefolius*; HPLC−UV; liquid chromatography; ginsenoside extraction**

## INTRODUCTION

The herb "ginseng" often refers to members in the family Araliaceae within the genera *Panax* (*Panax ginseng* and *Panax quinquefolius*) and *Eleutherococcus* (*Eleutherococcus senticosus*). The use of *P. ginseng* (Chinese ginseng) for medicinal purposes dates back over 5000 years, beginning with ancient traditional Chinese medicine. Native Americans also used *Panax quinquefolius* (American ginseng) as a health food. Current commercial interest is based upon the purported benefits of ginseng for general health, including positive effects on the endocrine, cardiovascular, immune, and central nervous systems (*1*, *2*), prevention of fatigue, oxidative damage, mutagenicity, stimulation of male copulatory behavior in rats (*3*), and cancer prevention (*4*−*6*).

Ginsenosides fall within a group of approximately 30 plant saponins present in the roots, leaves, berries, and other parts of the ginseng plant (*7*). The most abundant ginsenosides in American ginseng root tissue are the R-family members, which include Rb1, Rb2, Rc, Rd, Re, and Rg1 (**Figure 1**). The ginsenosides in this family have either 20(*S*)-protopanaxadiol or 20(*S*)-protopanaxatriol (both steroidal triterpenes) as aglycon, besides ginsenoside Ro (oleanolic acid). The 20(*S*)-protopanaxadiol ginsenosides (Rb1, Rb2, Rc, and Rd) have two sugar moieties (glycons) and the 20(*S*)-protopanaxatriol ginsenosides (Re and Rg1) have three glycons (*8*). The pharmacological activity of ginsenosides is based primarily upon the structural aspects of the molecule, which is a glycosylated steroidal triterpene (**Figure 1**). Although much of the research focuses on crude ginseng extracts, combinations of ginsenosides as well as individual ginsenosides have been attributed to having a diverse range of pharmacological activity. For example, individual ginsenosides such as Rb1 have been shown to act as phytoestrogens in breast cancer cells via activation of the estrogen receptors (*9*). Individual ginsenosides Rc and Rh2 have demonstrated inhibitory effects on the proliferation of human breast cancer cells (*10*, *11*), while ginsenoside Rg1 has been shown to serve as a functional ligand of the glucocorticoid receptor (*12*) and as a suppressor of oxidative stress (*13*).

A variety of extraction, purification, and analysis procedures have been utilized to obtain and quantify ginsenosides from leaves (*14*, *15*), berries (*16*), or, most commonly, roots (*17*−*19*, *20*, *22*). Most extraction procedures utilize methanol or ethanol (*18*, *19*) as solvents, either pure or as an aqueous solution

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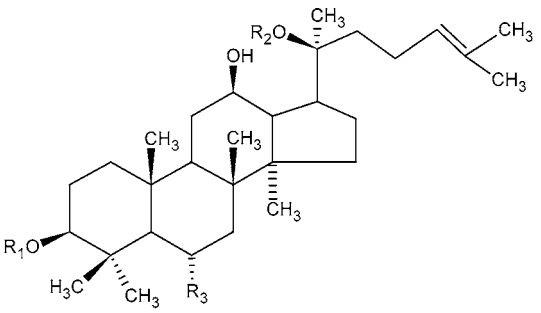

| Ginsenoside | R1 | R2 | R3 |
|---|---|---|---|
| Rb1 | -glc(2→1)glc | -glc(6→1)glc | -H |
| Rb2 | -glc(2→1)glc | -glc(6→1)ara (p) | -H |
| Rc | -glc(2→1)glc | -glc(6→1)ara (f) | -H |
| Rd | -glc(2→1)glc | -glc | -H |
| Re | -H | -glc | -O-glc(2→1)rha |
| Rg1 | -H | -glc | -O-glc |

Glc = glucose, ara (p) = alpha-L arabinopyranose, ara (f) = alpha-L arabinofuranose, rha = rhamnose.

**Figure 1.** Structure of the main neutral ginsenosides from the roots of *Panax quinquefolius*.

(*20*), and often coupled with heat, refluxing, or sonication (*21*−*24*). Subsequent purification can include cloud-point extraction/salting out (*25*), oxidative cleavage and trimethylsilylation (*21*), solid-phase extraction (*26*−*28*), or additional extraction with diethyl ether/*n*-butanol (*14*, *28*−*32*). Quantitative analysis most commonly employs high-performance liquid chromatography (HPLC) coupled to photodiode array (*20*), UV (*26*) or mass spectrometric detection *(33*, *34)*, although IR spectroscopy, gas chromatography−mass spectrometry (GC-MS) (*21*), and enzyme-linked immunosorbent assays (ELISA) (*14*, *35*) have also been used. The structural similarity of the ginsenosides has made separation and identification difficult, and sample preparation is often time-consuming (*19*, *21*, *29*, *36*, *37*), requiring additional steps that may decrease ginsenoside recovery. The primary objective of this research was to test four methods to establish a simplified, yet effective, protocol for the extraction of major ginsenosides from the roots of *P. quinquefolius* with minimal sample preparation.

## MATERIALS AND METHODS

The four methods tested here compared methanol and water (alone or in combination) as solvents in combination with sonication, heat, and refluxing for the extraction of ginsenosides. The effects of a second extraction (for methods C and D) and of lyophilization (of aqueous extracts only) on ginsenoside recovery were also evaluated. To simplify sample purification and preparation steps after extraction, samples were filtered once through a nylon membrane before HPLC analysis.

**Solvents and Standards.** Solvents (HPLC-grade water, methanol, and acetonitrile) were obtained from Fisher Scientific (Pittsburgh, PA). Ginsenosides Rg1, Re, Rb1, Rc, Rb2, and Rd were obtained from Indofine Chemical Co. (Hillsborough, NJ), and ginsenosides F2 and F3 were purchased from LKT Laboratories Inc. (St. Paul, MN). Stock solutions were prepared by adding 1.0−2.5 mg/mL of these ginsenosides to methanol prior to HPLC analysis and were stored at −20 °C. A standard mixture of Rg1, Re, Rb1, Rc, Rb2, and Rd was run by HPLC with each set of samples to calculate the ginsenoside content in root extracts (**Figure 2**). The concentration of each standard in the standard mix is specified at the end of the HPLC analysis section.

**Ginseng Roots and Extraction Methods.** Powdered *Panax quinquefolius* roots used in this work were obtained from the Ginseng Board (Wausau, WI) and were approximately 4 years old at time of harvest. Powdered ginseng roots were extracted with 100% methanol plus sonication at room temperature (method A), 70% methanol plus sonication at room temperature (method B), water at 90 °C with gentle agitation (method C), or refluxing in 100% methanol at 60 °C (method D). Extracts from method C were analyzed before or after lyophilization to assess the impact of this treatment on ginsenoside recovery. Three replicates were used in all experiments, and the extraction methods are detailed below.

**Methanol Extraction.** Dry powdered ginseng root tissue (5 g) was extracted for 0.5 h by 100% methanol (method A) or aqueous (70%) methanol (method B), with the slurries subjected to sonication at room temperature. Roots were also extracted for 1 h by refluxing with 45 mL of methanol at 60−65 °C (method D) or in a water bath/sonicator (model FS220; Fisher Scientific, Pittsburgh, PA). First and second methanol extractions were obtained as described below for the water extracts but with methanol instead. Methanol extracts were stored at −20 °C for no more than 3 days prior to HPLC analysis. Samples were filtered once through a 0.2 $\mu$m nylon filter before HPLC analysis.

**Water Extraction.** Dry powdered ginseng roots (5 g) were extracted for 1 h with 45 mL of deionized water in a water bath (model R76, New Brunswick Scientific Co., Inc., Edison, NJ) at ∼90 °C with gentle agitation (method C). After cooling, each sample was centrifuged (Marathon 3200R, Fisher Scientific, Thermo IEC, Needham Heights, MA) for 15 min at 2500*g* (23 °C), and the supernatant was decanted. A 10-mL aliquot (referred to hereafter as the first extraction) was taken from the supernatant and stored at −20 °C for no more than 3 days prior to analysis. The pellet was resuspended by vortexing in 25 mL of deionized water and then reextracted for an extra hour under the same conditions as the first water extraction. After cooling, each sample was again centrifuged and the supernatant was decanted. A 5-mL aliquot (referred to hereafter as the second extraction) was taken from the supernatant and stored at −20 °C for no more than 3 days prior to analysis. After the two aliquots were taken, the remaining supernatants from the first and second extractions were combined. An aliquot of the combined extracts was lyophilized (Labconco, Freezone 6, Labconco Corp., Kansas City, MO) and stored as powder at −20 °C for no more than 2 days prior to analysis. The lyophilized samples (0.5 g) were resuspended in 7.0 mL of 18 mΩ water, maintaining the same water-to-dry matter ratio as the prelyophilized samples. This lyophilized extract was compared to the combined extract not subjected to lyophilization to determine if this treatment step influenced ginsenoside recovery. All aliquots and remaining supernatants were taken into consideration for total ginsenoside recovery.

**Ginsenoside Recovery.** In a separate experiment, 1 g of ground ginseng roots was spiked with 1.0 mg/mL ginsenoside F2 to determine ginsenoside recovery after hot water extractions at 90 °C (method C) or methanol refluxing at 60−65 °C (method D). The extractions were completed as stated previously, except that the first extractions were done with 9 mL of solvent and the second extractions were done with 5 mL of solvent to match root:solvent ratios from previous experiments. No aliquots were taken following these extractions. The supernatants of the first and second extraction were combined and brought to 14 mL with either distilled water or 100% methanol. A clean spike solution, consisting of 0.264 mg/mL ginsenosides F2 added to distilled water, but not subjected to extraction, was used to calculate recovery of ginsenosides. Recovery was estimated by comparing the peak area of F2 in the clean spike to the peak area of F2 added to an extracted root sample as an internal standard.

**Ginsenoside F2 Stability.** HPLC-grade water was spiked with a known quantity of ginsenoside F2 and incubated for 2 h at 60 °C with no agitation. An aliquot was taken for HPLC analysis, and the remaining F2 solution was subjected to 90 °C for an additional 2 h with no agitation. At the end of the second incubation, another aliquot was analyzed by HPLC. These temperatures were chosen to mimic the extractions of methanol and water, respectively. The HPLC peak areas of the aliquots from the clean spikes heated to 60 and 90 °C were compared to the peak area of a clean spike not submitted to heat to verify the temperature effect on F2 stability.

**HPLC Analysis.** Ginsenoside analysis was performed on a Hitachi (Hitachi Technologies, Atlanta, GA) HPLC with an L-7100 gradient pump equipped with a degasser, an L-7250 autosampler, and an L-7455

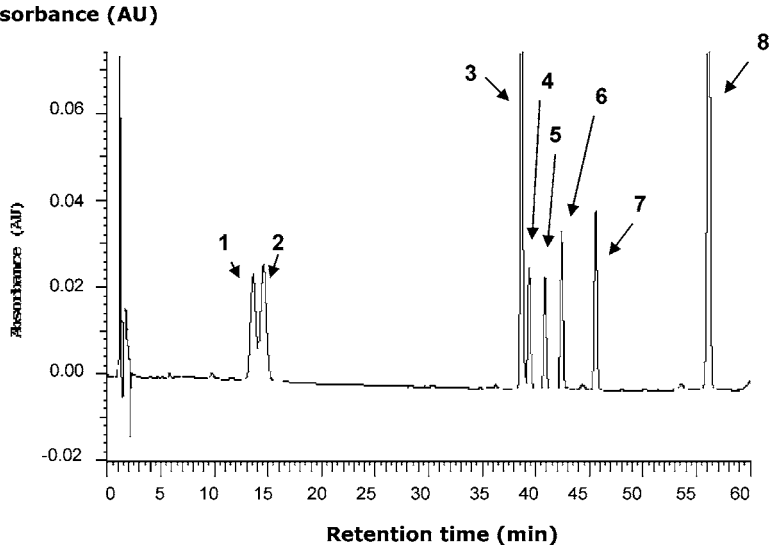


**Figure 2.** HPLC chromatogram of 20 $\mu$L of the following ginsenosides at concentrations ranging from 0.16 to 0.45 mg/mL: 1 = Rg1, 2 = Re, 3 = F3, 4 = Rb1, 5 = Rc, 6 = Rb2, 7 = Rd, and 8 = F2. Column was an end-capped Purosphere RP C-18 250 mm × 4.6 mm i.d. Mobile phase was a binary gradient of acetonitrile/water with flow rate = 1.3 mL/min and detection at 203 nm. Rg1 and Re eluted with acetonitrile at 21%, while the other ginsenosides were eluted with acetonitrile at 42%. The retention times (minutes) are as follows: Rg1, 13.63; Re, 14.59; F3, 38.67; Rb1, 39.39; Rc, 40.85; Rb2, 42.40; Rd, 45.57; and F2, 56.13.

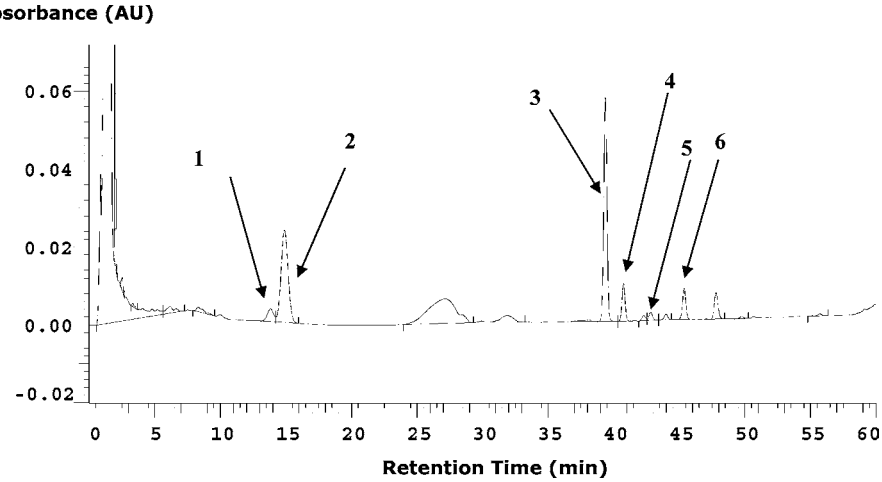


**Figure 3.** HPLC chromatogram of ginsenosides (1 = Rg1, 2 = Re, 3 = Rb1, 4 = Rc, 5 = Rb2, 6 = Rd) present in a ginseng root powder (at 71.4 mg/mL) extracted in water at 90 °C in two subsequent 1-h extractions, and filtered through a 0.2 $\mu$m membrane before HPLC analysis. The retention times (minutes) are as follows: Rg1, 13.84; Re, 14.91; Rb1, 39.96; Rc, 40.75; Rb2, 42.27; and Rd, 45.36.

diode-array detector (DAD). Data were collected with the Hitachi D-7000 HPLC System Manager software. Samples from all experiments were filtered through 25 mm, 0.2 $\mu$m nylon Millex-GN filters (Millipore Corp., Bedford, MA) attached to disposable 3-mL syringes prior to injection. The column was an end-capped Purosphere (Hitachi Technologies, Atlanta, GA) C18-RP 250 × 4.6 mm (5.0 $\mu$m pore size), kept at room temperature. The mobile phase, modified from a previous study (*38*), was a binary gradient of acetonitrile (A) and water, at a flow rate of 1.3 mL/min, as follows: 0−15 min, 21% A; 16−38 min, 30% A; 39−55 min, 42% A; 56−65 min, 90% A; and 66−80 min, back to 21% A before the next injection. The method used a cut injection system with 20 $\mu$L of both lead and rear volume, and detection performed at 203 nm for all ginsenosides (**Figures 2** and **3**). Individual ginsenosides from the extracts were identified and quantified by retention time and peak areas, respectively, as compared to pure standards of the following ginsenosides (= milligrams per milliliter): Rg1 = 0.30, Re = 0.16, Rb1 = 0.45, Rc = 0.30, Rb2 = 0.32, and Rd = 0.41 (**Figure 2**). Due to the similar structure of the ginsenosides under study, a standard curve (0.08−2.7 mg/mL) was created ($R$ = 0.999; data not shown) with ginsenoside Rb1 to verify the linearity and reproducibility of the assay. The concentrations chosen for the standard curve bracketed the ginsenoside levels of root tissues in this study.

**Statistical Analysis.** Data were analyzed by a one-way ANOVA or two-way ANOVA by use of the SPSS 10.0 software package. Tukey's post hoc analyses were used when significant differences were attained from the initial ANOVA and multiple comparisons were necessary. Standard error bars were calculated from three replicates, with an $\alpha$ level of 0.05 used to establish significance.

## RESULTS AND DISCUSSION

Previous research has revealed that different solvents yield various concentrations of the individual ginsenosides (*20*, *23*, *25*, *39*). Yield also varies depending on the physical/chemical approach (*22*, *23*, *37*, *40*) and extraction time used (*18*, *39*).

**Extraction Solvents and Methods.** The solvents and methods used here (**Figure 4**) will be referred to as methanol 100% + sonication at room temperature for 0.5 h (method A), methanol 70% + sonication at rt for 0.5 h (method B), water at 90 °C with gentle agitation for 1 h (method C), and methanol

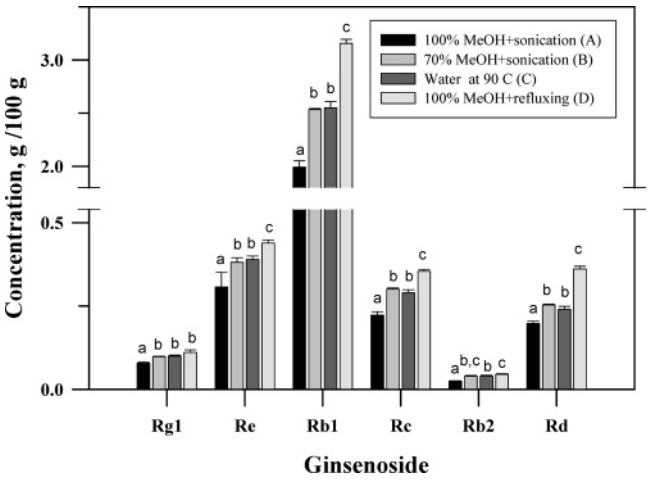


**Figure 4.** Concentration of ginsenosides (w/w) from ginseng roots extracted after a single extraction by method A (100% MeOH with sonication for 0.5 h at rt), method B (70% MeOH with sonication for 0.5 h at rt), method C (water at 90 °C with gentle agitation for 1 h), and method D (refluxing in 100% MeOH at 60–65 °C for 1 h). For all ginsenosides, in each treatment, $n = 3$; standard errors are indicated for each treatment compared for the ginsenoside extraction. Letters indicate significance between treatments within each individual ginsenoside, not among ginsenosides.

**Table 1.** Total Mass of Six R-Family Ginsenosides

| | ginsenoside extracted | | | |
|---|---|---|---|---|
| ginsenoside[a] | water extraction at 90 °C[b] (mg) | MeOH extraction at 60 °C[c] (mg) | % (w/w) in MeOH extraction[d] | rel. recovery % (w/w) of water extraction[e] |
| Rg1 | 6.21 (0.17) | 6.18 (0.43) | 0.13 | 100.5 |
| Re | 22.92 (0.89) | 24.67 (0.39) | 0.50 | 92.9 |
| Rb1 | 144.39 (7.35) | 185.12 (1.50)* | 3.70 | 78.0 |
| Rc | 15.86 (0.96) | 20.84 (0.24)* | 0.41 | 76.1 |
| Rb2 | 2.23 (0.31) | 2.66(0.09) | 0.05 | 83.8 |
| Rd | 13.27 (0.99) | 21.17 (0.48)* | 0.42 | 62.7 |
| total | 204.88 | 260.64 | 5.21 | 78.6 |

[a] The ginsenosides are presented in the order they eluted from the HPLC column. Values represent the sum of ginsenosides recovered from 5.0 g of dried ginseng roots by the first and second extractions and are expressed as the means ($n = 3$) and standard errors (in parentheses). [b] Method C. [c] Method D. Significant differences ($p < 0.05$) are denoted with an asterisk. [d] Ginsenoside content (w/w) was calculated on the basis of the two extractions of method D. [e] Relative recovery expresses the mass recovered by method C as a percentage of recovery by method D.

(100%) refluxing at 60−65 °C for 1 h (method D). The methods were evaluated by the amount of individual and total ginsenosides extracted, determined by HPLC. The sample preparation was kept to a minimum without matrix background interference in the identification and quantification of individual ginsenosides (**Figure 3**). Method D yielded a significantly greater ($p \leq 0.002$) concentration for all six R-family ginsenosides except for Rg1 and Rb2 (method B ≃ C = D) than the other methods (**Table 1**, **Figure 4**). Our results were similar to studies (*40*) where Soxhlet extraction of ginseng roots in 95% ethanol extracted more of the same six ginsenosides tested here than sonication and support other studies (*22*) where heat increased sonication effectiveness. Yet other studies (*41*) showed that sonication was better than heat, especially for extraction of malonyl ginsenosides, which are more sensitive to heat degradation and will convert to their corresponding neutral ginsenosides (*22*). Method C was the second best method but was similar to method B in ginsenoside extraction (**Figure 4**). Other methods (*17*, *42*) tested sonication of samples in 70% methanol at room temperature, here tested as method B, but outperformed by method D. Structural changes in ginsenosides occurred when raw (air-dried) ginseng was compared with red (steamed from 100 to 120 °C) ginseng (*30*). These authors reported that ginsenosides Rb1, Rb2, Rc, Rd, Re, and Rg1 decreased, while ginsenosides F4, Rg3, and Rg5 increased after steaming. The high total ginsenoside yield observed in our study after heating of the samples to 90 °C contradicts the results of Kim et al. (*30*). Method A consistently provided the lowest yields for all ginsenosides, being outperformed by the other three methods (**Figure 4**). This observation is consistent with results obtained from comparing aqueous methanol with 100% methanol as solvents (*20*, *23*, *43*). Wu et al. (*22*) also found methanol less effective than water-saturated butanol when each solvent was used in combination with ultrasound-assisted extraction. However, the authors acknowledged that the use of water-saturated butanol requires a higher temperature of extraction (118 °C vs 65 °C), which makes the sample more difficult to evaporate, and may cause thermal degradation. The methods tested here were similar or better than some conventional extractions, which take from 3 to 10 h (*18*, *22*, *28*, *39*).

**Total Ginsenoside Extraction. Table 1** shows the total amount of individual ginsenosides (milligrams) obtained from two extractions by methods C and D, individual and total ginsenoside content (w/w %), and relative recoveries determined by HPLC. Our results agree with those of others (*23*), which indicate that polar organic solvents, such as methanol used in method D [52 mg $g^{-1}$ DW (dry weight), or 5.21% (w/w)] are better than aqueous solvents, used in method C [41 mg $g^{-1}$ DW, or 4.1% (w/w)] for ginsenoside extraction. Total ginsenoside content from both extractions with method D was greater than that achieved from both extractions with method C, although significant differences were observed only for ginsenosides Rb1, Rc, and Rd ($p \leq 0.005$) (**Table 1**). Similar results were observed with ethanol + Soxhlet being better than water + Soxhlet for all ginsenosides except Rd (*25*). Other studies showed similar ginsenoside content (5.2% w/w) and recoveries (>95%) for *P. quinquefolium* roots extracted with 70% methanol (*21*). Variability in ginsenoside extraction could be due to type and concentration of solvent used, length of extraction, number of extractions, and method of tissue disruption. Other nonphysical/chemical factors could include environmental factors such as soil composition (*44*−*46*), light availability (*8*, *45*, *47*), season of harvest (*48*, *49*), genetic variability (*50*, *51*), age of root (*52*, *53*), drying procedures (*30*), and sections of root used (*26*, *54*).

**First versus Second Extraction.** The number of extractions performed for ginsenoside from roots range from a single extraction **(***38*) up to five extractions (*20*, *55*). Some studies (*20*) reported that the first extraction yielded the highest amount of ginsenosides from the tissue. Thus, methods C and D were used to evaluate the efficiency of a second extraction. The second extraction was performed on the pellet resulting from the first extraction. In this study, 11.8% of the total ginsenoside content was extracted during the second extraction with method C, and 12.3% of the total ginsenoside content was extracted during the second extraction with method D. These results are slightly higher than another study where only 8% of all ginsenosides were obtained during the second extraction with sonication (*20*). Another study (*55*) found that a fourth extraction removed only 0.4% of the total ginsenosides. Under industry standards, a minimum of 70% recovery is expected, and a single extraction here produced 87% or better for all ginsenosides tested. A longer first extraction could produce better results,

but methanol + reflux for 4 h (*28*) resulted in a lower total ginsenoside content [<3.3% (w/w) ginsenosides] than the one obtained here in two 1-h extractions (5.2%, **Table 1**). Wu et al. (*22*) reported that an 8-h traditional extraction by refluxing was not better than sonication for 1−2 h (*22*). The same relative trend observed in total ginsenoside content (**Table 1**) was observed for the second extraction by method D (for Rb1, Rc, and Rd) compared to method C (results not shown).

**Ginsenoside Recovery.** Methods C and D were also used to estimate extraction efficiency, by use of powdered ginseng roots spiked with ginsenoside F2. Ginsenoside F2 was chosen as an internal standard to calculate recovery due to its low concentration in roots (background levels subtracted) and lack of overlapping with retention time of other ginsenosides. Stability of F2 after extraction with method D was 111.1%, significantly greater than that after method C with (43.4%) or without (50.9%) lyophilization. The stability of F2 for the water extracts was surprisingly low and suggests that high temperatures increase its degradation. This temperature lability led to low calculated recoveries of ginsenosides extracted by method C. However, this was an artifact because individual ginsenosides peak areas did not differ between methods C and D.

Some ginsenosides, particularly the acidic ones, are more sensitive to heat and can be degraded during extraction (*20*, *22*, *37*, *54*). Also, research suggests that thermal degradation may result from prolonged heating of the sample (*39*). For example, while water-saturated *n*-butanol is a highly effective solvent for total ginsenosides, its boiling point is around 118 °C, at which degradation of heat-sensitive ginsenosides would be expected.

To examine the extent of heat degradation, water spiked with the F2 ginsenoside was treated sequentially with the two temperature regimes, pertinent to both methanolic refluxing (60 °C) and hot water (90 °C) extractions. There was no significant difference in F2 recovery between methanol spikes (control) and samples incubated for 2 h in methanol at 60 °C. However, when these samples were transferred to 90 °C for 2 h, complete degradation of F2 was observed (data not shown). Although F2 ginsenoside seems to be more temperature-labile than the R-family ginsenosides, the results suggest that extraction with method D minimizes heat degradation and provides a higher recovery than method C. If R-ginsenosides were to show a sensitivity to heat, comparable to F2, the lower recovery estimated for their water extraction (method C) compared to their methanolic extraction (method D) could be explained (**Table 1**). Therefore, the relative heat sensitivity of these ginsenosides could be estimated through their relative recoveries by method C. Recoveries from method C were >92% of the recoveries by method D for the ginsenosides Rg1 and Re but <84% of recoveries by method D for ginsenosides Rb1, Rc, and Rb2. Ginsenoside Rd showed the lowest relative recovery, being only 62.7% of the methanol extraction recovery (**Table 1**). This suggests that a heat lability trend for the R-family ginsenosides has not yet been established. Kim et al. (*30*) demonstrated that the ginsenoside profiles were altered after ginseng roots were steamed at 110 °C, resulting in the production of ginsenosides undetected in raw ginseng root, but with significantly lower recoveries for ginsenosides Rg1, Re, Rd, Rc, Rb2, and Rb1.

**F2 Stability.** A comparison between F2-spiked tissue samples extracted with 100% methanol at 60 °C or water at 90 °C resulted in a 46.9% recovery of F2 in water and 79.6% recovery in methanol. These results are consistent with the increased degradation of F2 reported in the Ginsenoside Recovery section. The specific effect of heat on ginsenoside F2 has yet to be determined, but other ginsenosides (acidic) more polar than F2 have been reported as heat-sensitive (*20*).

A possible matrix effect may have affected F2 stability, if recoveries of clean spikes (111.1%) are compared to recoveries of F2-spiked samples (76.6%). Sonication is recognized as a safer alternative to the extraction with heat (*20*, *22*), but the effectiveness may depend on the length of the extraction process (*23*), not tested here. Ginsenoside yields were significantly lower (~20−40%) when sonication alone was used for 15−45 min (*23*). In contrast, our 30-min sonication in 70% methanol (method B) provided a lower yield than extractions with method D but was similar to method C (**Figure 4**), indicating that aqueous methanol is more suited for extraction by sonication than pure methanol. Our results agree with several studies that have reported satisfactory recovery following sonication for 30 min (*20*, *33*, *56*). Further experiments are needed to determine how matrix interacts with solvents, physical treatments, and extraction time to optimize ginsenoside recovery.

In conclusion, the results presented here demonstrate that a single extraction by refluxing ground ginseng roots with 100% methanol at 60 °C for 1 h provided a simple and efficient (>87% recovery) method for the R-family ginsenosides. This extraction method (D) was significantly superior to both water at 90 °C (method C) and methanol at 100% (method A) or 70% (method B). Simple filtration of samples through a 0.2 $\mu$m nylon membrane considerably reduced sample preparation time without interfering with peak resolution during HPLC analysis. Heat degradation of ginsenosides during the methanolic refluxing extraction could not be discarded, but was clearly less than that observed for ginsenosides extracted with water at 90 °C. The relative sensitivity of the R-family ginsenosides to heat degradation was estimated and indicated that Rd was the most sensitive, followed by Rc, Rb1, and Rb2. Rg1 and Re were the most resistant to heat degradation. Ginsenoside F2, although eluting outside of the window of the R-family ginsenosides, is heat-labile and is not recommended as an internal standard for extractions above 60 °C.

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