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Determination of Ginsenosides R_{b1}, R_c, and R_e in Different Dosage Forms of Ginseng by Negative Ion Electrospray Liquid Chromatography−Mass Spectrometry

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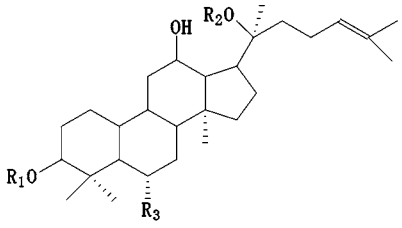
A method based on high-performance liquid chromatography (HPLC) and negative ion electrospray mass spectrometry (LC-MS) has been used to determine ginsenosides R_{b1}, R_c, and R_e in six different samples of ginseng. These included a liquid extract, capsules, tea bags, and an instant tea. It was found that four of the six samples had detectable levels of at least one of the ginsenosides. The liquid extract, capsules, instant tea, and tea bags labeled ginseng had ginsenosides. The labels on the two samples that did not have ginsenosides indicated that they were a mixture of green tea, licorice, and ginseng. Also, ^{13}C NMR was used to identify the types of complex carbohydrates present in the samples. One of the samples of tea bags had none of the ginsenosides, but did have complex carbohydrates found in most of the other samples. The instant tea had all three ginsenosides, but had no complex carbohydrates, only sucrose. The amounts of ginsenosides found in standard doses from six different sources of ginseng varied considerably. It was found that steeping a tea bag for a longer time than that recommended on the label produced a larger recovery of ginsenosides and that reusing a tea bag produced even higher recoveries.

KEYWORDS: Ginseng; ginsenosides; LC-MS; NMR

INTRODUCTION

The dried roots and rhizomes of ginseng have been used in Asia for at least 2000 years (*1*) and by native Americans long before the arrival of Europeans (*2*). In four recent years, ginseng has accounted for 15−20% ($300 million annually) of the market share of dietary supplements in the United States (*3*). There has been some confusion about the terminology and the distinction among Asian (Chinese or Korean) ginseng (*Panax ginseng* C.A. Meyer, from the family Aralaceae), North American ginseng (*Panax quinquefolius* Aralaceae), and so-called Siberian ginseng (*Eleutherococus senticosus*). The pharmacologic properties of ginseng are usually attributed to its triterpene glycosides, called ginsenosides (*4*). Structures are given in **Figure 1**. Among the 35 identified so far, 6 of them (R_{b1}, R_{b2}, R_c, R_d, R_e, and R_{g1}) account for 90% of the total (*2*, *4*). Some of them affect the activity of cytochrome P450 enzymes (*5*). Positive ion electrospray liquid chromatography−mass spectrometry (LC-MS) has been used to determine the amount of some of these (*6*) and to distinguish between Asian ginseng (*P. ginseng* C.A. Meyer) and North American ginseng (*P. quinquefolius*) (*2*). About 0.1% 24(*R*)-pseudoginsenoside F_{11} was found in North American ginseng and only ~0.0001% in Asian ginseng. About ~0.021% ginsenoside R_f was also in Asian ginseng, but none in the North American. Negative ion LC-MS has also been used (*7*, *8*). It was found to have better sensitivity (*7*) and to be able to profile the malonyl ginsenosides of different types of ginseng (*8*).



Compound	R_1	R_2	R_3	Formula	MW
R_{b1}	Glc-^{2}Glc	Glc-^{2}Glc-	Glc-^{6}Glc	$C_{54}H_{92}O_{23}$	1109
R_{b2}	Glc-^{2}Glc	Glc-^{2}Glc-	Ara(p)-^{6}Glc-	$C_{54}H_{90}O_{22}$	1079
R_c	Glc-^{2}Glc	Glc-^{2}Glc-	Ara(f)-^{6}Glc-	$C_{54}H_{90}O_{22}$	1079
R_d	Glc-^{2}Glc	H	Glc-	$C_{48}H_{82}O_{18}$	947
R_e	H	Rha-^{2}Glc-O-	Glc-	$C_{48}H_{82}O_{18}$	947
R_f	H	Glc-^{2}Glc-O	H	$C_{42}H_{72}O_{14}$	801
20-gluc-R_f	H	Glc-^{2}Glc-O	Glc-	$C_{48}H_{82}O_{19}$	963
R_{g1}	H	Glc-O-	Glc-	$C_{42}H_{72}O_{14}$	801

Figure 1. Ginsenoside structures.

Ginseng also contains complex carbohydrates and other compounds that can be extracted with hot water (*2*, *9*). These

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could be important in making the active ingredients bioavailable. They might also be useful in identifying a sample as true ginseng.

The objectives of the current study were to compare the amounts of three ginsenosides present in six different formulations of ginseng, to determine whether more ginsenosides can be extracted by steeping tea bags longer or by using the same tea bag twice, and to use NMR to compare different sources of ginseng for carbohydrate content.

MATERIALS AND METHODS

Ginsenoside standards and glycyrrhizic acid were from Sigma (St. Louis, MO). Acetonitrile was from Burdick & Jackson (Muskegon, MI). Ammonium formate (0.02 M) was prepared by mixing triply distilled NH_4OH (redistilled 28−30% NH_3) (GFS Chemicals, Powell, OH) and 88% formic acid (Fisher Chemicals, St. Louis, MO) in deionized water.

Sample Preparation. Ginsenoside standards were prepared in 1:4 CH_3CN/H_2O (v/v). Ginseng samples were obtained from local vendors and prepared according to instructions on the boxes except that deionized water was used instead of tap water. That is, the liquid extract (sample 1) was prepared by adding 30 drops (1.8 g) to 240 mL (1 cup) of deionized water. Instant tea (sample 2) was prepared by adding the contents of one packet (∼2 g) to 240 mL of hot water. Three different brands of ginseng tea bags (samples 3−5) were prepared by boiling deionized water, removing it from the hot plate, and adding 240 mL of it to one tea bag each (∼2 g) in individual beakers and letting them steep for 3 min. After 3 min, the tea bags were squeezed into the beaker. In an additional study, the two tea bags (samples 3−5) were steeped for 5 min, and in another, the bag was reused and steeped for 24 h, after pouring off the hot water and squeezing the tea bag after the first extraction. Samples 1, 2, 5, and 6 (the capsules) had much more than the 2 ppm of ginsenosides that was present in the most concentrated standard, so they were diluted 1/10 into the mobile phase before injection on the LC-MS. Samples 3 and 4 were not diluted. Samples 3 and 4 had ginseng listed as only a minor ingredient, after green tea and licorice. The packages and labels on samples 1, 2, 5, and 6 indicated that ginseng was the only herbal ingredient.

The instructions on the package containing the ginseng capsules (sample 6) were to take one capsule. Potentially, all of the ginsenosides in the capsule would be consumed. Thus, they were extracted as follows, to ensure extraction of all the ginsenosides. The contents of one capsule was placed into a centrifuge tube, and 10 mL of CH_3OH was added. The tube was shaken for 30 min in a mechanical shaker and then centrifuged, and the liquid was decanted and filtered. It was diluted 1/10 and analyzed by LC-MS.

LC-MS Analysis. LC-MS was performed on a Finnegan MAT LCQ Duo ion trap, equipped with a Spectra System P4000 pump, a UV6000 LP UV detector, and an AP3000 autosampler. The capillary temperature for the MS was 260 °C. Separations were done on a 2 × 150 mm i.d., 100 Å pore BDS Hypersil C18 column (Keystone Scientific, Schaumburg, IL). Injection volume was 20 μL, and the eluent flow rate was 0.2 mL/min. The solvent system consisted of solvent A, 0.02 M ammonium formate, pH 3, in water, and solvent B, acetonitrile. The gradient elution program was as follows: 0−1 min, 80% A; 1−26 min, 45% A, hold for 4 min, then 30−31 min, 15% A, hold for 5 min, then 36−45 min, 80% A.

^{13}C NMR Analysis. Instant tea samples were prepared by dissolving the contents of one package in ∼3 mL of D_2O. The ethanol in the ginseng liquid extract was evaporated off before the residue was dissolved in D_2O. Tea bags and capsules were extracted with boiling deionized water. The volume was reduced from ∼200 to 10 mL, and this was mixed in a 1:1 ratio with D_2O.

Carbon-13 spectra of samples were obtained on a Varian 400 MHz NMR, equipped with a broadband, tunable probe, a SunSparc computer, and VNMR software. At least 1000 transients were collected, using a 45° pulse width and a 1 s pulse delay, with proton decoupling. To determine the number of protons attached to each carbon, distortionless enhancement through polarization transfer (DEPT) spectra were obtained.

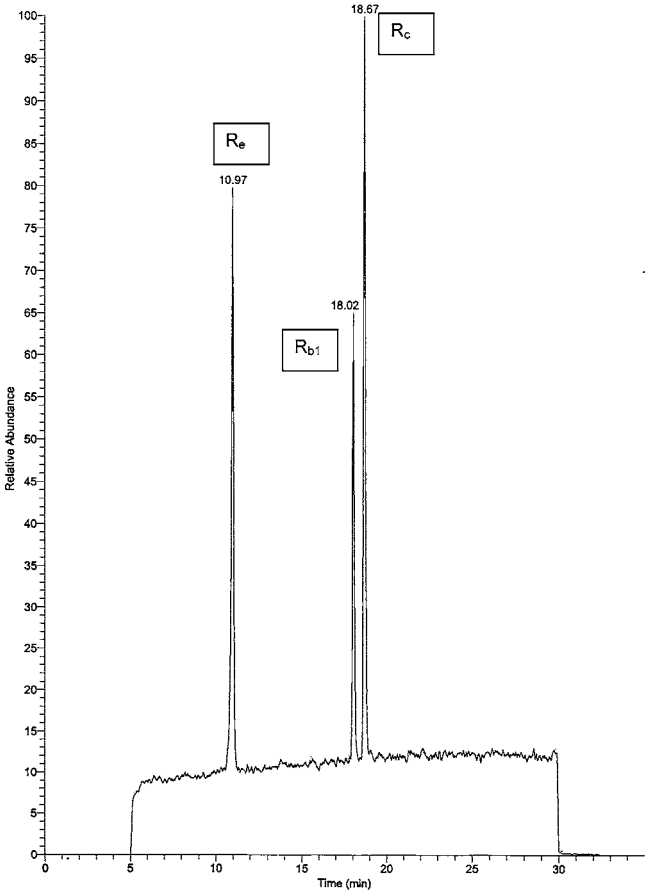

Figure 2. Negative ion electrospray LC-MS of ginsenoside standards.

Table 1. Ginsenoside Content of Different Samples

sample	R_b (μg/dose)	R_c (μg/dose)	R_e (μg/dose)
1, liquid extract	5969	2527	2059
2, instant tea	2757	1471	1285
3, tea bag mix[a]	ND[b]	ND	ND
4, tea bag mix[a]	0.096	0.065	0.128
5, tea bag	1680	542	1202
6, capsules	291	334	1917

[a] Package was labeled green tea, licorice, and ginseng. All other samples were labeled as having ginseng and no other herb. [b] None detected.

RESULTS AND DISCUSSION

Concentrations of 0.1−2 μg/mL (ppm) of each ginsenoside were injected on the LC-MS and the peak areas integrated. A sample total ion chromatogram of the 0.1 ppm standard is shown in **Figure 2**. The mass range scanned was *m*/*z* 700−1200. The masses of the different ginsenosides (800, 946, 1108, and 1078) were scanned over a much smaller range, 1 amu. The *m*/*z* 799−800 scan showed only noise, because there was no R_f or R_g standard. However, R_e (t_r = 10.56 min), R_{b1} (t_r = 17.65 min), and R_c (t_r = 18.3 min) each produced peaks. The calibration data were fit to a straight line using linear regression. The correlation coefficients, or r^2, were as follows: R_e, 0.997; R_{b1}, 0.995; and R_c, 0.997.

Six different sources of ginseng were analyzed after being prepared according to the instructions on the box (except for the capsule, which was supposed to be injested whole, one

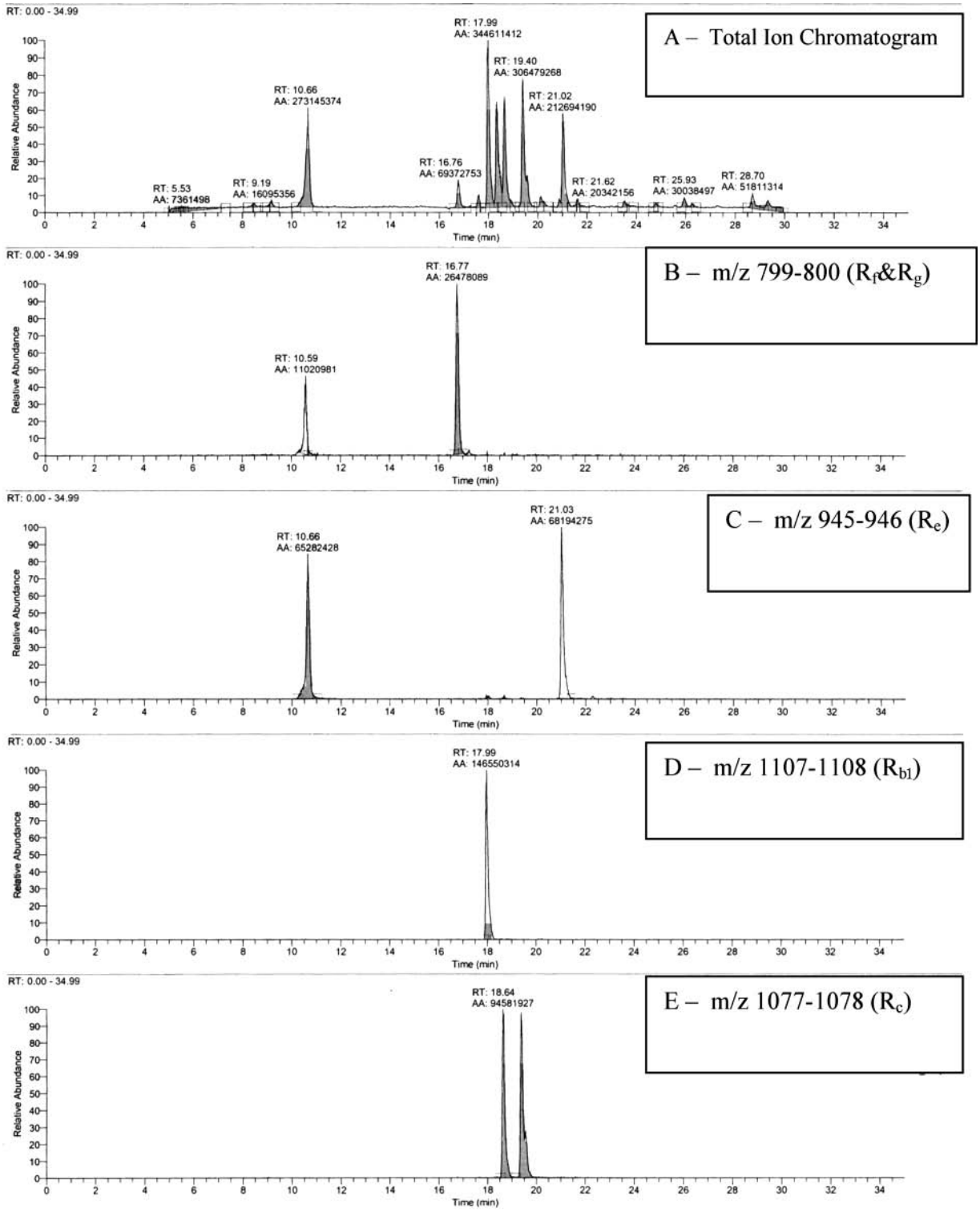

Figure 3. Negative ion electrospray LC-MS of liquid extract, sample 1: (**A**) total ion chromatogram of R_e, R_b, and R_c, eluting at 10.96, 18.01, and 18.67 min; (**B**) mass 799–800 (R_f and R_g); (**C**) mass 945–946 (R_e); (**D**) mass 1107–1108 (R_b); (E) mass 1077–1078 (R_c).

capsule per serving). The results are in **Table 1**, and a typical chromatogram is shown in **Figure 3**. **Figure 3A** shows the total ion chromatograms. **Figure 3B** shows R_f and R_g, whereas panels **C**−**E** of **Figure 3** show ginsenosides R_e, R_{b1}, and R_c. The liquid extract, one of the tea bags, the capsule, and the instant tea looked somewhat similar to each other. They shared not only the peaks due to R_e, R_{b1}, and R_c but also additional peaks, including a relatively large one that appeared just after R_c at 19.40 min. It had an *m*/*z* 1077.5 and so is probably due to R_{b2}, which was shown to elute after R_c by others (*8*, *9*). There is also a peak at 21.02, with *m*/*z* 945, which has been shown to be due to R_d (*8*, *9*). However, the sizes of the peaks were different in each sample, so it is possible that each sample had different efficacies, because they have different amounts of active ingredients.

It was also found that more ginsenosides could be extracted from sample 5 (ginseng tea) by steeping for 5 min. The amount of ginsenosides increased to 2552 μg for R_{b1}, 761 μg for R_c, and 1908 μg for R_e. The bag was reused and steeped for 24 h and even more was recovered: R_{b1}, 5969 μg; R_c, 2527 μg; and

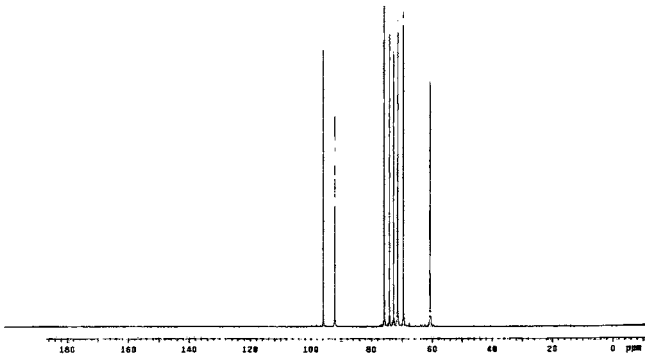

Figure 4. ^{13}C NMR of ginseng instant tea (sample 2) in D_2O.

R_e, 2059 μg. It should be noted that previously published methods used methanol or methanol and water to extract ginsenosides (*6*−*8*). However, the objective of this study was to determine what was in a typical dose as it would be consumed.

Sample 3, however, had no detectable R_{b1}, R_c, or R_e. None was detected after 5 min or 24 h of steeping. Sample 4 had only 0.096, 0.065, and 0.128 μg of the ginsenosides. Five minutes of steeping produced less R_{b1}, R_c, or R_e (0.083, 0.055, and 0.121 μg, respectively). The label on the box indicated that the tea bags contained green tea, licorice, and ginseng. The LC-MS analysis did indicate the presence of 1728 μg of glycyrrhizic acid (an ingredient of licorice) per bag, producing a peak at 19.97 min. Also, the ^{13}C NMR spectrum of the hot aqueous extract showed the presence of the same carbohydrates that were present in other ginseng samples.

Five different ginseng instant tea bags (sample 2) were analyzed in triplicate. The average amounts of the ginsenosides per tea bag were as follows: R_{b1}, 2757 μg; R_c, 1471.5 μg; R_e, 1285.3 μg. The average percent relative standard deviation (%RSD) of the triplicate injections was 2.67%. There was more variance in the results between different tea bags. The %RSDs between different tea bags were as follows: R_{b1}, 8.34%; R_c, 9.29%; R_e, 7.73%. However, nuclear magnetic resonance (NMR) analysis indicated that the complex carbohydrates present in the other samples were not present in the instant tea—only sucrose was detected. Also, the weight of the contents of the instant tea varied considerably. The %RSD was 11.4%. Thus, the amount of ginsenosides was better controlled than the total weight, which was primarily due to the amount of sucrose. The manufacturer was more careful with weighing the ginsenosides than with the sucrose. However, little other plant material was present. This is in contrast to sample 3, which had plenty of carbohydrates, but very little ginsenoside R_{b1} and no detectable R_c or R_e.

The ^{13}C NMR spectra in **Figure 4** show that the instant tea did not have complex carbohydrates, although LC-MS indicated that it had ginsenosides. The liquid extract had not only the ginsenosides and complex carbohydrates but also an unknown olefinic compound that produced peaks in the ^{13}C NMR at 131.7 and 135.2 ppm (one hydrogen attached to each carbon).

It has been shown that different samples of ginseng have different chemical compositions, although most contained characteristic ginsenosides and carbohydrates. More ginsenosides were recovered from tea bags that were steeped for 5 min than from those steeped for 3 min, and the tea bag could be reused to recover even more ginsenosides. Still, it was not clear whether some of the samples should be labeled as ginseng when some had little or no ginsenosides, whereas another had ginsenosides but no complex carbohydrates that might be important for bioavailability of the active ingredients. It would be useful to have internationally accepted definitions of ginseng supplements and teas.

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