Perspectives on the Quality Assurance of Ginseng Products in North America

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ABSTRACT

A validated and reproducible HPLC method was developed for the profiling and quantitative analysis of ginsenosides in commercial products available in North America. Analysis of 280 *Panax ginseng* and *Panax quinquefolius* products showed profiles indicative of the presence of ginsenosides in the majority of these products. However, the quantitative contents of the products vary greatly, not only in products of different formulations, but also of products within each of the formulations examined.

Introduction

Under the Dietary Supplement Health and Education Act (DSHEA) of 1994, ginseng products are marketed as dietary supplements in the U.S.A. Since DSHEA places the responsibility for the safety of these products on the manufacturers, ginseng products have not been subjected to mandated quality assurance (QA) standards. Consequently, product quality may differ from brand to brand, and even from lot to lot. An analysis of the quality of commercial ginseng products in North America thus appears to be in order. Of particular interest are the two major species of commerce, Asian ginseng (*Panax ginseng* C. A. Meyer [Araliaceae]), and North American ginseng (*Panax quinquefolius* L. [Araliaceae]). Both species contain similar ginsenosides, with more than thirty having been identified(1), and six of these, Rg_1, Re, Rb_1, Rc, Rb_2, and Rd reported to account for more than 90% of the saponin content of the root(2). A number of methods for their identification and quantitative analysis are available in the literature(3). However, methods such as colorimetry may overestimate results and cannot give information on individual ginsenoside levels, while gas chromatography has other reported difficulties (4, 5). Liquid chromatography(HPLC) methods have been the most successful and are now the most widely accepted analytical procedure (4-8). To assess the quality of commercial ginseng products available in North America, we updated, adapted and verified an HPLC method for the quantitation of the aforementioned major ginsenosides plus Rf, whose presence or absence is the basis for the differentiation between and Asian and North American ginseng.

Ginsenoside Rf is absent from the latter. The present paper describes the analytical method and results obtained in a dual-laboratory analysis of the various formulations of commercial ginseng products available in North America.

Materials and Methods

Test materials:

Commercially available ginseng samples (280) in various formulations (bulk powder, capsule, soft gel capsule, concentrated extract, liquid [including syrup, alcoholic and aqueous solution], tea, granule, tablet) were obtained from the commercial retail market in North America and provided as blinded numbered samples in standard containers with no identifying marks as to origin by the American Botanical Council for analysis at the University of Ottawa(UO) and at the University of Illinois at Chicago (UIC).

Instrumentation:

HPLC: A Beckman System Gold, consisting of a Beckman diode-array detector (module 168), a programmable binary solvent delivery system (module 126), an autosampler (module 502), a 486 Dell (IBM-compatible) computer for data processing, and Beckman System Gold software; and a Waters HPLC system consisting of model 510 EF pumps, model 717 autosampler, and model 486 UV-VIS detector set at 202 nm and equipped with a Millennium^R version 2.15 programmable system controller and data processing software were employed at UO and UIC, respectively.

LC-MS: Negative ion electrospray mass spectra were obtained using a Hewlett-Packard G1946A LC-MSD quadrupole mass spectrometer equipped with a Series 1100 HPLC system consisting of a binary pump, automatic solvent degasser and diode-array absorbnace detector. The electrospray capillary voltage was 3500 V, the nitrogen nebulizer gas pressure 40 p.s.i.g. and the nitrogen drying gas temperature was 300℃ at a flow-rate of 10 L/min. Mass spectra were acquired over the scan range m/z 700-1300 in *ca.* 4 sec/scan.

Reference standards:

Standard ginsenosides Rg_1, Re, Rb_1, Rc, Rb_2, and Rd were isolated from *Panax quinquefolius* roots and their identities confirmed by NMR and MS at the UIC laboratories; Rf was a gift from Dr. Y. C. Ma, Celex Laboratories Inc., Atholville, NB, Canada.

Solvents and chemicals:

Acetonitrile, methanol and butanol were all HPLC grade (BDH Inc., Toronto or Fisher Scientific, Springfield, NJ), trifluoroacetic acid 99% (Aldrich Chemical Co., Milwaukee, WI), distilled and

deionized water were obtained from a Milli-Q Reagent or a NANO pure® ultrapure water system.

Sample preparation:

Solid formulations, including capsules, powder, granules and tablets were processed by one of two methods:

Method 1 (UO): The sample (0.5-3 g, pulverize as needed) was extracted repeatedly at 55℃ with a 20% aqueous methanolic solution (3 x 20 mL) for 30 minutes each, the combined extract evaporated to dryness *in vacuo* at 40-55℃, the residue dissolved in water (2 x 5mL) and applied to an Extrelut column (EM Industries, Inc.). The column was eluted with *n*-BuOH saturated with water (75 mL), the eluate evaporated to dryness under vacuum (55-60℃), dissolved in MeOH (5.0 mL, HPLC grade) and filtered (Varian LC Sample Preparation filters, Lot. No. 160216) prior to injection into the HPLC system.

Method 2 (UIC): The sample (0.2-2 g, pulverize as needed) was weighed accurately into a 50 mL Erlenmeyer flask, MeOH (15 mL) added, stirred and macerated at room temperature overnight. The extract was filtered, the marc washed with MeOH (3 x 15 mL) and the combined MeOH fraction evaporated to dryness *in vacuo* at 45-50℃. This residue was redissolved in MeOH(4 x 2 mL), transferred to a 10-mL volumetric flask and brought up to volume with MeOH. The sample solution was filtered directly into the HPLC sample insert, using a Nylon Acrodisc 13 filter, just prior to HPLC analysis.

Soft gelatin capsules were processed by using method 1 above or by partitioning the contents (0.2-3 g) dissolved in 30 mL of a mixture of hexane: methanol:water(20:15:10). The hexane solubles were washed with MeOH : H_2O mixture (3 x 15 mL) and the washing combined with the aqueous MeOH fraction. The combined polar fraction was evaporated to dryness *in vacuo* at 45-50℃, and treated as described in method 2.

Liquid samples were processed by evaporating a MeOH solution of a measured aliquot to dryness, redissolving in 20 mL of a mixture of aqueous trifluoroacetic acid (0.05%) solution and MeOH (1:4), and filtering through a 0.2 μm solvent-resistant filter prior to HPLC. Alternately, an aliquot was weighed directly into a 10 mL volumetric flask, diluted with MeOH to volume and filtered directly into the HPLC sample insert just before HPLC analysis.

HPLC Analysis:

HPLC analyses were performed on a Beckman Ultrasphere ODS, 5 μm particle, 250 x 4.6 mm column, The solvent system consisted of isocratic water/acetonitrile (80:20;v/v) for 20 min followed by a 40 min linear gradient to 42% acetonitrile at a flow rate of 1.6 mL/min. UV detection was at 202-203 nm. Calibration curves were established with Rg_1, Re, Rf, Rb_1, Rc, Rb_2, and Rd standards for quantitative analyses.

Recovery of Ginsenosides:

Aliquots of selected standard ginsenosides (Rg_1, Rb_1, Rc and Rd) were added to samples of capsule, liquid, tea and syrup ginseng formulations and recovered by extraction using the same procedure as for the original sample. The extracts prepared from a sample with the standard ginsenosides added (spiked) and an unaltered sample were analyzed on HPLC and quantified as described to determine the recovery.

Peak Identity Confirmation:

In addition to their t_R values, the identities of the separated ginsenosides were confirmed by LC-MS for both the standard mixture and sample extracts. HPLC separations were carried out using a Beckman ODS column (2.0 x 250 mm) with a solvent gradient of acetonitrile and water as described above at a flow rate of 1.6 mL/min. Mass spectra were acquired over the scan range m/z 700-1300 in *ca.* 4 sec/scan. In addition, UV absorbance at 202 nm was simultaneously monitored using an absorbance detector between the HPLC column and the mass spectrometer.

Results and Discussion

A total of 210 Asian ginseng *(Panax ginseng)* products in 12 different formulations, and a total of 69 North American ginseng *(Panax quinquefolius)* products in 9 different formulations were evaluated for their ginsenoside content. Each encoded product was evaluated in duplicate at both the UO and UIC laboratories. Typical chromatographic profiles of Asian and North American ginseng root extracts showing baseline resolution are presented in Figures 1 and 2. Ginsenoside peak identity confirmation by LC-MS is shown in Figure 3 for an actual sample extract. As an example, Figure 4 shows the mass spectrum recorded at 44.2 min during the LC-MS analysis shown in Figure 3, which confirmed the peak as Rb_1. This identity confirmation contributed significantly to the confidence of the assay. The validity of the analytical method is further augmented by the recovery experiments. Due to the lack of availability of all standard ginsenosides in sufficient quantity, only Rg_1, Rb_1, Rc and Rd were employed in the recovery experiments. However, it can be seen from Table 1 that the recoveries of all standards was better than 94%. The response of the LC system to standard ginsenosides in various ranges of concentration was highly linear with an $r^2>0.99$ in all cases. The procedure enabled detection of 12 ng of Rg_2 and 22 ng Rg_1 at above background noise levels. Of particular interest is the similarity of the recovery data (see Table 1) for the capsules and liquid preparations recorded by the two laboratories, which employed different extraction methods for these two types of formulations. To test the ruggedness of our assay protocol, a comparative experiment employing the two methods for the extraction of ginsenosides from the same products was performed. As can be seen from the data presented in Table 2, the individual as well as total ginsenoside contents of these

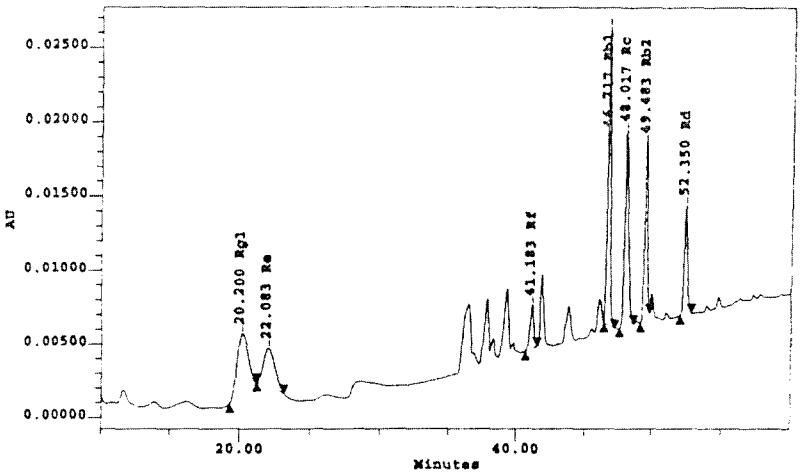

Fig 1. HPLC profile of a methanol extract from Asian ginsen *(Panax ginseng)* roots.

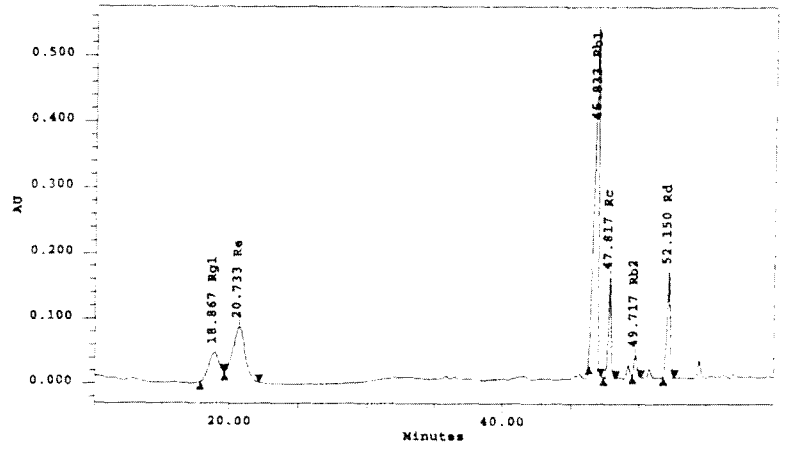

Fig 2. HPLC profile of a methanol extract from North American ginsen *(Panax ginseng)* roots.

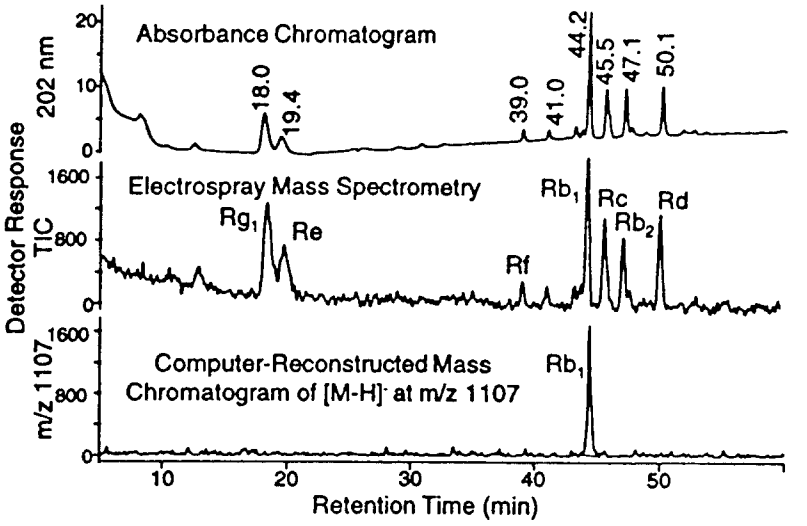

Figure 3. Electrospray LC-MS of a ginsenoside sample extract. Reversed phase HPLC with negative ion ESI MS

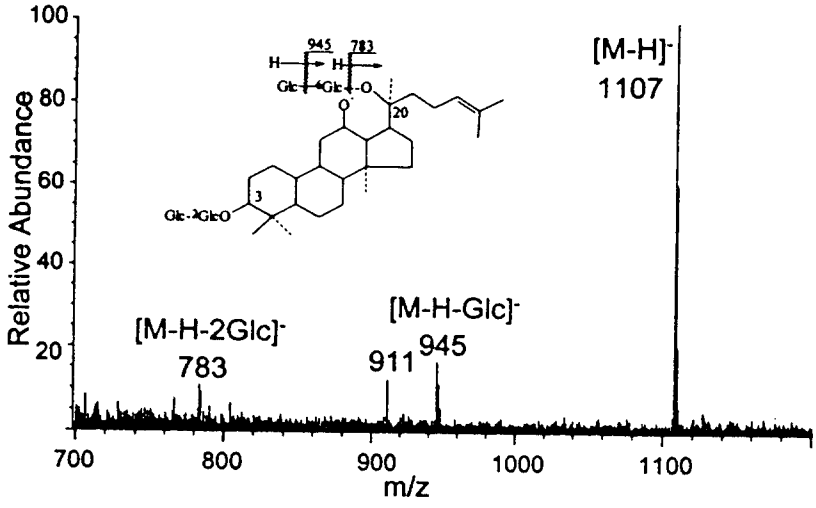

Figure 4. Negative ion electrospray mass spectrum of the peak eluting at 44.2 min in the LC-MS chromatogram shown in Figure 3.
Based on molecular weight, fragmentation patern and retention time, this peak was identified as Rb1

Table 1. Recovery of Ginsenosides(%)

Ginsenosides	Rg_1		Rb_1		Rc		Rd	
Laboratory [1]	OU	UIC	OU	UIC	OU	UIC	OU	UIC
Formulation :								
Capsules	104	103	102	104	107	105	103	106
Liquids	98	102	96	102	99	110	99	102
Teas	NT[2]	110	NT	101	NT	NT	NT	102
Syrups	96	NT	95	94	NT	96	100	99

[1]OU = University of Ottawa, UIC = University of Illinois at Chicago
[2]NT = Not tested

Table 2. Comparative Analysis of Extration Procedures

Method[1]	Ginsenoside Content(%)			
	Capsule Sample 1		Capsule Sample 1	
	BuOH	MeOH	BuOH	MeOH
Ginsenoside :				
Rg_1	0.13	0.12	0.40	0.43
Re	2.03	2.01	0.18	0.20
Rf	0.05	0.05	0.06	0.07
Rb_1	3.11	3.11	0.81	0.80
Rc	0.58	0.58	0.30	0.31
Rb2	0.06	0.06	0.20	0.21
Rd	0.78	0.78	0.16	0.16
Total	6.74	6.74	2.11	2.18

[1]BuOH = Extraction procedure #1 (UO); MeOH = Extraction method #2(UIC)

these two extracts are essentially identical, thus confirming the reproducibility of our protocol.

The qualitative HPLC chromatograms of the majority of the products showed ginsenoside profiles consistent with Figure 1 or 2. The quantitative ginsenoside contents of the 210 Asian ginseng products ranged from 0.00-13.54% in the various dosage forms (Table 3). The lack of consistency in the ginsenoside contents of these products is evident not only among different products of all types, but also among the products from within a given formulation type. Concentrated root extracts and capsules composed of powder ginseng roots or dried extracts contain the highest quantity of ginsenosides (average > 3%). Tablets and liquid dosage forms, on the other hand, have the lowest ginsenoside content (average<0.7%). Although aesthetically pleasing, the liquid products containing whole roots, constitute the poorest dosage form, with a total ginsenoside content range of 0.00-0.90%. The total ginsenoside content in 69 North American ginseng products ranges from 0.82-9.21%. As in the case of Asian ginseng, the lowest concentration of ginsenosides is found in the liquid and tablet for-

Table 3. Ginsenoside Content in Commercial ginseng Products in North America

Method[1]	Asian Ginseng *(Panax ginseng)*			North American Ginseng *(P. quinquefolius)*		
	No. of Samples	Ginsenosides(%) Total	Range	No. of Samples	Ginsenosides(%) Total	Range
I. Root Powder :						
Bulk	2	2.67	1.78 - 3.56	4	3.26	2.51 - 4.09
Capsule	54	3.06	0.02 - 9.32	24	5.22	0.09 - 8.01
Tea	2	3.88	2.39 - 5.36	2	9.21	6.40 - 12.02
Tablet	12	0.37	0.00 - 1.22	2	0.92	0.36 - 1.49
II. Root Extract	8	4.24	0.35 -13.15	-	-	-
Concentrate	27	3.60	1.36 -13.54	6	4.60	3.10 - 6.21
Capsule	57	1.22	0.00 - 3.24	1	1.89	1.89
Soft gel	9	0.98	0.29 - 1.90	2	1.59	0.40 - 2.79
Tablet	2	1.05	0.47 - 1.62	1	1.15	1.15
Liquid	25	0.68	0.01 - 3.56	27	0.82	0.03 - 4.82
Liquid with whole root	6	0.16	0.00 - 0.90	-	-	-
III. Root Extract with Root Powder						
Capsule	6	3.93	1.98 - 8.73	-	-	-

mulations of the North American ginseng products. These data suggest that the lack of establised GMP regulations is a major contributing factor to the current proliferation of products in which the ginsenoside contents vary widely and do not always meet lable claims. Fortunately, the US FDA, acting under the provisions of DSHEA, has published (1997) an advanced notice of rule making for CGMP for the manufacture of dietary supplements, including ginseng products, of uniform quality meeting label claims. Hopefully, the implementation of these rules in the near future will lead to enhanced quality ginseng products in North America.

References

1. M.Sakata *et al., Proceedings of the 3rd International Ginseng Symposium,* **8** (1980).
2. G.Sollorz, *Deutsch. Apoth. Ztg.* **125,** 2025 (1985).
3. T.Nagasawa, T.Yokozawa, Y.Nishino and H. Oura, *Chem.Pharm.Bull.,* **28,** 2059 (1980).
4. O.Sticher and F.Soldati, *Planta Med.,* **36,** 30,(1979).
6. F.Soldati and O.Sticher, *Planta Med.,* **38,** 348 (1980).
7. H.Kanazawa, Y.Nagata, Y.Matsushima, M.Tomoda and N. Takai, *J.Chromatogr.,* **507,** 327 (1990).
8. W-S. Lang, Z-C. Lou and P.P-H. But, J. *Chinese Pharm. Sci.* **3,** 133 (1993).
9. Y.Ma, L. Mai, L. Mallery, M. Doucet, *Chinese J. Med. Chem.* **6,** 11 (1996).