

American Botanical Council



GINSENG EVALUATION PROGRAM

Introduction

Ginseng comprises one of the largest sales categories of commercial herbal products in North America. For the past 25 years numerous advanced ginseng products have been sold in tea, capsule, tablet and extract forms. Consumers purchase ginseng in its various forms because they seek the presumed health benefits of the fabled root. The so-called tonic and adaptogenic properties, the apparent basis of much tradition and mythology, have been subjected to extensive scientific studies in both Asia and Europe. The primary types of ginseng on the US market are Asian ginseng (*Panax ginseng*), American ginseng (*P. quinquefolius*), and Eleuthero or Siberian ginseng (*Eleutherococcus senticosus*, formerly known as *Acanthopanax senticosus*). All are members of the plant family Araliaceae.

Veterans of the ginseng and general herb industries, as well as research scientists, have long suspected that some of the products claiming to contain ginseng may be mislabeled and/or adulterated. Prompted by this concern, ABC initiated, in 1993, a comprehensive study of commercial ginseng products sold throughout North America: the Ginseng Evaluation Program (GEP). This is the first time a study of this magnitude has been conducted on ginseng or any popular herbal product. Through GEP, ABC seeks to set a standard for future studies, increase consumer confidence in properly labeled brands, and increase awareness and responsibility on the part of the manufacturers of natural products.

What is the Ginseng Evaluation Program?

The Ginseng Evaluation Program is a long-term comprehensive study of commercial ginseng products sold throughout North America. Working with two leading university laboratories, GEP has analyzed more than 500 Asian, American, and Eleuthero (Siberian) ginseng products using High Performance Liquid Chromatography (HPLC) to profile and assay ginsenosides and/or eleutherosides. The results of these analyses are then used to determine the validity of manufacturer claims regarding identity and claimed levels of key chemical constituents.

In order to ensure fairness and objectivity, ABC has developed strict protocols for obtaining and storing products, shipping samples to the testing laboratories, as well as reviewing results. All products analyzed were purchased from retail establishments (including health food stores, grocery stores, drugstores, and ethnic grocery stores), mail order, multilevel marketing distributors, and/or health care professionals. ABC has not accepted in this program any ginseng products that were sent directly by the manufacturer, in order to preclude the possibility that uncharacteristic products could be evaluated.

GEP has three phases: the Regular Testing Phase, New Lot Testing Phase, and the Standardized Testing Phase. The Regular Testing Phase is the original idea behind the GEP. Products were purchased throughout the U.S. and Canada, analyzed up to four times, and given a status of Pass or Fail.

Upon completion of the analyses, ABC notified manufacturers with products that had failed of the results of the analyses for their products. Many of those manufacturers expressed concern that the products analyzed in GEP were older products that had since been improved or had been discontinued. In some cases manufacturers explained that they had discovered problems and corrected them. Many manufacturers urged ABC to test the new version of their product currently on the market. After assessing all the input and based on the fact that many of the original products were manufactured prior to the passing of Dietary Supplement Health and Education Act of 1994 (DSHEA), ABC decided to initiate the New Lot Testing Phase. For each product that had failed in the Regular Lot Testing Phase, a new lot was purchased and analyzed one time at one lab. Each of these products received a Pass or Fail status. The results for both the Original lot and the New lot will be published in the final GEP report.

In the process of acquiring products for the Regular Testing Phase, it became evident that many products claimed to be standardized and some of those claimed to be standardized to a specific level of ginsenosides. Not knowing what each manufacturer means by standardized, ABC decided to expand the GEP to review a small group of standardized *Panax ginseng* products. Fourteen different products were selected and 5 different lots of each product were purchased. These products do not receive a Pass or Fail status. Rather, the GEP report will include a comparison of the different standardized products plus a comparison of the different lots of the standardized products to determine any variation in the total quantity of key constituents and/or the relative ratios or profiles of these compounds. This second comparison will show if a standardized product is consistent on a lot-by-lot basis.

Laboratories & Methodologies

ABC has been working with the University of Illinois at Chicago (UIC) and University of Ottawa (UO). Both labs worked together to develop the methodologies for analysis of ginsenosides and eleutherosides. Both laboratories were involved in the analysis of the products. In addition, UIC isolated many of the necessary chemical reference standards used in the analyses.

Results

The dissemination of information in the GEP Report will have a significant impact on the health and well being of the general public as it impacts the future policy of research, manufacturing, and claims about natural products. The level of responsibility on the part of industry will continue to increase. In addition, due to the rapidly expanding interest in the use of herbal products, the industry and the public will see the great value in utilizing this pioneering study as a model to conduct further studies analyzing herbal dietary supplements.

The GEP reports will be featured in multiple issues of *HerbalGram*. <u>The first report appears in</u> the Summer 2001 issue of *HerbalGram* and discusses the consistency of "standardized" Asian ginseng products. The remaining reports will cover eleuthero (aka Siberian ginseng) and American and Asian ginseng products and whether or not they meet their label claims. In addition to the GEP reports, these special ginseng issues will include additional articles on ginseng covering such topics as characteristics, research, taxonomy, conservation, history, etc.

If you would like to order HerbalGram 52 containing the first GEP report and do not want to become a member at this time, call the ABC toll free order line at 800-373-7105.

Ginsenoside Methodology

HPLC Methods for Separation and Quantitative Determination of GinsenosidesUsed in the American Botanical Council's Ginseng Evaluation Program

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INTRODUCTION

Ginseng is one of the most widely used herbal drugs and is reported tohave a wide range of therapeutic and pharmacological activities (1-3). The twomajor species of commerce are *Panax ginseng* C.A. Meyer (Asian ginseng), and *Panax quinquefolius* L. (North American ginseng). Both speciescontain active ginsenoside saponins, but there are significant differences intheir identity and distribution. It has been observed that over thirtyginsenosides have been identified from *Panax* spp. (4), however six of these,Rg1, Re, Rb1, Rc, Rb2, and Rd constitute the major ginsenosides accounting forover 90% of the saponin content of ginseng root (5).

Methods for the identification and quantitative analysis of ginsenosides are available in the literature (6). However, methods such as colorimetry mayoverestimate results and cannot give information on individual ginsenoside levels, while gas chromatography has other reported difficulties (7, 8). HPLC methods have been the most successful and are now the most widely accepted analytical procedure (7-9). A definitive HPLC/MS method is now available (10).

Based on a reviewof verified and widely used High Pressure Liquid Chromatography (HPLC)methods reported in the scientific literature, such as the Soldati method (7,8),we updated, adapted and verified an HPLC method for the quantification of seven major ginsenosides including Rf, whose presence or absence is the basisfor the HPLC differentiation between Asian and North American ginseng, fromwhich Rf is absent. Rg2, also absent from North American ginseng, for whichpurported ginseng products were initially screened, was not assayed for because agood standard was not available in sufficient quantities.

MATERIALS AND METHODS

Extraction.

Commercially available ginseng samples in various formulations (capsule,soft gel capsules, syrup, liquid, powder, granules, tablets) were obtained from the commercial retail market in North America and provided by the AmericanBotanical Council as blinded numbered samples in standard containers with noidentifying marks as to origin.

Capsules, powder, granules (pulverized), tablets (pulverized):

At the University of Ottawa, typicallya 0.5-3g sample of the ginseng formulation was extracted repeatedly at 55 C with a 20% aqueous methanolic solution (3 x 20mL) for 30 minutes each. The combined extract was evaporated to dryness under reduced pressure at40-55 C, and the residue dissolved in water (2 x 5mL) and applied onto anExtrelut column (EM Industries, Inc.). The saponins were then eluted (after 15minutes) using butanol saturated with water (75mL). The butanol extract wasevaporated to dryness under vacuum (55-60 C), and the residue dissolvedin methanol (5.0mL, HPLC grade). This was filtered using Varian LC SamplePreparation filters (Lot. No. 160216) and (20uL) injected into the LC system.

At the UIC College of Pharmacy laboratory:0.2-2g of each sample was weighed accurately into a 50mL Erlenmeyer flask,15mL of methanol (NB) was added to mix then shaken and the mixture was kept atroom temperature overnight, then filtered and the residue washed with methanol(3 x 15mL). The filtrate was combined into a 500mL round bottom flask, andevaporated under vacuum at 45-50 C to dryness. Residue was taken up withmethanol (4 x 2mL) transferred to a 10mL volumetric flask and made up tomark with methanol prior to HPLC analysis. The sample solution was filtereddirectly into the HPLC sample insert, using a Nylon Acrodisc 13 filter, just beforeHPLC analysis.

Soft gelatin capsules:

At the University of Ottawa, softgel capsules were extracted similar to tablets, capsules, etc.

At the UIC College of Pharmacy laboratory, soft gels were emptied of contents, 0.2-3 g of each sample was weighedaccurately, then dissolved in a mixture of hexane:methanol:water (20:15:10), 30mL, thelower layer collected, the upper layer washed with methanol and water mixture (3x 15mL), combined with the lower layer, evaporated under vacuum at 45-50 C to dryness, then treated further as described for capsules.

For the extraction of syrupsand liquids:

At the University of Ottawa an amount (1-5mL) was transferred to a 50mL flask and mixed withmethanol (20mL). The solution was evaporated to dryness under vacuum and dissolvedin 20mL of a mixture of aqueous 0.05% triflouroacetic acid solution andmethanol (1:4) and filtered through a 0.2uL solvent-resistant filter.

At the UIC Collegeof Pharmacy laboratory: 2-6g of each sample was weighed accurately, directly into a 10mL volumetric flask, diluted with methanol and made up to themark prior to HPLC analysis. The sample solution was filtered directly into the HPLC sample insert just before HPLC analysis.

Recovery of Ginsenosides.

At the University of Ottawa, replicate analyses were performedon recovery of standard ginsenoside Rg1 in root sample, and of a mixtureof standard ginsenosides, Rg1, Re, Rb1, and Rd in different ginsengformulations: powder, capsule, softgel, syrup and liquid. Two equal amounts by weight of a particular ginseng formulation were taken and standard ginsenoside(s)added to one of these. Extraction procedures were identically performed on thetwo samples and the extract analyzed. Analysis on the unaltered extract wasfollowed by analysis of the extract containing standard(s). Recovery was determined by taking the difference between the total ginsenoside concentration(including added standard) and the detected ginsenoside in extracts without standardadded. This spiking procedure also allowed verification of the peak identity inextracts, since there was no development of split or shouldered peaks or change inonline spectra in the rising or declining portion of the peak.

Recovery studies were performed similarly at the UIC Collegeof Pharmacy laboratory; the number of recovery studies was limited by theamount of available pure ginsenoside standards; these are reported in Table1.

HPLC Analysis.

The HPLC used at the University of Ottawa was a Beckman System Gold, consisting of a Beckman Diode-array detector (module 168), a programmable binarysolvent delivery system (module 126), an autosampler (module 502), capable ofinjecting up to 94 samples, a 486 Dell (IBM-compatible) computer for dataprocessing, equipped with Beckman System Gold software. The equipment was certified semi-annually by Beckman technicians. Separations were achieved with Beckman ultrasphereODS, 5um particle, 250 x 4.6mm column. For ginsenoside analysis, the mobilephase was water (A) and acetonitrile (B) at a constant composition of 21%B from0-20 minutes and flow rate of 1.3 mL/min; and a gradient elution rising to 42%Bfrom 20-60 minutes. At 60-70 minutes, the column was flushed with 99%B thenreturned and equilibrated for 15 minutes with 21%B. Detection of ginsenosides wasachieved at 203nm.

At the UIC Collegeof Pharmacy laboratory, analysis was performed with a Waters HPLC system consisting of model 510 EF pumps, model 717 autosampler, and model 486 UV-VIS detector set at 202nm and equipped with a MillenniumR version 2.15 programmable system controller and data processing software; the column used was also aBeckman Ultrasphere ODS 5um particle, 250 x 4.6mm.

HPLC Conditions at Chicago lab:

Column: Beckman Ultrasphere ODS Column, 5m,250 x 4.6mm

Mobile: A: Acetonitrile B: Water Gradient elution: 0-20 min 20% A; 20-60 min 42% A; Flow rate: 1.6mL/min Wavelength: 202nm

Solvents and Standards.

At the University of Ottawa: Acetonitrile, methanol and butanol were all HPLC grade (BDHInc., Toronto) and distilled and de-ionized water was obtained from a Milli-QReagent Water system. The following ginsenosides, namely Rg1, Re, Rb1, Rc, Rb2, and Rd (provided by Prof. H. Fong, University of Illinois, Chicago), Rf andRg2 (provided by Dr. Y. C. Ma, Celex Laboratories Inc., Atholville, NB)representing over 90% of the total ginsenosides in roots, were maintained in a -20 Cfreezer until use and then prepared as pure standards at concentrations of0.01 -0.1 mg/mL in HPLC grade methanol. Analysis based on peak area (AU) wasperformed and the Response Factors (mg/mL/AU) calculated and built in to the method.Response Factors were continuously updated by bracketing standard mixture between anumber (3-6) of sample runs.

At the UIC Collegeof Pharmacy laboratory: Methanol and acetonitrile (HPLC Grade, FisherScientific), triflouroacetic acid 99% (Aldrich Chemical Company, Inc.), water wasobtained from a NANO pure^R ultrapure water system. Standard ginsenosidesRg1, Re, Rb1, Rc, Rb2 and Rd isolated and characterized by NMR in the UIClaboratories; Rf was a gift from Dr. Dennis V.C. Awang at MediPlant ConsultingServices.

RESULTS

Figures 1-4 show typical chromatographic profiles of NorthAmerican ginseng (*Panax quinquefolius*) and Asian ginseng (*Panaxginseng*) respectively showing baseline resolution of peaks.



Figure 1. Chromatogram of Panax quinquefolius, University of Ottawa

Figure 2. Chromatogram of Panax quinquefolius, UIC

Figure 3. Chromatogram of Panax ginseng, University of Ottawa

Figure 4. Chromatogram of Panax ginseng, UIC

Based on these separations, Table 1 shows recovery analysis of selected ginsenosides from powdered root, softgel, capsule, liquid and syrup formulations, respectively. Recoveries of all standards were > 85%.

Compound	Ginseng	Ginseng	Ginseng	Ginseng	Ginseng
	Root	Softgel	Capsule	Liquid	Syrup
	powder			formulations	
Ottawa					
Rg1	96	107.4	104.1	98.3	96
	(-)	(8.1)	(5.9)	(1.5)	(-)
Re		124.1	96.0	101.2	
		(5.5)	(3.7)	(1.7)	
Rf		109.8	96.8	85.9	
		(-)	(2.9)	(2.2)	
Rb1		108.3	102.3	95.5	95
		(6.9)	(5.9)	(2.5)	(-)
Rc		104.0	107.3	98.7	
		(1.1)	(6.3)	(1.6)	
Rb2		100.1	92.3	100.9	
		(1.7)	(3.17)	(0.9)	
Rd		106.4	103.1	99.0	100
		(3.9)	(7.4)	(3.1)	(-)
Chicago					
Rg1			103	102	
Rb1			104	102	94
Rc			105	110	96
Rd			106	102	99

Table 1. Mean recoveries (s.e.) of marker compounds from various formulations (%).

Note: For Ottawa, n = 4-5 where s.e. is given, n = 1 where nos.e. is given. For Chicago, n = 2.

The response of the LC system to standard ginsenosides invarious ranges of concentration was highly linear with an $r^2>0.99$ in all cases (Table 2a). The procedure enabled detection of 12ng of Rg2and 22ng Rg1 at above background noise levels.

Table 2a.

Ginsenoside	Concentration range (mg/mL)	\mathbf{r}^2
Rg1	1.10-110.	0.9996
Re	5.70-114.	0.9980
Rf	2.80-56.0	0.9991
Rg2	0.60-56.0	0.9997
Rb1	4.80-96.0	0.9905
Rc	1.02-102.0	0.9971
Rb2	3.00-300.	0.9972
Rd	1.70-85.00	0.993

Linearity of peak area to concentration (Ottawa)

Similar linear response data was obtained in the Chicago laboratory; seeTable 2b.

Table 2b. Calibration curves and detection limit(Chicago)

In order to check the linearrelationships between UV and absorption intensity and amount of ginsenosides, calibration curves are established for Rg1, Re, Rb1, Rc, Rb2, Rd.

Regression Analysis of Standard Ginsenosides: Correlation Coefficient r²

Ginsenoside	Concentration range (mg/mL)	r ²
Rg1	32.6-2100	1.000
Re	32.3-2060	1.000
Rb1	36.0-2300	1.000
Rc	18.3-1180	1.000
Rb2	28.6-1830	0.999
Rd	32.3-2070	1.000

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Superscript "R"means trademark.

NB: Chicago laboratory initially used butanol saturated with water untillate 1995. Samples done with butanol saturated water were also done withmethanol and no substantial differences were seen. So all reported samples from theChicago laboratory used methanol based extraction.HPLC Methods for Separation andQuantitative Determination of Ginsenosides Used in the American Botanical Council'sGinseng Evaluation Program.

Literature Search for Root Powder Minimums

Results of Literature Search as Basis for Root Powder Minimums (%) Used in the Ginseng Evaluation Program

ASIAN GINSENG (PANAX GINSENG)

	Root	Lateral	Root		Leaf	
	(Main)	Root	Fiber	Leaves	stalks	Stem
Chang, 1989 (2)	0.91					
Chuang, 1995 (3)						
	3.72-9.03					
Korean White						
Korean Red						
	3.90-7.19					
Ko, 1989 (4)			3.3-7.2			
Liu, 1992 (5)						
(rootstock, not normal root)	4 10					
	4.19					
Ma, 1995 (6)	1.20-3.95					
Soldati, 1980 (9)	1.35	3.53	6.15	5.19	0.77	0.76
Sollorz, 1985 (10)	0 (0 1 00	1 5 4 2 0 2	6.75-			
	0.09-1.80	1.54-2.93	8.56			

The minimum level of ginsenosides found in the literature search was 0.69%. This value was then adjusted for the coefficient of variation for the test method and for the representative amount of ginsenosides assayed. The result is the Root Powder Minimum (RPM) of 0.59% for Asian ginseng

	Root (whole)	Tea
Chang, 1989 (2)	3.09	
Chuang, 1995 (3)	8.65-24.00	
Ma, 1995 (6)	1.17-8.60	0.23-9.85
Sanada, 1978 (7)	6.2	
Soldati, 1980 (9)	1.70	

AMERICAN GINSENG (PANAX QUINQUEFOLIUS)

The minimum level of ginsenosides found in the literature search for American ginseng was 1.17%. This value was then adjusted for the coefficient of variation for the test method and for the representative amount of ginsenosides assayed. The result is the Root Powder Minimum (RPM) of 1.00% for American ginseng

ELEUTHERO, AKA SIBERIAN GINSENG (ELEUTHEROCOCCUS SENTICOSUS)

	Eleutheroside	Eleutheroside
	В	E
Bladt,1990 (1)		
Eleutherococci radix type A (China / Russia /	0.03-0.15%	0.04-0.13%
Korea)	0.000 0.120 / 0	0101 011070
<i>Eleutherococci radix</i> type B (China / Korea / Japan)	– (traces)	0.02-0.05%
E. senticosus (Korea) root	0.15-0.18%	0.13-0.15%
E. senticosus (Korea) Root bark	0.09–0.11%	0.09–0.10%
E. senticosus (Korea) Wood	_	0.07-0.08%
E. senticosus (Korea) Stem bark	0.01%	0.05%
Slacanin, 1991 (8)		
Samples 1–9 (China, Siberia)	0.055-0.178%	0.10-0.120%
Sample 10 (Korea)	_	1.23%

Since some types of eleuthero contain no eleutheroside B, the GEP team set no root powder minimum for eleutheroside B content. The minimum level of eleutheroside E found in the literature search for eleuthero was 0.02%. This value was then adjusted for the coefficient of variation for the test method. The result is the Root Powder Minimum (RPM) of 0.019% for eleuthero root powder.

For eleuthero root *bark* powder, the minimum level of eleutheroside E found in the literature search was 0.09%. This value was then adjusted for the coefficient of variation for the test method. The result is the Root Powder Minimum (RPM) of 0.088% for eleuthero root *bark* powder.

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The foundations, organizations, and companies listed below have demonstrated their belief in the significance of this project as evidenced by their support of more than \$700,000 in donations to this historic, unprecedented project of the American Botanical Council. In addition to contributions from supporters, ABC has invested more than \$500,000 of its own operating funds.

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Dietary Supplement Quality Initiative

The DSQI Web Site is the most publicly visible part of the Dietary Supplement Quality Initiative. Beyond our consumer-oriented activities is a commitment to develop comprehensive quality practices and standards for dietary supplements. We believe increased levels of research in combination with widely accepted standards will benefit those who use, prescribe and make dietary supplements. In promoting a science-based understanding of the health benefits associated with supplementation we believe the quality, safety and availability of dietary supplements can only increase.

The Institute for Nutraceutical Advancement

The INA is a non-corporate division of Denver-based Industrial Laboratories, an independent laboratory that provides analytical and consulting services to the natural products industry. The INA's mission is to support the production of high quality botanical products. To serve their mission, in 1998, they launched the INA Methods Validation Program (MVP), an international project designed to select, validate and publish scientific methods for use in analyzing raw botanical materials. The INA MVP is funded through contributions from 30 companies, including some of the industry's largest producers of dietary supplements and botanical raw materials. These 30 companies along with seven industry organizations and the FDA comprise the INA MVP Advisory Committee.