ChromaDex®

Application Note

As published in "The Handbook of Analytical Methods for Dietary Supplements"

0048 - Ginseng for Ginsenosides by HPLC

Botanical Name: *Panax ginseng; Panax quinquefolium*

Common Names: American ginseng (*Panax quinquefolium*), Asian ginseng, Chinese ginseng (*Panax ginseng*), Korean ginseng
NOTE: *Eleutherococcus senticosus* (Eleuthero) is often referred to as "Siberian ginseng"; however, it is not related to Asian or American ginseng, and the following analytical methods do not apply to Eleuthero.

Parts of Plant Used: Roots
NOTE: Some ground ginseng powders or extracts may be adulterated with ginseng leaves. This type of adulteration usually can be detected by visual inspection: the material will have a green tint, as opposed to the typical reddish brown tint obtained from the roots.

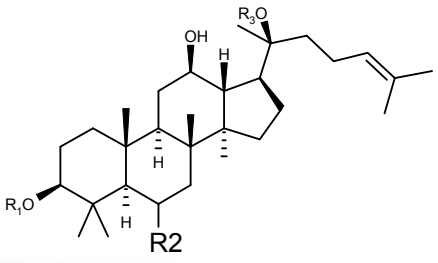
Uses: Both Asian and American ginsengs traditionally have been used as tonics to reduce effects of stress, to counteract fatigue, and to increase stamina.

Modes of Action:

There is very little clinical evidence that Asian and American ginsengs possess the pharmacological properties ascribed to them. Any activity that ginseng may have is generally attributed to a group of triterpenoid saponin glycosides commonly called ginsenosides. There are two main groups of ginsenosides, which are referred to as panaxadiols or panaxatriols, depending upon the number of hydroxyl groups attached to the saponin backbone. The ginsenosides are structurally related to sterols such as cholestanol. There is no known mode of action for the ginsenosides.

Chemical Markers:

Both Asian and American ginsengs are assayed for their ginsenoside content. More than 20 ginsenosides have been identified, but typically only seven are assayed: ginsenosides Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1. The remaining ginsenosides are present in very low concentrations. While both Asian and American ginsengs contain these same ginsenosides, the individual ratios are different between the two species. The structures of the marker compounds follow.



Ginsenoside	R_1	R_2	R_3
Rb1	Glc^2-Glc	H	Glc^6-Glc
Rb2	Glc^2-Glc	H	Glc^6-Ara(p)
Rc	Glc^2-Glc	H	Glc^6-Ara(f)
Rd	Glc^2-Glc	H	Glc
Re	H	O-Glc^2-Rha	Glc
Rf	H	O-Glc^2-Glc	H
Rg1	H	O-Glc	

10005 Muirlands Blvd., Suite G, Irvine, CA 92618 | Tel: +1-949-419-0288
Fax: +1-949-419-0294 | sales@chromadex.com | www.chromadex.com

Methods of Analysis

Most published analytical methods utilize reversed-phase HPLC with gradient elution and low-wavelength UV detection. Ginsenosides are very water soluble, owing to the presence of the sugar-group moieties; therefore, mobile phases are generally highly aqueous. Because ginsenosides do not possess a good chromophore, low-wavelength UV detection (203 to 205 nm) usually is used to take advantage of the absorptivity of the alkene group. Acetonitrile must be used as the organic modifier in the mobile phase to enable detection at these low wavelengths. Other more exotic detection techniques that are not dependent upon chromophores, such as evaporative light-scattering detection or MS, can be used.

Extraction of the ginsenosides from the matrix is of key importance. Ginsenosides have good solubility in aqueous extraction solvents, with decreasing solubility as the concentration of the organic modifier increases. Above about 40% organic concentration in the extraction solvent, incomplete recovery or inconsistent results occur, and multiple extractions must be performed on the sample. Acidic or basic extraction solvents also should be avoided, as hydrolysis of the glycosides can occur.

Method 1:

Court et al.[1] developed a gradient reversed-phase HPLC method for the determination of not only the seven listed ginsenosides, but also ginsenosides Rg2, Ro, F11, and gypenoside XVII in ground ginseng.

Sample Preparation:

Extract about 300 mg of ground ginseng root with 10 mL of methanol–water (70:30) in an ultrasonication bath for 15 minutes. Remove the solvent, and repeat the extraction of the residue twice. Dry the combined extracts under vacuum, and dissolve the residue in 2 mL of water containing m-cresol internal standard.

Chromatography:

Column: Waters Resolve C18, 150 × 3.9 mm, equipped with Symmetry C18 guard column.
Mobile phase: Solvent A = phosphate buffer, pH 5.81; solvent B = acetonitrile; solvent C = water.
Gradient:

Time (minutes)	%A	%B	%C
0–15	81–79	19–21	0
15–24.5	79–73.7	21–26.3	0
24.5–29	73.7–73	26.3–27	0
29–43	73–66	27–34	0
43–47	66–64	34–36	0
47–54	64–57	36–43	0
54–55	57–0	43–85	0–15
55–59	0	85	15

Flow rate: 1.15 mL/minute
Injection volume: 5 µL
Detection wavelength: 203 nm

Validation Data:

Linearity: Not specified
Accuracy: Not specified
Precision: 1.1% to 8.7% RSD, depending on ginsenoside (six determinations).
Selectivity: Peak identification of Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1 was determined against standards. In addition, selectivity was confirmed by comparing results with those obtained using a different chromatographic system.
Ruggedness: Not specified
Robustness: Not specified
LOD/LOQ: Not specified

Method 2:

The Institute for Nutraceutical Advancement (INA) developed a reversed-phase gradient HPLC method2 for the determination of ginsenosides in ginseng roots and extracts

Sample Preparation:

Extract a standardized extract (about 90 mg) or ground root (about 800 mg) with 25 mL of ethanol–water (50:50) by sonicating and shaking at 50oC. Cool, add about 10 mL of acetonitrile, and dilute the sample to 50 mL with water.

Chromatography:

Column: YMC ODS-A, 3 µm, 3.0 × 150 mm.
Mobile phase: Solvent A = water, solvent B = acetonitrile.
Gradient:

Time (minutes)	%A	%B
0–22	80–78%	20–22%
22–40	78–50%	22–50%
40–50	50–45%	50–55%
50–52	45–35%	55–65%

Flow rate: 0.6 mL/minute
Injection volume: 12 µL
Detection wavelength: 205 nm

Validation Data:

Linearity: 0.5 to 60 mcg/mL for each ginsenoside; correlation coefficient 0.99978 to 1.00000, depending on ginsenoside. RSD of response factors: 3.37% to 9.61%, depending on ginsenoside.
Accuracy: Ginseng extracts and root material were spiked with a ginseng extract of known ginsenoside content at the 150% and 200% target. The percent recoveries were calculated and ranged from 98.5 to 106.3.
Precision: 1.47% RSD for extract sample (total ginsenosides; six determinations).
1.74% RSD for root sample (total ginsenosides; six determinations).
Selectivity: Peak identification of Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1 was determined against standards. In addition, selectivity was shown using UV diode-array analysis.
Ruggedness: Three different laboratories analyzed six sets of samples. The percent RSD between the three laboratories for each sample was: Sample 1 = 5.6%; Sample 2 = 2.1%; Sample 3 = 4.6%; Sample 4 = 5.3%; Sample 5 = 5.0 %; and Sample 6 = 0.7%.
Robustness: Not determined
LOD/LOQ: LOD = 0.348 to 1.58 mcg/mL, depending on the ginsenoside; LOQ = 1.16 to 5.27 mcg/mL, depending on the ginsenoside.

Method 3:

Li and Fitzloff [3] developed an HPLC method with evaporative light-scattering detection (ELSD) to overcome the limitations of poor sensitivity associated with low-wavelength UV detection.

Sample Preparation:

Extract about 0.5 g of ground root with three 15-mL portions of methanol in a 50-mL volumetric flask using sonication and shaking at 25 to 30oC for 30 minutes. Combine the methanol extracts and dry. Reconstitute the residue in methanol, and bring to volume in a 10-mL volumetric flask.

Chromatography:

Column: Waters Spherisorb ODS-2 C18, 250 × 4.6 mm.
Mobile phase: Solvent A = water, solvent B = acetonitrile.
Gradient:

Time (minutes)	%A	%B
0–20	80	20
20–60	80–58	20–42
60–61	58–10	42–90

Flow rate: 1.6 mL/minute
Injection volume: 10 µL
Detection: Sedex 75 ELSD; evaporation chamber temperature = 35oC; nebulizing gas pressure = 3.4 bar.

Validation Data:

Linearity: 20 to 200 mcg/mL for each ginsenoside; correlation coefficient 0.9976 to 0.9998, depending on the ginsenoside.
Accuracy: No spiking studies were performed.
Precision: 4.23 to 9.89% CV, depending on ginsenosides; three determinations, control sample.
Selectivity: Peak identification of Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1 was determined against standards. In addition, selectivity was shown using UV diode-array analysis.
Ruggedness: Not determined
Robustness: Not specified
LOD/LOQ: LOD = 50 ng for each ginsenoside; LOQ = not specified.

Method 4:

Gomez-Serranillos et al.[4] developed a rapid, sensitive HPLC–UV method with solid-phase extraction (SPE) sample cleanup. The SPE step removes many interfering peaks, increasing the sensitivity of the method while allowing for a shorter run time.

Sample Preparation:

Extract ground ginseng root sample or powdered ginseng tablets with methanol–water (70:30) at room temperature for 30 minutes. Evaporate the solution and reconstitute the residue in water. Apply a portion of the aqueous solution to a Waters Sep-Pak C18 cartridge. Wash the cartridge with water, and follow by methanol–water (30:70). Elute the ginsenosides with methanol. Evaporate the eluate and reconstitute the residue with methanol–water (30:70).

Chromatography:

Column: Hypersil ODS, 5 m, 4.6 × 200 mm.
Mobile phase: Solvent A = water–acetonitrile (90:10), solvent B = acetonitrile.

Gradient:

Time (minutes)	%A	%B
0–20	75–50	25–50

Flow rate: 2.0 mL/minute
Injection volume: 10 µL
Detection wavelength: 205 nm

Validation Data:

Linearity: Not specified
Accuracy: Not specified
Precision: Not specified
Selectivity: Peak identification of Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1 was made against standards. In addition, selectivity was shown using UV diode-array analysis.
Ruggedness: Not specified
Robustness: The method was tried on three different brands of C18 columns with comparable results.
LOD/LOQ: Not specified

References:

1. Court WA, Hendel JG, Elmi J. Reversed-phase high-performance liquid chromatographic determination of ginsenosides in *Panax quinquefolium. J Chromatogr A.* 1996;755:11–7.
2. Institute for Nutraceutical Advancement. Panax and American ginseng assay by HPLC. Available at: http://www.nsf.org/business/ina/index.asp?program=INA.
3. Li WK, Fitzloff JF. HPLC analysis of ginsenosides in the roots of Asian ginseng (*Panax ginseng*) and North American ginseng (*Panax quinquefolium*) with in-line photodiode array and evaporative light scattering detection. *J Liq Chromatogr Relat Technol.* 2002;25(1):29–41.
4. Gomez-Serranillos MP, Palomino OM, Carretero E, et al. A new HPLC method for the analysis of ginsenosides in *Panax ginseng. Fitoterapia.* 1997;68(6):533–6.