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Application Note

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

0064 - Eleuthero (Siberian Ginseng) for Eleutherosides by HPLC

Botanical Name: *Eleutherococcus senticosus Maxim; Acanthopanax senticosus Harms*

Common Names: Ci-Wu-Jia, Eleuthero, pepperbush, Ussurian thorny

Parts of Plant Used: Roots

Uses: Adaptogen; immune-enhancing agent; a remedy for stress, fatigue, and depression.

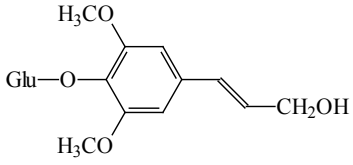
Modes of Action:

Several clinical trials have been performed to validate the activity of Siberian ginseng. The results are not consistent: some results showed that Siberian ginseng or a herbal formula with Siberian ginseng was effective and some results were negative.[1–4] Currently, no active components have been identified in Siberian ginseng.

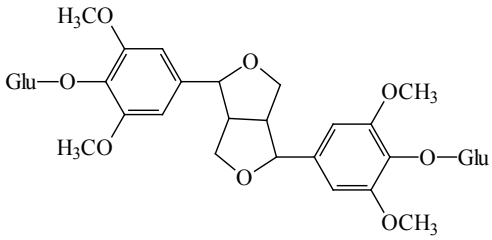
Chemical Markers:

Several types of compounds have been identified in Siberian ginseng roots and leaves including polysaccharides, phenolics (coumarins, phenylpropanoic derivatives, lignans), sterols and triterpene glycosides, thymidine, and isomaltol 3-O-α-D-glucopyranoside.[5–12] Most of the compounds isolated from Siberian ginsengs were named as eleutherosides, although they are different types of compounds. For examples, eleutheroside A (daucosterol) is a sterol, eleutheroside B (syringin) is a phenylpropanic derivative, eleutheroside B1 (isofraxetin) is a coumarin glycoside, eleutheroside E is a lignan diglycoside, eleutheroside E1 is a lignan monoglycoside, and eleutheroside I is a triterpene saponin. Since no bioactive components have been identified in Siberian ginseng and eleutherosides B and E are the major phenolic compounds in the root extract, they are used as marker compounds for quality control of Siberian ginseng extract. Usually in the market, Siberian ginseng is sold as a 0.8% extract of eleutherosides B and E.

Major Phenolics in Siberian ginseng



Eleutheroside B



Eleutheroside E

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Methods of Analysis

The compounds in Siberian ginseng root have been analyzed by various methods including TLC, HPLC, LC-MS, and micellar electrokinetic chromatography.[13–16] HPLC is the most popular method and has been widely used in the industry for analysis of eleutherosides B and E as a way of quality control of Siberian ginseng extract.

Extraction is key for accurate analysis of phytochemicals. Usually, methanol–water solutions are suitable extraction solvents.

Method 1:

The method found at www.nsfina.org was used.

Sample Preparation:

For root powder, transfer about 2 g to a 100-mL round-bottom flask and reflux with 50 mL of methanol–water (60:40) for 30 minutes. Filter the extraction solution into a 100-mL volumetric flask. Extract the residue a second time for 30 minutes using 50 mL of the same solvent and add to the first extraction solution. Dilute to volume with methanol–water (60:40).

Chromatography:

Column: Phenomenex Luna C18, 5 µm, 4.6 × 250 mm or YMC-Pack Pro C18, 4.6 × 250 mm (an equivalent column).
Mobile phase: Solvent A = water (adjusted to pH 2 with phosphoric acid), solvent B = acetonitrile.
Gradient:

Time (minutes)	%A	%B
0	90	10
2	90	10
20	70	30
25	70	30
27	90	10
45	90	10

Column temperature: 40°C
Flow rate: 0.8 mL/minute
Detection wavelength: 215 nm
Injection volume: 20 µL

Validation Data:

Not available, but this method is known to be a fully validated method.

Method 2:

The method at www.chromadex.com was used.

Sample Preparation:

Add about 1 g of Siberian ginseng extract to a 100-mL volumetric flask. Add 50 mL of 10% acetonitrile and shake until the extract is dissolved. Dilute to volume with 10% acetonitrile.

Chromatography:

Column: Phenomenex Luna C18(2) 3 µm, 150 × 4.6 mm.
Mobile phase: Solvent A = water–acetonitrile (90:10), solvent B = water–acetonitrile (60:40).
Gradient: 100%A to 100% B in 25 minutes
Flow rate: 1.0 mL/minutes.
Injection volume: 20 µL
Detection wavelength: 220 nm

Method 3:

The method of Yat et al.[17] was used.

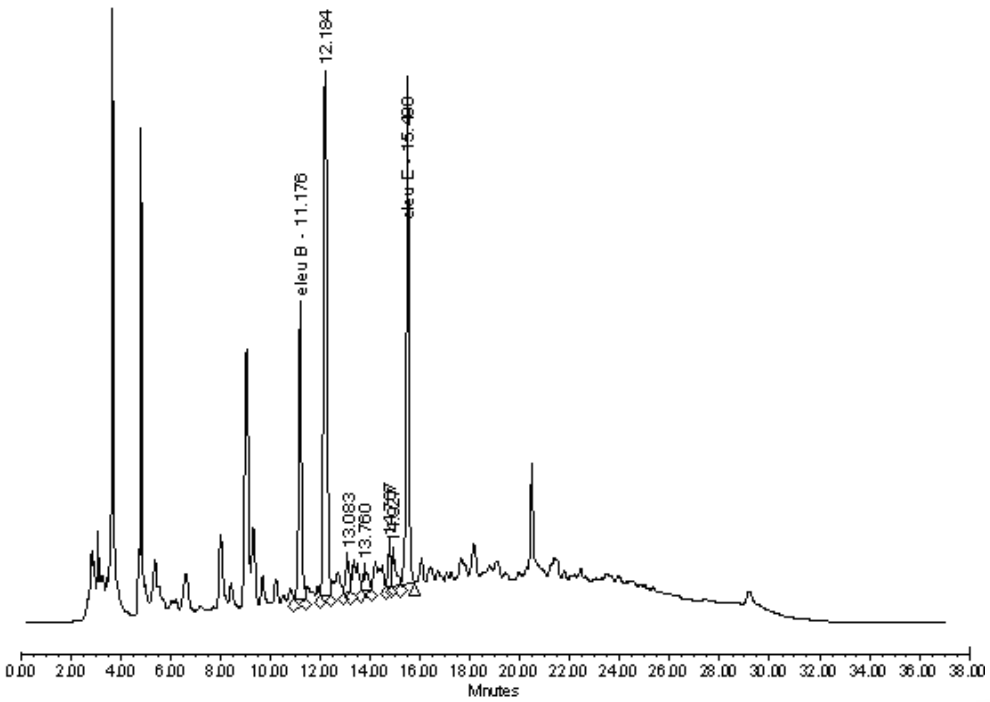
Sample Preparation:

Extract powdered root samples at 60°C with 20% aqueous methanol (2 × 30 mL) for 30 minutes each. Evaporate the combined extracts to dryness and dissolve in aqueous 0.05% trifluoroacetic acid–methanol (1:4).

Chromatography:

Column: Beckman Ultrasphere ODS, 5 µm, 250 × 4.6 mm, with a Beckman ODS precolumn, 5 µm, 45 × 4.6 mm.
Mobile phase: Solvent A = water (0.05% trifluoroacetic acid), solvent B = acetonitrile.
Gradient: 10%B to 50%B in 30 minutes.
Flow rate: 1.0 mL/minute
Injection volume: 20 µL
Detection wavelength: 220 nm
Column temperature: Ambient

Representative HPLC Chromatogram of Siberian Ginseng Extract Run by Method 1 using Phenomenex Luna C18(2) Column.



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