

ChromaDex[®]

Application Note

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

0050 - Goldenseal for Alkaloids by HPLC

Botanical Name: *Hydrastis canadensis L.*

Common Names: Eye-balm, goldenseal, ground raspberry, wild turmeric, yellow eye, yellow paint, yellow root

Parts of Plant Used: Dried rhizomes and roots

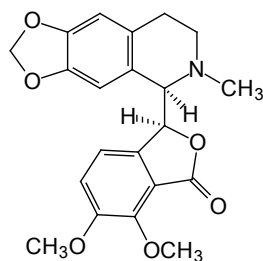
Uses: Treatment of infection; inflammation; congestion of lungs, throat, and sinuses; diarrhea; colds; and flu. Immune system booster.

Modes of Action:

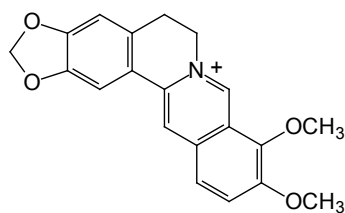
Although goldenseal is a well-known herbal medicine in North America, it has not been evaluated in many clinical trials. There is a general belief that the major alkaloids berberine, β -hydrastine, canadine, and canadine are the bioactive components in goldenseal and are responsible for its bioactivity.

Chemical Markers:

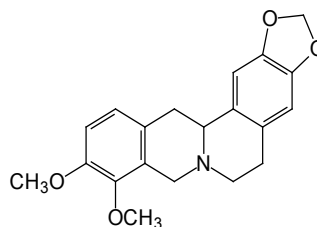
The isoquinoline alkaloids are the major chemical components in goldenseal root. The total content of alkaloids in goldenseal root is about 5% and the main alkaloids are berberine, hydrastine, canadine, and canadine.¹ Goldenseal also contains (-)-8-oxotetrahydrothalifendine, canadine acid, hydrastidine, isohydrastidine, 5-O-feruloylquinic acid butyl ester, 4-O-feruloylquinic acid butyl ester, chlorogenic acid, polyphenols, and fatty acids.²⁻⁷ When the volatile components of goldenseal were analyzed by GC-MS, several compounds including 2,3-dihydro-3,5-dihydro-6-methyl-4H-pyran-4-one and 5-(hydroxymethyl)-2-furane carboxaldehyde were identified.⁸ Usually hydrastine and berberine are used as chemical markers for quality control of goldenseal.



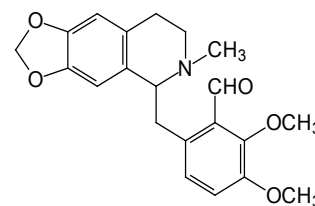
Hydrastine



Berberine



Canadine



Canadine

Methods of Analysis

HPLC is the primary method for the analysis of berberine and hydrastine in goldenseal root. Other methods include capillary electrophoresis–MS, TLC, and colorimetry.

Various extraction solvents have been used to extract alkaloids from goldenseal including methanol, 90% methanol, 70% methanol, and 50% methanol acidic solution. Based on the literature, they all extract berberine and hydrastine from goldenseal.

Method 1:

The HPLC method of Abourashed and Khan⁹ was used to determine the hydrastine and berberine content in powders, capsules, and tablets.

Sample Preparation:

Sonicate 100 mg of powder in 3 mL of methanol for 15 minutes, then centrifuge for 5 minutes at 1500 rpm. Transfer the supernate to a 10-mL volumetric flask and repeat this procedure twice. Combine the supernates and dilute to 10 mL for HPLC analysis.

Chromatography:

Column: Phenomenex Luna C18, 5 μ m, 150 \times 4.6 mm, and SecurityGuard cartridge system.

Mobile phase: Solvent A = 0.10 M sodium acetate–acetic acid (pH 4.0 in water), solvent B = acetonitrile–methanol (90:10).

Gradient:

Time (minutes)	%A	%B
0	80	20
20	40	60

Flow rate: 1.0 mL/minute

Injection volume: 10 μ L

Detection wavelength: 290 nm

Validation Data:

Linearity: 3.1 to 100 mcg/mL with a correlation coefficient of over 0.999 for berberine and hydrastine.

Accuracy: The percent recoveries were 98.38 and 98.36 for hydrastine and berberine, respectively.

Precision: 2.67% RSD for hydrastine and 0.86% RSD for berberine.

Selectivity: Peak identification was determined against standards.

Ruggedness: Not specified

Robustness: Not specified

LOD/LOQ: LOQ = 3.13 mcg/mL for both compounds, LOD = 1.0 mcg/mL for both compounds

Method 2:

The HPLC method Li and Fitzloff¹⁰ was used to determine the hydrastine and berberine content in powders, capsules, and tablets.

Sample Preparation:

Extract one capsule with 15 mL of 90% methanol (containing 1% acetic acid) by sonication for 60 minutes and let

stand overnight. Filter the solution and then extract with 15 mL of methanol (containing 1% acetic acid) by sonication for 30 minutes. Filter the solution into a flask and wash the residue with three 15-mL portions of methanol. Combine the extracts, and evaporate to dryness under reduced pressure. Dissolve the residue in 10 mL of methanol for HPLC analysis.

Chromatography:

Column: Supelco Discovery C18, 5 μ m, 250 \times 4.6 mm.

Mobile phase: Solvent A = water (containing 10% acetonitrile and 0.1% trifluoroacetic acid), solvent B = acetonitrile (containing 0.1% trifluoroacetic acid).

Gradient:

Time (minutes)	%A	%B
0	90	10
20	40	60

Flow rate: 1.0 mL/minute

Injection volume: 10 μ L

Detection wavelength: 225 nm

Column temperature: 20°C

Validation Data:

Linearity: 10 to 200 mcg/mL with a correlation coefficient over 0.999 for berberine and hydrastine.

Accuracy: The average percent recoveries were 98.25 and 104.2 for berberine and hydrastine, respectively.

Precision: Not specified

Selectivity: Peak identification was determined against standards.

Ruggedness: Not specified

Robustness: Not specified

LOD/LOQ: LOQ = 0.4 mcg/mL for both compounds, LOD = 0.1 mcg/mL for both compounds.

Method 3:

The HPLC method of Weber et al.¹¹ was used to determine berberine, hydrastine, canadine, and palmatine.

Sample Preparation:

Extract 0.3 to 2 g of powdered roots with 100 mL of acetonitrile–water–phosphoric acid (70:30:0.1 vol/vol/vol).

Sonicate the sample at ambient temperature for 5 minutes, shake on wrist-action shaker for 10 minutes, and then centrifuge at 2200 rpm for 5 minutes.

Chromatography:

Column: Zorbax Elipse XDB-C18, 80Å, 3.5 μ m, 150 \times 4.6 mm with Zorbax Eclipse XDB-C18, 80Å, 5 μ m, 12.5 \times 4.6 mm guard column.

Mobile phase: Solvent A = buffer solution (30 mM ammonium acetate–14 mM triethylamine, adjusted to pH 4.85 with acetic acid), solvent B = acetonitrile. A/B, 680/320 vol/vol, isocratic.

Flow rate: 1.0 mL/minute

Injection volume: 10 μ L

Detection wavelength: 230 nm

Column temperature: 30°C

Validation Data:

Linearity: For palmatine, 0.439 to 8.760 mcg/mL with a correlation coefficient of 0.99999; for berberine, 5.220 to 103.560 mcg/mL with a correlation coefficient of 0.99998; for hydrastine, 5.490 to 109.2 mcg/mL with a correlation coefficient of 0.99994; and for canadine, 0.462 to 9.060 mcg/mL with a correlation coefficient of 0.99996.

Accuracy: Palmatine = 92.3% recovery, berberine = 101.5%, canadine = 101.6%, and hydrastine = 101.9%.

Precision: The RSDs for all four alkaloids were less than 1.6%.

Selectivity: Peak identification was determined against standards.

Ruggedness: Less than 5% RSD between two chemists.

Robustness: Not specified

LOD/LOQ: Not specified

Method 4:

The HPLC method of Wang et al.1 was used to determine the hydrastine and berberine content in powders, capsules, and tablets.

Sample Preparation:

Extract 100 mg of root powder or extract with 35 mL of 50% methanol (containing 2% of concentrated hydrochloric acid) by sonication for 25 minutes and shaking for 15 minutes. Cool to room temperature and dilute to volume using 50% methanol (containing 2% concentrated hydrochloric acid).

Chromatography:

Column: Phenomenex Luna Phenylhexyl, 3 μ m, 150 \times 4.6 mm.

Mobile phase: Acetonitrile–isopropanol–water–phosphoric acid (34:10:56:0.2 vol/vol/vol/vol) with sodium lauryl sulfate added to achieve the final concentration of 5 mM.

Flow rate: 1.2 mL/minute

Injection volume: 10 μ L

Detection wavelength: 235 nm

Validation Data:

Linearity: For hydrastine, 2 to 1000 mcg/mL with a correlation coefficient of over 0.9996; and for berberine, 2 to 800 mcg/mL with a correlation coefficient of over 0.9992.

Accuracy: The percent recoveries were 98.40 and 102.9 for hydrastine and berberine, respectively.

Precision: 2.10% RSD for hydrastine and 1.48% RSD for berberine.

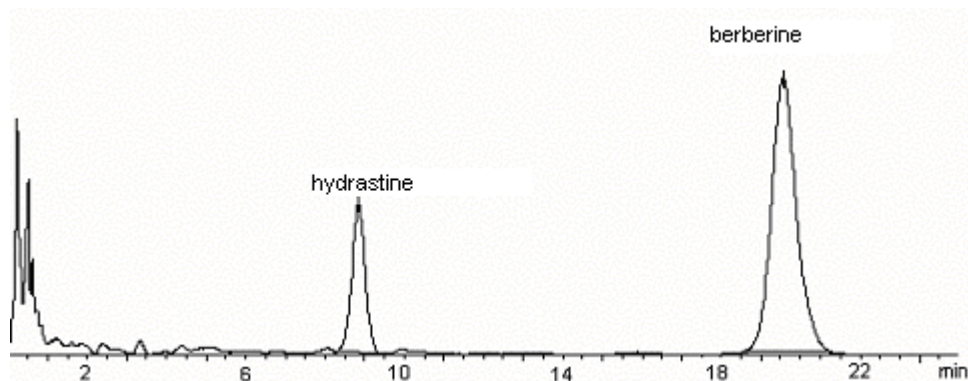
Selectivity: Peak identification was determined against standards.

Ruggedness: 0.49% between two chemists.

Robustness: Not specified

LOD/LOQ: LOQ = 2 mcg for both compounds

Representative HPLC Chromatogram of Goldenseal Run by Method 4.



References:

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