NSF International Standard for Botanical Dietary Supplement Ingredients: Goldenseal Root (*Hydrastis canadensis*)

1 General

1.1 Purpose

This Standard provides test methods and evaluation criteria for the dietary supplement ingredient goldenseal root (*Hydrastis canadensis*) to allow for the determination that this botanical ingredient is accurately identified, that the product contains the quantity of dietary ingredients and marker constituents as determined by the American Herbal Pharmacopoeia (AHP), that the ingredient does not contain unacceptable quantities of contaminants, conforms to the compliance criteria of the AHP, and can be used to facilitate GMP compliance.

1.2 Scope

This Standard contains requirements for dietary supplements that contain goldenseal root as an ingredient in a dietary supplement as defined as a dietary substance for use by man to supplement the diet by increasing the total dietary intake, or a concentrate, metabolite, constituent, extract, or combinations of these ingredients. With appropriate modifications to the testing methodology, this Standard can also apply to extracts of the ingredient.

Products and ingredients deemed a hazard to public health or safety by a regulatory agency having jurisdiction shall be excluded from the scope of this document.

2 Normative references

The following documents contain requirements, which by reference in this text, constitute requirements of this Standard.

AHP, American Herbal Pharmacopoeia and Therapeutic Compendium, *Goldenseal*, 2001[4]

AHP, American Herbal Pharmacopoeia, *Goldenseal Compliance Monograph*, 2008

Dietary Supplements Health and Education Act of 1994, (an amendment to the Federal Food, Drug and Cosmetic Act): Public Law 103-417 – October 25, 1994[1]

3 Definitions

Terms used in this Standard that have special technical meaning are defined here.

3.0 AHP Pharmacopoeial Standard: The primary standard of quality required to meet AHP compliance.

3.1 Roots and rhizomes: The unnderground portion of specific plants.

[1] Superintendent of Documents, U. S. Government Printing Office, Washington, D. C. 20401

3.2 Hydrastine and berberine: Two isoquinolone alkaloids contained in the roots and rhizomes of goldenseal used as markers for identity and quality.

3.3 Macroscopic Identification: A method of visual assessment based on the gross morphological (macroscopic) and sensory (organoleptic) characteristics of the material.

3.4 Organoleptic characterization: A method of assessment based on the sensory (organoleptic) characteristics of the material including color, taste, smell, texture, etc.

3.5 Annular scars: Raised fleshy tissue occurring on the upper part of a rhizome, usually formed into a ring, representing the foremer presence of the plant stem.

3.6 Medullary rays: A network of vascular tissue that conducts water and nutrients through the plant body in higher plants and forms a visual spoke-like pattern seen in the cross sections of roots.

3.7 Pith: The soft, spongelike, central cylinder of the stems and some roots of flowering plants, composed mainly of specialized tissue (parenchyma).

3.8 Fracture: The description of the manner in which a root, rhizome, or bark breaks.

3.9 Microscopic Identification: A method of assessment based on the identification of the microscpic structures and tissues of the plant material. Requires specialized training.

3.10 Quantitative standards: Standards for establishing the purity profile of the material.

3.11 Foreign Organic Matter: Consists of foreign elements of plant origin that are not derived from the specific plant species and part given in the definition.

3.12 Total Ash: Test designed to measure the amount of the residual substances when a sample is ignited under the conditions specified in the individual monograph or according to pharmacopoeial test methodologies.

3.13 Acid Insoluble Ash: Test designed to detect unacceptable quantities of certain minerals (e.g. from rock and dirt). The test measures the amount of ash insoluble to diluted hydrochloric acid according to pharmacopoeial test methodologies.

3.14 Loss of Moisture on Drying: Test designed to measure the moisture content in dry material. Determined on 1.000 g of the powdered material by drying in an oven at 105 ˚C for 2 h.

4 Ingredient requirements

4.1 AHP Pharmacopoeial Standard

Goldenseal Root (*Hydrastis canadensis* L.): Goldenseal root consists of the fresh or dried roots and rhizomes of *Hydrastis canadensis* L. containing not less than 2.0% hydrastine (C21H21NO6) and 2.5% berberine (C20H18NO4) calculated on a dry weight basis.

4.1.1 Macroscopic Identification

Goldenseal rhizome and root are traded both fresh and dried, in whole, cut, and

powdered forms. When fresh, the full-grown rhizome is knotted and sub-cylindrical, 1-6.5 cm in length and 2-10 mm in diameter. Fibrous rootlets are sparsely distributed on the upper surface of the rhizome and are thicker on the sides and lower surface. The roots make up approximately 70% and the rhizomes 30% of the underground portion. On average, the rhizome and roots of a single plant weigh 5-11 g. When freshly picked, they are a bright yellow both internally and externally. When dry, the rhizome is sub-cylindrical, knotted, contorted, 1-5 cm in length, and 2-6 mm in diameter. The external surface is brownish-gray to yellowish-brown in color and rough due to the raised, circumferential growth rings which are spaced approximately 1.5-3.1 mm apart. The upper surface has small, raised, annular scars where past stems emerged from the rhizome. These scars look like old wax seals, hence the common name. The fracture is short, brittle, clean, and resinous, revealing a smooth brownish-yellow or greenish-yellow internal surface and a yellowish-orange center. In cross section, the bark is approximately 0.5 mm thick. The wood, approximately 1 mm thick, is arranged radially with broad medullary rays. The pith is light yellowish-orange and is large in diameter compared to the wood. The dry root is 4-7 cm in length and 0.2-0.4 mm in diameter. The external surface is brownish-gray to yellowish-brown in color. The fracture is brittle and short; when magnified it has the appearance of broken beeswax. The internal surface is bright yellow to orange-yellow in younger roots, changing to greenish-yellow or dark yellowish-brown in older roots. Occasionally there is a reddish hue to the central part of the root. The bark is thick and the wood is arranged in a quadrangular fashion.

4.1.2 Organoleptic Characterization

Aroma: Characteristic and persistent. Taste: Acrid and astringent, possessing a marked and unique bitterness stimulating salivation. Powder: Bright yellow to brownish-yellow.

4.1.3 Microscopic Identification

4.1.3.1 Rhizome: Preparation in chloral hydrate: Parenchyma tissue dominates the rhizome in cross section. The rhizome has a thin, yellowish-brown cork consisting of several thin-walled cell layers. The secondary phloem consists of parenchyma cells only; these are generally thin-walled, though in the outer regions they may be somewhat thickened. The cells are rounded or polygonal in cross section and elongated in longitudinal view, and frequently contain yellow-brown, granular masses. Close to the cambium, semicircular regions of smaller cells indicate the sieve tubes and companion cells. Interior to the cambium and associated with the phloem bundles are found narrow cuneiform groups of vessels separated by wide medullary rays. The vessels are small with numerous slit-shaped pits, bordered pits, or helical secondary walls. Many of the pitted vessels are filled with yellow, amorphous, granular masses. The thin-walled cells of the pith, as well as other parenchyma cells, contain single or compound starch granules with either a round or slit-shaped hilum. Calcium oxalate crystals, sclereids, and fibers are absent throughout.

4.1.3.2 Root: Preparation in chloral hydrate: Parenchyma tissue dominates the root in cross section. The root is covered by a hypodermis of a single cell layer. The cortex consists of parenchyma cells only and is separated from the stele by a conspicuous primary endodermis, the cells of which often have sinuous walls. The stele shows the typical structure of an oligarch radial bundle. Sclerenchymatous cells and crystals are absent. The primary diagnostic characteristics are the pervading yellow color, the minute starch grains, the absence of calcium oxalate crystals, the nature of the elements of the wood, and the absence of sclerenchymatous cells.

4.1.3.3 Powder: Goldenseal root powder contains numerous, mostly single, nearly spheroidal starch grains from 2-15 µm in diameter, either free or in the parenchyma

cells; fragments of the fibro-vascular bundles mostly associated with starch-bearing parenchyma; vessels with pitted (simple and bordered) or helical secondary walls; and occasional fragments of tabular cork cells with yellowish- or reddish-brown walls and occasional granular masses attached to them. Powdered goldenseal root may be adulterated by goldenseal leaf. Goldenseal leaf powder is dark green and lacks the persistent characteristic odor and taste of the root. Pure goldenseal leaf or admixtures of root and leaf can be identified by the occurrence of leaf fragments with unicellular, thick-walled, acute trichomes (up to 600 µm in length) and epidermal cells with wavy or sinuous walls. On fragments from the lower epidermis of the leaf, anomocytic stomata are often found. Note: Goldenseal that has been improperly dried, stored, or that is old will have a greenish hue to it rather than a brilliant gold color.

4.2 Sampling, preparation, and analysis of samples - High Performance Thin Layer Chromatography (HPTLC) for the Identification of Goldenseal Root

4.2.2 Sample Preparation

In a test tube, 0.25 g of powdered drug is extracted in an ultrasonic bath at room temperature for 30 minutes with 4 mL of a methanol and water mixture (80:20). The suspension is filtered and the residue washed twice with 2 mL methanol. The filtrate and washings are combined and brought up to volume with methanol in a 20 mL volumetric flask. One mL of the solution is transferred into a small sample vial. This is the test solution. The solution is sensitive to light and heat and must be stored in the refrigerator in an amber vial. Hydro alcohol extracts can be applied to the plate directly. Dried extracts can be dissolved in an appropriate solvent (e.g. methanol) and stirred and applied to the plate directly

4.2.3 Standard Preparation

In a 50 mL volumetric flask, 25.0 mg (1R,9S)-b-hydrastine HCl, 1.0 mg hydrastinine HCl, 1.2 mg palmatine chloride, and 1.2 mg berberine chloride are dissolved in methanol. After dissolution is complete, the solution is brought up to volume with methanol. One mL of this solution is transferred into a small sample vial. This is the reference solution.

Note: Hydrastine is light and heat sensitive and readily decomposes.

4.2.4 Ninhydrin Reagent (optional)

Ninhydrin reagent is prepared by dissolving 0.6 g Ninhydrin in 190 mL isopropanol/5 mL acetic acid.

4.2.5 Chromatographic Conditions

4.2.5.1 Stationary Phase:

HPTLC plates 10 x 10 cm or 20 x 10 cm silica gel 60 F254.

Note: HPTLC plates allow for better separation, shorter development times, and less solvent. Standard TLC plates can also be used under the same conditions.

4.2.5.2 Solvent System:

Ethyl acetate:methanol:formic acid:water (50:10:6:3).

4.2.5.3 Sample Application:

5 µL test solution and 5 µL standard are applied each as a 8 mm band with 4 mm distance between bands. Application position should be 8 mm from lower edge of

plate.

4.2.5.4 Development:

10 x 10 cm or 20 x 10 cm Twin Trough Chamber, chamber pre-saturated for 15 minutes, 5 mL or 10 mL, solvent, respectively, per trough Developing distance 60 mm from lower edge of plate. Dry plate in a stream of cold air.

4.2.5.5 Detection:

a) UV 254 nm.

b) UV 366 nm.

c) Ninhydrin reagent (optional): Immerse plate in or spray plate with reagent for 2 seconds, then dry in a stream of cold air. Heat plate to 120 °C for two minutes. Evaluate under white light.

Table 1 - Rf values of standards and corresponding bands in goldenseal root

a) UV 254	**Observation**	**Approximate R_f value**
Berberine	Broad dark band	0.51
Hydrastine	Broad dark band (absent or only traces in leaf)	0.30
Hydrastinine*	Sharp but faint blue fluorescent band	0.06
Additional bands	Light black bands visible between hydrastinine and hydrastine	0.20
Palmatine **	Absent	0.38 (if present denotes adulteration)
b) UV 366		
Berberine	Broad and intense yellow band	0.51
Hydrastine	Broad dark blue band (absent or only traces in leaf)	0.30
Hydrastinine	Sharp fluorescent blue-white band	0.06
Palmatine	Absent	0.38 (if present denotes adulteration)
c) White light, ninhydrin reagent		
Berberine	Faint to marked yellow band	0.51
Hydrastine	Faint yellow band (absent or only traces in leaf)	0.30
Hydrastinine	Not visible (a brown band not corresponding to hydrastinine is visible)	0.06
Additional bands	Reddish brown bands visible between hydrastinine and hydrastine	0.20
Palmatine	Absent	0.38 (if present denotes adulteration)

* Hydrastinine is a degradation compound of hydrastine and can result from improper storage or aging.

** Palmatine is a compound present in a number of adulterants of goldenseal (e.g., *Berberis vulgaris, Coptis chinensis*, *Mahonia* spp., *Xanthorhiza*).

4.3 High Performance Liquid Chromatography (HPLC) for the Quantification of Berberine and Hydrastine

4.3.2 Sample preparation

Place 1.0 g of accurately weighed finely powdered goldenseal rhizome and root into an extraction thimble. Connect soxhlet extractor to a 500-mL round bottom flask. Add 200 mL* of methanol to the powder and extract for 6 hours or until the solvent is clear. The volume of the thimble should be at least one-half the volume of methanol. Cool to room temperature and transfer the extract to a 250- mL volumetric flask. Rinse the extraction apparatus with methanol and transfer the rinsings to the volumetric flask, diluting with methanol to volume. Hydro alcohol extracts can be dissolved in an appropriate solvent (e.g. methanol) by stirring and filtering if necessary. Kerri, any thing else needed here?

4.3.3 Standard preparation

Dissolve accurately weighed quantities of berberine chloride and hydrastine hydrochloride in a 1:1 mixture of water and methanol to obtain a solution containing approximately 0.2 mg/mL of each standard. The standard solution must be prepared fresh daily. Reference standards are available from Chromadex Inc, Irvine, CA and phytoproof reference standard, PhytoLabs, Germany.

Note: To account for the concentration of chlorides, the concentrations of berberine and hydrastine in the standard solution are calculated by multiplying the concentration of each reference standard.

4.3.4 Chromotagraphic conditions

Column:	Phenomenex Luna 2, C18, 5 µm, 4.6 x 150 mm or equivalent.
Column Temperature:	Ambient.
Mobile Phase: Isocratic:	27% acetonitrile, 73% 0.1M potassium dihydrogen phosphate.
Flow Rate:	1.8 mL/minute.
Detection:	235 nm.
Injection Volume:	10 µL.
Run Time:	10 minutes.

343.5 Calculation

Measuring the areas under the major peaks, calculate the percentage of berberine and hydrastine using the following formula:

100(CV/W)(ru/rs)

C = Concentration (mg/mL) of berberine or hydrastine in the reference standards used in the standard solution
calculated using the correction factors given above).
V = Final volume (mL) of the sample solution.
W =Weight (mg) of goldenseal sample used.
ru = Peak areas for berberine and hydrastine obtained from the sample solution.
rs = Peak areas for berberine and hydrastine obtained from the standard solution.

4.3.6 System suitability

The capacity factor determined from hydrastine and berberine peaks shall not be less than 3.0, the column efficiency shall not be less than 2.0, and the relative standard deviation for replicate injections shall not exceed 2.0%.

4.3.7 Linearity range

The linearity range was 20% to 200% of the target concentrations of hydrastine and berberine. The correlation coefficient was > 0.999 for both compounds.

4.3.8 Retention times (min)

Hydrastine: 4.75
Berberine 6.88

Note: This method was validated on goldenseal raw material. With specific modifications to the sample preparation as noted this method can also be used for the analysis of goldenseal extracts. For analyzing extracts retention times may change and the calculation has to be adjusted acoording to the purported concentration of the extract.

4.4 Quantitative standards

Foreign Organic Matter:	Not to exceed 2%.
Total Ash:	Not to exceed 9%.
Acid Insoluble Ash:	Not to exceed 5%.
Loss of Moisture on Drying:	Not to exceed 12.0% determined on 2 g of powdered goldenseal dried at 100 °C for 5 hours.

4.5 Storage

Follow general guidelines for storage of dry materials by packing in airtight containers that are protected from light, heat, moisture, and insect infestation.