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Belladonna Leaf

DEFINITION

Belladonna Leaf consists of the dried leaf and flowering or fruiting top of *Atropa belladonna* L. or of its variety *acuminata* Royle ex Lindley (Fam. Solanaceae). Belladonna Leaf yields NLT 0.35% of the alkaloids of belladonna leaf.

ASSAY

• **Procedure**

Phosphate buffer: 34.8 g of dibasic potassium phosphate in 900 mL of water. Adjust to a pH of 9.5 by the addition of 3 N hydrochloric acid or sodium hydroxide, with mixing.

Diluent: Dilute sulfuric acid (1 in 350)

Internal standard solution: 0.8 mg/mL of USP Homatropine Hydrobromide RS in *Diluent*. [Note—Prepare fresh on the day of use.]

Standard stock solution A: 1.0 mg/mL of USP Scopolamine Hydrobromide RS in *Diluent*

Standard stock solution B: Dissolve 20 mg of USP Atropine Sulfate RS in 25 mL of *Diluent* in a 50-mL volumetric flask, add 2.0 mL of *Standard stock solution A*, and mix. Add *Diluent* to volume. [Note—Prepare fresh on the day of use.]

Standard solutions: Pipet into three separate 60-mL separators 1.0-, 2.0-, and 3.0-mL portions, respectively, of *Standard stock solution A*, and add 9.0, 8.0, and 7.0 mL, respectively, of *Diluent*. Add 1.0 mL of *Internal standard solution*, then add 15 mL of chloroform, shake vigorously, allow the layers to separate, and discard the chloroform layer.

Sample solution: Moisten 10 g, previously reduced to a moderately coarse powder, with a mixture of 8 mL of ammonium hydroxide, 10 mL of alcohol, and 20 mL of ether, and extract the alkaloids by either *Method I* or *Method II* as follows. If necessary, reduce the volume of the extract to 100 mL by evaporation on a steam bath.

Extraction blank: Place 10 mL of *Diluent* in a 60-mL separator. Prepare as directed under *Standard solutions*, beginning with "then add 15 mL of chloroform". The blank chromatogram contains no significant interferences at the locus of atropine, scopolamine, or homatropine.

Method I: Place the moistened drug in a continuous-extraction thimble, and allow maceration to proceed overnight, then extract with ether for 3 h, or longer if necessary, to effect complete extraction.

Method II: Place the moistened drug in a small percolator, and allow maceration to proceed overnight. Percolate slowly with a mixture of three volumes of ether and one volume of chloroform. Continue the percolation until the residue from 3–4 mL of percolate last passed, when dissolved in dilute sulfuric acid (1 in 70) and treated with mercuric iodide TS, shows NMT a faint turbidity.

Transfer the extract from *Method I* or *Method II* to a separator with the aid of ether. Extract with five 15-mL portions of dilute sulfuric acid (1 in 70), filtering each portion drawn off into a 100-mL volumetric flask. Wash the filter with dilute sulfuric acid (1 in 70), and collect the washings in the flask. Add dilute sulfuric acid (1 in 70) to volume, and mix. Dilute 20.0 mL of the resulting solution with the same dilute acid to 100.0 mL. Pipet 10 mL of this solution into a 60-mL separator. Add 1.0 mL of *Internal standard solution*, then add 15 mL of chloroform, shake vigorously, allow the layers to separate, and discard the chloroform layer. [Note—If emulsions are formed, a mixed solvent consisting of chloroform-isopropyl alcohol (10:3) may be substituted for chloroform throughout the extraction procedure.]

Add another 15 mL of chloroform, and extract again, discarding the chloroform phase. Add 15 mL of *Phosphate buffer* and sufficient 1 N sodium hydroxide to yield a final pH between 9.0 and 9.5. Add 15 mL of chloroform, shake vigorously, and allow the layers to separate. Filter the organic phase through 10 g of anhydrous sodium sulfate (see *Reagents, Indicators, and Solutions—Sodium Sulfate, Anhydrous, Suitability for Alkaloid Assays*), previously washed with chloroform and supported in a funnel with a small pledget of glass wool, into a suitable container. Extract again with two 15-mL portions of chloroform, again collecting the clarified organic phase. Wash the sodium sulfate and the tip of the funnel with 5 mL of chloroform. Evaporate the combined organic phases under reduced pressure at a temperature below 45°, add 1 mL of chloroform, and mix to dissolve the alkaloids, taking care to wet the sides of the container.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 1.2-m × 4-mm glass; packed with 3% G3 on S1AB. [Note—The column may be cured and conditioned as specified under *Chromatography ⟨621⟩, General Procedures, Gas Chromatography*.]

Temperatures

Injection port: 240°

Detector: 240°

Column: 215°

Carrier gas: Dry helium

Flow rate: 65 mL/min

Injection volume: 5 µL

System suitability

Sample: *Sample solution*

Suitability requirements

Resolution: NLT 3.0 between atropine and homatropine peaks

Tailing factor: NMT 2.0 for atropine peak

Relative standard deviation: The analytical system is suitable for conducting this *Assay* if the relative standard deviation for the ratio, R_A, is NMT 2.0%, for the atropine peak in repeated injections.

Analysis

Samples: *Standard solutions* and *Sample solution*

Measure the areas, a_A, a_H, and a_S, of the atropine, homatropine, and scopolamine peaks, respectively, in each chromatogram of *Standard solution*, and calculate the ratios A_A and A_S:

$$A_A = a_A/a_H$$

$$A_S = a_S/a_H$$

Plot the standard curves of the values of R_A and R_S against the amounts, in milligrams, of atropine and scopolamine in the solutions. (The ratio of the molecular weight of atropine to that of anhydrous atropine sulfate is 0.8551, and the ratio of the molecular weight of scopolamine to that of anhydrous scopolamine hydrobromide is 0.7894.) Inject a portion of the *Sample solution* into the chromatograph, measure the peak areas, and calculate the area ratios, as with the *Standard solutions*. Record from the standard curves the quantities, in milligrams, of atropine and scopolamine in the weight of the specimen taken. Add the quantity, in milligrams, of atropine and scopolamine, and multiply by 50 to obtain the weight, in milligrams, of alkaloids in the portion of Belladonna Leaf taken.

Acceptance criteria: NLT 0.35% of the alkaloids of belladonna leaf

CONTAMINANTS

- **Articles of Botanical Origin ⟨561⟩, *Methods of Analysis, Acid-Insoluble Ash*:** NMT 3.0%
- **Articles of Botanical Origin ⟨561⟩, *Pesticide Residue Analysis*:** Meets the requirements
- **Belladonna Stems:** The proportion of belladonna stems over 10 mm in diameter does not exceed 3.0%.

SPECIFIC TESTS

- **Botanical Characteristics**

Macroscopic: Usually partly matted together, crumpled or broken leaves, together with some smaller stems and a number of flowers and fruits. The leaves are thin and brittle, mostly light green to moderate olive-green. The lamina is mostly 5–25 cm in length and 4–12 cm in width and possesses an ovate-lanceolate to broadly ovate outline, an acute to acuminate apex, an entire margin, an acute to somewhat decurrent base and slightly hairy surface, the hairs being more abundant along the veins; when broken transversely, it shows numerous light-colored dots (crystal cells) visible with a lens. The petiole is slender and usually up to 4 cm in length. The flowers possess a campanulate corolla with five small, reflexed lobes, purplish to yellowish purple, becoming faded to brown or dusky yellow or yellow; a green, five-lobed calyx; five epipetalous stamens; and a superior, bilocular ovary with numerous ovules. The fruit is subglobular, dark yellow to yellowish brown to dusky red or black, up to 12 mm in width, and sometimes subtended by the persistent calyx and containing numerous flattened, somewhat reniform seeds, the latter up to 2 mm in width. The stems are more or less flattened and hollow and finely hairy when young.

Microscopic

Leaf: The epidermis of the lamina possesses wavy anticlinal walls and a distinctly striated cuticle. Stomata are more numerous in the lower epidermis and are surrounded by three or four neighboring cells, one of which is smaller than the others. The nonglandular hairs are uniseriate and up to six-celled. Short club-shaped glandular hairs with a one-celled stalk and multicellular head and long glandular hairs with a uniseriate stalk and unicellular head occur on both epidermises. The mesophyll consists of a single layer of palisade parenchyma beneath which occurs spongy parenchyma, the latter with scattered cells filled with microcrystals. The midrib contains an arc of bicollateral bundles, collenchyma beneath upper epidermis, and scattered parenchyma cells with microcrystals.

Stem: The stem shows an epidermis with striated cuticle and few hairs; a distinct endodermis; small strands of long, thin-walled, slightly lignified pericyclic fibers; and a circle of bicollateral bundles. The parenchyma of the cortex and pith is interspersed with crystal cells.

Flower: The calyx possesses numerous glandular hairs with uniseriate stalks and one- to three-celled glandular heads. The corolla shows a papillose inner epidermis and an outer epidermis with glandular hairs similar to those of the calyx. The pollen grains, when mounted in chloral hydrate solution, are subspherical, 40 µm in diameter, tricolpate, having three germinal furrows and rows of pits between the ridges on the exine.

Fruit: The epicarp exhibits polygonal epidermal cells with a striated cuticle and stomata. The mesocarp consists of large pulp cells some of which contain rosette aggregate crystals of calcium oxalate.

Seed: The seed is characterized by an epidermis of large, wavy-walled cells with prominent ridges over the anticlinal walls.

Powdered Belladonna Leaf: Light olive-brown to moderate olive-green in color. The following are among the elements of identification: the separate microcrystals, the dark gray crystal cells, the cuticular striping of the epidermal cells, the vessels with ellipsoidal bordered pits,

the fibers of the stem, and occasional hairs and pollen grains. Rosette aggregates of calcium oxalate and fragments of the seed occur when the drug contains belladonna fruits. Examine Belladonna Leaf for hairs having a papillose cuticle and for raphides of calcium oxalate: their presence indicates adulteration.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers and avoid long exposure to direct sunlight. Preserve powdered Belladonna Leaf in light-resistant containers.

• **USP Reference Standards** ⟨11⟩

USP Atropine Sulfate RS
USP Homatropine Hydrobromide RS
USP Scopolamine Hydrobromide RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
BELLADONNA LEAF	Nam-Cheol Kim Scientific Liaison	BDSHM2020 Botanical Dietary Supplements and Herbal Medicines
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	BDSHM2020 Botanical Dietary Supplements and Herbal Medicines

Chromatographic Database Information: Chromatographic Database

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