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Rauwolfia Serpentina

DEFINITION

Rauwolfia Serpentina is the dried root of *Rauwolfia* (L.) Bentham ex Kurz (Fam. Apocynaceae), sometimes having fragments of rhizome and aerial stem bases attached. It contains NLT 0.15% of reserpine–rescinnamine group alkaloids, calculated as reserpine.

IDENTIFICATION

• **A. Chemical Identification**

[Note—In these analyses, use formamide treated as directed if it has an ammoniacal odor.]

Immobile solvent: Formamide and acetone (30:70)

Mobile solvent A: Isooctane, carbon tetrachloride, piperidine, and tertiary butyl alcohol (90:60:4:2)

Mobile solvent B: Chloroform, isooctane, and tertiary butyl alcohol (75:75:2)

Spray solution: 250 mg/mL of trichloroacetic acid in methanol

Standard solution: 200 mg/mL of USP Rauwolfia Serpentina RS in alcohol. [Note—Warm USP Rauwolfia Serpentina RS with alcohol at 55°–65° for 30 min, with occasional mixing. Cool, and filter.]

Sample solution: Reduce 10 g of Rauwolfia Serpentina root to a fine powder. Treat a 1-g portion as in the preparation of the *Standard solution*.

Analysis A: Line the sides of a chromatographic chamber suitable for ascending chromatography (see *Chromatography* ⟨621⟩) with blotting paper. Transfer *Mobile solvent A* to the bottom of the container, and cover the chamber. Immerse a 20-cm × 20-cm sheet of filter paper (Whatman No. 1 or equivalent) in the *Immobile solvent*, and blot between paper toweling. Allow the acetone solvent to evaporate completely. Apply 1-µL portions of the *Sample solution* and the *Standard solution* to a line 2.5 cm from the bottom of the filter paper. Allow to dry. Apply a 2-µL portion of the *Immobile solvent* to each spot, allow to dry, and suspend the paper so that it dips into the *Mobile solvent*. Cover the chamber and after 1 h, when the *Mobile solvent* has risen approximately seven-eighths of the height of the paper, remove the chromatogram and dry at 90° in a current of air. Spray the paper lightly and evenly with the *Spray solution*, and dry at 90° for 10 min.

Analysis B: Use the apparatus described in *Analysis A*, but containing a glass trough with 2 mL of ammonium hydroxide to saturate the atmosphere of the tank with ammonia (NH_3). Transfer *Mobile solvent B* to the bottom of the tank outside the trough. Complete the test as described in *Analysis A*, omitting the *Spray solution*. Examine both chromatograms under UV light, and note the fluorescent spots.

Acceptance criteria: In both chromatograms, the *Sample solution* yields spots corresponding in position and color to those from the *Standard solution*.

COMPOSITION

• **Procedure**

Apparatus: A medium-sized continuous-extraction apparatus provided with a 250-mL flask and a 35-mm × 80-mm thimble is convenient, although a smaller apparatus may be used.

[Note—Protect the flask and thimble and all solutions of Rauwolfia Serpentina alkaloids from direct or strong light.]

Solvents: Alcohol, chloroform, and 1,1,1-trichloroethane. [Note—Use 1,1,1-trichloroethane with a boiling range between 73° and 76°.]

Standard stock solution: Dissolve 20.0 mg of USP Reserpine RS in 25 mL of hot alcohol, cool, dilute with alcohol to 50.0 mL, and mix. When stored in a tightly-stoppered, light-resistant bottle in the dark, this solution is chromogenically stable for several weeks.

Standard solution: Dilute 5.0 mL of the *Standard stock solution* with alcohol to 100.0 mL, and mix before using.

Analysis: Extract 2.5 g of finely powdered Rauwolfia Serpentina, accurately weighed, in a continuous-extraction apparatus for 4 h. Use 100 mL of vigorously boiling alcohol as solvent, and a few boiling chips to prevent bumping.

Wash the extract into a 100-mL volumetric flask with alcohol, cool, dilute with alcohol to volume, and mix. Transfer 20.0 mL to a separator containing 200 mL of 0.5 N sulfuric acid, mix, and extract with three 25-mL portions of 1,1,1-trichloroethane. Lubricate stopcocks only with lubricants insoluble in trichloroethane or chloroform (polytef stopcocks are satisfactory). Drain the lower phase as completely as possible. Wash each of the 1,1,1-trichloroethane extracts in a second separator containing 50 mL of 0.5 N sulfuric acid, and discard the trichloroethane extracts.

Extract the weakly basic alkaloids from the first acid solution with 25-, 15-, 15-, 10-, 10-, and 10-mL portions of chloroform. Wash each chloroform extract with the acid in the second separator, then with two 10-mL portions of sodium bicarbonate solution (1 in 50) in two

additional separators. Filter the chloroform extracts through chloroform-washed cotton into a 100-mL volumetric flask containing 10 mL of alcohol. Dilute with alcohol to volume, and mix. Transfer duplicate 10.0-mL aliquots to glass-stoppered, 25-mL conical flasks, and mix with 4 mL of alcohol. Evaporate with gentle heating almost to dryness, place in a vacuum desiccator, and evaporate to dryness. Dissolve the residues by agitating with 5.0 mL of alcohol.

Transfer duplicate 5.0-mL aliquots of the *Standard solution* to flasks. Add 2.0 mL of 0.5 N sulfuric acid to one of the sample flasks and to one of the standard flasks (the blanks). Add to the other flasks 1.0 mL of 0.5 N sulfuric acid and 1.0 mL of sodium nitrite solution (3 in 1000). Mix the contents of each flask, and warm in a water bath at 50°–60° for 20 min. Cool, add to each flask 500 µL of sulfamic acid solution (1 in 20), and mix. After stabilization of the solution colors, determine their absorbances in 1-cm cells at 390 nm, relative to a blank consisting of a mixture of alcohol and water (2:1).

Calculate the quantity of reserpine–rescinnamine group alkaloids, in mg, in the sample taken:

$$\text{Result} = 5(A - A_0)/(S - S_0)$$

A = absorbance of the nitrite-treated sample

A_0 = absorbance of the sample blank

S = corresponding absorbance of the solution from the respective *Standard solution* aliquot

S_0 = corresponding absorbance of the blank from the respective *Standard solution* aliquot

Acceptance criteria: NLT 0.15% of reserpine–rescinnamine group alkaloids, calculated as reserpine

CONTAMINANTS

• **Articles of Botanical Origin ⟨561⟩, *Pesticide Residue Analysis*:** Meets the requirements

SPECIFIC TESTS

• **Microbial Enumeration Tests ⟨61⟩** and **Tests for Specified Microorganisms ⟨62⟩:** Rauwolfia Serpentina (as a ground root) meets the requirements of the tests for absence of *Salmonella* species.

• **Articles of Botanical Origin ⟨561⟩, *Methods of Analysis, Foreign Organic Matter*:** NMT 2.0% of stems and NMT 3.0% of other foreign organic matter

• **Articles of Botanical Origin ⟨561⟩, *Methods of Analysis, Acid-Insoluble Ash*:** NMT 2.0%

• **Loss on Drying ⟨731⟩**

Analysis: Dry at 100° to constant weight.

Acceptance criteria: It loses NMT 12.0% of its weight.

• **Botanical Characteristics**

Macroscopic

Unground Rauwolfia Serpentina root: This occurs as segments usually 5–15 cm in length (pieces sometimes shorter) and 3–20 mm in diameter. The pieces are subcylindrical to tapering, rather tortuous or curved, rarely branched, but bearing occasional twisted rootlets, which are larger, more abundant, and more rigid and woody on the thicker parts of the roots. Externally: light brown to grayish yellow to grayish brown; dull, rough, or slightly wrinkled longitudinally yet peculiarly smooth to the touch; occasionally showing small circular rootlet scars in the larger pieces, with some exfoliation of the bark in small areas to reveal the paler wood beneath. When scraped, the bark separates readily from the wood. Fracture short, but irregular, the longer pieces readily breaking with a snap, slightly fibrous marginally. The freshly fractured surfaces show a rather thin layer of grayish yellow bark, with the pale yellowish white wood constituting 80% of the radius. The smoothed transverse surface of larger pieces shows a finely radiate stele with three or more clearly marked growth rings; a small knoblike protuberance is frequently noticeable at the center. The wood is hard and of relatively low density. The odor is indistinct, earthy, and reminiscent of stored white potatoes.

Microscopic

Rauwolfia Serpentina root: A transverse section shows externally 2–8 alternating strata of cork cells, the strata with larger cells alternating with strata made up of markedly smaller cells (distinction from *R. canescens*). Each stratum composed of smaller cells includes 3–5 tangentially arranged cell layers; each stratum made up of larger cells includes 1–6 tangential layers. In a cross-sectional view, the largest central cells of the larger cell group measure 40–90 µm radially and up to 75 µm tangentially (although usually smaller); the cells of the smaller cell groups measure 5–20 µm radially and up to 75 µm tangentially. The walls are thin and suberized. The secondary cortex consists of several rows of tangentially elongated to isodiametric parenchyma cells, most being densely filled with starch grains; others (the short latex cells) occur singly or in short series and contain brown resin masses. The secondary phloem is relatively narrow and is made up of phloem parenchyma (bearing starch grains and less commonly tabular to angular calcium oxalate crystals up to 20 µm in length; also, occasionally, with some brown resin masses in outer cells and phloem rays) interlaid with scattered sieve tissue and traversed by phloem rays 2–4 cells in width. Sclerenchyma cells (stone cells and fibers) are absent in root (distinction from other species of *Rauwolfia*). Cambium is indistinct, narrow, dark, and wavering. The secondary xylem represents the large bulk of the root and shows

one or more prominent annual rings with a denser core of wood 500 µm across at the center. The xylem is composed of many wood wedges separated by xylem rays, and on closer examination reveals vessels in interrupted radial rows, much xylem parenchyma, many large-celled xylem rays, few wood fibers, and tracheids, all lignified-walled. The xylem fibers occur in both tangential and radial rows. The xylem rays are 1–12, occasionally up to 16, cells in width.

Rauwolfia Serpentina rhizome: This is similar to that of the root except for the presence of a prominent cortex, pericycle fibers, bicollateral vascular bundles, and a small central pith. The pericycle fibers occur singly or in groups of 2–5, and have thick, nonlignified walls and tapering, often lobed ends, with subterminal enlargements having thin walls and broad lumina. Vessel elements up to 485 µm are found. The xylem rays are 1–4 cells in width, with lignified and pitted walls. Internal phloem strands occur embedded in the outer region of the pith. The xylem fibers are somewhat less wavy than those of the root. The pith consists of starch parenchyma cells, among which are scattered short latex cells with yellowish contents stained brown with iodine TS.

Ground Rauwolfia Serpentina root: This is brownish to reddish gray in color. Present are very numerous starch grains (mostly simple, two- to three-compound, occasionally four-compound); simple grains spheroid, ovate, muller-shaped, plano- to angular-convex, or irregular; hilum simple, Y-shaped, stellate, or irregularly cleft; unaltered grains 6–34 µm (average 20 µm) in diameter, mostly in the lower range (maximum sizes larger than in *R. canescens* and *R. micrantha*); altered grains up to 50 µm in diameter; large unaltered grains show polarization cross clearly; calcium oxalate prisms and cluster crystals scattered, 10–15 µm in size; brown resin masses and yellowish granular secretion masses occur occasionally; isolated cork cells elongated, up to 90 µm in length; phelloderm and phloem parenchyma cells similar in appearance; vessels subcylindrical, up to 360 µm in length and 20–57 µm in diameter (narrower than in *R. canescens*) (the wall markings generally consist of simple pits, with bordered pits adjacent to xylem ray cells), the vessel end walls oblique to transverse, generally with openings in the end walls, some vessels showing tyloses; tracheids pitted, with moderately thick, tapering, beaded walls, with relatively broad lumina, polygonal in cross-section; xylem parenchyma cells with moderately thick walls with simple circular pits, cells polygonal in cross-section, bearing considerable starch; phloem and xylem-ray cells with pitted walls bearing much starch, sometimes with brown resin masses, xylem fibers with thick, heavily lignified walls showing small transverse and oblique linear pits and pointed simple to bifurcate ends, measuring 200–750 µm in length (shorter than in *R. micrantha* and *R. canescens*). No phloem fibers or sclereids are present in root (colorless nonlignified pericycle or primary phloem fibers, single or in small groups, may be present from rhizome or stem tissues).

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers, and store at controlled room temperature, in a dry place, secure against insect attack.

• **USP Reference Standards** ⟨11⟩
USP Rauwolfia Serpentina RS
USP Reserpine RS

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

Topic/Question	Contact	Expert Committee
RAUWOLFIA SERPENTINA	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

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