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Add the following:

^Siler Root Powder

DEFINITION

Siler Root Powder consists of the dried roots of *Saposhnikovia divaricata* (Turcz. ex Ledeb.) Schischk. (Fam. Apiaceae) reduced to a fine or very fine powder. The roots are collected in spring or autumn before or after the growth of flowering stems, and rootlets are removed. It contains NLT 0.32% of total chromones calculated as the sum of prim-O-glucosylcimifugin ($C_{22}H_{28}O_{11}$), cimifugin ($C_{16}H_{18}O_6$), 5-O-methylvisammioside ($C_{22}H_{28}O_{10}$), and sec-O-glucosylhamaudol ($C_{21}H_{26}O_{10}$), and NLT 0.18% of the sum of prim-O-glucosylcimifugin and cimifugin, all on the dried basis.

IDENTIFICATION

• A. [HPTLC FOR ARTICLES OF BOTANICAL ORIGIN \(203\)](#)

Standard solution A: 0.25 mg/mL of [USP 5-O-Methylvisammioside RS](#) and 0.5 mg/mL of [USP Cimifugin RS](#) in [methanol](#). Sonicate to dissolve.

Standard solution B: 100 mg/mL of [USP Saposhnikovia divaricata Root Dry Extract RS](#) in [methanol](#). Sonicate for 10 min, centrifuge, and use the supernatant.

Sample solution: About 0.5 g of Siler Root Powder (adjust the amount if necessary) in 5 mL of [methanol](#). Sonicate for 10 min, centrifuge, and use the supernatant.

Chromatographic system

(See standard parameters as defined in [HPTLC for Articles of Botanical Origin \(203\), Table 1](#).)

Application volume: 5 μ L, as 8-mm bands

Developing solvent system: [Cyclohexane](#), [methylene chloride](#), [acetone](#), [anhydrous formic acid](#), and [water](#) (3: 6: 8: 1.6: 1)

Derivatization reagent: 10% [sulfuric acid](#) in [alcohol](#). [NOTE—Prepare on the day of the test; it can be used for up to 1 week if stored in a tightly closed container in a cool place. Keep alcohol cold over ice, and add sulfuric acid slowly.]

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* as bands and dry in air. Develop in a saturated chamber, remove the plate from the chamber, and dry in air. Examine under UV light at 254 nm. Then treat the plate with *Derivatization reagent*, dry in air for 10 min, and examine under long-wavelength UV light.

System suitability

Under UV light at 254 nm: *Standard solution A* exhibits a bluish quenching band in the lower third due to 5-O-methylvisammioside and a bluish quenching band in the middle third due to cimifugin. In the lower third section, *Standard solution B* exhibits a bluish quenching band corresponding in R_F to 5-O-methylvisammioside in *Standard solution A* and a bluish quenching band below 5-O-methylvisammioside due to prim-O-glucosylcimifugin. In the middle third section, *Standard solution B* exhibits a bluish quenching band corresponding in R_F to cimifugin in *Standard solution A* and a quenching band below cimifugin due to sec-O-glucosylhamaudol.

Under long-wavelength UV light: *Standard solution A* exhibits a bright blue band in the lower third due to 5-O-methylvisammioside and a greenish blue band in the middle third due to cimifugin. In the lower third section, *Standard solution B* exhibits a bright blue band corresponding in R_F to 5-O-methylvisammioside in *Standard solution A*, a greenish blue band below 5-O-methylvisammioside due to prim-O-glucosylcimifugin, and a blue band right above 5-O-methylvisammioside due to nodakenin. In the middle third section, *Standard solution B* exhibits a greenish blue band corresponding in R_F to cimifugin in *Standard solution A*, and in the upper third section, two to three blue bands above cimifugin.

Acceptance criteria

Under UV light at 254 nm: The *Sample solution* exhibits the following bands corresponding to those in *Standard solution B*: in the lower third section, a bluish quenching band corresponding in R_F to 5-O-methylvisammioside in *Standard solution A* and a bluish quenching band below 5-O-methylvisammioside due to prim-O-glucosylcimifugin; in the middle third section, a bluish quenching band corresponding in R_F to cimifugin in *Standard solution A*, and in the upper third section, two to three blue bands above cimifugin.

to cimifugin in *Standard solution A* and a quenching band below cimifugin due to sec-*O*-glucosylhamaudol (a distinction from *Libonotis laticalycina* root).

Under long-wavelength UV light: The *Sample solution* exhibits the following bands corresponding to those in *Standard solution B*: in the lower half section, a bright blue band corresponding in R_f to 5-*O*-methylvisammioside in *Standard solution A*, a greenish blue band below 5-*O*-methylvisammioside due to prim-*O*-glucosylcimifugin, and a blue band right above 5-*O*-methylvisammioside due to nodakenin (a distinction from *Libonotis laticalycina* root); in the middle third section, a greenish blue band corresponding in R_f to cimifugin in *Standard solution A*; and in the upper third section, three to four blue bands (*L. laticalycina* root contains a total of five to six blue bands) above cimifugin.

• B. HPLC

Analysis: Proceed as directed in *Content of Chromones*.

Acceptance criteria: The *Sample solution* exhibits a peak at the retention time corresponding to 5-*O*-methylvisammioside in *Standard solution A* and peaks due to prim-*O*-glucosylcimifugin, cimifugin, and sec-*O*-glucosylhamaudol at retention times corresponding to those in *Standard solution B*. Beside the above mentioned peaks, no other peak between peaks of prim-*O*-glucosylcimifugin and sec-*O*-glucosylhamaudol is more intense than the peak of sec-*O*-glucosylhamaudol. The content ratios of each chromone relative to 5-*O*-methylvisammioside are within the ranges listed in [Table 2](#).

COMPOSITION

• CONTENT OF CHROMONES

Solution A: [Water](#)

Solution B: [Acetonitrile](#)

Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	87	13
6	85	15
9	85	15
11	82	18
17	79	21
18	75	25
25	73	27
33	5	95
46	5	95
46.1	87	13
55	87	13

Solvent: [Methanol](#) and [water](#) (8.5: 1.5)

Standard solution A: 0.05 mg/mL of [USP 5-*O*-Methylvisammioside RS](#) in *Solvent*

Standard solution B: 30 mg/mL of [USP Saposhnikovia divaricata Root Dry Extract RS](#) in *Solvent*, sonicate for 30 min. Before injection, pass through a suitable membrane filter of 0.22- μ m pore size.

Sample solution: Transfer about 500 mg of Siler Root Powder, accurately weighed, to a suitable flask, add 15.0 mL of *Solvent*, and close tightly. Accurately weigh the filled flask and sonicate for 45 min (NLT 200 W, NLT 40 kHz). Cool to room temperature and dry the flask, adding *Solvent* to adjust to the initial weight, if necessary. Before injection, pass through a suitable membrane filter of 0.22- μ m pore size and discard the first portion of the filtrate.