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**Add the following:**

## ^Chinese Skullcap Root

### DEFINITION

Chinese Skullcap Root consists of the dried roots of *Scutellaria baicalensis* Georgi (Family Lamiaceae) collected in spring or autumn by removing fibrous roots and rough skin. It contains NLT 11% of total flavone glucuronides calculated as the sum of baicalein 7-O-glucuronide (C<sub>21</sub>H<sub>18</sub>O<sub>11</sub>) and wogonin 7-O-glucuronide (C<sub>22</sub>H<sub>20</sub>O<sub>11</sub>) on the dried basis; and NMT 3.5% of total flavone aglycones calculated as the sum of baicalein (C<sub>15</sub>H<sub>10</sub>O<sub>5</sub>) and wogonin (C<sub>16</sub>H<sub>12</sub>O<sub>5</sub>) on the dried basis.

### IDENTIFICATION

• **A. HPTLC FOR ARTICLES OF BOTANICAL ORIGIN (203).**

**Standard solution A:** 1.0 mg/mL of [USP Baicalein 7-O-Glucuronide RS](#) and 0.5 mg/mL of [USP Baicalein RS](#) in [methanol](#). Sonicate to dissolve.

**Standard solution B:** 50 mg/mL of [USP Scutellaria baicalensis Root Dry Extract RS](#) in [methanol](#). Sonicate for 15 min, centrifuge, and use the supernatant.

**Sample solution:** 100 mg/mL of finely powdered Chinese Skullcap Root in [methanol](#). Sonicate for 15 min, centrifuge, and use the supernatant.

#### Chromatographic system

**Adsorbent:** Chromatographic silica gel F<sub>254</sub> mixture

**Application volume:** 3 µL, as 8-mm bands

**Relative humidity:** Condition the plate to a relative humidity of about 33% using a suitable device.

**Temperature:** About 25°

**Developing solvent system:** [Toluene](#), [ethyl acetate](#), [methanol](#), and [formic acid](#) (6:4:1:2)

**Developing distance:** 6 cm

**Derivatization reagent A:** 10 mg/mL of [2-aminoethyl diphenylborinate](#) in [methanol](#)

**Derivatization reagent B:** 50 mg/mL of [polyethylene glycol 4000](#) in [alcohol](#)

#### 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 the plate at 100° for 3 min. Treat the plate with *Derivatization reagent A*, and dry for 5 min with a current of cool air. Immediately treat the plate with *Derivatization reagent B*, dry for 5 min with a current of cool air, and examine under UV light at 365 nm.

#### System suitability

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

**Suitability requirements:** *Standard solution A* exhibits two dark bands, one due to baicalein 7-O-glucuronide in the lower-third section and one due to baicalein in the middle-third section. *Standard solution B* exhibits four dark bands: two corresponding in *R<sub>f</sub>* and color to the bands due to baicalein 7-O-glucuronide and baicalein in *Standard solution A*; one above baicalein 7-O-glucuronide due to wogonin 7-O-glucuronide; and one above baicalein due to wogonin. *Standard solution B* exhibits two blue bands below baicalein, a light-yellow band coeluted with wogonin 7-O-glucuronide, and a yellow band below baicalein 7-O-glucuronide.

**Acceptance criteria:** The *Sample solution* exhibits four dark bands: two corresponding in *R<sub>f</sub>* and color to the bands due to baicalein 7-O-glucuronide and baicalein in *Standard solution A* and *Standard solution B*; and one above baicalein 7-O-glucuronide due to wogonin 7-O-glucuronide and one above baicalein due to wogonin, both corresponding in *R<sub>f</sub>* and color to similar bands in *Standard solution B*. The *Sample solution* exhibits additional colorful bands including two blue bands below baicalein, a light-yellow band coeluted with wogonin 7-O-glucuronide, a yellow band below baicalein 7-O-glucuronide, and some other faint bands corresponding in *R<sub>f</sub>* and color to similar bands in *Standard solution B*. There are no significant red bands observed in the upper half of the chromatogram (the leaves of *S. lateriflora*, *S. scordiifolia*, *S. barbata*, and *S. indica* display multiple red bands in the upper-half section above baicalein).

• **B. HPLC**

**Analysis:** Proceed as directed in the test for *Content of Flavone Glucuronides and Flavone Aglycones*.

**Acceptance criteria:** The *Sample solution* exhibits the most intense peak corresponding to baicalein 7-O-glucuronide and a smaller peak corresponding to baicalein in *Standard solution A*. The *Sample solution* also exhibits peaks due to wogonin 7-O-glucuronide, wogonin, and two unidentified peaks with similar intensities as wogonin, between baicalein 7-O-glucuronide and wogonin 7-O-glucuronide, at retention times corresponding to the same constituents in *Standard solution B*. No other peak in the chromatogram between the relative retention time of 0.5 for baicalein 7-O-glucuronide and 1.2 for wogonin is more intense than the peak corresponding to wogonin. The content ratio of wogonin 7-O-glucuronide relative to baicalein 7-O-glucuronide is NLT 0.1 and NMT 0.3, and the content ratio of total flavone glucuronides to total flavone aglycones is NLT 3.0.

**COMPOSITION****• CONTENT OF FLAVONE GLUCURONIDES AND FLAVONE AGLYCONES**

[NOTE—Protect solutions from light and proceed under low actinic light. The standard solutions and the *Sample solution* are stable for 24 h at room temperature.]

**Solution A:** 0.1% [phosphoric acid](#) in [water](#)

**Solution B:** [Acetonitrile](#)

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

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	78	22
10	75	25
15	75	25
25	68	32
30	60	40
35	60	40
40	50	50
45	5	95
50	5	95
50.1	78	22
60	78	22

**Solvent:** [Methanol](#) and [water](#) (7:3)

**Standard stock solution:** 0.50 mg/mL of [USP Baicalein 7-O-Glucuronide RS](#) and 0.10 mg/mL of [USP Baicalein RS](#) in [methanol](#)

**Standard solution A:** 0.10 mg/mL of [USP Baicalein 7-O-Glucuronide RS](#) and 0.02 mg/mL of [USP Baicalein RS](#) from *Standard stock solution* diluted with *Solvent*

**Standard solution B:** 1 mg/mL of [USP Scutellaria baicalensis Root Dry Extract RS](#) in *Solvent*. Sonicate for 15 min, centrifuge, and pass through a suitable membrane filter of 0.45- $\mu$ m or finer pore size.

**Sample solution:** Transfer about 100 mg of finely powdered Chinese Skullcap Root accurately weighed into a suitable flask, add 50.0 mL of *Solvent*, and close tightly. Weigh the filled flask accurately, and sonicate for 30 min. Cool to room temperature and adjust to the initial weight by adding *Solvent*, if needed. Before injection, pass through a suitable membrane filter of 0.45- $\mu$ m or finer pore size, and discard the first portion of the filtrate.

**Chromatographic system**

(See [Chromatography \(621\)](#), [System Suitability](#).)

**Mode:** LC

**Detector:** UV 276 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing [L1](#)

**Column temperature:** 30°

**Flow rate:** 1.0 mL/min

**Injection volume:** 3  $\mu$ L

**System suitability**

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

**Suitability requirements**

**Resolution:** NLT 1.5 between the peak of baicalein 7-O-glucuronide and the small peak before it, *Standard solution B*

**Tailing factor:** NMT 2.0 for the baicalein 7-O-glucuronide and baicalein peaks, *Standard solution A*

**Relative standard deviation:** NMT 2.0% for the baicalein 7-O-glucuronide and baicalein peaks in repeated injections, *Standard solution A*

**Chromatogram similarity:** The chromatogram of *Standard solution B* is similar to the reference chromatogram provided with the lot of [USP Scutellaria baicalensis Root Dry Extract RS](#) being used.

**Analysis**

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

Using the chromatograms of *Standard solution A*, *Standard solution B*, and the reference chromatogram provided with the lot of [USP Scutellaria baicalensis Root Dry Extract RS](#) being used, identify the peaks corresponding to baicalein 7-O-glucuronide, wogonin 7-O-glucuronide, baicalein, and wogonin in the *Sample solution*.

Separately calculate the percentage of baicalein 7-O-glucuronide and wogonin 7-O-glucuronide against [USP Baicalein 7-O-Glucuronide RS](#) and the percentage of baicalein and wogonin against [USP Baicalein RS](#) in the portion of Chinese Skullcap Root taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times F \times 100$$

$r_U$  = peak area of the relevant analyte from the *Sample solution*

$r_S$  = peak area of baicalein 7-O-glucuronide or baicalein from *Standard solution A*

$C_S$  = concentration of [USP Baicalein 7-O-Glucuronide RS](#) or [USP Baicalein RS](#) in *Standard solution A* (mg/mL)

$V$  = volume of the *Sample solution* (mL)

$W$  = weight of Chinese Skullcap Root taken to prepare the *Sample solution* (mg)

$F$  = conversion factor for the relevant analyte (see [Table 2](#))

Calculate the content of the total flavone glucuronides as the sum of the percentages of baicalein 7-O-glucuronide and wogonin 7-O-glucuronide.

Calculate the content of the total flavone aglycones as the sum of the percentages of baicalein and wogonin.

**Table 2**

Analyte	Conversion Factor for Flavone Glucuronides	Conversion Factor for Flavone Aglycones
Baicalein 7-O-glucuronide	1.00	—
Wogonin 7-O-glucuronide	0.86	—
Baicalein	—	1.00
Wogonin	—	0.82

#### Acceptance criteria

**Total flavone glucuronides:** NLT 11% on the dried basis

**Total flavone aglycones:** NMT 3.5% on the dried basis

#### CONTAMINANTS

- [ARTICLES OF BOTANICAL ORIGIN \(561\)](#), [Limits of Elemental Impurities](#): Meets the requirements
- [ARTICLES OF BOTANICAL ORIGIN \(561\)](#), [Pesticide Residue Analysis](#): Meets the requirements
- [ARTICLES OF BOTANICAL ORIGIN \(561\)](#), [Test for Aflatoxins](#): Meets the requirements
- [MICROBIAL ENUMERATION TESTS \(2021\)](#): The total aerobic bacterial count does not exceed  $10^5$  cfu/g, the total combined molds and yeasts count does not exceed  $10^3$  cfu/g, and the bile-tolerant Gram-negative bacterial count does not exceed  $10^3$  cfu/g.
- [ABSENCE OF SPECIFIED MICROORGANISMS \(2022\)](#), [Test Procedures](#), [Test for Absence of Salmonella Species](#) and [Test for Absence of Escherichia coli](#): Meets the requirements

#### SPECIFIC TESTS

##### BOTANICAL CHARACTERISTICS

**Macroscopic:** The roots are conical and twisted, 8–25 cm long, 1–3 cm in diameter; the upper part rough with twisted longitudinal wrinkles or irregular reticular stripes; the lower part with longitudinal striations and fine wrinkles. The outer surface is brownish yellow or dark yellow with sparse and warty rootlet scars. Texture is hard and fragile, easily broken. The fracture is yellow but reddish brown in the center. The old roots have a dark-brown fracture with a withered or hollow center.

##### Microscopic

**Transverse section:** The cork consists of multiple layers of cells, up to 20 layers, mostly broken. The phelloderm is narrow with scattered sclereids. The phloem is broad with scattered sclereids in the outer part. The cambium is distinct. In the xylem, vessels are singly scattered or aggregated into groups and surrounded by lignified fiber bundles; xylem rays are broad. The parenchymatous cells contain starch granules and clusters of calcium oxalate. The central part of an old root usually appears with more than 1 cork tissue whorl.

- [ARTICLES OF BOTANICAL ORIGIN \(561\)](#), [Methods of Analysis, Foreign Organic Matter](#): NMT 1.0%
- [ARTICLES OF BOTANICAL ORIGIN \(561\)](#), [Methods of Analysis, Alcohol-Soluble Extractives, Method 1](#): NLT 18.0%
- [ARTICLES OF BOTANICAL ORIGIN \(561\)](#), [Methods of Analysis, Water-Soluble Extractives, Method 2](#): NLT 28.0%
- [ARTICLES OF BOTANICAL ORIGIN \(561\)](#), [Methods of Analysis, Total Ash](#): NMT 6.0%

- [ARTICLES OF BOTANICAL ORIGIN \(561\)](#), [Methods of Analysis, Acid-Insoluble Ash](#): NMT 1.0%
- [LOSS ON DRYING \(731\)](#).  
**Sample:** The roots, finely powdered  
**Analysis:** Dry the *Sample* at 105° for 5 h.  
**Acceptance criteria:** NMT 12.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light and moisture, and store at controlled room temperature.
  - **LABELING:** The label states the Latin binomial following the official name of the plant contained in the article. Dosage forms prepared with this article should bear the following statement: "Discontinue use and consult a healthcare practitioner if you develop symptoms of liver trouble, such as abdominal pain, dark urine, or jaundice (yellowing of the eyes or skin)."
  - **USP REFERENCE STANDARDS (11).**  
[USP Baicalein RS](#)  
[USP Baicalein 7-O-Glucuronide RS](#)  
[USP Scutellaria baicalensis Root Dry Extract RS](#)
- ▲ (USP 1-Dec-2019)

**Auxiliary Information:** Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
CHINESE SKULLCAP ROOT	<a href="#">Cuiying Ma</a> Senior Scientific Liaison	BDSHM2020 Botanical Dietary Supplements and Herbal Medicines

**Chromatographic Database Information:** [Chromatographic Database](#)

**Most Recently Appeared In:**

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