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Add the following:

▲Chinese Skullcap Root Powder

DEFINITION

Chinese Skullcap Root Powder consists of the dried roots of Chinese Skullcap (*Scutellaria baicalensis* Georgi) reduced to a fine or very fine powder. It contains NLT 11% of total flavone glucuronides calculated as the sum of baicalein 7-*O*-glucuronide ($C_{21}H_{18}O_{11}$) and wogonin 7-*O*-glucuronide ($C_{22}H_{20}O_{11}$) on the dried basis, and NMT 3.5% of total flavone aglycones calculated as the sum of baicalein ($C_{15}H_{10}O_5$) and wogonin ($C_{16}H_{12}O_5$) 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 Chinese Skullcap Root Powder in methanol. Sonicate for 15 min, centrifuge, and use the supernatant.

Chromatographic system

Adsorbent: Chromatographic silica gel F_{254} 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_F 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_F 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_F and color to similar bands in *Standard solution B*.

The *Sample solution* exhibits additional colorful bands including two blue bands below baiclein, 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_F 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-µm or finer pore size.

Sample solution: Transfer about 100 mg of Chinese Skullcap Root Powder 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-µm 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 × 25-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 3 µL

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 Powder 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 Powder 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: Yellowish-white or yellowish-green powder

Microscopic: Phloem fibers are fusiform, 60–250 µm long, 9–33 µm in diameter, with thick walls and fine pit canals, singly scattered or aggregated in bundles. Sclereids are subround, subsquare, or rectangular, with thick or very thick walls. Cork cells are brownish yellow and polygonal in surface view. Reticulate vessels are dominating, 24–72 µm in diameter. Lignified fibers are mostly broken with sparse oblique pits, about 12 µm in diameter. Starch granules are abundant, simple granules spheroidal, 2–10 µm in diameter, hilum distinct, compound granules composed of 2–3 simple granules.

• **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⟩

Analysis: Dry 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 POWDER	Cuiying Ma Senior Scientific Liaison	BDSHM2020 Botanical Dietary Supplements and Herbal Medicines

Chromatographic Database Information: Chromatographic Database

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