Herbal Medicines Compendium

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Sphaeranthus indicus Aerial Parts Dry Extract

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***Sphaeranthus indicus* Aerial Parts Dry Extract**

DEFINITION

The article consists of aerial parts of *Sphaeranthus indicus* L. (Family: Asteraceae) by extraction with a hydroalcoholic mixture. It contains NLT 90% and NMT 110% of the labeled amount of sphaeranthanolide, calculated on the dried basis.

POTENTIAL CONFOUNDING MATERIALS

None known

CONSTITUENTS OF INTEREST

Sesquiterpene glycoside: Sphaeranthanolide

Sterol glycoside: 7-hydroxyfrullanolide

IDENTIFICATION

- **A. Thin-Layer Chromatography**

Standard solution A: 1 mg/mL of USP Sphaeranthanolide RS in methanol

Standard solution B: Dissolve 0.1 g of USP *Sphaeranthus indicus* Aerial Parts Dry Extract RS in 10 mL of methanol with sonication for 10 min. Centrifuge and use the supernatant.

Sample solution: Mix 0.1 g of Dry Extract with 10 mL of methanol. Sonicate for 10 min, centrifuge, and use the supernatant.

Chromatographic system

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

Adsorbent: Use a suitable chromatographic material with an average particle size of 5 µm (HPTLC plates).

Application volume: 5 µL each of *Standard solution A*, *Standard solution B*, and *Sample solution*, as 8-mm bands

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

Developing solvent system: Dichloromethane and methanol (70: 9.2)

Developing distance: 7 cm

Derivatization reagent: Sulfuric acid reagent created by carefully adding 20 mL of sulfuric acid to 180 mL of ice-cooled methanol.

Analysis

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

Apply the *Samples* as bands to a suitable HPTLC plate and dry in air. Develop the chromatograms in a

saturated chamber, remove the plate from the chamber, and dry. Treat the plate with *Derivatization reagent* by dipping, heat for 5 min at 105°, and examine under visible light.

System suitability: Under visible light, the chromatogram of *Standard solution B* exhibits a dark brown band right above the origin in position and color similar to the sphaeranthanolide band in the chromatogram of *Standard solution A*. Six additional bands appear with increasing order of R_F: a broad dark brown band near the origin, a light purple band near one half of the lower half, a purple band right above the half, a strong blue band above the purple band, a blue/purple band, and a strong brown band near the front.

Acceptance criteria: Under visible light, the chromatogram of *Sample solution* exhibits a band due to sphaeranthanolide corresponding in color and R_F to the band in the chromatogram of *Standard solution A*. The following bands, with increasing R_F, correspond to the similar bands in the chromatogram of *Standard solution B:* a broad dark brown band near the origin, a light purple band in two thirds of the lower half, a purple band right above the half, a strong blue band, a blue/purple band, and a strong brown band near the front.

- **B. HPLC**

Analysis: Proceed as directed in the *Assay* for *Content of Sphaeranthanolide*.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits a peak at the retention time corresponding to the peak due to sphaeranthanolide in *Standard solution B*.

ASSAY

- **Content of Sphaeranthanolide**

Solution A: Acetonitrile

Solution B: Water

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	5	95
20	100	0
25	100	0
27	5	95
30	5	95

Standard solution A: 0.01 mg/mL of USP Sphaeranthanolide RS in methanol

Standard solution B: Dissolve 0.25 g of USP *Sphaeranthus indicus* Aerial Parts Dry Extract RS in 20 mL of methanol by shaking for 2 h at 60°. Cool to room temperature, pass through filter paper into a 50-mL volumetric flask, and fill with methanol. Dilute with methanol to obtain a solution with a concentration of about 0.5 mg/mL. Pass the solution through a membrane filter of 0.45-µm pore size, discarding the first few mL of the filtrate.

Sample solution: Transfer about 0.25 g of Dry Extract to a flask, add 20 mL of methanol, and shake for 2 h at 60°. Cool to room temperature and pass through filter paper into a 50-mL volumetric flask. Wash the flask and the residue on the filter with methanol, dilute with the washings, and mix. Transfer 1 mL of the solution to a 10-mL volumetric flask and dilute with methanol to obtain a solution with a concentration of about 5 mg/mL. Pass the solution through a membrane filter of 0.45-µm pore size,

discarding the first few mL of the filtrate.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Detector: UV 206 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1 (similar to Zorbax XDB C_{18})

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

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

Suitability requirements

Chromatogram similarity: The chromatogram of *Standard solution B* is similar to the reference chromatogram provided with the lot of USP *Sphaeranthus indicus* Aerial Parts Dry Extract RS being used.

Tailing factor: NMT 2.0 for the sphaeranthanolide peak, *Standard solution A*

Relative standard deviation: NMT 2.0%, *Standard solution A*

Analysis

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

Calculate the percentage of sphaeranthanolide in the portion of *Sphaeranthus indicus* Aerial Parts Dry Extract taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area for sphaeranthanolide from the *Sample solution*

r_S = peak area for sphaeranthanolide from *Standard solution A*

C_S = concentration of the USP Sphaeranthanolide RS in *Standard solution A* (mg/mL)

C_U = concentration of Dry Extract in the *Sample solution* (mg/mL)

Calculate the percentate of the labeled amount of sphaeranthanolide in the Dry Extract:

$$\text{Result} = (P/L) \times 100$$

P = content of sphaeranthanolide as determined above (%)

L = labeled amount of sphaeranthanolide (%)

Acceptance criteria: 90%–110% on the dried basis

CONTAMINANTS

• Elemental Impurities—Procedures <233>

Acceptance criteria

Arsenic: NMT 2.0 µg/g

Cadmium: NMT 0.5 µg/g

Lead: NMT 5 µg/g

Mercury: NMT 0.2 µg/g

• Articles of Botanical Origin, *General Method for Pesticide Residues Analysis* **<561>:** Meets the requirements

• **Microbial Enumeration Tests <61>:** 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 bacteria does not exceed 10^3 cfu/g.
• **Tests for Specified Microorganisms <62>:** Meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*

SPECIFIC TESTS

• **Loss on Drying <731>**
Sample: 1 g of *Sphaeranthus indicus* Aerial Parts Dry Extract
Analysis: Dry the *Sample* at 105° for 2 h.
Acceptance criteria: NMT 20%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture, and store at room temperature.
• **Labeling:** The label states the Latin binomial and the part(s) of the plant contained in the article.
• **USP Reference Standards <11>**
USP Sphaeranthanolide RS
USP *Sphaeranthus indicus* Aerial Parts Dry Extract RS
