Polygonum multiflorum Root

Proposed For Comment Version 0.2

**DEFINITION**
The article consists of the dried root of *Polygonum multiflorum* Thunb. with the currently accepted Latin name *Reynoutria multiflora* (Thunb.) Moldenke (Family Polygonaceae)\(^1\), collected in autumn or winter. It contains NLT 1.5% of 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside (C\(_{20}\)H\(_{22}\)O\(_9\)), on the dried basis; NLT 0.10% of total anthraquinones, calculated as the sum of emodin-8-O-β-D-glucoside (C\(_{21}\)H\(_{20}\)O\(_{10}\)), physcion-8-O-β-D-glucoside (C\(_{22}\)H\(_{22}\)O\(_{10}\)), emodin (C\(_{15}\)H\(_{10}\)O\(_5\)), and physcion (C\(_{16}\)H\(_{12}\)O\(_5\)), on the dried basis; and NLT 0.07% of total anthraquinone glycosides, calculated as the sum of emodin-8-O-β-D-glucoside and physcion-8-O-β-D-glucoside, on the dried basis.

**SYNONYMS**
*Fallopia multiflora* (Thunb.) Haraldson  
*Pleuropterus multiflorus* (Thunb.) Turcz. ex Nakai  
*Reynoutria multiflora* (Thunb.) Moldenke

**POTENTIAL CONFOUNDING MATERIALS**
*Pteroxygonum giraldii* Dammer & Diels, root  
*Polygonum cillinerve* (Nakai) Ohwi, root  
*Cynanchum auriculatum* Royle ex Wight, root  
*Polygonum cuspidatum* Sieb. & Zucc., root  
*Fagopyrum esculentum* Moench, root

**SELECTED COMMON NAMES**
Chinese: 何首乌 (He Shou Wu)  
English: Chinese Fleeceflower root, Fo-ti  
German: Chinesischer Flügelknöterich  
Japanese: カシュウ  
Korean: 하수오  
Spanish: Raiz de Fo-ti  
Swedish: Kinabinda

**CONSTITUENTS OF INTEREST**
Stilbenes: 2,3,5,4′-Tetrahydroxystilbene-2-O-β-D-glucoside  
Anthraquinones: Emodin-8-O-β-D-glucoside, physcion-8-O-β-D-glucoside, emodin, and physcion

**IDENTIFICATION**
- **A. BOTANICAL CHARACTERISTICS**
  - **Macroscopic:** Briquette or irregular spindle, 6–15 cm long, 4–12 cm in diameter (0.5–2.5 cm in diameter for *Polygonum cuspidatum*). Externally reddish-brown, shrunk and uneven with shallow grooves, transverse elongated lenticel-like protrusion, and fine rootlet scars. The fracture is pale yellowish-brown or pale reddish-brown and starchy; the phloem exhibits 4–11 subrounded allotype vascular bundles arranged in a ring, forming brocaded patterns; the xylem in the central part is larger with an occasionally woody core. The texture is dense, compact, and not easily broken.
  - **Microscopic:** Transverse section: The cork consists of several layers of cells filled with brown contents. The phloem is relatively broad, scattered with 4–11 subrounded allotype vascular bundles of collateral type, vessels are rare. The central cambium of the root is in a ring, with few vessels in the xylem, surrounded by some tracheids and a few lignified fibres. The parenchymatous cells contain starch granules and clusters of calcium oxalate.

- **B. THIN-LAYER CHROMATOGRAPHY**
  - **Standard solution A:** 0.5 mg/mL of USP 2,3,5,4′-Tetrahydroxystilbene-2-O-Beta-D-Glucoside RS and 0.1 mg/mL of USP Emodin RS in
methanol

**Standard solution B**: 60 mg/mL of USP *Polygonum multiflorum* Root Dry Extract RS in methanol. Sonicate for 15 min, centrifuge, and use the supernatant.

**Sample solution**: Transfer about 500 mg of *Polygonum multiflorum* Root, finely powdered, into 5 mL of methanol. Sonicate for 15 min, centrifuge, and use the supernatant.

**Chromatographic system**

(See *Chromatography <621>*, 11thin-Layer Chromatography.)

**Adsorbent**: Use a suitable chromatographic silica gel mixture with an average particle size of 5 µm (HPTLC plates).

**Application volume**: Standard solution A and Standard solution B: 2 µL, as 8-mm bands; Sample solution: 7 µL, as 8-mm bands.

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

**Developing solvent system**: Toluene, anhydrous ethanol, and glacial acetic acid (8:2:0.5)

**Developing distance**: 6 cm

**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 in a hood. Examine under UV light at 366 nm.

**System suitability**: Under UV light at 366 nm, Standard solution B exhibits, in the lower-half, a bright blue band corresponding in Rf, and color to the band of 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside in Standard solution A, and two faint brownish or red bands above the bright blue band. In the upper-half, Standard solution B exhibits two yellow bands, one corresponding in Rf, and color to the band of emodin in Standard solution A, and another one above emodin.

**Acceptance criteria**: Under UV light at 366 nm, the Sample solution exhibits, in the lower-half, a bright blue band corresponding in Rf, and color to the band of 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside in Standard solutions A and B (distinction from *Pteroxygonum giraldii* root, *Cynanchum auriculatum* root, and *Fagopyrum esculentum* root; *Polygonum cillinerve* root and *Polygonum cuspidatum* root exhibit a similar band due to polydatin, thus distinction from *P. cillinerve* root and *P. cuspidatum* root is achieved by Identification D, Stilbene Glycosides HPLC Profile), one blue-green band may appear immediately above the bright blue band, and two faint brownish or red bands corresponding in Rf, and color to similar bands in Standard solution B appear above the blue band; the one with lower Rf is due to emodin-8-O-β-D-glucoside. In the upper-half, the Sample solution exhibits two yellow bands due to emodin and physcion corresponding in Rf, and color to similar bands in Standard solution B (distinction from *Pteroxygonum giraldii* root, *Cynanchum auriculatum* root, and *Fagopyrum esculentum* root).

**C. Anthraquinones and Anthraquinone Glycosides HPLC Profile**

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

**Acceptance criteria**: The Sample solution exhibits a peak with a retention time corresponding to emodin in Standard solution A and peaks for emodin-8-O-β-D-glucoside, physcion-8-O-β-D-glucoside and physcion corresponding to the retention times for the same anthraquinones in Standard solution B. The peak for emodin is more intense than that for physcion; and the peak of emodin-8-O-β-D-glucoside is more intense than that for physcion-8-O-β-D-glucoside.

**D. Stilbene Glycosides HPLC Profile**

**Analysis**: Proceed as directed in the Assay for Content of 2,3,5,4'-Tetrahydroxystilbene-2-O-β-D-glucoside.

**Acceptance criteria**: The Sample solution exhibits only one principal peak with a retention time corresponding to 2,3,5,4’-tetrahydroxystilbene-2-O-β-D-glucoside (distinction from *P. cillinerve* root, which shows three principal peaks, one corresponds to polydatin; and *P. cuspidatum* root for which the principal peak corresponds to polydatin) in the Standard solution.

**ASSAY**

**Content of 2,3,5,4’-Tetrahydroxystilbene-2-O-β-D-glucoside**

**Solution A**: 0.1% Formic acid in water

**Solution B**: Acetonitrile

**Mobile phase**: See Table 1.

**Table 1**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>83</td>
<td>17</td>
</tr>
<tr>
<td>10</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>18</td>
<td>72</td>
<td>28</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>25</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>
Note—Protect from light and proceed under low actinic light. The Standard solution and Sample solution are stable for 24 h at room temperature.

Solvent: Methanol and water (7:3)

System suitability solution: 0.25 mg/mL of USP 2,3,5,4'-Tetrahydroxystilbene-2-O-Beta-D-Glucoside RS and 0.25 mg/mL of USP Polydatin RS in methanol

Standard solution: 0.25 mg/mL of USP 2,3,5,4'-Tetrahydroxystilbene-2-O-Beta-D-Glucoside RS in methanol

Sample solution: Accurately transfer about 200 mg of *Polypogon multiflorum* Root, finely powdered, into a suitable flask and accurately add 15 mL of Solvent. Weigh the filled flask with a precision of ± 0.1 mg and then reflux for 90 min. After cooling to room temperature, adjust to the initial weight by adding methanol. Before injection, pass through a 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 320 nm
Column: 4.6-mm × 25-cm; 5-μm packing L1 (similar to Shimadzu ODS C18 and Agilent Zorbax Stable Bond C18)
Column temperature: 35 ± 5°
Flow rate: 1.0 mL/min
Injection volume: 10 μL

System suitability
Samples: System suitability solution and Standard solution
Suitability requirements
Resolution: NLT 1.5 between 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside and polydatin peaks, System suitability solution. [Note—The approximate relative retention times of 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside and polydatin are 1.00 and 0.95, respectively.]
Tailing factor: NMT 1.5 for 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside, Standard solution
Relative standard deviation: NMT 2.0% for 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside, Standard solution

Analysis
Samples: Standard solution and Sample solution
Using the chromatogram of the Standard solution, identify the retention time of the peak for 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside in the Sample solution.
Calculate the percentage of 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside in the portion of *Polypogon multiflorum* Root taken:

\[
\text{Result} = \left( \frac{r_u}{r_s} \right) \times C_s \times \left( \frac{V}{W} \right) \times 100
\]

\[r_u = \text{peak area of 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside from the Sample solution}\]
\[r_s = \text{peak area of 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside from the Standard solution}\]
\[C_s = \text{concentration of USP 2,3,5,4'-Tetrahydroxystilbene-2-O-Beta-D-glucoside RS in the Standard solution (mg/mL)}\]
\[V = \text{volume of the Sample solution (mL)}\]
\[W = \text{weight of *Polypogon multiflorum* Root taken to prepare the Sample solution (mg)}\]

Acceptance criteria: NLT 1.5% on the dried basis

### CONTENT OF ANTHRAQUINONES

**Solution A:** 0.1% Formic acid in water

**Solution B:** Acetonitrile

**Mobile phase:** See Table 2.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>
[NOTE—Protect from light and proceed under low actinic light. The Standard solution and Sample solution are stable for 24 h at room temperature.]

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

**Standard solution B**: 6 mg/mL of USP *Polygonum multiflorum* Root Dry Extract RS in methanol, sonicate for 15 min, centrifuge, and pass through a membrane filter of 0.45-μm or finer pore size.

**Sample solution**: Accurately transfer about 200 mg of *Polygonum multiflorum* Root, finely powdered, into a suitable flask and accurately add 15 mL of methanol. Weigh the filled flask with a precision of ± 0.1 mg and then reflux for 60 min. After cooling to room temperature, adjust to the initial weight by adding methanol. Before injection, pass through a membrane filter of 0.45-μm or finer pore size, and discard the first portion of the filtrate.

**Chromatographic system**

(See *Chromatography <621>*; [1]*System Suitability*.)

**Mode**: LC

**Detector**: UV 280 nm

**Column**: 4.6 mm × 15 cm; 5-μm packing L1 (similar to Shimadzu ODS C18, Agilent Zorbax Stable Bond C18, and Thermo Syncronis C18)

**Column temperature**: 30 ± 5°

**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 *Polygonum multiflorum* Root Dry Extract RS being used.

- **Resolution**: NLT 1.5 between the peak of emodin-8-O-β-D-glucoside and the peak after it, Standard solution B

- **Tailing factor**: NMT 1.5 for emodin peak, Standard solution A

- **Relative standard deviation**: NMT 3.0% for emodin, Standard solution A

**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 *Polygonum multiflorum* Root Dry Extract RS being used, identify the retention times of the peaks corresponding to emodin, emodin-8-O-β-D-glucoside, physcion-8-O-β-D-glucoside, and physcion in the Sample solution. [NOTE—The approximate relative retention times of the analytes are provided in Table 3.]

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Approximate Relative Retention Times</th>
<th>Conversion Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emodin-8-O-β-D-glucoside</td>
<td>0.55</td>
<td>1.15</td>
</tr>
<tr>
<td>Physcion-8-O-β-D-glucoside</td>
<td>0.68</td>
<td>1.18</td>
</tr>
<tr>
<td>Emodin</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Physcion</td>
<td>1.08</td>
<td>1.04</td>
</tr>
</tbody>
</table>

Separately calculate the percentages of emodin-8-O-β-D-glucoside, physcion-8-O-β-D-glucoside, emodin, and physcion in the portion of *Polygonum multiflorum* Root taken:

\[
\text{Result} = \left( \frac{r_U}{r_S} \right) \times C_s \times \left( \frac{V}{W} \right) \times F \times 100
\]

\(r_U\) = peak area of the relevant analyte from the Sample solution
Calculate the content of total anthraquinones as the sum of emodin-8-O-β-D-glucoside, physcion-8-O-β-D-glucoside, emodin, and physcion.

Calculate the content of total anthraquinone glycosides as the sum of emodin-8-O-β-D-glucoside and physcion-8-O-β-D-glucoside.

**Acceptance criteria**

Total anthraquinones: NLT 0.10% on the dried basis

Total anthraquinone glycosides: NLT 0.07% on the dried basis

**CONTAMINANTS**

**Elemental Impurities—Procedures <233>** [2]

Acceptance criteria

**Arsenic:** NMT 2.0 µg/g

**Cadmium:** NMT 0.3 µg/g

**Lead:** NMT 5.0 µg/g

**Mercury:** NMT 0.2 µg/g

**Articles of Botanical Origin** [3], General Method for Pesticide Residues Analysis <561> [3]: Meets the requirements

**Microbial Enumeration Tests <61>** [4]: The total aerobic bacterial count does not exceed $10^5$ cfu/g, the total combined molds and yeasts count does not exceed $10^4$ cfu/g, and the bile-tolerant Gram-negative bacteria does not exceed $10^3$ cfu/g.

**Tests for Specified Microorganisms <62>** [3]: Meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*

**Articles of Botanical Origin** [3], *Aflatoxins* [6] <561> [3]: Meets the requirements

**SPECIFIC TESTS**

**Articles of Botanical Origin** [3], *Foreign Organic Matter* <561> [3]: NMT 2.0%

**Articles of Botanical Origin** [3], *Alcohol-Soluble Extractives, Method 1* <561> [3]: NLT 15.0%

**Articles of Botanical Origin** [3], *Water-Soluble Extractives, Method 2* <561> [3]: NLT 13.0%

**Loss on Drying <731>** [7]

Sample: 1.0 g of *Polygonum multiflorum* Root, finely powdered

Analysis: Dry the Sample at 105° for 5 h.

Acceptance criteria: NMT 13.0%

**Articles of Botanical Origin** [3], *Total Ash* <561> [3]

Analysis: 4.0 g of *Polygonum multiflorum* Root, finely powdered

Acceptance criteria: NMT 5.0%

**Articles of Botanical Origin** [3], *Acid-Insoluble Ash* <561> [3]: NMT 2.0%

**ADDITIONAL REQUIREMENTS**

**Packaging and Storage:** Preserve in well-closed containers, protected from light, moisture, and moths, and store at controlled room temperature.

**Labeling:** The label states the Latin binomial and the part(s) of the plant contained in the article.

**USP Reference Standards <11>** [8]

- USP Aflatoxins RS [6]
- USP Emodin RS
- USP Polydatin RS
- USP *Polygonum multiflorum* Root Dry Extract RS
- USP 2,3,5,4'-Tetrahydroxystilbene-2-O-Beta-D-Glucoside RS

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1 Source of current accepted botanical name used is The Plant List accessible at [http://www.theplantlist.org](http://www.theplantlist.org) [9].

Source URL (modified on 2015/02/12 - 1:44pm): https://hmc.usp.org/monographs/polygonum-multiflorum-root-0-2