**Polygonum multiflorum Root Dry Extract**

**Proposed For Development Version 0.1**

**Polygonum multiflorum** Root Dry Extract

**DEFINITION**
The article is prepared from the dried root of *Polygonum multiflorum* Thunb. (Family Polygonaceae), collected in autumn or winter, by extraction with a mixture of alcohol and water (7:3). The ratio of starting crude plant material to Dry Extract is about 5:1. It contains NLT 90.0% and NMT 110.0% of the labeled amount of 2,3,5,4’-tetrahydroxystilbene-2-O-β-D-glucoside (C_{20}H_{22}O_9); NLT 90.0% and NMT 110.0% of the labeled amount 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}); and NLT 90.0% and NMT 110.0% of the labeled amount of anthraquinone glycosides, calculated as the sum of emodin-8-O-β-D-glucoside and physcion-8-O-β-D-glucoside; all on the dried basis.

**POTENTIAL CONFOUNING MATERIALS**
- *Pteroxygonum giraldii* Damm. et Diels
- *Polygonum cillinerve* (Nakai) Ohwi
- *Cynanchum bungei* Decne.

**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. Thin-Layer Chromatography**

  **Standard solution A:** 0.5 mg/mL of USP 2,3,5,4’-Tetrahydroxystilbene -2-O-β-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:** 60 mg/mL of *Polygonum multiflorum* Root Dry Extract in methanol. Sonicate for 15 min, centrifuge, and use the supernatant.

  **Chromatographic system**
  (See Chromatography <621>, Thin-Layer Chromatography.)
  - **Adsorbent:** Use a suitable chromatographic silica gel mixture with an average particle size of 5 µm (HPTLC plates).
  - **Application volume:** 2 µL, as 8-mm bands
  - **Relative humidity:** Condition the plate to a relative humidity of about 33% using a suitable device.
  - **Developing solvent system A:** Ethyl acetate, methanol, and acetic acid (4: 1: 0.2)
  - **Developing solvent system B:** Hexanes and ethyl acetate (3:2)

  **Developing distance**
  - **Developing solvent system A:** 3.5 cm
  - **Developing solvent system B:** 7 cm

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

  **System suitability:** Under UV light at 366 nm, Standard solution B exhibits, in the lower-half section, a bright blue band corresponding to the band of 2,3,5,4’-tetrahydroxystilbene-2-O-β-D-glucoside in Standard solution A, an orange-red band due to emodin-8-O-β-D-glucoside right below the bright blue band, a blue band right below the orange-red band, and another orange-red band below the blue band; the first three bands are clearly separated. In the upper-half section, Standard solution B exhibits two yellow bands, one corresponding to the band of emodin in Standard solution A, and another band due to physcion above emodin.

  **Acceptance criteria:** Under UV light at 366 nm, the Sample solution exhibits, in the lower-half section, a bright blue
band corresponding to the band of 2,3,5,4’-tetrahydroxystilbene-2-O-β-D-glucoside (distinguished from Polygonum cillinerve root, Pteroxygonum giraldii root, and Cynanchum auriculatum root) in Standard solutions A and B, an orange-red band corresponding to the band of emodin-8-O-β-D-glucoside in Standard solution B right below the bright blue band, a blue band right below the orange-red band, and another orange-red band below the blue band, all corresponding to similar bands in R, and color to Standard solution B. In the upper-half section, the Sample solution exhibits two yellow bands (distinguished from Pteroxygonum giraldii root and Cynanchum auriculatum root), one corresponding to the band of emodin in Standard solutions A and B, and another band corresponding to a similar band in Standard solution B due to physcion. The Sample solution exhibits additional minor bands corresponding to similar bands in Standard solution B.

• B. Anthraquinones 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; the peaks related to anthraquinones for emodin-8-O-β-D-glucoside, physcion-8-O-β-D-glucoside, and physcion correspond to the same anthraquinones in Standard solution B and the reference chromatogram provided with the lot of USP Polygonum multiflorum Root Dry Extract RS being used. The Sample solution exhibits additional peaks corresponding to similar peaks in Standard solution B. The content ratio for emodin-8-O-β-D-glucoside to emodin is about 1.3: 6; the content ratio for physcion-8-O-β-D-glucoside to physcion is about 1.2: 5.

• C. 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 (distinguished from Polygonum cillinerve root, which shows three principal peaks and Polygonum 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>20</td>
<td>70</td>
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<td>100</td>
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<tr>
<td>31</td>
<td>83</td>
<td>17</td>
</tr>
<tr>
<td>40</td>
<td>83</td>
<td>17</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)
Standard solution: 0.25 mg/mL of USP 2,3,5,4’-Tetrahydroxystilbene-2-O-β-D-glucoside RS in methanol
System suitability solution: 0.25 mg/mL of USP 2,3,5,4’-Tetrahydroxystilbene-2-O-β-D-glucoside RS and 0.25 mg/mL of USP Polydatin RS in methanol
Sample solution: Accurately transfer about 60 mg of Polygonum multiflorum Root Dry Extract into a suitable stoppered conical flask and accurately add 10 mL of methanol. Weigh the filled flask with a precision of ± 0.1 mg and then sonicate for 15 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; packing L1 (similar to Shimadzu ODS C18 and Aglient Zorbax Stable Bond C18)
Column temperature: 35 ± 5°
Flow rate: 1.0 mL/min
Injection volume: 10 µL

System suitability
Sample: Standard solution
Suitability requirements
Resolution: NLT 1.5 between the peaks of 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside and polydatin, 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
Relative standard deviation: NMT 2.0% for 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside

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 Polygonum multiflorum Root Dry Extract taken:

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

\( r_U \) = peak area of 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside from the Sample solution
\( r_S \) = peak area of 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside from the Standard solution
\( C_s \) = concentration of USP 2,3,5,4’-Tetrahydroxystilbene-2-O-β-D-glucoside RS in the Standard solution (mg/mL)
\( V \) = volume of the Sample solution (mL)
\( W \) = weight of Polygonum multiflorum Root Dry Extract taken to prepare the Sample solution (mg)

Calculate the percentage of the labeled amount of 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside in the portion of Polygonum multiflorum Root Dry Extract taken:

\[
\text{Result} = \left( \frac{P}{L} \right) \times 100
\]

\( P \) = content of 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside determined above (%)
\( L \) = labeled amount of 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside (%)

Acceptance criteria: 90.0%–110.0% on the dried basis

- CONTENT OF ANTHRAQUINONES
  Solution A: 0.1% Formic acid in water
  Solution B: Acetonitrile
  Mobile phase: See Table 2.

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>
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<tr>
<td>10</td>
<td>0</td>
<td>100</td>
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<td>100</td>
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<td>70</td>
<td>30</td>
</tr>
<tr>
<td>19</td>
<td>70</td>
<td>30</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

**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 60 mg of *Polygonum multiflorum* Root Dry Extract into a suitable stoppered conical flask and accurately add 10 mL of methanol. Weigh the filled flask with a precision of ± 0.1 mg and then sonicate for 15 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 280 nm
- **Column:** 4.6 mm × 15 cm; 5-μm packing L1 (similar to Shimadzu ODS C18, Agilent Zorbax Stable Bond C18, and Thermo Synchronis C18)
- **Column temperature:** 30 ± 5°
- **Flow rate:** 1.0 mL/min
- **Injection volume:** 10 μL

**System suitability**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Standard solution A and Standard solution B</th>
</tr>
</thead>
</table>

**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 the peak of emodin, Standard solution A
- **Relative standard deviation:** NMT 2.0% 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. The approximate relative retention times are provided in Table 3.

**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 Dry Extract 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
- \( r_S \) = peak area of emodin from the Standard solution A
- \( C_S \) = concentration of USP Emodin RS in Standard solution A (mg/mL)
- \( V \) = volume of the Sample solution (mL)
- \( W \) = weight of *Polygonum multiflorum* Root Dry Extract taken to prepare the Sample solution (mg)
- \( F \) = conversion factor for the analytes as provided in Table 3.

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.

Calculate the percentage of the labeled amount of total anthraquinones in the portion of Polygonum multiflorum Root Dry Extract taken:

\[
\text{Result} = \left( \frac{P}{L} \right) \times 100
\]

\( P \) = content of total anthraquinones determined above (%)

\( L \) = labeled amount of total anthraquinones (%)

**Acceptance criteria:** 90.0%-110.0% on the dried basis

Calculate the percentage of the labeled amount of total anthraquinone glycosides in the portion of Polygonum multiflorum Root Dry Extract taken:

\[
\text{Result} = \left( \frac{P}{L} \right) \times 100
\]

\( P \) = content of total anthraquinone glycosides determined above (%)

\( L \) = labeled amount of total anthraquinone glycosides (%)

**Acceptance criteria:** 90.0%-110.0% on the dried basis

**CONTAMINANTS**

- **Elemental Impurities—Procedures <233>** [1]
  
  **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 [2], General Method for Pesticide Residues Analysis <561>**: Meets the requirements

- **Microbial Enumeration Tests <61>** [3]: The total aerobic bacterial count does not exceed 10⁵ cfu/g, the total combined molds and yeasts count does not exceed 10³ cfu/g, and the bile-tolerant Gram-negative bacteria does not exceed 10³ cfu/g.

- **Absence of Specified Microorganisms <62>** [4]: Meets the requirements of the tests for the absence of Salmonella species and Escherichia coli.

- **Articles of Botanical Origin [2], Aflatoxins <561>**: Meets the requirements

**SPECIFIC TESTS**

- **Loss on Drying <731>** [5]
  
  **Sample:** 1 g of Polygonum multiflorum Root Dry Extract
  
  **Analysis:** Dry the Sample at 105° for 5 h.
  
  **Acceptance criteria:** NMT 10.0%

- **Articles of Botanical Origin [2], Total Ash <561>**
  
  **Analysis:** 3.0 g of Polygonum multiflorum Root Dry Extract
  
  **Acceptance criteria:** NMT 7.0%

**ADDITIONAL REQUIREMENTS**

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

- **Labeling:** The label states the Latin binomial and the part(s) of the plant from which the article was prepared. It meets other labeling requirements under Botanical Extracts <565>.

- **USP Reference Standards <11>** [6]
  
  - USP Emodin RS
  - USP Polygonum multiflorum Root Dry Extract RS
  - USP 2,3,5,4’-Tetrahydroxystilbene-2-O-β-D-glucoside RS

**Source URL (modified on 2014/07/07 - 1:58pm):** https://hmc.usp.org/monographs/polygonum-multiflorum-root-dry-extract-0-1