Polygonum multiflorum Root Dry Extract

Proposed For Comment Version 0.2

Polygonum multiflorum Root Dry Extract

DEFINITION
The article is prepared from the dried root of Polygonum multiflorum Thunb. with the currently accepted Latin name Reynoutria multiflora (Thunb.) Moldenke (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 between 3:1 and 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 \( (C_{20}H_{22}O_9) \), on the dried basis; 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 \( (C_{16}H_{12}O_5) \), on the dried basis; and NLT 90.0% and NMT 110.0% of the labeled amount 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.

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

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-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:** 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>, §1: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:** 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 \( R_\text{f} \) 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 \( R_\text{f} \) 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 \( R_\text{f} \) 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.}
P. 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 *R* and color to similar bands in *Standard solution B* appear above the blue band; the one with lower *R* 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 *R* and color to similar bands in *Standard solution B* (distinction from *Pteroxygonum giraldii* root, *Cynanchum auriculatum* root, and *Fagopyrum esculentum* root).

**• B. 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.

**• 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 (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>
<tr>
<td>26</td>
<td>83</td>
<td>17</td>
</tr>
<tr>
<td>35</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.]*

**System suitability solution:** 0.25 mg/mL of USP 2,3,5,4'-Tetrahydroxystilbene-2-O-Beta-o-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-o-Glucoside 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>*, [1]:System Suitability.)

**Mode:** LC

**Detector:** UV 320 nm

**Column:** 4.6-mm x 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 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-Beta-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>
<td>50</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>14</td>
<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.]

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: Prepare the Sample solution using the same procedure as in the Assay for Content of 2,3,5,4'-Tetrahydroxystilbene-2-O-β-D-glucoside.
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 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. [Note—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:

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

\[r_U\] = 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 (see 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:

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

\[P = \text{content of total anthraquinone glycosides determined above (\%)}\]
\[L = \text{labeled amount of total anthraquinone glycosides (\%)}\]

Acceptance criteria: 90.0%–110.0% 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 10⁴ cfu/g, and the total combined molds and yeasts count does not exceed 10² cfu/g.

- **Tests for Specified Microorganisms** <62> [5]: 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**

- **Loss on Drying** <731> [7]
  - **Sample**: 1.0 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** [3], **Total Ash** <561> [3]
  - **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. 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> [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-o-Glucoside RS

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