Lonicera japonica Flower Dry Extract

Final Authorized Version 1.0

Lonicera japonica Flower Dry Extract

DEFINITION
The article is prepared from the dried flower buds or dried flowers in the early opening stage of Lonicera japonica Thunb. (Family Caprifoliaceae) collected in early summer and extracted with water or a hydroalcoholic mixture containing NMT 15% of alcohol. Extracts may be further processed by adding alcohol, filtering, evaporating, and then adding water, filtering, and evaporating. It contains NLT 90.0% and NMT 110.0% of the labeled amount of caffeoylquinic acids, calculated as the sum of neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, 3,4-di-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, and 4,5-di-O-caffeoylquinic acid on the dried basis; and NLT 90.0% and NMT 110.0% of the labeled amount of iridoids, calculated as the sum of sweroside, secoxyloganin, and centaurosides on the dried basis.

POTENTIAL CONFOUNDING MATERIALS
Lonicera macranthoides Hand.-Mazz, Flower
Lonicera hypoglauca Miq. Flower
Lonicera confusa DC. Flower
Lonicera fulvotomentosa P. S. Hsu & S. C. Cheng, Flower

CONSTITUENTS OF INTEREST
Caffeoylquinic acids: Neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, 3,4-di-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, and 4,5-di-O-caffeoylquinic acid
Flavonoids: Luteolin-7-O-glucoside and rutin
Iridoids: Secoxyloganin, sweroside, and centaurosides

IDENTIFICATION
• A. HPTLC
(See High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin <203> [3].)
Standard solution A: 0.2 mg/mL of USP Chlorogenic Acid RS, 0.25 mg/mL of USP Rutin RS, and 0.2 mg/mL of USP Luteolin-7-O-glucoside RS in methanol
Standard solution B: 10 mg/mL of USP Lonicera japonica Flower Dry Extract RS in methanol.
Sonicate for 10 min, and filter. Evaporate the filtrate at about 50°C under reduced pressure to dryness. Add 5 mL of water to dissolve the residue, and then add 10 mL of ethyl acetate, mix. After the solution separates into two layers, take the ethyl acetate layer. Repeat the extraction one more time. Combine the ethyl acetate extracts, evaporate the solvent at about 50°C under reduced pressure to dryness, and dissolve the residue in methanol equivalent to 1/5 of the initial volume of the USP Lonicera japonica Flower Dry Extract RS solution.
**Sample solution:** 100 mg of *Lonicera japonica* Flower Dry Extract in 10 mL of methanol. Sonicate for 10 min, and filter. Evaporate the filtrate at about 50° under reduced pressure to dryness. Add 5 mL of water to dissolve the residue, and then add 10 mL of ethyl acetate and mix. After the solution separates into two layers, take the ethyl acetate layer. Repeat the extraction one more time. Combine the ethyl acetate extracts, evaporate the solvent at about 50° under reduced pressure to dryness, and add 2 mL of methanol to dissolve the residue.

**Chromatographic system**

- **Adsorbent:** Chromatographic silica gel mixture with an average particle size of 5 µm
- **Application volume:** 5 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:** The upper layer solution of a mixture of *n*-butyl acetate, formic acid, and water (7:5:5)
- **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 to a suitable HPTLC plate and dry in air. Develop in a saturated chamber, remove the plate from the chamber, and dry in air. Treat the plate with Derivatization reagent A and allow to air-dry. Immediately treat the plate with Derivatization reagent B, allow to air-dry, and examine under UV light at 366 nm.

- **System suitability:** Under UV light at 366 nm, in the lower-third section, Standard solution B exhibits one blue fluorescent band corresponding in *R*<sub>f</sub> to the band due to chlorogenic acid and one yellow band above chlorogenic acid corresponding in *R*<sub>f</sub> to the band due to luteolin-7-O-glucoside in Standard solution A. One faint blue fluorescent band is below the chlorogenic acid band and another yellow band, due to rutin [2], is below the faint blue fluorescent band. In the middle-third section, Standard solution B exhibits three blue fluorescent bands: the band with the highest *R*<sub>f</sub> is due to 3,5-di-*O*-caffeoylquinic acid; the band with the middle *R*<sub>f</sub> is due to 4,5-di-*O*-caffeoylquinic acid; and the band with the lowest *R*<sub>f</sub> is due to 3,4-di-*O*-caffeoylquinic acid.

- **Acceptance criteria:** Under UV light at 366 nm, in the lower-third section, the Sample solution exhibits one blue fluorescent band corresponding in *R*<sub>f</sub> and color to the band of chlorogenic acid and one yellow band above chlorogenic acid corresponding in *R*<sub>f</sub> and color to the band of luteolin-7-O-glucoside in Standard solution A. One faint blue fluorescent band is below the chlorogenic acid band and another yellow band, due to rutin [2], is below the faint blue fluorescent band corresponding in *R*<sub>f</sub> and color to similar bands in Standard solution B. In the middle-third section, the Sample solution exhibits three blue fluorescent bands due to 3,5-di-*O*-caffeoylquinic acid, 4,5-di-*O*-caffeoylquinic acid, and 3,4-di-*O*-caffeoylquinic acid corresponding in *R*<sub>f</sub> and color to similar bands in Standard solution B.

**B. CAFFEEOYLQUINIC ACIDS HPLC PROFILE**

- **Analysis:** Proceed as directed in the Assay for Content of Caffeoylquinic Acids.

  **Acceptance criteria:** The Sample solution exhibits the most intense peak with a retention time corresponding to chlorogenic acid in Standard solution A and peaks at the retention times corresponding to neochlorogenic acid, cryptochlorogenic acid, 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid in Standard Solution B. The chlorogenic acid content is NMT 65% of the total caffeoylquinic acids.

**C. IRIDOIDS HPLC PROFILE**

- **Analysis:** Proceed as directed in the Assay for Content of Iridoids.
Acceptance criteria: The Sample solution exhibits a peak with a retention time corresponding to secoxyloganin in Standard solution A and two additional iridoid peaks of sweroside and centauroside at retention times corresponding to the same iridoids in Standard solution B.

ASSAY

• Content of Caffeoylquinic Acids

Solution A: 0.1% Phosphoric 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>86</td>
<td>14</td>
</tr>
<tr>
<td>8</td>
<td>81</td>
<td>19</td>
</tr>
<tr>
<td>14</td>
<td>81</td>
<td>19</td>
</tr>
<tr>
<td>34</td>
<td>69</td>
<td>31</td>
</tr>
<tr>
<td>35</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>39.5</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>40</td>
<td>86</td>
<td>14</td>
</tr>
<tr>
<td>48</td>
<td>86</td>
<td>14</td>
</tr>
</tbody>
</table>

[Note—Protect from light and proceed under low actinic light. The Standard solutions and the Sample solution are stable for 24 h at room temperature.]

Solvent: Methanol and water (7.5: 2.5)
Standard solution A: 0.30 mg/mL of USP Chlorogenic Acid RS in methanol
Standard solution B: 2.5 mg/mL of USP Lonicera japonica Flower Dry Extract RS in Solvent. Sonicate for 15 min, and pass through a membrane filter of 0.45-μm or finer pore size.

Sample solution: Accurately transfer about 25 mg of Lonicera japonica Flower Dry Extract to a suitable stoppered conical flask, and accurately add 10.0 mL of Solvent. Weigh the filled flask with a precision of ±0.1 mg, stopper, and then sonicate for 30 min. After cooling to room temperature, adjust to the initial weight by adding Solvent. 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> [4], System Suitability.)

Mode: LC
Detector: UV 327 nm
Column: 4.6-mm × 25-cm; 5-μm packing L1 (similar to Thermo Scientific Syncronis C18)
Column temperature: 15°
Flow rate: 0.7 mL/min
Injection volume: 2 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 for caffeoylquinic acids provided with the lot of USP *Lonicera japonica* Flower Dry Extract RS being used.

**Resolution:** NLT 1.5 between chlorogenic acid and cryptochlorogenic acid peaks, and 3,4-di- O-caffeoylquinic acid and 3,5-di-O-caffeoylquinic acid peaks, *Standard solution B*

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

**Relative standard deviation:** NMT 2.0% for the chlorogenic acid peak, *Standard solution A*

Analysis

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

Using the chromatograms of *Standard solution A* and *Standard solution B*, and the reference chromatogram for caffeoylquinic acids provided with the lot of USP *Lonicera japonica* Flower Dry Extract RS being used, identify the retention times of the peaks of neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, 3,4-di-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, and 4,5-di-O-caffeoylquinic acid in the *Sample solution*. [Note—See Table 2 for relative retention times. These values are not monograph requirements. They may vary due to differences in the chromatographic conditions allowed by the system suitability requirements.]

### Table 2

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Approximate Relative Retention Time</th>
<th>Conversion Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neochlorogenic acid&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.75</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Chlorogenic acid</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Cryptochlorogenic acid&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.05</td>
<td>1.00</td>
</tr>
<tr>
<td>3,4-Di-O-caffeoylquinic acid&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.65</td>
<td>0.92</td>
</tr>
<tr>
<td>3,5-Di-O-caffeoylquinic acid&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.88</td>
<td>0.77</td>
</tr>
<tr>
<td>4,5-Di-O-caffeoylquinic acid&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3.01</td>
<td>0.77</td>
</tr>
</tbody>
</table>

<sup>a</sup> (1R,3R,4S,5R)-3-[(E)-3-(3,4-Dihydroxyphenyl)acryloyl]oxy]-1,4,5-trihydroxycyclohexane-1-carboxylic acid.

<sup>b</sup> (1S,3R,4R,5R)-3-[(E)-3-(3,4-Dihydroxyphenyl)acryloyl]oxy]-1,4,5-trihydroxycyclohexane-1-carboxylic acid.

<sup>c</sup> (1S,3R,4S,5R)-4-[(E)-3-(3,4-Dihydroxyphenyl)acryloyl]oxy]-1,4,5-trihydroxycyclohexane-1-carboxylic acid.

<sup>d</sup> (1S,3R,4R,5R)-3,4-Bis[(E)-3-(3,4-dihydroxyphenyl)acryloyl]oxy]-1,5-dihydroxycyclohexane-1-carboxylic acid.

<sup>e</sup> (1S,3R,4S,5R)-3,5-Bis[(E)-3-(3,4-dihydroxyphenyl)acryloyl]oxy]-1,5-dihydroxycyclohexane-1-carboxylic acid.

<sup>f</sup> (1R,3R,4S,5R)-3,4-Bis[(E)-3-(3,4-dihydroxyphenyl)acryloyl]oxy]-1,5-dihydroxycyclohexane-1-carboxylic acid.

Separately calculate the percentages of neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid,
3,4-di-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, and 4,5-di-O-caffeoylquinic acid in the portion of *Lonicera japonica* Flower 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 chlorogenic acid from *Standard solution A*
\( C_s \) = concentration of USP Chlorogenic Acid RS in *Standard solution A* (mg/mL)
\( V \) = volume of the *Sample solution* (mL)
\( W \) = weight of *Lonicera japonica* Flower Dry Extract taken to prepare the *Sample solution* (mg)
\( F \) = conversion factor for the analyte (see Table 2)

Calculate the content of caffeoylquinic acids as the sum of the percentages of neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, 3,4-di-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, and 4,5-di-O-caffeoylquinic acid.

Calculate the percentage of the labeled amount of caffeoylquinic acids in the portion of *Lonicera japonica* Flower Dry Extract taken:

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

\( P \) = content of caffeoylquinic acids as determined above (%)
\( L \) = labeled amount of caffeoylquinic acids (%)

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

**• CONTENT OF IRIDOIDS**

**Solution A, Solution B, Mobile phase, Standard solution B,** and **Sample solution:** Prepared as directed in the Assay for Content of Caffeoylquinic Acids.

**Standard solution A:** 0.05 mg/mL of USP Secoxyloganin RS in methanol

**Chromatographic system:** Proceed as directed in the Assay for Content of Caffeoylquinic Acids except for the Detector.

**Detector:** UV 240 nm

**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 for iridoids provided with the lot of USP *Lonicera japonica* Flower Dry Extract RS being used.

**Resolution:** NLT 1.5 between the peak of secoxyloganin and the peak before it, *Standard solution B*

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

**Relative standard deviation:** NMT 2.0% for the secoxyloganin peak, *Standard solution A*

**Analysis**

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

Using the chromatograms of *Standard solution A* and *Standard solution B,* and the reference chromatogram for iridoids provided with the lot of USP *Lonicera japonica* Flower Dry Extract RS being used, identify the peaks of sweroside, secoxyloganin, and centauroside in the *Sample solution.*

[Note—The approximate relative retention times for the peaks of sweroside, secoxyloganin, and centauroside are 0.85, 1.00, and 1.80, respectively.]
Separately calculate the percentages of secoxyloganin, sweroside, and centauroside in the portion of *Lonicera japonica* Flower 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 secoxyloganin from Standard solution A
- \( C_S \) = concentration of USP Secoxyloganin RS in Standard solution A (mg/mL)
- \( V \) = volume of the Sample solution (mL)
- \( W \) = weight of *Lonicera japonica* Flower Dry Extract taken to prepare the Sample solution (mg)
- \( F \) = conversion factor for the analyte (1.00 for secoxyloganin, 1.03 for sweroside, and 0.89 for centauroside)

Calculate the content of iridoids as the sum of the percentages of secoxyloganin, sweroside, and centauroside.

Calculate the percentage of the labeled amount of iridoids in the portion of *Lonicera japonica* Flower Dry Extract taken:

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

- \( P \) = content of iridoids as determined above (%)
- \( L \) = labeled amount of iridoids (%)

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

**CONTAMINANTS**

- **Articles of Botanical Origin** <561>, [5] *Pesticide Residue Analysis* [5]: Meets the requirements
- **Microbial Enumeration Tests** <61> [6]: The total aerobic bacterial count does not exceed \( 10^4 \) cfu/g, the total combined molds and yeasts count does not exceed \( 10^2 \) cfu/g, and the bile-tolerant Gram-negative bacteria does not exceed \( 10^2 \) cfu/g.
- **Absence of Specified Microorganisms** <62> [7]: Meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*

**SPECIFIC TESTS**

**Limit of Triterpenoid Saponins**

- **Standard solution:** 5.0 mg/mL of USP *Lonicera macranthoides* Flower Dry Extract RS in methanol. Sonicate for 10 min, and filter.
- **Sample solution:** 50 mg of *Lonicera japonica* Flower Dry Extract in 10 mL of methanol. Sonicate for 10 min, and filter.

**Chromatographic system**

(See *High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin* <203> [3].)

- **Adsorbent:** Chromatographic silica gel mixture with an average particle size of 5 µm
- **Application volume:** 3 µL, as 8-mm bands
- **Relative humidity:** Condition the plate to a relative humidity of about 33% using a suitable device.
- **Developing solvent system:** The upper layer solution of a mixture of *n*-butanol, formic acid, and...
Developing distance: 6 cm
Derivatization reagent: 10% Sulphuric acid in ethanol

Analysis
― Samples: Standard solution and Sample solution
    Apply the Samples as bands to a suitable HPTLC plate and dry in air. Develop in a saturated chamber, remove the plate from the chamber, and dry in air. Treat the plate with Derivatization reagent, heat at 105° for 5 min, and examine immediately under UV light at 366 nm.
    System suitability: Under UV light at 366 nm, in the lower-third section, the Standard solution exhibits three clearly separated brown bands: the band with the highest R_f is due to dipsacoside B; the middle band is due to macranthoidin A; the band with the lowest R_f is due to macranthoidin B.
    Acceptance criteria: Under UV light at 366 nm, the Sample solution does not exhibit any bands corresponding in R_f and color to the bands due to dipsacoside B, macranthoidin A, and macranthoidin B in the Standard solution.

― Loss on Drying <731> [8]
    Sample: 1 g of Lonicera japonica Flower Dry Extract
    Analysis: Dry the Sample at 105° for 2 h.
    Acceptance criteria: NMT 5.0%

Additional Requirements
― Packaging and Storage: Preserve in well-closed containers, protected from light and moisture, and store in a cool place.
― 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 in Botanical Extracts <565>. [9]
― USP Reference Standards <11> [10]
    USP Chlorogenic Acid RS [1]
    USP Lonicera japonica Flower Dry Extract RS
    USP Lonicera macranthoides Flower Dry Extract RS
    USP Luteolin-7-O-glucoside RS
    USP Rutin RS [2]
    USP Secoxyloganin RS

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