Lonicera japonica Flower

Final Authorized Version 1.0

Lonicera japonica Flower

DEFINITION
The article consists of the dried flower buds or dried flowers in the early opening stage of Lonicera japonica Thunb. (Family Caprifoliaceae) collected before full flowering. It contains NLT 3.8% of caffeoylquinic acids, calculated as the sum of chlorogenic acid, 3,5-di-O-caffeoylquinic acid, and 4,5-di-O-caffeoylquinic acid on the dried basis; and NLT 0.80% of iridoids, calculated as the sum of sweroside, secoxyloganin, and centauroside on the dried basis.

SYNONYMS
Caprifolium chinensis S.Watson ex Loudon
Caprifolium japonicum (Thunb.) Dum.Cours.
Caprifolium roseum Lam.
Lonicera brachypoda Siebold

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

SELECTED COMMON NAMES
Chinese: 金银花 (Jin Yin Hua)
English: Japanese honeysuckle flower; gold and silver flower
French: clématite du japon
Japanese: キンギンカ
Korean: 금은화
Latin: Lonicerae Japonicae Flos
Spanish: Madreselva del Japón

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 centauroside
IDENTIFICATION

• A. BOTANICAL CHARACTERISTICS

  Macroscopic: Flower buds are clavate, tapered downwards, slightly curved; 2–3 cm long, about 3 mm in diameter in upper part, and 1.5 mm in diameter in lower part with densely pubescent surface; externally yellowish-white, greenish-white to yellowish-green, gradually darkening over time to a golden color. The foliaceous bracts are occasionally visible. The calyx is green, pubescent, 5-lobed at the apex, about 2 mm long. The corollas of opening flowers are tubular and 2-lipped at apex. The stamens are epipetalous, yellow, in groups of five, and the one ovary is glabrous.

  Microscopic: Pedicels are covered with numerous glandular hairs. The heads of glandular hairs are multicellular, turbinate, subround or slightly oblate, usually 30–70 μm in diameter, exceptionally up to 110 μm. The stalks of glandular hairs are unicellular or multicellular with up to five cells, usually 20–70 μm long, exceptionally up to 700 μm. Non-glandular hairs occur in two types: one with thick walls, unicellular, 45–900 μm long, 15–40 μm in diameter, with fine verrucae on the surface, some have conocephalous spiral; another type with thin walls, slender, curved or shrinkage, with fine verrucae on the surface. Clusters of calcium oxalate are usually 6–45 μm in diameter. Pollen grains are spherical, with three germinal pores, 60–90 μm in diameter.

• B. THIN-LAYER CHROMATOGRAPHY

  Standard solution A: 1.0 mg/mL of USP Chlorogenic Acid RS, 0.5 mg/mL of USP Rutin RS, and 0.5 mg/mL of USP Luteolin-7-O-glucoside RS in methanol.

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

  Sample solution: 500 mg of Lonicera japonica Flower, finely powdered, in 10 mL of methanol. Sonicate for 10 min, and filter. Evaporate the filtrate at about 50° under reduced pressure to dryness. Dissolve the residue with 5 mL of water, 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 dissolve the residue with 2 mL of methanol.

  Chromatographic system

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

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

    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: 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 the chromatograms in a saturated chamber, remove the plate from the chamber, and dry in air. Treat the plate with Derivatization reagent A and allow the plate to air-dry. Immediately, treat the plate with Derivatization reagent B, allow the plate to air-dry and examine under UV light at 366 nm.

  System suitability: Under UV light at 366 nm, in the lower-third section of the chromatogram for Standard solution B, there is one blue fluorescent band corresponding in R_f to the band due to
chlorogenic acid and one yellow band above chlorogenic acid corresponding in \( R_f \) to the band due to luteolin-7-O-glucoside in Standard solution A; one faint blue fluorescent band is below the chlorogenic acid band; another yellow band due to rutin \[2\] is below the faint blue fluorescent band. In the middle-third section, the chromatogram for Standard solution B exhibits three blue fluorescent bands: the band with the highest \( R_f \) is due to 3,5-di-O-caffeoylquinic acid; the band with the middle \( R_f \) is due to 4,5-di-O-caffeoylquinic acid; and the band with the lowest \( R_f \) is due to 3,4-di-O-caffeoylquinic acid.

**Acceptance criteria:** Under UV light at 366 nm, the Sample solution exhibits the most intense band corresponding in \( R_f \) and color to the band of chlorogenic acid in the chromatogram of Standard solution A and a yellow band above chlorogenic acid corresponding in \( R_f \) and color to the band due to luteolin-7-O-glucoside in Standard solution A. The Sample solution exhibits another yellow band in the lower-third section corresponding to rutin, and two blue bands in the middle-third section due to 3,5-di-O-caffeoylquinic acid and 4,5-di-O-caffeoylquinic acid corresponding in \( R_f \) and color to similar bands in the chromatogram of Standard solution B.

- **C. CAFFEOLYQUINIC ACIDS HPLC PROFILE**
  **Analysis:** Proceed as directed in the Assay for Content of Caffeoylquinic Acids.
  **Acceptance criteria:** The chromatogram of the Sample solution exhibits the most intense peak with a retention time corresponding to the retention time for chlorogenic acid in Standard solution A, and peaks at the retention times corresponding to 3,5-di-O-caffeoylquinic acid and 4,5-di-O-caffeoylquinic acid in the chromatogram of Standard solution B. It meets the content ratios listed in Table 2.

- **D. IRIODIDS 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 peaks related to iridoids for sweroside and centauroside at retention times corresponding to the same iridoids in Standard solution B.

**ASSAY**

- **CONTENT OF CAFFEOLYQUINIC 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 solution and the Sample]
Solution are stable for 24 h at room temperature.

**Standard solution A:** 0.30 mg/mL of USP Chlorogenic Acid RS [1] in methanol

**Standard solution B:** 2.5 mg/mL of USP Lonicera japonica Flower Dry Extract RS in 75% methanol.
Sonicate for 15 min, and pass through a membrane filter of 0.22-μm or finer pore size.

**Sample solution:** Accurately transfer about 100 mg of Lonicera japonica Flower, finely powdered, into a suitable stoppered conical flask and accurately add 10.0 mL of 75% methanol. Weigh the filled flask with a precision of ± 0.1 mg and then sonicate for 30 min. After cooling to room temperature, adjust to the initial weight by adding methanol. Pass through a membrane filter of 0.22-μm or finer pore size and discard the first portion of the filtrate.

**Chromatographic system**
(See Chromatography <621> [3], System Suitability.)

- **Mode:** LC
- **Detector:** UV 327 nm
- **Column:** 4.6-mm × 25-cm; 5-μm packing L1 (similar to Thermo Syncronis C18)
- **Column temperature:** 15 ± 1°
- **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 the peaks of chlorogenic acid and cryptochlorogenic acid, 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, 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 for 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—The relative retention times (RRT) provided in Table 2 are not monograph requirements. These are approximate values as 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 Times</th>
<th>Conversion Factor</th>
<th>Content Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neochlorogenic acid&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.75</td>
<td>1.00</td>
<td>minor peak&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chlorogenic acid&lt;sup&gt;i&lt;/sup&gt;</td>
<td>1.00</td>
<td>1.00</td>
<td>1.0</td>
</tr>
<tr>
<td>Cryptochlorogenic acid&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.05</td>
<td>1.00</td>
<td>minor peak&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Compound</td>
<td>peak Area</td>
<td>Area %</td>
<td>Remarks</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-----------</td>
<td>---------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>3,4-Di-O-caffeoylquinic acid</td>
<td>2.65</td>
<td>0.92</td>
<td>minor peak*</td>
</tr>
<tr>
<td>3,5-Di-O-caffeoylquinic acid</td>
<td>2.88</td>
<td>0.77</td>
<td>0.3–0.8</td>
</tr>
<tr>
<td>4,5-Di-O-caffeoylquinic acid</td>
<td>3.01</td>
<td>0.77</td>
<td>0.04–0.2</td>
</tr>
</tbody>
</table>

* (1R,3R,4S,5R)-3-{(E)-3-(3,4-Dihydroxyphenyl)acryloyl]oxy}-1,4,5-trihydroxycyclohexane-1-carboxylic acid.

b The peak area is lower than the peak area for 4,5-di-O-caffeoylquinic acid.

c (1S,3R,4R,5R)-3-{(E)-3-(3,4-Dihydroxyphenyl)acryloyl]oxy}-1,4,5-trihydroxycyclohexane-1-carboxylic acid.

d (1S,3R,4S,5R)-4-{(E)-3-(3,4-Dihydroxyphenyl)acryloyl]oxy}-1,4,5-trihydroxycyclohexane-1-carboxylic acid.

e (1S,3R,4R,5R)-3,4-Bis{(E)-3-(3,4-dihydroxyphenyl)acryloyl]oxy}-1,5-dihydroxycyclohexane-1-carboxylic acid.

f (1S,3R,4S,5R)-3,5-Bis{(E)-3-(3,4-dihydroxyphenyl)acryloyl]oxy}-1,5-dihydroxycyclohexane-1-carboxylic acid.

g (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 chlorogenic acid, 3,5-di-O-caffeoylquinic acid, and 4,5-di-O-caffeoylquinic acid in the portion of Lonicera japonica Flower 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 taken to prepare the Sample solution (mg)
- \( F \) = conversion factor for the analytes as provided in Table 2

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

**Acceptance criteria:** NLT 3.8% on the dried basis

- **CONTENT OF IRIDOIDS**

  **Solution A, Solution B, Mobile phase, Standard solution B, and Sample solution:** Proceed 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, 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. [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 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 taken to prepare the Sample solution (mg)
- \(F\) = conversion factors for analytes; 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.

**Acceptance criteria:** NLT 0.80% on the dried basis

**CONTAMINANTS**

- **Elemental Impurities—Procedures <233>**

**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**<5>, 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

• **Articles of Botanical Origin** [5], *Test for Aflatoxins* [9]<561>: Meets the requirements

**SPECIFIC TESTS**

• **Limit of Triterpenoid Saponins by TLC**

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

  **Sample solution:** 500 mg of *Lonicera japonica* Flower, finely powdered, in 10 mL of methanol. Sonicate for 10 min, and filter.

  **Chromatographic system**
  (See *Chromatography* <621> [3], *Thin-Layer Chromatography.*)

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

  **Application volume:** 3 μL, as 8-mm bands

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

  **Temperature:** 25°

  **Developing solvent system:** The upper layer solution of a mixture of *n*-butanol, formic acid, and water (4:1:5)

  **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 the chromatograms 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, the *Standard solution* exhibits, in the lower-third section, three clearly separated brown bands: the band with the highest \( R_f \) is due to dipsacoside B; the band with the middle \( R_f \) 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 at \( R_f \) and color corresponding to the bands due to dipsacoside B, macranthoidin A, and macranthoidin B in the *Standard solution*.

• **Articles of Botanical Origin** [5], *Foreign Organic Matter* [5]<561>: NMT 2.0%

• **Articles of Botanical Origin** [5], *Alcohol-Soluble Extractives, Method 1* [5]<561>: NLT 30.0%

• **Articles of Botanical Origin** [5], *Water-Soluble Extractives, Method 2* [5]<561>: NLT 35.0%

• **Loss on Drying** [731]<10>

  **Sample:** 2 g of *Lonicera japonica* Flower, finely powdered

  **Analysis:** Dry the *Sample* at 105° for 2 h.

  **Acceptance criteria:** NMT 12.0%

• **Articles of Botanical Origin** [5], *Total Ash* [5]<561>

  **Analysis:** 4 g of *Lonicera japonica* Flower, finely powdered

  **Acceptance criteria:** NMT 10.0%

• **Articles of Botanical Origin** [5], *Acid-Insoluble Ash* [5]<561>: NMT 1.0%

**ADDITIONAL REQUIREMENTS**

• **Packaging and Storage:** Preserve in well-closed containers, protected from light, moisture, and moths, and store in a cool place.
• **LABELING:** The label states the Latin binomial and the part(s) of the plant contained in the article.

• **USP Reference Standards <11>** [11]
- USP Aflatoxins RS [9]
- 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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