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Japanese Honeysuckle Flower Dry Extract

DEFINITION

Japanese Honeysuckle Flower Dry Extract is prepared from the dried flower buds or dried flowers in the early opening stage of *Lonicera japonica* Thunb. (Fam. Caprifoliaceae) collected in early summer and extracted with water or a hydroalcoholic mixture containing NMT 15% 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 centauroside on the dried basis. It may contain suitable added substances as carriers.

IDENTIFICATION

• A. [HPTLC FOR ARTICLES OF BOTANICAL ORIGIN \(203\)](#)

Standard solution A: 0.2 mg/mL of [USP Chlorogenic Acid RS](#), 0.25 mg/mL of [USP Rutin RS](#), and 0.1 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° 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 dissolve the residue in methanol equivalent to one-fifth of the initial volume of the [USP *Lonicera japonica* Flower Dry Extract RS](#) solution.

Sample solution: 100 mg of Japanese Honeysuckle 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 about 5 µm

Application volume: 5 µL, as 10-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: 8 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 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_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 and another yellow band, due to rutin, 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_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; the band with the lowest R_F 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_F and color to the band of chlorogenic acid and one yellow band above chlorogenic acid corresponding in R_F 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, is below the faint blue fluorescent band corresponding in R_f 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_f and color to similar bands in *Standard solution B*.

• **B. CAFFEYOYLQUINIC ACIDS HPLC PROFILE**

Analysis: Proceed as directed in the test 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 test 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 centaurosides at retention times corresponding to the same iridoids in *Standard solution B*.

COMPOSITION

• **CONTENT OF CAFFEYOYLQUINIC ACIDS**

Solution A: 0.1% phosphoric acid in water

Solution B: Acetonitrile

Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	86	14
8	81	19
14	81	19
34	69	31
35	10	90
39.5	10	90
40	86	14
48	86	14

[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 Japanese Honeysuckle Flower Dry Extract into 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 \(621\), System Suitability](#).)

Mode: LC

Detector: UV 327 nm

Column: 4.6-mm × 25-cm; 5-µm packing [L1](#)