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^Japanese Sophora Flower Bud

DEFINITION

Japanese Sophora Flower Bud consists of the dried flower bud of *Styphnolobium japonicum* (L.) Schott (syn. *Sophora japonica* L.) (Fam. *Fabaceae*). It contains NLT 14% and NMT 27% of total flavonol glycosides calculated as the sum of rutin ($C_{27}H_{30}O_{16}$), kaempferol-3-O-rutinoside ($C_{27}H_{30}O_{15}$), and isorhamnetin-3-O-rutinoside ($C_{28}H_{32}O_{16}$); NLT 13% and NMT 25% of rutin; and NLT 0.3% and NMT 1.0% of quercetin ($C_{15}H_{10}O_7$), all on the dried basis.

IDENTIFICATION

• A. HPTLC FOR ARTICLES OF BOTANICAL ORIGIN (203)

Standard solution A: 0.5 mg/mL of [USP Rutin RS](#) and 0.1 mg/mL each of [USP Isorhamnetin-3-O-Rutinoside RS](#) and [USP Quercetin RS](#) in [methanol](#). Sonicate to dissolve.

Standard solution B: 10 mg/mL of [USP Sophora japonica Flower Dry Extract RS](#) in [methanol](#). Sonicate for 10 min, centrifuge, and use the supernatant.

Sample solution: 10 mg/mL of finely powdered Japanese Sophora Flower Bud in [methanol](#). Sonicate for 10 min, centrifuge, and use the supernatant.

Chromatographic system

Adsorbent: [Silica gel with fluorescence indicator F₂₅₄](#), precoated plates, for HPTLC

Application volume: 2 µL, as 8-mm bands

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

Temperature: 20 ± 5°

Developing solvent system: [n-Butyl acetate](#), [methanol](#), [water](#), and [formic acid](#) (7.5: 2: 1: 1)

Derivatization reagent A: 10 mg/mL of [2-aminoethyl diphenylborinate](#) in [methanol](#)

Derivatization reagent B: 50 mg/mL of [polyethylene glycol 400](#) in [ethanol](#)

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, and remove the plate from the chamber. After drying in air, examine the plate under UV light at 254 nm. Then dry the plate further at 100° for 3 min. Treat the hot plate with *Derivatization reagent A*, and dry for 5 min under a current of cool air. Immediately treat the plate with *Derivatization reagent B*, dry for 5 min under a current of cool air, and examine under long wavelength UV light.

System suitability

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

Suitability requirements

Under UV light at 254 nm: *Standard solution A* exhibits a quenching band due to rutin and a faint quenching band due to isorhamnetin-3-O-rutinoside right above rutin in the lower-third section, and a faint quenching band due to quercetin in the upper-third section. *Standard solution B* exhibits a quenching band corresponding in R_f to rutin and two faint quenching bands right above rutin, one with a lower R_f corresponding to isorhamnetin-3-O-rutinoside in *Standard solution A*. In the upper-third section, *Standard solution B* exhibits a faint quenching band corresponding in R_f to quercetin in *Standard solution A*.

Under long wavelength UV light: *Standard solution A* exhibits an intense yellow band due to rutin and a green band right above rutin due to isorhamnetin-3-O-rutinoside in the lower-third section, and a yellow band due to quercetin in the upper-third section. *Standard solution B* exhibits an intense yellow band corresponding in R_f to rutin in *Standard solution A*; several faint yellow bands below rutin; a pair of green bands right above rutin, one with a lower R_f corresponding to isorhamnetin-3-O-rutinoside in *Standard solution A*; and a weak yellow band above the green bands. In the upper-third section, *Standard solution B* exhibits a yellow band corresponding in R_f to quercetin in *Standard solution A*.

Acceptance criteria

Under UV 254 nm: The *Sample solution* exhibits a quenching band corresponding in R_F to rutin; two faint quenching bands right above rutin, one with a lower R_F corresponding to isorhamnetin-3-O-rutinoside; and a faint quenching band corresponding in R_F to quercetin. No additional significant quenching band appears in the *Sample solution* (Japanese Sophora fruits exhibit three intense quenching bands, two above rutin and one below rutin).

Under long wavelength UV light: The *Sample solution* exhibits the following bands corresponding to similar bands in *Standard solution B*: an intense yellow band in the lower-third section corresponding in R_F to rutin in *Standard solution A*; several faint yellow bands below rutin; a pair of green bands right above rutin, one with a lower R_F corresponding to isorhamnetin-3-O-rutinoside in *Standard solution A*; a weak yellow band above the green bands; and a yellow band in the upper-third section corresponding in R_F to quercetin in *Standard solution A*. Other than the above-mentioned green bands, no significant green band appears in the *Sample solution* (Japanese Sophora fruits exhibit additional green bands above and below rutin).

• B. HPLC

Analysis: Proceed as directed in the test for *Content of Flavonol Glycosides and Quercetin*. Analyze the chromatogram between the relative retention time of 0.7 compared to rutin and the retention time of isorhamnetin.

Acceptance criteria: The *Sample solution* exhibits the most intense peak corresponding to rutin in *Standard solution A*, along with peaks due to kaempferol-3-O-rutinoside, isorhamnetin-3-O-rutinoside, quercetin, kaempferol, and isorhamnetin corresponding to the same constituents in *Standard solution B*. The two minor peaks of kaempferol and isorhamnetin may or may not be observed. No other peak in the analyzed chromatogram is more intense than kaempferol-3-O-rutinoside. The content ratio of rutin to total flavonol glycosides is about 90%. The content ratios of kaempferol-3-O-rutinoside, isorhamnetin-3-O-rutinoside, and quercetin to rutin are within the ranges listed in [Table 2](#).

COMPOSITION**• CONTENT OF FLAVONOL GLYCOSIDES AND QUERCETIN**

Solution A: 0.2% [formic acid](#) in [water](#)

Solution B: [Acetonitrile](#)

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

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	82	18
8	82	18
10	75	25
20	70	30
25	64	36
30	60	40
31	5	95
40	5	95

Solvent: [Methanol](#) and [water](#) (8:2)

Standard solution A: 0.30 mg/mL of [USP Rutin RS](#) in *Solvent*

Standard solution B: 2 mg/mL of [USP Sophora japonica Flower Dry Extract RS](#) in *Solvent*. Sonicate for 30 min, centrifuge, and pass through a suitable membrane filter of 0.45- μ m or finer pore size.

Sample solution: Transfer about 20 mg of finely powdered Japanese Sophora Flower Bud, accurately weighed, to a suitable flask. Add 10.0 mL of *Solvent* and close tightly. Weigh the filled flask accurately and sonicate for 30 min (140 W and 100 KHz). Cool to room temperature and, if necessary, adjust with *Solvent* to the initial weight. Before injection, pass through a suitable membrane filter of 0.45- μ m or finer pore size, and discard the first portion of the filtrate.

Chromatographic system