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Wild Chrysanthemum Flower Dry Extract

DEFINITION

Wild Chrysanthemum Flower Dry Extract is prepared from dried flower heads of *Chrysanthemum indicum* L. by extraction with hydroalcoholic mixtures. It contains NLT 90% and NMT 110% of the labeled amount of total caffeoylquinic acids calculated as the sum of chlorogenic acid ($C_{16}H_{18}O_9$), cryptochlorogenic acid ($C_{16}H_{18}O_9$), isochlorogenic acid A ($C_{25}H_{24}O_{12}$), and isochlorogenic acid C ($C_{25}H_{24}O_{12}$) on the dried basis; and NLT 90% and NMT 110% of the labeled amount of total flavone glycosides calculated as the sum of luteolin-7-O-glucoside ($C_{21}H_{20}O_{11}$) and linarin ($C_{28}H_{32}O_{14}$) on the dried basis. It may contain suitable added substances as carriers.

IDENTIFICATION

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

Standard solution A: 0.1 mg/mL each of [USP Chlorogenic Acid RS](#), [USP Linarin RS](#), [USP Luteolin 7-O-Glucoside RS](#), and [USP Isochlorogenic Acid A RS](#) in [methanol](#)

Standard solution B: 10 mg/mL of [USP Chrysanthemum indicum Flower Dry Extract RS](#) in 70% [methanol](#). Sonicate for 20 min, centrifuge, and use the supernatant.

Standard solution C: 10 mg/mL of [USP Chrysanthemum × morifolium Flower Dry Extract RS](#) in 70% [methanol](#). Sonicate for 20 min, centrifuge, and use the supernatant.

Sample solution: 10 mg/mL of Wild Chrysanthemum Flower Dry Extract in 70% [methanol](#). Sonicate for 20 min, centrifuge, and use the supernatant.

Chromatographic system

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

Application volume: 5 µL, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of 33 ± 5% using a suitable device.

Temperature: 20 ± 5°

Developing solvent system: [n-Butyl acetate](#), [formic acid](#), and [water](#) (15:8:8) prepared as follows. Shake for 5 s and let stand for 3 min until the solution is clearly separated into 2 layers. Use the upper layer.

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

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

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, 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 dry in air. Immediately treat the plate with *Derivatization reagent B*, dry in air, and examine under UV light at 365 nm.

System suitability

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

Suitability requirements: In the middle-third section, *Standard solution A* exhibits a blue band due to isochlorogenic acid A; in the lower-third section, *Standard solution A* exhibits an orange-yellow band due to luteolin-7-O-glucoside, a blue band due to chlorogenic acid below the orange-yellow band, and a green band due to linarin below chlorogenic acid. *Standard solution B* exhibits a blue band, an orange-yellow band, another blue band, and a green band corresponding in R_f to the bands due to isochlorogenic acid A, luteolin-7-O-glucoside, chlorogenic acid, and linarin, respectively, in *Standard solution A*; a weak yellow band in the upper-third section; and a blue band below isochlorogenic acid A. If a different plate is used, the linarin band is immediately below chlorogenic acid and may be yellow in *Standard solution B* due to overlap with some other compound.

Acceptance criteria: The *Sample solution* exhibits a blue band, an orange-yellow band, another blue band, and a green band corresponding in R_f to the bands due to isochlorogenic acid A, luteolin-7-O-glucoside, chlorogenic acid, and linarin, respectively, in *Standard solution A* and

Standard solution B. The *Sample solution* exhibits additional bands corresponding in R_f and color to similar bands in *Standard solution B*, which include a weak yellow band in the upper-third section; a blue band below isochlorogenic acid A; and some faint bands below linarin. If a different plate is used, the linarin band is immediately below chlorogenic acid and may be yellow in the *Sample solution* due to overlap with some other compound. The *Sample solution* does not exhibit a significant green band or a pair of adjacent green bands above luteolin-7-O-glucoside as observed in the chromatogram of *Standard solution C* (a distinction from *Chrysanthemum × morifolium* flower).

• B. HPLC

Analysis: Proceed as directed in the test for *Content of Caffeoylquinic Acids and Flavone Glycosides*.

Acceptance criteria: The *Sample solution* exhibits an intense peak corresponding to chlorogenic acid and a peak corresponding to isochlorogenic acid A in *Standard solution A*; a peak corresponding to luteolin-7-O-glucoside in *Standard solution B*; additional caffeoylquinic acid peaks corresponding to cryptochlorogenic acid, isochlorogenic acid B (which may be coeluted with an unknown peak), and isochlorogenic acid C; and an additional flavone glycoside peak corresponding to linarin in *Standard solution C*. Isochlorogenic acid A has a peak intensity higher than those of isochlorogenic acid B and isochlorogenic acid C. The *Sample solution* exhibits no significant peaks corresponding to apigenin-7-O-glucoside (which is close to isochlorogenic acid A) and apigenin-7-O-6"-malonylglucoside (which is close to linarin; a distinction from *Chrysanthemum × morifolium* flower, which displays significant peaks due to apigenin-7-O-glucoside and apigenin-7-O-6"-malonylglucoside).

COMPOSITION

• CONTENT OF CAFFELOYLQUINIC ACIDS AND FLAVONE GLYCOSIDES

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

Solution A: 0.1% [glacial acetic acid](#) in [water](#)

Solution B: 0.1% [glacial acetic acid](#) in [acetonitrile](#)

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

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
10	82	18
14	81	19
20	81	19
35	80	20
40	78	22
45	75	25
55	65	35
55.1	5	95
70	5	95
70.1	90	10
80	90	10

Solvent: [Methanol](#) and [water](#) (7:3)

Standard solution A: 0.1 mg/mL each of [USP Chlorogenic Acid RS](#) and [USP Isochlorogenic Acid A RS](#) in *Solvent*

Standard solution B: 0.05 mg/mL of [USP Luteolin 7-O-Glucoside RS](#) in *Solvent*

Standard solution C: 10 mg/mL of [USP Chrysanthemum indicum Flower Dry Extract RS](#) in *Solvent*. Sonicate for 30 min, and pass through a suitable membrane filter of 0.22-μm pore size.