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▲Wild Chrysanthemum Flower Powder

DEFINITION

Wild Chrysanthemum Flower Powder is the dried flower heads of *Chrysanthemum indicum* L. reduced to a fine or very fine powder. It contains NLT 0.30% 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 0.20% 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.

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: 500 mg of Wild Chrysanthemum Flower Powder in 25 mL of 70% methanol. Sonicate for 20 min, centrifuge, and use the supernatant.

Chromatographic system

Adsorbent: Silica gel with fluorescence indicator F_{254}, 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*,

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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 Caffeoylquinic 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.

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