

Status: Currently Official on 17-Feb-2025
Official Date: Official as of 01-Dec-2015
Document Type: Dietary Supplement Monographs
DocId: GUID-D7F6EAE8-A403-404C-A999-80CD5FE49814_1_en-US
DOI: https://doi.org/10.31003/USPNF_M36582_01_01
DOI Ref: 3op7u

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Powdered Hawthorn Leaf with Flower

DEFINITION

Powdered Hawthorn Leaf with Flower is Hawthorn Leaf with Flower reduced to a fine or very fine powder.

IDENTIFICATION

• **A. [THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST \(201\)](#).**

- Standard solution:** 0.1 mg/mL each of chlorogenic acid, rutin, [USP Hyperoside RS](#), and [USP Vitexin RS](#) in methanol. [NOTE—Reserve a portion of this solution for use in *Identification* test B.]
- Sample solution:** Transfer about 1 g of Powdered Hawthorn Leaf with Flower to a flask, and add 10 mL of methanol. Heat the flask on a water bath maintained at 65° for 5 min, cool, filter, and use the filtrate.
- Adsorbent:** 0.50-mm layer of chromatographic silica gel mixture (TLC plates)
- Application volume:** 10 µL
- Developing solvent system:** Ethyl acetate, glacial acetic acid, formic acid, and water (10:1.1:1.1:2.6)
- Derivatization reagent A:** 2-Aminoethyl diphenylborinate in methanol (1 in 100)
- Derivatization reagent B:** Polyethylene glycol 4000 in methanol (5 in 100)

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in the chapter, except to dry the plate at 105°, and spray the plate while still hot with 10 mL of *Derivatization reagent A* and then with 10 mL of *Derivatization reagent B*. Allow the plate to air-dry for 30 min, and examine under long-wave UV light (365 nm).

Acceptance criteria: The chromatogram of the *Standard solution* exhibits an intense orange zone (at *R_F* value of 0.3) due to rutin; a light blue fluorescent zone (at *R_F* value of 0.4) due to chlorogenic acid; a yellowish-orange zone (at *R_F* value of 0.55) due to hyperoside; and a yellowish-green zone (at *R_F* value of 0.65) due to vitexin. The chromatogram of the *Sample solution*, in addition to the zones due to rutin, chlorogenic acid, hyperoside, and vitexin, exhibits a yellowish-green zone (at *R_F* value of 0.35) due to vitexin-2-rhamnoside; a light blue fluorescent zone (at *R_F* value of 0.6) due to spiraeoside; and a light blue fluorescent zone near the solvent front (at *R_F* value of 0.9) due to caffeic acid. The chromatogram of the *Sample solution* also exhibits additional zones of weaker intensity.

• **B. HPLC**

- Solution A:** Tetrahydrofuran, acetonitrile, and methanol (92.4:3.4:4.2)
- Solution B:** 0.5% Phosphoric acid in water
- Mobile phase:** See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	12	88
12	12	88
25	18	82
30	18	82

Standard solution: Use the *Standard solution* reserved from *Identification* test A.

Sample solution: Transfer about 3 g of Powdered Hawthorn Leaf with Flower to a 100-mL round-bottom flask, add 60 mL of a mixture of methanol and water (4:1), and maintain under reflux for 1 h. Cool, filter, and collect the filtrate in a separate flask. Transfer the residue from the filter back to the flask, add 40 mL of a mixture of methanol and water (4:1), and maintain under reflux for 10 min. Cool, filter, and combine the filtrate with the filtrate obtained from the first extraction. Evaporate the solvent from the combined filtrates under vacuum to a volume of 20 mL. Dilute the resulting solution with a mixture of methanol and water (4:1) to 25.0 mL. Pass 5.0 mL of this solution through a freshly conditioned solid-phase extraction column containing 360 mg of packing L1, collect the eluate in a 10-mL volumetric flask, and dilute with a mixture of methanol and water (4:1) to volume.

Chromatographic system

(See [Chromatography \(621\), System Suitability](#).)

Mode: LC

Detector: UV 336 nm

Column: 4.0-mm × 10-cm; 5-μm packing L1

Column temperature: 25°

Flow rate: 1 mL/min

Injection volume: 5 μL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for chlorogenic acid, vitexin, rutin, and hyperoside are 0.26, 1.0, 1.16, and 1.4, respectively.]

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—The relative retention times for acetyl vitexin-2"-O-rhamnoside, vitexin, isovitexin, and vitexin-2"-O-rhamnoside are 1.53, 1.0, 0.73, and 0.67, respectively.]

Measure the retention times for the major peaks.

Acceptance criteria: The retention times of the peaks for chlorogenic acid, vitexin, rutin, and hyperoside from the *Sample solution* correspond to those of the *Standard solution*.

COMPOSITION

• CONTENT OF C-GLYCOSYLATED FLAVONES

Solution A: 0.5% Solution of phosphoric acid in water

Solution B: Tetrahydrofuran, isopropyl alcohol, and acetonitrile (10:8:3)

Mobile phase: *Solution A* and *Solution B* (22:3)

Standard solution: 0.3 mg/mL of [USP Vitexin RS](#) in *Solution B*, with heating if necessary

Sample solution: Transfer about 4 g of Powdered Hawthorn Leaf with Flower, accurately weighed, to a continuous-extraction apparatus fitted with a flask containing 80 mL of methanol, and extract for 5 h. Cool, remove the flask, and evaporate the solvent from the extract under vacuum to 40 mL. Transfer this solution to a 50-mL volumetric flask, and dilute with methanol to volume. Transfer 10.0 mL of the solution to a suitable flask fitted with a reflux condenser, add 4 mL of 25% hydrochloric acid, and maintain under reflux for 90 min. Cool, transfer the contents of the flask to a 50-mL volumetric flask, and dilute with methanol to volume.

Chromatographic system

(See [Chromatography \(621\), System Suitability](#).)

Mode: LC

Detector: UV 336 nm

Column: 4-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 5 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 3000 theoretical plates

Tailing factor: 0.8–2

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Measure the areas of the major peaks.

Calculate the percentage of C-glycosylated flavones, expressed as vitexin (C₂₁H₂₀O₁₀), in the portion of Powdered Hawthorn Leaf with Flower taken:

$$\text{Result} = (\Sigma r_U / r_S) \times (C_S / C_U) \times 100$$

Σr_U = sum of the peak areas of vitexin and isovitexin from the *Sample solution*, with relative retention times of about 1.0 and 0.85, respectively

r_S = vitexin peak area from the *Standard solution*

C_S = concentration of [USP Vitexin RS](#) in the *Standard solution* (mg/mL)

C_U = concentration of Powdered Hawthorn Leaf with Flower in the *Sample solution* (mg/mL)

Acceptance criteria: NLT 0.6% of C-glycosylated flavones, expressed as vitexin (C₂₁H₂₀O₁₀) on the dried basis

• CONTENT OF O-GLYCOSYLATED FLAVONES