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Add the following:

^European Elder Berry Dry Extract

DEFINITION

European Elder Berry Dry Extract is prepared from the quick-frozen ripened fruits of *Sambucus nigra* L. (Family Adoxaceae/Viburnaceae) by extraction with alcohol. It contains NLT 17% of total anthocyanosides calculated as the sum of the chloride salts of cyanidin-3-O-sambubioside-5-O-glucoside, cyanidin-3,5-di-O-glucoside, cyanidin-3-O-sambubioside, and cyanidin-3-O-glucoside on the anhydrous basis; and NMT 0.2% of cyanidin as chloride salt on the anhydrous basis. It contains NLT 90.0% and NMT 110.0% of the labeled amount of total anthocyanosides calculated as the sum of the chloride salts of cyanidin-3-O-sambubioside-5-O-glucoside, cyanidin-3,5-di-O-glucoside, cyanidin-3-O-sambubioside, and cyanidin-3-O-glucoside on the anhydrous 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 each [USP Chlorogenic Acid RS](#) and [USP Rutin RS](#) in methanol. Sonicate to dissolve.

Standard solution B: 50 mg/mL of [USP European Elder Berry Dry Extract RS](#) in 70% alcohol. Sonicate at about 50° for 30 min, centrifuge, and use the supernatant.

Sample solution: 50 mg/mL of European Elder Berry Dry Extract in 70% alcohol. Sonicate at about 50° for 30 min, centrifuge, and use the supernatant.

Chromatographic system

Adsorbent: Chromatographic silica gel

Application volume: 5 µL

Temperature: About 25°

Developing solvent system: [Ethyl acetate](#), [glacial acetic acid](#), [formic acid](#), and water (10:0.9:0.9:2)

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 at 100° for 3 min. Treat the plate with *Derivatization reagent A* and dry for 5 min in a current of cool air. Immediately treat the plate with *Derivatization reagent B*, dry for 5 min in a current of cool air, and examine under UV light at 366 nm and under white light.

System suitability

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

Suitability requirements: Under UV light at 366 nm, *Standard solution A* exhibits a yellow band due to rutin and a blue fluorescent band due to chlorogenic acid in the lower half of the chromatogram. *Standard solution B* exhibits a band corresponding in R_f and color to rutin and a band corresponding in R_f and color to chlorogenic acid in *Standard solution A*; a yellow band appears above chlorogenic acid and a weak blue band that is partially overlapped with chlorogenic acid appears between chlorogenic acid and the yellow band above. *Standard solution B* exhibits a yellow band in the upper-third section and several faint blue bands between the two aforementioned yellow bands. Under white light, *Standard solution B* exhibits a yellow band corresponding in R_f and color to rutin in *Standard solution A* and a couple of blue bands below rutin.

Acceptance criteria: Under UV light at 366 nm, the *Sample solution* exhibits a yellow band and a blue fluorescent band corresponding in R_f and color to rutin and chlorogenic acid in *Standard solution A* and *Standard solution B*. The *Sample solution* exhibits additional bands corresponding to similar bands in *Standard solution B*, including a yellow band above chlorogenic acid in the lower half of the chromatogram, a weak blue band that is partially overlapped with chlorogenic acid between chlorogenic acid and the yellow band above, a yellow band in the upper third section, and some or all the faint blue bands observed between the two aforementioned yellow bands. Under white light, the *Sample solution* exhibits a band corresponding in R_f and color to rutin in *Standard solution A* and a couple of blue bands below rutin (the European elder berry stems do not display the blue bands).

• B. HPLC

Analysis: Proceed as directed in the test for *Content of Anthocyanosides and Cyanidin*.

Acceptance criteria: The *Sample solution* exhibits a peak corresponding to cyanidin-3-*O*-glucoside in *Standard solution A*, a peak corresponding to cyanidin in *Standard solution B*, and peaks corresponding to cyanidin-3-*O*-sambubioside-5-*O*-glucoside, cyanidin-3,5-di-*O*-glucoside, and cyanidin-3-*O*-sambubioside in *Standard solution C*. The most intense peak corresponds to cyanidin-3-*O*-sambubioside. The peak that follows in intensity corresponds to cyanidin-3-*O*-glucoside. The cyanidin-3-*O*-sambubioside-5-*O*-glucoside peak is substantially smaller. Cyanidin-3,5-di-*O*-glucoside and cyanidin are minor peaks. Any other peak in the chromatogram is not more intense than the one corresponding to cyanidin-3,5-di-*O*-glucoside.

COMPOSITION

• CONTENT OF ANTHOCYANOSIDES AND CYANIDIN

[NOTE—Protect from light and moisture, and proceed under low-actinic light. *Standard solution A*, *Standard solution C*, and the *Sample solution* are stable for 48 h at 4°; *Standard solution B* is stable for 36 h at 4°.]

Solution A: [Water](#), [acetonitrile](#), and [trifluoroacetic acid](#) (19:1:0.06)

Solution B: [Methanol](#), [acetonitrile](#), [water](#), and [trifluoroacetic acid](#) (7:8:5:0.06)

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

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	80	20
20	60	40
30	10	90
35	10	90
36	80	20
40	80	20

Solvent 1: 2% [hydrochloric acid](#) in [methanol](#)

Solvent 2: 10 mL of [phosphoric acid](#) diluted with [water](#) to 100 mL

Standard stock solution A: 0.30 mg/mL of [USP Cyanidin-3-*O*-glucoside Chloride RS](#) in *Solvent 1*. Sonicate to dissolve.

Standard solution A: 0.060 mg/mL of [USP Cyanidin-3-*O*-glucoside Chloride RS](#) in *Solvent 2*, prepared from *Standard stock solution A*

Standard stock solution B: 0.10 mg/mL of [USP Cyanidin Chloride RS](#) in *Solvent 1*. Sonicate to dissolve.

Standard solution B: 0.002 mg/mL of [USP Cyanidin Chloride RS](#) in *Solvent 2*, prepared from *Standard stock solution B*

Standard solution C: 1 mg/mL of [USP European Elder Berry Dry Extract RS](#). Add 20% of final volume of *Solvent 1*, sonicate at least for 10 min to dissolve, and dilute with *Solvent 2* to volume. Before injection, pass through a membrane filter of 0.45-μm or finer pore size.

Sample solution: Transfer about 25 mg of European Elder Berry Dry Extract accurately weighed into a 25-mL volumetric flask. Add 5 mL of *Solvent 1*, sonicate to dissolve, and dilute with *Solvent 2* to volume. Before injection, 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 535 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 30°

Flow rate: 0.8 mL/min

Injection volume: 10 μL

System suitability

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

Suitability requirements