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Powdered Bilberry Extract

DEFINITION

Powdered Bilberry Extract is prepared from the ripe fruits of *Vaccinium myrtillus* L. (Fam. Ericaceae) using suitable solvents such as alcohol, methanol, or water or mixtures of these solvents. The ratio of the starting plant material to Powdered Extract is between 153:1 and 76:1. It contains NLT 36.0% of anthocyanosides, calculated as cyanidin-3-O-glucoside chloride, and NMT 1.0% of anthocyanidins, calculated as cyanidin chloride; both are calculated on the anhydrous basis.

IDENTIFICATION

• **A. [Thin-Layer Chromatographic Identification Test](#)**

Standard solution: 4 mg/mL of [USP Powdered Bilberry Extract RS](#) in methanol. Centrifuge, and use the clear supernatant.

Sample solution: 4 mg/mL of Powdered Bilberry Extract in methanol. Centrifuge, and use the clear supernatant.

Adsorbent: Use suitable thin-layer chromatographic plates coated with a layer of cellulose.

Application volume: 10 µL

Developing solvent system A: Glacial acetic acid, hydrochloric acid, and water (15:3:82)

Developing solvent system B: Glacial acetic acid and water (6:4)

Analysis

Samples: *Standard solution* and *Sample solution*

Use a saturated chamber. Develop the chromatograms using *Developing solvent system A*, and dry the plate with the aid of a current of warm air. Develop the chromatograms in the same direction using *Developing solvent system B*. Examine the plate under visible light.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits three main red bands with *R_f* values of approximately 0.55, 0.65, and 0.70 that are similar in position and color to the corresponding main bands in the chromatogram of the *Standard solution*.

• **B.** The retention times of the anthocyanoside peaks in the chromatogram of the *Sample solution* correspond to those in the chromatogram of *Standard solution C*, as obtained in the test for *Content of Anthocyanosides and Anthocyanidins*. The peaks due to delphinidin-3-O-galactoside chloride and delphinidin-3-O-glucoside chloride are the most intense peaks and are of similar intensity, and each is more intense than the peak due to cyanidin-3-O-glucoside chloride. The peaks due to cyanidin-3-O-galactoside chloride, delphinidin-3-O-arabinoside chloride, and cyanidin-3-O-glucoside chloride are of similar intensity. Each of the remaining anthocyanoside peaks is of lower intensity than the peak due to cyanidin-3-O-glucoside chloride.

COMPOSITION

Change to read:

• **CONTENT OF ANTHOCYANOSIDES AND ANTHOCYANIDINS**

Solvent: Methanol and hydrochloric acid (49:1)

Diluent: 85% phosphoric acid and water (1:9)

Solution A: Formic acid and water (1:9)

Solution B: Acetonitrile, methanol, formic acid, and water (45:45:20:80)

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

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	93	7
35	75	25
45	35	65
46	0	100
50	0	100
51	93	7

Time (min)	Solution A (%)	Solution B (%)
60	93	7

Standard stock solution A: 0.4 mg/mL of [USP Cyanidin-3-O-glucoside Chloride RS](#) in *Solvent*. [NOTE—Dissolve using sonication.]

Standard solution A: 0.08 mg/mL of [USP Cyanidin-3-O-glucoside Chloride RS](#) from *Standard stock solution A* in *Diluent*. [NOTE—This solution is stable for 48 h at 4°.]

Standard stock solution B: 0.5 mg/mL of [USP Cyanidin Chloride RS](#) in *Solvent*. [NOTE—Dissolve using sonication.]

Standard solution B: 0.01 mg/mL of [USP Cyanidin Chloride RS](#) from *Standard stock solution B* in *Diluent*. [NOTE—This solution is stable for 36 h at 4°.]

Standard solution C: Transfer 125 mg of [USP Powdered Bilberry Extract RS](#) to a 100-mL volumetric flask, add 25 mL of *Solvent*, sonicate to dissolve, and dilute with *Diluent* to volume. [NOTE—This solution is stable for 48 h at 4°.]

Sample solution: Transfer 125 mg of Powdered Bilberry Extract to a 100-mL volumetric flask, add 25 mL of *Solvent*, sonicate to dissolve, and dilute with *Diluent* to volume.

Chromatographic system

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

[NOTE—Use deactivated silanized HPLC vials.]

Mode: LC

Detector: UV-Vis 535 nm

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

Temperature

Refrigerated autosampler: 4°

Column: 30 ± 1°

Flow rate: 1 mL/min

Injection size: 10 μL

System suitability

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

Suitability requirements

Chromatogram similarity: The chromatogram from *Standard solution C* is similar to the Reference Chromatogram provided with [USP Powdered Bilberry Extract RS](#).

Resolution: NLT 0.8 between the delphinidin-3-O-arabinoside, malvidin-3-O-galactoside, and petunidin-3-O-▲arabinoside▲ (ERR 1-Jun-2020) peaks and NLT 1.0 for other components, *Standard solution C*

Tailing factor range: 0.8–2.0 for the cyanidin-3-O-glucoside chloride peak, *Standard solution A*

Relative standard deviation: NMT 2.0% for cyanidin-3-O-glucoside chloride peak in repeated injections, *Standard solution A*

Analysis

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

Using the chromatogram of *Standard solution C* and the Reference Chromatogram, identify the retention times of the peaks corresponding to the different anthocyanosides and anthocyanidins. The approximate relative retention times, relative to cyanidin-3-O-glucoside chloride, are provided for the anthocyanosides in [Table 2](#) and for the anthocyanidins in [Table 3](#).

Separately calculate the percentages of each anthocyanoside (see [Table 2](#)) in the portion of Powdered Extract taken:

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

r_U = peak response of each of the anthocyanosides in the *Sample solution*

r_S = peak response of cyanidin-3-O-glucoside chloride in *Standard solution A*

C_S = concentration of [USP Cyanidin-3-O-glucoside Chloride RS](#) in *Standard solution A* (mg/mL)

C_U = concentration of Powdered Extract in the *Sample solution* (mg/mL)

Table 2

Analyte	Relative Retention Time
Delphinidin-3-O-galactoside chloride	0.61
Delphinidin-3-O-glucoside chloride	0.73