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Powdered Black Pepper

DEFINITION

Powdered Black Pepper is Black Pepper reduced to powder or very fine powder. It contains NLT 2.5% of piperine, calculated on the dried basis.

IDENTIFICATION

• **A.** Powdered Black Pepper meets the requirements under *Specific Tests, Botanical Characteristics*.

• **B.** [HPTLC FOR ARTICLES OF BOTANICAL ORIGIN \(203\)](#).

Standard solution A: 0.9 mg/mL of [USP Piperine RS](#) in methanol

Standard solution B: 2.0 mg/mL of borneol in methanol

Standard solution C: 5 mg/mL of [USP Powdered Black Pepper Extract RS](#) in methanol. Sonicate for about 10 min, centrifuge, and use the supernatant.

Sample solution: Sonicate for 10 min about 0.5 g of Powdered Black Pepper in 5 mL of methanol, centrifuge, and use the supernatant.

Chromatographic system

Adsorbent: Chromatographic silica gel mixture with an average particle size of 5 µm (HPTLC plates)

Application volume: 15 µL of *Standard solution C*, 7 µL of the *Sample solution*, and 3 µL of *Standard solution A* and *Standard solution B*, as bands, 8 mm

Developing solvent system: Hexanes and ethyl acetate (5:3)

Derivatization reagent: A mixture of 17 mL of ice-cooled methanol, 2 mL of acetic acid, 1 mL of sulfuric acid, and 0.1 mL of anisaldehyde, mixed in this order

Analysis

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

Apply the *Samples* as bands. Use a saturated chamber, and condition the plate to a relative humidity of about 33% using a suitable device.

Develop until the solvent front has moved up about 7 cm from the lower edge of the plate. Remove the plate from the chamber, dry, and examine under UV light at 254 nm. Treat with the *Derivatization reagent*, heat for 5 min at 100°, and examine under white light.

Acceptance criteria: Under UV 254 nm, the chromatogram of the *Sample solution* exhibits an intense quenching band at R_f of about 0.15 corresponding to the piperine band in the chromatogram of *Standard solution A*, a quenching band at R_f of about 0.02, and three quenching bands of similar intensity equally spaced located between R_f of about 0.3 and 0.5. Under white light, the derivatized chromatogram of the *Sample solution* exhibits main bands similar in position and color to the main bands in the chromatogram of *Standard solution C*. These bands include a dark green band of the same color and R_f as the piperine band in *Standard solution A* (R_f of about 0.15), a weak violet band at R_f of about 0.47 below the position of the band due to borneol in *Standard solution B*, and a greenish band in the lower part of the chromatogram at R_f of about 0.07. Other minor bands may be observed in the *Sample solution* and *Standard solution C* chromatograms. No blue bands are detected in the chromatogram of the *Sample solution* at R_f of about 0.10 and 0.58 (distinction from long pepper).

• C. HPLC

Analysis: Proceed as directed in the test for *Content of Piperine*.

Acceptance criteria: The chromatogram of the *Sample solution* obtained at 343 nm exhibits a major peak at the retention time corresponding to piperine. Identify other piperamide peaks in the *Sample solution* chromatogram by comparison with the chromatogram of *Standard solution B* and the reference chromatogram provided with the lot of [USP Powdered Black Pepper Extract RS](#) being used. The *Sample solution* chromatogram shows an additional peak corresponding to piperyline. The chromatogram of the *Sample solution* obtained at 270 nm does not exhibit a peak due to (2*E*,4*E*)-*N*-isobutyldecadienamide at a relative retention time of 1.14 to the piperine peak (distinction from long pepper).

COMPOSITION

• CONTENT OF PIPERINE

Solution A: Dissolve 0.14 g of anhydrous potassium dihydrogen phosphate in 900 mL of water, and add 0.5 mL of phosphoric acid. Dilute with water to 1000 mL, mix, filter, and degas.

Solution B: Acetonitrile

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

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
18	55	45
25	20	80
28	20	80
35	55	45
40	95	5
45	95	5

[NOTE—Proceed under subdued light or using low-actinic glassware.]

Standard solution A: 0.1 mg/mL of [USP Piperine RS](#) in methanol

Standard solution B: Sonicate a portion of [USP Powdered Black Pepper Extract RS](#) in methanol to obtain a solution having a concentration of about 0.5 mg/mL. Before injection, pass through a membrane filter of 0.45-µm or finer pore size, discarding the first few mL of the filtrate.

Sample solution: Transfer about 2.0 g of Powdered Black Pepper, accurately weighed, to a 250-mL flask fitted with a reflux condenser. Add 50 mL of methanol, reflux on a water bath for about 20 min, allow to settle, and decant the supernatant. Repeat until the last extract is colorless. Combine the extracts, concentrate under vacuum, and adjust the volume to 100 mL using methanol. Before injection, pass through a membrane filter of 0.45-µm or finer pore size, discarding the first few mL of the filtrate.

Chromatographic system

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

Mode: LC

Detector: UV 343 nm and 270 nm

Column: 4.6-mm × 25-cm; 5-µm, 100 Å packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

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

Suitability requirements

Chromatogram similarity: The chromatogram obtained from *Standard solution B* is similar to the reference chromatogram provided with the lot of [USP Powdered Black Pepper Extract RS](#) being used.

Tailing factor: NMT 1.5 for the piperine peak, *Standard solution A*

Relative standard deviation: NMT 2.5% determined from the piperine peak in replicate injections, *Standard solution A*

Analysis

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

[NOTE—*Standard solution A*, *Standard solution B*, and the *Sample solution* are stable for 6 h at room temperature.]

Using the chromatograms of *Standard solution A*, *Standard solution B*, and the reference chromatogram provided with the lot of [USP Powdered Black Pepper Extract RS](#) being used, identify the retention times of the peaks corresponding to piperine and piperidine in the *Sample solution* chromatogram.

Calculate the percentage of piperine in the portion of Powdered Black Pepper taken:

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

r_U = peak area of piperine from the *Sample solution* chromatogram at 343 nm

r_S = peak area of piperine from the *Standard solution A* chromatogram at 343 nm

C_S = concentration of piperine in *Standard solution A* (mg/mL)

V = final volume of the *Sample solution* (mL)

W = weight of Powdered Black Pepper used to prepare the *Sample solution* (mg)

Acceptance criteria: NLT 2.5% on the dried basis

CONTAMINANTS

- [ARTICLES OF BOTANICAL ORIGIN, Limits of Elemental Impurities\(561\)](#): Meets the requirements
- [ARTICLES OF BOTANICAL ORIGIN, Pesticide Residue Analysis\(561\)](#): Meets the requirements