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Capsicum

DEFINITION

Capsicum is the dried ripe fruit of various *Capsicum* species (Fam. Solanaceae). It contains NLT 0.3% of total capsaicinoids, calculated as the sum of capsaicin, dihydrocapsaicin, nordihydrocapsaicin, nonivamide, decanylvanillinamide, and homocapsaicin; and the nonivamide content is NMT 5% of the total capsaicinoids; all calculated on the dried basis.

IDENTIFICATION

• **A. Thin-Layer Chromatography**

Standard solution A: 0.4 mg/mL of USP Capsaicin RS in methanol

Standard solution B: 0.4 mg/mL of USP Dihydrocapsaicin RS in methanol

Sample solution: Shake for 5 min about 0.5 g of Capsicum, finely powdered, in 5 mL of hexanes, and centrifuge. Use the supernatant.

Chromatographic system

Adsorbent: Chromatographic reversed-phase octadecyl silyl silica gel with an average particle size of 5 µm (HPTLC plates)

Application volume: 2 µL, as 8-mm bands

Developing solvent system: A mixture of methanol and water (8:2)

Derivatization reagent A: 0.25 mg/mL of dichloroquinonechlorimide in ethyl acetate

Derivatization reagent B: Ammonium hydroxide solution

Analysis

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

Apply the *Samples* as bands to a suitable high-performance thin-layer chromatographic plate. Use a saturated chamber, and condition the plate to a relative humidity of about 33% using a suitable device. Develop the chromatograms over a distance of 6 cm. Remove the plate from the chamber, dry, derivatize with *Derivatization reagent A*, and expose to vapors of *Derivatization reagent B* until blue bands develop. Examine under white light.

System suitability: *Standard solution A* shows a blue band at about one-third of the chromatogram, and *Standard solution B* shows a blue band at an R_F right below that from *Standard solution A*.

Acceptance criteria: The *Sample solution* exhibits a blue band at about one-third of the chromatogram, similar in position and color to the capsaicin band in the chromatogram of *Standard solution A*, and exhibits a blue band at an R_F right below that of capsaicin, similar in position and color to the dihydrocapsaicin band in the chromatogram of *Standard solution B*. Other bands may be observed in the *Sample solution* chromatogram.

• **B. HPLC**

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

Acceptance criteria: The *Sample solution* chromatogram exhibits the main capsaicinoid peak at the retention time corresponding to capsaicin in the chromatogram of *Standard solution A* and a peak of lower intensity corresponding to dihydrocapsaicin in the chromatogram of *Standard solution B*. The *Sample solution* chromatogram shows additional minor peaks corresponding to nordihydrocapsaicin, nonivamide, decanylvanillinamide, and homocapsaicin.

COMPOSITION

• **Content of Total Capsaicinoids**

Mobile phase: A mixture of acetonitrile and diluted phosphoric acid (1 in 1000) (2:3)

Standard solution A: 0.02 mg/mL of USP Capsaicin RS in methanol

Standard solution B: 0.01 mg/mL of USP Dihydrocapsaicin RS in methanol

Sample solution: To a glass centrifuge tube transfer about 0.5 g of Capsicum, powdered and accurately weighed, add 30 mL of methanol, shake for 15 min, and centrifuge. Transfer the supernatant to a 50-mL volumetric flask. To the residue add 10 mL of methanol, shake for 5 min, and centrifuge. Transfer the supernatant to the volumetric flask. Repeat the extraction one more time with 10 mL of methanol. Complete with methanol to volume, and mix. 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 281 nm

Column: 4.6-mm × 25-cm; end-capped, 5-µm, 150 Å, packing L11

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

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

Suitability requirements

Resolution: NLT 1.5 between the capsaicin peak and the nonivamide peak occurring at a retention time of 0.95 relative to 1.0 for capsaicin, *Sample solution*

Relative standard deviation: NMT 2.0% for the capsaicin peak in repeated injections, *Standard solution A*

Analysis

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

Identify the capsaicin and dihydrocapsaicin peaks in the *Sample solution* chromatogram by comparison with the chromatograms of *Standard solution A* and *Standard solution B*, respectively. Identify the peaks corresponding to nordihydrocapsaicin, nonivamide, decanylvanillinamide, and homocapsaicin using the approximate relative retention times provided in *Table 1*.

Table 1

Analyte	Approximate Relative Retention Time
Nordihydrocapsaicin	0.89
Nonivamide	0.95
Capsaicin	1.00
Decanylvanillinamide	1.34
Homocapsaicin	1.40

Calculate the percentage of capsaicin in the portion of Capsicum taken:

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

r_U = peak area of capsaicin from the *Sample solution*

r_S = peak area of capsaicin from *Standard solution A*

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

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

W = weight of Capsicum taken to prepare the *Sample solution* (mg)

Calculate the percentage of dihydrocapsaicin in the portion of Capsicum taken:

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

r_U = peak area of dihydrocapsaicin from the *Sample solution*

r_S = peak area of dihydrocapsaicin from *Standard solution B*

C_S = concentration of dihydrocapsaicin in *Standard solution B* (mg/mL)

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

W = weight of Capsicum taken to prepare the *Sample solution* (mg)

Calculate the percentage of nonivamide, expressed as capsaicin, in the portion of Capsicum taken:

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

r_U = peak area of nonivamide from the *Sample solution*

r_S = peak area of capsaicin from *Standard solution A*

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

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

W = weight of Capsicum taken to prepare the *Sample solution* (mg)

Calculate the sum of the percentages of nordihydrocapsaicin, decanylvanillinamide, and homocapsaicin, expressed as capsaicin, in the portion of Capsicum taken:

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

Σr_U = sum of peak areas of nordihydrocapsaicin, decanylvanillinamide, and homocapsaicin from the *Sample solution*

r_S = peak area of capsaicin from *Standard solution A*

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

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

W = weight of Capsicum taken to prepare the *Sample solution* (mg)

Calculate the content of total capsaicinoids as the sum of the percentages of capsaicin, dihydrocapsaicin, nordihydrocapsaicin, nonivamide, decanylvanillinamide, and homocapsaicin.

Acceptance criteria: NLT 0.3% on the dried basis

CONTAMINANTS

• **Elemental Impurities—Procedures ⟨233⟩**

Acceptance criteria

Arsenic: NMT 0.5 µg/g

Cadmium: NMT 1.0 µg/g

Lead: NMT 5.0 µg/g

Mercury: NMT 0.1 µg/g

Change to read:

• **▲Articles of Botanical Origin,** *Pesticide Residue Analysis* ⟨561⟩▲ (CN 1-May-2019) **:** Meets the requirements

• **Articles of Botanical Origin,** *Test for Aflatoxins*⟨561⟩**:** Meets the requirements

SPECIFIC TESTS

• **Limit of Nonivamide**

Analysis: Use the chromatograms and calculations obtained in the test for *Content of Total Capsaicinoids*.

Calculate the content of nonivamide as a percentage of total capsaicinoids:

$$\text{Result} = (PN/PTC) \times 100$$

PN = percentage of nonivamide as calculated in the *Content of Total Capsaicinoids*

PTC = percentage of total capsaicinoids as calculated in the *Content of Total Capsaicinoids*

Acceptance criteria: NMT 5% on the dried basis

• **Botanic Characteristics**

Macroscopic: Mature Capsicum fruit vary in length from a few cm to more than 10 cm; fruit diameter ranges from a few mm to a few cm; shape varies from elongate, ellipse, almost round, triangular, campanulate, tomato-like, or square (blocky); number of locules two, three, or four; the dissepiments being united at the base to a conical, central placenta; calyx margin is either entire, dentate, or intermediate dentate, and sometimes attached to a long, straight pedicel; the presence of calyx annular constriction, at junction of calyx and pedicel indicates *Capsicum chinense*; the seeds are light brown to weak yellowish-orange, suborbicular or irregular, flattened, from 2–4 mm in diameter, with a thickened edge and a prominent, pointed micropyle.

Microscopic: Powdered Capsicum shows fragments of epicarp cells, polygonal, triangular, rectangular, or irregular cells, with or without beaded walls; numerous fragments of thin-walled parenchyma cells of the pericarp containing oil globules and chromoplasts of the various colors similar to the color of the fruits; fragments of sclerenchymatous cells of the endocarp with slightly wavy, lignified walls and broad lumina, striations and pit canal distinct, cells appear bright white under a polarizing microscope; fragments of epidermal cells of testa, extremely thick wall, deeply sinuate, striations and pit canal distinct, cells appear bright yellowish-white under a polarizing microscope; fragments of parenchyma cells of the endosperm containing fixed oil and aleurone grains; and occasional fibrovascular elements and calyx tissues.

• **Articles of Botanical Origin,** *Foreign Organic Matter*⟨561⟩**:** NMT 1%, other than stems and calyces, the proportion of which does not exceed 3%

• **Loss on Drying ⟨731⟩**

Sample: 1.0 g of finely powdered Capsicum

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 11%

- **Articles of Botanical Origin,** *Total Ash*⟨561⟩**:** NMT 10.0%
- **Articles of Botanical Origin,** *Acid-Insoluble Ash*⟨561⟩**:** NMT 1.25%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. A few drops of chloroform may be added from time to time to prevent attack by insects.
- **Labeling:** Label each container to indicate which variety of Capsicum is contained therein.
- **USP Reference Standards** ⟨11⟩
  USP Capsaicin RS
  USP Dihydrocapsaicin RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
CAPSICUM	Nam-Cheol Kim Scientific Liaison	BDSHM2020 Botanical Dietary Supplements and Herbal Medicines

Chromatographic Database Information: Chromatographic Database

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