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Capsicum Tincture

DEFINITION
Capsicum Tincture is prepared as follows.

Capsicum	100 g
A mixture of Alcohol and Water (70:30) to (85:15), a sufficient quantity to make	1000 mL

Prepare the Tincture as directed for *Botanical Extracts ⟨565⟩, Tinctures, Maceration Process*. It contains NLT 0.02% (w/v) 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.

IDENTIFICATION

• **A. Thin-Layer Chromatography**

Standard solution A: 0.2 mg/mL of USP Capsaicin RS in methanol

Standard solution B: 0.2 mg/mL of USP Dihydrocapsaicin RS in methanol

Sample solution: Shake 10 mL of Tincture with 10 mL of hexanes, allow to separate, and use the lower layer.

Chromatographic system

(See *Chromatography ⟨621⟩, Thin-Layer Chromatography*.)

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

Application volume: 2 µL, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

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

Derivatization reagent A: 5 mg/mL of dichloroquinonechlorimide in methanol

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 develop the chromatograms over a distance of 6 cm. Remove the plate from the chamber, dry, derivatize with *Derivatization reagent A*, dry, expose to the vapors of *Derivatization reagent B* until blue bands develop, and then 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 just below that from *Standard solution A*. Separation between capsaicin and dihydrocapsaicin should be achieved with the reference standards; the bands appear at the intended location of the plate and they are visible as blue bands.

Acceptance criteria: The *Sample solution* chromatogram exhibits a blue band at about one-third of the chromatogram, corresponding in color and R_F to the capsaicin band in the chromatogram of *Standard solution A*, and exhibits a blue band at an R_F just 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.

STRENGTH

• **Content of Total Capsaicinoids**

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

Standard solution A: 0.1 mg/mL of USP Capsaicin RS in methanol

Standard solution B: 0.05 mg/mL of USP Dihydrocapsaicin RS in methanol

Sample solution: Dilute an accurately measured volume of Tincture in methanol (1:1), and mix. Before injection, pass through a membrane filter with a 0.45-µm or finer pore size, discarding the first few mL of the filtrate. [Note—The sample can be weighed and converted to

volume using the density of the Tincture.]

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

Flow rate: 1.0 mL/min

Column temperature: 30°

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 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 Tincture taken:

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

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* (g/mL)

C_U = concentration of Tincture in the *Sample solution* (mL/mL)

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

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

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* (g/mL)

C_U = concentration of Tincture in the *Sample solution* (mL/mL)

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

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

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* (g/mL)

C_U = concentration of Tincture in the *Sample solution* (mL/mL)

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

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

Σr_U = sum of the 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* (g/mL)

C_U = concentration of Tincture in the *Sample solution* (mL/mL)

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

Acceptance criteria: NLT 0.02%

OTHER COMPONENTS

• **Alcohol Determination,** *Method I* ⟨611⟩**:** NLT 90.0% and NMT 110.0% of the labeled amount of C_2H_5OH

CONTAMINANTS

Change to read:

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

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at room temperature.

• **Labeling:** The label states the official name of the article, the Latin binomial, and the part of the plant from which the article was prepared. Label it to indicate the content of capsaicinoids, the solvent mixture used for extraction, and the ratio of the starting crude plant material to Tincture.

• **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 TINCTURE	Nam-Cheol Kim Scientific Liaison	BDSHM2020 Botanical Dietary Supplements and Herbal Medicines

Chromatographic Database Information: Chromatographic Database

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