

CAPSAICIN and DIHYDROCAPSAICIN

5041

(1) C₁₈H₂₇NO₃ MW: 305.40
 (2) C₁₈H₂₉NO₃ 307.48

CAS: 404-86-4
 19408-84-5

RTECS: RA8530000
 RA5998000

METHOD: 5041, Issue 1

EVALUATION: PARTIAL

Issue 1: 15 May 1996

OSHA : no PELs
NIOSH: no RELs
ACGIH: no TLVs

PROPERTIES: (1) white solid; MP 65 °C; BP 210-220 °C @ 0.01 mm Hg; VP not significant @ 25 °C
 (2) white solid; VP not significant @ 25 °C

NAMES & SYNONYMS: (1) capsaicin: *trans*-8-methyl-*N*-vanillyl-6-nonenamide
 (2) dihydrocapsaicin: 8-methyl-*N*-vanillylnonanamide

SAMPLING		MEASUREMENT	
SAMPLER:	FILTER (13-mm glass fiber)	TECHNIQUE:	HPLC, FLUORESCENCE DETECTOR
FLOW RATE:	1 to 3 L/min	ANALYTES:	capsaicin and dihydrocapsaicin
VOL-MIN:	(1) 5L @ 0.01 mg/m ³ (2) 7 L @ 0.01 mg/m ³	EXTRACTION:	2 mL acetonitrile; ultrasonic bath, 10 min
-MAX:	1000 L	INJECTION VOLUME:	25 µL
SHIPMENT:	ship at 0 °C	MOBILE PHASE:	48% acetonitrile/52% water v/v; 1 mL/min
SAMPLE STABILITY:	at least 26 days @ 5 °C [1,2]	COLUMN:	3.9-mm ID x 15 cm stainless steel packed with 10-µm µ-Bondapak C ₁₈
BLANKS:	2 to 10 field blanks per set	DETECTOR:	fluorescence: excitation 281 nm; emission 312 nm
ACCURACY		CALIBRATION:	standard solutions of capsaicin and dihydrocapsaicin in acetonitrile
RANGE STUDIED:	not studied	RANGE:	(1) 0.050 to 100 µg/sample (2) 0.067 to 100 µg/sample
BIAS:	not determined	ESTIMATED LOD:	(1) 0.015 µg/sample (2) 0.02 µg/sample
OVERALL PRECISION (Ŝ_{r,T}):	not determined	PRECISION (Ŝ_r):	(1) 0.042 @ 0.13 to 17 µg/sample (2) 0.065 @ 0.11 to 3 µg/sample
ACCURACY:	not determined		

APPLICABILITY: This method has been used to analyze samples collected at a pickle and pepper processing plant [1]. Analyte concentrations in sample solutions are not expected to exceed 0.2 µg/mL when samples are collected in this type of environment.

INTERFERENCES: Capsaicin and dihydrocapsaicin exhibit baseline separation at concentrations of 0.2 µg/mL and less. At higher concentrations, baseline separation can be achieved by increasing the water in the mobile phase to about 55%. Nordihydrocapsaicin causes little interference during measurement of capsaicin because its abundance is relatively small in *Capsicum* fruit [3].

OTHER METHODS: HPLC methods for one or both analytes in solution have been published [3-8]. None have been published for air analysis.

REAGENTS:

1. Capsaicin, $\geq 98\%$ pure.*
2. Dihydrocapsaicin, $\geq 90\%$ pure.*
3. Water, distilled.
4. Acetonitrile, chromatographic quality.*
5. Capsaicin calibration and recovery stock solution, 0.8 mg/mL: Dissolve 20 mg of capsaicin in acetonitrile to make 25 mL solution. Store at 5 °C. Prepare fresh bimonthly.
6. Dihydrocapsaicin calibration and recovery stock solution, 0.8 mg/mL: Dissolve 20 mg dihydrocapsaicin in acetonitrile to make 25 mL solution. Store at 5 °C. Prepare fresh bimonthly.

* See SPECIAL PRECAUTIONS

EQUIPMENT:

1. Sampler: 13-mm glass fiber filter in 2-piece filter holder (Swinnex, Millipore Corp., or equivalent).
2. Personal sampling pump, 1 to 3 L/min, with flexible connecting tubing.
3. HPLC, fluorescence detector, ex 281nm, em 312 nm; recorder, integrator, and column (page 5041-1).
4. Vials, 4-mL, with PTFE-lined caps.
5. Graduated cylinder, 1-L, readable to 10 mL.
6. Volumetric flasks, 25- and 10-mL.
7. Syringes: 10-mL; 500- μ L, readable to 10 μ L; 100- μ L, readable to 1 μ L; 10 μ L, readable to 0.1 μ L.
8. Ultrasonic water bath.
9. Film, plastic, flexible, water resistant.
10. Syringe filters, 3-mm PTFE membrane, 0.45- μ m pore size, in polypropylene housing.
11. Tweezers.
12. Dust mask.

SPECIAL PRECAUTIONS: Capsaicin and dihydrocapsaicin are toxic, are classified as mutagens, and can destroy certain sensory nerve cells [9,10]. Inhalation of aerosols of these compounds will result in prolonged coughing or sneezing. Exposure by inhalation can take place during weighing operations; thus, a dust mask is recommended. Skin contact will cause a burning sensation. Ingestion can cause intolerable burning and gastrointestinal disorders.

Acetonitrile is toxic and is a fire hazard (flash point = 12.8 °C).

SAMPLING:

1. Calibrate each personal sampling pump with a representative sampler in line.
2. Attach the sampler to the personal sampling pump with flexible tubing.
3. Sample at an accurately known flow rate between 1 and 3 L/min for a total sample size of 7 to 1000 L.
NOTE: Limit the maximum loading of particulate matter on the filter to approximately 0.5 mg.
4. Seal ends of sampler with plastic film. Ship to laboratory in insulated container with bagged refrigerant.

SAMPLE PREPARATION:

5. Transfer the 13-mm glass fiber filter to a 4-mL vial.
6. Add 2 mL of acetonitrile to the vial and cap securely.
7. Place the sample vial into an ultrasonic bath and agitate for 10 min.
8. Filter the sample solution through a PTFE membrane filter.

CALIBRATION AND QUALITY CONTROL:

9. Calibrate daily with at least six working standards over the range of interest: 0.008 to 10 μ g/mL for capsaicin; 0.01 to 10 μ g/mL for dihydrocapsaicin.
 - a. Prepare working standards from calibration stock solution in acetonitrile.
NOTE: Working standards may be stored in tightly sealed vials at 5 °C for at least 9 weeks.
 - b. Analyze together with samples and blanks (steps 12 and 13).
 - c. Prepare calibration graphs for capsaicin and dihydrocapsaicin (peak area or height vs. μ g of analyte).
10. Determine recoveries (R) at least once for each lot of glass fiber filters in the calibration range (step 9).

Prepare three filters at each of five concentration levels plus three media blanks.

NOTE: Use separate filters for each analyte unless chromatographic conditions have been modified to permit baseline separation at concentrations >0.2 µg/mL (see APPLICABILITY and INTERFERENCES, Page 5041-1).

- a. Place 13-mm glass fiber filters into 4-mL vials.
 - b. With a microliter syringe, fortify each filter with recovery solution.
 - c. Allow the uncapped vials to stand overnight at room temperature.
 - d. Prepare and analyze with working standards (steps 5 through 8, and steps 12 and 13).
 - e. Prepare graph of R vs. µg of analyte recovered.
11. Analyze three quality control blind spikes and three analyst spikes for each analyte to ensure that the respective calibration graphs are in control.

MEASUREMENT:

12. Set high performance liquid chromatograph according to manufacturer's recommendations and to conditions given on page 5041-1. Inject 25-µL sample aliquot manually or with autosampler.

NOTE: If peak area is above the range of the working standards, dilute with acetonitrile, reanalyze, and apply appropriate dilution factor in calculations.

13. Measure peak area or height for each analyte.

CALCULATIONS:

14. Determine the mass, µg (corrected for R), of each analyte found on the filter (W) and the average media blank (B).

15. Calculate the concentration, C, of each analyte in the air volume sampled (L):

$$C = \frac{W - B}{V}, \text{ mg/m}^3$$

EVALUATION OF METHOD:

Average recoveries of capsaicin after fortification of 13-mm glass fiber filters with 0.13-, 0.28-, 0.58-, 1.1-, 2.9-, and 17-µg quantities of the compound were 1.02, 0.95, 0.98, 0.99, 1.04, and 1.00, respectively; precision (\bar{S}) was 0.042 (35 samples, pooled). After 28 days storage at 5 °C, the average recovery of 0.99-µg quantities of capsaicin from glass fiber filters was 0.96; S_r was 0.023 (6 samples). In addition, the average recovery of 0.99-µg quantities of capsaicin from glass fiber filters after 28 days storage at room temperature was 0.92; S_r was 0.052 (6 samples). These data for stored samples suggest that recovery and precision of measurement are improved when samples are stored at the lower temperature. Empty glass vials were fortified with 0.90-µg quantities of capsaicin and stored uncapped for three days at room temperature. The average recovery from the vials was 0.98; thus, the vapor pressure of capsaicin at room temperature is insignificant.

A standard solution of capsaicin in acetonitrile at a concentration of 0.5 µg/mL was found to be stable during 9 weeks storage at 5 °C. The container was sealed tightly to prevent evaporation of solvent during refrigeration.

Average recoveries of dihydrocapsaicin after fortification of 13-mm glass fiber filters with 0.11-, 0.28-, 1.1-, and 3.0-µg quantities of the compound were 0.94, 1.03, 0.99, and 0.93, respectively; precision (\bar{S}_r) was 0.065 (23 samples, pooled). After 26 days storage at 5 °C, the average recovery from glass fiber filters fortified with 0.88 µg of dihydrocapsaicin was 0.88; S_r was 0.047 (6 samples).

This method was not evaluated with controlled atmospheres in a laboratory. However, the method was employed for measurement of capsaicin and dihydrocapsaicin in air at a pickle pepper processing plant [1,11]. A curious phenomenon was the fact that in each of many of the samples the ratio of capsaicin to dihydrocapsaicin was less than 1:1. Generally, capsaicin is the capsaicinoid that occurs in *Capsicum* fruit in the greatest abundance [3].

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METHOD WRITTEN BY:

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