

AOAC Official Method 995.03
Capsaicinoids in Capsicums and Their Extractives
Liquid Chromatographic Method
First Action 1995

[Applicable for determination of 750-650000 Scoville Heat Units (SHU) of capsaicinoids in ground and crushed red pepper, chili pepper, ground cayenne pepper, ground jalapeno pepper, and red pepper oleoresins. Not applicable to chili powders or products containing oregano or thyme.]

(Caution: See Appendix B, safety notes for the safe handling of organic solvents and special chemical hazards-acetone, acetonitrile, and ethanol. See Material Safety Data Sheets, or equivalent, for each reagent. N-Vanillyl-nonanamide is an extreme irritant; do not inhale. Dispose of waste solvents in an appropriate manner compatible with applicable environmental rules and regulations.)

Method Performance:

See Table 995.03 for method performance data.

A. Principle

Test sample is extracted in warm ethanol using reflux condenser. Extract is filtered and injected into liquid chromatograph equipped with UV or fluorescence detector.

B. Apparatus (a) Liquid chromatograph (LC).--Equipped with I V integrator, 20 gL sample injector, and with UV detector set at 280 nm wavelength or fluorometer with excitation 280 nm and emission 325 nm. Operating conditions: temperature, ambient (20-25°C); flow rate, 1.5 mL/min., isocratic; relative retention times: N-vanillyl-n-nonanamide, 1.00; nordihydrocapsaicin, 0.90; capsaicin, 1.00; dihydrocapsaicin, 1.58. See Figure 995.03 for baseline separation of major capsaicinoids.

(b) LC column.--Stainless, C18, 150 x 4.6 mm id, packed with 5 micrometer particle size. Use guard column, if desired.

(c) Reflux condenser.

(d) Syringe filter.- 0.45 micrometer.

(e) C18 solid-phase extraction cartridge.

C. Reagents

(a) Ethanol-95% or denatured, suitable for chromatography

(b) Acetone.-ACS grade.

(c) LC mobile phase@Acetonitrile-water. Use LC grade solvents, or equivalent. Mix

400 mL acetonitrile with 600 mL H₂O containing 1% acetic acid (v/v). De-gas with helium or by other suitable technique.

(d) N-Vanillyl-n-nonanamide standard solutions. -N-Vanillyln-nonanamide standard, 99%, is available as synthetic capsaicin from Penta International Corp., 50 Okner Pkwy, Livingston, NJ 07039. Keep solutions out of direct sunlight. (1) *Standard solution A.*-0.15 mg/mL. Accurately weigh 75 mg N-vanillyl-n-nonanamide and transfer it into 500 mL volumetric flask. Dilute to volume with ethanol, and mix. Use standard solution A for analyzing all peppers except chili pepper. (2) *Standard solution B.*-0.015 mg/mL. Transfer 10 mL standard solution A into 100 mL volumetric flask, dilute to volume with ethanol, and mix. Use standard solution B when analyzing chili peppers.

D. Extraction

(a) Ground or crushed peppers.-Accurately weigh ca 25 g pepper into 500 mL boiling flask. Place 200 mL ethanol into same flask, add several glass beads, and attach flask to reflux condenser. Gently reflux test sample 5 h and then allow to cool. Filter 1-4 mL sample through 0.45 µm syringe filter into small glass vial. Use for LC analysis.

(b) Red pepper oleoresins.-Accurately weigh 1-2 g oleoresin into 50 mL volumetric flask. Increase weight of sample, if total capsaicinoid concentration is < 1 %.

Note: Do not allow any oleoresin to coat sides of flask.

Add 5 mL acetone, C(b), to flask and swirl contents of flask until test sample is completely dispersed (no oleoresin can coat bottom of flask when turning flask neck at 45° angle). Add five 3-5 mL portions ethanol, swirling flask during each addition. Dilute contents of flask to volume with ethanol and mix well.

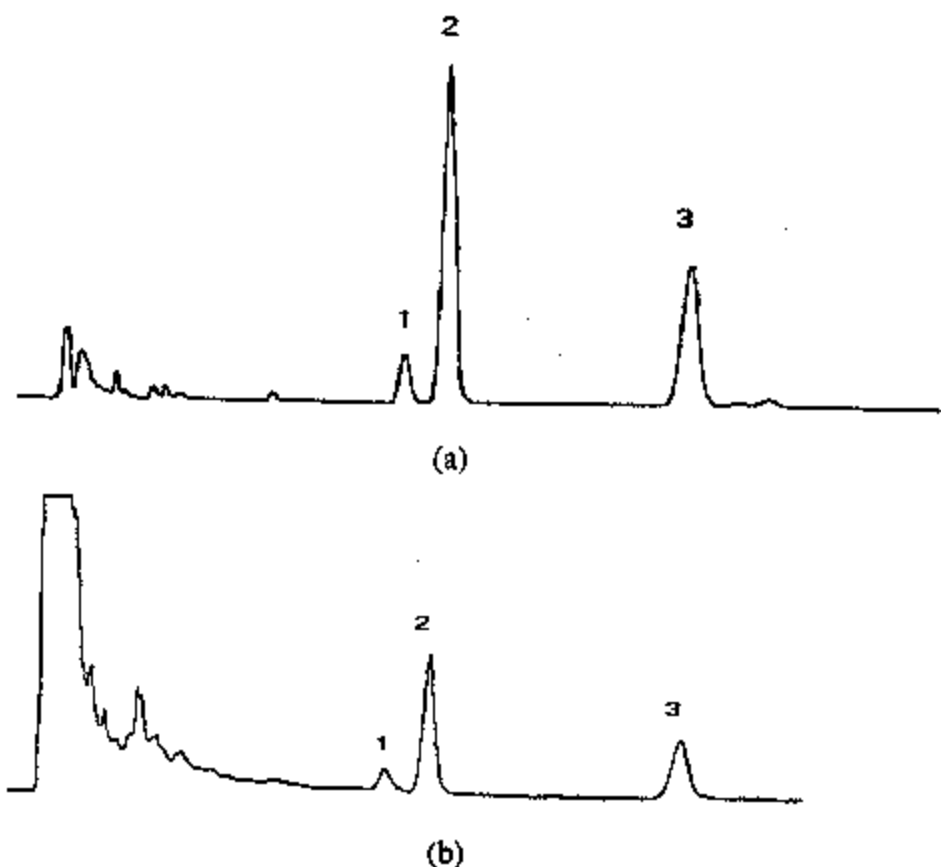


Figure 995.03-Red pepper extract analyzed by (a) fluorescence detection, and (b) UV detection.
Peak 1 = nordihydrocapsaicin; peak 2 = capsaicin; peak 3 = dihydrocapsaicin

Hold C18 solid-phase extraction cartridge over 25 mL volumetric flask or place cartridge on 10 mL glass syringe and hold over 25 mL volumetric flask. Transfer 5 mL solution from flask to cartridge or syringe. (Note: When using syringe, deliver solution to bottom of syringe so that sides of syringe are not coated with sample.) Pass aliquot through cartridge and collect in 25 mL flask. Wash cartridge 3 times with 5 mL ethanol, collecting washings in same flask. Dilute contents of flask to volume with ethanol and mix. Filter 1-4 mL solution through 0.45 micrometer syringe filter into small glass vial. Use for LC analysis.

E. LC Determination

Inject 20 microliters standard solution B, C(d)(2), onto LC column, when analyzing chili peppers. When analyzing other matrices inject 20 microliters standard solution A, C(d)(1). Re-inject standard solution at intervals of 6 sample injections, or less.

Inject in duplicate 20 microliter test sample from D onto LC column.

After <30 sample injections, purge LC column 30 min with 100% acetonitrile at 1.5 mL/min flow rate. Use LC mobile phase, C(c), for further analysis.

F. Calculation

Capsaicinoids contain 3 major compounds: nordihydrocapsaicin (N), capsaicin (C), and dihydrocapsaicin (D). Calculate capsaicinoids as sum of these compounds [N + C + D; in Scoville Heat Units (SHU); 1 microgram total capsaicinoids/g = ca 15 SHU], as follows: (a) *UV detection*

(1) Ground peppers and chili pepper.-

$$N = (P_n/P_s) \times (C_s/W_t) \times (200/0.98) \times 9300$$

$$C = (P_c/P_s) \times (C_s/W_t) \times (200/0.89) \times 16100$$

$$D = (P_d/P_s) \times (C_s/W_t) \times (200/0.93) \times 16100$$

where P_n, P_c, and P_d = average peak areas for nordihydrocapsaicin, capsaicin, and dihydrocapsaicin, respectively, from duplicate injections; P_s = average peak area of appropriate standard solution; C_s = concentration of standard solution, mg/mL; W_t = weight of test sample, g

(2) Red pepper oleoresins:

$$N = (P_n/P_s) \times (C_s/W_t) \times (250/0.98) \times 9300$$

$$C = (P_c/P_s) \times (C_s/W_t) \times (250/0.89) \times 16100$$

$$D = (P_d/P_s) \times (C_s/W_t) \times (250/0.93) \times 16100$$

(b) Fluorescence detection (1) Ground peppers and chili pepper.

$$N = (P_n/P_s) \times (C_s/W_t) \times (200/0.92) \times 9300$$

$$C = (P_c/P_s) \times (C_s/W_t) \times (200/0.88) \times 16100$$

$$D = (P_d/P_s) \times (C_s/W_t) \times (200/0.93) \times 16100$$

(2) Red pepper oleoresins:

$$N = (P_n/P_s) \times (C_s/W_t) \times (250/0.92) \times 9300$$

$$C = (P_c/P_s) \times (C_s/W_t) \times (250/0.88) \times 16100$$

$$D = (P_d/P_s) \times (C_s/W_t) \times (250/0.93) \times 16100$$

Reference: J. AOAC Int. (future issue).

(c) 1996 AOAC INTERNATIONAL