Method 21.3 (2026)

Pungency of Capsicums and Their Oleoresins (HPLC Method - Preferred)

Purpose: To determine pungency levels in crushed red pepper, chili pepper, jalapeno pepper, and red pepper oleoresins.

A. Apparatus:

1. Liquid chromatograph equipped with:
   a. integrator.
   b. 20 µL sample loop injection valve.
   c. fluorescence detector and/or ultraviolet detector.

2. Chromatographic column stainless steel, 150 x 4.6 mm id packed with 5µm LC-18 (available from Supelco, Inc., Bellefonte, PA) or equiv. Use guard column if desired.

3. Balance, readable to 0.01 g.

4. Pipets Volumetric - various sizes, Class A.

5. Glass beads.

6. Water cooled condenser, with 24/40 S/T joint.

7. Syringes, disposable 5 and 10 mL with Luer Lock tips.

8. Syringe Filters, 0.45 µm; nylon.

9. Volumetric Flasks, various sizes, Class A.

10. Round bottom flask, 500 mL size with 24/40 S/T joint.

B. Reagents:

1. Ethyl Alcohol (EtOH), 95% or denatured.

2. Acetone, ACS grade.

3. Mobile phase. Use LC grade solvents, or equivalent:
   a. HPLC grade water, with 0.1% acetic acid (v/v).
   b. Acetonitrile.
   c. Mix 400 mL of Acetonitrile with 600 mL of HPLC grade water with 0.1% acetic acid.
   d. De-gas mobile phase.

4. Standard: N-vanillyl-n-nonanamide, 99 + % (CAS Registry Number 2444-46-4) (available from Penta International Corporation, P.O. Box 1452, West Caldwell, N.J. (201) 740-2300) Code 03-08700 ****CAUTION-----EXTREME IRRITANT-----HANDLE WITH EXTREME CARE-------DO NOT INHALE****.

5. C-18 Sep-pak cartridge, 6 mL capacity (Millipore Inc.), or equivalent.

C. Preparation of Sample:

1. Ground or crushed peppers: Weigh accurately about 25 g pepper into a 500 mL boiling flask. Pipet 200 mL EtOH into the flask and add several glass beads to aid boiling. Reflux gently for 5 hours. Allow to cool. Filter 3-4 mL through a 0.45 µm syringe filter into glass vial. (Note 1)

2. Oleoresins: Accurately weigh 1 to 2 g oleoresin (increase sample size if total capsaicinoid concentration is below 1%) into 50 mL volumetric flask, being sure not to allow any oleoresin to coat the sides of the flask. Add 5 mL of acetone to flask and swirl acetone until sample is completely dispersed as evidenced by observing no oleoresin coating bottom of flask with neck at 45° angle. Slowly add EtOH, mixing completely by swirling with acetone while adding. Continue adding and mixing until solution becomes cloudy. Dilute to volume and mix well. Place C-18 SEP-PAK on a 10 mL syringe and hold over a 25 mL volume flask. Pipet 5 mL aliquot of sample mixture to syringe. Deliver aliquot to bottom of syringe so that sides of syringe are not coated with sample. Pass aliquot through SEP-PAK and collect in flask. Rinse SEP-PAK through the syringe with three 5 mL portions of EtOH, again collecting washings in flask. Dilute to volume with EtOH and mix. Filter through 0.45 µm syringe filter into a glass vial. (Note 2)

D. Procedure:

1. Prepare all standard solutions with EtOH. Keep solutions out of direct sunlight.
   a. Standard solution A-0.15 mg/mL. Accurately weigh and transfer 75 mg of standard into 500 mL volume flask, dissolve, dilute to volume, and mix.
   b. Standard solution B-0.015 mg/mL. Pipet 10 mL standard solution A into 100 mL volume flask, dilute to volume, and mix. (Use with chili peppers)
   c. Dilute standard solution C-0.00075 mg/mL. Pipet 5 mL of working standard solution B into 100 mL volumetric flask, dilute to volume, and mix. (Use with samples, which contain capsicum heat levels below 5000.)

2. Chromatographic Conditions:
   a. Mobile Phase: 40% Acetonitrile and 60% HPLC grade water with 1% Acetic Acid (v/v).
   b. Flow Rate: 1.5 mL/minute, isocratic.
   c. Column: LC-18 150 x 4.6 mm i.d., 5 µm particle size.

d. Injection Volume: 20 µL.
e. Detection - Excitation 280 nm, Emission 325 nm for fluorescence or 280 nm for ultraviolet. (Note 3)
Using a sample loop injection valve, inject, in duplicate, 20 µL of the prepared sample solution onto the column. Inject the appropriate standard solution before first sample injection and after no more than 6 sample injections.

3. Purge the column with 100% acetonitrile for 30 min at 1.5 mL/min after no more than 30 sample injections. Return to previous mobile phase for further determinations.

E. Calculations:

1. Scoville Heat Units (SHU) are the sum of SHU of the three major capsaicinoids. Calculate SHU as follows:
   a) Nordihydrocapsaicin, $SHU_N = (N/A) \times (Cs/C_X) \times (H_N/R_N)$.
   b) Capsaicin, $SHU_C = (C/A) \times (Cs/C_X) \times (H_C/R_C)$.
   c) Dihydrocapsaicin, $SHU_D = (D/A) \times (Cs/C_X) \times (H_D/R_D)$.
   d) Total $SHU_T = SHU_N + SHU_C + SHU_D$.

   Where: A = average peak area of standard;
   N, C, and D = average peak areas for respective capsaicinoids (nordihydrocapsaicin, capsaicin and dihydrocapsaicin) from duplicate injections;
   Cs = concentration of std in mg/mL;
   C_x = concentration of sample in extract expressed as, mg of sample/mL;
   H_N, H_C, and H_D = heat factors for respective capsaicinoids;
   R_N, R_c, and R_D = response factors of respective capsaicinoids relative to standard.

2. Accepted heat factors and response factors:

		UV	FLU
Nordihydrocapsaicin (N)	- $H_N = 9.3 \times 10^6$	$R_N = 0.98$	0.92
Capsaicin (C)	- $H_C = 16.0 \times 10^6$	$R_C = 0.98$	0.96
Dihydrocapsaicin (D)	- $H_D = 16.0 \times 10^6$	$R_D = 0.93$	0.93
N-vanillyl-n-nonanamide		R = 1.00	1.00

3. Relative retention times:

Nordihydrocapsaicin	0.90
N-vanillyl-n-nonanamide	1.00
Capsaicin	1.00
Dihydrocapsaicin	1.58

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F. Statistics:

Reproducibility relative standard deviation (RSD_R)

	SHU's	%
Chili Peppers	900-2750	9.6-11.6
Red Peppers	30140-41600	4.5-11.6
Oleoresins	305690-644060	7.6-14.8

G. Note:

1.) If directions are followed, there is little to no loss of ETOH.
2.) For oleoresins with more than 5% total capsaicinoids, it is recommended to pre-heat the sample at 100°C for at least 1 hr.
3.) Adjust the detector sensitivity or gain so that eluting peaks are not overloading detector.

H. Reference:

Official Methods of AOAC International (1996) 995.03 (43.1.43).
J. AOAC International (1996) 79 (3), 738-745.
J. Agricultural Food Chemistry (1983) 31, 1326.

I. Appendix: Alternative Rapid Extraction method B, Block Digestion

1. Apparatus:
    a. Lab-Line Multiblock heater, model 2056, 120V, or equivalent
    b. Lab-Line multitube module for 6 tubes, 25 mm
    c. Corning Culture Tube SCP, 25 x 150 mm and screw cap with seal

2. Reagent:
    a. Ethanol, 95% reagent or equivalent

3. Preparation of Sample:
    a. This method is only applicable for ground product and samples.

4. Procedure:
    a. Preheat the block to 75° C ± 5° C.
    b. Accurately weigh 2.0 ± 0.5 g of sample into the tube.
    c. Pipet 20.0 mL of ethanol into the culture tube and seal tightly with cap.
    d. Vortex tube to mix contents.
    e. Place the tube into the heating block and allow the sample to extract for 1 hour after the solution begins to boil.
    f. After extraction, remove tube(s) from the block heater, cool tubes to room temperature. **WARNING: DO NOT LOOSEN CAPS AT ELEVATED TEMPERATURE.**
    g. Filter sample extract through 0.45µm Nylon filter prior to HPLC analysis.

J. Appendix: Alternative Extraction method C, Ethanol saturated with sodium acetate

1. Apparatus:
    a. Erlenmeyer flask, 125 mL size with standard taper joint
    b. Stopper to fit flask
    c. Clamp or fixture to hold stopper
    d. Clamp to hold flask

2. Reagent:
    a. 95% ethanol saturated with sodium acetate (ca. 5 g/100 mL at room temperature, a few crystals should remain)

3. Preparation of sample:
    a. This method is only applicable for ground product and samples

4. Procedure:
    a. Weight 10.0 ± 0.2 g of sample into the flask.
    b. Add 100.0 mL of sodium acetate saturated 95% ethanol solution by pipet to the flask and stopper.
    c. Swirl initially to mix contents, then occasionally during the extraction.
    d. Place in water bath held at 60° C for three hours.
    e. Remove from water bath and cool to room temperature.
    f. Filter sample extract through 0.45µm Nylon filter prior to HPLC analysis.

K. Conversion of SHU from ASTA Method 21.3 (2026) to ASTA Method 21.3 (2004)

The ASTA Method 21.3 (2004) SHU can be estimated from the SHU as determined from ASTA Method 21.3 (2026) using the following equation:

$$SHU_{\text{ASTA Method 21.3 (2004)}} = SHU_{\text{ASTA Method 21.3 (2026)}} / 0.9431$$

L. Revision History

03/01/26 ASTA method 21.3 (2026) is a modification from ASTA method 21.3 (2004) which updates the response factor for Capsaicin. The updated response factors are based on the averages of five different laboratories.

04/23/26 Corrected equation in section K.