

# Hot Chili Peppers: Extraction, Cleanup, and Measurement of Capsaicin

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## Objectives

Capsaicin, the pungent ingredient of the red pepper or *Capsicum annuum*, is widely used in food preparation. More novel uses of capsaicin have been as a herpes treatment, a tear gas, and a deterrent to dog fights and swine cannibalism (1). In humans, the limit for taste is approximately 10 ppm, which is 70 times lower than that for piperine, the active spice in black pepper, and 1000 times lower than for zingerone, the active component in ginger (1).

The purpose of this experiment is to acquaint students with the active ingredients of hot chili pepper (capsaicin and dihydrocapsaicin) and the extraction, cleanup, and analysis of these chemicals (2) as a fun and informative analytical exercise. Our experiment is similar to those described by Betts (3) and by Batchelor and Jones (4), who analyzed hot pepper sauces. In our experiment students are directed to find the hottest pepper they can, testing the degree of “hotness” in the lab using HPLC–UV. Particular attention is paid to optimizing extraction of the peppers and assuring clearly resolved peaks for the target analytes. This experiment serves as a “contest lab”: the group with the hottest pepper as measured by capsaicinoid concentration wins a free lunch at a restaurant featuring hot food.

## Materials and Methods

### Sampling

Students were directed to obtain a pepper they thought likely to be “hot” and note the species and variety.

**Table 1. Data for Some Varieties of Hot Pepper**

Variety	Capsaicinoid Concentration/(mg/g) <sup>a</sup>			Scoville Heat Units <sup>b</sup>
	Capsaicin	Dihydro-capsaicin	Total	
Habanero	2.10	0.46	2.57	276,000
Jamaican	1.70	0.61	2.31	248,000
Habanero	1.26	0.47	1.73	186,000
Scotch Bonnets	1.22	0.35	1.57	170,000
Jamaican	0.99	0.55	1.54	165,000
Ring of Fire Cayenne	0.98	0.40	1.38	148,000
Jamaican Belle	0.43	0.32	0.75	80,000
Puca-Uchu Tarija	0.41	0.31	0.72	77,000
Scotch Bonnet	0.36	0.19	0.55	59,000
Jalapeno	0.25	0.13	0.38	41,000

<sup>a</sup>Wet weight basis.

<sup>b</sup>From calculated dry weight comparison based on a value of 16.1 for the Scoville heat value (SHU) for capsaicin/dihydrocapsaicin (4) and an 85% water content value for the fresh peppers used in this experiment.

### Extraction

Fresh pepper (10.0 g) was weighed, finely chopped, and ground for 10 minutes using a mortar and pestle. Then 30 mL of acetonitrile was added as an extraction solvent and the pepper was ground for a further 20 minutes. Insoluble plant material was removed by vacuum filtration. The final solvent extract was accurately measured and 1 mL was placed in a volumetric flask, diluted to the mark (typically 10 mL), and mixed well.

**CAUTION:** The extract is very noxious. See Hazards section.

### Cleanup

A C-18 (500 mg) solid-phase extraction (SPE) cartridge was conditioned with sequential treatments of acetonitrile, methanol, and water (2 column volumes each). Ten milliliters of the extract was then added to the column. Elution of the capsaicinoids from the SPE cartridge was accomplished with 4 mL of methanol followed by 1 mL of methanol containing 1% acetic acid. This was an optimized version of the method of Attuquayefio and Buckle (2).

### Analysis

The extract was analyzed by C-18 reverse-phase HPLC (PE 200 Series pump) with UV detection at 281 nm (PE 765A variable wavelength detector or PE 235C diode array). The mobile phase consisted of 77% methanol and 23% H<sub>2</sub>O. Quantitation was by external standardization with authentic standards (Aldrich).

### Hazards

The capsaicin extract is very toxic and will cause contact burns and severe irritation on exposed skin (5). All extraction steps should be carried out in a hood and gloves should be worn at all times. After the lab, students should carefully wash hands and any other exposed areas. Caution is particularly warranted when working with neat standards.

### Results and Discussion

Habanero and Jamaican peppers had the highest amount of capsaicin and are generally regarded as the “hottest” peppers available. The amounts of capsaicin and dihydrocapsaicin obtained using the extraction and cleanup method in this experiment were similar to literature values for these species of peppers (6). In comparison to the processed hot sauces (3, 4), raw peppers appear to be generally hotter as represented by the Scoville heat unit (SHU) (Table 1). This experiment demonstrated the need for thorough extraction and cleanup techniques for highly colored co-extractives in food materials. Students particularly enjoyed relating concentrations of capsaicinoids to their perceived valuation of “hotness”.

### Acknowledgment

This laboratory experiment was developed and carried out in the ANALEST research and training facility realized through the kind support of Perkin Elmer Canada, Analytical Instruments Division.

### <sup>W</sup>Supplemental Material

The student handout and instructions for the teaching assistant or laboratory instructor are available in this issue of *JCE Online*.

### Literature Cited

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