

Capsaicinoids

Capsaicin, (trans 8-methyl-N-vanillyl-6-nonenamide), is a major pungent lipophilic alkaloid of *Capsicum* fruits (e.g., chili pepper and paprika). Capsaicin is used as a food additive in various spicy cuisines. The hotness of a pepper depends upon the amount of capsaicin (and related capsaicinoids – Figure 1) it contains. Capsaicin and dihydrocapsaicin comprise 80-90% of the total capsaicinoids found in peppers (typically 0.01-1% by weight) located mainly within the white ribs (palcenta) and seeds of the fruit. Capsaicin is seventy times hotter than piperine (black pepper) and 1000 times hotter than zingerone (ginger). The heat level of a pepper is measured in Scoville units – named after Wilbur Scoville who developed his subjective organoleptic (dilution taste) test in 1912 while working at the Parke Davis pharmaceutical company. Interestingly, a typical bell pepper is rated at 0-100 Scoville units, the habanero pepper is ~300,000 while pure capsaicin is rated at 16,000,000 Scoville units. Capsaicin is also used for therapeutic purposes to treat a number of peripheral painful conditions including rheumatoid arthritis and diabetic neuropathy (Fusco and Giacobazzo (1997)). Dermatological ointments used to treat itchy skin, psoriasis, shingles and muscle pain contain 0.025% capsaicin.

How capsaicin produces its biological effects is very interesting. C-fiber sensory afferent (nociceptive) neurons, which contain substance P, mediate a wide variety of physiological responses including chemogenic pain, thermoregulation, and neurogenic inflammation. Initial exposure to capsaicin intensely activates these C-fiber neurons causing the release of inflammatory mediators resulting in pain, burning, perspiration, rhinitis, lacrimation, gastrointestinal and dermatological irritation. Higher doses and prolonged exposure, however, actually causes desensitization of these neurons (the reason why many people become accustomed to spicy food) (Biro *et al.*, (1997)). Desensitization accounts for the selective analgesic effects of capsaicin and is the basis for its therapeutic application in the treatment of the diseases mentioned above.

The mechanism of action of capsaicin is complex. Capsaicin and many other vanilloids (e.g., its ultrapotent diterpene analog resiniferatoxin obtained from *Euphorbia* plants) are agonists of the vanilloid receptor(s) (VR1 etc.) located within the neuronal membrane (Sterner and Szallasi (1999); Szallasi and Blumberg (1996)). Stimulation of VR1 causes the entry of

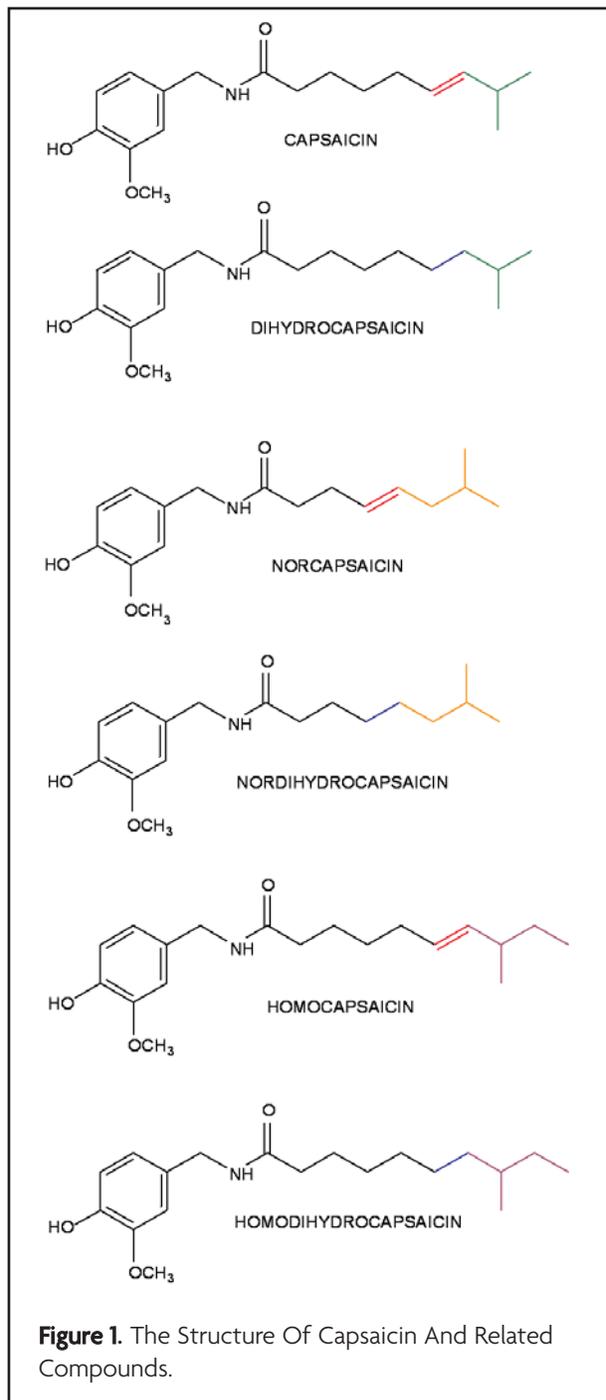


Figure 1. The Structure Of Capsaicin And Related Compounds.

calcium into the neuron, release of neurotransmitter and the activation of secondary cascades. Excessive entry of calcium into the neuron, however, can lead to neurodegeneration. The cytotoxic effect of exposure to high concentrations of capsaicin is still under investigation (Surh and Lee (1995, 1996)).

Capsaicin has been previously measured using HPLC-UV, CZE-UV, GC-MS following HPLC purification, and HPLC with amperometric electrochemical detection (Iwai *et al.*, (1976, 1979); Kawada *et al.*, (1985); Laskaridou-Monnerville (1999); Weaver and Awde (1986)). Presented here is a routine, stable, selective and highly sensitive HPLC-coulometric electrochemical array assay capable of accurately measuring capsaicin and its related metabolites (see Acworth and Gamache (1996); Acworth *et al.*, (1997) for more information). The ability to generate a “metabolic fingerprint” of the sample and its use in assessing product stability, product profiling, possible contamination, and authenticity is also discussed.

Materials and Methods

The isocratic analytical system consisted of a pump, autosampler, thermostatic chamber, a four channel CoulArray® detector and an UV/vis detector placed before the array.

LC Conditions:

Column: MD-150 (3 x 150mm; 3µm).
 Mobile Phase: 50mM Ammonium Acetate, pH 4.4 with acetic acid; 45% Acetonitrile.
 Flow Rate: 0.8 mL/min.
 Temperature: Ambient
 Injection Volume: 20 µL.

Detectors and Conditions:

Electrochemical Detector: Model 5600A, CoulArray with Model 5010 Analytical Cell.
 Applied Potentials: +150, +450mV vs. Pd.
 UV Detector: Standard UV detector
 Wavelength: 235 and 280nm

Results and Discussion

The separation of capsaicin, dihydrocapsaicin and nordihydrocapsaicin was completed within 15 minutes and was free from contamination (Figure 2). Analysis of an ASTA (American Spice Trade Association) sample showed that electrochemical detection was ~35 times more sensitive than UV detection (Figure 3). Analysis by electrochemical detection was linear up to 100ppm but this could be extended well beyond 1000ppm by use of the UV detector.

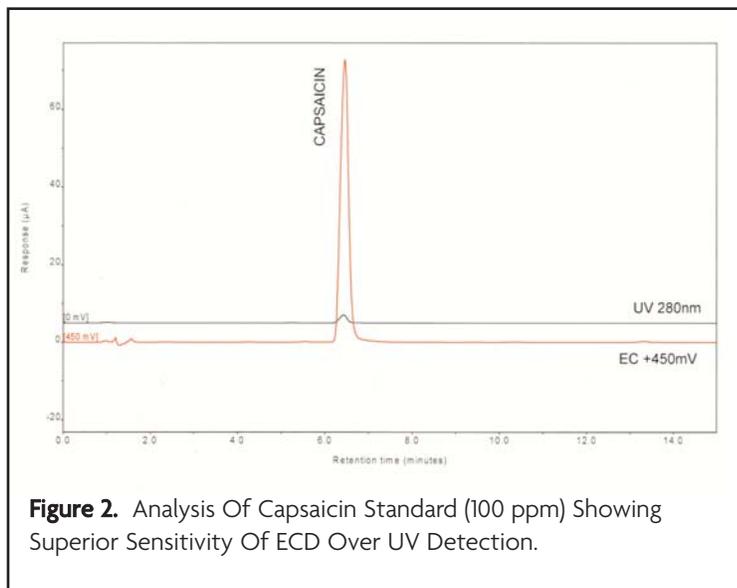


Figure 2. Analysis Of Capsaicin Standard (100 ppm) Showing Superior Sensitivity Of ECD Over UV Detection.

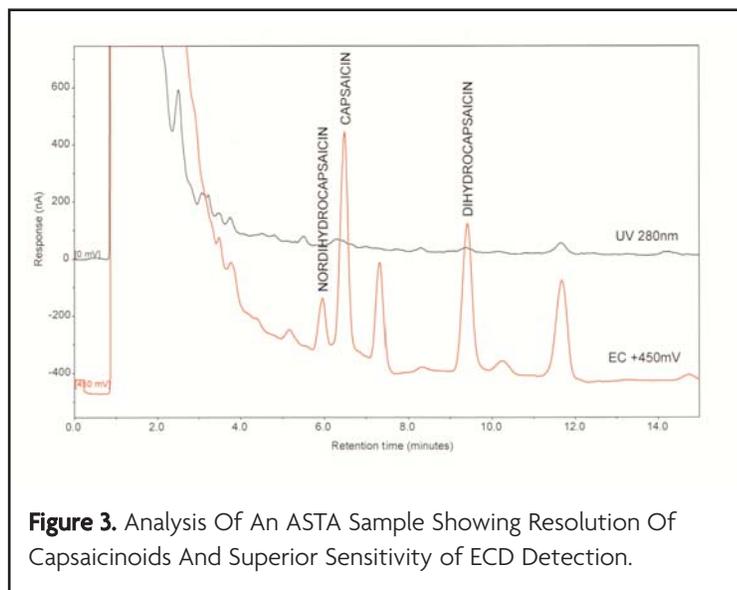


Figure 3. Analysis Of An ASTA Sample Showing Resolution Of Capsaicinoids And Superior Sensitivity of ECD Detection.

In a separate study, the pattern of chili pepper metabolites (both known and unknown) was measured using gradient HPLC coupled to an array of sixteen coulometric sensors. Figure 4A shows a chromatogram of a supercritical fluid extract while Figure 4B shows a chromatogram of the residue. There is an incredible amount of useful information contained within the pattern of metabolites. This can be used to measure product shelf life, adulteration and material source (Gamache *et al.*, (1995)), contamination (Acworth and Gamache (1997)), formulation of blends, analysis of competitive products and content of natural products.

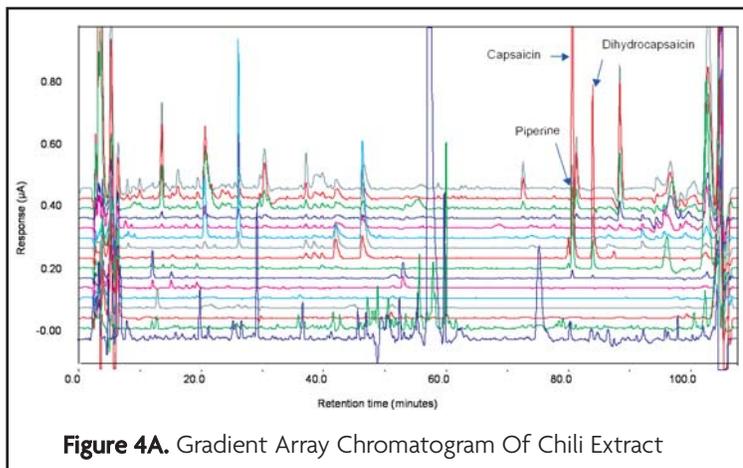


Figure 4A. Gradient Array Chromatogram Of Chili Extract

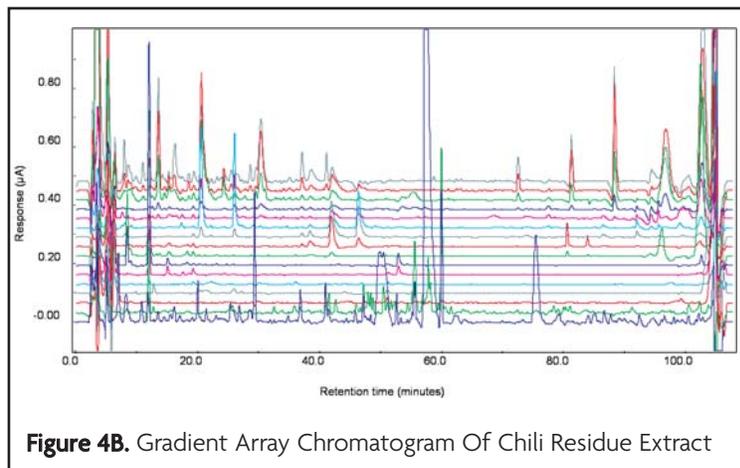


Figure 4B. Gradient Array Chromatogram Of Chili Residue Extract

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Ordering Information

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