Determination of Capsaicinoids in Chili Pepper Using HPLC-ECD

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Key Words
Capsaicinoids, HPLC-ECD, Lipophilic Alkaloids

Goal
To develop a fast and sensitive HPLC-electrochemical detection (ECD) method for separating capsaicinoids

Introduction
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.

Figure 1. The structure of capsaicin and related compounds.
Capsaicin is also used for therapeutic purposes to treat a number of peripheral painful conditions including rheumatoid arthritis and diabetic neuropathy. 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). 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. Stimulation of VR1 causes the entry of 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.

Capsaicin has been previously measured using HPLC-UV, CZE-UV, GC-MS following HPLC purification, and HPLC with amperometric electrochemical detection. Presented here is a routine, stable, selective and highly sensitive HPLC-coulometric electrochemical array assay capable of accurately measuring capsaicin and its related metabolites. 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 Thermo Scientific™ Dionex™ CoulArray™ Coulometric Array Detector and an UV/vis detector placed before the array.

**LC Conditions**

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<td>Column</td>
<td>C18, 3 × 150 mm, 3 µm</td>
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<tr>
<td>Mobile Phase</td>
<td>50 mM Ammonium Acetate, pH 4.4 with acetic acid; 45% Acetonitrile</td>
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<tr>
<td>Flow Rate</td>
<td>0.8 mL/min</td>
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<tr>
<td>Temperature</td>
<td>Ambient</td>
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<tr>
<td>Injection Volume</td>
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Results and Discussion

Targeted: The separation of capsaicin, dihydrocapsaicin and nordihydrocapsaicin was completed within 15 min 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 100 ppm but this could be extended well beyond 1000 ppm by use of the UV detector.
Additional Information—Profiling

In a separate profiling 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, contamination, formulation of blends ESA, now part of Thermo Scientific, Natural Products Book; Part Number 70-1437), analysis of competitive products and content of natural products.

References

Ordering Information
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