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A MODULAR HPLC SYSTEM FOR ROUTINE ANALYSIS OF CAPSAICIN FROM HOT SAUCES

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INTRODUCTION

Capsaicin (Figure 1), (8-methyl-N-vanillyl-6-nonenamide) is an active component of chili peppers, which are plants belonging to the genus Capsicum. Capsaicin and several other related compounds such as dihydrocapsaicin (Figure 1) are called capsaicinoids and are produced as secondary metabolites by chili peppers. Pure capsaicin is a hydrophobic, colorless, odorless, crystalline-to-waxy compound.

Because of the burning sensation caused by capsaicin when it comes in contact with the mucous membranes found in the mouth, it is frequently used in food products to give them added spice or heat (pungency). In high concentrations, it can also cause a burning effect on sensitive areas, of skin such as the backs of fingers and hands, from handling and processing chili peppers.

Capsaicin can also now be found in topical lotions, where it acts as a pain reliever for minor aches and pains that may be associated with backaches, strains, sprains, and arthritis.

The degree of heat found within a food can be measured on the Scoville scale, however, high performance liquid chromatography (HPLC) analysis is a more accurate method of calculating heat pungency caused by capsaicin content in a food product.

This application note describes the analysis of capsaicin and dihydrocapsaicin by HPLC by using a modular chromatographic system comprised of a Waters 2707 Autosampler, 1525 Binary HPLC Pump, 2489 Dual Wavelength UV/VIS Detector and 1500 Series column oven. The resulting method possessed excellent area count reproducibility, retention time reproducibility, reliable quantitation, and low carryover performance, demonstrating the excellent performance of this modular system.

EXPERIMENTAL

Capsaicin and dihydrocapsaicin were extracted by the following procedure from six commercial hot sauces. A 10 mL aliquot of each hot sauce was transferred into a 50 mL centrifuge tube, 2 mL of dichloromethane was added, and the tube and its contents were vortexed for approximately 1 minute. Then the test tube was placed in a centrifuge for 10 minutes at 3000 RPM, following centrifugation, 1 mL of extract was withdrawn from the tubes and evaporated to dryness using nitrogen gas. The material left in the test tube was then reconstituted in 2 mL of methanol. Sample extracts were passed through a 0.45 mm syringe tip filter prior to HPLC analysis.

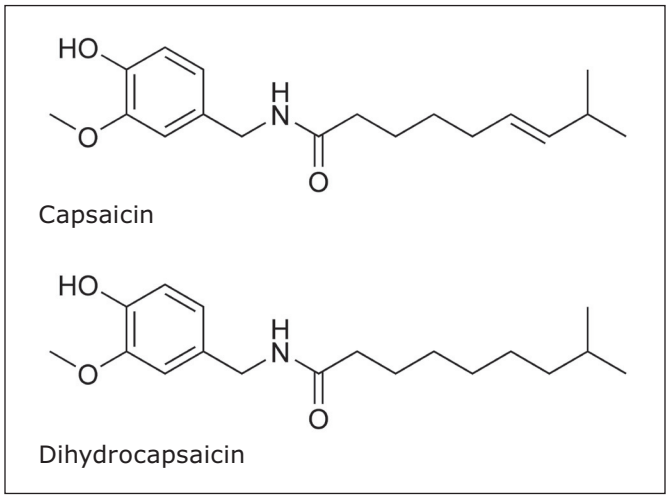

Figure 1. Chemical structures of capsaicin and dihydrocapsaicin.

HPLC Conditions

HPLC system:	Waters 1525 Binary HPLC Pump
	2707 Autosampler
Column:	Waters Atlantis® T3
	3 µm, 4.6 x 100 mm
Detector:	2489 Dual Wavelength UV/Vis
Data:	Empower™ 2 Software
Sample temp.:	Ambient
Column temp. :	40 °C
Injection volume:	25 µL
Mobile phase A:	Water
Mobile phase B:	Methanol
Flow rate:	1.50 mL/min

Linear gradient:

Time	%A	%B
	60	40.0
8.00	15.0	85.0
10.00	1.00	99.0
13.00	1.00	99.0
15.00	60.0	40.0

RESULTS AND DISCUSSION

A total of six samples were analyzed; all were commercial-grade hot sauces bought from a local supermarket: two red pepper-based, two habanero pepper-based, one jalapeno pepper-based and one cayenne pepper-based sauce.

Injections of sample extracts from each sauce were bracketed by six replicate injections of capsaicin and dihydrocapsaicin standards (Sigma-Aldrich Chemical Co.). Methanol/water blanks were injected throughout the sample set to assess carryover. Examples of the chromatograms for the two standards and a sample are found in Figures 2, 3, and 4.

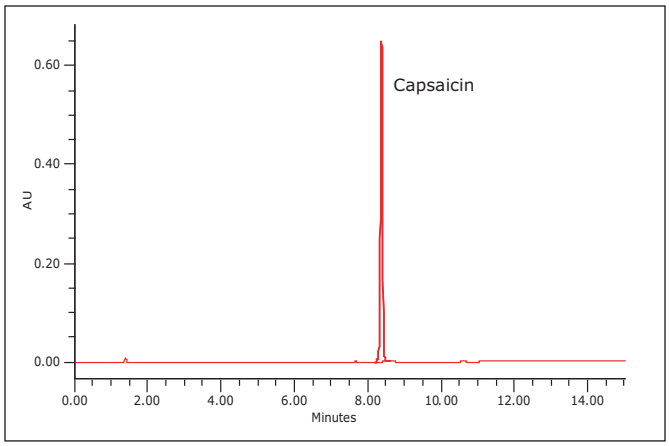

Figure 2. Capsaicin standard from Sigma.

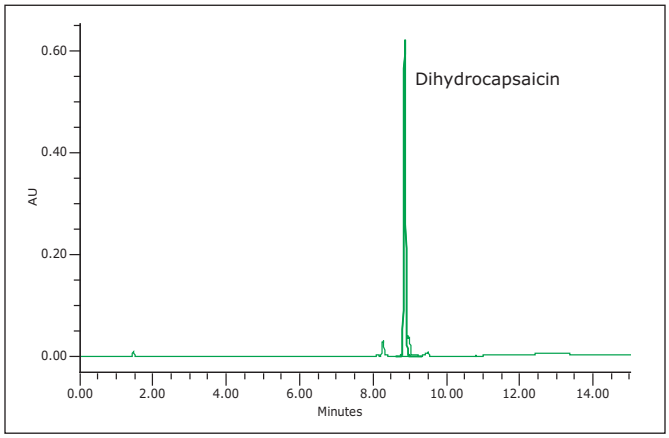

Figure 3. Dihydrocapsaicin standard from Sigma.

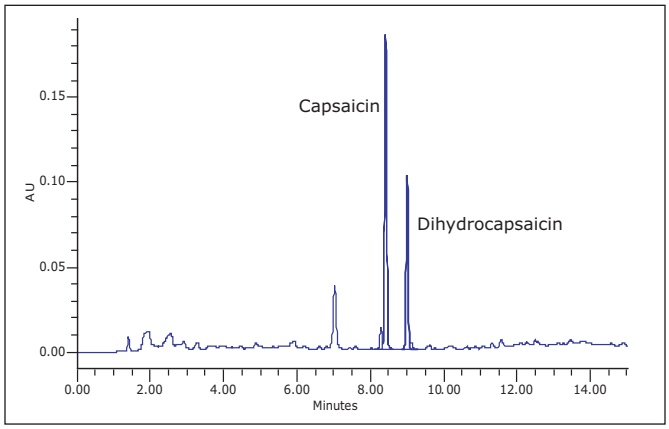

Figure 4. HPLC separation of capsaicin and dihydrocapsaicin from a red pepper-based hot sauce.

The assay reproducibility with this methodology for capsaicin and dihydrocapsaicin showed a %Relative Standard Deviation (%RSD) for area less than 0.30%, and a %RSD for retention time less than 0.10%.

It was confirmed that capsaicin and dihydrocapsaicin were present in all of the hot sauces analyzed. The amounts were calculated from a six-point linear calibration curve and are displayed in Table 1. Standard concentrations ranged from 0.028 to 0.84 mg/mL for both compounds. Excellent linearity was observed for both capsaicin (R2 = 0.999963) and dihydrocapsaicin (R2 = 0.999961), (Figures 5 and 6).

	Capsaicin content	Dihydrocapsaicin content	Calculated Scoville heat units	Scoville heat units according to manufacturer
Red pepper-based sauce	0.228 mg/mL	0.122 mg/mL	3,420	2500 to 5000
Jalapeno-based sauce	0.069 mg/mL	0.037 mg/mL	1,035	600 to 1200
Habanero-based sauce	0.479 mg/mL	0.067 mg/mL	7,185	7000 to 8000
Cayenne-based sauce	0.046 mg/mL	0.030 mg/mL	690	747
Red pepper-based sauce	0.266 mg/mL	0.039 mg/mL	3,990	3600
Habanero-based sauce	0.323 mg/mL	0.098 mg/mL	4,845	Not stated by manufacturer

Table 1. Results of HPLC analysis of capsaicin and dihydrocapsaicin.

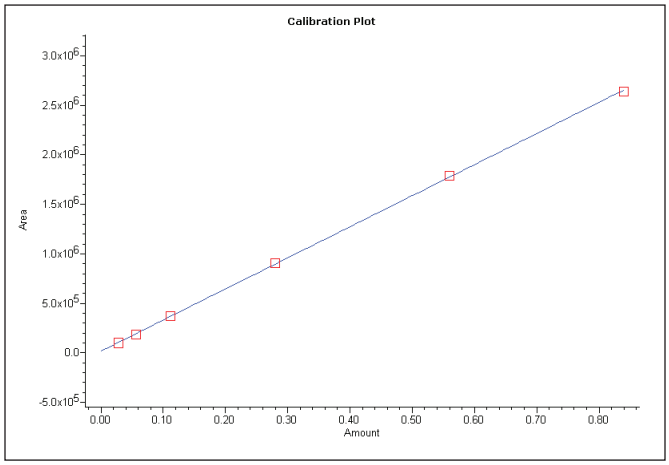

Figure 5. Capsaicin calibration curve.

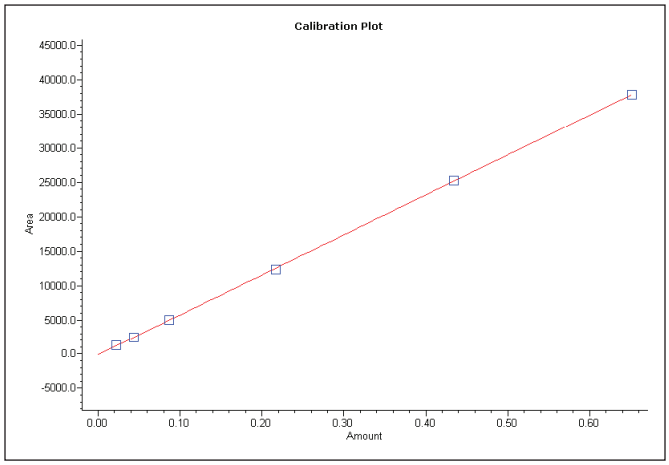

Figure 6. Dihydrocapsaicin calibration curve.

Using a wash solvent of 90:10 water/methanol, extraction solvent blanks run after the most concentrated standard showed no measurable carryover (Figure 7), proving the methodology has a low probability of showing false positives.

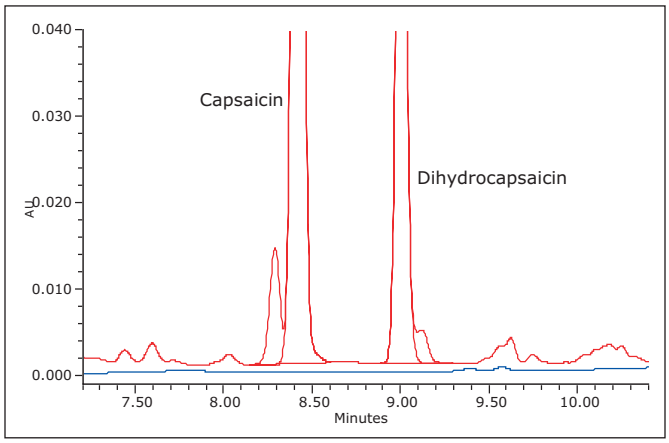

Figure 7. Water/methanol blank (blue) run after last unknown (red).

The Scoville Organoleptic Test was the first method used to determine pungency of a pepper,[1] however it is very imprecise due to the fact that it relied on human subjectivity. In Wilbur Scoville's method, a solution of the pepper extract is diluted in sugar water until its heat is no longer detectable to a panel of usually five tasters; the degree of dilution gives its measure on the Scoville scale.

For example, capsicum, sweet pepper, or a bell pepper (all containing no capsaicin) all have Scoville ratings of zero, meaning no heat is detectable even undiluted. However, when the hottest chiles – such as a habanero pepper – have a rating of 200,000 or more, this extract has to be diluted 200,000-fold before the capsaicin present is undetectable.[2]

According to Tainter and Grenis,[2] a measurement of one part capsaicin per million (1 mg/kg) is the equivalency of about 15 Scoville units. Using HPLC, this approximation gives 20 to 40% lower amounts than the Scoville method would have.

With HPLC, manufacturers can now accurately measure the amount of capsaicin in their products, whether it be a hot sauce or a topical lotion used as a pain reliever. By using the described HPLC method, a consumer product manufacturer can more accurately monitor and control the Scoville rating in each batch of product.

CONCLUSION

- Quantitation and confirmation of capsaicin and dihydrocapsaicin from commercial-grade hot sauces was demonstrated using a modular HPLC system.
- Retention reproducibility was less than 0.10 %RSD for both compounds.
- Calibration curves were linear for both capsaicin and dihydrocapsaicin, and area count repeatability of injections was less than 0.30 %.
- The habanero-based sauces showed higher concentrations of capsaicin and dihydrocapsaicin than the red pepper, cayenne pepper, and jalapeno pepper hot sauces.
- The calculated Scoville heat units were within the range of the stated units found on the bottle of the sauces.
- Blanks showed no carryover, demonstrating the excellent carryover management of the 2707 Autosampler.
- A simple-to-use modular HPLC system, consisting of the 2707 Autosampler, the 1525 Binary HPLC Pump, and the 2489 UV/VIS Detector, is an excellent choice for this analysis and for other analyses in the QC laboratories of the food and beverage industry.

References

1. The Journal of the American Pharmacists Association 1912; 1:453–4.

2. Tainter, DR, Grenis, AT. Spices and Seasonings, Wiley-IEEE. 2001; p.30.

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