APPLICATION NOTE

Liquid Chromatography

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Analysis of Capsaicin and Dihydrocapsaicin in Chili Peppers Using the PerkinElmer Altus HPLC System with PDA Detection

Introduction

Capsaicinoids are the compounds that produce the pungency, aroma and flavor of chili peppers. The two most abundant capsaicinoids in chili peppers are capsaicin (8-methyl-N-vanillyl-trans-6-nonenamide) and dihydrocapsaicin. Combined, these two make up close to 90% of the most pungent varieties of capsaicinoids, with capsaisin making up about 71%.[1] The capsaicin content of peppers is one of the parameters that determine their commercial quality. The amount of capsaicin can vary, depending on the light intensity and temperature at which the plant is grown, the age of the fruit, and the position of the fruit in the plant.[2]

Besides their widespread uses in foods, capsaicinoids are increasingly being used as the active component in pharmaceuticals and have been used as an analgesic against arthritis pain and inflammation.[3] They have also been reported to be active against neurogenic inflammation (as in pepper sprays) and have shown protective effects against high cholesterol levels and obesity.[4, 5]

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Considering the increased use of capsaicinoids in both foods and pharmaceuticals, there is an increasing demand for their accurate quantitation as part of monitoring the quality of chili peppers. In this regard, this application focuses on the extraction, HPLC separation and quantitation of capsaisin and dihydrocapsaicin in two store-bought chili pepper powders. Method conditions and performance data, including linearity and repeatability, are presented.

Experimental

Hardware/Software

For all chromatographic separations, a PerkinElmer Altus™ HPLC System was used, including the A-10 solvent/sample module with integrated vacuum degasser, A-10 column module and A-10 PDA detector. All instrument control, analysis and data processing was performed using the Waters® Empower® 3 chromatography data software (CDS) platform.

Method Parameters

The HPLC method parameters are shown in Table 1.

Table 1. HPLC Method Parameters.

HPLC Conditions	
Column:	PerkinElmer Brownlee™ Validated C18 3 µm, 4.6 x 100-mm (Part# N9303552)
Mobile Phase:	Solvent A: Water Solvent B: Methanol Solvent Program:
Analysis Time:	4.0 min.
Flow Rate:	1.0 mL/min. (3000 psi)
Oven Temp.:	25 °C
Detection:	Altus A-10 PDA Excitation: 222 nm
Injection Volume:	10 µL
Sampling (Data) Rate:	10 pts./sec

	Time (min)	Flow Rate (mL/min)	%A	%B	%C	%D
1	Initial	1.0	20.0	80.0	0.0	0.0
2	4.0	1.0	20.0	80.0	0.0	0.0

Solvents, Standards and Samples

All solvents and diluents used were HPLC grade and filtered via 0.45-µm filters.

The capsaicinoid standards were obtained from Sigma Aldrich, Inc® (Allentown, PA) and consisted of capsaicin and dihydrocapsaicin. A stock 200-ppm standard was made using methanol as diluent. The lower level standards were then prepared from this stock solution.

Two chili powder samples were purchased at a local store. They were labeled *Cayenne1* and *Jalapeño1*. Each chili powder was prepared by adding 2.0 g into 20-mL methanol. The solutions were placed in a water bath at 60 °C for 2 hours.[6] After cooling to room temperature, the samples were then centrifuged at 5000 rpm for five min. The supernatants were then filtered, diluted 1:1 with methanol and injected.

Prior to injection, all calibrants and sample extracts were filtered through 0.45-µm filters to remove small particles.

Results and Discussion

Figure 1 shows the chromatographic separation of the 100-ppm capsaicinoid standard (100-ppm Std) using the optimized conditions described above. The analysis time was under four minutes.

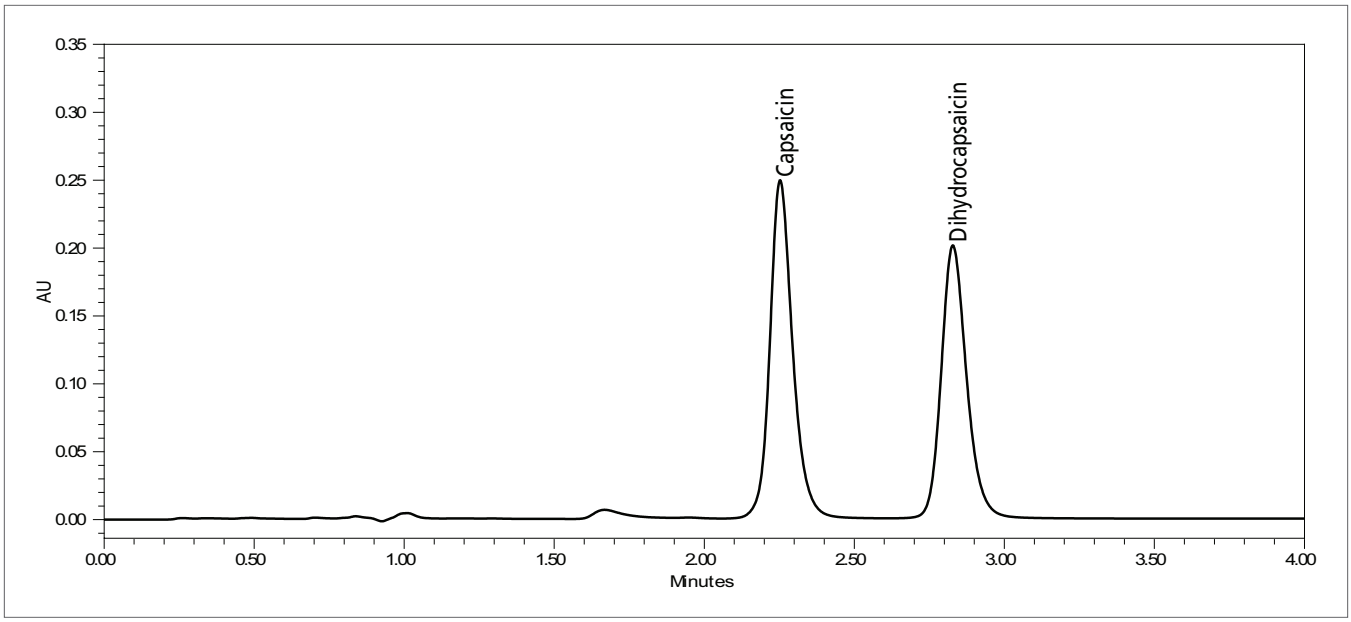

Figure 1. Chromatogram of the 100-ppm capsaicinoid standard (100-ppm Std).

Figure 2 shows the overlay of 10 replicate 100-ppm Std injections, demonstrating exceptional reproducibility. The retention time %RSD for capsaicin was 0.067%.

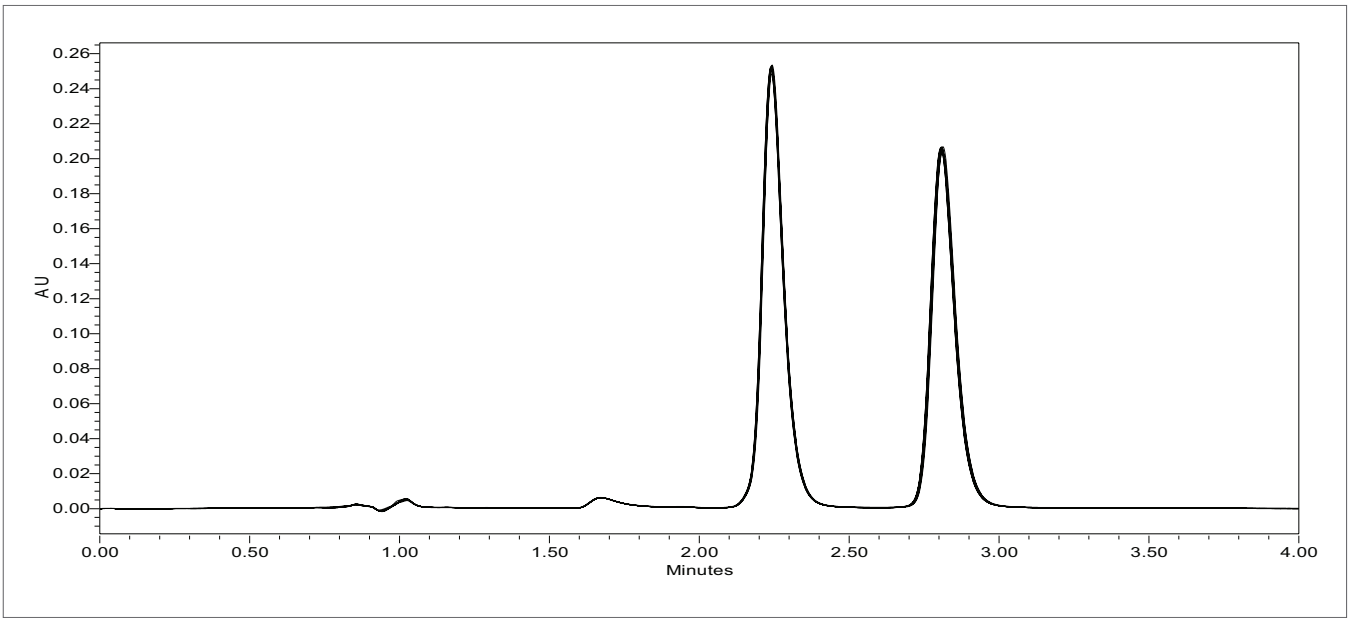

Figure 2. Overlay of 10 replicates of the 100-ppm Std.

Figure 3 and Figure 4 show the calibration results for capsaicin and dihydrocapsaicin over a concentration range of 1 to 100 ppm. Both capsaicinoids followed a linear (1st order) fit and had R^2 coefficients > 0.999 (n = 3 at each level).

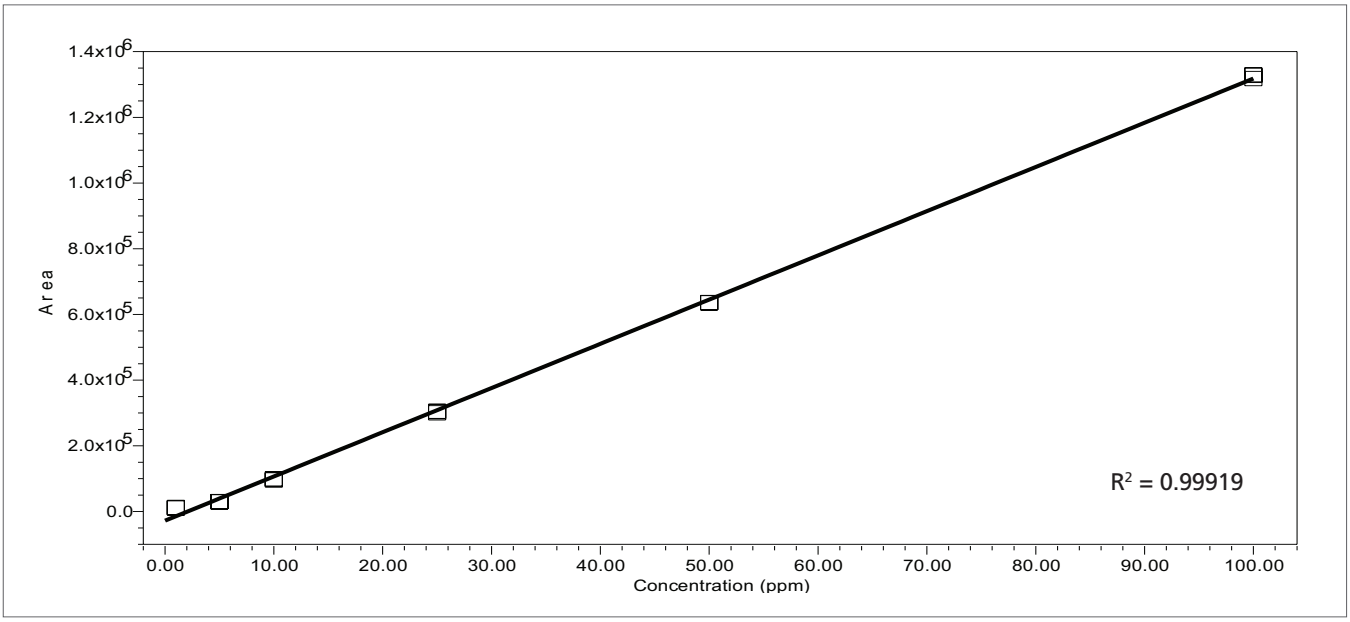

Figure 3. Results of 6-level calibration set for capsaicin.

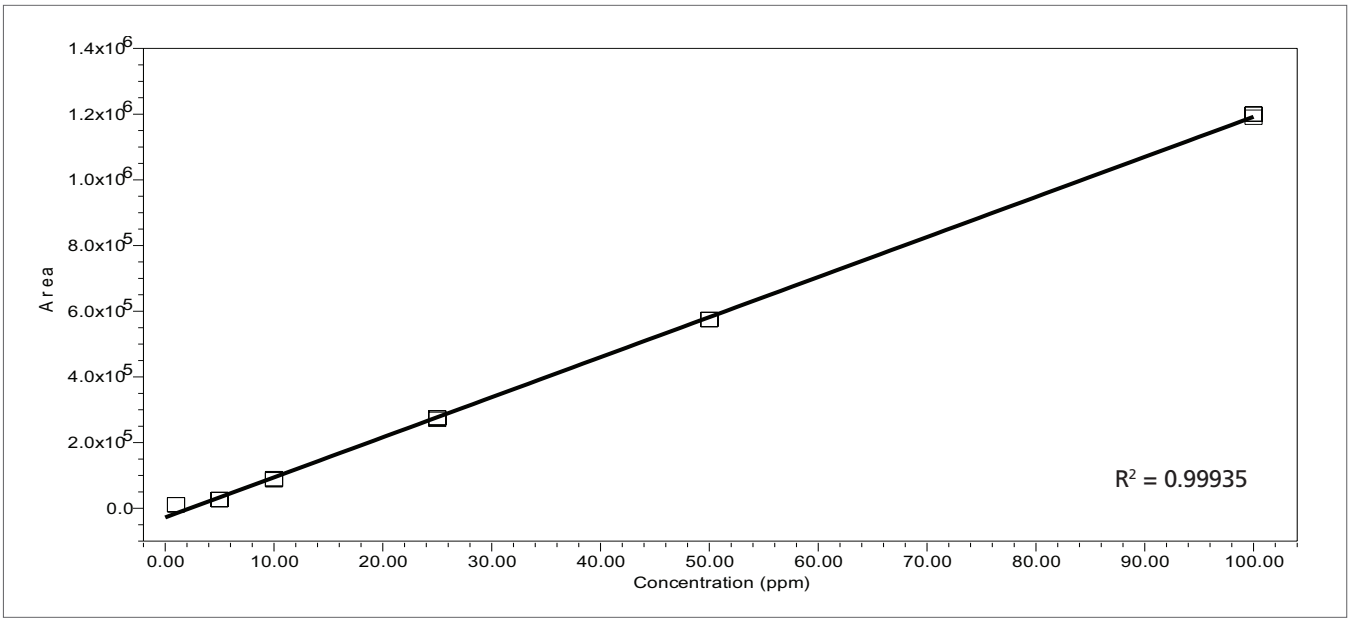

Figure 4. Results of 6-level calibration set for dihydrocapsaicin.

Using the same chromatographic conditions, two chili pepper powder samples were then analyzed: *Cayenne1* and *Jalapeño1*. The chromatographic results are shown in Figure 5. Comparing the chromatograms of these chili pepper samples with the 100-ppm Std, it can be observed that both samples contain capsaicin and dihydrocapsaicin.

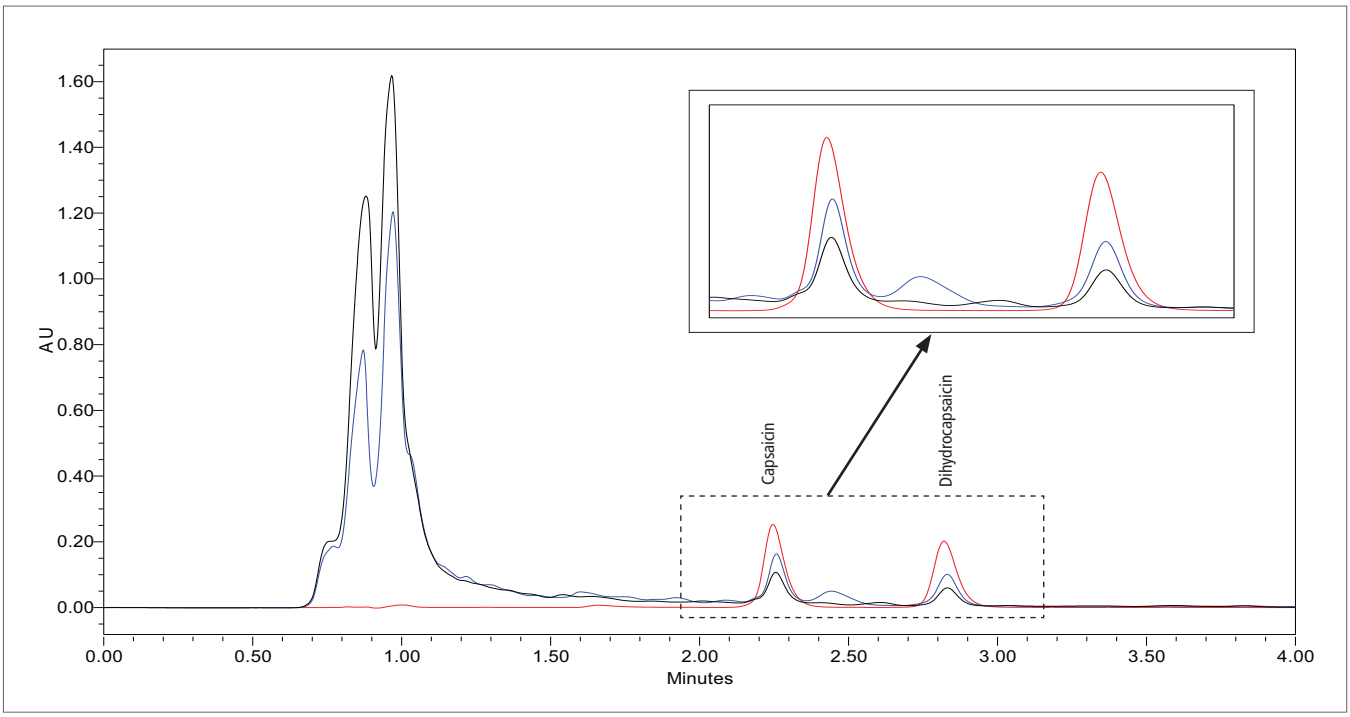

Figure 5. Overlaid chromatograms of Cayenne1 (blue), Jalapeño1 (black), and 100-ppm Std (red).

Based on the standard calibration plots and the 20-fold sample dilution during sample preparation, the quantitative results for each chili powder sample are shown in Table 2. The Cayenne1 pepper powder was shown to contain approximately two times more capsaicin and dihydrocapsacin than the Jalapeño1 pepper powder. The results from this experiment correspond with the Scoville scale, used to measure the pungency of peppers, where cayenne peppers are rated significantly more pungent than jalapeño peppers.[7]

Table 2. Quantitative Results.

Pepper Powder	Capsaicin (µg/g)	Dihydrocapsaicin (µg/g)
Cayenne1	1030	771
Jalapeño1	694	428

Conclusion

This work has demonstrated the effective chromatographic separation and quantitation of two capsaicinoids using a PerkinElmer Altus HPLC System with PDA detection. The results exhibited very good retention time repeatability as well as excellent linearity over the tested 1-100 ppm concentration range. Although a PDA detector was used for possible component characterization/confirmation, a UV detector could also be used for this analysis.

From a food quality perspective, there is an ever growing emphasis on food monitoring. This is especially the case pertaining to capsaicinoids for their use as active components in pharmaceutical products. With this in mind, the results of this work also demonstrated the effective and robust analysis of capsaicinoids in two store-bought chili pepper powders, identifying the particular analytes contained in each of the two samples, as well as comparing their profiles, both chromatographically and quantitatively.

References

1. S. Losuge, M. Furuta. *Agricultural and Biological Chemistry.* 34, 248, 1970.
2. Z. Othaman, Y. Ahmed, M. Habila, A. Ghafar. *Molecules.* 16, 8919, 2011.
3. D. Deal, T. Schnitzer, E. Lipstein, J. Seibold, R. Stevens, M. Levy. *Clinical Therapeutics.* 13, 383, 1999.
4. J. Szocsany. *Neuropeptides.* 38, 377, 2004.
5. R. Kempaiah, H. Manunatha, K. Drinivasan. *Molecular Cellular Biology.* 275, 7, 2005.
6. J. Juangsamoot, C. Ruangviriyachai, S. Techawongstien, S. Chanthai. *International Food Research Journal.* 19, 3, 1217, 2012.
7. "http://www.chilliworld.com/factfile/scoville_scale.asp," [Online]. [Accessed 13 April 2015].

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