Profiling capsaicinoids in spicy foods and food products using the Agilent 1290 Infinity LC system and Agilent ZORBAX Poroshell 120 2.7 µm columns with UV/Vis diode array and fluorescence detection

Application Note

Food

Author

Michael Woodman
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, Delaware 19808
USA

Abstract

The Agilent 1290 Infinity LC system has significant capabilities for a wide range of HPLC and UHPLC applications. It exhibits a broader power range (for example, the combination of pressure and flow capabilities), and the flexibility to operate a wide range of column dimensions and particle sizes than any other commercially available system. Advanced optical design in the diode array detector allows a wide dynamic range and high sensitivity, both of which are critical in the monitoring of natural and synthetic food flavorings.

The combined benefits are demonstrated by a separation of primary capsaicinoid components found in chili pods, sauces and spices. Capsaicin is the primary component recognized as the "hot" component of chili peppers, members of the capsicum family. The capsaicinoid class is comprised of eight or more compounds that variably contribute a heat component to flavor. Capsaicin or synthetic capsaicin (nonivamide) has also been used in topical creams and applications to relieve arthritis, itching, neuropathy and other ailments. The core structure is phenolic and primary variation is found in the hydrophobic alkyl chain.

The high pressure capability of the system allows the use of methanol, and acetonitrile, to explore the selectivity of the two solvents. Various column configurations, including porous 1.8 µm and superficially porous 2.7 µm materials, were evaluated. The structure of capsaicin is shown in Figure 1.

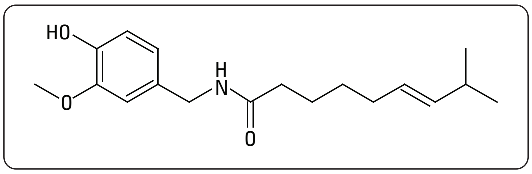


Figure 1
The structure of capsaicin ((E)-N- (4-hydroxy-3-methoxybenzyl) - 8-methylnon-6-enamide).

The distinctive spectrum of capsaicin allows one to use the diode array detector to interrogate peaks in capsaicinoid regions to determine if the compound is likely to be an active component. The UV ratio for 229 nm/280 nm is nominally 2.4 (Figure 2).

After optimization of the standard mixture, a number of extracted samples were chromatographed (Figure 3).

Cayenne and other pure chili spices and fresh pods were easily analyzed with UV detection. Low level samples such as American paprika and complex blends such as chili and curry powders were more likely to show interferences. The solution to this problem was the Agilent 1200 Series fluorescence detector (G1321A) as shown in Figure 4. The remarkable selectivity and sensitivity of the FLD minimized or eliminated non-capsaicinoid peaks from the analyte region.

Configuration

- Agilent 1290 Infinity Binary Pump with Integrated Vacuum Degasser (G4220A)
- Agilent 1290 Infinity Autosampler (G4226A)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1200 Series Diode Array Detector (G1315C)

Conclusion

The flexible solvent and column selection features, and high pressure capability, of the system allows one to use highly efficient columns to rapidly develop separations with remarkable resolution while conserving solvent over the use of 4.6 mm id columns. The added selectivity of fluorescence detection provides solutions to unexpected interferences that would otherwise have required redevelopment of the separation method.

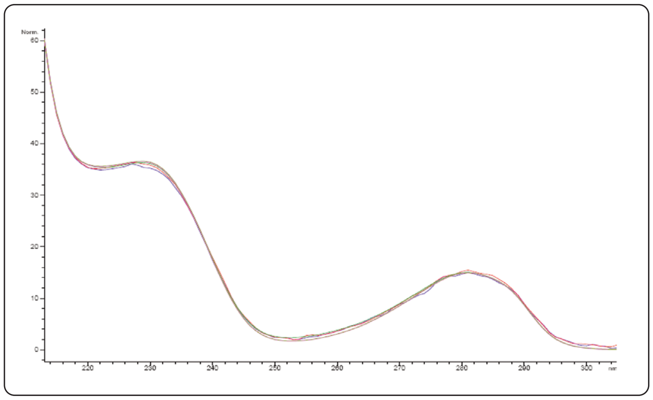


Figure 2
Overlaid extracted UV spectra of capsaicinoid compounds in chili.

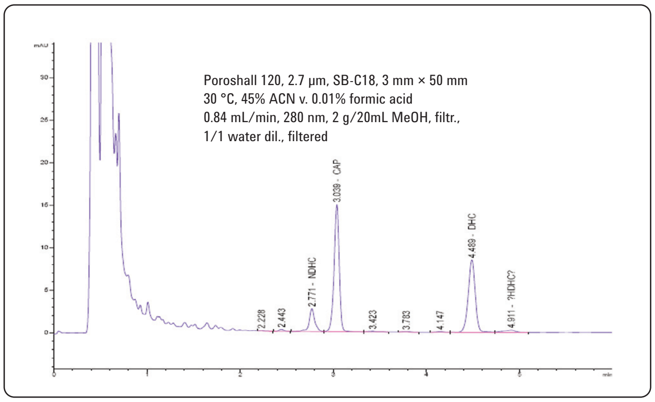
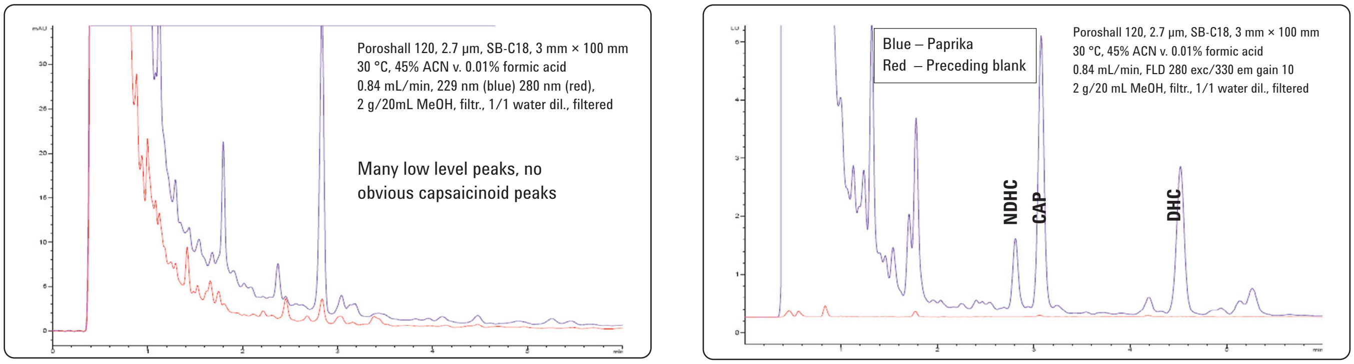

Poroshall 120, 2.7 µm, SB-C18, 3 mm × 50 mm
30 °C, 45% ACN v. 0.01% formic acid
0.84 mL/min, 280 nm, 2 g/20mL MeOH, filtr.,
1/1 water dil., filtered

Figure 3
Separation of cayenne dried ground spice (CAP- capsaicin, NDHC – nordihydrocapsaicin, DHC – dihydrocapsaicin).


Poroshall 120, 2.7 µm, SB-C18, 3 mm × 100 mm
30 °C, 45% ACN v. 0.01% formic acid
0.84 mL/min, 229 nm (blue) 280 nm (red),
2 g/20mL MeOH, filtr., 1/1 water dil., filtered

Many low level peaks, no obvious capsaicinoid peaks

Blue – Paprika
Red – Preceding blank

Poroshall 120, 2.7 µm, SB-C18, 3 mm × 100 mm
30 °C, 45% ACN v. 0.01% formic acid
0.84 mL/min, FLD 280 exc/330 em gain 10
2 g/20 mL MeOH, filtr., 1/1 water dil., filtered

Figure 4
Comparison of UV and fluorescence signals for American Paprika extract (two frames).

www.agilent.com/chem/1290

July 1, 2010
Publication Number 5990-5992EN

Agilent Technologies