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Note

Rapid high-performance liquid chromatographic method for the determination of very low capsaicin levels

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A method is presented which permits the detection and quantification of capsaicin and dihydrocapsaicin to a level of 100 Scoville units. A reversed-phase, isocratic high-performance liquid chromatographic (HPLC) system using a C_8 column with UV detection at 200 nm is employed.

EXPERIMENTAL

Apparatus

The liquid chromatograph used was an Altex Model 332 equipped with two Model 110A pumps and a Model 210 injection valve fitted with a 20-μl loop. The detector was a Hewlett-Packard Model 1040A diode array, coupled with an HP85B computer, an HP2225 printer and an HP7470A plotter. A C_8, 5-μm, 150 mm × 4.6 mm I.D. column was employed (Supelco).

Reagents

Mobile phase. A 1.85-g amount of sodium pentanesulphonate (Regis) was dissolved in 400 ml HPLC-grade water (Caledon Labs., Georgetown, Canada) and then diluted to 1 l with HPLC-grade methanol (Caledon Labs.).

Solvents. ACS-grade acetone, denatured ethanol (DAG 2A) and methanol–water (50:50) (HPLC grades) are required for sample preparation.

Standards. Grade II capsaicin (Sigma) was used as the standard.

Chromatographic conditions

The detector was set at 200 nm with a bandwidth of 4 nm. A column flow-rate of 2.0 ml/min was used.

Sample preparation

If necessary, samples must be ground to pass a U.S.20-mesh screen. Samples (10.00 g) were refluxed for 1 h with 100 ml acetone in a 250-ml boiling-flask connected to a 300-mm water-cooled condenser. After cooling, the samples were vacuum filtered through No. 41 Whatman filter paper, then the acetone was removed using a rotary film evaporator. The residue was redissolved in denatured ethanol and the volume adjusted to 50.0 ml. A 5-ml sample aliquot was then diluted to 10.0 ml with

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methanol–water (50:50). This solution was filtered through a 0.5-μm disposable filter and injected.

RESULTS AND DISCUSSION

An "on-the-fly" UV scan of capsaicin (Fig. 1) shows a maximum absorbance at 201 nm. By selecting a detector wavelength of 200 nm, an approximate 17 times increase in sensitivity over 280 nm can be achieved with similar detector conditions.

Previous recovery studies using a similar system[1] have shown that the selected solvent, acetone, gives a fast, efficient extraction of capsaicinoids. The acetone must be removed to eliminate a large, tailing solvent peak in sample chromatograms. The final dilution using methanol–water eliminates leading-edge tailing and poor resolution apparent when injecting a solvent "stronger" than the eluting solvent[2].

For calibration, standard capsaicin was dissolved in denatured ethanol, and diluted with methanol–water to a final concentration of 5 μg/ml (0.1 μg in 20 μl). The standard capsaicin is a mixture of the capsaicinoids nordihydrocapsaicin, capsaicin and dihydrocapsaicin. Each component was calculated using the appropriate individual response factor where capsaicin = 1.00, nordihydro = 1.05, and dihydro = 1.02 (refs. 1 and 3).

Fig. 2 is a chromatogram of standard capsaicin, showing the resolution between the three capsaicinoids. Fig. 3 is a chromatogram of standard capsaicin diluted to a concentration of 7.06 ng per 20 μl. This concentration extracted from a sample would represent a calculated value of 56 Scoville units using the factor of $16.1 \cdot 10^6$ Scoville units for pure capsaicin and pure dihydrocapsaicin[4]. Fig. 4 shows a chromatogram of Spanish paprika with a calculated value of 150 Scoville units. Fig. 5 shows a chromatogram of American paprika with a calculated value of 290 Scoville units. Also, chromatograms from analyses of samples containing cumin and oregano show sufficient levels of resolution between the capsaicinoids of interest and other spice components to permit heat determinations well below 1000 Scoville units.

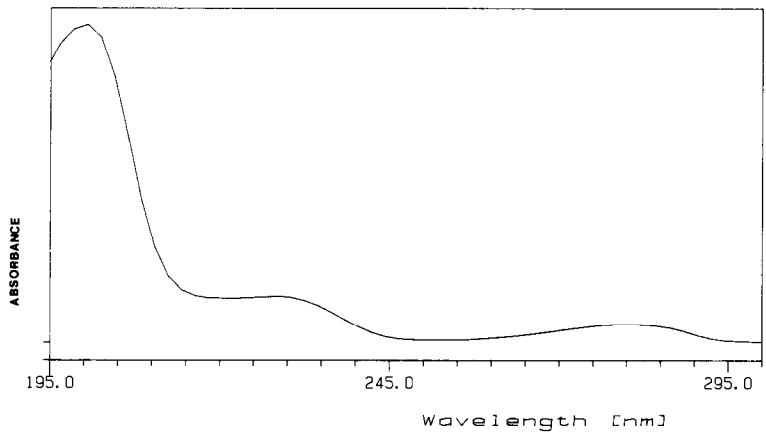

Fig. 1. "On-the-fly" UV scan of capsaicin.

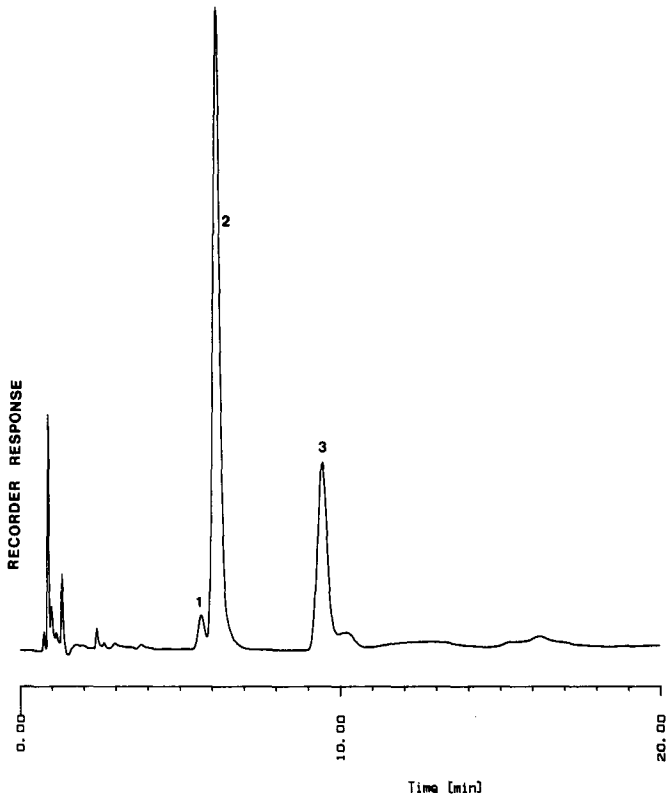

Fig. 2. HPLC chromatogram of standard capsaicin. Peaks: 1 = nordihydrocapsaicin; 2 = capsaicin; 3 = dihydrocapsaicin.

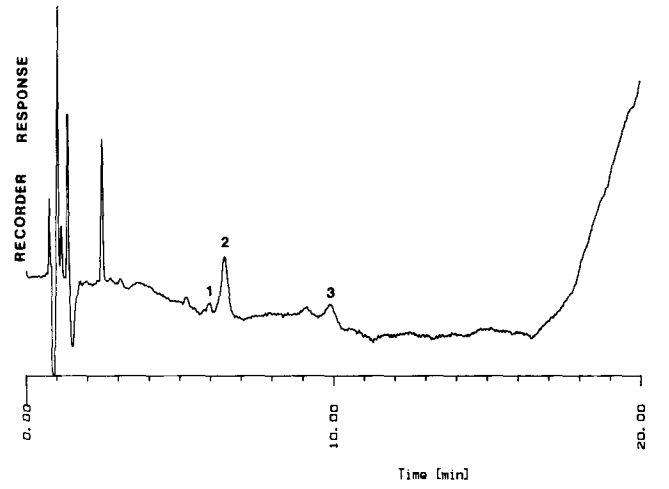

Fig. 3. HPLC chromatogram of standard capsaicin representing 56 Scoville units. Peaks as in Fig. 2.

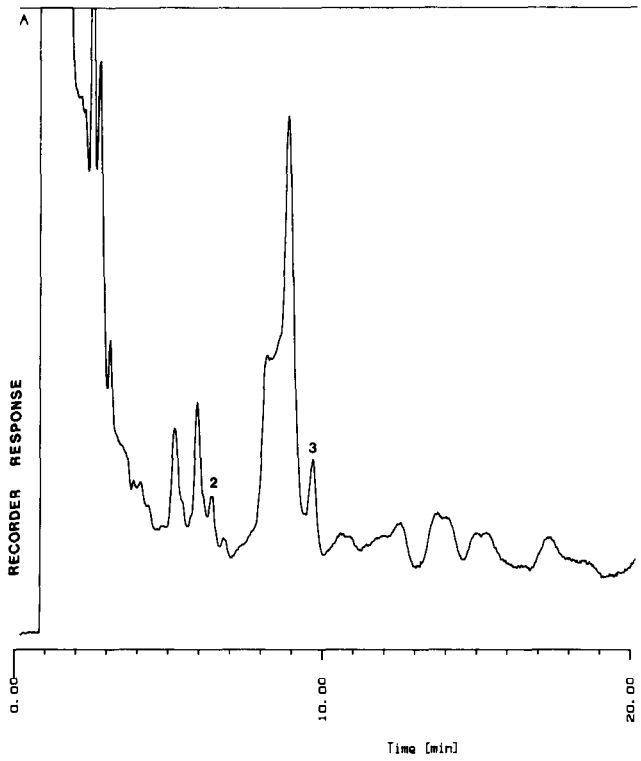

Fig. 4. HPLC chromatogram of Spanish paprika. Peaks as in Fig. 2.

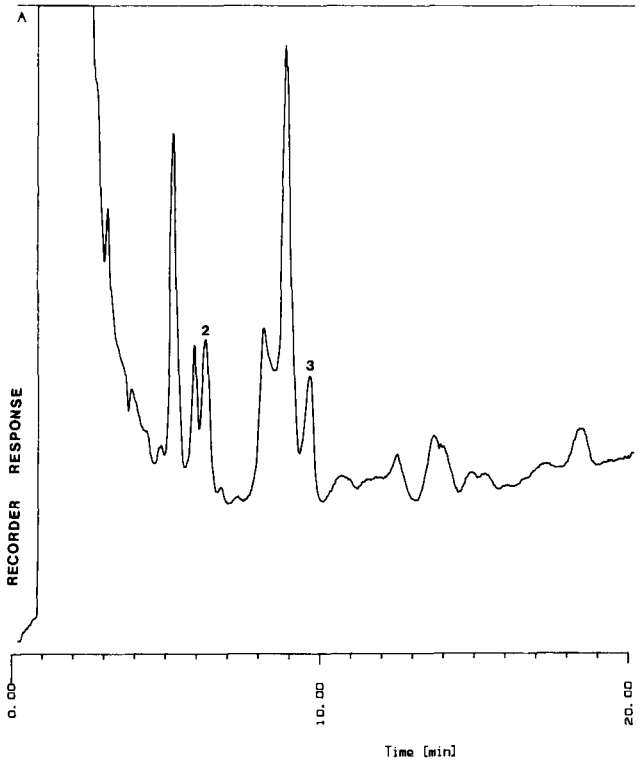

Fig. 5. HPLC chromatogram of American paprika. Peaks as in Fig. 2.

REFERENCES

1 K. M. Weaver, R. G. Luker and M. E. Neale, *J. Chromatogr.*, 301 (1984) 288.
2 J. F. Lawrence, *Organic Trace Analysis by Liquid Chromatography*, Academic press, New York, 1981, p. 34.
3 P. G. Hoffman, M. C. Lego and W. G. Galetto, *J. Agric. Food Chem.*, 31 (1983) 1326.
4 P. H. Todd, M. G. Bensinger and T. Bifter, *J. Food Sci.*, 42 (1977) 660.