

CHROM. 17,000

Note

Rapid quality control procedure for the determination of Scoville heat units and the detection of chillies in black pepper, via high-performance liquid chromatography

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Previously published methods for capsaicin analysis have been noted by Woodbury¹ and Hoffman *et al.*², and piperine analysis via high-performance liquid chromatography (HPLC) has been demonstrated by Rathnawaite and Buckle³, Galetto *et al.*⁴ and Verzele *et al.*⁵.

In this study, we have used a reversed-phase C₁₈ column with a mobile phase of methanol-water containing sodium pentane sulfonate and acetic acid. This solvent is less dangerous and cheaper than that used by Woodbury¹ which contained acetonitrile and perchloric acid. Further, UV detection at 280 nm obviates the need for the specialized fluorescence detector used by Woodbury and still permits detection of very low levels of capsaicinoids.

EXPERIMENTAL

Apparatus

The liquid chromatograph used was an Altex Model 322 equipped with a Model 110A pump and a C₁₈ (I.P.) column, 5 µm particle size, 250 × 4.6 mm (Altex), fitted with a rheodyne 20-µl syringe-loading sample loop injector. Detection was at 280 nm with a Beckman Analytical Optical Unit, cell dimensions 10-mm path length, 8-µl volume, used at 0.04 a.u.f.s. Data acquisition was by an Altex Model C=RIA recording integrator.

Reagents

Mobile phase. Sodium pentane sulfonate (Eastman Kodak) (0.95 g) was dissolved in 160 ml HPLC reagent grade water (Baker) and mixed with 335 ml HPLC reagent grade methanol (Caledon Labs., Georgetown, Ontario) and 5 ml distilled-in-glass acetic acid (Caledon Labs.).

Standards. Standard solutions were prepared from reagent grade capsaicin (Sigma), piperine (Sigma) and the three major capsaicinoids (synthesized by McCormick & Co., Hunt Valley, MD, U.S.A. (see Discussion for compounds used).

Sample preparation

Whole spices were ground to pass U.S. 20 mesh; oleoresins were warmed and

thoroughly mixed. The analyte solutions were prepared by refluxing 10.00 g prepared spice with 100 ml acetone for 1 h and filtering, or by dissolving 0.1 g oleoresin capsicum in 100 ml acetone. The prepared solutions were diluted (4 ml to 10 ml) with methanol-water of the same composition as the mobile phase (i.e. 70:30).

Chromatographic conditions

The standard capsaicinoids and piperine were satisfactorily separated (Fig. 1) using the described solvent at a flow-rate of 1 ml/min at ambient temperature.

RESULTS AND DISCUSSION

The three major capsaicinoids present in "hot" capsicum species (chillies) shown⁶ to constitute 98% of the total capsaicinoids are capsaicin, dihydrocapsaicin and nordihydrocapsaicin.

Fig. 1 shows the chromatogram of a mixture of pure piperine, capsaicin, dihydrocapsaicin and nor dihydrocapsaicin. Fig. 2 shows the chromatogram of a sample of a typical analysis of chillies. The absence of piperine is clearly shown.

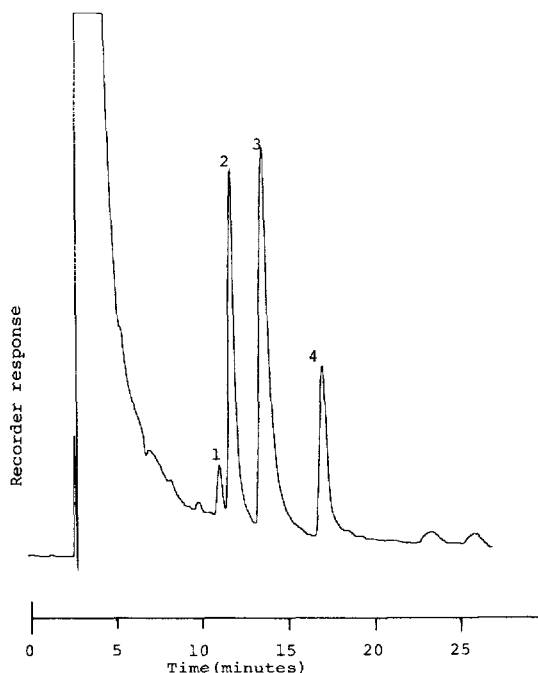


Fig. 1. Chromatogram illustrating the separation of capsaicinoids and piperine. Peaks: 1 = nordihydrocapsaicin, 2 = capsaicin, 3 = piperine, 4 = dihydrocapsaicin.

Detectable levels of the capsaicinoids and piperine were determined by diluting to 0.3–2.4 $\mu\text{g}/20 \mu\text{l}$ (15.0–120.0 ppm) and 0.1–1.0 $\mu\text{g}/20 \mu\text{l}$ (5.0–50.0 ppm) respectively and chromatographing under the given conditions. The relationship between area counts and μg injected was found to be linear in the both cases ($r = 0.999$ and 1.000).

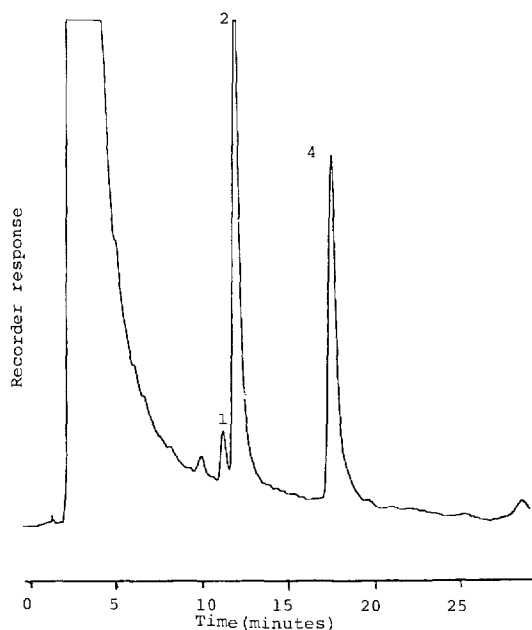


Fig. 2. Chromatogram of typical extract of chillies (peak numbers as in Fig. 1).

respectively). The limiting concentration of capsaicinoids detectable by the method given was 25 ppm (based on a preset minimum area rejection level of 1500 units).

Recovery of added capsaicinoids was found to be in the range 98–101% (Table I).

Relative response factors for nor dihydrocapsaicin, capsaicin and dihydrocap-

TABLE I
RECOVERY OF ADDED CAPSAICINOID

<i>Amount in sample (mg)</i>	<i>Amount added (mg)</i>	<i>Total (mg)</i>	<i>Found (mg)</i>	<i>Recovery (%)</i>
698.5	126.8	825.3	826.9	100.2
698.6	196.5	895.1	891.1	99.6
698.4	294.4	992.8	978.3	98.5

TABLE II
STATISTICAL ANALYSIS OF VARIATION OF CAPSAICINOID RATIO FOUND

<i>Capsaicinoid</i>	<i>Mean</i>	<i>Standard deviation</i>	<i>Mean \pm 1.96 S.D.</i>
Nordihydrocapsaicin	5.18	1.84	1.58– 8.78
Capsaicin	57.9	5.8	46.5 –69.3
Dihydrocapsaicin	36.7	5.1	26.7 –46.7

saicin were found to be in the ratio 1.05:1.00:1.02, which is in close agreement (1.10:1.00:1.04) with those found by Hoffman².

The "Scoville" heat units of a sample of chilli or its extract is calculated by determining the percentages of the three capsaicinoids, multiplying each of them by the Scoville heat value as determined by Todd *et al.*⁶ and summing. External standards calibration was used, using capsaicinoids of known purity.

"Synthetic" capsaicin (N-vanillyl-n-nonamide) which may be used to spike chilli extracts, was found to coelute with capsaicin. However, if present, the calculated Scoville heat units would be erroneously high, since the Scoville heat units of N-vanillyl-nonamide have been shown by Todd⁶ to be 9.2 million, compared with the value of 16.1 million for capsaicin itself. A survey of 22 samples (whole spices and oleoresins) shows that the three major capsaicinoids occur in fairly constant proportions. A statistical analysis of the ratios found is given in Table II. An estimate can be made as to whether or not a sample has been spiked with "synthetic" capsaicin.

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