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Note

New method for the determination of capsaicin by using multi-band thin-layer chromatography

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Gibbs' reagent (2,6-dichloro-*p*-benzoquinone chlorimine) is very sensitive for the quantitative assay of capsaicin, but its use requires complete elimination of all interfering substances[1]. Methods of separation (and subsequent determination) of capsaicin from such interfering substances as carotenoids and fats by paper chromatography[2], thin-layer (TLC)[3,4] and gas–liquid chromatography[5,6] have been described. However, these methods either result in partial purification or require special equipment not always available for routine analysis. An attempt has therefore been made to develop an accurate, sensitive and reproducible method for determining capsaicin from chillies. The original method described by Gibbs[7] for the colorimetric estimation of phenols has been further improved and is used for the estimation of capsaicin after its separation by multi-band TLC.

EXPERIMENTAL

Materials and apparatus

Silica gel G (E. Merck, Darmstadt, G.F.R.), type 60, kieselguhr G (Merck), activated charcoal (Aktivkohle, purum, Darco G-60, Fluka A.G., Buchs, Switzerland) and distilled methanol were used. A mixture of absolute methanol and acetic acid (49:1) was used as developing solvent, and a 0.01 % solution of Gibbs' reagent (Merck) in acetone (analytical grade) was used as spray reagent. A saturated solution of Gibbs' reagent in distilled water (freshly prepared) and 0.05 *M* borate buffer solution of pH 9.4 were used for colour development. Standard capsaicin was obtained from K & K Laboratories (New York, U.S.A.) and used as a solution (1 mg/ml) in acetone.

Preparation of multi-band TLC plates

Plates were prepared by using a spreader modified as shown in Fig. 1. Two close-fitting plastic discs (D) were inserted into the body of the spreader, dividing it into three compartments (A, B and C). Two plastic supports (S) were fitted above the two discs in a way such that the space was just sufficient between the disc and the

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support for the metallic roller in the spreader to slide within. The sizes of compartments A, B and C were 15.6, 1.9 and 2.5 cm, respectively. The adsorbent in compartment B was kieselguhr G containing 8% of activated charcoal; compartments A and C contained silica gel G.

Slurries were prepared for the three compartments, by taking one part of the adsorbent in two parts of water. These slurries were poured into the compartments simultaneously, and layers of 0.5 mm thick were prepared on plates (20 × 20 cm) in the usual way (Fig. 2); the layers were allowed to dry in air for 15 min and then activated at 100° for 50 min.

Preparation of chilli extract

An appropriate amount (2–10 g) of chilli powder was completely extracted with absolute methanol for at least 6 h. The extract was then evaporated to dryness in a rotary flash evaporator at 40°, and the residue was dissolved in chloroform–methanol (1:1), and the final volume made to 5 or 10 ml. An aliquot containing less than 80 μg of capsaicin was applied to the activated layer with a semi-automatic spotting device, and the chromatogram was developed with absolute methanol–acetic acid (49:1). After development, the plate was dried in air, and the capsaicin spots were located by spraying very lightly with the 0.01% solution of Gibbs' reagent in acetone. The acetone was allowed to evaporate in air, and the plate was then sprayed very lightly with the borate buffer solution and kept in the dark for some minutes; the faint blue spots that appeared below the solvent front ($R_F = 0.8$) were marked immediately and scraped into centrifuge tubes. In each of these tubes was placed 3 ml of borate buffer solution, then the tubes were shaken on an electric shaker for 5 min before the addition to each of 0.5 ml of a freshly prepared saturated aqueous solution of Gibbs' reagent. Each tube was shaken vigorously for 5 min and then kept in the dark for 30 min for development of colour. The supernatant liquid was transferred carefully into a graduated tube, residual colour in the silica gel was repeatedly extracted with 2-ml portions of the borate buffer solution and the final volume was made up to 10 ml; the absorbance of the blue complex was measured at 600 nm with a Beckman DU quartz spectrophotometer.

A calibration graph was prepared with standard capsaicin (0–80 μg). This graph (see Fig. 3) showed that the Beer–Lambert law was obeyed over the stated range.

TABLE I

RECOVERY OF STANDARD CAPSAICIN AFTER SEPARATION BY MULTI-BAND TLC

Capsaicin spotted (μg)	*O.D. of capsaicin by direct method*	*Capsaicin recovered (μg)*	*O.D. of capsaicin recovered*	*Recovery of capsaicin*
10	0.042	9.5	0.040	95.0
20	0.080	19.2	0.077	96.2
30	0.124	28.3	0.117	94.3
40	0.160	37.0	0.148	92.5
50	0.204	47.3	0.193	94.6
60	0.240	56.7	0.227	94.5
70	0.278	67.2	0.267	95.7
80	0.320	74.5	0.298	93.1

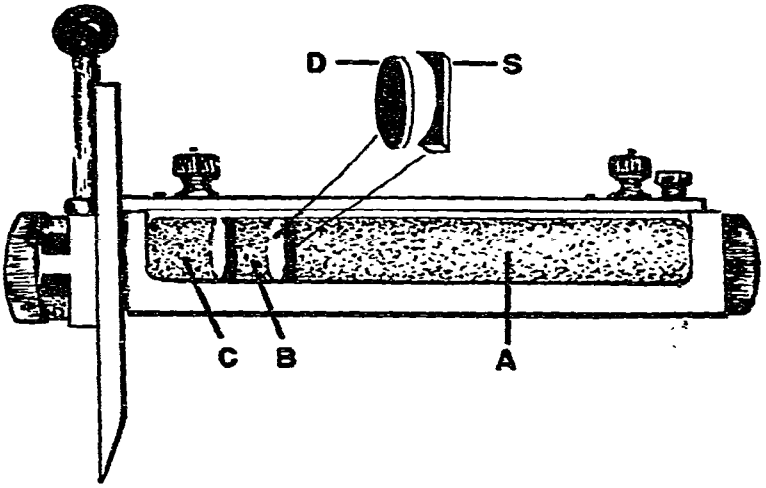

Fig. 1. The modified spreader. A, B and C are the compartments. Two plastic supports (S) are fitted above the two discs (D).

Fig. 2. Multi-band TLC plate after pouring of slurries.

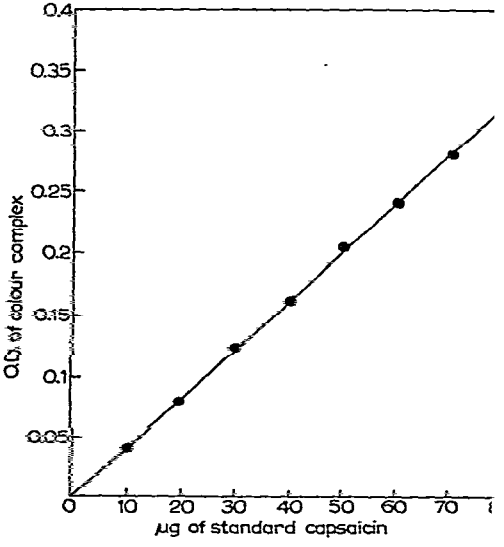

Fig. 3. Calibration curve prepared by plotting O.D. against concentration of capsaicin by direct method with Gibbs' reagent.

RESULTS AND DISCUSSION

Table I gives the recovery of standard capsaicin after multi-band TLC. Comparison of these values with those obtained by direct colorimetric estimation (see Fig. 3) shows that recovery of the standard capsaicin ranged from 92.5 to 96.2%.

Another recovery experiment was carried out by adding 500 μg of standard capsaicin to a chilli extract (1 ml), the capsaicin content of which was determined simultaneously. The results in Table II show the recovery of 97.2 and 103.5%.

TABLE II

RECOVERY OF CAPSAICIN FROM FORTIFIED CHILLI EXTRACT AFTER SEPARATION BY TLC

Volume spotted (ml)	*Capsaicin content of original extract (μg)*	*Amount of capsaicin added (μg)*	*Capsaicin content determined after fortification (μg)*	*Recovery of capsaicin (%)*
0.02	21	10	32.1	103.5
0.04	42	20	60.2	97.2

The method we have used for separating capsaicin by multi-band TLC requires no preliminary purification of chilli extract; the one-step separation method is less tedious, and more rapid and accurate, than those of procedures used hitherto.

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