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Note

Analysis of caffeine and trigonelline using high-performance liquid chromatography

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Several analyses using high-performance liquid chromatography (HPLC) have been published for caffeine[1–6]. Analytical procedures for trigonelline are less numerous[6–9]. This paper reports an HPLC method for these compounds which is suitable for the routine analysis of regular and caffeine-free samples of coffee and tea.

MATERIALS AND EQUIPMENT

Samples were prepared using the anion exchanger Dowex 1-X4 (100–200 mesh) and a Millipore filter (0.45 μm). The chromatographic column was a Partisil 10 SCX column (25 cm × 4.6 mm I.D.) (packed by Reeve Angel, Clifton, N.J., U.S.A.).

The HPLC equipment used was a Micromeritics 725 auto-injector, a Spectraphysics 740 pump, a Spectraphysics 8200 filterphotometer at either 254 or 280 nm and a Spectra-Physics system 1 integrator. The eluent was prepared by dissolving potassium citrate in demineralized water, bringing this solution to the desired pH with H_2SO_4 and subsequently mixing it with methanol.

ANALYTICAL PROCEDURE

Sample preparation

Samples of coffee and tea were extracted with, and samples of instant coffee dissolved in, demineralized water to give concentrations of caffeine and trigonelline in the range 3–500 mg/l. 2.0 ml of these solutions were applied to a column (I.D. 0.8 cm) containing 1.0 g of Dowex anion exchanger. The column was eluted with demineralized water and the effluent made up to 25.0 ml. Aliquots of these effluents were filtered over the Millipore filter and 10 μl of the filtrate injected into the chromatograph.

Chromatography

For the chromatographic separation caffeine, trigonelline, theobromine and an unidentified peak found in roasted coffee samples were taken into consideration. The following parameters were investigated: salt concentration, pH and methanol content of the eluent. Capacity factors (k') were calculated using the formula:

$$k' = \frac{t_R - t_{R0}}{t_{R0}}$$

where t_R = retention time of peak studied and t_{R0} = retention time of unretained peak (value given in the column test sheet by Reeve Angel).

The salt concentration of the eluent was varied by changing the potassium citrate concentration. The results are shown in Fig. 1 (pH 3.0 and 10% (v/v)

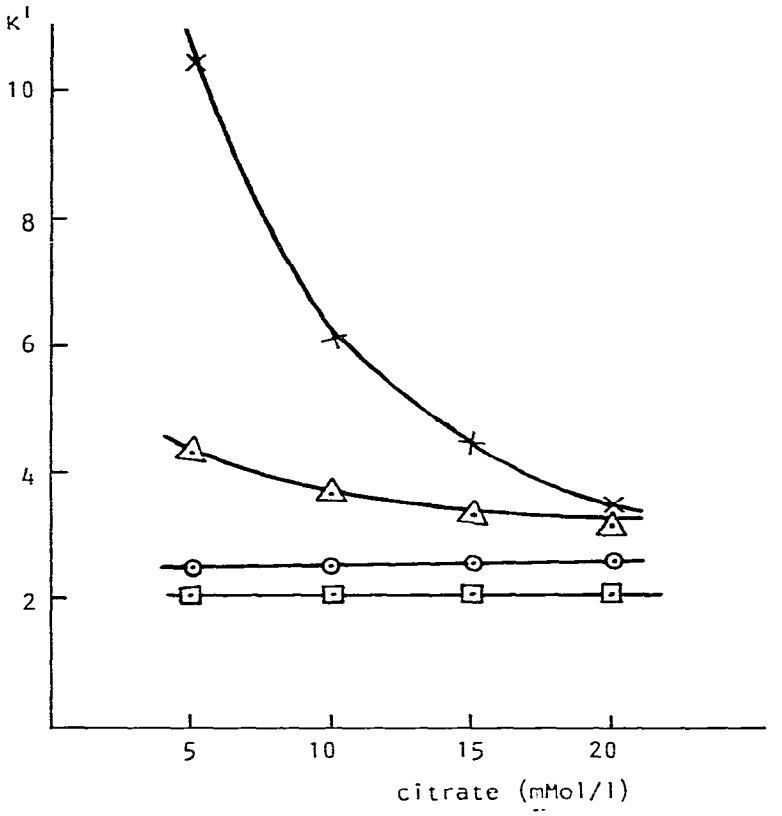

Fig. 1. Capacity factor *versus* citrate concentration of the eluent. ×, Unknown peak of roasted coffee; △, trigonelline; ○, caffeine; □, theobromine.

methanol). The influence of pH on capacity factors is shown in Fig. 2 (0.015 *M* potassium citrate and 10% (v/v) methanol) and the influence of methanol concentration in Fig. 3 (0.015 *M* potassium citrate and pH 3.0).

It may be supposed that caffeine and theobromine are chromatographed by an adsorption mechanism at the silica core of the cation exchanger as their capacity factors are influenced only by methanol concentration, not by salt concentration nor pH. The published pK_b values[10,11] for caffeine (pK_b 13.0) and theobromine (pK_b 13.9) also make an ion-exchange mechanism unlikely.

The preferred eluent was an aqueous solution of 15 m*M* potassium citrate, pH 3.0 and 10% (v/v) methanol. The flow-rate of the eluent was taken to be *ca.*

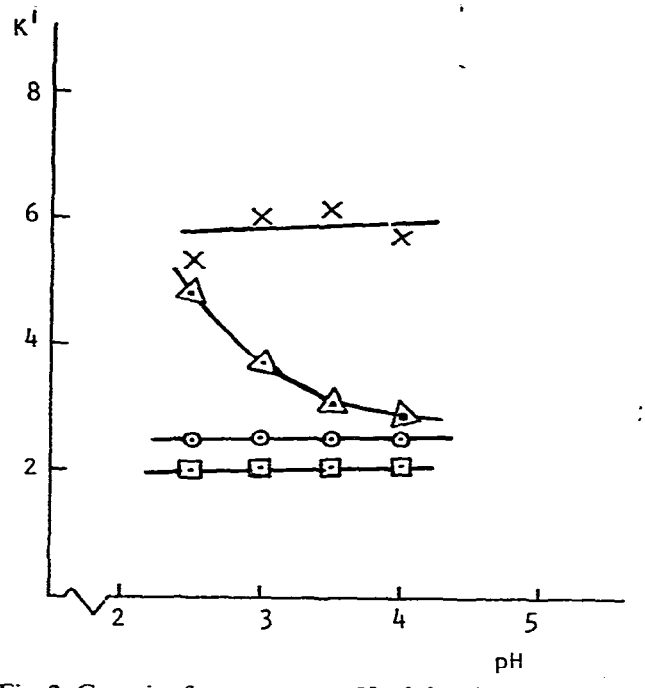

Fig. 2. Capacity factors *versus* pH of the eluent. ×, Unknown peak; △, trigonelline; ○, caffeine; □, theobromine.

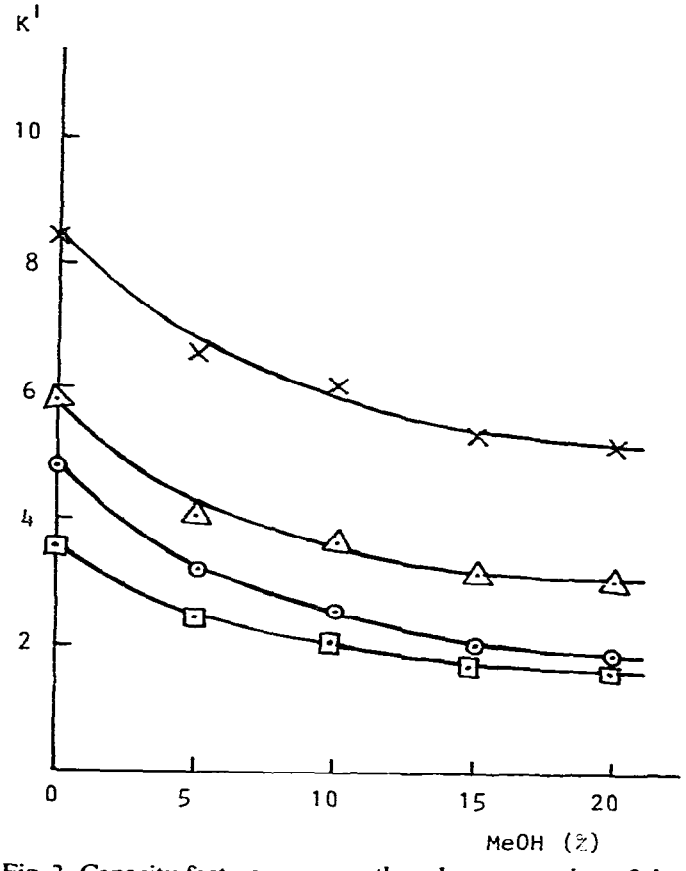

Fig. 3. Capacity factors *versus* methanol concentration of the eluent. ×, Unknown peak; △, trigonelline; ○, caffeine; □, theobromine.

1.1 ml/min. A test chromatogram under these conditions is shown in Fig. 4 (theobromine, 0.05 g/l; caffeine, 0.1 g/l; trigonelline, 0.2 g/l). The plate numbers and

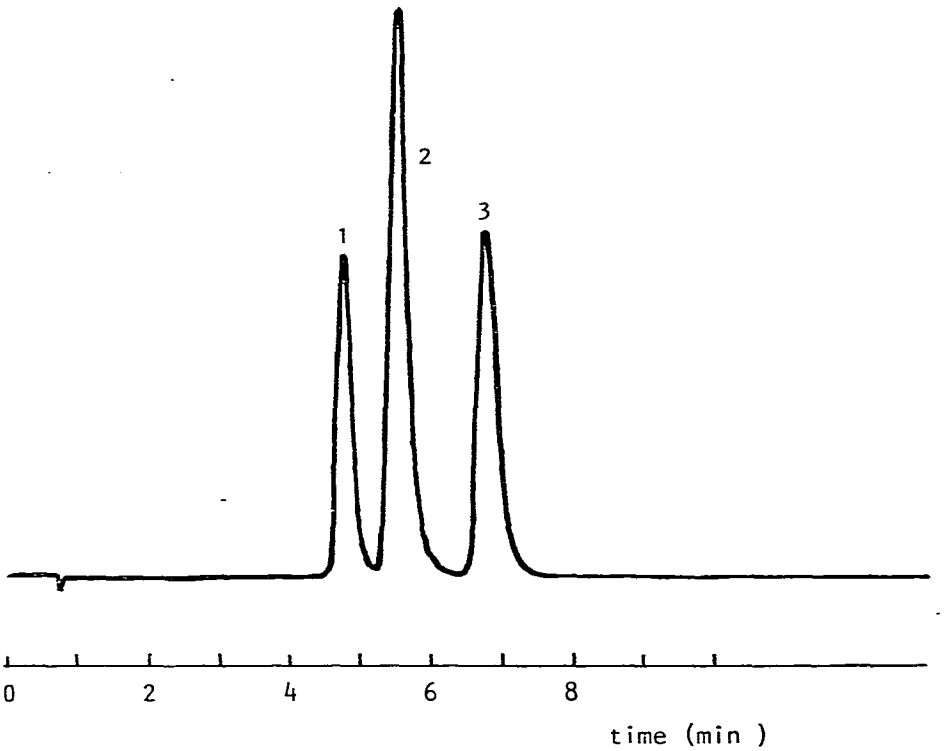

Fig. 4. Chromatogram of a test mixture. 1, Theobromine; 2, caffeine; 3, trigonelline.

resolution factors for this chromatogram are given in Table I. Plate numbers are calculated from the formula $N = 5.54\ (t_R/w)^2$
and resolution factors from the formula

$$R_s = \frac{t_{R_2} - t_{R_1}}{W_1 + W_2}$$

where W = peak width at half height.

TABLE I

PLATE NUMBERS AND RESOLUTION FACTORS

Compound	*Plate number*	*Resolution factor*
Theobromine	3.0×10^3	Theobromine–caffeine, 1.6
Caffeine	2.6×10^3	Caffeine–trigonelline, 2.4
Trigonelline	3.1×10^3	

A chromatogram of a sample of regular roasted coffee is shown in Fig. 5. The caffeine and trigonelline peaks in this chromatogram were identified on the basis of

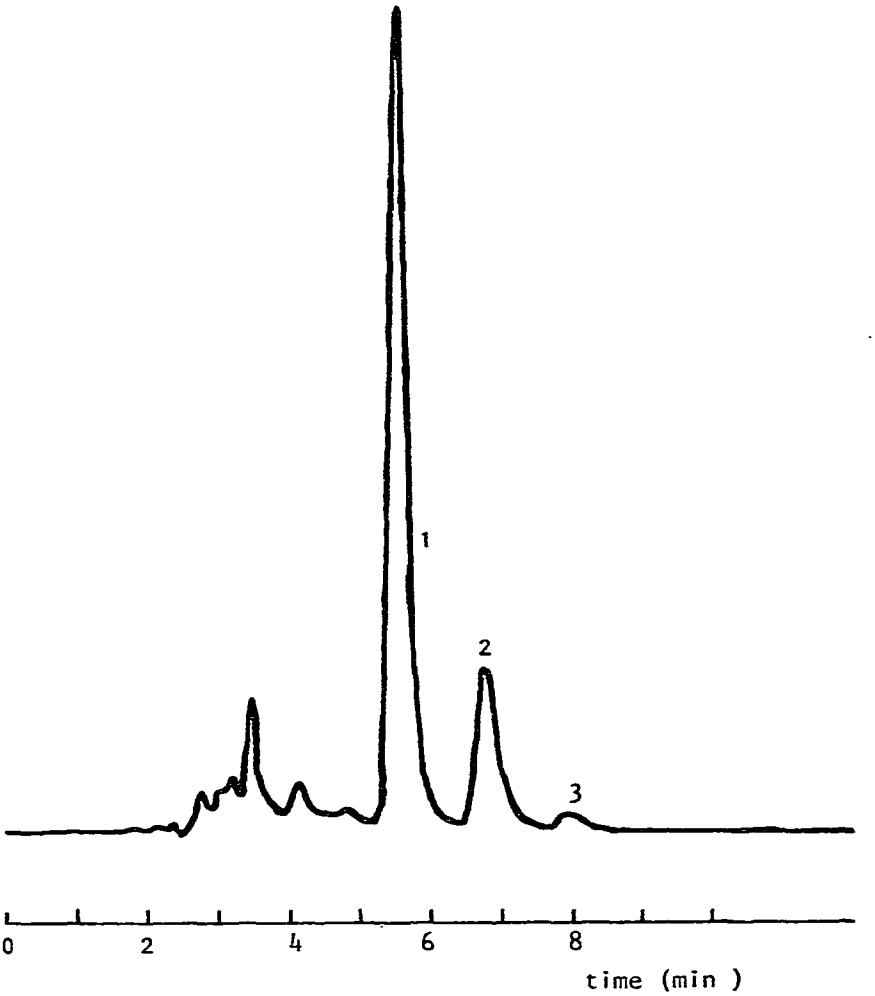

Fig. 5. Chromatogram from a roasted coffee sample. 1, Caffeine; 2, trigonelline; 3, unknown peak.

retention volumes and also by collecting the effluents of these peaks and comparing them with authentic samples by thin-layer chromatography.

Detection and calibration

For caffeine, linear detector responses (at 280 nm) were obtained for injected amounts of up to 500 ng. Analysing six samples of roasted coffee and using external standards, relative standard deviations were 1.7% for caffeine and 3.6% for trigonelline. The recovery of the analytical procedure is better than 99.7% for both caffeine and trigonelline.

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