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Note

Use of microbore high-performance liquid chromatography for the determination of caffeine, theobromine and theophylline in cocoa

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In recent years there has been growing interest in the microbore high-performance liquid chromatography (HPLC) technique[1–5]. This technology involves the use of small internal diameter HPLC columns (1–2 mm). These columns require a substantially lower flow-rate and have a lower sample capacity than conventional HPLC columns. Solvent consumption is decreased by about 80–90% therefore allowing an initial savings in solvent and a subsequent savings in solvent disposal. The lower sample capacity allows for smaller sample size without a loss of efficiency[4] since these columns essentially follow theory developed for 3.9 mm I.D. HPLC columns.

There have been many studies published on the theoretical aspects of microbore HPLC whether in an isocratic or gradient elution mode but few have been published giving practical examples of this technology. This paper describes the determination of caffeine, theobromine and theophylline in cocoa using microbore HPLC.

MATERIALS AND METHODS

HPLC equipment

The HPLC equipment consisted of an M6000A solvent delivery system (Waters Assoc.) equipped with a flow control accessory which permitted microliter per minute flow-rates. The detector used was a Model 440 UV detector (Waters Assoc.) at 280 nm equipped with a 2-μl flow cell. The HPLC injector was a Model 7113 microinjector (Rheodyne).

HPLC columns and mobile phase

The HPLC column used was a Whatman Micro-B ODS-3 (25 cm × 1 mm I.D.) while the mobile phase was water–methanol–concentrated acetic acid (68:31:1, v/v/v) at a flow-rate of 60 μl/min.

Standards

Caffeine, theobromine and theophylline were purified by sublimation, dried *in vacuo* and dissolved in water for a final concentration of 0.1 μg/μl. These standards were stored in the refrigerator and diluted as needed.

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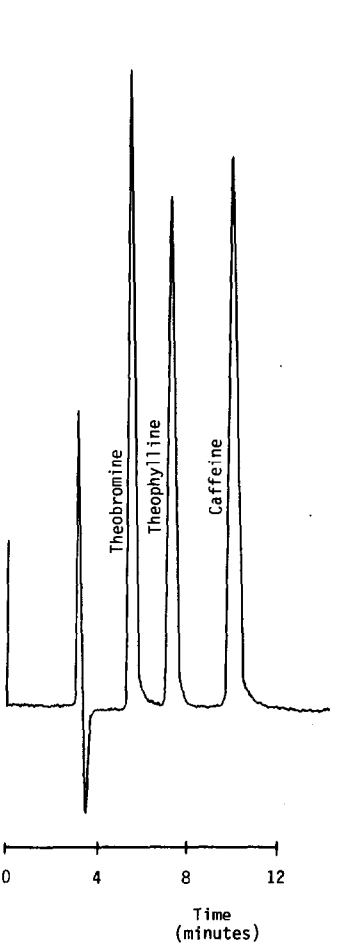
Sample preparation

The AOAC method for the determination of caffeine and theobromine in cocoa and milk chocolate was modified for smaller sample amounts. An amount of 10 mg (± 0.1 mg) was weighed into a 10-ml flask. Approximately 5 ml of water was added and the resulting solution heated at 90°C for 25 min. This step requires continual attention or the flask can easily be heated to total dryness. After 25 min the solution is allowed to cool to room temperature and brought up to a final volume of 5 ml with additional distilled water. When using defatted milk chocolate approximately 20 mg of sample is used. Prior to analysis, samples are filtered through a 0.2-μm filter to remove any particulate matter that might interfere with the final determination.



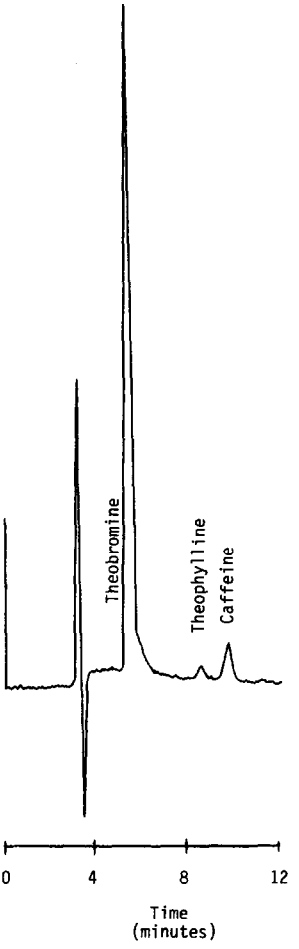


Fig. 1. Chromatogram of xanthine standards. Column, Micro-B ODS-3; mobile phase, methanol–water–acetic acid (68:31:1); flow-rate, 60 μl/min; UV detector, 280 nm.

Fig. 2. Chromatogram of cocoa extract. Conditions as in Fig. 1.

Analysis

A volume of 1 μl of both standard and sample was injected. Final concentrations were calculated by comparing the peak height of the standard with the peak height of the sample. Fig. 1 shows the chromatograms of the three standards while Fig. 2 shows a chromatogram of a cocoa extract.

RESULTS AND DISCUSSION

The method described in this paper was evaluated for accuracy and precision. Four samples of cocoa were extracted and analyzed for caffeine and theobromine using this microbore technique. These samples had previously been analyzed by the official AOAC technique[6] and had a known composition. Table I summarizes the data for this determination.

Precision studies were also accomplished on both sample extracts and standard solutions. These data are summarized in Table II.

TABLE I

COMPARISON OF MICROBORE AND CONVENTIONAL HPLC RESULTS

$n = 4$.

	Microbore	*Conventional*
Theobromine (%)	2.38	2.45
Caffeine (%)	0.20	0.20

TABLE II

PRECISION STUDY FOR STANDARDS AND SAMPLE

$n = 5$.

	Standard		*Sample*	
	Conc. (μg)	*C.V. (%)*	*Conc. (μg)*	*C.V. (%)*
Theobromine	0.1	2.33	23	4.07
Caffeine	0.1	1.04	2	1.85

The results indicate that the method has excellent precision. A plot of peak height *versus* concentration of compound injected indicates that the data are linear over a range of 0.1 to 1.0 μg with regression coefficients of greater than 0.98. The lower limit of detection at 2 times signal-to-noise is 100 pg per injection for theobromine and theophylline and 150 pg per injection for caffeine.

The results demonstrate the practical use of the microbore technique in the food industry for the determination of caffeine, theobromine and theophylline. It allows for decreased solvent consumption and decreased sample size. The experimental results indicate that it compares favourably with data generated using more conventional HPLC columns. This study used HPLC equipment that was modified to accommodate microbore requirements. It would have additional benefits if an analyst were doing experiments with a limited amount of sample.

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