CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Caffeine In Beverages

Purpose
Determination of caffeine in beverages.

Caffeine (F1) is a mild central nervous system stimulant, generally regarded as safe by the FDA. However, individual tolerances vary and unfavorable side effects may occur if excessively large quantities are ingested. Since caffeine is a naturally occurring component of many plants from which food products are derived, there is a considerable need for convenient, reliable analytical methods for monitoring caffeine levels in these materials. This is particularly true in the case of beverages, since those produced from coffee, tea, kola nuts, and cocoa beans typically contain high levels of caffeine. Most coffees (except those which are specially processed), teas and many soft drinks fall into this category.

Conditions
System: BAS 400 Liquid Chromatograph
Column: BAS 3 µm Phase II ODS (100 x 3.2 mm) (PN MF-6213)
Detector: BAS UV-8 fixed wavelength (254 nm)
Mobile Phase: 19% (v:v) methanol, 81% 0.01 M KH_2PO_4 (pH 2.3). Flow rate was 0.8 mL/min.
Detection Limit: 1 ng injected caffeine (S/N = 6).

Sample Preparation
The calibration curve (F2) was constructed by injecting 20 µL volumes of standard solutions appropriately diluted. Absorbances were calculated by comparing peak heights to full-scale deflection. Samples of Pepsi-Cola® were diluted 1:20 with distilled water and injected in 20 µL volumes.

Notes
Caffeine standards and caffeine in Pepsi-Cola® were readily detected at 254 nm (F3).

Caffeine has absorbance maxima at 204 and 272 nm; additional sensitivity might be achieved at these wavelengths.

This determination of caffeine also can be performed on the BAS 200 Problem Solver.

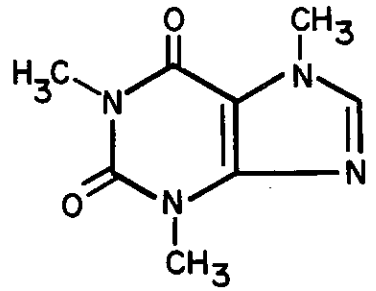

Figure 1. Structure of caffeine.

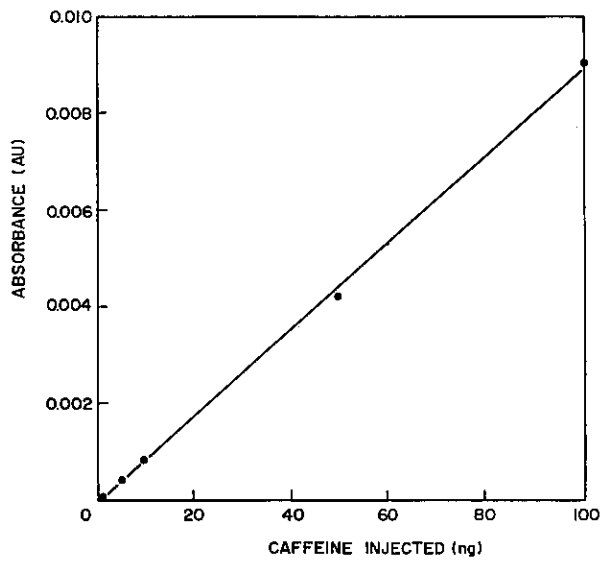

Figure 2. Calibration curve for caffeine standards. Each point represents the mean of 3 determinations.

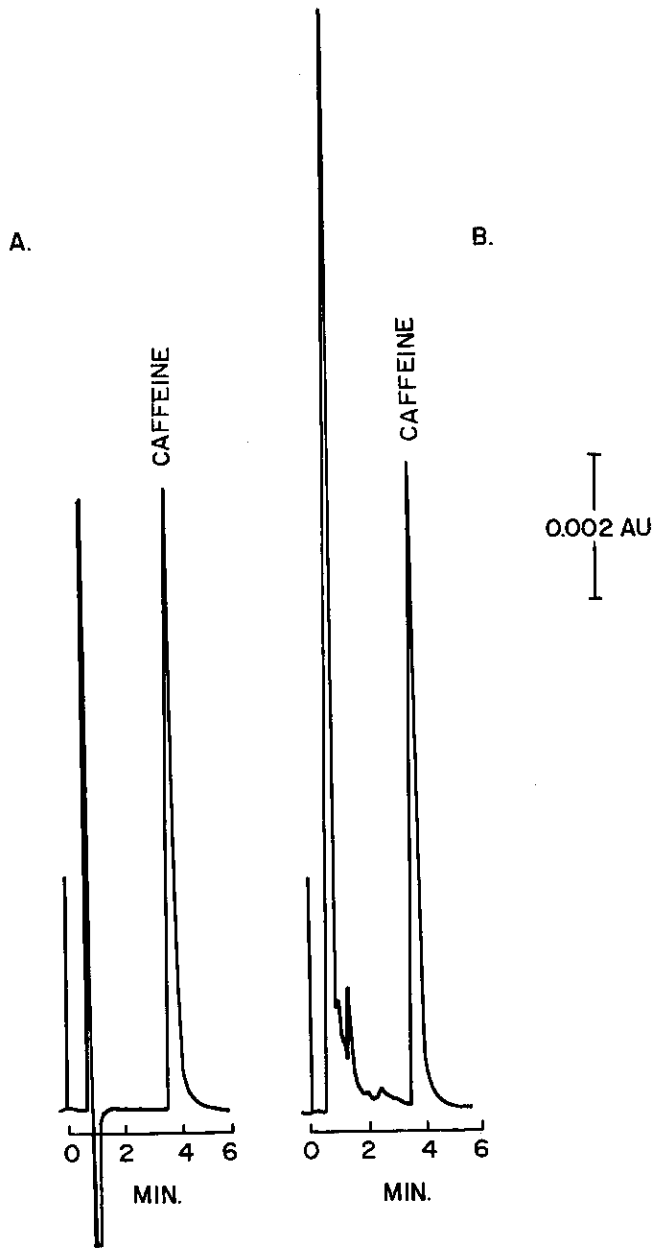

Figure 3. Chromatograms of caffeine standard (A) and Pepsi-Cola® (B). The beverage sample contained 102 µg/mL caffeine (36.1 mg/can)