

Comparison of Gas Chromatographic and Spectrophotometric Methods of Determination for Caffeine in Coffees and Teas

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Caffeine (CAF) levels in instant regular coffees, instant decaffeinated coffees, and instant teas are determined and compared using spectrophotometric and gas chromatographic methods of determination. Modification of a previously reported spectrophotometric assay for CAF in biological fluids is necessary to reduce interference from theophylline (THP) and theobromine (THB). Readjustment of the buffer pH and the introduction of an intermediate buffer wash were found to effectively limit interference from THB and THP. The gas chromatographic assay involves extraction of CAF into benzene followed by evaporation of the benzene and redissolution of the CAF into chloroform for injection into the gas chromatograph. The spectrophotometric method consistently gave higher levels of CAF than were found by the gas chromatographic method for the decaffeinated and regular coffees. However, these differences were not observed for the teas. Using the results from the gas chromatographic assay, two decaffeinated coffees were found to be greater than 97% CAF free. CAF levels in regular coffees were found to vary as much as 32% within the group of five brands studied.

Caffeine (CAF) is a widely used self-administered stimulant found in coffee, tea, and some cola beverages. Its usage as derived from these sources is widely reported to result in significant actions on the central nervous system, kidney, and cardiovascular system. Little, however, is known concerning the intestinal absorption of CAF from these various types of beverages.

Prior to initiating studies to compare the absorption characteristics of CAF from coffees, teas, and colas, a study was undertaken to determine a suitable and rapid means of determination of CAF in small volumes (100–200 μ l) of beverage sample. The need to analyze small sample volumes was a prerequisite in design of a suitable assay procedure. A recognized method of determining drug absorption profiles in animals entails perfusing the drug solution (here, brews of coffees and teas) through the intestine of anesthetized rats and determining (via sampling 100 μ l of solution) the amount of drug remaining to be absorbed (Doluisio, 1969). Reported methods of determination of CAF include a variety of spectrophotometric assay procedures (Ishler, 1948; Cornish and Christman, 1957; Newton, 1969; Somorin, 1973; Association of Official Analytical Chemists, 1970), gravimetric methods (Martinek and Wolman, 1955; Marine Font and Sancho Riera, 1973), Kjeldahl procedures (Wolman, 1955; Moores and Campbell, 1948; Association of Official Agricultural Chemists, 1965), and liquid chromatographic procedures (Wolford, 1971; Nelson, 1973; Murgia, 1973). Although many of these methods have been available for some time, the applicability of these procedures for the determination of small quantities of CAF dissolved in small sample volumes is limited.

Recently, Marks and Kelley (1973) used a spectrophotometric method originally developed for the determination of CAF in small sample volumes of biological fluids (Axelrod and Reichenthal, 1953) to find the levels of CAF in coffee, tea, and cola samples. The applicability of this procedure was further tested by determining the CAF levels in several coffee and tea samples and comparing the results to those obtained from a gas chromatographic procedure developed in our laboratory. These preliminary studies showed rather large differences in the CAF levels found by the two assay procedures. In certain

cases, the differences were of such a large magnitude that further detailed studies to compare the two assay methods were undertaken.

EXPERIMENTAL SECTION

Apparatus. A Hewlett-Packard gas chromatograph Model 5711 A with a flame-ionization detector was used. Peak integration was measured with a Hewlett-Packard electronic chart integrator, Model 7123 A. Dual 6-ft glass columns, 0.25 in. o.d., were packed with 2.5% SE-30 on Chromosorb G, DMCS 80–100 mesh. The N_2 flow rate was 60 ml/min. Oven, injector, and detector temperatures were maintained at 190, 250, and 250 °C, respectively. Attenuations ranged from 10×8 for regular coffees and teas to 10×1 for decaffeinated coffees.

Coffees and Teas. Instant coffees and instant teas (all domestic) were purchased locally in the Albuquerque, N.M. area during 1975. A 2.0-g sample of coffee or 0.5-g sample of tea was dissolved with heat and stirring into distilled water, and the volume adjusted to 200 ml. These concentrations approximate what would be considered an average consumption level for a cup of instant coffee or tea as might be prepared in the home.

Procedure. *Spectrophotometric Methods.* The spectrophotometric method of Axelrod and Reichenthal (1953) was employed with modifications to reduce interference from theophylline (THP) and theobromine (THB). Two-tenths milliliter of coffee or tea sample, 1.0 ml of citrate buffer, pH 3.0, and 17 ml of benzene were placed into a 50-ml glass centrifuge tube fitted with a Teflon-lined cap. The samples were shaken for 20 min and centrifuged. Fifteen milliliters of the benzene layer was transferred to another 50-ml centrifuge tube and 5.0 ml of pH 3.0 citrate buffer was added. After shaking and centrifuging as before, 10 ml of the benzene layer was shaken with 6 ml of 5 N HCl, and the acid layer read at 273 $m\mu$ in a Perkin-Elmer Model 124 double-beam spectrophotometer.

Gas Chromatographic Method. Two-tenths milliliter of coffee or tea sample, 1.0 ml of citrate buffer, pH 3.0, and 12 ml of benzene were added to a 50-ml glass centrifuge tube fitted with a Teflon-lined cap. The samples were shaken for 20 min and centrifuged. A 5-ml aliquot of the benzene layer was transferred to a tapered 15-ml glass centrifuge tube, and the sample evaporated to dryness under vacuum at an oven temperature of 59 °C. The samples were then redissolved with 100 μ l of chloroform

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Table I. Recovery of Caffeine (CAF) in the Presence of THP and THB Using Unmodified and Modified Spectrophotometric Methods

Composition of sample ^b	Apparent CAF, % recovd ^a		Level of significance of differences of means
	Unmodified assay	Modified assay	
CAF (115 mg)	100 ^c	100 ^c	
CAF (115 mg) + THP (115 mg)	144.5 ± 4.2 (<i>p</i> < 0.0025) ^d	103.7 ± 1.3 (<i>p</i> < 0.05) ^d	<i>p</i> < 0.001
CAF (115 mg) + THP (115 mg) + THB (70 mg)	165.7 ± 7.8 (<i>p</i> < 0.0025) ^d	106.5 ± 3.0 (<i>p</i> < 0.05) ^d	<i>p</i> < 0.001

^a Mean ± standard deviation of three determinations. ^b All volumes adjusted to 200 ml. ^c Represents control samples. ^d Level of significance in comparison to control samples.

Table II. Recovery of Caffeine (CAF) Added to 1.0 g of Instant Coffee Using the Gas Chromatographic Assay Method

Amt. added, mg	Total CAF recovd, mg	Net CAF recovd, mg	% recovd of added CAF ^a
0	26.2 ± 0.4	0	
14	40.8 ± 0.2	14.6 ± 0.2	101.4 ± 0.4
35	61.1 ± 1.1	34.9 ± 1.1	99.8 ± 1.8
49	78.0 ± 1.1	51.7 ± 1.1	103.6 ± 1.5
70	98.2 ± 2.9	72.0 ± 2.9	102.1 ± 3.0

^a Mean of three determinations ± standard deviation.

and a 2- μ l sample was injected onto the column. A series of CAF standards was prepared daily and run during the same time period as the coffee and tea samples. Retention time for CAF was 3.8 min. Absolute reproducibility of injected volumes was measured to be 2.1% based on full scale peak readings using a Hamilton 10- μ l syringe, Model 19000 A. The absolute recovery of CAF from aqueous samples was measured to be 94.4%.

RESULTS AND DISCUSSION

Initial studies using the method of Axelrod and Reichenthal (1953) proved to be unsatisfactory due to the potential for interference from THP and THB, both of which are also found in beverages containing CAF. A comparison of the results obtained using their assay and the modified assay reported here is shown in Table I. The addition of THP and THB to aqueous solutions of CAF resulted in marked and significant increases in recovery of apparent CAF utilizing the method of Axelrod and Reichenthal unmodified. However, a significant reduction in the recovery of apparent CAF was observed utilizing the modified spectrophotometric assay, with increases of only 3.7 and 6.5% over control values for the addition of THP or a combination of THP and THB, respectively. Re-adjustment of the buffer pH from 7.0 to 3.0 and the introduction of an intermediate buffer wash effectively aided in reducing interference from THP and THB, without significantly altering the extent of extraction of CAF from the aqueous solution.

To validate the applicability of the gas chromatographic assay for the quantization of CAF in coffees and teas, two studies were performed. First, 0–70 mg of CAF was added to a solution containing 1.0 g of instant coffee in 200 ml of water. The peak area measurements for CAF extracted from instant coffee and from water as a function of the milligrams of CAF added were found to be linear with the slopes parallel (Figure 1). Assuming CAF was extracted to the same extent from coffee as from water, the slopes would be parallel, differing only in the intercept value on the y axis. This intercept value, therefore, represents the concentration of CAF in the coffee sample before addition of CAF. The observed peak area of 34 for the y intercept represents a concentration of 26.2 mg of CAF/1.0 g coffee sample. The overall net recovery of CAF added to 1.0 g of coffee averaged 101.7% (Table II).

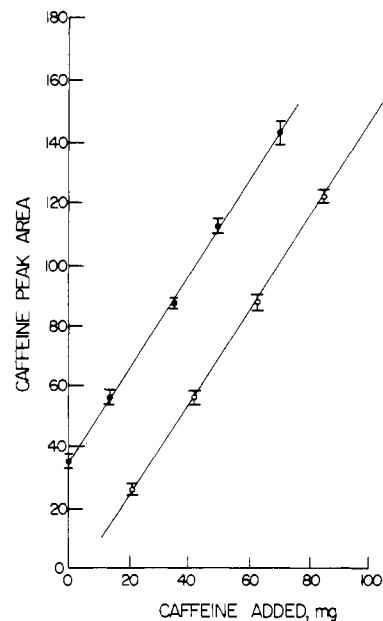


Figure 1. Peak area measurements for CAF added to a 1.0-g sample of coffee (●) and to water (○).

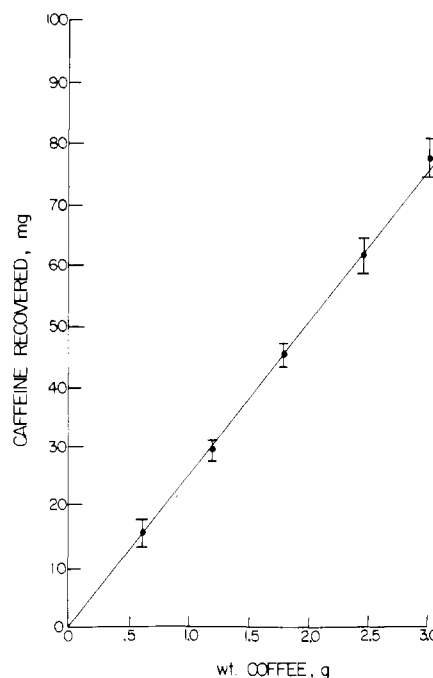


Figure 2. Recovery of CAF from coffee as a function of the weight of coffee analyzed.

Second, the recovery of CAF from aqueous dilutions of instant coffee ranging from 0.5 g/200 ml to 3.0 g/200 ml was studied. A plot of milligrams of CAF recovered as a function of grams of coffee present was found to be linear with a slope of 25.3 mg of CAF/1.0 g of coffee and an

Table III. Caffeine (CAF) in Instant Coffees Using a Gas Chromatographic and a Modified Spectrophotometric Assay Method

Products	CAF, mg/2.0 g sample	
	Gas chromatographic assay	Spectrophotometric assay
Regular coffees ^a		
Coffee C1	58.3 ± 2.6	67.0 ± 2.5
Coffee C3	76.0 ± 1.8	82.2 ± 1.6
Coffee C4	67.8 ± 0.9	72.3 ± 1.3
Coffee C5	63.9 ± 2.1	70.1 ± 3.1
Coffee C8	54.8 ± 1.3	58.7 ± 2.5
Decaffeinated coffees ^b		
Coffee C2	2.25 ± 0.24	7.88 ± 0.85
Coffee C10	1.62 ± 0.16	9.72 ± 1.57
Coffee C12	1.25 ± 0.18	9.95 ± 0.14

^a Mean of four determinations ± standard deviation.

^b Mean of three determinations ± standard deviation.

Table IV. Caffeine (CAF) in Instant Teas Using a Gas Chromatographic and a Modified Spectrophotometric Assay Method

Products	CAF, mg/0.5 g sample ^a	
	Gas chromatographic assay	Spectrophotometric assay
Tea T1	29.2 ± 0.65	29.9 ± 0.60
Tea T4	33.4 ± 1.0	34.2 ± 0.65
Tea T6	33.0 ± 1.3	33.0 ± 0.90

^a Mean of four determinations ± standard deviation.

intercept passing through the origin (Figure 2). These findings compare favorably with those observed in Figure 1 for the level of CAF in a 1.0-g coffee sample (26.2 mg of CAF/1.0 g of coffee) and support the applicability of the proposed gas chromatographic assay for CAF as outlined in this current study.

Tables III and IV show a direct comparison of the mean values ± standard deviation for CAF in coffee and tea samples as determined from the gas chromatographic and modified spectrophotometric methods. Values for CAF in coffee are reported as per 2.0-g sample whereas values for CAF in tea are reported as per 0.5-g sample.

An important finding is that CAF levels for the decaffeinated coffees are several magnitudes higher using the spectrophotometric assay in comparison to the gas chromatographic assay. The differences were significant at the $p < 0.001$ level for all three samples studied. On the average, the spectrophotometric method gave 7.5 mg more CAF/2.0 g sample than did the gas chromatographic assay. Recently, Burg (1975) suggested that a value of 3 mg of CAF/cup of instant decaffeinated coffee (with a range of 2–8 mg as determined by spectrophotometric methods) be taken as a standard value. It would appear from the present studies that this value is somewhat high, with a more realistic range being 1–3 mg/cup, based on the results of the gas chromatographic method of determination.

These differences observed in the two analytical methods for CAF levels in decaffeinated coffees were also found in the determination of CAF in instant regular coffees, but not in instant teas (Tables III and IV). Mean differences for CAF in the two assay procedures ranged from 3.9 mg/2.0 g in coffee sample C8 to 8.7 mg/2.0 g in coffee sample C1. All regular coffees showed significant differences between the mean values reported in Table III

at the $p < 0.05$ level. Instant teas, however, showed no significant differences between the mean values reported in Table IV ($p > 0.05$) and an excellent correlation between the two assay methods was observed in these cases. It is therefore apparent that a substance found in all the coffees studied, which gives rise to higher than normal absorbance readings, is not likewise present in the instant teas. The possibility that the interfering substance is either THP or THB can be ruled out based on the fact that the modified spectrophotometric assay method used here effectively reduces interference from THP and THB (Table I). Also, teas are reported to contain higher levels of THP than coffees, and no significant difference between the two assay methods was observed for the instant teas.

Of significance is the finding that the CAF level in regular instant coffees varied by as much as 32% (samples C3 and C8) in the group of samples studied, based on the results of the gas chromatographic assay. This difference was found to be significant at the $p < 0.001$ level and is consistent with the wide variation in CAF levels in instant regular coffees reported in a survey by Burg (1975).

Based on label and advertising claims, decaffeinated coffees are said to be 97% CAF free. This claim was evaluated by comparing CAF levels in brands C5 and C10 (both supplied by one processor) and in brands C3 and C12 (also supplied by one processor) utilizing the data from the more reliable gas chromatographic assay. By direct ratio, brand C10 was found to be 97.5% CAF free in comparison to brand C5 and brand C12 was found to be 98.5% CAF free in comparison to brand C3. Thus, both brands of decaffeinated coffee were found to exceed manufacturers' claims of 97% CAF free.

The application of gas chromatography to the analysis of CAF in small sample volumes of coffees and teas allows for rapid determinations with high reproducibility and specificity over a wide range of CAF levels. In particular, the specificity and sensitivity of the gas chromatographic method of determination at low CAF levels, such as are found in decaffeinated coffees, would appear to offer distinct advantages over the spectrophotometric method compared here.

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