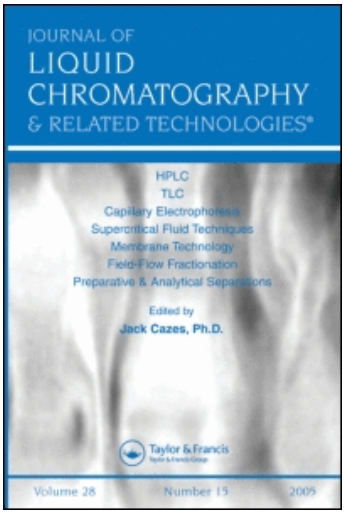

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Comparative HPLC and GLC Determination of Caffeine in Different Food Products

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COMPARATIVE HPLC AND GLC DETERMINATION OF CAFFEINE IN DIFFERENT FOOD PRODUCTS

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ABSTRACT

Two chromatographic (HPLC and GLC) methods are described for direct analysis of caffeine, and some related xanthine derivatives in various kinds of food products and beverages. For HPLC, a 10 uC_{18} reverse phase column and for GLC, a non polar OV17 column were used. Caffeine and/or other alkaloids are effectively extracted with $CHCl_3$ containing 5% isopropanol from NH_4OH alkalinized prepartions. The extract was used for direct analysis by any of the proposed methods. Better resolution and more sensitivity are achieved by the HPLC procedure. Detectability in both HPLC and GLC is at the nanogram levels and the two methods can be applied for caffeine determination in decaffeinated products as well as in minicoffee tablets and in beverages.

INTRODUCTION

Caffeine-containing products represent one of the most popular food formulations all over the world (1). Concern about the possible adverse health effects of caffeine on the consumer

has resulted in the development of decaffeinated products (containing about 1-3% of the original amount of caffeine).

Many analytical approaches have been reported for the analysis of caffeine and other related xanthine alkaloids (theophylline and theobromine) in biological fluids and in coffee, tea and cocoa products (1-26). Some GLC procedures involve either multiple extraction steps, derivatization steps, or use of gas chromatography - mass spectrometry (3-5). Several spectrophotometric and HPLC methods have been developed for the analysis of caffeine or other methylated xanthines in special materices, though reported (6) that most of these methods require a specific preparation of sample and considerable time for completion. Besides, some of the reported methods cannot separate mixtures of some xanthine alkaloids in a reasonable time period (7-9). Klassen and Stavric (6) on the other hand, described an HPLC method for rapid analysis of caffeine and seven of its metabolites in plasma, urine, milk and saliva in a single operation using a $5uC_{18}$ reverse phase column. Chloroform-isopropanol (85:15) was the solvent used for extraction of these compounds from the investigated samples. Other methods for the HPLC determination of caffeine in biological fluids were also devised (10-13).

As concerning food products, many HPLC methods, were reported for the analysis of its caffeine content; in coffee, tea, beverages and choclatè preparations (1,14-17); in decaffeinated instant coffee and tea (18,19) and in drugs (20-22).

It was reported, however, that most of the methods devised for the analysis of formulations with full caffeine content, cannot be reliably applied for the determination of caffeine in decaffeinated products due to their much lower caffeine content. An official AOAC method (2) and some other methods (23) on the other hand, exist for determination of caffeine in decaffeinated coffee only.

The objective of this study is to develop a versatile and accurate HPLC method for the direct determination of caffeine and other xanthine alkaloids in food products, including all types of coffee, different brands of tea and choclate preparations, cola seeds and soft drinks supposed to contain it. The results are compared with those obtained by GLC procedure.

EXPERIMENTAL

Apparatus:

I A Water Associates (Milford, MA) Liquid Chromatograph was used for the HPLC analysis. The instrument was equipped with a 6000 A pump, U6K injector, Lambda Max 481 Detector and a M 730 Data Module. A reversed-phase column (uBondapak C_{18}, 8 mm x 10 cm, 10μ, Waters Associates) was used for the analysis.

II Gas Chromatograph : Varian-Model 3700 with Dual FID. A coiled glass columns (1.5 m x 4 mm i.d.) packed with 3% OV 17 on chromosorb W.H.P., 50-100 mesh was used for the analysis.

Food Materials

29 Different food products collected from the local market in Riyadh city; these include 9 tea brands; 10 coffee brands (green and roasted) including instant soluble coffee, minicoffee tablets; 2 decaffeinated instant coffee; 3 choclate products; 2 types of cola seeds and 2 kinds of soft drinks.

Standard Alkaloids

Caffeine, theobromine and theophylline were (BDH, Biochemicals), dried in a vacuum oven at 60°C before use. Standard caffeine solution was done by dissolving 25 mg in 25 ml $CHCl_3$ (for GLC) or in 10 ml of the mobile phase (for HPLC).

METHODS

Chromatographic Conditions:

I - HPLC

Several preliminary trials for HPLC separation of caffeine, theophylline and theobromine were carried out. Best resolution was achieved by using a mobile phase composed of acetonitrile and water with 2% acetic acid with the following gradient sequence:

Time (min)	Flow (ml/min)	Acetonitrile	Water
initial	2.0	10	90
10	2.0	40	60
15	2.0	10	90

The UV detector was adjusted at 274 nm.

II - GLC

Different conditions using different polarity columns for the direct GLC separation of the three xanthine alkaloids were tried. The best conditions were achieved on the non polar OV 17 stationary phase under the following operating conditions:

Column temperature 120°C programmed to 220°C with a rate of 10°/min; injection port and detector temperatures, 250°C; Carrier Gas, N_2 with a flow of 40 ml/min; gases for the FID, H_2 at 30 ml/min and air with 300 ml/min.

Qualitative identification in both techniques depends on retention time comparison with standard xanthine alkaloids and by spiking each sample with the expected alkaloid. Quantitative analysis was carried out by using the integrated area under each peak and the percent of each alkaloid was deduced from the respective standard curve using the external standard quantitation method.

Linearity of both methods was tested by injection of increasing amounts of the standard solutions; the GLC method was linear from 0.2 µg to 10 µg caffeine while in HPLC, the method was linear from 0.3 µg to 25 µg of the alkaloid.

Caffeine content in selected food materials was also determined by other methods viz. spectrophotometric (24) non-aqueous titration (25) and gravimetric procedure (26).

Extraction Procedures:

I - For Solid Materials:

Accurately weigh 2 g of powdered material (different brands of tea, coffee, cola seeds and choclate) into a 25 ml beaker. Add about 2 ml NH_4OH (1:2) dropwise and mix thoroughly to moisten the powder. Leave for 30 min and pack through a small extraction thimble or in small filter paper into a small soxhlet. Extract continuously with chloroform containing 5% isopropanol (50 ml) for about 40 min. Filter and distill the solvent at low temperature, under reduced pressure. Dissolve the residue in pure chloroform, transfer to a 25 ml volumetric flask and complete to volume with the same solvent.

For minicoffee tablets, weigh accurately two tablets, grind alkalinize with dilute NH_4OH and proceed as mentioned above for extraction of the alkaloids.

II - For Soft Drinks:

Measure accurately 50 ml of the product into a 250 ml separating funnel, render alkaline (to pH 8-9 using pH paper) with ammonia solution and extract with chloroform containing 5% isopropanol (3 x 100 ml). Wash the extract with few mls of water and dehydrate by filtering over anhydrous sodium sulfate. Distill the solvent under reduced pressure, dissolve the residue in few mls of chloroform, transfer to a 10 ml volumetric flask and complete to volume with the same solvent.

For GLC analysis, inject aliquot volumes of the prepared solutions (from 0.2 to 2 µl) so as to bring the peak height of caffeine within 2/3 of the chromatographic paper.

For HPLC analysis, distill 10 ml of the prepared solution of the solid materials (equivalent to 0.8 g powder) or the 10 ml of the soft drink extract, dissolve the residue in 10 ml of the mobile phase; use 5 µl for the analysis.

The caffeine content was calculated in g% for solid samples and in mg% for liquid samples.

Recovery study

Add proper volume of the caffeine standard solution to each sample so that caffeine content of spiked sample will approximately double. Determine caffeine content of spiked sample (in triplicate) by both techniques as described above and calculate percent recovery (Table 1).

RESULTS AND DISCUSSION

The adopted HPLC conditions showed much better resolution of the three alkaloids when compared by GLC as it is evident from Fig. 1 & 2.

On the other hand, the recovery study (Table 1) revealed quantitative recovery (99-104%) by the HPLC procedure; by GLC, however, the recovery was lower (80-95%), a fact which can be

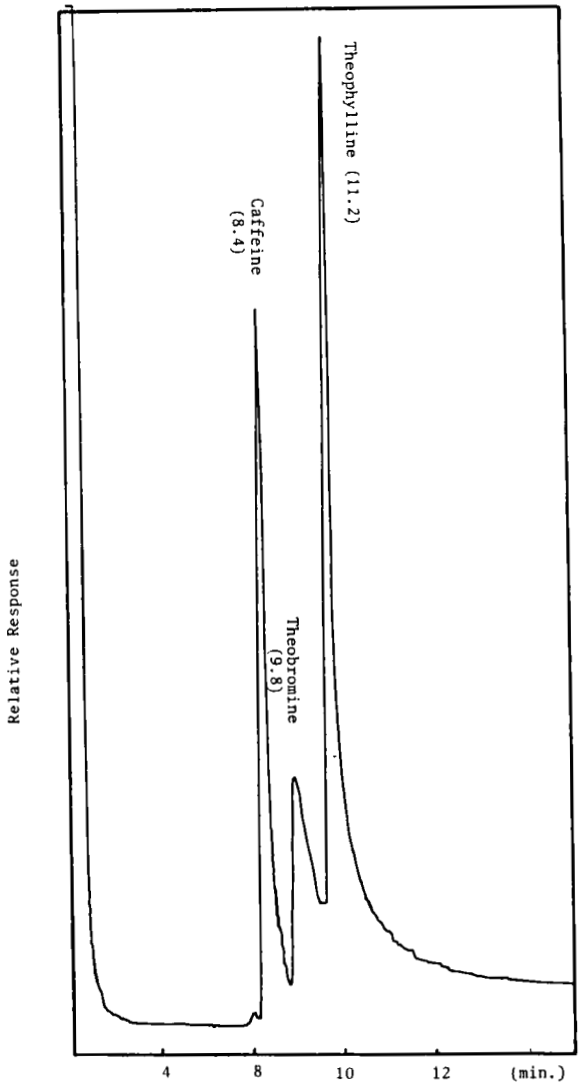

Fig. 1. GLC of standard mixture of Caffeine, Theobromine and Theophylline
Column, coiled, glass 1.5 m X 4 mm i.d. packed with 3% OV 17 on Chromosorb W.H.P., temp. 120°c programmed, 10°/min. to 240°c; Carrier gas, N_2 with 40 ml/min; detector, FID at 250°c.

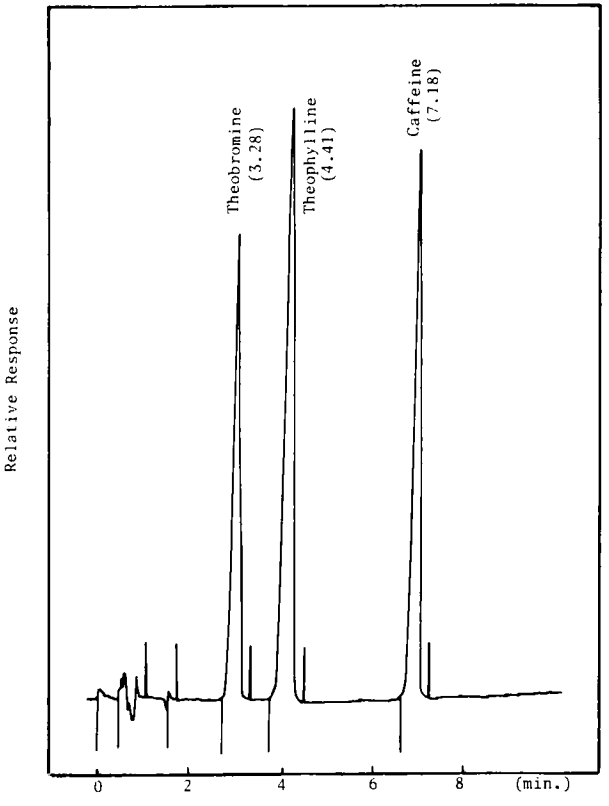

Fig. 2. HPLC chromatogram of standard mixture of Caffeine, Theobromine and Theophylline.

Column : μ Bondapak C_{18}, 10 cm X 8 mm i.d.; mobile phase, gradient composed of acetonitrile/ water with 2% acetic acid; gradient sequence is shown in the text; detector UV at 274 nm.

TABLE 1

Comparative Recovery % of Caffeine
(Results are the average of determinations in triplicate)

mg added	mg Found		Recovery %	
	HPLC	GLC	HPLC	GLC
10	9.9	8	99	80
20	20.1	18	100.05	90
40	40.0	38	100	95
50	50.2	47	100.4	94
70	69.9	66	99.85	94
80	80.01	75	100.01	93.75
100	100.1	80	100.1	80

attributed to the detector response and/or to the relatively poor resolution of the eluted components.

Table 2 shows a comparative determination of caffeine by the proposed HPLC and GLC methods and by spectrophotometric, non-aqueous titration and gravimetric procedures.

In HPLC, the precision of the method was estimated by analysing six samples of roasted coffee and using the external standard where the relative standard deviation for caffeine was found to be 1.5%. Moreover, the recovery of the analytical procedure was good (99-104%). The method proved adequate sensitive for the determination of caffeine in the samples studied.

However, GLC and non aqueous titration gave lower results possibly due to the variation in detector response and presence of interfering substances respectively while the spectrophotometric and gravimetric methods gave in certain samples higher results, possibly due to their non specificity.

TABLE 2

Comparative Determinations of Caffeine
(Results are the average of determinations in triplicate)

Sample	Caffeine % HPLC	GLC	Spectro.	Non aq.	Grav.
Tea brand	3.3	2.4	3.5	2.6	3.25
Coffee (green)	1.0	0.98	1.15	0.9	1.40
Coffee (roasted)	2.06	2.00	2.06	1.98	2.35
Kola seeds (fresh)	1.7	1.35	1.69	1.21	1.30

It is interesting to report that the used solvent (chloroform containing 5% isopropanol) was found to be efficient and somewhat specific for extraction of the xanthine alkaloids free from other non alkaloidal contaminants. The so obtained extract can be directly applied to both chromatographic techniques without the need for any further purification steps (c.f. previously reported methods).

From the data cited in Table 3, it is evident that there is a variation of caffeine contents in the different brands; being from 1.3-3.3% in tea samples; 0.76-1.00% in green coffee; 1.0-2.06% in roasted coffee; 2.52% in minicoffee tablets; 0.01-0.08% in decaffeinated coffee samples (This range values may be due either to differences in varieties of tea leaves or coffee beans or to differences in extraction rates); 1.9% in air-dried kola seeds and 1.7% in the fresh samples; 0.02-0.08% in choclate powder and 0.004-0.008% in soft drinks.

TABLE 3

Comparative assays of Caffeine in different Food Products under investigation.
(Results are the average of determinations in triplicate).

Sample	Caffeine % HPLC	Caffeine % GLC	Sample	Caffeine % HPLC	Caffeine % GLC
I TEA BRANDS					
1	2.24	2.32	2	1.30	1.25
3	2.65	2.50	4	2.84	2.40
5	3.30	2.40	6	2.92	2.50
7	2.78	2.16	8	2.52	2.10
9	3.10	2.80			
II COFFEE BRANDS					
a) Green					
1	0.76	0.71	2	1.00	0.98
b) Roasted					
1	1.70	1.64	2	2.06	2.00
3	1.00	0.96	4	1.14	1.08
c) Soluble Coffee					
1	1.69	0.95	2	1.30	1.30
3	2.24	0.93	4	1.11	1.63
d) Caffeine Free (instant)					
1	0.80	0.02	2	0.01	0.01
e) Mini Coffee Tablets					
	2.52	2.16			
III COLA SEEDS					
a) Fresh	1.70	1.35			
b) Dry	1.90	1.38			
IV CHOCKLATE POWDER					
1	0.08	0.08	2	0.02	0.022
3	0.02	0.023			
V SOFT DRINKS (mg%)					
1	4.00	2.40	2	8.00	5.6

It is obviously expected that the green coffee beans should contain higher caffeine content than the corresponding roasted coffee as caffeine is sublimable alkaloid.

In contrary to the above fact we found that caffeine content was higher in the roasted coffee (Table 3). This can be attributed to the possible presence of caffeine partially in combination form with tannins in the green samples, which may impair its complete extraction by the used solvent.

In conclusion, among the methods adopted, HPLC offers the most simple, versatile, accurate and sensitive technique. It is suitable for decaffeinated as well as full-caffeine products, choclates and soft drinks. The sensitivity of the assay procedure reached to less than 0.4 ug caffeine (relative standard deviation, 1.5% and recovery % reached up to 100%). Moreover, the use of $CHCl_3$ containing 5% isopropanol for direct extraction of caffeine from slightly alkalinized preparations (pH 8-9) using ammonia, resulted in the maximum yield of caffeine with the minimum amount of non-caffeine constituents which when left does not interfere by any means in the quantitation of this alkaloid.

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