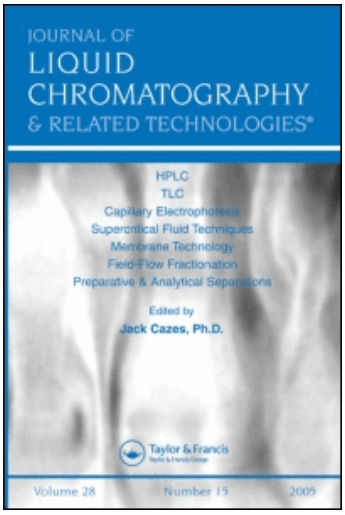

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Determination of Theobromine, Theophylline, and Caffeine in Various Food Products Using Derivative UV-Spectrophotometric Techniques and High-Performance Liquid Chromatography

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DETERMINATION OF THEOBROMINE, THEOPHYLLINE, AND CAFFEINE IN VARIOUS FOOD PRODUCTS USING DERIVATIVE UV-SPECTROPHOTOMETRIC TECHNIQUES AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Accurate, sensitive and precise derivative ultraviolet spectrophotometric and high-performance liquid chromatographic (HPLC) methods are described for the simultaneous determination of three natural methyl xanthines (theobromine, theophylline and caffeine) in various food products. The derivative UV-spectrophotometry, with zero crrosing technique of measurement was selected for determination of theobromine and theophylline. First-derivative procedure (D1) was adopted for theobromine determination in the presence of theophylline and caffeine; and second-derivative procedure (D2) was adopted for theophylline determination in the presence of theobromine and caffeine. Caffeine was determined by compensation method using the zero-

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order spectrophotometry. The second method was based on HPLC on a reversed phase column, using a mobile phase of 0.01 M sodium acetate solution: acetonitrile (85:15%) at PH 4.0 with detection at 272 nm. Both methods show good linearity, precision and reproducibility. The developed methods proved to be applicable for the determination of methyl xanthines in various food products.

The validity of the proposed methods were tested by analyzing laboratory-prepared mixtures containing the three components in various proportions and by analyzing several food products spiked with a known concentration of the three components.

INTRODUCTION

Xanthine derivatives are widely employed as central nervous system stimulants (caffeine), diuretics (theobromine and theophylline), and as respiration stimulants and smooth muscle relaxants (theophylline). The effects produced by various non alcoholic beverages and drinks such as tea and coffee depend mainly on their natural xanthine contents.

Several analytical methods have been reported for the determination of natural xanthines (theobromine, theophylline and caffeine) in various food products, beverages, and several pharmaceutical preparations. Among these methods are gas chromatography[1-5] UV-spectrophotometry[6], densitometry[7], spectrochromatography[8], phosphorimetry[9,10], colorimetry[11,12], derivative-spectrophotometry[13-19] and high-performance liquid chromatography, (HPLC)[20-27]. With the exception of some HPLC procedures, the above mentioned methods lack the capability of quantifying the three natural xanthines in the same sample simultaneously.

This paper reports two methods based on selective derivative UV-spectrophotometry and HPLC assay procedure for determining theobromine (TB), theophylline (TP) and caffeine (CA) without previous separation step. The optimum experimental parameters for each method are described. The paper describes also the applicability of the proposed methods for the determination of the above mentioned natural xanthines in various food products such as tea, coffee, cocoa, chocolate and cola drinks

EXPERIMENTAL

Reagents

Pharmaceutical grade samples of theobromine (TB), theophylline (TP), caffeine (CA), and ethyl paraben (EP, employed as internal standard) were obtained from Merck, Darmstadt, Germany. All other chemicals were of analytical- reagent grade. Methanolic solutions of the drugs were protected from light during the analysis. The mobile phase for HPLC was prepared by mixing 0.01M sodium acetate (NaAC) solution and acetonitrile (HPLC grade) in a ratio of 85:15 and adjusting the pH to 4.0 with glacial acetic acid. The mobile phase was filtered by passing it through a 0.45U membrane filter (Millipore, Bedford, MA, USA) and thoroughly degassing prior to use.

Instrumentation

Zero-order UV, D1 and D2 spectra were measured in 1-cm matched quartz cells using a Shimadzu-UV-visible recording spectrophotometer (Model UV 240) equipped with a Shimadzu derivative accessory unit operating in the first and second-derivative modes. The instrument parameters for zero-order, D1

and D2 spectra were: spectral slit width, 2nm; scan speed, 20nm sec^{-1}; recorder chart speed, 20nm cm^{-1}; wavelength range, 190-300 nm; and ordinate minimum and maximum settings, 0-2 (zero-order spectra); $\pm$0.25 (D1); and $\pm$0.05 (D2). The wavelength calibration was checked by using a holmium oxide-filter, against air.

A chromatographic system composed of a solvent-delivery pump (Model 114M, Beckman Instrument, Int., Geneva, Switzerland); a variable-wavelength UV detector (Model 165, Beckman); an injector with 20 ul loop size (Beckman); and an integrator-plotter (SP 4270, USA) was used. Analysis was carried out isocratically on a 5-um (250 x 4.6 mm i.d.) reversed-phase lichrocart C-8 column (Merck, Hohenbrunn, Germany). The flow rate was 2.0 ml min^{-1}; chart speed 0.25 cm min^{-1}; detector wavelength 272 nm; and sensitivity range 0.02 a.u.f.s.

Procedures

Determination of Theobromine, Theophylline and Caffeine

Accurately weighed amounts of Ca. 50 mg of TB or TP were separately transferred into 50-ml calibrated flasks and adjusted to volume with methanol (1.0 mg ml^{-1}). Similarly, accurately weighed amount of Ca. 100 mg of CA was transferred into 50-ml calibrated flask and diluted to volume with methanol (2.0 mg ml^{-1}). A working internal solution of EP (2.0 mg ml^{-1}) was prepared in similar manner for the HPLC analysis.

Spectrophotometric Methods

In separate 10-ml calibrated flasks, different aliquots 50-300ul (TB or TP) or 50-150 ul (CA) of the stock solutions were

diluted to volume to obtain standard solutions of TB, TP or CA (5-30 ug ml^{-1}). Dilution was either with distilled water (for the zero-order spectrophotometric studies), 0.1M sodium hydroxide (for D1-spectrophotometry), or 0.1M hydrochloric acid (for D2-spectrophotometry). The D1-spectrum for each solution was recorded over the wavelength range 190-300 nm against 0.1M sodium hydroxide and the trough amplitude (D1) at 248 nm (TB) was measured. The D2-spectra were recorded against 0.1M hydrochloric acid and the trough amplitude (D2) at 278 nm (TP) was measured. The zero-order spectrum for each solution of TB, TP and CA was recorded against distilled water and the maximal absorption at 275 nm was measured.

HPLC Method

In separate 1.5-ml disposable plastic microcentrifuge vials, different aliquots of TB, TP or CA stock solutions (5-20 ul) were mixed with 35 ul of the working internal standard solution (EP). The final volume was adjusted to 1 ml with methanol to obtain standard solutions (5-20 ug ml^{-1} TB and TP) or (10-40 ug ml^{-1} CA). The samples were vortex-mixed for 30 s. An aliquot (20 ul) was then injected on to the column at ambient temperature and eluted with the mobile phase under the indicated chromatographic conditions.

Preparation of tea and coffee samples

An accurately weighed amount of Ca.1.0 g of tea or ground coffee was transferred into a 250-ml beaker and boiled for 10 min with 50-ml distilled water and then filtered. To the filterate, sufficient amount of lead acetate was added to precipitate tannin and other polyphenolic compounds followed by gradual addition of dilute sulphuric acid until no more precipitate (lead sulfate) was

formed. The filterate was then transferred into 100-ml volumetric flask and the solution was made to volume with distilled water.

Preparation of Cocoa and Chocolate Samples

An accurately weighed amount of Ca.1.0 g of cocoa powder or chocolate cake, cut into small pieces, was boiled with 50-ml aqueous sodium carbonate solution, containing 5-ml amyl alcohol, for 10 min^{1}. The mixture was then cooled and centrifuged for 10 min. The aqueous layer was then transferred into 100-ml volumetric flask and adjusted to volume with distilled water.

Preparation of Cola Drink Samples

An aliquot (1.0 ml) of decarbonated cola drink solution was transferred into 100-ml volumetric flask and the solution was adjusted to volume with distilled water.

Spectrophotometric Analysis of Food Products

An aliquot (1.0 ml) of the above prepared samples was transferred separately into 10-ml volumetric flask and adjusted to volume with distilled water. From this diluted solution, 300 ul aliquot was transferred into 10-ml calibrated flask and further diluted to volume as previously mentioned under spectrophotometric methods.

For spiked food products, the above procedure was followed exactly but with the transfer of 300 ul aliquots from the stock solutions of TB, TP and CA into the calibrated flasks prior to volume adjustment to 10 ml.

HPLC analysis of food products

An aliquot (1.0 ml) of the above prepared samples was transferred separately into 10-ml volumetric flask and adjusted to

volume with distilled water. From this solution, 20 ul aliquot was transferred into 1.5-ml disposable plastic microcentrifuge vials and treated as previously described under HPLC method.

For spiked food products, the above procedure was repeated but with the addition of 20 ul aliquots from the stock solutions of TB, TP and CA into the microcentrifuge vials prior to volume adjustment to 1.0 ml with methanol.

RESULTS AND DISCUSSION

Spectrophtometric Analysis

The zero-order UV-spectra of theobromine (TB), theophylline (TP) and caffeine (CA) show an extensive overlap of the spectral bands of these drugs in the region 270-275 nm (Fig. 1). Because of this extensive overlap, conventional UV-spectrophotometry cannot be used for individual quantification of these drugs in combination. When first-and second-derivative UV-spectra are recorded for the three drugs, sharp bands of great amplitudes (Fig. 2,3) are produced which permit more selective identification and determination of theobromine and theophylline respectively. As discussed elswhere[28] the choice of the optimum wavelength is based on the fact that the contribution of each component to the overall derivative signal is zero at the wavelength at which the other component has the maximum absorption. Therefore, the first-derivative amplitude (D1) at 248 has been chosen for determination of TB in the presence of TP and CA. Fig. 4 shows the first-derivative spectra of Tb solutions in 0.1M sodium hydroxide at different concentrations in presence of TP and CA.

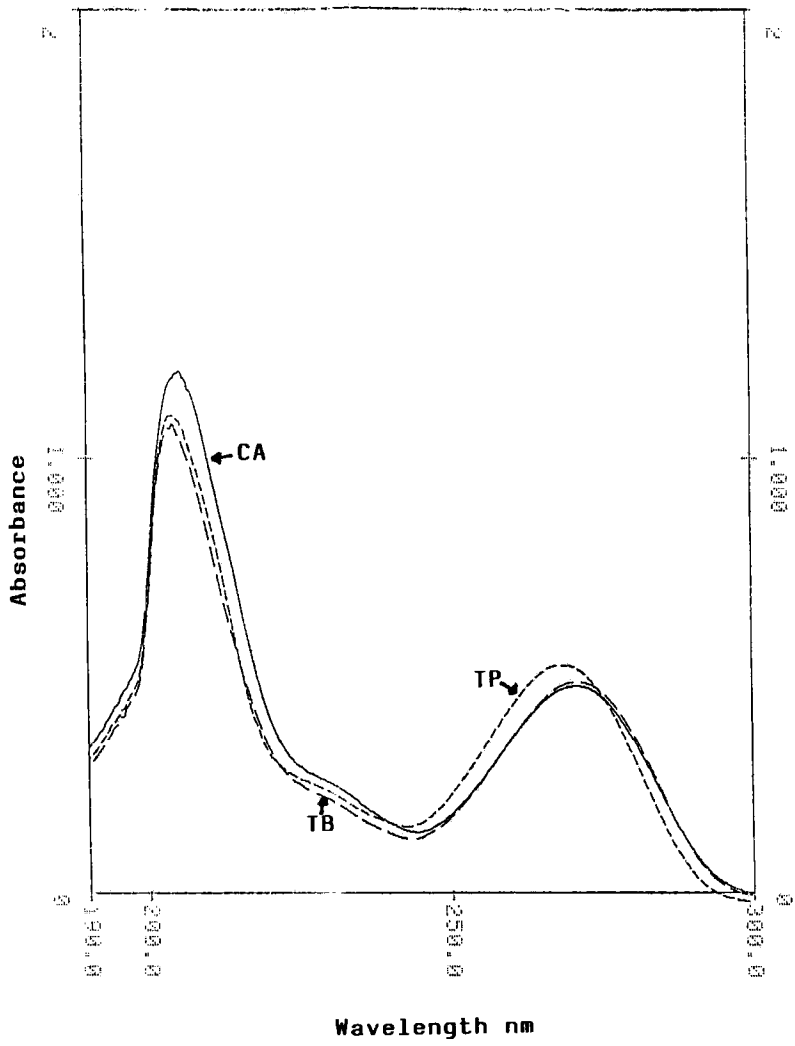

FIGURE 1. Zero-order UV-spectra of theobromine (TB) 10 ug ml^{-1}, theophylline (TP) 10 ug ml^{-1} and caffeine (CA) 10 ug ml^{-1} in distelled water

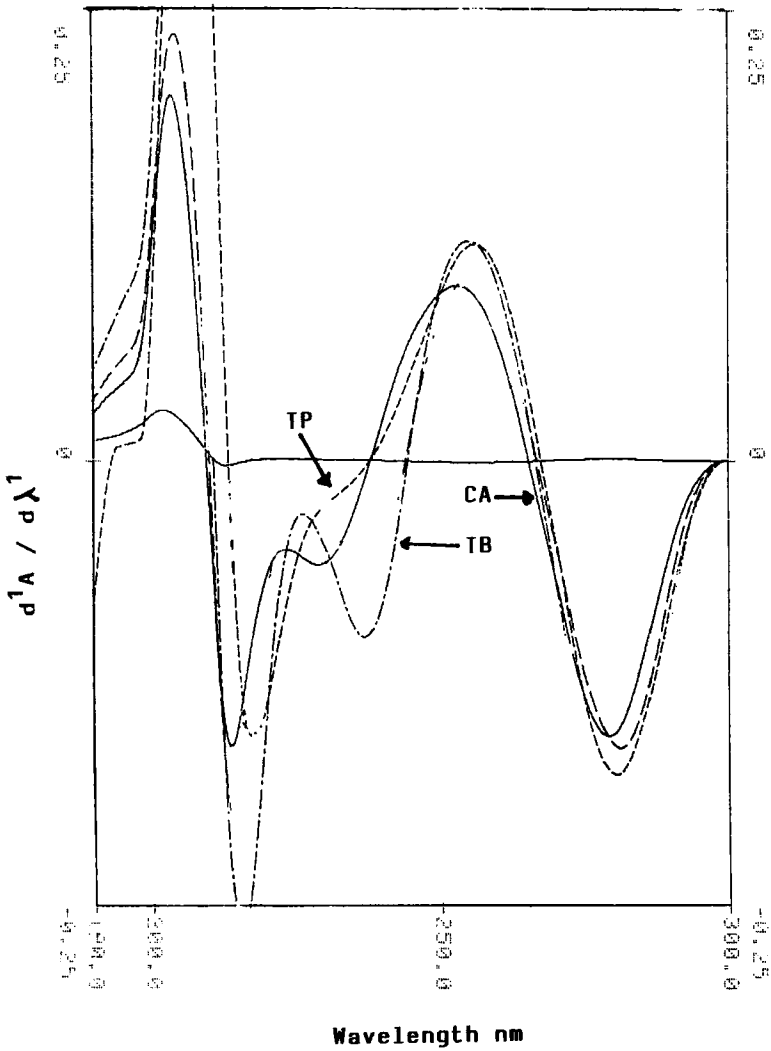

FIGURE 2. First-derivative UV-spectra of theobromine (TB) 10 ug ml^{-1}, theophylline (TP) 10 ug ml^{-1} and caffeine (CA) 10 ug ml^{-1} in 0.1M sodium hydroxide.

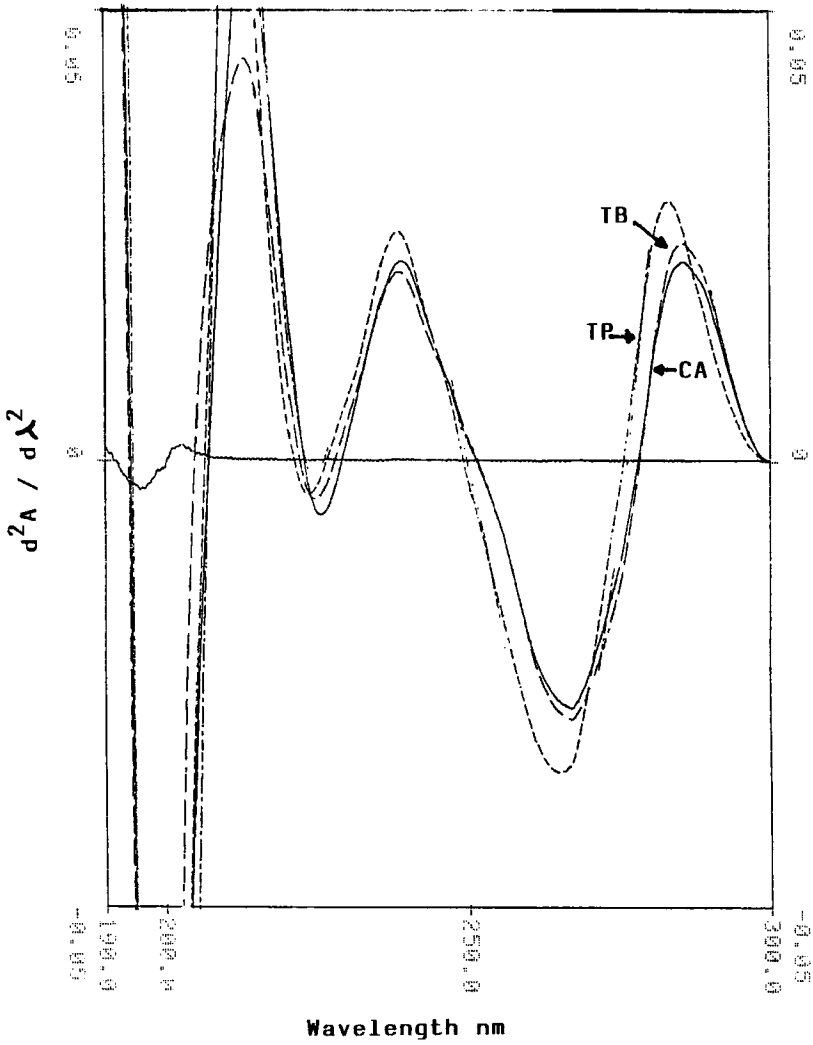

FIGURE 3. Second-derivative UV-spectra of theobromine (TB) 10 ug ml^{-1}, theophylline (TP) 10 ug ml^{-1} and caffeine (CA) 10 ug ml^{-1} in 0.1M hydrochloric acid.

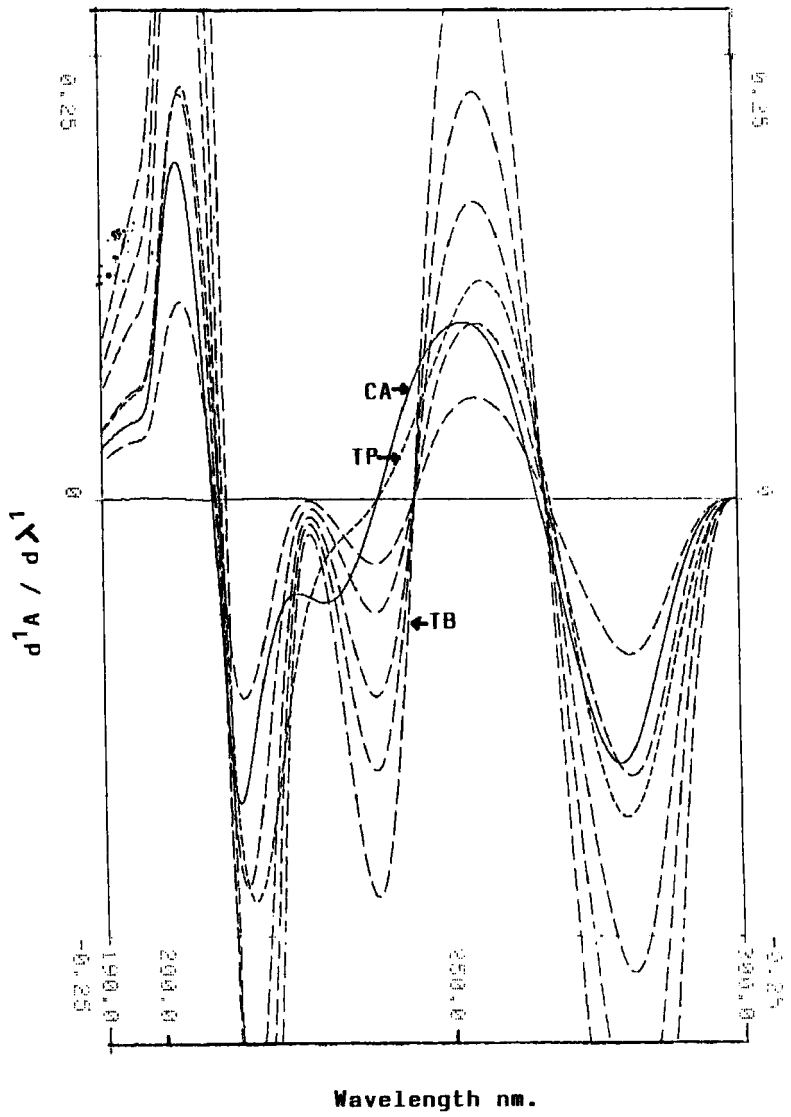

FIGURE 4. First-derivative UV-spectra of theobromine (TB) at different concentrations; 5, 10, 20 and 30 ug ml^{-1}, theophylline (TP) 10 ug ml^{-1} and caffeine (CA) 10 ug ml^{-1} in 0.1M sodium hydroxide.

The second-derivative amplitude (D2) at 278 nm has been chosen for determination of TP in the presence of TB and CA. Fig. 5 shows the second-derivative spectra of TP solutions in 0.1M hydrochloric acid at different concentrations in presence of TB and CA. For caffeine analysis in a mixture containing the three components, the contents of TB and TP in the mixture were first determined using first and second-derivative spectrophotometry as previously described. A solution containing the determined concentration of TB and TP was then prepared in distilled water and used as a blank for the determination of CA in the mixture by compensation technique. Measurement of the zero-order spectrum of the mixture was carried out at 275 nm. The zero-order absorbance spectrum obtained was due to CA content in the mixture only. Quantification of each component was achieved by plotting selected trough amplitudes from the first-derivative spectra (TB) or selected peak amplitudes from the second-derivative (TP) or zero-absorbance spectra (CA) against component concentration. Linear correlations over the concentration range 5-30 ug ml^{-1} for each component were obtained (Table 1). Least squares regression analysis was carried out for the slope, intercept, and the correlation coefficient (r). The relative standard deviation (RSD%) calculated for separate determination of each component was 0.83%-1.32%, indicating good precision and reproducibility of the spectrophotometric measurements. The precision of the developed spectrophotometric methods was confirmed by analysing six synthetic mixtures with different proportions of the three components. The recovery of each component is shown in Table 2 indicating good precision.

The proposed methods have been applied satisfactorily for analysing TB, TP and CA in various samples of tea, coffee, cocoa,

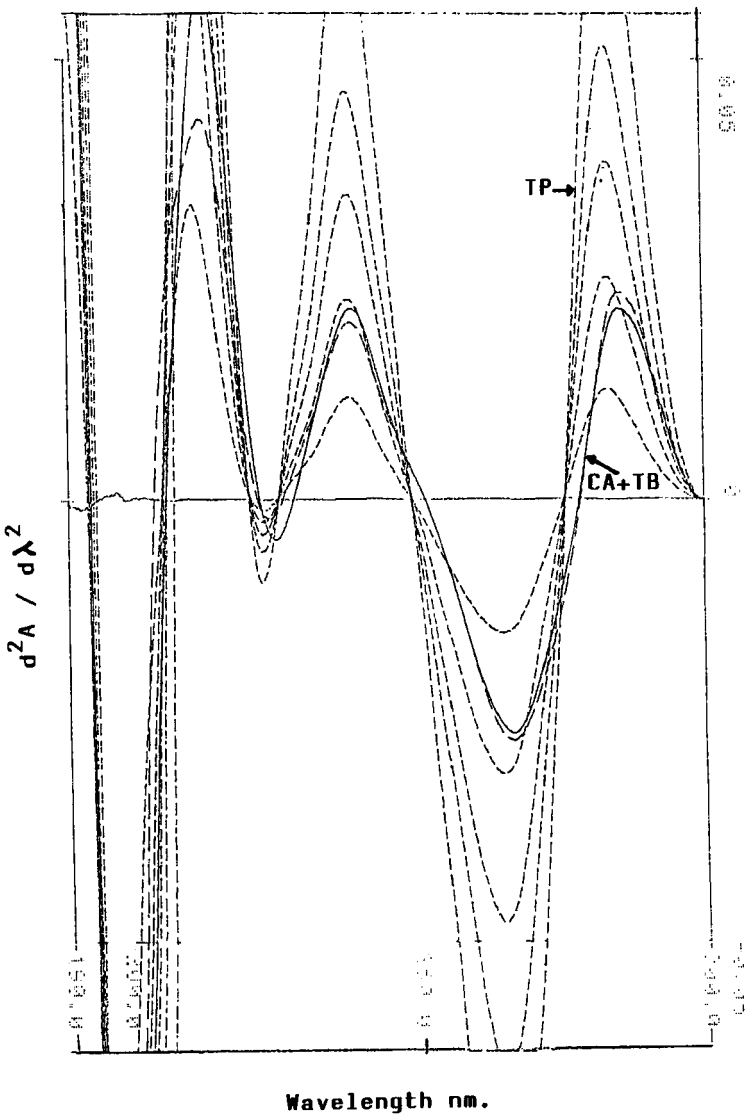

FIGURE 5. Second-derivative UV-spectra of theophylline (TP) at different concentrations; 5, 10, 20 and 30 ug ml^{-1}, theobromine (TB) 10 ug ml^{-1} and caffeine (CA) 10 ug ml^{-1} in 0.1M hydrochloric acid.

TABLE 1: Equations• for calibration graphs (n=5) for the determination of CA, TB, and TP in suitable solutions by zero, first or second-derivative UV-spectrophotometry

Drug	Equation	Correlation Coefficient !	Concentration range ug ml^{-1}	RSD%
CA	Y= 0.0659+0.0171X	0.9920±0.0065	5-30	1.32
TB	Y=-0.0021+0.0076X	0.9983±0.0007	5-30	1.12
TP	Y= 0.0003+0.0012X	0.9998±0.0021	5-30	0.83

• The equation is defined as Y = a + bX, where Y is the trough or peak amplitude at selected wavelength and X is the concentration range

! ±SD

CA= Caffeine

TB= Theobromine

TP= Theophylline

TABLE 2: Determination of CA, TB and TP in laboratory prepared mixtures zero, first-or second-derivative UV-spectrophotometry

CA:TB:TP	CA ug ml^{-1} (A^{275nm})			TB ug ml^{-1} (D1 method)			TP ug ml^{-1} (D2 method)		
	Taken	Found	R.E.%*	Taken	Found	R.E.%*	Taken	Found	R.E.%*
1:1:1	10	09.94	-0.6	10	10.03	+0.30	10	10.1	+1.0
2:3:4	20	20.20	+1.0	30	30.37	+1.23	40	29.70	-0.7
3:2:1	30	29.86	-0.47	20	20.15	+0.75	10	09.97	-0.7
4:3:2	40	39.50	-1.25	30	29.85	-0.50	20	19.86	-0.7
5:4:3	50	50.85	+1.70	40	39.62	-0.95	30	30.37	+1.7
3:5:4	30	30.33	+1.10	50	49.50	-1.00	40	39.74	-0.6

• R.E.% = Relative error

CA= Caffeine

TB= Theobromine

TP Theophylline

$A275^{nm}$= Zero-order spectrophotometry

D1= First-derivative spectrophometry

D2= Second-derivative spectrophotometry.

TABLE 3: Assay results for the analysis of CA, TB, and TP in various food products samples by zero, first, or second-derivative UV-spectrophotometry

Food sample	CA%*	TB%*	TP%*
Ceylon tea	3.10±0.80	1.07±0.08	0.07±0.03
Green tea	1.93±0.27	0.70±0.03	1.03±0.06
Roasted coffee	1.40±0.43	---	---
Unroasted coffee	1.45±0.22	---	---
Fresh coffee (unroasted)	1.10±0.08	0.07±0.02	0.09±0.05
Instant coffee	1.60±0.15	---	---
Cocoa	1.31±0.33	4.10±0.93	---
Chocolate	0.25±0.07	4.80±1.15	---
Pepsi cola	7.15±0.87	1.80±0.07	---

* Mean of five determinations ± S.D.

CA= Caffeine

TB= Theobromine

TP= Theophylline

chocolate and cola drinks. Preparation of food samples, as previously mentioned under procedures, was necessary as tannins, polyphenolic compounds and other constituents were found to interfere with the derivative UV-spectrophotometric spectra and elimination of these constituents was essential to avoid such interference. The results are shown in Table 3. It is clear from the Table that theophylline is not present in most of these food products. Theobromine is present in a higher concentration than caffeine in cocoa and chocolate.

In order to assess the validity of the proposed methods for assaying natural xanthines in various food products, food samples spiked with a known concentration of CA, TB, and TP (20 ug

TABLE 4: Assay results for the analysis of CA, TB, and TP in various food products samples spiked with 30 ug ml^{-1} of CA, TB, and TP by zero, first, or second-derivative UV-spectrophotometry

Food sample	CA%*	TB%*	TP%*
Ceylon tea	103.70±1.53	101.23±1.03	101.03±1.09
Green tea	102.07±0.75	101.03±0.75	100.73±1.00
Roasted coffee	101.63±0.73	100.13±1.22	099.93±0.07
Unroasted coffee	101.89±0.95	100.10±0.05	100.72±0.93
Fresh coffee (unroasted)	101.20±0.23	100.10±0.05	100.72±0.93
Instant coffee	101.89±1.83	100.05±1.27	100.53±1.02
Cocoa	101.55±2.33	104.56±1.23	098.33±2.37
Chocolate	100.83±1.63	106.23±2.75	101.63±2.01
Pepsi cola	108.25±2.53	101.63±0.69	100.52±1.09

* Mean of five determinations ± S.D.

CA= Caffeine

TB= Theobromine

TP= Theophylline

(N.B: % Recovery of the spiked amount of CA, TP and TB plus original amount present in food samples)

ml^{-1}) were analysed. Satisfactory results were obtained for the recovery of each component (Table 4).

Chromatographic Analysis

Prior to the assessment of CA, TB, or TP and in order to effect simultaneous elutions of these compounds under isocratic conditions, extensive studies were carried out. Emphasis was devoted to establish appropriate HPLC parameters, namely the mobile phase composition, setting the HPLC instrument and selection of a suitable HPLC column. It was found that the ideal

mobile phase needed to achieve the best resolution of CA, TB, TP and EP (internal standard) in reasonable time with no tailing was 0.01M sodium acetate (NaAC) solution and acetonitrile in a ratio of 85:15 at a pH of 4.0. The retention times for TB, TP, CA, and EP were found to be 2.83, 3.68, 5.95 and 7.00 min, respectively (Fig. 6) at a flow rate of 2.0 ml min^{-1}. The best column tested was a reversed-phase C-8 Lichrocart 250x4.6 mm olumn (Merck, Hohenbrunn, Germany). The effluent was monitered at 272 nm with a sensitivity range of 0.02 a.u.f.s.

Quantification of the chromatograms of either TB, TP, or CA was performed using the peak-height ratio (PHR) of each component relative to the internal standard (EP). Statistical analysis adopting the least squares method showed a linear correlation between the (PHR) and the concentration of each compound. Linear regression equations, concentration ranges and correlation coefficients are presented in Table 5. The correlation coefficients obtained were better than 0.9950. The relative standard deviation (RSD%) calculated for separate determination of each drug was 0.75-1.07%, indicating good precision and reproducibility of HPLC procedure.

The precision of the HPLC procedure was further evaluated by analysis of aqueous samples of component mixtures at three different concentrations. The results obtained are sumarized in Table 6. The results shown in this Table indicate good precision and reproducibility of the developed method.

The applicability of the proposed HPLC procedure for analysing CA, TB, and TP in various food products samples was studied. The results are shown in Table 7. It is clear from the

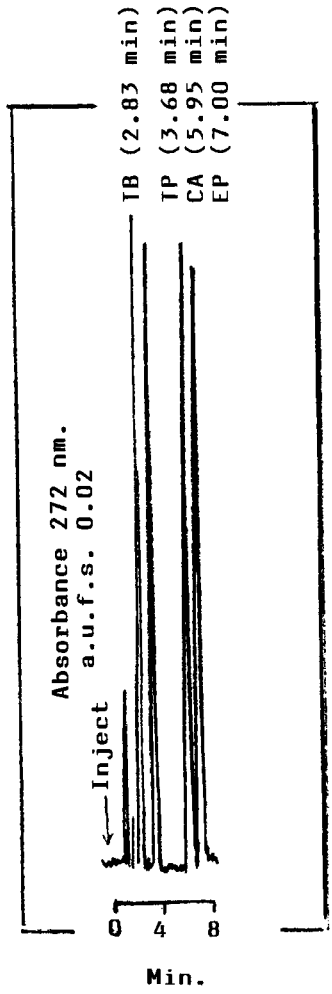

FIGURE 6. HPLC chromatogram of 20 ul injection containing 20 ug ml^{-1} theobromine (2.83 min), 20 ug ml^{-1} theophylline (3.68 min), 40 ug ml^{-1} caffeine (5.95 min) and 70 ug ml^{-1} ethyl paraben (7.00 min).

TABLE 5: Equations* for calibration graphs (n=5) for the determination of TB, TP and CA in aqueous solutions using the HPLC procedure

Compound	Equation	Correlation cofficient!	Concentration range ug ml^{-1}	RSD%
TB	Y=-0.0117+0.0560X	0.9965±0.0016	05-20	1.07
TP	Y=-0.0107+0.0530X	0.9982±0.0014	05-20	0.92
CA	Y=-0.0188+0.0270X	0.9957±0.0021	10-40	0.75

* The equation is defined as Y = a + bX, where Y is the peak height ratio and X is the concentration range (ug ml^{-1})

!± SD.

TB= Theobromine.

TP= Theophylline.

CA= Caffiene

HPLC= High-performance liquid chromatography.

TABLE 6: Precision of the HPLC method for the determination of TB, TP, and CA in aqueous samples of component mixtures

Concentration (ug ml^{-1})	Measured concentration (ug ml^{-1})*	CV%	Bias%+
TB			
5	05.15±0.063	1.22	3.00
15	15.59±0.390	2.50	3.93
20	19.88±0.47	2.36	-0.60
TP			
5	05.06±0.02	0.40	1.20
15	14.66±0.44	2.98	-2.27
20	19.75±0.55	2.80	-1.25
CA			
10	10.26±0.23	2.27	2.60
30	30.71±0.96	3.13	2.37
40	39.57±1.17	2.95	1.08

CV= Coefficient of variation
* Mean ± SD of five samples for each conentration
TB= Theobromine
TP= Theophylline
CA= Caffeine
HPLC= High-performance liquid chromatography.

Table that the percentage of each xanthine component in the tested food samples matches exactly that obtained using UV-spectrophotometric procedures. Food samples of tea or coffee prepared by boiling the weighed amount with water without further precipitation and separation steps were analysed by HPLC. No interference from tea or coffee constituents such as tannins and polyphenolic compounds was noticed and the results obtained were very similar to those obtained from samples prepared as previously described where these constituents were eliminated.

TABLE 7: Assay results for the analysis of CA, TB, and TP in various food products samples by HPLC

Food Sample	CA%	TB%	TP%
Ceylon tea	3.45±0.45	1.23±0.31	0.09±0.03
Green tea	2.03±0.43	0.93±0.35	1.23±0.12
Roasted coffee	1.40±0.77	---	---
Unroasted coffee	1.63±0.37	---	---
Fresh coffee (unroasted)	1.32±0.23	0.09±0.04	0.07±0.03
Instant coffee	1.72±0.32	---	---
Cocoa	1.45±0.54	4.32±1.13	---
Chocolate	0.34±0.12	5.02±0.86	---
Pepsi cola	7.54±1.03	1.67±0.12	---

* Mean of five determinations ± S.D.

CA= Caffeine

TB= Theobromine

TP= Theophylline.

HPLC= High-performance liquid chromatography.

(N.B: Tea or coffee samples prepared without further precipitation and seperation steps gave results as above).

The validity of the proposed method for food analysis was further confirmed by spiking food samples with a known concentration of CA, TB and TP (20 ug ml^{-1}). The results obtained for the recovery of each component are satisfactory indicating good precision of the HPLC procedure (Table 8).

CONCLUSIONS

Caffeine, theobromine and theophylline have been assayed in various food products using two different techniques. The

TABLE 8: Assay results for the analysis of CA, TB, and TP in various food products samples spiked with 20 ug ml^{-1} of CA, TB, and TP by HPLC

Food sample	CA%*	TB*	TP%*
Ceylon tea	104.00±1.67	101.35±1.00	102.03±1.03
Green tea	101.73±0.83	101.33±1.03	101.75±1.09
Roasted coffee	102.03±1.03	100.67±0.75	100.05±1.03
Unroasted coffee	101.69±0.63	100.03±1.23	100.32±1.72
Fresh coffee (unroasted)	101.62±0.72	100.03±0.72	100.30±0.62
Instant coffee	102.03±1.05	098.75±0.75	099.93±0.75
Cocoa	102.13±0.97	105.12±0.87	098.73±3.22
Chocolate	102.57±1.07	105.62±1.93	102.34±1.62
Pepsi cola	108.03±1.36	102.09±1.84	101.07±1.03

* Mean of five determinations ± S.D.

CA= Caffeine

TB= Theobromine

TP= Theophylline

HPLC= High-performance liquid chromatography

(N.B: %Recovery of the spiked amount of CA, TP and TB plus original amount present in food samples).

derivative UV-spectrophotometric method is simple, rapid and sensitive. It is suitable for routine analysis in control laboratories of plant extracts, food products and even biological fluids. The HPLC method was shown to be simple, rapid, specific and may offer advantages over the derivative spectrophotometric method for selective determination of intact xanthine components in various food samples in the presence of variety of other food constituents such as tannins, polyphenolic compounds and others. Previous precipitation, separation and purification steps are not necessary. This is not the case using the convenient spectrophotometric

methods. Moreover, the HPLC procedure seems promising in quality control analysis of xanthine components in pharmaceutical dosage forms and biological fluids.

REFERENCES

1. Ishida, H.; Sekine, H.; Kimura S. and Sekiya, S. "G-C determination of theobromine in foods", 27, 75, 1986.

2. Chen, J.; "Determination of caffeine in tea, coffee and cola drinks by G.C.", Sepu, 5, 376, 1987.

3. Khayyal, S.E., "Estimation of theophylline and theobromine in pharmaceutical preparations and natural products", J. Drug Res. 15, 79, 1984.

4. Duthil, J.M.; Mignot, C. and Vallon, J.J., "Rapid extraction of caffeine from urine on C18 cartrige and determination by G.C.", Anal. Lett., 22, 2627, 1989.

5. Abdel-Moety, E.M., "First-derivative spectrophotometric and GLC determination of caffiene in foods and pharmaceuticals", Z. Lebensm. Unters.-Forsch., 186, 412, 1988.

6. Skhiladze, N.R., "Spectrophotometric method for quantitative determination of caffeine in the hulls of ripe tea seeds", 22, 491, 1987.

7. Wallash, M.I. and Salama, O., Densitometric determination of theophylline", Anal. Lett., 22, 827, 1989.

8. Srivostava, R.A.K., "Spectrochromatographic estimation of caffeine and theobromine in tea", Indian Drugs, 22, 438, 1985.

9. Perry, L.M.; Shao, E.Y. and Winefordner, J.D., "Room temperature phosphorimetric studies of caffeine and theophylline", Talanta, 36, 1037, 1989.

10. Bateh, R.P. and Winefordner, J.D., "Pharmaceutical formulation analysis using room temperature phosphorescence", J. Pharm. Biomed. Anal. 1, 113, 1983.

11. Agrawal, A.K. and Sharma, S.K., "Spectrophotometric determination of caffeine as its murexide", J. Inst. Chem., 58, 146, 1986.

12. Kuznetsov, D.I. and Semenova, L.I, "Determination of caffeine", Pishch. Prom. St., 9, 65, 1989.

13. Bermejo, A.; Lopez-Rivadulla, M.; Ferrandez, P. and Concheiro, L., "Application of derivative spectroscopy to the determination of plasma theophylline in the presence of phenobarbitone", Anal. Toxicol., 9, 76, 1985.

14. Sun, J.; Chen, R.; Shang, H.; Han, J.; Chen, C.; Su, M. and Li, Z., "Assay of sod. benzoate and caffeine injection, comparison of reversed phase HPLC and derivative spectrophotometric methods", Yaowu Fenxi Zazhi, 6, 270, 1986.

15. El Henawee, M.M.; Mohamed, H.S.; El-Balkiny, N. and Ayad, M.M., "UV-derivative spectrophotometric determination of chlorpheneramine HCl in combination with anhydrous caffeine", Spectrosc. Lett. 23, 273, 1990.

16. Weng, X., "Determination of caffeine and sodium benzoate injection by differential derivative spectrophotometry", Yaowu Fenxi Zazhi, 8, 187, 1988.

17. Deng, P.; Li, H.; Lu, A and Dai, Y., "Determination of caffeine contents of cola drinks by second derivative spectrophotometry", Shipin Kexue, 98, 5155, 1988.

18. Dong, S.; Xu, P. and An, D., "Determination of caffeine content in syrup vitamine B_1 and glycerophosphate using first derivative ratio method", Yaoxue Xuebao, 23, 441, 1988.

19. Onur, F. and Acar, N., "Determination of caffeine and paracetamol in pharmaceutical preparation by first derivative spectrophotometry", FABAD Farm Bilimler Derg, 14, 1, 1989.

20. Vergens, M.F. and Alary, J., "Determination of natural xanthines by HPLC", Talanta, 33, 997, 1986.

21. Baltassat-Millet, F.; Ferry, S. and Dorche, J. "Determination of caffeine in drug plants", Ann. Pharm. Fr., 38, 127, 1980.

22. Turcant, A.; Cailleux, P. and Allain, P., "Separation of xanthine derivative by HPLC and application to plasma analysis", J. Liquid Chromato. 8, 1537, 1980.

23. Yan, Z.; Wang, F., "Determination of hypoxanthine, xanthine and uric acid by HPLC", Sepu, 7, 95, 1989.

24. Alary, J. and Vergnes, M.F., "Quantitative analysis of drugs containing caffeine using HPLC", Ann. Pharm. Fr. 42, 249, 1984.

25. Belliardo, F.; Martelli, A. and Valle, M.G., "HPLC determination of caffeine and theophylline in paullinia cupana Kunth (Guarana) and cola seed samples", Z. Lebensm.-Unters.-Forsch. 180, 398, 1985.

26. Terada, H. and Sakabe, Y., "HPLC determination of theobromine, theophylline and caffeine in food products", J. Chromatog., 291, 453, 1984.

27. Rainbow, S.J.; Dawson, C.M. and Tickner, T.R., "Non extraction HPLC method for simultaneous measurement of theophylline and caffeine in human serum"., Ann. Clin. Biochem., 26, 527, 1989.

28. Morelli, B., "Zero-crossing derivative spectrohpotometry for the determination of mixtures of cephaloridine and cephalothin in pure and dosage form", J. Pharm. Sci., 77, No. 7, 615, 1988.