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# Derivative Spectrophotometric Determination of Caffeine in Some Beverages

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Caffeine content was determined in cola, coffee and tea by second and third order derivative spectrophotometry without using any separation or background correction technique and reagent. The method is based on the measurement of the distances between two extremum values (peak to peak amplitudes) in the second order (cola) and third order (coffee and tea) derivative spectra of the sample solution. Calibration curves were constructed for the 2.0-10.0  $\mu\text{g ml}^{-1}$  concentration range. As a reference method, reversed phase high performance liquid chromatographic procedure was developed. Commercially available beverages were analyzed by the two methods and the results were statistically compared by using t- and F-tests.

**Key Words:** Caffeine, Beverages, Derivative Spectrophotometry

## Introduction

Caffeine is widely distributed in pharmaceutical preparations and beverages.

The need for a fast and selective determination method is obvious, especially when routine determinations are required. Many analytical methods have been developed for the determination of caffeine and the quality control of products containing caffeine. For the determination of caffeine in beverages, various analytical techniques including titrimetry<sup>1-5</sup> spectrophotometry<sup>3,6-13</sup>, polarography<sup>11</sup>, GC<sup>12-14</sup>, and HPLC<sup>15-21</sup> have been reported.

Derivative spectrophotometric methods have been developed for the assay of caffeine in some pharmaceutical preparations<sup>22-26</sup>. Titrimetric methods suffer the disadvantage of using a large amount of sample and undergoing interference from many redox reagents with the determination. With the chromatographic methods, the use of expensive equipment and the demand for more operator attention prevent their application in small industrial laboratories where only a few analyses are performed each day. The polarographic method requires long analysis and suffers interferences from electrochemical impurities present in the food sample, whereas the enzymatic technique is generally considered to be too specialized for ordinary chemical laboratory use. Spectrophotometry is a fast and simple method for caffeine determination, but it cannot be used in samples with complex matrices because of background correction and techniques have been proposed such as thermal decomposition, direct ultraviolet irradiation alkaline treatment and an enzymatic

method<sup>27,28</sup>. Some visible spectrophotometric methods often require tedious pre-separation techniques to remove possible interferences from colored materials. Derivative spectrophotometry is a useful technique for extracting qualitative and quantitative information from spectra composed of unresolved bands<sup>29</sup>, and for eliminating the effect of baseline shifts and baseline tilts<sup>30</sup>. Derivative spectrophotometry is now a reasonably priced standard feature of modern microcomputerized UV/Vis spectrophotometry.

The purpose of this work is to develop a direct and simple UV-spectrophotometric method for the determination of caffeine in beverages without using pre-separation or background correction procedures. Cola, coffee and tea were chosen owing to their high caffeine content. Several cola, coffee and tea samples were analyzed for their caffeine content and the results were compared with those obtained by the HPLC method.

## Experimental

### Apparatus

For derivative spectrophotometric measurement a Philips 8740 UV-VIS spectrophotometer and 10 mm quartz cells were used. All spectra were recorded from 190 nm to 350 nm with 2 nm slit width, 500 nm min<sup>-1</sup> scan speed and very high smoothing. Chromatographic analyses were performed using a Waters HPLC system consisting of a model 510 delivery system, a model 481 variable wavelength spectrophotometer (at 270 nm) and a computer. A 10  $\mu$ m Bondapak C<sub>18</sub> (3.9 mm x 30 cm) RP column and an isocratic mobile phase containing MeOH-water (30:70) were used. The flow rate was 1.5 ml min<sup>-1</sup> (pSI=2600). Quantitative analysis was accomplished by internal standard calibration.

### Materials

Caffeine and acetaminophen (internal standard) (Doğu) were used as received. HPLC grade methanol and other analytical grade chemicals were purchased from E. Merck. Milli Q water was used.

Caffeine stock solution (100  $\mu$ g ml<sup>-1</sup>) was prepared by dissolving 10 mg of caffeine in 100 ml of water.

This solution was freshly prepared and was diluted to obtain standard solutions for the preparation of calibration curves.

Internal standard (acetaminophen) solution (100  $\mu$ g ml<sup>-1</sup>) was prepared.

### Analytical Procedures

#### Preparation of sample solution

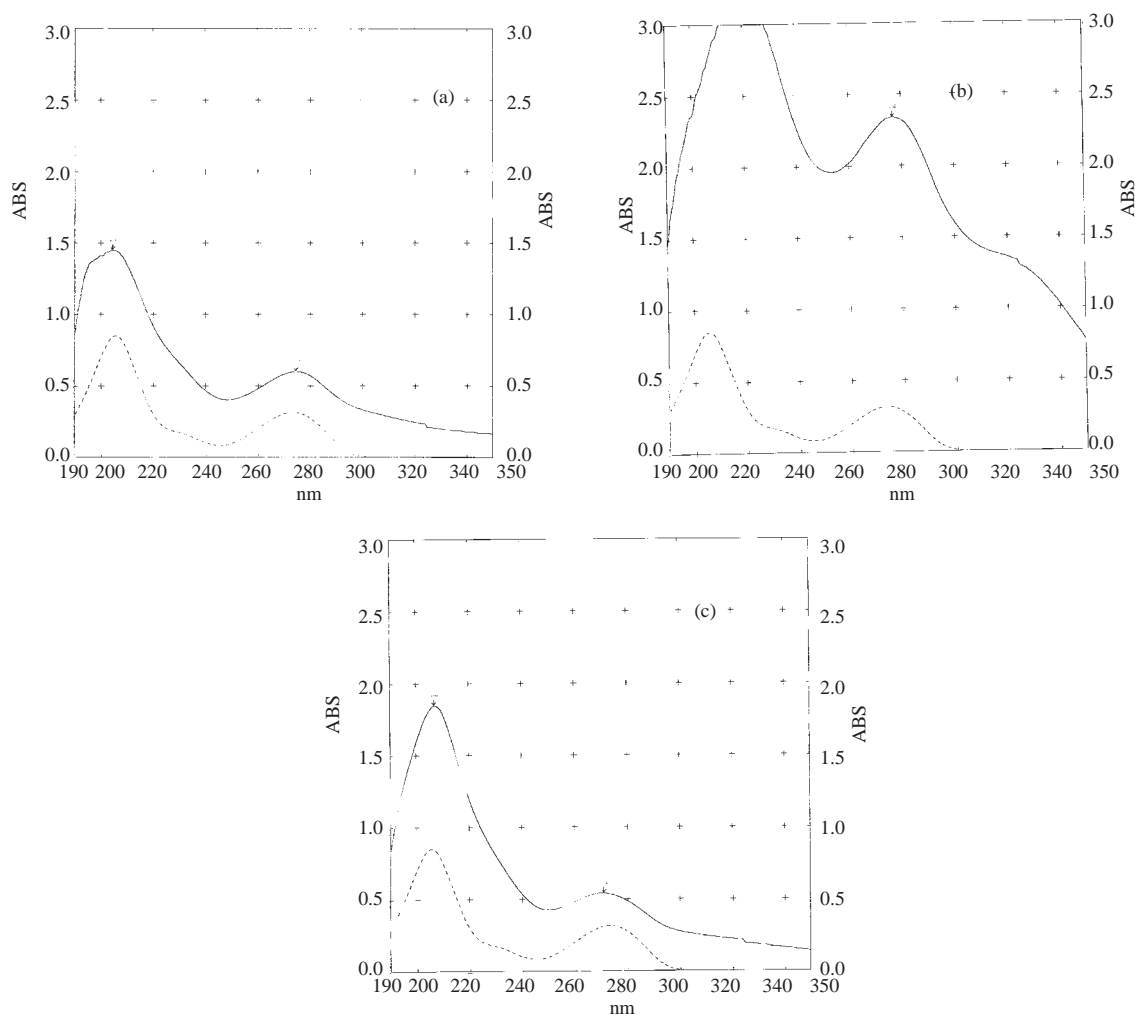
Samples were purchased from the local markets for analysis. The portions of each sample were analyzed using two methods. In order to degas the cola, the sample solution was mixed for 20 min by with a magnetic stirrer. Soluble coffee (1.5-2.0 g) was homogenized with 100 ml of water using a magnetic stirrer for about 5 min. To a portion of tea 1-2 g was added 100 ml of water and it was boiled until the volume was reduced to 40-50 ml. The mixture was cooled, diluted to 100 ml with water and filtered through a dry filter paper. The first 20 ml was discarded and the filtrate was used for the analysis.

The samples were then diluted to provide the necessary working concentration (2.0-10.0  $\mu$ g ml<sup>-1</sup>).

### Preparation of Calibration Graphs

The second and third order derivative absorption spectra of caffeine standard solutions ( $2.0 - 10.0 \mu\text{g ml}^{-1}$ ) were recorded against water between 190 and 350 nm.

In the second order derivative spectrum, the extrema of 246.7 nm and 234.1 nm were used for the quantitative determination of caffeine in cola. In the third order derivative spectrum, the extrema of 286.7 nm and 265 nm were used for coffee, and the extrema of 286.7 nm and 300.3 nm were used for tea. Regression equations of the calibration graphs were calculated by the method of least squares.



**Figure 1.** Absorption spectra of (a) cola (—); (b) coffee (—); (c) tea (—); and caffeine (- - -) solutions. Concentration:  $6 \mu\text{g ml}^{-1}$ , Reference: Water

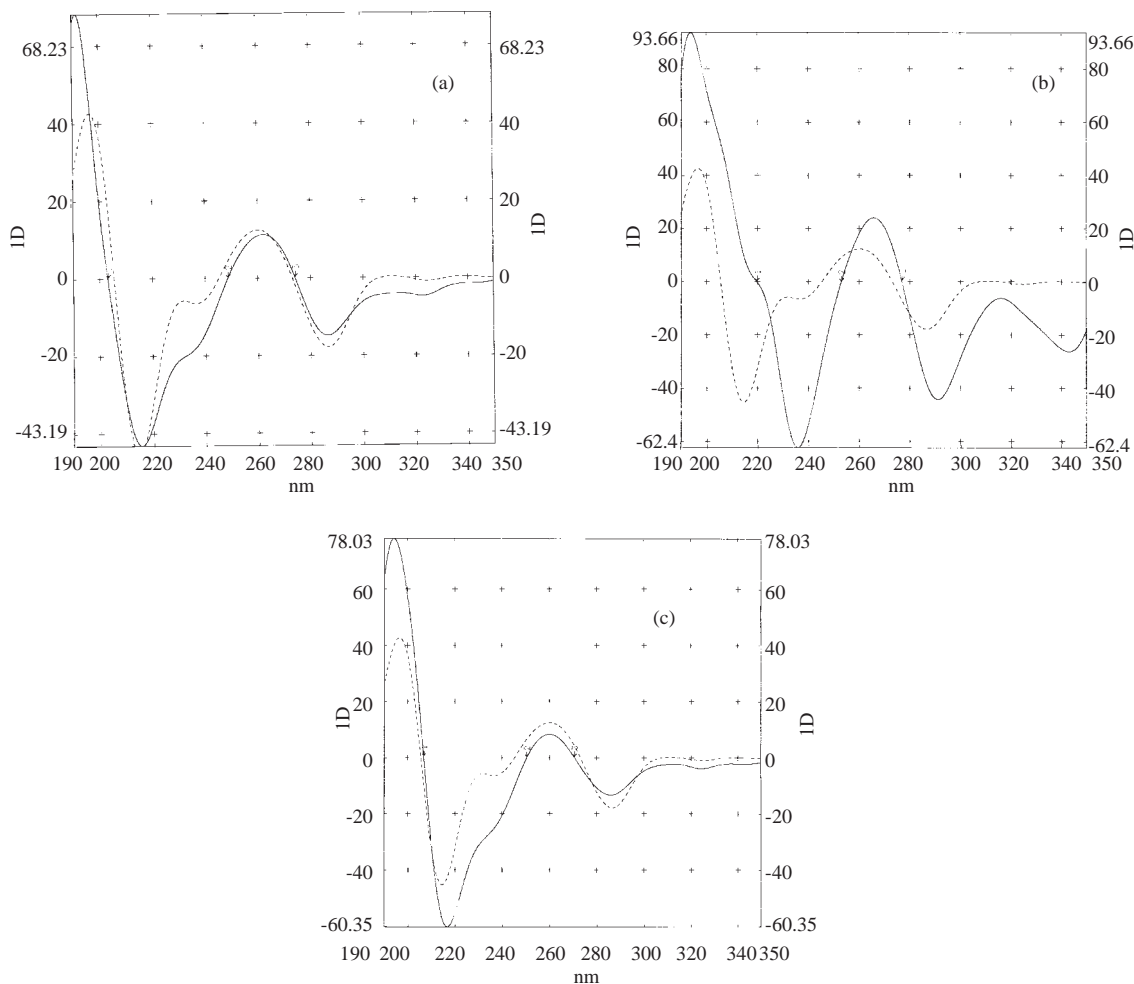
### Sample Analysis

Second and third order derivative spectra of sample solutions were recorded against water. Peak-to-peak measurements were made at 232.7-245.2 nm in the second derivative spectrum for cola, 268.5-289.5 nm in the third derivative spectrum for coffee, and 286.0-300.0 nm in the third derivative spectrum for tea samples.

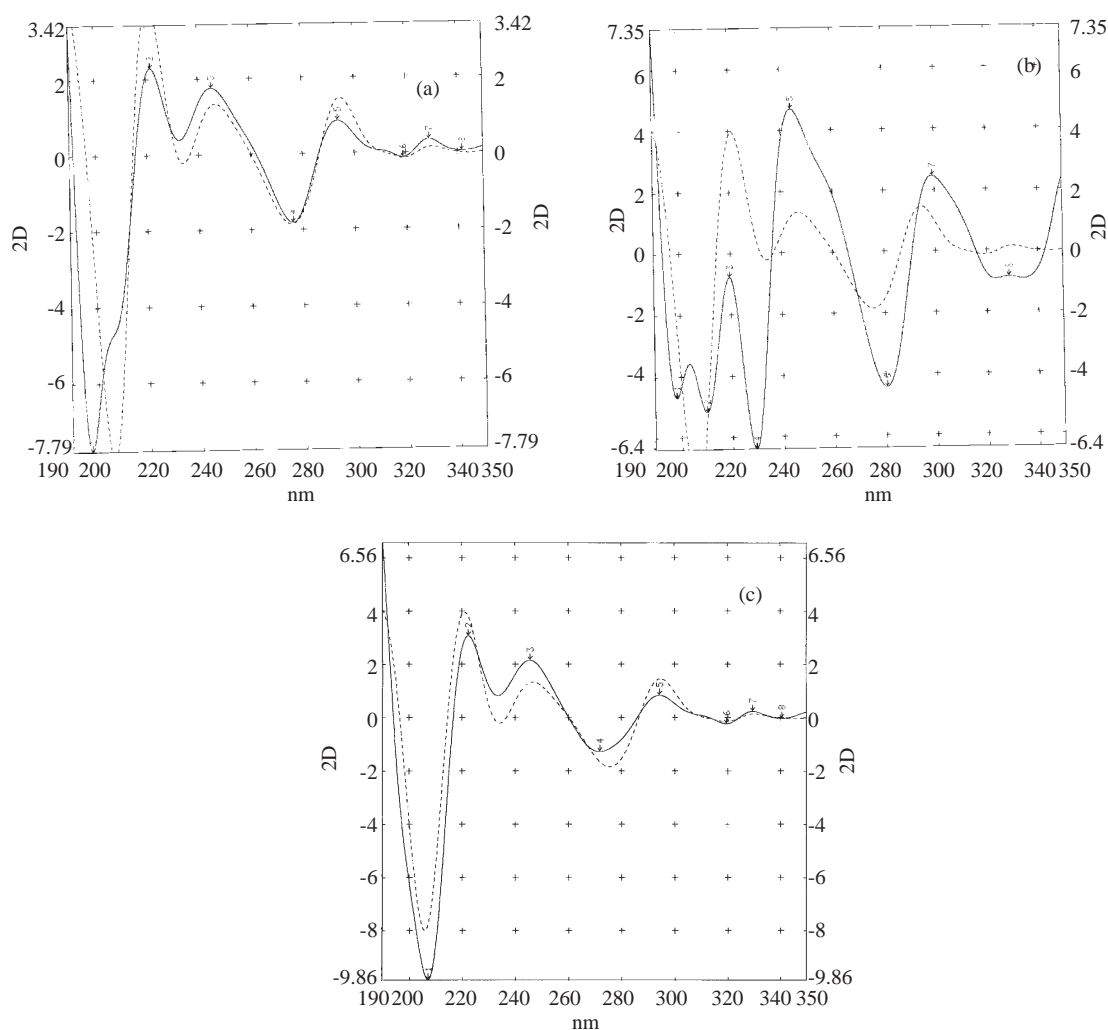
The concentration of caffeine in the sample solutions was deduced by means of the regression equation of the related calibration graph.

## Results and Discussion

A UV-spectrophotometric method cannot be used directly for the determination of caffeine in beverages owing to the matrix effect of UV-absorbing substances in the sample matrix. This effect is clearly seen in Fig. 1, which shows the absorption spectra of cola, coffee and tea extract, together with the spectra of standard caffeine solutions prepared in water. To overcome this difficulty, some tedious background correction techniques have been used<sup>27,28</sup>. In contrast, the derivatization of the absorption spectrum and measurement of the distance between two neighboring extremum values allow the elimination of matrix effects, because the variable background absorptions overlapping the analyte peaks are smoother in derivative spectra. Figs. 2, 3 and 4 show first, second and third order derivatives of the absorption spectra given in Fig. 1. As seen, matrix effects are eliminated by derivation of the absorption spectra, although extremum wavelengths are not exactly same as those obtained with standard caffeine solutions.



**Figure 2.** First order derivative spectra of (a) cola (—); (b) coffee (—); (c) tea (—); and caffeine (- - -) solutions. Concentration:  $6 \mu\text{g ml}^{-1}$ , Reference: Water



**Figure 3.** Second order derivative spectra of (a) cola (—); (b) coffee (—); (c) tea (—); and caffeine (- - -) solutions. Concentration:  $6 \mu\text{g ml}^{-1}$ , Reference: Water

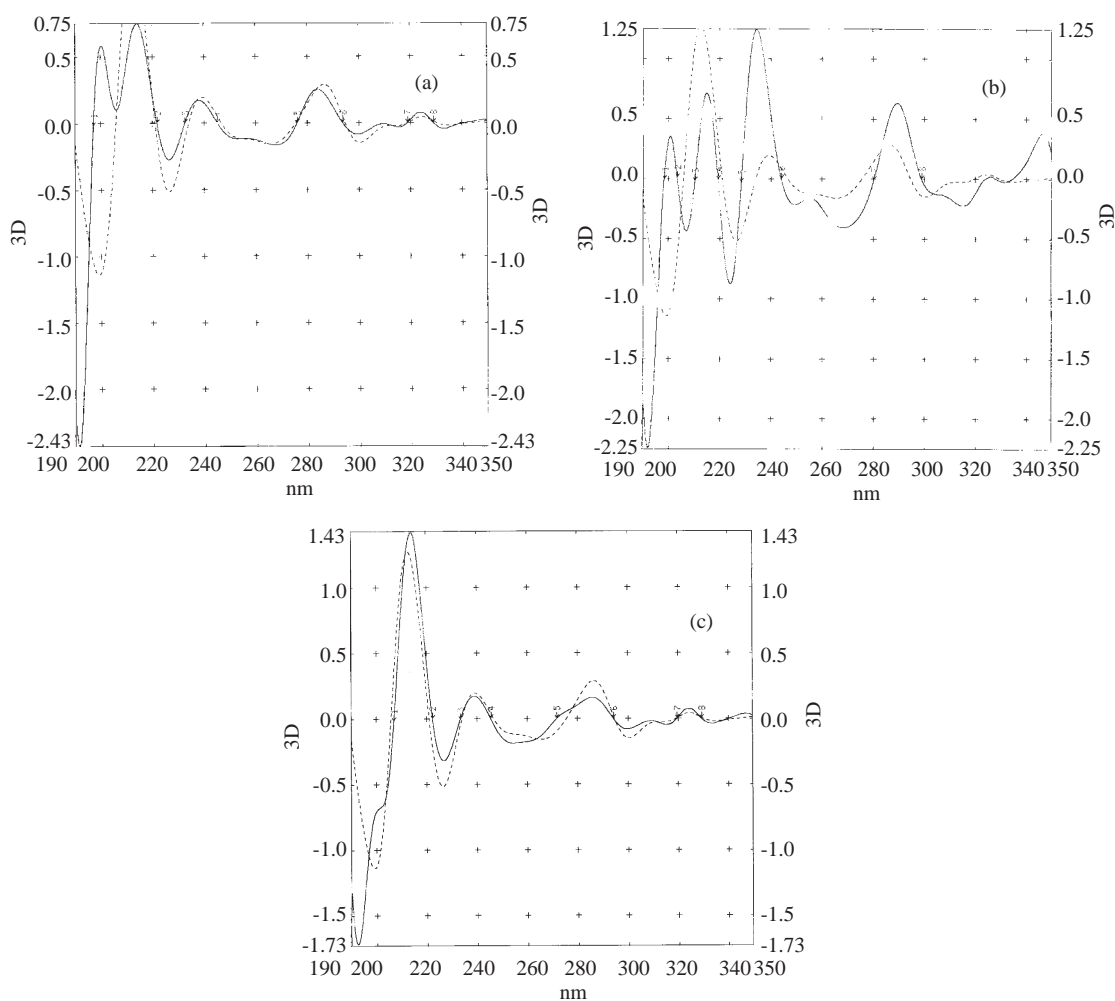
Linear relationships were obtained between the caffeine concentration ( $2.0\text{-}10.0 \mu\text{g ml}^{-1}$ ) and peak-to-peak amplitudes ( $\overline{D}_3$ ) of third order spectra and ( $\overline{D}_2$ ) of second order spectra at the wavelengths stated above. The regression equations were as follows:

$$(\overline{D}_3) = 0.0731c + 0.01 \quad (r = 0.9999) \quad (\text{Coffee}) \quad (c: \mu\text{g ml}^{-1})$$

$$(\overline{D}_3) = 0.071c + 0.01 \quad (r = 0.9999) \quad (\text{Tea})$$

$$(\overline{D}_3) = 0.25 c + 0.03 \quad (r = 0.9998) \quad (\text{Cola})$$

The contents of caffeine in six samples were determined by the proposed method. For the comparison of the derivative spectrophotometric method with HPLC samples were also analyzed on a  $\text{C}_{18}$  reversed-phase column using  $\text{MeOH-H}_2\text{O}$  (30:70 v/v) mobile phase. In the chromatogram obtained by this procedure, the retention time of caffeine is 5.14 min. As the internal standard substance, acetaminophen was used, having a retention time of 3.03 min. The assay results obtained by both methods were statistically compared at 95% confidence level. As shown in Table 1, there was no significant difference between the mean values and precisions of the two methods.



**Figure 4.** Third order derivative spectra of (a) cola (—); (b) coffee (—); (c) tea (—); and caffeine (- - -) solutions. Concentration:  $6 \mu\text{g ml}^{-1}$ , Reference: Water

**Table 1.** Comparison of the results for caffeine determination by the proposed method and the HPLC method

	Mean $\pm$ t.s / $\sqrt{n}$		
	Cola ( $\mu\text{g ml}^{-1}$ )	Coffee (%)	Tea (%)
Derivative Spectrophotometric Method	$149.32 \pm 0.68$	$1.36 \pm 0.03$	$1.53 \pm 0.03$
HPLC Method	$150.06 \pm 1.38$	$1.35 \pm 0.04$	$1.51 \pm 0.05$
t-test of significance*	0.71	0.45	0.70
F-test of significance*	4.05	1.78	4.00

\*t = 2.23; F = 5.05 for p = 0.05 and  $n_1 = n_2 = 6$

In conclusion, the derivative spectrophotometric method is relatively easy, fast and cheap for the determination of the caffeine content of cola, coffee and tea. Because it does not require expensive solvents and reagents, it may be recommended for the rapid, precise and sensitive quantification of caffeine in these

products. The major instrument required is a modern, microcomputerized UV/VIS spectrophotometer, which can be purchased at a reasonable price.

This method also takes less time than the others, and the absorption values of the solutions are stable for at least 2 days. The method can be extended to caffeine determination in various beverages, provided that the proper wavelengths for peak-to-peak measurements are accurately selected. Method detection limits (MDLs) were  $2 \mu\text{g/g}$ ,  $1.5 \mu\text{g ml}^{-1}$ ,  $2 \mu\text{g/g}$  of caffeine for tea, cola and coffee, respectively.

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