

## **Original Research Article**

### **Extraction and Determination of Caffeine Concentrations in Coffee, tea and chocolate milk available in Saudi markets**

#### **Abstract**

In this work a study was performed using UV/Vis spectrophotometer to determine the concentrations of caffeine in coffee, tea and chocolate milk available in local market in Riyadh, Saudi Arabia. Quantitative analysis was carried using dichloromethane as extracting solvent. Results showed that the minimum caffeine level was observed in the chocolate milk (16.38 ppm) and the highest caffeine content observes in coffee (32 ppm). Results obtained for samples agree well with those found by the official limit set by the US Food and Drugs Administration.

#### **Keywords**

Caffeine, dichloromethane, Absorbance, UV/vis spectrophotometer

#### **Introduction**

Caffeine is the main alkaloid found in many kinds of foods and drinks [1] and thus the determination of caffeine is required in food laboratories in order to inform the consumers about the characteristics and concentration. There are several methods proposed in the literature for the determination of caffeine in foods, based on ultraviolet UV –visible spectrophotometry [2], HPLC [3-5] or mass spectrometry [6].

Caffeine is found in various kinds of foods and drinks that we consume in daily life [1]. About 200 mg of caffeine contains pharmacological effect. At this level, it stimulates the central nervous system, decreases fatigue leading to clearer flow of thoughts, sustained intellectual effort and a more perfect association of ideas with a better appreciation of sensory stimuli in man [7].

And their concentration in vivo is a key mark for various disorders including heart disease, carcinogenesis, kidney malfunction and asthma [8]. Therefore, establishing a rapid and cheap analytical method for the determination of caffeine in foods and drinks has an interest for a wide range of physiological effects on the human body and quality controls [9].

In this paper, a method for measuring caffeine content in coffee, tea and chocolate milk are reported using UV/vis spectrophotometer, which is available in most laboratories. Moreover, the methods are easy, fast and cheap for the determination of the caffeine contents in samples. The methods include characterizing pure caffeine in dichloromethane, and extracting caffeine from samples using dichloromethane.

## **Material and Methods**

### **Preparation of caffeine standard solutions**

A 100-ppm stock standard solution of caffeine was prepared by dissolving 25 **micrograms** of caffeine in 250 mL purified dichloromethane ( $\text{CH}_2\text{Cl}_2$ ) in a volumetric flask (250 mL). Working standards solutions were prepared by pipetting 5, 10, 15, 20, 25 and 30 mL, respectively aliquots of the stock standard solution into separate volumetric flasks (100 mL) and diluted to volume with purified dichloromethane to produce concentrations of 5, 10, 15, 20, 25 and 30 mg/L, respectively standard solution. The absorbance of each solution was measured at absorption maximum of 270 nm using 10 mm quartz cuvette. The absorbance values were then plotted against concentrations to generate a standard calibration curve [10].

### **Quantitative caffeine determination**

Quantitative analysis of caffeine was performed by **PD-303UV/Vis Spectrophotometer**. The  $\lambda$  max was determined by scanning the standard solution from 200-600 nm and the obtained results gave an absorption spectrum, which was characterized by a single intensive absorption band located in the UV range at  $\lambda$  max = 270 nm. Standard linear calibration curve was run to obtain the linear range of sample analysis. The standard calibration curve was linear over the range (5-30) ppm caffeine with equation ( $y = 0.0714x + 0.3845$ ). The quantitative amount of caffeine in samples (ppm) was then determined using the standard curve [11].

### **Caffeine extraction procedure from coffee**

In a large beaker 10 g of powdered coffee was weighted followed by the addition of 100 mL of boil distilled water then covered with watch glass for 10 minutes to steep. The precipitate was filtered, and the coffee extraction was repeated twice with 25 mL of boiling water then pressed the precipitate. 20 g of sodium chloride with 1g of calcium hydroxide was added to the filtrate and heated with stirring for 15 minutes. The solution was filtered by filter paper then cooled at room temperature. The filtrate was transferred to separatory funnel with 25mL of **dichloromethane**  $\text{CH}_2\text{Cl}_2$ . The non -aqueous layer was removed to a clean volumetric flask. Another (25 mL) portion of dichloromethane was added to the aqueous solution in the separating funnel and the extraction procedure was repeated twice and the dichloromethane layers were combined. The extract was dried with 2 g of anhydrous magnesium sulfate and the volume was made up to 100 mL with the solvent. The absorbance of the resulting solution was measured on UV/Vis spectrophotometer at 270 nm using 10 mm quartz cuvette [12-14].

### **Caffeine extraction procedure from tea leaves**

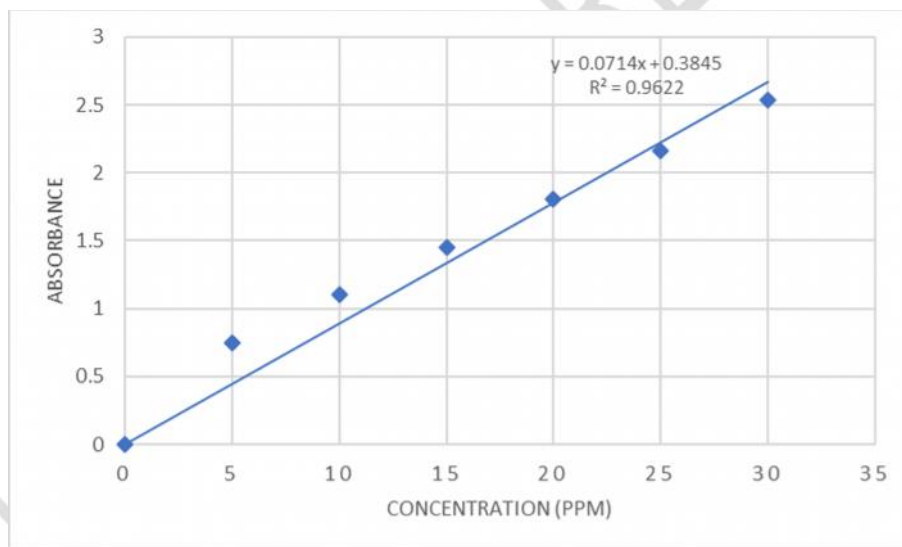
The above procedure of coffee extraction was repeated with 11.2g of tea leaves.

### Caffeine extraction procedure from chocolate milk

An aliquot 5mL of the milk chocolate was taken with a 10 mL pipette and was placed into a 125 mL separating funnel followed by the addition of 10 mL distilled water and 20 mL of dichloromethane. The milk chocolate was extracted by inverting the funnel at least three times, venting the funnel after each inversion. The non -aqueous layer was removed to a clean 100 mL volumetric flask. Another (20 mL) portion of dichloromethane was added to the aqueous solution in the separating funnel and the extraction procedure was repeated twice and the dichloromethane layers were combined. This volume was made up to 100 mL with the solvent. The absorbance of the resulting solutions was then measured on UV/Vis spectrophotometer at 270 nm using 10 mm quartz cuvette [11].

### Result and discussion

The UV–vis absorption spectrum of caffeine in dichloromethane was found to be in the region of 272 and 274.7 nm at room temperature. The standard linear calibration curve obtained from the standard solution analysis is presented in Fig.1. It showed a good linear relationship between the absorbance and concentrations of the standard solutions [11].

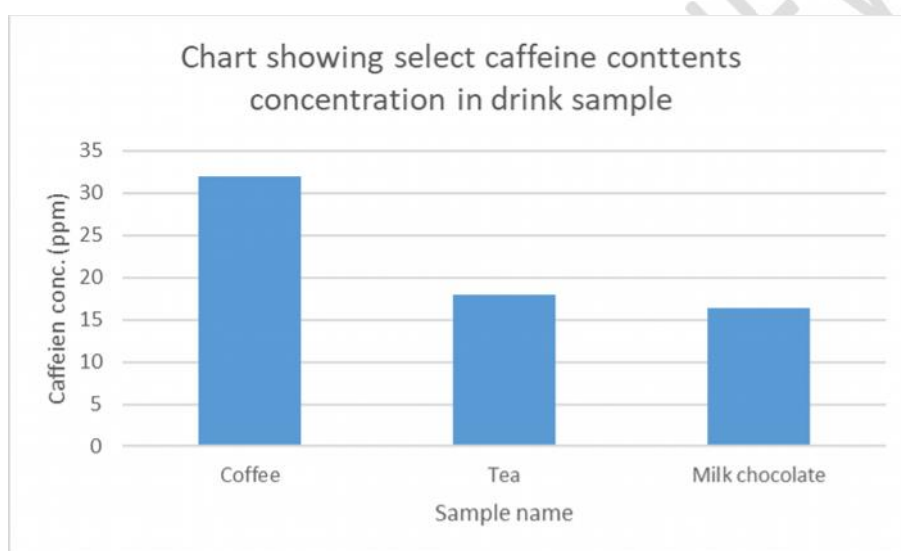


**Fig. 1 Calibration curve for standard caffeine**

Using the proposed methods, the percentage of caffeine in coffee, tea and chocolate milk were determined. The caffeine content levels in samples are presented in Table 1 and Fig.2

Sample name	Caffeine conc.(ppm)	Range (ppm) <sup>[15]</sup>
Coffee	32 ppm	30-50 ppm
Tea	18 ppm	30-90 ppm
Milk chocolate	16.38 ppm	1-15 ppm

**Table 1: caffeine contents concentration of drink sample.**



**Fig.2 Chart showing Select caffeine contents concentration in the drink sample.**

The minimum caffeine level was found in chocolate milk (16.38 ppm) which is slightly high than the published data range (1-15 ppm) and the highest was found in coffee (32.00 ppm) which is slightly below the published data range (30-50) [15].

The literature reported the moderate caffeine consumption of 300 mg/day, is considered generally safe [11]. Caffeine content in samples varies by type from 16-32 ppm of caffeine per serving however the US Food and Drug Administration (FDA, 2006) limits the maximum amount to 6 mg (200 ppm). The results indicating that the level of caffeine in the samples analyzed in this study is below the maximum allowable limits set by the above food regulatory bodies [11].

### **Conclusion**

The current method developed on UV/vis spectrophotometer is relatively easy, fast, cheap and highly sensitive for the determination of caffeine content in coffee, tea and chocolate milk. Moreover, chemicals and instruments needed to carry out the analysis by proposed methods are those which are available in most common laboratories.

## Reference

- 1- Singh, D. K., & Sahu, A. (2006). Spectrophotometer determination of caffeine and theophylline in pure alkaloids and its application in pharmaceutical formulations. *Journal of Analytical Biochemistry*, 349, 176–180.
- 2- Alpdogan, G., Karbina, K., & Sungur, S. (2002). Derivative spectrophotometerdetermination of caffeine in some beverages. *Turkish Journal of Chemistry*, 26, 295–302.
- 3-Brannstrom, M., & Edenteg, K. (2002). Determination of caffeine in Vietnamese coffee using HPLC. Umea, Sweden: Department of Analytical Chemistry.
- 4-Casal, S., Oliveira, M. B., & Ferreira, M. A. (2000). HPLC/Diode-array applied to thermal degradation of trigonelline, nicotinic acid and caffeine in coffee. *Journal of Food Chemistry*, 68, 481–485.
- 5- Ortega-Burrales, P., Padilla-Weigand, R., & Molina-Diaz, A. (2002). Simultaneous determination of paracetamol and caffeine by flow injection solid phase spectrometry using C18 silica Gel as a sensing support. *Journal of Analytical Science*, 18, 1241–1246.
- 6- Lauritsen, F.R. and Ketola, R.A. (1997) *Anal. Chem.* 69, 4917- 4922.
- 7-Talab, S., (2017), Analysis of caffeine in soft drinks consume in Saudi Arabia by UV spectroscopy, *Indian journal of applied research*, 7 (12), 645-646.
- 8- Zhang, Q.-L., Lian, H.-Z., Wang, W.-H., & Chen, H.-Y. (2005). Separation of caffeine and theophylline in poly (dimethylsiloxane) micro channel electrophoresis with electrochemical detection. *Journal of Chromatography A*, 1098, 172–176.
- 9- Abebe Belay, *et al.*, (2008) Measurement of caffeine in coffee beans with UV/vis spectrometer, *Food chemistry*, 108, 310-15.
- 10- Rogers, P.J. and C. Dernoncourt, (1998). Regular caffeine consumption: A balance of adverse and beneficial effects for mood and psychomotor performance. *Pharmacol. Biochem. Behav.*, 59(4): 1039-45.
- 11- Tautua, A; Martin, W.B; Diepreye, E.R.E. (2014), Ultra-violet Spectrophotometric Determination of Caffeine in Soft and Energy Drinks Available in Yenagoa, Nigeria. *Advance Journal of Food Science and Technology.*, 6(2),155-158.
- 12- Murray, D.S.; Hansen, P.J., (1995), *J. Chem. Educ.*, (72) 851.
- 13- Hampp, A., (1996), *J. Chem. Educ.*, (73) 1172.
- 14- Hill, Barbaro; (2000), Experiments in Organic Chemistry CPC, John Thompson; Lane Community College, Carol Handy PCC.
- 15- Shearer,J; Graham,T.E., (2014), Performance Effects and Metabolic Consequences Of Caffeine and Caffeinated Enerhy Drink Consumption On Glucose Disposal. *Nutrition Reviews.*,72(1),121-136.