

Research Article

Ultra-violet Spectrophotometric Determination of Caffeine in Soft and Energy Drinks Available in Yenagoa, Nigeria

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Abstract: This study was carried out to determine the pH and levels of caffeine in eight brands of carbonated and energy drinks available in local market in Yenagoa, Nigeria. Quantitative analysis of caffeine was performed by a simple and fast standard UV spectrophotometric method, using carbon tetrachloride as the extracting solvent. Results showed that the pH of the beverages were slightly acidic ranging from 5.92-6.44. The minimum caffeine level was observed in the carbonated soft drink Coca Cola (43.71±0.55 ppm), while the energy drink, Red Bull sample showed the highest caffeine content (58.31±0.35 ppm). The carbonated soft drinks showed caffeine levels in the range of 43.71 and 45.83 ppm with average concentration of 44.52 ppm, whereas in the energy drinks it ranged from 47.56 to 58.31 ppm with a mean concentration of 52.24 ppm. The caffeine content in all the beverage samples analyzed in this study are well below the maximum allowable limits set by the US Food and Drugs Administration.

Keywords: Beverage drinks, caffeine, UV spectrophotometry

INTRODUCTION

Caffeine is a bitter white crystalline xanthine alkaloid that acts as a mild psychoactive stimulant drug. It also possesses a weak diuretic action. It is found in varying quantities in the seeds, leaves, or fruits of many plants species (Andrews *et al.*, 2007; Dionex, 2007; Wanyika *et al.*, 2010; Violeta *et al.*, 2010). The most common sources of caffeine are coffee, cocoa beans, cola nuts and tea leaves.

In humans, caffeine acts as a central nervous system stimulant, hence it is used both recreationally and medically to reduce physical fatigue and restore mental alertness when unusual weakness or drowsiness occurs (Nehlig *et al.*, 1992; Maidaon *et al.*, 2012). It is metabolized in the liver into three primary metabolites, paraxanthine (84%), theobromine (12%) and theophylline (4%) (Aurnaud, 1987) (Fig. 1).

Caffeine is also a common ingredient of soft drinks such as cola and energy drinks where it is deliberately added as a flavoring agent and to make the drinks addictive. Caffeine content in soft drinks varies by brand from 10 to 50 mg of caffeine per serving (Violeta *et al.*, 2010); however the US Food and Drug Administration (FDA, 2006) limits the maximum amount in carbonated beverages to 6 mg/oz. Therefore caffeine content allowed in soft drinks may be in the range between 30 and 72 mg/355 mL (12 oz) or 8.45-20.28 mg/100 mL (NSDA,1999).

Energy drinks including Red Bull, Bullet, Power Horse, Monster, are non-alcoholic beverages containing

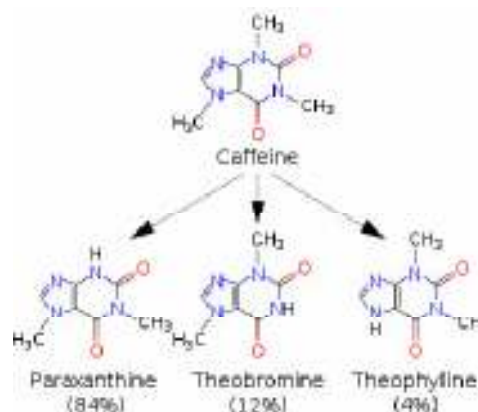


Fig. 1: Caffeine and its main metabolic products (Wanyika *et al.*, 2010)

caffeine, herbal extracts such as guarana, ginseng and ginkgo biloba, B vitamins, amino acids such as taurine, amino acid derivatives such as carnitine and sugar derivatives, including glucuronolactone and ribose (Boyle and Castillo, 2006). Energy drinks are reported to contain as much as 80-300 mg of caffeine and 35 g of processed sugar per 8-oz serving (Clauson *et al.*, 2008).

In recent years, a number of these energy drinks have been introduced to the Nigerian market. Their use in combination with alcohol by young adult consumers claims to improve performance and boost energy. This could further increase the health risk to consumers.

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A wide variety of methods have been employed including High Performance Liquid Chromatography (HPLC) being the method of choice by many researchers in determining the caffeine contents of beverages (Camargo and Toledo, 1999; Bispo *et al.*, 2002; Mumin *et al.*, 2006; Nour *et al.*, 2008; Violeta *et al.*, 2010; Wanyika *et al.*, 2010), however HPLC is a high-priced and resource consuming technique that is not typically found in most universities in developing countries such as Nigeria. Therefore, the present study was undertaken to analyze and quantify the caffeine content of some soft and energy drinks found in Yenagoa market in Nigeria using a fast alternative analytical method that uses UV spectroscopy.

MATERIALS AND METHODS

All the glassware were soaked overnight with chromic acid solution and washed thoroughly with water and detergent, then rinsed with deionised water dried in the oven and transferred to a desiccator before use. The chemicals and reagents used in this study were of analytical grade and no further purification was carried out and was obtained from BDH Chemical Limited, England. Pure anhydrous caffeine was obtained from Coca Cola PLC, Nigeria. Different kinds of commonly consumed soft and energy drinks including Pepsi Cola, Diet Coca Cola, Mountain Dew, Coca Cola, Bullet, Power Horse, Lucozade Boost and Red Bull were purchased from the local market in Yenagoa, Nigeria.

pH determination: Beverages pH were determined by using Sartorius pH meter.

Preparation of caffeine standard solutions: A 100 ppm stock standard of caffeine was prepared by dissolving 25 mg of caffeine in 250 mL purified carbon tetrachloride (CCl₄) in a volumetric flask (250 mL). Working standards were prepared by pipetting 10, 20, 30, 40 and 50 mL, respectively aliquots of the stock standard solution into separate volumetric flasks (100 mL) and diluting to volume with purified carbon tetrachloride to produce concentrations of 10, 20, 30, 40 and 50 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.

Caffeine extraction procedure: An aliquot (5 mL) of the drink sample was drawn with a 10 mL pipette and placed into a 125 mL separating funnel followed by the addition of distilled water (10 mL), then 20% aqueous Na₂CO₃ solution (1 mL) and analytical grade CCl₄ (20 mL). The caffeine was extracted by inverting the funnel

at least three times, venting the funnel after each inversion. The non-aqueous CCl₄ layer was removed to a clean 50 mL volumetric flask. Another 20 mL portion of CCl₄ was added to the aqueous solution in the separating funnel and the extraction procedure was repeated twice more and the CCl₄ solvent layers combined. This volume was made up to 50 mL with the solvent. This procedure was repeated for all the drink samples. The absorbance of the resulting solutions was then measured on UV/Vis spectrophotometer at 270 nm using 10 mm quartz cuvette.

Quantitative caffeine determination: Quantitative analysis of caffeine was performed by a 6405 Jenway UV/Vis Spectrophotometer. The λ 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 λ max = 270 nm. Standard linear calibration curve was run to obtain the linear range of sample analysis, correlation factor was with accepted value = (0.996) and the standard calibration curve was linear over the range (10-60) ppm caffeine with equation ($y = 0.035x + 0.100$).

The quantitative amount of caffeine in samples (ppm) was then determined using the standard curve.

RESULTS AND DISCUSSION

The standard linear calibration curve obtained from the standard solutions analysis is presented in Fig. 2. It showed a good linear relationship between the absorbance and concentrations of the standard solutions.

The pH and caffeine content levels in beverage samples are presented and illustrated in Table 1 and Fig. 3.

pH values in Table 1 show that all beverages were slightly acidic, with Red Bull having the lowest pH

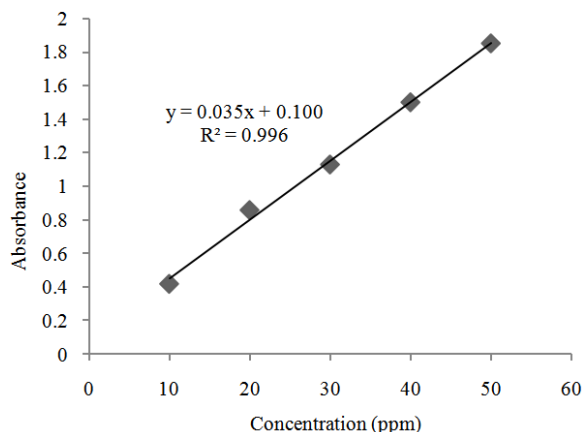


Fig. 2: Calibration curve for standard caffeine

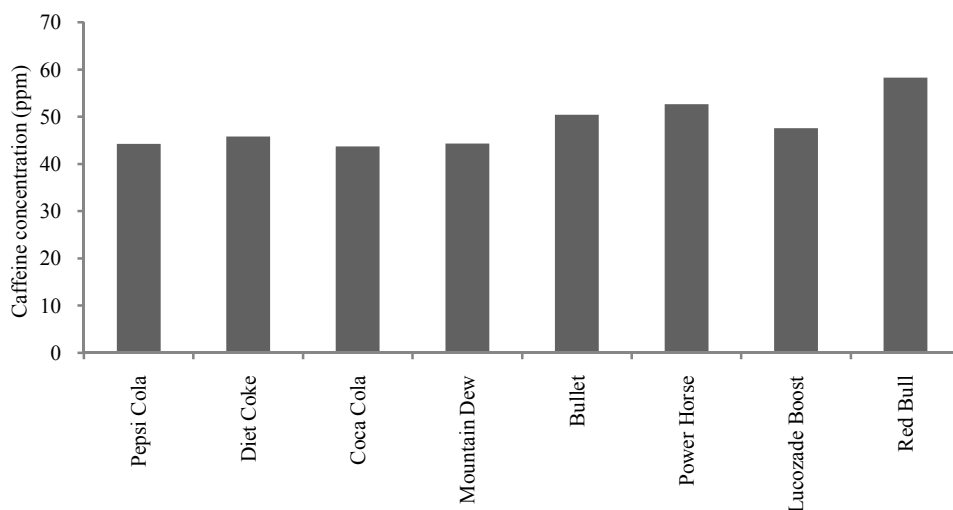


Fig. 3: Chart showing caffeine contents in the beverage samples

Table 1: pH and caffeine contents of beverage samples

Sample type	Sample name	pH±S.D. ^a	Caffeine conc.±S.D. ^a (ppm or mg/L)
Carbonated soft drinks	Pepsi cola	5.52±1.89	44.22±1.02
	Diet coke	5.79±2.12	45.83±1.05
	Coca cola	5.50±0.97	43.71±0.55
	Mountain dew	5.54±0.65	44.31±1.03
Energy drinks	Bullet	5.92±1.12	50.42±0.45
	Power horse	5.85±2.03	52.65±0.68
	Lucozade boost	5.79±1.04	47.56±0.44
	Red bull	6.44±0.76	58.31±0.35

^a: Mean±standard deviation (n = 3); S.D.: Standard deviation

(6.44) and Bullet having the highest (5.92). The reason behind the low pH values could be as a result of the presence of CO₂ gas or other acids such as phosphoric acid, malic acid, ascorbic acid, citric acid and tartaric acid used as preservatives by the manufactures of these beverages (Bassiouny and Yang, 2005; Ashurst, 2005). These acids inhibit the growth of microorganisms such as bacteria, mould and fungi which may contaminate beverages. Studies revealed that drinking acidic beverages over a long period can erode tooth enamel and predisposes the consumer to dental disease (Marshall *et al.*, 2003; Bassiouny and Yang, 2005). Our results were corroborated by Lin *et al.* (2003) who reported that all kinds of soft drinks are acidic and that cola drinks specifically make our bodies poor in oxygen (Lin *et al.*, 2003).

The minimum caffeine level was observed in the carbonated soft drink Coca Cola (43.71±0.55), while the energy drink, Red Bull sample showed the highest caffeine content (58.31±0.35 ppm). The caffeine content in the in the carbonated soft drinks group ranged between 43.71 and 45.83 ppm; while in the energy drink samples it ranged from 47.56 to 58.31 ppm. The average caffeine quantity in the energy drinks was found to be 52.24 ppm; while in the carbonated soft drink samples the mean caffeine contents was lower (44.52 ppm). It was also observed that the caffeine level of the Diet Cola (45.83±1.05) was

slightly higher compared to the regular cola drinks (Pepsi cola and Coca-cola). This is in agreement with previous work reported by Mei *et al.* (2012).

Moderate caffeine consumption of 300 mg/day, is considered generally safe (Rogers and Derroncourt, 1998; Smith, 2005). This amount typically corresponds to 4 cans of energy drink. Caffeine content in beverage drinks varies by brand from 10 to 60 mg of caffeine per serving (Violeta *et al.*, 2010); however the US Food and Drug Administration (FDA, 2006) limits the maximum amount in carbonated beverages to 6 mg/oz (200 ppm). Therefore caffeine content allowed in soft drinks may be in the range between 30 and 72 mg/355 mL (12 oz) or 8.45-20.28 mg/100 mL (NSDA, 1999).

Obviously, the levels of caffeine in the beverage samples analyzed in this study are well below the maximum allowable limits set by the above food regulatory bodies.

CONCLUSION

It is shown from the results of this study that the concentration of caffeine in the beverage drinks obtained from the local market in Yenagoa was significantly lower than the maximum authorized level. However since caffeine is an additive substance and because of health concerns arising from its consumption, it seems appropriate that warning labels,

indication of the presence and amounts of caffeine should accompany all caffeinated beverages. None of the drinks evaluated in this study were so labelled.

The UV/VIS spectrophotometric method employed in this study for the quantification of caffeine in beverages was found to be relatively easy, fast and cheap. The major instrument required is a modern computerized UV/VIS spectrophotometer which can be acquired at an affordable price. This analytical method may therefore, be recommended for the rapid, accurate and sensitive quantification of caffeine in beverages by any educational institutions in developing countries.

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