

**DETERMINATION OF CAFFEINE CONTENT IN NON-ALCOHOLIC
BEVERAGES AND ENERGY DRINKS USING HPLC-UV METHOD**

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ABSTRACT

The purpose of this study was to determine the amount of caffeine in non-alcoholic energy drinks and prepared teas using reverse phase HPLC. Caffeine was extracted from 19 different types of non-alcoholic beverages and prepared teas sampled from supermarkets in Nairobi Central Business District, Kenya. These were analyzed alongside a caffeine standard of 99 % purity by use of HPLC-UV detector at the wavelength of 272nm, Supelco HS C18 column 25 cm x 4.6 cm x 5 µm, oven temperature of 40 °C, mobile phase 80:20 (v/v) of methanol: water and mobile phase flow rate of 1.5mL/min. For quantitation purposes, serial dilution of the caffeine standard gave correlation coefficient (r) of 0.9993 and the retention time of 2.11±0.03 minute. Percentage recovery of caffeine from the column ranged from 89.78 to 105.59%. Limits of detection and quantitation were found to be 0.279 and 0.931 µg/mL respectively. It was found that Burn[®], XL energy drink[®] and Red Bull[®] had the highest amount of caffeine. It was however noted that though most of the non-alcoholic beverages had high caffeine content they had no label claim.

KEY WORDS: Reverse Phase High Performance Liquid Chromatography (HPLC), Ultra violet visible (UV/VIS), caffeine, non-alcoholic beverages

INTRODUCTION

Caffeine, the common name for 1, 3, 7-trimethylxanthine, is the most widely used psychoactive drug in the world (Barone & Roberts, 1996). Approximately 80% of the world's population consumes it on a daily basis, and

continuous research is being carried out to determine its health benefits and consequences. The amount of caffeine consumed daily in foods and beverages varies widely. A typical cup of brewed coffee contains approximately 100mg of caffeine and a cup of green tea contains 20-30mg of caffeine. Caffeine consumption from

all sources reaches 210-238mg/person per day in the USA and Canada and more than 400mg per person per day in Sweden and Finland (Klatsky et al., 1993; Barone & Roberts, 1996).

Caffeine acts as a mild central nervous system stimulant (Chou & Bell, 2007), which is completely absorbed after ingestion within 30 to 45 minutes and its effects substantially diminish within about 3 hours. Coffee, tea and soft drinks are the major sources of caffeine in the diets of US adults, with coffee being the primary and most potent source (Knight et al., 2004; Frary et al., 2005). Many over-the-counter medications, such as cold and allergy tablets, headache medicines, diuretics, and stimulants also contain caffeine, although they lead to relatively minimal intake (Schreiber et al., 1988).

Caffeine is a central nervous system and metabolic stimulant (Nehling et al., 1992) and is used both recreationally and medically to reduce physical fatigue and restore mental alertness when unusual weakness or drowsiness occurs. Caffeine stimulates the central nervous system first at the higher levels, resulting in increased alertness and wakefulness, faster and clearer flow of thought, increased focus, and better general body coordination, and later at the spinal cord level at higher doses (Bolton & Gary, 1981).

Some researchers have however suggested several psychiatric syndromes that are associated with high caffeine consumption, among them anxiety disorders, diuretic effects, caffeine induced sleep disorder, caffeine intoxication, caffeine withdrawal, and caffeine dependency syndrome (Winkelmayer et al., 2005). Caffeine has been associated with coronary heart disease (Yano et al., 1977), cardiovascular diseases (Dawber et al., 1974), hypertension and hypoglycemia (Watson & Kerr, 1999; Winkelmayer et al., 2005).

There is a growing individual desire to know the amount of caffeine consumed from non-alcoholic beverages. In epidemiological studies, it has been assumed that one cup of coffee contains at least 100 mg of caffeine, and soft drinks, such as colas, contain 10-50 mg of caffeine per 12-ounce serving (Christian & Brent, 2001).

Kenya is a major producer of coffee in Africa, and caffeine consumption is ranked second after tea in Kenya. Sources of caffeine consumed in Kenya range from packaged non-alcoholic beverages to over the counter caffeinated drugs. There is lack of information on the amount of caffeine consumed by an adult Kenyan in one serving of a mug of brewed coffee or a packaged caffeinated soda. Many consumers of soft and energy drinks are totally unaware of caffeine content in these drinks. Similarly, commercially available non-alcoholic beverages served in Kenya do not have product label on the packaging material and do not even declare caffeine as part of the ingredients.

In Kenya, the major source of caffeine is coffee. Kenyans consume only 3,000 tons of 50, 000 tons of coffee grown in the country annually (Douglas, 2009). However, the trend is changing with increase in coffee consumption in urban centers.

The present study determined the caffeine contents of commonly consumed non-alcoholic drinks sold in supermarkets in Nairobi Kenya using reverse phase high performance liquid chromatography with UV/VIS detector.

METHOD

Chemicals and Apparatus

A caffeine standard 99 % pure purchased from Prolabo, ECC, Manchester M, Discovery[®] HS C-18 column 25 cm x 4.6mm x 5 µm Cat. No. 568523 from Supelco, Sigma-Aldrich Co. USA and analytical grade methanol of 99.9% purity from Panreac Quimica SAU, Barcelona, and HPLC grade water Fischer Scientific were used.

The stocks solutions and the extracts were mixed in a sonicator (Bandelin, Sonorex RK 255 transistor Berlin-German) and UV-Vis spectrophotometer (UV-1700, Pharmaspec Shimadzu, Tokyo Japan) was used to determine the wavelength of maximum absorption for caffeine. HPLC fitted with UV/VIS detector (Shimadzu Corporation, Kyoto Japan) was used for the determination of amount of caffeine from the extracts.

Stock solutions

A stock solution of 1.0mg/ml of caffeine was prepared by weighing 100 mg of caffeine into a 100ml volumetric flask. This was dissolved in distilled water to make up volume to 100mL. Working solution of 0.05mg/mL, 0.1mg/mL, 0.5mg/mL were made by pipetting 5, 10 and 50mL of stock solution into a 100ml volumetric flask and topping up the volume to 100mL with HPLC analytical grade water. These serial dilutions of the standards were filtered using 0.45 μ m membrane filters and used in the construction of the calibration curve.

The mobile phase was prepared daily by mixing 80 volumes of methanol and 20 of HPLC water. This was then filtered under vacuum through 0.45 μ m pore size nylon filters and put in the HPLC mobile phase reservoir.

Sample Preparation

Nineteen samples of non-alcoholic energy drinks and prepared teas were degassed in a sonicator for 10 minutes and 5mL of each pipetted into a 100 mL amber flask and diluted 20 times with mobile phase (methanol: water at 80:20 v/v). An aliquot of 2-5mL diluted sample was filtered through membrane filter of 0.45 μ m pore diameter to remove any particulate matter. The filtrate was ready for determination of caffeine content by HPLC.

UV/Visible Spectra Scan for caffeine

Dilute caffeine standard stock solution was scanned at a range of 190 to 350nm wavelength with UV-Vis spectrophotometer (UV-1700, Pharmaspec Shimadzu, Tokyo Japan) to generate complete absorbance spectra and identify wavelength of maximum absorbance for subsequent use in HPLC-UV/VIS detector.

Determination of caffeine content by HPLC-UV method

The HPLC column was conditioned for 20 minutes with the mobile phase and the programmed oven temperature of 40°C before any analysis was carried out. HPLC-UV/VIS method was created with the following parameters, oven temperature of 40 °C, mobile phase flow rate of 1.5 mL/ minute, detector wavelength of 272nm and injection volume of 20 μ L.

A blank sample composed of the extraction solution was injected severally until the baseline was good. Then, serially diluted standards were injected in triplicates, and the retention time, peak areas and heights were recorded. After cleaning the system with blank solution, diluted sample was injected three times and the peak height and area with retention time corresponding to that of the standard was recorded. The chromatograms for caffeine standard and a sample of Coca cola beverage are shown in Figures 1 and 2 respectively.

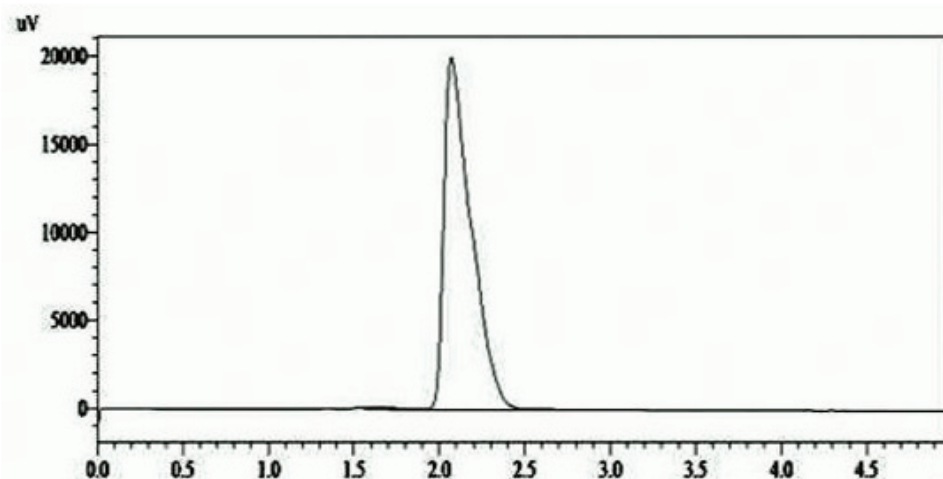


Figure 1. 10 ppm caffeine standards chromatogram, with retention time at 2.1 minutes

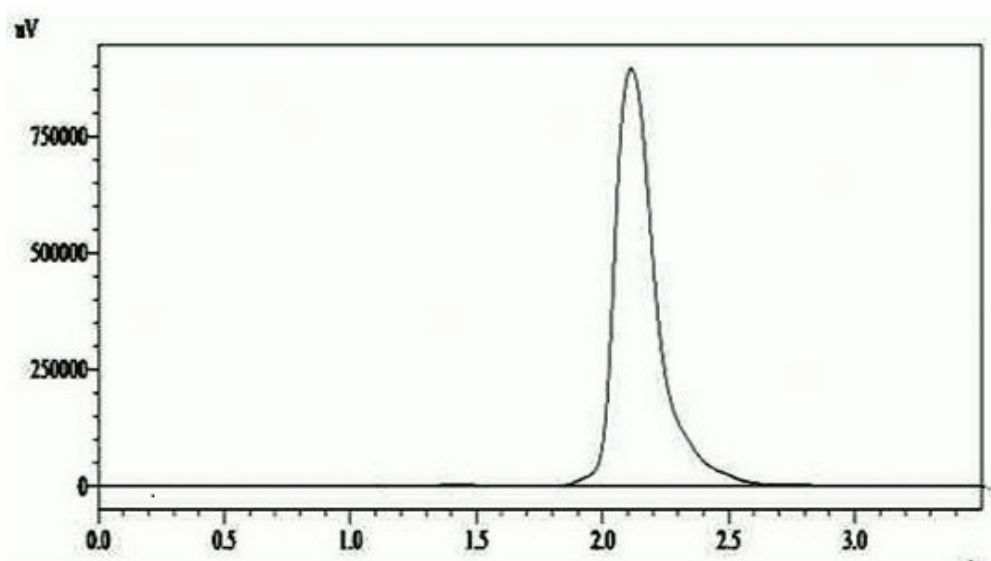


Figure 2. Corresponding peak for caffeine from Coca cola drink. The retention time is 2.1 minutes

RESULTS

The wavelength of maximum absorbance for caffeine was found to be 272nm. This was then programmed in the HPLC-UV detector for the analysis of caffeine content in non-alcoholic beverages and prepared teas. The mean retention time for caffeine was found to be 2.11 minutes with a standard deviation of ± 0.03 minutes. After analysis of the serial dilutions of the caffeine standard the coefficient of correlation was found to be 0.9993, and the equation of the linear regression was

sufficiently fit for quantitative determination of caffeine in the samples. This is shown in Figure 3.

The limit of detection (LOD) and quantitation (LOQ) of the analytes were determined based on the standard deviation of the detector response (α) and the slope (m) of the calibration curve. The following formulas were used; $3\alpha/m$ and $10\alpha/m$ for LOD and LOQ respectively (ISO 11843-2:2003). The limit of detection and quantitation of caffeine according to the method used were found to be 0.279 and 0.931 $\mu\text{g/mL}$, respectively as shown in Table 1.

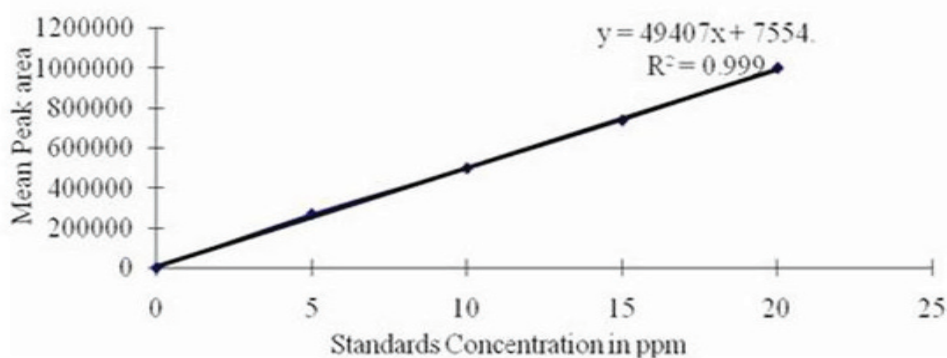


Figure 3. Linear regression curve for the serially diluted standards of caffeine

Table 1. Summary of precision and validation data for caffeine in non-alcoholic beverages obtained with reverse phase HPLC (n=12)

Validation Parameter	Amount in ($\mu\text{g/mL}$)
Limit of detection (LOD)	0.279
Limit of quantitation (LOQ)	0.931

The limit of detection and quantitation of the method were determined by $3\alpha/m$ and $10\alpha/m$, where α stands for standard deviation of the detector response and m is the slope of the linear regression curve. The detector response was obtained from 6 injections of 10ppm caffeine standard and then this concentration was divided by the average peak area.

The ruggedness of the HPLC method was determined by analysis of a known concentration of the standard working solution in the same chromatographic system and column on three different days. This is shown in Table 2. The prepared mixture of standard was injected twelve times each day for a period of three days by the same analyst. The retention times and peak areas symmetry were found to be consistent during the three days analysis with the 0.009% and 0.302% relative standard deviations for the peak area and retention times respectively. The comparable detector responses obtained on different days indicate that the method is capable of producing consistent data for routine analysis.

Analysis of 19 samples found that XL energy drink had the highest amount of caffeine with 98.06mg in a 250mL volume. This was closely followed by Burn which had 97.55mg per 250ml. The results for the other samples analyzed are as shown in Table 3. Tango orange, 7UP and Rani Float were found not to contain caffeine.

DISCUSSION

Scientific literature is rich with a wide range of values for caffeine content in food products. In United States the following standard values are suggested by several authors, coffee (5 oz) 85 mg for ground roasted coffee, 60 mg for instant and 3 mg for decaffeinated, 18 mg for instant colas. Caffeine consumption survey data are limited. In US approximately a mean daily caffeine intake for consumers is 4 mg/kg and among children younger than 18 years of age who are consumers of caffeine-containing foods, the mean daily caffeine intake is about 1 mg/kg (Barrone & Roberts, 1996).

Caffeine use is associated with several distinct psychiatric syndromes such as caffeine intoxication, withdrawal, dependence, caffeine-induced sleep disorder, and caffeine-induced anxiety disorder. In certain people, caffeine consumption causes adverse health effects, such as anxiety, palpitations, irritability, difficulty sleeping and stomach upsets. Other studies have indicated moderate caffeine intake could lower consumers' risk of liver disease (Frery et al., 2005). The American Dietetic Association and the UK Food Standards Agency recommend not more than 300 mg of caffeine per day (Frery et al., 2005). This recommendation is particularly useful to pregnant women who have greater

Table 2. Day to day peak area and % recovery of 10ppm caffeine standard

Number of days	1	2	3
*Mean Area \pm SD	524112 \pm 3505	529257 \pm 4966	525715 \pm 3842
Percent RSD	0.007	0.009	0.007

SD = standard deviation; RSD = relative standard deviation.

*Mean of 10ppm spectral peak areas for caffeine standard injected daily was used to monitor the reproducibility of the method.

Table 3. Summary of caffeine contents in mg/ total can volume in mL of various beverages

Non-alcoholic beverage	Caffeine conc. In mg per can
XL energy drink 250 ml	98.06
Burn 250 ml	97.55
Red Bull 250ml	93.54
Shark 250ml	91.84
Bomba 250ml	91.20
Atomic 250ml	90.52
B-52 250ml	79.45
Safari ice tea 310 ml	66.29
Pepsi Maxi 500ml	46.26
Bullet 250ml	44.71
Africola 500ml	42.25
M-150 150ml	38.50
Vigor Power ice 250ml	36.84
Coca cola 330ml	33.27
Strong Horse 250ml	6.19
Tango cherry 330ml	0.23
Tango orange 330ml	0
7UP 500ml	0
Rani float	0

risk of miscarriage or giving birth to low birth weight babies.

Because of the health concerns associated with caffeine consumption, it is important that the manufacturers of caffeinated non-alcoholic beverages and teas append appropriate warning labels declaring presence and amount of caffeine. Studies show that 30 mgs or less of caffeine can alter self-reports of moods and affect behavior and 100 mg per day can lead to physical dependence and withdrawal symptoms upon abstinence.

Caffeine is an addictive drug, which alters the brain's natural state and stimulates it in a manner similar to the amphetamines, cocaine and heroin. One challenge with caffeine

addiction is that most people don't think of caffeine as a drug.

Determination of caffeine content in non-alcoholic beverages and prepared teas is a very important analytical process to safeguard the well-being of the people who are vulnerable to the adverse effects of caffeine.

CONCLUSIONS

Reverse phase high performance liquid chromatography method for determination of caffeine content in non-alcoholic beverages is simple, accurate and robust. The amount of caffeine in non-alcoholic beverages is critical especially when one what to control consumption of caffeine. The data obtained in this study is important in the local setting because most of these non-alcoholic beverages list caffeine in their product label without withholding the amount contained. Therefore, one can easily control caffeine intake if caffeine is declared as an ingredient and at the same time the amount is indicated in the packaging materials.

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