Research Article

Determination of Caffeine in Different Brands of Tea and Coffee Marketed in South Eastern Nigeria

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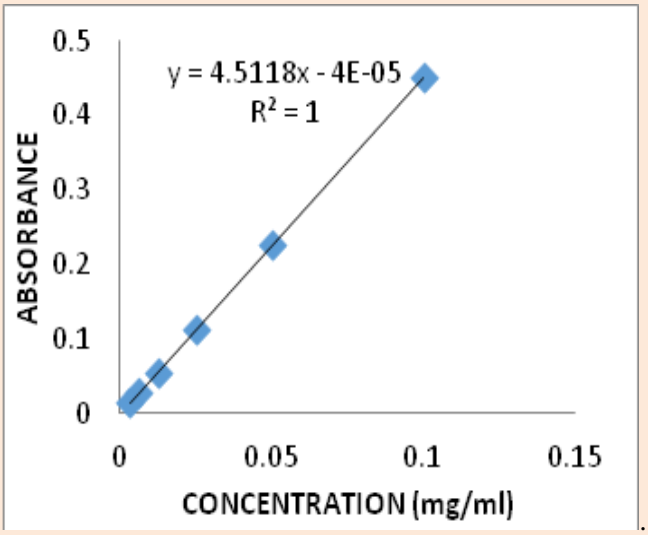
Abstract

Caffeine is consumed for different purposes and is obtained from different sources as beverages and medication. Therefore, unintended over dosage can occur. Although intoxication due to over dosage of caffeine rarely causes fatality on its own, it can lead to accidents that result in fatalities or severe injuries in drivers and machinery operators. In Nigeria also, where counterfeiting and faking of products is widespread, it is necessary to ascertain the labeled claim of product periodically for public safety and wellbeing. Samples of different tea and coffee brands were purchased from chain stores in South Eastern Nigeria. Quantities equivalent to 6 g of each brand were weighed out and extracted using liquid-liquid extraction. Absorbances of solutions were measured using UV/Vis spectrophotometer and the actual concentration determined using Beer's plot. An internal standard was used to measure the efficiency of the extraction procedure. Results showed that while the caffeine contents of some of the bags were normal, some supposedly caffeine free and decaf products had too high a concentration of caffeine which could affect people who for medical reasons took them in place of other caffeinated beverages. This suggests that some decaffeinated products are not actually as decaffeinated as they should be.



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Introduction

Caffeine has been used for many years. As a food ingredient, it is one of the most widely consumed [1]. It can be found naturally in cocoa beans, coffee beans, kola nuts, guarana berries and tea leaves including yerba mate. It can also found in common beverages as well as cocoa containing chocolate and in different types of medications [2–3]. Almost all of the caffeine meant for consumption as food in the US is imported in the form of coffee and tea in [4]. Primarily, coffee and tea leaves are majorly the common sources of daily consumed caffeine. Other sources include soft drinks and energy drinks [1]. The recent increase in the use of caffeinated sport drinks, juices, waters, energy drinks and other functional beverages have increased the market for caffeinated beverages [5]. Caffeine has been used in beverages consumed to achieve mental alertness and boost energy [6] Medically, caffeine is used as a CNS stimulant [7], as an adjuvant in the treatment of apnea associated with preterm birth also known as apnoea of prematurity [8], in the treatment of migraine in combination with ergotamine [9], as an analgesic adjuvant [10] and for diuresis [11]. As a result, it is possible to overdose with caffeine while consuming different caffeinated beverages and medications for different purposes at different times during the day.

When consumed in moderation, caffeine is safe [12]. Only few cases of fatality has been reported [13]. Caffeine intoxication is thought to majorly results from the over consumption of caffeine containing medications, not caffeinated foods or beverages [14]. However untoward effects resulting from excessive consumption of caffeine, leading to dependence or withdrawal effects [15] and possibly hypertension [16] call for moderation. Caffeine intoxication manifest as restlessness, tremors, anxiety, and tarchycardia. Some of these symptoms may not result in emergencies but may lead to injuries and accidents that may result in fatalities. Body weight and sensitivity to caffeine play a major role in determining the onset of caffeine intoxication. Generally, it is recommended that consumption of less than 400 mg/day will guide against intoxication symptoms [17].

The aim of the study is to quantitate and validate the caffeine content of different brands of caffeine containing beverages while the objective is provide the consumer, regulatory agencies and policy makers with adequate data for decision making.

Experimental

Materials and Reagents

Pure caffeine sample (anhydrous) was obtained from Pauco Pharmaceuticals, Awka, Nigeria. Absorbances were measured with Shidmadzu UV- spectrophotometer (Japan). All chemicals and activated charcoal was obtained from BDH (Germany). Top tea, Tetley tea, lipton tea, Nescafe (classic), Koffiehius (full roast grain), Frisco Vital (chamomile with rooibus&honeybush tea) Nescafe (gold blend) decaff and Café enrista were all purchased from a Shoprite, a chain store in Nigeria.

Preparation of basic lead acetate

First, 5 g of lead acetate was weighed into a 500 ml beaker, 20 g of lead oxide was added to the beaker containing the lead acetate 250 ml of boiling water was added to the mixture and stirred continuously. The resultant mixture was heated to boil for 5 minutes on a hot plate, the mixture was then filtered using a filter paper, and the filtrate obtained was dispensed into a conical flask and appropriately labeled.

Preparation of dilute sulphuric acid

Using a 10 ml measuring cylinder, 10 ml of concentrated sulphuric acid was measured out, the sulphuric acid was poured gently into a 1000 ml beaker containing 1000 ml of distilled water. The dilute sulphuric acid thus obtained, was then appropriately labeled.

Extraction of caffeine from beverage

Six grams of the beverage was weighed using an electronic weighing balance into a 500ml beaker, to the content in the beaker, 300 ml of boiling distilled water was added. The resultant mixture was placed in a water bath, and allowed to digest for 15 minutes while stirring intermittently.

Using a filter paper, the resultant mixture was filtered, while still kept hot over a water bath. To the filtrate, 100 ml of basic lead acetate was added and the resultant precipitate formed was filtered off while the mixture was still left hot, using a filter paper. Then, to the filtrate obtained in above, 50 ml of dilute sulphuric acid was added. The mixture obtained above was filtered using a filter paper, to the filtrate obtained above, 2 g of activated charcoal was added and the mixture stirred using a glass rod so as to decolourize the mixture. The mixture was placed on a hot plate and allowed to concentrate by evaporating the water to at least one-half of its initial volume, the mixture above was then filtered while hot using a filter paper and allowed to cool over ice packs. The filtrate obtained above was placed in a separating funnel and using 30ml of chloroform, the caffeine contained in the water extract was extracted.

The chloroform extraction was repeated twice, and the whole chloroform extract was combined, measured and stored in an airtight dispensing bottle. The whole procedure was repeated four times, so as to obtain five results for each of the brand of beverages.

Preparation of internal standard

Using an electronic weighing balance, 0.2 g of caffeine was weighed into a 500ml beaker, to the content in the beaker 320 ml of boiling distilled water was added. The resultant solution was placed in a water bath and heated to boil for 15 minutes.

Using a filter paper, the resultant solution was filtered, while still kept hot over a water bath. To the filtrate, 100 ml of basic lead acetate was added and the resultant mixture was filtered using a filter paper while still kept hot on a water bath. To the filtrate obtained above, 50 ml of dilute sulphuric acid was added and the mixture obtained above was filtered using a filter paper. To the filtrate obtained above, 2 g of activated charcoal was added and the mixture stirred using a glass rod. The mixture was placed on a hot plate and allowed to concentrate by evaporating the water to at least one-half of its initial volume; the mixture above was filtered while hot using a filter paper and cooled using ice packs. The filtrate obtained above was placed in a separating funnel and using 30ml of chloroform, the caffeine contained in the water extract was extracted.

The above procedure was repeated twice and the whole chloroform residue was combined, measured and stored. The whole procedure above was repeated four times to obtain five results.

Preparation of standard solutions for the beer-lamberts plot

Using an electronic weighing balance, 0.4g of pure caffeine sample was dissolved in 100ml of chloroform in a 250 ml beaker. From the above solution formed, 1ml was withdrawn and diluted to 10ml using chloroform in a conical flask. Then, from the above solution formed, a two-fold serial dilution was performed to obtain a range of concentrations between 0.1 mg/ml and 0.003125 mg/ml.

Results and Discussion

The absorbances of the standard solutions were used to prepare a calibration plot **Figure 1** which is linear with an R^2 value of 1. The standard deviation of the intercept was determined using LINEST function in Excel to be 0.000495331 and the limits of detection (LOD) and quantitation (LOQ) were determined using the equation LOD=kSDa/b and LOQ= kSDa/b where k=3 for LOD and 10 for LOQ. The LOD was determined to be 0.00033 mg while the LOQ was 0.0011 mg.

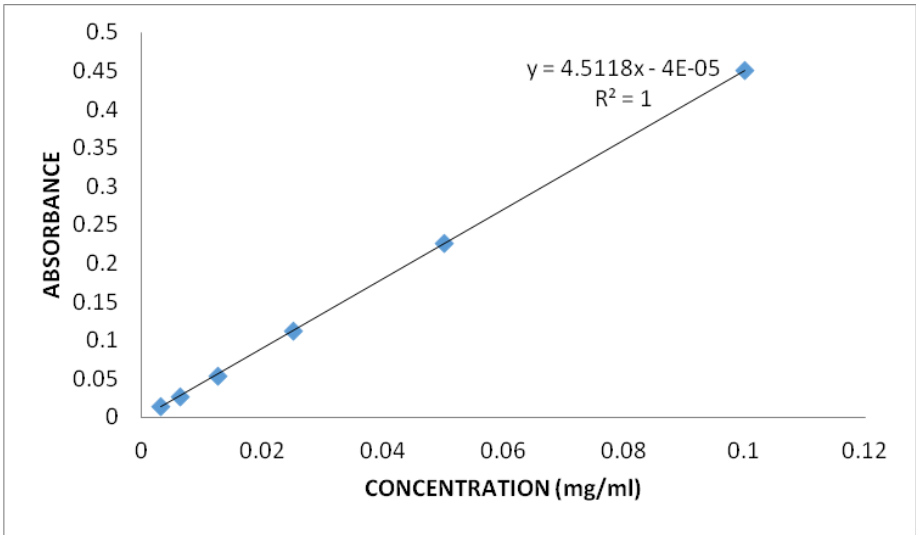

Figure 1 Graph of absorbance against concentration of the standard solutions.Using the regression analysis equation the different concentrations of the caffeine containing chloroform extracts was obtained from their absorbance (A) respectively

Table 1 below displays the absorbances of the different brands of chloroform extract, their concentrations in mg/ml, their concentration in mg present in 6g (1 teaspoon for coffee and 3 bags for teas) of the samples and their equivalent concentration in mg present in each teabag or teaspoonful of the coffee. Hence, to obtain the quantity of caffeine present in a teabag the amount of caffeine present in 6g was divided by 3. **Table 2** below shows the recommended level of caffeine that should be generally contained in the following beverages namely; tea, coffee and decaffeinated coffee.

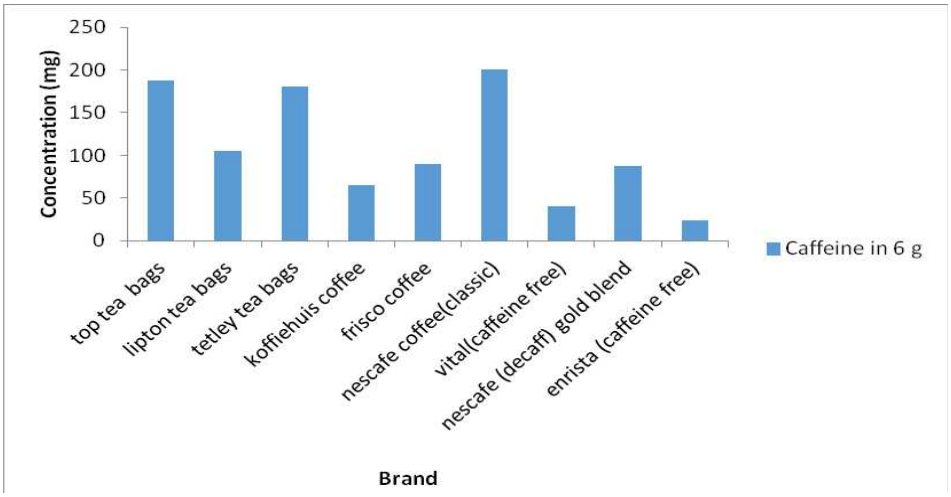

Figure 2 Bar chart showing the equivalent amount of caffeine present in the different brands of beverages for every 6g of sample as well as for each teabag or teaspoon taken

Table 1 Showing the absorbances and concentration different brands of beverages

Brands	Absorbance (λ=254nm)						Concentration		
	A1	A2	A3	A4	A5	A(AVG)	CONCN (mg/ml) per 6g	Per 6g	Per bag (mg)
top tea bags	1.011	0.752	0.876	0.813	0.789	0.8482	0.188021	188	63
lipton tea bags	0.47	0.555	0.516	0.498	0.329	0.4736	0.104983	105	35
tetley tea bags	0.791	0.736	0.938	0.832	0.779	0.8152	0.180706	181	60
koffiehuis coffee	0.224	0.245	0.419	0.311	0.277	0.2952	0.065437	65	
frisco coffee	0.376	0.365	0.513	0.387	0.398	0.4078	0.090397	90	
nescafe coffee (classic)	0.899	0.971	0.769	0.913	1.105	0.9314	0.206464	201	
Vital (caffeine free)	0.182	0.224	0.087	0.197	0.222	0.1824	0.040433	40	
nescafe (decaff) gold blend	0.259	0.359	0.548	0.421	0.387	0.3948	0.087516	88	
enrista (caffeine free)	0.086	0.157	0.084	0.098	0.122	0.1094	0.024251	24	
Internal Stnd.	0.731	0.826	0.861	0.976	1.113	0.9014	0.199814	200	

Table 2 Showing normal caffeine content in teas and coffee beverages (FDA, 2007)

Item	Item Size	Caffeine(mg)
Coffee	150 ml	60-150
Tea	150 ml	40-80
Coffee(decaf)	150 ml	2-5

From the results obtained (Table 1), it could be seen that there was no loss of internal standard, as reflected by the amount recovered (199.8 mg) since 200 mg of pure caffeine was used as the internal standard. This shows that the extraction process was efficient; hence, there was no need to adjust for lost values obtained for the different brands as to reflect the actual caffeine content.

The quantity of caffeine extracted from 6g of tea leaves was high compared to that from some coffee brands with the exception of Nescafe classic (Table 1 and Figure 1). For instance, 188 mg was extracted from top tea, 105 mg from Lipton, while 180.7 mg from Tetley. Nescafe classic had the highest concentration of caffeine at 200.6 mg/6 g, a value that was expected. However, most tea drinkers use only one tea bag at a time. Since the amount of caffeine recorded for tea was for 3 tea bags approximately, the actual values of caffeine per tea bags reduces to 63 mg, 60 mg and 35 mg for Top tea, Tetley and Lipton tea brands respectively. These values fall within the range for teas in table for Top tea and Tetley while it was below the desired range for Lipton tea suggesting that Lipton tea was either adulterated or poorly processed. It was reported that the amount of caffeine present in a cup of Lipton tea steeped for about 3 minutes and 5 minutes are 38 mg and 47 mg respectively [18]. Considering that the Lipton tea used for this study was boiled for 30 minutes a time long enough to enable exhaustive extraction of caffeine from the tea bags, it goes to confirm that the Lipton tea does not have adequate quantity of caffeine which suggests adulteration.

Unexpectedly, the other coffee brands had lower values of caffeine with koffiehuis and frisco having a value of 90.4 mg and 65.4 mg respectively. However, both values fall within the ranges. On the other hand, the content of caffeine in Nescafe classic was above the upper limit per 150 ml.

The recommended daily intake of caffeine according to different regulatory bodies is as follows: pregnant women <300 mg [19], Adults ≤400 mg/day [20], Children 6-12yrs, 45-85 mg [19], 2.5 mg/kg/day for ≥12yrs [19]. Considering that some people use more than 2 teaspoonful (400 mg) per cup and many cups of coffee per day especially in Europe, during the winter, it may be necessary to investigate the correlation between the consumption of coffee and accidents that occur in factories and warehouse especially at night among machine operators and other factory worker.

Although decaffeinated coffee and caffeine free beverages claim to be caffeine free, significant quantities of caffeine albeit lower than those from earlier groups were extracted from 6 g of the samples. For instance, 40.4 mg of caffeine was extracted from vital, while 24.3 mg was extracted from enrista which are supposed to be caffeine free. Disturbingly, Nescafe decaff had more caffeine, 87.5 mg, than there is in Frisco and almost the same amount with Koffiehuis.

It could be argued that the value of caffeine in the decaff is less than half as there is in classic but the quantity was still not safe for any person that chose decaff for any health reasons. Apart from healthy adults who are free to take up to 400 mg of caffeine per day, it is recommended that unlealthy adults, pregnant women, children and adolescents should be more conservative in their intake of caffeinated drinks [5]. Therefore, this result shows that the health of these groups of individuals may be affected by inadvertent overdosage and possible drug-drug interaction.

Conclusions

In conclusion, it could be said that labeled claims do not always represent true composition of active components a product. It should therefore not be taken for granted that the composition of beverages will remain the same after they have been licensed for sale especially when a functional food like caffeine is involved. Periodic surveillance by regulatory agencies is recommended

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