

Determination and Characterization of Caffeine in Tea, Coffee and Soft Drinks by Derivative Spectrophotometry and High Performance Liquid Chromatography

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Abstract: Caffeine is a stimulant drug which is commonly found in several beverages like tea, coffee, cocoa and colanuts. Depending on the dose, it can be a mild central nervous system stimulant. In this paper, the quantification of the caffeine content of twelve beverage samples were reported using UV/ Vis spectrophotometry and the results were compared with the reversed phase HPLC method. Solubilization of pure caffeine was done in three solvents namely: dichloromethane (DCM), ethylacetate and water. The spectra were derivatised to obtain the first and second order derivatives. The results showed that dichloromethane had the highest resolution at λ_{max} of 274nm compared to water (272nm) and ethylacetate (273nm). Isolation of caffeine from samples was carried out by liquid-liquid extraction using dichloromethane as an extracting solvent. The caffeine extracts were analyzed using PG T80 UV/Visible spectrophotometer series. The percentage of caffeine in the twelve samples analyzed were as follows: Napacafe coffee (8.90%) > Nescafe coffee (7.80%) > Lipton Yellow tea (7.64) > Top tea (6.76%) > *Cola acuminata* (3.90%) > Extra joss energy powder (3.26%) > *Cola nitida* (2.60%) > Cocoa beans (0.88%) > Minicoffee kopiko sweet (0.49%) in the solid beverage samples and it was found that caffeine content of planet energy drink (9.28%) > Pepsi cola (7.55%) > Coca-Cola (7.20%) in the liquid samples. The results also indicated that caffeine can be extracted more at the boiling temperature (100°C) (as found in Top tea, Nescafe coffee and Napacafe coffee with values: 11.72%, 13.25% and 14.03%) than at 30°C with 6.76%, 7.80% and 8.90% respectively). Generally, higher concentrations of caffeine were obtained with the UV/Vis spectrophotometric method (i.e 6.76, 3.26 and 3.90µg/g) compared to HPLC method (2.76, 1.00 and 1.49µg/g). This shows that caffeine has high affinity for DCM and thus a better solubilization solvent than ordinary water mixed with acetonitrile.

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Introduction

Caffeine (1,3,7-trimethylxanthine or 3,7-dihydro-1,3,7- trimethyl-1*H*-purine-2,6-dione) is a purine alkaloid of the methylxanthine family that occurs naturally in the leaves, seeds or fruits of tea, coffee, cocoa, colanut and more than 60 other plants (Abdul Mumin et al., 2006; Nour Violeta et al., 2008) Pure caffeine is odourless and has a bitter taste (M.J. O'Neil et al., 2001; J.A.T. Pennington., 2005). Caffeine is one of the legally permitted additives in beverages, with a tolerated upper limit of 0.015% (w/w) (Ministerio de Agricultural et al., 1982).

Recreationally, caffeine is used to provide a boost of energy or a feeling of heightened alertness. It is often used to stay awake longer. The pleasant stimulant feeling, which often occurs at low doses, may be replaced by psychological symptoms, which resemble anxiety and depressive neuroses at high doses. Those with more severe psychological problems may have their symptoms exaggerated with excessive caffeine usage (Khan K et al., 2006).

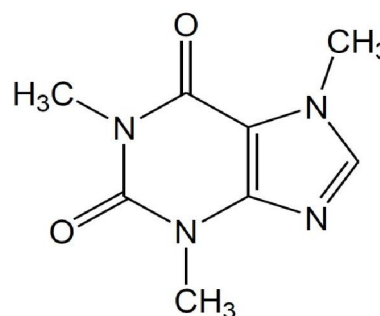


Figure 1: Structure of Caffeine

Caffeine is added to soft drinks as a flavoring agent, and from dietary sources is the most frequently and widely consumed central-nervous-system stimulant today. Nevertheless, it was classified as a drug of abuse by the International Olympic Committee (IOC) when present in urine at concentration levels of more than 12 µg/mL. The high levels of caffeine consumption have been implicated in various

disorders including the increase of gastric-acid secretion, kidney malfunction, heart disease (cardiac arrhythmia), and disturbances of the central nervous system such as seizures and delirium. However, caffeine is utilized as a co adjuvant in many pharmaceutical formulations (Bispo M.S et al., 2002).

The reported caffeine content in the main dietary sources varies significantly: 93.0 – 163.5 mg per cup in ground coffee, 46.7 – 67.6 mg per cup in instant coffee, 30.2 – 67.4 mg per cup in bag tea and 0.32 – 0.54 mg/g in dark sweet chocolate. The differences have been attributed to the variety of coffee bean or tea leaf, method of preparation (e.g. the brewing of coffee and tea), volume of a cup and analytical methods utilized for caffeine determination. Although the level of caffeine in chocolate is less variable, it still depends on the origin of the beans. In the case of carbonated beverages the variability occurs among brands, since most of the caffeine content in these products is added from other natural sources, i.e. less than 5% of the total present caffeine is from cola nuts (Camargo M.C.R et al., 1999).

Cola like drinks account for 80 to 90% of the caffeine added to foods today. These products with caffeine artificially stimulate the body and increase the heart rate. While this artificial stimulation temporarily arouses the intellect and fatigue seems to disappear, it is short lived. The excess stimulation depletes the body of vital energy as it struggles to deal with this poison that has entered its system. In the amounts presently being consumed, it can cause insomnia, nervousness, irritability, anxiety and disturbances in the heart rate and rhythm. There are other effects from the consumption of caffeine, including increased incidence of bladder and stomach cancer, raised blood pressure and it aggravates diabetes and damages the lining of the stomach (Khan K. et al., 2006).

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 (Dzneladze Z et al., 1987; Laskowski K et al., 1965) spectrophotometry; polarography, GC and HPLC have been reported. Derivative spectrophotometric methods have been developed for the assay of caffeine in some pharmaceutical preparations. 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 295. Derivative spectrophotometry is a useful technique for extracting qualitative and quantitative information from spectra composed of unresolved bands (Abdel-Moety E M. et al., 1988), and for eliminating the effect of baseline shifts and baseline tilts (Deng P. et al., 1989). Derivative spectrophotometry is now a reasonably priced standard feature of modern micro-computerized UV/Vis spectrophotometry.

Experimentals

Samples

All the tea and coffee samples were of commercial origin purchased from the local supermarkets of the University of Lagos (UNILAG) except raw colanuts and cocoa beans which were acquired from a local market at Atan, a suburb of Ota, in Ogun state, Nigeria.

Materials and Chemicals

A double beam PG T80 UV/Visible spectrophotometer using bandwidth (0.5, 1, 2 and 5nm), 10mm micro quartz cells. (wavelength range 240 nm to 300 nm.), Agilent 1200 HPLC system using reversed phase column (5 μ m 250mm X 4.6mm C₁₈ reverse phase column (supelcosil)) microbalance (Mettler At250, 0.1/0.01 mg), Chemical balance (BP 210S, d = 0.1 mg, Max 210 g) for measuring mass of tea leaves, measuring cylinder, magnetic stirrer, glass filter (2 'Pyrex', England), beakers, thermometer, separatory funnel, funnel, ice bath, distilled water, dichloromethane (assay: 99.6%, Aldrich Germany), ethyl acetate (assay: 99%, Indian), caffeine (M.W. 194.19 g/mol, Aldrich Germany).

Analytical procedures

Caffeine stock solution (100 μ g/ml) was prepared by dissolving 10.00mg of caffeine standard in 100ml of water, dichloromethane, and ethylacetate respectively. The mixture was stirred using the magnetic stirrer for 30 minutes and the absorbance measurement determined with wavelength verification (From 240-300nm). Solutions were freshly prepared and diluted to obtain working standards for the preparation of calibration curves.

Sample preparation and extraction procedure

50.00mg each of tea and instant coffee was accurately weighed in an 100ml beaker. 50ml of

distilled water heated to about 30°C was then added and mixture stirred for 30 minutes with magnetic stirrer. The mixture was filtered through a glass filter and cooled to room temperature. 15 ml of dichloromethane was poured into the infusion and stirred for 10 minutes with magnetic stirrer. The aqueous phase and organic phase were then separated by separatory funnel. The procedure was repeated three more times to ensure efficient removal of the total caffeine in the samples. The total caffeine content in the beverage samples were then determined by UV spectrometry.

Another set of samples were extracted using distilled water (At temp: 100°C).

Soft and energy drink samples preparation

Each of the beverages was poured into a 250 ml beaker and degassed by stirring on a hot plate with magnetic stirrer. The homogenized solution was then

filtered. 50 ml each of the filtrate were measured into a beaker and 15 ml of dichloromethane was added. The mixture was gently swirled on the hotplate for about five minutes. The resultant mixture was separated with separatory funnel and the organic layer decanted into an erlenmeyer flask.

15 ml of DCM was added and subsequent extraction was repeated three more times. Finally, the total caffeine content was determined with a double beam spectrophotometer. (PG T80 series of UV-Visible Spectrophotometer).

Results And Discussion

Spectra Results

The absorption spectra of pure caffeine in three different solvents were measured using UV-visible spectrometer. The spectra were recorded at a spectral band width of 1 nm and fast scan speed.

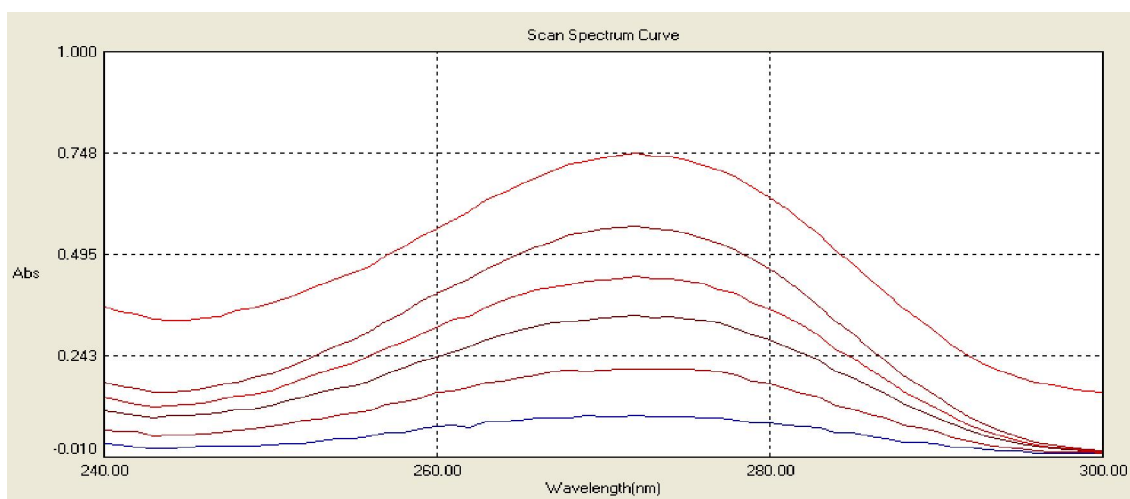


Figure 2: Absorption spectrum of caffeine standards in water (D_0)

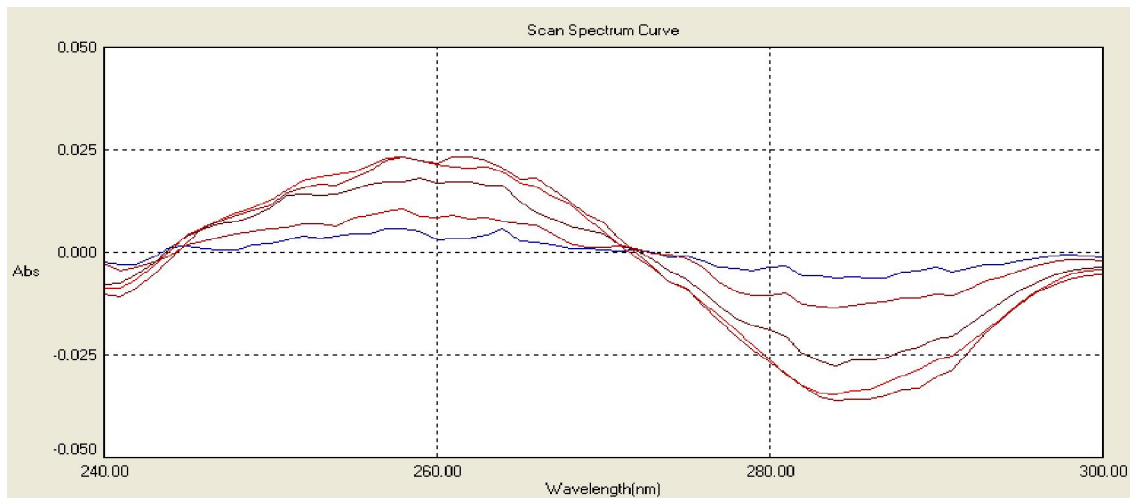


Figure 3: First derivative overlay spectrum of caffeine standards in water (D_1) (spectra range of between 245 – 300 nm with λ_{max} of 272 nm)

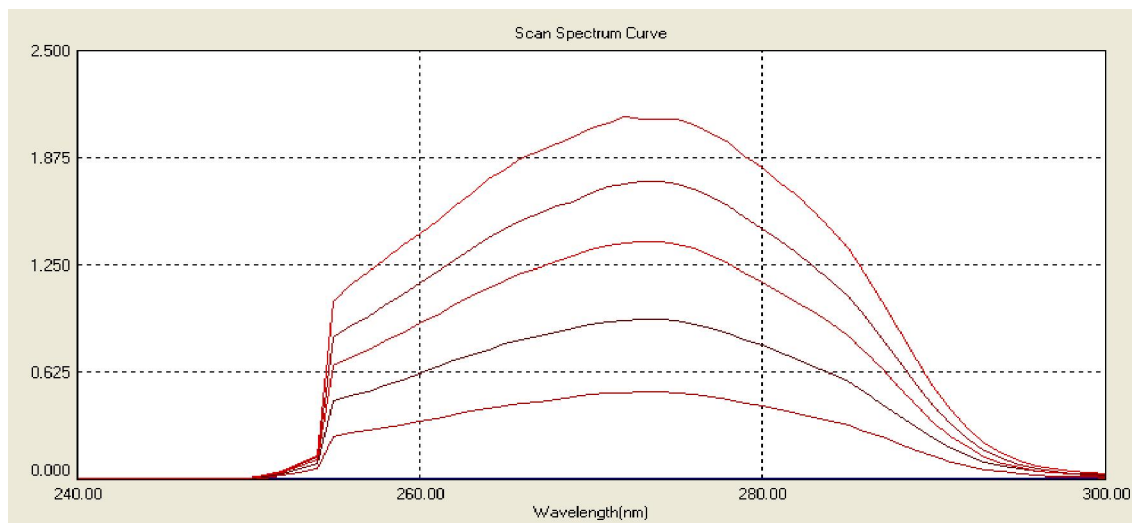


Figure 4: Absorption spectrum of caffeine standard in ethylacetate (D_0)

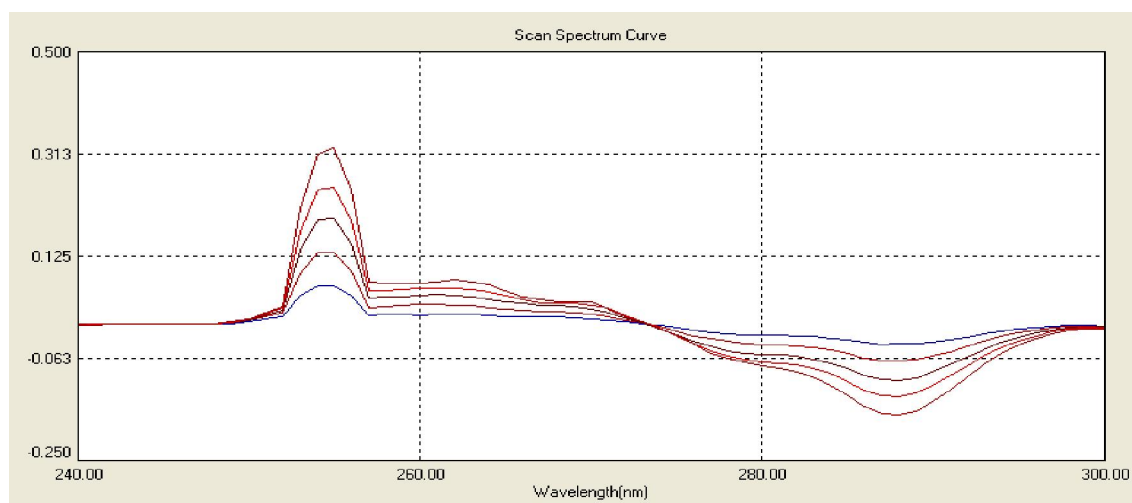


Figure 5: First derivative overlay spectrum (D_1) of caffeine standards in ethylacetate

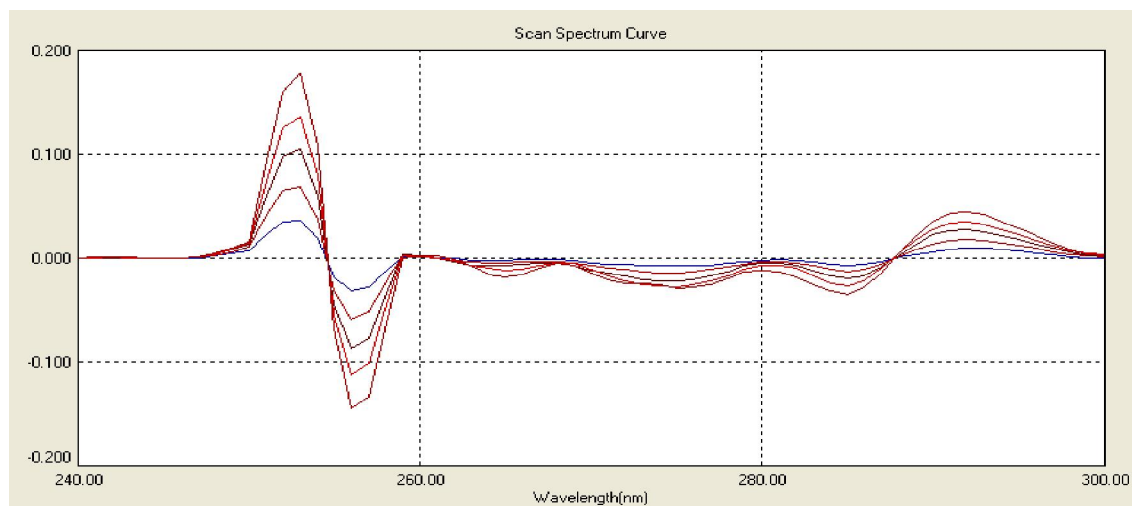


Figure 6: Second derivative spectrum (D_2) of caffeine standards in ethyl acetate. (λ_{max} of 273nm)

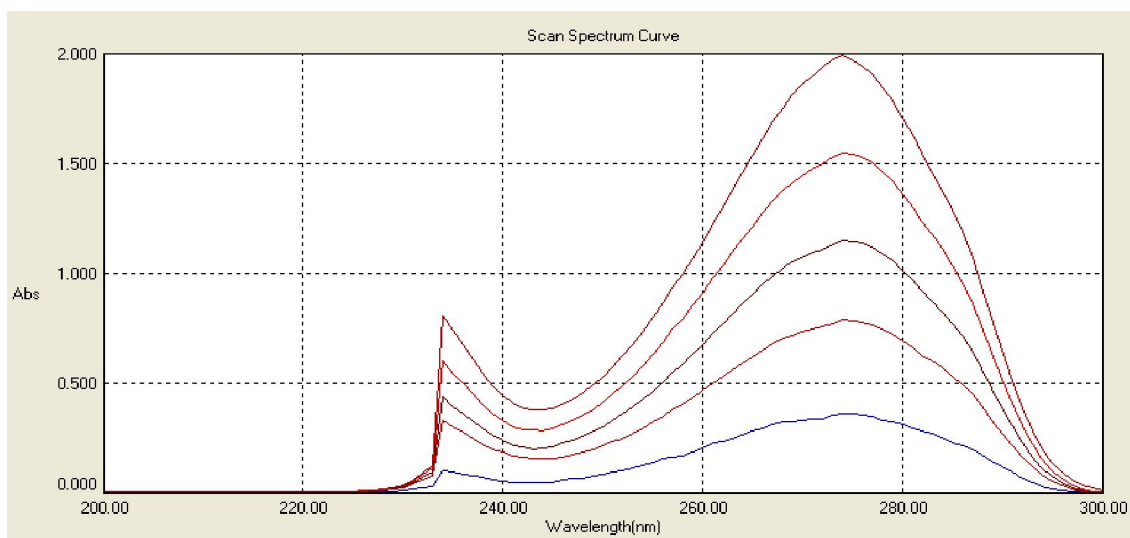


Figure 7: Absorption spectrum of pure caffeine in dichloromethane. (D_0)

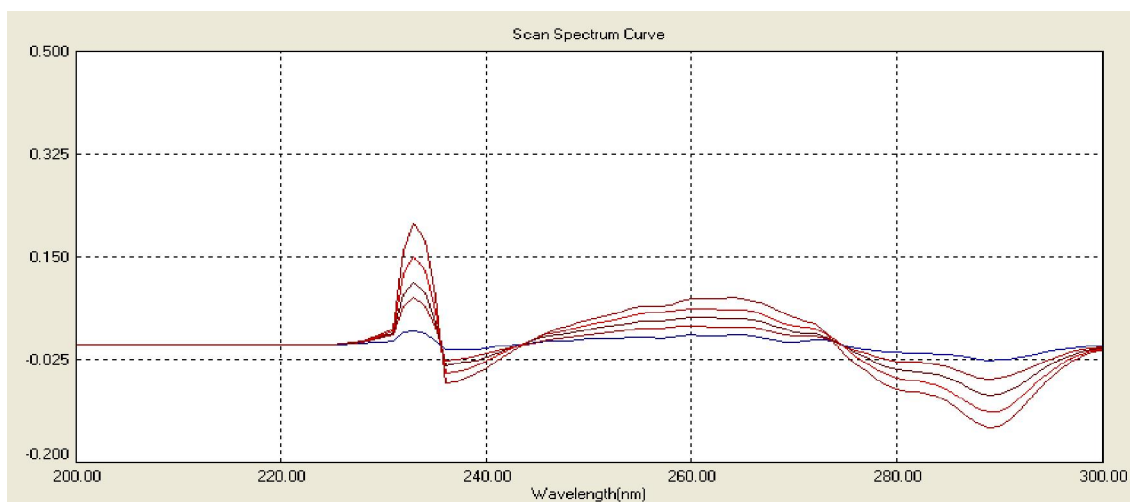


Figure 8: First Derivative overlay spectrum (D_1) of caffeine standards in dichloromethane (λ_{max} of 274nm)

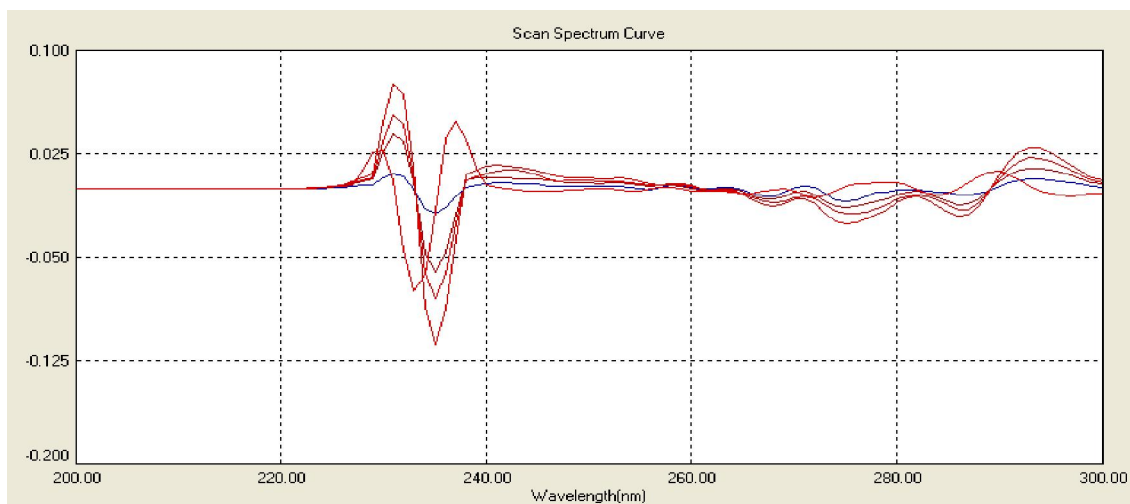


Figure 9: Second Derivative overlay spectrum (D_2) of caffeine standards in dichloromethane

Validation of the method

The calibration graph of absorbance versus the concentration of pure caffeine in water, ethylacetate and dichloromethane were plotted as shown in the Figure 4.9, 4.10 and 4.11 respectively.

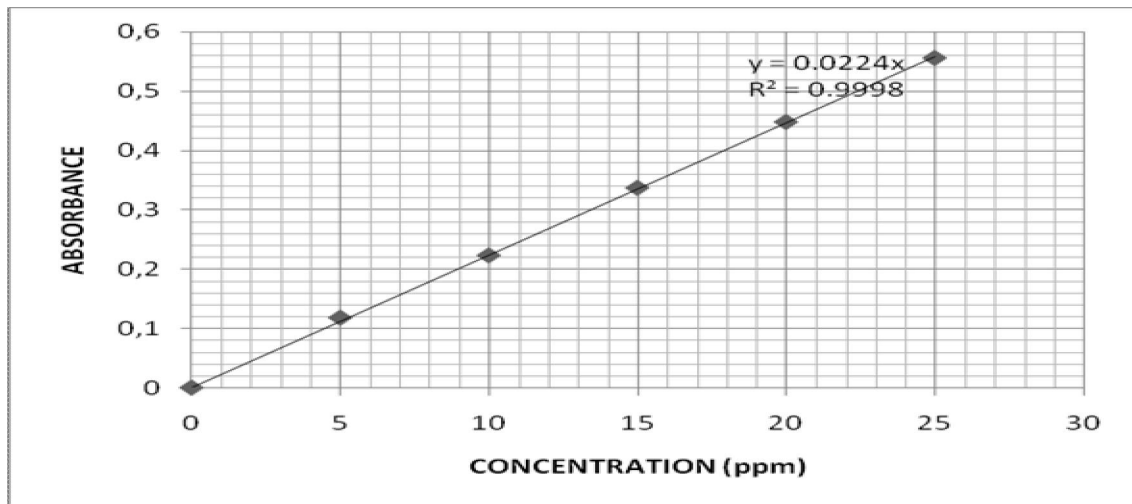


Figure 10: Calibration graph of caffeine in water

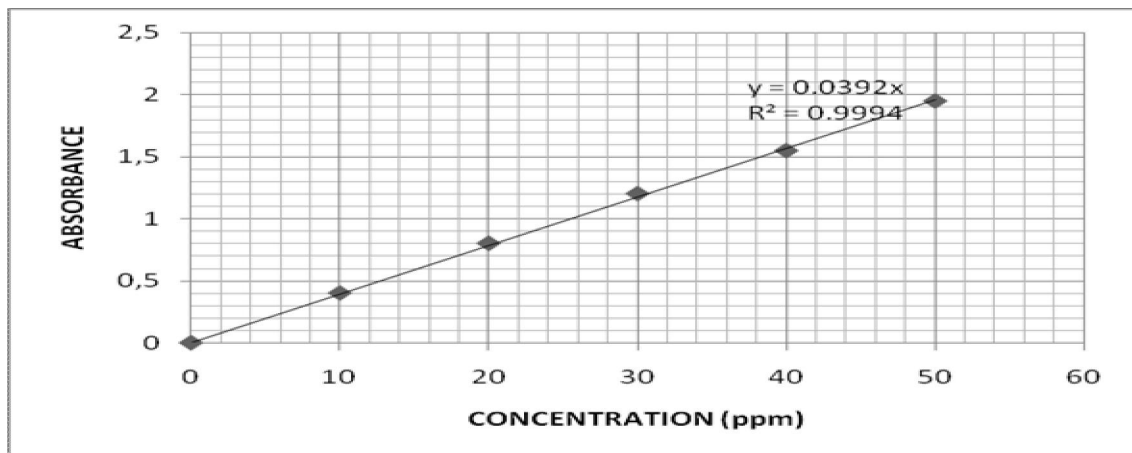


Figure 11: Calibration graph of caffeine in ethylacetate

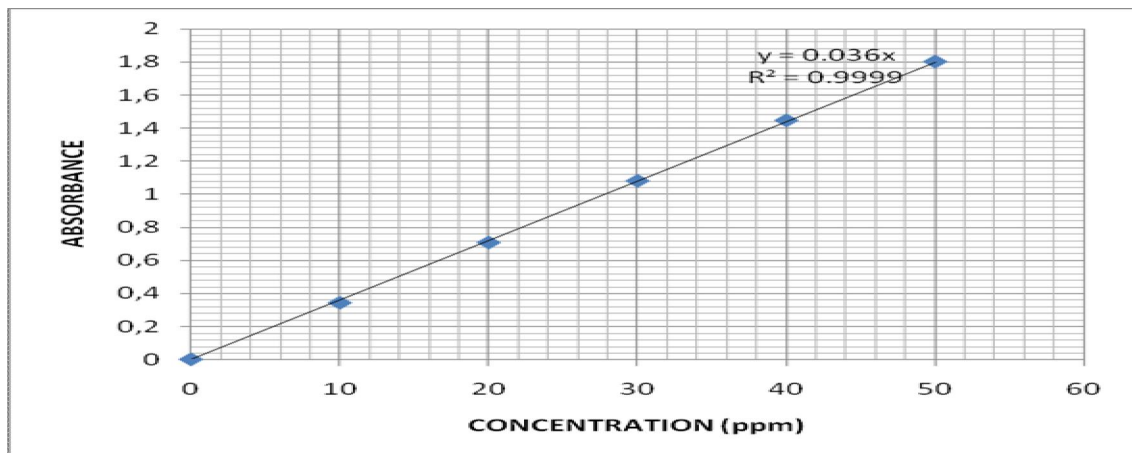


Figure 12: Calibration graph of caffeine in dichloromethane

A good linear relationship was observed for a wide concentration range. This shows that absorbance is directly proportional to the concentration.

Determination of caffeine contents in beverage samples

Caffeine content in tea leaves cannot be determined directly using UV-Visible spectrophotometer, due to the matrix effect of UV absorbing substances (Guzin, 2002).

Therefore, it was necessary to develop a method to overcome this difficulty. The method developed was to dissolve caffeine in water and then extract, using dichloromethane.

To determine caffeine from tea leaves, the extractions were repeated four times until the content of caffeine was found negligible as observed in figure 13.

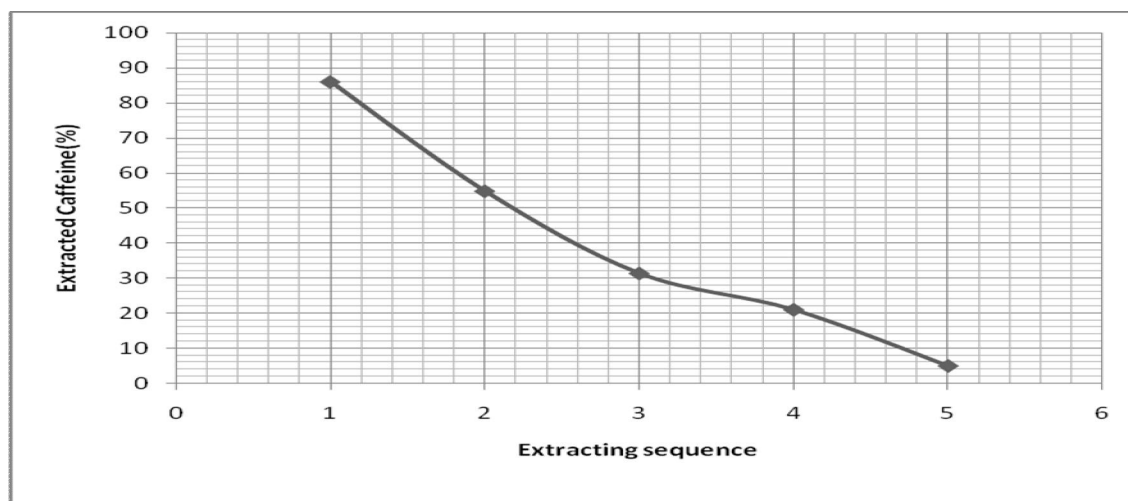


Figure 13: Extraction sequence graph of caffeine standard using dichloromethane

Table 1: Experimental result of determination of caffeine in beverage samples at two different temperatures (30 °C and 100 °C) (n=3).

30°C				100 °C			
S/N	Sample	Absorbance	Concentration	% Caffeine	Absorbance	Concentration	% Caffeine (w/w)
1	Top Tea	2.435±0.001	67.64±0.030	6.76±0.003	4.221±0.001	117.24±0.017	11.72± 0.003
2	Lipton Yellow Tea	2.753±0.003	76.46±0.017	7.64±0.017	4.450±0.005	123.62±0.017	12.36±0.002
3	Nescafe coffee	2.809±0.002	78.02±0.046	7.80±0.005	4.769±0.001	132.47±0.030	13.25±0.003
4	Napacafe coffee	3.203±0.004	88.98±0.017	8.90±0.002	5.050±0.001	140.29±0.017	14.03±0.002
5	Extra joss powder.	1.174±0.001	32.61±0.030	3.26±0.003			
6	Kopiko sweet	0.179±0.006	4.97±0.030	0.49±0.003			
7	Cocoa beans	0.317±0.001	8.80±0.017	0.88±0.002			
8	Cola nitida	0.935±0.005	25.96±0.017	2.60±0.002			
9	Cola acuminata	1.404±0.003	38.99±0.017	3.90±0.002			
10	Planet Energy Drink	3.341±0.001	92.81±0.025	9.28±0.003			
11	Pepsi cola	2.718±0.005	75.51±0.035	7.55±0.004			
12	Coca-Cola	2.591±0.007	71.98±0.017	7.20±0.005			

Table 1 presents the experimental result for the determination of caffeine at two different temperatures. (30°C and 100°C). The result in the table indicates that at 30°C, the percentage of caffeine in Napacafe coffee sample with 8.90% is the highest for all the solid samples (tea, Extrajoss energy powder, kola nuts, cocoa beans, and mini coffee kopiko sweet). Minicoffee kopiko sweet sample had the least caffeine content (0.497%). Next to the coffee with the a highest caffeine content is the tea. It can be observed that

Lipton yellow tea has a higher caffeine content of 7.646% compared to the top tea with 6.70%.

From the table, it can also be observed that the two species of cola nuts have different amount of caffeine even though they are both of the same family. *cola acuminata* (3.899%) has the higher content of caffeine than *cola nitida* with 2.596% w/w.

In the liquid beverage samples planet energy drink has the highest caffeine content while Coca-Cola beverage has the lowest (Table 2).

From the beverage samples tested at the boiling temperature (100°C) similar to those at 30° higher caffeine content were obtained. Top tea has the least caffeine content of the four tea and coffee samples while Napa café coffee has the greatest caffeine content. Comparing the content of caffeine at 30°C and boiling temperature, the percentage of caffeine at boiling temperature is greater (Table 2). Caffeine content in tea leaves reported in this experimental work is in the range of values reported (1-8%) by Abdu et al. (2006) and the caffeine contents of marketed tea by Misra et al. (2009).

Chromatogram Results Of A Standard And Some Selected Samples

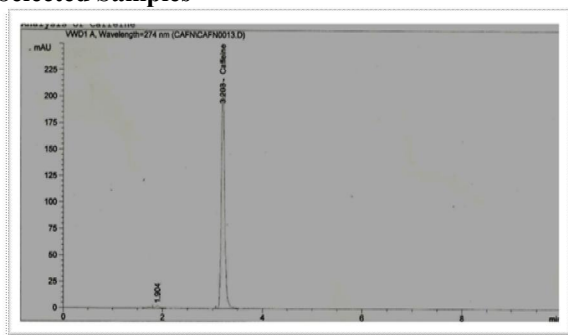


Figure 14 chromatogram of caffeine standard 25 ppm

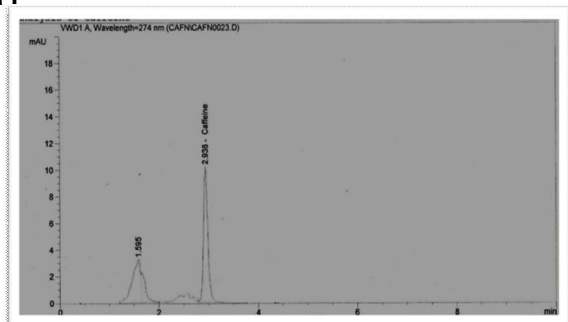


Figure 16: Chromatogram Of Lipton Yellow Tea Sample

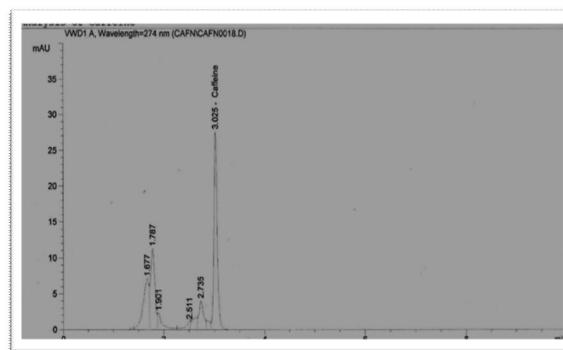


Figure 17: Chromatogram of Nescafe Coffee Sample

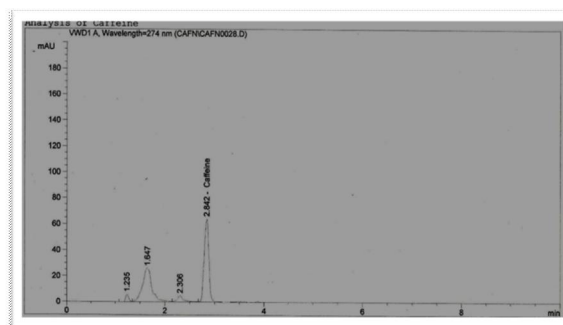


Figure 18: Chromatogram of Coca-Cola Sample

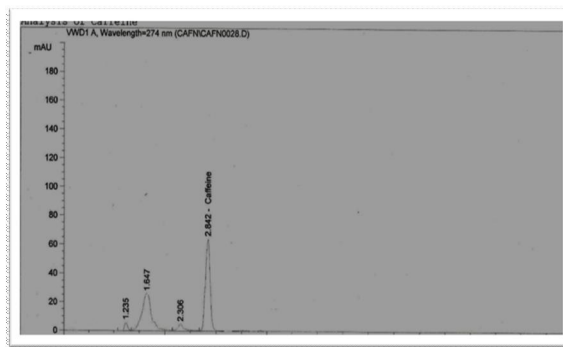


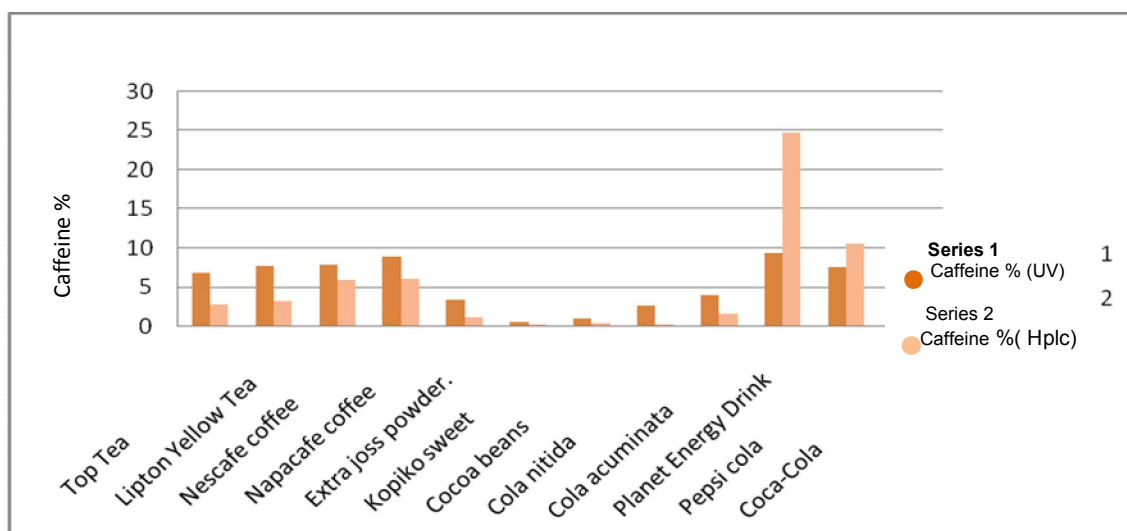
Figure 19: Chromatogram of Cola acuminata Sample

Table 2: Result of caffeine content in beverage samples using HPLC

Sample Name	Area	Concentration (ppm)	% Caffeine (w/w)
Top Tea	113.698	2.726	2.726
Lipton Yellow Tea	128.411	3.078	3.078
Nescafe coffee	246.332	5.905	5.905
Napacafe coffee	253.325	6.073	6.073
Extra joss powder.	41.744	1.001	1.001
Kopiko sweet	8.815	0.211	0.211
Cocoa beans	10.097	0.242	0.242
Cola nitida	8.422	0.202	0.202
Cola acuminata	62.067	1.488	1.488
Planet Energy Drink	1032.165	24.743	24.743
Pepsi cola	438.619	10.514	10.514
Coca-Cola	419.545	10.057	10.057

Table 3: Result of comparison of caffeine content in beverage samples by UV/Vis spectrophotometer and HPLC

Sample Name	UV/Vis spectrophotometric method (%)	HPLC method (%)
Top Tea	6.76	2.726
Lipton Yellow Tea	7.64	3.078
Nescafe coffee	7.80	5.905
Napacafe coffee	8.90	6.073
Extra joss powder.	3.26	1.001
Kopiko sweet	0.49	0.211
Cocoa beans	0.88	0.242
Cola nitida	2.60	0.202
Cola acuminata	3.90	1.488
Planet Energy Drink	9.28	24.743
Pepsi cola	7.55	10.514
Coca-Cola	7.20	10.057

**Figure 20: Comparison of caffeine levels in beverage samples obtained by UV/Vis spectrophotometer and HPLC**

Conclusion

The order of caffeine concentration in the beverage samples were: Napa café coffee > Nescafe coffee> Lipton yellow> extra joss energy drink powder > cola nut> coca beans > kopiko sweet for the solid samples and planet energy drink > Pepsi cola > Coca-Cola for thee liquid sample.

The highest caffeine content was found in the coffee (8.9%) and Planet energy drink (9.28%) while the lowest amount was observed in mini coffee kopiko sweet (0.49%) and cocoa beans (0.88%).

The results at two different temperatures reveal that, caffeine is better extracted at boiling temperature (100⁰C) as in Top tea, Lipton yellow, Nescafe and Napacafe with greater values of 11.72, 12.36, 13.25 and 14.03% respectively than at 30⁰C with lower progression values of 6.76, 7.64, 7.80 and 8.90% and more extraction time will still favour more.

In conclusion, the derivative spectrophotometric method is relatively easy, fast and cheap for the determination of the caffeine content of cola, coffee, tea and other sample. 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, micro-computerized UV/VIS spectrophotometer, which can be purchased at a reasonable price.

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