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Physicochemical properties of teas sold in Abuja, Nigeria, and evaluation of their caffeine content using HPLC

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Abstract
Tea is commonly consumed in Nigeria. Caffeine, a major constituent in tea, has some beneficial pharmacological properties, but can negatively affect human health if consumed excessively. The objective of the study was to evaluate some physicochemical properties and caffeine content of teas marketed in FCT, Abuja, Nigeria. Ten commercial brands of teas (8 black teas and 2 green teas) were assessed for weight variation, moisture content and pH tests using standard methods. Extraction of caffeine was carried out and the identity determined by thin layer chromatography and melting points, respectively. High performance liquid chromatography (HPLC) method for analysis of caffeine was developed, validated, and applied to determine caffeine content in the tea brands. Results of the weight, moisture content and pH tests of the samples ranged from 2.07–2.33 g, 5.65–11.0 % and 4.9–5.5, respectively. Caffeine was isolated from all the samples and showed same R_f value (0.46) with that of the reference standard. Melting points ranged from 236.0–238.5 °C. Caffeine content ranged from 12.25–21.76 mg/g for black teas and 13.35–15.05 mg/g for green teas. The study provides information on the stability, acidity and caffeine content in some commercially available tea brands.

Keywords: Tea; Caffeine; Moisture content; pH; HPLC

INTRODUCTION

Tea is a popular and widely consumed beverage around the world [1]. In Nigeria, it is a traditional and common beverage taken by most families. Drinking tea is a common social activity and has long been an important fluid component of the diet [2]. Consumption of tea has become increasingly popular and this is due to the introduction of new flavours and reported studies of the healthy benefits of tea [3,4]. The main types of tea are green, white, oolong, black, and purer teas depending on the fermentation of the raw leaves of *Camellia sinensis* L. [5]. One of the pharmacologically active constituent and quality indicators of tea is caffeine, a methylated xanthine alkaloid structurally identified as 1,3,7-trimethyl xanthine (Figure 1) [6]. Caffeine is reported to have many beneficial pharmacological effects on the body including antioxidant, anti-inflammatory, antimicrobial, anticancer, diuretic properties and lower risk of neurodegenerative diseases development but also not without some attendant risk factors

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[1,7]. The food based dietary guidelines (FBDG) of Nigeria, in considering dietary caffeine sources, discouraged high tea intake because of inhibition of iron bioavailability and increase phosphorus levels [8]. A review article by Nawrot *et al.* [9] reported that a daily caffeine intake of up to 400 mg/day was not associated with adverse effects in healthy adults [9]. Reports from studies carried out in Nigeria showed that Ultraviolet–Visible (UV–Vis) spectroscopy was a more frequently employed technique for the quantification of caffeine from different sources [10–12]. Besides the fact that the method is not very sensitive and precise, there are also no recent reports that investigated only tea beverages in Nigeria. The aim of this study is to evaluate some physiochemical parameters of commercially available teas in the Nigerian market, and determine the caffeine content per tea bag using a developed and validated HPLC method.

EXPERIMENTAL METHODS

Weight measurements were carried out on a Mettler Toledo ME303E. Phosphate buffers (pH 4, 7 and 9, Merck, Germany) were used for calibration prior to measurement on a Mettler Toledo, SevenExcellence^{TM}, Malaysia. Silica gel GF_{254} pre coated TLC plate (Qingdao Marine Chemical, Inc., Qingdao, P.P. China) was used for thin layer chromatography. A melting point apparatus (A&E Lab, DMP-400, United Kingdom) was used to determine the melting point. An Agilent HPLC-DAD 1200 series system was used for the HPLC chromatographic analysis. All chemicals and reagents used for the study were of analytical grade. Methanol (HPLC grade), caffeine reference standard (USP) were used for the HPLC study. Distilled-deionized water was used in preparation of the stock and working solutions.

Sample collection. Ten (10) different brands of teas were purchased from supermarkets within the Federal Capital Territory (FCT) Abuja. The brands were purchased in July 2021, and analysed before their expiry dates. The brands were deliberately selected to reflect locally produced, foreign produced, black and green teas. The tea brands were coded with the letters A to J (Table 1). The tea packets were assessed for information such as brand name, batch no, manufacturer's name and address, manufacture and expiry dates, NAFDAC registration number and net weight of tea bag

Physicochemical properties

Weight variation. Fifteen (15) tea bags of each brand were randomly taken from each packet and weighed individually using an analytical balance (Mettler Toledo ME303E). The average weight and the percentage weight variation from the mean value were calculated for each brand.

Moisture content. The moisture content of the tea samples was determined according to the method of Association of Official Analytical Chemists (AOAC) [13]. Tea samples measuring 2 g were heated at 105 ^{0}C in disposable aluminum pans for two hours in the oven, and cooled thereafter in a desiccator for 30 min. The loss of weight was used to calculate the moisture content of the sample.

Determination of pH. Adopting the home tea preparation method, a tea bag sample was infused in 100 ml of hot table water and allowed to cool. Phosphate buffers (pH 4, 7 and 9; Merck, Germany) were used to calibrate the pH meter and buffer 7 was used for verification (Mettler Toledo, SevenExcellence^{TM}, Malaysia). A solution of each of the samples maintained at 26.8 °C was measured and the pH reading was recorded.

Extraction procedure.

Isolation of caffeine from tea. This was carried out according to the method of Pradhan *et al.* [14] and the contents of 15 tea bags from each brand weighing approximately 30 g were placed in a beaker and 250 ml of distilled water was added to it. The mixture was heated on a

water bath at 100 °C for 30 min and filtered. To the hot filtrate, 10 ml 0.1 M sodium carbonate was added to solubilize the tannins present. The resulting solution was transferred into a separating funnel and partitioned with dichloromethane (1:1). The organic layer was collected, dried with pellets of sodium sulphate and concentrated on a rotary evaporator to obtain a pale white compound which was re-crystallized with ethanol, to yield a white crystalline powder.

Detection and identification of caffeine in tea samples. The thin layer chromatograms of caffeine reference standard (USP) and the extracted compound from each of the tea samples were compared. The caffeine reference standard and the extracted compound were dissolved in ethyl acetate, spotted on a silica gel GF_{254} pre-coated TLC plate (Qingdao Marine Chemical, Inc., Qingdao, P.P. China) and developed in a TLC glass tank with a solvent system of hexane:ethyl acetate (2:1). The plate was viewed under ultraviolet light (254 nm) and R_f values calculated. The melting point of the extracted compound was determined using a melting point apparatus (A&E Lab, DMP-400, United Kingdom).

Determination of caffeine in tea samples by High Performance Liquid Chromatography (HPLC)

***Instrumentation*.** HPLC chromatographic analysis was carried out on an Agilent HPLC-DAD 1200 series system equipped with a G1312B Binary pump SL (Agilent), manual injector (Agilent manual syringe, P/N5190-1501, 50µl - FN, LC tip) with a 20 µl loop volume, Diode array detector (G1315C DAD SL). A reversed-phase C_{18} column (Phenomenex, 125 mm × 4.6 mm i.d, 5µm) was used for the separation. The aqueous mobile phase used was freshly prepared, filtered (membrane filter, 0.45 µm × 44 mm, Millipore) and sonicated with an ultrasonic water bath. Separation was via isocratic mode with methanol and 1% acetic acid in water (35: 65). The flow rate was at 1.0 ml/min and the wavelength of detection was set at 278 nm with a run time of 7 min. Data was acquired and evaluated with the Chemstation® software.

***Method validation*.** The HPLC method used was validated for linearity, precision, limit of detection (LOD), limit of quantification (LOQ) and accuracy (percentage recovery), according to the International Conference for Harmonization (ICH) guidelines [15]. Concentrations of the standard solution in the range (5–80 µg/ml) were injected into the HPLC system in triplicates. Linear regression analysis was calculated by the least square regression method. Five replicate injections of 10 µg/ml standard solution were taken to determine intra- and inter-day repeatability. Five replicates of the test solutions in the range 5–80 µg/ml were injected and the LOD and LOQ measured based on the standard deviation of the response and the slope. Accuracy studies were carried out using three different concentrations of caffeine standard analyzed in triplicates. Samples of known concentrations were analyzed for their content and the measured values compared to the expected values, were expressed in terms of percentage recovery. Separation of the caffeine was optimised by exploring different ratios of methanol, water and acetic acid as the mobile phase.

***Preparation of standard caffeine and working standard solution*s.** A stock solution concentration of 100 µg/ml of caffeine standard was prepared by accurately weighing 10 mg of caffeine reference standard into a 100 ml volumetric flask, dissolved and made up to volume with the mobile phase. The stock solution was further diluted to give working standards of 5, 10, 20, 40 and 80 µg/ml.

Calibration graph. Concentrations of the standard solution in the range (5–80 µg/ml) were prepared and injection into the HPLC system was done in triplicate. Linear regression analysis was calculated by the least

square regression method. The chromatogram for the caffeine standard is shown in Figure 3. Five concentrations 5, 10, 20, 40 and 80 µg/ml measured in triplicates were used in plotting the calibration graph.

Sample preparation for HPLC. Extraction of caffeine from tea samples was done with hot water as solvent according to the method of Nhan and Phu [16]. Two (2) grams of each sample was weighed and transferred into a beaker. 100 ml of distilled-deionized water was then added to each sample and heated over a water bath at 100°C for 30 min. The solution was allowed to cool, filtered, transferred to a 250 ml volumetric flask and made up to volume with distilled-deionized water. Five (5) ml of the filtrate was then taken into a 50 ml volumetric flask and diluted to volume with the mobile phase. The resulting solution was then filtered (membrane filter; 0.45 µm × 13 mm, Millipore), and placed into amber-coloured vials for analysis.

RESULTS AND DISCUSSION

Physicochemical parameters of tea samples and isolation of caffeine. The product information for the purchased sample tea brands are shown in Table 1. Eight of the brands (A, B, C, E, G, H, I, J) were black teas while two (D, F) were green teas, according to the label claim. The results of the weight variation, moisture content, and pH tests are shown in Table 2. The mean weight ranged between 2.07–2.33 g for sample A–F. The samples had a moisture content range between 5.65–11.0 %, the lowest being sample A and the highest sample F. Similar studies had reported moisture contents of 3.9–9.5 % for black tea [17] and 3.31–6.65 % for green tea [18]. The report from another study showed 70 % of commercial tea samples having a moisture content of 6.6 % or less and 30 % samples containing up to 8 % which the authors postulated would have a negative effect on the shelf life of the product [19]. Moisture content is one of the most important properties and thus a frequent physicochemical analysis performed on food products [20]. Analysis of moisture content is very important in the food industry to control the quality, stability and shelf life of the product. For better quality, the moisture content should be controlled at 2.5–6.5 %. [21]. A study reported that the method of steaming followed by pan roasting and tray drying at controlled conditions would achieve a moisture content of <5 % in green teas [22]. The tea solutions had pH values ranged from 4.9–5.0 and 5.4–5.5 for black and green tea, respectively as shown on Table 2. Research has shown that low pH is the primary determinant of a beverage's erosive potential and will favor higher infusion leading to the increase in the activity of catechins [23]. The pH values of black tea solutions in this study agree with the previous work by of Simpson *et al*. who reported pH value of 4.9 for the black tea and concluded that black tea was of low acid composition in comparison with many of the beverages consumed and showed no erosive potential to teeth [24]. In the present study, the water used to prepare the teas showed a pH value of 6.95, confirming that the water had no effect on the pH of the tea solutions. Isolation of caffeine was achieved from the different tea brands (samples used for the weight variation test) with sample A having the highest yield (2.18 g) and sample D the lowest (0.78g). The variation in the weights of the isolated caffeine may be attributed to the efficiency of the extraction method employed. The TLC chromatogram (Figure 2) qualitatively identified all the samples as caffeine, with an R_f of 0.46 calculated for all the observed spots from samples and reference standard. TLC is a quick and inexpensive method for the separation and identification of components of a mixture and also to determine the purity of a compound. The authentication of caffeine was further confirmed by the melting points of the samples and standard, as shown in Table 3. The melting points of the isolated caffeine

powder from the samples were in the range of 236.0–238.5 °C. These values compared well with that of the reference standard, confirming the presence and purity of caffeine in all the tea brands investigated.

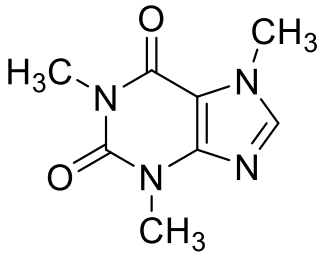

Fig 1. Structure of caffeine

Table 1. Label information of the sample tea brands

Sample Code	Sample Brand Names	Type of Tea	Batch Number	Manufacturing Date	Expiry date	Country of Manufacture	NAFDAC number
A	Top tea	Black	209221	28/07/2021	01/10/2022	Nigeria	01-1286
B	Lipton	Black	212992	20/07/2021	19/01/2023	Nigeria	01-0132
C	Hilltop	Black	KST1126	06/2021	04/2023	Nigeria	01-9669
D	Qualitea	Green	D20260	10/2020	10/2023	Srilanka	01-7026
E	Highland	Black	006	06/2020	04/2022	Nigeria	01-5768
F	Legend	Green	NG/06/2020	06/2020	06/2023	Srilanka	LG-ALI-01-0694L
G	Twinnings	Black	0000535587260	NS*	16/09/2022	United Kingdom	NS
H	Vivo-finest	Black	015/2020-21	09/2020	09/2023	United Arab Emirates	C1-3648
I	Selen	Black	ANS/SNL20/001-D	02/2020	01/2023	Nigeria	D1-9876
J	Oriba special	Black	221/223	03/2019	03/2022	Nigeria	NS

*NS- Not stated

Table 2. Physicochemical properties of the sample tea brands

Sample code	Mean weight + SD (g)	Moisture content (%)	pH* + SD (25.8 °C)
A	2.07 ± 0.04	5.65	4.85 ± 0.03
B	2.29 ± 0.02	6.40	4.81 ± 0.01
C	2.16 ± 0.03	6.35	4.93 ± 0.01
D	2.27 ± 0.03	7.90	5.54 ± 0.01
E	2.13 ± 0.03	9.20	4.99 ± 0.03
F	2.14 ± 0.03	11.00	5.40 ± 0.01
G	2.33 ± 0.04	8.65	4.94 ± 0.03
H	2.11 ± 0.03	7.85	4.91 ± 0.01
I	2.19 ± 0.00	8.70	5.03 ± 0.02
J	2.21 ± 0.02	8.30	5.01 ± 0.02

pH of water was 6.95 ± 0.038

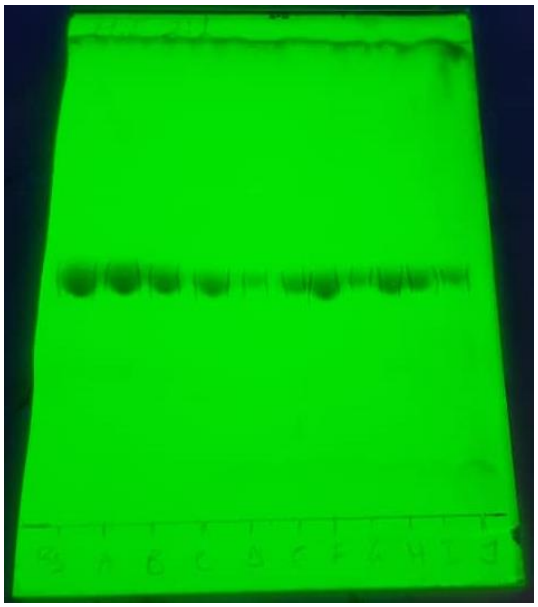

Fig 2. TLC of caffeine reference standard (RS) and isolates from samples (A-J) in hexane: ethyl acetate (2:1)

Table 3. Weights and melting points of isolated caffeine powder from the sample tea brands

Sample code	Weight (g)	Melting point*(°C)
A	2.182	236.8–237.2
B	1.664	236.0–237.2
C	1.484	238.0–238.5
D	0.775	236.5–238.2
E	1.435	237.0–238.2
F	1.551	236.1–237.5
G	1.964	236.3–236.9
H	1.363	236.2–237.5
I	1.199	236.9–237.9
J	1.293	237.1–237.6

* Melting point of caffeine reference standard is 236.2–237.5

Table 4. Linearity results of caffeine reference standard

Concentration (µg/ml)	Peak area
5	285.194
10	532.880
20	1089.402
40	2238.600
80	4200.863

Table 5. Precision results of caffeine reference standard at 10 µg/ml

	Inter-day		Intra-day	
No	Retention time	Peak Area	Retention time	Peak Area
1	4.65	520.880	4.65	520.880
2	4.65	536.116	4.66	533.939
3	4.65	538.705	4.65	532.880
4	4.65	533.649	4.66	534.985
5	4.66	527.519	4.65	526.991
Average	4.65	531.374	4.65	529.935
Std Dev	0.004	7.183	0.005	5.934
% RSD	0.082	1.352	0.117	1.120

Table 6. Accuracy results of caffeine reference standard

Sample	Concentration added (µg/ml)	Concentration found (µg/ml)	Recovery (%)
1	5	4.872	97.4
2	20	20.12	100.6
3	80	79.09	98.9
Mean			99.0
SD			1.570
% RSD			1.586

Table 7. Concentration and amount of caffeine in the sample tea brands

Sample Code	Peak Area (Mean ± SD)	Caffeine concn. (µg/ml)	Caffeine (mg/g)	Amount of caffeine (mg) in 2g (tea bag)
A	753.01 ± 18.31	13.74	17.18	34.35
B	845.16 ± 8.40	15.49	19.35	38.71
C	642.42 ± 16.46	11.64	14.55	29.11
D	591.64 ± 22.83	10.68	13.35	26.70
E	621.18 ± 10.20	11.24	14.05	28.10
F	667.79 ± 11.49	12.12	15.05	30.10
G	944.47 ± 3.21	17.37	21.76	43.42
H	810.61 ± 21.62	14.83	18.54	37.07
I	578.16 ± 8.50	10.42	13.03	26.06
J	543.25 ± 2.44	9.76	12.25	24.41

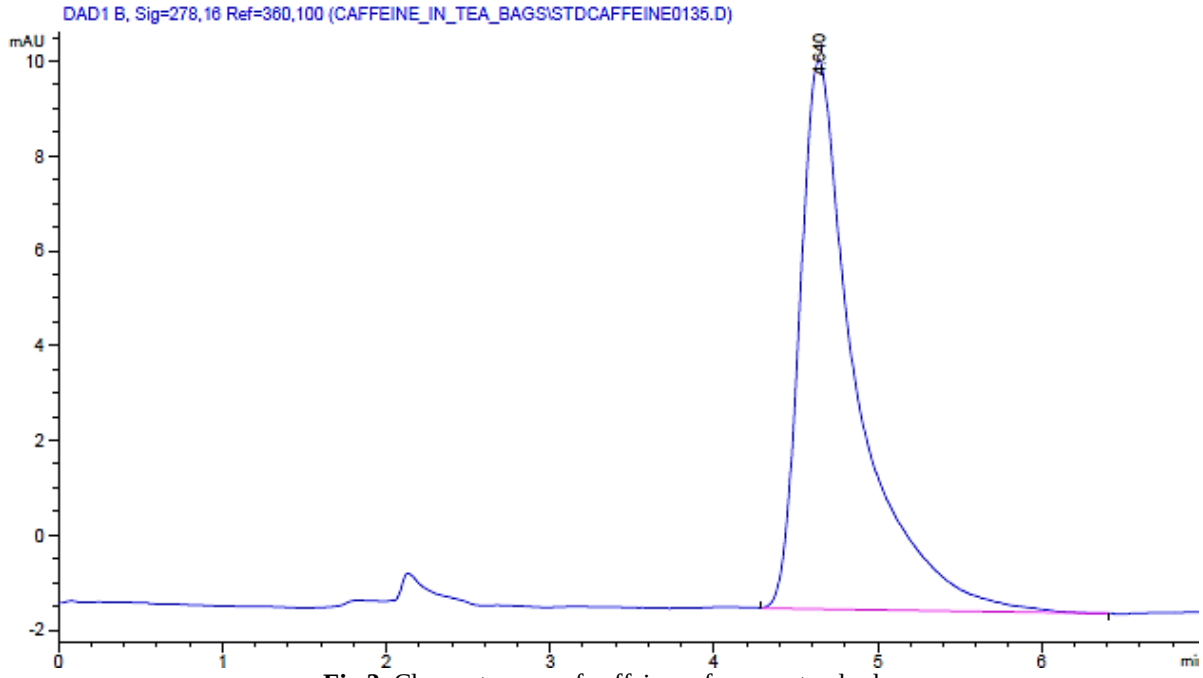

Fig 3. Chromatogram of caffeine reference standard

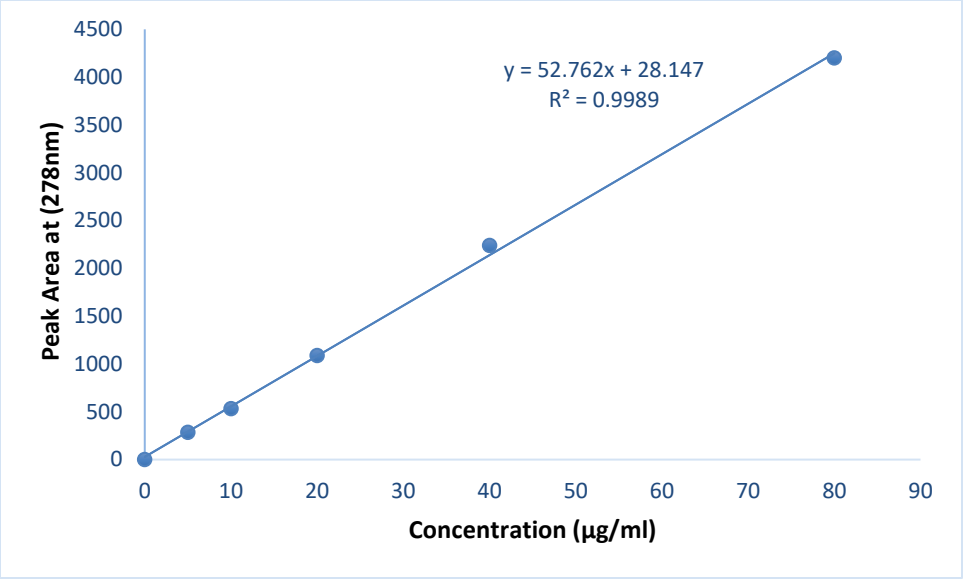

Fig 4. Calibration curve for caffeine standard

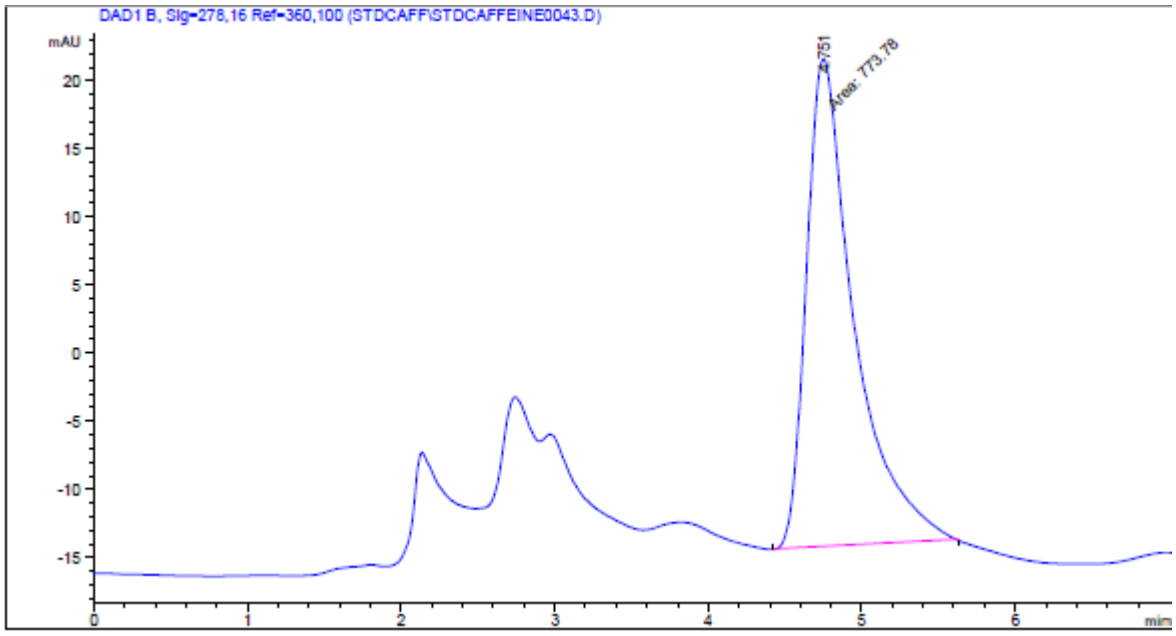

Fig 5. Chromatogram of caffeine extracted from a tea sample

HPLC method validation

Linearity. A good linear relationship was observed between the areas under the peak and concentrations of the standard in the range tested (Figure 4, Table 4). The slope, intercept and regression coefficient were calculated to be 52.762, 28.147 and 0.9989, respectively.

Sensitivity. The sensitivity of the method was measured in terms of LOD and LOQ. The LOD and LOQ tests gave values of 2.079 µg/ml and 6.931 µg/ml, respectively.

Precision and accuracy. The inter- and intra-day precision studies were satisfactory with % RSD of 1.35 % and 1.11 %, respectively as

shown in Table 5. The results obtained from the accuracy test gave a percentage recovery range of 97.4–100.6% and a mean recovery of 99.0 % (Table 6), demonstrating that the method was accurate and adequate for the quantitation of caffeine in the commercial teas.

Caffeine content in commercial tea samples. The separation of caffeine was optimized in a solvent system of methanol and water with 1% (v/v) acetic acid (35:65) and the identification was confirmed for the samples with a retention time of 4.6 min (Figure 5). The developed and validated HPLC method was used in determining the caffeine content in the commercially available tea samples.as shown in Table 7. The caffeine content in samples from each tea bag studied ranged from 12.25–21.76 mg/g for black teas (24.41–43.42 mg/ 2g tea bag) and 13.35–15.05 mg/g for green teas (26.70–30.10 mg/ 2g tea bag). Boros *et al*. reported similar values in their study with a range of 13.48–23.44 mg/g for black teas and 10.23–22.75 mg/g for green teas [25]. The variation in caffeine content across the tea brands may be due to varied geographical sources of *Camellia sinensis*, growing conditions, harvest seasons, and the processing techniques employed. There was no notable difference in caffeine content between the foreign-produced and locally-produced teas. The amount of caffeine extracted from the teas may also depend on the method of preparation and the steeping/brewing time of the tea bags in this study.

Conclusion. The study determined the caffeine contents in tea brands sold in F.C.T, Abuja, Nigeria, and confirmed its identity via TLC and melting point tests. The moisture content of the samples showed that some teas with values >7.0 may have shorter shelf lives and lower stability. The pH values obtained showed no erosive potential to teeth. The validated isocratic HPLC method with a short run time of 7 min offers a simple and sensitive method for the quantitative evaluation of caffeine in tea bags. The study provides useful information on the quality and content of caffeine in the commercial teas and serves as a guide for consumers to not exceed the limit of 400 mg/ day United State FDA's regulation of caffeine containing products.

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