

DETERMINATION OF CAFFEINE LEVELS IN GREEN TEA AND SOFT DRINKS BY UV-VISIBLE SPECTROSCOPY: A REVIEWGanesh Akula^{1*}, Bandoj Nikhitha², Manumula Jusya Vani³, Jonnada Swetha⁴, Saniya Begum⁵, Joganolla Sri Vidya⁶^{*1}Department of Chemistry, Surabhi Dayakar Rao College of Pharmacy, Rimmanaguda, Gajwel, Siddipet, Telangana-502312.^{2,3,4,5,6}Surabhi Dayakar Rao College of Pharmacy, Rimmanaguda, Gajwel, Siddipet, Telangana.

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ABSTRACT

To determine the content of caffeine in the beverages like green tea, black tea, and soft drink which are commercially available in the local market with the help of a UV-Visible spectrophotometer. Chloroform was used as the solvent and concentrations of caffeine were measured at the wavelength of 274 nm. Using chloroform as an extractant, the caffeine was extracted from them, and the UV-Visible spectrophotometer was used to measure it both quantitatively and qualitatively. Caffeine, a naturally occurring stimulant, is widely consumed worldwide in various forms such as tea, coffee, and soft drinks. It plays a significant role in human daily life, impacting cognitive functions, alertness, and metabolic activities. The standard solutions of caffeine from the range 2-30 ppm were prepared in the chloroform, show the linearity with the correlation coefficient of 0.99. From the calibration curve, the concentration of caffeine was determined in various brands. It is observed that black tea contains the maximum caffeine content followed by green tea and soft drinks. In this study the maximum caffeine content found out was (45.6mg/g) in the Nice black tea sample and the minimum (0.161mg/ml) in Pepsi soft drink sample. The caffeine content reported here is higher than that of previous studies may be due to the modified approach treatment temperature (90-100°C) and longer brewing time (5 minutes) in this study. The use of the UV-Visible spectrophotometer is also an alternative to the HPLC technique, thereby the working cost could be brought down.

INTRODUCTION

Caffeine is one of the most widely consumed psychoactive substances worldwide. It is naturally found in coffee, tea, cocoa, and various plant sources. Over the years, caffeine consumption has expanded to include a range of artificially produced beverages such as soft drinks and energy drinks. Despite its well-known stimulant properties, excessive caffeine intake has been linked to adverse health effects, including insomnia, increased heart rate, and dependence.^[1] A purine alkaloid, caffeine (1,3,7-trimethylxanthine) is mostly found in a variety of drinks. Therefore, it is crucial to measure the amount of caffeine included in different foods. Caffeine is an ingredient in the most popular drinks consumed worldwide, including tea, coffee, and

soft drinks. Soft drink brands differ in how much caffeine they contain, although the US Food and Drug Administration (FDA) limits the amount to no more than 6mg/oz of fluid or 200mg/L. As a result, caffeine analysis is necessary to guarantee that beverages contain the right amount of caffeine and to comply with legal requirements.^[2] Establishing a more accurate, straightforward, quick, and affordable analytical method is crucial for monitoring caffeine levels in food and beverages so that its physiological effects on the human body's metabolism can be studied. These effects include raising blood pressure, stimulating the central nervous system, and controlling the quality of food.^[3,4]

Chemical properties of caffeine

Biological source: It is the dried ripe seeds of *Coffearabica* Linn.

Family: Xanthine alkaloids (Rubiaceae)

Chemical Formula: $C_8H_{10}N_4O_2$

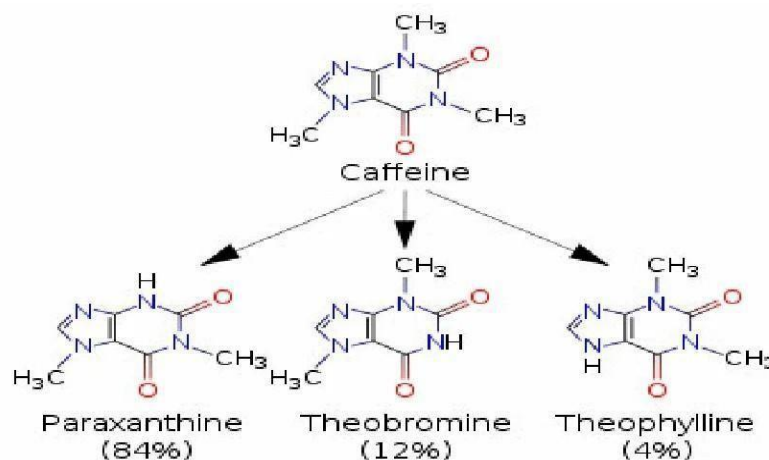


Fig. 1: Caffeine and its main metabolic products.^[5]

Caffeine is a bitter white crystalline alkaloid from the methylxanthine group. The systematic name of caffeine is 1,3,7-trimethylxanthine. The molar mass of caffeine is 194.19 g/mol. and its density is 1.2 g/mL. It has low solubility in cold water. Its solubility is somewhat better in hot water, ethyl-acetate, pyrimidine, pyrrole and acetone. It is very well dissolved in ether, petroleum ether, benzene and chloroform.^[6]

Caffeine has a molecular formula of $C_8H_{10}N_4O_2$. It is found in the seed, leaves and fruits of some plant in varying quantities. In plant, caffeine acts as a natural pesticide that paralyzes and kills certain insects feeding on the plants. It also can enhance the reward memory of pollinators. Caffeine also limits germination of seeds near the plant that could grow to compete for resources. In humans, it stimulates the central nervous system, heart rate, and respiration. However, negative side effects from using caffeine can occur and include anxiety, increased blood pressure, and diminished fine motor skill.

Tea is the most commonly used soft beverage in the household. It acts as a stimulant for central nervous system and skeletal muscles. This is why tea removes fatigue, tiredness and headache. The principal constituent of tea, which is responsible for all these properties is the alkaloid-caffeine. Originally it was thought that caffeine is responsible for the taste and flavour of tea but, it found that the pure caffeine is tasteless.

In this experiment, the instrument that we use is UV-Visible spectroscopy is one of the most popular analytical techniques because it is very versatile and able to detect nearly every molecule. With UV-Visible spectroscopy, the UV-Visible light is passed through a sample and the transmittance of light by a sample is measured. From the transmittance (T), the absorbance can be calculated as $A = -\log(T)$. An absorbance spectrum is obtained that shows the absorbance of a

compound at different wavelengths. The amount of absorbance at any wavelength is due to the chemical structure of the molecule.

MATERIALS AND METHODS

Chemicals: The chemicals used in this study include caffeine standard ($C_8H_{10}N_4O_2$) from Sigma-Aldrich (Sigma Aldrich Chemie GmbH, Munich, Germany), chloroform ($CHCl_3$) and sodium Carbonate(Na_2CO_3) obtained from Merck (Merck, Darmstadt, Germany). All reagents used in this study were of analytical grade and all solutions were prepared by using distilled water.

Instrument: The UV/Vis spectrophotometer (Secomam, UVi LightXTD5, Ales, France) was used for the analysis of caffeine in different samples of teas, soft and energy drinks.^[7]

Materials: Samples of green tea and soft drinks were purchased from the local market. The green tea sample of Tetley was in the form of a tea bag. All the chemical used in analytical grade. The chemical like chloroform, Caffeine, sodium carbonate anhydrous, de-ionised water are used. For the UV/Visible study the spectrophotometer model Double Beam Spectrophotometer AU- 2702 Systronics (India) limited is used.

METHODOLOGY

Preparation of Stock solution (Caffeine): A 100ppm standard caffeine stock solution is prepared. Several standards of caffeine were prepared from the stock solution ranging from 2 to 30ppm. The standards are scanned from 200 to 400nm in the UV spectrum, yielding a maximum absorbance at 276nm.

Preparation of tea samples: A 250mL beaker was filled with 500mg of dried, crushed, and sieved tea. 100mL of boiling distilled water was then added. Rather of the

40°C indicated in the prior method, the solution was left on a boiling water bath for five minutes at a temperature of 90 to 100°C. Next, the solution was filtered. To precipitate the tannins, the solution was combined with anhydrous sodium carbonate. The earlier research disregarded this phase. After another filtering to get rid of the tannins, 10ml of the solution is extracted at least three times using 10ml of chloroform. After extraction all the fractions were combined and the fraction after dilution is analyzed with the spectrophotometer. The absorbances of the prepared solutions were measured at 276nm. The % of Caffeine content was calculated by using formula:

$$\% \text{Caffeine} = \frac{\text{Test Absorbance} \times 5 \times \text{Potency/Standard}}{\text{Absorbance} \times 100}$$

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 (10mL), then 20% aqueous Na₂CO₃ solution (1mL) and analytical grade carbon tetrachloride (20 mL). The caffeine was extracted by inverting the funnel at least three times, venting the funnel after each inversion. The non-aqueous carbon tetrachloride layer was removed to a clean 50 mL volumetric flask. Another 20 mL portion of carbon tetrachloride was added to the aqueous solution in the separating funnel and the extraction procedure was repeated twice more and the carbon tetrachloride 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.

Preparation of soft drink sample: Transfer approximately 5 milliliters (after degassing) of a suitable soft drink sample (Sting) into a 125 milliliter separatory funnel. Instead of using carbon tetrachloride, add 10ml of distilled water, 1ml of 20% aqueous Na₂CO₃ and then 10ml of chloroform (since caffeine is more soluble in it). After five minutes of vigorous shaking, transfer the lower, non-aqueous layer to the volumetric flask. All layers are mixed and examined following three extractions.

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 (1060) ppm caffeine with equation ($y = 0.035x + 0.100$). The quantitative amount of caffeine in samples (ppm) was then determined using the standard curve.

pH determination: Beverages pH were determined by using Sartorius pH meter.^[8] The main objective of this research is to know the caffeine level in soft and energy drinks high or low then the published value or the FDA recommended value. The ideal (neutral) pH of the mouth ranges from 6.5-7.5. A pH of 5.5 is considered to be the threshold level for the development of dental decay. Both soft drinks and sports drinks have been shown to have a pH between 2.5 and 3.5. Demineralization of tooth enamel will occur more rapidly if the pH drops below the critical 5.5 level for long periods of time and if the pH is dropped below the critical level frequently. All kinds of soft drinks are acidic and that cola drinks especially make our bodies poor in Oxygen.^[9]

Analytical Method Development and Validation: The measurement of caffeine was done using a UV spectroscopic technique. By comparing the devised method to USP and ICH criteria, its specificity, linearity, accuracy, recovery, robustness, and LOD/LOQ were evaluated.

Range/Linearity: The stock solution was diluted to create solutions with varying concentrations between 10 ppm and 50ppm. For solutions with varying concentrations at λ max, absorbance was recorded, and a calibration curve was created by graphing concentration on the X-axis versus absorbance on the Y-axis.

- 1. Precision:** Using a UV spectrophotometer, the composition of six samples from the same batch was examined. 2.1. Intraday Precision: The percentage Relative Standard Deviation (%RSD) for the Analysis within a day (24 hours), at different time intervals, was calculated in order to determine the intraday precision. 2.2. Interday Precision: Relative Standard Deviation (RSD) was computed for the study of six replicates over the course of three consecutive days in order to determine interday precision.
- 2. Accuracy:** Separately, 10 milliliters of the 80%, 100%, and 120% standard solution were extracted in order to conduct an accuracy investigation. Every concentration was made twice, and each was examined three times.
- 3. Ruggedness:** Ruggedness was studied to measure the reproducibility of method under variation in condition other than laboratory.
- 4. Robustness:** UV spectrophotometer was set to 276nm \pm 2nm, and test samples were analyzed in triplicate using UV spectroscopy to examine robustness.
- 5. LOD AND LOQ:** The lowest concentration of the analyte in a sample that can be identified by an analytical process is known as its limit of detection; this concentration need not be measured to an exact value. $\text{LOD} = 3.3 \times \text{SD/Slope}$
- 6.** The lowest concentration of the analyte in a sample that can be detected as an exact number with

appropriate accuracy and precision is known as the limit of quantification for an analytical method.^[10]

$$LOQ = 10 * SD / Slope$$

RESULTS AND DISCUSSION

The standard linear calibration curve obtained from the standard solutions analysis is presented in 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 pH values show that all beverages were slightly acidic, with Red Bull having the lowest pH 6.44 and Bullet having the highest pH 5.92.

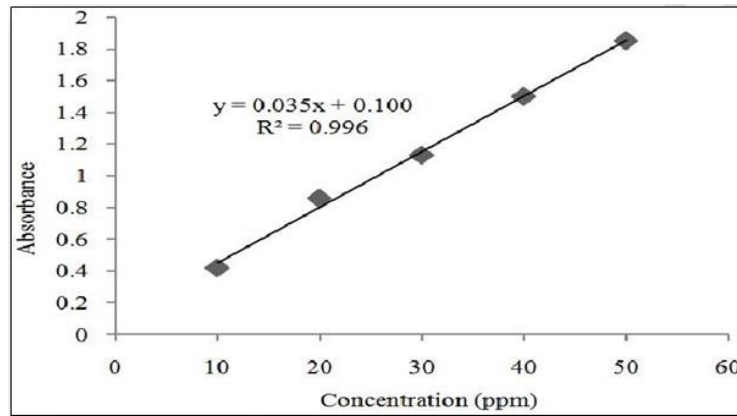


Fig. 2: Calibration curve for standard caffeine.

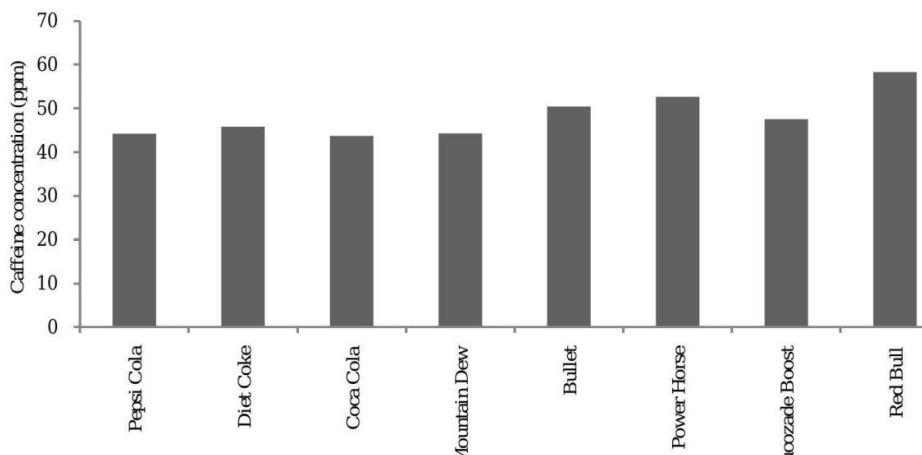


Fig. 3: Chart showing caffeine contents in the beverage samples.

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
	Luozade 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

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.^[8] 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.^[11] Our results were reported that all kinds of soft drinks are acidic and that cola drinks specifically make our bodies poor in oxygen.^[12]

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. 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). Moderate caffeine consumption of 300 mg/day, is considered generally safe.^[13]

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.^[14] 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 (12oz) or 8.45-20.28 mg/100 ml.^[15]

According to the European Food Safety Authority (EFSA) (2015), single doses of caffeine that do not raise safety concerns recommended for adults are up to 200 mg. About 3 mg per kilogram of body weight (mg/kg bw) are generally appropriate for a healthy adult population. Single doses of 100 mg of caffeine (about 1.4 mg/kg bw) especially if consumed before bedtime. A safety level of 3 mg/kg bw of caffeine is also recommended for children and adolescents. A cup of black tea (220 ml) approximately contains 50 mg of caffeine so one could consume up to 4 cups per day. Since caffeine content depends on the type of tea and since portion size varies within and between countries one should be careful with caffeine intakes.^[16]

Table 2: Result of caffeine content under different brands.^[17]

Beverage Type	Brand	Caffeine Content (mg/100ml)	Typical Serving Size (ml)	Total Caffeine per Serving (mg)	pH Level
Green Tea	Lipton Green Tea	12	240	29	6
	Twinings Green Tea	10	240	24	6.2
	Tazo Green Tea	15	240	36	5.9
	Ito En Oi Ocha	16	240	38	6.1
Soft Drink	Coca-Cola Classic	9.6	355	34	2.5
	Pepsi	10.7	355	38	2.5
	Mountain Dew	15.2	355	54	3
	Dr Pepper	12	355	43	2.9
	Sprite (Caffeine-Free)	0	355	0	3.3
	7Up (Caffeine-Free)	0	355	0	3.4

Table 3: Experimental values of caffeine in different category and their comparison with earlier studies.^[18]

S.no	Category	Name of beverages	Caffeine content in the body	Caffeine content reported by other researchers
1	Green Tea	Lipton Green Tea ¹⁹	23.60 mg/g	13.672mg/g
2		Himalayan Green Tea ²⁰	25.30 mg/g	7.856mg/100g
3	Black Tea	Taaza Tea ²⁰	30.50 mg/g	7.826mg/100g
4		Nice Tea	45.60 mg/g	-----
5	Soft Drinks	ThumsUp ⁹	0.178 mg/ml	10.69 -42.17mg/L
6		Pepsi ¹⁹	0.161 mg/ml	19.9mg/L

CONCLUSION

We used UV-Visible spectrophotometry to modify the approach, which may have contributed to the greater caffeine level we found in the local green tea and soft drink samples.

The UV visible spectrophotometric method is a sensitive, accurate, and reliable technique that was used in this work to quantitatively analyze the caffeine contents in green tea and soft drinks. When compared to HPLC and other analytical procedures, it is also a less expensive and time-consuming method.

The suggested UV spectrophotometric approach is easy to use, quick, precise, and accurate, as shown by the results and statistical parameters. The research shows the

caffeine content in Tetley is 0.4% and sting is 2.5% which is in range of promising limit by the company Tetley (i.e., 0.6%) and Sting (i.e. 2.88%).

The higher caffeine content reported by us from the local samples of green tea, black tea and soft drink may be due to the modified method with the help of UV-Visible spectrophotometry. Although in the previous studies the factors like temperature and brewing time were ignored. Since in our study, it mostly focuses on the higher temperature and longer brewing time result the higher caffeine content. UV Visible spectrophotometric method applied in this study for the quantitative analysis of the caffeine concentrations of green tea, black tea and soft drink is sensitive, precise and correct method. It is also an inexpensive and time saving technique instead of

HPLC and other analytical techniques with accurate results. Furtherly it is going to be the most widely used technique.

The results of the current study led to a conclusion that the caffeine content should be indicated on the product labels especially due to the great popularity and easy accessibility of caffeine-containing drinks. Since caffeine can be a cause for potential health concerns, precise quantities stated on the labels of caffeinated beverages should be highlighted in the interest of those who drink them. It is necessary to work on raising awareness among those who drink caffeinated beverages about the amounts of caffeine they consume.

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