

## RESEARCH ARTICLE



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**Received:** 23.12.2020

**Accepted:** 11.10.2021

**Published:** 03.11.2021

**Citation:** Garg AK (2021) Quantitative Analysis of Caffeine in the Green Tea, Black Tea and Soft Drink Using UV-Visible Spectrophotometer. Indian Journal of Science and Technology 14(37): 2860-2864. <https://doi.org/10.17485/IJST/V14I37.2241>

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**Funding:** Department of Science and Technology (DST), New Delhi for providing the grant for the research facilities in the College through DST-FIST program ref. SR/FST/College-318/2016(C)

**Competing Interests:** None

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Published By Indian Society for Education and Environment ([iSee](https://www.indjst.org/))

**ISSN**

Print: 0974-6846

Electronic: 0974-5645

# Quantitative Analysis of Caffeine in the Green Tea, Black Tea and Soft Drink Using UV-Visible Spectrophotometer

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## Abstract

**Objective** -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 of Hapur with the help of a UV Visible spectrophotometer. **Method**- To find out the content of caffeine, six different brands of green tea, black tea, and soft drink were purchased from the local market in Hapur. The caffeine was extracted from them using chloroform as an extractant and assessed qualitatively and quantitatively with the help of a UV Visible spectrophotometer. The maximum absorbance of caffeine in chloroform occurs at 273 nm. 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. **Findings**- In this study the maximum caffeine content find out was (45.6 mg/g) in the Nice black tea sample and the minimum (0.161 mg/ml) in Pepsi soft drink sample. **Novelty**-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 (05 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.

**Keywords:** Caffeine; brewing time; beverages; UV visible spectrophotometer; extraction

## 1 Introduction

Caffeine (1,3,7-trimethylxanthine) is a purine alkaloid and is largely present in several beverages. Hence it becomes essential to quantify the caffeine in various food items.

The beverages such as tea, coffee and soft drinks are the most widely consumed in the world have caffeine. The amount of caffeine in soft drinks varies among brands which are regulated by the US Food and Drugs Administration (FDA) to no more than 6 mg/oz fluid or 200 mg/L. Therefore, analysis of caffeine is required to ensure proper caffeine levels in beverages and to meet regulatory standards<sup>(1)</sup>.

It is important to monitor caffeine in beverages and food by establishing a more precise, simple, fast and low-cost analytical method in order to study its physiological

effects on the human body's metabolism, including stimulating the central nervous system, increasing blood pressure and control food quality<sup>(2,3)</sup>. The excessive use of caffeine can also be unsafe<sup>(4)</sup>.

Due to the wide occurrence of caffeine in the variety of products and interference of the matrix elements of sample, a large number of extraction methods have been proposed for the caffeine. In these methods the aqueous solution of tea or beverages is extracted with the solvents like dichloromethane, chloroform, acetone<sup>(5,6)</sup>. The caffeine then passes to the organic solvent due to its high solubility in them. The use of other procedures was also suggested by several workers<sup>(7,8)</sup>.

In the modified method using solid-phase extraction, the interfering tea pigments were effectively removed by passing the sample through a Sep-Pak C18 cartridge filter<sup>(9)</sup>. The use of magnetic ionic liquids<sup>(10)</sup>, supercritical fluid extraction (SFE)<sup>(11)</sup>, Ultrasonic assisted extraction<sup>(12)</sup> methods were also developed.

Several chemical and physical methods have been developed for the determination of caffeine in coffee and other beverages. The most widely used methods for the determination of caffeine in beverages include various analytical techniques such as Fourier Transform infrared,<sup>(12)</sup> Raman spectroscopy,<sup>(13)</sup> UV Visible spectrophotometer,<sup>(14–16)</sup> HPLC,<sup>(17,18)</sup> GC with mass<sup>(19,20)</sup>. In the present paper the UV Visible spectrophotometer is used to quantify the caffeine in various beverages since it is a cheap, economic and fast method.

## 2 Method

### 2.1 Chemicals and apparatus

Different samples of green tea and black tea (Lipton Tea, Himalayan Tea, Taaza Tea, Nice Tea) and soft drinks (Thums Up, Pepsi) were purchased from the local market in Hapur. The green tea sample of Lipton was in the form of a tea bag whereas Nice black tea was in the form of dried leaves. The details of samples with their brands and category are given in Table 1. All the samples were within their shelf life.

All the chemicals used were of analytical grade. The chemicals like chloroform (Fisher), caffeine 99.9% (Sigma Aldrich), sodium carbonate anhydrous (Merck), de-ionized water (Merck) was used.

For the UV/Visible study the spectrophotometer model Specord-210 Plus from Analytical Jena, Germany is used.

**Table 1.** Brand used to determine the Caffeine content under different category

S. No.	Category	Name of Brand
1	Green Tea	Lipton Green Tea
2		Himalayan Green Tea
3	Black Tea	Taaza Tea
4		Nice Tea
5	Soft Drink	Thums Up
6		Pepsi

### 2.2 Sample preparation

#### 2.1.1 Standard Caffeine

A standard stock solution of caffeine is prepared at 100 ppm. Several standards of caffeine from the stock solution were prepared from the range of 2-30 ppm. The UV spectrum is obtained by scanning the standards from the range 250 nm to 400 nm. The maximum absorbance is obtained at 273 nm.

#### 2.2.2 Preparation of tea samples

500 mg of tea (dried, ground and sieved) was placed in a 250 mL beaker, and 100 mL of boiling distilled water was added to it. The solution was kept on a boiling water bath for 5 min at temperature 90-100°C instead of 40°C mentioned in previous method<sup>(21)</sup>. Solution was then filtered. The solution was mixed with the anhydrous sodium carbonate to precipitate the tannins. This step was neglected in the previous studies<sup>(5,6)</sup>. It is filtered again to remove tannins and 10 ml of the solution is extracted with the 10 ml of chloroform at least three times. After extraction all the fractions were combined and the fraction after dilution is analyzed with the spectrophotometer.

### 2.2.3 Preparation of soft drink sample

Pipet an appropriate soft drink sample (Thums Up, Pepsi) about 5 ml (after degassing) into a 125-ml separatory funnel. Add 10 ml of distilled water, 1 ml of 20% aqueous  $\text{Na}_2\text{CO}_3$  followed by 10 ml of chloroform (due to more solubility of caffeine in it), instead of Carbon tetrachloride used previously by Tautua A et al<sup>(15)</sup> in his study. Shake the mixture for 5 minutes, then transfer the lower (non-aqueous) layer to the volumetric flask. After three extractions all layers are combined and analyzed. Similar process was repeated for the other soft drink brands as well.

## 3 Result and discussion

The spectrum for the various standard solutions of caffeine (2-30 ppm) were recorded with the help of UV Visible spectrophotometer. The maximum absorbance of caffeine is found out at 273 nm. The absorbance values for standard caffeine solutions at 273 nm are used to plot the calibration curve. The calibration curve for the caffeine is constructed from 2 to 30 ppm show the value of regression coefficient 0.99 given in Figure 1. The calibration curve shows a good correlation between the standard conc. of caffeine and absorbance at 273 nm. Using this curve, the concentration of caffeine in the different samples of black tea, green tea and cold drink is determined. The experimental caffeine level in the various samples is given in the Table 2 and compared with value of caffeine reported in earlier studies. The minimum caffeine level was observed in the carbonated soft drink from 0.161-0.178 mg/ml, while the black tea showed the highest caffeine content 30.50-45.60 mg/g. The green tea showed the content of caffeine 23.60 -25.30 mg/g. The amount of caffeine in the samples of soft drink was found out between the 0.161-0.178 mg/ml (17.8-16.1 mg/100ml) is greater than reported by the Letic NG et al<sup>(22)</sup> (11.91 mg/100 ml Pepsi and 9.69 mg/100 ml Coca Cola). The higher content is possibly due to the longer extraction time in our study. The value of caffeine in the green and black tea reported in our study is also higher than those reported earlier in their study by Rehman R et al<sup>(14,16,22)</sup>. The higher caffeine content reported in this paper may be due to longer brewing time and temperature (5 minutes and 90°C-100°C respectively). These factors were not considered in the other studies<sup>(14-16)</sup>. Thus, the use of UV visible spectrophotometer can be the easy, accurate and economic process to find the concentration of caffeine in various food beverages.

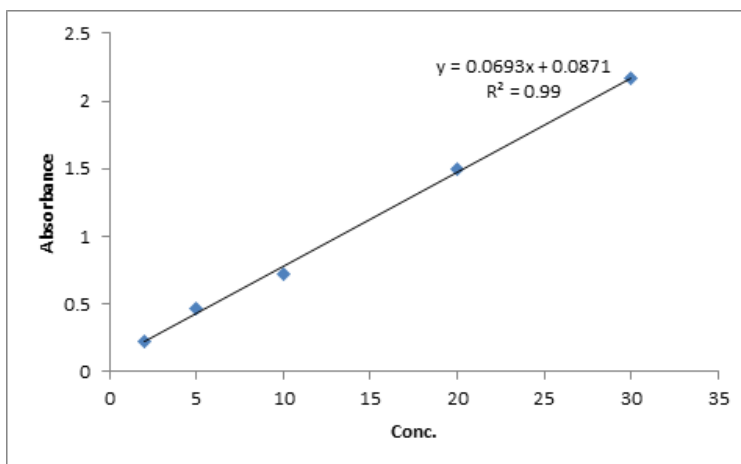


Fig 1. Calibration curve for caffeine standards solutions

Table 2. Experimental values of caffeine in different category and their comparison with earlier studies

S. No.	Category	Name of beverages	Caffeine content in this study	Caffeine content reported by other researchers
1	Green Tea	Lipton Green Tea	23.60 mg/g	13.672mg/g (Rehman R et al) <sup>(16)</sup>
2		Himalayan Green Tea	25.30 mg/g	7.856mg/100g (Letic NG et al) <sup>(22)</sup>
3	Black Tea	Taaza Tea	30.50 mg/g	7.826mg/100g (Letic NG et al) <sup>(22)</sup>
4		Nice Tea	45.60 mg/g	—
5	Soft Drink	Thums Up	0.178 mg/ml	10.69 mg/L-42.17mg/L (Khalid A et al) <sup>(14)</sup>
6		Pepsi	0.161 mg/ml	19.9 mg/L (Rehman R et al) <sup>(16)</sup>

## 4 Conclusion

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. In the previous studies the factors like temperature and brewing time were ignored. Since in our study, 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.

## Acknowledgement

The author is highly thankful to the department of Science and Technology (DST), New Delhi for providing the grant for the research facilities in the College through DST-FIST program ref. SR/FST/College-318/2016(C).

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