

# EXTRACTION, IDENTIFICATION AND ESTIMATION OF CAFFEINE IN GREEN AND BLACK TEA SAMPLES BY A SIMPLE UV-VISIBLE SPECTROSCOPIC METHOD

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## ABSTRACT

Caffeine is a widely consumed psychoactive compound present in coffee, tea, soft drinks, energy drinks, and chocolates. It is a diuretic and a central nervous system (CNS) stimulant. Apart from stimulating the CNS, it exerts beneficial effects in combination with other substances like L-theanine and catechins. Caffeine was extracted from green tea and black tea samples using various solvents. The maximum yield of caffeine was obtained from dichloromethane extracts. The extracted caffeine was identified by TLC and FTIR. A simple UV spectroscopic method was developed and validated for the estimation of caffeine in green tea and black tea. The wavelength maximum of caffeine was obtained at 272 nm in distilled water. The Beer-Lambert law was obeyed in the concentration range of 0.5 to 35  $\mu\text{g mL}^{-1}$  for caffeine. The linearity, accuracy, precision, robustness, and ruggedness, limit of detection and limit of quantification of this method are within the limits of ICH guidelines. This method was found to be precise as % RSD was less than two. The LOD and LOQ values were 0.03  $\mu\text{g mL}^{-1}$  and 0.091  $\mu\text{g mL}^{-1}$ , respectively. The percentage purity of caffeine from green tea and black tea samples was found to be 98.32% and 99.42 %. Thus, the proposed method was found to be rapid, specific, precise and accurate for the routine analysis of caffeine in green and black tea samples.

**Keywords:** Extraction, dichloromethane, TLC, caffeine, UV method, validation

## INTRODUCTION

Caffeine (1,3,7-trimethylxanthine) is a naturally occurring phytochemical present in tea leaves. Its gastrointestinal absorption is rapid and comprehensive, with 99% being absorbed within 45 minutes of intake<sup>1</sup>. Caffeine, a central nervous system (CNS) stimulant, is likely the world's most commonly consumed pharmacologically active chemical<sup>2</sup>. Green tea is prepared from *Camellia sinensis* leaves and buds without exposing them to the withering and oxidation processes that are used to make oolong tea and black tea. Oolong, yellow, white, and green teas are less oxidized than black tea, which is a variety of tea<sup>3</sup>. Green tea was first produced in China, but it is now also produced and manufactured in other East Asian nations<sup>4</sup>. Compared to other teas, black tea typically has a stronger flavor. The tea leaves are used to make all four varieties<sup>5</sup>.

An acute overdose of caffeine, usually in excess of 250 mg can result in a state of CNS over stimulation called caffeine intoxication<sup>6</sup>. Caffeine's effects on the body can start as soon as 15 minutes after consumption and last for hours<sup>7</sup>. Caffeine is highly addictive, increases stress level and accelerates aging and wrinkles<sup>8</sup>. To avoid the negative effects of excessive doses, an analytical technique that can rapidly quantify caffeine is required.

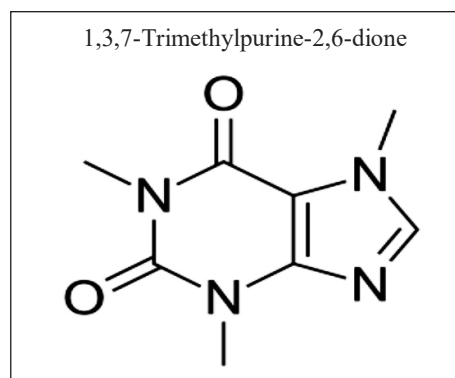


Fig. 1: Structure of caffeine

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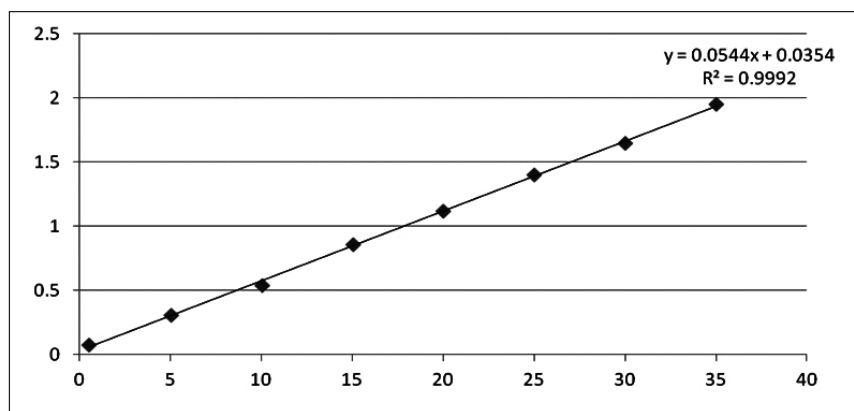


Fig. 2: Calibration curve of caffeine

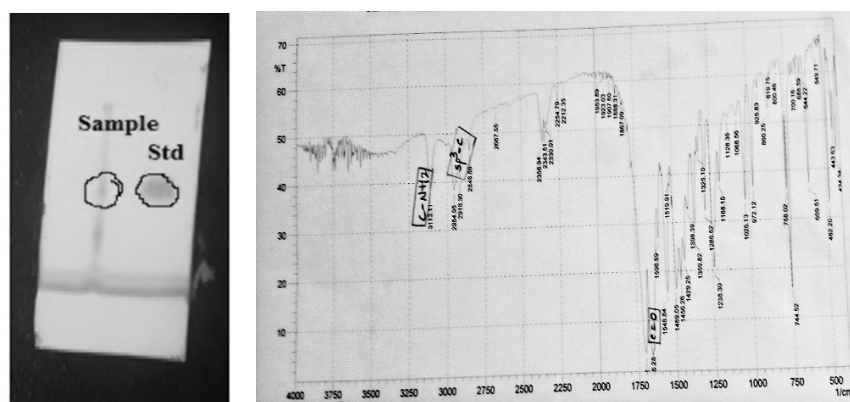


Fig. 3: Thin layer chromatogram and FTIR spectrum of caffeine

## MATERIALS AND METHODS

### Chemicals

Green tea and black tea leaves were obtained from the local market. Standard caffeine was obtained from SD Fine Chemicals Ltd., Mumbai, India. Analytical grade *n*-hexane, ethyl acetate, ethanol and distilled water were used in this study.

### Instruments

A double beam UV-VIS spectrophotometer [Elico sl 210] connected to a computer with spectra manager software was used. CONTECH electronic balance was used for weighing standard and sample substances.

### Preparation of standard and sample solutions

A stock solution of 1 mg mL<sup>-1</sup> was prepared by dissolving 10 mg of caffeine in a small volume of boiling water and then made up to the mark with distilled water in a 10 mL volumetric flask. Aliquots of standard and samples were made from this stock solution.

## Extraction of caffeine from green and black tea samples

Caffeine was extracted from green and black tea samples by using three different solvents.

### Ethyl acetate extraction

10 g of sample was weighed and 100 mL of distilled water was added to it and kept on water bath at 70 °C for 40 min. Then, the solution was filtered to obtain the infusion. To the infusion, 250 mL of ethyl acetate was added and the two layers were separated using a separating funnel. To the ethyl acetate layer, 10% of 40 mL citric acid was added. The citric acid layer was separated and kept for drying to obtain caffeine.

### Dichloromethane extraction

Tea infusion was prepared and 15 mL of dichloromethane was added and shaken vigorously using a separating funnel. On standing, water and dichloromethane layers were separated and the dichloromethane layer was dried to get caffeine extract.

### Acetone extraction

The saturated solution of infusion was prepared by adding pre determined amount of salt and filtered. To the filtrate, 15 mL of acetone was added and shaken vigorously in a separating funnel and allowed for separation of the two layers. Thus, water layer and acetone layer were separated by salting out technique.

### Qualitative estimation of caffeine obtained from the samples

The caffeine extracted from green and black tea samples was subjected to phytochemical testing, TLC and Fourier transform infrared spectroscopy (FTIR).

Table I: Recovery studies of caffeine

Standard and sample	% Level	% Recovery
5+10 µg mL <sup>-1</sup>	50%	99.31%
10+ 10 µg mL <sup>-1</sup>	100%	99.6%
15+10 µg mL <sup>-1</sup>	150%	99.6%

**Table II: Intra and interday precision of caffeine**

Intraday precision		Interday precision	
Conc [ $\mu\text{g mL}^{-1}$ ]	Abs[nm]	Conc [ $\mu\text{g mL}^{-1}$ ]	Abs[nm]
10	0.4953	10	0.4953
10	0.4967	10	0.4951
10	0.4962	10	0.4962
10	0.4955	10	0.4955
10	0.4954	10	0.4954
10	0.4958	10	0.4967
Mean	0.4958	Mean	0.49576
SD	0.000495	SD	0.000599
%RSD	0.0009	%RSD	0.00120

### Thin layer chromatography

The sample was dissolved in methanol. Silica gel G used as stationary phase. The mobile phase used was *n*-hexane: ethyl acetate: ethanol [2.5:1.5:0.4 V/V/V].

### Fourier transform infrared spectroscopy

100 mg of sample and potassium bromide were mixed together in a mortar and pestle. The pellet was processed for analysis.

## RESULTS

### Extraction of caffeine from green and black tea samples

The yields of caffeine from green tea in dichloromethane, ethyl acetate and acetone extracts were found to be 0.85%, 0.72% and 0.81%, respectively. The yields of caffeine from black tea in dichloromethane, ethyl acetate and acetone were found to be 1%, 0.82% and 0.95%, respectively.

### Qualitative estimation of caffeine obtained from the samples

In TLC plate, the caffeine zones were indicated by a dark-brown colour and standard caffeine served as

a positive control. Depending on the retention time in TLC and functional groups, C-NH<sub>2</sub>, Sp<sup>3</sup>-C, C=O found in FTIR the extracted samples were identified as caffeine, as shown in Fig. 3.

### Determination of $\lambda_{\text{max}}$ of caffeine

10  $\mu\text{g mL}^{-1}$  standard solution of caffeine in distilled water was scanned within 200 to 400 nm wavelength, range to detect  $\lambda_{\text{max}}$  of caffeine. It was found to be 272 nm.

### Linearity

Linearity is the capacity of the method to produce test outcomes that are proportionate to the concentration of the analyte in the sample. Linearity range of 0.5 to 35  $\mu\text{g mL}^{-1}$  obeyed Beer-Lambert's law. Calibration curve with concentration versus absorbance was plotted and the standard curve of caffeine was linear and exhibited good correlation coefficient ( $R^2 = 0.999$ ), as shown in Fig. 2.

### Accuracy

Accuracy was evaluated by adding standard solution to sample solution at 50%, 100%, 150% concentration levels. The absorbance was measured at 272 nm wavelength. % recovery was determined and the results are shown in Table I.

### Precision

It is the agreement level between a series of measurements made using different samples of the same homogeneous material. Six replicates of 10  $\mu\text{g mL}^{-1}$  solution were prepared and the absorbances were noted at 272 nm and the % RSD was found to be within limits, as shown in Table II.

### Robustness

Deliberate changes in the method are made such as change in wavelength. 10  $\mu\text{g mL}^{-1}$  of standard solution was prepared and it was scanned at  $272 \pm 2$  nm wavelength and the results are shown in Table III.

**Table III: Robustness data of caffeine**

Sr. No.	Conc. in $\mu\text{g mL}^{-1}$	Absorbance @ 270nm	Absorbance @ 272nm	Absorbance @ 274nm	Mean	% RSD
1.	10	0.4816	0.5398	0.5123	$0.5112 \pm 0.29$	0.5695
2.	10	0.4808	0.5398	0.5102	$0.5102 \pm 0.03$	0.05856
3.	10	0.4800	0.5398	0.5123	$0.5107 \pm 0.03$	0.05857

**Table IV: Ruggedness data of caffeine**

Sr. No.	Concentration	Analyst 1	Analyst 2	Mean	%RSD
01.	10 µg mL <sup>-1</sup>	0.4952	0.4932	0.49315±0.0003	0.000755
02.	10 µg mL <sup>-1</sup>	0.4924	0.4929	0.4629±0.00035	0.000765

**Ruggedness**

It is the degree to which results from sample analysis can be reproduced under various conditions, such as a different equipment or analyst. The absorbance was checked under different conditions and results are represented in Table IV.

**LOD:** It is the least amount of analyte in a sample that can be identified but not necessarily quantified under the specified experimental conditions. It was found to be 0.03 µg mL<sup>-1</sup>.

**LOQ:** It is the minimum amount of analyte in the sample that can be quantitatively measured under the specified experimental conditions with acceptable precision and accuracy. It was found to be 0.091 µg mL<sup>-1</sup>.

**Percentage purity of caffeine:** The percentage purity of caffeine in green and black tea samples are as shown in Table V.

**Table V: Percentage purity of caffeine from different samples**

Sr. No.	Sample	%Purity
01.	Green tea	98.32%
02.	Black tea	99.42%
03.	Green tea infusion	99.31%
04.	Black tea infusion	98.07%

**DISCUSSION**

In previous literature, caffeine was extracted using solvents like ethyl acetate, citric acid and other organic solvents<sup>9</sup>. Here, caffeine was extracted from green tea and black tea samples using three different solvents. The yield of caffeine was maximum from dichloromethane in both the samples. The extracted caffeine was identified using thin-layer chromatography TLC and FTIR. The TLC developing moving phase efficiently separates caffeine (R<sub>f</sub>= 0.5)<sup>10</sup>.

Caffeine can be estimated by different analytical techniques. Spectrophotometry is a simple analytical tool for the determination of various compounds. It is very simple, rapid and economical method for routine use. Very few spectroscopic methods have been reported for the estimation of caffeine in black tea<sup>11</sup>. Thus, the developed method can be adopted for quality control of caffeine obtained from diverse sources as well as for black tea and green tea infusions. This method can estimate the caffeine accurately in less time with more precision at low cost.

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