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Original Article

# NOVEL UV SPECTROSCOPIC METHOD FOR QUANTIFICATION OF CAFFEINE IN MARKETED ENERGY DRINKS

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**ABSTRACT**

**Objective:** This study is performed to quantitatively estimate caffeine in marketed energy drinks by using UV-Visible spectroscopic method.

**Methods:** This experiment was performed on various soft drinks and energy drinks available in the local market of India to determine the caffeine concentration. The quantitative method used was simple, easy UV-Visible spectrophotometric method by using carbon dichloromethane as diluent at 274 nm. UV-Vis spectroscopy is an analytical technique that measures the amount of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample in comparison to a reference or blank sample.

**Results:** Among all the samples i.e. soft or energy drinks taken for this experiment sample 1 has low concentration of caffeine and the highest concentration was observed in sample 3.

**Conclusion:** Caffeine in an energy drink provides a stimulant effect, it gives energy. At lower levels, as it's typically used in soft drinks, it has less of a stimulant effect and is used mainly for its taste profile. However the concentration of caffeine should be within the limits specified. Excessive consumption of caffeine may lead to anxiety, caffeine dependence, increased urination, and may cause insomnia. Energy drinks can contain high levels of caffeine but are unlikely to be hazardous unless consumed with alcohol. This research is very important analytical process to safeguard the well being of people who are unaware to adverse effects of caffeine.

**Keywords**: Caffeine, Soft drinks, Energy drinks, UV visible spectrophotometer, Dichloromethane


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## INTRODUCTION

Caffeine is a white crystalline xanthenes alkaloid i.e. methyl xanthenes group which is bitter in taste [1, 2]. The IUPAC name of caffeine is 1, 3, 7-trimethylpurine-2, 6-dione. Caffeiene molar mass and density are 194.19 g/mol and 1.2g/ml respectively. In cold water caffeine has low solubility and it has better solubility in hot water, pyrrole, acetone, pyrimidine, and ethyl-acetate. Caffeine has very well solubility in petroleum ether, chloroform, benzene and ether. Chemical structure of caffeine is given in the fig. 1 below.

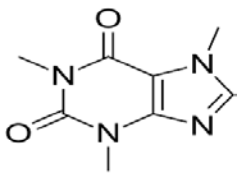

**Fig. 1: Chemical structure of caffeine**

Caffeine is present in leaves and seeds of different plants. Mostly it is derived from the tea leaves, coffee, cocoa or cola seeds. Big source of caffeine is tea. Higher concentrations of caffeine are present in black or oolong tea when compared with other kind of tea such as green tea. Tea was found in china and was initially used for medical purposes. It is extracted from *Camellia sinensis*, a plant that is indigenous to India and china. Tea has Flavanoids and imparts anti-inflammatory and neuroprotective activity. In Asia green tea is most popular tea. It has large amount of caffeine, tastes bitter and is made of unfermented leaves. Black tea tastes much bitter and is made with fermented leaves. It has polyphenols such as Flavanoids and has highest amount of caffeine. Flavanoids are effective against harmful agents [3].

In soft drinks like colas caffeine is most common ingredient. It is intentionally added to these drinks for the reason of their taste and to make the drink addictive. Depending on the drink type the content of caffeine in soft drinks varies which can be from 10 mg to 50 mg per serving portion [4]. The maximum amount of caffeine in carbonated drinks is restricted to 6 mg/oz by the USFDA [5]. In soft drinks the allowed amount of caffeine is in the range of 30 to 72 mg/335 ml or 8.45 to 20.28 mg/100 ml [6].

The popular drinks which contain caffeine are tea, coffee, soft and energy drinks. The most commonly used psychoactive compound worldwide is caffeine [7]. The amount of caffeine present in a cup of coffee is 100 mg. Even decaf coffee contains caffeine which can be 12 mg. The amount of caffeine present in a cup of tea is 85 mg. After 15 min of ingestion the effect of caffeine on human body may appear and can last for hours [8].

**Fig. 2: Soft drinks**

People will not have mood changes with consumption of less than 300 mg of caffeine. Consumption of caffeine causes dehydration due to its diuretic activity. Metabolism of caffeine takes place in liver by

p450 oxidase enzyme system and the metabolites produced are paraxanthine (84%), theobromine (12%), and theophylline (4%) [9, 10]. Each of these metabolites has effects on the body which are paraxanthine will increase the amount of free fatty acids in blood plasma, theobromine will increase the urine volume, and theophylline will causebronchi smooth muscle relaxation and treat asthma [11, 12].

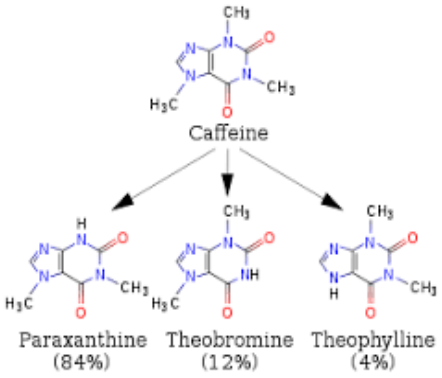


**Fig. 3: Metabolites of caffeine**

Advantages of caffeine consumption are [13-15]

- Reduces physical fatigue
- Used to treat migraine in combination with certain pain relievers
- Induces hair growth
- Protects from eyelid spasm and cataract
- Reduces risk of some types of cancer like liver cancer or colon cancer, skin cancer
- Reduces risk of type II diabetes and Parkinson's disease

The main purpose of this study is to determine the concentration of caffeine in different soft drinks and energy drinks available in local market of India using simple uv-visible spectrophotometer.

## MATERIALS AND METHODS

### Chemicals

Chemicals utilized in this experiment were pure caffeine ($C_8H_{10}N_4O_2$), dichloromethane ($CH_2Cl_2$), sodium carbonate ($Na_2CO_3$) of analytical grade gifted by SDFC(s d fine chem. Limited, Mumbai, India).

### Sample collection

Different types of soft drinks and energy drinks were purchased from the local market of India.

### Instrument

Double beam UV-Visible Spectrophotometer was used for determination of caffeine in soft drinks and energy drinks.

### Wavelength selection

The wavelength of caffeine at which it has maximum absorbance was known by scanning in the range of 190-400 nm. The **λ** max of caffeine was found out to be at 274 nm. This wavelength was chosen for further analysis.

### Calibration solutions preparation

Standard stock solution of caffeine was prepared by weighing 100 mg of caffeine. The weighed caffeine was transferred into a 100 ml volumetric flask. The caffeine solubility in the suitable diluents was checked. The weighed caffeine was dissolved in the diluent; the diluent selected here for this study was dichloromethane. The volume was made up to the mark with the dichloromethane. This is the stock solution of 1000 ppm concentration. From the stock solution, working standard solution of concentration 100 ppm was made. Further dilutions of 2, 4, 6, 8, 10 and 12 ppm concentration solutions were prepared from the stock solution. All the concentrations were scanned from 190-400 nm and maximum absorbance was observed at 274 nm by using quartz cuvettes and their absorbance was known. Calibration curve of absorbance v/s concentration was plotted.

### Extraction of caffeine from the samplesand preparation of sample solutions

- Various soft and energy drinks were purchased from the local market.
- In a 25 ml volumetric flask sodium carbonate solution was prepared by dissolving 20g of sodium carbonate in 25 ml of distilled water.
- Into a separating funnel 5 ml of drink sample was drawn by adding distilled water and to this 1 ml of sodium carbonate solution and 20 ml of carbon dichloromethane were added.
- The caffeine from the sample was extracted by inverting the separating funnel and venting the funnel at least 3 times after each inversion.
- The non-aqueous layer i.e. dichloromethane layer was transferred to a clean 50 ml volumetric flask.
- To the separating funnel containing aqueous solution another 20 ml of dichloromethane was added and the extraction was repeated twice.
- All the dichloromethane layers were combined.
- Caffeine from all the drink samples was extracted by following the same procedure and the absorbance of the solutions was measured at 274 nm in uv-visible spectrophotometer by using quartz cuvettes.

## RESULTS

**Table 1: Standard caffeine solution and sample solutions**

| Concentration (ppm) | Absorbance |
|---|---|
| 2 | 0.1523 |
| 4 | 0.3101 |
| 6 | 0.4838 |
| 8 | 0.6305 |
| 10 | 0.7990 |
| 12 | 0.9542 |
| Sample 1 | 0.9593 |
| Concentration | 12.03352954 |
| Sample 2 | 1.5316 |
| Concentration | 19.16041958 |
| Sample 3 | 2.011 |
| Concentration | 25.13041973 |
| Sample 4 | 1.3399 |
| Concentration | 16.77316671 |

Table 1 depicts the absorbance of caffeine standard solution of concentrations 2, 4, 6, 8, 10 and 12 ppm measured at 274 nm and also the absorbance of different sample solutions at the same wavelength.

The standard calibration curve was plotted from the caffeine standard solutions as shown in fig. 2 and the regression value was found out to be 0.9997.

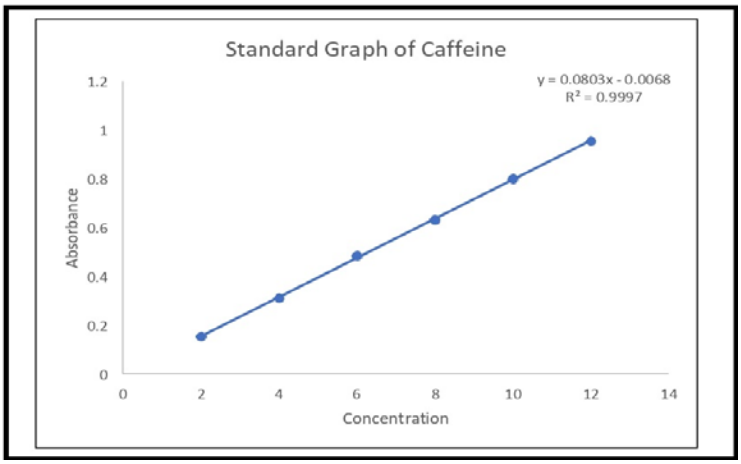


**Fig. 4: Standard calibration curve**

## DISCUSSION

The main objective of this study is to determine the concentration of caffeine in various energy and soft drinks available in the market. During the extraction of caffeine from the energy and soft drinks, high concentration of sodium carbonate was added. The purpose of adding sodium carbonate in the extraction process is that caffeine, along with the unwanted organic acids, is deprotonated. Deprotonated caffeine along with other unwanted acids is obtained. The caffeine is found to have maximum solubility in dichloromethane. Once deprotonated, caffeine can be obtained by further dissolving it in dichloromethane, whereas other undesired substances cannot as they are immiscible in it.

The absorbance of obtained caffeine along with the diluent dichloromethane was checked in uv-visible spectrophotometer. The absorbance is directly proportional to the concentration of solution. If the absorbance of the solution is high that indicates that the concentration of caffeine is more in that sample.

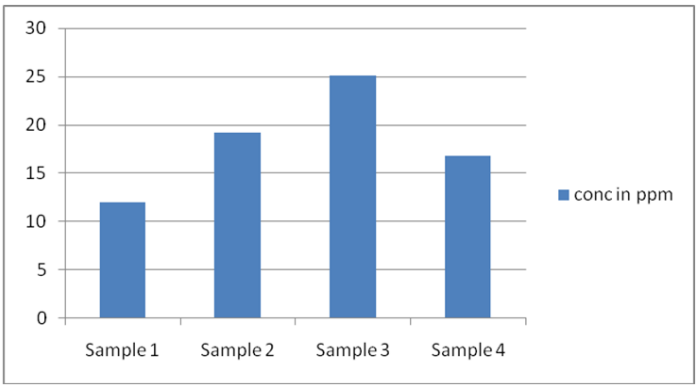


**Fig. 5: Graph of concentration of soft and energy drinks**

Concentration of caffeine should be within the prescribed limits. If the concentration of caffeine in the samples is as mentioned on the drinks then it is within the limits. The concentration is known by using simple, easy and accurate analytical method i.e. uv-visible spectrophotometer. Calibration curve was constructed with standard caffeine solutions and from the graph the concentration of the samples was known. Sample 1 is found to have lowest concentration of caffeine and it can be marketed. Lower the concentration lower is the stimulant activity. Sample 3 is found to have highest concentration of caffeine. Higher the concentration higher is the stimulant activity.

## CONCLUSION

Extraction of caffeine from the energy and soft drinks available in the market and determination of concentration of caffeine in them can be done by many analytical methods but in this research uv-visible spectrophotometer was used because it is relatively fast, easy, inexpensive, and highly sensitive and gives accurate concentration of caffeine. This research is very important analytical process to safeguard the well being of people who are unaware to adverse effects of caffeine. Sample 1 has low concentration of caffeine and sample 3 has high concentration of caffeine.

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Nil

## AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

## CONFLICT OF INTERESTS

Declared none

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