Avens Publishing Group
J Food Processing & Beverages
September 2017 Vol.:5, Issue:1


Open Access Research Article
Journal of Food Processing & Beverages

# Spectrophotometric Determination of Caffeine in Selected Pakistani Beverages

**Zahid Shar*, Hadia Shoaib, Naila Anwar, Muneeba Zubair, Aisha Hussain and Khizra Khan**

*Dr. M.A Kazi Institute of Chemistry University of Sindh, Jamshoro, Pakistan*

***Address for Correspondence**
Zahid Shar, Dr. M.A Kazi Institute of Chemistry University of Sindh, Jamshoro, Pakistan, Tel: +923337011526; E-mail: zahidshar_2009@yahoo.com

**Submission:** 25 April, 2017
**Accepted:** 05 September, 2017
**Published:** 25 September, 2017



**Keywords:** Caffeine; UV-Visible Spectrophotometry; Pakistani beverages; Energy drinks

## Abstract

Amount of caffeine in beverages gained much attention due to its various toxicological effects. However, limited data is available especially for store brands beverages. In order to assess the caffeine content, thirty different samples of soft drinks, energy drinks and tea samples were collected from Hyderabad, Pakistan. Quantification of caffeine was done by using in-house validated UV-Visible spectrophotometer. Accuracy and precision of the methods was checked by standard addition method. Tea sample (Tapal danedar) contains the highest amount of caffeine (111 µg/mL) with an average value of 89 µg/mL. Energy drinks (Mad Croc Cola) contain next higher amount of caffeine (90 µg/mL) with an average value of 47 µg/mL. The pH of all analyzed samples was found to be in the acidic range (2.88-5.01) which is hazardous for adolescent teeth. This data will provide useful information for Pakistani regulatory authorities to set standards for additives in different types of beverages.

## Introduction

Caffeinated soft drink consumption has increased enormously in recent times, and approximately 80% of the world's population consumes caffeinated product every day [1]. Increased popularity in caffeinated products increase the demand of energy drinks (ED) with higher amount of caffeine [2]. The attractiveness and recognition of these beverages are due to the fact that caffeine, helps in staying awake and improve mental alertness after fatigue [3,4]. Caffeine is a naturally occurring alkaloid belong to methylxanthine class and found in many different plants leaves and fruits [5-7]. IUPAC name of caffeine is 1,3,7 Trimethylpurine-2,6-dione and its structure is shown in Figure 1.

Caffeine has drawn more attention in the past decades, due to its physiological effects beyond that of its stimulatory effect [8,9]. Caffeine directly effects central nervous, cardiovascular, gastrointestinal, respiratory, and renal systems [10-14]. Caffeine is only psychoactive substance which is widely consumed by humans [15-17]. In cases of overdosing of caffeine or in combination with alcohol, narcotics and some other drugs, produce a toxic effect, sometimes with lethal outcome [7,18-20].

The absence of regulatory oversight has resulted in aggressive marketing of energy drinks with higher amount of caffeine, targeted primarily toward young males, for psychoactive, performance-enhancing and stimulant purposes [1]. There are increasing reports of caffeine intoxication from energy drinks, and it seems likely that problem with caffeine dependence increasing day by day [21]. In children and adolescents who are not habitual caffeine users, vulnerability to caffeine intoxication may be markedly increased due to an absence of pharmacological tolerance [22]. Several studies suggest that energy drinks may serve as a gateway to other forms of drug dependence [23,24]. Due to its toxicological effects, caffeine regulation in soft drinks is gaining importance (aqsa, 2015) and many countries have already set the maximum limits in different kinds of beverages [24-34] (Table 1).

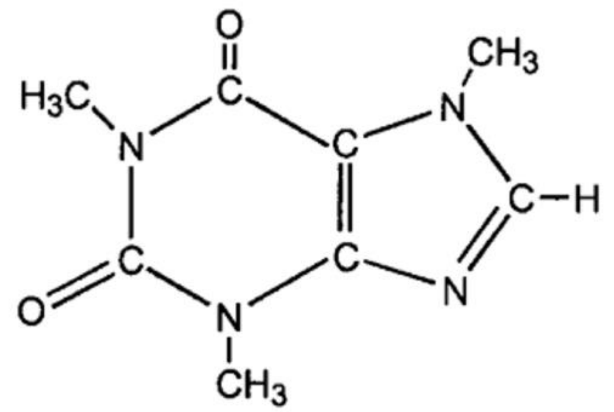

**Figure 1:** Chemical structure of caffeine.

**Table 1:** Regulation of caffeine in beverages.

| Country | Beverages | Maximum limit |
|---|---|---|
| United states | Cola type beverages | 200 mg/L |
| Australia | Cola-type soft drinks and flavored syrups | 145 mg/L |
| New Zealand | | 200 mg/Kg |
| Canada | Cola type beverages | 200 mg/L |
| European union | Cola type beverages | 150 mg/L |
| South Korea | Cola type beverages | Less than 400 mg/L |
| Taiwan | Cola type beverages | 320 mg/L |
| Mexico | Cola type beverages | 200 mg/L |
| Brazil | Cola type beverages | 350 mg/L |
| Chile | Cola type beverages | 500 mg/L |
| Pakistan | Cola type beverages | No regulation |

ISSN: 2332-4104

**Table 2:** Recoveries of caffeine from spiked beverages samples.

| Sample name | Amount of caffeine in sample (µg/mL) | Amount of caffeine added (µg/mL) | Amount of caffeine found (µg/mL) | Recovery percentage (%) |
|---|---|---|---|---|
| Pepsi | 19 µg/mL | 10 µg/mL | 27±3.1 µg/mL | 93 % |
| Red bull | 36 µg/mL | 10 µg/mL | 45±2.8 µg/mL | 98 % |
| Lipton | 110 µg/mL | 10 µg/mL | 117±2.1 µg/mL | 97% |
| LOD[1] | 0.006 µg/mL | | | |
| LOQ[2] | 0.06µg/mL | | | |

[1] limit of detection $(LOD) = 3.3 \times \frac{\sigma}{Slope}$

[2.] Limit of quantification $(LOQ) = 10 \times \frac{\sigma}{Slope}$

**Table 3:** Caffeine contents and pH levels of analyzed Pakistani beverages.

| Sample type | Sample name | Caffeine concentration | PH |
|---|---|---|---|
| Carbonated drinks | Pepsi | 19 µg/mL | 2.92 |
| | Pepsi diet | 14 µg/mL | 3.76 |
| | 7 up | N.D | 3.57 |
| | Pakola | N.D | 3.92 |
| | Coca cola | 6 µg/mL | 2.90 |
| | Cock diet | 13 µg/mL | 3.17 |
| | Marinda | N.D | 4.01 |
| | Slice | N.D | 3.12 |
| | Apple sidra | 12 µg/mL | 3.31 |
| | Big apple | N.D | 3.24 |
| | Dew | 11 µg/mL | 3.80 |
| | Sprite | N.D | 3.62 |
| | 7up diet | ND | 3.64 |
| | Gourmet | 2 µg/mL | 3.31 |
| Energy drinks | Sting | 40 µg/mL | 3.90 |
| | Red bull | 36 µg/mL | 3.70 |
| | speed | 56 µg/mL | 3.52 |
| | Mad croc cola | 90 µg/mL | 2.88 |
| | Mad croc orange | 11 µg/mL | 3.56 |
| | Booster | N.D | 3.41 |
| Tea | TapalDanedar | 111 µg/mL | 4.91 |
| | Tapal family mixture | 94 µg/mL | 4.80 |
| | Lipton | 110 µg/mL | 4.91 |
| | Tezzdam | 87 µg/mL | 4.47 |
| | Supreme | 78 µg/mL | 4.83 |
| | Green tea | 52 µg/mL | 5.01 |

The energy drinks like 'Red Bull' and 'Sting' by PepsiCo are emerging industry in Pakistan [35]. Energy drinks are consumed by all age group but the most vulnerable to its use and ill effects are adolescents and young adults. This could be potentially a disastrous public health issue in a country like Pakistan where adolescents and young adults make the majority of the population. There is increasing interest regarding the potential health effects of energy drink (ED) consumption [35]. The aim of the present study was to investigate the caffeine content in different kinds of beverages and their pH levels available in Pakistan.

## Materials and Methods

### Chemicals and instrumentation

All reagents used in this study were of analytical or HPLC grade and all solutions were prepared by using disttled water. Caffeine standard was purchased from Sigma-Aldrich (Sigma-Aldrich Chemie GmbH Munich, Germany). A Perkin Elmer (Germany) double-beam UV/VIS spectrophotometer with 1-cm matched quartz cells was used for all absorbance measurements. A pH-meter, Thermo Orion (Thermo scientific fisher, USA), with combined electrodes was employed for measuring pH values. Analytical balance (Kern and sohn, GmbH, Germany) was used for weighing purpose throughout the study. Bath sonicator(Fisher scientific, UK) was used for sonication purposes.

### Wavelength selection

Wavelength at which caffeine absorb maximum was determined by scanning the range of 120-400 nm. Wavelength at which Caffeine absorb maximum was found to be 271 nm, which was selected for further analysis, which is same as reported by other authors [36,37].

### Preparation of standards

Caffeine stock standard solution of 500 µg/mL was prepared by dissolving 0.05 gram of caffeine standard obtained from Sigma-Aldrich (Sigma-Aldrich, Chemie GmbH Munich, Germany)in 80 mL

ISSN: 2332-4104

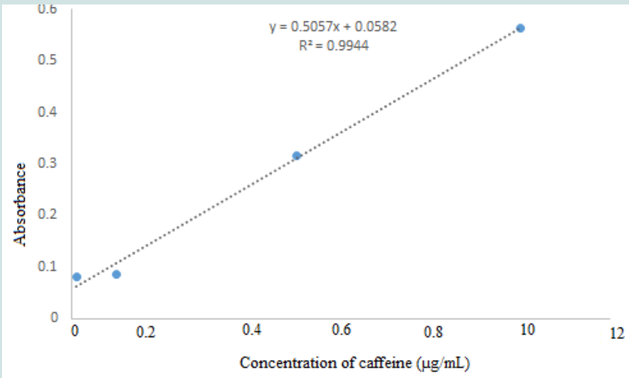


**Figure 2:** Calibration curve obtained by using caffeine standards.

distilled water and sonicated for 10 min. Then solution was transferred to 100 mL volumetric flask and the volume was completed to mark by distilled water. The stock solution was stored in dark place at +4 °C. Working standard solutions of 0.01, 0.1, 0.5, 1, 10 µg/mL were prepared by suitable dilution of stock solution. Calibration curve was obtained by plotting absorbance versus standard concentration of caffeine (Figure 2).

### Sampling and sample preparation

Thirty different kinds of carbonated drinks including regular and diet cola, energy drinks and tea samples were purchased from local supermarkets of Hyderabad, Pakistan. Once bottles were opened, the drinks were degassed by sonication, homogenized and filtered. Then each sample was filtered through a 0.45 µm syringe filter with a 5 mL syringe. Filtered drink sample of one millilitre was diluted with hundred millilitre of distilled water.

Two grams of tea samples were weighed and put into 250 mL beakers. Hundred millilitre of boiling distilled water was added and let to stand for five minutes with stirring; the solution was cooled and filtered into conical flasks. One millilitre of filtrate were pipetted into clean 100 mL of volumetric flasks and made to the mark with the distilled water. The samples were filtered through 0.45 µm syringe filter. Caffeine content in each sample was determined by using UV-Visible spectrophotometer.

### Spiking

Known amount of standard (10 µg/mL) was added to different matrix (carbonated drink, energy drink and tea) in order to check the accuracy and precision of the method.

### Detection and quantification limits

Limit of detection and limit of quantification were calculated by using calibration curve method based on standard deviation of the response and the slope value as follows

$$LOD = 3.3 \times \frac{\sigma}{Slope} \qquad \text{Eqn (1)}$$

$$LOQ = 10 \times \frac{\sigma}{Slope} \qquad \text{Eqn (2)}$$

## Results and Discussion

### Method validation

Method performance characteristics such as linearity, accuracy and precision, limit of quantification (LOQ), limit of detection (LOD), and specificity were evaluated according to European Union guidelines [38]. The method was found to be linear in the range of 0.01-10 µg/mL in both spiked feed extract as well as in pure solvent, which shows that this method is valid in determining the low levels of the caffeine in beverages. Calibration equation with $R^2$ values for caffeine is given in (Figure 2).

Recoveries and precision (repeatability, expressed as relative standard deviation (RSD%) were determined by analyzing spiked samples in triplicate at the spiking levels of 10 µg/mL Recoveries in the range of 93-98% with RSDs ( 1.5%) were obtained.

Limit of quantification (LOQ) and limit of detection (LOD) were calculated from calibration curve method by using equation 1 and 2. LOD and LOQ values were found 0.006 µg/mL, 0.06 µg/mL respectively (Table 2). The specificity of the method was tested by the analysis of blank samples, No any peak were observed in beverages, indicating that there were no matrix compounds that might give a false positive signal in the samples.

### Determination of caffeine

The validated method was used to determine the concentration of caffeine in real beverages samples (carbonated soft drinks, energy drinks and different kinds of tea). The highest caffeine concentration (111 µg/mL) was found in tea sample brand name Tapal Danedar (Table 3).

Caffeine content in tea depends upon the environmental conditions during the growth of the tea plant and type of species [12]. Lowest concentration of caffeine was found in green tea sample 52 µg/mL as show in Table 3.The concentration of caffeine in tea samples obtained in present study was much lower than the values reported from the Sudanese tea samples [36]. This lower value might be of different plant species [12].

The next higher concentration of caffeine 90 µg/mL was found in energy drink sample brand name mad croc cola. It was observed that the caffeine concentrations in energy drinks is higher than the carbonated soft drinks, since their mean concentrations level were of 47 µg/mL and11 µg/mL respectively. Our results of caffeine content in different beverages samples are similar to another study conducted on carbonated drinks [39].

The pH of carbonated soft drinks and tea samples ranged from 2.88 to 5.01. Carbonated soft drinks have a relatively low pH which makes soft drinks not suitable for people with stomach ulcers. Low pH has effects on the enamel which can affect teeth development in infants [40]. *In vitro* studies have shown that soft drinks with low pH can cause dental erosion in permanent and deciduous teeth [41]. Decrease in pH has also been associated with increase in dental erosion [42]. Low pH is as a result of addition of acidulants such as phosphoric acid and citric acid .The acid is used in soft drink product as a key factor in the taste profile of a drink as it balances the sweetness and helps to inhibit microbial growth [43,44].

## Conclusion

UV-Visible method for quantification of caffeine in beverages was found to be simple, precise, sensitive and accurate. In spite of

ISSN: 2332-4104

the number of drink samples analyzed is small, the data presented in this study gave a preliminarily information about caffeine levels in tea, carbonated and energy drinks frequently consumed in Pakistan. However, the energy drinks contained appreciable levels of caffeine, hence should be avoided by children at an early age to avoid caffeine dependence. The pH of the drinks was low. This is attributed to the addition of acidulants which makes carbonated drinks and energy drinks harmful to infants and people suffering from ulcers. Despite the fact that caffeine is a permitted food additive, excessive use should be minimized.

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