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Determination of Caffeine content by HPLC method in Energy and Soft Drinks available in Indian market

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ABSTRACT

Caffeine, a ubiquitous stimulant consumed globally, necessitates accurate quantification for quality control and consumer safety, particularly in beverages like energy drinks, soft drinks, and juices. The widespread consumption of caffeine containing products has sparked considerations about potential health effects, emphasizing the need to precisely measure caffeine levels. High Performance Liquid Chromatography (HPLC) is the preferred method for determining caffeine concentration, offering unparalleled accuracy and reliability. The HPLC technique employs a C18 reversed-phase chromatographic column, which provides exceptional separation and resolution of caffeine from other components. The mobile phase, comprising a mixture of methanol and water, facilitates the efficient elution of caffeine, while UV detection at 274nm enables precise quantification. This methodology offers numerous advantages, including excellent sensitivity, reproducibility, and low limits of detection and quantification. A comparative analysis of caffeine content among various brands revealed significant variations, highlighting the importance of standardized quantification methods. Energy drinks contained higher amounts of caffeine than soft drinks, with some exceeding recommended daily intake levels. The concentration of caffeine in various energy and soft drinks ranged from 6.19 to 43.08 mg/100ml. This finding underscores the need for regulatory monitoring and quality control measures to ensure consumer safety.

Keywords: Caffeine; Energy drinks; Chromatogram; Food and drug administration; High performance liquid chromatography; Quality control; Quantitative analysis; Soft drinks; Non-alcoholic beverages; Consumer safety; Methanol; Retention time.

1. Introduction

Energy drinks are functional, non-alcoholic beverages designed to improve people's psychophysiological reactions. Caffeine, D-glucuronolactone, B vitamins, taurine, carbohydrates, and herbal extracts like ginseng and guarana are a few examples [1]. A naturally occurring stimulant, caffeine belongs to a class of molecules called methylxanthines and can be found in the leaves, seeds, or fruits of more than 63 plant species worldwide [2,3]. Coffee, tea, guarana, cola nuts, and cocoa are the main sources of caffeine. These goods vary in their caffeine content, with guarana (4–7%) having the greatest levels, followed by tea leaves (3.5%), coffee beans (1.1–2.2%), cola nuts (1.5%), cocoa beans (0.03%), and mate tea leaves (0.89–1.73%) [4].

To improve and preserve the drink's quality while also advancing food safety, several substances are added to it, including various food additives, including preservatives and antioxidants. Food additives are also frequently utilized to extend the shelf life and enhance the energy drink's organoleptic quality [5]. Both recreationally and medicinally, caffeine is utilized as a central nervous system and metabolic stimulant to lessen physical exhaustion and regain mental clarity when unexpected weakness or drowsiness arises [6]. Caffeine is typically eliminated from the body after a few hours of use and does not build up over time [7].

With centuries of safe use in foods and drinks, caffeine is considered one of the thoroughly researched substances in the food supply. The U.S. Food and Drug Administration (FDA) declared caffeine in cola beverages to be "Generally Recognized as Safe" (GRAS) in 1959 [8]. The Federal food and drug regulatory authority regulates the consumption of caffeine as a food ingredient. Legally, food and beverage producers must only list caffeine on the

label of their products after it has been added [9]. For people with psychological issues, excessive coffee consumption can exacerbate symptoms such as headaches, anxiety, irritability, and insomnia. Frequent excessive intake of caffeine has been linked to cardiovascular illness, decreased cerebral blood flow, nervous system abnormalities, impaired liver function, increased gastric secretion, and harmful effects when used with alcohol, opiates, and other medications [10].

Several analytical methods, including UV-Vis spectrophotometry [11, 12], potentiometry, amperometry, as well as separation methods such as Liquid Chromatography LC [13], Ion Chromatography, and High Performance Thin Layer Chromatography HPTLC [14], have been used to evaluate the concentration of caffeine in beverages.

1.1. Objectives of the Study

a) To develop a precise and accurate method for determining caffeine levels.

b) To address specific concerns related to caffeine content in products and ensure consumer safety.

c) To potentially identify any discrepancies between declared and actual caffeine levels.

d) To ensure accurate labeling, assess compliance with regulations.

e) To help ensure manufacturer comply with the standards set by regulatory bodies.

2. Materials and Methods

2.1. Materials

HPLC grade Methanol, commercial standard of anhydrous caffeine and HPLC grade water.

2.2. Methods

HPLC Instrument: LC-2010AHT (Schimadzu Corporation), Column: C18 Column, pore size 5µm, internal diameter 4.6nm and length 250 mm, Flow rate: 1 ml/min, Column temperature: 25^0C, UV detector set at: 274 nm, Mobile phase: HPLC grade Water and Methanol (55:45), Sample injection volume: 10 µl.

Mobile Phase Preparation: The mobile phase was composed of water (55%) and methanol (45%). About 225 ml of methanol was mixed with 275 ml HPLC grade water in a volumetric flask with proper shaking. The mixture was then sonicated for 30 mins in a Sonicator.

Preparation of Standard Solution: 10 ml of methanol was added to 20mg of anhydrous caffeine in 20 ml of volumetric flask and vortexed to mix well. Later, it was diluted up to the mark and mixed well.

Preparation of Sample Solution: All the samples were sonicated for two hours at a room temperature. After sonication, 5 ml of methanol was mixed with 1 ml of sample (soft drinks or energy drinks) in 10 ml volumetric flask and vortexed to mix well. Later, it was diluted up to the mark and mixed well.

3. Results

In this study, caffeine in various brands of soft and energy drinks was studied using HPLC. The optimum rate of flow for the eluent was set at 1.0 ml/min with caffeine standard and the above rate was kept consistent for all sample

determination. According to literature survey, we fixed wavelength for determination of caffeine at 274nm. Standard caffeine with a concentration of 1000 ppm was loaded in the column. In optimized condition, a peak was obtained at retention time of 4.714 min for caffeine content. The complete experiment was performed at room temperature. All the samples (Energy drinks and soft drinks) were diluted by pipetting 1ml of sample to 10 ml with HPLC grade Methanol. After diluting samples, they were loaded in sample injection vial and then 10 µl of each sample was injected by automated HPLC instrument. Lab solutions CS liquid chromatography data integrity software was used to set all instructions. All samples gave the peak due to caffeine at the same retention time as standard caffeine. The retention time, area under peak and concentration of caffeine in mg/100 ml for standard and various samples is tabulated in Table 1.

Table 1. Experimental observation of HPLC method in various energy and soft drinks

S. No.	Sample	Retention Time	Area	Concentration mg/100ml
1	Standard	4.714	24950364	1000
2	ED1	4.738	154462	6.19
3	ED2	4.733	798840	32.017
4	ED3	4.735	968149	38.8
5	ED4	4.737	761122	30.5
6	ED5	4.733	1009039	40.4
7	ED6	4.737	1075005	43.08
8	ED7	4.738	834043	33.42
9	ED8	4.215	1018837	40.834
10	SD1	-	-	-
11	SD2	4.745	322695	12.9

Figure 1 demonstrates the caffeine concentration in mg/100 ml in various energy and soft drinks in the form of bar graph.

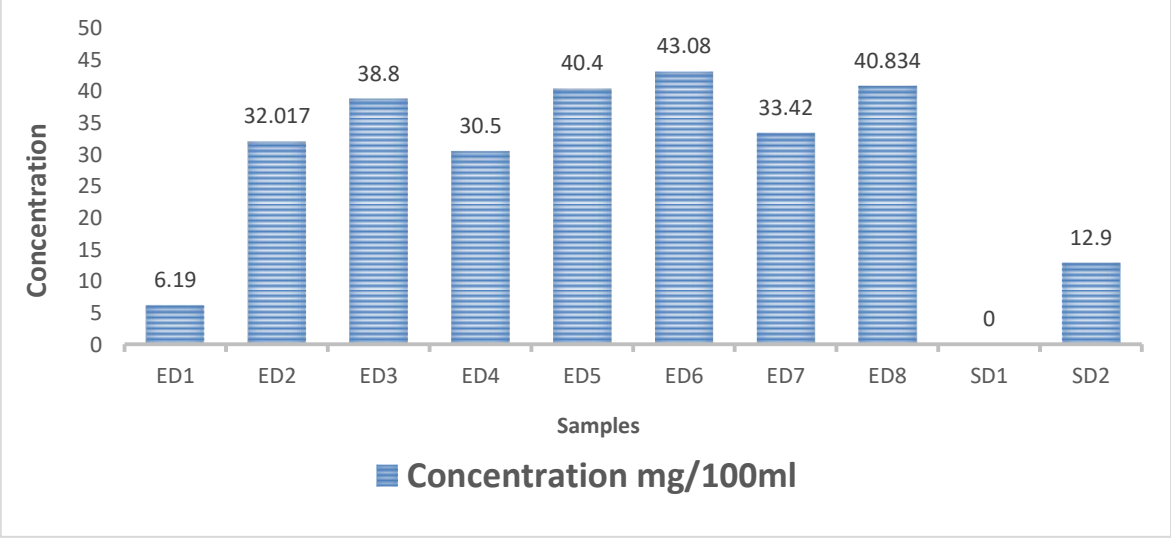

Figure 1. Caffeine concentration in various energy and soft drinks

Figure 2 is the chromatogram of standard caffeine injected under similar experimental conditions as that of sample.

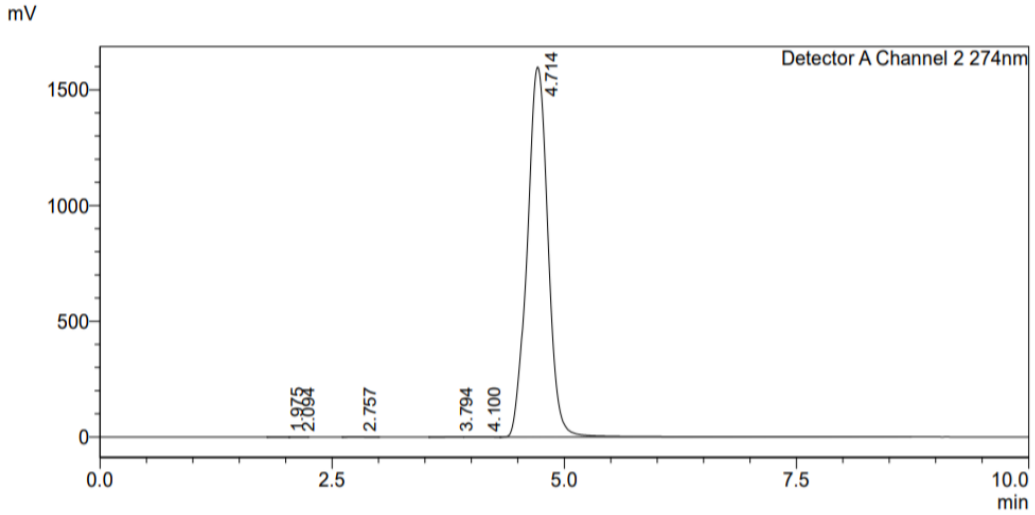

Figure 2. Chromatogram of standard caffeine

Figure 3 is a representative chromatogram of energy drink injected under similar experimental conditions as that of standard caffeine.

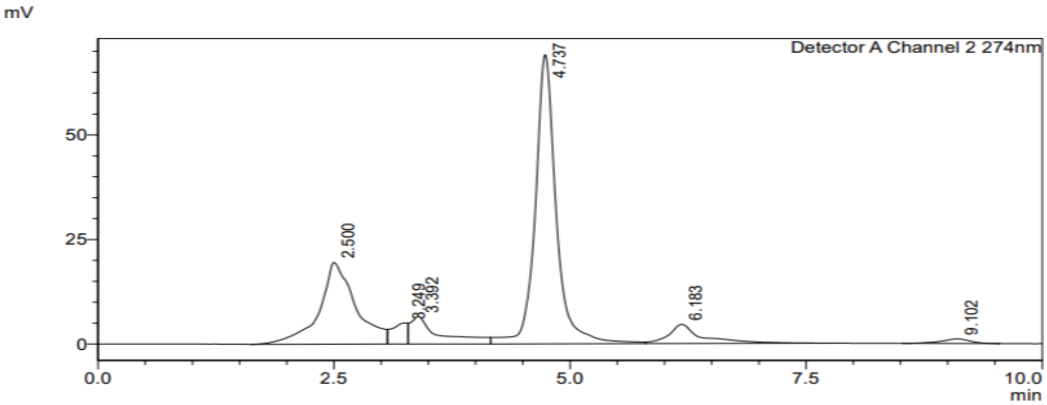

Figure 3. Chromatogram of ED6

Figure 4 is a representative chromatogram of soft drink injected under similar experimental conditions as that of standard caffeine.

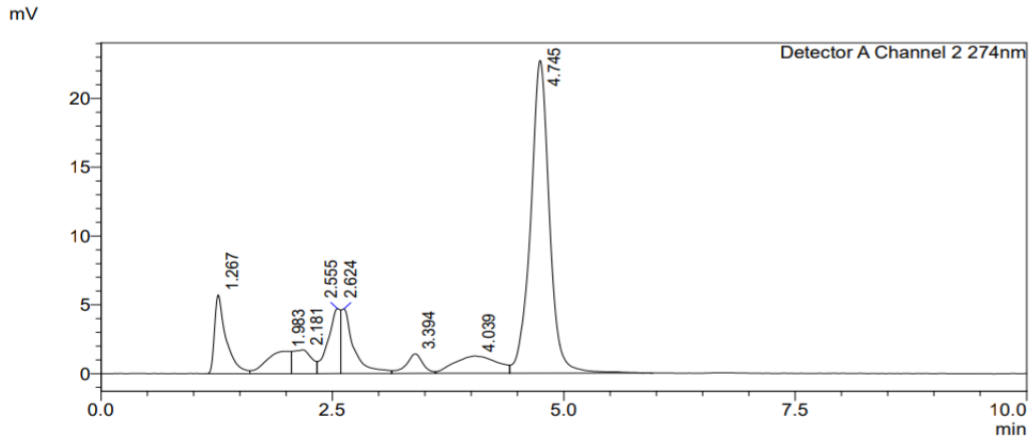

Figure 4. Chromatogram of SD2

4. Conclusion

HPLC analysis demonstrates that majority of the energy and soft drinks contained caffeine at varied level. The concentration of caffeine in various energy and soft drinks ranged from 6.19 to 43.08 mg/100ml. ED6 sample showed the highest concentration of caffeine whereas ED1 sample showed the lowest concentration of caffeine. No peak in the chromatogram of sample SD1 was matched to the peak of standard caffeine indicating no evidence of caffeine in it.

According to the Food safety and standards authority of India (FSSAI) and FDA regulations, the acceptable range of caffeine in soft drinks and energy drinks is between 145 and 300ppm. The brand ED1, ED4 and SD2 have caffeine concentration well within the limits set by FSSAI and FDA.

Future Recommendations: Brands should offer products with lower caffeine levels to cater to health conscious consumers and address concerns about excessive caffeine intake. Government authorized agency should take control and regular monitoring to check the level of caffeine in all brands of energy and soft drinks. It should implement stricter regulations regarding caffeine content, mandatory disclosures, and consumption limits for energy and soft drinks. It should focus on consumer protection and regulatory compliance.

Declarations

Source of Funding

This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing Interests Statement

The authors declare that they have no conflict of interest.

Consent for publication

The authors declare that they consented to the publication of this study.

Authors' contributions

Contribution is equal among authors.

Ethical approval and consent to participate

Not applicable for this study.

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