Analysis Of Caffeine And Artificial Sweeteners As Active Ingredients In Popular Energy Drinks Available In Indian Market For Forensic Prospects

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Abstract
Energy drinks (EDs) are beverages designed to boost energy, alertness, and concentration. They typically contain caffeine, sugar, vitamins, and other ingredients like taurine, ginseng, and B vitamins, though exact ingredient amounts are often undisclosed. These drinks are popular among people looking for a quick energy boost, especially in situations requiring extended periods of wakefulness or physical activity. However, they can have side effects, particularly when consumed in large quantities or mixed with other drinks. They are typically marketed to enhance physical or cognitive performance and promote weight loss by increasing energy expenditure, owing to the presence of active ingredients of these drinks. This study aimed to measure the concentrations of such active ingredients of energy drinks using HPLC and based on the analysis result, assess whether the product label claims stand true or not and whether the product complies with FSSAI standards. Samples from ten different ED brands were analyzed using HPLC for determining levels of active ingredients of EDs, i.e., caffeine and artificial sweeteners and explore its scope in forensic science. The study found significant discrepancies and non-compliance with standards across all brands with high prevalence, well above the recommended values in all the samples, suggesting potential health risks and highlighting consumer fraud from a forensic perspective.

Keywords: energy drinks, HPLC, caffeine, sugar, artificial sweeteners, forensic science

Introduction

Caffeinated energy drinks (EDs) are popular beverages believed to enhance physical and cognitive performance. However, it is unclear if benefits come from ingredients other than caffeine, which is one of the active ingredients of these drinks. Typical contents of EDs include caffeine, taurine, B-vitamins, herbs, sugars, artificial sweeteners and other compounds like antioxidants and preservatives to extend its shelf life.[1] In the United States, ED manufacturers often classify their products as dietary supplements, avoiding the requirement to disclose quantities of active ingredients[1] but as of 2024, the rules have changed and such malpractice of adulteration and misbranding have been prohibited by the FDA and the manufacturers must ensure that the safety and labelling of the products is accurate or else they are liable for actions taken by FDA.[2] These drinks aim to improve concentration, endurance, and performance. Therefore, it is crucial to have extensive scientific and nutritional research to support their safety and effectiveness, making it important to profile their ingredients.[3,4]

In India, as of September 2021, the Food Safety and Standards Authority of India (FSSAI) permits caffeine content in caffeinated drinks (including EDs) to be between 145-300 ppm. The concern extends beyond caffeine and sugar content to other additives in EDs, which are marketed as functional foods with supplements like ginseng, taurine, etc.[5] Water, sugar and caffeine are the primary ingredients of energy drinks out of which, caffeine is the key psychoactive ingredient and in combination with sugar, it provides ergogenic effects.[5] These beverages are promoted as ergogenic and mind-stimulating but often do not disclose all ingredients or their quantities on the labels.[6] This raises issues of potential consumer fraud regarding whether the claimed benefits are delivered, if all listed ingredients are present, if there are undisclosed ingredients, and if these drinks are safe for long-term consumption. This has prompted scientists, healthcare professionals, and regulatory bodies to scrutinize the effects of ingredients other than caffeine and verify the brands' claims.[1] Although energy drinks are not widely studied in forensic science, their potential toxicity, use as consumption markers, and risks of adulteration and contamination make them relevant in forensic contexts.[5] Forensic analysis can

reveal insights into the quality, composition, and regulatory compliance of these beverages. Using scientific techniques, food forensic scientists can verify the authenticity, safety, and compliance of energy drinks in the market.[5]
The objective of this work was to determine levels of caffeine and artificial sweeteners in ten commercial EDs through HPLC analysis and assess whether the product label claims stand true or not and whether the product complies with FSSAI standards and use these results to find its scope in food forensics.

Methodology

For sample collection, the e-commerce websites and apps were used for filtering out the top 10 brands and making purchases. A total of 100 non-alcoholic energy drink samples, comprising 10 samples each from 10 different brands, were procured from various online and offline stores in Prayagraj, Uttar Pradesh at different time period and stored at 4°C in sterile conditions. The samples were tested at Instrumental Laboratory, Rohilkhand Laboratory and Research Center, Bareilly, Uttar Pradesh in 2022. At the time of testing, the samples were firstly degassed using ultrasonicator, followed by sample preparation. All reagents and reference materials used in this study were of HPLC analytical grade and all the lab wares and instruments used were sterile. Different mobile phases were used for extraction of different analytes[7,8] which have been enlisted in the table 1 below:

Table 1: *Mobile phases for extraction of different analytes using HPLC[7,8]*

Sr. No.	Analyte	Mobile Phase A	Mobile Phase B	Ratio of mobile phase A:B
1.	Caffeine	Methanol	Milli-Q water	70:30
2.	Artificial sweeteners (saccharin, aspartame, acesulfame-K)	Phosphate Buffer	Methanol	80:20

Later, the samples were subjected to HPLC for analysis. The HPLC instrument used was Agilent HPLC-1260 Infinity II with an autosampler, Agilent Zorbax Eclipse Plus C18 column (5 µm, 4.6 x 250 mm)**.**

Table 2: *HPLC instrument condition for analysis of standards of analytes*

Sr. No.	Analyte	Flow rate (ml/min)	Run time (in min.)	Injection vol. (in µL)	Detector	Column Oven Temp.	λ_{max} (in nm)	Retention time of standard (in min.)	Average Retention time of sample (in min.)
1.	Caffeine	1.0	5	20	DAD	Ambient	272	2.77	2.81
2.	Artificial sweeteners	1.0	32	20	UV	Ambient	220	Saccharin-3.1 Acesulfame-K-4.7 Neotame-7.37	Saccharin- 3.0 Acesulfame-K-4.8 Neotame-7.35

The UV maximum absorbance value of the sample had to match that of the standard. The results were calculated using Agilent OpenLAB software to control the system and process the data. After analysis, the average concentration of the analytes of these 10 samples per brands was taken into consideration and the obtained processed data was then tabulated (table 3,4) for comparison with FSSAI standards and product label claims.

Results and discussion

The data obtained from HPLC analysis of caffeine and artificial sweeteners are presented as an average value below in tabulated form for comparison of actual concentration of analytes in the samples of brand B-1 to B-10 with that of concentrations as per FSSAI standards and those mentioned on the product label.

Table 3: *Concentration of caffeine in different brands (B1-B10) of energy drinks*

Brand	Caffeine conc. in sample		
	As per FSSAI	As per product label	Analysis result
	Conc. (ppm)	Conc. (ppm)	Avg. conc. (ppm)
B-1	300	300	347.131
B-2	300	800	271.96
B-3	300	288	258.31
B-4	300	246	372.02

B-5	300	230	227.28
B-6	300	288	358.21
B-7	300	246	237.83
B-8	300	320	363.75
B-9	300	230	239.75
B-10	300	300	278.12

Table 4: *Concentration of artificial sweeteners in different brands (B1-B10) of energy drinks*

Brand	Artificial sweetener conc. in sample						
	As per FSSAI			As per product label	Analysis result (average conc.)		
	Saccharin conc. (ppm)	Acesulfame-K conc. (ppm)	Neotame conc. (ppm)	Conc. (ppm)	Saccharin conc. (ppm)	Acesulfame-K conc. (ppm)	Neotame conc. (ppm)
B-1	100	300	33	NA	00.00	00.00	00.00
B-2	100	300	33	NA	4932.10	2.98	12.38
B-3	100	300	33	NA	384.72	00.00	00.00
B-4	100	300	33	NA	955.50	0.45	00.00
B-5	100	300	33	NA	306.78	1.31	00.00
B-6	100	300	33	NA	1867.80	28.31	3.25
B-7	100	300	33	NA	5648.82	29.31	5.35
B-8	100	300	33	NA	1554.49	0.43	00.00
B-9	100	300	33	NA	00.00	95.39	00.00
B-10	100	300	33	NA	8269.84	00.00	00.00

The analysis of caffeine concentration in different brands of energy drinks (B1-B10) reveals discrepancies between FSSAI standards, product labels, and actual analysis results. While the FSSAI standard is 300 ppm, product labels vary widely, with brand B-2 listing 800 ppm concentration of caffeine and B-5 and B-9 listing just 230 ppm. The analysis result also show a range of concentrations, with B-4 showing the highest at 372.02 ppm and B-5 the lowest, at 227.28 ppm. Notably, actual average concentration of samples of brand B-2 is much lower than its label claim, while B-4, B-6, and B-8 have higher actual concentrations than stated on their labels. These findings highlight inconsistencies between label claims, regulatory standards, and actual product content. Overall, the average concentrations show that samples of brand B-2, B-3, B-5, B-7, B-9 and B-10 have the concentration of caffeine within acceptable range.

The analysis of artificial sweetener concentrations in various brands reveals several discrepancies when compared to FSSAI standards and product labels. While FSSAI standards have listed saccharin, acesulfame-K, and neotame concentrations to be 100 ppm, 300 ppm, and 33 ppm respectively, the analysis results differ significantly. Average concentration of saccharine in samples of brand B-10 was found to be 8269.84 ppm, which was 82.7 times higher than the FSSAI limit. The concentration of saccharine in brands B-7, B-2, B-6, B-8, B-4, B-3 and B-5 also exceeded the limit by 56.49, 49.32, 18.68, 15.54, 9.56, 3.85, and 3.07 times, respectively. Average acesulfame-K concentrations in samples of brand B-9 and B-7 were notably higher than others at 95.39 ppm and 29.31 ppm respectively, while neotame concentrations in B-2 and B-7, although detected, remained below the FSSAI limit. Acesulfame-K and neotame levels were generally within or below the FSSAI limits across all brands, indicating a more controlled use of these sweeteners. These findings highlight a significant overuse of saccharin in several products, raising concerns about compliance with regulatory standards.

Discussion

Energy drinks contain active ingredients like caffeine, sugar, taurine, B vitamins, and ginseng, designed to boost energy, alertness, and performance.[1] While these ingredients offer temporary benefits, excessive consumption or mixing with other stimulants can lead to adverse health effects. Accurate labeling and regulatory compliance are essential for ensuring consumer safety. The scope of energy drink analysis in food forensics includes detecting and quantifying ingredients like caffeine and artificial sweeteners to ensure compliance with regulatory standards. It also involves identifying adulteration, contamination, and mislabeling, thus protecting consumer health and preventing fraud. This analysis is crucial for verifying product authenticity and maintaining food safety.

Conclusion

This study underlines the critical role of forensic science, especially food forensics through the analysis of caffeine and artificial sweetener concentrations in energy drinks popular in the Indian markets where the results revealed substantial discrepancies between the standard and actual values, while the product label does not declare the presence of these ingredients. Detection of such cases where product authenticity and regulatory compliances are dealt with, are important from forensic perspective for protecting consumers from potential health hazards and food forensics thus plays a pivotal role in maintaining food safety and preventing fraud.

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