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ICP-MS Analysis Of Selected Heavy Metals In Energy Drinks Popular In The Indian Market For Forensic Prospects

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Abstract

Food forensics is a scientific discipline that studies food origin, adulteration, and contamination through the use of scientific methods. In the same context, energy drinks also need to be analyzed from food forensics perspective, as they are consumed with an intent to provide energy to the body, owing to the presence of various macro and micro nutrients in it, like carbohydrates in from of sugars, caffeine, added vitamins, and other minerals, which also add to the popularity of these drinks. However, regular consumers of these drinks stand a chance of being more susceptible to be negatively impacted by the presence of certain metallic components present in these products. These metallic elements, especially heavy metals, can cause chronic and acute toxicity by various modes of action in both children and adults. The prerequisite for food authentication include reliable and precise analytical techniques due to the extreme competition in production of these drinks which leads to a high chance of consumer fraud. In this study, quantitative assessment of 10 heavy metals (Nickel (Ni), Copper (Cu), Zinc (Zn), Arsenic (As), Molybdenum (Mo), Cadmium (Cd), Tin (Sn), Barium (Ba), Mercury (Hg), Lead (Pb)) in energy drink samples of 10 different brands was carried out using microwave assisted acid decomposition and quantitation through ICP-MS instrument which is capable of analyzing several samples rapidly and permitted the identification of multiple metallic elements present in those samples which may be useful for investigations of food frauds in various legal issues. The study results however showed the presence of the selected heavy metals within the prescribed limit by FSSAI.

Introduction

Food forensics is a scientific discipline focused on studying food origin, adulteration, and contamination, using scientific approaches of forensic investigations and food technology.[1] With the growing demand for safe, high-quality food, energy drinks (EDs) are increasingly being examined for more than just their caffeine and sugar content. Recent studies, have shifted attention toward the profiling of heavy metals in EDs, which can pose significant health risks.[2,3] Heavy metals such as arsenic (As), lead (Pb), cadmium (Cd), antimony (Sb), copper (Cu), chromium (Cr), and nickel (Ni) are natural components of the earth's crust and are naturally present in our food but in small concentrations.[4] These metals however, can cause acute and chronic toxicity, upon accumulation in the body through consumption of contaminated food or water, along with other sources.[2] In context

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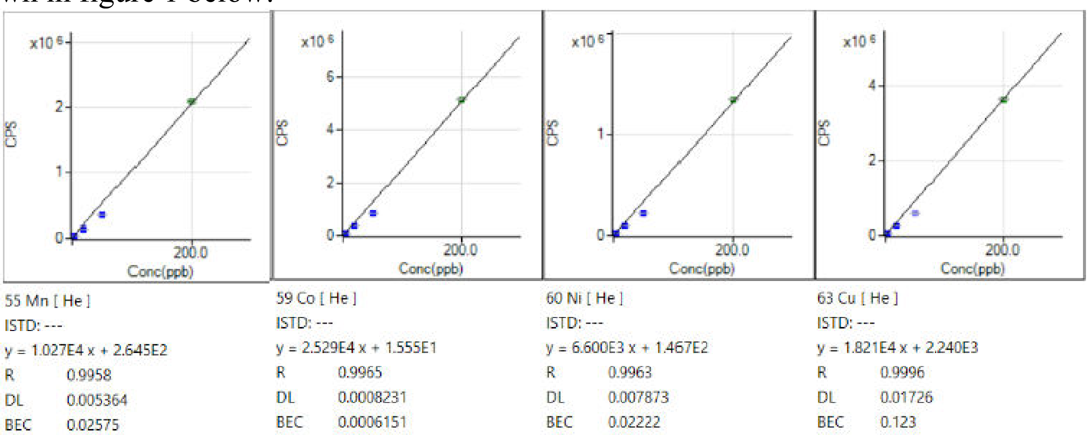
of energy drinks, the contamination of the contents can occur through various means, including the migration of metals from packaging materials[5] or residues from the ingredients.[6,7]

The analysis of heavy metals in EDs is crucial not only for food safety but also for forensic investigations, as these metals are significant environmental pollutants with potential health risks. Various analytical techniques, including atomic absorption spectrometry (AAS), X-ray fluorescence spectrometry, and inductively coupled plasma mass spectrometry (ICP-MS), are used to detect these metals.[1] Among these, ICP-MS is particularly effective due to its high sensitivity and ability to measure multiple elements at ultra-trace levels.[1] Techniques such as solid phase extraction (SPE) are commonly used to enhance sensitivity and reduce matrix interference and ICP-MS has become essential tools in the accurate quantification of trace heavy metals, contributing to the ongoing innovation in food forensics.[8]

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 standard and sample preparation. All reagents and reference materials used in this study were of ICP-MS/Trace metal grade and all the lab wares and instruments used were sterile. The procedure for determination of total acid-extractable concentrations of Nickel (Ni), Copper (Cu), Zinc (Zn), Arsenic (As), Molybdenum (Mo), Cadmium (Cd), Tin (Sn), Barium (Ba), Mercury (Hg) and Lead (Pb) in energy drinks using microwave assisted acid decomposition ICP-MS was followed.[9,10]

Firstly, standard stock solution of 1000 ppm concentration was prepared for As, Sn, Se, Mo, Hg, Sb, Zn, Cu and Cd. These standard solutions were diluted using 2% nitric acid (ultra pure) at different concentrations (0.2 ppm, 0.05 ppm, 0.02 ppm, 0.005 ppm, 0.001 ppm, 0.0005 ppm, 0.0001 ppm- all elements except Hg and 0.02 ppm, 0.005 ppm, 0.002 ppm, 0.0005 ppm, 0.0001 ppm, 0.00005 ppm, 0.00001 ppm - Hg) and were then mixed together to obtain calibration curves for heavy metals and Hg, shown in figure 1 below:



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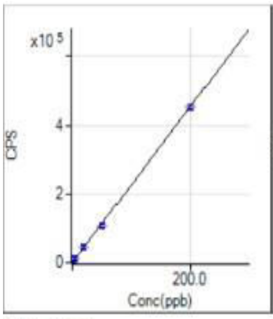
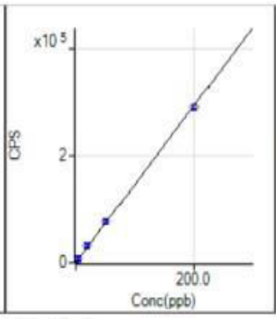
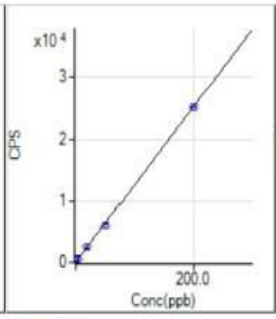


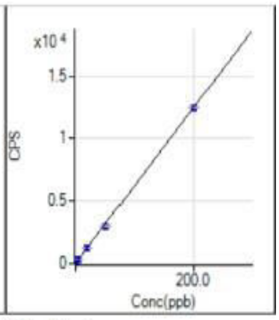
66 Zn [He]
ISTD: ---
y = 2.261E3 x + 4.645E2
R 0.9999
DL 0.05772
BEC 0.2054



75 As [He]
ISTD: ---
y = 1.461E3 x + 3.667E0
R 0.9999
DL 0.004275
BEC 0.00251



78 Se [He]
ISTD: ---
y = 1.259E2 x + 2.667E1
R 0.9999
DL 0.6877
BEC 0.2117



82 Se [He]
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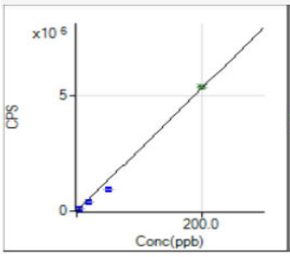
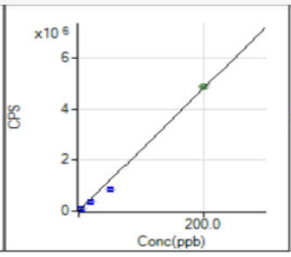
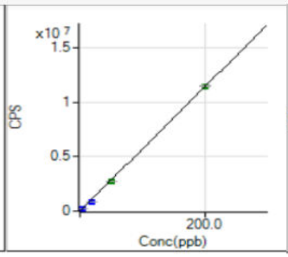
y = 6.193E1 x + 5.333E1
R 0.9999
DL 0.1834
BEC 0.8612



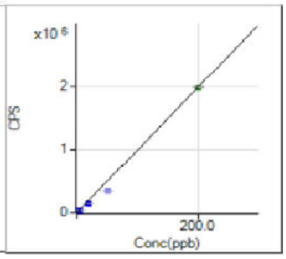
206 [Pb] [He]
ISTD: ---
y = 2.662E4 x + 2.556E2
R 0.9971
DL 0.003706
BEC 0.009599



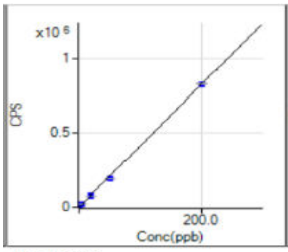
207 [Pb] [He]
ISTD: ---
y = 2.399E4 x + 2.211E2
R 0.9970
DL 0.0008677
BEC 0.009219



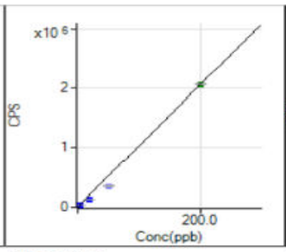
208 Pb [He]
ISTD: ---
y = 5.701E4 x + 5.100E2
R 0.9996
DL 0.00137
BEC 0.008946



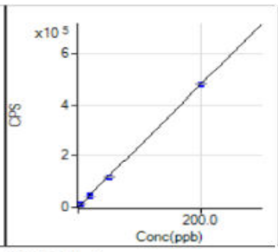
95 Mo [He]
ISTD: ---
y = 9.907E3 x + 4.889E1
R 0.9995
DL 0.005559
BEC 0.004935



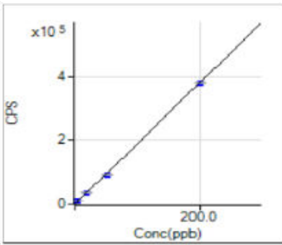
111 Cd [He]
ISTD: ---
y = 4.108E3 x + 1.000E0
R 0.9998
DL 0.0007303
BEC 0.0002434



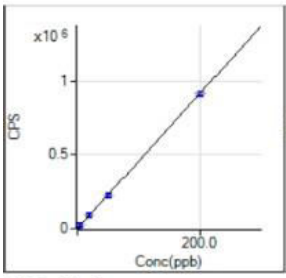
118 Sn [He]
ISTD: ---
y = 1.030E4 x + 3.111E1
R 0.9995
DL 0.001483
BEC 0.00302



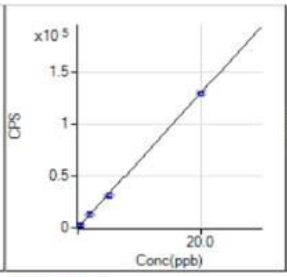
121 Sb [He]
ISTD: ---
y = 2.394E3 x + 6.667E0
R 1.0000
DL 0.01447
BEC 0.002785



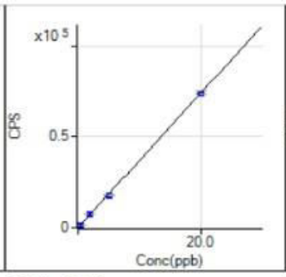
123 Sb [He]
ISTD: ---
y = 1.895E3 x + 2.444E1
R 1.0000
DL 0.0238
BEC 0.0129



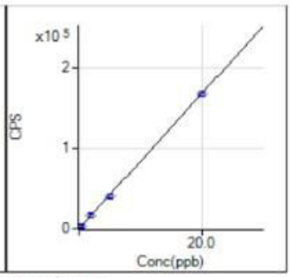
137 Ba [He]
ISTD: ---
y = 4.572E3 x + 1.978E2
R 1.0000
DL 0.01456
BEC 0.04326



200 Hg [He]
ISTD: ---
y = 6.463E3 x + 1.357E2
R 1.0000
DL 0.005134
BEC 0.02099



201 Hg [He]
ISTD: ---
y = 3.697E3 x + 7.733E1
R 1.0000
DL 0.006091
BEC 0.02092



202 Hg [He]
ISTD: ---
y = 8.383E3 x + 1.627E2
R 1.0000
DL 0.004532
BEC 0.01941

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Figure 1: ***Calibration curves for the selected heavy metals***

For sample preparation, after degassing, samples were poured in Teflon® lined digestion vessels, placed in microwave digester (Multiwave GO Plus, Anton Paar) and using multi-step programming of temperature-time program, they were subjected to high temperature (120°C, 150°C) and pressure (300 psi) for digestion of organic contents in the sample to reduce matrix interference during sample analysis by ICP-MS. Samples were then transferred to Tarson tubes and diluted to a known volume using ultrapure Milli-Q water, which were then loaded in tray and subjected to ICP-MS instrument (Agilent ICP-MS (7800 series)) for elemental analysis. ICP-MS apparatus set-up conditions have been enlisted in the table 1 below:

Table 1: ***Set-up conditions of ICP-MS instrument***

Parameter	Condition
RF Power (W)	1550
Carrier gas (l/min)	1.029
Plasma gas flow (l/min)	15
Auxiliary gas flow (l/min)	1.0
Spray chamber	Water cooled double pass
Sample chamber temperature (°C)	2
Lens voltage (V)	8.2
Mass resolution	0.8
Integration time points/ms	3
Points per peak	3
Replicates	3
Carrier gases	
Argon gas (high purity 99.99%) (kPa)	630
Helium gas (ultra high purity- 99.999%) (kPa)	120

The concentration of the analytes of interest was automatically calculated by the software of the ICP-MS instrument. The following steps were performed for each element: The count rates were corrected according to the correction functions chosen. For the count rates measured in the zero members, calibration and test solutions were normalized on the count rates of the internal standard. The calibration function was calculated. By the use of the count rates, the calibration function and the dilution factor the concentrations of the elements were calculated.

The mass fraction (w) of the element (in ppm or mg/kg of sample), was calculated using mass fraction of the element in the test solution, (a, in µg/L), made-up volume of the digestion solution (V, in ml), dilution factor of test solution (F), initial sample mass (m, in gm); in following formula:

$$w = \frac{a \times V \times F}{m \times 1000}$$

For analytical quality control, blank solutions and reference samples of comparable matrix having reliably known contents of the elements to be determined were analyzed in parallel with all the series of samples analyzed. The reference samples were subjected to all the steps in the method, starting from the digestion.

Results and discussion

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The data obtained from ICP-MS analysis of Ni, Cu, Zn, As, Mo, Cd, Sn, Ba, Hg and Pb are presented as an average value below in table 2 for comparison of actual concentration of elements in the samples of brand B-1 to B-10 with that of concentrations recommended by FSSAI (Food Safety and Standards Authority of India) standards.

Table 2: ***Concentration of heavy metals in different brands (B1-B10) of energy drinks***

Element analyzed	Average concentration (in ppb)										
	FSSAI std.	Brands									
		B1	B2	B3	B4	B5	B6	B7	B8	B9	B10
Ni	**20**	0.19	0.45	0.47	0.28	0.34	0.25	0.23	0.38	0.13	0.08
Cu	**50**	1.35	0.98	1.45	1.24	0.72	0.89	0.94	0.05	0.07	0.68
Zn	**5000**	2.52	6.96	4.81	14.95	12.58	4.49	2.92	0.75	0.36	0.74
As	**10**	0.06	0.07	0.10	0.14	0.11	0.05	0.05	0.07	0.11	0.10
Mo	**40**	0.14	0.21	0.48	0.48	0.15	0.40	0.61	0.20	0.03	0.15
Cd	**3**	0.01	0.01	0.03	0.02	0.02	0.02	0.01	0.01	0.03	0.05
Sn	**40**	0.12	0.09	0.31	0.11	0.09	0.08	0.08	0.40	0.45	0.10
Ba	**700**	2.71	2.79	1.47	2.40	2.41	1.79	2.41	1.15	0.16	0.26
Hg	**1**	0.06	0.06	0.07	0.08	0.04	0.04	0.08	0.09	0.10	0.06
Pb	**10**	0.89	0.41	0.48	0.22	0.31	0.21	0.15	0.06	0.10	0.08

The results showed that all analyzed brands had heavy metal concentrations which were well below the FSSAI limits. For instance, Ni levels ranged from 0.08 ppb in B10 to 0.47 ppb in B3, far below the 20 ppb limit. Cu concentrations were between 0.05 ppb in B8 and 1.45 ppb in B3, against a 50 ppb limit. Zn levels varied, with B4 showing the highest concentration at 14.95 ppb, still much lower than the 5000 ppb standard. Concentrations of As were also minimal, with the highest being 0.14 ppb in B4, compared to the 10 ppb limit. Mo levels peaked at 0.61 ppb in B7, well under the 40 ppb threshold. Cd, Sn, Ba, Hg, and Pb also remained significantly lower than their respective limits, with the highest detected values being 0.05 ppb (Cd in B10), 0.45 ppb (Sn in B9), 2.79 ppb (Ba in B2), 0.10 ppb (Hg in B9), and 0.89 ppb (Pb in B1). While most elements were present at low concentrations across all brands, Zn shows the most variability. Although there were slight correlations between Cd and Pb levels in some brands but there doesn't appear to be a strong or consistent relationship between the elements across the board. Overall, the analysis confirms that the heavy metal content in these energy drinks is within safe consumption limits as per FSSAI standards.

Discussion

In the present study, the results show that the concentrations of heavy metals were well within the recommended limits by FSSAI. Similar results were found in the studies on energy drinks in Lagos Nigeria[2], Poland[7] and Brazil[11] where the concentrations of different heavy metals analyzed in the studies were well within the limits mentioned in the norms followed in the respective countries of the researchers. The comparatively higher concentration of Zn in the samples and consistent concentration trend in Cd and Pb was also seen by researchers from Lagos Nigeria[2].

Conclusion

This study underlines the critical role of forensic science, especially food forensics through the qualitative and quantitative analysis of various heavy metals in energy drinks popular in the Indian markets where the results showed that the actual concentrations of these elements all the brands complied with the standard values. The trace metals in canned drinks must be monitored expansively and intermittently to ensure product authenticity and safeguard consumers' health. Detection of such

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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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