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QUALITATIVE AND QUANTITATIVE PROFILES OF MAJOR MACRONUTRIENT ELEMENTS PRESENT IN ENERGY DRINKS POPULAR IN INDIA USING ICP-MS

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Abstract
Energy drinks market is increasingly popular in India, where they have been marketed with claims to provide instant boost of energy. They have been equally popular subject of study by food scientists as well where major contents like caffeine, sugars, etc. present in them have been extensively studied by the researchers but less focus has been paid on their elemental composition. The elemental profiling of these drinks becomes a subject of interest also because the consumers consistently consuming these drinks are more susceptible to be negatively impacted by the presence of certain elemental components present in these products, especially because of their high concentrations. Present study investigates the qualitative and quantitative profiles of macro nutrient elements present in energy drinks from ten popular brands in India. Macronutrient elements are required in large amounts for normal and vital functioning of physiological processes in body. In this study, quantitative assessment of 5 such macronutrient elements (Sodium (Na), Magnesium (Mg), Aluminum (Al), Potassium (K), Calcium (Ca)) 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 findings revealed several brands exceeded the permissible limits set by the Food Safety and Standards Authority of India (FSSAI) for key elements, raising significant health concerns.

Key words: *Macronutirent, ICP-MS, energy drinks, elemental profiling, FSSAI*

1. Introduction

Energy drinks are primarily composed of carbohydrates, vitamins, and a variety of minerals, serving as a source of bodily energy[1]. These drinks have rapidly gained global popularity as functional beverages designed to enhance physical and cognitive performance through the inclusion of stimulants, vitamins, and other bioactive compounds. Initially targeting athletes, these drinks have expanded their market to encompass adolescents, young adults, and even the elderly, driven by aggressive marketing and changing consumer lifestyles. While they are associated with increased energy and endurance, their diverse formulations and high stimulant content raise concerns about potential health risks.

Most studies on energy drinks focus on caffeine content, limited research has examined their metal composition. Metallic elements, although essential for enzyme metabolism and maintaining physical health, can become detrimental when consumed in excess, especially for individuals who habitually consume these beverages[2]. The analysis of various elements in energy drinks serves multiple purposes, primarily focusing on their nutritional significance and potential toxicity. Aluminum (Al) is monitored due to its toxicity, even in trace amounts. Essential minerals such as calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na) are assessed for their nutritional contributions, with their concentrations varying from major to minor or trace levels[3]. Calcium plays a crucial role in bone health, while magnesium is vital for enzymatic functions. Potassium and sodium are key electrolytes involved in maintaining fluid balance and nerve function[3]. Understanding the presence and concentration of these elements is essential for evaluating the health implications of energy drink consumption. The analysis of metal content in food samples is highly significant as it offers valuable insights into their nutritional composition, facilitates the development of food composition databases, and ensures the monitoring of antinutritional elements or potentially toxic substances[4]. The packaging material used for energy drinks, including polyethylene terephthalate (PET) and glass bottles, as well as aluminum and steel cans, plays a significant role in influencing the final product's characteristics[5]. Metal leaching during manufacturing can introduce impurities[6], and the levels of metals in energy drinks are influenced by raw materials, production processes, and additives[5].

Analytical techniques such as flame atomic absorption spectrometry (FAAS)[7,8], electrothermal atomic absorption spectrometry[9], microwave plasma atomic emission spectrometry[10], inductively coupled plasma optical emission spectrometry (ICP-OES)[11,12] and inductively coupled plasma mass spectrometry (ICP-MS)[13] have been employed to quantify metallic elements in food and beverages. In this study, the concentrations of aluminum (Al), calcium (Ca), magnesium (Mg), sodium (Na), and potassium (K) were determined in 100 energy drink samples from 10 popular Indian brands, packaged in aluminum cans and PET bottles, using ICP-MS.

2. Methodology

2.1. *Instrumentation*

An ultrasonic bath was used for the degassing of soft drink samples. 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.

Inductively coupled plasma mass spectrometer (ICP MS) (Agilent ICP-MS (7800 series)) was used to quantify macronutrient elements (Na, Mg, Al, K and Ca) in energy drinks samples. The determination of macro and micronutrients were carried out under the conditions shown in 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.
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.

2.2. *Reagents and solutions*

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 aluminum (Al), calcium (Ca), magnesium (Mg), sodium (Na), and potassium (K) in energy drinks using microwave assisted acid decomposition ICP-MS was followed[14,15].
Firstly, standard stock solution of 1000 ppm concentration was prepared for Al, Ca, Mg, Na, K and Hg. 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).

2.3. *Sample collection*

Energy drinks (100 samples; 10 samples each from 10 different brands) contained in PET and glass bottles were procured from various online and offline stores in Prayagraj, Uttar Pradesh at different time period and stored at 4°C in sterile conditions until digestion. The samples were tested at Instrumental Laboratory, Rohilkhand Laboratory and Research Center, Bareilly, Uttar Pradesh in 2022.

3. Results and discussion

Tables 2-11 show the concentrations of the five elements determined by ICP MS in energy drink samples from brands B1-B10. These elements were present in high concentrations (Na, Mg, K, Ca and Al). These concentration levels were expected, because the first four elements are considered element macronutrients, or are present in high concentrations in food and are needed in extremely large amounts for the metabolism[16], whereas, Al turns out to be undesirable micronutrient element which was also present in exceptionally high amounts in energy drinks stored in metal cans. The concentrations of all the analytes of choice for this study were present in high concentrations, as opposed to the permissible levels set by Food Safety and Standards Authority of India (FSSAI).

Table 2: ***Concentration of macronutrient elements in samples from brand B1***

Permissible value as per FSSAI		**200 ppm**	**50 ppm**	**30 ppb**	**--**	**20-75 ppm**
Mfg. Month	**Sample**	**Na [He] ppm**	**Mg [He] ppm**	**Al [He] ppb**	**K [He] ppm**	**Ca [He] ppm**
Feb, 2022	**E1**	481.88	92.38	172.03	360.43	293.70
Feb, 2022	**E2**	231.34	87.01	160.66	224.31	104.61
Apr, 2022	**E21**	369.19	80.62	157.69	250.18	122.16
Apr, 2022	**E22**	147.69	65.01	148.67	141.07	78.25
Jun, 2022	**E41**	256.49	62.04	75.65	128.03	80.74
Jun, 2022	**E42**	78.09	30.03	71.82	96.10	61.58
Aug, 2022	**E61**	168.04	57.05	69.18	89.07	51.95
Aug, 2022	**E62**	73.48	23.03	53.03	80.03	50.32
Oct, 2022	**E81**	57.97	20.20	23.01	40.03	40.56
Oct, 2022	**E82**	58.68	14.90	22.41	41.19	39.22

Table 3: ***Concentration of macronutrient elements in samples from brand B2***

Permissible value as per FSSAI		**200 ppm**	**50 ppm**	**30 ppb**	**--**	**20-75 ppm**
Mfg. Month	**Sample**	**Na [He] ppm**	**Mg [He] ppm**	**Al [He] ppb**	**K [He] ppm**	**Ca [He] ppm**
Feb, 2022	**E3**	498.99	136.78	155.60	490.10	308.64
Feb, 2022	**E4**	303.30	92.46	153.86	358.03	170.09
Apr, 2022	**E23**	330.07	114.98	93.79	381.09	270.47
Apr, 2022	**E24**	154.67	85.98	92.71	290.08	129.35
Jun, 2022	**E43**	249.84	106.06	88.90	210.36	202.06
Jun, 2022	**E44**	133.15	49.36	81.61	150.05	84.34
Aug, 2022	**E63**	193.08	85.70	42.71	190.81	181.43
Aug, 2022	**E64**	98.12	25.84	39.36	96.26	80.95
Oct, 2022	**E83**	33.32	15.25	13.58	104.01	47.47
Oct, 2022	**E84**	38.00	13.60	11.29	91.07	43.00

Table 4: ***Concentration of macronutrient elements in samples from brand B3***

Permissible value as per FSSAI		**200 ppm**	**50 ppm**	**30 ppb**	**--**	**20-75 ppm**
Mfg. Month	**Sample**	**Na [He] ppm**	**Mg [He] ppm**	**Al [He] ppb**	**K [He] ppm**	**Ca [He] ppm**
Feb, 2022	**E5**	547.52	200.05	205.66	398.64	289.17
Feb, 2022	**E6**	414.91	118.60	156.68	351.08	240.17
Apr, 2022	**E25**	482.01	160.04	157.49	311.58	231.02
Apr, 2022	**E26**	371.51	89.90	120.01	290.65	176.41
Jun, 2022	**E45**	340.18	95.89	85.07	250.39	183.67
Jun, 2022	**E46**	268.35	51.83	69.29	211.50	120.84
Aug, 2022	**E65**	232.47	63.26	49.30	185.26	105.94

Aug, 2022	**E66**	187.51	41.08	41.60	141.37	83.02
Oct, 2022	**E85**	141.22	30.07	29.96	78.44	61.17
Oct, 2022	**E86**	136.08	28.06	27.44	76.38	58.19

Table 5: ***Concentration of macronutrient elements in samples from brand B4***

Permissible value as per FSSAI		**200 ppm**	**50 ppm**	**30 ppb**	**--**	**20-75 ppm**
Mfg. Month	**Sample**	**Na [He] ppm**	**Mg [He] ppm**	**Al [He] ppb**	**K [He] ppm**	**Ca [He] ppm**
Feb, 2022	**E7**	477.20	257.82	556.06	514.76	679.16
Feb, 2022	**E8**	391.07	218.20	505.10	493.06	512.16
Apr, 2022	**E27**	380.85	190.16	549.86	460.15	481.19
Apr, 2022	**E28**	304.46	154.64	500.90	341.01	457.05
Jun, 2022	**E47**	310.84	114.01	472.89	375.04	348.01
Jun, 2022	**E48**	256.00	91.01	454.62	260.40	309.16
Aug, 2022	**E67**	190.49	60.16	288.00	198.17	271.15
Aug, 2022	**E68**	156.42	40.81	210.03	157.01	225.02
Oct, 2022	**E87**	113.61	43.26	91.05	78.09	90.08
Oct, 2022	**E88**	80.32	39.53	58.90	69.37	85.41

Table 6: ***Concentration of macronutrient elements in samples from brand B5***

Permissible value as per FSSAI		**200 ppm**	**50 ppm**	**30 ppb**	**--**	**20-75 ppm**
Mfg. Month	**Sample**	**Na [He] ppm**	**Mg [He] ppm**	**Al [He] ppb**	**K [He] ppm**	**Ca [He] ppm**
Feb, 2022	**E9**	408.52	186.20	130.63	539.43	335.73
Feb, 2022	**E10**	356.01	157.08	97.31	470.02	220.46
Apr, 2022	**E29**	362.59	134.00	104.75	425.04	299.06
Apr, 2022	**E30**	301.00	104.62	87.09	301.76	140.21
Jun, 2022	**E49**	323.74	86.61	62.42	259.25	182.13
Jun, 2022	**E50**	272.16	64.22	56.09	223.85	100.01
Aug, 2022	**E69**	134.01	43.27	46.11	164.22	108.06
Aug, 2022	**E70**	103.24	32.25	40.47	114.07	67.44
Oct, 2022	**E89**	95.41	20.04	20.39	90.06	51.12
Oct, 2022	**E90**	91.48	16.81	20.29	87.28	48.93

Table 7: ***Concentration of macronutrient elements in samples from brand B6***

Permissible value as per FSSAI		**200 ppm**	**50 ppm**	**30 ppb**	**--**	**20-75 ppm**
Mfg. Month	**Sample**	**Na [He] ppm**	**Mg [He] ppm**	**Al [He] ppb**	**K [He] ppm**	**Ca [He] ppm**
Feb, 2022	**E11**	409.058	119.204	9.877	419.927	247.119
Feb, 2022	**E12**	370.332	83.467	9.254	383.149	112.049
Apr, 2022	**E31**	320.164	86.156	11.951	342.561	189.666
Apr, 2022	**E32**	281.836	65.155	11.473	204.538	98.798
Jun, 2022	**E51**	212.096	49.076	8.683	254.532	103.308
Jun, 2022	**E52**	161.819	41.023	8.287	138.486	80.429
Aug, 2022	**E71**	154.359	42.069	7.891	194.459	79.457
Aug, 2022	**E72**	110.333	36.067	7.852	116.443	64.175
Oct, 2022	**E91**	62.233	23.065	8.394	87.121	61.362
Oct, 2022	**E92**	62.069	20.063	6.426	85.120	57.214

Table 8: ***Concentration of macronutrient elements in samples from brand B7***

Permissible value as per FSSAI		**200 ppm**	**50 ppm**	**30 ppb**	**--**	**20-75 ppm**
Mfg. Month	**Sample**	**Na [He] ppm**	**Mg [He] ppm**	**Al [He] ppb**	**K [He] ppm**	**Ca [He] ppm**
Feb, 2022	**E13**	357.19	146.04	29.94	433.00	407.34
Feb, 2022	**E14**	217.47	83.52	21.32	266.21	188.66
Apr, 2022	**E33**	221.88	90.15	6.25	284.12	258.33
Apr, 2022	**E34**	185.09	51.04	8.41	197.48	139.97
Jun, 2022	**E53**	129.00	58.40	11.09	151.06	114.40
Jun, 2022	**E54**	90.80	42.02	8.36	103.25	94.36
Aug, 2022	**E73**	67.36	43.44	10.23	84.13	71.33
Aug, 2022	**E74**	49.48	35.00	11.20	52.37	60.14
Oct, 2022	**E93**	23.30	20.43	19.72	30.20	31.34
Oct, 2022	**E94**	22.03	19.74	19.31	29.36	31.05

Table 9: ***Concentration of macronutrient elements in samples from brand B8***

Permissible value as per FSSAI		**200 ppm**	**50 ppm**	**30 ppb**	**--**	**20-75 ppm**
Mfg. Month	**Sample**	**Na [He] ppm**	**Mg [He] ppm**	**Al [He] ppb**	**K [He] ppm**	**Ca [He] ppm**
Feb, 2022	**E15**	548.99	175.11	95.51	488.09	318.64
Feb, 2022	**E16**	333.00	117.41	73.06	338.02	188.01
Apr, 2022	**E35**	431.01	134.98	83.73	374.08	276.42
Apr, 2022	**E36**	254.13	103.24	62.71	297.15	132.05
Jun, 2022	**E55**	349.12	108.08	68.08	198.38	212.06
Jun, 2022	**E56**	147.30	89.36	41.65	140.21	104.24
Aug, 2022	**E75**	273.02	85.74	52.74	171.52	154.00
Aug, 2022	**E76**	138.11	45.84	34.36	106.23	75.40
Oct, 2022	**E95**	114.02	28.09	23.52	124.02	78.47
Oct, 2022	**E96**	89.32	25.25	21.01	96.36	71.05

Table 10: ***Concentration of macronutrient elements in samples from brand B9***

Permissible value as per FSSAI		**200 ppm**	**50 ppm**	**30 ppb**	**--**	**20-75 ppm**
Mfg. Month	**Sample**	**Na [He] ppm**	**Mg [He] ppm**	**Al [He] ppb**	**K [He] ppm**	**Ca [He] ppm**
Feb, 2022	**E17**	489.36	109.81	103.70	368.00	127.03
Feb, 2022	**E18**	311.01	90.34	82.65	310.00	87.36
Apr, 2022	**E37**	399.01	114.00	98.81	300.83	102.66
Apr, 2022	**E38**	287.62	74.62	73.11	216.67	81.21
Jun, 2022	**E57**	371.06	106.61	92.71	315.07	91.92
Jun, 2022	**E58**	200.01	52.09	59.42	204.85	76.43
Aug, 2022	**E77**	259.32	83.27	63.33	269.35	85.35
Aug, 2022	**E78**	177.60	51.81	21.29	188.63	68.63
Oct, 2022	**E97**	186.20	60.04	9.69	176.26	34.00
Oct, 2022	**E98**	178.03	48.62	10.80	152.85	31.60

Table 11: ***Concentration of macronutrient elements in samples from brand B10***

Permissible value as per FSSAI		200 ppm	50 ppm	30 ppb	--	20-75 ppm
Mfg. Month	**Sample**	**Na [He] ppm**	**Mg [He] ppm**	**Al [He] ppb**	**K [He] ppm**	**Ca [He] ppm**
Feb, 2022	**E19**	493.01	119.20	98.52	511.46	355.46
Feb, 2022	**E20**	351.09	99.51	87.35	357.49	304.23
Apr, 2022	**E39**	332.60	90.07	101.07	483.54	271.35
Apr, 2022	**E40**	302.06	81.03	61.43	280.12	260.36
Jun, 2022	**E59**	292.01	113.47	72.00	397.93	247.12
Jun, 2022	**E60**	256.03	64.01	40.54	136.15	192.05
Aug, 2022	**E79**	231.84	73.15	32.19	91.53	186.31
Aug, 2022	**E80**	187.02	52.07	33.58	78.56	130.67
Oct, 2022	**E99**	190.36	67.17	19.20	63.44	79.18
Oct, 2022	**E100**	122.90	48.90	18.92	59.02	74.03

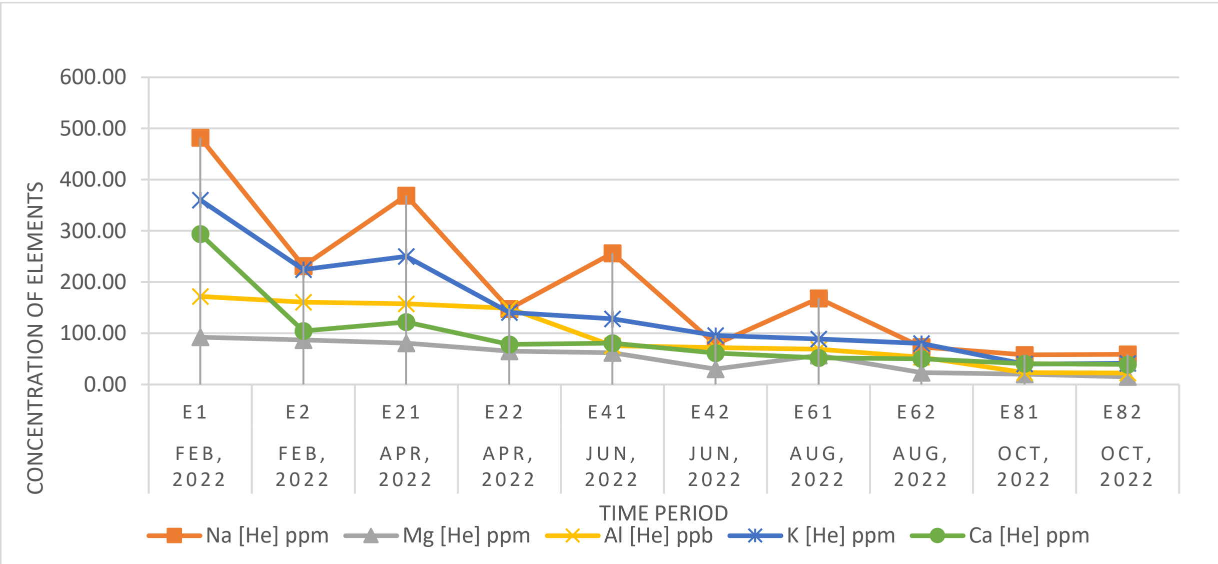

Figure 1: ***Trend in concentration of macronutrient elements in samples from brand B1***

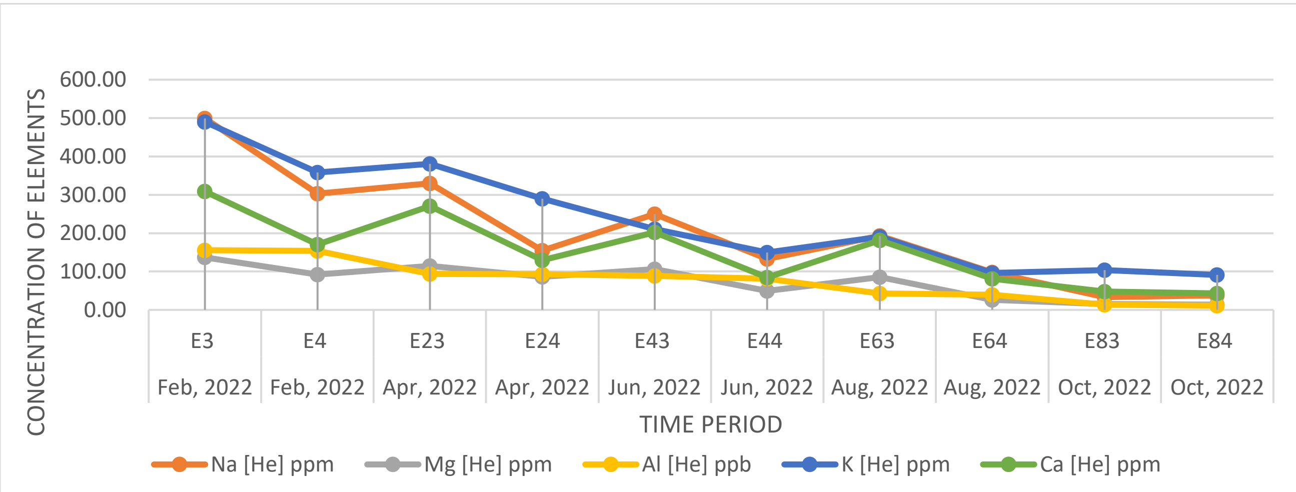

Figure 2: ***Trend in concentration of macronutrient elements in samples from brand B2***

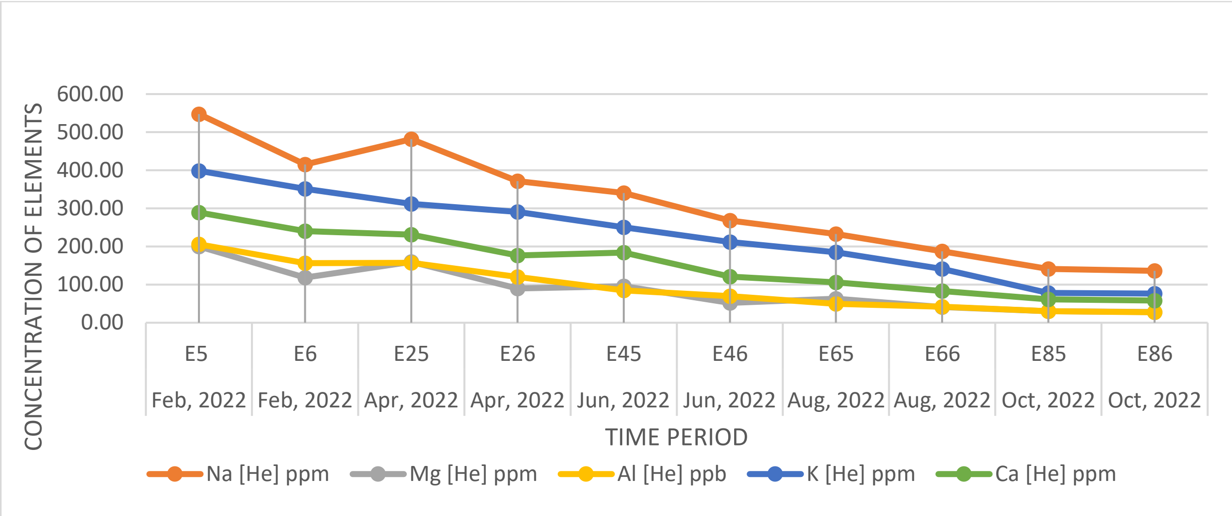

Figure 3: ***Trend in concentration of macronutrient elements in samples from brand B3***

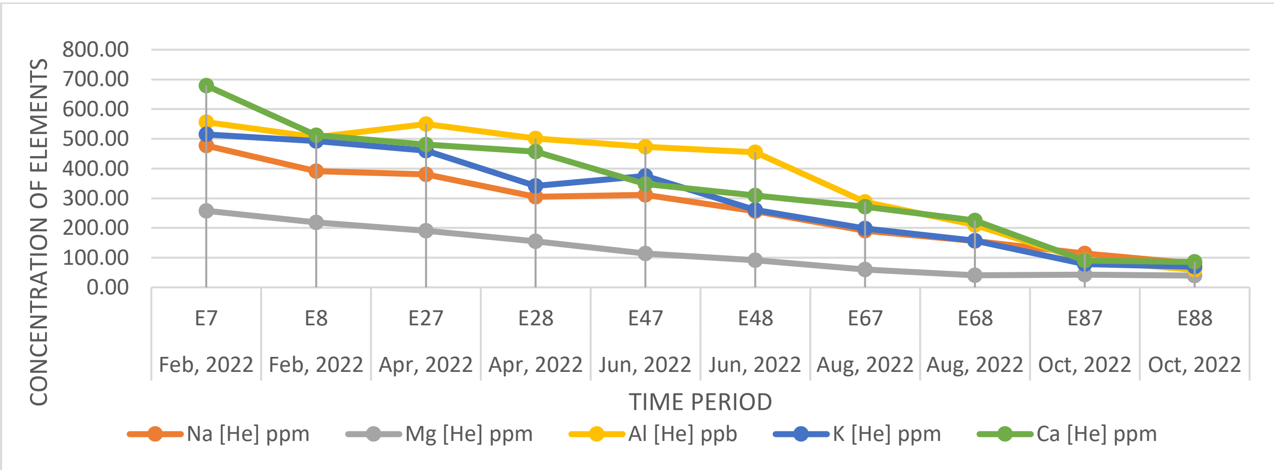

Figure 4: ***Trend in concentration of macronutrient elements in samples from brand B4***

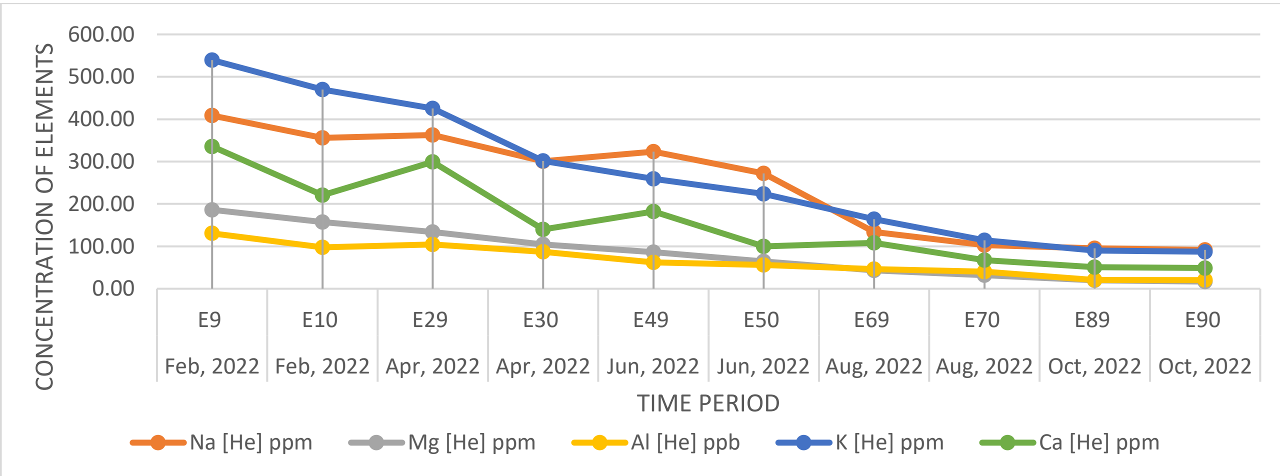

Figure 5: ***Trend in concentration of macronutrient elements in samples from brand B5***

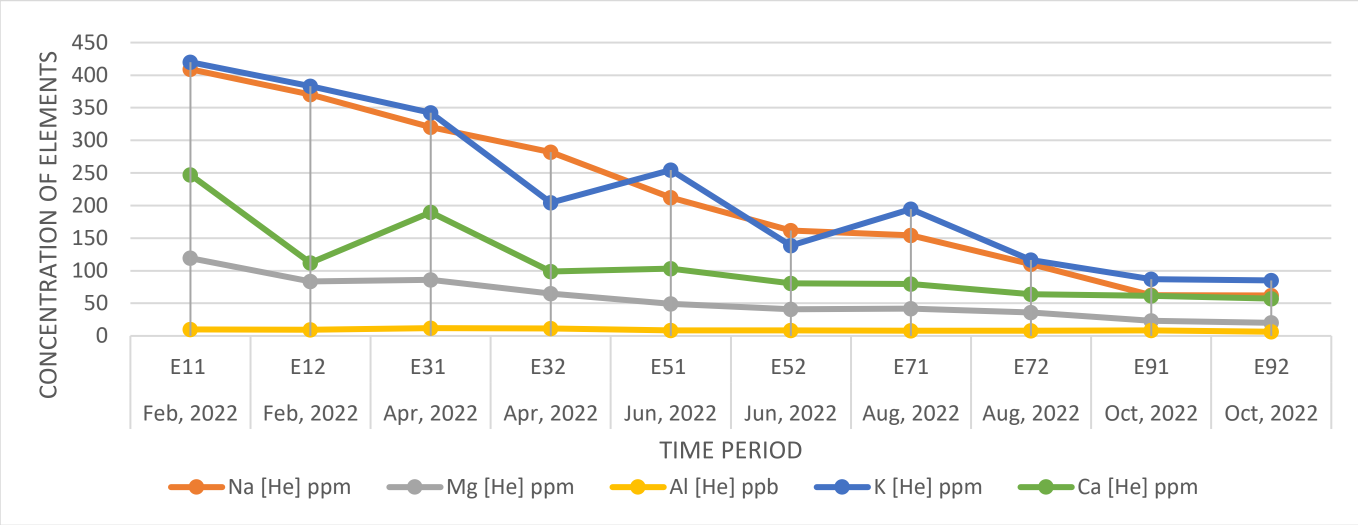

Figure 6: ***Trend in concentration of macronutrient elements in samples from brand B6***

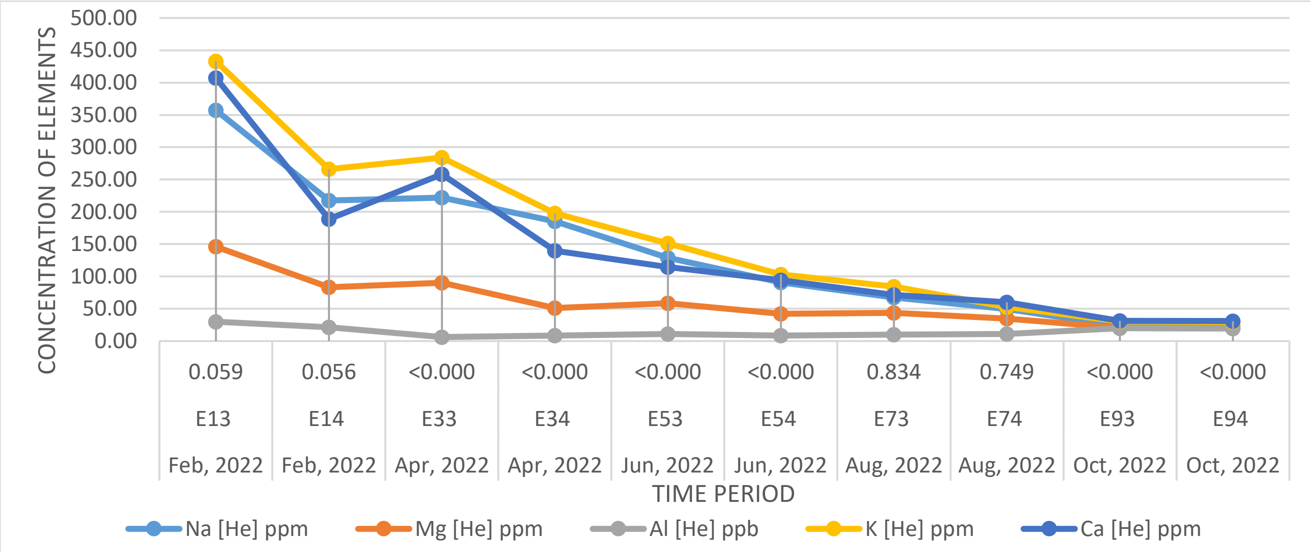

Figure 7: ***Trend in concentration of macronutrient elements in samples from brand B7***

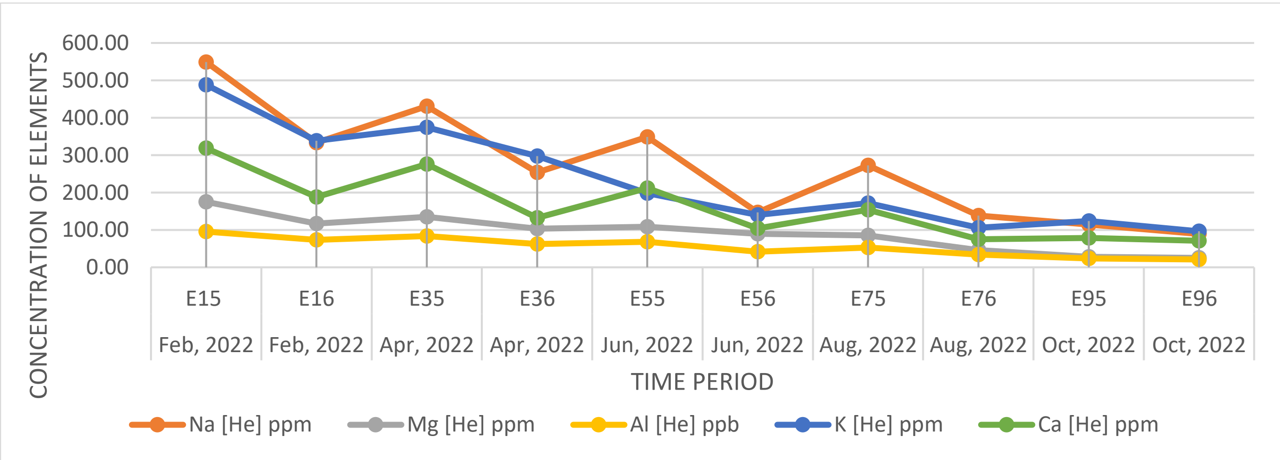

Figure 8: ***Trend in concentration of macronutrient elements in samples from brand B8***

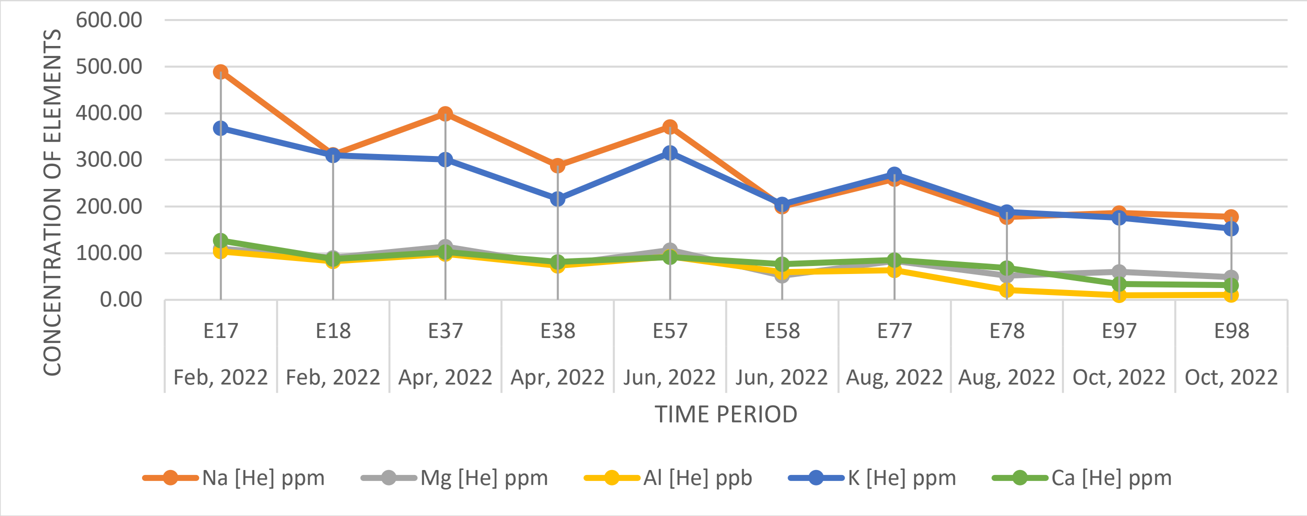

Figure 9: ***Trend in concentration of macronutrient elements in samples from brand B9***

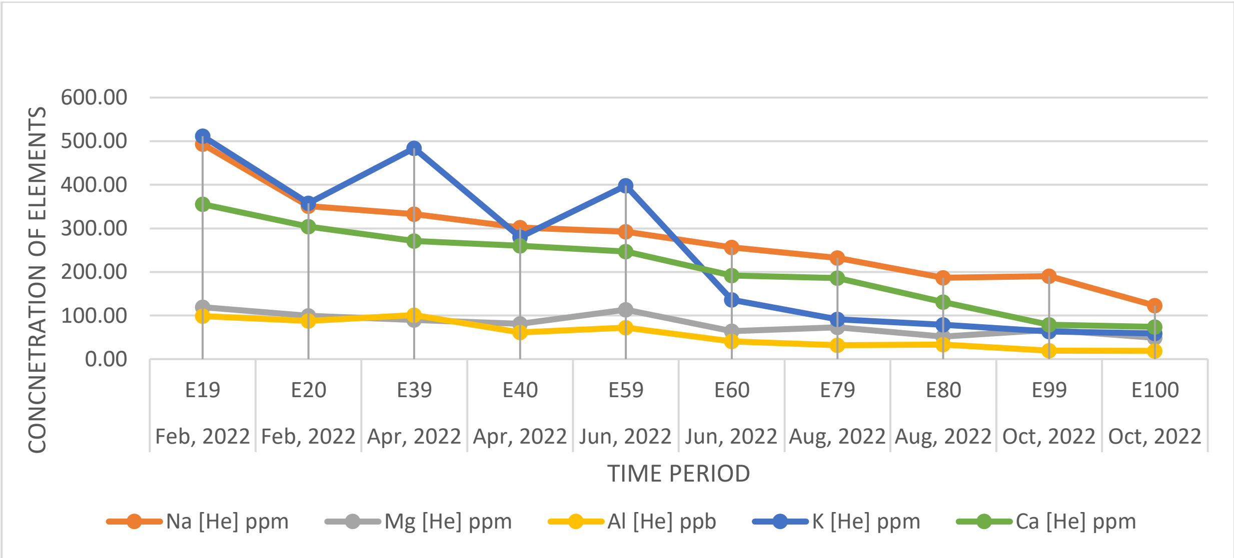

Figure 10: ***Trend in concentration of macronutrient elements in samples from brand B10***

The analysis of macronutrient concentrations in energy drink samples from ten different brands revealed significant variations in sodium (Na), magnesium (Mg), aluminum (Al), potassium (K), and calcium (Ca) levels. Sodium concentrations across brands ranged from a maximum of 548.99 ppm (E15, B8) to a minimum of 22.03 ppm (E94, B7). Brand B8 exhibited the highest sodium concentration, whereas brand B7 showed the lowest. All brands had at least one sample exceeding the permissible FSSAI limit of 200 ppm, indicating non-compliance.

Magnesium levels varied between 257.82 ppm (E7, B4) and 13.60 ppm (E84, B2). The highest concentrations were observed in brand B4, while the lowest was found in brand B2. Many samples from multiple brands exceeded the FSSAI limit of 50 ppm, highlighting non-compliance.

Aluminum content spanned from 556.06 ppb (E7, B4) to 6.25 ppb (E33, B7). Brand B4 recorded the highest aluminum concentration, far exceeding the permissible limit of 30 ppb, whereas B7 had the lowest. Most samples in brands B4 and B5 significantly exceeded the limit, indicating a potential health risk. Another interesting trend which was seen was that the samples which were packaged in PET bottles (brands B6 and B7) had Al concentrations well within the permissible limits set by FSSAI whereas samples from other brands that were packaged in aluminum cans showed significantly high concentrations of Al in their samples.

Potassium levels ranged from 539.43 ppm (E9, B5) to 29.36 ppm (E94, B7). The highest concentration was found in brand B5, while B7 had the lowest levels. Since no FSSAI standard is specified for potassium, compliance was assessed only against the product label.

Calcium concentrations were highest at 679.16 ppm (E7, B4) and lowest at 31.05 ppm (E94, B7). Brand B4 showed excessive calcium levels, surpassing the permissible range of 20-75 ppm in most of its samples, while brand B7 had the lowest concentration. Several other brands, including B1, B2, and B3, also exhibited samples exceeding the upper FSSAI limit, indicating non-compliance.

Previous research[17,18] has also provided valuable context for this study which highlight the influence of packaging materials on the elemental content of energy drinks, specifically aluminum cans. These studies draw attention to the potential leaching of elements such as aluminum into the beverage, which is critical when interpreting the findings of this study. Similarly, another research in Poland[19] observed elevated levels of calcium and magnesium in energy drinks, which parallels some of the findings in this research.

Overall, sodium, magnesium, aluminum, and calcium levels frequently surpassed the permissible limits set by FSSAI, indicating inconsistencies in formulation across brands. These deviations from regulatory standards highlight potential health concerns and emphasize the need for strict quality control measures in the energy drink industry.

4. Conclusion

The present study on energy drinks 10 popular brands in India has revealed significant variations across different brands. The study found that Na, Mg, Al, K and Ca levels frequently exceeded FSSAI regulatory limits, raising concerns about compliance and potential health risks. Na, Mg, Ca and K concentrations in several brands surpassed the permissible limit by FSSAI suggesting inconsistencies in formulation and non-compliance with FSSAI standards. Al contamination was notably higher in brands using aluminum cans for packaging, reinforcing concerns regarding elemental leaching from container materials.

These findings emphasize the need for stringent regulatory lapse and quality control measures in the energy drink industry to ensure consumer safety. The observed variations, coupled with similarities to previous studies across the world, highlight the broader implications of packaging materials and ingredient inconsistencies. Future research should focus on further investigating the impact of packaging on elemental contamination, as well as potential health effects associated with excessive consumption of these macronutrients. By addressing these concerns, regulatory authorities and manufacturers can work toward ensuring safer and more standardized formulations for energy drinks in the Indian market.

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