Simultaneous Determination of Caffeine, Preservatives and Antioxidants in Energy- and Soft-Drinks Commercially Available in Bangladesh

Md. Samiul Islam, Md. Shahadat Hossain, Subrata Bhadra and Abu Shara Shamsur Rouf

Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh

Received: March 30, 2016; Accepted: June 14, 2016; Published (web): June 20, 2016

ABSTRACT: This study describes a validated assay method to determine caffeine, benzoic acid, methylparaben, propylparaben, butylparaben, butylatedhydroxyanisole, and butylatedhydroxytoluene simultaneously by reversed phase high performance liquid chromatographic (HPLC) method. The separation of compounds was achieved on a C_{18} column in a gradient of acetonitrile and diluted sulfuric acid (pH=2.3) and quantification was performed by using a UV-detector set at 265 nm. The method was found to be linear over the concentration range of 60-90 ppm (R^2=0.995), 200-300 ppm (R^2=0.993), 16-24 ppm (R^2=0.990), 16-24 ppm (R^2=0.994), 16-24 ppm (R^2=0.997), 48-72 ppm (R^2=0.993), and 32-48 ppm (R^2=0.994) for caffeine, benzoic acid, methylparaben, propylparaben, butylparaben, butylatedhydroxyanisole, and butylatedhydroxytoluene, respectively. Sixty energy- and soft-drink samples were analyzed where about 53% were found to contain caffeine among which 16% exceeded the tolerance limit (>200 ppm) set by USFDA. Moreover, 65% of analyzed samples contained benzoic acid and 10% of those samples exceeded the maximum allowable limit (>600 ppm) set by joint expert committee for food additives (JECFA).

Key words: HPLC, Caffeine, Benzoic acid, Parabens, Energy drinks, Soft drinks, USFDA, JECFA.

INTRODUCTION

At present concern about the importance of food and beverage quality has been growing, specially due to the increase in the incidence of diseases that are directly or indirectly related to nutritional habits. Therefore, analyses of food additives came into focus for the assessment of their harmful potentials and quantitative or qualitative value of risks related to their uses.[1] Beverages, specially, energy-drinks and soft-drinks are becoming a part of the daily life among the people of all ages in Bangladesh with the trend of industrialization of the society and are very popular to quench thirst mostly during the hot summer. Moreover, successful reiterative commercials through TV or other media by the distributors, they are becoming one of the fast-moving consumer goods (FMCG) in our country. Now-a-days, these sorts of drinks are devoured all year round just for light refreshment or even as a dessert after a heavy meal. According to Encyclopedia Britannica, soft drinks are non-alcoholic beverages, usually but not necessarily carbonated, with some sweeteners, edible acids and flavoring agents; whereas energy drinks are also beverages containing stimulants, chiefly caffeine, taurine, guarana and ginseng which are marketed as energy provider to improve physical activities and mental alertness. To manufacture these beverages, different types of food additives, especially

Correspondence to: Abu Shara Shamsur Rouf,
Email: rouf321@yahoo.com

preservatives and antioxidants are commonly used. They play a vital role for maintaining food quality and its characteristics as well as promoting food safety.[2-5] However, the inappropriate or excessive use of these food additives could lead to adverse effects on health such as metabolic acidosis, convulsions and hyperpnoea, particularly in children and pregnant women.[1] Therefore, these types of additives and their contents used in each food item always play the most important roles for food products investigations all over the world.

Caffeine (CAF), chemically 1,3,7-trimethyl-xanthine, is widely used in foods and beverages as stimulant.[6,7] It is a white crystalline powder with a chemical formula of $C_8H_{10}N_4O_2$. It is bitter in taste and occurs in many plants such as coffee beans, tea leaves and cocoa nuts.[2,8] Due to the presence of CAF, manufacturers always proclaim that energy drinks can relieve fatigue, restore energy and promote alertness. Actually, it is an addictive stimulant that stimulates the central nervous system (CNS) and acts as a mild diuretic.[2,6] Numerous investigational reports have indicated that intake of CAF in an overdose (>200 ppm) is associated with many clinical diseases such as coronary heart disease, myocardial infarction, cancers (urinary tract, kidney, and pancreas), anxiety, and fibrocystic breast disease.[2,8-10] Based on the regulation of USFDA, the concentration of CAF in soft drink can not exceed 200 ppm and the label of "containing caffeine" is required if the drink is not caffeine free.[2]

On the other hand, food preservatives are also widely used to preserve the characteristics like appearance, odor, and taste of food and to preserve it for a long period of time.[11] Benzoic acid (E210), sodium benzoate (E211), potassium benzoate (E212) or calcium benzoate (E213) belong to the category of conditionally permitted preservatives which can protect food against deterioration caused by microorganisms. The toxicity of benzoic acid (BA), as well as of its derivatives, is low, but in high recurrent doses (>600 ppm), it produces irritations of the digestive mucous membrane and depresses some digestive enzymes like pepsin, trypsin, polypeptides and D-amino acid oxidase.[12] Parabens, which are chemically p-hydroxybenzoic esters, have also been widely used as preservatives at a concentration of not more than 500 ppm in water-based drinks.[13] Their antimicrobial activities increase with increasing chain length of alkyl moiety, although the esters of longer alkyl chains are of limited applications due to their lower solubility in water.[14] For that reason, combinations of methylparaben (MP), propylparaben (PP) and butylparaben (BP) are often used together. But concern has been expressed over the use of paraben mixture for their estrogenic nature, because of their binding capacity to estrogen receptors that can regulate estrogen responsive gene products.[15] On the other hand; some literatures revealed that propylparaben alone can adversely affects the hormonal secretion and the male reproductive functions. However, propylparaben and butylparaben can also decrease sperm function and alter metabolic hormones.[16]

Butylatedhydroxyanisole (BHA, E320) is another commonly used additive consisting of a mixture of two isomeric organic compounds, 2-tert-butyl-4-hydroxyanisole and 3-tert-butyl-4-hydroxyanisole. The primary role of BHA in food science is antioxidant in the concentration of not more than 100 ppm in both food beverages and animal feeds.[14] It is frequently used in combination with other antioxidants, particularly butylatedhydroxytoluene (BHT, E321), which is a derivative of phenol. The European and U.S. regulations allow up to 100 ppm to be used as a food additive.[17] Literatures survey showed that both BHA and BHT can cause different symptoms like headache, flushing, asthma, back pain and diaphoresis; whereas in another experiment on rats revealed that they could be human carcinogens if they exceed their maximum allowable level.[18,19]

In the year 2012, Institute of Public Health of Bangladesh tested a total of 5,322 marketed samples of 50 food items and found 80-99 % of those (drinks, yoghurt, milk powder) compounded,[20] which clearly indicates that food security remains an elusive goal in our country although the country is nearly self-sufficient in food production. According to a report

published in one of the leading newspapers of Bangladesh "The Daily Star" - individuals who consumed two or more soft drinks per week had an 87% increased risk for pancreatic cancer, compared with those who did not.[21,22] Moreover, on August 31, 2012, Asia News Network published a report stated that countries like France, Denmark, Norway and Argentina had already banned sales of high-caffeine beverages.[23] So as a country like Bangladesh, a land of almost 160 million people, it is very unfortunate that still today it has no regulations on the sales of energy drinks. Moreover, energy drinks and soft drinks that are commonly sold in Bangladesh, due to lack of strict monitoring system, do not list all the ingredients in the labels and sometimes hides the exact added amount in the products. Therefore, it becomes very hard to determine exactly how much CAF and other additives people are consuming per day. The overall scenario becomes worse as there is no restriction on volume of consumption as well as lack of warnings on the products that could be "excessive intake can be dangerous". So our aim was firstly to investigate the presence of these seven additives (Figure 1) i.e. CAF, BA, MP, PP, BP, BHA and BHT in 60 top selling beverages in Bangladesh which were purchased from the local market in between March and April, 2013 and secondly, to check if their concentrations are meet within the maximum allowable limit set by different regulatory bodies or not.

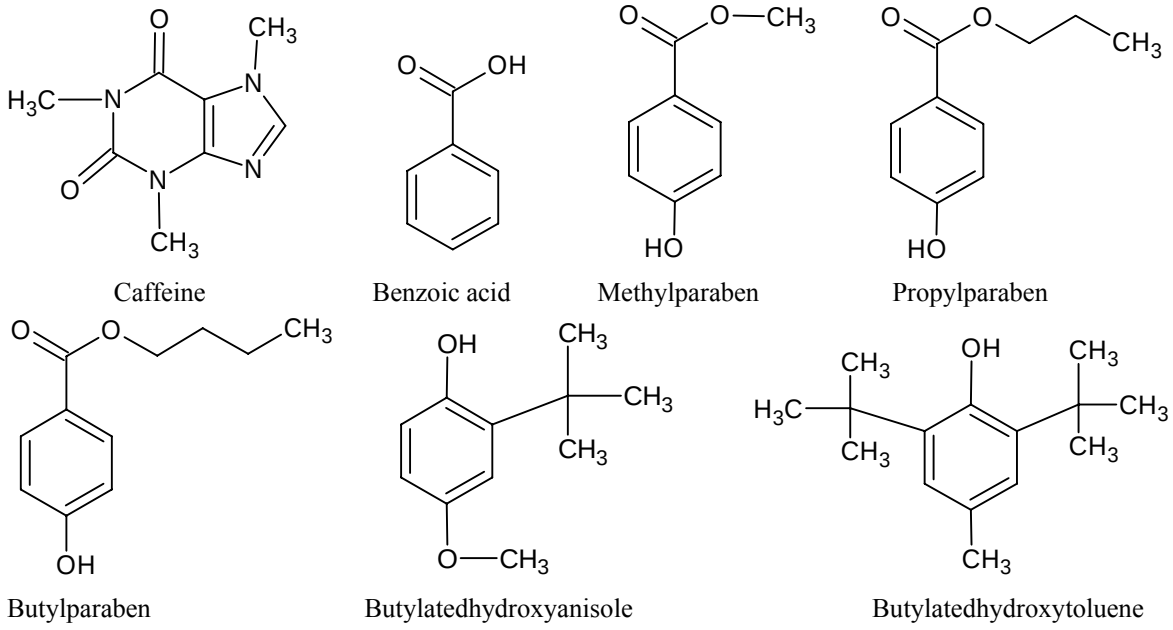

Figure 1. Structure of additives analyzed in energy- and soft-drinks

MATERIALS AND METHODS

Products selection. A total of 60 beverages were purchased from different confectionaries, departmental stores, supermarkets and local markets of Dhaka. These are listed in table 1.

Chemicals and reagents. Caffeine (BASF, Germany), benzoic acid (Fluka, Switzerland), methylparaben (Sigma-Aldrich Co, Germany), propylparaben (Sigma-Aldrich Co, Germany), butylparaben (Sigma-Aldrich Co, Germany), butylatedhydroxyanisole (Acros-Organics, USA) and butylatedhydroxytoluene (Across-Organics, USA) were used as standards. HPLC grade acetonitrile (RCI Labscan Ltd, Thailand), sulfuric acid (95-97%) (Sigma-Aldrich Co, Germany) and distilled water were used to prepare mobile phase and standard solutions.

Instrumentation and chromatographic conditions. Analysis was carried out using an HPLC (Shimadzu LC-20 AT, Japan) equipped with a UV/Vis detector (Shimadzu SPD 20A) and a vacuum degasser (Shimadzu DGU 20 A3). A 20 µL sample solution was analyzed using a Capcell Pak C_{18} (150 mm × 4.6 mm i.d., 5 µm particle size) column with acetonitrile and aqueous solution of sulfuric acid (pH=2.3) from ratio of 15:85 to 85:15 (expressed as %v/v) as mobile phase in a gradient flow and at a flow rate of 2.0 mL/min. The detecting wavelength was 265 nm. The gradient elution was as follows: (% of acetonitrile): 0–10 min in 15%, 10.01–11 min in 60%, 11.01–13 min in 70%, 13.01–16 min in 80%, and 16.01–18 min in 15%.

Table 1. Types of purchased beverages.

Product types	Locally produced	Locally distributed	Imported products	Total quantity
Energy Drinks	15	3	8	26
Soft Drinks	10	24	-	34
Total	25	27	8	60

Label claiming information of those products is summarized in Table 2.

Table 2. Products labeled with CAF, preservatives and antioxidants.

Sources of products	Number of products	CAF containing products	Preservatives containing products	Antioxidants containing products
Locally produced	25	12	16	3[a]
Locally distributed	27	13	20	-
Imported	8	5	1	-
Total	60	30	37	3

[a]claimed ascorbic acid as antioxidant.

Preparation of experimental solutions

Diluting solution. Acetonitrile and aqueous solution of sulfuric acid (pH= 2.3) in a ratio of 15:85 was used as diluting solution to dissolve the standards.

Standard solution. Accurately measured CAF, BA, MP, PP, BP, BHA and BHT were taken in a clean and dry 25 mL volumetric flask to prepare the standard solution having the concentration of 75, 250, 20, 20, 20, 60 and 40 ppm, respectively.

Sample preparation. Products were filtered through Whatman filter papers (No.1) and an aliquot of 20 µl was injected and respective chromatograms were recorded. Products found to contain higher concentrations of CAF or BA, in respect of the corresponding linearity ranges, were diluted by using the aforementioned diluting solution to bring them down within the linearity ranges. All standards and samples were stored at 25 ± 2^0C until further use.

Analytical method validation

System suitability. To assess system suitability of the proposed method, the repeatability, theoretical plates, tailing factors and retention times of six replicate injections of working standards were used and %RSD values were calculated in each case.

Linearity. The linearity was evaluated at five different concentration points over the concentration ranges of 80% to 120% of nominal test concentration by analyzing triplicate injections of seven working standards. Analyzed ranges of CAF, BA, MP, PP, BP, BHA and BHT were from 60-90 ppm, 200-300 ppm, 16-24 ppm, 16-24 ppm, 16-24 ppm, 48-72 ppm and 32-48 ppm, respectively. Three calibration curves were prepared for five different concentrations

of each standard component and the linearity of the method was determined by regression analysis.

Sensitivity. According to ICH Q2(R1) recommendations, limit of detection (LOD) and limit of quantitation (LOQ) were calculated for each of the selected standards in accordance with the 3.3s/m and 10s/m criteria, respectively; where 's' is the standard deviation of the response and 'm' is the slope of the calibration curve.

Accuracy. Accuracy was determined by using the method of standard additions, where different ranges of predetermined amounts of working standards were added to the sample solutions and they were analyzed to get the corresponding percent recoveries (mean ± %RSD of three replicates).

Precision. Repeatability (intra-day precision) and intermediate precision (inter-day precision) of the method were determined by using the nominal standard solutions of seven compounds. Sample solution was analyzed in six replicates on the same day (intra-day precision) and daily for six times over a period of three days (inter-day precision) and the results were expressed as %RSD of the measurements.

Ruggedness. Ruggedness of the proposed method was determined by analyzing six replicates of nominal standard solutions of seven standards by two analysts in the same laboratory to check the reproducibility of the test results. The % recovery and standard deviation were calculated in both cases.

Robustness. To determine the robustness of the current method, the effect of changes in flow rate of mobile phase, pH of the sulfuric acid solution and column temperature were studied at 1.8 and 2.2 $mLmin^{-1}$ instead of 2.0 $mLmin^{-1}$, pH 2.1 and pH 2.5 instead of pH 2.3 and 20ºC and 30ºC instead of 25ºC, respectively. The %RSD of robustness testing under these conditions was calculated in all the cases.

RESULTS AND DISCUSSION

Method validation

System suitability. The results (Mean ± %RSD of six replicates) of the chromatographic parameters are shown in table 3, which suggest that the performance of the system was good.

Linearity. The regression equations were calculated as Y = A + BX, where Y is peak area and X is the concentration in ppm of the standard solutions of seven standards separately. The correlation coefficients of the respective standard solutions in the prescribed ranges shown in table 4 that proved excellent linearity of the proposed method.

Sensitivity. The LOD and LOQ of seven standards of the proposed method were calculated and are presented in table 5.

Accuracy. The results of percent recoveries are shown in table 6, which indicates excellent accuracy of the proposed method.

Precision. The results are shown in table 7, which indicate excellent both intra-day and inter-day precisions of proposed method.

Table 3. Chromatographic characteristics of system suitability solution.

Standards	Peak area (Mean ± %RSD)	Tailing factor (Mean ± %RSD)	Theoretical plate (Mean ± %RSD)	Retention time (Mean ± %RSD)
CAF	1879339 ± 0.638	0.818 ± 0.518	28989 ± 0.497	3.568 ± 0.033
BA	986340 ± 0.137	1.081 ± 0.679	15413 ± 0.116	11.622 ± 0.048
MP	1291739 ± 0.559	1.249 ± 0.464	22244 ± 0.781	12.295 ± 0.052
PP	956580 ± 0.242	1.243 ± 0.396	16376 ± 0.345	13.023 ± 0.039
BP	838704 ± 0.274	1.209 ± 0.281	15455 ± 0.354	13.331 ± 0.052
BHA	180395 ± 0.301	1.248 ± 0.335	10547 ± 0.645	13.559 ± 0.051
BHT	136402 ± 0.622	1.025 ± 0.509	9434 ± 0.558	16.718 ± 0.021

Table 4. Regression equations of seven standards and their correlation coefficients.

Standards	Concentration range (ppm)	Linear equation Y = A ± (%RSD) + B ± (%RSD)X	Correlation coefficient (R^2)
CAF	60-90	Y = -36664.3 ± (15.36) + 29809.7 ± (2.68)X	0.995
BA	100-300	Y = -437643.7 ± (0.612) + 5694.8 ± (0.31)X	0.993
MP	16-24	Y = -130101.3 ± (4.746) + 72960.3 ± (0.23)X	0.990
PP	16-24	Y = -120608 ± (3.218) + 53420 ± (0.815)X	0.994
BP	16-24	Y = -89116.3 ± (10.65) + 46796.3 ± (1.08)X	0.997
BHA	48-72	Y = 21946.3 ± (46.36) + 2693.73 ± (5.76)X	0.993
BHT	32-48	Y = -34679.67 ± (32.68) + 4368.03 ± (5.72)X	0.994

Table 5. LOD and LOQ of the standards.

Standards	LOD (ppb)[a]	LOQ (ppb)[a]
CAF	2.66	16.7
BA	2.70	8.10
MP	0.215	0.645
PP	0.207	0.621
BP	0.199	0.597
BHA	2.13	12.8
BHT	1.42	8.52

[a]ppb = parts-per billion.

Ruggedness. The results (% recovery ± %RSD of six assay samples) are presented in table 8, indicating the ruggedness of the proposed method.

Robustness. The %RSD of robustness testing under different conditions is shown in table 9, which indicates that the proposed method is robust.

Quantitation of the marketed preparations

Though our main concern of this research work was to find out the presence of caffeine including six other additives commonly used to boost up the shelf lives of different food products and then to quantify them precisely, but numerous preliminary approaches had been revealed that all of 60 products, collected from different departmental shops, may contain MP, PP, BP, BHA and BHT less than the LOD and LOQ to be determined by this method. Therefore, our steps regarding this field were then confined only on CAF and BA.

The peaks of standard CAF, BA, MP, PP, BP, BHA and BHT were found at the retention times abut 3.56, 11.62, 12.29, 13.02, 13.33, 13.56 and 16.72 min, respectively, and their peak purity profiles indicated that they were totally free from all sorts of interactions at the wavelength of 265 nm. The HPLC chromatograms of seven standards, a representative of all 60 products (sample no. 13) and blank are shown in figures 2,3 and 4 respectively.

Out of 60 products, 32 products (53%) found to be CAF positive of which 15 were locally produced, 12 were locally distributed and 5 were imported. Moreover, 39 products (65%) found to be BA positive among which 19 were locally produced, 19 were locally distributed and 1 was imported. We have organized the obtained results in two distinct categories; one is "CAF or BA positive" indicates that the number of samples

Table 6. Results of accuracy testing.

Standards	Amount used (ppm) equivalent to (80% - 120%), respectively	Amount recovered (ppm)	% Recovery (Mean ± %RSD)
CAF	60	59.72	99.53 ± 0.051
	67.5	67.77	100.41 ± 0.084
	75	74.68	99.57 ± 0.215
	82.5	82.34	99.81 ± 0.231
	90	90.33	100.37 ± 0.133
BA	200	199.67	99.83 ± 0.194
	225	224.49	99.77 ± 0.114
	250	250.31	100.12 ± 0.228
	275	274.59	99.85 ± 0.171
	300	300.41	100.14 ± 0.147
MP	16	15.86	99.10 ± 0.947
	18	18.14	100.79 ± 0.994
	20	19.82	99.13 ± 0.947
	22	22.17	100.77 ± 0.816
	24	24.22	100.93 ± 0.455
PP	16	15.88	99.22 ± 0.819
	18	18.13	100.70 ± 0.859
	20	19.82	99.10 ± 0.934
	22	21.84	99.27 ± 0.848
	24	24.21	100.88 ± 0.459
BP	16	15.85	99.06 ± 0.953
	18	18.13	100.70 ± 0.855
	20	19.84	99.18 ± 0.935
	22	22.17	100.76 ± 0.814
	24	23.82	99.25 ± 0.978
BHA	48	48.45	100.94 ± 0.454
	54	53.64	99.33 ± 0.682
	60	60.55	100.92 ± 0.455
	66	65.60	99.39 ± 0.632
	72	71.30	99.03 ± 0.487
BHT	32	31.81	99.40 ± 0.676
	36	36.29	100.77 ± 0.828
	40	40.31	100.77 ± 0.717
	44	43.66	99.23 ± 0.916
	48	47.65	99.26 ± 0.939

Table 7. Summary of intra-day and inter-day precision data.

Standards	Spike level (%)	Intra-day (Mean ± %RSD)	Inter-day peak area (Mean ± %RSD)		
			1st day	2nd day	3rd day
CAF	100	1878429 ± 0.606	1881115 ± 0.756	1875702 ± 0.798	1870434 ± 0.824
BA	100	984180 ± 0.172	987306 ± 0.179	985925 ± 0.201	985299 ± 0.192
MP	100	1289906 ± 0.624	1289621 ± 0.802	1286818 ± 0.650	1282195 ± 0.815
PP	100	954564 ± 0.205	956627 ± 0.177	955835 ± 0.185	954988 ± 0.163
BP	100	836370 ± 0.289	838178 ± 0.321	837689 ± 0.396	837561 ± 0.448
BHA	100	180895 ± 0.303	180016 ± 0.546	179908 ± 0.477	179441 ± 0.599
BHT	100	135402 ± 0.755	136418 ± 0.619	136094 ± 0.488	135852 ± 0.977

Table 8. Results of ruggedness study.

Standards	Amount taken (ppm)	Analyst 1		Analyst 2	
		Amount found (ppm) (Mean ± SD)	% Recovery (Mean ± %RSD)	Amount found (ppm) (Mean ± SD)	% Recovery (Mean ± % RSD)
CAF	75	74.68 ± 0.161	99.57 ± 0.216	74.64 ± 0.350	99.52 ± 0.469
BA	250	250.31 ± 0.570	100.12 ± 0.228	250.28 ± 0.642	100.11 ± 0.257
MP	20	19.83 ± 0.188	99.13 ± 0.947	19.82 ± 0.234	99.12 ± 1.178
PP	20	19.82 ± 0.182	99.1 ± 0.934	19.79 ± 0.239	98.98 ± 1.205
BP	20	19.84 ± 0.186	99.18 ± 0.935	19.81 ± 0.145	99.05 ± 0.733
BHA	60	60.55 ± 0.276	100.92 ± 0.456	60.53 ± 0.259	100.89 ± 0.429
BHT	40	40.31 ± 0.289	100.77 ± 0.717	40.35 ± 0.211	100.88 ± 0.522

Table 9. Results of robustness study of caffeine and benzoic acid.

Parameters	Variations	Standard CAF			Standard BA		
		Amount added (ppm)	Amount recovered (Mean ± SD)	Retention time (min) (Mean ± %RSD)	Amount added (ppm)	Amount recovered (Mean ± SD)	Retention time (min) (Mean ± %RSD)
Mobile phase flow rate	1.8 mL/min	75	74.65 ± 0.12	3.715 ± 0.041	250	250.2 ± 0.2	11.778 ± 0.025
	2.0 mL/min	75	74.5 ± 0.21	3.566 ± 0.043	250	250.05 ± 0.47	11.622 ± 0.035
	2.2 mL/min	75	74.4 ± 0.35	3.423 ± 0.058	250	249.98 ± 0.82	11.466 ± 0.022
Mobile phase pH	SA(pH=2.1): ACN	75	74.53 ± 0.19	3.463 ± 0.132	250	250.16 ± 0.27	11.466 ± 0.214
	SA(pH=2.3): ACN	75	74.68 ± 0.16	3.566 ± 0.043	250	250.31 ± 0.57	11.622 ± 0.035
	SA(pH=2.5): ACN	75	74.48 ± 0.23	3.365 ± 0.12	250	250.12 ± 0.37	11.268 ± 0.236
Column temperature	20ºC	75	74.67 ± 0.09	3.666 ± 0.068	250	250.28 ± 0.41	11.716 ± 0.039
	25ºC	75	74.43 ± 0.09	3.566 ± 0.043	250	250.29 ± 0.18	11.622 ± 0.035
	30ºC	75	74.61 ± 0.09	3.365 ± 0.045	250	250.04 ± 0.27	11.126 ± 0.045

ACN = acetonitrile; SA = sulfuric acid.

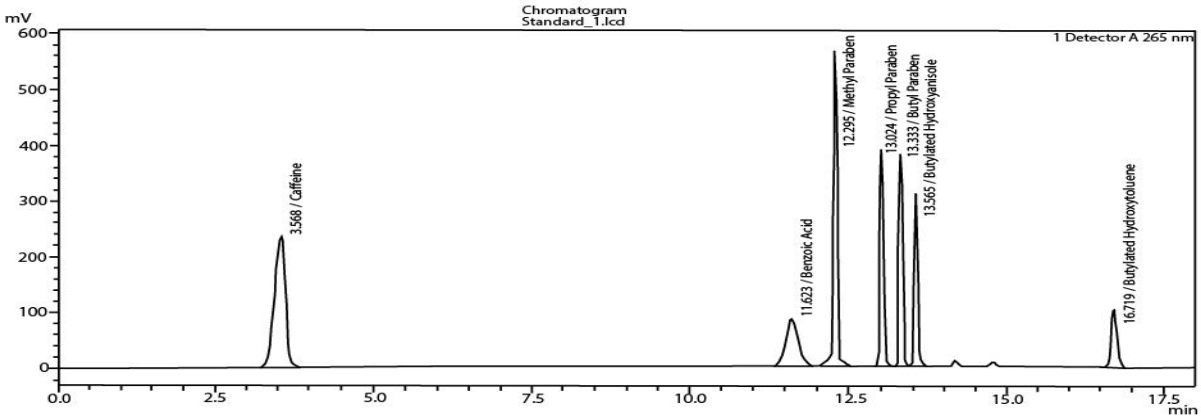

Figure 2. Chromatogram of the seven standards.

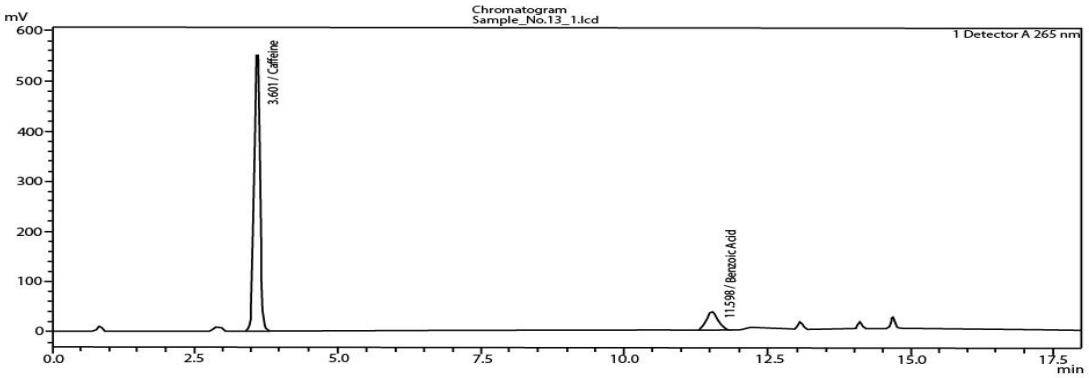

Figure 3. Chromatogram of a representative product.

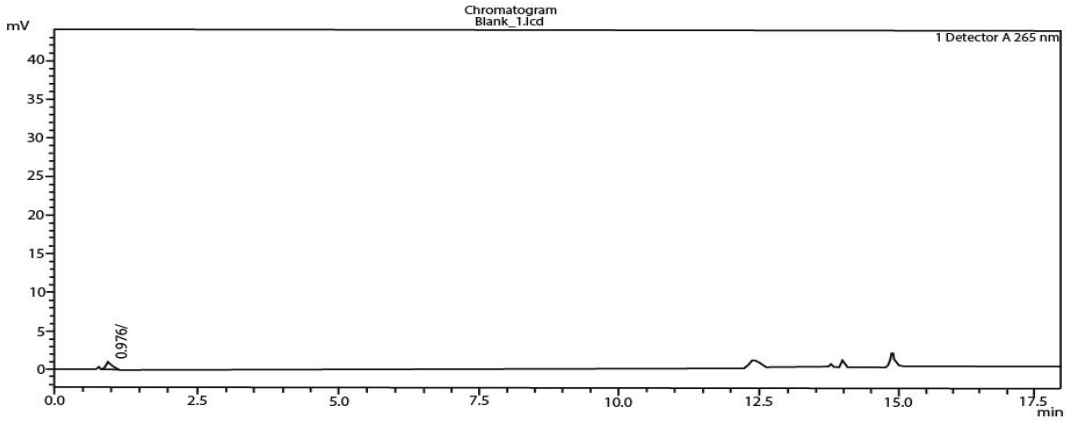

Figure 4. Chromatogram of blank.

Table 10. Market scenario of CAF and BA contents.

	Number of samples	CAF positive	CAF Content found (ppm)	CAF>200 ppm	BA positive	BA Content found (ppm)	BA>600 ppm
On the basis of sources							
Locally produced	25	15	16-138	-	19	74-1340	2
Locally distributed	27	12	53-130	-	19	100-6261	2
Imported	8	5	202-244	5	1	145	-
On the basis of types							
Energy Drinks	26	17	75-244	5	17	106-6261	4
Soft Drinks	34	15	16-102	-	22	74-170	-

showed positive responses towards CAF or BA and have peak responses greater than their respective LOQ values, the other one is "CAF>200 ppm" or "BA>600 ppm" as presented in table 10, which indicates the number of samples exceeded their respective maximum tolerance levels.

Although a total of 9 products found to contain excess amount of CAF and BA; 4 of them shown alarmingly higher amount of BA (1112, 1340, 5872 and 6261 ppm, respectively), which were even ten folds than that of the recommended range (600 ppm, set by JECFA).

Higher levels of CAF containing energy drinks could put certain susceptible people at risk of dangerous, even life-threatening consequences and adversely effects on blood pressure, heart rate and brain function. Literature also revealed that excess CAF might cause disruption of sleep pattern, nervousness, anxiety, restlessness, insomnia, gastrointestinal upset and tremor.[24] Figure 5 presents a brief overview of our investigated CAF containing products.

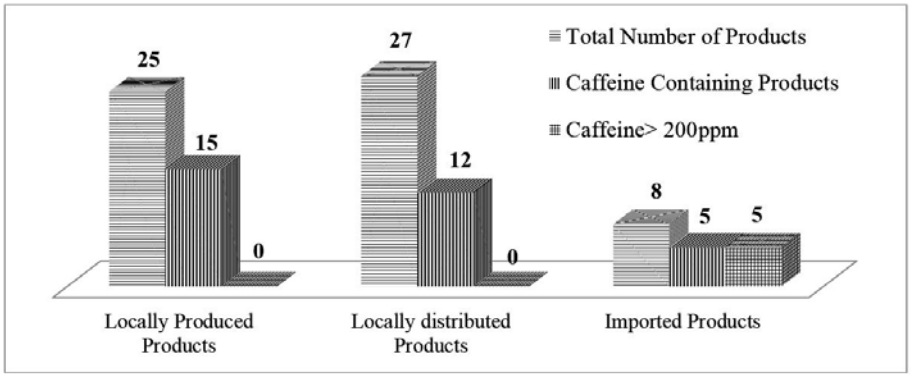

Figure 5. Overview of CAF containing products based on their origins.

Classification based on the type of products i.e. energy- and soft-drinks, shown that 65% of energy drinks and 44% of soft drinks were CAF positive among which 19% (5 out of 26) of energy drinks were found to contain more than 200 ppm (Figure 6).

On the other hand, BA is responsible for irritations of the digestive mucous membrane and depresses some digestive enzymes if exceeds the recommended range.[12] Figure 7 emphasizes on the scenario of our collected BA containing products.

Classification based on the type of products i.e. energy- and soft-drinks, shown that 65% of energy drinks and 65% of soft drinks were BA positive among which 15% (4 out of 26) of energy drinks were found to contain more than 600 ppm (Figure 8).

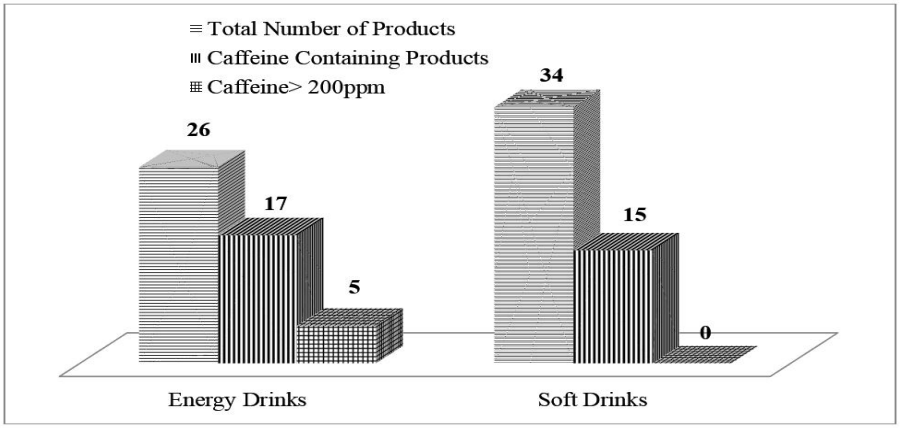

Figure 6. Overview of CAF containing products based on their types.

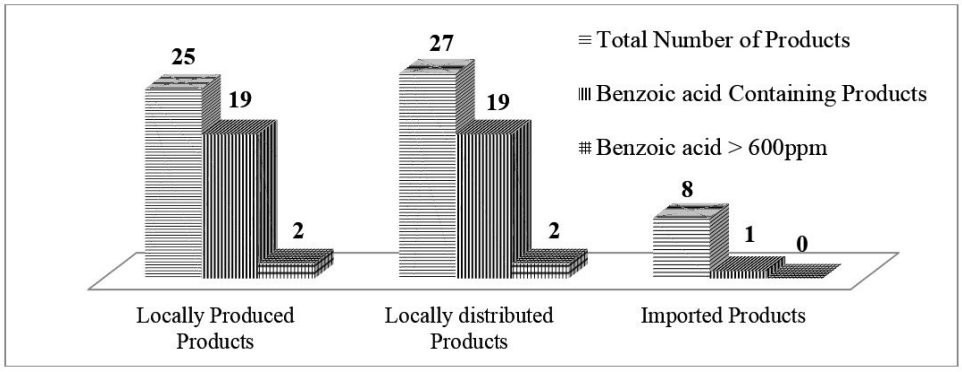

Figure 7. Overview of BA containing products based on their origins

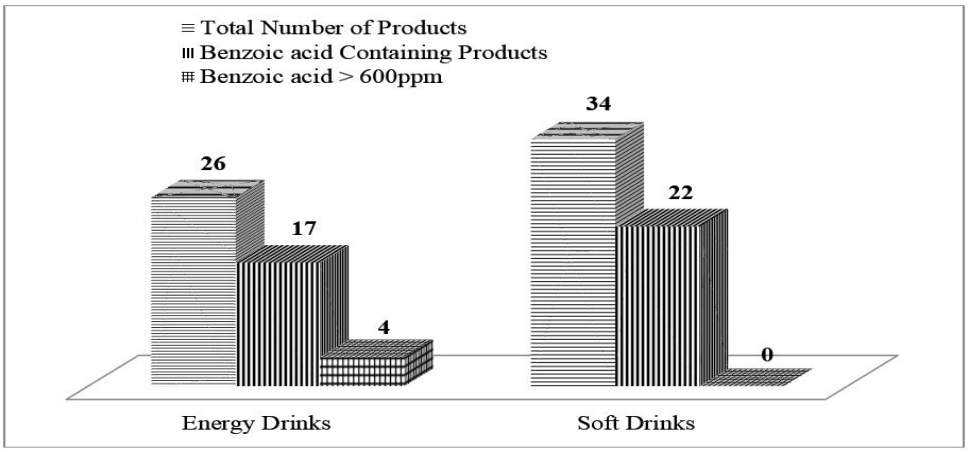

Figure 8. Overview of CAF containing products based on their types.

As we have already mentioned in the introduction section that the food security in our country is at stake and unfortunately our investigated products also revealed the same scenario. So, it is the high time to set strict rules to stop the wide-spread access of these products across the country by the respective authorities and should conduct an all-out investigation to stop this subtle corruption by the crooked producers.

CONCLUSION

Now a days, food and beverage safety is a global concern and our present study clearly reveals a small part of that bitter scenario in Bangladesh. The overall Bangladeshi market conditions of energy drinks and soft drinks in terms of the presence of caffeine and preservatives are severely deteriorated. This unwanted condition is further boosted up due to the lacking of strict controls and regulations on the usage limit of these additives. So that, it is the high time to take immediate and proper measures by the concerned regulatory authorities for the sake of the country men; unless otherwise it would be a disaster in public health sector in the near future.

ACKNOWLEDGEMENT

This research work has been supported by Higher Education Quality Enhancement Project (HEQEP), AIF, Round-III, Window-2, CP-3245, Award No. 26, University Grants Commission (UGC), Bangladesh.

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