

Composition of Energy Drink Samples in Bangladesh

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Abstract

The commercial energy drink samples of 10 different brands were analyzed to study the level of alcohol (if any), sodium benzoate, caffeine, vitamin B, and carbohydrates content. Samples were degassed by ultrasonicator, and CO₂ was found to be in a range of 1.27 ± 0.05 to 4.10 ± 0.10 mL per 100 mL sample. The amount of caffeine, sodium benzoate, glucose, and vitamin B were studied by a UV-Visible spectrophotometer. The wavelength of absorption maxima was 224, 272, 489, 445, and 292 nm, respectively, for sodium benzoate, caffeine, glucose, vitamin B2, and vitamin B6. The correlation coefficients of the calibration curves were found to be 0.998, 0.992, 0.998, 0.997, and 0.989 for caffeine, glucose, sodium benzoate, vitamin B2 and vitamin B6, respectively. The alcohol content was studied by gas chromatography equipped with a flame ionization detector. Ethanol was not found to be present in any sample. The caffeine and sodium benzoate content in different samples was found in the range of 147.84 ± 0.53 to 846.78 ± 2.64 and 52.92 ± 6.23 to 1575.37 ± 4.99 µg/mL, respectively. The sugar content in different samples was found to be 16.16 ± 4.25 to 338.33 ± 0.75 mg/mL. Vitamin B2 was found to be 13.21 ± 0.08 and 1.04 ± 0.002 µg/mL, respectively in Bacchus and Wild Brew samples and Vitamin B6 was found to be 261.17 ± 8.38 and 115.88 ± 0.31 µg/mL in Red Bull and Bacchus samples, respectively. The pH of the samples was found to be in the range of 2.79 ± 0.04 to 4.88 ± 0.04.

Keywords: Beverage, energy drinks, gas chromatography, spectrophotometer

I. Introduction

Energy drinks are carbonated beverages that can provide an extra boost in energy, promote wakefulness, maintain alertness, and provide cognitive and mood enhancement. Energy drinks are a group of beverages containing different ingredients such as caffeine, sugar, sodium benzoate, citric acid, sodium citrate, vitamin B, vitamin E, amino acids, herbal stimulants, coloring agents etc.

Caffeine has been used widely for its stimulating effects as psychoactive drug.¹ The main mechanism of action of caffeine is to act as an adenosine receptor blocker in the brain.² But excess intake of caffeine can bring harmful effects to human especially for pregnant mother and children.^{3,4} An overdose of caffeine may cause side effects such as headaches, nausea, sleeplessness, jitters and heart palpitations.⁵

Due to the antimicrobial characteristic and the advantage of low cost sodium benzoate has been used as a preservative in a wide variety of products. To avoid the drawback of its astringent taste another preservative like potassium sorbate can be added with sodium benzoate.⁶⁻⁸ The use of sodium benzoate as a preservative can cause asthmatic attacks, hives or other allergic reactions for the people who are hypersensitive to sodium benzoate. The use of sodium benzoate in combination with citric acid or ascorbic acid can form benzene, a cancer causing chemical associated with leukemia and other blood cancers.⁹

Energy drinks typically contain high dose of sugar. Glucose is the key source of energy in human body through aerobic respiration. Glucose can be absorbed directly during digestion into the bloodstream and is used as an energy source in cells.

However, excess sugar intake can cause obesity, diabetes, and can spike insulin levels.¹⁰

The most common of the B vitamins are B2 (riboflavin), B3 (niacin), B6 (pyridoxine), and B12 that are incorporated into energy drink formulations. The consumption of large amounts of B vitamins claimed the increases mental alertness, focus and improves mood.¹¹

At present, the rising prevalence of energy drinks consumption in Bangladesh is demanding concern to the scientists due to the negative health consequences including adverse cardiovascular effects, risk-seeking behaviors, metabolic, renal, and dental conditions.^{12,13} Since energy drinks are especially very popular to the young people, our attention is focused to assess the compliance of the levels of caffeine, glucose, sodium benzoate, vitamin B and alcohol (if present) in commercial energy drinks available in Bangladesh.

II. Experimental

Sample collection

Energy drink samples of ten different brands, mainly Braver, Red Bull, Royal Tiger, Speed, Power, 3Horse, Bacchus, Hulk, Oscar and Wild Brew were collected from different shops of Azimpur, New Market and Zigatola of Dhaka city. For each brand three samples of different batches were collected.

Chemicals and solvents

All chemicals and reagents used in the study were of analytical grade. Phenol (Merck, Mumbai, India), sulfuric acid (98%, w/w, BDH, U.K.), methanol (99.5%, w/w, Sigma-Aldrich), ethanol (99.8%, w/v, Sigma-Aldrich) and distilled deionized water were used during the present work.

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Standard compounds

Analytical standard reference for sodium benzoate, vitamin B and caffeine were collected from Drug Administration Bangladesh, extra pure D-(+)-glucose, riboflavin (vitamin B2) and pyridoxine hydrochloride (vitamin B6) stored at 0°C in a refrigerator. D-(+)-glucose (extra pure) was purchased from Aldrich Chemical Co. Ltd.

Instruments

A double beam Ultraviolet-visible spectrophotometer (Shimadzu UV-1800, Japan), GC-FID (Shimadzu GC-2025, Japan), Electric balance (FR-200, NDO-450ND, Japan), Ultrasonicator (Powersonic 610), Analytical balance (AL104, Mettler Toledo, USA), Vortex Machine (Q01, VWR International, Germany) and pH meter (Hanna pH 211) were used in the present work.

Preparation of standard solutions

The primary standard solution of caffeine was prepared in a 100 mL volumetric flask by dissolving 0.01g of caffeine in the distilled deionized water. Then the working standard solutions of caffeine were prepared (50.0, 40.0, 20.0, 10.0, 8.0, 5.0, 4.0, and 2.0 µg/mL) in deionized distilled water from the primary standard solution. 100 µg/mL of the primary standard solution of sodium benzoate was prepared in distilled deionized water. The primary standard solution of

sodium benzoate was diluted to 50.0, 40.0, 20.0, 10.0, 5.0, 2.5, 2.0, 1.0 and 0.5 µg/mL of working standard solutions. Similarly, the working standard solutions (0.5, 1, 5, 10, 20, 40, 50, 100 and 500 µg/mL) of riboflavin (vitamin B2) were also prepared by diluting primary standard solution (100 µg/mL) of riboflavin (vitamin B2). Absorbance of caffeine, sodium benzoate and riboflavin (vitamin B2) was measured by a double beam UV-Visible spectrophotometer at 272, 225 and 445 nm, respectively. The calibration curves were made for caffeine, sodium benzoate and riboflavin (vitamin B2), respectively (Figure 1a, 1c & 1d).

For the preparation of 100 mg/L primary standard solution, the standard glucose was dissolved in distilled deionized water. The primary standard solution was diluted in distilled deionized water and 80, 60, 50, 30 and 15 mg/L of working standard solutions were prepared. The total carbohydrates in the primary standard solutions were determined by modified Molisch's test (absorbance measured at 489 nm).^{14,15} The standard calibration curve was drawn by plotting the absorbance vs. concentrations of the primary standard solutions of glucose (Figure 1b).

For pyridoxine hydrochloride (vitamin B6), the primary standard solution (500 µg/mL) was made by dissolving the certified standard pyridoxine hydrochloride in deionized distilled water.¹⁶ The working standard solutions (250, 200, 100, 80, 40, 20, 10, 5, 2, and 1 µg/mL) of vitamin B6 were prepared.

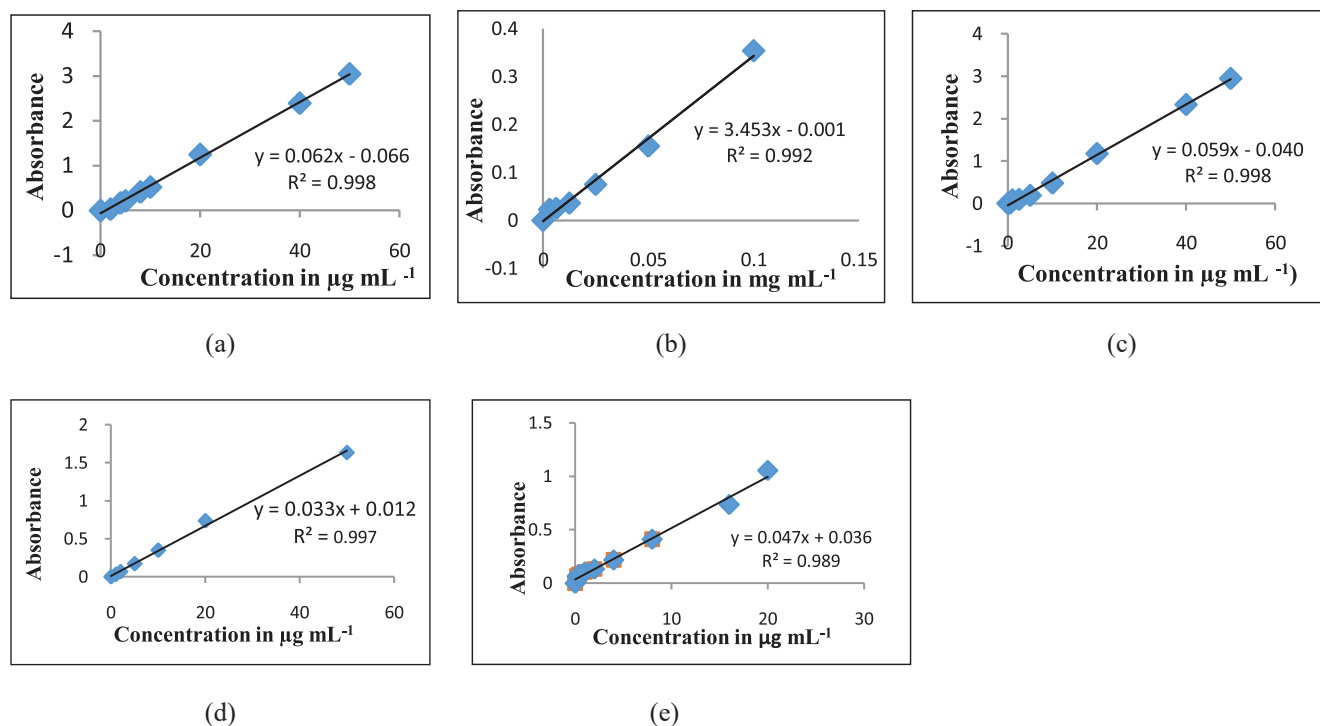


Fig 1. Calibration curves of standard (a) Caffeine, (b) Glucose, (c) Sodium benzoate, (d) Vitamin B2 and (e) Vitamin B6.

2 mL of each working standard solution was taken in a 10 mL volumetric flask and then 5 mL of methanol and 0.7 mL of glacial acetic acid were added, respectively. Finally distilled deionized water was added up to the marked. The final concentration of each solution was 5 times diluted. So the final concentration of 250, 200, 100, 80, 40, 20, 10, 5, 2, and 1 $\mu\text{g/mL}$ were converted to 50, 40, 20, 16, 8, 4, 2, 1, 0.4 and 0.2 $\mu\text{g/mL}$, respectively. For making blank solution in a 10 mL volumetric flask same amount of methanol (5 mL) and glacial acetic acid (0.7 mL) was taken and up to the marked by distilled deionized water. Absorbance of the working standard solutions was measured by a double beam UV-visible spectrophotometer at 292 nm where the blank solution was taken as reference. Then the calibration curve (Figure 1e) of absorbance vs. concentrations was drawn.

Preparation of samples

The cleaned and dried 100 mL volumetric flask was used to measure energy drinks samples. Then 0.22 μm HPLC grade filter paper was used for filtration the sample. Energy drinks sample was degassed by an ultrasonic bath. The volume of the degassed sample (Table 2) was recorded by using burette. The samples were diluted with distilled deionized water to measure absorbance by UV-visible spectrophotometer.

Determination of pH value

Energy drink sample from each batch was taken in a beaker and 30 mL of distilled deionized water was added. Then of pH was determined using calibrated Microprocessor pH meter.

Analysis of ethanol in energy drinks by GC-FID

Ethanol in energy drink sample was analyzed by using gas chromatography (GC) equipped with a flame ionization detector (FID). Nitrogen gas was used as carrier and makeup gas. For flame hydrogen and air were used. Separations were performed on capillary WCOT quartz HP-5 column (30 m long, 0.25 mm inner diameter & film thickness 0.25 μm). The column temperature was programmed as initial temperature of 40°C; hold time 4 min, then was increased at 10°C min^{-1} to 100°C; post run time 3 min. The detector and injector temperatures were 290°C and 280°C, respectively. The injector was auto and injection volume was 1 μL . The injection mode was splitless-split with split ratio of 80:20. The retention time of ethanol peak was detected at 6.4 min (Figure 3).

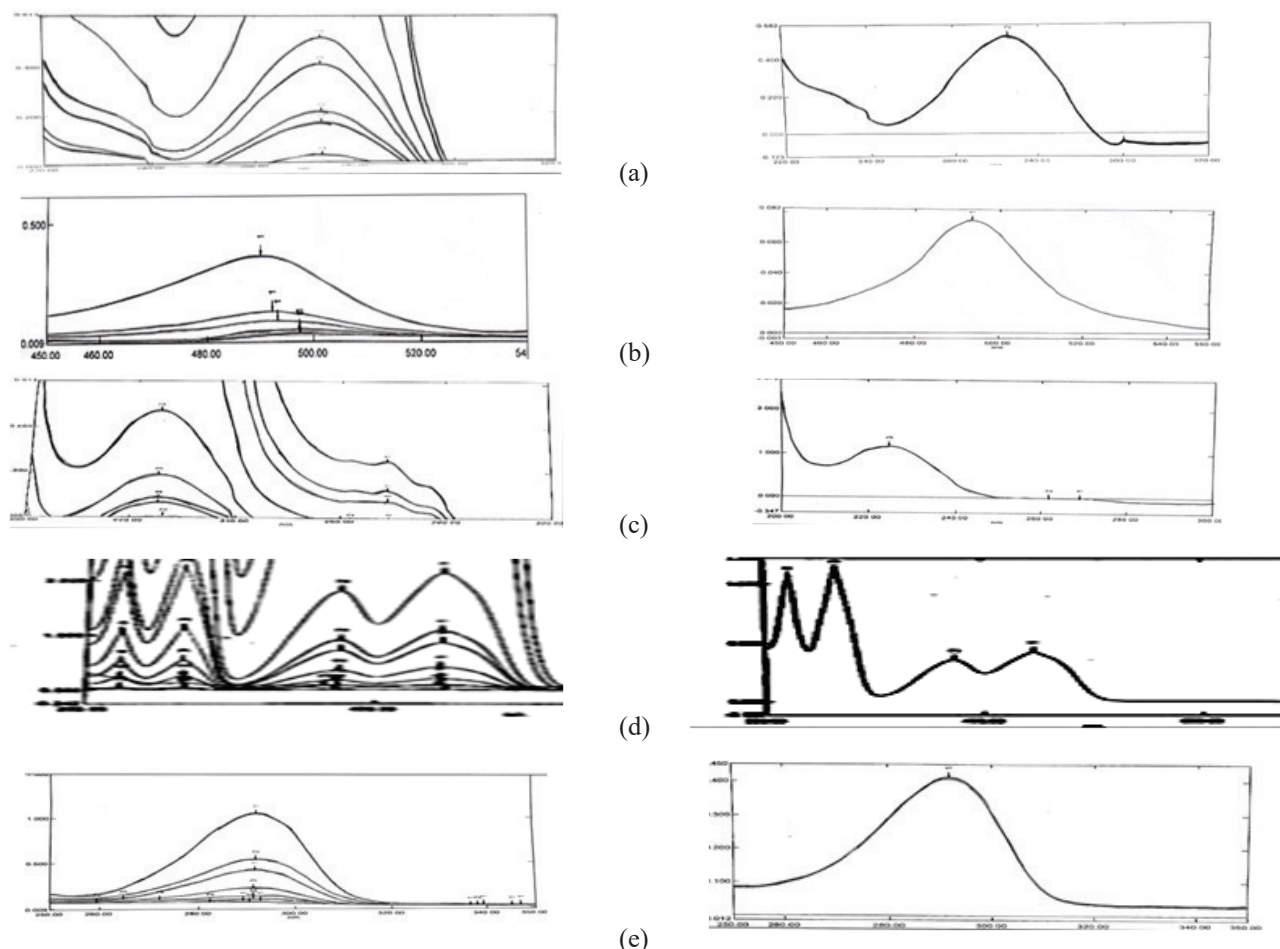


Fig. 2. Overlain (at different concentrations) and UV-visible spectrum of standard caffeine (a), glucose (b), sodium benzoate (c), vitamin B2 (d) and vitamin B6 (e).

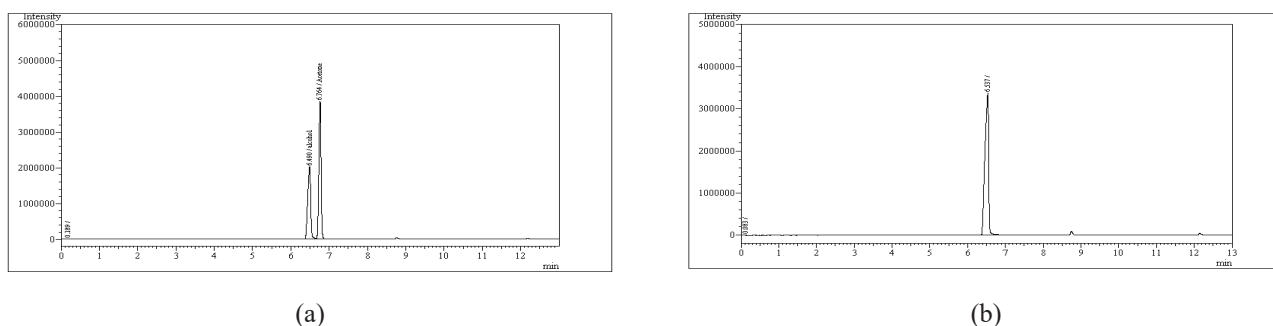


Fig 3. GC-FID chromatogram of ethanol (a) and energy drink sample (b).

Recovery experiment

The standard solutions of caffeine, glucose and sodium benzoate were spiked in distilled deionized water at three different concentration levels (25 $\mu\text{g/mL}$ of caffeine; 50 $\mu\text{g/mL}$ of glucose; and 25 $\mu\text{g/mL}$ of sodium benzoate) for recovery experiment. Eight replicates were done for each substance (Table 3).

III. Results and Discussion

Table 1. Amount of caffeine, sugar and sodium benzoate in energy drink samples.

Sample	Caffeine		Sugar		Sodium Benzoate	
	Average Amount \pm SD ($\mu\text{g/mL}$), n= 3	RSD (%)	Average Amount \pm SD (mg/mL),n= 3	RSD (%)	Average Amount \pm SD ($\mu\text{g/mL}$), n= 3	RSD (%)
Braver	725.32 \pm 1.90	0.26	117.88 \pm 3.97	3.36	298.52 \pm 2.52	0.84
Red Bull	388.20 \pm 2.11	0.54	117.25 \pm 0.85	0.73	52.92 \pm 6.23	11.78
Royal Tiger	194.50 \pm 2.42	1.24	325.96 \pm 0.29	0.09	52.92 \pm 6.23	1.78
Speed	212.21 \pm 0.23	0.11	338.33 \pm 0.75	0.22	403.54 \pm 6.27	1.55
Power	262.76 \pm 1.01	0.38	338.33 \pm 0.75	0.22	403.54 \pm 6.27	1.55
3Horse	212.93 \pm 0.04	0.02	18.42 \pm 0.05	0.27	ND	ND
Bacchus	846.78 \pm 2.64	0.31	162.59 \pm 1.10	0.68	1575.37 \pm 4.99	0.32
Hulk	237.52 \pm 0.79	0.33	208.84 \pm 1.75	0.84	443.62 \pm 0.67	0.15
Oscar	523.43 \pm 0.17	0.03	167.45 \pm 2.69	1.61	473.17 \pm 16.09	3.39
Wild Brew	147.84 \pm 0.53	0.36	16.16 \pm 4.25	6.29	58.14 \pm 2.70	4.64

Notes: SD-Standard Deviation, ND-Not Detected, RSD-Relative Standard Deviation& “n” indicates number of replications

Most energy drinks have similar ingredients: water, sugar, caffeine, certain vitamins, minerals and preservatives such as sodium benzoate and citric acid. The maximum absorption (max) of the cleaned-up extracts of all the samples were found at 272, 489, 224, 445 and 292 nm for caffeine, glucose, sodium benzoate, vitamin B2 and vitamin B6, respectively. Limit of detection (LOD) of caffeine, glucose, sodium benzoate, vitamin B2 and vitamin B6 were 0.1, 2, 1, 1 and 0.1 $\mu\text{g/mL}$, respectively (Fig 2) showed sensitivity of the modified method. Caffeine content (Table 1) in different samples were found to be in a range of 147.84 \pm 0.53 to 846.78 \pm 2.64 $\mu\text{g/}$

mL, whereas FDA specified the maximum limit of caffeine is 200 $\mu\text{g/mL}$.¹⁷ The amounts of sodium benzoate (Table 1) in the samples were found to be in a range of 52.92 \pm 6.23 to 1575.37 \pm 4.99 $\mu\text{g/mL}$. For sodium benzoate, the acceptable daily intake (ADI) is 0-5 mg/kg body weight is specified by the expert Committee on Food Additives.¹⁸ Sugar content in different energy drink samples were found to be in a range of 16.16 \pm 4.25 to 338.33 \pm 0.75 mg/mL (Table 1). High carbohydrates containing energy drinks causes obesity to the consumers.¹⁹ The American Heart Association (AHA) recommends no more than 6 teaspoons (25 grams) of added

sugar per day for women and 9 teaspoons (38 grams) for men.²⁰ According to European Food Safety Authority (EFSA) energy drinks which contain over 150 mg/L of caffeine must be clearly labeled as having 'high caffeine content'.²¹ Almost all of the energy drink samples of the present work was found to be contained high levels of caffeine (147.84 ± 0.53 to $846.78 \pm 2.64 \mu\text{g} / \text{mL}$) and also high sugar (16.16 ± 4.25 to

$338.33 \pm 0.75 \text{ mg/mL}$) content. The pH of commercial brand energy drinks samples were found to be in the range of 2.79 ± 0.04 to 4.88 ± 0.04 (Table 2). The acidity of beverages can effect dental erosion if $\text{pH} < 4.0$.²² Only 3Horse ($\text{pH} 4.88 \pm 0.04$) and Wild brew ($\text{pH} 4.35 \pm 0.05$) samples pH level was more than 4.

Table 2: Amount of dissolved CO₂ gas, vitamin B2, vitamin B6, pH and alcohol (ethanol) in energy drink samples.

Sample	Volume of dissolved CO ₂ Average \pm SD (mL in 100 mL Sample), n= 3	Vitamin B2 Average \pm SD ($\mu\text{g/mL}$), n= 3	Vitamin B6 Average \pm SD($\mu\text{g/mL}$), n= 3	pH Average \pm SD, n= 3	Ethanol
Braver	2.71 ± 0.08	ND	ND	3.04 ± 0.04	ND
Red Bull	1.62 ± 0.07	ND	261.17 ± 8.38	3.51 ± 0.06	ND
Royal Tiger	1.37 ± 0.04	ND	ND	2.92 ± 0.03	ND
Speed	1.27 ± 0.05	ND	ND	3.01 ± 0.06	ND
Power	2.30 ± 0.09	ND	ND	2.96 ± 0.06	ND
3Horse	3.00 ± 0.09	ND	ND	4.88 ± 0.04	ND
Bacchus	1.46 ± 0.04	13.21 ± 0.08	115.88 ± 0.31	2.79 ± 0.04	ND
\Hulk	2.02 ± 0.08	ND	ND	3.20 ± 0.07	ND
Oscar	4.10 ± 0.10	ND	ND	3.86 ± 0.03	ND
Wild Brew	3.52 ± 0.22	1.04 ± 0.002	ND	4.35 ± 0.05	ND

Notes: SD-Standard Deviation, ND-Not Detected & "n" indicates number of replications

The dissolved CO₂ was found to be in a range of 1.27 ± 0.05 to $4.10 \pm 0.10 \text{ mL}$ per 100 ml sample. The dissolved carbon dioxide in carbonated drinks can influence the activity of taste receptors in the mouth and thus the neuromotor responses.²³ Out of ten different samples vitamin B2 and B6 were found to be present in two samples. Vitamin B2 was found to be 13.21 ± 0.08 and $1.04 \pm 0.002 \mu\text{g/mL}$ respectively, in Bacchus and Wild Brew samples and Vitamin B6 was found to be 261.17 ± 8.38 and $115.88 \pm 0.31 \mu\text{g/mL}$ in Red Bull and Bacchus samples, respectively.

People can confuse to consume energy drinks because if it contain alcohol. The presence of ethanol in energy drinks is an important topic. Some research was satisfied the presence of alcohol less than 0.5%. The FDA considers beverages containing less than 0.5% alcohol to be "non-alcoholic".²⁴

The retention time of standard ethanol was found at 6.4 min but there was no such peak in that retention time was observed in any samples (Figure 3).

Recovery

The accuracy of the analytical method was assessed by the recovery result. The mean recovery of caffeine at $25 \mu\text{g/mL}$ spiking level was $95.53 \pm 1.82\%$, for D(+)glucose the mean recovery was $96.85 \pm 1.53\%$ at the spiking level of $50 \mu\text{g/mL}$ and the mean recovery of sodium benzoate at $25 \mu\text{g/mL}$ spiking level was $98.59 \pm 0.49\%$ (Table 3). The relative standard deviation for the recovery of caffeine, glucose and sodium benzoate were 1.19, 1.59 and 0.50 %, respectively. The recovery results were satisfactory with the acceptable range.²⁵

Table 3. Results of recovery experiment for caffeine, glucose and sodium benzoate.

Food Additives	Spiking Level (µg/mL)	Recovery (%)	Mean Recovery (%), n= 8	RSD (%)
Caffeine	25	98.24	95.53± 1.82	1.91
		93.55		
		94.72		
		96.32		
		95.48		
		98.36		
		93.68		
		93.92		
		97.40		
		96.80		
Glucose	50	97.80	96.85± 1.53	1.59
		97.40		
		93.80		
		95.80		
		99.00		
		96.80		
		99.04		
		98.92		
		98.64		
		97.48		
Sodium Benzoate	25	98.84	98.59± 0.49	0.50
		98.36		
		98.72		

Notes: RSD-Relative Standard Deviation& “n” indicates number of replications

IV. Conclusion

The described GC-FID and UV-Visible spectrophotometric methods for determination of ethanol, caffeine, glucose, sodium benzoate, and B vitamins are satisfactory for a wide range of concentration. The methods are easy to carry out for the routine analysis of ethanol, sodium benzoate, caffeine, glucose and B vitamins in energy drinks within a short analysis time.

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