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Evaluation of Caffeine in Soft and Energy Drinks by Means of UV/Visible Spectrophotometer

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Abstract

The UV-VIS spectrometry is a method frequently used for routine caffeine determination in beverages. Caffeine absorb at UV-VIS region with maximum absorption band at 271 nm. In the present work we undertook various experiments to determine the pH and levels of caffeine concentration in ten soft drinks and five energy drinks available in local market in Albania. pH levels were measured by pH meter. Calibration solutions were prepared in the concentration range of 1-25 ppm from a 100 ppm stock solution. Concentration of caffeine in drinks was performed by a simple and fast standard UV spectrophotometric method at 271 nm. The minimum caffeine content of soft drinks was observed in *Brand-5* (24.55 mg/L), while the highest concentration of caffeine was observed in *Brand-9* (79.46 mg/L). Unlike the soft drinks, the concentration of caffeine in energy drinks were slightly higher with minimum amount at *Brand-4* (62 mg/L) and maximum at *Brand-5* (152 mg/L). The pH range of soft drinks were (2.51 to 3.34) and in energy drinks (2.33 to 3.49)

Keywords*:* Caffeine, UV/Visible Spectrophotometer, Soft and energy drinks

1. Introduction

1,3,7-trimethylxanthine widely known as Caffeine is a naturally alkaloid usually found in coffee beans and tea leaves or fruits [1]. Around sixty plant species contain caffeine [2]. Coffee plant is one of the common sources of caffeine, other sources of caffeine are found in the leaves of the tea, kola drinks, energy drinks, chocolates, cocoa beans, yaupon holly leaves, to quote only few [3]. For the first time, caffeine was isolated in laboratory as pure compound by German chemist Ferdinand Runge in 1819 [4]. It is a white odorless organic compound, powder crystalline and has bitter taste and react as stimulant drug in human body [5]. Mentioning some physical properties, the density of caffeine is 1.2 g/cm³, melting point of caffeine is 237 °C and the boiling point is 178 °C. Regarding solubility, is very low in water, moderate in ethyl acetate, pyrimidine, pyrrole, acetone, and very high in petroleum ether, ether, benzene and chloroform [6]. While coffee and tea beverages naturally contain caffeine and other derivatives, caffeine is found in varying quantities as an ingredient in many carbonated soft drinks including colas, Pepsi beverages, and in energy drinks its content varies from 10 to 50 mg of caffeine per serving. Caffeine is added as flavoring agent and is the most common additive found in soft and energy drinks in way to make them more addictive [7]. Caffeine has taken special attention in the past decades concerning its physiological and stimulatory effect. Caffeine as alkaloid directly affect at central nervous system as stimulant. It is worth to mention that the Food and Drug Administration (FDA) describe caffeine as a generally recognized as safe (GRAS) substance. Notwithstanding, FDA specifies that the maximum quantity in soft and energy drinks is limited to 0.02% (FDA 2006) [8]. As matter of fact, the highest legal amount of caffeine allowed in a 350 mL can of various drink is about 71 mg [8][a,b]. Regarding EFSA (European Food Safety Authority), Caffeine should not exceeded daily consumption for the adults up to 400 mg, 200 mg for pregnant women and less than 200 mg for children (however, is depended form their weight) [8][c]. Caffeine has drowned special interest of consumers and health professionals thanks to its wide consumption in the diet by a large percentage of the

population and its pharmacological effects in humans [9]. In this context, the physiological effects on human body systems have been stated by different researchers, including the central nervous, respiratory, gastrointestinal, cardiovascular, and renal systems, mentioning some of them [10]. Amongst several agencies, International Olympic Committee (IOC) has classified caffeine as a drug and when caffeine concentrations are found to be higher than 12 μg/mL in urine, is considered as abuse and punishments have been taken [11]. More in detail, considerable studies have proven caffeine to be a stimulant to human's central nervous system [12]. Also, it increases heart beat rate, dilate blood vessels and elevate levels of free fatty acids and glucose in plasma. In light of this, when amount of caffeine consumption is higher than amount allowed by FDA/EFSA, causes numerous body irregularity such as insomnia, nervousness, nausea, ear ringing, derillum and tremulosness. It is highly important avoiding overdose because in combination with alcohol, and some other drugs, these compounds generate a toxic effect and sometimes, even though in small occasion, lethal outcome [13,14]. In addition to, caffeine facilitates the conduction velocity in the heart which is responsible for the contractility of the heart and blood vessels. However, caffeine may significantly reduce cerebral blood flow by constricting of cerebral blood vessels. Although many negative consequences, when caffeine is consumed at acceptable level, provides a diuretic effect due to elevating the blood flow and glomerular filtration rate of the kidneys. Stomach problems like heart burn is an issue for some subjects' gastrointestinal system after consuming caffeine. Problems are reported for skeletal muscles and are predominantly increasing tremors incident [12,14]. Several methods exist for determining the caffeine content in tea, coffee, and beverages which are reported in literature. Including UV-Visible spectrophotometry, potentiometry, high performance liquid chromatography (HPLC), ion chromatography, high performance thin layer chromatography (HPTLC), capillary electrophoresis, micellar capillary electrophoresis, gas chromatography, and solid-phase microextraction gas chromatography [15 - 22]. The UV-VIS is a tool frequently used for routine caffeine determination in beverages for its effectiveness and is cheap compared with other techniques mentioned above. Caffeine absorb at UV-VIS region with maximum absorption band at 271 nm [23]. Accordingly, the aim of this work was determination of caffeine quantity in numerous soft and energy drinks available in Albanian market due to increasing consumption of above mentioned drinks in our daily life and then observing whether the concentration is at acceptable level allowed by FDA. In light of this we determined the amount of caffeine in ten different brands of soft drinks and five energy drinks by means of UV/VIS Spectrophotometer.

2. Material and methods

2.1 Chemicals and instrument

All glassware were washed with distilled water and then dried overnight in oven. Soft and energy drink were taken in different Albanian supermarket. The caffeine pure powder was provided from Sigma Aldrich. Biochrom Libra S22 UV/Vis Spectrophotometer was scanned from 10 – 400 nm. The pH meter SI Analytic lab 845 was used for pH measurement of drinks.

2.2 Standard solution preparation

The stock solution of Caffeine was prepared by dissolving 10 mg of pure caffeine in 1000 ml of distilled water to obtain 10 ppm caffeine solution. Standard solution was prepared by pipetting 0; 1; 1,5; 2 ml stock solution into 10 ml volumetric flask and then filled with distilled water up to the mark. The absorbance of each standard solution was measured at absorption maximum of 270 nm three time for each solution using 10 mm quartz cuvettes.

2.3 Sample preparation

All soft and energy drinks were decarbonated prior of using for absorbance measurement. More in detail, all sample were left under vigorous stirring in room temperature for several hours in order to release all carbon dioxide present in drinks. A beverage portion was drawn by pipette and filter off, then 1 ml of filtered sample was placed in 10 ml flask and was diluted with water. This procedure was repeated for all soft and energy drinks samples. The absorbance was measured three times for each sample at 270 nm in quartz cuvettes. Note of worth, the pH of each sample was measured after decarbonated.

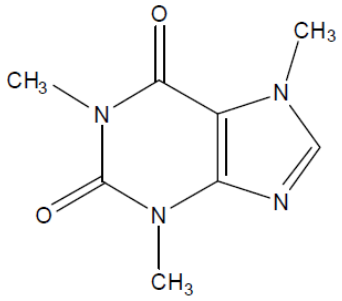

Figure 1. Chemical formula of caffeine

3. Results and Discussion

In fig 2 is presented absorption spectra of caffeine standard solution and the spectra of one sample from energy drinks and one from soft drink

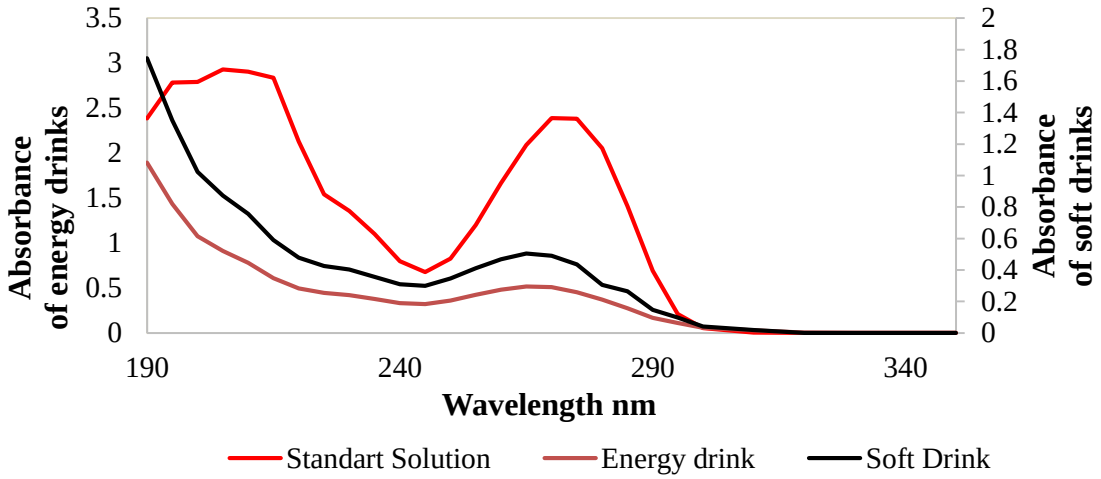

Figure 2. Absorption spectra of caffeine standard solution, energy drinks and soft drinks

The peak of caffeine standard solution is at 270 nm which is associated as n → π* electronic transition of caffeine [26]. This band at 270 nm is related with the C = O chromophore absorption. Concerning the absorption spectra of samples, the maximum absorption is at 265 nm. As it was expected, this shifted of maximum absorption is due to presence of other compound in sample and solvent that absorb in this region and strongly interfere [27].

In Figure 3 is plotted the calibration curve of caffeine, which was prepared by using standard solutions of caffeine in the concentration range of 0-2.5 ppm and absorbance was measured on a UV/Vis spectrometer at wavelength 270 nm, as shown in Table.1. Moreover, the corresponding equation and the correlation coefficient are given.

Table 1. Caffeine content of standard solution and the absorbance measurements

Caffeine Content ppm	Absorbance 271nm
0.0	0.0000
1.0	0.0218
1.5	0.0338
2.0	0.0450

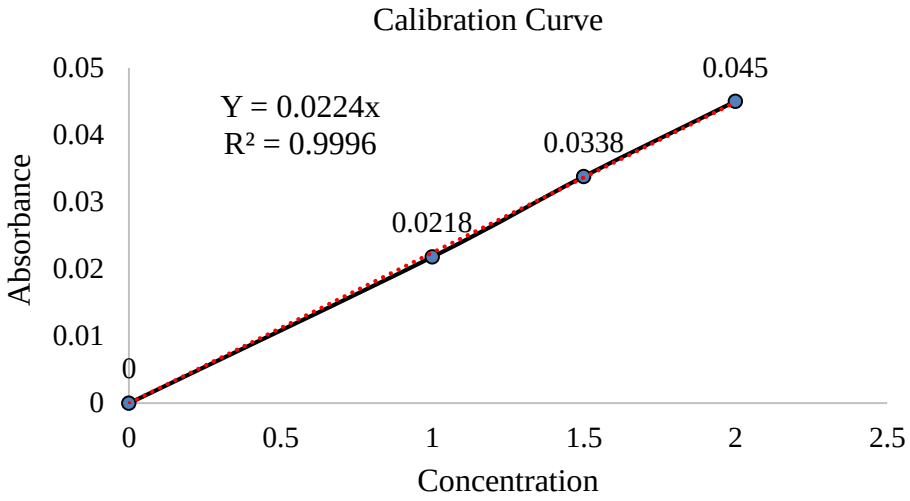

Figure 3. The calibration curve of caffeine standard solution

One of the main objective of this research was to observe the caffeine amount in soft and energy drinks collected from Albanian market and whether the quantity of caffeine is high or low of the FDA /EFSA recommended value[8] [a,b,c].

In the table below are given the corresponded concentration and the pH values of soft drinks. The maximum level of caffeine was found in Brand 5 which was 79.46 ppm and has a pH value 3.34 so it means Brand 5 has moderate acid pH and based on caffeine amount it is strongest central nervous system stimulant so it can be avoided from the consumer in market [8][a,b,c]. The minimum level of caffeine was found to be in Brand 6 which was 24.55 ppm and pH value is the amongst lowest compared to the other soft drinks so it means this brand is strong acidic. However, it can be sold in market and is a weak central nervous system stimulant regarding caffeine content. The US food and Drug Administration (FDA, 2006) limits maximum quantity of caffeine in soft drinks in range between 30-72 mg in 355 ml, and the EFSA limits maximum daily consumption about 3 mg/kg for adults, 5.7 mg/kg for habitual consumption and around 1.5 mg/kg for pregnant women and children [8][a]. In addition to, the most acidic drink is brand 3 with pH value at 2.51 and the less acidic is brand 5.

Table 2. Caffeine content of soft drinks

Sample	Absorbance 271 nm	Caffeine content mg/L	Con mg/L with dilution factor	pH
Brand 1	0.111	4.955	49.55	2.91
Brand 2	0.130	5.804	58.04	2.59
Brand 3	0.108	4.821	48.21	2.51
Brand 4	0.127	5.670	56.70	2.78
Brand 5	0.178	7.946	79.46	3.34
Brand 6	0.055	2.455	24.55	2.70
Brand 7	0.060	2.679	26.25	2.78
Brand 8	0.126	5.625	56.25	3.01
Brand 9	0.136	6.071	60.71	2.68
Brand 10	0.088	3.929	39.29	2.63

Taking into account the maximum limits of caffeine allowed from FDA and EFSA for the soft drinks, we found that the quantity is at the acceptable range and none of the drinks is in possession of higher amount of caffeine than FDA/EFSA regulation [8].

Presence of CO_2 in either soft or energy drinks is frequently considered as main contributor for the low pH values. It is worth to mention that other acids used as preservatives such as phosphoric acid, citric acid and tartaric acid, malic acid, ascorbic acid contribute to the acidic nature of these drinks. Due to presence of these acids in drinks, various microorganisms such as bacteria, fungi which may find a way to contaminate beverages, do not find an appropriate condition thanks to low pH level. Numerous studies have shown that consuming beverages daily can cause different dental problems [24,25].

Statistical analysis of soft drinks is shown in table below. The minimum of value Standard Deviation that subsequently correspond to the minimum of Variance is for brand 5. From the other side the maximum value of Standard Deviation and Variance is for brand 2.

Table 3. Statistical analysis of soft drinks

Sample	Mean	Standard Deviation	Variance
Brand 1	49.55	0.0818	0.0067
Brand 2	58.04	0.1081	0.0117
Brand 3	48.21	0.0888	0.0079
Brand 4	56.70	0.1509	0.0228
Brand 5	79.46	0.0458	0.0021
Brand 6	24.55	0.1248	0.0156
Brand 7	26.25	0.0953	0.0091
Brand 8	56.25	0.1276	0.0163
Brand 9	60.71	0.0655	0.0043
Brand 10	39.29	0.0754	0.0057

In the table below are given the corresponded concentration and the pH values of energy drinks.The highest concentration of caffeine was found in Brand 5 which was 152.68 ppm and has a pH value 3.38 so it means Brand 5 has moderate acid pH and based on caffeine amount it is strongest central nervous system stimulant so it can be avoided from the consumer in market. The lowest caffeine concentration was found to be in Brand 4 which is 62.05 ppm and pH value is the highest compared to the other energy drinks so it means this brand is less acidic, thus, it can be sold in market and is a weak central nervous system stimulant. Highlighting, the most acidic drink is brand 2 with pH value at 2.33 and the less is brand 4 as was mention above.

Table 4. Caffeine content of energy drinks

Sample	Absorbance 271 nm	Caffeine Content mg/L	Con mg/L with dilution factor	pH
Brand 1	0.141	6.2950	62.950	3.26
Brand 2	0.170	7.5890	75.890	2.33
Brand 3	0.170	7.5890	75.890	3.31
Brand 4	0.139	6.2050	62.050	3.49
Brand 5	0.342	15.268	152.68	3.38

As somehow it was expected the quantity of caffeine in energy drinks is higher compared with soft drinks. However, lower than maximum limited from FDA and EFSA (Range from about 80 – 150 mg per serving) [8]. Note of worth, when caffeine concentration exceeded 150 ppm should be mention in label.

Statistical analysis of energy drinks are given in table 5. Accordingly, the minimum of value Standard Deviation correspond minimum of Variance is for brand 1. In contrast, the maximum value of Standard Deviation and Variance is for brand 4.

Table 5. Statistical analysis of energy drinks

Sample	Mean	Standard deviation	Variance
Brand 1	62.95	0.036	0.0013
Brand 2	75.89	0.081	0.0067
Brand 3	75.89	0.105	0.0112
Brand 4	62.05	0.174	0.0304
Brand 5	152.68	0.153	0.0237

4. Conclusion

Analyzing the data gathered from our experiment approach, it is shown that the content of caffeine in soft drinks is not higher than the maximum limits authorized from the international authority of drug regulation. Even though the caffeine quantity in energy drinks are slightly higher than soft drinks, concentration is within maximum limits allowed form the international authority. Taking into consideration problems arising from the consumption of caffeine, even at permitted level, is suggested for the producer to mention the presence of caffeine quantity and health concerns in label, since we found out that those data are missing to the most selected drinks for this study. Determination of caffeine was carried out by means of spectrophotometer UV-VIS as cheapest, fast, and excellent accuracy.

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