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Determination of Caffeine in Soft and Energy Drinks Available in Market by using UV/Visible Spectrophotometer

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Abstract

This experiment did to determine the pH, levels of caffeine concentration in five soft drinks and four energy drinks available in local market of Pakistan. pH were measured by pH meter. Quantitative estimation of caffeine concentration in drinks was performed by a simple and fast standard UV spectrophotometric method (Perkin Elmer lambda 35 UV/Vis spectrometer) using carbon tetrachloride as the extracting solvent at 270 nm wavelengths. The minimum caffeine level of soft drinks was observed in Brand-3(10.69 mg/serving), while Brand-5 showed the highest caffeine content (42.17 mg/serving) showing a range from 10.6 to 42.17 mg/serving. The concentration of caffeine in all energy drink samples are well below the maximum allowable limits set by the food regulatory bodies, except E2. The E2 has greater calculated concentration then the labelled concentration. The minimum caffeine level of energy drinks was observed in E4 (32.04 mg/L) while E2 showed highest caffeine level in energy drinks (101.705 mg/serving) showing range from 32.04 mg/serving to 101.705 mg /serving . And the pH range of these soft drinks were (2.29 to 3.02) and in energy drinks (2.85 to 3.28).

Keywords: Caffeine; UV spectrophotometer; Comparison of soft and energy drinks

Introduction

Caffeine is a most common ingredient of energy drinks. It is added as a flavouring agent and to make the drinks addictive [1]. Caffeine is a bitter in taste, white crystalline xanthine alkaloid that acts as a psychoactive stimulant drug and a mild diuretic. Almost sixty plant species are known to contain caffeine [2]. Common sources of caffeine are the "bean" (seed) of the coffee plant; in the leaves of the tea bush; and in kola nuts. Some other sources include yaupon holly leaves, South American holly yerba mate leaves, seeds from Amazonian maple guarana berries [3]. Columbia In 1819, the German chemist Friedrich Ferdinand Runge first time isolated pure caffeine in laboratory [4] Caffeine is one of the world's most widely used drugs. Many anthropologists believe people used caffeine start from Stone Age. Caffeine was first extracted from coffee in 1821 [5]. Caffeine is a naturally occurring substance found in the leaves, seeds or fruits of over 63 plants species worldwide and is part of a group of compounds known as methyl xanthine's. The most commonly known sources of caffeine are coffee, cocoa beans, cola nuts and tea leave [6]. Caffeine is a naturally occurring substance found in humans, caffeine is a central nervous system (CNS) stimulant [7]. It has the effect of temporarily warding off drowsiness and restoring alertness. Beverages containing caffeine, such as coffee, tea, soft drinks and energy drinks, enjoy great popularity [8]. Caffeine is the world's most widely consumed psychoactive substance. Adults receive nearly three quarters of their daily caffeine from coffee. Children receive one half of their caffeine from soft drinks Energy drinks represent a fast-growing beverage market.

Different energy drinks having different amount of caffeine and its range is from 50-300 mg. Most people experience no behavioural effects with less than 300 mg caffeine. Sleep is more sensitive and can be disrupted by 200 mg caffeine [9]. The caffeine content in your average cup of coffee is around 100 mg. Decaffeinated coffee isn't actually caffeine-free, and can contain up to 12 mg of caffeine. Your average cup of tea contains 85 mg of caffeine. A single can of commercially available energy drink can have anywhere between 80 and 280 mg of caffeine depending on the can size. Green tea is close behind with 60 mg of caffeine, followed by white tea with 55 mg. Slim-fast chocolate drinks come in at 20 mg of caffeine in a single serving [10]. Caffeine is metabolized in the liver into three primary metabolites: Para xanthine (84%), Theo bromine (12%), and theophylline (4%) [11] Caffeine is metabolized in the liver by the cytochrome P450 oxidase enzyme system (specifically, the 1A2 isoezyme) into three metabolic dimethyl xanthine's (Figure 1) which each have their own effects on the body [12].

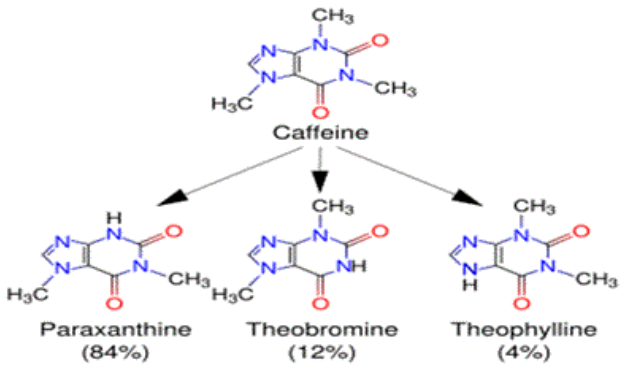

Figure 1: Caffeine and its metabolites.

Caffeine structure and its metabolism

Para xanthine (84%) Increase free fatty acid levels in the blood plasma.

Theo bromine (12%) increases urine volume.

Theophylline (4%) Relaxes smooth muscles of the bronchi, and is used to treat asthma [13].

An acute overdose of caffeine, usually in excess of 250 milligrams (more than 2-3 cups of brewed coffee), can result in a state of central nervous system overstimulation called caffeine intoxication [14]. The effects of caffeine on the body may begin as early as 15 minutes after in jesting and last up to hours [15]. Caffeine is highly addictive, caffeine increase stress level, caffeine accelerates aging and wrinkles [16]. Caffeine intake of 150-300 mg after a 10 h fast increased urinary calcium excretion 2-3 h after exposure in adolescent men and women [17].

Dehydration is a major drawback of caffeine consumption, and results from the drugs ability to increase urine production. In addition to dehydration, caffeine causes some people to get jittery stomachs or "coffee stomach"-which can be quite uncomfortable and mask any potential benefits [18].

100-200 mg dose of caffeine result in increased alertness and wakefulness, faster and clearer flow of thought, increased focus, and better general body coordination [19]. Caffeine makes people more alert, less drowsy, and improves coordination. Combined with certain pain relievers or medicines for treating migraine headache [20]. In a large 217,883 person study, those that consumed caffeine from any source had less kidney stone formation than those that did not consume caffeine [21].

Aims

Author selected this topic determination of caffeine in drinks available in market because now a day's energy and soft drinks play vital role in our daily life and become necessity of our life that's why I want to know all benefits and disadvantages of these drinks. So author determined the amount of caffeine in 9 brands of soft and energy drinks by using UV/VIS Spectrophotometer to obtained practical knowledge in the use of basic UV/VIS Spectrophotometer equipment.

- The main purpose of this research is to raise the awareness of negative effects of caffeine on human health because many companies' sales their products by highlighting few advantages of caffeine so companies should be labelled the caffeine content in drinks and also its effects as well as benefits.

Literature

Alpdogan et al. [22] concluded the caffeine concentration range in coca cola is 149.32 ± 0.68 mg/ml by using derivative spectrophotometric method and use Philips 8740UV/VIS Spectrophotometer at 232.7-245.2 nm and the caffeine concentration range of coffee is1.36 ± 0.03% at 268.5-289.5 nm and in the tea range of caffeine concentration is the 1.53 ± 0.03 % at 286.0-300.0 nm.

Alghamdi et al. [23] determined the content levels of some food additives (Aspartame, Caffeine, Sodium benzoate) in 29 different beverage samples commercially available in Riyadh local markets by using UV spectrophotometric method (Perkin-Elmer, USA). The caffeine contents in energy drink samples ranged from 22.64 ppm to 34.96 ppm.

Wanyika et al. [24] discussed the levels of caffeine in certain coffee (Nescafe, africafe, Dorman's) and tea (chai maramoja, kericho gold, sasini, final's premium) brands found in the Kenyan market were determined using High performance liquid chromatography (hplc) and UV/ Vis spectrophotometer (Shimadzu) at 274 nm which gave a concentration of 471 .73 ± 1 96.92 ppm.

Kalra et al. [25] studied the concentration of Caffeine of seven brands of soft drinks with the use of an analytical method, U.V Spectrophotometer Shimanzu 1800 Compact. which will tell us the best brand amongst different brands containing caffeine at 271.2 nm, highest concentration of caffeine was found in Power-ex (46 µg/ml) and the lowest concentration of caffeine in XXX (19.5 µg/ml).

Mohammed et al. [26] has done research on ten brands of beverages (soft and energy drinks) consumed in Basrah governorate/Iraq, and he were determinate its pH, trace minerals and caffeine contents by using UV/VIS spectrophotometer (shimadzu AA 630-12) at 254 nm wavelength. The caffeine concentration in beverages are these Kalaschnikow 103.13 ± 1.14, 2 Boom Boom 102.56 ± 1.11, Power horse 94.53 ± 0.10, O290.89 ± 1.02, Pit bull 79.99 ± 1.00, Pepsi 80.00 ± 1.34, Wild tiger 79.94 ± 0.22 , Mountain dew 44.08 ± 0.34.

Hillary et al. [27] studied the different brands of soft drinks and juices were randomly sampled from different stores in Eldoret town, the caffeine levels were found to be in the range of 1.43 mg/L and 40.51 mg/L.

Mufakkar et al. [28] has done research on the eight brands of soft drinks and determine the caffeine concentration by using Ultraviolet spectroscopy at 272 nm. The highest concentration of caffeine was found in Sting 500 mL (560.29 µg/mL). The lowest concentration of caffeine was found in 7 up 500 mL (29.71 µg/mL).

Hossain et al. [29] investigated to carry out to determine the pH, levels of caffeine and reducing sugar contents in five energy drinks available in local market in Rajthahi, Bangladesh by using UV/VIS Spectrophotometer. The pH of the beverages was perfectly acidic ranging from 2.85 to 3.11. The minimum caffeine level was observed in Brand-4 (40.34 mg/serving), while Brand-5 showed the highest caffeine content (244.57 mg/serving) showing a range from 40.34 to 244.57 mg/serving.

Material and Methods

Instrument

UV/VIS spectrometer Perkin Elmer lambda35. The double beam spectrophotometer having the range 190-1100 nm and bandwidth: 0.4-4 nm (variable).

pH determination

Beverages pH was determined by using Sartorius pH meter.

Preparation of stock solution

- All glassware was washed with distilled water.
- Then glassware was dried in oven at 105°C.

- A 100 ppm stock standard of caffeine was prepared by dissolving 20 mg caffeine in 250 ml carbon tetra chloride in 200 ml volumetric flask.

Preparation of standard solution

Working standards were prepared by pipetting 0.1,0.2,0.3,0.4,0.5 ml respectively aliquots of stock standard solution into a separate volumetric flasks of 100 ml and dilute it with carbon tetra chloride and forms 10,20,30,40,50 mg/L standards solution.

The absorbance of each solution was measured at absorption maximum of 270 nm using 10 mm quartz cuvettes.

Caffeine extraction procedure

- The brands of soft and energy drinks were taken by different shops.
- Then the sodium carbonate solution is prepared by dissolving 20 g sodium carbonate into distilled water in 25 ml volumetric flask.
- Then separating funnel was taken and adjusts it in the stands with beakers.
- Then 5 ml of drink sample was drawn in the separating funnel by addition of distilled water and add 1 ml of sodium carbonate solution in the separating funnel and add 20 ml of carbon tetra chloride in it.
- The caffeine was extracted by inverting funnel at least three times venting the funnel after each inversion.
- The non-aqueous carbon tetra chloride layer was removed to a clean 50 ml volumetric flask.
- Another 20 ml portion of carbon tetra chloride was added to aqueous solution in separating funnel and extraction procedure was repeated twice and carbon tetra chloride layers combined.

This procedure was repeated for all drink samples (Table 1). The absorbance of resulting solutions was measured on UV/Vis Spectrophotometer at 270 nm using 10 mm quartz cuvette (Figure 2).

Caffeine equivalent con mg/L	Absorbance
10	0.332
20	0.713
30	1.073
40	1.463
50	1.785

Table 1: Absorbance of resulting solutions at 270 nm.

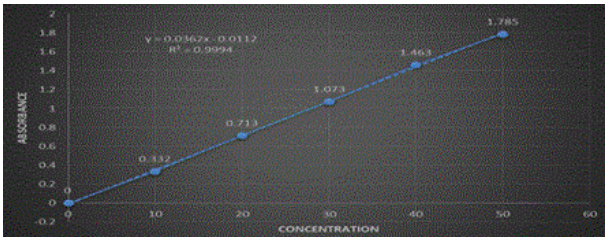

Figure 2: Standard calibration curve.

The regression line is Y=0.036x-0.0112

Dilution factor=flask volume/sample volume=50/5=10

Results and Discussions

Caffeine concentration and pH

The main objective of this research is to know the caffeine level in soft and energy drinks high or low then the published value or the FDA recommended value.

The Brand 2 has the highest pH 3.02 value among all soft drinks so it means it is less acidic among all soft drinks. And Brand 5 has lowest pH 2.29 among all soft drinks it means it is more acidic among all soft drinks. The soft drinks having pH range 2.29 to 3.02.

The ideal (neutral) pH of the mouth ranges from 6.5-7.5. A pH of 5.5 is considered to be the threshold level for the development of dental decay. Both soft drinks and sports drinks have been shown to have a pH between 2.5 and 3.5. Demineralization of tooth enamel will occur more rapidly if the pH drops below the critical 5.5 level for long periods of time and if the pH is dropped below the critical level frequently. All kinds of soft drinks are acidic and that cola drinks especially make our bodies poor in oxygen.

The caffeine concentration range in soft drinks is 10.69-42.17 ppm. And the concentration of Brand 1 is 37.62 at 270 nm. Similarly caffeine concentration in Brand 2 was found to be 12.345 ppm, caffeine concentration in Brand 3 was found to be 10.69 ppm, caffeine concentration in Brand 4 was found to be 19.11 ppm and caffeine concentration was found to be 42.17 ppm.

The highest caffeine concentration was found to be in Brand 5 which is 42.17 ppm and it has lowest pH value so it means Brand 5 is most acidic among all soft drinks. So it is strongest central nervous system stimulant. So it can be discontinued in market.

The lowest caffeine concentration was found to be in Brand 3 which is 10.69 ppm and pH values are high so it means these brands are less acidic so it can be sold in market and a weak central nervous system stimulant. The US food and Drug Administration (FDA, 2006) limits maximum amount of caffeine in soft drinks is 200 pm in 6 mg/oz. Therefore caffeine content allowed in soft drinks may be in range between 30-72 mg in 355 ml (Table 2).

Brands names	Conc. in mg/L	Conc. in mg/L with dilution factor	pH
Brand 1	3.762	37.620	2.45
Brand 2	1.234	12.340	3.02
Brand 3	1.069	10.690	2.57
Brand 4	1.911	19.110	2.71
Brand 5	4.217	42.170	2.29

Table 2: Caffeine content allowed in soft drinks.

Statistical analysis of soft drinks

The variance and standard deviation of Brand 1 was 3.8088 and 1.95161 respectively. The variance and standard deviation of Brand 2 was 0.34445 and 0.5869 respectively. The variance and standard

deviation of Brand 3 was 0.9522 and 0.5869 respectively. The variance and standard deviation of Brand 4 was 0.605 and 0.77782 respectively. The variance and standard deviation of Brand 5 was 1.87986 and 1.37108 respectively (Table 3).

Mean=Sum of X values/N(Number of values)

Standard deviation= S=√(X-M)^2/n-1

Variance=s2

Brands name	Mean	Variance	Standard deviation
Brand 1	37.62	3.8088	1.95161
Brand 2	12.345	0.34445	0.5869
Brand 3	10.69	0.9522	0.97581
Brand 4	19.11	0.605	0.77782
Brand 5	42.17	1.87986	1.37108

Table 3: Variance and standard deviation.

Caffeine concentration snd pH

The pH range in energy drinks is 2.85-3.28. The Brand 9 having pH 2.85 it means it is highest acidic among all energy drinks and E1 have highest pH which is 3.28 so it means it less acidic among all energy drinks.

The low pH values could be as a result of presence of carbon dioxide, phosphoric acids, malic acid, tartaric acid used as preservatives by manufactures of these beverages.

The acids inhibit growth of microorganism, bacteria, fungal may contaminate beverages.

E4>E3>E2>E1

The caffeine concentration range in energy drinks are 32.05-101.905 ppm and the concentration of E1 59.95 ppm. Similarly caffeine concentration in E2 was found to be 101.705 ppm, caffeine concentration in E3 was found to be 46.185 ppm, caffeine concentration in E4 was found to be 32.05 ppm.

The highest caffeine concentration was found to be in E2 which is 101.705 ppm and it has lowest pH value so it means E2 is most acidic among all energy drinks. So it is strongest central nervous system stimulant. So it can be discontinued in market.

The lowest caffeine concentration was found to be in E4 which is 32.05 ppm and pH values are high (Table 4) so it means these brands are less acidic so it can be sold in market and a weak central nervous system stimulant (Table 5).

Brands names	Con in mg/L	Con in mg/L with dilution factor	pH
E1	5.999	59.990	3.28
E2	10.170	101.700	3.22
E3	4.618	46.180	2.98
E4	3.205	32.050	2.85

Table 4: Caffeine concentration range in energy drinks.

Brands name	Mean	Variance	Standard deviation
E1	59.995	0.15125	0.38891
E2	101.705	7.48845	2.7365
E3	46.185	29.87645	5.46594
E4	32.05	3.645	1.90919

Table 5: Variance and standard deviation of energy drinks.

Statistical analysis of energy drinks

The variance and standard deviation of E1 was 0.15125 and 0.38891 respectively. The variance and standard deviation of E2 was 7.48845 and 2.7365 respectively. The variance and standard deviation of E3 was 29.87645 and 5.46594 respectively. The variance and standard deviation of E4 was 3.645 and 1.90919 respectively.

Comparison of labelled and calculated concentrations

The E1 had been labelled concentration was 300 mg per 250 ml and calculated 299.95 mg per 250 ml. The E2 is the first brand which have ginseng that cause low tension, relieve stress, Stimulate metabolism (Figure 3). It is manufacture by king beverages and its calculated caffeine content is 508.5 mg per 250 ml and labelled concentration was 450 ppm/250 ml. The E3 had labelled concentration was 300 mg/250 ml and calculated 230.9 mg per 250 ml so it is less than its labelled caffeine content amount (Table 6). The E4 had labelled concentration was 200 mg per 500 ml and calculated amount of caffeine 160.27 mg per 500 ml it is less than its labelled caffeine content amount (Table 7).

Brands name	Serving size (ml)	Calculated con in ppm	Labelled con in ppm
E1	250	299.95	300
E2	250	508.5	450
E3	250	230.9	300
E4	500	160.27	200

Table 6: Calculated amount of caffeine in energy drinks.

Concentration in ppm 250 ml=concentration in ppm per 5 ml × 50

Concentration in ppm per 500 ml=concentration in ppm per 5 ml × 100

Brands name	serving size in (ml)	Con.in ppm
Brand 1	250	188
Brand 2	250	61.72
Brand 3	500	106.9
Brand 4	250	95.55
Brand 5	250	210.85

Table 7: Calculated amount of caffeine in beveragers.

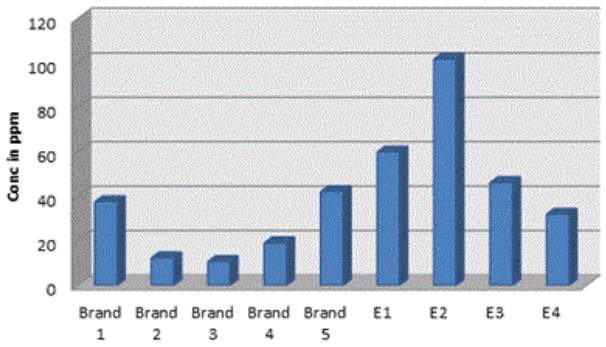

Figure 3: Comparision betwwen soft drinks and energy drinks.

Conclusion

Determination of caffeine content in non-alcoholic beverages and energy drinks is very important analytical process safeguard the well-being of people who are unaware to adverse effects of caffeine. In soft drinks the Brand 5 have highest concentration of caffeine that is 42.17 ppm and Brand 3 having low concentration of caffeine 10.69 ppm and in energy drinks Brand 2 having high concentration of caffeine that is 101.705 ppm. Brand 9 having low concentration of caffeine that is 32.05 ppm among all energy the process of determination of caffeine in drinks can done by many analytical method but in this research UV/VIS Spectrophotometer was used because it is relatively easy, fast, cheap, highly sensitive and give accurate concentration of caffeine.

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