



## **Determination of Caffeine in Soft and Energy Drinks Available In Market By Using U.V/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 wave length. 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 E<sup>2</sup>. The E<sup>2</sup> has greater calculated concentration than the labeled concentration. The minimum caffeine level of energy drinks was observed in E<sup>4</sup> (32.04 mg/L) while E<sup>2</sup> showed highest caffeine level in energy drinks (101.705mg/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).*

**Key words:** Caffeine, UV Spectrophotometer, Comparison of soft and energy drinks

Received 12.03.2016

Revised 20.04.2016

Accepted 10.05.2016

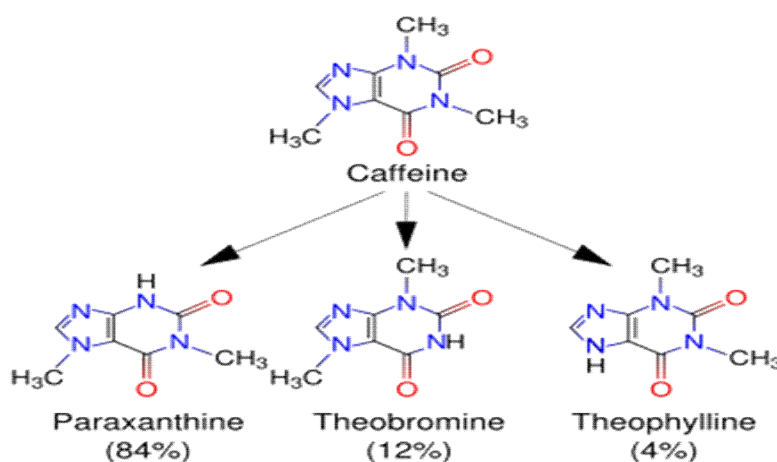
### **INTRODUCTION**

Caffeine is a most common ingredient of energy drinks. It is added as a flavoring 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.

Formula:	C <sub>8</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub>
Molar mass	194.19 g/mole
Boiling point:	178 °C
IUPAC ID:	1,3,7-Trimethylpurine-2,6-dione
Melting point	235 °C
Density	1.23 g/c

Different energy drinks having different amount of caffeine and its range is from 50-300 mg. Most people experience no behavioral 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 100mg. Decaffeinated coffee isn't actually caffeine-free, and can contain up to 12mg of caffeine. Your average cup of tea contains 85mg of caffeine. A single can of commercially available energy drink can have anywhere between 80 and 280mg of caffeine depending on the can size. Green tea is close behind with 60mg of caffeine, followed by white tea with 55mg. Slim-fast chocolate drinks come in at 20mg 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 isoenzyme) into three metabolic dimethyl xanthine's which each have their own effects on the body [12].



### 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

I selected this topic determination of caffeine in drinks available in market because now a days 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 I 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 labeled the caffeine content in drinks and also its effects as well as benefits.

## MATERIAL AND METHODS

### INSTRUMENT

UV/VIS spectrometer Perkin Elmer lambda 35. The double beam spectrophotometer having the range 190-1100 nm and bandwidth: 0.4-4nm (variable).

### PH DETERMINATION

Beverages PH were determined by using Sartorius pH meter

### PREPARATION OF STOCK SOLUTION

- All glassware were washed with distilled water
- Then glassware were dried in oven at 105 degree Celsius
- A 100ppm stock standard of caffeine was prepared by dissolving 20 Mg caffeine in 250ml carbon tetra chloride in 200ml volumetric flask

### PREPARATION OF STANDARD SOLUTION

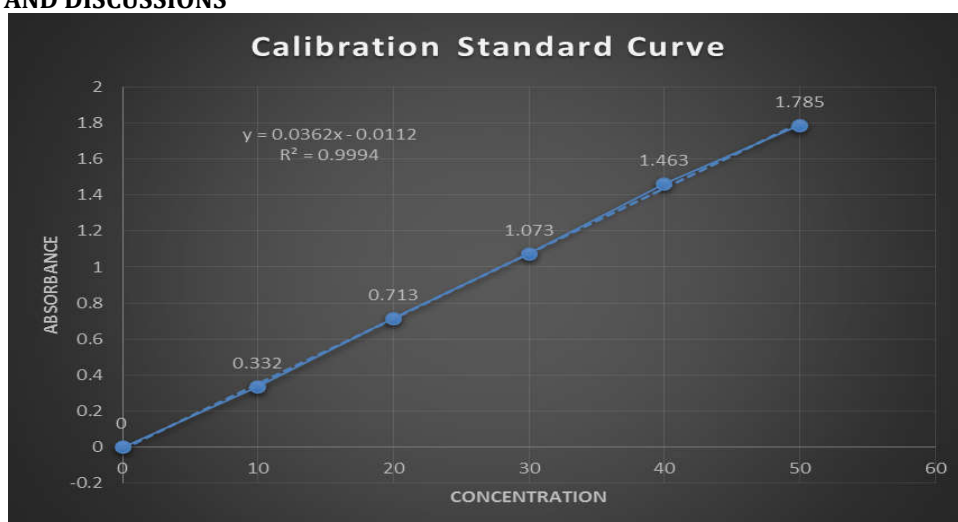
Working standards were prepared by pipetting 0.1, 0.2, 0.3,0.4,0.5 ml respectively aliquots of stock standard solution into a separate volumetric flasks of 100ml and dilute it with carbon tetra chloride and forms 10, 20,30,40,50 mg/L standards solution.

The absorbance of each solution was measured at absorption maximum of 270nm using 10mm 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 20g sodium carbonate into distilled water in 25ml volumetric flask.
- Then separating funnel was taken and adjusts it in the stands with beakers.
- Then 5ml of drink sample was drawn in the separating funnel by addition of distilled water and add 1ml 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 The absorbance of resulting solutions was measured on UV/Vis Spectrophotometer at 270 nm using 10mm quartz cuvette.

## RESULTS AND DISCUSSIONS



The regression line is  $Y = 0.036x - 0.0112$

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

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

The caffeine concentration ranges in soft drinks are 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 12.345ppm, caffeine concentration in Brand 3 was found to be 10.69ppm, caffeine concentration in Brand 4 was found to be 19.11ppm and caffeine concentration was found to be 42.17 ppm

The highest caffeine concentration was found to be in Brand 5 which is 42.17ppm 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 6mg/oz. therefore caffeine content allowed in soft drinks may be in range between 30-72mg in 355ml.

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

#### 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.

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

Standard deviation =  $S = \sqrt{\frac{\sum (X-M)^2}{n-1}}$

Variance =  $s^2$

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

#### CAFFEINE CONCENTRATION AND P.H

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 32.05-101.905ppm. And the concentration of E1 59.95ppm. Similarly caffeine concentration in E2 was found to be 101.705ppm, caffeine concentration in E3 was found to be 46.185ppm, caffeine concentration in E4 was found to be 32.05ppm.

The highest caffeine concentration was found to be in E2 which is 101.705 ppm and it has lowest P.H 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 so it means these brands are less acidic so it can be sold in market and a weak central nervous system stimulant.

Brands names	Con in mg/L	Con in mg/L with dilution factor	pH
E <sup>1</sup>	5.999	59.990	3.28
E <sup>2</sup>	10.170	101.700	3.22
E <sup>3</sup>	4.618	46.180	2.98
E <sup>4</sup>	3.205	32.050	2.85

### 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.

Brands name	Mean	Variance	Standard deviation
E <sup>1</sup>	59.995	0.15125	0.38891
E <sup>2</sup>	101.705	7.48845	2.7365
E <sup>3</sup>	46.185	29.87645	5.46594
E <sup>4</sup>	32.05	3.645	1.90919

### COMPARISON OF LABELED AND CALCULATED CONCENTRATIONS

The E1 had been labeled concentration was 300mg 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 .It is manufacture by king beverages and its calculated caffeine content is 508.5mg per 250ml and labeled concentration was 450 ppm per 250 ml .The E3 had labeled concentration was 300mg per 250 ml and calculated 230.9mg per 250 ml so it is less than its labeled caffeine content amount .The E4 had labeled concentration was 200mg per 500 ml and calculated amount of caffeine 160.27mg per 500ml it is less than its labeled caffeine content amount.

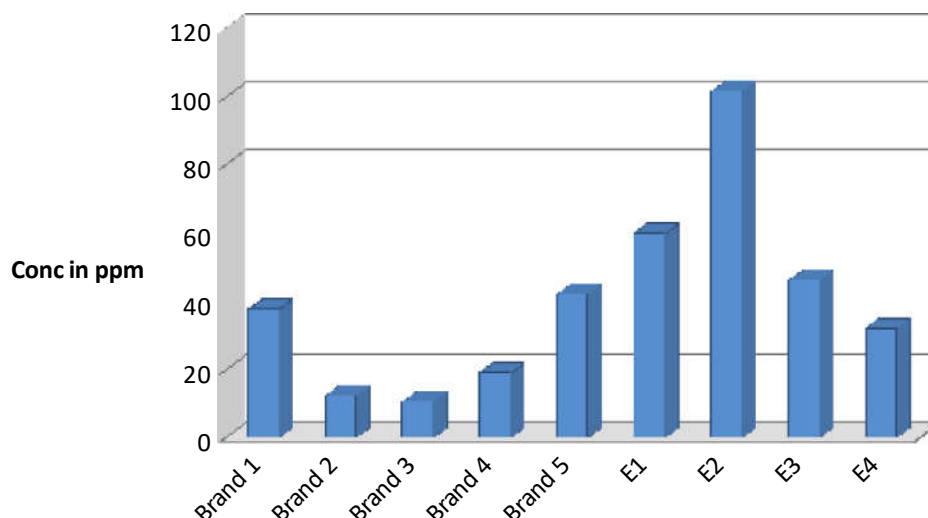
Brands name	Serving size (ml)	Calculated con in ppm	Labeled con in ppm
E <sup>1</sup>	250	299.95	300
E <sup>2</sup>	250	508.5	450
E <sup>3</sup>	250	230.9	300
E <sup>4</sup>	500	160.27	200

Concentration in ppm 250ml = concentration in ppm per 5ml \*50

Concentration in ppm per 500ml= concentration in ppm per 5ml \*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

### Comparison b/w Sof drinks and energy drinkg



### CONCLUSION

Determination of caffeine content in non-alcoholic beverages and energy drinks is very important analytical process safeguard the wellbeing 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 32.05ppm 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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#### **CITATION OF THIS ARTICLE**

Sohail Ahmad, Asma Khalid, Nadia Parveen, Ayesha Babar, Rida Aslam Lodhi, Badar Rameez, Amber Shafi, Nadia Noor, Faiza Naseer. Determination of Caffeine in Soft and Energy Drinks Available In Market By Using U.V/Visible Spectrophotometer. *Bull. Env. Pharmacol. Life Sci.*, Vol 5 [8] July 2016: 15-21



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