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# QUANTITATIVE DETERMINATION OF CAFFEINE IN IMPORTED ENERGY DRINKS OF LAHORE, PAKISTAN

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#### Abstract

Caffeine is a central nervous system stimulant and an efficient psychoactive stimulant medication when taken in moderate amount. Recently, caffeine has become one of the main substances in the composition of energy drinks. Moreover, various compounds also used to preserve energy drinks, especially citric acid. In present study, the levels of caffeine concentration were determined in six imported energy drinks - Lucozade, Bullet, Monster, Black Bruin, Red Bull and 3 Horses - available in local market of Pakistan, to ensure that whether the companies meet the caffeine concentration content of recommendation (400 mg/mL) or not. pH level, percentage acidity and sugar content of energy drinks were also determined. Among the six samples, the maximum amount of caffeine was found in Black Bruin (9.96 mg/100 mL) and the lowest in Lucozade (8.36 mg/100 mL), showing a range from 9.96 to 8.36 mg/100 mL. The % age of acidity were found to be 1.536, 1.792, 1.664, 1.344, 1.376 and 1.620 for Lucozade, Bullet, Monster, Black Bruin, Red Bull and 3 Horses respectively. pH meter was used to determine the pH at 20 °C, and result were 3.18, 3.82, 3.93, 3.58, 3.67 and 3.41 for Lucozade, Bullet, Monster, Black Bruin, Red Bull and 3 Horses respectively, showing a range of 3.18 to 3.93. In all energy drinks, caffeine concertation was found below the maximum allowable limit recommended by FDA. Furthermore, it is found that even two serving size packs of any selected sample will have no health impact on individual.

# **Keywords**

Caffeine, Energy Drinks, FDA, Lahore



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#### 1. Introduction

Caffeine (1,3,7-trimethylxanthine) is a white crystalline natural alkaloid with a bitter taste and widely used as a mellow psychoactive stimulant medication and has a low diuretic activity. It is found in differing amounts in leaves, seeds and products of numerous types of plants (Nour et al., 2010; Wanyika et al., 2010; Andrews et al., 2007). The espresso, cola nuts, tea leaves and cocoa beans are regular wellsprings of caffeine. A germen chemist extracted caffeine in 1819 at laboratory level (Anna & Kurek, 2013; Jarvis, 2002). The central nervous, muscular and cardiovascular systems in human stimulates by caffeine intake, thus during the unusual weakness it is used to eliminate physical fatigue and restore mental health (Maidon et al., 2012; Claudio et al., 2013). It is believed that ancient Chinese used tea leaves to made tea in stone ages (2737 B.C.) The plant-derived foods including guarana, coffee beans, kola nuts, tea leaves, and cocoa beans introduced the caffeine in human diet (Barone and Roberts, 1996). As caffeine gives the basic energizing boost, therefore, in 1960s emerged as dietary supplement in Asia and Europe on the demand of consumer (Reissig et al., 2009). Recently, coffee and energy drinks has become major contributor of caffeine in human's food chain (Mitchell et al., 2015; Richards and Smith 2016). Caffeine has beneficiary impact on human including reduce in physical fatigue, smooth muscle relaxation and improve in athletic performance in some cases (McLellan et

al., 2005; Lisko et al., 2017). The caffeine ingestion of more than 400 mg/day by a woman can enhance the risk of detrusor instability (unstable bladder) and even 200 to 400 mg caffeine consumption may increase the detrusor instability for woman with pre-existing bladder symptom (Arya et al., 2000). The consistent acute doses of caffeine > 250 mg/person can also induce an increase in blood pressure (systolic 5-15 mmHg, diastolic 5-10 mmHg) in both genders irrespective of the race, age, habitual caffeine ingestion and blood pressure status (Nawrot et al., 2003). In sound grownups, a maximum 400 mg caffeine intake per day is viewed as protected; intense clinical poisonous quality starts at 1g, and 5 to 10g can be deadly (Amos et al., 2014). The maximum caffeine intake  $\leq 400$  mg/day in adults,  $\leq 300$ mg/day in pregnant women and  $\leq 2.5$  mg/kg-day children remain appropriate recommendations.

Once caffeine is ingested, it is readily absorbed by gastrointestinal tract and entered the bloodstream. After ingestion, caffeine takes only 1-1.5h to reach the maximum level in blood and distributed throughout the body (Arnaud *et al.*, 1999).

Among beverages, energy drinks are most popular drinks in adolescents. Caffeine is the basic functional ingredient of energy drinks, many of them consist of 70 to 80mg per 8-oz serving pack. The stimulant activity, bitter taste and the addictive behavior make the caffeine attractive ingredient of energy drinks.

Commonly, energy drinks used to gain energizing boost during physical fatigue, however, addictive behavior of caffeine could be harmful (Seifert *et al.*, 2011).

In liver, the cytochrome P450 oxidase enzyme system (specifically, the 1A2 isoenzyme) metabolizes caffeine into three different metabolites (Figure 1). Each of these three primary metabolic dimethyl xanthine; paraxanthine (84%), theobromine (12%) and theophylline (4%) have its own effect on human body (Bolton et al., 1981). Paraxanthine increases the levels of free fatty acid in blood plasma, theobromine increases the level of urine volume and theophylline uses to relax smooth muscles of the bronchi and to treat asthma (Newton et al., 1981).

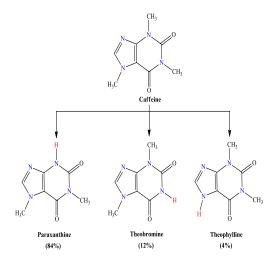


Figure 1: Structural Formula Of Caffeine And Primary Dimethyl Xanthine

Energy drinks are fast growing beverages and are most popular among adolescents. Caffeine is the main ingredient of energy drinks and has benefit as well as negative impact for human health. Therefore, many studies have been reported for the determination of caffeine in energy drinks around the globe. The main aim of the present research work was to determine the level of caffeine concentrations in imported energy drinks available in local market of Lahore, Pakistan. Determination of the levels of pH, acidity and BRIX index of selected energy drinks are also part of this manuscript.

#### 2. Materials And Methods

# 2.1 Materials and Instrumentation

The caffeine (sigma reference standard, sigma Aldrich) activated charcoal (sigma Aldrich), double distilled water, phenolphthalein solution (sigma Aldrich) and sodium hydroxide NaOH (1 N) were provided by Food and Biotechnology Research Center, PCSIR Lahore. Six samples of energy drinks of different imported brands namely Lucozade, Bullet, Monster, Black Bruin, Red Bull and 3 Horses were collected from native market of Lahore, Pakistan. All glassware were washed with distilled water and dried in oven at 100°C. Electronic balance (Electric Mettler Toledo adjust, show AL 204) was used for mass measurement of materials. UV-visible double beam spectrophotometer (Lambda 25, Perkin Elmer) was used to determine the caffeine concentrations. pН values measured with WTW 1F10-220 Inolab Level 1 Multiparameter Meter without Probe 110 V. The sugar content of energy drinks was determined with high quality sweetness test refractometer

sugar measuring reader meter (range 0-20% brix).

2.2 Determination of caffeine concentration
Concentration of caffeine in energy drinks was
determined by reported methodology (Dutta et
al, 2014). Schematic illustration was given in.
Figure 3.

# 2.2.1 Stock solution preparation

Standard stock solution of caffeine was prepared by dissolving 10 mg caffeine in double distilled water in a 100 mL volumetric flask up to the mark.

# 2.2.2 Standard Preparations

For the preparation of working standards, the aliquots of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 mL from standard stock solution were taken in separate volumetric flasks and diluted up to 100 times by using double distilled water (Table 1).

Table 1: Standard Stock Preparations Of Caffeine

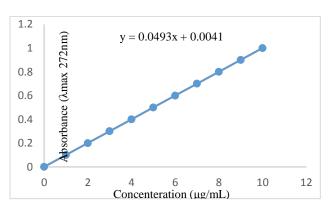
Sr. No.	Standard stock	Distilled water
	solution (mL)	(mL)
1	02	98
2	04	96
3	06	94
4	08	92
5	10	90
6	12	88
7	14	86
8	16	84
9	18	82
10	20	80

Aliquots of the standard preparations were taken in 10 mm quartz cuvettes and examined for the absorbance of caffeine by using UV-vis spectrophotometer at 272 nm wavelength because caffeine exhibits maximum absorbance

at 272 nm wavelength and is in accordance with similar studies (Aslam *et al.*, 2019). Table 2 and Figure 2 shows the absorbance values and calibration curve for standard preparations of caffeine.

**Table 2:** Absorbance of Caffeine for Standard Stock Solutions

Sr. No.	Concentration	Absorbance	
Sr. No.	(µg)	(nm)	
0	0	0	
1	1	0.10	
2	2	0.20	
3	3	0.30	
4	4	0.40	
5	5	0.50	
6	6	0.60	
7	7	0.70	
8	8	0.80	
9	9	0.90	
10	10	1.00	



**Figure 2:** Absorbance Of Standard Preparations Of Caffeine

The calibration curve was plotted between the concentration (µg) and absorbance. The concentration was taken along the x-axis and absorbance along the y-axis (Khalid *et al.*, 2016). Caffeine content of energy drinks was determined by using the following equation,

which is derived from the above calibration curve.

y = 0.049 + 0.004

x = y - 0.004/0.049

here.

x = concentration

y = absorbance

# 2.2.3 Sample preparation

Preparation of samples was carried out in three steps, firstly, de-carbonation of all energy drink sample was done by removing carbon dioxide. For this purpose, the sample bottles were shacked after regular intervals and kept open for 3 days to remove whole carbon dioxide. Secondly, all sample of energy drinks were decolorized by adding 0.1 gm activated carbon / Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> in each sample (10 mL) of energy drink and kept for five minutes. Thirdly, in case of activated charcoal, each sample was filtered with the filter paper but in case of Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> the filtration is not required, and samples are used as such. To determine the absorption of caffeine, sample aliquots were taken in quartz cuvettes and observed with UV-vis spectrophotometer at  $\lambda_{max}$  272 nm.

# 2.2.4 Role of calibration curve

The caffeine concentrations of energy drinks were analyzed with the help of standard curve as reference (Khalid *et al.*, 2016).

#### 2.3 Determination of pH

The pH values of energy drink samples were determined by using WTW 1F10-220 Inolab Level 1 Multiparameter Meter without Probe, 110 V.

# 2.4 Determination of Brix Level

The BRIX level (symbol °Bx) of all sample energy drinks was determined with the help of Digital Brix Refractometer. A drop of energy drink sample was placed on the slide of the BRIX meter and analyzed. Slide of the BRIX meter was cleaned appropriately with cotton wool each time before using.

# 2.5 Determination of Acidity

The determination of the acidity was done by simple acid base titration, based on the concentration of citric acid. Sample solution of the energy drink (10mL) and distilled water (90mL) was taken, subsequently 1 - 2 drops of phenolphthalein were added as indicator. The prepared sample salutation was titrated against the NaOH standard solution (0.1N). Following equation was used to calculate the %age acidity:

Acidity(%age)

 $= \frac{\text{Standard solution used (mL)x Normality x 0.064 x 100}}{10}$ 

#### 3. Results and Discussion

Six imported energy drinks available in Lahore, namely Lucozade, Bullet, Monster, Black Bruin, Red Bull and 3 Horses were used to analyze their caffeine content by UV-vis spectrophotometer, acidity through acid base titration, pH level at 20 °C via pH meter and BRIX level by digital Brix refractometer.

UV vis. Absorption method was used to determine caffeine concentration by taking distilled water as reference. Results shown that the caffeine concentration in imported energy drinks ranged from 8.36 to 9.96 mg/100 mL.

The highest content of caffeine (9.96 mg/100 mL) was observed in Black Bruin. Lucozade shown the lowest caffeine content (8.36 mg/100 mL). Similarly, the observed content of caffeine in Bullet was 8.84 mg/100 mL, while in Brand Monster the caffeine content was 8.82 mg/100 mL. Furthermore, Caffeine content in Red Bull, 3 Horses, Bullet and Monster was 9.42 mg/100 mL, 9.38 mg/100 mL, 8.84 mg/100 mL and 8.82 mg/100 mL respectively (Table III). The highest content of caffeine was observed in Black Bruin; therefore, it is the strongest stimulant to the central nervous system among all sample.

The Food and Drug Administration (FDA) characterizes caffeine as a generally recognized as safe ingredient. However, the maximum content in carbonated drinks is restricted to 0.02% or no more than 200 ppm by FDA (Dutta *et al.*, 2014). The maximum legitimate measure of caffeine permitted within 355 mL (12 oz) cane of energy beverage is around 71 mg. In this way, Bullet, Black Bruin and Red Bull can contain the highest caffeine level up to 50 mg/250 mL, Lucozade and 3 Horses must be below than 66 mg/330 mL and Monster 71 mg/355 mL. Here all the energy drinks did not cross the limit set by FDA (Table III).

**Table 3:** Found Caffeine Concentrations

Brand Name	Calculated conc. (mg/100mL)	Calculated conc. per bottle (mg/mL)	
Lucozade	8.36	27.60/330	
Bullet	8.84	22.10/250	
Monster	8.82	31.31/355	
Black	9.96	24.90/250	

Bruin		
Red Bull	9.42	23.55/250
3 Horses	9.38	30.96/330

# 3.1 Comparison of Labeled and Calculated Concentrations of Caffeine

There are too much large differences in calculated and labeled contents of caffeine for all energy drink samples. Hence, all brands mislead the customer by labeling wrong content of caffeine (Table 4).

**Table 4:** Comparison Of Labeled And Calculated Caffeine Concentrations

Brand Name	Calculated conc.	Labeled conc.	
	(mg/100mL)	(mg/100mL)	
Lucozade	8.36	30	
Bullet	8.84	30	
Monster	8.82	30	
Black	9.96	15	
Bruin	7.90	13	
Red Bull	9.42	30	
3 Horses	9.38	32	

# 3.2 pH

The measured pH value for the Brand Monster is 3.93, which was the highest among all energy drinks, hence, it implies that it is least acidic among all beverages. The least pH value observed for Lucozade which is 3.18 and it implies that is highly acidic. So, pH ranging from 3.18 to 3.93 at 20 °C for all samples of energy drinks (Table 5). Different acids used as preservatives are responsible for these pH values (Table 8). The oral pH ranged from 6.5 to 7.5. If pH falls up to critical level (5.5) it may cause tooth rot (Hossain *et al.*, 2015). All energy drink

samples were happened acidic in nature. The preservatives such as acids and carbon dioxide used in energy drinks lower the oral pH values (Table 3).

**Table 5:** pH of Energy Drinks

Sample	pН	Temperature °C
Lucozade	3.18	20.1
Bullet	3.82	20
Monster	3.93	20.3
Black Bruin	3.58	20
Red Bull	3.67	20
3 Horses	3.41	20

#### 3.3 BRIX level

The measured BRIX level for each of the energy drink sample is shown in Table VI. The results shown that the BRIX level ranged from 7 to 13 BRIX units. Sample 3 Horses had least sugar content i.e., BRIX level 7 while sample Bullet had the highest sugar content i.e., 13 BRIX units. Frequent utilization of sugar-containing beverages can increase danger of dental caries.

The high content of sugar in energy drinks make challenging for spit to remove this item from the tooth and hence the pH stays more acidic for a more drawn-out timeframe. Refined sugars assumed a significant part in the dental caries (Hossain *et al.*, 2015).

Table 6. BRIX levels of energy drinks

Sample name	BRIX (units)
Lucozade	8
Bullet	13
Monster	12
Black Bruin	12
Red Bull	10
3 Horses	7

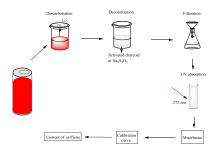
# 3.4 Acidity

Titration is a simple, financially suitable and solid technique to be utilized in day by day and routine lab research work (Van Staden *et al.*, 2002). These are the reasons why we used such technique to do this imperative investigation.

The determined percentage acidity for all 6 sample energy drinks is given in (Table VII). The content of citric acid in all selected sample energy drinks ranged between 1.344% - 1.792%. The percentage acidity determined for imported Brands of Energy drinks i.e., Lucozade, Bullet, Monster, Black Bruin, Red Bull and 3 Horses is 1.536%, 1.792%, 1.664%, 1.344%, 1.376% and 1.620% respectively. The use of citrus extract in energy drinks generally reflects levels of acidity, regularly found in common organic products (Orange 1 %, grapefruit 1.5 % and lemon 2.5 %) (Brima *et al.*, 2014). Our results in this investigation for the percentage of acidity are comparable with natural fruits.

**Table 7:** The %age of acidity

Sample	Acidity
Name	%
Lucozade	1.536
Bullet	1.792
Monster	1.664
Black	1.344
Bruin	1.344
Red Bull	1.376
3 Horses	1.620



**Figure 3:** Schematic Illustration For Determination Of Caffeine Concentration

# 4. Conclusion

Caffeine is one of the fundamental substances inculpated in the manufacture of energy drinks. Caffeine containing energy drinks are widely used now-a-days in youngsters at an alarming amount and rate. Due to psychoactive property of caffeine, it is of great significance to get knowledge, about such types of energy drinks because caffeine is widely consumed in all over showed world. Results that concentration of caffeine was present in Black Bruin and less amount of caffeine was present in Lucozade. Caffeine concentrations in all sample energy drinks were below the limit set by FDA but wrong content of caffeine was labeled on all selected energy drink cane. The provincial food authorities are suggested to take action against these brands for their misleading labeled contents of ingredients.

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**Table 8: Ingredients Of Imported Energy Drinks Under Study** 

Monster	Bullet	Lucozade	Black Bruin	Red Bull	3 Horses
Monster Energy Ltd. Ireland	Sun Mark Ltd. England	Cott Beverages, UK	Daren-Oguz Food, Turkey	Red Bull, UK	Not mentioned (made in Netherlan ds)
Carbonated water Sucrose, glucose syrup	Carbonated water Sugar, Glucose, Fructose syrup	Carbonated water Glucose syrup (13%), Sweeteners (Aspartame, Acesulfame K)	CO <sub>2</sub> Glucose, Fructose syrup	Carbonated water Glucose, Fructose	CO <sub>2</sub> Glucose, Fructose syrup
Citric acid Sodium citrate	Citric acid, Acidifier Acidity regulator (E331)	Citric acid, Lactic acid Sodium citrate	Citric acid Sodium citrate	Citric acid	Sodium citrate
Caffeine Taurine (0.4%) Color (anthocy anins) Natural and artificial flavor	Caffeine (0.03%) Taurine (0.38%) Coloring (E150C) Flavorings	Caffeine - Color (Sunset yellow, Ponceau 4R)	Caffeine (150mg/L) Taurine (800mg/L) Ammonia caramel, Riboflavin elol	Caffeine (0.03%) Taurine (0.4%) Caramel	Caffeine (0.032%) Taurine (0.04%) Caustic Sulphite caramel (E150B)
Inositol Vitamins (B3, B2, B12)	Inositol Vitamins (Pantothe nic acid B5, B6, B12), Nicotina mide	- Vitamin (C antioxidant)	Inositol (100mg/L) Vitamins (B3, Pantotheni c acid B5, B6, B2, B12)	Inositol (100mg/L) Vitamins (B2, B3, Pantothe nic acid B5, B6, B12)	Inositol Vitamins
Glucuronolactone	Glucuronolactone (0.01%)	-	Glucuronolactone	Glucuronolactone	Glucuronolactone
D-Glucuronolactone Panax Ginseng Root Extract (0.08%)	-	-	Ginseng Extract	-	-
L-Carnitine L- Tartrate (0.4%)	-	-	-	-	-
Maltodextrin Preservatives (Sorbic acid, Benzoic acid) Sodium chloride	Dextrose -	Potassium sorbate	-	Sodium carbonate Magnesium carbonate	-
Guarana (0.02%)	-	-	-	-	-