

ESTIMATION OF CAFFEINE CONTENT FROM SOFT AND ENERGYDRINKS OBTAINED FROM REGIONAL MARKET BY UV SPECTROSCOPY AND TLC.

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Abstract: This research is for to estimate the concentration of caffeine in soft drinks and energy drinks. In pure form, caffeine is a crystalline white powder moderately soluble in water and in a wide range of organic solvent such as ethanol, ethyl acetate, methanol, dichloromethane, chloroform etc. The caffeine is determined by using UV-Visible spectroscopy method using dichloromethane as an extracting solvent at 273nm wavelength. The method was found to be fast, simple, cost effective and environmental friendly for the determination of caffeine in soft drinks with satisfactory results. Caffeine is found in many beverages such as coffee, tea, energy drinks and cola, which make the drinks addictive. Caffeine content in various energy drinks and beverage varies from 10 to 50 mg of caffeine per serving; however the US Food and Drug Administration (FDA, 2006) limits the maximum amount in carbonated beverages to 6 mg/oz. Large amount of caffeine consumption can cause physiological and psychiatrically dependence. The aim of this study is to determine the concentration of caffeine in soft drinks and energy drinks brands available in India to ensure whether the caffeine concentration in the follow soft drinks & energy drinks as per FDA recommendation or not. And other purpose of this research is to find erosion property of caffeine by determining buffer capacity. This research also helps to identify purity of caffeine by TLC (By measuring R_f value of sample compared with standard caffeine).

Keywords: Caffeine, soft drinks & energy drinks, UV Spectroscopy, TLC.

INTRODUCTION: Caffeine is a frequently used ingredient of energy drinks. It is added as a flavoring agent and to make the drinks addictive. Caffeine is a bitter in taste, white crystalline xanthenes alkaloid that acts as a psychoactive stimulant drug and a mild diuretic. It is an alkaloid of methylxanthine family. The methylxanthines caffeine (1,3,7-trimethylxanthine), theobromine (3,7-dimethylxanthine), and theophylline (1,3-dimethylxanthine) can be normally found in cola nuts, coffee beans, cocoa beans, tea leaves, mate leaves and other kinds of plants. Caffeine serves as an ingredient in many carbonated soft drinks including colas, pepper-type beverages, and citrus beverages. Pure caffeine occurs as Odorless, white, fleecy masses, glistening needles or powder. Caffeine has drawn more attention in the past decades due to its physiological effects beyond that of its stimulatory effect. The Food and Drug Administration (FDA) defines caffeine as a generally recognized as safe (GRAS) substance. However, FDA specifies that the maximum amount in carbonated beverages is limited to 0.02% (FDA 2006) [8,9]

STRUCTURE OF CAFFEINE:

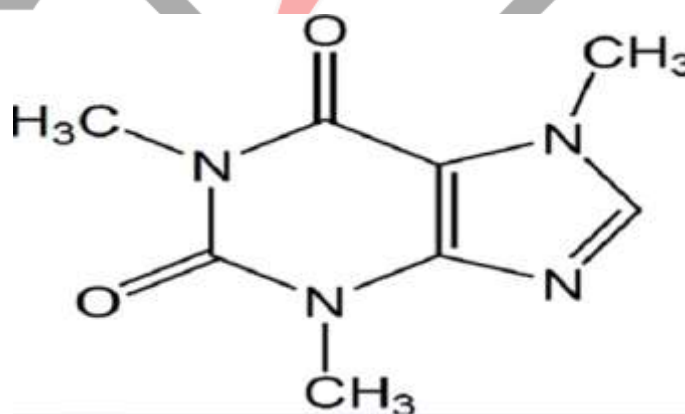
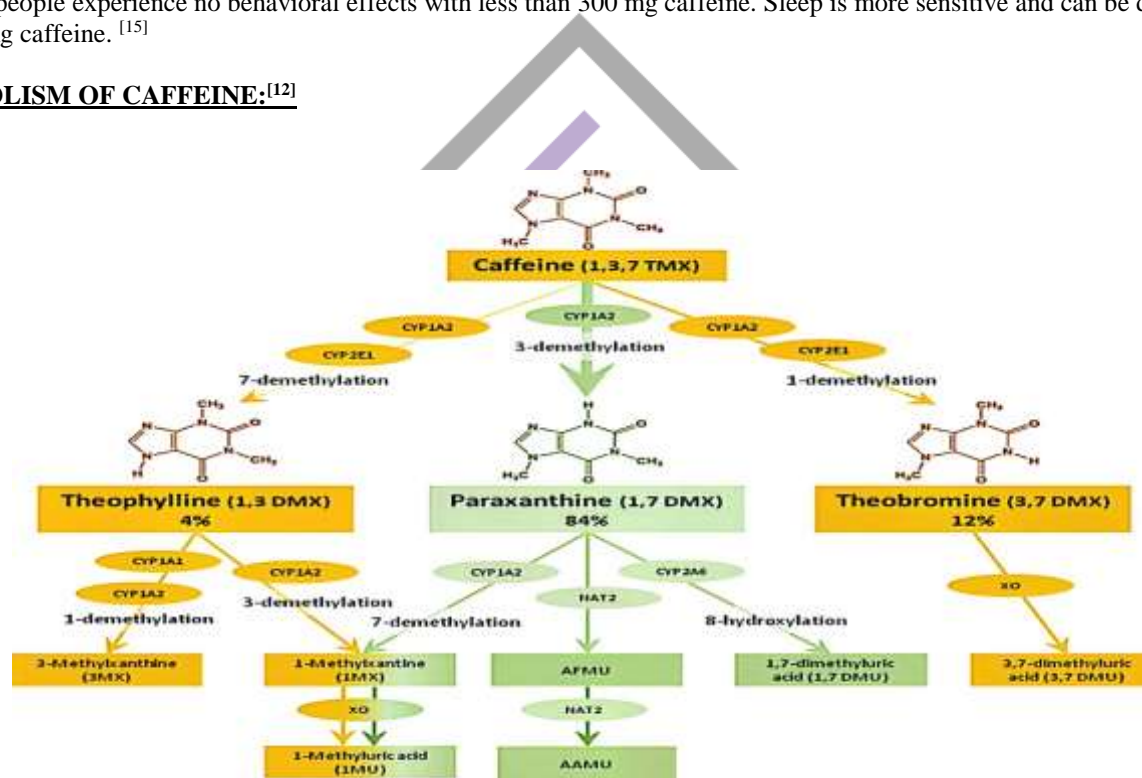


Figure no.1 Structure of caffeine

PROPERTIES OF CAFFEINE:^[12]**Table no:1** properties of caffeine

Formula	$C_8H_{10}N_4O_2$
Molar mass	194.19 g/mole
Boiling Point	178°C
IUPAC Name	1,3,7-Trimethylpurine-2,6-dione
Melting Point	235°C
Density	1.23g/c

- Therefore, the highest legal amount of caffeine allowed in a 355 mL (12oz) can of soft drink is about 71mg.
- Caffeine has attracted the interest of consumers and health professionals alike due to its wide consumption in the diet by a large percentage of the population and its pharmacological effects in humans (Mandel 2002).^[8]
- The International Olympic Committee (IOC) defined caffeine as a drug and abuse is indicated when athletes have urine caffeine concentrations higher than 12µg/mL.^[8]
- 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.^[15]

METABOLISM OF CAFFEINE:^[12]**Figure no.2** Metabolism of caffeine

- Caffeine is metabolized in the liver into three primary metabolites: Para xanthine (84%), Theobromine (12%), and theophylline (4%) 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.
- 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.
- The effects of caffeine on the body may begin as early as 15 minutes after ingesting and last up to hours. Caffeine is highly addictive, caffeine increase stress level, caffeine accelerates aging and wrinkles.
- 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.
- 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.
- 100-200 mg dose of caffeine result in increased alertness and wakefulness, faster and clearer flow of thought, increased focus, and better general body co ordination.
- Caffeine makes people more alert, less drowsy, and improves coordination.

- Combined with certain pain relievers or medicines for treating migraine, headache. 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.
- Caffeinated soft drink consumption has increased enormously in recent times, and approximately 80% of the world's population consumes caffeinated product every day.
- Increased popularity in caffeinated product increase the demand of energy drinks (ED) with higher amount of caffeine. The attractiveness and recognition of these beverages are due to the fact that caffeine, helps in staying awake and improve mental alertness after fatigue.
- Caffeine is a naturally occurring alkaloid belongs to methylxanthine class and found in many different plants leaves and fruits. IUPAC name of caffeine is 1,3,7 Trimethylpurine-2,6-dione.
- The absence of regulatory oversight has resulted in aggressive marketing of energy drinks with higher amount of caffeine, targeted primarily toward young males, for psychoactive, performance-enhancing and stimulant purposes.
- There are increasing reports of caffeine intoxication from energy drinks, and it seems likely that problem with caffeine dependence increasing day by day.
- In children and adolescents who are not habitual caffeine users, vulnerability to caffeine intoxication may be markedly increased due to an absence of pharmacological tolerance.

GOALS (OBJECTIVES):

- I 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 I determined the amount of caffeine in 9 brands of soft and 3 brands of energy drinks by using UV/VIS Spectrophotometer to obtained practical knowledge in the use of basic UV/VIS Spectrophotometer equipment.^[12]
- 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.

EXPERIMENTAL WORK:

METHOD: UV-Visible spectroscopy

REAGENT:

- Caffeine
- Dichloromethane
- Distilled water
- 20% sodium carbonate

STANDARD PREPARATION:

PREPARATION OF STOCK SOLUTION:

A 1000ppm stock solution of caffeine was prepared by dissolving 100mg of caffeine in 100ml of dichloromethane. From which further dilutions takes place 100ppm & 25ppm by withdrawing 5ml in 50ml & 6.25ml in 50ml dichloromethane respectively.

PREPARATION OF ALIQUOTS:

Aliquots are prepared from 25ppm solution of caffeine in dichloromethane. Withdrawing 1.6ml, 2.4ml, 3.2ml, 4.0ml, 4.8ml and make up to 10ml by dichloromethane. And prepared 4ppm, 6ppm, 8ppm, 10ppm, 12ppm respectively.

SAMPLE PREARATION:

Extract of drinks samples, present in the sample cell, was dissolved in 5mL of dichloromethane to form the sample solution. Reading was performed at a wavelength of 273 nm.

CAFFEINE EXTRACTION PROCEDURE:

The brands of soft and energy drinks were taken by different shops.

- Cold drinks/energy drinks (5ml) taken in separating funnel.
- Then separating funnel was taken and adjusts it in the stands with beakers.
- Add distilled water (10ml) in separating funnel.
- Add 1ml of 20% sodium carbonate solution. (20gm sodium carbonate in distilled water).
- Then add analytical grade dichloromethane as an extracting solvent in separating funnel.
- The extraction procedure repeated 4 times with the extracting solvent (10ml+10ml+10ml+10ml).
- The caffeine was extracted by inverting the funnel.
- Dichloromethane was removed in 50ml volumetric flask.

- The non aqueous solvent is taken in volumetric flask and makeup with 50ml solvent (dichloromethane).
- This procedure was repeated for all the drinks samples.
- The absorbance of resulting solution was then measured on UV-Visible spectrophotometer at $273\lambda_{\text{max}}$.^[15]

DATA AND CALIBRATION CURVE:

Table no:2 Calibration curve: λ_{max} - 273nm

Caffeine standard concentration (ppm)	Absorbance
4	0.2700
6	0.3943
8	0.5026
10	0.6415
12	0.7638

Figure 3 calibration curve of caffeine

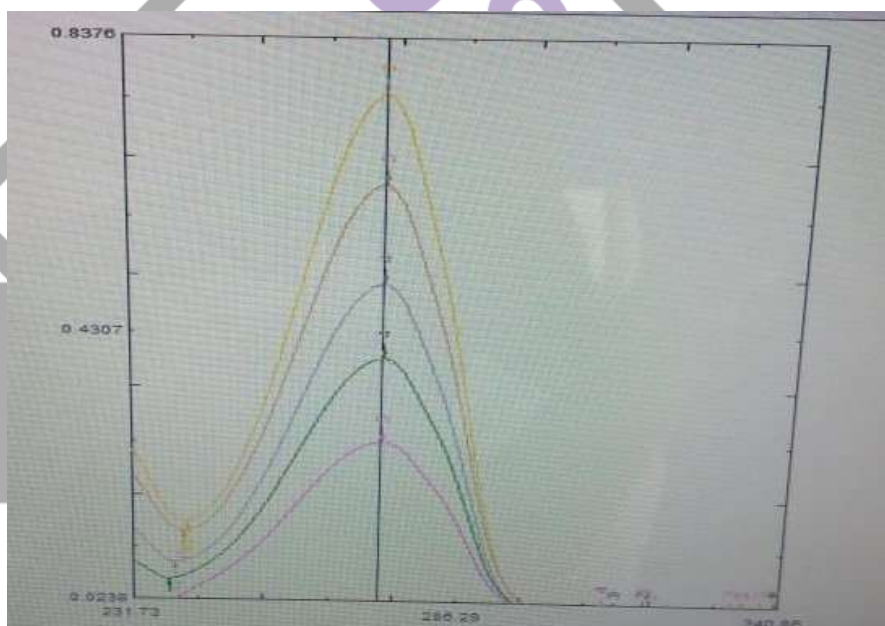
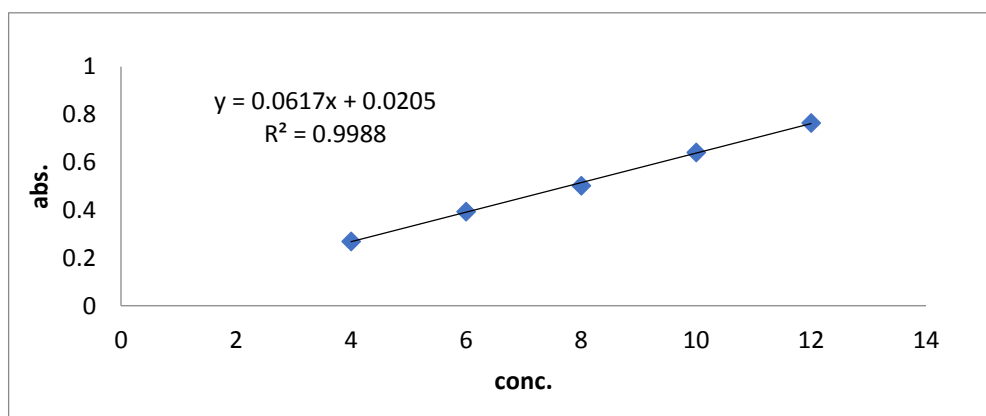


Figure 4 linear representation of caffeine



TLC PREPARATION:**SAMPLE PREPARATION:**

Extraction of caffeine with dichloromethane[10ml of sample(soft drinks/energy drinks) +1ml 5% sodium carbonate(5gm in distilled water) + 0.1ml of 0.2M NaOH + 3ml of dichloromethane.



Dichloromethane was removed after vigorous shaking of separating funnel. The layer is removed & adds anhydrous sodium sulfate (3 gm).



Then again add 2ml of dichloromethane for extraction. Shake the funnel and removed the non aqueous solvent & filtered.



Evaporate to dryness 50°C on hot plate or water bath.



Dry extract dissolve in 2ml dichloromethane. Then the extract cooled in ice.



Then spot applied on TLC plate & compared with standard caffeine solution.



Then the spot observed in UV Light in UV Chamber.

MOBILE PHASE PREPARATION:

➤ Ethyl acetate (85ml): methanol (10ml): ammonium hydroxide (dilute ammonia) (5ml)

BUFFER CAPACITY AND PH DETERMINATION:

- 100ml sample is taken & titrated with 1M NaOH in increment of 0.2- 0.3ml & the volume of 1M NaOH required to bring the pH to 5.5 (critical pH) was noted.
- Sample further titrated with 1M NaOH. Add 0.2-0.3ml to pH-7 (neutral pH).
- The total titrable acidity was expressed as the volume of 1M NaOH required neutralizing the sample.
- Titration was repeated in triplicates for all samples to check for reproducibility & to give a mean value for that sample
- Larger the amount of 1M NaOH required to raise the initial pH 5.5-7.0
- The greater will be the erosive potential of that particular sample.^[17]

Figure 5 (tlc of standard caffeine and sample 5)

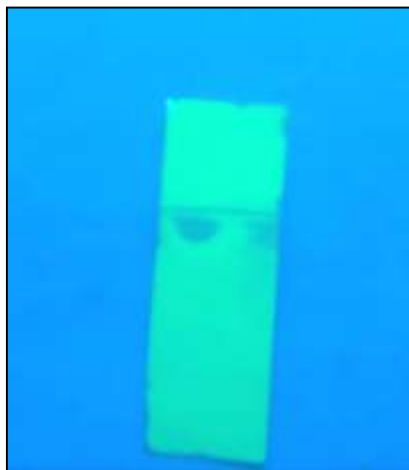


Figure 6 tlc of samples (std caffeine, sample 2 (F), sample 3 (P),sample 1(T),sample 4(S))

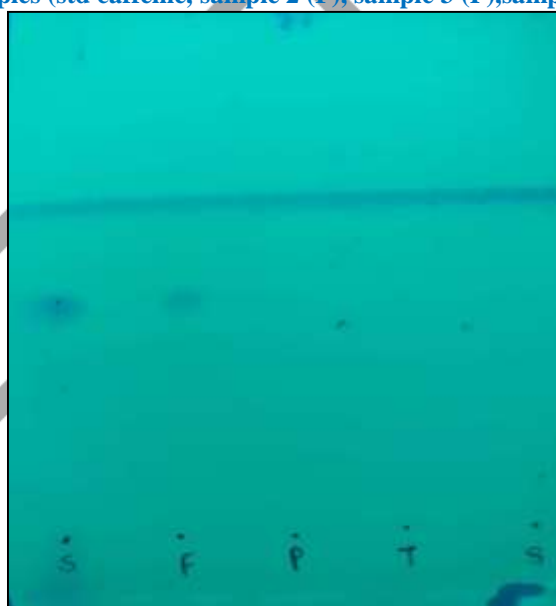
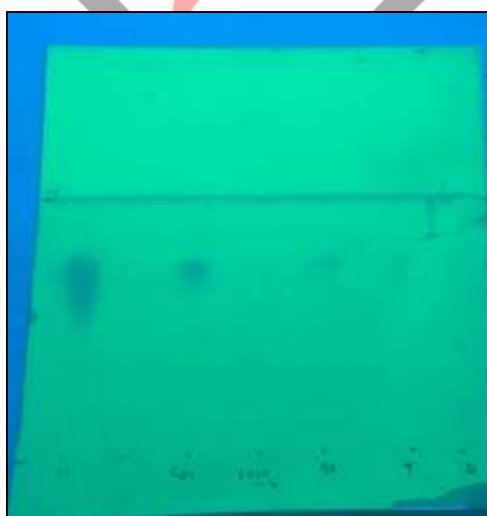


Figure 7 (tlc of std caffeine, sample 8(So), sample 10(st), sample 6(coc), sample 7(T), sample 9(R))



pH MEASUREMENT:

Table no:3 The samples pH was determined by the pH meter.

SAMPLES	pH
Sample 1	2.61
Sample 2	3.43
Sample 3	2.25
Sample 4	3.03
Sample 5	2.32
Sample 6	2.62
Sample 7	1.70
Sample 8	1.82
Sample 9	3.80
Sample 10	3.10

CALCULATIONS:**CONCENTRATION OF CAFFEINE:**

- IN PPM($\mu\text{g/ml}$):
 $Y = mx + c$
 - $y = 0.061x + 0.020$ put the value of y = absorbance of sample in this formula.
 - The value obtained x is your concentration in ppm ($\mu\text{g/ml}$).
- IN MILIGRAM(mg):
 - Conc. of caffeine (mg) = Concentration in ppm* dilution factor
 Here, dilution factor is 5 (50/10).

Rf VALUE DETERMINATION:

$$RF = A/B$$

WHERE,

- ❖ A=DISTANCE TRAVELLED BY SAMPLE/ STANDARD.
- ❖ B=DISTANCE TRAVELLED BY MOBILE PHASE.

EXAMPLE:

Distance travelled by caffeine (std) = 4cm.
Distance travelled by mobile phase = 5.2cm

$$R_F = 0.76$$

(FOR CAFFEINE STANDARD)

RESULT:

Caffeine determination in soft drinks (carbonated drinks)/ energy drinks. The absorbance, concentration (in mg), pH value, Rf value etc. are presented in below [table no:4](#)

Sr no.	SAMPLES	ABSORBANCE	CONCENTRATION (mg)	CONC. (µg)	pH	Rf VALUE
1	Sample 1	0.4307	33.5mg	6.7	2.61	0.61
2	Sample 2	0.2378	17.5mg	3.5	3.43	0.67
3	Sample 3	0.5239	41mg	8.2	2.25	0.61
4	Sample 4	0.0611	3.3mg	0.673	3.03	0.25
5	Sample 5	0.9167	73.5mg	14.7	2.32	0.75
6	Sample 6	0.6330	50.2mg	10.04	2.62	0.71
7	Sample 7	0.8697	69.6mg	13.92	1.70	0.76
8	Sample 8	0.0688	4mg	0.8	1.82	0.75
9	Sample 9	0.3270	25.16mg	5.032	2.74	0.19
10	Sample 10	1.3623	110.02mg	22	3.80	0.61
11	Sample 11	1.1900	95.90mg	19.18	3.10	0.76

CONCLUSION:

Determination of caffeine content in non-alcoholic beverage & drinks is very important. High consumption of caffeine leads to adverse effect. So, Determination of caffeine from soft drinks is necessary. The caffeine content found from the soft drinks is more than the specified limit (FDA LIMIT).

So, the UV-Visible spectroscopy and TLC are most suitable method for determination of caffeine. This is more suitable, accurate and economical method.

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