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OBTAINING AND INVESTIGATE OF CAFFEINE FROM CONTRASTING TEA SPECIMEN USING LIQUID-LIQUID EXTRACTION

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Abstract:

Caffeine is a found in a coffee. It is a white crystalline purine derivatives which is a methylaxanthine alkaloid caffeine extracted and charecterized tea leaves and coffee beans. Isolation was done by liquid-liqued extraction caffeine was conceleved for a wide range of readers interested in the effect of human health ,nutrition and biological effect of the caffeine food beverage . caffeine is commonly used stimulates athelets hence it is important to estimate the amount of caffeine in tea samples .A sample and caffeine in tea samples with respect to region of cultivate .the body tries to maintain equliburium between adenosine and caffeine leading to increased caffeine consumption by individuals to produce desied effect. The classification of caffeine extract method defined as two type in suplimation method And Abstract method.

Key words:

Find out caffeine liquid liquid extraction different method

Introduction:

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

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[5]. Caffeine is a naturally occurring substance found in humans, caffeine is a central nervous system (CNS) stimulant[6]. It has the effect of temporarily warding off drowsiness and restoring alertness indicates that caffeine metabolism is the rate-limiting factor in its plasma clearance [7]. Caffeine metabolism occurs primarily in the liver, catalyzed by hepatic microsomal enzyme .

Metabolism [9]. dimethylxanthines, uric acids, di- antrimethylallantoin, and uracil derivatives. In humans 3-ethyl demethylation to paraxanthine is the primary route of metabolism. This first metabolic step accounts for approximately 75–80 percent of caffeine metabolism and involves cytochrome P4501A2. Paraxanthine is the dominant metabolite in humans, rising in plasma to concentrations 10 times those of theophylline or theobromine. Caffeine is cleared more quickly than paraxanthine, so 8 to 10 hours after caffeine ingestion, paraxanthine levels exceed caffeine levels in plasma.

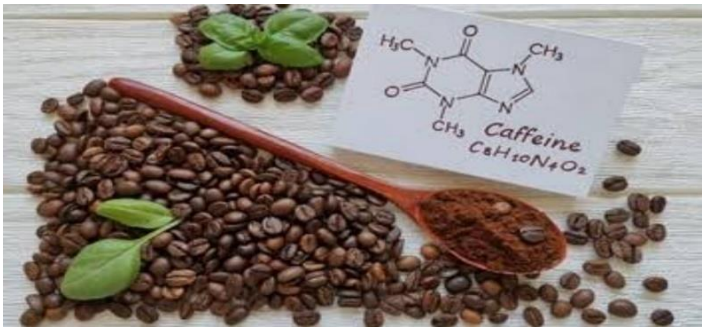
The fact that the human body converts 70–80 percent of caffeine into paraxanthine with no apparent toxic effects following caffeine doses of 300–500 mg/day suggests that paraxanthine's toxicological potency is low. Formation of paraxanthine and its excretion in the urine appears to be the major pathway for caffeine extraction[10].

Tea is the most commonly and widely used soft beverage in the household it act as a stimulant for central nervous system skeletal muscles.its also increase the capacity of thinking it is also using for lowering body temperature.the principle constituent of tea ,which is responsible for all these properties,is the alkaloid-caffeine. The amount of caffeine in tea leaves various from sample to sample caffeine (1,3,7-trimethylxanthine) is a plant alkaloid with a chemical structure of C8H10N4O2 and a molecular weight 194.19 in pure form.

Caffeine molecule:

Formula	C8H10N4O2
Molar mass	94.19g/mole
Boiling point	178degree Celsius
IUPAC ID	1,3,7-trimethylpurine-2,6-dione
Melting point	235degree Celsius
Density	1.23g/c

Caffeine structure:



Experimental method :

Aim:

Caffeine is naturally occurring molecule found in various plants world wide and founding beverage such as tea coffee and soft drinks.

It is used to measure caffeine in a drinks for quality control comparison across different brand.

Chemicals used:

Dichloromethane and anhydrous sodium sulfate and sodium hydroxide where used in extraction of caffeine from different tea samples acetic acid is used to precipitate the casein from fats sodium hydroxide and hydrochloric acid where used for modifying the PH of solution ammonium sulphate was used throughout this study. Different sample of tea where collected from local vender.

Materials required:

- tea bags
- beaker(500ml)
- hot plate
- separating funnel

1.Suplimation Method:

sublimation of caffeine from tea samples :

Initially take tea samples and record the weight of this tea bags. Take 500ml beaker and 200ml of distilled water to it. Now place the sample tea bags in the beaker. Boil the content in the beaker vigorously using a hot plate. Allow the mixture to cool for 5minutes and then decand the mixture in to another beaker. Gently squeeze the tea bags to liberate the rest of the water. Cool the aqueous solution to near room temperature.

Continue cooling in an ice box, the tea must be cool (20 degree Celsius) before

Coming in contact with dichloromethane .Extract the solution three times with 30 ml portions of dichloromethane .Do not get dichloromethane on your hands .The solution is poured in to a separating funnel and 20 ml of dichloromethane is added to it . The mixture will separate in to two layers –the top is the tea layer and bottom layer is the dichloromethane since it is denser then tea remove the funnel from stand and keep your fingers on the stopper and carefully shake the separating funnel.vent the separating funnel periodically to releavevapaour pressure created the inside the funnel, when the contents shaken place the separating funnel back on the ring stand and let the two layers separate drain the bottom layer in to a conical flask because now the caffeine is extracted in to the dichloromethane layer cover the mouth of conical flask to avoid evaporation of solution .

Dry the combined dichloromethanesolluion with unhydrous sodium sulfite add about one teaspoon of the drying agent until it no longer clumps together at bottom of the flask .Mix well and leave it for 10 minutes. Decand the dichloromethane in to a conical flask (100). Evaporate the dichloromethane solvent in a hot water both .when all the solvent is removed you observe a resedue of yellowish green-white crystalline caffeine .Take the conical flask containing cryatalline caffeine .Sublime the crude caffeine at atmospheric pressure by placing the flask directly on a preheated hot plate. Caffeine melts at 230 degree Celsius and sublimes at 178degree celcius Collect your sublimed caffeine by keeping a test tube on the mouth of the conical flask. White vapour of caffeine sticking on to the test tube on the conical flask and walls of the conical flask is observed now cool the conical flask take a clean watch glass and record its weight in a weigh balance.

1.Isolation of caffeine from tea leaves:

1.Take 5 tea bags and record the weight of these tea bags

2.Take 500 ml of beaker adds 200 ml of distilled water to it.now place the 5 tea bags in the beaker

3.Boil the contents in the beaker vigorously using a hot plate

4. Allow the mixture to cool for 5 minutes and then decant the mixture in to another beaker.

5. Gently squeeze the tea bags to liberate the rest of water .

2.Extraction step:

- The tea solution is bured in to a separating funnel and 2ml of dichlomethane is added to it . the mixture will be separate in to two layers is the top layer is the layer and bottem layer is the dichlomethane since it is denser then tea
- Remove the funnel from the stand and keep your fingers on the stopper
- And carefully shake the separating funnel
- When the contents have been syfficiently shaken place the separating funnel back on the ring stand and let the two layer separate
- Drain the bottem layer in to a conical flask because now the caffeine is extracted in to the dichloromethane layer cover the mouth of the conical flask
- To avoid evaporation of solution
- Repeat steps through twice.
- Decant the dichloromethane in to a conical flask (100ml).Evoparate the dichloromethane solvent in a hot water both
- When all the solvent is removed you observe a residue of yellowish green – white crystalline caffeine

3.Sublimation step:

- Take the conical flask containing crystalline caffeine
- Sublime the crude caffeine at atmospheric pressure by placing the flask directly on a preheated hot plate caffeine melts at 238 degree celcius and sublines at 178 degree celcius
- Collect your sublimed caffeine by keeping a test tube on the mouth of the conical flask
- White vapour of caffeine sticking on to the test tube and the walls of the conical flask is observed

- Caffeine was successfully extracted from a bag of tea leaves
- The caffeine percentage in the tea solution is 2.01%
- The mass of caffeine extracted was 0.5g
- The melting point of caffeine is 209degree celcius

2.Extraction method:

APPARATUS REQUIRED:

• Analytical balance

• Weighing boats

• Spatula

• 200ml volumetric flask x1

• Micropipette - ideally 1ml to 10ml size

• Compatible Pipette tips

• 50ml volumetric flask

•Hotplatte stirrer

• Stirrer bar

• 250ml beaker

• 100ml measuring cyclinder

• Separating funnel

• 250ml Conical flasks (one per sample to be tested)

• Dropper pipettes

Reagents required:

• Caffeine (pure) for standard preparation

• purified water

• Lead acetate

• Chloroform

PROCEDURE:

First of all, 50 grams of tea leaves were taken as sample and 150 ml of water was added to it in a beaker. Then the beaker was heated up to extreme boiling. The solution was filtered and lead acetate was added to the filtrater, leading to the formation of a curdy brown coloured precipitate. We kept on adding lead acetate till no more precipitate has been formed. Again solution was filtered. Now the filtrate so obtained was heated until it had become 50 ml. Then the solution left was allowed to cool. After that, 20 ml. of chloroform was added to it. Soon after, two layers appeared in the separating funnel. The residue left behind was caffeine. Then we weighed it and recorded the observations. Similar procedure was performed with different samples of tealeaves and quantity of caffeine was observed in them.

METHOD:

(i**) Prepare a standard** 1,000 ppm stock solution of caffeine; use an analytical balance to weigh 198.2mg of caffeine and make this up to 200ml using purified volumetric flask.

(ii) **Prepare calibration standards**: Using a pipette add 25ml, 12.5ml, 10ml, 7.5ml, 5ml and 2.5ml to each of 6x 50ml volumetric flasks. Make the standards up to the 50ml volume using purified water. These amounts will create 100ppm, 50ppm, 40ppm, 30ppm, 20ppm and 10ppm calibration standards respectively (ppm = mg/L)

(iii) **Prepare the samples;** (a) For instant coffee; add 2g of granules to a 250ml beaker and add 200ml boiling purified water. Stir at 500 rpm on a magnetic stirrer for 30 seconds, then leave to cool to room temperature without further stirring.

(b) For tea; add 3.2g of dried tea leaves to a 250ml beaker and add 200ml boiling purified water. Stir at 500 rpm on a magnetic stirrer for 30 seconds, then leave to cool to room temperature without further stirring.

(c) For pre made soft drinks (e.g. cola, energy drinks) no preparation is required, skip ahead to step 4 .

(iv) **Extract caffeine from the samples;** take 50ml of the calibration standard or sample and it to a separating funnel. Use the measuring cylinder to add 25ml of dichloromethane.Invert the separating funnel 3 times, then vent to avoid pressure build- up. Put the funnel in a stand and allow the layers to separate, before removing the dichloromethane layer to a labelled conical flask.Return the calibration standard or sample to the separating funnel and repeat twice more, until 3x 25ml dichloromethane

layers have been combined in the conical flask.

(v) **Measure the calibration curve;** use a dropper pipette to add the calibration standards to the quartz cuvette for measurement.First measure purified water only as a blank, then measure each of the calibration standards in increasing order of concentration.Tabulate the results of caffeine concentration in ppm vs. absorbance at 260nm.

(vi) **Calculate the calibration curve**; use a spreadsheet to create a line graph of the calibration curve results. Find the linear regression equation of the calibration curve, $y = mx + c$ (where y = absorbance and x = concentration)

(vii) **Measure the samples;** Use a dropper pipette to add your first sample to the (cleaned and dried) cuvette. Take a measurement and record the absorbance at 260nm. Repeat for each sample, taking care to clean and dry the cuvette carefully between samples.

(viii) **Calculate the results;** using the $y = mx + c$ equation from your calibration graph, you can calculate the caffeine concentration of your samples. Substitute Y for the absorbance value recorded for that sample, keep M and C constant, and rearrange to solve for X .For some models of spectrophotometer, such as the Jenway 7305, a concentration mode is available which allows the instrument to do this calculation for you based on your $y = mx + c$ equation, so the readout on the spectrophotometer will be in ppm directly.

• 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.

Uses of caffeine :

In medicine it is ued to stimulate central nervous system and to increase flow of urine becous of its stimulating effect, caffeine has been used to reliavefatigue.But it is dangerous and one may collaps if not consumers it under certain limit .The caffeine in coffee can help stimulate hair growth and stop hair loss .

Advantage:

- Caffeine can improve memory.
- Decrease fatigue improve your mental functioning, study after study suggest.
- It can improve your short term memory and act as a central nervous system stimulant
- Caffeine has been shown to protect against a host of problems

Disadvantage:

Caffeine affects adenosine receptors in the brain. Coffee also contains polyphenol antioxidants, and these, too, act on various pathways, Studies have suggested that drinking coffee may help enhance some thinking skills and slow the mental decline that comes with age.

SKIN CANCER:

1. Some scientists have suggested that caffeine may guard against certain skin cancers. One team found that caffeine applied directly to the skin of mice helped prevent damaging ultraviolet (UV) light from causing skin cancer.

2. Others have linked the consumption of three cups of caffeinated coffee a day with a 21 percent lower risk of developing basal cell carcinoma in women, and a 10 percent lower risk in men, compared with drinking less than one cup per month.

Kidney stones:

1. A study of 217,883 participants analyzed the association between caffeine intake and the risk of developing kidney stones.

2.Those who consumed more caffeine had a lower risk of developing kidney stones. Mouth, throat, and other cancers.

3.In a study of 968,432 men and women, participants who drank than 4 cups of coffee a day had a 49-percent lower risk of death from oral cancer, compared with those who drank no coffee at all or only an occasional cup.

Other possible cancer-related benefits include:

• A lower risk of endometrial cancer

• A reduced risk of prostate cancer

• Protection against head and neck cancer

• Protection against the recurrence of breast cancer

STROKE

1. Data for 34,670 women in Sweden without a history of cardiovascular disease indicated that women who drank more than one cup of coffee per day had a 22 to 25- percent lower riskTrusted Source of stroke compared with women who drank less. Low or no coffee drinking appeared to be linked to an increased risk of stroke.

DEPRESSION:

1.A high caffeine intake may worsen symptoms of anxiety and depression. Research published in 2016 found that, in 234 middle school students in Korea, a higher caffeine intake was linked toTrusted Source higher weight, lower academic achievement, and a higher risk of severe depression.

2.However, whether the caffeine leads to depression or depression causes people to consume more caffeine remains unclear.

BLOOD PRESSURE:

1. People with type 2 diabetes report that their blood glucose levels rise after consuming caffeine.

2.There is some evidence that caffeine may impair insulin action, leading to a small but detectable rise in blood sugar levels, particularly after meals. Pregnancy

3. Studies have suggested that more than 300 mg a day of caffeine, or the amount equal to around three cups of coffee, could lead to:

• loss of pregnancy

• delayed fetal growth

• abnormal fetal heart rhythm

4..According to the National Institutes of Health (NIH), the weeks before pregnancy also count. Research shows that if both parents consume more than two caffeinated drinks a day in the weeks before they conceive, a loss of pregnancy may be more likelyTrusted Source.

5.Women should limit their caffeine intake to 200 mg or less during pregnancy.

Breast-feeding

1.Caffeine passes into breast milk in small amounts, and it may build up in the nursing infant.

2.Infants whose mothers drink large amounts of caffeinated beverages may be jittery and have trouble sleep.

Result and discussion:

• 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 ofdistilled 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.

Determination of caffeine content in non-alcoholic beverages and energy drinks is very importantanalytical process safeguard the wellbeing of people who are unaware to adverse effects of caffeine .Insoft drinks the Brand 5 have highest concentration of caffeine that is 42.17 ppm and Brand 3 having lowconcentration of caffeine 10.69 ppm and in energy drinks Brand 2 having high concentration of caffeinethat is 101.705 ppm.

Caffeine equivalent con mg/l	Absorbence
10	0.332
20	0.713
30	1.073
40	1.463
50	1.783

Reference

[1] .Adrews K, Schweitzer A, Zhao C, Holden JM,Roseland JM, Brandt M, Dwyer J, Picciano M, Saldanha L, Fisher K, Yetley E, Betz J and Douglass L. The caffeine content of dietary supplements commonly purchased in the U.S.: Analysis of 53 products having caffeine containing ingredients. Anal Bioanal Chem 2007;389(1):231-239.

[2] .Palatini. P., Ceolotto, G., Ragazzo, F., Dorigatti, F., Saladini, F., Papparella, I., Mos, L., Zanata, G. and Santonastaso, M. Genotype modified the association between coffee intake and the risk of hypertension. CYP1A , (2) :(2009).

[3] Nathanson, J.A "Caffeine and related methylxanthines: possible naturally occurring pesticides".,Science ,J.226 (4671): 184–7.(1984).

(3)Jarvis, Gail The Rise and Fall of Cocaine Cola. 1st ed. pp. 302-311 (May 21, 2002).

[4] Jarvis,Gail The Rise and Fall of Cocaine Cola.1st ed. 302-311(May 21,2002).

[5]Anna, K. and Kurek, J.P. "Natural Products and Pharmaceuticals" UAM, Poznań 2nd ed. pp. 227 –256 (2013).

[6] Arnaud MJ. Metabolism of caffeine and other components of coffee. In: Garattini

S, ed. Caffeine, Coffee, and Health. New York: Raven Press. (1993) Pp. 43–95.

{7}Grant DM, Campbell ME, Tang BK, Kalow W. Biotransformation of caffeine by microsomes from human liver. Kinetics and inhibition studies. Biochem

Pharmacol 36 (1987) 1251–1260

[8]George J, Murphy T, Roberts R, Cooksley WGE, Halliday KW, Powell LW. Influence of alcohol and caffeine consumption on caffeine elimination. Clin Exp PharmacolPhysiol 13 (1986) 731–736.

[9] Stavric B. Methylaxanthines: Toxicity to humans, 3. Theobromine, paraxanthine and the combined effects of methylxanthines. Food Chem Toxicol 26 (1988) 725– 733.