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DETERMINATION AND ANALYSIS OF CAFFEINE FROM VARIOUS TYPES OF TEA SAMPLE USING LIQUID LIQUID EXTRACTION TECHNIQUE

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Abstract:

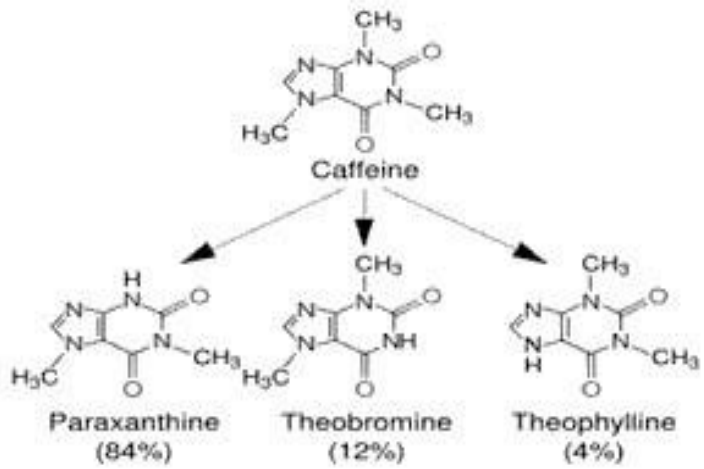
Caffeine is probably the most frequently ingested pharmacologically active substances in the world the possibility that caffeine ingestion adversely affects human health. Based on the data reviewed , it is concluded that for the healthy adult population, moderate daily caffeine intake at a dose level upto 400 mg day^{-1} is not associated with adverse effects such as general toxicity, cardiovascular effects ,effects on bone status . caffeine is consumed by over 80% percentage of U.S adults caffeine exerts its effects by blocking adenosine receptors effects on physical performance on a vast array of physical performance metrics such as time –to-exhaustion, time –trial ,muscles strength and endurance, and high intensity spirts typical of team sports evident following doses that exceed about 200 mg caffeine increases alterness and reduces fatigue caffeine improves performance on vigilance tasks and simple tasks that require sustained response .excessive consumption can lead to problems ,especially in sensitive individuals.

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] In 1819, the German chemist Friedrich Ferdinand Runge first time isolated pure caffeine in laboratory [3] Caffeine is one of the world's most widely used drugs. Many anthropologists believe people used caffeine start from Stone Age.. 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.[4] Caffeine is a naturally occurring substance found in humans, caffeine is a central nervous system (CNS) stimulant.[5] It has the effect of temporarily warding off drowsiness and restoring alertness.

Caffeinated drinks is the most consumed psychoactive substance (a substance that stimulates the central nervous system) and therefore a drug. It was estimated that about 80% of the world's population consume caffeine every day. Caffeine is so popular, because of its stimulating effect on the brain. The consumption of caffeine may alleviate fatigue, increase the feeling of wakefulness and improve concentration and focusing. However, according to the researches 5 grams of caffeine (equivalent to 30-40 cups of regular coffee) may cause the death of the consumer.

Caffeine is metabolized in the liver into three primary metabolites: Para xanthine (84%), Theo bromine (12%), and theophylline (4%) [6] Caffeine is metabolized in the liver by the cytochrome P450 oxidase enzyme system (specifically, the 1A2 isoezyme) into three metabolic dimethyl xanthine's which each have their own effects on the body [7].



1.1. Caffeine structure and its metabolism

Paraxanthine (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 [8] 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.[9] The effects of caffeine on the body may begin as early as 15 minutes after in jesting and last up to hours.[10]. Caffeine is highly addictive, caffeine increase stress level, caffeine accelerates aging and wrinkles [11]. 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 [12].

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. [13]. Caffeine makes people more alert, less drowsy, and improves coordination. Combined with certain pain relievers or medicines for treating migraine headache [14].

Material and Method:

Caffeine is a naturally occurring molecule found in various plants worldwide, and found in beverages such as tea, coffee and soft drinks. The amount of caffeine present in drinks can be measured by UV spectroscopy.

The full method below, as originally outlined by the Jenway team at Bibby Scientific, can be used to measure caffeine in drinks for quality control, comparison across different brands or as an educational introduction to working with UV-Vis spectroscopy.

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.

(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 water in a 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 buildup. 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.

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.

Application of caffeine:

* Caffeine can improve memory, decrease fatigue, improve your mental functioning, study after study suggests. It can improve your short-term memory and speed up your reaction times, according to a study presented in 2005 at the Radiological Society of North America. Caffeine acts as a central nervous system stimulant. When it reaches your brain, the most noticeable effect is alertness. You'll feel more awake and less tired, so it's a common ingredient in medications to treat or manage drowsiness, headaches, and migraines.

* Coffee cuts suicide risk. A 2013 study by Harvard's School of Public Health found that those who drank two to three cups of caffeinated coffee a day cut their suicide risk by 45 percent — possibly because caffeine's stimulant effect helps boost people's moods.

* It lowers the risk of oral cancers. Older adults who drank four or more 8-ounce cups of regular coffee daily were half as likely to die of mouth and upper throat cancer. Decaf had a weaker effect, while no protection was found with tea.

Disadvantages of caffeine

Brain function

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:

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.

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:

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

Those who consumed more caffeine had a lower risk of developing kidney stones.

Mouth, throat, and other cancers

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

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 25percent lower riskTrusted Source of stroke compared with women who drank less.

RESULTS AND 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 methods but in this research UV/VIS Spectrophotometer was used because it is relatively easy, fast, cheap, highly sensitive and give accurate concentration of caffeine.

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

The regression line is Y = 0.036 x -0.0112 Dilution

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

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