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Determination of Caffeine in different Beverages and their Respective Tests

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Abstract—*Caffeine ($C_8H_{10}N_4O_2$) is a very common substance in most drinks, such as coffee, tea, Coca-Cola etc. It is white crystalline stimulant drug. Adults or even students will drink a cup of coffee to restore alertness and warding off drowsiness. Caffeine was extracted from different beverages such as coffee, tea etc. Extraction of caffeine was proved by Thin Layer Chromatography. We also did some research on other chemical tests for caffeine. The methodology involved qualitative analysis by Thin Layer Chromatography. Extracted was 90% pure as it contains some kind of impurities.*

1. INTRODUCTION

Caffeine is a chemical substance originally extracted from seeds, nuts and leaves of a no. of plants. Caffeine is methylxanthine alkaloid which is colorless powder at room temp.; it is odorless but does have a slightly bitter taste [1]. Caffeine finds its use in pharmaceutical industry as caffeine sodium benzoate and caffeine citrate. We somehow introduce caffeine into our bodies by the regular intake of coffee, tea and other beverages. Almost all beverages available in the market contain some amount of caffeine in them. European food safety authority had stated that single doses of caffeine up to 200mg and daily intakes of up to 400 mg do not raise safety concerns for adults, but above this specified limit, caffeine is detrimental to human health. Sensitivity to caffeine depends on many factors such as how often and how much coffee is consumed, body weight, and the individual physical condition. Other factors include the type of bean, the grind, and the preparation and processing methods used.[2] Caffeine can be found in numerous drinks like coffee, green tea, soft drinks and various drugs and can be extracted by using different chemical methods. Some analytical methods for quantization of methylxanthines are ultra violet spectroscopy, thin layer chromatography, gas chromatography, high performance liquid chromatography, capillary electrophoresis. [3] We have done qualitative analysis of caffeine by Thin Layer Chromatography.

2. HEALTH EFFECTS OF CAFFEINE

Today, most of us are addicted to any of the caffeinated drinks maybe it is coffee, tea or a coke. A lot of people are unaware of the fact that green tea and black tea too contains some amount of caffeine. Caffeine has both short term and long term effects on human health. Consuming it in excessive amount can lead to insomnia, nervousness, indigestion and headaches. High blood pressure patients must avoid caffeine as it increases the blood pressure. People who are diabetic must reduce the intake of caffeine as it increases blood sugar levels. Pregnant women who consume caffeine are at the risk of anxiety and depression.[4] Women having polycystic ovaries syndrome are suggested to avoid caffeine. Clinical studies have shown that coffee or caffeine have cardiovascular effects, general toxicity and effects on calcium balance and bone status. [5] Caffeine might be helpful in increasing physical performance and alertness, but it will always be a legal drug which can deteriorate human body functioning if overdosed.

3. METHODOLOGY

(a) Extraction of caffeine from raw coffee power: A raw coffee solution was prepared by using 7g of fine grind draw coffee. 150 ml of distilled water and 3.0g of sodium carbonate was added to increase the solubility of Caffeine (alkaloid). We boiled and brewed it by following the instruction on the Coffee package. The raw coffee solution was cooled down to room temperature.

Solvent Extraction: We rinsed all the apparatus with chloroform and transferred the raw coffee solution to separating funnel. 30 ml of chloroform was added and swirled vigorously (don't shake the mixture because an emulsion will form) and the mixture was allowed to stand. We separated out the bottom chloroform layer to a beaker and this step was repeated for 3 times, calcium sulphate was added to the separated chloroform to remove water. The mixture was shaken well until fluffy, cloudy effect is witnessed. We weighted the beaker which is going to hold the filtrate, filtered out the excess calcium sulphate. We Put the beaker with filtrate into hot water bath to evaporate chloroform, weighed the powder and calculated the amount of powder extracted. The results are stated in table 1.

(B) Qualitative analysis of caffeine by Thin Layer Chromatography (TLC):

Thin-layer chromatography or TLC is a solid-liquid form of chromatography where the stationary phase is normally a polar absorbent and the mobile phase can be a single solvent or combination of solvents. TLC is a simple, quick, and inexpensive procedure that gives a quick answer as to how many components are in a mixture. [7] It can be applied in different areas of analysis: pharmaceuticals and drugs, clinical chemistry, forensic chemistry, biochemistry, food analysis, environmental analysis, natural products chemistry, synthetic organic chemistry and other areas. [6]

Method: We Dissolved the extract and pure caffeine into distilled water separately and prepared the TLC plate (silica gel coating, and cut it to 3 cm x 10 cm). We used a pencil to draw a horizontal line 0.5 cm above the bottom of the TLC plate and spotted the extracted caffeine and pure caffeine by 10µL micro-capillary tubes separately on the TLC plate. The TLC plate was immersed into the solvent (a mixture of 20:1 of ethyl acetate: ethanoic acid). Chromatogram was developed, the results were visualized with a UV lamp; using a pencil to circle the spot seen under UV light. The refracted values obtained by performing the test are stated in table 2.

4. TESTS AND RESULTS

The result of caffeine estimation in black tea, green tea and coffee are set out in tables. A glance at these tables indicates wide variation in caffeine content. Since variability of caffeine content depends on factors such as variety of tea and coffee, therefore there is a variation in content of caffeine.

Table 1: Mass of extract obtained from raw coffee solution

Sample	Amount of sample(g)	Amount of caffeine after solvent extraction (g)	% of Crude caffeine
Coffee	7	0 .173	2.47%
Taj Mahal	7	0.79	1.128%
Green Tea	7	0.07	1%

Table 2: Thin Layer Chromatography

Samples	R_f values
(A) Green Tea	0.23
(B) Taj Mahal	0.30
(C) Coffee	0.121
(D) Standard Caffeine Solution	0.124

5. DISCUSSION

Caffeine extraction is a useful as it tells us about the amount of caffeine that we regularly take on our daily basis. A method has been developed for the extraction for the extraction, purification of caffeine from tea, coffee, green tea and some other beverages. Caffeine from the tea and coffee was extracted by liquid-liquid extraction. The purified caffeine was then analyzed by Thin Layer Chromatography. The TLC results proved that caffeine was successfully extracted from the beverages. It has a very high accuracy. The order of caffeine was found to be coffee > black tea > green tea.

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