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Isolation & analytical technique of caffeine from different sources: A review

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Abstract
Caffeine is the world's most widely consumed drug with its main source found in coffee. We evaluated the caffeine content of caffeinated and decaffeinated specialty coffee samples obtained from coffee shops. Caffeine was isolated from the coffee by liquid-liquid extraction and analyzed by gas chromatography with nitrogen-phosphorus detection. In this study, the coffees sold as decaffeinated were found to have caffeine concentrations less than 17.7 rag/dose. There was a wide range in caffeine content present in caffeinated coffees ranging from 58 to 259 rag/dose. The mean (SD) caffeine content of the brewed specialty coffees was 188 (36) mg for a 16-oz cup. Another notable find is the wide range of caffeine concentrations (259-564 rag/dose) in the same coffee beverage obtained from the same outlet on six consecutive days. Caffeine is a chemical found in coffee, tea, cola, guarana, mate, and other products. Caffeine is one of the most commonly used stimulants among athletes. Taking caffeine, within limits, is allowed by the National Collegiate Athletic Association (NCAA). Urine concentrations over 15 mg/mL are prohibited. It takes most people about 8 cups of coffee providing 100 mg/cup to reach this urine concentration. The aim of this study is to determine the concentration of caffeine in reputed tea types and coffee. The Technique used here is Liquid-Liquid Extraction to extract caffeine. The Study also focused whether we can extract a significant amount of caffeine using different extracting solvents and different bases.

Keywords: Coffee, uses, properties, analytical technique, extraction

Introduction

Caffeine (1, 3, 7-trimethylxanthine) is the world's most widely consumed drug with its main source found in coffee [1]. The plasma half-life ranges from 2.3 to 12 h [3] with caffeine plasma concentrations reaching peak levels at 45 rain to 2 h after ingestion [1]. The half-life of caffeine is increased for those with liver disease, in newborns, and during Pregnancy [1]. Estimates of daily caffeine consumption in the United States in 1978 indicated that approximately 200 mg was consumed daily by adults over the age of 18 with coffee accounting for about 75% of the total caffeine consumed [2]. An average cup of coffee contained approximately 85 mg of caffeine; hence average consumption was slightly over two cups of coffee per day. Individual consumption varied considerably, with those in the 99th percentile consuming 563 mg of caffeine [2]. This is equivalent to approximately seven cups of coffee per day. Caffeine is rapidly absorbed in the stomach and small intestine and metabolized primarily in the liver. It is excreted in 24-h urine as dimethylxanthines, uric acid derivatives, and 2.4% caffeine [3].

Although there are beneficial effects of caffeine ingestion, there may also be potentially harmful effects. There has been considerable study of the effects of caffeine on the cardiovascular system. In one study in which doses of 45-360 mg of caffeine were administered, both systolic and diastolic pressures in- creased, with a significant heart rate increase after the 360-mg caffeine dose [4]. In another study, it was concluded that anxiety may be increased with doses of 300 mg or higher [5], and a separate study found increased anxiety with as little as 125 mg of caffeine [6]. Care should be exercised when consuming caffeine with medications such as bronchodilators (both stimulate the central nervous system), quinolones (increases caffeine levels leading to excitability and nervousness), and anti-anxiety drugs (lessens effects of drug) [7].

Coffee, an infusion of ground, roasted coffee beans, is reported to be among the most widely consumed beverages in the world. Although coffee is lauded for its aroma and flavor, its caffeine content likely plays a role in its popularity.

In fact, coffee is a complex chemical mixture reported to contain more than a thousand different chemicals, including carbohydrates, lipids, nitrogenous compounds, vitamins, minerals, alkaloids and phenolic compounds [8]. The majority of studies on the health effects of coffee consumption in humans are observational. Concerns about potential health risks of coffee and caffeine consumption raised by epidemiological research in the past were likely ex acerbated by associations between high intakes of coffee and unhealthy behaviors such as cigarette smoking and physical inactivity. [9] More recently, coffee consumption has been associated with reductions in the risk of several chronic diseases. [10, 12]

Caffeine is an alkaloid found in tea, one of the most popular caffeinated beverages consumed worldwide [13]. As an organic substance with at least one nitrogen atom, caffeine is classified as an alkaloid. It is a member of the methylxanthines chemical class, along with theophylline and theobromine, which are closely related substances [14]. Tea shrubberies containing the Caffeine, also have acidic compound namely tannins, cellulosic materials, pigments and chlorophyll. To isolate the Caffeine from these tea shrubberies and leaves, it must be available in free basic forms Caffeine is invigorating due to cyclic backbone arrangement analogous to structure of a base named purine of DNA [15].

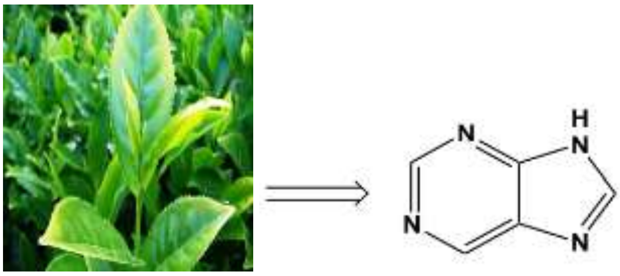


Fig 1: Chemical structure of caffeine

Caffeine is one of the most valuable stimulant compounds found in over 63 plant sand it is mostly found in coffee, tea leaves (theine) and cocoa plants [16]. It is a naturally occurring substance found in various parts of the plant including the bark, fruits, flowers, roots and leaves. The chemical formula of caffeine is $C_8H_{10}N_4O_2$, and its systematic name is 1, 3, 5-trimethylxanthine [17]. Acacia nilotica is found in several parts of the world. It grows in arid and semi-arid areas. It was first described by the Carl Linnaeus in 1773 as Acacia nilotica. There are almost 1380 species of acacia spread around the world with most of them found in Australia. It thrives well in tropics and subtropical regions. More than 40 species of acacia are found in India and are referred to as 'Babul." In Africa it is found in North West countries such as Senegal, northern Africa, south west and east Africa. In east Africa, it is majorly spread in Ethiopia, Sudan and Kenya [18].

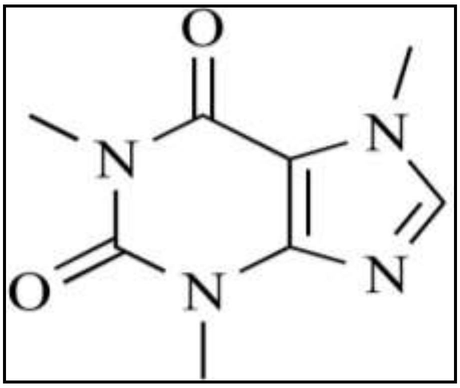
Caffeine was first isolated from coffee in 1819 by a German chemist, Friedlieb Ferdinand Runge. Its pure form is an odourless white solid with a melting point of 235-238 °C. Caffeine is classified as a heterocyclic purine base with two or more nitrogen elements in its rings. Caffeine is weakly basic, and its solubility in water at room temperature is quite low (2 g/100 ml) but increases dramatically in boiling water (66 g/100 ml. The main methylxanthine in tea is the stimulant caffeine. Other methylxanthines found in tea are two chemically similar compounds, theobromine and theophylline, which play a major role in the long-term popularity of non-alcoholic beverages and foods such as coffee, tea, cocoa, chocolate and a variety of soft drinks. It is combined with painkillers (such as aspirin or acetaminophen) and a chemical called ergotamine for treating migraine headaches [19].

Health Benefits of Caffeine
Research indicates that caffeine may help protect human brain cells, which lowers the risk of developing some diseases, such as Parkinson'
Regular cups of coffee may stimulate the gallbladder and reduce the risk of gallstones.
Caffeine causes the blood vessels to constrict, which may help relieve some headache pain
Coffee reduces inflammation and may help prevent certain heart related illnesses
Treats Migraine
Relieves Asthma Attack
Increases the potency of analgesics.
Caffeine is also used for weight loss and type 2 diabetes.
Very high doses are used, often in combination with ephedrine, as an alternative to illegal stimulants.
Reduced risk of mortality and chronic diseases [20].

Fig 2: Tea leaves & powder

Properties of Caffeine
➢ Systematic name: 1,3,7-trimethyl-1H-purine- 2,6(3H,7H)-Dione.
➢ Another name: 1,3,7-trimethylxanthine & 1,3,7-trimethyl-2,6-dioxopurine.
➢ Molecular formula: $C_8H_{10}N_4O_2$.
➢ Molecular mass: 194.19 g/mole.
➢ Melting point: 238 °C.
➢ Solubility in water: slightly soluble [21]

Caffeine is sparingly soluble in most polar solvents but is highly soluble in less polar solvents. The melting point is 234 °C-239 °C and the chemical formula is $C_8H_{10}N_4O_2$. It is an intensely bitter, white powder in its pure state. Caffeine is an alkaloid of the methylxanthine family, which also includes the similar compounds theophylline and theobromine



Structure of Caffeine

Sources of Caffeine

Food sources	The amount of caffeine included in some common foods and beverages are:	Caffeine occurs naturally in the leaves, seeds, or fruit of more than 60 plant species, including:	Consuming too much caffeine can cause unpleasant side effects in some people, including Trusted Source:
• tea • coffee • chocolate • gum • jelly beans • waffles • water • syrup • marshmallows • sunflower seeds • other snacks	• One 8-ounce cup of coffee: 95 to 200 mg One 12-ounce can of cola: 35 to 45 mg One 8-ounce energy drink: 70 to 150 mg One 8-ounce cup of tea: 14 to 60 mg	• coffee beans tea leaves and buds kola nuts cacao beans guarana seeds yerba mate leaf	• sleeping problems headache nausea jitteriness increased heart rate stomach upset anxiousness

Uses of Caffeine

Coffee is one of the most consumable beverages around the world today, often to provide a burst of energy when needed. On average, 90% of adults consume caffeine on a daily basis from beverages ranging from coffee, soda, tea, and others. There are numerous benefits and drawbacks to caffeine consumption. Because caffeine is a stimulant, it can provide energy, decrease fatigue, and enhance motor performance [22]. Additionally, caffeine can help to maintain attention when needed. Coffee is often consumed by college students and other adults for this increase in energy and attention, so the amount of caffeine in various types of coffee is certainly of interest to the community. Caffeine is a naturally occurring substance found in the leaves, seeds or fruits of over 63 plants species worldwide [23, 24, 25, 26]. The levels of endogenous caffeine and theobromine were much higher in buds and young leaves of *Coffea arabica* L. than in fully developed leaves. Biosynthesis of caffeine from 14C-labeled adenine, guanine, xanthosine, and theobromine was observed, whereas other studies have indicated that there is no detectable incorporation of label into caffeine when theophylline and xanthine are used as substrates for in vivo feeds with leaves of *C. Arabica* [27]. Caffeine is a contamination indicator of domestic water because it is anthropogenic origin and it is detected in both waste and surface water [28]. Surface water is contaminated due to waste water from septic disposal and pharmaceutical disposal areas [29, 30]. Paxeus and Schroder (1996) [32] reported that 37 ug/l of caffeine in to Swedish sewage treatment plant. The recommended daily dose of Caffeine for stimulation is 200 mg/day [31]. A dose of 10 g is lethal, which is equivalent to about 100 cups of coffee [32, 33]. Recent epidemiological studies have seen an association between consumption of caffeine and risk of miscarriage [34]. High doses of caffeine are associated with various disorders affecting the central nervous system and cardiovascular system as well as increased gastric secretion and poor liver function [35-37].

Methods of Material

Materials Required

Lab Apparatus: 500 mL Beaker, 250 mL Erlenmeyer flask, Funnel, Distilled Water, separating funnel, Separatory funnel stand, stirring rod, Whatman filter paper, Watch glass.

Reagents and Materials

Tea Bags (or) Coffee bags, Sodium Carbonate (Na_2Co_3), Dicloromethane (Ch_2Cl_2)

Preparation of Sample

7 grams, of tea (or) coffee were taken in 150 ml of distilled water and 5 g of sodium carbonate is added. The solution was then heated and was kept at 100C for 15 min. Then, the solution was cooled and filtered using Whatman filter paper

Caffeine Extraction Procedure
This sample was placed into a separating funnel and 6 ml of dichloromethane (DCM) was added. The caffeine was extracted by inverting the funnel at least three times, venting the funnel after each inversion. Vigorous shaking will produce an intractable emulsion, while extremely gentle mixing will fail to extract the caffeine. The bottom layer containing dichloromethane (DCM) was removed to a clean flask, leaving behind the layer of water and the extraction procedure was repeated twice more and the solvent layers combined.

Separation of Caffeine
The dichloromethane was evaporated from the extract by heating the flask on mantle or by covering with perforated aluminium foil and leave it for some time and allow it to get evaporated and it was recovered in the other beaker using Heat Reflux Extraction method. The residue obtained was whitish powder which was considered to be pure caffeine. The mass of flask with residue was measured on electronic scale.

Analysis Techniques for Caffeine
Thin Layer Chromatography (TLC)
There are different types of chromatographic methods such as paper chromatography, thin-layer chromatography, column chromatography, gas chromatography, etc. They have the same principle:

1. Different solutes have different solubility in a solvent/different solute have different degrees of tendency to be dissolved in the same solvent.
2. As the solution (contains the solvent with the dissolved solutes) moves along a stationary solid surface (a solid surface), different solutes adsorbed onto the solid surface in different extent as they have different degree of adsorption characteristics (due to the different degrees of dissolve tendency)
3. The “less soluble” solute will be retained first, and the “more soluble” solutes will be retained afterwards.
4. (Note: No two substances have the same solubility and adsorption characteristics.)
5. Different solutes will then be separated on the different positions of the solid surface.
6. Retention Factor (RF) of each component is calculated as follow

$$Rf = \frac{\text{Grams of caffeine Recovered Distance travelled by the component substance from the baseline}}{\text{Distance travelled by the solvent from the baseline}}$$

Pure caffeine and the extract are analyzed in the same TLC plate and compare any differences of their Rf.

Spike Test
By adding known amount of standard caffeine in distilled water and raw coffee solution, then carry out solvent extraction. By comparing the extraction results, we can analyze the recovery percentage of the spiked caffeine and efficiency of solvent extraction.

Iodometric Back Titration
Iodometric Back Titration Caffeine reacts with excess accurately known amount of iodine in acidic environment, forming insoluble precipitate. Then the insoluble precipitate is removed by filtration. Using titration by a standard sodium thio-sulphate solution with starch solution as indicator, we can determine the amount of remaining iodine, and thus the amount of caffeine can be found. Here are the chemical equations:

$$C_8H_{10}N_4O_2 + 2I_2 + KI + H_2SO_4 \rightarrow C_8H_{10}N_4O_2.HI.I_4 + KHSO_4$$
$$I_2 + 2\ Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6$$

The Analysis Technique used here is iodometric back titration. The procedure is as follows: Caffeine solution was prepared using sulphuric acid as an acidic medium. Iodine was added to it and then the solution was titrated against sodium thiosulphate till the solution becomes pale brown in color. Then starch was added as indicator after the solution becomes pale brown. Again, the solution is titrated against sodium thiosulphate and the endpoint of the reaction is dark blue to colorless solution. Before the addition of starch the color of the solution is pale brown but after the addition of starch indicator the color of the solution changes to dark blue which then after titration with sodium thiosulphate the color of the solution turns colorless The solution is colorless that means the end point is reached and now calculating the amount of unreacted iodine with caffeine we can easily calculate the amount of reacted iodine with caffeine and hence by stoichiometry the amount of caffeine or the concentration of caffeine is determined.

Murexide test
1. In a watch glass, small amount of a sample with 2-3 drops of concentrated hydrochloric acid is mixed. Use a glass rod for mixing
2. Then we add a few small crystals of potassium chlorate and mix well.
3. Heat the watch glass until the sample is dry.
4. Allow to cool.
5. Add a drop of ammonium hydroxide solution. The sample should turn purple.

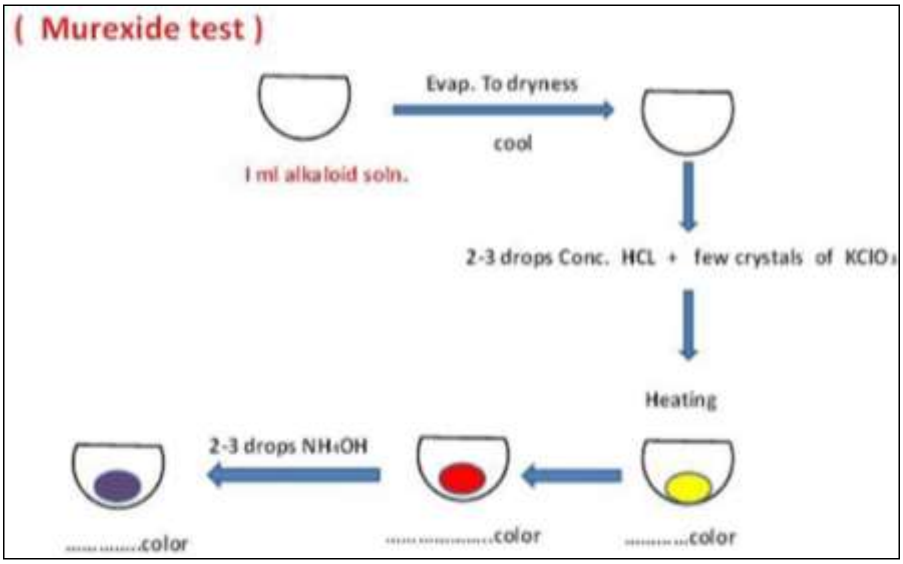


Fig 3: Murexide test

Conclusion

A method has been developed for the extraction, purification of caffeine from tea and coffee. Caffeine from tea and coffee was extracted by liquid- liquid extraction followed by recrystallization. The purified caffeine was then analysed by High performance liquid chromatography. Effective characterization of caffeine was achieved by determining IR spectrum, and employing a melting point apparatus and differential scanning calorimeter. The serious concern about potential use of caffeine for pathogenic effects has made it one of the most broadly studied drugs. It provides clinicians with the information they require in order to understand, diagnose and treat the effects of caffeine consumption in their patients.

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