



Isolation of Caffeine from Carbonated Beverages

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Abstract

The work presented on the isolation of naturally occurring alkaloid from carbonated beverages. The extensive presence of caffeine in different plants plays an important role in the long-standing acceptance of caffeine-containing products. Caffeine (3,7-dihydro-1, 3,7-trimethyl-1H-purine-2,6-dione or 1,3,7-trimethylxanthine) is an alkaloid belongs to Methylxanthine family. Liquid-liquid extraction methods were used in the assay of research work. Chloroform was taken as extracting solvent. Solid residue of caffeine was recrystallized from 95% ethanol using 5 mL/gram (5 mL per gram). It is declared to raise caffeine, effects a number of different drugs include Paracetamol, Benzodiazepines and Aspirin and amount of plasma free Fatty acids increases. While inform that in regular sleeping interaction caffeine take place and raise the absorption of certain drugs. Changes in drug metabolizing enzymes, acts as an agent in a microsomal system of the body. The highest amount of caffeine dry crystal is extracted in sting sample while the 7up sample is free from caffeine.

Keywords: Caffeine, liquid-liquid extraction, carbonated beverages, Crystals. Chloroform

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■ Introduction

Caffeine (3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione or 1,3,7-trimethylxanthine) is an alkaloid belongs to Methylxanthine family. The chemical formula of caffeine is (C₈H₁₀N₄O₂) and the systematic name of the caffeine is 1,3,5-

trimethylxanthine. Pure caffeine exists as odorless, fleecy masses, white, glimmering needles of powder. The molecular weight of caffeine is 194.19 gm, and its melting point is 236 °C. At atmospheric pressure the caffeine sublimates at 178 °C, pH of Caffeine is 6.9 in a 1% solution, its specific gravity is 1.2, volatility is

0.5%, and vapor pressure of caffeine is 760 mm of Hg at 178°C. The solubility of caffeine in water is 2.17%, vapor density is 6.7. The extensive presence of caffeine in different plants plays an important role in the long-standing acceptance of caffeine-containing products. Caffeine naturally exists in coffee beans, tea leaves and cocoa beans/leaves. People intake, caffeine in tea, chocolate, cocoa, and in various soft drinks. The amount of caffeine varies in different products, the maximum amounts are found in Guarana (4-7%), after that tea leaves (3.5%), cola nuts (1.5%), coffee beans (1.1-2.2%) and cocoa beans (0.03%). The structure formula of caffeine [1] Show in below [Figure 1]. Caffeine is traditionally used for its stimulatory effects. It stimulates heart, central nervous system and acts as a diuretic and increases brain activity. However, if caffeine is present in maximum amount in human being it can cause several unwanted symptoms such as tachycardia, tremor, seizures, gastrointestinal problem and even death. So it is necessary to check caffeine in foods and beverages for normal activities of human beings [2]. In the past decades Caffeine has drawn more attention due to its more consumption and physiological effects it causes stimulatory effect. The level of caffeine in human salivary, which show the extent of absorption, peaks around 40 min after caffeine consumption. Different physiological effects on the cardiovascular, respiratory, central nervous, gastrointestinal and renal systems have been noted Mild elevation in blood pressure is caused by caffeine. In addition, effect of caffeine's diuretic is widely known [3].

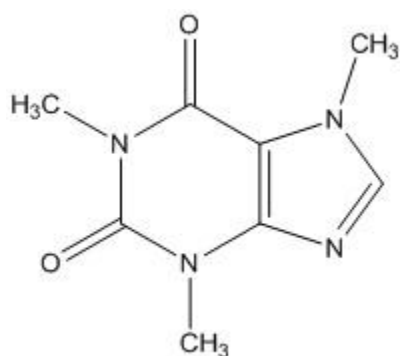


Figure 1. Structure of Caffeine

Caffeine metabolism exists inside the liver, where molecules of caffeine are metabolized by cytochrome P450122 (CYP1A2) by three main demethylation reactions (Carrillo and Benitez). More than 95% of caffeine metabolism the CYP1A2 enzyme are responsible, from carbon #3 removal of methyl (-CH₃) group is the important demethylation reaction to demethylation common metabolism reaction, severally(carriti & Benitez, 200). This would look to show increase metabolism of caffeine due to up-regulation of CYP1A2 enzyme in wonted caffeine user, an indication achievable caffeine tolerance; it also mean that non consumer of caffeine are much vulnerable to higher caffeine medicine, due to drug are not clear rapidly [4].

Caffeine amount in different food and beverage have been analyzed including coffee, carbonated beverages, Tea and chocolate products. Ten years ago, on large-scale the caffeine contents of carbonated beverages was study handled where the caffeine contents of 24 fountains, 20 prepackaged national-brand, and 16 prepackaged private-label store-brand carbonated beverages were imposed; the store-brand beverages were limited to products from 4 stores. On the label of carbonated beverages, caffeine value are not written then the consumers are left relatively uninformed attention the amount of caffeine contained in these beverages. The maximum amount caffeine in carbonated beverages is allowed different governmental bodies. The U.S. Food and Drug Admin the amount of caffeine limit in carbonated beverages to a maximum of 0.02%. Thus, the maximum amount of caffeine allowed in a 355 ml (12 Oz) can of soft drink is roughly 72 mg. Similarly, Canada the caffeine limit to cola-variety beverages at a level of 200 ppm or about 71 mg/12 Oz (Dept. Of Justice 2007). In cola-type beverages the maximum amount of caffeine must not exceed 145 mg/kg or about 51 mg/12 Oz, In Australia different analytical methods are used for the determination of caffeine content in carbonated beverages and foods. These techniques are based on UV-visible spectrophotometry, high-performance liquid chromatography (HPLC), gas chromatography (GC), thin-layer chromatography/mass spectrometry (TLC/MS), Fourier transforms

infrared spectrometry (FTIR), ion chromatography and capillary electrophoresis (CE) [2]. Here we presented the isolation of naturally occurring alkaloid from carbonated beverages.

■ Experimental

Apparatus

Graduated cylinder, stirrer, spirit lamp, tripod stand, separating funnel, and stand, Apparatus for suction filtration, water bath, and funnels.

Chemicals

Chloroform (CHCl_3), ethanol ($\text{C}_2\text{H}_5\text{OH}$), distilled water, carbonated beverages (Pepsi, Red bull, Coca cola etc.).

Carbonated beverages Samples

Different samples of carbonated beverages were taken from local market of Mardan. These carbonated beverages were sting, Coca Cola, Pepsi cola, 7up, Diet Sunkist, Pepsi diet, Red bull, and Mountain dew.

Procedure A

100 mL sample of carbonated beverages was placed in a 250 mL Erlenmeyer flask and then 100 mL of chloroform was added to it. The mixture was stirred for about 10 minutes. After stirring the mixture was transferred to a separating funnel. The solubility of caffeine in chloroform is quite high at room temperature. The caffeine was transferred to chloroform layer. Two separate layers were formed. The upper layer is aqueous while chloroform layer is lower because chloroform is denser than water. The organic layer (chloroform layer) was transferred to a weighted, 25 mL flask and the mixture was evaporated to dryness on the steam bath. Solid residue of caffeine was recrystallized from 95% ethanol using 5 mL/gram (5 mL per gram). Structure of caffeine crystal are given in (Figure 2).

Procedure B

200 mL of carbonated was take in 500 mL Erlenmeyer flask and add 200 mL of chloroform to Erlenmeyer flask. Stirred the mixture up to 10

minutes. Then transferred to a separating funnel. In a separating funnel formed two different layers. One is organic layer so the organic layer was transferred to a weighted, 50ml flask and the mixture was evaporated to dryness on the steam bath. Solid residue of caffeine was recrystallized from 95% ethanol using 5 mL/gram (5 mL per gram).



Figure 2 Crystal of caffeine

■ Results and Discussion

Organoleptic

The work presented on the isolation of naturally occurring alkaloid from carbonated beverages. 100 and 200 mL sample of carbonated beverages was placed in a 250 mL Erlenmeyer flask and then 100 mL of chloroform was added to it. The mixture was stirred for about 10 minutes. After stirring the mixture was transferred and Solid residue of caffeine crystal was recrystallized from 95% ethanol using 5 mL/gram (5 mL per gram). The different results of caffeine presented in Table 1 and Table 2. The highest amount of caffeine extracted from sample sting 170 mg/100 mL shown in Figure 3. While different carbonated beverages contain wide range of caffeine only the sample 7up do not contain any content of caffeine shown in Figure 3 and Table 1. Also the amount of caffeine found in 200 mL of different carbonated beverages possess sting highest caffeine 300 mg/200 mL shown in

Table 2 and Figure 4. While the other sample show According to [5] reported that caffeine are extracted from beverages by using liquid-liquid extraction and detect through gas chromatography. [6] Reported that the amount of caffeine in different non-alcoholic beverages was analyzed through HPLC technique, which determined that Coke (4.15 compared with 3.13 mg/fl Oz) has less caffeine content than Diet Coke. While [1] showed that Simple High Performance Liquid Chromatographic (HPLC) technique is use for the determination of caffeine amount in 15 different beverage samples of the Sudan local markets, which are commercially available. Range of caffeine content in carbonated soft drinks is about 32.4 ppm to 133.3 ppm with normal concentration of 96 ppm. n non similar results as compared to one another.

Table 1. The amount of caffeine found in 100 ml of different carbonated beverages

S.No	Sample	Caffeine(mg/100ml)
01	Sting	170 mg
02	Coca cola	25 mg
03	Pepsi cola	29 mg
04	7up	00 mg
05	Diet Sunkist	28 mg
06	Pepsi diet	22 mg
07	Red bull	55 mg
08	Mountain dew	29 mg

Also [3] showed that the amount of caffeine in 56 national-brand and 75 private-label store-brand carbonated beverages determined by using of

high-performance liquid chromatography. Caffeine amounts ranged from 4.9 mg/12 Oz (IGA Cola) to 74 mg/12 Oz (Vault Zero) is calculated. Several public national-brand carbonated beverages analyzed their caffeine table of contents are Diet Coke (46.3 mg/12 Oz), Coca-Cola (33.9 mg/12 Oz), Diet Pepsi (36.7 mg/12 Oz), Pepsi (38.9 mg/ 12 Oz), Diet Dr. Pepper (44.1 mg/12 Oz), Dr. Pepper (42.6 mg/12 Oz), Diet Mountain Dew (55.2 mg/12 Oz) and Mountain Dew (54.8 mg/12 Oz). Wal-Mart store-brand beverages, caffeine content are Sam’s Diet Cola (13.3 mg/12 Oz), Sam’s Cola (12.7 mg/12 Oz), Dr. Thunder (30.6 mg/12 Oz), and Mountain Lightning (46.5 mg/12 Oz) and Diet Dr. Thunder (29.9 mg/12 Oz).14 other are analyses which contain caffeine less amount than national brand counterparts reported that in the past period caffeine has drawn more attention due to its maximum consumption and physiological effects beyond that of its stimulatory effect.

Table 2. The amount of caffeine found in 200 ml of different carbonated beverages

S.No	Sample	Caffeine(mg/200ml)
01	Sting	300 mg
02	Coca cola	50 mg
03	Pepsi cola	58 mg
04	7up	00 mg
05	Diet Sunkist	56 mg
06	Pepsi diet	44 mg
07	Red bull	110 mg
08	Mountain dew	58 mg

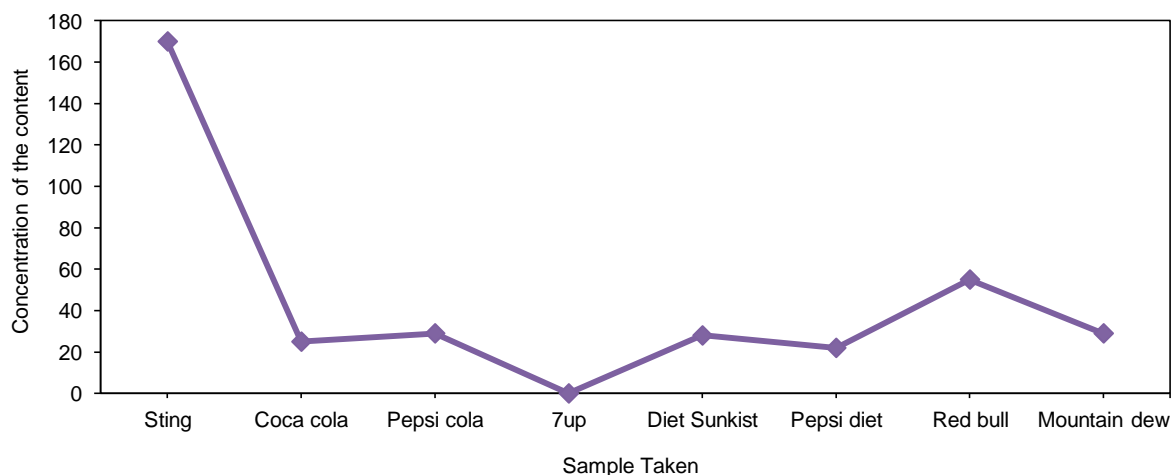


Figure 3: The Caffeine found in 100 ml of different carbonated beverages

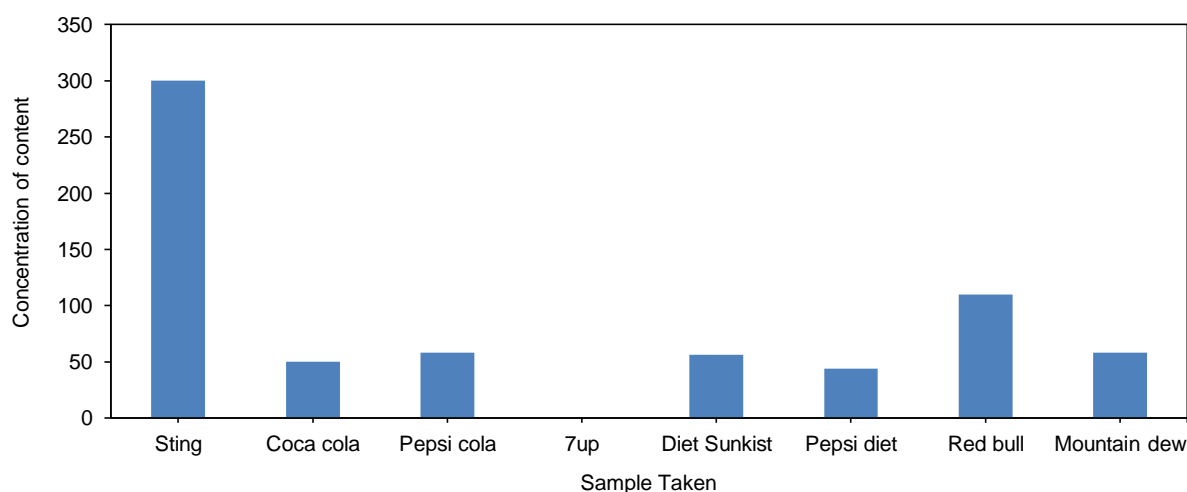


Figure 4: The Caffeine found in 200 ml of different carbonated beverages.

While [7] found that Caffeine is a natural substance present in the seeds, fruits or leaves of over sixty three plant species global. According to [8] that it is declared to raise caffeine effects of a number of different drugs include Paracetamol, Benzodiazepines and Aspirin and amount of plasma free Fatty acids increase. While [9] inform that in regular sleeping interaction caffeine take place and raise the absorption of certain drugs. Changes in drug metabolizing enzymes, acts as an agent in a microsomal system of the body. Also [10] concluded that the Cause of "food allergy" Caffeine are involved, as Common word for an upset condition which includes symptoms such as palpitations, vomiting, anxiety and panic attacks and headache. The [11] reported that the body absorbed Caffeine rapidly. Salivary of the human indicates the extent of caffeine absorption, peaks just about 40 min after caffeine consumption.

In [12] showed that Several physiological effects on the central nervous, cardiovascular, respiratory, gastrointestinal and renal systems have been noted For example, Hartley and others (2004) suggested that caffeine causes a moderate elevation in blood pressure level. While [3] reported that Different states have stated that in carbonated beverages the maximum level of caffeine allowed. The Drug Admin and U.S. Food, caffeine limits in carbonated beverages maximum of 0.02%. In the Australia country, the amount of caffeine content in beverages like cola-types beverages not maximum

than 145 mg/12 Oz or approximately 51 mg/12 Oz as long as in New Zealand, the level of caffeine in cola-types beverages is up to 200 mg/kg or approximately 71 mg/12 oz. The [2] reported that in beverages, caffeine is legally allowable with abide higher limit of 0.015% (w/w). In spite of [13] showed that the effect of caffeine on phosphodiesterase, they are insecticides naturally. In [14] reported that the caffeine in a few humans, cause gastric symptoms and also tachycardia. Also [15] confirm that when caffeine ingested through the mouth, it goes to the stomach and then to the GI tract, into the bloodstream, it is rapidly absorbed and dispersed in the body, pass through with the blood-brain barrier and into the brain [16-18]. While [19] showed that it shows positive activity between maternal consumption of the caffeine and fetal growth retardation or birth rate decrease. During the research it is found that women whose consumption of caffeine is 0-10mg per day his child weighing 116g greater than whose who consume 71-140 mg per day.

■ Conclusion

The liquid-liquid extraction method is used for determination of caffeine in beverages was prove simple, precise and obtained acceptable results. Concluded that the order of caffeine in carbonated beverages was Sting > Red bull >

Diet Sunkist > Pepsi cola > Mountain Dew > Pepsi diet > Coca cola > 7up. The highest amount of caffeine dry crystal is extracted in sting sample while the 7up sample is free from caffeine. The amount of caffeine present in sting (180mg/100mL) while the lower level of caffeine found in Coca Cola (30mg/100mL). The Seven Up (7up) is free of caffeine. So Seven Up is less effective than other beverages.

■ Conflict of Interest

The authors declare no conflict of interest.

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