Isolation and Characterization of Caffeine from Soft Drinks

Dr. K. Priya Rajini [1], Dr. C. Agatha Christie [2], Dr T. alwin [3], Mrs. L.lisy[4], Mr. P.Ashish Wilfred[5]
[1,2,3,4,5]*Department of Chemistry and Research Centre, Nanjil Catholic College of Arts and Science kaliyakkavilai ,Kanyakumari District-629153- TamilNadu, India*

Abstract **- The work presented on the isolation of naturally occurring alkaloid from carbonated beverages. Caffeine is a frequently used ingredient of energy drinks. It is added as a flavoring agent and to make the drinks addictive. Caffeine is a bitter in taste, white crystalline xanthenes alkaloid that acts as a psychoactive stimulant drug and a mild diuretic. Caffeine is (3, 7-dihydro-1, 3, 7-trimethyl-1H-purine-2, 6-dione or 1, 3, 7-trimethylxanthine) an alkaloid belongs to Methylxanthine family. Liquid-liquid extraction methods were used in the assay of research work. Ethylene acetate was taken as extracting solvent. Solid residue of caffeine was recrystallized from 95% ethanol using 5 mL/gram. The mostly available soft drinks are used for the isolation of caffeine; the highest amount of caffeine dry crystal is extracted in red bull sample while the 7up sample was very poor caffeine. And this paper is the detailed study of caffeine, Not only the isolation of caffeine but also the physical characteristic as well UV and IR spectral details were explained.**

Index Terms **- liquid-liquid extraction, soft drinks, cola drinks.**

I.INTRODUCTION

The investigation has been carried out so as to clarify the level of concentration of caffeine in some of the more popular soft drinks. Caffeine is one of the chemical materials which have many negative side effects if its level has become elevated in the human body and it leads to different types of diseases. It is a major factor in causing osteoporosis, it also contributes to causing several types of cancers such as, colon, stomach, prostate, pancreas, and gall bladder cancers, also it causes anemia. Finally, caffeine causes a rise in gastric pH causing ulcers in the stomach and the duodenum.

Caffeine is a pharmacologically active substance and depending on the dose, can be a mild central nervous system stimulant. Caffeine does not accumulate in the body over the course of time and is normally excreted within several hours of consumption [1]. Caffeine serves as an ingredient in many carbonated soft drinks including colas, pepper-type beverages, and citrus beverages. Pure caffeine occurs as Odorless, white, glistening needles of powder. Caffeine has drawn more attention in the past decades due to its physiological effects beyond that of its stimulatory effect. The Food and Drug Administration (FDA) defines caffeine as a generally recognized as safe (GRAS) substance. However, FDA specifies that the maximum amount in carbonated beverages is limited to 0.02% (FDA 2006) [2, 3]·In its pure form, caffeine is a white crystalline powder that tastes very bitter. It is medically useful to stimulate the heart and also serves as increasing the rate of urine excretion.

Caffeine is an alkaloid of the methylxanthine family. In its pure state, it is an intensely bitter white powder. Its chemical formula is $C_8H_{10}N_4O_2$, its systematic name is 1, 3, 5-trimethylxanthine [4] and its chemical formula is shown below.

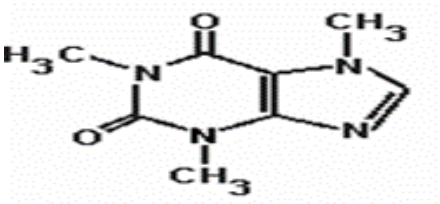

Fig1. Structure of caffeine

Pure caffeine occurs as odorless, white, fleecy masses, glistening needles of powder., Its molecular weight is 194.19g, melting point is 236 ^{0}C, point at which caffeine sublimes at 178^{0}C atmospheric pressure, pH is 6.9 (1% solution), specific gravity is 1.2, volatility is 0.5%, vapor pressure is 760 mm Hg at 178 ^{0}C, solubility in water is 2.17%, vapor density 6.7 [5]. Extraction is a method used for the separation of organic compound from a mixture of compound. This technique selectively dissolves one or more

compounds into an appropriate solvent. Liquid–liquid extraction (LLE) is a basic technique in chemical laboratories, where it is performed using a variety of apparatus, from separate funnels to counter current distribution equipment called as mixer settlers. This type of process is commonly performed after a chemical reaction as part of the work-up, often including an acidic work-up [6]. In the case of Caffeine extraction from soft drinks, the solubility of caffeine in water is 22mg/ml at 25°C, 180mg/ml at 80°C, and 670mg/ml at 100°C. Here the organic solvent Ethylene acetate is used to extract caffeine from aqueous extract of soft drinks because caffeine is more soluble in ethylene acetate (140mg/ml) than it is in water (22mg/ml). The ethylene acetate - caffeine mixture can then be separated on the basis of the different densities of ethylene acetate and water because ethylene acetate is much denser than water and insoluble in it. The procedure was as follows: 25 ml of soft drinks sample was taken and boiled for 15 minutes along with the addition of 6gm of sodium carbonate which acts like a base which reacts with tannins to form sodium salts of tannins [7]. After separation of organic layer from the separating funnel it is then kept for evaporation so as to evaporate the ethylene acetate present in it. The sodium sulphate is used for increase the settling reaction time for the caffeine isolated from the carbonated beverage. After the addition of sodium sulphate, the sample is filtered and allowed for crystallization. Recrystallisation is a fast and easy way to purify the caffeine. Ethanol was used for recrystallisation as solvent. The Success of extraction involving a natural product is often expressed as percentage recovery [8]. In this present study, we have extracted caffeine from different soft drinks like Red bull, Pepsi swag, Coca cola, Mountain dew and 7 up and also characterized by boiling point, melting point, UV and IR spectral analysis. In Bangladesh, there is no authentic data about caffeine content in soft drinks, then only we also determined the concentration of caffeine in various available soft drinks, especially cola drinks. The proposed method is simple, rapid and has significant advantages over other spectrophotometric methods as well as other HPLC methods.

II. PROCEDURE

A.Material

Sodium carbonate (Merck, Germany), ethylene acetate (sigma aldrich) sodium sulphate (Merk) and various brands of soft drinks like Red bull, Pepsi swag, Coca cola, Mountain dew, and 7 up were collected from local market.

B. Methods

Take 200 ml of different soft drinks in various beakers. To this add 2-3 gm of sodium carbonate salt allowed for stirring in magnetic stirrer with constant temperature (200-300^{o}C) continued for 20-30 minutes and allowed for cooling in an ice water bath. Cooled solution was filled in the dried clean 250 ml separating funnel and mixed with 20-25 ml of ethylene acetate. The sample solution was shaken well and allowed to stand for 5 minutes 2 layers are formed. The less organic layer was separated and mixed with 2-3 gm of sodium sulphate. The sodium sulphate added sample solution was filtered by Whitman filter paper, filtered sample was allowed to settle for overnight. The white crystalline caffeine sample was obtained, the same procedure followed for isolation of caffeine from other soft drinks. Then all this samples were recrystalysed using ethyl alcohol and water mixture.

III CHARACTERIZATION OF CAFFEINE

The prepared samples were subjected to physical characterization such as Melting point, the melting point of different extracted pure samples after purification was carried out in a digital melting point apparatus (Electro thermal, IA9100). The average melting points of the samples was 235^{0}C, Boiling point (Capillary tube method) , FT IR spectral analysis was done by Magna IR 550 spectrophotometer UV absorption spectrum of extracted and purified crystalline caffeine samples were studied by different absorbance against different wavelength using a UV-absorption spectrophotometer from SHIMADZU Corporation, Japan; Model: UV -1601. The average λmax was found to be 200-275 nm. Purity of the recrystalised sample was studied by % recovery method. The reproducibility of this method was also checked by determining the percentage recovery of known amount of standard caffeine sample. For example if X be the actual content of caffeine in the sample and 5ppm standard caffeine was added then the content become (X+5) ppm and if the observed concentration is X', then the percentage recovery is given by

$$\% \text{ Recovery } = X/(X+5) \text{ X } 100 \%$$

The percentage recovery is called the purified percent recovery or crude percent recovery. The extraction with the highest percent recovery is considered the most successful extraction.

IV RESULT AND DISCUSSION

A, Physical Characterization

The extraction procedure using ethylene acetate proved to be highly efficient and simple. The total amount of caffeine present initially in the different samples and their melting poet, boiling point and % recovery are listed in the table given below

Samples	Caffeine (mg)	Melting point ^{0}C	Boiling point°C	% recovery
Red bull	80	235	178	94
Mountain dew	55	233	176	91
Pepsi swag	37.5	230	178	88
Coca cola	34	210	175	87
7 up	0.1	200	170	1.96

Table1. Physical characterization of samples

B. UV Spectroscopy:

Spectrophotometer provides a sensitive method for the detection and measurement of caffeine. The UV absorption spectrum of caffeine exhibits a pair of absorption bands peaking at 205 nm and 273 nm with a characteristic absorption shoulder between them. Typically, caffeine content is determined by measuring the absorbance at 275 nm. Soft drinks contain a wide variety of substances, many of which absorb UV light at 275 nm [9]. Consequently, the direct measurement of the caffeine absorbance in soft drinks is not possible and one must first separate the caffeine from other components before making the absorbance measurement [10]. Although Spectrophotometer is a fast and simple method it is not possible to determine caffeine directly in coffee beans by conventional UV absorption measurement due to the spectral overlap [11]. We select UV- Visible spectrophotometer chose method for the determination of caffeine, because UV- Visible spectrophotometer is the most widely used qualitative and quantitative determination and separation method. This method is popular because it is non-destructive and unlike gas chromatography may be applied to thermally liable compounds. Moreover, it is also a very sensitive technique as it incorporates a wide range of detection methods [12, 13].

Samples	λ_{max1}	λ_{max2}
Red bull	202	274
Mountain dew	205	281
Pepsi swag	206	271
Coca cola	203	279
7 up	-	-

Table2. UV Spectral studies of samples

C. Fourier Transforms Infrared Spectroscopy (FTIR):

FTIR is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high-spectral-resolution data over a wide spectral range [14]. This confers a significant advantage over a dispersive spectrometer, which measures intensity over a narrow range of wavelengths at a time. Different caffeine was characterized by absorption bands at 2860-2850 cm^{-1} which are assigned to C=H aromatic stretching vibration. The peaks at 3150 to 3100 cm^{-1} are assigned to -CH_2 and –C-H aliphatic stretching vibration. The bands at 1630 cm^{-1} and 1645 cm^{-1} indicates the non conjugated deformation of C=C stretching in benzene ring. The band at 1695 to 1690 cm^{-1} indicates cyclic ketone C = O groups are present in caffeine nucleus. The bands at 1240 to 1245 cm^{-1} can be attributed to C-N out of plane bending vibration of benzene ring. The bands at 2240 cm^{-1} to 2266 cm^{-1} indicates the non conjugated stretching vibrations of nitrogen, it also indicates two nitrogen presents in the caffeine structure. The FTIR spectrum shows the various atoms present in the caffeine nucleus.

Samples	Red bull	Mountain dew	Pepsi swag	Coca cola	7 up
Bands	v (cm^{-1})	v (cm^{-1})	v (cm^{-1})	v (cm^{-1})	v (cm^{-1})
C=H	2860	2861	2860	2866	2850
C-H	3150	3100	3100	3150	3100
C=C	1645	1640	1645	1645	1640
C=O	1695	1695	1693	1690	1690
C-N	1240	1246	1240	1244	1240
C=N	2240	2265	2240	2250	2220

Table 3. IR Spectral studies of samples

V CONCLUSIONS

The liquid-liquid extraction method is used for determination of caffeine in carbonated beverages was prove simple, precise and obtained acceptable results. Concluded that the order of caffeine in carbonated beverages was Red bull > Mountain Dew > Pepsi swag > Coca cola > 7up. The highest amount of caffeine dry crystal was extracted from the entire sample while the

7up sample was very poor amount caffeine. The amount of caffeine present in Red bull (80mg/100mL) while the lower level of caffeine found in Coca Cola (34mg/100mL) So, Seven Up is less effective than other soft drinks. Caffeine is very rich in antioxidants; it is the most widely used beverage all over the world, it also has medicinal properties. This study will be carried out to check the amount of caffeine in used soft drinks. Caffeine is the most commonly used psychoactive drug in the world. It is a pharmacological active substance and depending on the dose, can be a mild central nervous system stimulant. Approximately 80% of the world's Population Consumes Caffeine on daily basis. The Series of experiments that have been conducted, we can conclude that the caffeine content of RED BULL is relatively high as compared to other soft drinks and therefore we can also state that the caffeine is highly soluble in Ethylene acetate as compared to other solvents. FTIR spectral, and UV spectral studies also shows the characteristics absorption bands of prepared samples are similar to standard caffeine samples, as well as it obeys all the properties of caffeine. Caffeine present in the soft drinks is easily separated out and easy to make analysis. Caffeine is become the most dangerous food substance in the world. The uncontrolled use of caffeine causes dangerous to human health. So, make a limited use of soft drinks and maintain good health.

REFERENCES

[1] J.J. Barone and H.R Roberts, "Caffeine Consumption", Food Chemistry and Toxicology, McGraw-Hill, Newyork, 34, 119, 1996.

[2] I.Gerald, D.E Arthur and A .Adedayo, Determination of caffeine in Beverages. A Review American journal of Engineering Research; 3(8): 124-137. 2014.

[3] R. Rehman and S. Ashraf: Analysis of caffeine contents in commercial beverages and tea samples of Pakistan using UV visible spectrophotometry. Bulgarian chemical communications; 49(4): 823-828. 2017.

[4] M. J Arnaud, The Pharmacology of Caffeine, Prog Drug, 31, 273 ,1987

[5] G.L Clementz, and J. W Dailey, Psychotropic effects of caffeine, Amer. Fan Physician. 37 167, 1988

[6] S. Venkatesh, M.M Swamy, Y.S. R Reddy, B. Suresh and M.Sethuraman A simple method for determination of caffeine content in tea samples. Journal Ancient Science of Life,14(1-2),35-38. 1994.

[7] G. Serdar, E. Demir and M. Sokmen, Recycling of tea waste: Simple and effective separation of caffeine and catechins by Microwave Assisted Extraction (MAE). International Journal of Secondary Metabolite. INT. J. Sec. Metabolite,4(2),78-89. 2016.

[8] A. Chaudhary, S. Sarkar, A. Chaudhary, S. Bardhan, P. Mandal and M. Chaudhary Tea waste management: A case study from West Bengal, India. Indian Journal of Science and Technology, 9(42), 89790, 2016.

[9] A. Babatunde,T. Oluseyi ,O. Kehinde and O.Andrew: Determination and characterization of caffeine in Tea, Caffeine and Soft Drinks by Derivative Spectrophotometry and High Performance Liquid Chromatography. Biomedicine and Nursing 3(2). 2017.

[10] G. Serdar, E. Demir, M. Sokmen New approaches for effective microwave assisted extraction of caffeine and catechins from green tea. Journal International Journal of Secondary Metabolite, 3,3-13. 2016.

[11] Q.L .Zhang, H..Z Lian, W.H Wang and H.Y Chen, Separation of caffeine and theophylline in poly (dimethylsiloxane) micro channel electrophoresis with electrochemical detection. Journal of Chromatography A, 1098, 172–176. 2005.

[12] G. Serdar, E. Demir and M. Sokmen, Comparison of some extraction methods for isolation of catechins and caffeine from Turkish green tea. Journal international journal of secondary metabolite, 2, 16-25. 2015.

[13] S. Parvathy, A. luiz and J. Varkey, Chemical analysis of caffeine content in tea and coffee samples. Asian journal of science and applied technology,3(1),1-4. 2014.

[14] A. Amoozegaran and M. Javaherian Comparative Evaluation of caffeine extraction in waste tea as a potential low-cost source relative to dry tea in different solvents. Natural products-An Indian journal, 10(1), 27-29. 2014.