2024
4(2), 695

Journal of
Molecular Chemistry

Extraction of caffeine and sodium benzoate from different brands of cold drinks available in market

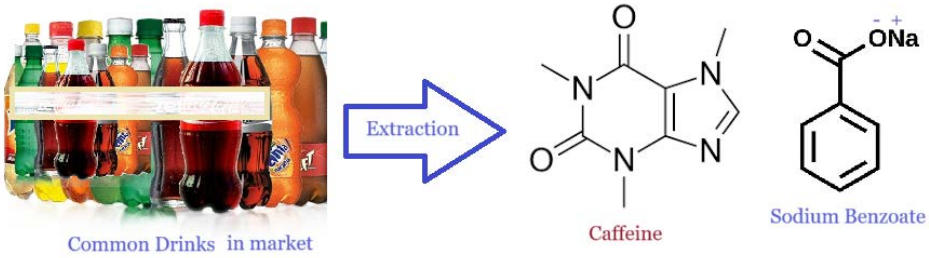
Priyanka Yadav, Saurabh Kumar, Savita Bargujar*

Department of Chemistry, Ramjas College, University of Delhi, Delhi, India

Submitted on: 12-Dec-2023, Accepted and Published on: 20-Apr-2024

Article

ABSTRACT



Caffeine is an alkaloid present in beverages and soft drinks that are available on the market. It belongs to the methylxanthine family. An acceptable daily intake (ADI) of caffeine is 0.4-1.4 mg/kg of body weight for adolescents (10-18 years). It has an instant psychoactive effect on our minds. It has the ability to put off sleep, increase alertness, and improve attention in studies. Besides these effects, it brings various health concerns with it. It disturbs the sleep cycle and leads to different types of diseases, such as osteoporosis and several types of cancer. On the other hand, sodium benzoate is often used as a preservative in soft drinks to prevent decomposition by undesirable changes. An acceptable daily intake (ADI) of sodium benzoate is 0-5 mg/kg of body weight. But daily intake can develop adverse effects on the skin, such as rash, non-immunological contact urticaria, metabolic acidosis, and asthma. So, the determination of caffeine and sodium benzoate in soft drinks is important from a biological point of view, as such drinks are very common among youth. In this work, the presence of caffeine and sodium benzoate was determined in different brands of cold drinks available on the market. There are many techniques for their determination, but the present work has been done by liquid-liquid extraction of caffeine and sodium benzoate. A qualitative analysis of the extracted caffeine was done by the murexide test. A qualitative analysis of the extracted sodium benzoate was performed with the $FeCl_3$Test. Further, quantitative analysis of sodium benzoate was done via acid-alkalimetry. The analysis performed showed that Sting has the highest caffeine content as well as sodium benzoate, while no caffeine was extracted from Sprite. Rev was found to contain the least sodium benzoate.

Keywords: Liquid-liquid Extraction, Murexide Test, $FeCl_3$ Test, Acid-alkalimetry Titration

INTRODUCTION

Cold drinks, which are common beverages, are consumed by people of all ages especially common among school and college going students. It is taken as an energy drink, as a substitute for coffee and tea and its consumption is increasing day by day. It has become part of the youth lifestyle. The reason that such drinks provide instant refreshment is due to the presence of caffeine. Caffeine has an instant psychoactive effect on our minds.[1] It has the ability to put off sleep, increase alertness, and improve attention in studies. Besides these effects, it brings various health concerns with it. Caffeine (3,7-dihydro-1,3,7-trimethyl-1Hpurine-2,6-dione, or 1,3,7-trimethylxanthine) is an alkaloid belonging to the methylxanthine family.[2] The chemical formula of caffeine is ($C_8H_{10}N_4O_2$), and the systematic name of caffeine is 1,3,7-trimethylxanthine. Pure caffeine exists as odourless, fleecy masses of white, glimmering needles of powder. The molecular weight of caffeine is 194.19 g, and its melting point is 236℃. At atmospheric pressure, caffeine sublimes at 178 ℃; the pH of caffeine is 6.9 in a 1% solution; its specific gravity is 1.23 %; and the vapour pressure of caffeine is 760 mm of Hg at 178 ℃. The solubility of caffeine in water is 2.17%. The negative effects of the alkaloid in moderate and high doses are alterations (1) in the sleep cycle and (2) affective states. In the first case, children's metabolism increases at night. Their sleep cycle demands stability and consistency, as well as more hours in bed than adults. As observed, any alteration in the sleep cycle indirectly compromises the adequate mental and physical development of children. The ingestion of caffeine is one of the leading factors interfering with these processes.[3] It would be important to know if there are variations depending on different doses. Alterations in affective states such as anxiety and depression produced by caffeine are directly proportional to the

*Corresponding Author: Dr. Savita Bargujar
Email: savitabargujar@ramjas.du.ac.in

URN:NBN:sciencein.jmc.2024.695

Published by: ScienceIn Publishing
https://pubs.thesciencein.org/jmc

amount ingested.[4] Since children's body mass is lower than that of adults, they consume more caffeine but in fewer products. This effect seems to be more harmful in the case of affective states. Children's emotional health seems to be one of the aspects affected by caffeine intake and its dosage influence. Therefore, this information may suggest that children are more sensitive to the effects of the alkaloid, although the cause remains unclear. Besides these effects, if its level is elevated in the human body, 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 cancer;[5] and it causes anaemia. Finally, caffeine causes a rise in gastric pH, causing ulcers in the stomach and duodenum.

Carbonated drinks have become one of the favourite drinks among youth because they taste good and are fresh.[6] Because it is stored for a certain period of time, this drink is often given additional preservatives to maintain its quality for a longer period of time. A preservative is a chemical substance that is added to products such as packaged food items, beverages, pharmaceutical drugs, and many other products to prevent decomposition by undesirable chemical changes. The two most common preservatives used in soft drinks are sodium benzoate and potassium sorbate whose daily intake can develop adverse effects on the skin, such as rash and asthma.[7] It increases the rate of DNA damage. When these preservatives are consumed for a long time, sodium benzoate in the presence of vitamin C (present in our body) is converted to benzene. Benzene is recognized by the IARC (International Agency for Research on Cancer) as carcinogenic to humans.

The main purpose of this project is to identify the presence of caffeine in different brands of cold drinks by liquid-liquid extraction and checking it by the 'Murexide Test'. On the other hand, to identify the presence of sodium benzoate in different brands of soft drinks by liquid-liquid extraction and checking it via the $FeCl_3$Test. Further, there are various local brands of soft drinks that are randomly consumed by students and locals. Thus, these soft drinks need to be analyzed.

MATERIALS AND METHODS

Samples

Different brands of cold drinks commonly consumed by locals and youth were taken. The main focus was to take up those drinks that contain caffeine, as asserted by their respective companies. The soft drinks that did not contain caffeine in them were also taken to analyze whether they were caffeine-free, as reported. Brands named Coca-Cola, Sprite, Rev, and Sting were taken for qualitative analysis of their caffeine content. Sprite, Coca-Cola, Sting, Limca, and Rev were taken for qualitative as well as quantitative analysis of sodium benzoate.

Method

The liquid-liquid extraction method is based on the principle that a solute can divide itself between two completely immiscible solvents. The ratio of the concentration of a solute between two different phases is called the distribution coefficient. Since the purpose of the project was to extract caffeine from cold drinks whose main content was water, chloroform was chosen as the extracting solvent, as caffeine was more soluble in chloroform than water. The same solvent was used to extract sodium benzoate as benzoic acid from soft drinks.

Qualitative analysis

Extraction of caffeine

100 ml of each cold drink was taken in a beaker. 2g of sodium bicarbonate (Na_2CO_3) was added to it. It was stirred using a magnetic stirrer for 2 minutes. Then, the contents of the beaker were emptied into a separate funnel. 10 ml of chloroform ($CHCl_3$) was added to it and shaken properly so that caffeine got extracted into the organic solvent. It was then left undisturbed so that two layers could be separated on the basis of their densities. Since chloroform has a higher density than water, it settled in the bottom layer. This layer was taken out of a beaker. The remaining portion was again extracted with 10 ml of chloroform twice so as to increase the amount extracted. Then chloroform was distilled using steam distillation, and the yellowish-white residue left behind in the round bottom flask was caffeine. The remaining portion was again extracted with 10 ml of chloroform twice so as to increase the amount extracted. For this, a very small amount of potassium chlorate ($KClO_3$) was added to the round bottom flask containing caffeine, and a few drops of dilute hydrochloric acid (HCl) were also added. It was then heated until it was dried. Then, a few drops of ammonium hydroxide (NH_4OH) were added to it. The contents of the round-bottom flask turned pink, which confirmed that the white residue left was caffeine.[8]

Extraction of sodium benzoate

10 ml of each soft drink was taken in a beaker and acidified with 15 ml of concentrated hydrochloric acid to turn the benzoic compound back into benzoic acid, which is not soluble in water but dissolved in organic solvent ($CHCl_3$). Then the content was transferred to the separating funnel, and 10 ml of chloroform was added and stirred properly by releasing the pressure regularly so that sodium benzoate got extracted into the organic solvent. It was then left undisturbed for a few minutes so that two layers could be separated on the basis of their densities. As chloroform is denser than water, it thus settled at the bottom layer, which was taken out in a beaker. The remaining portion was again extracted with 10 ml of chloroform twice in order to increase the amount of sodium benzoate extracted. Then added 15 ml of 10% NaOH to alkalize the solution so that all the benzoate was present as its water-soluble salt, followed by the addition of a 10 ml saturated solution of NaCl to increase the level of ionization, and kept aside undisturbed for the next 2 hours. After that, the solution was acidified with 2-3 ml of conc. HCl to turn benzoic compounds back into benzoic acid, which is not soluble in water but dissolved in $CHCl_3$. Then the content was heated over a wire gauge, and white residue was obtained, which was transferred to the watch glass, and $FeCl_3$ solution was added, which turned a light brown color, showing the presence of sodium benzoate.[9]

Quantitative analysis of sodium benzoate

Preparation of a standard oxalic acid solution

0.6304g of oxalic acid was transferred into a 100 ml standard volumetric flask. A test tube of distilled water was added to dissolve, and the volume was made to 100 ml by adding distilled

water up to the mark on the neck of a standard volumetric flask and shaking it upside down to get a homogeneous solution.

Standardization of NaOH with oxalic acid solution

The burette was rinsed and filled with a NaOH solution. The initial reading of the burette was noted. 10 ml of standard oxalic solution was pipetted out in a 100 ml conical flask, and 2 drops of phenolphthalein indicator were added. It was titrated against the NaOH solution until the color of the solution changed from colorless to light pink. The titration was repeated to get three concordant readings.

Determination of sodium benzoate level in given soft drinks

10 ml of soft drink was dissolved in 30 ml of distilled water, and then 2-3 drops of phenolphthalein indicator were added. It was titrated with NaOH until a pink color was obtained. The same procedure was repeated for different brands of soft drinks. The initial and final burette readings were recorded, and the percentage of sodium benzoate present in them was calculated.

Characterization of caffeine and sodium benzoate

The caffeine and sodium benzoate were characterized using Infrared spectroscopy.

RESULTS AND DISCUSSIONS

The following tables 1 and 2 are the results of the qualitative analysis of caffeine and sodium benzoate.

Table 1. Qualitative analysis of caffeine in Coca cola, Sprite, Rev and Sting.

Brand of cold drink	Murexide Test	Caffeine
Coca cola	Positive	Present
Sprite	Negative	Absent
Rev	Positive	Present
Sting	Positive	Present

Table 2. Qualitative analysis of sodium benzoate in Coca cola, Sprite, Limca, Rev and Sting.

Brand of cold drink	$FeCl_3$ Test	Sodium Benzoate
Coca cola	Negative	Absent
Sprite	Positive	Present
Limca	Positive	Present
Rev	Positive	Present
Sting	Positive	Present

The following are result of the quantitative analysis of sodium benzoate.

Table 3. Standardization of NaOH solution.

Initial burette reading (ml)	Final burette reading (ml)	Volume of NaOH used (ml)
0.0	9.8	9.8
9.8	19.8	10.0
19.8	29.8	10.0
29.8	39.8	10.0

Normality of standard oxalic acid (N_2)= 0.6304*1000*2/126.07*100

Using equation,

$N_1V_1 = N_2V_2$

N_1 is normality of NaOH solution

V_1 is volume of NaOH solution used= 10ml

N_2 is normality of standard oxalic acid

V_2 is volume of standard oxalic acid=10ml

N_1= N_2V_2/V_1 = 0.6304*2*1000*10/126.07*100*10 = 0.1N = N(titrant) = N(NaOH)

Table 4. Determination of sodium benzoate level in different brands of cold drinks.

% sodium benzoate = $(N*V_1*ME*DF*100)/(V_2*1000)$

N(titrant)= 0.1N

V_1(titrant)= volume of NaOH used

ME=mass equivalent=molecular mass/n-factor

Molecular mass of sodium benzoate=144.11g

n- factor=1 ME=144.11/1=144.11

Dilution factor=30/10=3 V_2(sample)=10ml

Brand of cold drink	V (titrant) (ml)	% of sodium benzoate
Sting	5.4	2.33
Sprite	2.0	0.86
Limca	2.6	1.12
Rev	1.9	0.82

Table 5. The amount of caffeine found in 100 ml of different brands of cold drinks.

Brand of cold drink	Caffeine (mg/100ml)
Coca cola	25
Sprite	0
Rev	20
Sting	55

Table 6. Characteristic Infrared-Absorption bands of sample and standard for caffeine.

Bonds	Standard	Sample
C-H	2955.24	2954.30
C=C	1550.10	1550.45
C=O	1701.20	1701.40
C-N	1239.35	1240.10
C=N	1660.80	1660.47

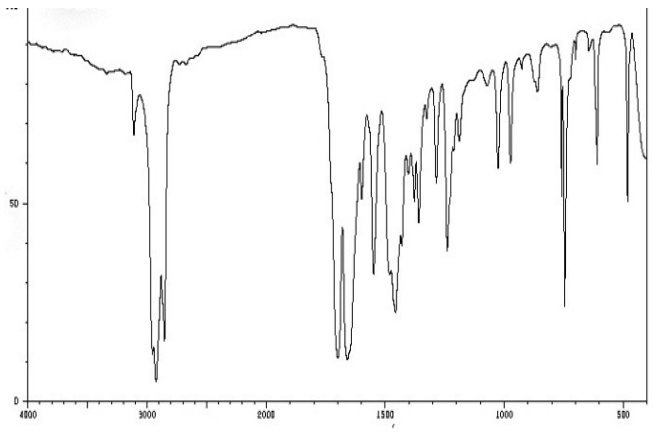


Figure 1: Infrared spectrum of caffeine sample

Table 7. Characteristic Infrared-Absorption bands of sample and standard for sodium benzoate.

Bonds	Standard	Sample
C-H	2950.35	2950.45
C=C	1540.67	1539.68
C-C	1400.40	1400.97
C=O	1706.80	1706.56

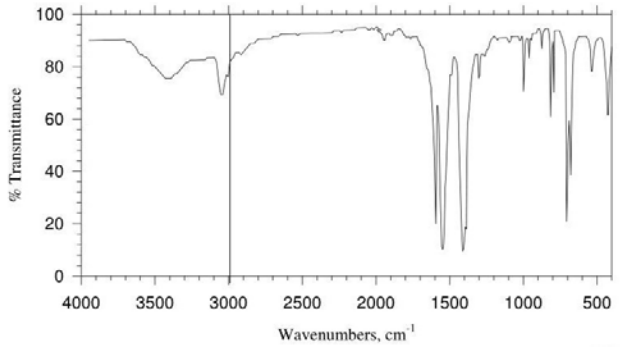


Figure 2: Infrared spectrum of sodium benzoate sample

Different drinks (mainly cold or soft drinks) available in the market has been evaluated by different researchers for presence of different compounds including pesticides,[10] preservatives, and other components having adverse effects on the human health including asthma, digestive system problems, toxicity, cancers,[11] effect on kidney or excretory system. The quantity of preservatives such as sodium benzoate and energy enhancers such as caffeine have different effect on human health.[12] As youth have been reported to be frequent consumer of these products, the higher consumed amount of these might affect health adversely.[13] The liquid-liquid extraction processed used in this study indicated normal to high presence of these compounds in different products. The presence of respective compounds has been confirmed by IR spectroscopy.

Conclusion

The liquid-liquid extraction process was used for qualitative analysis of caffeine content in different brands of cold drinks. Murexide Test was performed to confirm that the white residue extracted was caffeine. It was found that the amount of caffeine extracted from Sting was highest (55 mg/100ml), while no caffeine was extracted from Sprite (per 100 ml of cold drink). Coca -cola (25 mg/100ml) and Rev (20 mg/ml) had almost the same amount of caffeine content but less than Sting. Further, qualitative analysis of the extracted sodium benzoate was done by $FeCl_3$ test. It was found that Sting had the highest amount of sodium benzoate (233µg/ml) and Rev had the highest amount of sodium benzoate (82 µg/ml). Limca had 112µg/ml, while Sprite had 86 µg/mL of sodium benzoate.

Since caffeine has various health concerns associated with it, especially in children aged 12-18. According to reports, caffeine consumption should not exceed 100 mg for adolescents between the ages of 12-18 (two cans of soda).[14] Since its intake cannot be completely stopped, it can be limited for good health. Further, it has been clearly specified by researchers that such caffeinated drinks containing caffeine as high as 72 mg/250 ml of Sting are not for children below 12 years old. Such drinks disturb the sleep cycle, which in turn affects the mental health of children.

Acknowledgments

Authors acknowledge the support from college for providing the necessary facilities for this project.

Conflict of Interest

This study was not supported by any funding from any agency. There is no conflict of interest for this work.

References

1. E.B. Klaassen, R.H.M. De Groot, E.A.T. Evers, et al. The effect of caffeine on working memory load-related brain activation in middle-aged males. *Neuropharmacology* **2013**, 64, 160–167.
2. A. Chaudhary, D. Mathur, R. Gaba, R. Pasricha, K. Sharma. Greening up organic reactions with caffeine: applications, recent developments, and future directions. *RSC Adv.* **2024**, 14 (13), 8932–8962.
3. C. Gardiner, J. Weakley, L.M. Burke, et al. The effect of caffeine on subsequent sleep: A systematic review and meta-analysis. *Sleep Med. Rev.* **2023**, 69.
4. T. Kawada. Habitual caffeine consumption, genetic polymorphisms of cytokines and sleep quality. *Sleep Med.* **2023**, 106, 132.
5. A. Grundy, S. Sandhu, J. Arseneau, et al. Lifetime caffeine intake and the risk of epithelial ovarian cancer. *Cancer Epidemiol.* **2022**, 76, 102058.
6. S. Pattison, R. Rusin, Y. Bai. Caffeine Consumption among College Students: Its Effect on Sleep Hours and Academic Achievement. *J. Acad. Nutr. Diet.* **2016**, 116 (9), A13.
7. S.L. Azuma, N.K.A. Quartey, I.W. Ofosu. Sodium benzoate in non-alcoholic carbonated (soft) drinks: Exposure and health risks. *Sci. African* **2020**, 10.
8. D.V. Bermejo, P. Luna, M.S. Manic, et al. Extraction of caffeine from natural matter using a bio-renewable agrochemical solvent. *Food Bioprod. Process.* **2013**, 91 (4), 303–309.
9. L. Xue, L. Chen, J. Dong, et al. Dispersive liquid-liquid microextraction coupled with surface enhanced Raman scattering for the rapid detection of sodium benzoate. *Talanta* **2020**, 208, 120360.
10. S. Kumar, R. Bhuvaneshwari, S. Jain, et al. A Systematic Review on Pesticide-loaded Nanocapsules: A Sustainable Route for Pesticide Management to Enhance Crop Productivity. *Curr. Nanosci.* **2023**, 20 (3), 280–297.
11. R. Kumar, B.S. Chhikara, S.E. Zeybekler, et al. Nanotoxicity of multifunctional stoichiometric cobalt oxide nanoparticles (SCoONPs) with repercussions toward apoptosis, necrosis, and cancer necrosis factor (TNF-α) at nano-biointerfaces. *Toxicol. Res. (Camb).* **2023**, 12 (5), 716–740.
12. J.L. Temple, A.M. Dewey, L.N. Briatico. Effects of acute caffeine administration on adolescents. *Exp. Clin. Psychopharmacol.* **2010**, 18 (6), 510–520.
13. N. Yasuma, K. Imamura, K. Watanabe, et al. Association between energy drink consumption and substance use in adolescence: A systematic review of prospective cohort studies. *Drug Alcohol Depend.* **2021**, 219, 108470.
14. R. Soós, Á. Gyebrovszki, Á. Tóth, S. Jeges, M. Wilhelm. Effects of caffeine and caffeinated beverages in children, adolescents and young adults: Short review. *Int. J. Environ. Res. Public Health* **2021**, 18 (23).