Est. 1984

ORIENTAL JOURNAL OF CHEMISTRY
An International Open Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2021, Vol. 37, No.(3):
Pg. 663-666

Brief communication

Determination of Caffeine in Beverages Found in Bangladeshi Market by High Performance Liquid Chromatography (HPLC)

G. M. M. ANWARUL HASAN[1]* and ANUJ KUMER DAS[2]

[1]Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dr. Qudrat-E-Khuda Road, Dhaka 1205, Bangladesh.
[2]Hi-Tech Health Care Ltd. Banani, Dhaka-1263, Bangladesh.
*Corresponding author E-mail: pd-cbirmdp@bcsir.gov.bd

http://dx.doi.org/10.13005/ojc/370320

(Received: April 09, 2021; Accepted: June 11, 2021)

ABSTRACT

Caffeine, a phycostimulant is present in several foods and drinks. In the present study, beverages of different brands in Bangladeshi market were analyzed for caffeine by high-performance liquid chromatography (HPLC) using methanol-water (40:60, v/v) as mobile phase. Caffeine content ranged from 16.33-19.33 mg/can in soft drinks and 45.66-47.33 mg/can in energy drinks respectively. These data indicated that the levels of caffeine in Bangladeshi soft drinks and energy drinks are within the ranges reported from similar products in other countries.

Keywords: Caffeine, HPLC, Beverages, Bangladesh.

INTRODUCTION

Caffeine (1,3,7-tryethylxanthine), an alkaloid compound of xanthine naturally present in many plants[1]. The presence of caffeine is found in many foods and beverages and also the most consumed legal psycho stimulant nowadays[2,3]. For apnea of prematurity, caffeine is thought as the frontline therapeutic agent[4]. Caffeine is considered as safe compared to other older drugs[5]. The internally proposed dosage for caffeine base are 10 mg/kg load dose and 2.5 mg/kg maintenance dose respectively[6,7]. Caffeine is consumed through coffee, tea and beverages.

Caffeine has several effects including stimulation of alertness, sleeping problem, short-term memory improvement and specific drugs effectiveness. Therefore, caffeine amount should be detected using a reliable method in beverages found in Bangladeshi market. Caffeine has several pharmacological effects including central nervous system stimulation, elimination and drowsiness. Mental alertness, fatigue, and athletic performance can be improved through caffeine[8,9]. Caffeine is used to treat neurasthenia and coma[10,11]. It is also used to treat weight loss[12], glucose tolerance[13], type II diabetes[14], Parkinson's disease and cancer risk reduction[15]. Cleavage of double stranded DNA reparation was found to be inhibited by caffeine[16,17]. Viral infection can be reduced through caffeine *in vitro*[18,19]. Caffein is also considered as

chemopreventive and anti-inflammatory agent where it is protective agent against cirrhosis and hepatic fibrosis, two chronic inflammatory diseases which can lead to Liver cancer development causing diseases such as cirrhosis and hepatic fibrosis can be protected by caffeine as it has chemo preventive and anti-inflammatory properties[20,21]. Although caffeine has some pharmacological effects but excessive usage can stimulate heartbeat, problem in stomach, memory, and sleeping difficulties[22]. Consumption of more than 500 mg (in one drink), will lead to poisoning. Because of excessive coffee drinking, during 1995 to 2008 there have dead reports of 52.515 peoples in the world[23]. According to FDA (Food Drug Administration), for adults the permissible dose is <400 mg/day. Therefore, the highly caffeine containing beverages need to be decaffeinated to reduce the risk of caffeine poisoning[24].

Researchers used various methods such as HPLC; UV-Vis based spectrophotometric method to analyze caffeine in beverages. HPLC has become the preferred method because of its accuracy, selectivity and high speed. In this study, caffeine content is determined in available beverages of Bangladesh through high performance liquid chromatography.

MATERIALS AND METHODS

Chemicals and materials

Analytical grade chemicals were used in this study. Doubly distilled water was used for solution preparation. Analytical grade methanol was sourced from Merck (Darmstadt, Germany) analytical grade water was obtained from Millipore (Molfheim, France). Disposable syringe filters of 0.45 µm pore size were bought from Macherey-Nagel (Düren, Germany).

Equipments

HPLC Instrument (UltiMate® 3000 HPLC system; Dionex, Thermofisher Scientific, USA) consisted of AcclaimTM 120 C18 5μm (4.6 X 250 mm) with UV-Vis detector. For data processing, Chromeleon® 6.80 SP5 Chromatography Management Software (Dionex) was used.

Sample preparation

Standard of caffeine was sourced from Aldrich, Germany with 99% purity. Soft and energy drinks of different brands were purchased from supermarkets of Dhaka City, Bangladesh. All samples were analyzed in triplicate. Samples were sonicated for 10 min in an ultrasonic bath for 10 min and injected into a liquid Chromatograph. Then the samples were diluted with mobile phase containing methanol: water (40:60 v/v). Then, 2-5 mL of diluted samples was filtered through 0.45µm membrane filter. Finally, the filtrates were injected into HPLC system for caffeine content determination.

Stock and working solution preparation

100 ppm of caffeine stock solution was prepared. For this, 10 mg of pure caffeine was dissolved in 100 mL of HPLC analytical grade water. Stock solution was diluted with analytical grade water serially to prepare standards. Calibration curve was constructed using the serial dilutions.

Chromatographic conditions

The mobile phase used in this study was methanol: water (40:60, v/v). After filtration, filtrates were sonicated for 5 minutes. Chromatographic analysis was performed at 1.0 mL/min flow rate and 20.0 µL of sample volume. A 10 min run time was used and Chromatograms obtained at 272 nm. Caffeine responsive peaks were detected by comparison of the retention time with the standards. Statistical analysis SPSS software (Window version 18) and Excel 2007 software were used for statistics. Results are presented in the form of Mean ± SD after statistical analysis.

RESULTS AND DISCUSSION

Determination of Retention Time (tR) for Caffeine

From this experiment, standard caffeine average (tR) and the sample mean tR was 5.15 and 5.14 min respectively. According to these values, it was confirmed that both of the samples were almost similar. Thereby, conclusion can be drawn that analyzed samples contained caffeine. The both chromatograms are shown in Fig. 1 and 2 respectively.

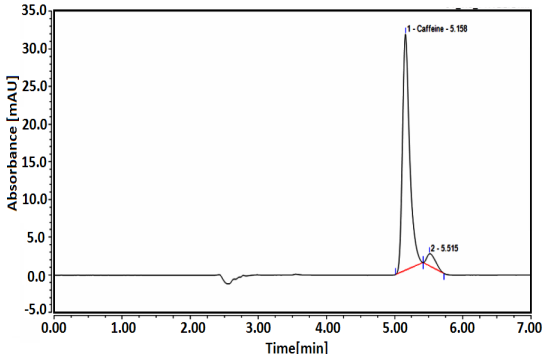

Fig. 1. Chromatogram of caffeine standard

Determination of caffeine content

In this study, 10 beverage samples including 6 soft drinks and 4 energy drinks were analyzed. Table 1 presents the caffeine levels determined in beverages. From these results, it was found that the average caffeine content in soft drinks were 17.22 mg/250 mL can while average caffeine content found in energy drinks was 46.75 mg/250 mL can. The results showed that the caffeine content in soft drinks was relatively lower than the energy drinks.

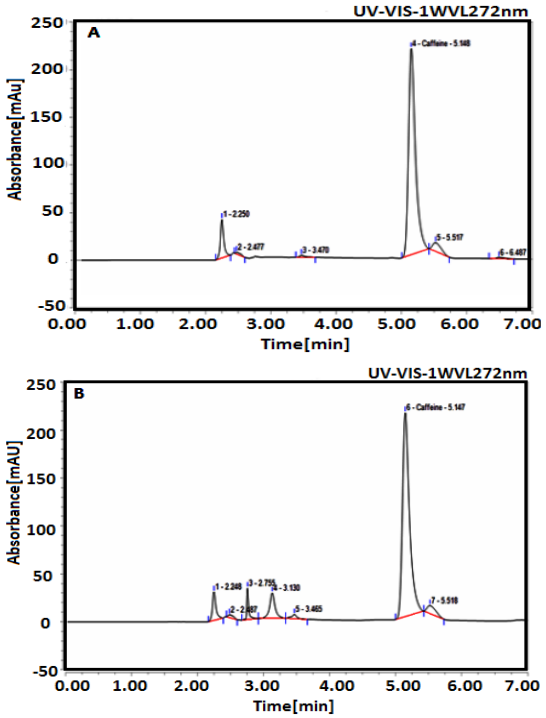

Fig. 2. Chromatogram of Soft drinks (A) and Energy drinks (B)

Although different factors may affect the amount of caffeine in beverages but the values obtained from this current study is in the range reported in the previous litaratures. The results also show that the caffeine contents in the analyzed products are much lower than that the internally proposed dosage for caffeine. Hence, this study supports that, in one serving (250 mL) of soft or energy drinks contained lower caffeine content (>50 mg) and there should not have any caffeine related health risks after drinking the soft drinks and energy drinks found in Bangladeshi market. For the safety awareness, manufacturers should strictly maintain the caffeine levels and declare the amount present in specific beverages.

Table 1: Caffeine content (mg/can) in various beverages

Items	Mean mg/250 mL can	SD
Soft Drinks A	16.3333	1.69967
Soft Drinks B	15.3333	1.69967
Soft Drinks C	15.3333	1.24722
Soft Drinks D	18	0.8165
Soft Drinks E	19	1.63299
Soft Drinks F	19.3333	1.24722
Energy Drink A	45.6667	1.69967
Energy Drink B	45.6667	2.49444
Energy Drink C	48.3333	3.68179
Energy Drink D	47.3333	1.24722

The trade names of the products used must be written

ACKNOWLEDGEMENT

The study was part of R & D work of "Milk, Dairy and Fermented Product Research Section" of Institute of Food Science and Technology (IFST), BCSIR. All the instruments used in this study were supported by ADP project allocated by Ministry of Science of Technology, Bangladesh.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Spiller, M. A. The chemical components of coffee. *Prog Clin Biol Res.*, **1984**, *158*, 91-147.
2. Reissig, C.J.; Strain, E.C.; Griffiths, R.R. Caffeinated energy drinks-A growing problem, *Drug and Alcohol Dependence.*, **2009**, *99*, 1–10.
3. Goldman, R.D. *Caffeinated energy drinks in children Canadian Family Physician.*, **2013**, *59*, 947-948.

4. Zhao, J.; Gonzalez, F.; Mu, D. Apnea of prematurity: from cause to treatment. *European Journal of Pediatrics*., **2011**, *170*, 1097–1105.
5. Pacifici, G.M. Clinical pharmacology of theophylline in preterm infants: effects, metabolism and pharmacokinetics. *Current Pediatric Reviews*., **2014**, *10*, 297–303.
6. Aldridge, A.; Aranda, J.V.; Neims, A.H. Caffeine metabolism in the newborn. *Clinical Pharmacology and Cerapeutics*., **1979**, *25*, 447–453.
7. Natarajan, G.; Lulic-Botica, M.; Aranda, J.V.; Pharmacology review clinical pharmacology of caffeine in the newborn. *Neoreviews*., **2007**, e214–e221.
8. Cronstein, B.N. Caffeine, a drug for all seasons. *Journal of hepatology*., **2010**, *53*, 207-8.
9. Ballard, S.L.; Wellborn-Kim, J.J.; Clauson, K.A. Effects of commercial energy drink consumption on athletic performance and body composition. *The Physician and sportsmedicine*., **2010**, *38*,107-17.
10. Sereshti, H.; Samadi, S. A rapid and simple determination of caffeine in teas, coffees and eight beverages. *Food Chemistry*., **2014**, *158*, 8-13.
11. Guo, S.; Zhu, Q.; Yang, B.; Wang, J.; Ye, B. Determination of caffeine content in tea based on poly (safranine T) electroactive film modified electrode. *Food chemistry*., **2011**, *129*, 1311-4.
12. Modi, A.A.; Feld, J.J.; Park, Y.; Kleiner, D.E.; Everhart, J.E.; Liang, T.J.; Hoofnagle, J.H. Increased caffeine consumption is associated with reduced hepatic fibrosis. *Hepatology*., **2010**, *51*, 201-9.
13. Strassnig, M.; Brar, J.S.; Ganguli, R. Increased caffeine and nicotine consumption in community-dwelling patients with schizophrenia. *Schizophrenia research*., **2006**, *86*, 269-75.
14. Aljofan, M.; Gaipov, A. *Metformin: a stroke of luck*., **2019**,*16*, em143.
15. de Oliveira, P.F.; de Andrade, K.J.B.;Paula, M.C.F.; Acésio, N.O., da Silva Moraes, T.; Borges, P.S.F.; Barcelos, G.R.M; Tavares, D.C. Bixin protects hepatocytes against 1, 2-dimethylhydrazine-induced genotoxicity but does not suppress DNA damage and pre-neoplastic lesions in the colon of Wistar rats. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*., **2014**, *759*, 37-42.
16. Tsabar, M.; Eapen, V.V.; Mason, J.M.; Memisoglu, G.;Waterman, D.P.; Long, M.J.; Bishop, D.K.; Haber, J.E. Caffeine impairs resection during DNA break repair by reducing the levels of nucleases Sae2 and Dna2. *Nucleic acids research*., **2015**, *18*, 6889-901.
17. Alao, J.P.; Sunnerhagen, P. The ATM and ATR inhibitors CGK733 and caffeine suppress cyclin D1 levels and inhibit cell proliferation. *Radiation Oncology*., **2009**, *4*, 1-7.
18. Aljofan, M.; Lo, M.K.; Rota, P.A.; Michalski, W.P.; Mungall, B.A. off label antiviral therapeutics for henipaviruses: new light through old windows. *Journal of antivirals & antiretrovirals*., **2010**,*1.*
19. Aljofan, M.; Sganga, M.L.; Lo, M.K.; Rootes, C.L.; Porotto, M.; Meyer, A.G.; Saubern, S.; Moscona, A.; Mungall, B.A. Antiviral activity of gliotoxin, gentian violet and brilliant green against Nipah and Hendra virus *in vitro*. *Virology Journal*., **2009**, *6*, 1-3.
20. Cornelis, M.C.; Byrne, E.M.; Esko, T.; Nalls, M.A.; Ganna, A.; Paynter, N,; Monda, K.L.; Amin, N.; Fischer, K.; Renstrom, F.; Ngwa, J.S. Genome-wide meta-analysis identifies six novel loci associated with habitual coffee consumption. *Molecular psychiatry*., **2015**, *20*, 647-56.
21. Sumi, T.; Shirakami, Y.; Shimizu, M.; Kochi, T.; Ohno, T.; Kubota, M.; Shiraki, M.; Tsurumi, H.; Tanaka, T.; Moriwaki, H. Epigallocatechin-3-gallate suppresses hepatic preneoplastic lesions developed in a novel rat model of non-alcoholic steatohepatitis. *Springer Plus.,* **2013**, *2*, 1-0.
22. Tjay, H.; Rahardja, K. Essential Drug, It's Benefits, uses and Side effect. Jakarta, Indonesia: Elexmedia Komputindo., **2007.**
23. Freedman, N.D.; Park, Y.; Abnet, C.C.; Hollenbeck, A.R.; Sinha, R. Association of coffee drinking with total and cause-specific mortality. *N Engl J Med*., **2012**, *17*, 1891-904
24. Arwangga, A.F.; Asih, I.A.; Sudiarta, I.W. Analisis kandungan kafein pada kopi di Desa Sesaot Narmada menggunakan spektrofotometri UV-Vis. *Jurnal Kimia*., **2016**, *10*, 110-4.