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

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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 plants1. The presence of caffeine is found in many foods and beverages and also the most consumed legal psycho stimulant nowadays2,3. For apnea of prematurity, caffeine is thought as the frontline therapeutic agent4. Caffeine is considered as safe compared to other older drugs5. The internally proposed dosage for caffeine base are 10 mg/kg load dose and 2.5 mg/kg maintenance dose respectively6,7. 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 caffeine8,9. Caffeine is used to treat neurasthenia and coma10,11. It is also used to treat weight loss12, glucose tolerance13, type II diabetes14, Parkinson's disease and cancer risk reduction15. Cleavage of double stranded DNA reparation was found to be inhibited by caffeine16,17. Viral infection can be reduced through caffeine in vitro18,19. 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 properties⁴₀,⁴₁. Although caffeine has some pharmacological effects but excessive usage can stimulate heartbeat, problem in stomach, memory, and sleeping difficulties⁴₂. 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 world⁴₃. 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 poisoning⁴₄.

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 (Molheim, 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.
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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.

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                 | 1.63929|
| 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|

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 literatures. 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.

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Conflict of interest

The authors declare no conflict of interest.

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