ORIGINAL PAPER

DETERMINATION OF CAFFEINE CONTENTS IN THE MOST COMMONLY USED BEVERAGES IN THE KINGDOM OF SAUDI ARABIA USING HIGH-EFFICIENCY LIQUID CHROMATOGRAPHY

MAHA ABDALLAH ALNUWAISER[1]

Manuscript received: 20.06.2021; Accepted paper: 25.08.2021;
Published online: 30.12.2021.

Abstract. *This study aims to determine the levels of caffeine in beverages commonly consumed in Saudi Arabia.10 samples collected from Riyadh market. Caffeine level was determined using high-efficiency liquid chromatography (HPLC). Three types of samples were analyzed. The caffeine concentration ranged from 0.446 to 6.374 mg / 1000 mL in coffee samples, and from 0.053 to 8.897 mg/1000 mL in tea samples. In soft drinks the caffeine amount was in the range of 1.466 - 4.698 mg / 1000 mL. Green tea (Alwazah tea) had the highest caffeine content among all the studied drinks (i.e., 8.897 mg/1000 mL). This study is the first in terms of caffeine levels, applied for a variety of beverages in Saudi Arabia. On the other hand, this investigation highlights the useful results for controlling caffeine intake or setting safe limits for it in drinks consumed in the Kingdom, and for nutritional education as well.*

Keywords: *caffeine; HPLC; soft drinks; energy drinks; tea; coffee; daily intakes.*

1. INTRODUCTION

Caffeine (1,3,7-trimethylxanthine, Fig. 1) is an alkaloid of the methylxanthine family, that have been found in more than 60 species of plants such as tea and cocoa leaves, cocoa beans, coffee and Cola trees, being considered a valuable potential source of beneficial bioactive compounds with polyphenols [1-7]. Caffeine is the responsible substance for the flavor of caffeinated drinks [8]. The main sources of caffeine are coffee (Coffea spp.), tea (*Camellia sinensis*), guarana (*Paullinia cupana*), maté (*Ilex paraguariensis*), cola nuts (*Cola vera*), and cocoa (*Theobroma cacao*). The amount varies for different products [9] Caffeine is a central nervous system stimulant that can restore a person's alertness. That is why caffeinated drinks are very popular all over the world. However, its excessive intake is harmful to the human body [10]. Consuming caffeine in large quantities can cause some diseases, such as headaches, heart disease, cancer, and impaired renal function [11]. Several research studies have been published confirming the high effectiveness of caffeine in many very important health problems such as diabetes, obesity, cancer and cirrhosis of the liver. One study published in the Journal of the American Society of Clinical Oncology confirmed that heavy coffee consumption can significantly reduce the recurrence of cancer. Recently published studies confirmed that caffeine consumption can significantly increase the risk of developing liver cirrhosis [12], diabetes and obesity. They are the most common health problems in the Kingdom of Saudi Arabia .Where coffee is the first drink in the Kingdom of Saudi Arabia. A large portion of Saudis suffer from one or both diseases. Caffeine added as a flavoring in soft drinks and is one of most common food sources that stimulate the CNS.

[1] Princess Nourah bint Abdulrahman University, Department of Chemistry, P.O. Box 28445, 11437 Riyadh, Saudi Arabia. E-mail: maha.alnuwaiser80@gmail.com

However, it has been rated by the International Olympic Committee (IOC) as an anesthetic of abuse when present in urine at concentrations greater than 12 mg/mL. It is also used as an adjunct in many pharmaceutical formulations [13]. The amount of caffeine reported in major food samples vary greatly such as 93.0 - 163.5 mg/cup in ground coffee, 46.7 - 67.6 mg/cup in instant coffee, 30.2 - 67.4 mg/cup in tea bag and 0.32 - 0.54 mg. This difference is due to the preparation, type of coffee and tea, cup size and the methods of caffeine analysis used. In soft drinks, the difference is by type of brand, and the caffeine used in these products mostly comes from other natural sources [14]. Various laboratory techniques were used to investigate/measure the caffeine concentration in the studied samples, including high-performance liquid chromatography (HPLC) [15] and gas chromatography-mass spectrometry (GC-MS) [16], liquid chromatography-mass spectrometry (LC-MS) [17], capillary electrophoresis (CE) [18]; high performance liquid chromatography is a method of high sensitivity and ultra-high accuracy [19].

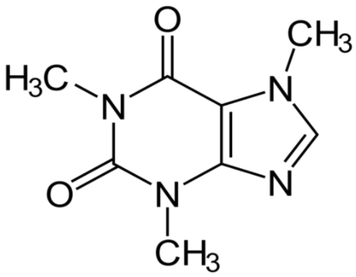


Figure 1. Chemical structure of caffeine.

Cola drinks account for more than 80% of the caffeine in food additives. These caffeinated products have a high ability to artificially stimulate the body and increase the heart rate. This artificial stimulation causes the layers of fatigue to disappear temporarily, but it lasts for a short time. So excessive stimulation drains vital energy from the body while fighting the poison that has entered his system. In the quantities currently consumed, insomnia causes irritability and anxiety, nervousness and heart rhythm disturbances. There are other effects of caffeine consumption [20]. Since the consumption of soft drinks is a widespread habit all over the world, so these food products are considered to be of great economic and social importance. As with any food, the composition of soft drinks is regulated by legislation [21]. In accordance with Directive 2000/13/EC, quinine and/or caffeine used as a flavoring in foodstuffs is written by name in the list of ingredients immediately after the term “flavor”. In addition, in accordance with Directive 2002/67/EC of July 18, 2002, caffeinated beverages above 150 mg/L must also present a warning message on the label followed by an indication of the caffeine content. The identification of caffeine compounds in beverages gaseous and energy to ensure food safety and quality control is mandatory [22]. This study aimed to evaluate the compatibility of caffeine concentration levels in the different brands of commercial drinks most commonly used from the local market in the Kingdom of Saudi Arabia. The analytical data obtained from three groups under study (three types of coffee, three types of tea and four types of soft and energy drinks) were used.

2. MATERIALS AND METHODS

The collected samples were coffee (baja, lavazza, dunkin). Tea (Alwazah, Khadeer Jizani) and energy and soft drinks samples bison, cocacola, pepsi, red bull. All products were purchased from the local market of Riyadh, Saudi Arabia**.**

2.1. SAMPLE PREPARATION

2.1.1. Tea and coffee

The three types of tea (Alwazah, khadeer and Jizani) as described by Camargo et al. [23] were extracted by his method with some modifications. One gram of tea was extracted with 100 mL of boiled distilled water. The coffee was prepared from three different types of coffee (baja, lavazza and dunkin) by mixing 1 gram with 100 mL of distilled water at room temperature, and leaving it for 24 hours. The tea or coffee extract was filtered (Whatman filter paper, no. 1). 100 mL of either extract was mixed with 100 mL of dichloromethane using a glass separating funnel (250 mL); The substrate was withdrawn (repeated three times) and then the extract was left in a steam room to dry the dichloromethane. Caffeine extracted from 1000 mL of high-purity methanol was taken and read by high-efficiency liquid chromatograph.

2.1.2. Soft and energy drinks

Determination the level of caffeine in energy and soft drinks can be made by using the procedure proposed by Khalid et al. [24] slightly modified, in which the caffeine can be extracted from dink sample with dichloromethane. In this respect the drink sample are mixed with an equal volume of saturated sodium bicarbonate (8%), *i.e.*, 25:25; 50 mL of either mixture was then trated with 100 mL dichloromethane using a glass separating funnel. The substrate was withdrawn (in triplicate) and then the extract was left in a vapor chamber to dry the dichloromethane. The extracted caffeine was taken by 1000 mL of highly purified methanol and read by a high-efficiency liquid chromatograph.

2.1.3. Standard solution preparation

Standard samples were prepared at different concentrations such as 5, 10, 15, 20, 25 ppm by serial dilution of standard substance (caffeine 1mg freebase/1mL methanol) lipomed company.

2.2. CROMATOGRAPHIC ANALYSIS

The chromatographic analyses were performed on a Shimadzu HPLC system consisting of an isometric LC-20aB pump, a SIL-20A injector, a vacuum gas remover, and an SPD-20AV UV detector (Shimadzu, Kyoto, Japan) connected to a data acquisition and processing system. The used class column was Shim Pak. VP-ODS (inner diameter of 4.6 mm and a length of 250 mm) was set oven temperature at 30°C ± 1°C Oven model CTO-20A. The used mobile phase was a water:methanol mixture (50:50 V/V) at a flow rate of 0.5 mL/min.

3. RESULTS AND DISCUSSION

The HPLC method provides stable retention times of 7.05 min. The retention time of caffeine concentrations in standard solutions was (5, 10, 15, 20, 25). It gave an excellent linear result, with a relative standard deviation (RSD) of 0.052%. The titration graph is also shown in Fig. 2 of the titration formula below 10 ppm to 25 ppm. A calibration curve was then generated according to the response obtained Fig. 3.

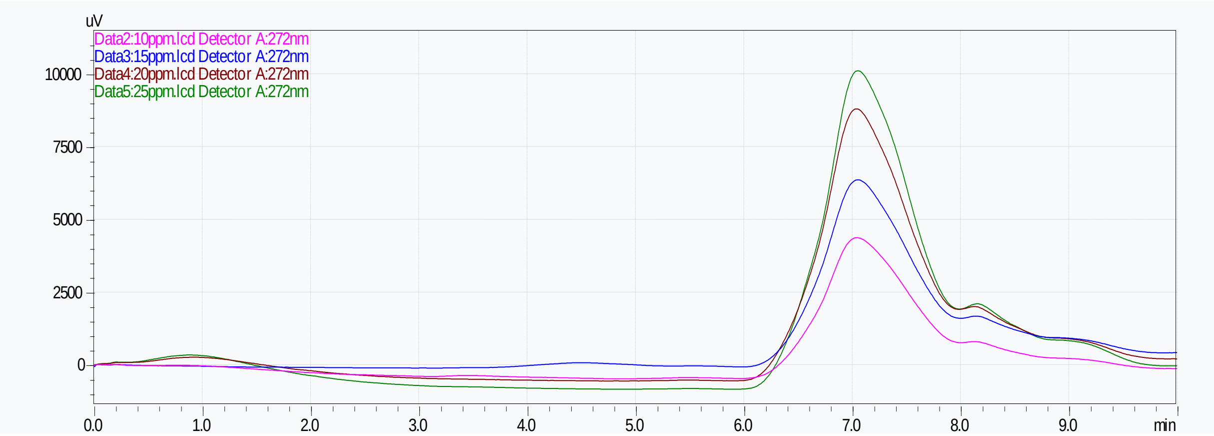
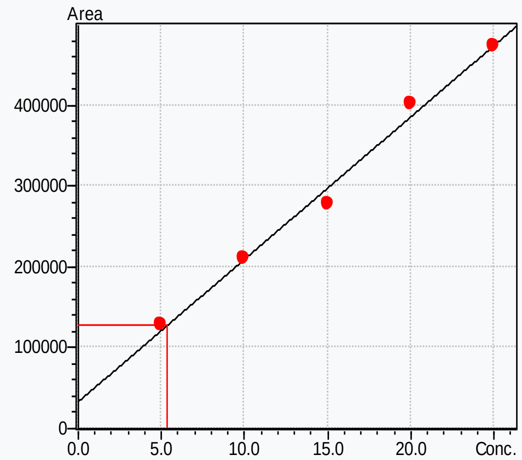


Figure 2. Standard samples.



Y=17643.1X+33267.5
$\hat{r}^2$=0.990 r =0.995

Figure 3. Calibration curve.

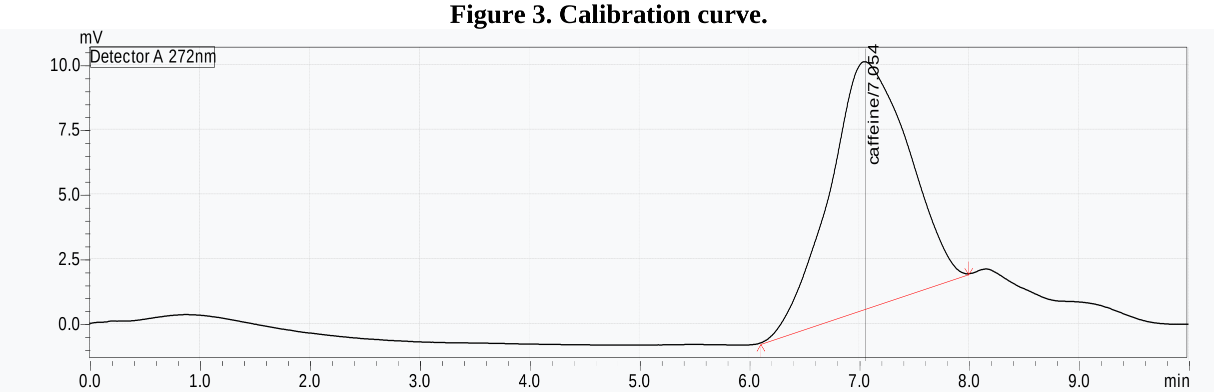


Figure 4. Tea chromatogram.

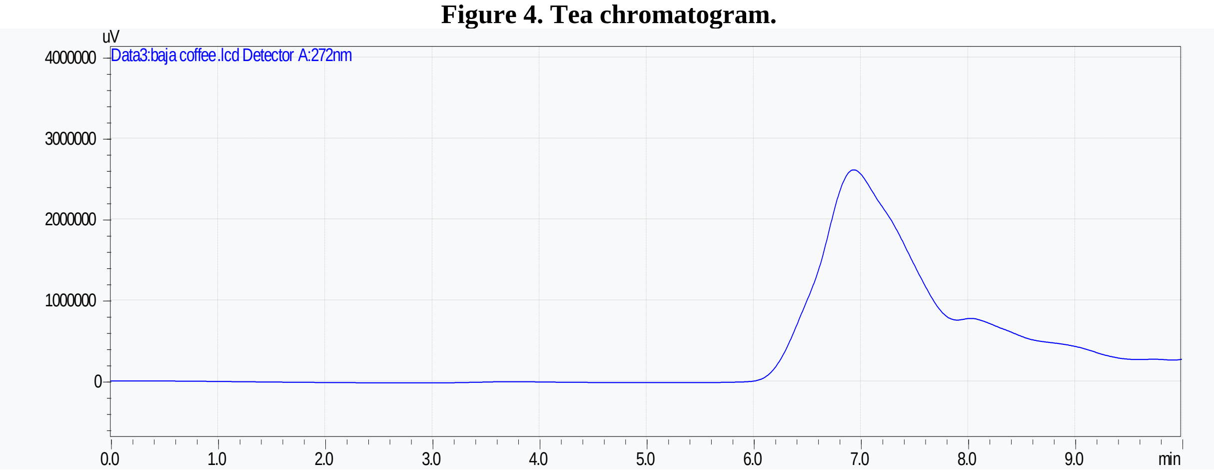


Figure 5. Coffee chromatogram.

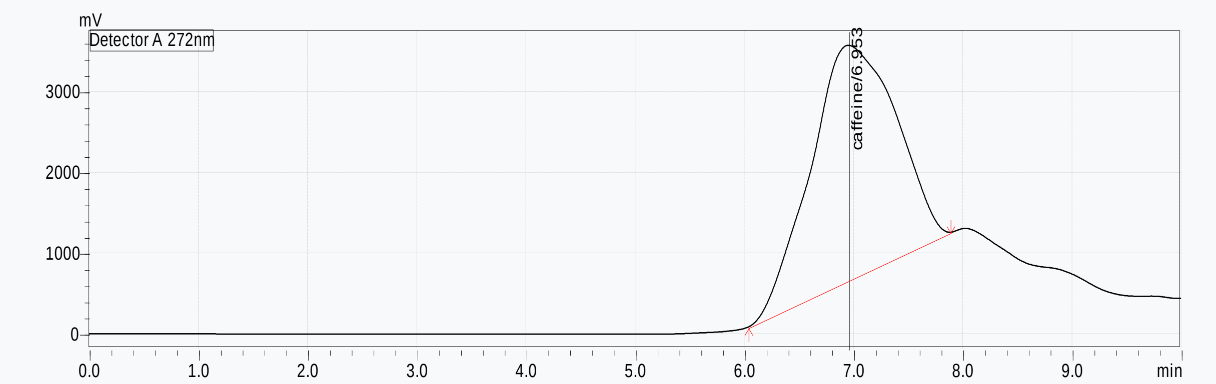


Figure 6. Soft drink chromatogram.

The method has been validated to determine the caffeine concentration in three types of coffee, the three tea samples, the soft drink samples, and the four energy drinks, and compare their concentrations in the above-mentioned weights, Where the results in tea samples ranged between 0.053 ppm in Jizani tea, 7.102 ppm in Khadeer tea, and 8.897 ppm in Alwazah tea. These results are consistent with some previous studies of black, green, and blue tea samples. The results of black tea reported in [25] were 9.6 ppm, green tea 9.5 ppm, and for blue tea it not indicated any value. In this respect, can be concluded that no values are available for blue tea, with the exception of this study.

Table 1. Coffeine concentration

No.	Sample	Concentration [ppm]
1	Tea Jizani	0.053
2	Tea khadeer	7.102
3	Tea Alwazah	8.897
4	Coffee baja	6.374
5	Coffee Lavazza	5.800
6	Coffe Dunkin	0.446
7	Bison	4.698
8	Red Bull	3.795
9	Pepsi	1.623
10	Coca cola	1.466

Table 1 shows the concentration of caffeine in the studied samples. In soft drinks and energy drinks, caffeine contents in energy drinks ranged from 3.795 to 4.698 ppm. The lowest caffeine content was observed in the Red Bull energy drink sample, while the Bison sample showed the highest caffeine content. However, the analyzed soft drink samples had significantly lower caffeine content, ranging from 1.466 ppm in Coca-Cola to 1.623 ppm in Pepsi. These results are consistent with previous studies [26]. Soft drinks around the world vary in caffeine content; depending on the type of brand, the average content of soft drinks is present (10 mg/100 mL) [27]. Currently, the US Food and Drug Administration (FDA) sets restrictions on tolerable levels of caffeine in soft drinks (20 mg/100 mL). It is clear that the level of caffeine in the samples of the analyzed beverages, in which they are marketed Saudi Arabia is well below the above food industry guidelines.

4. CONCLUSION

From the above, green tea contains a higher level of caffeine than other types of tea and coffee. The study also showed that energy drinks (Bison, Red Bull) contained a high percentage of caffeine in all samples under a study. High-efficiency liquid chromatography was used to estimate the level of caffeine in coffee samples, tea, soft drinks, and energy drinks, which showed the results of this study.

Acknowledgments: *This research was funded by the Deanship of Scientific Research at Princess Nourah bint Abdulrahman University through the Fast-track Research Funding Program.*

REFERENCES

[1] Tefera, M., Gedo, A., Tessema, M., Admassie, S., *Food Chemistry*, **21**, 156, 2016.
[2] Hou, J., Li, H., Wang, L., Zhang, P., Zhou, T., Ding, H., Ding, L., *Talanta*, **146**, 34, 2016.
[3] Pur, M.R.K., Hosseini, M., Faridbod, F., Ganjali, M.R., *Microchimica Acta*, **184**, 3529, 2017.
[4] Knight, C.A., Knight, I., Mitchell, D.C., Zepp, J.E., *Food and Chemical Toxicology*, **42**(12), 1923, 2004.
[5] Radulescu, C., Tarabasanu-Mihaila, C., *Rev. Chim (Bucharest)*, **65**(1), 25, 2004.
[6] Radulescu, C., Hossu A.M., *Rev. Chim (Bucharest)*, **56**(7), 742, 2005.
[7] Barbes, L., Barbulescu, A., Radulescu, C., Stihi, C., *Romanian Report in Physics*, **62**(3), 877, 2014.
[8] Borghei, Y.S., Hosseini, M., Ganjali, M.R., *Microchimica Acta*, **184**, 2671, 2017.
[9] Smith, J., Loxley, K., Sheridan, P., Hamilton, T.M., *Journal of Chemical Education*, **93**, 1776, 2016.
[10] Salehnia, F., Hosseini, M., Ganjali, M.R., *Microchimica Acta*, **184**, 2157, 2017.
[11] Sabet, F.S., Hosseini, M., Khabbaz, H. et al, M.R., *Food Chemistry*, **220**, 527, 2017.
[12] Rosato, V., Guercio, V. et al., *European Journal of Cancer Prevention*, **30**(3), 204, 2021.
[13] Borghei, Y.S., et al, *Sensors and Actuators B: Chemical*, **248**, 133, 2017.
[14] Knight, C.A., Knight, I., Mitchell, D.C., *Canadian Journal of Dietetic Practice and Research*, **67**(2), 96, 2006.
[15] Sancho Cubero, A., *Estudio Comparativo del Contenido de Cafeína en Diferentes Bebidas*, Universidad de Zaragoza, 2013, Proyecto Fin de Carrera. Available online https://zaguan.unizar.es/record/10069/files/TAZ-PFC-2013-028.pdf.
[16] Perrone, D., Donangelo, C.M., Farah, A., *Food Chemistry*, **110**(4), 1030, 2008.
[17] Huck, C.W., Guggenbichler, W., Bonn, G.K., *Analytica Chimica Acta*, **538**, 195, 2005.
[18] Laasonen, M., et al, *Analytical Chemistry*, **75**, 754, 2003.
[19] Pura, J.N., Nagalakshmi, S., *Journal of Agricultural and Food Chemistry*, **45**, 3973, 1997.
[20] Khan, K. et al, *Journal of Applied Sciences*, **6**(4), 831, 2006.
[21] Alghamdi, A.H., Alghamdi, A.F., Alwarthan, A.A., *Journal King Saud University*, **18**(2), 99, 2005.
[22] Zen, J.M., Ting, Y.S., Shih, Y., *Analyst*, **123**, 1145, 1998.
[23] Camargo, M.C., Toledo, M.C., Farah, H.G., *Food Additives and Contaminants*, **16**, 79, 1999.
[24] Khalid, A. et al., *Fam Med Med Sci Res*, **5**(4), 1, 2016.
[25] Tfouni, S.A.V., et al, *Food Science and Technology*, **38**(4), 661, 2018.
[26] Nour, V., Trandafir, I., Ionica, M.E., *Scientific Study & Research Chemistry & Chemical Engineering, Biotechnology, Food Industry*, **11**(3), 351, 2010.
[27] International Food Information Council Foundation, *Questions and Answers About Energy Drinks and Health*, 2013. http://www.foodinsight.org/Resources.