Original Article

Determination of heavy metals and anthraquinone in black tea

Narges Chamkouri[*1], Sanaz Karimpour[2], Jamile Deris[2], Zahra Koolivand[3] , Seyed Mohammad Ali Malaekeh[2], Fariba Deris[2]

[1] Department of Basic Sciences, Faculty of Medicine, Abadan University of Medical Sciences, Abadan, Iran
[2] Abadan University of Medical Sciences, Abadan, Iran
[3] Department of Laboratory Sciences, School of Allied Medical Sciences, Abadan University of Medical Sciences, Abadan, Iran

Abstract

Introduction

A rapid and simple method was investigated for determining the concentration of some contaminants including heavy metals such as lead (Pb), cadmium(Cd), chromium (Cr), and anthraquinone in black tea samples in Iran.

Methods

Heavy metals such as Pb, Cd, and Cr in tea samples were detected using inductively coupled plasma optical emission spectrometry (ICP-OES) after microwave acid digestion (MAD). The anthraquinone in infusion tea samples was determined via liquid chromatography–photodiode array detector after the dispersive liquid-liquid microextraction method (DLLME).

Results

Recoveries of all the tea samples in heavy metals and anthraquinone were in the range of 75% - 89% and 71%-96, respectively. The results of recoveries are in the range of the Association of Official Analytical Chemists (AOAC) and the European Union. RSD in the MAD and DLLME methods was less than 5%. The mean concentrations of Pb, Cd, Cr, and anthraquinone were 0.724, 0.021, 2.98 ppm, and 2.27 ppb.

Conclusion

Overall our results showed that MAD and DLLME methods could successfully determine the concentration of some contaminants such as Pb, Cd, Cr, and anthraquinone in black tea samples.

Introduction

Black tea is one of the most common types of tea, known worldwide for its pleasant aroma, taste, and positive physiological functions [1,2]. Tea is a rich source of some essential dietary metals for human intake. Tea leaves contain polyphenols such as epigallocatechin 3-gallate (EGCG) that have antioxidant [3], cholesterol-decreasing[4], hepatoprotective [5], and anticancer activities [6,7]. The content of heavy metals in foods is important because of their possible toxic nature. The growing interest in drinking tea may be linked to its polyphenol antioxidative activity which defies the harmful permeation of environmentally generated free radicals [1]. The human body needs both metallic and non-metallic elements within allowable limits for growth and good

health. Many elements play an essential role in metabolic processes and contribute to the general health of humans. For example, trace levels of copper are indispensable for human health [8]. Anthraquinone is a polycyclic aromatic hydrocarbon and could cause urinary diseases, liver, and thyroid glands [9]. It was first identified by laboratories in Europe in 2011 [10]. Several methods for the determination of anthraquinone in plants, including extraction and maceration have been conducted before HPLC [11-13]. There are few studies for determining anthraquinone in tea samples [11, 12] and its determination has been suggested by using gas chromatography (GC) coupled with a tandem mass spectrophotometer (MS/MS). However, an MS detector is relatively costly and not usually available in laboratories, and thus an inexpensive instruments such as HPLC are required. Accordingly, it is essential to determine contamination such as heavy metals and anthraquinone in commonly consumed beverages in the world and relevant products for a better understanding of their nutritive importance. Thus, the present study aimed to determine the concentration of some contaminants such as heavy metals and anthraquinone in widely consumed black tea brands in the Iranian market.

Methods

Chemicals and instruments

All reagents were of HPLC grade and analytical grade and purchased from Merck (purity higher than 99%). The concentration of anthraquinone was determined by HPLC coupled with a DAD detector. Heavy metals in tea samples were tested by ICP-OES (Varian) and Argon (99.99%) as carrier gas. The digestion of samples was carried out in a microwave oven, Milestone MLS 1200 Mega model (Sorisole, Italy). All of the standard solutions were stored at 4°C and brought to the ambient temperature just before use. Throughout the experimental runs, all the solvents and real samples were filtered and diluted before analysis by ICP-OES and HPLC-DAD. In HPLC, the mobile phase was methanol and water with a ratio of 60:40 and a flow rate of 1.0 mL/min for 20 min. The volume of each injection was 20 μL. The present study was conducted on the most widely consumed black tea brands in Iran (10 samples of different black tea brands in Iran).

Microwave acid digestion of heavy metals in tea samples

After drying to a constant weight, the tea samples were ground into powder, dried at 60°C for 5 h, and subsequently cooled down to room temperature. Approximately 1g of dried tea powder was weighed and poured into the digestion vessel with 6.0 mL of concentrated HNO_3 and 1.0 mL of hydrogen peroxide (H_2O_2). Finally, the concentration of lead, cadmium, and chromium was determined by ICP-OES after microwave digestion. The sample preparation of the method is shown in Figure 1.
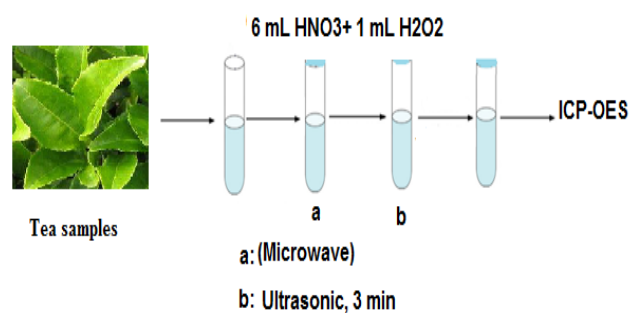
Figure 1. Sample preparation procedure

Extraction procedures of anthraquinone in tea samples

3 g of tea samples and 200 mL of water wawereoiled for 30 min and then filtered. After that 30 mL of the tea infusion aliquot was used to extract with 2 mL of 1% acetone in n-hexane. In this study, various ratios of the solvents were tested for obtaining a high resolution. The best symmetry of the peak area was obtained in the mobile phase containing methanol and water with a ratio of 60:40. The sample extraction procedures of anthraquinone are shown in figure 2.

Results and discussion

Optimization of the digestion procedure for heavy metals in tea samples

Microwave acid digestion (MAD) has advantages including less pollution and analyte evaporation, lower acid consumption, shorter digestion time, and significant blank value reduction [14]. Finally, the concentration of lead, cadmium, and chromium was determined in tea samples by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) after microwave digestion. The tea samples were macerated and homogenized in an agate mortar and subsequently, around 1.0 g was weighed directly on polytetrafluorethylene (PTFE) flasks after adding HNO_3 and H_2O_2. In this method, different mixtures of reagents using HNO_3 and H_2O_2 were investigated. The H_2O_2 and HNO_3 were used to reactive oxygen and catalytically active
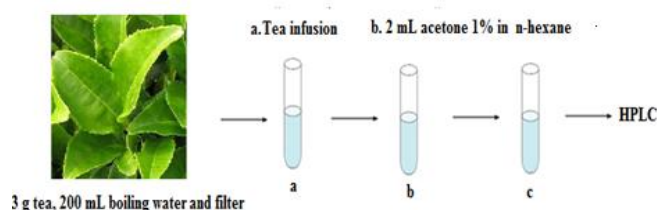

Figure 2. Sample extraction procedure

Table 1. Recoveries for the heavy metals after microwave digestion in different mixtures.

Element	I	II	III	IV	V
Cd	63	66	72	82	76
Cr	58	61	65	79	70
Pb	60	75	80	88	81

NO_2 which improve the tea samples digestion. Finally, HNO_3 and H_2O_2 digestion agents were used (volume ratio 6:1) under high pressure to improve the digestion of the samples. The ICP-OES results of tea samples after MAD are given in Table 1.

Optimization of DLLME parameter for anthraquinone in tea samples

The important properties of extraction solvent in DLLME procedure included low volatility, high extraction, and low solubility in water. For this purpose, several solvents, such as acetone, n-hexane, chloroform, acetone/hexane, and acetone/chloroform with volumes of 500-2000 μL were tested as extracting medium. Highest recoveries were obtained at 1990 μL of acetone/hexane. For investigating the effect of dispersive solvents on recoveries, acetonitrile, methanol, and ethanol with volume (0-20 μL) was selected. The highest recoveries of dispersive solvents were 10 μL of methanol. The extraction time were investigated between 5-20 min. Finally, extraction of time had no significant effect on recoveries.

Another probable effective factor in DLLME method is the effect of ionic strength on BPA extraction recovery. For this purpose, sodium chloride, at concentrations of 0-5 % w/v, was used. The highest extraction recovery obtained in the absence of of sodium chloride.

Method evaluation

To evaluate the method, the relative standard deviation (RSD), the limit of detection (LOD), and recovery were tested. Mean recoveries of method obtained in the ranges of 71% to 95%. The linearity obtained in the range of 2–180 ppb with the correlation coefficient of R2 =0.9975. The precision of the method was investigated in term of relative standard deviations (RSDs), and was less than 5 %. The results are shown in Table 2.

Application of the proposed method in real sample analysis

Tea samples were analyzed using the proposed methods (figure 1 and figure 2). In the present work, the above-mentioned conditions of the concentrations of some contamination such as Cr, Cd, Pb, and anthraquinone were evaluated in 10 samples of black tea in Iran. The results of heavy metal concentrations such as Cr, Cd, and Pb are summarized in Table 3.

Table 2. Method evaluation of tea samples

Parameters	R	LOD (ng.Kg^{-1})	RSD (%)	Recovery (%)
Cd	0.993	85.2	4.1	82
Cr	0.987	63.8	5.0	79
Pb	0.999	77.9	3.2	88
Anthraquinone	0.998	1.03	2.9	71-96

Table 3. Concentrations of lead, cadmium, and chromium in the black tea samples (n=2).

Real samples	Anthraquinone (ppb)	Recovery (%)
A	6.3	78
S	12.08	92
M	No Detect	No Detect
D	No Detect	No Detect
K	0.94	96
I	No Detect	No Detect
GH	No Detect	No Detect
B	9.88	90
L	7.84	96
SI	5.02	87

Figure 2, shows analysis of tea samples according to procedures of anthraquinone. Figures 3 and 4 present the chromatograms of standard sample of anthraquinone and tea sample extract. It can be seen that retention time of anthraquinone was at around 10.02 min. The recovery of anthraquinone in tea samples are listed in Table 4.

The lowest lead concentration was found in sample M, while sample A had the highest content. In this study, the lead concentration was significantly lower than that of determined by Al-Othman et al. [17]. According to their results, the range of lead concentrations in the tea samples were 3.9 mg/kg and 8.7 mg/kg in Saudi Arabia. The lead concentrations in the black tea samples were in the range of 1.88 - 5.63 mg/kg, similar to
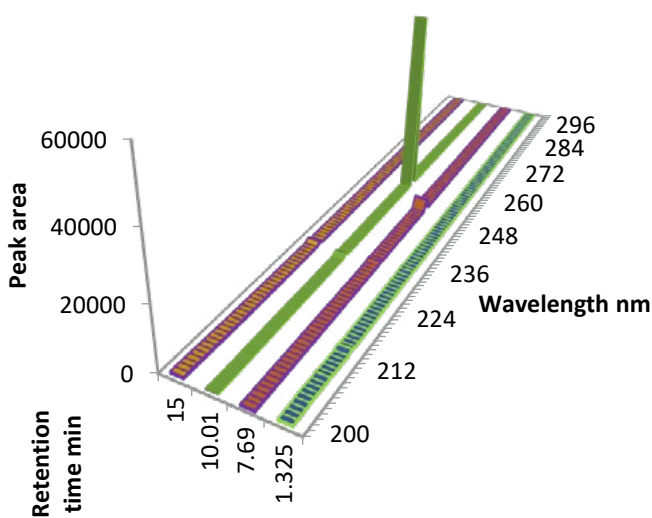

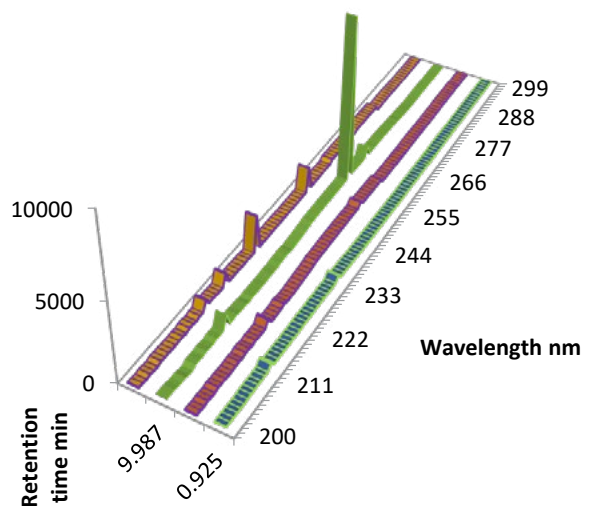Figure 3. Chromatogram of a anthraquinone in standard solution of 20 μg/L

Figure 4. Chromatogram of a anthraquinone in infusion tea samples

Table 4. Recovery of anthraquinone in infusion tea samples (n=2)

Samples	Pb (ppm)	Cd (ppm)	Cr (ppm)	Anthraquinone (ppb)
A	4.9	0.02	2	12.08
S	1.5	0.01	2	6.32
M	0.01	0.11	9.1	No Detect
D	0.03	0.01	2	No Detect
K	0.4	0.01	2	0.94
I	0.04	0.01	2	No Detect
GH	0.09	0.01	2	0.86
B	0.08	0.01	2	0.71
L	0.09	0.01	2	0.83
SI	0.1	0.01	2	1.02

those in black tea samples from Turkey (1.46-5.64 mg/kg) [18], lower than those from India and Iran [19-21] , and higher than those from Bangladesh, Ceylon, Russia, and Serbia. The high lead content in these five tea samples may be attributed to the use of older tea leaves [19] and the contamination with dust particles during processing [22]. The differences in the concentrations of lead in the tested tea samples may be attributed to the following factors: the age of the plant; the mineral composition of the soil, in which the plant grows; season, the time of sample collection; and the climatic conditions. Khakhathi et al. [23] found that Cr levels increased in the order of herbal tea, green tea, and black tea. The chromium concentration ranged in samples from 2.0 to 9.1 mg/kg. Most of the samples had a concentration below 2 mg/kg and the highest concentration was observed in sample M (9.1 mg/kg). The mean Cr concentration obtained in this work was lower than that found in African and Asian countries [23-25], but far higher than that found in Iran [26]. Srividhya et al.[27] reported that the reason for the high concentration of Cr is the use of rollers during black tea production.

In this work, the mean Cd concentration was 0.021 mg/kg (range: 0.01-0.11 mg/kg). The lowest Cd concentration was found in 7 samples (<0.01), while the highest was found in sample M. The Cd concentration of black tea in India was 0.89 mg/kg which was higher than that of in our study. The phosphate fertilizer in soil may cause cadmium contamination in crops [28].

Our results compared well with the literature values reported for tea samples from some countries. For example, cadmium concentrations in the China tea samples were close to those in tea samples from Argentina. However, the concentrations were lower than those in black teas produced in Taiwan and India. Table 5 summarizes the content of elements in this study against the literature. The concentration of Cd was at the same level in China, Argentina, Taiwan, and this study. Lead concentration was the same in China and current work but the contents in Argentina, China, and Taiwan were lower than

Table 5. Compression of toxic metals in black tea from different literature.

Country	Cd	Cr	Pb	Reference
Taiwan	0.07	7.92	2.01	[29]
Argentina	0.03	1.91	1.78	[30]
India	0.89	704.	2.31	[31]
China	0.057	0.80	4.4	[32]
Iran	-	0.06	-	[26]
Current study	0.21	2.71	4.89	-

current study. In this study, the concentrations of Cd and Pb was less than the standard limits recommended by WHO (3 mg/kg and 10 mg/kg) [33]. As obtained results, the concentrations of Pb, Cd and Cr in tea were lower than limit values of safety standards and Ministry of Agriculture of the People's Republic of China (5, 1,and 5 mg/kg), respestively [34-35]. The results of the two methods lied within the allowable range as in the AOAC and European Union [15-16,36].

Conclusion

In this study, a new rapid simple method was investigated to determine the concentration of some contaminants in black tea samples. The results lay a foundation for preventing contamination in human from tea samples and will help establish regulations to control the concentration of some contaminants in tea samples. Finally, our results showed that two methods were successfully applied to determine the concentration of some contaminants such as heavy metals and anthraquinone in black tea samples. In previous reports, costly instruments such as GC-MS and tandem MS were used for the detection of anthraquinone, usually not available in laboratories. According to the results of these two methods, the concentrations of contaminants were within allowable limits and were thus safe for human consumption. Some of the advantages of this work were that there was no report on anthraquinone determination and it was limited to anthraquinone concentration on black tea infusion. Overall our results showed that MAD and DLLME methods could successfully be applied for the concentration of some contaminants such as heavy metals and anthraquinone in black tea samples.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

References

1. Ostrowska J, Stankiewicz A, Skrzydlewska E. Antioxidative properties of green tea. Bromatol Toxicol Chem. 2001;2:131.

2. Zhu QY, Hackman RM, Ensunsa JL, Holt RR, Keen CL. Antioxidative activities of oolong tea. Journal of Agricultural and Food Chemistry. 2002;50(23):6929-34.

3. Chandra S, Gonzalez de Mejia E. Polyphenolic compounds, antioxidant capacity, and quinone reductase activity of an aqueous extract of Ardisia compressa in comparison to mate (Ilex paraguariensis) and green (Camellia sinensis) teas. Journal of agricultural and food chemistry.   2004;52(11):3583-9.

4. Maron DJ, Lu GP, Cai NS, Wu ZG, Li YH, Chen H, et al. Cholesterol-lowering effect of a theaflavin-enriched green tea extract: a randomized controlled trial. Archives of internal medicine. 2003;163(12):1448-53.

5. Hasegawa R, Chujo T, Sai-Kato K, Umemura T, Tanimura A, Kurokawa Y. Preventive effects of green tea against liver oxidative DNA damage and hepatotoxicity in rats treated with 2-nitropropane. Food and chemical toxicology. 1995;33(11):961-70.

6. Fujiki H. Green tea: Health benefits as cancer preventive for humans. The Chemical Record. 2005;5(3):119-32.

7. Bettuzzi S BM, Rizzi F, Castagnetti G, Peracchia G, Corti A. Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: a preliminary report from a one-year proof-of-principle study. Cancer Res. 2006;66(2):1234-1240. doi:10.1158/0008-5472.CAN-05-1145.

8. Davis AC, Wu P, Zhang X, Hou X, Jones BT. Determination of cadmium in biological samples. Applied Spectroscopy Reviews. 2006;41(1):35-75.

9. Program NT. NTP toxicology and carcinogenesis studies of EMODIN (CAS NO. 518-82-1) Feed Studies in F344/N Rats and B6C3F1 Mice. National Toxicology Program technical report series. 2001;493:1.

10. Yusiasih R, Pitoi MM, Ariyani M, Koesmawati TA, Maulana H. Anthraquinone in Indonesian infusion tea: analysis by HPLC–UV and risk assessment. Chemical and Biological Technologies in Agriculture. 2019;6(1):19.

11. Wang X, Zhou L, Luo F, Zhang X, Sun H, Yang M, et al. 9, 10-Anthraquinone deposit in tea plantation might be one of the reasons for contamination in tea. Food chemistry. 2018;244:254-9.

12. Hayward DG, Wong JW, Park HY. Determinations for pesticides on Black, Green, Oolong, and White Teas by gas chromatography triple-quadrupole mass spectrometry. Journal of agricultural and food chemistry. 2015;63(37):8116-24.

13. 2006. BHAUNLoM.

14. Salahinejad M, Aflaki F. Toxic and essential mineral elements content of black tea leaves and their tea infusions consumed in Iran. Biological trace element research. 2010;134(1):109-17.

15. F Ap. : guidelines for Standard METHOD performance requirements OMoAoA.

16. 2010. EGdopram.

17. Al-Othman ZA, Yilmaz E, Sumayli HM, Soylak M. Evaluation of trace metals in tea samples from Jeddah and Jazan, Saudi Arabia by atomic absorption spectrometry. Bulletin of environmental contamination and toxicology.

2012;89(6):1216-9.

18. Tokalıoğlu Ş, Kartal Ş. Bioavailability of soil-extractable metals to tea plant by BCR sequential extraction procedure. Instrumentation Science & Technology. 2004;32(4):387-400.

19 . Natesan S, Ranganathan V. Content of various elements in different parts of the tea plant and in infusions of black tea from southern India. Journal of the Science of Food and Agriculture. 1990;51(1):125-39.

20. Shokrzadeh M, Saberyan M, Saeedi Saravi S. Assessment of lead (Pb) and cadmium (Cd) in 10 samples of Iranian and foreign consumed tea leaves and dissolved beverages. Toxicological and Environmental Chemistry. 2008;90(5):879-83.

21. Zazouli MA, Bandpei AM, Maleki A, Saberian M, Izanloo H. Determination of cadmium and lead contents in black tea and tea liquor from Iran. Asian Journal of Chemistry. 2010;22(2):1387.

22. Karak T, Bhagat R. Trace elements in tea leaves, made tea and tea infusion: A review. Food Research International. 2010;43(9):2234-52.

23. Mandiwana KL, Panichev N, Panicheva S. Determination of chromium (VI) in black, green and herbal teas. Food Chemistry. 2011;129(4):1839-43.

24. Marcos A, Fisher A, Rea G, Hill SJ. Preliminary study using trace element concentrations and a chemometrics approach to determine the geographical origin of tea. Journal of Analytical Atomic Spectrometry. 1998;13(6):521-5.

25. S. Sreenivasan NM NNMaRSFC, 746 2008.

26. Kasrai M, Shoushtarian M, Bozorgzadeh M. Determination of trace elements in tea leaves by neutron activation analysis. Journal of Radioanalytical and Nuclear Chemistry. 1977;41(1-2):73-9.

27. Srividhya B, Subramanian R, Raj V. Determination of lead, manganese, copper, zinc, cadmium, nickel and chromium in tea leaves. Int J Pharm Pharm Sci. 2011;13:257-8.

28. De Meeus C, Eduljee G, Hutton M. Assessment and management of risks arising from exposure to cadmium in fertilisers. I. Science of the total Environment. 2002;291(1-3):167-87.

29. Shen F-M, Chen H-W. Element composition of tea leaves and tea infusions and its impact on health. Bulletin of Environmental Contamination and Toxicology. 2008;80(3):300-4.

30. Dybczynski R, Danko B, Kulisa K, Chajduk-Maleszewska E, Polkowska-Motrenko H, Samczynski Z, et al. Final certification of two new reference materials for inorganic trace analysis. Chemia analityczna. 2004;49(2):143.

31. Chajduk E. Elemental analysis of black and green tea leaves by instrumental neutron activation analysis. Chemia Analityczna. 2009;54(5):841.

32. Zhong W-S, Ren T, Zhao L-J. Determination of Pb (Lead), Cd (Cadmium), Cr (Chromium), Cu (Copper), and Ni (Nickel) in Chinese tea with high-resolution continuum source graphite furnace atomic absorption spectrometry. Journal of food and drug analysis. 2016;24(1):46-55.