

Determination of Cu, Cd, Ni, Pb and Zn in Edible Oils Using Reversed-Phase Ultrasonic Assisted Liquid–Liquid Microextraction and Flame Atomic Absorption Spectrometry¹

Maryam Mohebbi^a, Rouhollah Heydari^{b, *}, and Majid Ramezani^a

^aDepartment of Chemistry, Faculty of Sciences, Arak Branch, Islamic Azad University, Arak, Iran

^bRazi Herbal Medicines Research Center, Lorestan University of Medical Sciences, PO Box 68149-89468, Khorramabad, Iran

*e-mail: rouhollahheydari@yahoo.com

Received May 27, 2016; in final form, November 21, 2016

Abstract—A simple and green reversed-phase ultrasonic assisted liquid–liquid microextraction method for determination of Cu, Cd, Ni, Pb and Zn in edible oils was developed. Detection was carried out by flame atomic absorption spectrometry. The influence of main parameters including ultrasonic time and temperature, disperser solvent, volume of extracting solvent and centrifuging time on the extraction efficiency of target analytes were investigated and optimized. In the proposed method, a few microliters of water (containing 3%, v/v, nitric acid) as extracting solvent was injected into the oil sample and mixture transferred to ultrasonic bath. Then, the mixture was centrifuged in order to accelerate in phase separation. Finally, the aqueous phase was removed and delivered to flame atomic absorption spectrometer. Calibration curves for all metals were linear in the range of 5–100 ng/mL. The limit of detections for Cu, Cd, Ni, Pb and Zn were 0.8, 0.3, 0.5, 1.5 and 0.5 ng/mL, respectively. Relative standard deviation (RSD) values were in the range of 0.6–1.9%. The recoveries were in the range of 95.2–101.2% with RSD values ranging from 0.8 to 1.9%. The proposed method was applied successfully for the determination of interested metals in commercial edible oils.

Keywords: reversed-phase liquid–liquid microextraction, ultrasound, edible oils, metal ions

DOI: 10.1134/S1061934818010069

Edible oils play an essential role in the metabolic reactions of the human body. Also, the human body uses oils in the diet for different purposes including energy source, supply the essential fatty acids which body cannot produce, help to absorb vitamins and biological regulators [1]. Edible oils contain traces of metals, whose concentrations are the criteria for assigning quality, stability and freshness of oil [2]. The trace metals in oil can originate from several sources such as soil or fertilizers used during plant growth or production and storage [2–5]. Presence of trace metals may have a significant effect on promoting the oxidation reactions which result in the formation of toxic components [6]. The most of these metals are highly toxic for living organisms and have a long half-life. Therefore, regarding human health and oil quality, the determination of trace metals in edible oils is essential [7, 8]. Direct analysis of oil samples using flame atomic absorption spectrometry (FAAS) is not possible due to low concentration of metals and high viscosity of samples. To overcome these problems, various sample preparation techniques were used such as dilution with suitable organic solvents, mineralization

by dry or microwave digestion in oxidizing acids and conversion into oil-in-water emulsions [9]. Sample dilution is simple and rapid, but it increases the limit of detection and risks of analyte loss and contamination [10, 11]. For these reasons, development of suitable sample preparation techniques which can concentrate the desired analytes and reduce the sample viscosity are very interesting for the oil quality control and food analysis.

Different approaches has been carried out for the detection of trace metals in oils such as graphite furnace atomic absorption spectrometry (GF-AAS), FAAS, inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), cathodic stripping potentiometry and photometric determination [12–19]. Among these techniques FAAS has been preferred due to several advantages including simplicity, availability and low cost [20].

Liquid–liquid microextraction (LLME) methods have been attracted attention in recent years as an alternative to classical liquid–liquid extraction procedures [21–24]. Numerous LLME methods were applied for metal determination via formation of metal–ligand complexes [25–27]. Most of these

¹ The article is published in the original.

methods were used to extract of metals from aqueous samples. However, a few reports on the measurement of metals in edible oils are available [3, 10, 12, 15, 16, 19].

The aim of this study was to develop a direct and rapid reversed-phase ultrasonic assisted liquid-liquid microextraction (**RP-UALLME**) method for the determination of several metals including Cu, Cd, Ni, Pb and Zn in edible oils.

EXPERIMENTAL

Chemicals and materials. Methanol, acetone, ethanol, acetonitrile, copper(II) nitrate trihydrate, lead(II) nitrate, zinc nitrate tetrahydrate and nitric acid were purchased from Merck Chemical (Darmstadt, Germany). Cadmium nitrate tetrahydrate and nickel(II) nitrate hexahydrate were obtained from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were used without further purification. Deionized water was supplied using a Millipore system (Millipore, USA). Oil samples were obtained from local supermarkets (Arak, Markazi).

Instrumentation. The flame atomic absorption spectrometer (Model AA-680, Shimadzu, Kyoto, Japan) equipped with a deuterium background correction system and an air-acetylene flame was used under the conditions recommended for the studied metals. An ultrasound bath (VGT-1730 QTD, GT SONIC, China) was used for the ultrasonic process. A vortex mixer (Genie-2, Scientific Industries, Italy) and an Eppendorf centrifuge (ALC 4232, Hamburg, Germany) were used for mixing and separation of oil and aqueous phases, respectively. A Perkin Elmer ICP-OES (Optima 7300 V HF, USA) and microwave (MarsX, 1200 W, 2450 MHz, CEM Corp. USA) spectrometers were used for analysis of olive oil sample by reference method [16].

Preparation of standard and sample solutions. Stock solutions of metals (1000 $\mu\text{g/mL}$) were prepared by dissolving their nitrate salts in deionized water. Working standard solutions were prepared by diluting suitable volumes of stock solution in methanol. Calibration curves were constructed using sunflower oil samples spiked with metals solutions prepared in methanol subjected to the proposed method under the optimized conditions. Concentration range of metals for calibration was 5–100 ng/mL .

Procedure. 10 mL of oil sample was transferred into a 15 mL conical polypropylene centrifuge tube. 200 μL of water containing 3% nitric acid as extracting solvent was added to the tube, the mixture was vortexed for 30 s and placed in an ultrasonic bath at 60°C for 10 min. Finally, phase separation was completed by centrifuging the solution at 4000 rpm for 3 min, and aqueous phase was removed using a micro-syringe. Separated aqueous phase was diluted to 500 μL using deionized water and delivered to FAAS instrument.

RESULTS AND DISCUSSION

The parameters that can influence the extraction efficiency of interested metals such as extracting solvent volume, disperser solvent, ultrasonic time and temperature and centrifuging time using the proposed method were evaluated and optimized.

Optimization of the RP-UALLME method. In this study, a 3% (v/v) nitric acid solution was selected as extracting solvent to extract metals from oil samples, similar to previous reports [3, 17]. Several solvents including ethanol, methanol, acetone and acetonitrile were chosen as disperser solvents. Therefore, 300 μL of nitric acid solution (3%, v/v) as extracting solvent was mixed with 1 mL of these solvents and mixture injected into 10 mL of oil sample. As observed from the results, disperser solvent has no positive effect on the analytes extraction. In the other words, maximum extraction efficiencies were achieved even without any disperser solvent. Solubility of extracting solvent in oil phase can be increased in the presence of disperser solvents. Consequently, volume of recovered extracting solvent reduces which leads to decrease in the extracting solvent ability to extract the analytes. Later on, no disperser solvent was used in method optimization process.

Effect of volume of extracting solvent on the extraction efficiency of interested metals was investigated in the range of 0.2–1.5 mL. An increase in extracting solvent volume from 0.2 up to 0.4 mL has no significant effect on the extraction efficiencies of analytes. After 0.4 mL, with the increase in the extracting solvent volume due to dilution effect, the analytes signals decreased. Therefore, 0.2 mL was selected as the optimum extracting solvent volume for subsequent experiments.

Ultrasonic waves are driving force in the proposed extraction method. Ultrasound generates a turbulence state in sample solution which increases the contact surface between oil and aqueous phases. This phenomenon can accelerate the mass transfer of analytes from oil phase to aqueous phase. For this reason, ultrasonic time was evaluated in the range of 1–30 min. Figure 1 shows the effect of ultrasonic time on the extraction efficiency of the studied metals. It is observed all analytes signals were increased up to 10 min and then decreased or leveled off.

Another important parameter in the proposed method is ultrasonic bath temperature that was investigated in the range of 20–80°C. As can be seen in Fig. 2, an increase in temperature till 60°C increases the extraction efficiencies of Cd, Ni, Pb and Zn with a slow slope. While Cu signal indicates larger change. Therefore, 60°C was selected as the optimum ultrasonic bath temperature for further experiments.

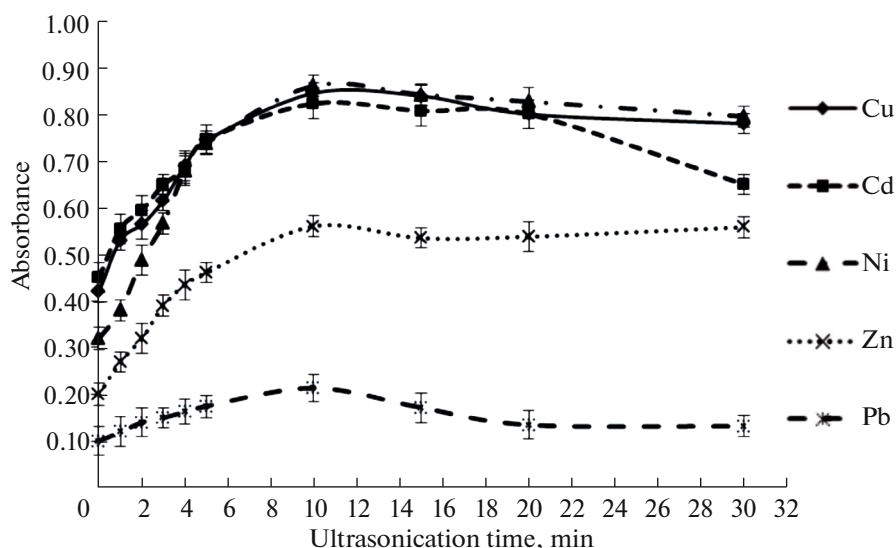


Fig. 1. Effect of ultrasonication time on the extraction efficiencies. Extraction conditions: extracting solvent, 3% (v/v) nitric acid; extracting solvent volume, 200 μ L; ultrasonication temperature, 60°C; centrifuging time, 3 min. Analytes concentrations: Cu, Cd, Ni, Zn, 5 ng/mL; Pb, 10 ng/mL.

Centrifuging can be influenced the rate and completeness of phase separation. In this study, centrifuging time was investigated in the range of 1–5 min. Maximum analytes signals were obtained at 3 min (4000 rpm). Therefore, 3 min was chosen as the optimum centrifuging time.

Method evaluation. Under the optimized conditions, validation parameters of the proposed method such as linearity, limit of detection (LOD), limit of quantitation (LOQ), precision (repeatability and reproducibility) and accuracy were determined. Linearity of the method was evaluated using extract of standard solutions of target metals at different concentrations under the optimized conditions. Correlation coefficients (R^2) for five calibration curves were larger than 0.9953 which approved the linearity of the proposed method in the studied concentration ranges. The LOD and LOQ were defined as concentrations with $S/N = 3$ and $S/N = 10$, respectively. The LOD and LOQ values are listed in Table 1.

Results of repeatability and reproducibility of the proposed method at three concentration levels are detailed in Table 2. Intra- and inter-day RSD values for five metals were less than 1.9 and 2.7%, respectively. The accuracy of the proposed method was investigated by determining the relative recovery of spiked metals in oil samples at three concentration levels. Table 2 lists the obtained relative recoveries from the analysis of spiked samples. As can be seen, relative recoveries were in the range of 95.2–101.2%. The results show that the oil matrix does not significant

effect on the extraction process and appropriate recoveries are obtained at the working range.

Method performance and real samples analysis. In order to investigate of method performance several edible oil samples were analyzed using the proposed method under the optimized conditions. In order to avoid the matrix effects on the analytical signals, metals determinations were performed using standard addition method. The results were listed in Table 3.

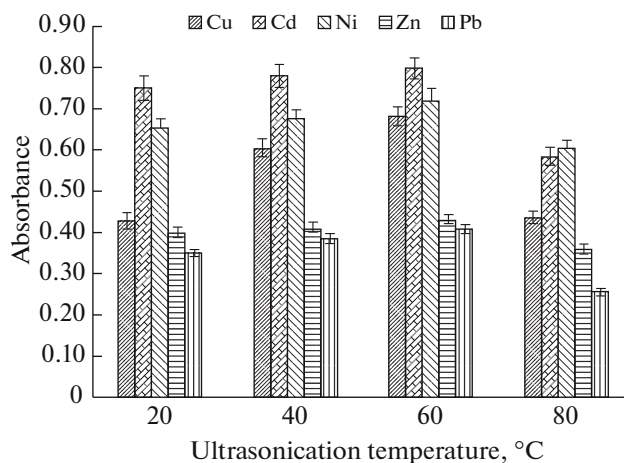


Fig. 2. Effect of ultrasonication temperature on the extraction efficiencies. Extraction conditions: extracting solvent, 3% (v/v) nitric acid; extracting solvent volume, 200 μ L; ultrasonication time, 10 min; centrifuging time, 3 min. Analytes concentrations: Cu, Cd, Ni, Zn, 5 ng/mL; Pb, 10 ng/mL.

Table 1. Analytical parameters for the proposed method

Metal	R^2	Slope	Intercept	LOD, ng/mL	LOQ, ng/mL
Cu	0.9953	3.9000	0.1910	0.7	2.6
Cd	0.9996	8.8020	0.2239	0.3	1.1
Ni	0.9958	6.0851	0.1877	0.5	1.6
Pb	0.9997	1.9030	0.0889	1.5	5.2
Zn	0.9967	5.6535	0.6261	0.5	1.7

The proposed method can be successfully used to determine the studied metals in edible oil samples.

Method accuracy was investigated using analysis of olive oil sample by a previously reported method. The obtained results using the proposed and reference methods are presented in Table 4. The results do not show any significant difference between two methods.

The analytical figures of merit the proposed method were compared with several reported methods in the literature (Table 5). As can be seen from these results, the obtained LODs and RSDs for studied metals using the proposed method were modified than

other reported methods with similar detection system. Although sensitivity and reproducibility data for ICP-MS are better than the obtained results by the proposed method, but because of high cost is not available in every laboratory.

CONCLUSIONS

In this study, a new method namely reversed-phase ultrasonic assisted liquid-liquid microextraction (RP-UALLME) was successfully developed for determination of Cu, Cd, Ni, Pb and Zn in edible oils by FAAS. Usually, the metal determination methods in oil samples require sensitive instruments such as ICP-OES, ICP-MS and GF-AAS which are expensive and not available in many laboratories. Also, use of ultrasonic process reduces the extraction time. Regard to the mentioned advantages, the proposed method is rapid, simple, cost-effective and environmentally friendly. In this method no organic solvent was used during the extraction process. The above mentioned advantages and good analytical results make the method an appropriate technique for the determination of Cu, Cd, Ni, Pb and Zn in the routine analysis of edible oils.

Table 2. Obtained precision and accuracy data for spiked olive oil samples using RP-UALLME method

Metal	Added, ng/mL	Found, ng/mL	RSD, %		Recovery, %
			intra-day ($n = 5$)	inter-days ($n = 15$)	
Cu	25.0	40.8	1.3	1.4	95.2
	50.0	65.1	1.4	1.4	96.2
	100.0	118.2	0.8	1.0	101.2
Cd	25.0	36.4	1.6	1.8	97.6
	50.0	62.5	1.2	1.4	101.0
	100.0	110.2	0.6	1.2	98.2
Ni	25.0	47.4	1.9	2.1	97.6
	50.0	72.2	1.8	2.1	98.4
	100.0	122.5	1.0	1.7	99.5
Pb	25.0	33.8	1.6	1.8	100.4
	50.0	57.2	1.2	1.6	97.0
	100.0	108.9	1.0	1.1	100.2
Zn	25.0	48.2	1.8	1.9	100.8
	50.0	71.2	1.9	2.7	96.4
	100.0	122.8	1.6	2.0	99.8

Table 3. Results for metals determination in edible oils (obtained content, ng/mL \pm SD^a) using the proposed method

Oil sample	Cu	Cd	Ni	Pb	Zn
Olive	17.0 \pm 0.5	12.0 \pm 0.6	23.0 \pm 0.6	8.7 \pm 0.1	23.0 \pm 0.4
Sunflower (A)	27.0 \pm 0.5	13.0 \pm 0.4	23.0 \pm 0.5	<LOQ	29.0 \pm 0.7
Sunflower (B)	32.0 \pm 0.6	13.0 \pm 0.5	27.0 \pm 0.6	<LOQ	23.0 \pm 0.5
Soybean	22.0 \pm 0.5	18.0 \pm 0.5	24.0 \pm 0.5	6.4 \pm 0.1	16.0 \pm 0.4
Grape	37.0 \pm 0.6	12.0 \pm 0.5	31.0 \pm 0.6	<LOQ	30.0 \pm 0.6
Sesame	30.0 \pm 0.4	15.0 \pm 0.4	67.0 \pm 0.7	4.5 \pm 0.1	16.0 \pm 0.4

^aStandard deviation.**Table 4.** The results (concentration, ng/mL \pm SD) of olive oil analysis using the proposed and reference methods ($n = 3$)

Method	Cu	Cd	Ni	Pb	Zn
RP-UALLME–AAS ^a	17.0 \pm 0.5	12.0 \pm 0.6	23.0 \pm 0.6	8.7 \pm 0.1	23.0 \pm 0.4
MAD–ICP–OES ^b [16]	17.2 \pm 0.4	11.7 \pm 0.4	22.1 \pm 0.4	8.9 \pm 0.3	21.8 \pm 0.3

^aReversed-phase ultrasonic assisted liquid-liquid microextraction–atomic absorption spectrometry.^bMicrowave assisted digestion–inductively coupled plasma optical emission spectrometry.**Table 5.** Analytical parameters of the proposed and reported methods for the determination of metals in edible oils

Metal	Sample preparation method	Detection	LOD, ng/mL	LOQ, ng/mL	RSD, %	Recovery, %	Reference
Cu	EIEB ^a	ICP–OES	–	–	<5	97–130	[3]
	Extraction with acidic water	GF–AAS	–	–	<15	71–117	[10]
		FAAS	–	–	<13	93.6–100.4	[12]
	UAE ^b	FAAS	–	–	<1.3	97.2–102.1	[15]
	Extraction as complex	FAAS	–	–	<1.8	95.2–101.2	This work
Cd	MAD ^c	ICP–OES	50	–	<2	–	[16]
	RP–UALLME	FAAS	0.3	1.1	<1.8	97.6–101.0	This work
Ni	UAE	FAAS	–	–	<11	95.0–97.3	[12]
	MAD	ICP–OES	260	–	<2	–	[16]
	MAD	GF–AAS	5	–	<5	–	[16]
	RP–UALLME	FAAS	0.5	1.6	<2	97.6–99.5	This work
Zn	UAE	FAAS	–	–	<12	96.0–101.2	[12]
	EIEB	ICP–MS	0.18	0.6	0.9	87.0–108.0	[28]
	MAD	ICP–OES	90	–	<2	–	[16]
	RP–UALLME	FAAS	0.5	1.7	<2.7	96.4–100.8	This work
Pb	EIEB	ICP–MS	0.004	0.013	2	89.0–108.0	[29]
	MAD	ICP–OES	220	–	<2	–	[16]
	RP–UALLME	FAAS	1.5	5.3	<1.8	97.0–100.4	This work

^aEIEB—extraction induced by emulsion breaking, ^bUAE—ultrasonic assisted extraction, ^cMAD—microwave assisted digestion.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support of Islamic Azad University, Arak Branch.

REFERENCES

1. Khan, H., Fida, M., Mohammadzai, I.U., and Khan, M., *J. Chin. Chem. Soc.*, 2007, vol. 54, p. 737.
2. Chu, Y.L. and Jiang, S.J., *J. Chromatogr. A*, 2011, vol. 1218, p. 5175.
3. Bakircioglu, D., Bakircioglu Kurtulus, Y., and Yurtsever, S., *Food Chem.*, 2013, vol. 138, p. 770.
4. Benincasa, C., Lewis, J., Perri, E., Sindona, G., and Tagarelli, A., *Anal. Chim. Acta*, 2007, vol. 585, p. 366.
5. Mena, C., Cobrera, C., Lorenzo, M.L., and Lopez, M.C., *Sci. Total Environ.*, 1996, vol. 181, p. 201.
6. Decker, E., Elias, R., and McClements, D.J., *Oxidation in Foods and Beverages and Antioxidant Applications*, Cambridge: Woodhead, 2010.
7. Murillo, M., Benzo, Z., Marcano, E., Gomez, C., Garaboto, A., and Marin, C., *J. Anal. Atom. Spectrom.*, 1999, vol. 14, p. 815.
8. Guldas, M., *J. Food Nutr. Res.*, 2008, vol. 47, p. 92.
9. Cassella, R.J., Brum, D.M., Lima, C.F., and Fonseca, T.C.O., *Fuel*, 2011, vol. 90, p. 1215.
10. De Leonardis, A., Macciola, V., and De Felice, M., *Int. J. Food Sci. Technol.*, 2000, vol. 35, p. 371.
11. Pehlivan, E., Arslan, G., Gode, F., Altun, T., and Özcan, M.M., *Grasas Aceites (Sevilla, Spain)*, 2008, vol. 59, p. 239.
12. Anwar, F., Kazi, T.G., Saleem, R., and Bhangar, M.I., *Grasas Aceites (Sevilla, Spain)*, 2004, vol. 55, p. 160.
13. Dugo, G., La Pera, L., Pollicino, D., and Saitta, M., *J. Agric. Food Chem.*, 2003, vol. 51, p. 5598.
14. Ooms, R. and Vanpee, W., *J. Am. Oil Chem. Soc.*, 1983, vol. 60, p. 957.
15. Baran, E.K. and Yasar, S.B., *J. Am. Oil Chem. Soc.*, 2010, vol. 87, p. 1389.
16. Cindric, I.J., Zeiner, M., and Steffan, I., *Microchem. J.*, 2007, vol. 85, p. 136.
17. López-García, I., Vicente-Martínez, Y., and Hernández-Córdoba, M., *Talanta*, 2014, vol. 124, p. 106.
18. Shishov, A.Y., Bulatov, A.V., Moskvina, A.L., and Moskvina, L.N., *J. Anal. Chem.*, 2014, vol. 69, p. 1159.
19. He, Y.M., Zhao, F.F., Zhou, Y., Ahmad, F., and Ling, Z.X., *Anal. Methods*, 2015, vol. 7, p. 4493.
20. Lepri, F.G., Chaves, E.S., Vieira, M.A., Ribeiro, A.S., Curtius, A.J., De Oliveira, L.C.C., and De Campos, R.C., *Appl. Spectrosc. Rev.*, 2011, vol. 46, p. 175.
21. Lord, H. and Pawliszyn, J., *J. Chromatogr. A*, 2000, vol. 902, p. 17.
22. Amelin, V.G. and Lavrukhin, D.K., *J. Anal. Chem.*, 2016, vol. 71, p. 359.
23. Hosseini, M., Heydari, R., and Alimoradi, M., *Talanta*, 2014, vol. 130, p. 171.
24. Heydari, R. and Zarabi, S., *Anal. Methods*, 2014, vol. 6, p. 8469.
25. Berton, P., Vera-Candioti, L., Goicoechea, H.C., and Wuilloud, R.G., *Anal. Methods*, 2013, vol. 5, p. 5065.
26. Wang, Y., Liu, Y., Han, J., Wang, L., Chen, T., and Ni, L., *Anal. Methods*, 2015, vol. 7, p. 2339.
27. Suryavanshi, V.J., Pawar, R.R., Anuse, M.A., and Mulik, G.N., *Anal. Methods*, 2015, vol. 7, p. 2497.
28. He, Y.M., Chen, J.J., Zhou, Y., Wang, X.J., and Liu, X.Y., *Anal. Methods*, 2014, vol. 6, p. 5105.
29. Khan, N., Kazi, T.G., Shah, F., Afridi, H.I., Baig, J.A., and Soomro, A.S., *J. AOAC Int.*, 2013, vol. 96, p. 447.