

## Development of ultrasound-assisted emulsification microextraction for the determination of trace zinc and copper by flame atomic absorption spectrometry

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### Abstract

In this work, we developed a method based on ultrasound-assisted emulsification microextraction (USAEME) for the determination of zinc and copper by flame atomic absorption spectrometry (FAAS). The method is based on the use of the organic solvent carbon tetrachloride ( $\text{CCl}_4$ ) as an extraction solvent. In order to obtain a high enrichment factor, the effect of different parameters affecting the complex formation and extraction conditions (such as the type and volume of the extraction solvent, pH, the chelating agent amount, extraction time, extraction temperature and ionic strength) were tested. Under optimum conditions, the eight replicates mixture of the  $100 \text{ ngmL}^{-1}$  and  $50 \text{ ngmL}^{-1}$  for Zn(II) and Cu(II) ions, gave a mean absorbance of 0.055 and 0.061, with a relative standard deviation (RSD) of  $\pm\%3.2$  and 2.9, respectively. The equations for the lines were  $A = 0.4921C + 0.0027$  ( $R = 0.9998$ ) and  $A = 1.0701C + 0.0032$  ( $R = 0.9997$ ), respectively. The limit of detection for Zn (II) and Cu(II) ions were 1.06 and  $1.4 \text{ ngL}^{-1}$ , respectively. The calibration graph was linear in the range of  $3.0\text{--}2000.0 \text{ ngmL}^{-1}$  and  $2.0\text{--}850.0 \text{ ngmL}^{-1}$  for Zn and Cu respectively. In the proposed procedure, enhancement factors were 9.51 and 6.25 for Zn and Cu, respectively. This proposed method was successfully applied in the analysis of four real environmental water samples and good spiked recoveries over the range of 98.4–103.0% were obtained. This is the first research using USAEME for simultaneous determination of Zn and Cu in water.

**Keywords:** Preconcentration; ultrasound-assisted emulsification microextraction silver; water pollution; zinc; copper.

### Introduction

The polluting nature of heavy metals has recently received considerable attention. Zinc and copper which are recognized as an extremely environmental toxic and carcinogenic metal can be easily dissolved and transported by water. These are highly toxic to animals, plants, and humans even at low concentrations.

Considering this, health organizations have established permissible limits for Zn and Cu in human food and drinking water [1-3].

Generally, there are many analytical techniques available for the sample preparation. Several procedures have been developed for the separation and preconcentration of pesticide from the water sample matrix, such as quick,

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easy, cheap, effective, rugged and safe (QuEChERS) [1] and solid phase microextraction (SPME) [2]. The main objectives of sample preparation are analyte concentration and sample cleanup.

Flame atomic absorption spectrometry (FAAS) has been widely used for the determination of trace metal ions because of the relatively simple and inexpensive equipment required. However, direct determination of metal ions at trace levels by FAAS is limited not only due to the insufficient sensitivity, but also because of the matrix interference [4]. Several methods have been proposed for separation and pre-concentration of trace metals including: Liquid-liquid extraction (LLE), coprecipitation, solid phase extraction (SPE), and cloud point extraction (CPE). However, liquid-liquid extraction (LLE), as the old pre-concentration and separation technique in analytical chemistry, is time-consuming and requires large amounts of organic solvents [5-8]. As compared to LLE, SPE offers simpler operation, a higher enrichment factor, and ease of automation, but the amounts of elution solvents are still relatively large. CPE is a comparatively simple, cheap and less toxic method, but it too suffers from several limitations. For instance, because of the viscosity of the surfactant-rich phase, samples being prepared in this way cannot be directly injected into conventional analytical instruments [9,10].

Jean not and Cantwell developed a liquid-phase microextraction (LPME) technique in 1996, which is based on the analyst partition between a drop of organic solvent (extraction phase) and the aqueous sample bulk [11]. Several different types of LPME have been developed, including single drop microextraction (SDME), hollow fiber

LPME and homogeneous liquid-liquid extraction (HLLLE). Microextraction techniques are fast, simple, inexpensive, environmentally friendly and compatible with many analytical instruments. Nevertheless, some drawbacks, such as instability of the droplet and relatively low precision are often reported [12]. Recently, Assadi and co-workers developed a novel microextraction technique, termed as dispersive liquid-liquid microextraction (DLLME). This technique is based on the formation of tiny droplets of the extractant in the sample solution using a water-immiscible organic solvent (extractant) dissolved in a water-miscible organic dispersive solvent. Its main drawback is the necessity of using a third component (disperser solvent), which usually decreases the partition coefficient of analytes into the extraction solvent [13].

Ultrasonic energy, when applied to solutions, causes acoustic cavitation: that is, bubble formation and implosion. The collapse of bubbles formed by ultrasonic energy results in the generation of extremely high temperatures and pressures at the interface of the collapsing bubble and another phase, leading to enhanced chemi-cal reactivity [14]. The combination of microextracting systems and ultrasound radiation provides an efficient pre-concentration technique, named as ultrasound-assisted emulsification-microextraction (USAEME). USAEME is based on the implosion bubbles generated by the cavitation phenomenon, which produces intensive shock waves in the surrounding liquid and high-velocity liquid jets. Such microjets can cause droplet disruption in the vicinity of collapsing bubbles and thus, improve emulsification by generating a smaller droplet size of the dispersed phase right

after disruption. Submicron droplet size leads to significant enlargement of the contact surface between both immiscible liquids, improving the mass-transfer between the phases [14, 15]. In USAEME, the appropriate extraction solvent is rapidly injected by syringe into aqueous samples containing analytes. After sonication, a cloudy solution is formed. Then, this cloudy solution is centrifuged and the fine droplets sediment at the bottom of the conical test tube are formed. The determination of analytes in the sedimented phase can be performed by instrumental analysis. In fact, this pre-concentration technique has been developed by Regueiro et al. [16] for the determination of synthetic musk fragrances, phthalate esters and lindane in aqueous samples. We extended its application to inorganic analysis, and have obtained the consistent conclusion.

This paper describes the first application of ultrasound-assisted emulsification–microextraction for the determination of trace Zinc and Copper in water samples without the addition of the dispersive solvent, thereby overcoming DLLME disadvantages. In order to obtain a high enrichment factor, the effect of different parameters affecting the complex formation and extraction conditions (such as the type and volume of the extraction solvent, pH, the chelating agent amount, extraction time, extraction temperature and ionic strength) were tested. USAEME can be employed as a simple and efficient extraction and pre-concentration procedure for heavy metals in aqueous samples with satisfactory results.

## **Experimental**

### *Instrumentation*

An Atomic Absorption spectroscopy (400p-novAA, Analytik Jena,

Germany) was used for all the absorption measurements (flow rate acetylene 1.5 liter per minute, flow rate air 3.5 liter per minute, 240.7 and 324.8 nm wavelength, and slit width 0.2 nm and 1.2 for Zinc and Copper, respectively). All pH measurements were carried out using a digital pH meter model 780 (Metrohm, Switzerland). The solution was stirred with a magnetic heater-stirrer (IKAMAG–RET, Germany). A centrifuge (Sigma Model 3-30K, Germany) was used to accelerate the phase separation process. An ultrasonic bath with temperature control (FALC instruments S.V.l Treviglio, Italy) model LBS2 was used to assist the emulsification process of the microextraction technique.

### *Reagents and solutions*

All chemical reagents were of analytical grades, and deionised water was used for the preparation of all solutions. The carbon tetrachloride, 1, 2-chlorobenzene, the salt of Zinc and Copper were purchased from the Merck Company (Darmstadt, Germany). The other chemicals were prepared from Sigma-Aldrich (St Louis, MO, USA). A stock solution of Ag was prepared from calculated amounts of silver nitrate at a concentration of 1000 milligrams per liter in 0.01 M HNO<sub>3</sub>. Working solutions were prepared daily by appropriate dilution of stock solutions.

### *USAEME procedure*

We determined the putting up of separate eight milliliters of aqueous solution containing 4.0 µg of Zinc and 2.0 µg copper in a test tube and, then, we added 1 mL phosphate buffer 0.1 M with pH = 10.0 and also 1 mL of 1% w/v sodium chloride and, finally, they were mixed thoroughly. Then, 20.0 µl carbon tetrachloride (as extraction solvent) is injected into the sample

solution by syringe. The test tube is placed in the ultrasonic sink for 15 min at a temperature of 50 °C. As a result, the system appears in cloudy mode due to the spread of fine particles carbon tetrachloride within the aqueous solution. Emulsions were then disrupted by centrifugation at 4,000 rpm for 5 min, until the emulsion was formed at the bottom of the cone tube. After that, the aqueous phase was separated and the settled phase was dissolved in 0.8 mL of 0.1 M methanolic nitric acid, and then injected to absorb it. All adsorbents were measured against the control solution prepared by this method.

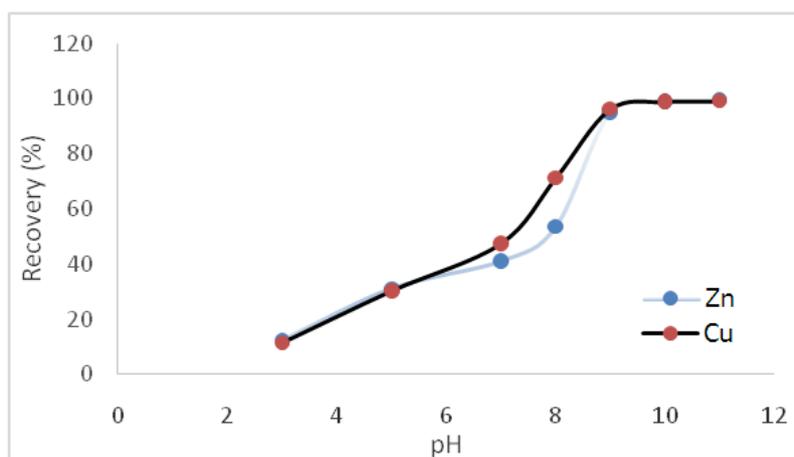
### Results and discussion

In this study, the combination of USAEME with FAAS was developed for the determination of trace amounts of Zn and Cu in water samples. Several

factors that may affect the extraction process, such as the type and volume of the extraction solvent, pH, extraction time, extraction temperature, and ionic strength were optimized. The enhancement factor was defined as the ratio of the slope of the calibration curve for the LL-USAEME procedure to that obtained without preconcentration.

#### Effect of pH

The pH of the sample solution is one of the important factors affecting the formation of nonionic compounds and subsequent extraction. The effect of the pH on the extraction of Zn and Cu was studied in the pH range of 3-11. As shown in Figure 1, the highest Ag signal intensity was obtained at pH 10-11. Hence, pH=10 was adopted for subsequent experiments.



**Figure 1.** Effect of pH on the extraction of Zn and Cu using the USAEME method

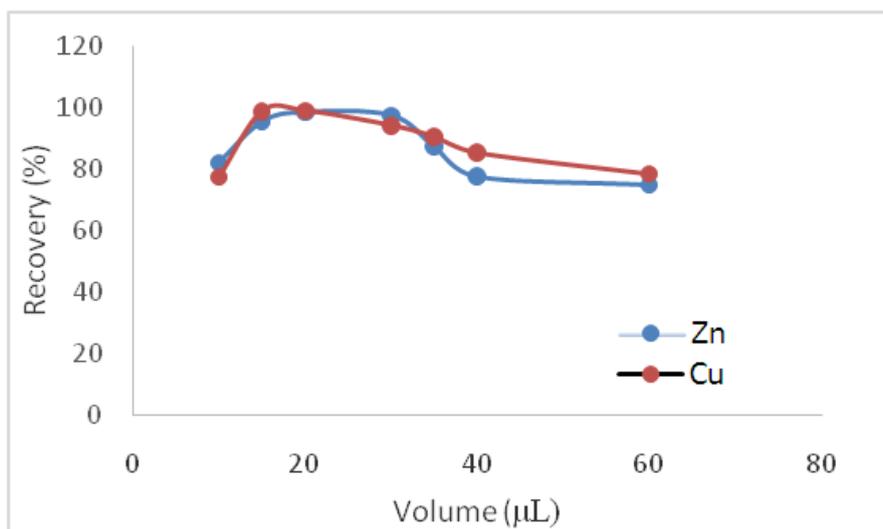
#### Selection type and volume of the extraction solvent

The type of extraction solvent is an essential consideration in USAEME for efficient extraction. It should present higher density than water, high extraction capability of the analytes and low solubility in water. Dichloromethane ( $\text{CH}_2\text{Cl}_2$ ), chloroform

( $\text{CHCl}_3$ ), 1,2-dichlorobenzene (1,2-DCB) and carbon tetrachloride ( $\text{CCl}_4$ ) were studied as an extraction solvent. The effect of these solvents on the extraction efficiency of USAEME was investigated using 30.0  $\mu\text{L}$  of each solvent. After the addition of  $\text{CHCl}_3$  and  $\text{CH}_2\text{Cl}_2$  not only the cloudy state was formed, but also there was no

sedimented phase at the bottom of the test tube after centrifugation. This effect can be explained by the higher solubility of these solvents in water as compared to the other tested solvents. Three replicate tests were performed for each of these solvents under the same conditions. The results have shown that the extraction efficiency of  $\text{CCl}_4$  (> 98%) is higher than 1, 2-DCB (93%). Therefore,  $\text{CCl}_4$  was selected as

extraction solvent for the subsequent experiments. In order to examine the effect of the extraction solvent volume, different volumes of  $\text{CCl}_4$  (10-60  $\mu\text{L}$ ) were used as an extraction solvent to the same USAEME procedure. It was observed that the highest extraction efficiency was obtained with 15.0-30.0  $\mu\text{L}$  of  $\text{CCl}_4$  (Figure 2). Therefore, 20.0  $\mu\text{L}$  of  $\text{CCl}_4$  was used for further experiments.

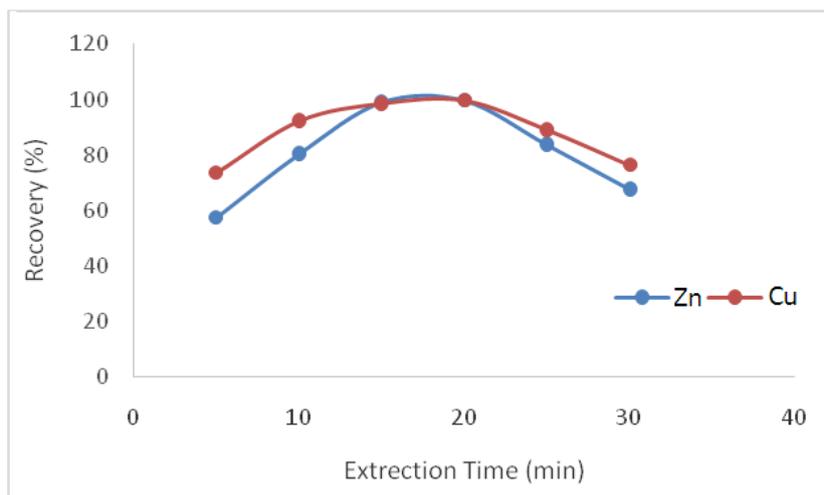


**Figure 2.** Effect of the extraction solvent volume ( $\text{CCl}_4$ ) on the USAEME of Zn and Cu

#### *Effect of extraction time*

In the process of UASEME, the interface between the extraction solvent and the sample was enormously enlarged by the formation of a cloudy solution and the dispersion of the solvent extraction into the aqueous solution. The ultrasound extraction time is defined as a time between the addition of the extraction solvent and the end of the sonication stage. The

effect of extraction time on the extraction efficiency was examined by varying the extraction time from 5 to 30 min. The results showed that the extraction efficiency increased with the increase of the extraction time to 15 min and then fixed to 20 minutes. Reduction in the extraction efficiency was observed after 20 min, therefore extraction time of 15 min was used for further experiments.



**Figure 3.** Effect of the volume of extraction time on the extraction of Zn and Cu

#### *Effect of extraction temperature*

The temperature could affect solubility of the organic solvents in water as well as the emulsification phenomenon. Thus, temperature affected the mass-transfer process and the extraction efficiency. The effect of extraction time was tested by keeping other laboratory conditions in the range of 40.0 to 50.0 °C. The results showed that the highest extraction efficiency was obtained at 45 °C. At higher temperatures, CCl<sub>4</sub> was partially dissolved into the aqueous bulk, leading to the reduction of the extraction efficiency. Hence, 50 °C was used for further experiments.

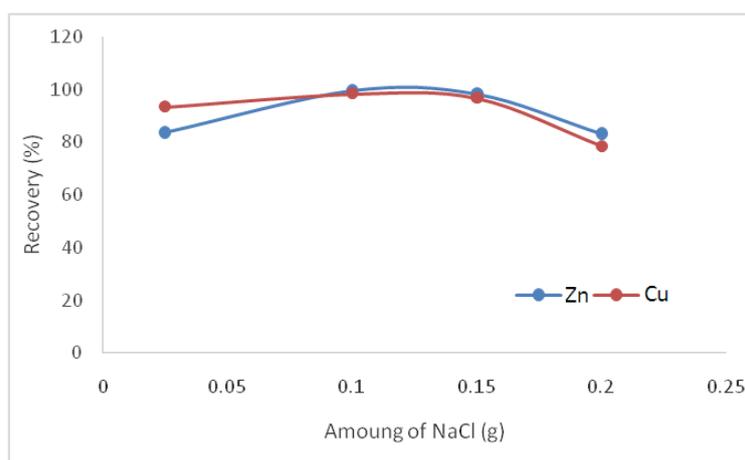
#### *Effect of stirring rate*

The stirring rate is an important parameter that enhances the kinetics of chelate formation and its extraction. According to the film theory of convective-diffusive mass transfer in the USAEME system, faster sample agitation causes the lower thickness of diffusion film in the aqueous phase. The optimum stirring rate was evaluated at different stirring rates, between 1000 and 6000 rpm at a constant extraction time of 5 min. As results, up to 4000 rpm, the absorbance signal rose as the stirring rate was increased. Afterwards, it remained

constant. Higher stirring rate was not used, because at such rates, the spattering damaged the micro drops. Hence a stirring rate of 4000rpm was adopted for subsequent experiments.

#### *Effect of Salt*

For investigating the influence of ionic strength on the performance of USAEME, various experiments were performed by adding varying NaCl amounts from 0.025 to 0.2 gr. Other experimental conditions were kept constant. By increasing the NaCl from 0.05 to 0.15, the volume of the sedimented phase increases slightly. The results showed that the salt addition has no significant effect on the enrichment factor, perhaps because of the two opposite effects of salt added in USAEME of Zn and Cu: one involves increasing the volume of the sedimented phase, which decreases the enrichment factor, and the other is the salting-out effect that increases the enrichment factor. Therefore, the enrichment factor is nearly constant by increasing the amount of sodium chloride, and the extraction experiments were carried out without adding salt. Therefore, 1 mL NaCl 10% was used in all further experiments.



**Figure 4.** Salting out effect on Zn and Cu percent recovery

#### *Interference effects*

In view of the high selectivity provided by flame atomic absorption spectrometry, the only interference may be attributed to the preconcentration step, in which the cations may react with  $\text{CCl}_4$  which may lead to decrease in extraction efficiency. The studied interferences were those related to the preconcentration step, cations that may react with  $\text{CCl}_4$  or species that may react with analytes and decrease the extraction efficiency. In other words, the possible interferences of common coexisting ions on the determination of Cu and Zn were investigated to demonstrate the selectivity of the

developed UASEME method. In these experiments, to 0.8 ml of solution containing  $0.4 \mu\text{g}$  of Zinc and  $2.4 \mu\text{g}$  of copper, the added coexisting ions were treated according to the recommended procedure. The tolerance limits of the coexisting ions, defined as the largest amount causing a change in the absorbance of Cu and Zn not higher than 5%, are given in Table 1. The results showed a good tolerance of coexisting ions studied in the samples. It stated that the developed method was applicable to the analysis of Cu and Zn in some environmental samples as shown further.

**Table 1.** Investigating the effects of interfering ions on Cu and Zn adsorption

Ions	Mole Ratio (Ion/analytes)	
	Zn	Cu
$\text{H}_2\text{PO}_4^-$	4000	4000
$\text{HPO}_4^{2-}$	4000	4000
$\text{Ca}^{2+}, \text{Mg}^{2+}$	1200	1300
$\text{Zn}^{2+}$	1100	1200
$\text{Pb}^{2+}$	500	200
$\text{Al}^{3+}$	100	50
$\text{Cd}^{2+}$	200	300
$\text{Fe}^{3+}$	370	500
$\text{Pd}^{2+}$	500	500
$\text{Cr}^{3+}$	350	200

*Analytical figures of merit*

Under the optimized conditions, calibration curves were constructed for the determination of Cu and Zn according to the USAEME procedure. The eight replicates mixture of the 100 ngmL<sup>-1</sup> and 50 ngmL<sup>-1</sup> for Zn(II) and Cu(II) ions, gave a mean absorbance of 0.055 and 0.061, with a relative standard deviation (RSD) of  $\pm$ 3.2 and 2.9, respectively. The equations for the lines were  $A = 0.4921C + 0.0027$  ( $R = 0.9998$ ) and  $A = 1.0701C + 0.0032$  ( $R = 0.9997$ ), respectively. In these equations, A is the absorbance value, C is the concentration of Zn and Cu (ng mL<sup>-1</sup>) and R is the correlation coefficient. The calibration graph was linear in the range of 3.0–2000.0 ngmL<sup>-1</sup> and 2.0–850.0 ngmL<sup>-1</sup> for Zn and Cu, respectively. The limit of detection defined as  $3S_B/m$  (where  $S_B$  is the standard deviation of the blank and m is the slope of the calibration graph) for Zn (II) and Cu(II) ions were 1.06 and 1.4 ngL<sup>-1</sup>, respectively. By definition, sensitivity to the atomic absorption spectrum is the concentration of the element that absorbs one percent of the radiation. The sensitivity for eight measurements of 10 and 100  $\mu$ gL<sup>-1</sup> of silver was 1.55

ngL<sup>-1</sup>. The relative standard deviation (RSD) for eight measurements of 0.20  $\mu$ gL<sup>-1</sup> of silver was  $\pm$ 2.3%. In the proposed procedure, enhancement factors were 9.51 and 6.25 for Zn and Cu, respectively.

*Accuracy of the Method*

The proposed method was used for the determination of Zn and Cu in several water samples. The results and the recoveries for spiked samples are given in Table 2. The recovery of spiked samples which was satisfactory was confirmed using the addition method, indicating the capability of the system in the determination of Cu and Zn. A good agreement was obtained between the added and measured analyte amounts. The recovery values calculated for the added standards were always higher than 98%, thus confirming the accuracy of the procedure and its independence from the matrix effects. Thus, the results of the analysis of the samples showed that the proposed method can be reliably used for the determination of Cu and Zn in different matrices with good recoveries in the range of 98.4–103.0%.

**Table 2.** Determination of Ag in three kind of environmental water samples and relative recovery of spiked Zn and Cu in environmental water samples

Sample	Found	Added	Found	Recovery(%)
Konjunchem	Zn:4.90 $\pm$ 0.18	5.0	10.02 $\pm$ 0.39	102.4
River Water	Cu:5.49 $\pm$ 0.25	5.0	11.09 $\pm$ 0.42	103.0
Seymareh	Zn:5.42 $\pm$ 0.21	5.0	10.37 $\pm$ 0.41	99.0
River Water	Cu:6.38 $\pm$ 0.23	5.0	11.41 $\pm$ 0.43	100.3
Haftchesmeh	Zn:4.61 $\pm$ 0.16	5.0	9.68 $\pm$ 0.38	101.4
Water wells	Cu:9.73 $\pm$ 0.41	5.0	14.65 $\pm$ 0.58	98.4

*Comparison of analytical performance data with literature*

A comparison of the demonstrated method with the others reported literature is shown in Table 3. As it can be seen from the data in this table, the recommended UASEME method is superior to previously reported methods in all terms which were presented. It can be seen from this table that the extraction time in the USAEME-FAAS procedure is very short. The present

technique also provides good enrichment factor. From Table 3, it was found that USAEME- FAAS procedure is a suitable procedure to simultaneous extraction and preconcentration of several analytes. Also, a lower enrichment factor and higher LOD than other methods may have been the result of the determination system and the fact that this method used a larger sample volume.

**Table 3.** Comparison of the proposed method with other methods for pretreatment and determination of silver

Methods	DL ( $\mu\text{g L}^{-1}$ )	EF*	Sample preparation time / min	References
CPE-FAAS	0.27	55-64	45	[18]
IL-DLLME-FAAS	0.71-0.93	40-62	7	[19]
DLLME- ICP OES	0.23-0.55	---	10	[20]
CPE- ICP OES	1.0-6.3	9-10	65	[21]
USAEME- FAAS	0.0010-0.0014	6-9.5	15	Proposed method

\*Enrichment Factor

**Conclusion**

In this paper, we introduced a USAEME method for the analysis of trace amounts of Zinc and copper in river water and water wells samples. The USAEME procedure has numerous advantages such as: low cost, low toxic, simplicity of operation, rapidity and high selectivity. In addition, it is important to point out that USAEME is a low organic solvent consuming extraction technique, which turns it into a low cost and also an environmentally friendly technique. In this method, the consumption of the toxic organic solvent (at microliter level) was minimized without affecting the sensitivity of the method.

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**Conflict of interest**

No potential conflict of interest relevant to this article was reported.

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