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Feasibility evaluation of trace amount of zinc in urine samples using atomic absorption solidified floating organic drop micro-extraction technique

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Abstract

Background: The present study was carried out with the aim of extracting trace amounts of zinc in urine samples with ultrasound-assisted emulsification solidified floating organic drop micro-extraction (USAE-SFODME) method by flame atomic absorption spectrometry (FAAS). The efficiency was investigated using the solvent extraction volume, extraction pH, time sonication and temperature extraction. The present study was conducted aiming to respond on the efficiency of SFODME technique in extracting inorganic analytes in biological samples**.**

Materials and Methods: This was an experimental research with several steps. After preparation standard solution of zinc, USAE-SFODME technique was used for extracting zinc cation from urine samples. This method involves centrifuge, buffer and ligand adding, sonication, extraction of analyte and finally analysis with FAAS. Excel 2010 software was used in this study in order to plot the graphs.

Results: Extraction of zinc was performed under optimized conditions of 2 ml 1-(2-Pyridylazo)-2-naphthol (PAN), 90 µl 1-dodecanol, pH = 5.5, for 20 minutes at 35 ˚C. Recovery, the regression coefficient, and relative standard deviation (RSD) were obtained as 96.6% and 99.0%, respectively. RSD for tree concentration 0.8 $\mu g ml^{-1}$ Zn cation (Zn^{2+}) was 3.4%. The limit of detection (LOD) was found to be 0.426 $\mu g ml^{-1}$.

Conclusions: Using green solvents, downsizing the samples, replacement of toxic reagents use, and lack of needing the preparation of the samples are the most important advantages of this technique. USAE-SFODME has a successful development in determining trace amounts of zinc in urine samples which can be performed in chemical laboratories with rather ordinary equipment.

Keywords: Zinc, Urine, Biological Monitoring

Introduction

Heavy metals are extensively used in several processes for example in mines and production industries. When heavy metals are accumulating in air, water or soil, the exposure risks of workers and the

individuals living in the polluted areas is increased (1). Zinc is an element existing in the nature and exposure to extensive amounts of zinc has occurred through food, water or inhaling polluted air in the workplace. Low amount of zinc is essential for health, however, exposing to its large amounts can be harmful (2). Zinc is one of the essential metals for appropriate functioning of enzymes in the body, having an important role in the growth and proper metabolism (3). Nevertheless, inadequate absorption of zinc can cause disorders in the cognitive performance system, and increase its absorption, in addition, its accumulation is related to some brain diseases like Alzheimer and Parkinson's disease (4). The most prevalent toxic effect of inhaling zinc fumes was called "metal fume fever", with its symptoms including shivering, fever, muscular pain, nausea, and vomiting (5-7). The results of the study by Aminian et al. on 188 workers in galvanizing industry exposed to zinc pollutants showed that the incidence of respiratory symptoms was significantly higher among the exposed group compared to the control group (8). Discharging zinc is via urine and stool, hence, exposure assessment of zinc on Chinese casting workers showed that increasing exposure to zinc has a significant relation with increasing zinc in urine (9). Researchers indicate that plasma is a weak selection for biological monitoring of zinc, and evaluating the amount of zinc in urine samples is preferred to other methods (10). Atomic absorption spectrometry (AAS) is a common laboratory monitoring instrument for determination of zinc in biological samples, including bone, liver, hair, blood, and urine (11). In the area of biological monitoring, various techniques have been introduced. The new technique considered in extracting and analyzing metal cation includes single drop microextraction (SDME) (12), consisting of continues-flow microextraction, hollow fiber liquid-phase microextraction (HF-LPME) (13), dispersive liquid-liquid microextraction (DLLME) (14), and the solidified floating organic drop microextraction (SFODME) was introduced recently (15, 16). The other method used is SFODME. Due to shortening the extraction time, low amount of organic solvent, appropriate precision, and accuracy, SFODME has rapidly absorbed the attention of researchers in this respect. The extraction solvent used in SFODME method has the lighter weight compared to water and its melting point is near ambient temperature. Not only this solvent has low pollution, but it is also considered as a green solvent. This method is used in tracking organic pollutants and metal ions in the environment (17). SFODME technique has had a successful development in determining the organic and inorganic analytes from environmental samples. However, the researchers of the present study could not find any studies among the literature regarding the inorganic analytes in biological samples. Hence, the present study was conducted with the aim to respond on the efficiency of SFODME technique in extracting inorganic analytes in biological samples. Therefore, zinc cations were selected in order for extraction and determination from urine samples with ultrasound-assisted emulsification solidified floating organic drop micro-extraction (USAE-SFODME) technique. As for the first time, zinc cation was extracted from the urine samples with this technique.

Material and Methods

Reagents: Standard zinc solution with the density of 1000.0 $\mu g ml^{-1}$ was purchased from Merck Co., Germany. Standard working solutions were made on the daily basis from diluting the appropriate rate of standard zinc solution. Chelating material 1-(2-Pyridylazo)-2-naphthol (PAN) made by Merck Co. was purchased, and after solving a suitable amount of it in pure ethanol, the solution with the concentration of 0.0001

$moll^{-1}$ was prepared. Nitric acid and sodium hydroxide were used in this study to adjust pH of the samples. The used extraction solvent in the present study is 1-dodecanol from Merck Co.

Instrumentation: AAS (Analytik Jena AG, AAS vario 6, Germany) was used in this study for the required experiments. Zinc hallow cathode lamp with the analytic wavelength of 213.9 nm was selected according to the manufacturer's instructions. The ultrasound device (Bandelin Co., USA) with adjustable temperature and time range, and laboratory centrifugal equipment (ROTOFIX 32A, HETTICH, Germany) were used respectively for emulsion function and fast separation of water and organic phases.

Hamilton micro-syringe was used manually for injecting the extraction solvent to centrifugal tubes containing the sample mixture and PAN and also injecting diluted extracted concentrate to the AAS. Polypropylene bottles were selected for the sampling and maintaining the samples. PH-meter (3510, JENWAY Co., UK) was also used for measuring pH of the samples.

Procedure: During the required procedures, 24-hour urine samples were taken from a volunteer for the present study to prepare spiked urine samples. After collection, the samples were kept in a refrigerator, which was under filtration process before starting the test. The glassware was kept in nitric acid 10% for 24 hours before being used and washed afterward with the distilled water. First, 10 ml urine sample of a healthy volunteer with the density of 800.0 μgml^{-1} of standard Zn^{2+} was spiked in a 15 ml centrifugal tube. Then it was mixed with 2 ml of PAN solution. To determine the effect of PAN ligand volume on extracting Zn^{2+}, different PAN amounts (0.5-2.5 ml) were evaluated, since pH is one of the important factors in the formation of Zn^{2+} metal complex with PAN ligand. The effect of a pH range of 2-10 in pre-concentration of Zn^{2+} was evaluated in this experiment. Nitric acid and sodium hydroxide were used to adjust pH of the samples. Then, 90 µl of 1-dodecanol was added using Hamilton micro-syringe, and the solution was placed in the ultrasound device in 35 ˚C for 20 minutes for the extraction. In the next step, for more assurance of the extraction, it was placed in a centrifugal device with the speed of 2000 rpm for 5 minutes. The evaluation was performed for the time and temperature factors within the ranges of 5-40 minutes and 15-45 ˚C, respectively. After the centrifugal process, the test tube was rapidly placed in the ice-containing bath for 5 minutes. The extraction solvent collected in the test tube with the chelating solution in emulsion form at the top of the sample solution was frozen in the ice-containing bath. Then, the extracting concentrate was easily taken by a small spatula, placed in another vial and rapidly melted. It was then diluted by injecting 500 µl of pure ethanol, and manually injected to the AAS. Finally the graphs were plotted using Excel 2010 software.

Results

Effect of amount of PAN ligand: According to figure 1, 2 ml was considered as the optimized amount of PAN ligand in extracting Zn^{2+}. The rates lower and higher than 2 ml have lower effects on the extraction efficiency.

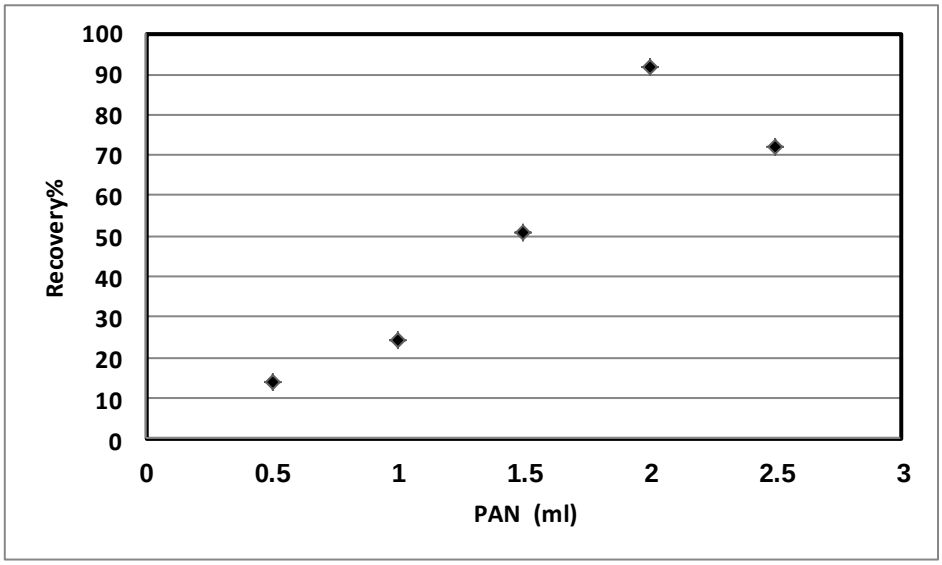

Figure 1: Effect of 1-(2-Pyridylazo)-2-naphthol (PAN) ligand rate on extracting Zn cation (Zn^{2+}) by USAE-SFODME method. Extraction conditions included: 10 ml of urine sample, spiking 0.8 $\mu g ml^{-1}$ of standard Zn^{2+}, 90 µl of 1-dodecanol, pH = 5.5, temperature = 35 ˚C, sonication time = 20 minutes.

Effects of the rate of pH: By increasing pH to amounts higher than 5.5, the competition of Zn^{2+} was reduced as compared to H ions. Thus, the extraction efficiency was also reduced. Hence, pH = 5.5 was selected for analyzing the actual samples (Figure 2).

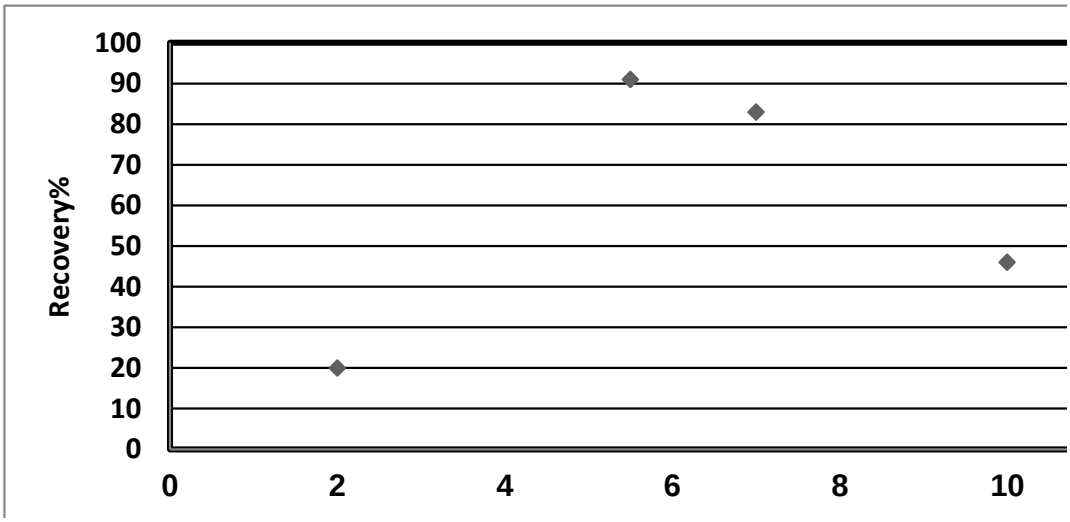

Figure 2: Effect of pH on extracting Zn cation (Zn^{2+}) by USAE-SFODM method. Extraction conditions included: 10 ml of urine sample, spiking 0.8 $\mu g ml^{-1}$ of standard Zn^{2+}, 2 ml of 1-(2-Pyridylazo)-2-naphthol (PAN) ligand, 90 µl 1-dodecanol, temperature = 35˚C, sonication time = 20 minutes.

Effect of sonication time: According to figure 3, the study results in analyzing the sonication time showed that the time of 20 minutes provided the highest efficiency in extracting Zn^{2+}.

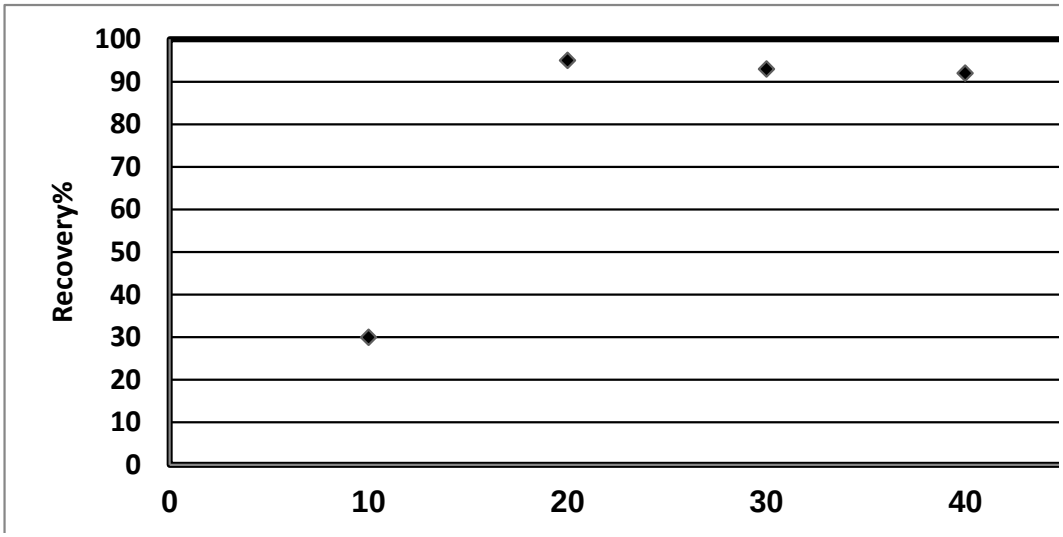

Figure 3: Effect of sonication time on extracting Zn cation (Zn^{2+}) by USAE-SFODM method. Extraction conditions included: 10 ml of the urine sample, spiking 0.8 $\mu g ml^{-1}$ of standard Zn^{2+}, 2 ml of 1-(2-Pyridlazo)-2-naphthol PAN ligand, 90 µl 1-dodecanol, pH = 5.5, temperature = 35 ˚C.

Effect of temperature: The study results in analyzing different temperatures indicated that the temperature value of 35 ˚C had the highest effect on increasing efficiency (Figure 4).

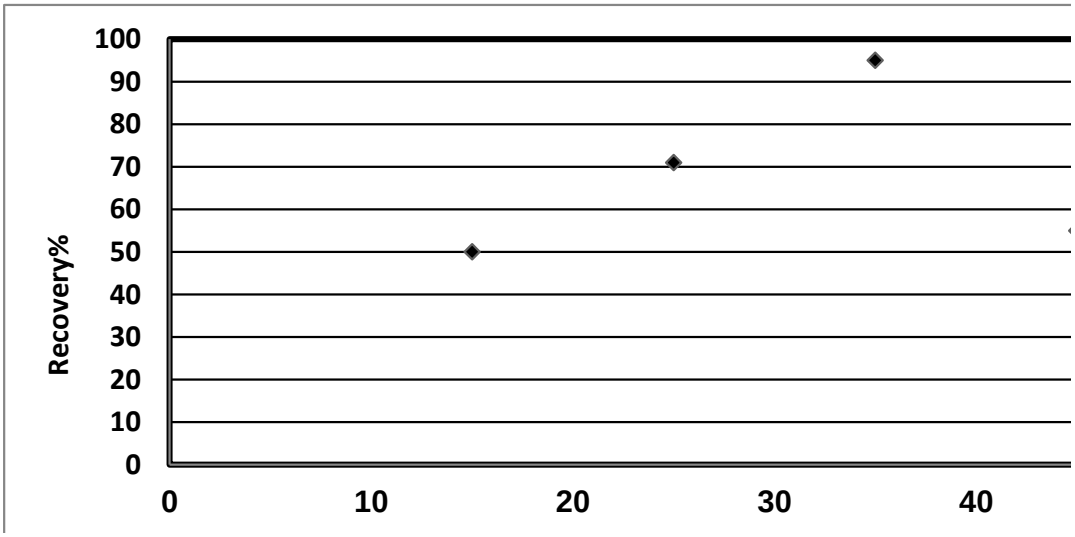

Figure 4: Effect of temperature on extracting Zn cation (Zn^{2+}) by USAE-SFODME method. Extraction conditions included: 10 ml of urine sample, spiking 0.8 $\mu g ml^{-1}$ of standard Zn^{2+}, 2 ml of 1-(2-Pyridylazo)-2-naphthol (PAN) ligand, 90 µl 1-dodecanol, pH = 5.5, sonication time = 25 minutes.

Effect of volume of extraction solvent: According to figure 5, to achieve the optimized rates, the extraction solvent within the range (30-60-90-120µl) was analyzed. 90 µl 1-dodecanol showed the highest effect on extraction efficiency. Therefore, this volume rate was selected for the following evaluations.

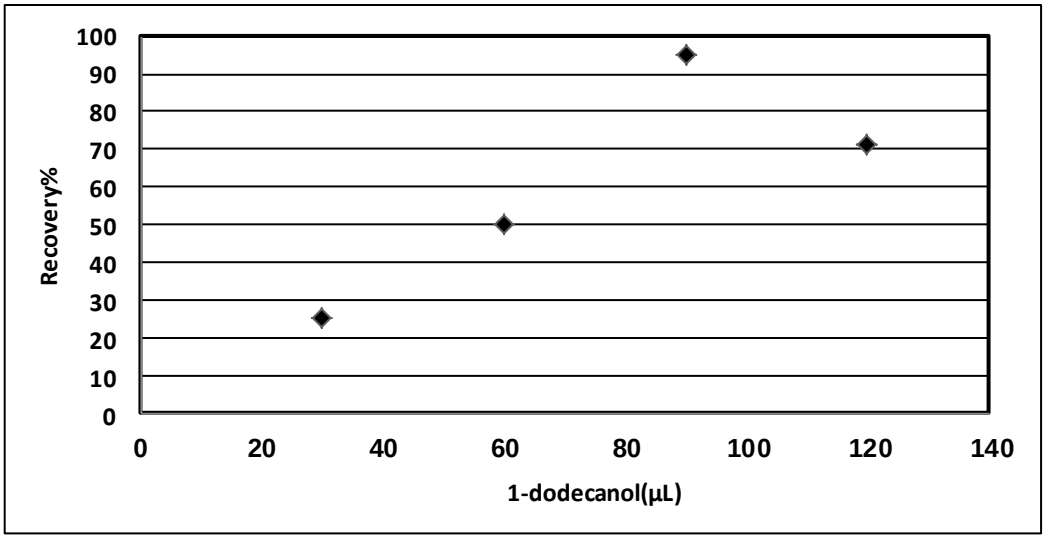

Figure 5: Effect of the amount of 1-dodecanol on extracting Zn cation (Zn^{2+}) by the USAE-SFODME method. Extraction conditions included: 10 ml of urine sample, spiking 0.8 $\mu g ml^{-1}$ of standard Zn^{2+}, 2 ml of 1-(2-Pyridylazo)-2-naphthol (PAN) ligand, pH = 5.5, sonication time = 25 minutes.

RSD and Recovery: Samples of deionized water (Merc Co., Germany) and urine samples were used to determine the accuracy of USAE-SFODME, as three standard concentrations of 0.3, 0.6 and 0.8 $\mu g ml^{-1}$ of Zn^{2+} were prepared by spiking the deionized water. Then, urine samples containing Zn^{2+} and spiked samples obtained in optimum conditions were examined. Finally, the efficiency and RSD were determined, as shown in table 1. The results indicate that the proposed method of USAE-SFODME has the suitable required accuracy.

Table 1: Extracting Zn cation (Zn^{2+}) from deionized water and urine (the standard deviations have been obtained from 3 repetitions with confidence interval of 95%)

Sample	Added ($\mu g l^{-1}$)	Obtained ($\mu g l^{-1}$) Mean ± SD*	Recovery (%)	RSD** (%)
Deionized water	-	ND***		
	0.8	0.786±0.005	98.3	1.7
	0.6	0.580 ±0.010	97.6	2.4
	0.3	0.276 ±0.010	95.0	5.0
Urine sample	_	ND***		
	0.8	0.773 ± 0.020	96.6	3.4
	0.6	0.570 ± 0.015	95.5	4.5
	0.3	0.276 ± 0.016	92.2	7.8

*** SD:** Standard deviation
** **RSD:** Relative standard deviation
*** **ND:** Not Detected

Table 2: LOD, LOQ and calibration curve parameters

Recovery (%)	96.6
Correlation coefficient	0.99
LOD*	0.426 µgml^{-1}
LOQ**	0.897 µgml^{-1}
Slope	0.2961

*** LOD:** Limit of detection
**** LOQ:** Limit of quantification

Calibration curve and limit of detection (LOD): The calibration curve was obtained with 6 concentrations of zinc solution as 0.00, 0.05, 0.10, 0.50, 1.00, and 2.50 µgml^{-1}. The calibration equation was obtained as y = 0.2961x + 0.0269. y and x are the absorption rate and concentration of zinc, respectively. The calibration equation indicated a suitable linearity with the correlation coefficient of r = 0.99 for Zn^{2+} (Figure 6). The LOD and limit of quantification (LOQ) were respectively 0.426 and 0.897 µgml^{-1} (Table 2).

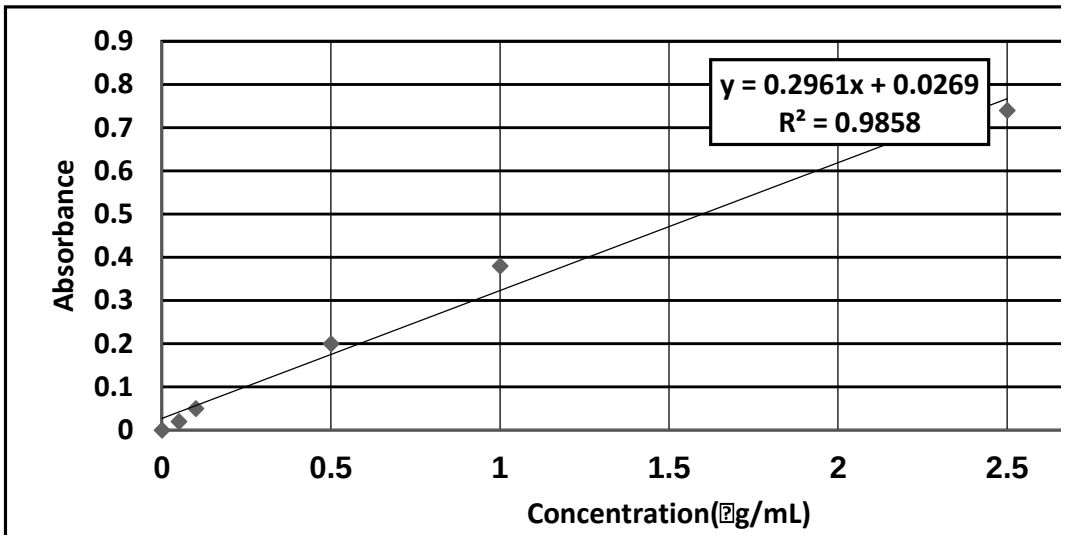

Figure 6: Calibration curve and linear equation for zinc standards with concentrations 0.05, 0.10, 0.50, 1.00 and 2.50 µgml^{-1}. The correlation coefficient of r = 0.99 indicates appropriate conditions for the standard rates regarding zinc analyte

Discussion

USAE-SFODME technique has already been used for environmental samples and Zn^{2+} extraction is analyzed by this technique for different water samples, hence, the successful efficiency of the proposed method was verified for the water samples (18). The matrix for urine samples is a little more complex compared to water samples, however, the complexity does not provide difficulties for the USAE-SFODME method. The proposed solution to eliminate the difference was performing filtration before starting the test (19). PAN was used as a chelating agent for separation and pre-concentration of the metal cation (20). The optimum volume of PAN as the ligand was 2 ml in this study. This can be due to the fact that the ligand may be extracted in the volumes higher than 2 ml, and therefore the capability of extraction phase and also extraction efficiency are reduced. Selecting appropriate extraction solvent is quite important for optimizing the extraction conditions. 1-dodecanol has low solubility in water and low volatility and melting point near the ambient temperature (21), hence it is used as the extraction solvent in this study. Increasing the volume of the

extraction solvent increases the absorption rate of the analyte. After reaching the maximum absorption, increasing the volume of the extraction solvent will have no effect on the rate of absorption, and it will remain fixed. The appropriate volume of the solvent was 90 µl in the present study. A reduction was observed in the extraction of zinc cation by increasing the amount of solvent higher than 90 µl. Using ultrasound waves and centrifugal action provides the possibility of formation of smaller drops of the organic solvent in the water phase in a shorter time. Shaking the ultrasound causes the formation of organic micro-drops and increasing the contact surface between the extraction solvent and the analyte. In the present study, increasing the ultrasonic time to 20 minutes increased the efficiency, hence this period was chosen as the optimal ultrasonic time. Temperature is an effective factor on the solubility of organic solvents in water and it affects the trend of mass transfer and extraction efficiency. The results of the study showed that sonication for 20 minutes in 35 °C has the highest effects in absorbing zinc analyte. The rate of zinc absorption was reduced by increasing the temperature and sonication time which can cause reduction in the extraction efficiency. The value of 5.5 was the most suitable pH in the present study, and a reduction in the extraction efficiency was observed by increasing or decreasing the mentioned rate. Most of the studies for SFODME technique refer to organic analytes, and few studies have been carried out on inorganic substances. Water has been studied in most of the studies considering the proposed method (22) and these is the lack of studies regarding the analysis of metal cation in urine samples with SFODME technique.

The present study was conducted on the analysis of Zn^{2+} in urine biological matrix. The finding of this study was compared to the findings of the study by Jingjun Ma et al. on the analysis of Zn^{2+} in water samples. In this study, the optimum conditions of Zn^{2+} extraction in water samples were PAN volume = 2 ml, volume of extraction solvent (1-dodecanol) = 90 µl, sonication time = 5 minutes, sonication temperature = 40 °C, and pH = 5. In the study by Q Chang et al. on determination of the trace amounts of copper in water samples using the SFODME method, the RSD for ten replicate measurements of 20 and 400 $\mu g l^{-1}$ of copper was 3.83% and 2.65%, respectively (15). Conformity and close results were observed in most of the effective parameters on zinc extraction from water samples with the present study.

Conclusion

In the present study, the SFODME method was used due to its simplicity and inexpensive procedure with capability of minimizing the exposure to toxic solvents. In addition, this novel method has not been widely used to determine toxic heavy metals in human biological samples. There was no specific limitation on the use of this method.

The present study proved the considerable success for the efficiency of USAE-SFODME technique in the extraction of Zn^{2+} in biological urine samples. Regarding suitable advantages like smaller sample size, shortening the analysis time, lack of requiring toxic solvents, using green solvents, cheaper price and appropriate accuracy, the proposed technique can be effective in extraction and determination of the trace amount of Zn^{2+} in urine samples.

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Conflict of interest: None declared.

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