Postprint of: Elahi F., Arain M. B., Ali Khan W., Haq H., Khan A., Jan F., Castro-Muñoz R., Boczkaj G., Ultrasound-assisted deep eutectic solvent-based liquid–liquid microextraction for simultaneous determination of Ni (II) and Zn (II) in food samples, FOOD CHEMISTRY Vol. 393, (2022), 133384, DOI: 10.1016/j.foodchem.2022.133384


# Ultrasound-assisted deep eutectic solvent-based liquid-liquid microextraction for simultaneous determination of Ni (II) and Zn (II) in food samples

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**Abstract**

A new approach was developed for the simultaneous pre-concentration and determination of Ni (II) and Zn (II) in food samples. This method is based on ultrasound-assisted liquid-liquid micro extraction using hydrophobic deep eutectic solvent (DES) and 1,10-phenanthroline as chelating agent. The effect of several parameters, such as pH, selection and volume of DES, amount of chelating agent, time of sonication and centrifugation, was studied. Under optimized conditions, the developed procedure offered exceptional sensitivity and linearity. The limit of detection was approximately 0.029 µg/Kg and 1.5 µg/Kg for Ni (II) and Zn (II), respectively. The proposed method was applied for the pre-concentration and determination of Ni (II) and Zn (II) in hydrogenated edible oils, fishes, and milk samples. The results of this study were compared with reported methods in the literature revealing its advantages.

**Keywords:** Metals extraction; Food Samples; sample preparation; Deep Eutectic Solvent; FAAS; mineralization

## 1. Introduction

Heavy metals are well-known chemical pollutants present in various types of food. These metals usually exist at very low concentration levels making their continuous monitoring more challenging (Khan, Arain, & Soylak, 2020). For instance, Nickel (Ni) is toxic for the human body and can cause allergy, heart and kidney problems, lung and nasal cancer (Genchi, Carocci, Lauria, Sinicropi, & Catalano, 2020). However, recent researches show that Ni (II) may have some beneficial effects (Shraim, Ahmad, Rahman, & Ng, 2022). Zinc is another essential element present in food. Its deficiency causes slow wound healing, vision problems, decrease growth rate, diarrhea, and Wilson disease. Moreover, zinc plays a key role in homeostasis, apoptosis, the immune system, and bone formation (Chasapis, Ntoupa, Spiliopoulou, & Stefanidou, 2020). On the contrary, the excess of Zn may also cause a serious threat to human life and a high risk of prostate cancer, brain lethargy, and gastrointestinal problems, such as vomiting, nausea, diarrhea (Plum, Rink, & Haase, 2010).

To determine Zn (II) and Ni (II) in foods samples at a very low concentration, various pre-concentration techniques have been developed for the simultaneous pre-concentration and determination of nickel and zinc, such as co-precipitation (Komjarova & Blust, 2006), solid-phase extraction (Roldan et al., 2003), liquid-liquid extraction (Mansur, Rocha, Magalhães, & dos Santos Benedetto, 2008), and sequential extraction procedure (Alomary & Belhadj, 2007). Unfortunately, these methods present drawbacks in terms of inefficiency, prolonged time, use of toxic chemicals, high costs, and provide insufficient sample cleanup (Khan, Arain, Yamini, et al., 2020; Khan, Yamini, Baharfar, & Arain, 2019).

Deep eutectic solvents (DES) are a new class of green solvents introduced by Smith et al.(Smith, Abbott, & Ryder, 2014). DESs seem to be the best alternative to conventional solvents. Their usefulness was proved for several applications in separation techniques (Haq et al., 2021b; Momotko, Łuczak, Przyjazny, & Boczkaj, 2021). Generally, the preparation of DES is easy, cheap, ecologically safe, less toxic and biologically acceptable (Harifi-Mood, Mohammadpour, & Boczkaj, 2020; Kumar et al., 2020; Makoś & Boczkaj, 2019; Makoś, Fernandes, Przyjazny, & Boczkaj, 2018). Due to the aforementioned advantages, DES-based pre-concentration methods are getting great attention and a vast number of articles have been published in recent years (Altunay & Tuzen, 2021; Elik, Demirbaş, & Altunay, 2022; Haq et al., 2022). This latter method is based on hydrophobic deep eutectic solvents (hDES) which were, for the first time, used for liquid-liquid micro-extraction in 2015 (van Osch, Zubeir, van den Bruinhorst, Rocha, & Kroon, 2015). In 2018, hDES were initially implemented for metal extraction from an aqueous medium (Schaeffer, Martins, Neves, Pinho, & Coutinho, 2018). hDESs are very effective for metal pre-concentration, displaying outstanding distribution coefficients specially for divalent metal ions (Van Osch, Dietz, Warrag, & Kroon, 2020). However, this new potential aspect of hDESs still needs more research to expand their applicability, especially in the field of analytical chemistry.

The present work timely proposes a new hydrophobic deep eutectic solvent liquid-liquid micro-extraction procedure for the pre-concentration of Ni (II) and Zn (II), followed by analysis using Flame atomic absorption spectroscopy (FAAS). This method was successfully applied for the analysis of Ni (II) and Zn (II) in hydrogenated edible oils, milk, and fishes.

## 2. Material and Methods

### *2.1 Instrumentation*

A centrifuge (Model 2206A, China) was used for phase separation. A pH electrode (Professional Meter PP-15 with a glass-electrode, Germany) was used for pH adjustment. Power-Sonic 405 ultrasonic bath (Hwashin Technology, Seoul, Korea) with microwave power 1000 W and frequency 750 GHz was used for sonication. The quantitative analysis was carried out with a flame atomic absorption spectrometer Perkin Elmer AAnalyst 700 Model (Norwalk, CT, USA).

### *2.2 Reagents and Solutions*

Analytical grade choline chloride, ethylene glycol, phenol, malonic acid, tetrabutylammonium chloride, decanoic acid, and tetraoctylammonium chloride were used for the synthesis of DESs without prior purification or solution formation. Analytical grade zinc chloride and nickel chloride were purchased from Sigma Aldrich (St. Louis, MO, USA) and used for the preparation of the standard metal solution. A 1, 10-phenanthroline stock solution (0.1 M) was prepared in deionized pure water. Acetic acid, ammonia, sodium acetate, ammonium acetate, ammonium chloride phosphoric acid, tetra butyl ammonium chloride, and disodium hydrogen phosphate were purchased from Sigma Aldrich (St. Louis, MO, USA) and used for buffers preparation. Analytical grade pure methanol was purchased from Sigma Aldrich (St. Louis, MO, USA) was used as received for dilution. Nitric acid, hydrochloric acid, potassium permanganate, sulphuric, and perchloric acid were also purchased from Sigma Aldrich (St. Louis, MO, USA) and used for the digestion of food samples. Ultrapure water was used as the working medium.

*2.3 Synthesis of DESs*

Five different types of DESs were evaluated for the pre-concentration of the target analytes from the food matrix. DESs with different functionalities were selected for tailoring their properties for the pre-concentration process. Hydrophobic DESs were tested to achieve higher recovery of the targeted analyte. DESs can be prepared in different molar ratios, however, in this study, DESs were prepared with a molar ratio corresponding to the eutectic point providing their lower viscosity and higher mass transfer at these conditions. DES1 was prepared from choline chloride and ethylene glycol with a molar ratio of 1:1. DES2 was prepared from choline chloride and phenol with a molar ratio of 1:2, while DES3 was prepared from choline chloride and malonic acid with a molar ratio of 1:3. DES4 was prepared from tetrabutylammonium chloride and decanoic acid with a molar ratio of 1:2. Finally, DES5 was prepared from tetraoctylammonium chloride and decanoic acid with a molar ratio of 1:2.

*2.4 Sampling and digestion*

In spectroscopic analysis of metals, acid digestion is one of the most significant steps of the entire analytical procedure. It has a substantial effect on the recovery of various analytes in highly complex matrices. Digestion is also helpful to achieve the optimal sample preparation method with clearer background (low noise level) (Bader, 2011; Uddin et al., 2016). Hydrogenated edible oil, milk, and fish samples were studied for pre-concentration and analysis of Ni (II) and Zn (II). All the samples were digested according to the recommended methods in the literature (Alomary & Belhadj, 2007; Begum, Bari, Jamaludin, & Hussin, 2012; Haq et al., 2021b). Triplicate samples were used for the analysis of target analytes. Hydrogenated edible oils (Shama vanaspati ghee) were purchased from the local market of District Mardan, Pakistan. Experimentally, composite samples (5 g) of hydrogenated edible oil were heated at 800 °C for 10 min in a furnace to decompose the organic matter. The obtained residues were dissolved in 5 mL hydrochloric acid (2M). Then, the solutions were filtered, and the resulting filtrate was diluted with deionized water up to 25 mL (Purohit & Devi, 1995). Milk samples were oven-dried at 70 °C. Each composite sample (2 g) of dried milk was digested. The digestion was performed by adding 3.5 mL of nitric acid (70%) and 1.0 mL of $H_2O_2$ (30% v/v) to the sample and heating it at 90 °C and 750-watt microwave power for 10 min. The conditions were gradually varied to 180 °C temperature and 1000-watt microwave power for 10 min. After cooling, the digested samples were transferred into 10 mL volumetric flasks and diluted to volume with nitric acid (1% v/v) (Abdulkhaliq, Swaileh, Hussein, & Matani, 2012). The fish sample was digested according to the procedure previously described and considered effective enough for this purpose (Fashi, Yaftian, & Zamani, 2017). Composite samples with edible parts (meat) of different fishes were prepared. Briefly, Siluriformes fish (catfish) samples (5 g) were transferred to a digestion flask containing 1 mL of deionized water, 1 mL of nitric acid, 1 mL of chloric acid, and 5 mL of sulfuric acid with 2 drops of potassium permanganate (1% w/v). The resulting mixture was heated at 150 °C until a clear solution was obtained – preliminary series of experiments revealed that 25 minutes endure effective digestion. The solution was kept for a while to cool and then diluted with ultrapure water to 50 mL.

*2.5 Optimization of the microextraction procedure*

Ni (II) and Zn (II) standard solutions were added to 10 mL sample in a falcon tube with a final concentration of 19.4 µg/Kg for each metal. Buffer solutions (pH 6) (2 mL) and 1, 10-phenanthroline (1 % w/v) (0.6 mL) were added to the matrix. The tube was tightly closed and shaken properly by hand. Subsequently DES (8 mL) was added, followed by sonication for 3 min at 25 °C. Finally, the mixture was centrifuged for 2 min with 4000 rpm to separate the aqueous and rich DES phase. The lower layer was discarded by a micropipette while the DES phase was collected. The collected DES phase was makeup with $CH_3OH$ up to 3 mL mark and analyzed with FAAS.

*2.6 Calculation of percent recovery and validation assays*

Percent recovery (% R) was evaluated as a reference to determine the appropriate values of pre-concentration parameters in the optimization studies. % R was calculated according to the formula below.

$$\% R = \frac{\mathrm{Cd}}{\mathrm{Ce}} \times 100 \qquad (1)$$

where Cd is the concentration determined in the spiked real sample while Ce is the expected concentration in the spiked real sample.

The % R was calculated as $C_d/C_e \times 100$, where $C_d$ is the determined concentration and $C_e$ is the expected concentration.

LOD and LOQ were calculated using the following formulas.

$$LOD = \frac{3 \times \mathrm{SD}}{m} \qquad (2)$$

$$LOQ = \frac{10 \times \mathrm{SD}}{\mathrm{m}} \qquad (3)$$

where LOD is limit of detection, SD is the residual standard deviation of regression lines, m is the slope of the calibration curve, and LOQ is the limit of quantification (Chan, Lee, Lam, & Zhang, 2004).

The pre-concentration factor (PF) was calculated as the concentration ratio of the analyte in the final extract (DES phase) ready for its determination and in the initial solution according to previously described method (Kazi et al., 2012). The pre-concentration factor was evaluated by using the following equation (Asgharinezhad et al., 2015; Asl, Yamini, Rezazadeh, & Seidi, 2015).

$$PF = \frac{Cf}{Ci} \quad (4)$$

where *Cf* and *Ci* are the final and initial concentration of analytes in the DES phase (receiving phase), and donor phase, respectively. The analyte concentration was determined in sample solution before extraction (Ci) and after extraction in the DES phase (Cf).

Relative standard deviation was calculated using the following formula

$$RSD\ (\%) = \frac{\mathrm{SD}}{\mathrm{Ca}} \times 100 \quad (5)$$

where SD is the standard deviation and Ca is the average concentration of analyte.

2.7 *Determination by FAAS*

The DES extract was diluted with methanol up to 5 mL. The resulting samples were finally analyzed using FAAS. For this, standard solutions for Ni (39.88-997.0 µg/Kg) and Zn (9.97-797.6 µg/Kg) were prepared. High-capacity auto samplers along with WinLab32™ software were used. Air-acetylene flame was used for excitation. Hollow cathode lamp with single wavelength (Ni 232.0 nm, Zn 213.9 nm) was used as a light source. Gas flow rate was 2 L $min^{-1}$ for air and 2 L $min^{-1}$ for acetylene for each metal. Absorbance was determined for standard solutions of Ni (II) and Zn (II) ions and plotted as a calibration curve.

## 3. Results and Discussion

### 3.1 Selection of parameters for optimal sample preparation

*3.1.1 Effect of pH*

pH represents a key parameter in the transfer of the target analyte from the aqueous phase into the DES phase (Haq et al., 2021a). pH significantly affects the formation of the metal-ligand complex. As for 1,10 phenanthroline, it favors the formation of metal complexes with divalent metal ions in a slightly acidic medium (Lee, Kolthoff, & Leussing, 1948). Herein, the effect of pH on the transfer of analytes from the aqueous phase to the DES phase was studied. In this experiment, the % R of analyte was determined at different pH values in the range of 2-10. The pH was adjusted by using a buffer of respective pH. A citrate buffer was used for making a buffer with pH 2, while a phosphate buffer was used for pH 4. Acetate buffer was used for pH 6. Buffer with pH 8-10 was prepared from $NH_4OH$ and $NH_4Cl$. A comparison of obtained results is presented in Figure 1. The maximum % R was observed at pH 6 and thus selected as optimum pH.

*3.1.2 Solvent selection and optimization*

The selection of a suitable solvent represents important aspect for developing an analytical method. DESs, as a new class of green solvents, were used for pre-concentration due to their least toxicity, easy formation, high selectivity, low cost, biodegradable nature, and easy availability (Makoś & Boczkaj, 2019; Makoś, Fernandes, et al., 2018; Makoś, Przyjazny, & Boczkaj, 2018). In this research, various types of hydrophobic deep eutectic solvents (hDES) were tested for the recovery of the target analytes. Differently from many other approaches, this method is based on the extraction of analytes after the digestion stage. Preliminary experiments for this study revealed that selected DESs are much more effective to extract metals from mineralized samples compared with direct extraction of metal-containing moieties from primary samples. This becomes an important feature of DESs as some of them are able to extract, or simply dissolve, metals as well as metal oxides. This property is a big advantage comparing to classic organic solvents (Richter & Ruck, 2019; Söldner, Zach, & König, 2019). It is worth mentioning that selected approach provides a one very important feature; for example, the mineralization step ensures the elimination of most matrix effects that could affect extraction reproducibility. In this case, the primary sample matrix is reduced to simple inorganic species. The extraction by DES allows obtaining additional selectivity while eliminating the matrix effects. This should allow using one universal calibration for each analyte – independently from the type of sample. All the DESs were tested at different pH (2-8). The results for different DESs are illustrated in Figure 2. It can be seen that the maximum recovery was obtained with choline chloride–phenol (1:2) and tetrabutylammonium chloride-decanoic acid (1:2). As tetrabutylammonium chloride-decanoic acid is comparatively more environmentally friendly, it was selected as extracting solvent for this method. The optimum volume of DES was also determined by changing its volume from 0.4 to 1.2 mL. The maximum % R was obtained using 0.8 mL of DES. Therefore, this latter quantity of DES extractant was selected as the optimum volume for this experiment. Figure S1 provides the results for the optimization of DES volume.

1,10-phenanthroline is a common chelating agent and is readily used for complex formation with Ni (II) and Zn (II) (Kruse & Brandt, 1952; Norman & Xie, 2004). Hence, the 1,10-phenanthroline was used as a chelating agent for the effective pre-concentration of metal cations through DES. To determine the optimum concentration of chelating agent, 1,10-phenanthroline concentration was varied from 20.2 to 100.2 mg/L for 15 mL sample (concentration of analyte

19.4 µg/Kg). Figure S2 shows the results for the optimization of chelating agent concentration, revealing that 60 mg/L was found as optimum concentration of 1,10-phenanthroline.

Since ultra-sonication plays a key role in the mass transfer of the target analyte from the aqueous to DES phase, the influence of sonication time was also evaluated by varying the sonication time from 0.5 to 4 min. Importantly, a maximum recovery for both analytes was achieved at 2 min sonication , thus it was selected as the optimum time of sonication. Similarly, the effect of centrifugation time on the pre-concentration recovery of target analytes was performed by changing the parameter in the range of 0.5-4 min. For 2 min centrifugation, maximum recovery was obtained for both analytes, being selected as the optimum time for centrifugation. Figure 3 shows results for sonication and centrifugation time.

## 3.2 Method validation and application for analysis of real samples

### *3.2.1 Analytical performance of the developed method*

The analytical characteristics were evaluated under optimum conditions. The calibration curves were linear in the range of 39.88-997 µg/Kg and 9.97-797.6 µg/Kg for Ni (II) and Zn (II), respectively. Calibration curves for both analytes were determined in two concentration ranges, as follows: Range 1 for lower concentrations ranging from 39.88 to 99.4 µg/Kg and 9.97-79.76 µg/Kg with a coefficient of determination ($R^2$) of 0.9823 and 0.9865 for Ni (II) and Zn (II), respectively. Range 2 for higher concentrations ranging from 99.4 to 997 µg/Kg and 79.76-797.6 µg/Kg with a coefficient of determination ($R^2$) of ca. 0.9942 and 0.9934 for Ni (II) and Zn (II), respectively. Data presented in figures S3 and S4 indicate satisfactory linearity of the method.

Limit of detection (LOD) and limit of quantification (LOQ) were determined according to the standard protocol by the Europe Union reference laboratory for calculation of LOD and LOQ in feed and food (Wenzl et al., 2016). The LOD values for Ni (II) and Zn (II) were calculated as

0.029 μg/Kg and 1.542 μg/Kg, respectively, while LOQ was estimated as 0.097 μg/Kg and 1.17 μg/Kg for Ni (II) and Zn (II), respectively, in real samples applying pre-concentration factor. The relative standard deviation was 3.09 % for Ni (II) and 5.1 % for Zn (II).

The pre-concentration improves the efficiency and selectivity of an analytical method (Lum, Tsoi, & Leung, 2014). This becomes relevant since food matrices are usually complex causing a wide variety of potential interferences. In general, pre-concentration has two major implications: (1) the analyte is enriched and (2) matrix effects are minimized or even suppressed (Alampanos & Samanidou, 2021; Simpson Jr, Quirino, & Terabe, 2008). The pre-concentration (also known as enrichment) is a procedure in which the target species are quantitatively moved from large sample into a small volume of solvent. Occasionally, it is done using first pre-concentration on a solid sorbent, followed by desorption of analytes. As a result, the analyte concentration is raised to detectable or determinable levels. The pre-concentration factor was calculated as ratio of the analyte in the final extract (i.e., DES phase) and in the initial solution. The pre-concentration factor was determined as 20 for both analytes.

*3.2.2 Interference study*

As it is well known food is a complex matrix that contains a large number of cations, anions as well as biomolecules. To evaluate the interference effect and selectivity of the DES medium for Ni (II) and Zn (II), different concentrations of cations and anions were added to the sample solution containing a known concentration of Ni (II) and Zn (II) and performed the pre-concentration procedure. Eight different cations and anions were added at higher concentrations. According to the results compiled in Table S1, the developed method is highly selective for the pre-concentration and determination of Ni (II) and Zn (II). % R was found between 92.49- 99.02 % and 95.43-98.93% for Ni (II) and Zn (II), respectively. Based on high % R in the presence of interfering ions, it was concluded that this method is highly selective for the pre-concentration Ni (II) and Zn (II).

*3.2.3 Applicability of the method*

The developed method was applied to real digested fish and hydrogenated edible oil samples for the determination of Ni (II) and Zn (II). To evaluate the validity of the obtained results, spike tests were carried out on the samples. Representative samples were prepared for each sample matrix from different sources, which were analyzed in triplicate. First samples were analyzed without a spike using newly developed method. In hydrogenated oil samples, the Ni (II) concentration was below the LOQ. The samples were spiked with Ni (II) standard solutions to obtain expected concentration of 49.85 µg/Kg and 99.70 µg/Kg. Interestingly, the % R was found to be as high as 103.1-103.5 % with RSD 3.4-3.5 % for n=3. The same approach was done for zinc, however, in the case of Zn (II), the concentration found in non-spiked sample was 25 µg/Kg. The % R for Zn (II) was between 97.9-100% with RSD 2.8-3.8% for n=3.

In fish samples, the Ni (II) concentration was below the LOD, thus same level of concentrations for spiked samples was implemented. In this case, % R was found to be as high as 101.7-102.9% with RSD 2.6% for n=3. As for Zn (II), the concentration for non-spiked samples was found 21 µg/Kg and % R for spiked samples was determined to be 95.27-96.2 % with RSD 4.2-4.8 % for n=3.

For composite milk samples, the Ni (II) concentration was 15 µg/Kg. % R was found to be between 102.6-103.3 % with RSD 1.6-1.8 % for n=3. In same samples, the Zn (II) concentration was determined as 35 µg/Kg while % R was between 101.4-103.9 % with RSD 2.4-2.7 % for n=3.

Ni (II) and Zn (II) concentration in edible oil depend on their source, soil texture, plant breed, along with the refining and processing stages (Bevis & Hestrin, 2020; Sadeghzadeh, 2013). Compiled data are presented in Table 2. This part of the study confirmed that developed method provides an exceptional recovery of Ni (II) and Zn (II) among the different studied matrices. Wide compatibility with hydrophilic and hydrophobic matrix makes this approach a promising tool for precise determination of zinc and nickel for food control purposes.

*3.2.4 Comparison with existing methods*

The results of the developed method were compared with other reported protocols (see Table 1). More interestingly, several methods for the pre-concentration of Nickel and Zinc in food samples were compared with our new assay. These reported methods include micro-emulsification as sample preparation with FAAS analysis (Nunes et al., 2011), ultrasound-assisted liquid–liquid extraction with high-resolution continuum source atomic absorption spectrometry (Trindade, Dantas, Lima, Ferreira, & Teixeira, 2015), solid-phase extraction with inductively coupled plasma optical emission spectroscopy (ICP-OES) (Feist & Mikula, 2014), cloud point extraction with FAAS analysis (Galbeiro, Garcia, & Gaubeur, 2014), deep eutectic solvent-based liquid-liquid microextraction (Haq et al., 2021b), and magnetic solid-phase extraction (Sodan, Höl, Çaylak, & Elçi, 2020). These methods are relatively associated with one or more issues, such as toxicity of chemicals, use of excessive amounts of solvents, time-consuming, low sensitivity, costly and multi-step pre-concentration procedures.

This newly developed method is simple and highly selective. As mentioned earlier, pre-concentration takes place after the digestion stage, thus it is possible to highlight the advantages of the developed DES-based method at this step only. For instance, no heat is required at the pre-concentration stage, but the most worthwhile advantage of this method is the successful usage of green solvents instead of classic organic solvents (Fabjanowicz, Kalinowska, Namieśnik, & Płotka-Wasylka, 2018). Few examples of typical organic solvents are 1-hexyl-3-methylimidazolium hexafluorophosphate [HMIM][$PF_6$, acetone, N,N′-bis(2-salicylaldiminato)-1,8-diamino-3,6-dioxaoctane (Rajabi, Asemipour, Barfi, Jamali, & Behzad, 2014), cyclohexylamine (Sorouraddin, Farajzadeh, & Okhravi, 2017), naphthalene modified with organic-solution-processable functionalized nano graphene (Moghimi, 2014), neodecanoic acid, Versatic 10 (Ichlas & Purwadaria, 2017), acetone and 1-undecanol, diethyldithiocarbamate (Amirkavei, Dadfarnia, & Shabani, 2013), and 1-(2-Pyridylazo)-2-naphthol (PAN) (Bidabadi, Dadfarnia, & Shabani, 2009), etc. The impressive features of DESs are related to their high availability of components and easy preparation, biodegradability, minimal toxicity, low volatility and costs (Arain, Yilmaz, & Soylak, 2016; Galbeiro et al., 2014; Haq et al., 2021a; Kohli & Mittal, 2018; Makoś, Przyjazny, et al., 2018). The ICP-OES based methods display comparatively better LOD; however, such methods need more complicated instrumentation. A comparison of Ni and Zn concentration levels found in the tested samples of the present work have been compared with concentration levels in similar food matrices determined by other analytical methods, as reported in Table S2.

This method is an important “step forward” with respect to already published approaches. Compared to a previous study in which DES was also used as an extractant for Zn (II) determination (Haq et al., 2021b), in this case, phenol, as a hydrogen bond donor, was replaced by decanoic acid improving the greenness of DES. It follows from the much less toxic character of decanoic acid compared to phenol. Furthermore, this method is based on simultaneous pre-concentration of two analytes. This newly developed approach also provides a wide range of applicability, high sensitivity and linearity range.

## 4. Conclusions

This work is a green solvent-based micro-extraction method for the simultaneous pre-concentration of Ni (II) and Zn (II) for FAAS analysis. A green hydrophobic deep eutectic solvent (based on tetrabutylammonium chloride and decanoic acid 1:2) was used for the pre-concentration of the target analytes. Particularly, ultrasonication was used to form nanodroplets of extractant and thus obtaining high pre-concentration efficiency. The developed method exhibits specific advantages in terms of broad linear range, simultaneous and short pre-concentration time, cost-effectiveness, low LOD, and easiness of operation. The method was found to be compatible with different matrices under the same analytical parameters. It is obtained by both – simplification of matrix by mineralization and selectivity of extraction based on selected DES. Thanks to its high selectivity, this method showed no interference from the commonly existing cations and anions in the matrix. When compared with already reported methods, it reveals appropriate results with many advantages over the conventional methods. It was applied to different food samples including hydrogenated edible oil, milk, and fish samples, demonstrating comparable results with highly sensitive methods based on ICP-MS.

## Acknowledgements

Prof. Grzegorz Boczkaj and Hameed Ul Haq gratefully acknowledge the financial support from the National Science Centre, Warsaw, Poland – decision no. UMO-2018/30/E/ST8/00642.

Prof. Muhammad Balal Arain gratefully acknowledge the financial support from the Higher Education Commission of Pakistan (NRPU No.20-3925/R&D/NRPU/HEC/2014), PAK-US Science and Technology Cooperation (Pak-US No 6-4/PAK-US/HEC/2015/04), and Pakistan Science Foundation joint research projects with MSRT, Iran No. PSF-MSRT/ENV/KP-AWKUM. R. Castro-Muñoz acknowledges the financial support from Polish National Agency for Academic Exchange (NAWA) under Ulam Programme (Agreement No. PPN/ULM/2020/1/00005/U/00001).

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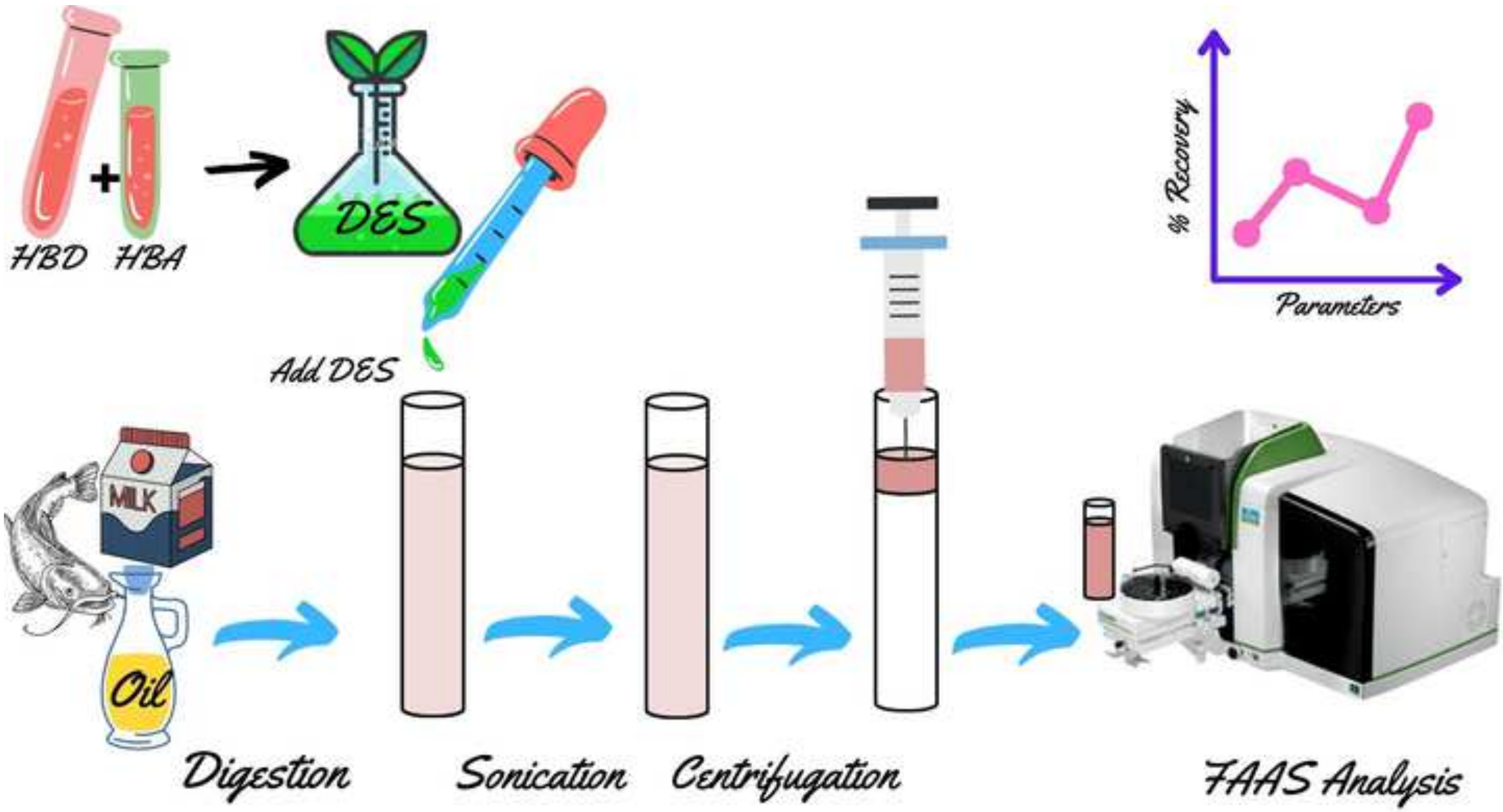
## Credit authorship contribution statement

**Fazal Elahi:** Formal analysis, methodology. **Muhammad Balal Arain:** Conceptualization, Methodology, Validation, Investigation, Supervision, Project administration, Funding acquisition. **Wajid Ali:** Writing - original draft. **Hameed Ul Haq:** Conceptualization, Investigation, Writing - original draft, Investigation, Writing - review & editing**. Asif Khan:** Formal analysis. **Roberto Castro-Muñoz:** Writing - review & editing. **Grzegorz Boczkaj*:** Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Graphic abstract





**Tables**

Table 1. Comparative study

| Analytical method | Detection tool | LOD | | Linearity range | | RSD (%) | | Matrix with Ni\|Zn | References |
|---|---|---|---|---|---|---|---|---|---|
| | | Ni | Zn | Ni | Zn | Ni | Zn | | |
| Micro-emulsion | [a]FAAS | 580 (µg/L) | 120 (µg/L) | 100-4500 (µg/L) | 100-4500 (µg/L) | 5-11 | 5-9 | Edible oils | (Nunes et al., 2011) |
| [b]UAE-LLE | FAAS | 210 (µg/L) | 40 (µg/L) | 300-2000 (µg/L) | 300-2000 (µg/L) | 2.0 | 3.6 | Edible oils | (Trindade et al., 2015) |
| [c]SPE | [d]ICP OES | 2.60 | 1.50 | 0.1–2 (µg/L) | 0.1–2 (µg/L) | 1.3 | 1.3 | Food | (Feist and Mikula, 2014) |
| SPE | ICP OES | 0.016 (µg/L) | --- | 0.33-16.66 (µg/L) | --- | 1.3 | --- | Baby food | (Ozdemir et al., 2019) |
| [e]CPE | FAAS | 2.6 | 2.3 | 2.5–160 | 5.0–80 | 2-2.6 | 2-2.6 | Food | (Galbeiro et al., 2014) |
| SPE | ICP OES | 0.019 (µg/L) | --- | 0.2-10 (µg/L) | --- | 3 | --- | Food | (Özdemir et al., 2021) |
| [f]DES-LLME | FAAS | --- | 0.041 (µg/Kg) | --- | 0.25-15 (µg/Kg) | --- | 1.7 | Fishes | (Haq et al., 2021) |
| [h]MSPE | FAAS | 9.6 (µg/L) | 1.2 (µg/L) | 13-80 (µg/L) | 3-27 (µg/L) | 1.1-9.2 | 1.1-9.2 | Soil, Leaves | (Sodan et al., 2020) |
| DES-LLME | FAAS | 0.029 (µg/Kg) | 1.54 (µg/Kg) | 39.8-997 (µg/Kg) | 9.97-798 (µg/Kg) | 3.09 | 5.1 | Milk, Oil, Fish | *This work* |

[a]FAAS: Flame atomic absorption spectrometer, [b]UAE-LLE: Ultrasound- assisted emulsification liquid-liquid extraction, [c]SPE: Solid phase extraction, [d]ICP-OES: Inductively coupled plasma optical emission spectroscopy, [e]CPE: Cloud point extraction [f]DES-LLME: Deep eutectic solvent, liquid-liquid micro extraction, [h]MSPE: Magnetic solid phase extraction.

Table 2. Determination of Ni (II) and Zn (II) in hydrogenated oil, fishes, and milk samples.

| Sample | Metal | Analyte added (µg/Kg) | Analyte found (µg/Kg) | % Recovery | % RSD (n=3) |
|---|---|---|---|---|---|
| Hydrogenated oil | Ni | 0.00 | < LOD | ---- | |
| | | 49.85 | 51.4 | 103.1 | ±3.4 |
| | | 99.70 | 103.2 | 103.5 | ±3.5 |
| | Zn | 0.00 | 25.0 | ---- | |
| | | 49.85 | 73.3 | 97.9 | ±2.8 |
| | | 99.70 | 124.8 | 100.0 | ±3.8 |
| Fishes | Ni | 0.00 | < LOD | ---- | |
| | | 49.85 | 51.3 | 102.9 | ±2.6 |
| | | 99.70 | 101.4 | 101.7 | ±2.6 |
| | Zn | 0.00 | 21.0 | ---- | |
| | | 49.85 | 68.2 | 96.2 | ±4.8 |
| | | 99.70 | 115.5 | 95.27 | ±4.2 |
| Milk | Ni | 0.00 | 15.0 | ---- | |
| | | 49.85 | 66.6 | 102.6 | ±1.6 |
| | | 99.70 | 118.5 | 103.3 | ±1.8 |
| | Zn | 0.00 | 35.0 | ---- | |
| | | 49.85 | 88.2 | 103.9 | ±2.7 |
| | | 99.70 | 136.6 | 101.4 | ±2.4 |

**Figures**

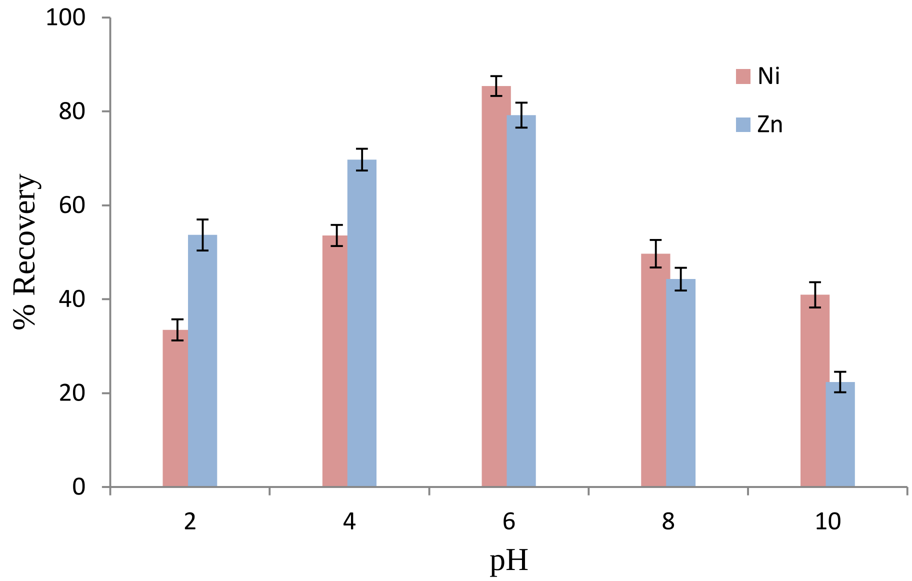



Fig 1. Optimization of pH. Sample volume: 15 mL, DES: 0.8 mL, Ni (II): 19.4 µg/Kg, Zn (II): 19.4 µg/Kg, Buffer volume: 1 mL, Ligand: 5.3 mg/L, Sonication: 2 min, Centrifugation: 2 min.

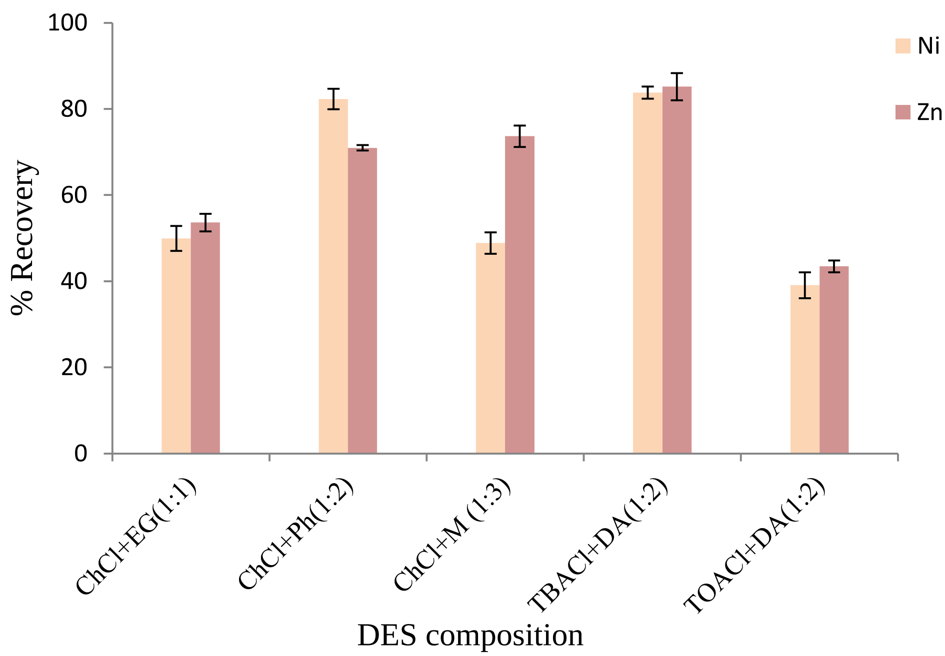



Fig 2. Selection of DES. Sample volume: 15 mL, DES: 0.8 mL, Ni (II): 19.4µg/Kg, Zn (II): 19.4µg/Kg, Buffer volume: 1 mL, Ligand: 5.3 mg/L, Sonication: 2 min, Centrifugation: 2 min.

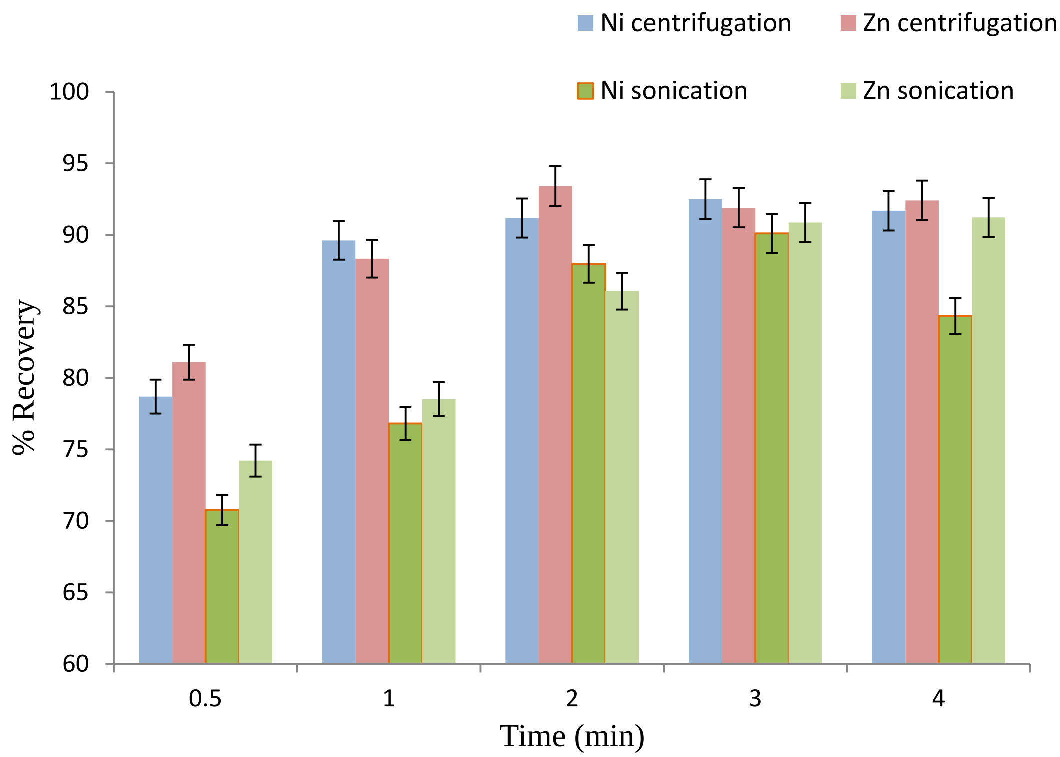



Fig 3. Time optimization for sonication and centrifugation. Sample volume: 15 mL, DES: 0.8 mL, Ni (II): 19.4 µg/Kg, Zn (II): 19.4 µg/Kg, Buffer volume: 1 mL, Ligand: 5.3 mg/L.

**Supplementary Data**

# Ultrasound-assisted deep eutectic solvent-based liquid-liquid microextraction for simultaneous determination of Ni (II) and Zn (II) in food samples

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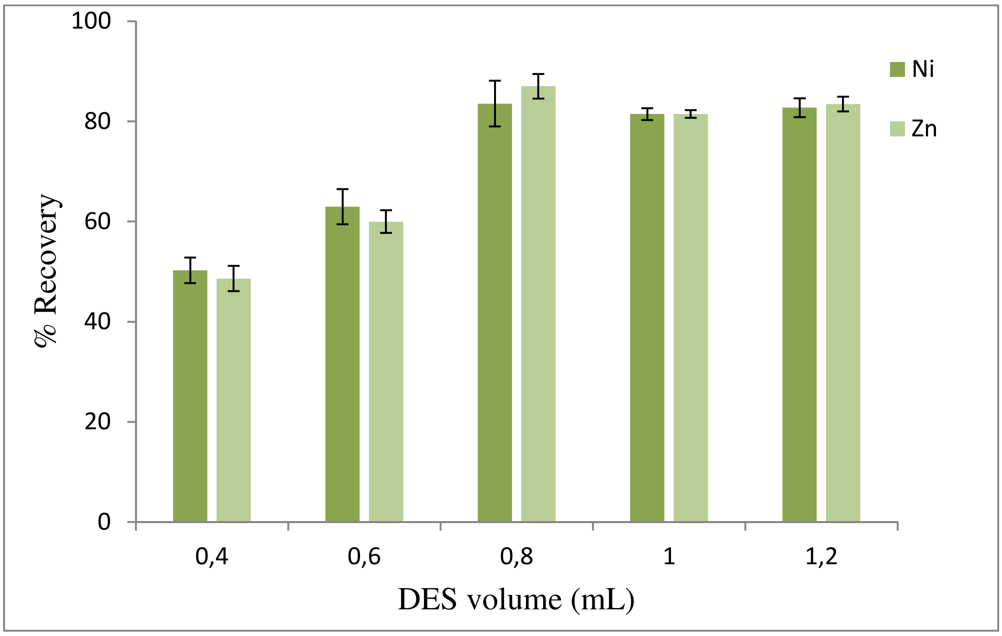


Fig S1. Effect of DES volume. Sample volume: 15 mL, Ni (II): 19.4 µg/Kg, Zn (II): 19.4 µg/Kg, Buffer volume: 1 mL, Ligand: 5.3 mg/L, Sonication: 2 min, Centrifugation: 2 min.

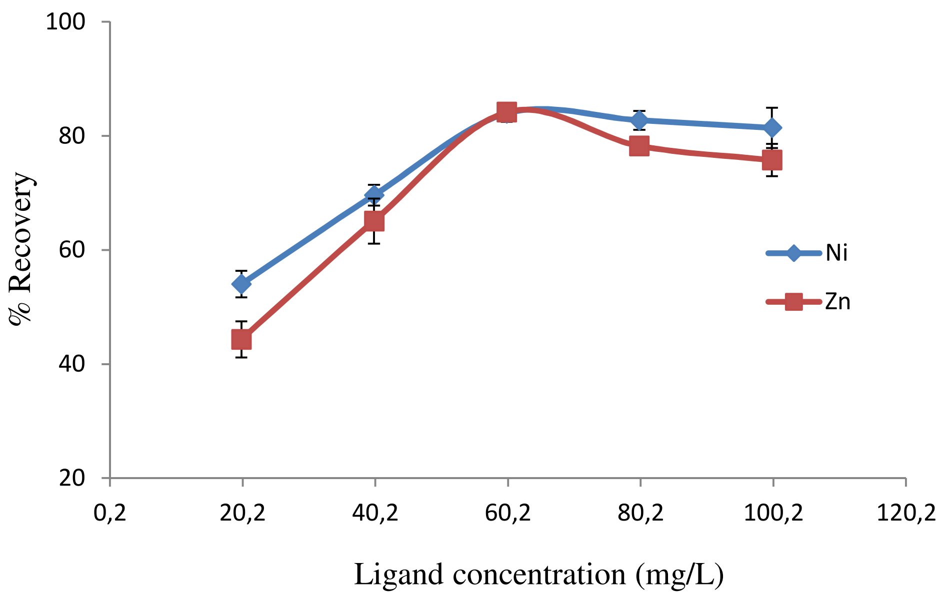


Fig S2. Optimization of chelating agent. Sample volume: 15 mL, DES: 0.8 mL, Ni (II): 19.4 µg/Kg, Zn (II): 19.4 µg/Kg, Buffer volume: 1 mL, Sonication: 2 min, Centrifugation: 2 min.

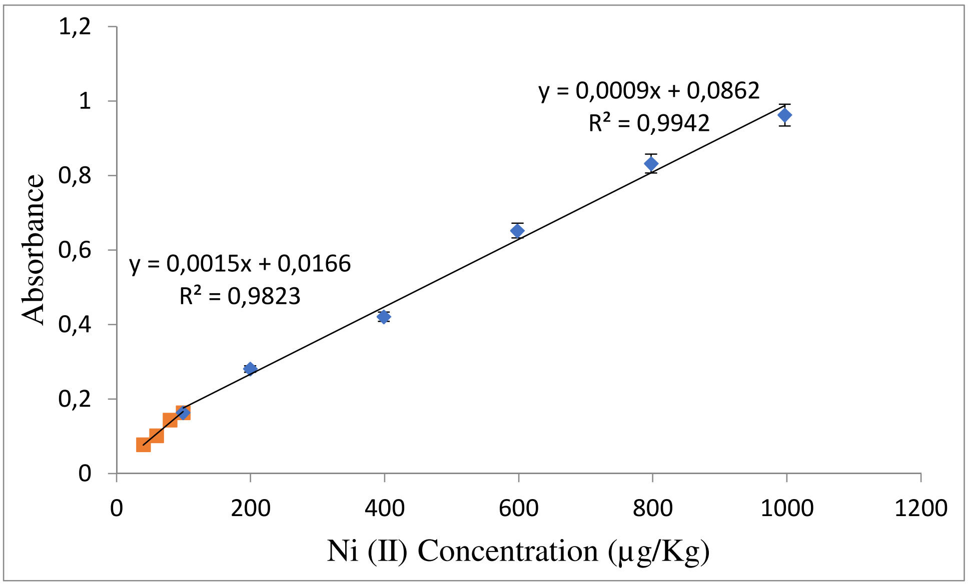


Fig S3. Calibration curve for Ni (II). Sample volume: 15 mL, DES: 0.8 mL, Zn (II): 19.4 µg/Kg, Buffer volume: 1 mL, Ligand: 5.3 mg/L, Sonication: 2 min, Centrifugation: 2 min.

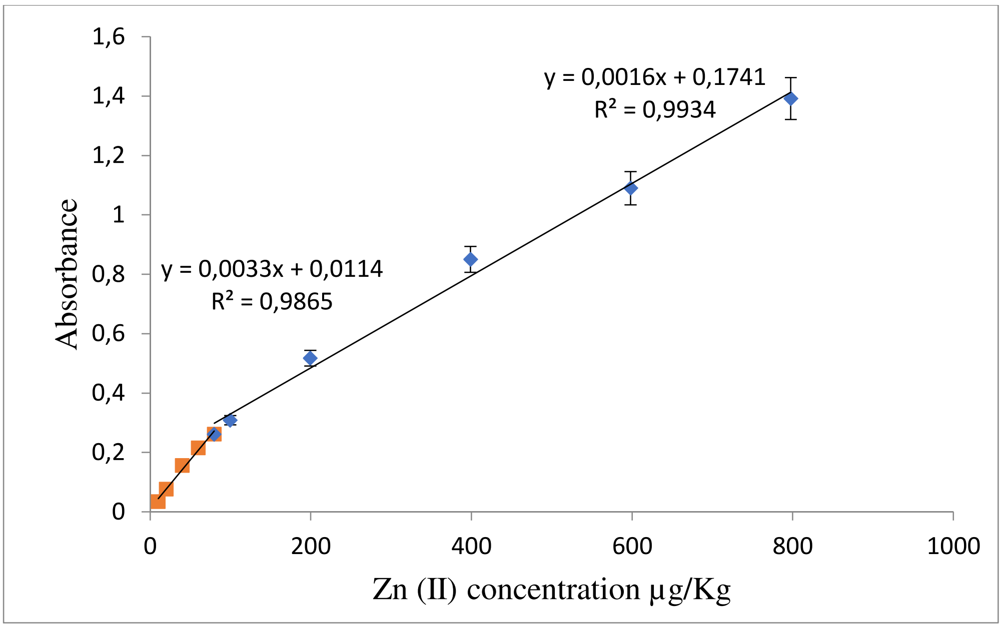


Fig S4. Calibration curve for Zn (II). Sample volume: 15 mL, DES: 0.8 mL, Ni (II): 19.4 µg/Kg, Buffer volume: 1 mL, Ligand: 5.3 mg/L, Sonication: 2 min, Centrifugation: 2 min.

Table S1. Effect of co-existing ions on the extraction recovery of Ni (II) and Zn (II)

| Ions | Added as | Tolerance limit (mg/L) | % Recovery Ni | Zn |
|---|---|---|---|---|
| $Cl^{-1}$ | NaCl | 1000 | 95.43 | 98.72 |
| $SO_4^{-2}$ | $Na_2SO_4$ | 2000 | 94.05 | 97.93 |
| $Pb^{+2}$ | $Pb(NO_3)_2$ | 20 | 98.84 | 96.49 |
| $Fe^{+2}$ | $FeSO_4.7H_2O$ | 10 | 92.49 | 95.62 |
| $Cd^{+2}$ | $Cd\ (NO_3)_2$ | 15 | 93.85 | 95.43 |
| $Na^{+1}$ | NaCl | 1000 | 98.24 | 98.93 |
| $K^{+1}$ | KCl | 1000 | 97.83 | 98.47 |
| $Mg^{+2}$ | $Mg(NO_3)_2{\cdot}6H_2O$ | 2000 | 99.02 | 97.64 |

Table S2. Comparison of Ni (II) and Zn (II) concentrations in real samples, LOD, Matrix and method of analysis.

| Matrix | Sample type | LOD | | Concentration | | References |
|---|---|---|---|---|---|---|
| | | Ni (II) | Zn (II) | Ni (II) | Zn (II) | |
| Edible oil | Soybean oil | 580 μg/Kg | 120 μg/Kg | 2.74 μg/Kg | < LOD | (Nunes et al., 2011) |
| | Olive oil | | | < LOD | 4.30 μg/Kg | |
| | Sunflower oil | | | < LOD | < LOD | |
| Edible oil | Aqueous vegetable oil | 210 μg/L | 40 μg/L | 0.0436 μg/Kg 0.0438 μg/Kg | 0.2436 μg/Kg 0.2497 μg/Kg | (Trindade, Dantas, Lima, Ferreira, & Teixeira, 2015) |
| Food | Fruits | 2.60 μg/Kg | 1.50 μg/Kg | 3.25 μg/Kg | 3.79 μg/Kg | (Feist & Mikula, 2014) |
| Baby food | Dry baby milk | 0.016 μg/Kg | --- | 0.032 μg/Kg | --- | (Ozdemir, Kilinc, & Oner, 2019) |
| | Dry baby milk (organic) | | | < LOD | --- | |
| | Dry baby milk with fruit | | | < LOD | --- | |
| | Infant food with fruit | | | < LOD | --- | |
| Food | Tuna fish candidate reference material | 2.6 μg/L | 2.3 μg/L | 9.90 μg/L | 9.83 μg/L | (Galbeiro, Garcia, & Gaubeur, 2014) |
| | Hemodialysis solution | | | 23.2 μg/L | 23.7 μg/L | |
| Food | Cow milk | 0.019 μg/Kg | 0.2 μg/Kg | < LOD | --- | (Özdemir, Yalçın, & Kılınç, 2021) |
| | Dry baby milk | | | < LOD | --- | |
| | Tuna fish | | | <LOD | --- | |
| Fishes | Fish | --- | 0.041 μg/Kg | --- | < LOD | (Haq et al., 2021) |
| | Eel | | | --- | 0.042 μg/Kg | |
| Soil, Leaves | Tibet Soil | 9.6 μg/g | 1.2 μg/g | 31.1 μg/g | 58.0 μg/g | (Sodan, Höl, Çaylak, & Elçi, 2020) |
| | Strawberry Leaves | | | 2.6 μg/g | 24 μg/g | |
| *Food* | Hydrogenated oil | 0.029 μg/Kg | 1.54 μg/Kg | *< LOD* | *25.0* μg/Kg | *This work* |
| | Fishes | | | *< LOD* | *21.0* μg/Kg | |
| | Milk | | | *15* μg/Kg | *35* μg/Kg | |

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