

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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1 **Abstract**

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

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

14 **1. Introduction**

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

27 To determine Zn (II) and Ni (II) in foods samples at a very low concentration, various pre-
28 concentration techniques have been developed for the simultaneous pre-concentration and
29 determination of nickel and zinc, such as co-precipitation (Komjarova & Blust, 2006), solid-
30 phase extraction (Roldan et al., 2003), liquid-liquid extraction (Mansur, Rocha, Magalhães, &
31 dos Santos Benedetto, 2008), and sequential extraction procedure (Alomary & Belhadj, 2007).
32 Unfortunately, these methods present drawbacks in terms of inefficiency, prolonged time, use of
33 toxic chemicals, high costs, and provide insufficient sample cleanup (Khan, Arain, Yamini, et al.,
34 2020; Khan, Yamini, Baharfar, & Arain, 2019).

35 Deep eutectic solvents (DES) are a new class of green solvents introduced by Smith et al. (Smith,
36 Abbott, & Ryder, 2014). DESs seem to be the best alternative to conventional solvents. Their

37 usefulness was proved for several applications in separation techniques (Haq et al., 2021b;
38 Momotko, Łuczak, Przyjazny, & Boczkaj, 2021). Generally, the preparation of DES is easy,
39 cheap, ecologically safe, less toxic and biologically acceptable (Harifi-Mood, Mohammadpour,
40 & Boczkaj, 2020; Kumar et al., 2020; Makoś & Boczkaj, 2019; Makoś, Fernandes, Przyjazny, &
41 Boczkaj, 2018). Due to the aforementioned advantages, DES-based pre-concentration methods
42 are getting great attention and a vast number of articles have been published in recent years
43 (Altunay & Tuzen, 2021; Elik, Demirbaş, & Altunay, 2022; Haq et al., 2022). This latter method
44 is based on hydrophobic deep eutectic solvents (hDES) which were, for the first time, used for
45 liquid-liquid micro-extraction in 2015 (van Osch, Zubeir, van den Bruinhorst, Rocha, & Kroon,
46 2015). In 2018, hDES were initially implemented for metal extraction from an aqueous medium
47 (Schaeffer, Martins, Neves, Pinho, & Coutinho, 2018). hDESs are very effective for metal pre-
48 concentration, displaying outstanding distribution coefficients specially for divalent metal ions
49 (Van Osch, Dietz, Warrag, & Kroon, 2020). However, this new potential aspect of hDESs still
50 needs more research to expand their applicability, especially in the field of analytical chemistry.

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

55



56 **2. Material and Methods**

57 *2.1 Instrumentation*

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

63 *2.2 Reagents and Solutions*

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

76



77 2.3 Synthesis of DESs

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

89 2.4 Sampling and digestion

90 In spectroscopic analysis of metals, acid digestion is one of the most significant steps of the
91 entire analytical procedure. It has a substantial effect on the recovery of various analytes in
92 highly complex matrices. Digestion is also helpful to achieve the optimal sample preparation
93 method with clearer background (low noise level) (Bader, 2011; Uddin et al., 2016).
94 Hydrogenated edible oil, milk, and fish samples were studied for pre-concentration and analysis
95 of Ni (II) and Zn (II). All the samples were digested according to the recommended methods in
96 the literature (Alomary & Belhadj, 2007; Begum, Bari, Jamaludin, & Hussin, 2012; Haq et al.,
97 2021b). Triplicate samples were used for the analysis of target analytes. Hydrogenated edible
98 oils (Shama vanaspati ghee) were purchased from the local market of District Mardan, Pakistan.
99 Experimentally, composite samples (5 g) of hydrogenated edible oil were heated at 800 °C for 10



100 min in a furnace to decompose the organic matter. The obtained residues were dissolved in 5 mL
101 hydrochloric acid (2M). Then, the solutions were filtered, and the resulting filtrate was diluted
102 with deionized water up to 25 mL (Purohit & Devi, 1995). Milk samples were oven-dried at 70
103 °C. Each composite sample (2 g) of dried milk was digested. The digestion was performed by
104 adding 3.5 mL of nitric acid (70%) and 1.0 mL of H₂O₂ (30% v/v) to the sample and heating it at
105 90 °C and 750-watt microwave power for 10 min. The conditions were gradually varied to 180
106 °C temperature and 1000-watt microwave power for 10 min. After cooling, the digested
107 samples were transferred into 10 mL volumetric flasks and diluted to volume with nitric acid
108 (1% v/v) (Abdulkhaliq, Swaileh, Hussein, & Matani, 2012). The fish sample was digested
109 according to the procedure previously described and considered effective enough for this purpose
110 (Fashi, Yaftian, & Zamani, 2017). Composite samples with edible parts (meat) of different fishes
111 were prepared. Briefly, Siluriformes fish (catfish) samples (5 g) were transferred to a digestion
112 flask containing 1 mL of deionized water, 1 mL of nitric acid, 1 mL of chloric acid, and 5 mL of
113 sulfuric acid with 2 drops of potassium permanganate (1% w/v). The resulting mixture was
114 heated at 150 °C until a clear solution was obtained – preliminary series of experiments revealed
115 that 25 minutes endure effective digestion. The solution was kept for a while to cool and then
116 diluted with ultrapure water to 50 mL.

117 *2.5 Optimization of the microextraction procedure*

118 Ni (II) and Zn (II) standard solutions were added to 10 mL sample in a falcon tube with a final
119 concentration of 19.4 µg/Kg for each metal. Buffer solutions (pH 6) (2 mL) and 1, 10-
120 phenanthroline (1 % w/v) (0.6 mL) were added to the matrix. The tube was tightly closed and
121 shaken properly by hand. Subsequently DES (8 mL) was added, followed by sonication for 3
122 min at 25 °C. Finally, the mixture was centrifuged for 2 min with 4000 rpm to separate the



123 aqueous and rich DES phase. The lower layer was discarded by a micropipette while the DES
124 phase was collected. The collected DES phase was makeup with CH₃OH up to 3 mL mark and
125 analyzed with FAAS.

126 *2.6 Calculation of percent recovery and validation assays*

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

$$130 \quad \% R = \frac{C_d}{C_e} \times 100 \quad (1)$$

131 where C_d is the concentration determined in the spiked real sample while C_e is the expected
132 concentration in the spiked real sample.

133 The % R was calculated as C_d/C_e×100, where C_d is the determined concentration and C_e is the
134 expected concentration.

135 LOD and LOQ were calculated using the following formulas.

$$136 \quad LOD = \frac{3 \times SD}{m} \quad (2)$$

$$137 \quad LOQ = \frac{10 \times SD}{m} \quad (3)$$

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

141 The pre-concentration factor (PF) was calculated as the concentration ratio of the analyte in the
142 final extract (DES phase) ready for its determination and in the initial solution according to
143 previously described method (Kazi et al., 2012). The pre-concentration factor was evaluated by
144 using the following equation (Asgharinezhad et al., 2015; Asl, Yamini, Rezazadeh, & Seidi,
145 2015).



146
$$PF = \frac{C_f}{C_i} \quad (4)$$

147 where C_f and C_i are the final and initial concentration of analytes in the DES phase (receiving
148 phase), and donor phase, respectively. The analyte concentration was determined in sample
149 solution before extraction (C_i) and after extraction in the DES phase (C_f).

150 Relative standard deviation was calculated using the following formula

151
$$RSD (\%) = \frac{SD}{Ca} \times 100 \quad (5)$$

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

153 *2.7 Determination by FAAS*

154 The DES extract was diluted with methanol up to 5 mL. The resulting samples were finally
155 analyzed using FAAS. For this, standard solutions for Ni (39.88-997.0 $\mu\text{g/Kg}$) and Zn (9.97-
156 797.6 $\mu\text{g/Kg}$) were prepared. High-capacity auto samplers along with WinLab32™ software
157 were used. Air-acetylene flame was used for excitation. Hollow cathode lamp with single
158 wavelength (Ni 232.0 nm, Zn 213.9 nm) was used as a light source. Gas flow rate was
159 2 L min^{-1} for air and 2 L min^{-1} for acetylene for each metal. Absorbance was determined for
160 standard solutions of Ni (II) and Zn (II) ions and plotted as a calibration curve.

161

162 **3. Results and Discussion**

163 **3.1 Selection of parameters for optimal sample preparation**

164 *3.1.1 Effect of pH*

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

176 *3.1.2 Solvent selection and optimization*

177 The selection of a suitable solvent represents important aspect for developing an analytical
178 method. DESs, as a new class of green solvents, were used for pre-concentration due to their
179 least toxicity, easy formation, high selectivity, low cost, biodegradable nature, and easy
180 availability (Makoś & Boczkaj, 2019; Makoś, Fernandes, et al., 2018; Makoś, Przyjazny, &
181 Boczkaj, 2018). In this research, various types of hydrophobic deep eutectic solvents (hDES)
182 were tested for the recovery of the target analytes. Differently from many other approaches, this
183 method is based on the extraction of analytes after the digestion stage. Preliminary experiments
184 for this study revealed that selected DESs are much more effective to extract metals from



185 mineralized samples compared with direct extraction of metal-containing moieties from primary
186 samples. This becomes an important feature of DESs as some of them are able to extract, or
187 simply dissolve, metals as well as metal oxides. This property is a big advantage comparing to
188 classic organic solvents (Richter & Ruck, 2019; Söldner, Zach, & König, 2019). It is worth
189 mentioning that selected approach provides a one very important feature; for example, the
190 mineralization step ensures the elimination of most matrix effects that could affect extraction
191 reproducibility. In this case, the primary sample matrix is reduced to simple inorganic species.
192 The extraction by DES allows obtaining additional selectivity while eliminating the matrix
193 effects. This should allow using one universal calibration for each analyte – independently from
194 the type of sample. All the DESs were tested at different pH (2-8). The results for different DESs
195 are illustrated in Figure 2. It can be seen that the maximum recovery was obtained with choline
196 chloride–phenol (1:2) and tetrabutylammonium chloride-decanoic acid (1:2). As
197 tetrabutylammonium chloride-decanoic acid is comparatively more environmentally friendly, it
198 was selected as extracting solvent for this method. The optimum volume of DES was also
199 determined by changing its volume from 0.4 to 1.2 mL. The maximum % R was obtained using
200 0.8 mL of DES. Therefore, this latter quantity of DES extractant was selected as the optimum
201 volume for this experiment. Figure S1 provides the results for the optimization of DES volume.
202 1,10-phenanthroline is a common chelating agent and is readily used for complex formation with
203 Ni (II) and Zn (II) (Kruse & Brandt, 1952; Norman & Xie, 2004). Hence, the 1,10-
204 phenanthroline was used as a chelating agent for the effective pre-concentration of metal cations
205 through DES. To determine the optimum concentration of chelating agent, 1,10-phenanthroline
206 concentration was varied from 20.2 to 100.2 mg/L for 15 mL sample (concentration of analyte



207 19.4 $\mu\text{g/Kg}$). Figure S2 shows the results for the optimization of chelating agent concentration,
208 revealing that 60 mg/L was found as optimum concentration of 1,10-phenanthroline.
209 Since ultra-sonication plays a key role in the mass transfer of the target analyte from the aqueous
210 to DES phase, the influence of sonication time was also evaluated by varying the sonication time
211 from 0.5 to 4 min. Importantly, a maximum recovery for both analytes was achieved at 2 min
212 sonication, thus it was selected as the optimum time of sonication. Similarly, the effect of
213 centrifugation time on the pre-concentration recovery of target analytes was performed by
214 changing the parameter in the range of 0.5-4 min. For 2 min centrifugation, maximum recovery
215 was obtained for both analytes, being selected as the optimum time for centrifugation. Figure 3
216 shows results for sonication and centrifugation time.

217 **3.2 Method validation and application for analysis of real samples**

218 *3.2.1 Analytical performance of the developed method*

219 The analytical characteristics were evaluated under optimum conditions. The calibration curves
220 were linear in the range of 39.88-997 $\mu\text{g/Kg}$ and 9.97-797.6 $\mu\text{g/Kg}$ for Ni (II) and Zn (II),
221 respectively. Calibration curves for both analytes were determined in two concentration ranges,
222 as follows: Range 1 for lower concentrations ranging from 39.88 to 99.4 $\mu\text{g/Kg}$ and 9.97-79.76
223 $\mu\text{g/Kg}$ with a coefficient of determination (R^2) of 0.9823 and 0.9865 for Ni (II) and Zn (II),
224 respectively. Range 2 for higher concentrations ranging from 99.4 to 997 $\mu\text{g/Kg}$ and 79.76-797.6
225 $\mu\text{g/Kg}$ with a coefficient of determination (R^2) of ca. 0.9942 and 0.9934 for Ni (II) and Zn (II),
226 respectively. Data presented in figures S3 and S4 indicate satisfactory linearity of the method.

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



230 0.029 $\mu\text{g/Kg}$ and 1.542 $\mu\text{g/Kg}$, respectively, while LOQ was estimated as 0.097 $\mu\text{g/Kg}$ and 1.17
231 $\mu\text{g/Kg}$ for Ni (II) and Zn (II), respectively, in real samples applying pre-concentration factor. The
232 relative standard deviation was 3.09 % for Ni (II) and 5.1 % for Zn (II).

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

244 *3.2.2 Interference study*

245 As it is well known food is a complex matrix that contains a large number of cations, anions as
246 well as biomolecules. To evaluate the interference effect and selectivity of the DES medium for
247 Ni (II) and Zn (II), different concentrations of cations and anions were added to the sample
248 solution containing a known concentration of Ni (II) and Zn (II) and performed the pre-
249 concentration procedure. Eight different cations and anions were added at higher concentrations.
250 According to the results compiled in Table S1, the developed method is highly selective for the
251 pre-concentration and determination of Ni (II) and Zn (II). % R was found between 92.49- 99.02
252 % and 95.43-98.93% for Ni (II) and Zn (II), respectively. Based on high % R in the presence of

253 interfering ions, it was concluded that this method is highly selective for the pre-concentration Ni
254 (II) and Zn (II).

255 *3.2.3 Applicability of the method*

256 The developed method was applied to real digested fish and hydrogenated edible oil samples for
257 the determination of Ni (II) and Zn (II). To evaluate the validity of the obtained results, spike
258 tests were carried out on the samples. Representative samples were prepared for each sample
259 matrix from different sources, which were analyzed in triplicate. First samples were analyzed
260 without a spike using newly developed method. In hydrogenated oil samples, the Ni (II)
261 concentration was below the LOQ. The samples were spiked with Ni (II) standard solutions to
262 obtain expected concentration of 49.85 $\mu\text{g/Kg}$ and 99.70 $\mu\text{g/Kg}$. Interestingly, the % R was
263 found to be as high as 103.1-103.5 % with RSD 3.4-3.5 % for $n=3$. The same approach was done
264 for zinc, however, in the case of Zn (II), the concentration found in non-spiked sample was 25
265 $\mu\text{g/Kg}$. The % R for Zn (II) was between 97.9-100% with RSD 2.8-3.8% for $n=3$.

266 In fish samples, the Ni (II) concentration was below the LOD, thus same level of concentrations
267 for spiked samples was implemented. In this case, % R was found to be as high as 101.7-102.9%
268 with RSD 2.6% for $n=3$. As for Zn (II), the concentration for non-spiked samples was found 21
269 $\mu\text{g/Kg}$ and % R for spiked samples was determined to be 95.27-96.2 % with RSD 4.2-4.8 % for
270 $n=3$.

271 For composite milk samples, the Ni (II) concentration was 15 $\mu\text{g/Kg}$. % R was found to be
272 between 102.6-103.3 % with RSD 1.6-1.8 % for $n=3$. In same samples, the Zn (II) concentration
273 was determined as 35 $\mu\text{g/Kg}$ while % R was between 101.4-103.9 % with RSD 2.4-2.7 % for
274 $n=3$.

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

281 *3.2.4 Comparison with existing methods*

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

294 This newly developed method is simple and highly selective. As mentioned earlier, pre-
295 concentration takes place after the digestion stage, thus it is possible to highlight the advantages
296 of the developed DES-based method at this step only. For instance, no heat is required at the pre-
297 concentration stage, but the most worthwhile advantage of this method is the successful usage of



298 green solvents instead of classic organic solvents (Fabjanowicz, Kalinowska, Namieśnik, &
299 Płotka-Wasyłka, 2018). Few examples of typical organic solvents are 1-hexyl-3-
300 methylimidazolium hexafluorophosphate [HMIM][PF₆, acetone, N,N'-bis(2-salicylaldiminato)-
301 1,8-diamino-3,6-dioxaoctane (Rajabi, Asemipour, Barfi, Jamali, & Behzad, 2014),
302 cyclohexylamine (Sorouraddin, Farajzadeh, & Okhravi, 2017), naphthalene modified with
303 organic-solution-processable functionalized nano graphene (Moghimi, 2014), neodecanoic acid,
304 Versatic 10 (Ichlas & Purwadaria, 2017), acetone and 1-undecanol, diethyldithiocarbamate
305 (Amirkavei, Dadfarnia, & Shabani, 2013), and 1-(2-Pyridylazo)-2-naphthol (PAN) (Bidabadi,
306 Dadfarnia, & Shabani, 2009), etc. The impressive features of DESs are related to their high
307 availability of components and easy preparation, biodegradability, minimal toxicity, low
308 volatility and costs (Arain, Yilmaz, & Soylak, 2016; Galbeiro et al., 2014; Haq et al., 2021a;
309 Kohli & Mittal, 2018; Makoś, Przyjazny, et al., 2018). The ICP-OES based methods display
310 comparatively better LOD; however, such methods need more complicated instrumentation. A
311 comparison of Ni and Zn concentration levels found in the tested samples of the present work
312 have been compared with concentration levels in similar food matrices determined by other
313 analytical methods, as reported in Table S2.

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

321 **4. Conclusions**

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

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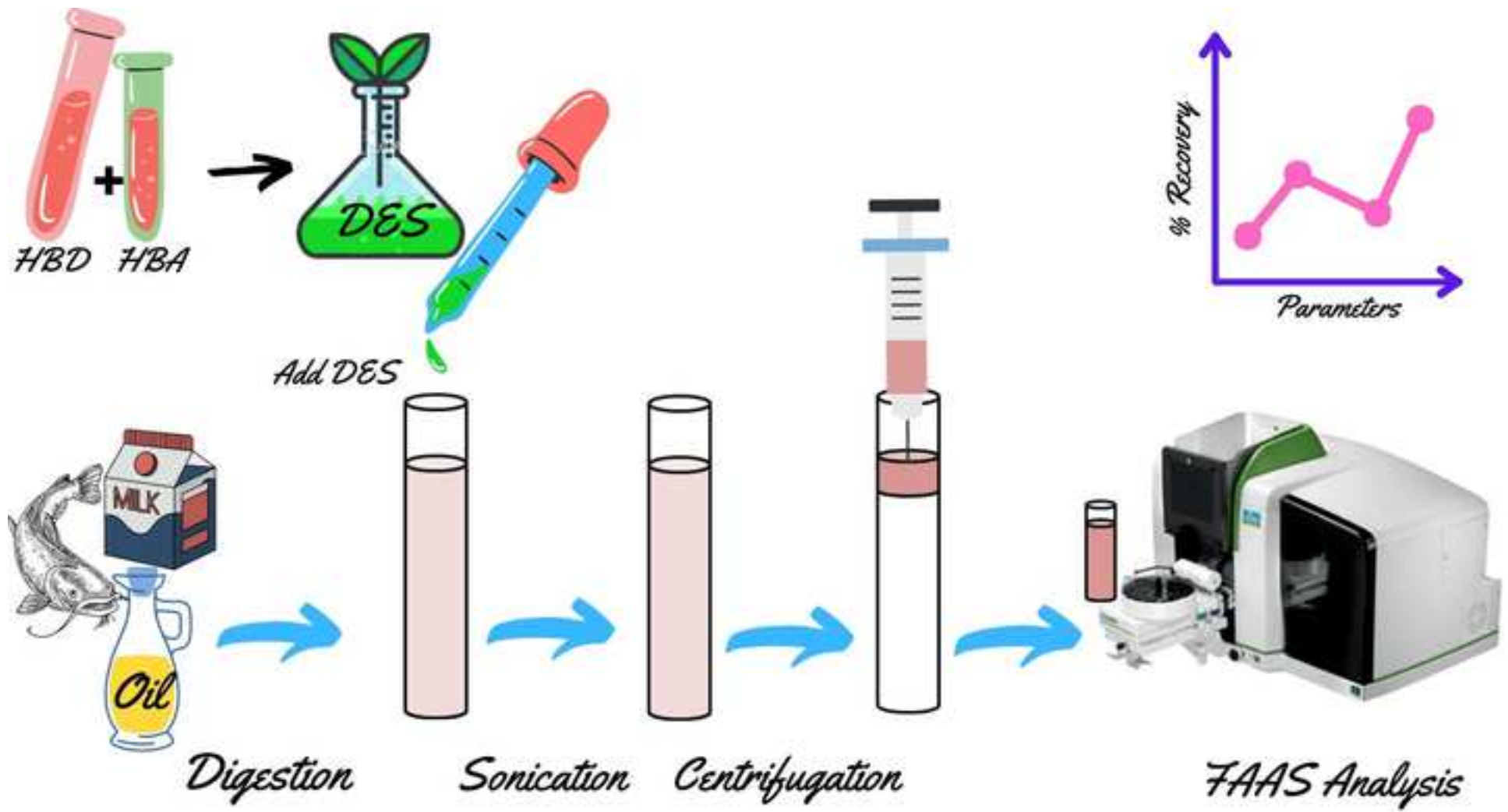
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Credit authorship contribution statement

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.



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	<i>This work</i>

^aFAAS: Flame atomic absorption spectrometer, ^bUAE-LLE: Ultrasound- assisted emulsification liquid-liquid extraction, ^cSPE: Solid phase extraction, ^dICP-OES: Inductively coupled plasma optical emission spectroscopy, ^eCPE: Cloud point extraction ^fDES-LLME: Deep eutectic solvent, liquid-liquid micro extraction, ^hMSPE: Magnetic solid phase extraction.

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

Sample	Metal	Analyte added ($\mu\text{g/Kg}$)	Analyte found ($\mu\text{g/Kg}$)	% 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

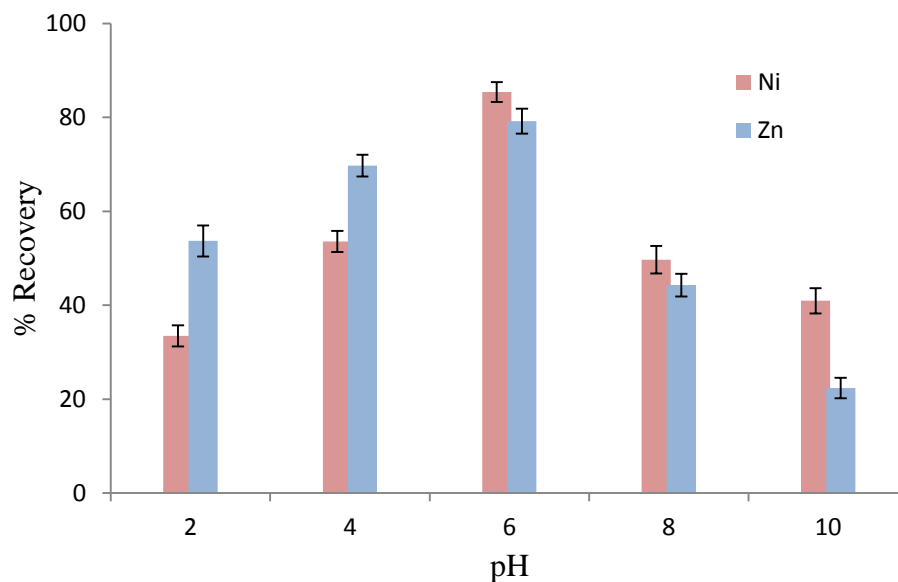


Fig 1. Optimization of pH. Sample volume: 15 mL, DES: 0.8 mL, Ni (II): 19.4 $\mu\text{g}/\text{Kg}$, Zn (II): 19.4 $\mu\text{g}/\text{Kg}$, Buffer volume: 1 mL, Ligand: 5.3 mg/L, Sonication: 2 min, Centrifugation: 2 min.

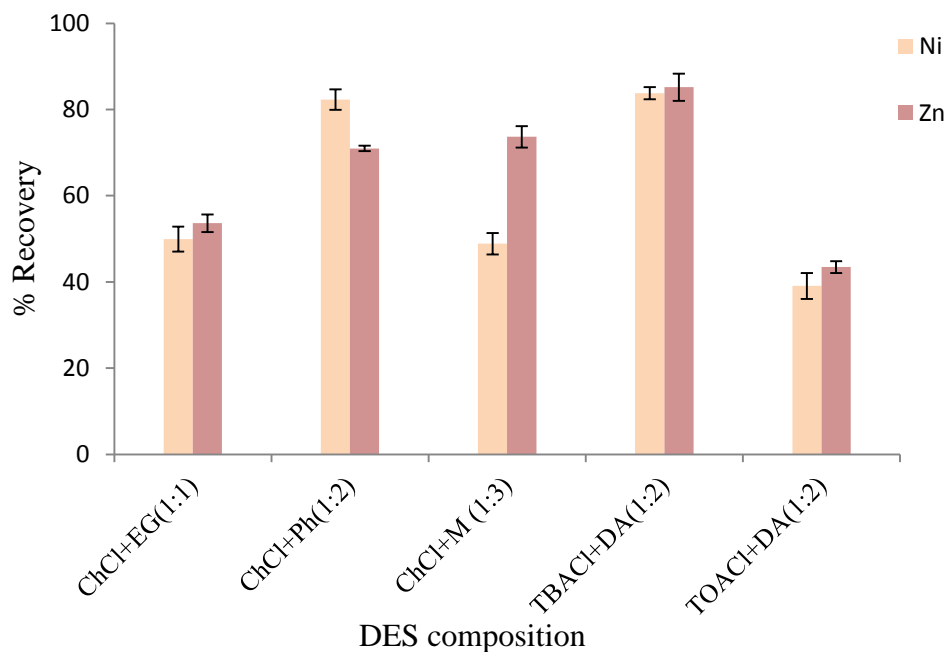


Fig 2. Selection of DES. Sample volume: 15 mL, DES: 0.8 mL, Ni (II): 19.4 $\mu\text{g}/\text{Kg}$, Zn (II): 19.4 $\mu\text{g}/\text{Kg}$, Buffer volume: 1 mL, Ligand: 5.3 mg/L, Sonication: 2 min, Centrifugation: 2 min.

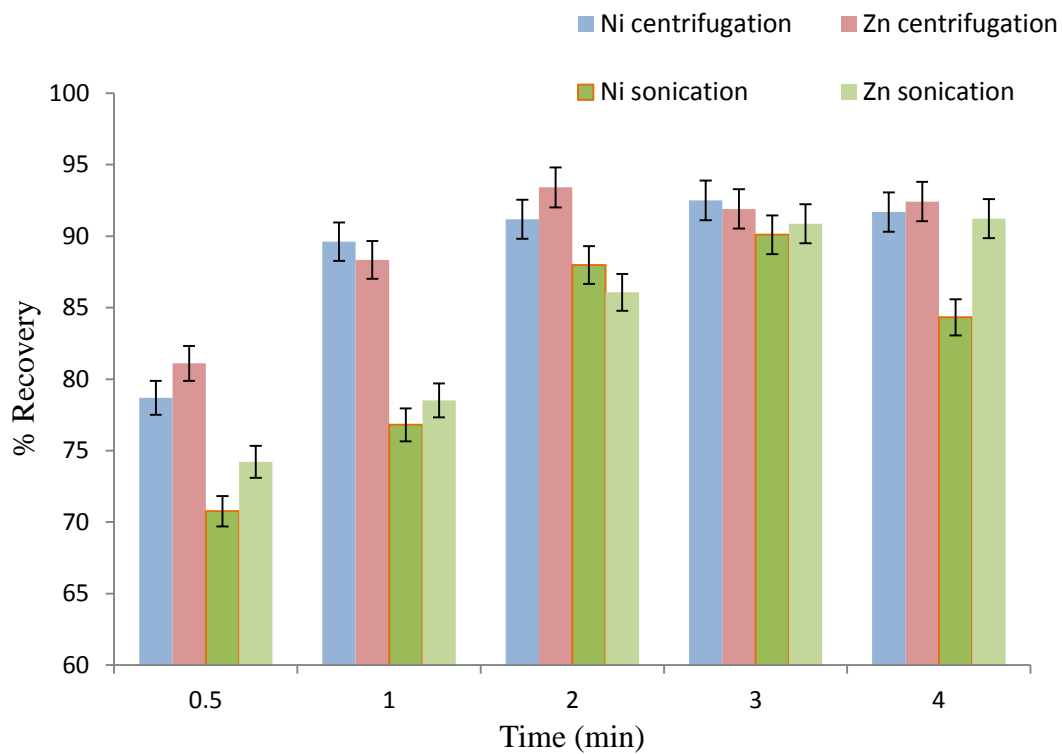


Fig 3. Time optimization for sonication and centrifugation. Sample volume: 15 mL, DES: 0.8 mL, Ni (II): 19.4 $\mu\text{g/Kg}$, Zn (II): 19.4 $\mu\text{g/Kg}$, Buffer volume: 1 mL, Ligand: 5.3 mg/L.

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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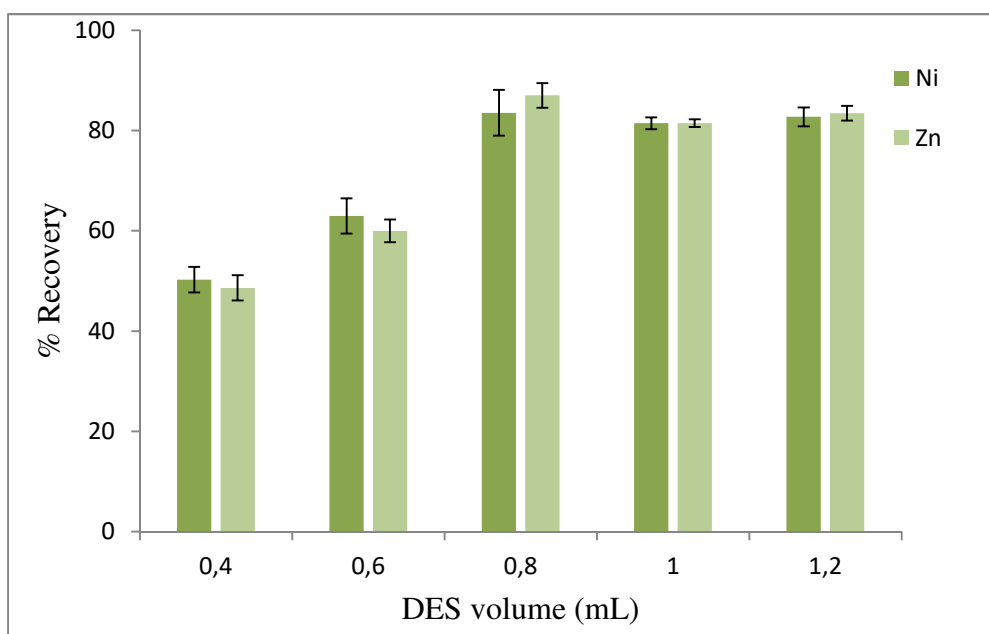


Fig S1. Effect of DES volume. Sample volume: 15 mL, Ni (II): 19.4 $\mu\text{g/Kg}$, Zn (II): 19.4 $\mu\text{g/Kg}$, Buffer volume: 1 mL, Ligand: 5.3 mg/L, Sonication: 2 min, Centrifugation: 2 min.

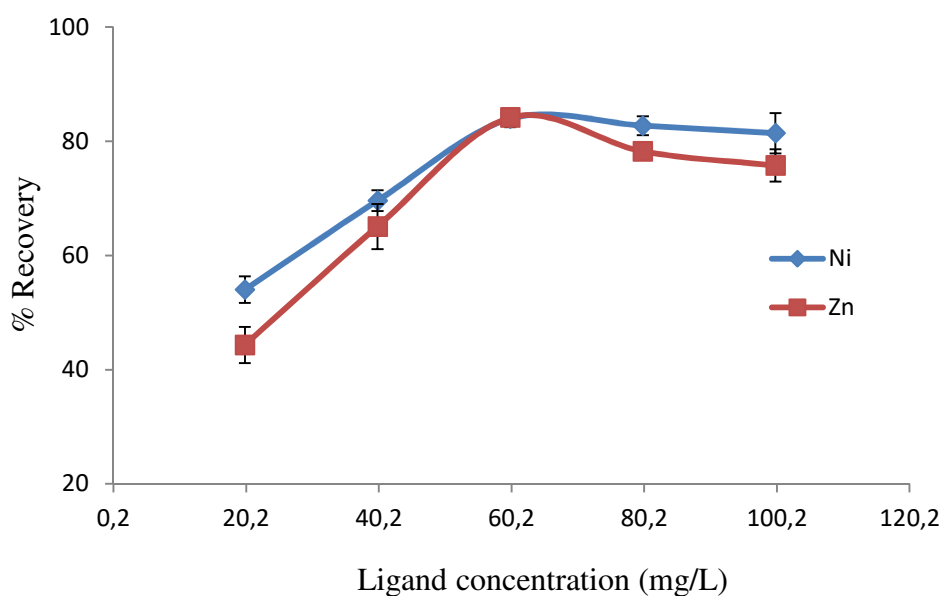


Fig S2. Optimization of chelating agent. Sample volume: 15 mL, DES: 0.8 mL, Ni (II): 19.4 $\mu\text{g/Kg}$, Zn (II): 19.4 $\mu\text{g/Kg}$, Buffer volume: 1 mL, Sonication: 2 min, Centrifugation: 2 min.

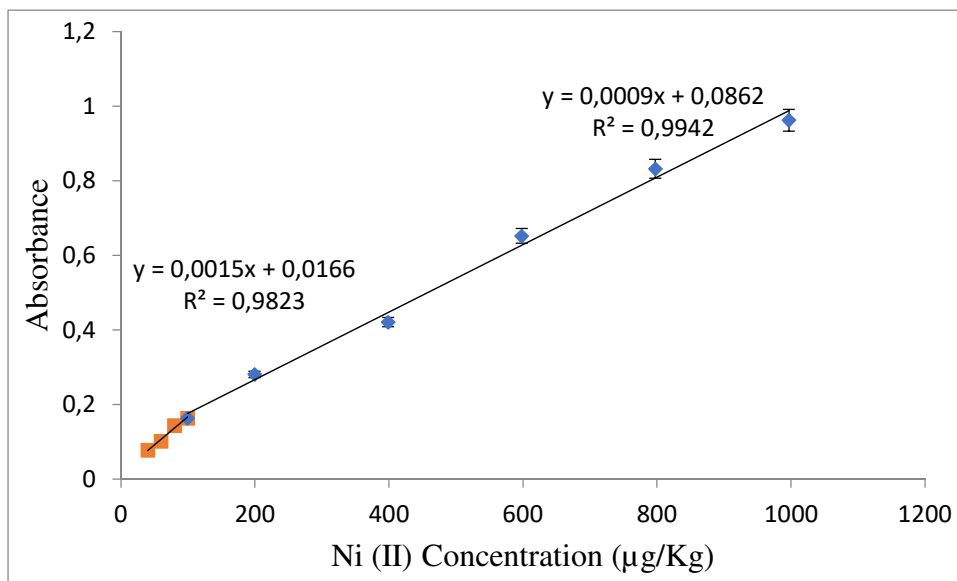


Fig S3. Calibration curve for Ni (II). Sample volume: 15 mL, DES: 0.8 mL, Zn (II): 19.4 µg/Kg, Buffer volume: 1 mL, Ligand: 5.3 mg/L, Sonication: 2 min, Centrifugation: 2 min.

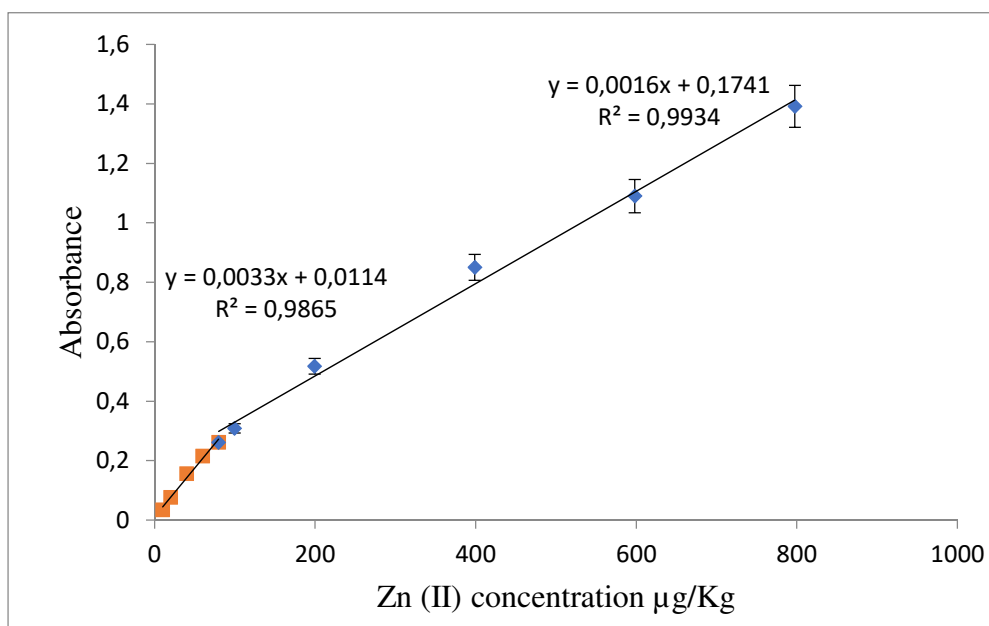


Fig S4. Calibration curve for Zn (II). Sample volume: 15 mL, DES: 0.8 mL, Ni (II): 19.4 µg/Kg, Buffer volume: 1 mL, Ligand: 5.3 mg/L, Sonication: 2 min, Centrifugation: 2 min.



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 ₂ SO ₄	2000	94.05	97.93
Pb ⁺²	Pb(NO ₃) ₂	20	98.84	96.49
Fe ⁺²	FeSO ₄ ·7H ₂ O	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 ·6H ₂ O	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	120	2.74	< LOD	(Nunes et al., 2011)
	Olive oil	$\mu\text{g/Kg}$	$\mu\text{g/Kg}$	< LOD	4.30 $\mu\text{g/Kg}$	
	Sunflower oil			< LOD	< LOD	
Edible oil	Aqueous			0.0436	0.2436	(Trindade, Dantas, Lima, Ferreira, & Teixeira, 2015)
	vegetable oil	210	40	$\mu\text{g/Kg}$	$\mu\text{g/Kg}$	
		$\mu\text{g/L}$	$\mu\text{g/L}$	0.0438	0.2497	
		$\mu\text{g/Kg}$	$\mu\text{g/Kg}$	$\mu\text{g/Kg}$	$\mu\text{g/Kg}$	
Food	Fruits	2.60	1.50	3.25	3.79 $\mu\text{g/Kg}$	(Feist & Mikula, 2014)
		$\mu\text{g/Kg}$	$\mu\text{g/Kg}$	$\mu\text{g/Kg}$		
Baby food	Dry baby milk			0.032	---	(Ozdemir, Kilinc, & Oner, 2019)
	Dry baby milk (organic)	0.016	---	$\mu\text{g/Kg}$	---	
	Dry baby milk with fruit	$\mu\text{g/Kg}$		< LOD	---	
	Infant food with fruit			< LOD	---	
Food	Tuna fish candidate reference material			9.90	9.83	(Galbeiro, Garcia, & Gaubeur, 2014)
		2.6	2.3	$\mu\text{g/L}$	$\mu\text{g/L}$	
		$\mu\text{g/L}$	$\mu\text{g/L}$			
	Hemodialysis solution			23.2 $\mu\text{g/L}$	23.7 $\mu\text{g/L}$	
Food	Cow milk			< LOD	---	(Özdemir, Yalçın, & Kılınç, 2021)
	Dry baby milk	0.019	0.2	< LOD	---	
	Tuna fish	$\mu\text{g/Kg}$	$\mu\text{g/Kg}$	< LOD	---	
Fishes	Fish		0.041	---	< LOD	(Haq et al., 2021)
	Eel	---	$\mu\text{g/Kg}$	---	0.042 $\mu\text{g/Kg}$	
Soil, Leaves	Tibet Soil			31.1 $\mu\text{g/g}$	58.0 $\mu\text{g/g}$	(Sodan, Höl, Çaylak, & Elçi, 2020)
		9.6	1.2			
		$\mu\text{g/g}$	$\mu\text{g/g}$			
	Strawberry Leaves			2.6 $\mu\text{g/g}$	24 $\mu\text{g/g}$	
<i>Food</i>	Hydrogenated oil			< LOD	25.0 $\mu\text{g/Kg}$	<i>This work</i>
	Fishes	0.029	1.54	< LOD	21.0 $\mu\text{g/Kg}$	
	Milk	$\mu\text{g/Kg}$	$\mu\text{g/Kg}$	15 $\mu\text{g/Kg}$	35 $\mu\text{g/Kg}$	



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