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

Fazal Elahi[1], Muhammad Balal Arain[2], Wajid Ali[1], Hameed Ul Haq[3], Asif Khan[1], Faheem Jan[4], Roberto Castro-Muñoz[3,5], Grzegorz Boczkaj[3,6,*]

[1]Department of Chemistry, Abdul Wali Khan University Mardan, 23200, KP, Pakistan. Email: wajidalikhan890@gmail.com

[2]Department of Chemistry, University of Karachi, Karachi 75270, Pakistan. Email: bilal_ku2004@yahoo.com

[3]Gdansk University of Technology, Faculty of Civil and Environmental Engineering, Department of Sanitary Engineering, 80 – 233 Gdansk, G. Narutowicza St. 11/12, Poland. E-mail: grzegorz.boczkaj@pg.edu.pl / hameed.haq@pg.edu.pl

[4]School of Materials Science and Engineering, University of Science and Technology of China, Shenyang 110016, Liaoning, People's Republic of China

[5]Tecnologico de Monterrey, Campus Toluca, Avenida Eduardo Monroy, Cárdenas 2000 San Antonio Buenavista, 50110 Toluca de Lerdo, Mexico

[6]EkoTech Center, Gdansk University of Technology, G. Narutowicza St. 11/12, 80-233 Gdansk, Poland

*Corresponding author: Dr Grzegorz Boczkaj, Assoc. Prof., PhD. Sc. Eng. Gdansk University of Technology, Faculty of Civil and Environmental Engineering, Department of Sanitary Engineering, 80 – 233 Gdansk, G. Narutowicza St. 11/12, Poland. Fax: (+48 58) 347-26-94; Tel: (+48) 697970303; e-mail: grzegorz.boczkaj@gmail.com or grzegorz.boczkaj@pg.edu.pl

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

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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

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.

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 H_2O_2 (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

7

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 CH_3OH 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
$$\% R = \frac{Cd}{Ce} \times 100 \qquad (1)$$

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

132 concentration in the spiked real sample.

133 The % R was calculated as $C_d/C_e \times 100$, 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
$$LOD = \frac{3 \times SD}{m} \qquad (2)$$

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

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

147   where *Cf* and *Ci* 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 (Ci) and after extraction in the DES phase (Cf).

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 µg/Kg) and Zn (9.97-

156   797.6 µg/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    $2\ \text{L min}^{-1}$ for air and $2\ \text{L min}^{-1}$ 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

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

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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

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207 19.4 µg/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 µg/Kg and 9.97-797.6 µg/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 µg/Kg and 9.97-79.76

223 µg/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 µg/Kg and 79.76-797.6

225 µg/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

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230   0.029 µg/Kg and 1.542 µg/Kg, respectively, while LOQ was estimated as 0.097 µg/Kg and 1.17

231   µg/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

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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 µg/Kg and 99.70 µg/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 µg/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 µg/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 µg/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 µg/Kg while % R was between 101.4-103.9 % with RSD 2.4-2.7 % for

274 n=3.

14

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

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298   green solvents instead of classic organic solvents (Fabjanowicz, Kalinowska, Namieśnik, &

299   Płotka-Wasylka, 2018). Few examples of typical organic solvents are 1-hexyl-3-

300   methylimidazolium hexafluorophosphate [HMIM][PF$_6$, 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.

16

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.

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346 **References**

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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.
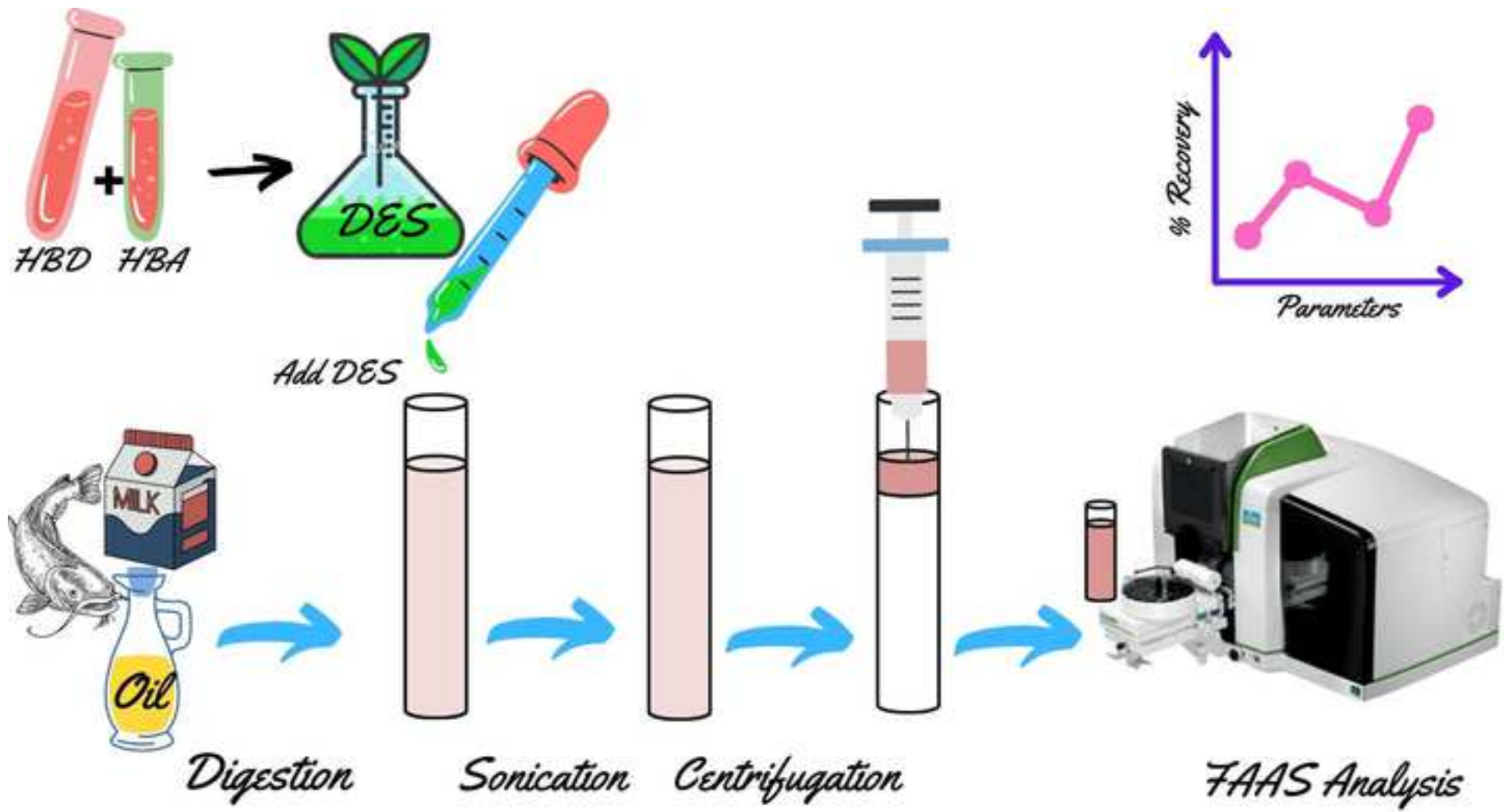
Tables

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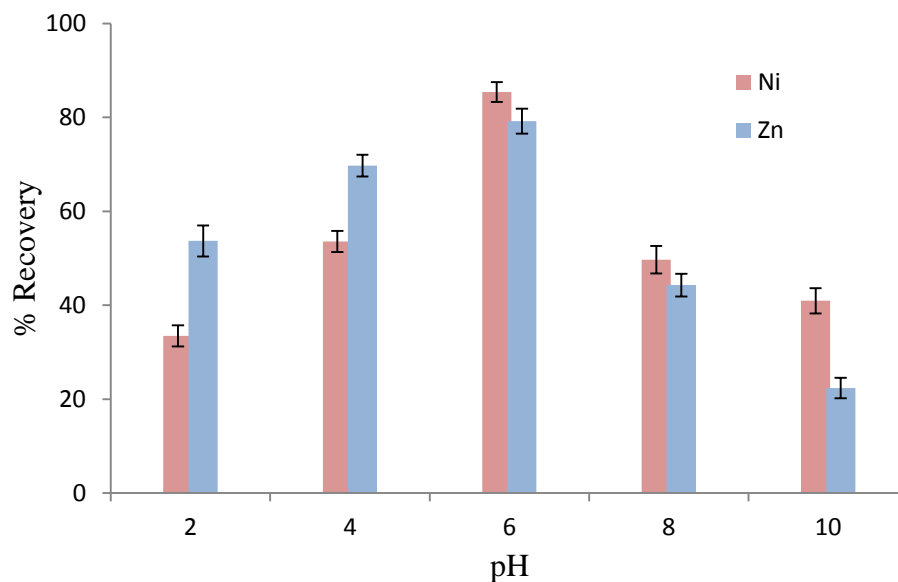
Fig 1. Optimization of pH. Sample volume: 15 mL, DES: 0.8 mL, Ni (II): 19.4 µg/Kg, Zn (II): 19.4 µg/Kg, Buffer volume: 1 mL, Ligand: 5.3 mg/L, Sonication: 2 min, Centrifugation: 2 min.
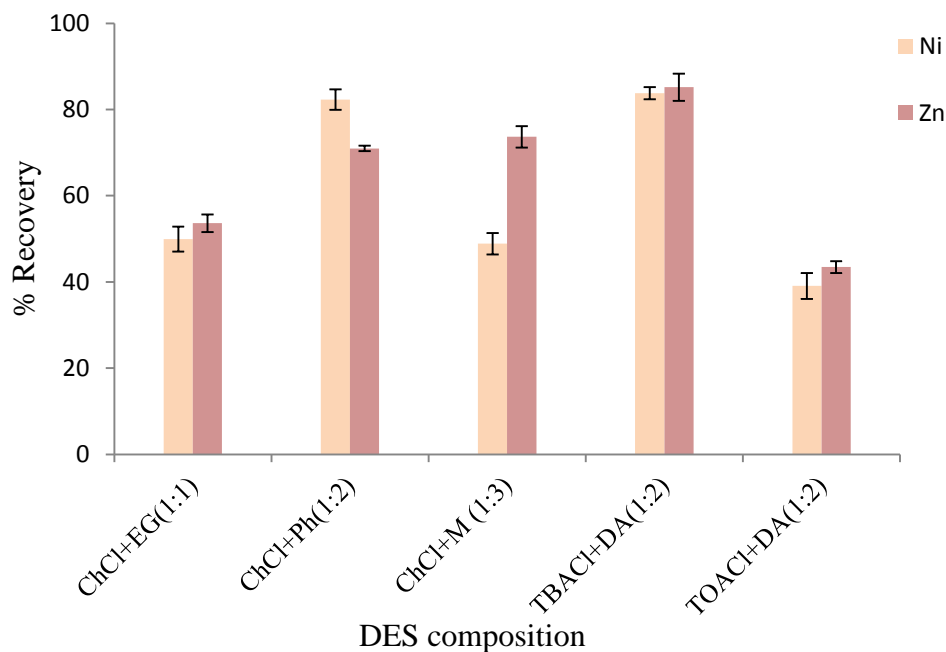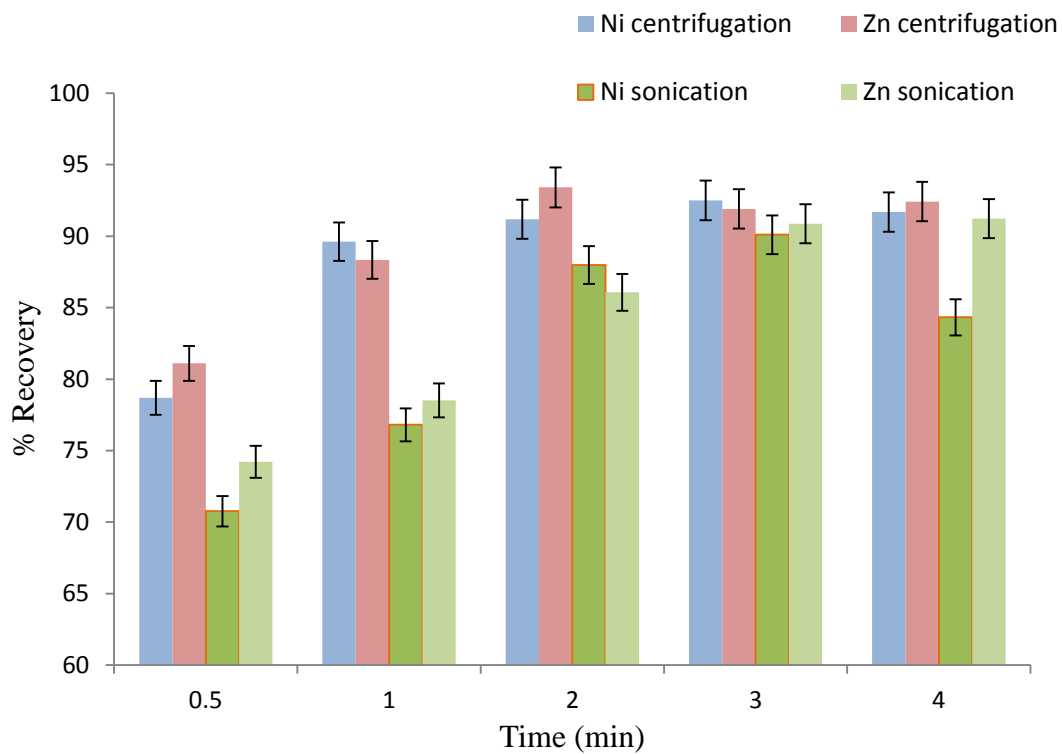

Fig 2. Selection of DES. Sample volume: 15 mL, DES: 0.8 mL, Ni (II): 19.4µg/Kg, Zn (II): 19.4µg/Kg, Buffer volume: 1 mL, Ligand: 5.3 mg/L, Sonication: 2 min, Centrifugation: 2 min.

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

Fazal Elahi[1], Muhammad Balal Arain[2], Wajid Ali[1], Hameed Ul Haq[3], Asif Khan[1], Faheem
Jan[4], Roberto Castro-Muñoz[3,5], Grzegorz Boczkaj[3,6,*]

[1]Department of Chemistry, Abdul Wali Khan University Mardan, 23200, KP, Pakistan.
Email: *wajidalikhan890@gmail.com*

[2]Department of Chemistry, University of Karachi, Karachi 75270, Pakistan. Email:
bilal_ku2004@yahoo.com

[3]Gdansk University of Technology, Faculty of Civil and Environmental Engineering,
Department of Sanitary Engineering, 80 – 233 Gdansk, G. Narutowicza St. 11/12, Poland. E-
mail: *grzegorz.boczkaj@pg.edu.pl / hameed.haq@pg.edu.pl*

[4]School of Materials Science and Engineering, University of Science and Technology of
China, Shenyang 110016, Liaoning, People's Republic of China

[5]Tecnologico de Monterrey, Campus Toluca, Avenida Eduardo Monroy, Cárdenas 2000 San
Antonio Buenavista, 50110 Toluca de Lerdo, Mexico

[6]EkoTech Center, Gdansk University of Technology, G. Narutowicza St. 11/12, 80-233
Gdansk, Poland

*Corresponding author: Dr Grzegorz Boczkaj, Assoc. Prof., PhD. Sc. Eng. Gdansk University
of Technology, Faculty of Civil and Environmental Engineering, Department of Sanitary
Engineering, 80 – 233 Gdansk, G. Narutowicza St. 11/12, Poland. Fax: (+48 58) 347-26-94;
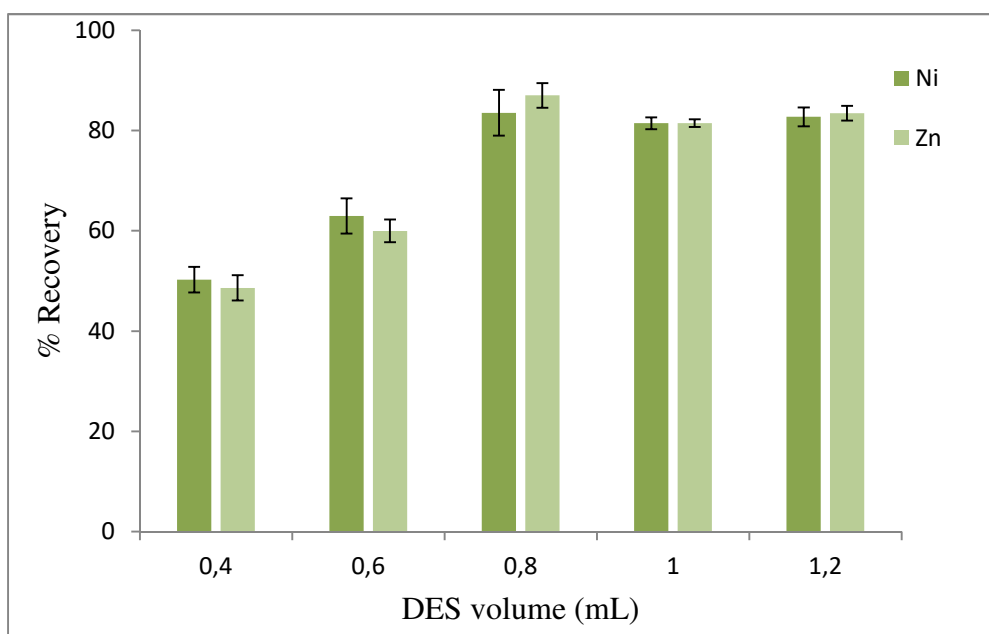Tel: (+48) 697970303; e-mail: grzegorz.boczkaj@gmail.com or grzegorz.boczkaj@pg.edu.pl*

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.
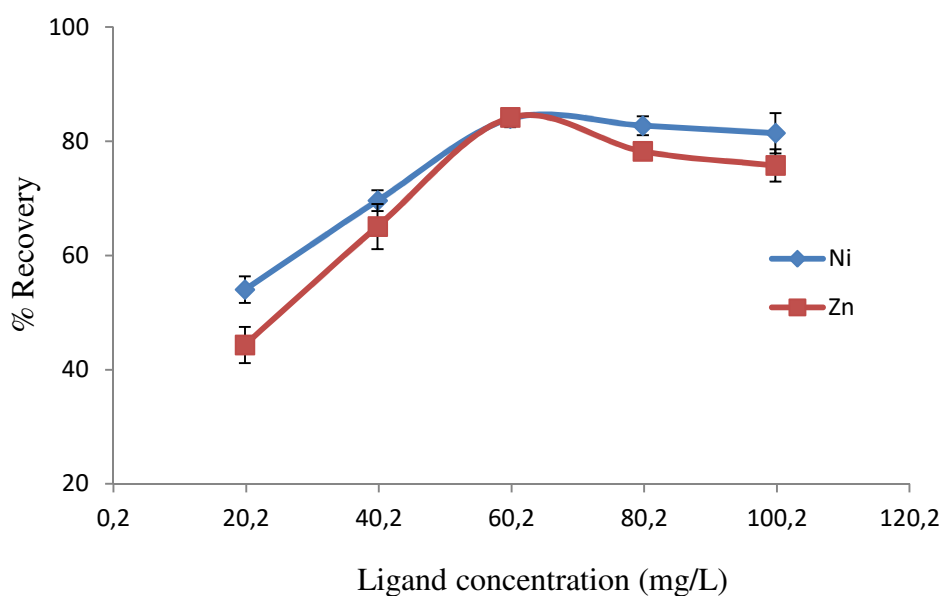

Fig S2. Optimization of chelating agent. Sample volume: 15 mL, DES: 0.8 mL, Ni (II): 19.4 µg/Kg, Zn (II): 19.4 µg/Kg, Buffer volume: 1 mL, Sonication: 2 min, Centrifugation: 2 min.
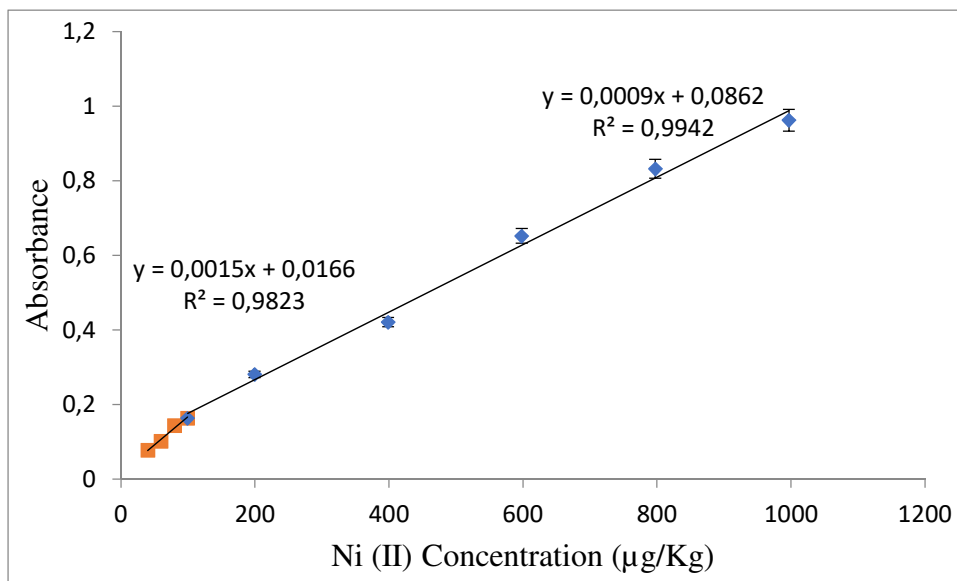
Fig S3. Calibration curve for Ni (II). Sample volume: 15 mL, DES: 0.8 mL, Zn (II): 19.4 µg/Kg, Buffer volume: 1 mL, Ligand: 5.3 mg/L, Sonication: 2 min, Centrifugation: 2 min.
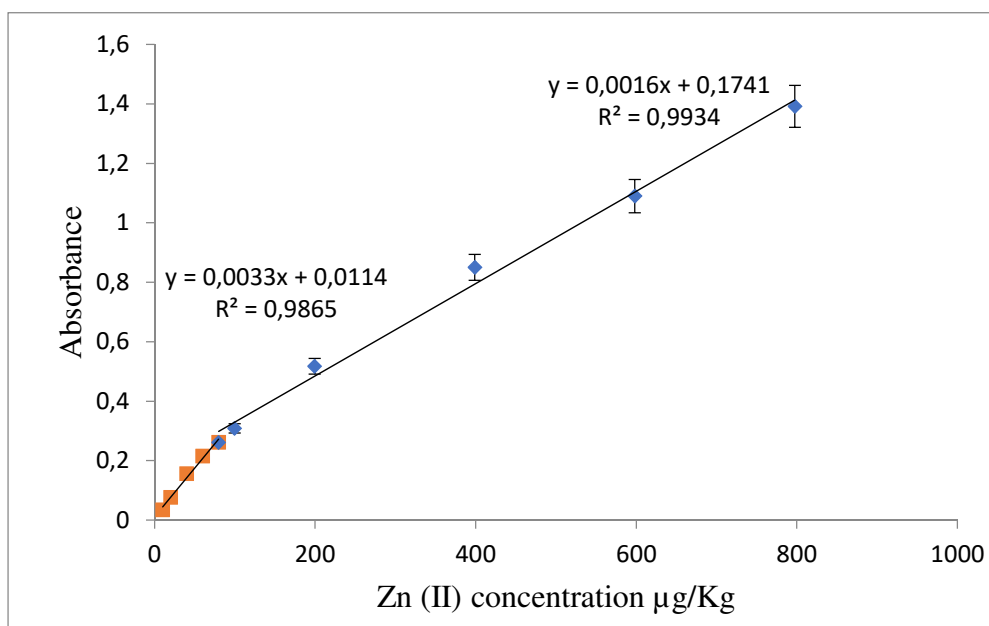

Fig S4. Calibration curve for Zn (II). Sample volume: 15 mL, DES: 0.8 mL, Ni (II): 19.4 µg/Kg, Buffer volume: 1 mL, Ligand: 5.3 mg/L, Sonication: 2 min, Centrifugation: 2 min.

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	% Recovery 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	210 µg/L	40 µg/L	0.0436 µg/Kg	0.2436 µg/Kg	(Trindade, Dantas, Lima, Ferreira, & Teixeira, 2015)
	vegetable oil			0.0438 µg/Kg	0.2497 µg/Kg	
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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