

Deep eutectic solvent (DES) with silver nanoparticles (Ag-NPs) based assay for analysis of lead (II) in edible oils

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Abstract

This paper presents an application of silver nanoparticles impregnated by Deep Eutectic Solvents (DES) as ultrasonication aided microextraction system for lead (II) determination in edible oils. The paper presents a systematic optimization of method parameters and examples of its application for analysis of real samples. Maximum recovery for lead (II) extraction was obtained for choline chloride and phenol with a 1:2 molar ratio. Optimum extraction conditions for 2g oil sample post-digested solution (10 mL, pH=2) require 1mL of Ag-nanoparticles solution (0,1mM) and per each 500 μ L of DES and tetrahydrofurane. The limit of detection (LOD) and quantification (LOQ) were 0.28 μ g/L and 0.94 μ g/L. The developed method covers the entire range of expected levels of lead concentration in oil samples -parts per billion levels to higher ones. This method is many folds faster (only 6.5 minutes/sample are needed) as well as more sensitive comparing to already reported methods.

Keywords:

Food analysis; Green chemistry; Lead (II) extraction; Edible oils; FAAS; sample preparation; analytical chemistry; food contaminants.

1 **1. Introduction**

2 Edible oils are the major contributors in daily food intake serving as a rich source of vitamin E
3 and mono-unsaturated fatty acids for human nutrition including the repair of worn out tissues and
4 new cells formation as well as a useful source of energy (Musa et al., 2012). Olive oil is present
5 in Mediterranean diet as its important component. Its presence in the diet was correlated with
6 lowering of the probability of several health issues such as coronary heart diseases and certain
7 kinds of cancer, such as colon and breast cancer (Zeiner et al., 2005). Regarding their use,
8 vegetable oils are used in different areas, such as kitchen and food processing, cosmetics,
9 pharmaceuticals, and chemical industries. The use of edible oil in foods is also associated with
10 trace elements, especially heavy metals since these oils are coming from natural resources (e.g.,
11 vegetable oils). Due to the metabolic role of metals and the possibility of identifying oil
12 adulteration, the determination of the toxic elements in edible oils is important (Dugo et al.,
13 2004). The metal content in vegetable oils is one of the most important quality criteria. In foods,
14 several organoleptic properties, such as taste, color, and smell, are negatively influenced by the
15 high levels of metal ions. Lead is a toxic element and WHO has identified lead (II) as 1 out of
16 the 10 chemicals of major public health concerns. U.S. Public Health Service established a daily
17 permissible lead intake for children of 300 $\mu\text{g}/\text{day}$ from all sources. Additionally, WHO
18 recommended a tolerable intake for adults of 600 $\mu\text{g}/\text{day}$ accumulative in all foods and drinks
19 (Sharrett et al., 1982). Since lead accumulates inside human tissues, even its low concentration in
20 foods is of great interest. Lead can also be deposited in the bones disturbing calcium metabolism.
21 For instance, inorganic lead (II) causes behavioral effects, mortality, worsening of renal role,
22 hypertension, reduced fertility and adversarial consequences of pregnancy, delay in sexual
23 maturation, dental impairments of cognitive development and intelligence, among others. Also,

24 lead exposure is considered one of the most serious and cumulative environmental pollutants
25 (Yusof & Ahmad, 2002). The dispersion of lead in the environment takes place through air and
26 water, as well as accumulated forms in food making it possible to reach human organisms and
27 cause extensive toxicity (Klaassen et al., 1986). Inorganic lead (II) ions can binds with the -SH
28 group in their proteins and act as an inhibitor of enzymes (Pourrut et al., 2011). Lead can also be
29 deposited in the bones disturbing calcium metabolism (Kovacs & Ward, 2020).

30 The determination of trace elements in edible oils is important because the metabolic role of
31 metals (Zeiner et al., 2005) as well as possibilities for adulteration detection and oil
32 characterization. Considering all the related drawbacks to lead exposure, there is a strong need
33 for determining its content in food systems. Herein, one of the “hot topics” in sample
34 preparation, which fulfills the requirements of green chemistry, is related to the application of
35 deep eutectic solvents (DESs). Recently, DESs are widely used for the extraction of
36 hydrocarbons, aromatic compounds, and other biomolecules (Haq et al., 2021a; Makoś,
37 Fernandes, et al., 2018). As reported elsewhere, DESs have similar features as ionic liquids;
38 however, DESs have some major advantages over ionic liquids including simplicity, low cost of
39 synthesis, easy biodegradation, non-toxicity, and less use of raw material (Fernandes et al., 2019;
40 Haq et al., 2021a). While various inorganic nanoparticles used in analytical methods have opened
41 new avenues for sensing, purification, and quantitative analysis. Therefore, the present work
42 presents a synergistic effect of the interaction of nanoparticles, thus silver nanoparticles (Ag-
43 NPs), combined with the advantages of DESs as green solvents for sample preparation. In this
44 way, a very sensitive and simple micro-extraction method based on Ag-NPs and an ultrasound-
45 assisted DES system was developed. The method was implemented in the quantitative analysis of



46 lead content in edible oils using flame atomic absorption spectroscopy (FAAS). To finalize, the
47 developed method is compared with other methods reported in the literature.

48

49 **2. Materials and methods**

50 **2.1. Chemicals and reagents**

51 Analytical grade reagents were used in this study. All chemicals (if not indicated differently)
52 were purchased from Sigma Aldrich. A stock standard (1000 mg/L) of lead (II) was prepared
53 from lead acetate, Regarding DES synthesis, choline chloride (ChCl), a quaternary ammonium
54 salt, was used as a hydrogen bond acceptor (HBA), while phenol (Ph) was used as hydrogen
55 bond donor (HBD). The Ag-NPs were synthesized using sodium tetrahydroborate (NaBH_4) and
56 silver nitrate (AgNO_3). The aprotic solvent, such as tetrahydrofuran (THF), was purchased from
57 Bangkok 10330, Thailand. Deionized water was obtained from HX 7000 SD M-Q Merck.

58

59 **2.2. Instrumentation**

60 Lead analysis was done through PerkinElmer AAnalyst 700 Model (Norwalk, CT, USA). A
61 centrifuge (model 2206A, China) was used for phase separation. A pH meter with a glass
62 electrode (Arwa AD 8000) was used for the pH adjustment of the sample. For the synthesis of
63 Ag-NPs, an ultrasonic bath (power sonic 405, China) was used.

64

65 **2.3. Preparation of silver nanoparticles**

66 The preparation of Ag-NPs was performed based on the procedure described by (Peng et al.,
67 2013). Briefly, a 100 mL solution of 0.1 mM of silver nitrate was prepared in a flat bottom flask
68 and then vibrantly shaken for mixing. After this, sodium borohydride (0.012 g) was added to the



69 solution. The resulting mixture was shaken for the next 30 minutes over a magnetic stirrer. The
70 prepared mixture remained in static conditions (without mixing) overnight in a dark place. The
71 resulting Ag-NPs suspension was stable for several months at ordinary temperatures without any
72 color change or aggregation. This suspension containing Ag-NPs was further used for the
73 extraction of lead (II) ions in edible oil. For each experiment, freshly prepared Ag-NPs were
74 used.

75 **2.4. Preparation of DESs and buffers**

76 Different combinations of DESs at different molar ratios were prepared using ChCl, like an HBA
77 compound. On the other hand, five types of HBD were tested, including malonic acid, ethylene
78 glycol, glycol, phenol, and urea, assaying their maximum metal recovery. These HBDs were
79 mixed with ChCl at molar ratios of 1:1, 1:2, and 1:3. All the DES solvents were prepared under
80 identical conditions. In a 50 mL polypropylene tube, the components of DES were mixed and
81 then heated at 60 °C in a water bath for 5 minutes. The mixtures were stirred with vortex for 2
82 min to make homogeneous mixtures and thus obtaining DESs. For each experiment, freshly
83 prepared DES solvents were prepared and directly used without any dilution or purification.

84 Buffer systems ranged from pH 2 to 10 were prepared. The buffer, having pH 2, was prepared by
85 dissolving phosphoric acid (purity 85%) and 3.118 g of disodium hydrogen phosphate in 100 mL
86 of deionized water. Similarly, the buffer, having pH 4, was prepared by dissolving 5.76 mL
87 acetic acid and 1.54 g sodium acetate in 100 mL deionized water; while the buffer of pH 6 was
88 prepared by dissolving 0.5 mL acetic acid (purity > 99%) and 11.7 g ammonium acetate in 100
89 mL deionized water. For buffer pH 8, 0.8 mL ammonium hydroxide was dissolved in 100 mL
90 deionized water with 10.7 g ammonium chloride, and the buffer pH 10 was prepared by
91 dissolving 20 mL deionized water, containing 4.5 g ammonium chloride, with 35 mL of 10 M



92 ammonia solution in 30 mL deionized water. Both solutions were previously mixed and diluted
93 up to 100 mL. 2 mL of buffer solution in the reagents mixture was found enough for maintaining
94 a stable pH value.

95 **2.5. Samples collection and preparation**

96 Three different commercial brands of edible oil were selected as a benchmark for determining
97 lead (II) concentration and validate the method. Here, sesame, olive, and canola oils were
98 acquired from Dalda oil, ghee industries Pvt limited, and Seasons oils Pvt limited, respectively.
99 Each sample was prepared independently in triplicate. For metal analysis, an oil sample (2.0 g)
100 was preliminarily digested by adding 2 mL HNO₃ and 1 mL H₂O₂ in a quartz tube and
101 subsequently heated at 120 °C. When the sample was near to dry, an additional 2 mL HNO₃ and
102 1 mL H₂O₂ were added to the sample. Finally, the sample was diluted with deionized water
103 making a final solution of 10mL. The resulting final solution was used for metal extraction.

104 **2.6. Analytical procedure for determination of lead in edible oil samples**

105 2 mL of metal standard solution (100 mg/L) was added to edible oil samples and digested. After
106 dilution, the total sample volume was 15 mL. For maintaining the pH, a buffer solution sample
107 (2 mL) was added to 1 mL of Ag-NPs (0.1 mM). The mixture was later stirred for 2 min and then
108 500µL of DES was added. The mixture was again stirred using a vortex for 1.5 min. Afterward,
109 THF (500 µL) was added to the sample solution; and the mixture was placed in an ultrasonic
110 bath for 1 min. The mixture was centrifuged at 3500 rpm for 2 min to obtain a complete phase
111 separation. After centrifugation, the DES layer was separated and obtained at the bottom. Such
112 extracted layer was diluted with ethanol up to 5 mL. Finally, HNO₃ was added to the solution
113 with a final concentration of 0.1M to avoid coagulation. Scheme of the developed procedure is
114 provided as graphic abstract figure.



115 **2.7. Quantitative analysis and quality assurance**

116 For quantitative analysis and quality assurance, different analytical parameters were determined
117 including the limit of detection (LOD) and limit of quantification (LOQ). Such parameters were
118 calculated following the JRC technical report on estimation of LOD and LOQ in foods (Wenzl et
119 al., 2016).

120 The LOD and LOD were determined using the blank samples. In other words, native samples
121 were used without spiking (Pseudo-blank). These samples were analyzed in ten replicates under
122 repeatability conditions. The variability of signal values, expressed as standard deviation, was
123 used for the estimation of LOD and LOQ. Both parameters were calculated, as below:

$$124 \quad \text{LOD} = 3 \times \frac{\text{SD}}{m} \quad (1)$$

$$125 \quad \text{LOQ} = 10 \times \frac{\text{SD}}{m} \quad (2)$$

126 where LOD and LOQ are the limits of detection and quantification, respectively; SD is the
127 standard deviation of blank signals, and m is the slope of the calibration curve.

128 For determining the accuracy of this new analytical method, the procedure M 1-92 from
129 American Oil Chemists' Society (AOCS) was followed, which is an updated procedure prepared
130 in collaboration with the International Union of Pure and Applied Chemistry (IUPAC) and the
131 International Organization for Standardization (ISO)(Liao et al., 2006).

132 Reliability and accuracy of the method, as the relative standard deviation (RSD), was determined
133 by applying six replicate determinations of 5 µg/L of analyte to deionized water, in which the %
134 RSD value of the recovery was found to be 4.5%.

135 The linearity of this new method was determined by adding a series of standards solutions from
136 the stock solution. Here, a defined amount of blank with a final concentration ranging from 5 to



137 150 µg/L was used according to the expected working range. Triplicate samples for each
138 concentration were analyzed.

139 **3. Results and discussion**

140 To introduce a green method for lead (II) extraction in edible oil, the suitable DES solvent was
141 preliminarily selected. It is reported in the literature that DES solvents are extensively used for
142 the extraction of biomolecules from natural products (Corrêa et al., 2021; Dwamena, 2019);
143 however, very few methods are available using DESs for the extraction of toxic metals in more
144 complex samples, such as edible oil. On the other hand, Ag-NPs are used for heavy metal uptake
145 (Sumesh et al., 2011). In this work, Ag-NPs along with adsorbing metal was extracted through
146 DES, which is a new approach to exploring new aspects of DES with Ag-NPs.

147 **3.1. Selection of DES system**

148 The extraction efficiency of the target analyte is mainly affected by the composition and the
149 nature of DES. The DES used for extraction must meet several requirements, such as high
150 affinity for analytes, liquid state at RT, no interference in analytical signal, different density than
151 that of water, high stability, and low solubility in an aqueous medium (Haq et al., 2021a; Makoś,
152 Przyjazny, et al., 2018). The DES system usually consists of two components, its 1st part consists
153 of quaternary ammonium salt while the second part consists of HBD. The mole ratio of the
154 quaternary ammonium salt and HBD has a significant impact on the applications of DES for
155 metal extraction. Therefore, different DES systems with different molar ratios were tested in this
156 work. ChCl was separately mixed with different types, including phenol, ethylene glycol, urea,
157 glycerol, and malonic acid, using different mole ratios. At this point, the optimization of the
158 HBD and HBA ratio is crucial for the applications of DES for extraction procedures. The
159 effectiveness of extraction generally decreases with an increase in HBD in the resulting DES. On

160 the other hand, mass transfer during extraction is increased by decreasing the density and
161 viscosity of DES (Hou et al., 2017; Razi Asrami et al., 2020). To determine the optimum molar
162 ratio DES, different HBD and HBA ratios were tested evaluating their maximum recovery. In
163 this way, a high recovery value was obtained with ChCl and phenol with a mole ratio of 1:2,
164 which was selected for further experiments. The results are illustrated in **Figure 1**.

165
166 **Figure 1.** Evaluation and selection of DES for maximum recovery of lead (II) ions. ChCl:
167 Chlorine chloride, M: Malonic acid, EG: Ethylene glycol, G: Glycerol, Ph: Phenol, U: Urea.

168
169 Phenol is an aromatic compound and capable of delocalizing negative charges across its entire
170 ring system. Considering its pK a value of 9.99, it is capable to form stronger hydrogen bonding
171 than other alcohols. The calculated interaction energies for clusters with similar hydrogen-
172 bonding patterns reveal that intermolecular interaction in phenol clusters is slightly stronger than
173 in water clusters. However, the fusion of phenol and water clusters leads to similar stability to
174 that of H₂O clusters (Parthasarathi et al., 2005). It is clear from the above mentioned facts, that
175 phenol presents a strong hydrogen bonding and fulfill the requirments of HBD in DES
176 formation. To evaluate the efficiency of different DESs, different HBDs were tested for Pb (II)
177 extraction. DES based on phenol as HBD was found advantageous for the extraction of Pb (II)
178 from edible oil samples.

179

180 **3.2. Optimization of extraction parameters**

181 3.2.1. Volume optimization of DESs



182 DES prepared with ChCl and phenol with molar ratio (1:2) was directly used without any further
183 purification or dilution. Since DES directly influences the extraction of lead (II), its optimum
184 concentration was determined for this specific procedure. Under the optimum condition, the DES
185 volume effect was studied ranging from 250 μL to 1250 μL for the recovery of lead (II). The
186 results indicate that, with a DES volume of 500 μL , a maximum recovery was obtained. Thus,
187 500 μL volume of DES was selected for onward studies. The results of this study are illustrated
188 in Figure S1.

189

190 3.2.2. pH effect

191 In adsorption/chelate formation and continuous extraction, pH plays a significant role (Haq et al.,
192 2021a). The solution pH is one of the parameters having a strong influence on the heavy-metal
193 ion uptake; this is due to the fact that the surface charge density of the adsorbent and the metallic
194 species depend on the pH (Haq et al., 2021b; Kubilay et al., 2007). Here, the lead (II) recoveries
195 were examined in the range of pH 2 to pH 8. The results revealed that a maximum recovery for
196 lead (II) was obtained at pH 2, as shown in Figure S2, indicating that a regular drop in the
197 recovery of lead (II) was found with a further increase after pH 2. Therefore, pH 2 was selected
198 for further studies.

199 At higher pH, Ag-NPs are more stable but less capable of metal adsorption, while at lower pH,
200 Ag-NPs are less stable but more capable of metal adsorption (Hosseini et al., 2016; Molleman &
201 Hiemstra, 2017). As the pH increases, the prolongation of the adsorbent surface decreased,
202 leading to a reduction in the electrostatic attraction between the metal ions species and the
203 adsorbent surface (Fernando & Zhou, 2019; Molleman & Hiemstra, 2017); this results in a
204 consequent decrease in the percentage removal of metal ions.



205

206 3.2.3. Optimization of THF addition

207 THF is an aprotic solvent, which was used in these studies to make phase separation easier. The
208 outcomes of this paper revealed that, while it indeed enhances the phase separation, it also
209 increases the % recovery of the analyte. These aprotic solvents tend to interact more with water
210 than DES. After interaction with THF molecules, water molecules decline their interaction with
211 DES, resulting in their self-aggregation and separation. In other words, THF acts as a
212 dehydrating agent. The most plausible mechanism of DESs self-aggregation involves π - π
213 overlap between the aromatic ring of phenol, followed by hydrogen bonding between functional
214 groups of DES and other charge transfer interactions (Haq et al., 2021a; Khezeli et al., 2015).

215 The volume effect of THF ranged from 250 μ L to 1500 μ L was studied for the % recovery of
216 lead (II) (see Figure S3), where the maximum recovery was obtained with a THF volume of 500
217 μ L. For further studies, a THF volume of 500 μ L was carefully used as an optimum value.

218

219 3.2.4. Effect of Ag-NPs concentration

220 For the separation of different metals, Ag-NPs have been used as a good adsorbent in the field of
221 research. Here, the excess of Ag-NPs is needed to provide maximum surface area for metal
222 adsorption. The mechanism of metal ion interaction with Ag-NPs is described and detailed in
223 section 3.3. In this study, the effect of the amount of Ag-NPs (0.1mM) was studied in the range
224 of 250 μ L to 2000 μ L. The results showed that the lead (II) signal response was increasing with
225 the increase of the amount of Ag-NPs up to 500 μ L and it remained constant. Therefore, 500 μ L
226 of Ag-NPs has been selected as the optimum value for lead (II) determination. The effect of Ag-
227 NPs on lead (II) recovery is illustrated in figure S4.

228

229 3.2.5. Effect of ultra-sonication time

230 The efficiency of the extraction process was greatly affected by ultra-sonication time. Our study
231 suggests that both THF amount and ultra-sonication waves caused the aggregation of the DES in
232 the aqueous phase and the reduction in extraction time. In general, it was noticed that the
233 conversion of these droplets of DES into tiny droplets contributed to reaching the equilibrium
234 state, resulting in increased extraction efficiency. At constant experimental conditions, the effect
235 of ultra-sonication time was studied from 1 to 5 min at 25 °C (see Figure S5). When the ultra-
236 sonication time was extended to 2 min, the extraction efficiency was stabilized. Therefore, 2
237 minutes of ultra-sonication time was selected for the subsequent experiments.

238 **3.3. Development of quantitative method/validation**

239 3.3.1. Interference study of different ions

240 To analyze lead content in food and environmental samples, an important issue relates to the
241 presence of interfering ions which could affect to some extent the analytical signal. The goal
242 during the development of this new method was to select the selective extraction conditions of
243 (Ooi & Ng, 2018), back extraction methods (Arpa & Aridaşir, 2018), solid-phase extraction
244 (Rahnama & Ghadiri, 2015), membrane techniques (Mesli & Belkhouche, 2018), adsorption
245 methods (Mahmoud et al., 2010), flow-injection on-line adsorption (Salonia et al., 1999) and co-
246 precipitation methods (Komjarova & Blust, 2006). The performance of the developed DES
247 impregnated Ag-NPs method was compared with liquid-phase microextraction methods, which
248 were used for lead (II) determination in edible oil, as shown in **Table 1**. Among all the reported
249 methods, cloud point extraction is the most dominantly used technique for lead extraction due to
250 its low cost, high enrichment factor, environment-friendly and minimal cost (Babae et al.,



251 2019). However, these methods are associated with some critical issues, such as a long time of
252 the procedure related to heating and centrifugation stages. Even though these methods achieve
253 good recovery after multi-cycle experiments (Galbeiro et al., 2014), while our proposed method
254 is quicker and provides a very good recovery obtained in a single cycle. The time for the
255 extraction procedure after digestion was estimated for the different reported methods in the
256 literature, which is compiled in **Table 1**. The estimated time for our extraction procedure was as
257 short as 6.5 minutes while estimated time values for other methods were 35 min (Coelho et al.,
258 2008), 87 min (Blanchet-Chouinard & Larivière, 2018), 217 min (Gouda & Zordok, 2018), 35
259 min (Citak & Tuzen, 2012), 60 min (Kazi et al., 2012), 16 min (Rahnama & Ghadiri, 2015) and
260 40min (Citak & Tuzen, 2010). At this point, it is noted that the newly proposed method is many
261 folds faster than the already reported methods. Application of AG-NPs with DES as extractant
262 with addition of THF provides further improvement also in the field of lead extraction based on
263 DESs in comparison to already published approaches (Karmini et al. 2016; Soyлак & Koksал,
264 2019; Habila et al. 2020.). Preliminary studies for this paper indicated that in case of sole use of
265 DES about 30 minutes of extraction is needed for acceptable recovery. Nanoparticle scale silver
266 reveals to provide rapid extraction. It follows from very fast kinetics of sorption. The
267 performance of this method is quite good due to its low LOD and LOQ values and high pre-
268 concentration factor. This method opens a new aspect in the application of DES coupled with
269 Ag-NPs for lead extraction.

270 **Table 1.** Comparative study of the new method with reported methods in the literature.
271



272 3.3.4. Application of the method for the analysis of real samples

273 Since edible oils are an essential part of the human diet, the developed procedure was applied to
274 the analysis of several categories of edible oils, such as sesame, olive, and canola oil obtained
275 from commercial brands of the district Mardan, Pakistan. In this approach, the standard addition
276 method, which is commonly used for AAS, was used. Known concentrations of lead (II) were
277 gradually added to real samples and percent recovery was determined in each case. The results of
278 the recovery performance are presented in **Table 2**. The recovery for real samples was between
279 97-107%. Since the concentration in real samples was revealed to be much higher than the limit
280 established by this method, the samples were diluted with methanol right after extraction. In
281 these experiments, three different types of edible oils were tested for lead (II) concentration,
282 exhibiting a lead (II) concentration of 1.21 mg/L, 1.26 mg/L and 1.19 mg/L for olive, sesame, and
283 canola oil, respectively. This method was sensitive and valid for determining very low
284 concentrations. To summarize, it can be stated that the performed experiments confirmed that the
285 developed procedure is reliable for the determination of lead (II) in different commercial edible
286 oil samples.

287 lead from the matrix, which was fully obtained making this method highly selective. DESs are
288 highly selective in extraction procedures (Rad et al., 2019); however, in this case, preliminary
289 studies revealed that additional selectivity must be provided. Thus, the application of Ag-NPs
290 was further studied to assure adequate accuracy of the quantitative analysis; for this, a standard
291 addition method was used.

292 To determine the effect of external ions on lead (II) extraction, known concentrations of different
293 ions were added in separate sets of the experiment. The interference of $\text{Na}^+(\text{NaNO}_3)$, $\text{K}^+(\text{KCl})$,
294 $\text{Mg}^{2+}(\text{Mg}(\text{NO}_3)_2)$, $\text{Cd}^{2+}(\text{CdCl}_2)$, $\text{Zn}^{2+}(\text{ZnNO}_3)_2$, $\text{Co}^{2+}(\text{CoCl}_2)$, $\text{Ni}^{2+}(\text{NiCl}_2)$, $\text{SO}_4^{2-}(\text{K}_2\text{SO}_4)$,



295 $Pb^{2+}(PbCl_2)$ was investigated. The recovery of the lead (II) in presence of interfering ions was
296 found to be higher than 90%. These results are compiled in supporting information (Table S1).
297 Importantly, it was proved that the interfering ions have no significant effect on the lead (II)
298 extraction/analysis and the proposed microextraction method fits the purpose.

299 3.3.2. Analytical performance of the method

300 According to the methodology described in section 2.7, LOD and LOQ values were calculated
301 as 0.28 and 0.94 $\mu\text{g/L}$, respectively. **Figure 2** shows the calibration curve, which has an
302 equation as follows: $Y = 0.0037x + 0.0026$, displaying an acceptable coefficient of
303 determination (R^2) of 0.9931. The linearity range was established from 5 to 140 $\mu\text{g/L}$. The
304 relative standard deviation calculated for six repetitions of lead (II) analysis (at a concentration
305 level of 5 $\mu\text{g/L}$) was 4.5%, showing the repeatability and reproducibility of the developed
306 method.

307

308 **Figure 2.** Calibration curve of lead (II) at different concentrations.

309

310 3.3.3. Comparison with other methods

311 Many methods have been reported in the literature for lead extraction and analysis, including
312 cloud point extraction (Blanchet-Chouinard & Larivière, 2018), liquid-liquid microextraction

313

314 **Table 2.** Determination of lead in edible oil samples.

315

316 Several papers recently reported presence of lead ions in oil samples. For example, it was
317 reported as lead (II) concentrations of 0.06-0.21 mg/Kg, 0.08-1.12 mg/Kg, and 0.06-0.08 mg/Kg

318 for rapeseed, soybean, and linseed oil, respectively (Szyczewski et al., 2016). In other study lead
319 (II) concentrations of 6-15 $\mu\text{g/Kg}$, 7.3-21 $\mu\text{g/Kg}$, and 3.4-16 $\mu\text{g/Kg}$ were reported for canola,
320 corn, and soybean oil, respectively (Allen et al., 1998). In vegetable hydrogenated oil, a lead
321 concentration of 15.92 $\mu\text{g/L}$ was reported by (Abbasi et al., 2009), while (Ng, 2010) documented
322 0.71 mg/Kg of lead in black olive oil and 0.75 mg/Kg in green olive oil. In more recent work,
323 (Zhuravlev et al., 2015) evaluated and determined a lead concentration in different types of oil,
324 e.g., 0.006 mg/Kg in corn oil, 0.014 mg/Kg in olive oil, 0.016 mg/Kg in refined sunflower oil,
325 0.062 mg/Kg sunflower oil, and 0.027 mg/Kg in soybean oil. It can be seen that the range of
326 reported values strongly differs in the type and origin of oil samples. It is worth mentioning that
327 this developed method covers the entire range of expected levels of lead concentration from low
328 parts per billion levels to higher ones after dilution. The modern instruments are equipped with
329 an automatic module for sample dilution; therefore, this approach fully fits the purpose of lead
330 determination in edible oils.

331 **4. Conclusions**

332 In this new method, lead (II) ions were efficiently extracted and determined from different
333 commercial edible oil products. Deep eutectic solvents based on ChCl and phenol with a 1:2
334 molar ratio demonstrated a maximum recovery for lead (II) extraction. It was concluded that Ag
335 nano particles can adsorb Pb (II) and facilitate the Pb (II) mobility from aqueous phase to DES
336 phase. This stage is very fast, as nanoparticles ensure very good kinetics of sorption.
337 The sonication process accelerates the formation of nano-sized fine droplets and as a result
338 increases the contact surface area between the extracting solvent (DES) and Pb(II). The
339 optimized method implies simplicity, ease of operation, short extraction times, and high
340 enrichment factor. Quantitative recovery (97-105%) from spiked samples demonstrated the



341 suitability of the optimized method for the quality control of the analyzed samples. The LOD and
342 LOQ were 0.28 $\mu\text{g/L}$ and 0.94 $\mu\text{g/L}$, respectively with an RSD value of 4.5% and a pre-
343 concentration factor of 25 which are comparatively improved comparing other methods reported
344 in the literature. The optimized procedure is green, simple and requires a small volume of
345 extraction solvent. The comparison of the developed method with already-existing analytical
346 procedures confirmed its advantages, such as shortening the time of analysis and sensitivity. To
347 finalize, the outcomes of this new assay are comparable to those obtained by the ICP-MS
348 technique with more simplicity, better accuracy, high enrichment factor, less time-consuming,
349 and environment-friendly. Further improvements in method development, also in green
350 chemistry aspects, should focus on minization of the scale of procedure. This studies confirmed
351 that the optimized procedure is perfectly useful for routine analysis of food especially edible oil
352 samples containing traces of Pb(II) ions.

353

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Conflict of interest

The authors declare no conflict of interest.

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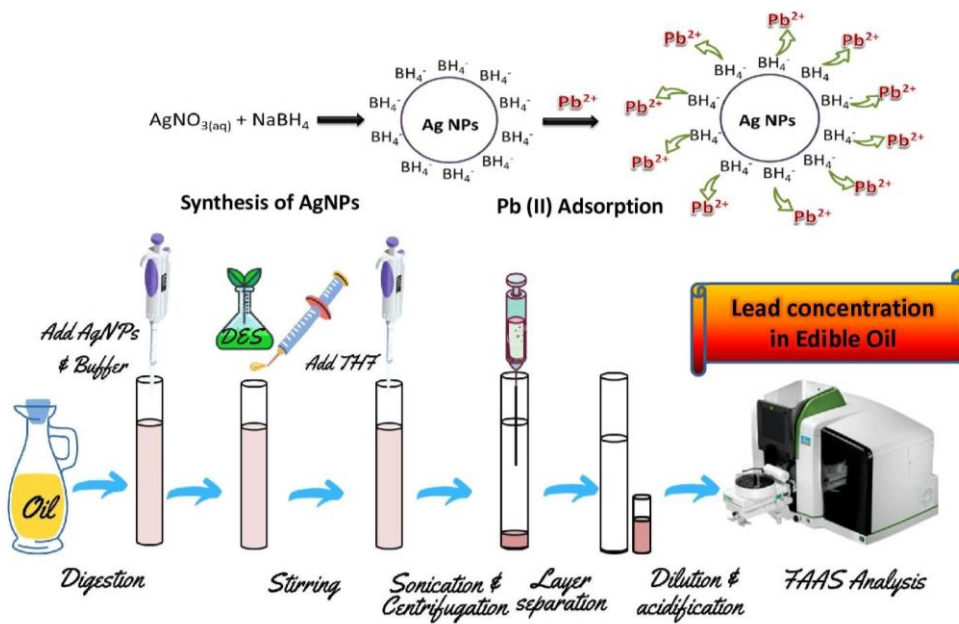
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Graphic abstract

New version:



Figures

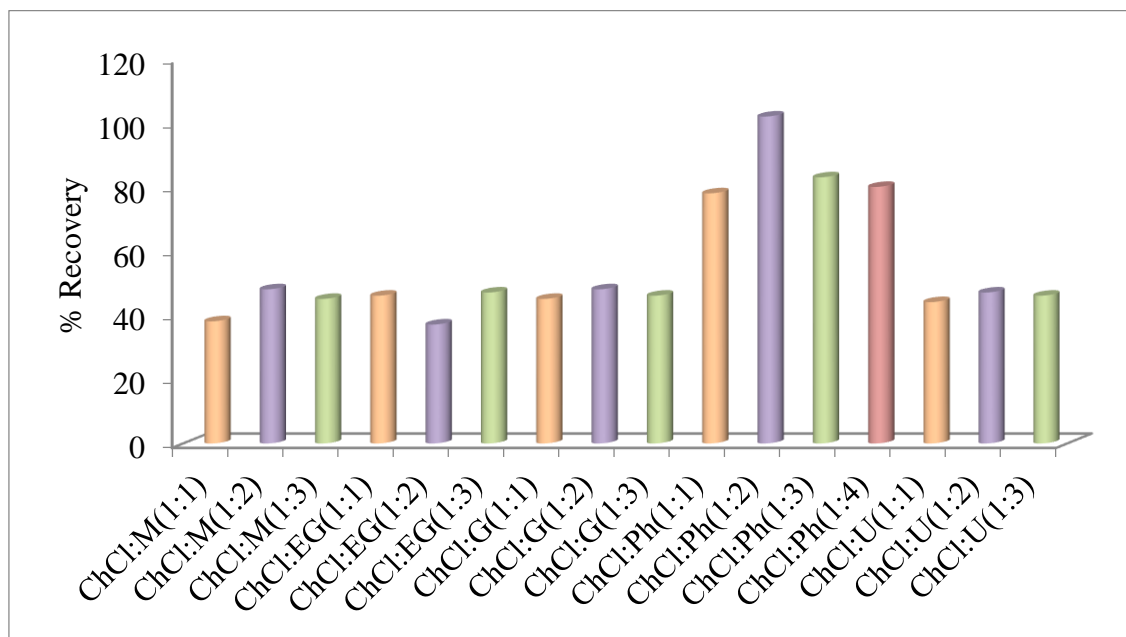


Figure 1. Evaluation and selection of DES for maximum recovery of lead (II) ions. ChCl: Chlorine chloride, M: Malonic acid, EG: Ethylene glycol, G: Glycerol, Ph: Phenol, U: Urea.

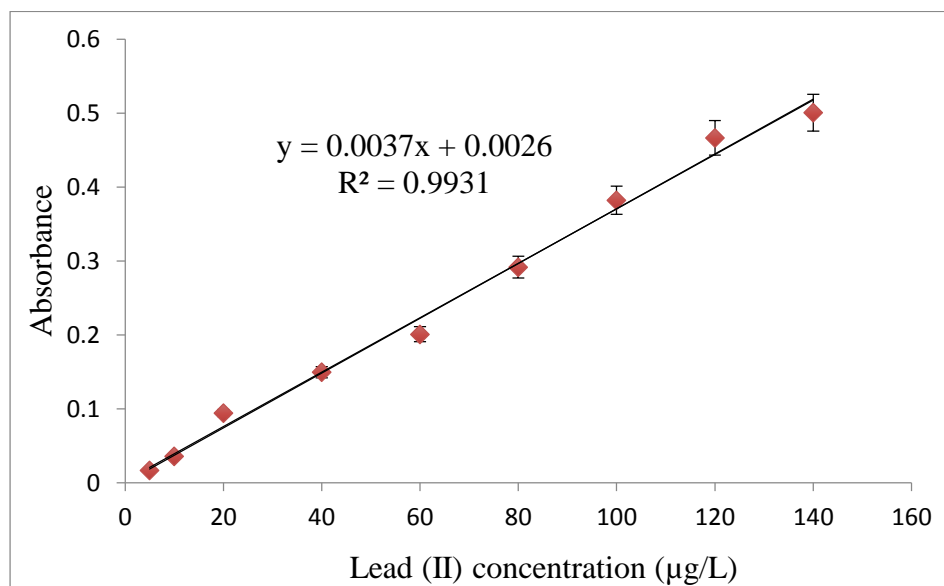


Figure 2. Calibration curve of lead (II) at different concentrations.

Tables

Table 1. Comparative study of the new method with reported methods in the literature.

Method	Detection techniques	LOD (µg/L)	LOQ (µg/L)	Linearity (µg/L)	RSD (%)	Estimated time of sample preparation* (minutes)	References
Cloud point extraction	TS-FF-AAS	0.43	1.44	5-50	8.7	>35	(Coelho et al., 2008)
Cloud point extraction	ICP-OES	0.8	2.60	---	13	>67	(Blanchet-Chouinard & Larivière, 2018)
Solid phase extraction	FAAS	0.2	1	1-60	3.2	>180	(Gouda & Zordok, 2018)
Cloud point extraction	FAAS	1.33	6.65	20-320	3.06	>35	(Citak & Tuzen, 2012)
Cloud point extraction	FAAS	0.26	0.86	20-100	1.88	>60	(Kazi et al., 2012)
SADSPE	FAAS	1.3	4.30	4-100	5	>21	(Rahnama & Ghadiri, 2015)
Cloud point extraction	FAAS	3.42	11.31	5-10	4.8	>45	(Citak & Tuzen, 2010)
DES-based extraction	FAAS	2.4	7.9	5-60	0.9-4.3	37	(Soylak & Koksak, 2019)
DES-based extraction with Fe ₃ O ₄ sorbent	FAAS	0.4	2	2-250	1.8	>75	(Karmini et al. 2016)
DES-based extraction with Ag-NPs	FAAS	0.28	0.92	5-140	4.5	6.5	<i>Present work</i>

*after digestion

SADSPE; Solvent-assisted dispersive solid-phase extraction, FAAS; Flame atomic absorption spectroscopy, ICP-OES; Inductively coupled plasma optical emission spectroscopy, TS-FF-AAS; Thermo spray flame furnace atomic absorption, DES; Deep eutectic solvent

Table 2. Determination of lead (II) in edible oil samples.

Samples	Lead spiked ($\mu\text{g/mL}$)	Lead found ($\mu\text{g/mL}$)	% Recovery
Olive oil	--	1.21 ± 0.01	-----
	2.5	3.90 ± 0.02	105.12
	5.0	6.64 ± 0.02	106.92
Sesame oil	--	1.26 ± 0.03	-----
	2.5	3.65 ± 0.017	97.07
	5.0	6.11 ± 0.04	97.60
Canola oil	--	1.19 ± 0.01	-----
	2.5	3.62 ± 0.01	98.10
	5.0	6.19 ± 0.04	100.00



Deep eutectic solvent (DES) with silver nanoparticles (Ag-NPs) based assay for analysis of lead (II) in edible oils

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Abstract

This paper presents an application of silver nanoparticles impregnated by Deep Eutectic Solvents (DES) as ultrasonication aided microextraction system for lead (II) determination in edible oils. The paper presents a systematic optimization of method parameters and examples of its application for analysis of real samples. Maximum recovery for lead (II) extraction was obtained for choline chloride and phenol with a 1:2 molar ratio. Optimum extraction conditions for 2g oil sample post-digested solution (10 mL, pH=2) require 1mL of Ag-nanoparticles solution (0,1mM) and per each 500 μ L of DES and tetrahydrofurane. The limit of detection (LOD) and quantification (LOQ) were 0.28 μ g/L and 0.94 μ g/L. The developed method covers the entire range of expected levels of lead concentration in oil samples -parts per billion levels to higher ones. This method is many folds faster (only 6.5 minutes/sample are needed) as well as more sensitive comparing to already reported methods.

Keywords:

Food analysis; Green chemistry; Lead (II) extraction; Edible oils; FAAS; sample preparation; analytical chemistry; food contaminants.

1 **1. Introduction**

2 Edible oils are the major contributors in daily food intake serving as a rich source of vitamin E
3 and mono-unsaturated fatty acids for human nutrition including the repair of worn out tissues and
4 new cells formation as well as a useful source of energy (Musa et al., 2012). Olive oil is present
5 in Mediterranean diet as its important component. Its presence in the diet was correlated with
6 lowering of the probability of several health issues such as coronary heart diseases and certain
7 kinds of cancer, such as colon and breast cancer (Zeiner et al., 2005). Regarding their use,
8 vegetable oils are used in different areas, such as kitchen and food processing, cosmetics,
9 pharmaceuticals, and chemical industries. The use of edible oil in foods is also associated with
10 trace elements, especially heavy metals since these oils are coming from natural resources (e.g.,
11 vegetable oils). Due to the metabolic role of metals and the possibility of identifying oil
12 adulteration, the determination of the toxic elements in edible oils is important (Dugo et al.,
13 2004). The metal content in vegetable oils is one of the most important quality criteria. In foods,
14 several organoleptic properties, such as taste, color, and smell, are negatively influenced by the
15 high levels of metal ions. Lead is a toxic element and WHO has identified lead (II) as 1 out of
16 the 10 chemicals of major public health concerns. U.S. Public Health Service established a daily
17 permissible lead intake for children of 300 $\mu\text{g}/\text{day}$ from all sources. Additionally, WHO
18 recommended a tolerable intake for adults of 600 $\mu\text{g}/\text{day}$ accumulative in all foods and drinks
19 (Sharrett et al., 1982). Since lead accumulates inside human tissues, even its low concentration in
20 foods is of great interest. Lead can also be deposited in the bones disturbing calcium metabolism.
21 For instance, inorganic lead (II) causes behavioral effects, mortality, worsening of renal role,
22 hypertension, reduced fertility and adversarial consequences of pregnancy, delay in sexual
23 maturation, dental impairments of cognitive development and intelligence, among others. Also,

24 lead exposure is considered one of the most serious and cumulative environmental pollutants
25 (Yusof & Ahmad, 2002). The dispersion of lead in the environment takes place through air and
26 water, as well as accumulated forms in food making it possible to reach human organisms and
27 cause extensive toxicity (Klaassen et al., 1986). Inorganic lead (II) ions can binds with the -SH
28 group in their proteins and act as an inhibitor of enzymes (Pourrut et al., 2011). Lead can also be
29 deposited in the bones disturbing calcium metabolism (Kovacs & Ward, 2020).

30 The determination of trace elements in edible oils is important because the metabolic role of
31 metals (Zeiner et al., 2005) as well as possibilities for adulteration detection and oil
32 characterization. Considering all the related drawbacks to lead exposure, there is a strong need
33 for determining its content in food systems. Herein, one of the “hot topics” in sample
34 preparation, which fulfills the requirements of green chemistry, is related to the application of
35 deep eutectic solvents (DESs). Recently, DESs are widely used for the extraction of
36 hydrocarbons, aromatic compounds, and other biomolecules (Haq et al., 2021a; Makoś,
37 Fernandes, et al., 2018). As reported elsewhere, DESs have similar features as ionic liquids;
38 however, DESs have some major advantages over ionic liquids including simplicity, low cost of
39 synthesis, easy biodegradation, non-toxicity, and less use of raw material (Fernandes et al., 2019;
40 Haq et al., 2021a). While various inorganic nanoparticles used in analytical methods have opened
41 new avenues for sensing, purification, and quantitative analysis. Therefore, the present work
42 presents a synergistic effect of the interaction of nanoparticles, thus silver nanoparticles (Ag-
43 NPs), combined with the advantages of DESs as green solvents for sample preparation. In this
44 way, a very sensitive and simple micro-extraction method based on Ag-NPs and an ultrasound-
45 assisted DES system was developed. The method was implemented in the quantitative analysis of



46 lead content in edible oils using flame atomic absorption spectroscopy (FAAS). To finalize, the
47 developed method is compared with other methods reported in the literature.

48

49 **2. Materials and methods**

50 **2.1. Chemicals and reagents**

51 Analytical grade reagents were used in this study. All chemicals (if not indicated differently)
52 were purchased from Sigma Aldrich. A stock standard (1000 mg/L) of lead (II) was prepared
53 from lead acetate, Regarding DES synthesis, choline chloride (ChCl), a quaternary ammonium
54 salt, was used as a hydrogen bond acceptor (HBA), while phenol (Ph) was used as hydrogen
55 bond donor (HBD). The Ag-NPs were synthesized using sodium tetrahydroborate (NaBH_4) and
56 silver nitrate (AgNO_3). The aprotic solvent, such as tetrahydrofuran (THF), was purchased from
57 Bangkok 10330, Thailand. Deionized water was obtained from HX 7000 SD M-Q Merck.

58

59 **2.2. Instrumentation**

60 Lead analysis was done through PerkinElmer AAnalyst 700 Model (Norwalk, CT, USA). A
61 centrifuge (model 2206A, China) was used for phase separation. A pH meter with a glass
62 electrode (Arwa AD 8000) was used for the pH adjustment of the sample. For the synthesis of
63 Ag-NPs, an ultrasonic bath (power sonic 405, China) was used.

64

65 **2.3. Preparation of silver nanoparticles**

66 The preparation of Ag-NPs was performed based on the procedure described by (Peng et al.,
67 2013). Briefly, a 100 mL solution of 0.1 mM of silver nitrate was prepared in a flat bottom flask
68 and then vibrantly shaken for mixing. After this, sodium borohydride (0.012 g) was added to the



69 solution. The resulting mixture was shaken for the next 30 minutes over a magnetic stirrer. The
70 prepared mixture remained in static conditions (without mixing) overnight in a dark place. The
71 resulting Ag-NPs suspension was stable for several months at ordinary temperatures without any
72 color change or aggregation. This suspension containing Ag-NPs was further used for the
73 extraction of lead (II) ions in edible oil. For each experiment, freshly prepared Ag-NPs were
74 used.

75 **2.4. Preparation of DESs and buffers**

76 Different combinations of DESs at different molar ratios were prepared using ChCl, like an HBA
77 compound. On the other hand, five types of HBD were tested, including malonic acid, ethylene
78 glycol, glycol, phenol, and urea, assaying their maximum metal recovery. These HBDs were
79 mixed with ChCl at molar ratios of 1:1, 1:2, and 1:3. All the DES solvents were prepared under
80 identical conditions. In a 50 mL polypropylene tube, the components of DES were mixed and
81 then heated at 60 °C in a water bath for 5 minutes. The mixtures were stirred with vortex for 2
82 min to make homogeneous mixtures and thus obtaining DESs. For each experiment, freshly
83 prepared DES solvents were prepared and directly used without any dilution or purification.

84 Buffer systems ranged from pH 2 to 10 were prepared. The buffer, having pH 2, was prepared by
85 dissolving phosphoric acid (purity 85%) and 3.118 g of disodium hydrogen phosphate in 100 mL
86 of deionized water. Similarly, the buffer, having pH 4, was prepared by dissolving 5.76 mL
87 acetic acid and 1.54 g sodium acetate in 100 mL deionized water; while the buffer of pH 6 was
88 prepared by dissolving 0.5 mL acetic acid (purity > 99%) and 11.7 g ammonium acetate in 100
89 mL deionized water. For buffer pH 8, 0.8 mL ammonium hydroxide was dissolved in 100 mL
90 deionized water with 10.7 g ammonium chloride, and the buffer pH 10 was prepared by
91 dissolving 20 mL deionized water, containing 4.5 g ammonium chloride, with 35 mL of 10 M



92 ammonia solution in 30 mL deionized water. Both solutions were previously mixed and diluted
93 up to 100 mL. 2 mL of buffer solution in the reagents mixture was found enough for maintaining
94 a stable pH value.

95 **2.5. Samples collection and preparation**

96 Three different commercial brands of edible oil were selected as a benchmark for determining
97 lead (II) concentration and validate the method. Here, sesame, olive, and canola oils were
98 acquired from Dalda oil, ghee industries Pvt limited, and Seasons oils Pvt limited, respectively.
99 Each sample was prepared independently in triplicate. For metal analysis, an oil sample (2.0 g)
100 was preliminarily digested by adding 2 mL HNO₃ and 1 mL H₂O₂ in a quartz tube and
101 subsequently heated at 120 °C. When the sample was near to dry, an additional 2 mL HNO₃ and
102 1 mL H₂O₂ were added to the sample. Finally, the sample was diluted with deionized water
103 making a final solution of 10mL. The resulting final solution was used for metal extraction.

104 **2.6. Analytical procedure for determination of lead in edible oil samples**

105 2 mL of metal standard solution (100 mg/L) was added to edible oil samples and digested. After
106 dilution, the total sample volume was 15 mL. For maintaining the pH, a buffer solution sample
107 (2 mL) was added to 1 mL of Ag-NPs (0.1 mM). The mixture was later stirred for 2 min and then
108 500µL of DES was added. The mixture was again stirred using a vortex for 1.5 min. Afterward,
109 THF (500 µL) was added to the sample solution; and the mixture was placed in an ultrasonic
110 bath for 1 min. The mixture was centrifuged at 3500 rpm for 2 min to obtain a complete phase
111 separation. After centrifugation, the DES layer was separated and obtained at the bottom. Such
112 extracted layer was diluted with ethanol up to 5 mL. Finally, HNO₃ was added to the solution
113 with a final concentration of 0.1M to avoid coagulation. Scheme of the developed procedure is
114 provided as graphic abstract figure.



115 **2.7. Quantitative analysis and quality assurance**

116 For quantitative analysis and quality assurance, different analytical parameters were determined
117 including the limit of detection (LOD) and limit of quantification (LOQ). Such parameters were
118 calculated following the JRC technical report on estimation of LOD and LOQ in foods (Wenzl et
119 al., 2016).

120 The LOD and LOD were determined using the blank samples. In other words, native samples
121 were used without spiking (Pseudo-blank). These samples were analyzed in ten replicates under
122 repeatability conditions. The variability of signal values, expressed as standard deviation, was
123 used for the estimation of LOD and LOQ. Both parameters were calculated, as below:

$$124 \quad \text{LOD} = 3 \times \frac{\text{SD}}{m} \quad (1)$$

$$125 \quad \text{LOQ} = 10 \times \frac{\text{SD}}{m} \quad (2)$$

126 where LOD and LOQ are the limits of detection and quantification, respectively; SD is the
127 standard deviation of blank signals, and m is the slope of the calibration curve.

128 For determining the accuracy of this new analytical method, the procedure M 1-92 from
129 American Oil Chemists' Society (AOCS) was followed, which is an updated procedure prepared
130 in collaboration with the International Union of Pure and Applied Chemistry (IUPAC) and the
131 International Organization for Standardization (ISO)(Liao et al., 2006).

132 Reliability and accuracy of the method, as the relative standard deviation (RSD), was determined
133 by applying six replicate determinations of 5 µg/L of analyte to deionized water, in which the %
134 RSD value of the recovery was found to be 4.5%.

135 The linearity of this new method was determined by adding a series of standards solutions from
136 the stock solution. Here, a defined amount of blank with a final concentration ranging from 5 to



137 150 µg/L was used according to the expected working range. Triplicate samples for each
138 concentration were analyzed.

139 **3. Results and discussion**

140 To introduce a green method for lead (II) extraction in edible oil, the suitable DES solvent was
141 preliminarily selected. It is reported in the literature that DES solvents are extensively used for
142 the extraction of biomolecules from natural products (Corrêa et al., 2021; Dwamena, 2019);
143 however, very few methods are available using DESs for the extraction of toxic metals in more
144 complex samples, such as edible oil. On the other hand, Ag-NPs are used for heavy metal uptake
145 (Sumesh et al., 2011). In this work, Ag-NPs along with adsorbing metal was extracted through
146 DES, which is a new approach to exploring new aspects of DES with Ag-NPs.

147 **3.1. Selection of DES system**

148 The extraction efficiency of the target analyte is mainly affected by the composition and the
149 nature of DES. The DES used for extraction must meet several requirements, such as high
150 affinity for analytes, liquid state at RT, no interference in analytical signal, different density than
151 that of water, high stability, and low solubility in an aqueous medium (Haq et al., 2021a; Makoś,
152 Przyjazny, et al., 2018). The DES system usually consists of two components, its 1st part consists
153 of quaternary ammonium salt while the second part consists of HBD. The mole ratio of the
154 quaternary ammonium salt and HBD has a significant impact on the applications of DES for
155 metal extraction. Therefore, different DES systems with different molar ratios were tested in this
156 work. ChCl was separately mixed with different types, including phenol, ethylene glycol, urea,
157 glycerol, and malonic acid, using different mole ratios. At this point, the optimization of the
158 HBD and HBA ratio is crucial for the applications of DES for extraction procedures. The
159 effectiveness of extraction generally decreases with an increase in HBD in the resulting DES. On

160 the other hand, mass transfer during extraction is increased by decreasing the density and
161 viscosity of DES (Hou et al., 2017; Razi Asrami et al., 2020). To determine the optimum molar
162 ratio DES, different HBD and HBA ratios were tested evaluating their maximum recovery. In
163 this way, a high recovery value was obtained with ChCl and phenol with a mole ratio of 1:2,
164 which was selected for further experiments. The results are illustrated in **Figure 1**.

165
166 **Figure 1.** Evaluation and selection of DES for maximum recovery of lead (II) ions. ChCl:
167 Chlorine chloride, M: Malonic acid, EG: Ethylene glycol, G: Glycerol, Ph: Phenol, U: Urea.

168
169 Phenol is an aromatic compound and capable of delocalizing negative charges across its entire
170 ring system. Considering its pK a value of 9.99, it is capable to form stronger hydrogen bonding
171 than other alcohols. The calculated interaction energies for clusters with similar hydrogen-
172 bonding patterns reveal that intermolecular interaction in phenol clusters is slightly stronger than
173 in water clusters. However, the fusion of phenol and water clusters leads to similar stability to
174 that of H₂O clusters (Parthasarathi et al., 2005). It is clear from the above mentioned facts, that
175 phenol presents a strong hydrogen bonding and fulfill the requirements of HBD in DES
176 formation. To evaluate the efficiency of different DESs, different HBDs were tested for Pb (II)
177 extraction. DES based on phenol as HBD was found advantageous for the extraction of Pb (II)
178 from edible oil samples.

179

180 **3.2. Optimization of extraction parameters**

181 3.2.1. Volume optimization of DESs



182 DES prepared with ChCl and phenol with molar ratio (1:2) was directly used without any further
183 purification or dilution. Since DES directly influences the extraction of lead (II), its optimum
184 concentration was determined for this specific procedure. Under the optimum condition, the DES
185 volume effect was studied ranging from 250 μL to 1250 μL for the recovery of lead (II). The
186 results indicate that, with a DES volume of 500 μL , a maximum recovery was obtained. Thus,
187 500 μL volume of DES was selected for onward studies. The results of this study are illustrated
188 in Figure S1.

189

190 3.2.2. pH effect

191 In adsorption/chelate formation and continuous extraction, pH plays a significant role (Haq et al.,
192 2021a). The solution pH is one of the parameters having a strong influence on the heavy-metal
193 ion uptake; this is due to the fact that the surface charge density of the adsorbent and the metallic
194 species depend on the pH (Haq et al., 2021b; Kubilay et al., 2007). Here, the lead (II) recoveries
195 were examined in the range of pH 2 to pH 8. The results revealed that a maximum recovery for
196 lead (II) was obtained at pH 2, as shown in Figure S2, indicating that a regular drop in the
197 recovery of lead (II) was found with a further increase after pH 2. Therefore, pH 2 was selected
198 for further studies.

199 At higher pH, Ag-NPs are more stable but less capable of metal adsorption, while at lower pH,
200 Ag-NPs are less stable but more capable of metal adsorption (Hosseini et al., 2016; Molleman &
201 Hiemstra, 2017). As the pH increases, the prolongation of the adsorbent surface decreased,
202 leading to a reduction in the electrostatic attraction between the metal ions species and the
203 adsorbent surface (Fernando & Zhou, 2019; Molleman & Hiemstra, 2017); this results in a
204 consequent decrease in the percentage removal of metal ions.



205

206 3.2.3. Optimization of THF addition

207 THF is an aprotic solvent, which was used in these studies to make phase separation easier. The
208 outcomes of this paper revealed that, while it indeed enhances the phase separation, it also
209 increases the % recovery of the analyte. These aprotic solvents tend to interact more with water
210 than DES. After interaction with THF molecules, water molecules decline their interaction with
211 DES, resulting in their self-aggregation and separation. In other words, THF acts as a
212 dehydrating agent. The most plausible mechanism of DESs self-aggregation involves π - π
213 overlap between the aromatic ring of phenol, followed by hydrogen bonding between functional
214 groups of DES and other charge transfer interactions (Haq et al., 2021a; Khezeli et al., 2015).

215 The volume effect of THF ranged from 250 μ L to 1500 μ L was studied for the % recovery of
216 lead (II) (see Figure S3), where the maximum recovery was obtained with a THF volume of 500
217 μ L. For further studies, a THF volume of 500 μ L was carefully used as an optimum value.

218

219 3.2.4. Effect of Ag-NPs concentration

220 For the separation of different metals, Ag-NPs have been used as a good adsorbent in the field of
221 research. Here, the excess of Ag-NPs is needed to provide maximum surface area for metal
222 adsorption. The mechanism of metal ion interaction with Ag-NPs is described and detailed in
223 section 3.3. In this study, the effect of the amount of Ag-NPs (0.1mM) was studied in the range
224 of 250 μ L to 2000 μ L. The results showed that the lead (II) signal response was increasing with
225 the increase of the amount of Ag-NPs up to 500 μ L and it remained constant. Therefore, 500 μ L
226 of Ag-NPs has been selected as the optimum value for lead (II) determination. The effect of Ag-
227 NPs on lead (II) recovery is illustrated in figure S4.



228

229 3.2.5. Effect of ultra-sonication time

230 The efficiency of the extraction process was greatly affected by ultra-sonication time. Our study
231 suggests that both THF amount and ultra-sonication waves caused the aggregation of the DES in
232 the aqueous phase and the reduction in extraction time. In general, it was noticed that the
233 conversion of these droplets of DES into tiny droplets contributed to reaching the equilibrium
234 state, resulting in increased extraction efficiency. At constant experimental conditions, the effect
235 of ultra-sonication time was studied from 1 to 5 min at 25 °C (see Figure S5). When the ultra-
236 sonication time was extended to 2 min, the extraction efficiency was stabilized. Therefore, 2
237 minutes of ultra-sonication time was selected for the subsequent experiments.

238 **3.3. Development of quantitative method/validation**

239 3.3.1. Interference study of different ions

240 To analyze lead content in food and environmental samples, an important issue relates to the
241 presence of interfering ions which could affect to some extent the analytical signal. The goal
242 during the development of this new method was to select the selective extraction conditions of
243 (Ooi & Ng, 2018), back extraction methods (Arpa & Aridaşir, 2018), solid-phase extraction
244 (Rahnama & Ghadiri, 2015), membrane techniques (Mesli & Belkhouche, 2018), adsorption
245 methods (Mahmoud et al., 2010), flow-injection on-line adsorption (Salonia et al., 1999) and co-
246 precipitation methods (Komjarova & Blust, 2006). The performance of the developed DES
247 impregnated Ag-NPs method was compared with liquid-phase microextraction methods, which
248 were used for lead (II) determination in edible oil, as shown in **Table 1**. Among all the reported
249 methods, cloud point extraction is the most dominantly used technique for lead extraction due to
250 its low cost, high enrichment factor, environment-friendly and minimal cost (Babae et al.,



251 2019). However, these methods are associated with some critical issues, such as a long time of
252 the procedure related to heating and centrifugation stages. Even though these methods achieve
253 good recovery after multi-cycle experiments (Galbeiro et al., 2014), while our proposed method
254 is quicker and provides a very good recovery obtained in a single cycle. The time for the
255 extraction procedure after digestion was estimated for the different reported methods in the
256 literature, which is compiled in **Table 1**. The estimated time for our extraction procedure was as
257 short as 6.5 minutes while estimated time values for other methods were 35 min (Coelho et al.,
258 2008), 87 min (Blanchet-Chouinard & Larivière, 2018), 217 min (Gouda & Zordok, 2018), 35
259 min (Citak & Tuzen, 2012), 60 min (Kazi et al., 2012), 16 min (Rahnama & Ghadiri, 2015) and
260 40min (Citak & Tuzen, 2010). At this point, it is noted that the newly proposed method is many
261 folds faster than the already reported methods. Application of AG-NPs with DES as extractant
262 with addition of THF provides further improvement also in the field of lead extraction based on
263 DESs in comparison to already published approaches (Karmini et al. 2016; Soyлак & Koksал,
264 2019; Habila et al. 2020.). Preliminary studies for this paper indicated that in case of sole use of
265 DES about 30 minutes of extraction is needed for acceptable recovery. Nanoparticle scale silver
266 reveals to provide rapid extraction. It follows from very fast kinetics of sorption. The
267 performance of this method is quite good due to its low LOD and LOQ values and high pre-
268 concentration factor. This method opens a new aspect in the application of DES coupled with
269 Ag-NPs for lead extraction.

270 **Table 1.** Comparative study of the new method with reported methods in the literature.
271



272 3.3.4. Application of the method for the analysis of real samples

273 Since edible oils are an essential part of the human diet, the developed procedure was applied to
274 the analysis of several categories of edible oils, such as sesame, olive, and canola oil obtained
275 from commercial brands of the district Mardan, Pakistan. In this approach, the standard addition
276 method, which is commonly used for AAS, was used. Known concentrations of lead (II) were
277 gradually added to real samples and percent recovery was determined in each case. The results of
278 the recovery performance are presented in **Table 2**. The recovery for real samples was between
279 97-107%. Since the concentration in real samples was revealed to be much higher than the limit
280 established by this method, the samples were diluted with methanol right after extraction. In
281 these experiments, three different types of edible oils were tested for lead (II) concentration,
282 exhibiting a lead (II) concentration of 1.21 mg/L, 1.26 mg/L and 1.19 mg/L for olive, sesame, and
283 canola oil, respectively. This method was sensitive and valid for determining very low
284 concentrations. To summarize, it can be stated that the performed experiments confirmed that the
285 developed procedure is reliable for the determination of lead (II) in different commercial edible
286 oil samples.

287 lead from the matrix, which was fully obtained making this method highly selective. DESs are
288 highly selective in extraction procedures (Rad et al., 2019); however, in this case, preliminary
289 studies revealed that additional selectivity must be provided. Thus, the application of Ag-NPs
290 was further studied to assure adequate accuracy of the quantitative analysis; for this, a standard
291 addition method was used.

292 To determine the effect of external ions on lead (II) extraction, known concentrations of different
293 ions were added in separate sets of the experiment. The interference of $\text{Na}^+(\text{NaNO}_3)$, $\text{K}^+(\text{KCl})$,
294 $\text{Mg}^{2+}(\text{Mg}(\text{NO}_3)_2)$, $\text{Cd}^{2+}(\text{CdCl}_2)$, $\text{Zn}^{2+}(\text{ZnNO}_3)_2$, $\text{Co}^{2+}(\text{CoCl}_2)$, $\text{Ni}^{2+}(\text{NiCl}_2)$, $\text{SO}_4^{2-}(\text{K}_2\text{SO}_4)$,



295 $Pb^{2+}(PbCl_2)$ was investigated. The recovery of the lead (II) in presence of interfering ions was
296 found to be higher than 90%. These results are compiled in supporting information (Table S1).
297 Importantly, it was proved that the interfering ions have no significant effect on the lead (II)
298 extraction/analysis and the proposed microextraction method fits the purpose.

299 3.3.2. Analytical performance of the method

300 According to the methodology described in section 2.7, LOD and LOQ values were calculated
301 as 0.28 and 0.94 $\mu\text{g/L}$, respectively. **Figure 2** shows the calibration curve, which has an
302 equation as follows: $Y = 0.0037x + 0.0026$, displaying an acceptable coefficient of
303 determination (R^2) of 0.9931. The linearity range was established from 5 to 140 $\mu\text{g/L}$. The
304 relative standard deviation calculated for six repetitions of lead (II) analysis (at a concentration
305 level of 5 $\mu\text{g/L}$) was 4.5%, showing the repeatability and reproducibility of the developed
306 method.

307

308 **Figure 2.** Calibration curve of lead (II) at different concentrations.

309

310 3.3.3. Comparison with other methods

311 Many methods have been reported in the literature for lead extraction and analysis, including
312 cloud point extraction (Blanchet-Chouinard & Larivière, 2018), liquid-liquid microextraction

313

314 **Table 2.** Determination of lead in edible oil samples.

315

316 Several papers recently reported presence of lead ions in oil samples. For example, it was
317 reported as lead (II) concentrations of 0.06-0.21 mg/Kg, 0.08-1.12 mg/Kg, and 0.06-0.08 mg/Kg

318 for rapeseed, soybean, and linseed oil, respectively (Szyczewski et al., 2016). In other study lead
319 (II) concentrations of 6-15 $\mu\text{g/Kg}$, 7.3-21 $\mu\text{g/Kg}$, and 3.4-16 $\mu\text{g/Kg}$ were reported for canola,
320 corn, and soybean oil, respectively (Allen et al., 1998). In vegetable hydrogenated oil, a lead
321 concentration of 15.92 $\mu\text{g/L}$ was reported by (Abbasi et al., 2009), while (Ng, 2010) documented
322 0.71 mg/Kg of lead in black olive oil and 0.75 mg/Kg in green olive oil. In more recent work,
323 (Zhuravlev et al., 2015) evaluated and determined a lead concentration in different types of oil,
324 e.g., 0.006 mg/Kg in corn oil, 0.014 mg/Kg in olive oil, 0.016 mg/Kg in refined sunflower oil,
325 0.062 mg/Kg sunflower oil, and 0.027 mg/Kg in soybean oil. It can be seen that the range of
326 reported values strongly differs in the type and origin of oil samples. It is worth mentioning that
327 this developed method covers the entire range of expected levels of lead concentration from low
328 parts per billion levels to higher ones after dilution. The modern instruments are equipped with
329 an automatic module for sample dilution; therefore, this approach fully fits the purpose of lead
330 determination in edible oils.

331 **4. Conclusions**

332 In this new method, lead (II) ions were efficiently extracted and determined from different
333 commercial edible oil products. Deep eutectic solvents based on ChCl and phenol with a 1:2
334 molar ratio demonstrated a maximum recovery for lead (II) extraction. It was concluded that Ag
335 nano particles can adsorb Pb (II) and facilitate the Pb (II) mobility from aqueous phase to DES
336 phase. This stage is very fast, as nanoparticles ensure very good kinetics of sorption.
337 The sonication process accelerates the formation of nano-sized fine droplets and as a result
338 increases the contact surface area between the extracting solvent (DES) and Pb(II). The
339 optimized method implies simplicity, ease of operation, short extraction times, and high
340 enrichment factor. Quantitative recovery (97-105%) from spiked samples demonstrated the



341 suitability of the optimized method for the quality control of the analyzed samples. The LOD and
342 LOQ were 0.28 $\mu\text{g/L}$ and 0.94 $\mu\text{g/L}$, respectively with an RSD value of 4.5% and a pre-
343 concentration factor of 25 which are comparatively improved comparing other methods reported
344 in the literature. The optimized procedure is green, simple and requires a small volume of
345 extraction solvent. The comparison of the developed method with already-existing analytical
346 procedures confirmed its advantages, such as shortening the time of analysis and sensitivity. To
347 finalize, the outcomes of this new assay are comparable to those obtained by the ICP-MS
348 technique with more simplicity, better accuracy, high enrichment factor, less time-consuming,
349 and environment-friendly. Further improvements in method development, also in green
350 chemistry aspects, should focus on minization of the scale of procedure. This studies confirmed
351 that the optimized procedure is perfectly useful for routine analysis of food especially edible oil
352 samples containing traces of Pb(II) ions.

353

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Conflict of interest

The authors declare no conflict of interest.

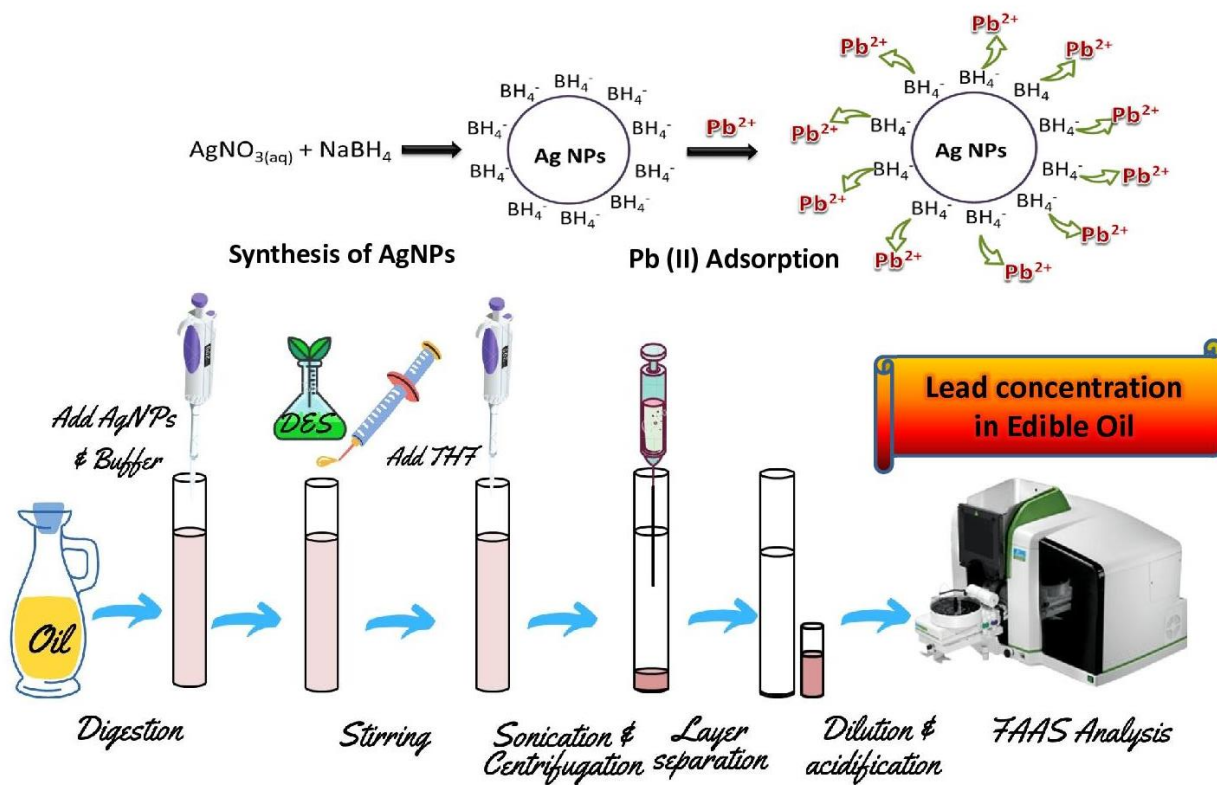
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Graphic abstract



Figures

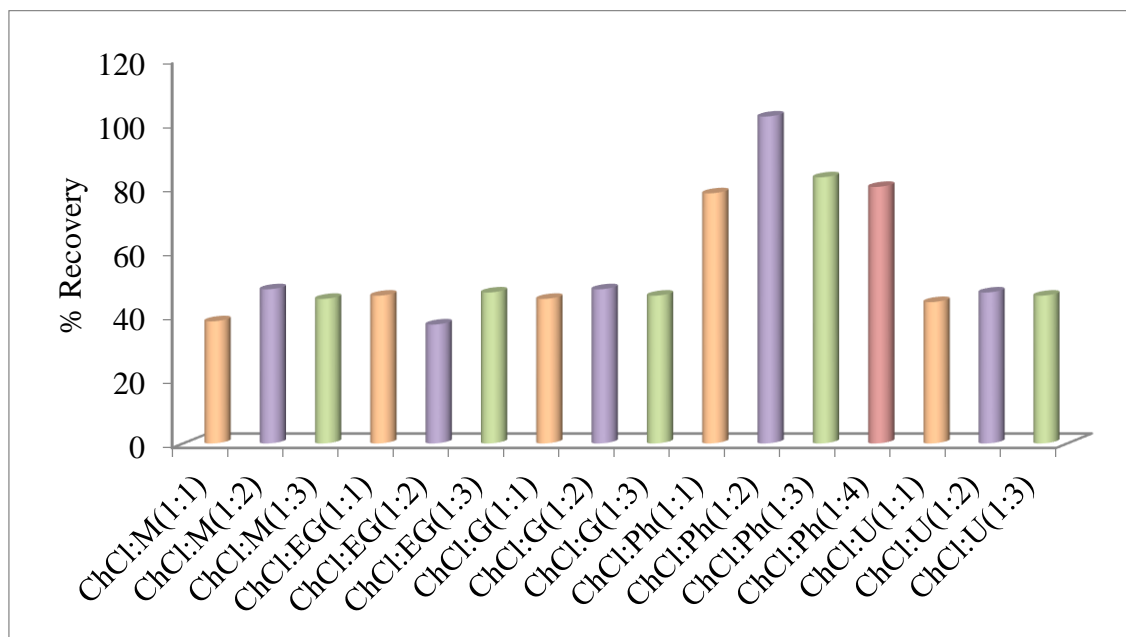


Figure 1. Evaluation and selection of DES for maximum recovery of lead (II) ions. ChCl: Chlorine chloride, M: Malonic acid, EG: Ethylene glycol, G: Glycerol, Ph: Phenol, U: Urea.

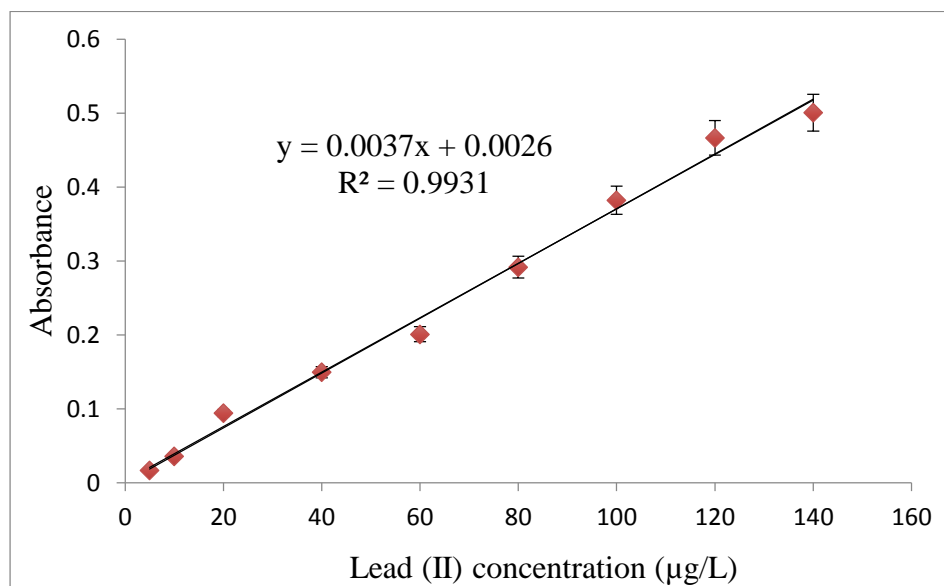


Figure 2. Calibration curve of lead (II) at different concentrations.

Tables

Table 1. Comparative study of the new method with reported methods in the literature.

Method	Detection techniques	LOD (µg/L)	LOQ (µg/L)	Linearity (µg/L)	RSD (%)	Estimated time of sample preparation* (minutes)	References
Cloud point extraction	TS-FF-AAS	0.43	1.44	5-50	8.7	>35	(Coelho et al., 2008)
Cloud point extraction	ICP-OES	0.8	2.60	---	13	>67	(Blanchet-Chouinard & Larivière, 2018)
Solid phase extraction	FAAS	0.2	1	1-60	3.2	>180	(Gouda & Zordok, 2018)
Cloud point extraction	FAAS	1.33	6.65	20-320	3.06	>35	(Citak & Tuzen, 2012)
Cloud point extraction	FAAS	0.26	0.86	20-100	1.88	>60	(Kazi et al., 2012)
SADSPE	FAAS	1.3	4.30	4-100	5	>21	(Rahnama & Ghadiri, 2015)
Cloud point extraction	FAAS	3.42	11.31	5-10	4.8	>45	(Citak & Tuzen, 2010)
DES-based extraction	FAAS	2.4	7.9	5-60	0.9-4.3	37	(Soylak & Koksak, 2019)
DES-based extraction with Fe ₃ O ₄ sorbent	FAAS	0.4	2	2-250	1.8	>75	(Karmini et al. 2016)
DES-based extraction with Ag-NPs	FAAS	0.28	0.92	5-140	4.5	6.5	<i>Present work</i>

*after digestion

SADSPE; Solvent-assisted dispersive solid-phase extraction, FAAS; Flame atomic absorption spectroscopy, ICP-OES; Inductively coupled plasma optical emission spectroscopy, TS-FF-AAS; Thermo spray flame furnace atomic absorption, DES; Deep eutectic solvent

Table 2. Determination of lead (II) in edible oil samples.

Samples	Lead spiked ($\mu\text{g/mL}$)	Lead found ($\mu\text{g/mL}$)	% Recovery
Olive oil	--	1.21 ± 0.01	-----
	2.5	3.90 ± 0.02	105.12
	5.0	6.64 ± 0.02	106.92
Sesame oil	--	1.26 ± 0.03	-----
	2.5	3.65 ± 0.017	97.07
	5.0	6.11 ± 0.04	97.60
Canola oil	--	1.19 ± 0.01	-----
	2.5	3.62 ± 0.01	98.10
	5.0	6.19 ± 0.04	100.00

Supplementary data

Deep eutectic solvent (DES) with silver nanoparticles (Ag-NPs) based assay for analysis of lead (II) in edible oils

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Supplementary figures

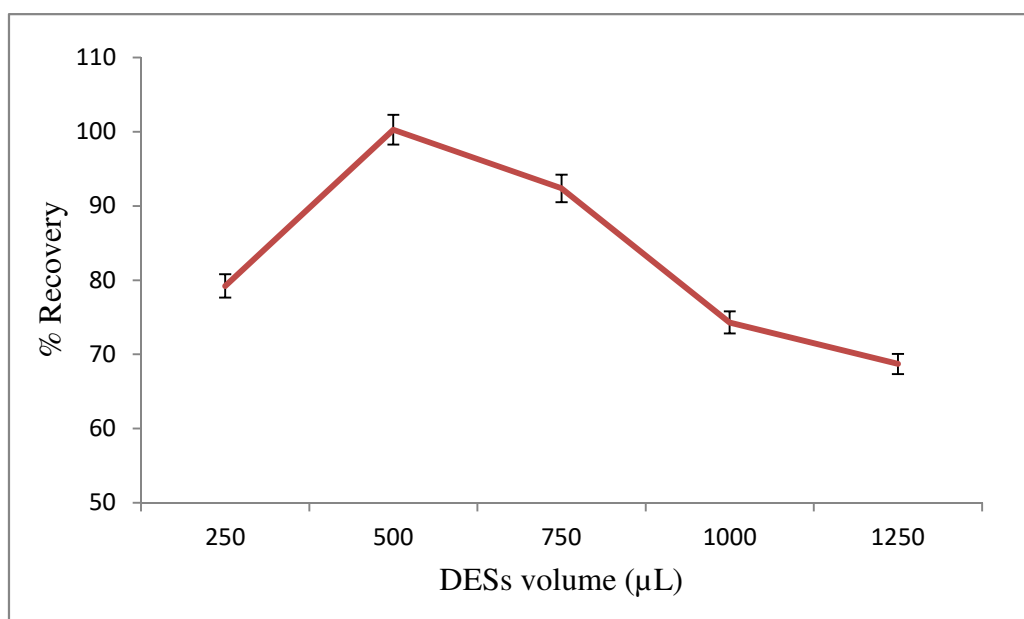


Figure S1: Volume optimization of DESs

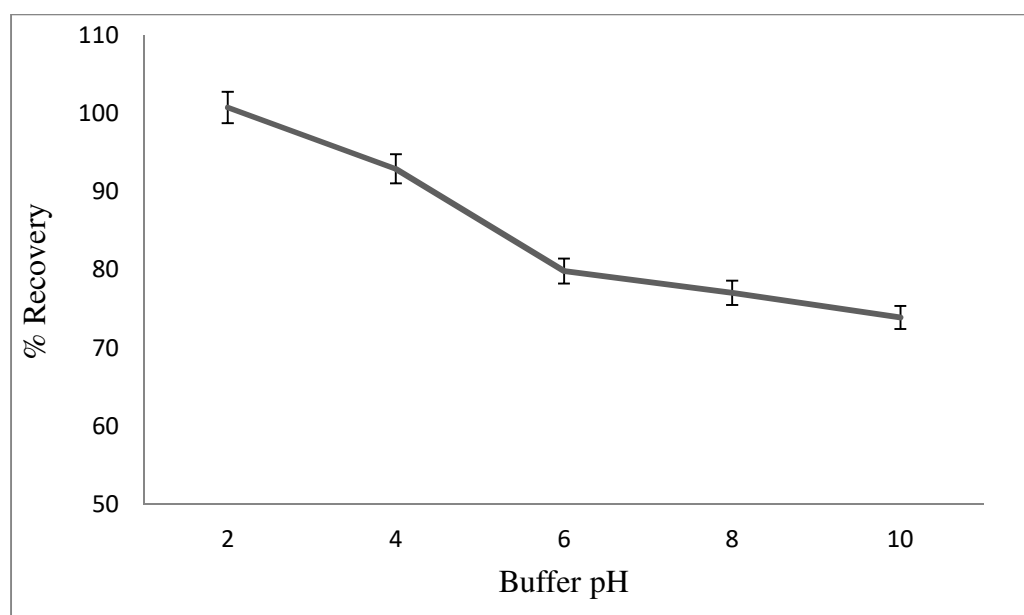


Figure S2: Optimization of pH



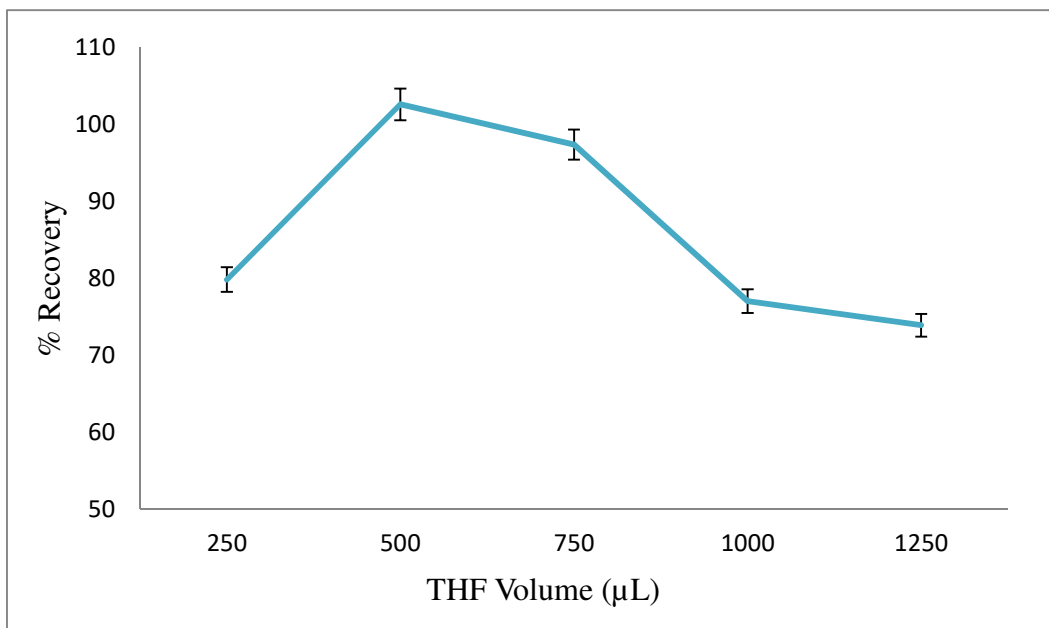


Figure S3: Optimization of THF

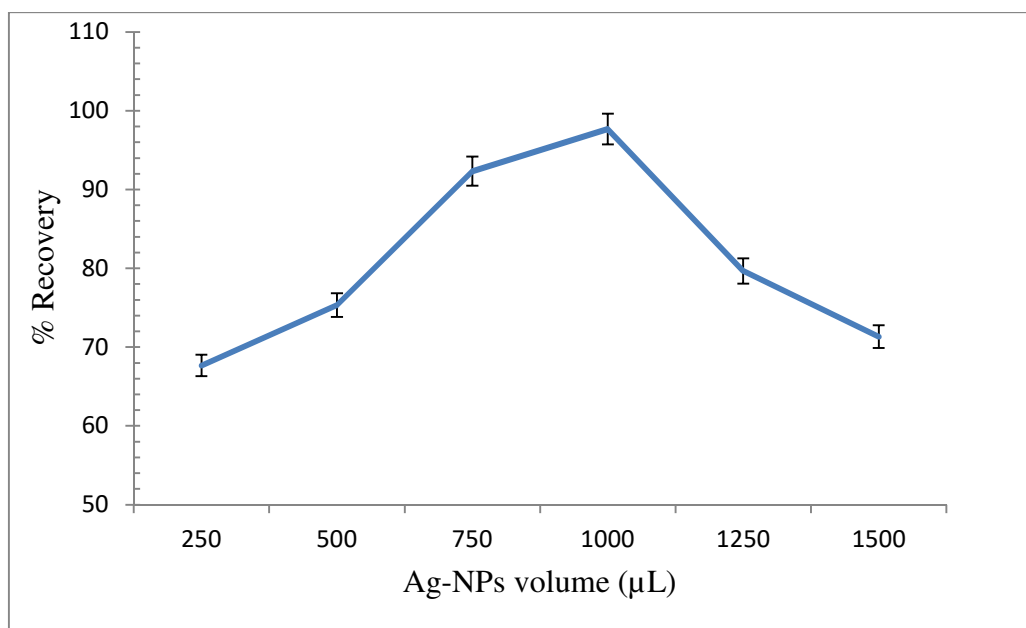


Figure S4: Optimization of Ag-NPs concentration

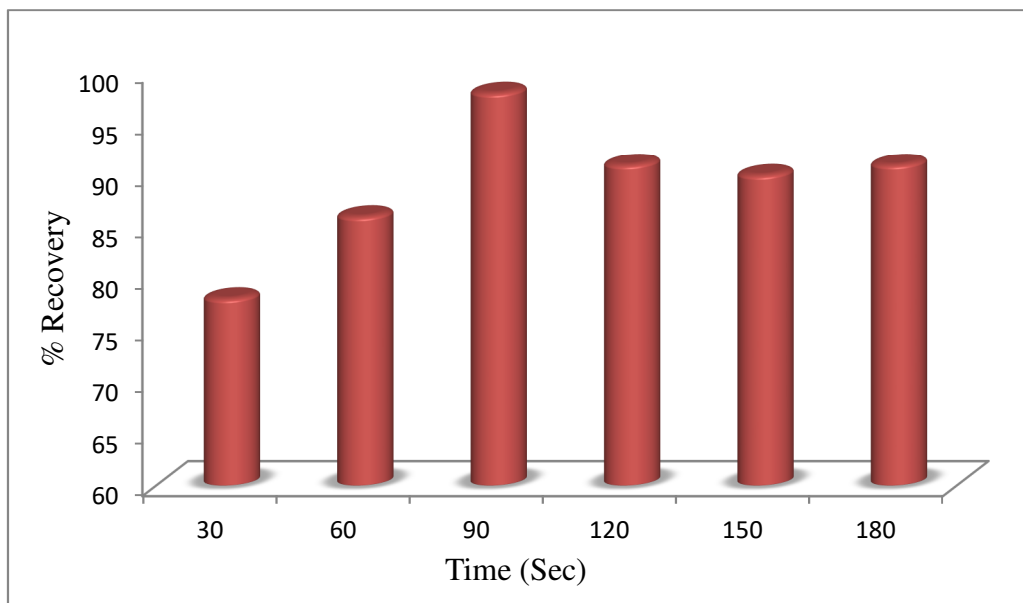


Figure S5: Optimization of sonication time for maximum recovery

Supplementary table

Table S1: Effects of interfering ions on the recovery of lead (II)

Ions	Added as	Concentration (mg/L)	Foreign ion	Recovery
Na ⁺	NaNO ₃	2000	500	99.75
K ⁺	KCl	1000	250	100.62
Cd ²⁺	CdCl ₂	10	2.5	94.86
Pb ²⁺	Pb(NO ₃) ₂	10	5	92.04
Mg ²⁺	Mg(NO ₃) ₂ ·6H ₂ O	500	1250	96.73
Co ²⁺	CoCl ₂ ·6H ₂ O	1000	250	91.21
Ni ²⁺	NiCl ₂ ·6H ₂ O	20	5	105.73
SO ₄ ²⁻	K ₂ SO ₄	2000	500	90.61

