

29 **Highlights**

- 30 ✓ Magnetic DES: Toner powder@aliquat 336 / lauric acid:menthol as extractant
- 31 ✓ Robust and time saving miniaturized sample preparation method
- 32 ✓ Validated method reliable for routine analysis of Ni and Cu in water and food
- 33 ✓ Short extraction time (8 min) and high preconcentration factor (125) were obtained
- 34 ✓ Sensitive (ppb level), selective and stable method towards complex sample matrix

35

36 **1. Introduction**

37 Copper is one of the heavy metals that has a red color and has been found in certain
38 amounts in soil, water and air. As a result, its presence was confirmed also in plants and animal's
39 bodies. Emission of this element from different industrial processes such as dyeing, oil, paper,
40 plating, copper is released in amounts that can be dangerous for aquatic and terrestrial
41 ecosystems (Seidi, & Alavi, 2019). Although copper is one of the essential elements for humans
42 in terms of providing enzymatic activity and taking part in red blood cell formation, excessive
43 copper levels can lead to diseases e.g epilepsy, dementia, depression and autistic disorder,
44 (Shrivias, & Jaiswal, 2013). For this reason, there are some restrictions on copper intake and the
45 recommended intake of copper by the World Health Organization is 0.5 mg Kg^{-1} per day body
46 weight(Council, 2000, Olivares et al., 1998, Cross et al., 2005).

47 Nickel (Ni) is a metal used in various materials such as dental prosthesis, coatings,
48 computer components, pigments, and ceramics. Release of nickel into water occurs as a result
49 of the dissolution of soil and rocks, the biological functions of some living things and industrial
50 activities (Kravkaz Kuşçu, Bayraktar, & Tunçer, 2022). Excessive nickel levels in the living
51 system causes skin and respiratory system diseases (Elahi et al., 2022). Based on surveys
52 performed on animals, it has been suggested that the daily intake of nickel for humans should
53 be $<100 \mu\text{g/day}$ (Nielsen, 2021). Considering the harmful effects of Ni and Cu and the related
54 restrictions, the necessity of determining trace amounts of these metals from foodstuffs comes
55 to the fore.

56 Different analytical methods are available for Cu(II) and Ni (II) determination including
57 flame atomic absorption spectrometry (Jalbani, & Soylak, 2015), inductively coupled plasma-
58 mass spectrometry (Wang et al., 2015), voltammetry (Primo, Buffon, & Stradiotto, 2021), UV-
59 VIS spectrophotometry (Eshaghi, Vafaeinezhad, & Hooshmand, 2016), and liquid
60 chromatography (De Oliveira Trinta et al., 2020) in different food and water samples. Flame

61 atomic absorption spectrometry is very useful for the detection and quantification of heavy
62 metals from various matrices such as environmental, food, and water samples because of its
63 high sensitivity, low cost, and relative convenience compared to other methods (Tuzen et al.,
64 2020). Despite of the above mentioned advantages, direct analysis of heavy metals from various
65 samples is challenging with flame atomic absorption spectrometry due to possible interference
66 of matrix, structure of the samples concerned and presence of interfering species. To overcome
67 this problem, a reliable and highly selective separation and enrichment method should be used
68 before the determination phase. Although traditional extraction techniques have an important
69 place among the sample preparation methods, the interest of researchers has recently shifted to
70 microextraction techniques as they offer various possibilities such as saving the time needed
71 for effective extraction and minimization of organic solvents usage (Jayasinghe et al., 2022;
72 Faraz et al., 2021).

73 DLLME is important as it is a simple and cost-effective microextraction technique that
74 allows simultaneous analysis of multiple analytes (Elik et al., 2023b, Haq et al., 2022)).
75 Although deep eutectic solvents (DESs) have comparable properties with ionic liquids in terms
76 of their stability, and tenability, they are superior to ionic liquids as they are cheaper, and easier
77 to synthesize (Makoś et al., 2018; Mbous et al., 2017; Haq et al., 2023a). DESs provide unique
78 intermolecular interactions with specific analytes, thus “tuning” the selectivity of the designed
79 separation system (Momotko et al., 2022, Momotko et al., 2021, Khajavian et al., 2022; Elik et
80 al., 2023a, Faraz et al., 2021, Haq et al., 2021). Their superior properties were proved in several
81 analytical procedures (Haq et al., 2023b, Ullah et al., 2022). Recently, researchers have
82 combined extraction solvents with magnetic materials to increase extraction efficiency
83 (Chisvert et al., 2017; An, Rahn, & Anderson, 2017). One of the important examples of
84 magnetic materials used in microextraction techniques are ferrofluids (Altunay et al., 2023).
85 Ferrofluids combined with the DLLME are rapidly injected into the sample, while dispersive

86 solvent is dissolved in the aqueous phase, resulting in the formation of small droplets of
87 extractant. It causes a large surface area for mass transfer between sample and extractant. In
88 this case, the extraction rate is accelerated and the extraction time is minimized. Another
89 advantage is that they are easily separated from the sample after extraction due to their magnetic
90 properties and there is no need to use complex devices and methods for their separation (Nayebi,
91 & Shemirani, 2021).

92 This study aimto prepare and test ferrofluid based on DESs for selective extraction and
93 analysis of Ni(II) and Cu(II) ions in different water and food samples. As a result of the ongoing
94 studies, a fast, simple and economical analytical method was developed. This method does not
95 include either a heating step or a centrifugation step. Effective separation of the extractant was
96 achieved using a magnetic stir bar. Important microextraction parameters of the sample
97 preparation procedure were investigated and optimized in details. The method was validated by
98 using certified reference materials (CRMs) such as GBW10015 Spinach, GBW10016 Tea and
99 GBW10019 Apple.

100 **2. Experimental**

101 **2.1. Apparatus**

102 The pH of the extraction solution was measured using a Metrohm 691 pH meter (Herisau,
103 Switzerland). A magnetic heating plate was used for the preparation of ferrofluid based DESs.
104 An orbital shaker (Multi Bio RS-24, BioSan, Berlin, Germany) was used dispersion of the
105 ferrofluid-based DES in the sample solution. A neodymium magnet was used to accelerate the
106 separation of magnetic DES from the aqueous solution. Ultrapure water was obtained using a
107 Milli-Q system (ICW-3000, Merck, Darmstadt, Germany). The digestion step was carried out
108 by microwave system (Milestone Ethos D model, Sorisole-Bg, Italy). The analysis step was
109 carried out with a flame atomic absorption spectrometry instrument (AAS-6300, Shimadzu,
110 Kyoto, Japan) with D₂ background correction.. The measurements were performed at two



111 wavelengths (Ni-232nm and Cu-324.8nm). The lamp current used for these wavelengths was
112 10mA and 3.0mA, respectively. The spectral bandwidths for the two measurements were 0.2nm
113 and 0.5nm. The flow rates of acetylene and air during the experiment were set at 1.8 and 8.0
114 mL min⁻¹, respectively.

115 **2.1. Chemicals and reagents**

116 The chemicals utilized in the research were of analytical grade and were employed without any
117 additional purification procedures. Standard solutions of Ni(II) and Cu(II) ions, each with a
118 concentration of 500 mg L⁻¹, were prepared by dissolving their nitrate salts in deionized water.
119 The experimental solutions and calibration standards were created by employing a sequential
120 dilution formula. Once prepared, these solutions were stored in a refrigerator at a temperature
121 of 4 °C. Acetone, tetrahydrofuran, acetonitrile, methanol, ethanol, and methyl violet were
122 obtained from Sigma-Aldrich (Barcelona, Spain). Borate, phthalate, citrate and TRIS buffer
123 solutions were used to fix the pH in the range from 3 to 9. Aliquat-336 (95.0 %, Merck,
124 Germany), butyric acid (99%, Merck), thymol, lactic acid (≥ 95.0 %, Sigma Aldrich), lauric
125 acid (≥98%, Sigma Aldrich), menthol (≥99%, Sigma Aldrich) and *o*-xylene (≥ 98, Merck) were
126 used in the preparation of ferrofluid based DESs. Toner powder was purchased from Thermo
127 Scientific (Norway).

128 **2.3. Sampling**

129 Bottled waters were bought from local markets in Sivas/Türkiye. Waste water samples were
130 collected from the industrial zone in Sivas/Türkiye. Well water was collected from an
131 agricultural region located in Sivas, Turkey. The river water was obtained from Kızılırmak
132 passing through Sivas. The spring waters were collected from the hot spring area in in
133 Sivas/Türkiye. The collected water samples were first filtered through a 0.45 µm pore size
134 membrane filter (cellulose membrane filter, Whatman®, USA) and then stored in the
135 refrigerator at 4 °C. All of the food samples including black tea, rice flour, wheat, green pepper,



136 spinach, apple, eggplant, pomegranate, parsley, mint, tomato, mushroom and potato were
137 purchased from local grocery stores and markets in Sivas/Türkiye. The collected food samples
138 were first washed with distilled water, the edible parts were cut with the help of a knife, and
139 then dried in the oven. The dried samples were homogenized with a laboratory blender. Then,
140 microwave digestion method was applied to these powdered samples.

141 **2.4. Microwave based sample digestion**

142 Food samples were digested according to the recommended methods in the literature
143 (Abdulkhaliq et al., 2012, Elahi et al., 2022). The microwave digestion steps used for food
144 samples are summarized below. First 1 g of the food samples was transferred into Teflon tubes
145 containing concentrated HNO₃ (20 mL) and concentrated H₂O₂ (5mL). Next, microwave
146 digestion was carried out with gradual change in temperature. For first 2 min the temperature
147 was 60 °C, with microwave power 250 W. In the second step temperature was 100 °C for 5 min
148 with microwave power 250 W. In third step 150 °C for 2 min with microwave power 500 W.
149 In the fourthstep temperature was 200 °C for 3 min with microwave power 600 W. The residue
150 obtained after microwave digestion was diluted to 10 mL with the water. All samples were
151 prepared in triplicate with sample blanks. In case ofwater samples analysis, a 100 mL of the
152 collected water samples were heated on heating plate. Volume of water sample was reduced to
153 a 10 mL. The remaining 10 mL sample was used for the recommended procedure.

154 **2.5. Preparation of ferrofluid based DESs**

155 In this study, the ferrofluid based DESs were prepared using the previously reported method in
156 literature (Mohebbi et al., 2021; Zarei, Nedaei, & Ghorbanian, 2018). Four different types of
157 DESs were prepared. Menthol was used as the hydrogen bond acceptor (HBA), while butyric
158 acid, thymol, lactic acid, and lauric acid served as the hydrogen bond donors (HBD). These
159 HBA and HBD compounds were added to beakers with their respective mixed molar ratios, as
160 specified in Table 1. The beakers were then heated on a magnetic stirrer heating plate at 80°C



161 until a clear liquid was formed. This resulting liquid was employed as a supporting solvent for
162 the production of a ferrofluid. To accomplish this, 1 g of toner powder was mixed with a 10 mL
163 of o-xylene in a tube, followed by sonication at room temperature for 10 min. The mixture was
164 subsequently heated to approximately 70°C for 2 h . Afterward, magnets were employed to
165 separate the magnetic particles from the aqueous solution, which were then properly washed
166 with ultrapure water and ethanol. The particles were subsequently dried in an oven and heated
167 up to 80°C. To prevent the agglomeration of the magnetic particles, 1.0 mL of aliquat-336 was
168 added. Then, a 150 mg of the obtained magnetic particles were introduced into a glass vial. A
169 1.0 mL portion of the prepared DES was added to the mixture, followed by stirring at 50°C for
170 3 h. Finally, the resulting ferrofluid was tested as the extracting solvent for the DLLME
171 procedure. After the ferrofluid-based DES-4 was separated from the aqueous solution, different
172 solvents such as ethanol, methanol, tetrahydrofuran, acetonitrile and acetone were investigated
173 to both dilute the viscous liquid formed and to strip the analytes from the magnetic phase. All
174 solvents were tested in equal volume.

175 **2.6. MAGDES-OS-DLLME method**

176 The magnetic deep eutectic solvent based orbital shaker-assisted dispersive liquid-liquid
177 microextraction (MAGDES-OS-DLLME) method was applied to the digested samples by
178 following the experimental steps belowInitially, a 10 mL of the digested samples were
179 transferred to vial that already contained a solution of Ni(II) and Cu(II) ions each with a
180 concentration of 25 $\mu\text{g L}^{-1}$. Secondly, a 0.1 M citrate buffer solution was added to adjust desired
181 pH (6.0). In third step, a methyl violet was added (final solution was 75 $\mu\text{mol L}^{-1}$) to ensure the
182 complexation. Fourth, a 0.8 mL ferrofluid-based DES-4 was added to the sample solution to
183 separate the metal-ligand complex. In fifth step, conical tubes were shaken at 1200 rpm for 8
184 min on orbital shaker and for dispersion of the ferrofluid-based DES-4 in the sample solution.
185 Six, a magnetic stir bar was dipped into the tube and the ferrofluid containing the analytes was



186 transferred along with the magnetic stir bar to other tubes. Seven, tetrahydrofuran (300 μ L) was
187 added followed by vortexing for 30 seconds. Finally, the resulting solution (approximately 1.0
188 mL) was analyzed by flame atomic absorption spectrometry. The experimental steps of
189 MAGDES-OS-DLLME are presented in Figure 1.

190 **2.7. Method optimization and calculation of validation parameters**

191 The method was optimized to obtain the highest recovery of the Ni(II) and Cu(II) ions. The
192 main extraction factors including pH, concentration of complexing agent, ferrofluid-based
193 DESs type and volume, mixing type, shaking time, type and volume of desorption solvent, KCl
194 amount and volume of sample were investigated in details. The extraction recovery % of the
195 DLLME was calculated by the following Equation.

$$196 \text{ Extraction recovery (\%)} = [C_{final} V_{final} / C_o V_o] \times 100$$

197 Where C_{final} - concentration of Ni(II) and Cu(II) ions determined by the developed method, C_o -
198 concentration in the sample before applying the method, V_{final} - the final volume and V_o - the
199 initial volume.

200 Detection limit (LOD) and quantification limit (LOQ) for the method were calculated by using
201 the formulas $[3S_{blank}/m]$ and $[10S_{blank}/m]$ respectively. Where S_{blank} is the standard deviation for
202 the blank solution and m is the slope of the standard curve.

203 The precision of the method was calculated as relative standard deviation (RSD%) and
204 calculated as $[RSD\% = \frac{SD}{Mean\ value} \times 100]$. Where SD is the standard deviation based on three
205 replicate determinations.

206 Enrichment factor (EF) was calculated as $[EF = \frac{m_f}{m_i}]$, where m_i is the slope of the calibration
207 graph obtained before the MAGDES-OS-DLLME and m_f is the slope of the calibration graph
208 obtained after the MAGDES-OS-DLLME.



209 The pre-concentration factor (PF) was evaluated by using the equation $[PF = \frac{V_f}{V_i}]$, where V_f is the
210 final volume of sample and V_i is the initial volume of the sample.

211 The tolerance limit for the interfering species on the extraction and determination was calculated
212 using the formula $[\text{Matrix species amount, } \mu\text{g L}^{-1}] / [\text{Analyte amount, } \mu\text{g L}^{-1}]$ for highest
213 concentration of matrix species that didn't cause error at determination step.

214 In CRM the t_{exp} was calculated using the following formula $[t_{\text{exp}} = \frac{|\mu - \bar{x}| \sqrt{N}}{s}]$. Where t_{exp} , s , N , \bar{x}
215 and μ were statistical values, the standard deviation, the number of independent determinations,
216 the experimental mean value, and the certified value, respectively.

217

218 **3. Results and discussion**

219 **3.1. Optimization of parameters**

220 A selective and sensitive microextraction step should be applied in order to quantify Ni(II) and
221 Cu(II) in food and water samples. To increase the extraction efficiency of the MAGDES-
222 DLLME procedure, a univariate optimization strategy was optimized. All runs were performed
223 in triplicate and the percent relative standard deviation was calculated for each experiment run.

224 **3.1.1. Effect of pH**

225 In case of extraction process from aqueous solutions, it is desirable to provide selective
226 interaction of the target analytes with the extractant added to the sample. In this context, a one
227 of the most significant factor affecting selective interaction is the pH of the aqueous solution
228 (Haq et al., 2021). Chemical species in aqueous solution may exist in different forms such as
229 neutral form or anions, cations, hydroxides, depends on the pH of sample medium. As a result,
230 the interaction between extractant and target analytes will be strongly varied depending on pH.
231 Taking these factors into consideration, the potential impact of the pH of the aqueous medium
232 on the recovery percentage of Ni(II) and Cu(II) ions was examined within the pH range of 2-

233 10. Figure S1 illustrates that the highest extraction recovery was achieved at pH 6. The decrease
234 in extraction recovery, especially in the basic region, is attributed to the increased hydroxide
235 formation of these metals. Due to the increase in the hydroxide concentration, the complexation
236 of the analyte ions cannot be completed due to the attack of hydroxide ions on the cationic
237 region of the chelating agent (methyl violet). As a result, for subsequent microextraction
238 studies, the pH of the medium was adjusted to 6.0 using a 0.1 M citrate buffer solution.

239 **3.1.2. Effect of concentration of complexing agent**

240 The extraction process was facilitated when the metal ions could be complexed with a suitable
241 chelating agent. In light of these facts, methyl violet was tested for complexation of Ni(II) and
242 Cu(II) ions. Another factor affecting effective complexation is the concentration of the
243 complexing agent. Methyl violet should be added to the aqueous solution in sufficient quantity
244 to quantitatively complex the metal ions in the medium. Therefore, the effect of concentration
245 of methyl violet on the recovery of Ni(II) and Cu(II) ions was investigated by changing the
246 concentration of methyl violet from $10 \mu\text{mol L}^{-1}$ to $150 \mu\text{mol L}^{-1}$. The results in Figure S2 reveal
247 that $75 \mu\text{mol L}^{-1}$ of methyl violet was sufficient to achieve quantitative recoveries of both
248 analytes. It is worth to mention, that, the extraction recovery of the analytes was almost stable
249 at higher methyl violet concentration values. Based on these considerations, a concentration of
250 $75 \mu\text{mol L}^{-1}$ of methyl violet was selected as the optimal concentration for the DLLME
251 procedure

252 **3.1.3. Effect of ferrofluid-based DES type and volume**

253 The most important parameter to achieve the desired goal in microextraction studies was the
254 type of extraction solvent. The extraction solvent should be specific (selective) and have high
255 extraction efficiency. Furthermore, the extraction solvent should not be miscible with the
256 sample solution. Based on these expectations, four ferrofluid-based DESs were prepared and



257 tested for the extraction of Ni(II) and Cu(II). The results of this part of the study were presented
258 in Figure S3a. The maximum extraction recovery for both analytes was obtained using the
259 ferrofluid-based DES-4 (toner powder@aliquat 336@lauric acid:menthol). Therefore, the
260 ferrofluid-based DES-4 was selected as extraction solvent DLLME studies.

261 Next, the volume of ferrofluid-based DES-4 extractant was optimised.. The addition of
262 excessive extraction solvent causes an increase in the magnetic phase in the final volume and
263 thus decrease the concentration of the analytes. The increase in the magnetic phase volume
264 causes excessive use of dispersive solvents. In this section, the effect of the volume of
265 ferrofluid-based DES-4 on the extraction recovery of Ni(II) and Cu(II) ions was investigated in
266 the volume range of 0.2-1.5 mL. Analysis of the results presented on Figure S3b reveals that
267 the best extraction recovery for both analytes was obtained when 0.8 mL of ferrofluid-based
268 DES-4 was used. Thus, this optimized value was used in the further studies..

269 **3.1.4. Effect of mixing type and time**

270 In order to to obtain effective extraction, i.e. effectively complex the Ni(II) and Cu(II) from the
271 sample, the ferrofluid-based DES-4 must be completely dispersed in the medium. Therefore,
272 after adding ferrofluid-based DES-4 to the aqueous solution, mixing steps including orbital
273 shaking, vortexing, hand mixing and sonication were compared. According to the results in
274 Figure S4a, the maximum recovery for both analytes was achieved when orbital shaking was
275 applied. Another parameter that affects the effective distribution of the ferrofluid-based DES-4
276 in the aqueous solution is the shaking time. The effect of orbital shaking time on the extraction
277 recovery of Ni(II) and Cu(II) ions was studied from 1 min to 15 min. Figure S4b shows that the
278 8 min shaking time was enough for quantitative recovery of analytes. Therefore, 8 min of orbital
279 shaking was used in the further studies..

280 **3.1.5. Effect of desorption solvent type and volume**



281 After the ferrofluid-based DES-4 was separated from the aqueous solution, different solvents
282 such as ethanol (EtOH), methanol (MeOH), tetrahydrofuran (THF), acetonitrile (ACN) and
283 acetone were investigated to both dilute the viscous liquid formed and to strip the analytes from
284 the magnetic phase. All solvents were tested in equal volume. Based on the results presented in
285 Figure S5a, THF was chosen as the suitable desorption solvent. Next, the THF volume was
286 optimized. While increasing the volume of solvent, firstly the desorbed amount of analyte
287 increases up to full recovery, but further excess volume of the solvent causes decrease of analyte
288 concentration. On the other side, too low volume of desorption solvent makes the analytes
289 recovery difficult. The impact of varying THF volume between 0 and 750 μL was investigated.
290 Figure S5b reveals that 300 μL of THF was sufficient to achieve maximum recoveries. Thus,
291 300 μL of THF was used in the further studies..

292 **3.1.6. Effect of KCl amount**

293 In DES-based microextraction studies, the ionic strength of the aqueous solution can have
294 different effect on the recovery of analyte. The addition of salt increases the ionic strength of
295 the aqueous phase and causes the salting effect of the extraction solvents to shift the dispersion
296 balance towards the anhydrous phases, thus maximizing the extraction recovery and phase
297 separation. Consequently, the influence of different concentrations of KCl ranging from 0 to 15
298 (w/v%) on the retrieval of analytes was examined (see Figure S6). The samples spiked with
299 KCl don't have any significant effect on the extraction efficiency of analytes. Thus, KCl
300 solution was not used in the further studies..

301 **3.1.7. Effect of sample volume**

302 To maximize the PF of the method, it was necessary to optimize the volume of sample used in
303 microextraction stage. The PF was determined by comparing the initial volume of the sample
304 with the final volume after extraction. The extraction recoveries (approximately 95%) for both



305 analytes were stable from 25 mL to 125 mL (see Figure S7). Furthermore, it was observed that
306 at higher sample volumes, there was a significant decrease in the extraction recoveries for both
307 analytes. For 125 mL sample volume, 95% recovery was achieved so this value was used in
308 further studies..

309 **3.2. Validation studies**

310 For routine application of DLLME method for real samples, validation parameters such as
311 linearity range, LOD, LOQ, EF, PF, matrix effect, precision, accuracy and sensitivity of the
312 MAGDES-OS-DLLME method must be evaluated under optimized conditions.

313 **3.2.1. Aspects of quantitative analysis**

314 Different concentrations of Ni(II) and Cu(II) ions standard were added to the sample solutions
315 and linearity studies were performed for each analyte. As a result of the study, linearity for
316 Ni(II) and Cu(II) ions were 0.1-330 $\mu\text{g L}^{-1}$ and 0.5-250 $\mu\text{g L}^{-1}$, respectively. LOD and LOQ
317 calculated were 0.03 $\mu\text{g L}^{-1}$ and 0.10 $\mu\text{g L}^{-1}$ for Ni(II) ions and 0.15 $\mu\text{g L}^{-1}$ and 0.46 $\mu\text{g L}^{-1}$ for
318 Cu(II) ions.. In this context, the PF of the method was 125. Moreover, the EF for Ni(II) and
319 Cu(II) were 108 and 97, respectively. Detailed information and comprehensive data can be
320 found in Table12.

321 The method was validated using CRM. GBW10015-Spinach, GBW10016-Tea, and
322 GBW10019-Apple were analyzed for Ni (II) and Cu (II). In GBW10015 Spinach (Ni), the
323 calculated Ni concentration in Spinach was 0.86 mg kg^{-1} , and the reference value was 0.92 mg
324 kg^{-1} . The calculated percent recovery was 93.4%, indicating that the method was quite accurate
325 for this element in this reference material. The t-exp value was 0.84, which suggests a good
326 agreement between the experimental mean and the certified value. In GBW10015 Spinach (Cu),
327 for Cu in Spinach, the calculated value was 8.5 mg kg^{-1} , while the reference value was 8.9 mg
328 kg^{-1} . Thus, the percent recovery was 95.0%, indicating a high level of accuracy. The t-exp value



329 was 1.12, which confirms good agreement. The results of GBW10016 Tea and GBW10019
330 Apple for both Ni and Cu gave high percent recovery% and t-exp values close to 1 indicate that
331 the method was providing accurate results, confirming high applicability of the method in
332 routine analysis of real samples..

333 **3.2.2. Precision**

334 The MAGDES-OS-DLLME method was examined for both intraday and inter-day variations
335 at different concentrations of analytes within the specified working range. The concentrations
336 tested included low ($5 \mu\text{g L}^{-1}$), medium ($100 \mu\text{g L}^{-1}$), and high ($200 \mu\text{g L}^{-1}$) levels. In the
337 intraday study, the added concentrations of analyte ions were investigated with five repetitive
338 extractions on the single day. In the interday study, the same concentrations were studied with
339 five repetitive extractions on three consecutive days. In addition, the accuracy of the MAGDES-
340 OS-DLLME method was tested by performing a recovery study for the concentrations added
341 in these studies. The RSD% for Ni (II) ion in the intraday and interday study were in the range
342 of 2.8-3.3% and 3.1-3.6%, respectively. In addition, the RSD for Cu (II) ion in the intraday and
343 interday study were in the range of 2.4-3.5% and 2.9-3.9%, respectively. Furthermore, it
344 provided quantitative recoveries (see Table 1) for both analytes.

345 **3.2.3. Accuracy**

346 Some certified reference materials (GBW10015 spinach, GBW10016 tea and GBW10019
347 apple) were analyzed in this study with the MAGDES-OS-DLLME method. Five replicates of
348 each reference material were analyzed and the results averaged. Experimental results from
349 analysis of GBW10015 spinach, GBW10016 tea and GBW10019 apple were $0.86 \pm 0.16 \text{ mg Kg}^{-1}$
350 ¹ for Ni/ $8.9 \pm 0.4 \text{ mg Kg}^{-1}$ for Cu, $3.3 \pm 0.18 \text{ mg Kg}^{-1}$ for Ni/ $18.6 \pm 0.7 \text{ mg Kg}^{-1}$ for Cu and
351 $0.13 \pm 0.04 \text{ mg Kg}^{-1}$ for Ni/ $2.5 \pm 0.2 \text{ mg Kg}^{-1}$ for Cu, respectively. At a 95% confidence level, the
352 obtained results were in good agreement with the reference values. Also, the t-exp (0.56-1.24)



353 of all studies was smaller than the t-tabulate (2.31). These findings suggest that the observed
354 results exhibit no statistically significant difference when compared to the expected values. In
355 this particular aspect of the research, the recovery rates for Ni (II) ranged from 92.9% to 97.1%,
356 while the recovery rates for Cu (II) ranged from 95.0% to 97.8%. Detailed results were given
357 in Table 2.

358

359 **3.2.4. Matrix effect**

360 The matrix effect on the MAGDES-OS-DLLME method under optimized conditions was
361 investigated in the following part of the study. This study was carried out on the food samples
362 because it can create stronger matrix effects comparing to relatively simple water samples. On
363 the other hand, the mineralization step of the procedure, significantly simplifies the matrix
364 proceeded to extraction. First, the anions and cations listed in Table S2 were added to the food
365 sample in different proportions. Then, the recovery, RSD% and tolerance limit of the related
366 species were estimated for the target analytes. According to Table S2, the RSD% for both
367 analytes was lower than 2.6%. Quantitative recoveries (92-99%) were also obtained for both
368 analytes. High tolerable limits (up to 10000) were obtained in the presence of studied anions
369 and cations. Results shows that the MAGDES-OS-DLLME is highly selective and stable for
370 matrix interferences for both analytes.

371 **3.3. Application of method- real samples analysis**

372 The MAGDES-OS-DLLME method was employed to determine the targeted analytes in water
373 and food samples. To assess the method's accuracy in analyzing water samples, a two-level
374 standard addition approach was employed, involving additions of 10 and 100 $\mu\text{g L}^{-1}$
375 concentrations. Both Ni and Cu were not detected in bottled water, spring water-1 and mineral
376 water. The highest Ni ($14.2 \pm 1.6 \mu\text{g L}^{-1}$) and Cu ($9.2 \pm 0.2 \mu\text{g L}^{-1}$) contents were detected in



377 spring water-2 and well-water, respectively. Furthermore, recoveries for both analytes were in
378 the range of 92.5-98.3% and 93.0-98.6%, respectively (Table 5a). Using the MAGDES-OS-
379 DLLME method, the Ni could not be detected in food samples including rice flour, wheat and,
380 and pomegranate. In addition, the highest Ni content ($5.12 \pm 0.09 \mu\text{g g}^{-1}$) was detected in
381 spinach. The Cu was detected in all food samples. In addition, the highest Cu content ($44.2 \pm$
382 $0.8 \mu\text{g g}^{-1}$) was detected in green pepper. Finally, all results were within WHO's acceptable
383 limits.

384 **3.4. Comparative study**

385 The important analytical parameters of the MAGDES-OS-DLLME method were compared
386 with different micro-extraction studies using a similar technique for quantitative analysis. In
387 terms of linearity, LOD, RSD%, EF/PF, and estimated extraction procedure time, the developed
388 method underwent assessment and was compared to other reported methods. A detailed
389 comparison of the data can be found in Table S3. The extraction time of the developed method
390 (only 8 mins) was lower than all the compared methods. Also, the linearity of the method was
391 comparable to other methods. In addition, the LOD of the method was lower than other
392 procedures. The PF obtained for both analytes was higher in most microextraction methods.

393 **4. Conclusions.**

394 The MAGDES-OS-DLLME method was employed for extraction of Ni(II) and Cu(II) from
395 water and food samples. Among the studied ferrofluid-based DESs, the one made of toner
396 powder@aliquat 336 and lauric acid:menthol (molar ratio 1:2) was selected as extraction
397 solvent. The obtained magnetic DES extract is collected by magnetic stir bar, followed by
398 analytes desorption by 300 μL of THF. Final determination step is performed by means of
399 FAAS. To the best of our knowledge, this study is the first ferrofluid DES-based analytical
400 method reported so far for the simultaneous determination of Ni(II) and Cu(II). In addition,



401 negligible matrix effect and short extraction time were obtained with the developed method.
402 Extensive validation studies, proved the usefulness of the MAGDES-DLLME-FAAS method
403 in routine analysis of water and food samples.. Based on the obtained results, it has been shown
404 that the MAGDES-DLLME-FAAS provide high reproducibility, low LODs, high PF and low
405 matrix effect. Analysis of real samples revealed presence of controlled metals, but in all cases
406 reported values were below WHO limits.

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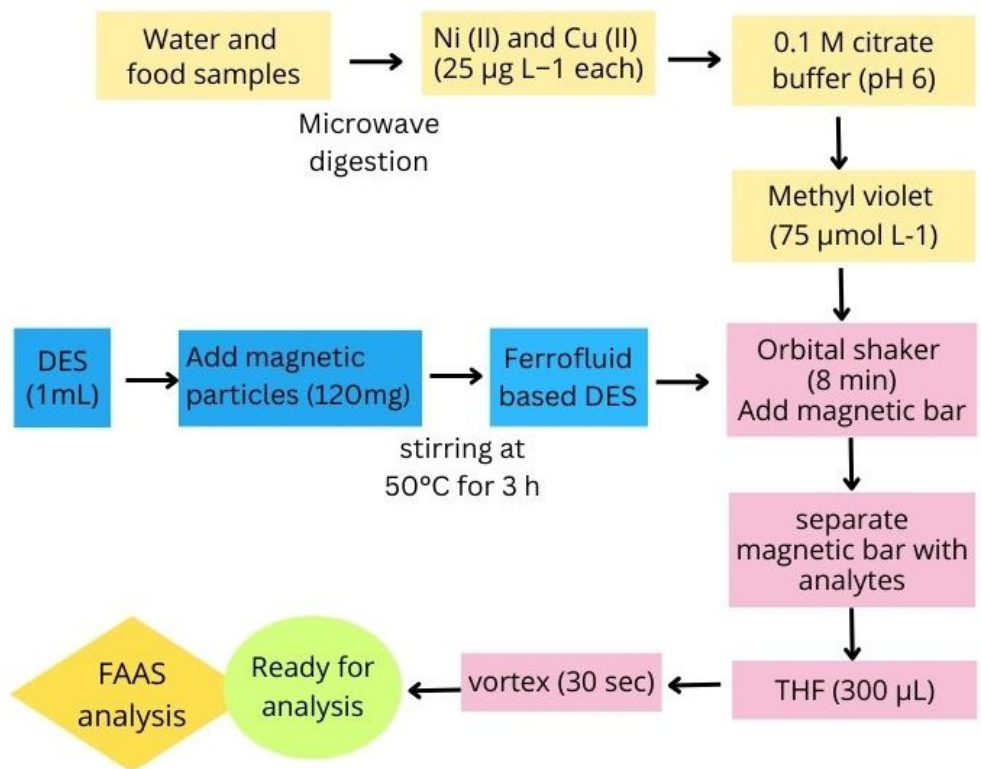
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551 Figure 1. Schematic diagram for the MAGDES-OS-DLLME method

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553

554 Table 1. Analytical characteristics of the MAGDES-OS-DLLME method.

Analyte s	Linearit y ($\mu\text{g L}^{-1}$)	LO D ($\mu\text{g L}^{-1}$)	LO Q ($\mu\text{g L}^{-1}$)	EF	PF	a,b Recovery (%)			a,b RSD (%)		
						5 $\mu\text{g L}^{-1}$	100 $\mu\text{g L}^{-1}$	200 $\mu\text{g L}^{-1}$	5 $\mu\text{g L}^{-1}$	100 $\mu\text{g L}^{-1}$	200 $\mu\text{g L}^{-1}$
Ni(II)	0.1-330	0.03	0.1	135. 9	12 5	96.4 ^a (93.1) ^b	97.3(95.2)	97.8 (96.1)	3.1 ^a (3.4) ^b	2.8 (3.1)	3.3 (3.6)
Cu(II)	0.5-250	0.15	0.46	112. 5	12 5	94.1 (94.8)	95.4 (95.5)	97.2 (96.7)	2.4 (2.9)	2.8 (3.3)	3.5 (3.9)

555 a: intraday studies (N=5) b: interday studies (N=5x3)

556 LOD: limit of detection

557 LOQ: Limit of quantification

558 EF: Enrichment factor

559 PF: Preconcentration factor

560 RSD: Relative standard deviations

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564 Table 2. Analysis of reference materials using the MAGDES-OS-DLLME method (N=5).

Reference material	Ni				Cu			
	Referenc e value (mg kg^{-1})	Calculate d (mg kg^{-1})	Recover y (%)	*t- exp	Referenc e value (mg kg^{-1})	Calculate d (mg kg^{-1})	Recover y (%)	*t- exp
GBW1001 5 Spinach	0.92±0.1 2	0.86±0.16	93.4	0.8 4	8.9±0.4	8.5±0.8	95.0	1.1 2
GBW1001 6 Tea	3.4±0.30	3.3±0.18	97.1	1.2 4	18.6±0.7	18.2±1.2	97.8	0.7 4
GBW1001 9 Apple	0.14±0.0 5	0.13±0.04	92.9	0.5 6	2.5±0.2	2.4±0.2	96.2	1.1 5

565 * $t_{\text{exp}} = \frac{|\mu - \bar{x}|}{s} \sqrt{N}$, where t_{exp} , s, N, \bar{x} and μ were statistical value, the standard deviation, number of independent

566 determinations, the experimental mean value, and the certified value, respectively.

Table 3. Results from the analysis of the water samples using the MAGDES-OS-DLLME method (N=3)

Water samples	Ni			Cu	
	Spiked, Ni(II), Cu(II), $\mu\text{g L}^{-1}$	Calculated, $\mu\text{g L}^{-1}$	Recovery, %	Calculated, $\mu\text{g L}^{-1}$	Recovery, %
Bottled water	-	$\leq\text{LOD}$	-	$\leq\text{LOD}$	-
	10	$9.7\pm 0.5^*$	97.0	9.5 ± 0.5	95.0
	100	98.3 ± 3.4	98.3	97.2 ± 4.1	97.2
Wastewater	-	5.4 ± 0.2	-	2.6 ± 0.2	-
	10	15.0 ± 1.1	96.1	12.0 ± 0.9	94.0
	100	102.9 ± 4.8	97.6	99.3 ± 4.4	96.7
River water	-	11.8 ± 1.6	-	3.3 ± 0.8	-
	10	21.5 ± 2.3	96.7	12.6 ± 1.2	93.0
	100	107.7 ± 5.2	95.9	100.2 ± 3.6	96.9
Spring water-1	-	$\leq\text{LOD}$	-	$\leq\text{LOD}$	-
	10	9.7 ± 0.8	97.0	9.6 ± 0.7	96.0
	100	96.3 ± 4.1	96.3	98.5 ± 4.2	98.5
Spring water-2	-	14.2 ± 1.6	-	4.7 ± 0.3	-
	10	23.7 ± 2.8	95.0	14.4 ± 0.8	97.0
	100	11.9 ± 4.9	97.7	103.1 ± 5.2	98.4
Mineral water	-	$\leq\text{LOD}$	-	$\leq\text{LOD}$	-
	10	9.6 ± 0.4	96.0	9.3 ± 0.1	93.0
	100	98.1 ± 3.7	98.1	95.7 ± 3.8	95.7
Well-water	-	8.2 ± 0.9	-	9.2 ± 0.2	-
	10	17.5 ± 1.3	93.0	18.8 ± 1.1	96.0
	100	103.8 ± 4.6	95.6	107.4 ± 4.6	98.2

*Mean value \pm standard deviation based on three replicate determinations

1 Table 4. Results from the analysis of the food samples using the MAGDES-OS-DLLME method
 2 (N=3)

Food samples	Ni		Cu	
	Calculated, $\mu\text{g g}^{-1}$	RSD, %	Calculated, $\mu\text{g g}^{-1}$	RSD, %
Black tea	$0.75 \pm 0.01^*$	1.3	24.4 ± 0.6	2.5
Rice flour	$\leq\text{LOD}$	-	28.7 ± 0.9	3.1
Wheat	$\leq\text{LOD}$	-	5.9 ± 0.2	3.4
Green pepper	2.26 ± 0.03	1.3	44.2 ± 0.8	1.8
Spinach	5.12 ± 0.09	1.8	10.3 ± 0.3	2.9
Apple	0.26 ± 0.01	3.8	3.9 ± 0.1	2.6
Eggplant	0.38 ± 0.01	2.6	6.2 ± 0.2	3.2
Pomegranate	$\leq\text{LOD}$	-	11.3 ± 0.5	4.4
Parsley	1.95 ± 0.05	2.7	26.5 ± 0.6	2.2
Mint	2.04 ± 0.01	2.6	11.6 ± 0.4	3.5
Tomato	0.33 ± 0.01	3.0	6.9 ± 0.1	1.5
Mushroom	1.58 ± 0.03	2.2	2.3 ± 0.1	4.3
Potato	1.92 ± 0.04	2.1	81.3 ± 2.9	3.6

3 *Mean value \pm standard deviation based on three replicate determination

4