

1           **Application of chemometric modeling for ionic liquid-based ultrasonic-assisted**  
2           **dispersive liquid-liquid microextraction: Analysis of fosetyl-aluminum in fruit and**  
3           **vegetable samples**

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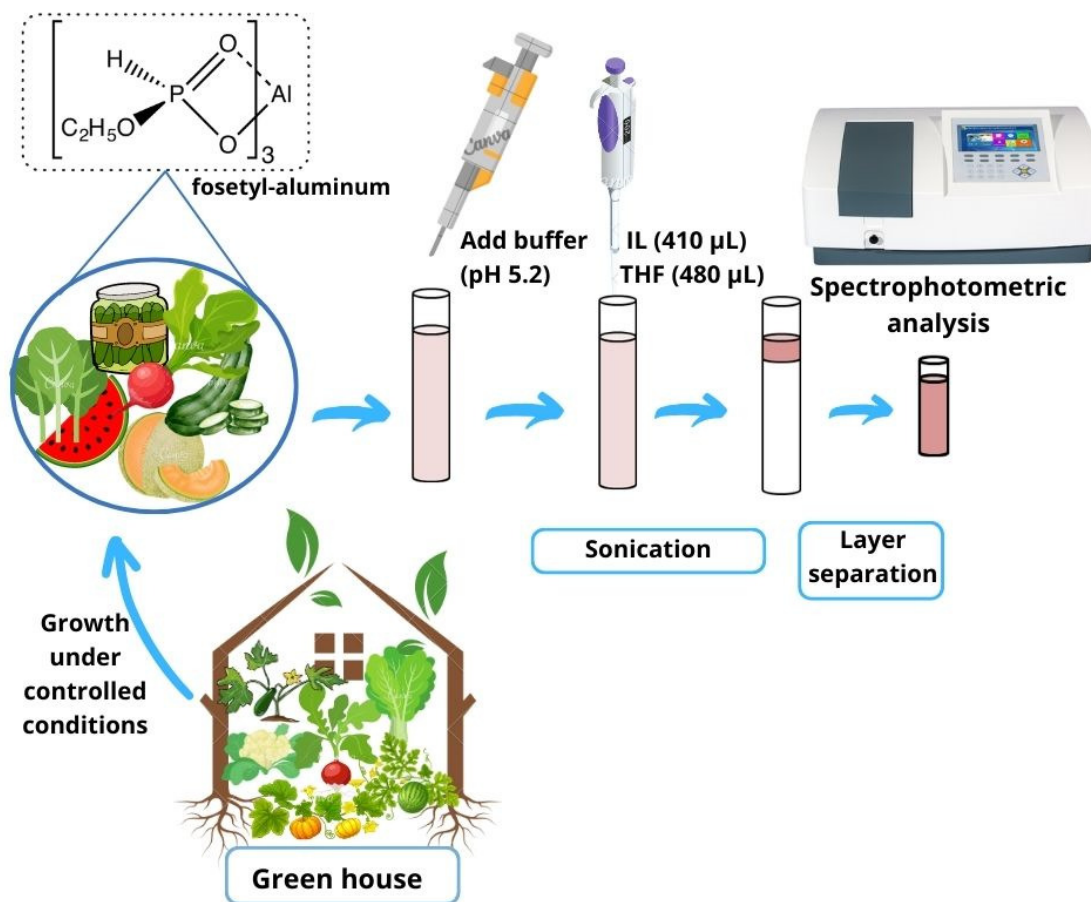
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## Graphical abstract



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19      **Highlights**

- 20      • Ionic liquid-based pre-concentration method for fosetyl-aluminum analysis in foods.
- 21      • Increased mass transfer via DLLME for fungicide isolation from plant material.
- 22      • Fully validated method with proved applicability to real samples and routine analysis.
- 23      • A highly sensitive, selective, and robust assay for environmental monitoring.
- 24      • Fast analyte isolation followed by spectroscopic final determination stage

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26

27 **Abstract**

28 This manuscript presents a new method for selective extraction and determination of fosetyl-  
29 aluminum in fruits and vegetable samples based on ultrasonic-assisted dispersive liquid-liquid  
30 microextraction method using ionic liquids (IL-UA-DLLME). A UV-Visible  
31 spectrophotometer was used for detection and quantification. Plants used for sample  
32 collection were grown under controlled conditions in a greenhouse. Central composite design  
33 (CCD)-response surface methodology (RSM) analysis was used for the optimization of  
34 significant factors (volume of IL, pH, ultrasonication time, and THF volume). Under optimal  
35 conditions, the limit of detection and limit of quantification of the IL-UA-DLLME procedure  
36 were  $1.5 \text{ ng mL}^{-1}$  and  $5.0 \text{ ng mL}^{-1}$  respectively with relative standard deviation 1.9-3.3%. The  
37 developed IL-UA-DLLME procedure demonstrated linearity within the concentration range  
38 of  $5\text{-}600 \text{ ng mL}^{-1}$  with an  $R^2$  value of 0.9914. The enrichment factor was 114 with a  
39 recovery% of 94.2-98.6% ( $n=3$ ) at optimal conditions. The IL-UA-DLLME assay was used  
40 for the analysis of fosetyl-aluminum in a variety of food samples and was found highly  
41 selective and efficient.

42 **Keywords:** Food analysis; Food contamination; Organomettalic compounds analysis; Sample  
43 preparation; Trace analysis.

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## 50 **1. Introduction**

51 As the population continues to grow at a rapid pace, the issue of nutrition has emerged as  
52 a major concern in our era. To enhance the quality and quantity of crops, farmers are widely  
53 using pesticides to solve the issues related to presence of undesirable organisms (Tudi et al.,  
54 2021). Statistics indicate that herbicides account for 47.5% of all pesticides utilized globally,  
55 while insecticides make up 29.5%, fungicides 17.5%, and the remaining 5.5% represent other  
56 methods of pest control (Sharma et al., 2019). Fungal plant pathogens are capable of causing  
57 significant reductions in crop yield across all agricultural systems globally (Wan de Wouw et  
58 al., 2021). The extensive utilization of chemical pesticides has adverse effects on the  
59 environment, leading to concerns about pollution. The accumulation of these pesticides on  
60 living organisms, pollution of soil and water, and destruction of beneficial organisms are  
61 some of the environmental problems caused by their widespread use (Bohinc et al., 2019).  
62 Fungal diseases such as mold and mildew can significantly diminish crop yields, making  
63 fungicides essential for agriculture and ensuring food safety (Zubrod et al., 2019). To prevent  
64 risks related to phytopathogens and increase productivity, fungicides are extensively used  
65 (Shahid et al., 2020). Despite their beneficial effects on preventing damage caused by  
66 phytopathogens, the detrimental impact of fungicides on soil microbiota's composition and  
67 functions is a significant concern for both plant and human health along the food chain  
68 (Shahid et al., 2021).

69 Fungicides, which belong to a large group of pesticides, are frequently used in high-yield  
70 agriculture to protect plants against the detrimental effects of phytopathogens and enhance  
71 crop production. These chemicals are utilized to combat a wide range of fungal diseases and  
72 prevent plant infections. It is applied to agricultural production for the preservation of root  
73 crops, vegetables, and fruits, or as a direct treatment for ornamental plants, trees, field crops,  
74 cereals, and grasses. In a study conducted by Kiselev et al. (2022), it was discovered that



75 fungicides with long-lasting effects, developed for use on potato plants, are capable for  
76 effectively suppressing disease development and increasing potato yield. Additionally, these  
77 preparations gradually release pesticides into the soil during precipitation or irrigation,  
78 reducing the abrupt release of these chemicals. The researchers also noted that these new  
79 formulations enable the reduction of pesticide application rates, minimizing the risk of  
80 pesticide dispersion and accumulation in the biosphere (Pérez-Lucas, Vela et al. 2019, Tudi,  
81 Daniel Ruan et al. 2021).

82 Fosetyl-aluminum is a systemic fungicide that is utilized to manage numerous fungal  
83 diseases in plants, such as downy mildew, Phytophthora, and Pythium. It is a phosphonate-  
84 derived substance that is usually administered as either a foliar spray or soil drench (Han et  
85 al., 2012). Although fosetyl-aluminum has been used for many years, there have been  
86 concerns about its potential environmental and health impacts. Some studies have suggested  
87 that fosetyl-aluminum may be toxic to aquatic organisms and may accumulate in the human  
88 body. There have been also a reports on health effects for humans, such as skin and eye  
89 irritation (Han et al., 2012, Rouabhi, 2010). Fosetyl-aluminum is generally considered to have  
90 low acute toxicity to mammals, including humans. However, chronic exposure or high levels  
91 of exposure may have adverse effects. Studies on laboratory animals have shown that high  
92 doses of fosetyl-aluminum can cause reproductive and developmental toxicity, including  
93 effects on fertility and fetal development. Additionally, there have been concerns raised about  
94 potential carcinogenic effects. Skin contact, inhalation of spray mists, or ingestion of  
95 contaminated food or water are potential routes of exposure. Short-term exposure may cause  
96 irritation to the skin, eyes, and respiratory system (Authority, Arena et al. 2018, Gormez,  
97 Golge et al. 2022).

98 Several analytical methods are available for fosetyl-aluminum analysis including HPLC,  
99 flow injection analysis (Sadiq and Hammood, 2022), ion chromatography (Rajski et al.,



100 2018), liquid chromatography-triple quadrupole mass spectrometer (López-Ruiz et al., 2020),  
101 liquid chromatography-tandem mass spectrometry (Chamkasem, 2017). However, sample  
102 pretreatment/sample preparation is required before analysis. Sample preparation is an  
103 important step in analytical methods, where extraction is a commonly used procedure to  
104 enhance sensitivity and selectivity. This method involves isolating and concentrating analytes  
105 from complex sample matrices like food or biological fluids before analysis (Ullah et al.,  
106 2022). Extraction can significantly increase the sensitivity of analytical methods as it reduces  
107 matrix effects (Makoś et al., 2018, Haq et al., 2021). Furthermore, extraction can reduce the  
108 effects of the sample matrix on the analysis and save time and resources by reducing the  
109 volume of the sample matrix (Haq et al., 2023).

110 Ionic liquids (ILs) are a particular type of salts that possess distinctive characteristics such  
111 as low volatility, high thermal stability, and adjustable polarity. Due to these characteristics,  
112 ILs have been increasingly used as solvents or extractants in various extraction techniques,  
113 especially in liquid-liquid extraction. ILs have high solubility for organic and inorganic  
114 compounds, making them an effective extractants for a diverse range of samples (Han et al.,  
115 2012). Unlike many traditional solvents, ILs are often less toxic, non-volatile, and non-  
116 flammable, making them more environmentally friendly. By altering the chemical structure of  
117 the cation or anion, ILs can be customized to exhibit high selectivity for particular analytes.  
118 This attribute renders them appropriate for extracting analytes from intricate matrices (Llaver  
119 et al., 2021). ILs have high thermal stability and do not undergo phase separation or  
120 degradation at high temperatures or in the presence of water or other polar solvents  
121 (Huddleston et al., 2001). ILs can be easily recovered and reused multiple times, making them  
122 a cost-effective and sustainable alternative to traditional solvents (Chiappe et al., 2016). ILs  
123 are compatible with many analytical instruments and do not require additional derivatization  
124 or extraction steps (Farajzadeh et al., 2020; Rykowska et al., 2018).



125 Central Composite Design (CCD) is an important experimental design technique widely  
126 used to optimize analytical methods (Rasheed et al., 2023). It allows for a systematic and  
127 efficient exploration of the design space by carefully selecting a limited number of  
128 experiments. By incorporating a balanced combination of factorial, axial, and center points,  
129 CCD ensures coverage of a broad range of factor settings, facilitating the identification of  
130 optimal operating conditions (Chen et al., 2020). CCD is especially useful for detecting and  
131 modeling nonlinear relationships between variables. It effectively captures curvature and  
132 interaction effects through the inclusion of axial points, resulting in a more precise  
133 representation of the response surface. This capability enhances the understanding of complex  
134 variable relationships and aids in determining the best combination of factors for optimization  
135 (Bahram et al., 2016, Sharma et al., 2022, Chen et al., 2020, Ngan et al., 2014).

136 A novel approach was developed for the extraction and analysis of fosetyl-aluminum in  
137 fruit and vegetable samples, utilizing the ultrasonic-assisted dispersive liquid-liquid  
138 microextraction technique with an ionic liquid. This method was designed based on the  
139 properties and applicability of extraction and is noted for its high sensitivity, selectivity, and  
140 versatility over a broad range of concentrations.

## 141 **2. Materials and methods**

### 142 **2.1. Instrumentation**

143 A UV-Visible spectrophotometer (Shimadzu 1800 model, Kyoto, Japan) was used for  
144 absorbance measurements. A cuvette (Fisher, Germany) made from quartz glass (volume 500  
145  $\mu\text{L}$ ) was used as sample holder for spectrophotometric measurements. Microwave system  
146 (Milestone Ethos, Italy) was used for the digestion of fruit and vegetable samples. Ultra-pure  
147 water (18.2 M $\Omega$ ) was obtained from Milli-Direct Q3 system (Millipore, Bedford, MA, USA).  
148 An ultrasonic bath (SK5210LHC Kudos, Shanghai, China) was used for sonication. A pH  
149 meter (model 630 Metrohm, Switzerland) with digital pH measuring input for the intelligent





150 pH electrodes from Metrohm, was used for the pH adjustment of samples. The combination  
151 electrode consists of two main parts: a pH-sensitive glass membrane and a reference electrode  
152 (Ag/AgCl electrode immersed in KCl). A centrifuge (Universal-320 model, Hettich  
153 Universal, England) was used to separate the IL phases from the sample solution.

## 154 2.2. Chemicals and solutions

155 The chemicals and reagents used in this research were obtained from Sigma (St. Louis,  
156 MO, USA) and Merck (Darmstadt, Germany). All chemicals were of analytical purity and no  
157 further purification step was applied. The stock solution ( $500 \text{ mg L}^{-1}$ ) of fosetyl-aluminum  
158 was prepared by dissolving the appropriate amount of its solid reagent (Merck) in the water.  
159 Working and calibration solutions of fosetyl-aluminum were prepared by daily dilution of the  
160 stock solution. Tributyl-tetradecylphosphonium chloride (Sigma,  $[\text{P}_{4,4,4,14}]\text{Cl}$ ) ionic liquid  
161 was used as the extraction solvent. Tetrahydrofuran (THF) (Merck) was used to disperse IL in  
162 the sample solution. Citrate buffer solution (0.1 M pH 5.2) was prepared by dissolving  
163 appropriate amount of sodium citrate dihydrate and citric acid in water.

## 164 2.3. Sampling

165 This study was conducted in the greenhouse of Sivas Cumhuriyet University,  
166 Department of Crop and Animal Production, using a plastic pots with a capacity of 3 kg. The  
167 research followed a randomized plot design, with 3 replications. A soil samples were  
168 collected from a depth of 0-20 cm and had a clay loam texture, calcareous (13.8%), salt-free  
169 (0.026%), low organic matter content (1.77%), slightly alkaline pH (7.89), low phosphorus  
170 content ( $48.8 \text{ kg ha}^{-1}$ ) and sufficient potassium content ( $1099.5 \text{ kg ha}^{-1}$ ). In the study, melon,  
171 watermelon, collards, gherkin, cauliflower, radish, and zucchini were used as test plants. As  
172 basic fertilization, nitrogen  $150 \text{ mg Kg}^{-1}$  ( $\text{CaNO}_3 \cdot 4\text{H}_2\text{O}$ ), phosphorus  $100 \text{ mg Kg}^{-1}$ , and  
173 potassium  $125 \text{ mg Kg}^{-1}$  ( $\text{KH}_2\text{PO}_4$ ) were applied for all plants with planting. After the plants



174 emerged and reached a certain size, the fungicide containing 80% Fosetyl-Al was added 3  
175 times in total, at weekly intervals. 50 days after sowing, the plants were harvested by cutting  
176 them from the soil surface. The harvested plants included melons, watermelons, collards,  
177 gherkins, cauliflowers, radishes, and zucchinis, which were then left to dry in the shade at  
178 room temperature. The dried plants were subsequently grinded using a plant grinding mill,  
179 with 1 g of each plant weighed and combined with 10 mL of distilled water (at a ratio of  
180 1:10). The mixture was then subjected to maceration, wherein it was shaken for 24 h in a  
181 shaker. Following the maceration process, the mixture was filtered with No. 1 Whatman blue  
182 band filter paper, and the water was evaporated using a rotary evaporator at 40°C.

#### 183 **2.4. Experimental design**

184 To optimize and design the experimental parameters and perform ANOVA analysis of  
185 the analytical data, trial version 11.0.3.0 of the Design-Expert® package from STATISTICA  
186 was used. A central composite design-response surface methodology approach was used for  
187 the optimization of extraction-affecting factors for fosetyl-aluminum. Four parameters  
188 including IL volume (300-900 µL), pH (4-8), ultrasound time (2-10 min), and THF volume  
189 (100-500 µL) were optimized using a three-level CCD model. Total 30 experiments  
190 (including 6 central experiments) designed by CCD model were performed. Optimized  
191 parameters, their units, symbols, and their lowest-highest limits are given in Table S1.

#### 192 **2.5. IL-UA-DLLME procedure**

193 The experimental steps of the IL-UA-DLLME procedure are as follows. First, 10 mL of the  
194 digested samples were added to the conical tubes. After this step, the pH of the sample  
195 solution was adjusted to pH 5.2 using 0.1 M citrate buffer solution. In order to extract the  
196 fosetyl-aluminum in the sample solution, first 410 µL of [P<sub>4,4,4,14</sub>]Cl (as extraction solvent)  
197 and then 480 µL of THF (as the dispersing solvent) were added to the obtained mixture.  
198 Conical tubes were placed in an ultrasonic bath and sonicated for 2.5 min at room temperature



199 to effectively disperse the [P<sub>4,4,4,14</sub>]Cl in the sample solution. At this stage, the [P<sub>4,4,4,14</sub>]Cl  
200 phase containing fosetyl-aluminum was collected on top of the aqueous solution. The  
201 [P<sub>4,4,4,14</sub>]Cl phase was transferred to microcuvettes using a syringe and absorbance  
202 measurements were made using UV-spectrophotometer at 289 nm. All these studies were  
203 carried out in parallel with the sample blank and standard spiked samples.

## 204 **2.6. Calculations of recovery and validation assay**

205 The percent recovery was utilized as a reference in the optimization studies to choose  
206 appropriate values for the extraction parameters. The percent recovery was calculated using  
207 the following equation 1.

$$208 \quad \text{Percent recovery} = \frac{C_e}{C_a} \times 100 \quad (1)$$

209 In the above equation, C<sub>e</sub> is the concentration experimentally determined in the model sample  
210 and C<sub>a</sub> is the actual/expected concentration.

211 To evaluate the matrix effect of components, the absorbance of the fosetyl-aluminum in the  
212 matrix standard and the absorbance of the the fosetyl-aluminum in the solvent standard at the  
213 same concentration level were used (Rutkowska et al., 2018).The matrix effect was calculated  
214 using the following equation 2.

$$215 \quad \text{Matrix effect} = \left( \frac{\text{absorbance}(\text{matrix standard})}{\text{absorbance}(\text{solvent standard})} - 1 \right) \times 100 \quad (2)$$

216 In analytical chemistry, the relative standard deviation (RSD%) is frequently used to describe  
217 the reproducibility of an assay. RSD% for this method was calculated using the following  
218 equation 3.

$$219 \quad RSD(\%) = \frac{sy/x}{C_m} \times 100 \quad (3)$$

220 In the above equation, sy/x is the residual standard deviation and C<sub>m</sub> is the  
221 mean concentration in real samples.

222 In line with the aforementioned method, the enrichment factor (EF) was determined as the  
223 ratio between the concentration of the analyte in the final phase, which is prepared for  
224 analysis, and that in the initial solution. The EF was calculated using the following equation 4.

$$225 \quad EF = \frac{C_f}{C_i} \quad (4)$$

226 The equation mentioned above uses  $C_f$  to represent the final concentration and  $C_i$  to represent  
227 the initial concentration of fosetyl-aluminum in the acceptor phase (IL phase) and donor  
228 phase, respectively. To determine the limit of detection (LOD) and limit of quantification  
229 (LOQ), the following formulas (5 and 6) was utilized.

$$230 \quad LOD = \frac{3 \times sy/x}{m} \quad (5)$$

$$231 \quad LOQ = \frac{10 \times sy/x}{m} \quad (6)$$

232 In the above equations,  $sy/x$  is the residual standard deviation of regression line and  $m$  is the  
233 slope of the calibration curve.

### 234 **3. Results and discussion**

#### 235 **3.1. Optimization of the extraction parameters using a central composite design**

236 The CCD is a commonly utilized method for experimental design in the process of  
237 optimizing analytical methods. Response surface methodology (RSM) is used to develop a  
238 mathematical model that describes the relationship between the response (i.e., the analytical  
239 signal) and the independent variables (i.e., the factors affecting the analytical method). The  
240 CCD is a common experimental design technique for optimizing analytical methods. The  
241 CCD comprises three categories of points: factorial, axial, and center points. Factorial points  
242 are ordinary experimental points utilized to ascertain the primary and interaction effects of  
243 independent variables. Axial points aid in estimating the curvature of the response surface,  
244 while center points are employed to estimate errors in the model. The CCD was used for the  
245 optimization of important analytical parameters and statistical analysis of the obtained results.



246 The CCD model was used for the optimization of four analytical parameters, IL volume, pH,  
247 ultrasonic time, and THF volume. The parameters were labeled as (A) for IL volume, (B) for  
248 pH, (C) for ultrasonic time, and (D) for THF volume. Results were obtained for recovery of  
249 fosetyl-aluminum. The design layout for the method using the CCD model is given in Table  
250 S2.

251 The suitability of the CCD was determined by assessing various statistical parameters  
252 such as the coefficients of determination ( $R^2$ ), adjusted  $R^2$  and predicted  $R^2$ , the p-value, and  
253 the lack-of-fit (LOF) test. The effect of the optimized parameters on the extraction of fosetyl-  
254 aluminum was evaluated using ANOVA statistical analysis, and the results are presented in  
255 Table 1. The high values of  $R^2$ , adjusted  $R^2$ , and predicted  $R^2$  suggest that the proposed model  
256 is well-suited to the experiment. The predicted  $R^2$  value is in reasonable agreement with the  
257 adjusted  $R^2$  value, with a difference of 0.0077, which indicates that the CCD methodology is  
258 being properly followed. The p-value, which should be less than 0.04 at the 95% confidence  
259 level, is  $<0.0001$  for this experiment, indicating that the parameters of the CCD have a  
260 significant effect. The statistical analysis reveals that the proposed model is well-suited to the  
261 experiment, as evidenced by the  $R^2$  (0.9984), adjusted  $R^2$  (0.9969), and predicted  $R^2$  (0.9910)  
262 values which are close to 1. The p-values for the model terms indicate that A, B, C, D, AB,  
263 AD, BC, BD, CD,  $A^2$ ,  $B^2$ ,  $C^2$ , and  $D^2$  are all significant. Lack of Fit for the proposed method  
264 is not significant. The final equation in terms of coded factors

$$\begin{aligned} 265 \text{ Recovery (\%)} = & +73.49 + 0.2722A - 5.74B + 1.54C + 5.88D - 3.16AB - 1.67AC - 4.47AD \\ 266 & + 3.14BC - 1.34BD + 2.77CD - 4.28A^2 - 7.23B^2 + 8.32C^2 + 7.02D^2 \end{aligned}$$

267 Furthermore, the effect of the signal-to-noise ratio on the CCD was evaluated using  
268 adequate precision. To achieve statistical significance, the adequate precision must exceed 4.  
269 According to the results presented in Table 1, the obtained adequate precision (105.91) was  
270 significantly greater than the critical value. Figure 1 shows the actual vs predicted values  
271 graph.



272 3D surface plots were used to plot the effect of binary interactions of optimized factors  
273 on the recovery of fosetyl-aluminum. The effect of the IL volume versus pH on the recovery  
274 of fosetyl-aluminum was given in Figure 2a. It can be seen that acceptable recoveries were  
275 achieved, especially when the pH was less than 6. Interestingly, phase separation could not be  
276 achieved due to decreased activity of IL binding sites in the basic region. The effect of  
277 ultrasonic time versus IL volume on the recovery of fosetyl-aluminum was presented in  
278 Figure 2b. To some extent, the recovery of fosetyl-aluminum was quantitative when  
279 ultrasonic time and IL volumes were in the range of 8-10 min and 300-500  $\mu\text{L}$ , respectively.  
280 In particular, the recovery of fosetyl-aluminum was not quantitative at high ionic liquid  
281 volumes. This may be attributed to insufficient sonication to achieve distribution in the  
282 sample solution with increasing IL volume. The effect of THF volume versus IL volume on  
283 the recovery of fosetyl-aluminum was shown in Figure 2c. THF (as a dispersive solvent)  
284 helped to increase its interaction with fosetyl-aluminum by effectively dispersing IL in the  
285 sample solution. In this way, the fosetyl-aluminum in the sample solution was easily  
286 transferred to the IL phase. Due to this phenomenon, quantitative recoveries were obtained  
287 when THF volume and IL volume were in the range of 380-490  $\mu\text{L}$  and 330-450  $\mu\text{L}$ ,  
288 respectively.

289 In the optimization step, CCD was applied to maximize the recovery of fosetyl-  
290 aluminum. According to the CCD, the maximum recovery was obtained using IL volume (410  
291  $\mu\text{L}$ ), pH (5.2), ultrasonic time (2.5 min), and THF volume (480  $\mu\text{L}$ ). After five replicates, the  
292 experimental recovery of fosetyl-aluminum was as high as 93.9%, which agrees with the  
293 predicted recovery (93.4%) of the CCD with a 0.945 of desirability function (see Figure 3).  
294 Therefore, these extraction conditions were selected as optimum values for the other studies  
295 such as validation and analysis.

### 296 **3.2 Analytical parameter of the IL-UA-DLLME procedure**



297 Basic analytical parameters of the IL-UA-DLLME procedure were estimated using  
298 optimized extracting conditions (IL volume 410  $\mu\text{L}$ , pH 5.2, ultrasonic time 2.5, and THF  
299 volume 480  $\mu\text{L}$ ). The linearity of the method was observed within the concentration range of  
300 5-600  $\text{ng mL}^{-1}$  with a high coefficient of determination ( $R^2$ ) of 0.9914. The limit of detection  
301 (LOD) and limit of quantification (LOQ) were calculated to be 1.5  $\text{ng mL}^{-1}$  and 5.0  $\text{ng mL}^{-1}$ ,  
302 respectively. The percentage recovery for actual samples was between 94.2-98.6%, with an  
303 EF of 114. The RSD was between 1.9-3.3%. The analytical performance of the IL-UA-  
304 DLLME procedure is shown in the Table 2. The robustness of the method was tested for 10%  
305 changes in basic analytical parameters (IL volume, pH, ultrasonic time, THF volume), and an  
306 effective recovery ( $\geq 93.8\%$ ) was obtained.

### 307 **3.3. Selectivity of the IL-UA-DLLME procedure-matrix species**

308 The matrix effect is an essential consideration in developing a new analytical method  
309 and understanding its impact on the method can help in optimizing the method to provide  
310 accurate and reliable results. In a new analytical method, it is crucial to assess the matrix  
311 effect to ensure that the method can accurately measure the analytes of interest in the sample  
312 matrix. In this method, the matrix effect of the most commonly existing 19 different types of  
313 cations, anions, and organic compounds were studied. The tolerance limit was calculated as  
314 “matrix species amount ( $\text{ng mL}^{-1}$ )/ fosetyl-aluminum amount ( $\text{ng mL}^{-1}$ ). A tolerance limit test  
315 is required for an analytical method to determine the method's ability to measure a specific  
316 analyte accurately and precisely within a predefined range. The tolerance limit test helps in  
317 assessing the method's ability to meet the acceptance criteria and the regulatory requirements  
318 for the specific application. Tolerance limit for the selected ions  $\text{Na}^+$  (20000  $\text{ng mL}^{-1}$ ),  $\text{Ca}^{2+}$   
319 (20000  $\text{ng mL}^{-1}$ ),  $\text{SO}_4^{2-}$  (20000  $\text{ng mL}^{-1}$ ),  $\text{CO}_3^{2-}$  (15000  $\text{ng mL}^{-1}$ ),  $\text{F}^-$  (15000  $\text{ng mL}^{-1}$ ),  $\text{C}_2\text{O}_4^{2-}$   
320 (10000  $\text{ng mL}^{-1}$ ),  $\text{Mg}^{2+}$  (10000  $\text{ng mL}^{-1}$ ),  $\text{Fe}^{2+}$  (10000  $\text{ng mL}^{-1}$ ),  $\text{Cd}^{2+}$  (4000  $\text{ng mL}^{-1}$ ), and  
321  $\text{Pb}^{2+}$  (1000  $\text{ng mL}^{-1}$ ) was quite high. In case of ions presence, the recovery was 96-99% with



322 RSD 1.7-2.6%. For other organic species like boscalid (1000 ng mL<sup>-1</sup>), metconazole (500 ng  
323 mL<sup>-1</sup>), tebuconazole (200 ng mL<sup>-1</sup>), spiroxamine (200 ng mL<sup>-1</sup>), cycloheximide (100 ng mL<sup>-1</sup>),  
324 chlorothalonil (100 ng mL<sup>-1</sup>), carbendazim (100 ng mL<sup>-1</sup>), azoxystrobin (50 ng mL<sup>-1</sup>), and  
325 triadimefon (50 ng mL<sup>-1</sup>) the recovery was 94-97% with RSD 1.9-2.2%. This study reveals  
326 that this method is highly selective and no considerable interference was observed. The  
327 summary of this study is given in Table S3.

### 328 **3.4. Precision and robustness of IL-UA-DLLME procedure**

329 Precision in the context of analytical methods refers to the degree of agreement or  
330 reproducibility between repeated measurements of the same sample under identical  
331 experimental conditions. In other words, precision is a measure of how closely individual  
332 measurements of a sample agree with each other. Inter-day and intra-day experiments were  
333 performed for the estimation of the precision and accuracy of the assay. Three concentrations  
334 of fosetyl-aluminum (10, 300, and 500 ng mL<sup>-1</sup>) were tested for precision of the method (see  
335 Table S4). The recover for intra-day (N=5) experiments was 94.8-97.8% with 2.5-3.8% of  
336 RSD. For inter-day precision, a total of 15 experiments were performed on three consecutive  
337 days (n=3x5). The recovery for inter-day experiments was 93.8-96.1% with 3.8-4.7% of RSD.

338 The robustness of an analytical method refers to the ability of the method to remain  
339 unaffected by small variations in experimental conditions, such as changes in temperature,  
340 pH, or sample preparation. An analytical method can produce consistent and reliable results  
341 even when small changes in experimental conditions are introduced. Robustness is typically  
342 evaluated by deliberately varying the experimental conditions within a certain range and  
343 observing the effect on the analytical results. The degree of variation that the method can  
344 tolerate while still producing reliable results is called the method's robustness (Ferreira et al.,  
345 2017). Robustness was estimated for IL volume±10%, pH±10%, ultrasonic time±10% and  
346 THF volume±10% (see Table S5). The volume of IL was in the range of 400-450 µL. pH was





347 changed in a range of 4.7-5.7. Ultrasonic time was varied in a range of 2.25-2.75 min. THF  
348 volume was varied in the range of 430-530  $\mu\text{L}$ . Results reveal that minor changes in  
349 extraction conditions does not significantly affect the recovery of the IL-UA-DLLME  
350 procedure for fosetyl-aluminum. It was concluded that the IL-UA-DLLME procedure is  
351 robust for mild change (10%) in extraction parameters.

### 352 **3.5. Application of IL-UA-DLLME method for food and vegetable samples**

353 To validate the developed method, the IL-UA-DLLME procedure was used for the  
354 analysis of fosetyl-aluminum in fruit and vegetable samples. Melon, watermelon, collards,  
355 gherkin, cauliflower, radish, and zucchini were used as test plants. Fruits and vegetables were  
356 obtained from plants grown under controlled conditions in a greenhouse. All samples were  
357 spiked with concentrations of  $100 \text{ ng mL}^{-1}$  and  $300 \text{ ng mL}^{-1}$  of fosetyl-aluminum. The IL-  
358 UA-DLLME procedure was then applied to these samples under optimized conditions. Five  
359 replicate samples were used for analysis to get reliable results. For the reliability of the results  
360 obtained, the same samples were also analyzed by independent method (Tóth et al., 2022).  
361 Comprehensive results are given in Table 3. Recovery of fosetyl-aluminum was determined in  
362 zucchini (94.7-96.2%), radish (97.1-98.6%), cauliflower (92.5-95.0%), gherkin (93.8-96.4%),  
363 collards (95.5-97.6), watermelon (91.7-95.9), and melon (96.3-98.8%). Results reveal that the  
364 IL-UA-DLLME procedure is applicable for fruit and vegetable samples.

### 365 **3.6. Comparison with previous studies**

366 In this study, important parameters (analytical methods, LOD, linearity range, %RSD, and  
367 matrix) of this method were compared with recently reported methods in the literature. The  
368 summary of this study is presented in Table 4. Only a few methods have been reported for  
369 fosetyl-aluminum analysis in food samples. Lopez-Ruiz et al 2020. developed a method for  
370 the analysis of fosetyl-aluminum in human blood serum by liquid chromatography-triple  
371 quadrupole mass spectrometer. However, this method required complicated instrumentation,



372 and the overall procedure is more complex. Furthermore, this method required more time (40  
373 minutes) for sample preparation (López-Ruiz et al., 2020). Raski et al. developed a method  
374 based on ion chromatography for the analysis of fosetyl-aluminium in fruits and vegetables  
375 (Rajski et al., 2018). The method is simple and robust however less sensitive, applicable only  
376 at ppm level. RSD value is high (17%) and applicable within a limited range of concentration  
377 (0.01-0.1 mg L<sup>-1</sup>). Buiarelli et al. has described a different analytical approach for the  
378 detection of fosetyl-aluminum in airborne particulate matter, which involves hydrophilic  
379 interaction liquid chromatography coupled with tandem mass spectrometry. This method  
380 required more time (60 minutes time) for sample preparation. This method required advanced  
381 instrumentation (Buiarelli et al., 2018). Chamkasem et al. introduced a liquid  
382 chromatography/tandem mass spectrometry approach for quantifying the presence of fosetyl-  
383 aluminum in grapes. This method required advanced instrumentation and a complicated  
384 sample preparation procedure. Furthermore, LOD for this method is quite higher than this  
385 new method (Chamkasem, 2017). Li et al. established an analytical method using a procedure  
386 of extraction coupled with hydrophilic interaction liquid chromatography-tandem mass  
387 spectrometry to detect fosetyl-aluminum in wheat flour (Li et al., 2021). The method is  
388 applicable for a wide range of concentrations (10–2000 µg Kg<sup>-1</sup>) with a preconcentration  
389 factor of 114. However, it involved complicated and advanced instrumentation and required  
390 more time for sample preparation. Sadiq and Hammood have documented a procedure that  
391 employs continuous flow injection and indirect photometric detection for detecting fosetyl-  
392 aluminum in commercial formulations. However, this method applies to only commercial  
393 formulations and may not apply to complex matrices. Secondly, the method is more time-  
394 consuming and required advanced instrumentation (Sadiq and Hammood, 2022).

395 The results of this study show that the IL-UA-DLLME procedure is the most sensitive  
396 method among available approaches for same type of samples. It involves simple



397 instrumentation like a UV-Visible spectrophotometer and an easy extraction procedure. There  
398 are no complicated steps and the least time is required for the extraction procedure.  
399 Furthermore, this method uses IL which is considered as a green solvent system. The method  
400 has been applied to multiple fruit and vegetable samples and was found highly selective.  
401 Furthermore, for sample preparation, plants and vegetables were grown under controlled  
402 conditions in a greenhouse which is another addition to the sample preparation process. Based  
403 on the results obtained it was evidently proved that this method can be effectively used for  
404 fosetyl-aluminum in food samples.

#### 405 **4. Conclusions**

406 In conclusion, the proposed method using IL-based UA-DLLME coupled with  
407 chemometric modeling was successfully applied for the analysis of fosetyl-aluminum in  
408 various fruit and vegetable samples. The use of this method demonstrated several advantages,  
409 including high selectivity and sensitivity, short analysis time, and low consumption of organic  
410 solvents. The results obtained for the optimized experimental conditions were in good  
411 agreement with the expected values, indicating good accuracy and reliability of the proposed  
412 model. The IL-UA-DLLME method was characterized by wide linearity (5-600 ng mL<sup>-1</sup>), low  
413 limit of detection (1.5 ng mL<sup>-1</sup>) and limit of quantification (5.0 ng mL<sup>-1</sup>), enrichment factor of  
414 114, very good precision (RSD in the range of 1.9-3.3% ) as well as robustness. Stable and  
415 effective extraction conditions were developed, ensuring high and repeatable recovery values  
416 (94.2-98.6%). Therefore, the IL-UA-DLLME method can be considered as a reliable and  
417 efficient alternative method for the determination of fosetyl-aluminum in fruit and vegetable  
418 samples possible to be applied in routine analysis.

#### 419 **Compliance with Ethical Standards**

420 None.

#### 421 **Informed Consent**



422 Not applicable.

## 423 **References**

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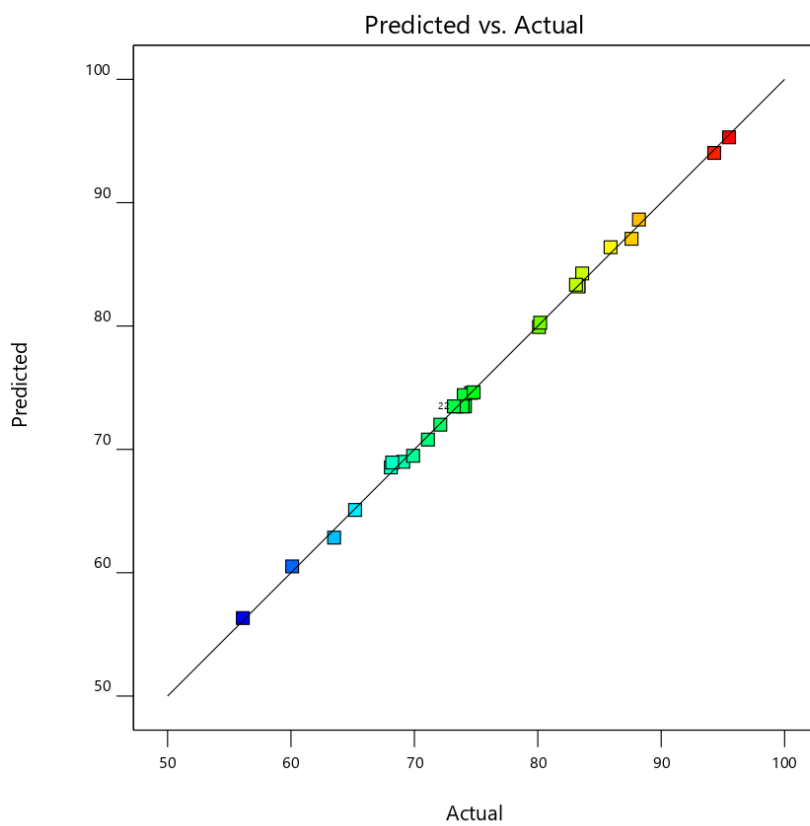
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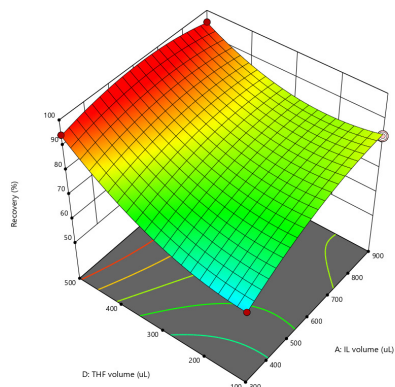
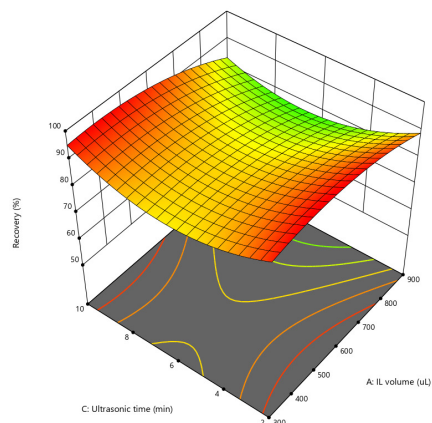
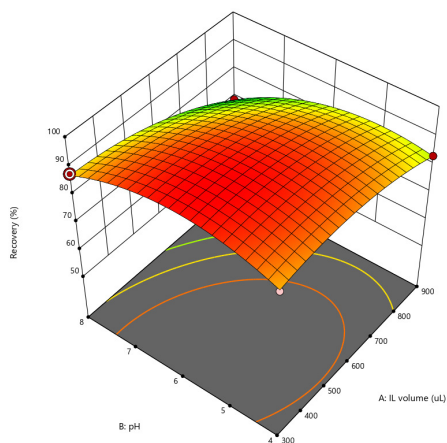
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Figure 1. Agreement between experimental data and CCD's prediction data

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(a)

(b)

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(c)

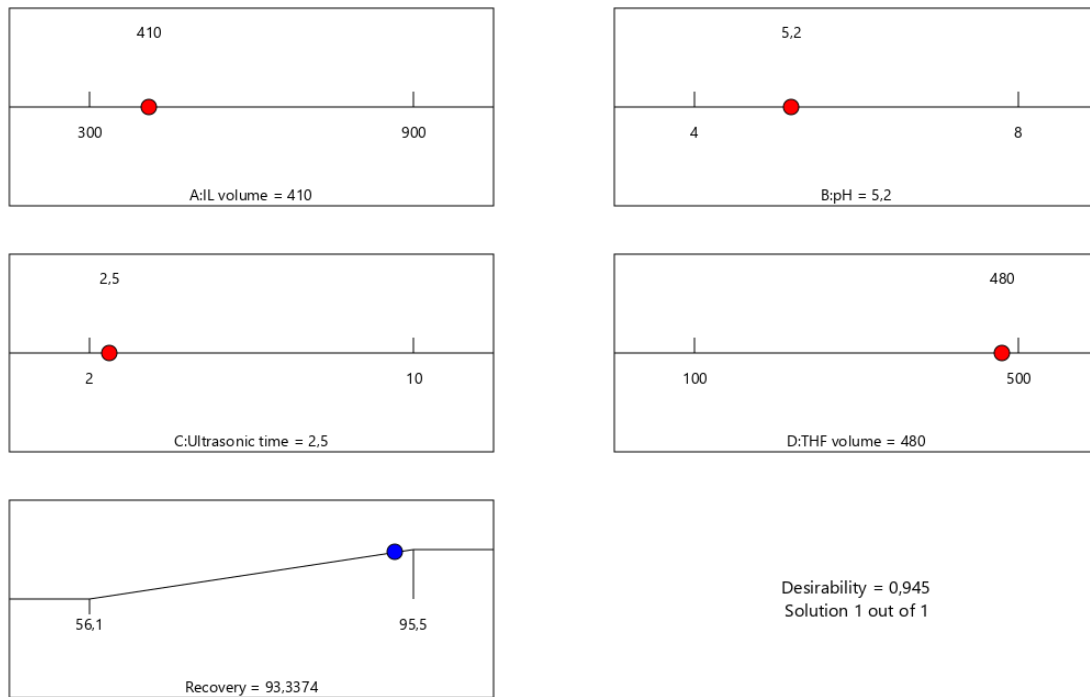
547 Figure 2 (a-c). 3D surface response plot for optimized variables, (a) IL volume and pH; (b) IL volume  
 548 and ultrasonic time; (c) IL volume and THF volume

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Figure 3. The optimum values predicted by the model for the variables

557 Table 1. ANOVA for quadratic model

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Source	Sum of Squares	Mean Square	F-value	p-value	
Model	2550,60	182,19	672,76	< 0.0001	significant
A	1.33	1.33	4.93	0.0423	
B	593.98	593.98	2193.38	< 0.0001	
C	42.63	42.63	157.41	< 0.0001	
D	623.04	623.04	2300.72	< 0.0001	
AB	160.02	160.02	590.92	< 0.0001	
AC	44.89	44.89	165.77	< 0.0001	
AD	320.41	320.41	1183.18	< 0.0001	
BC	157.50	157.50	581.61	< 0.0001	
BD	28.62	28.62	105.69	< 0.0001	
CD	123.21	123.21	454.98	< 0.0001	
A <sup>2</sup>	47.40	47.40	175.03	< 0.0001	
B <sup>2</sup>	135.33	135.33	499.73	< 0.0001	
C <sup>2</sup>	179.47	179.47	662.73	< 0.0001	
D <sup>2</sup>	127.78	127.78	471.87	< 0.0001	
Lack of Fit	3.41	0.3407	2.60	0.1515	not significant
<i>Fit Statistics</i>					
R <sup>2</sup>	0.9984		Predicted R <sup>2</sup>	0.9910	
Adjusted R <sup>2</sup>	0.9969		Adeq. Precision	105.9189	

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572 Table 2. Analytical performance of the IL-UA-DLLME procedure

Parameters	Value
Working range, ng mL <sup>-1</sup>	5-600
Coefficient of determination (R <sup>2</sup> )	0.9914
LOD, ng mL <sup>-1</sup>	1.5
LOQ, ng mL <sup>-1</sup>	5.0
EF	114
*Recovery%	94.2-98.6
*RSD%	1.9-3.3

573 \* At concentrations of 10, 300 and 500 ng mL<sup>-1</sup> of fosetyl-aluminum (n=3).

574 LOD: Limit of detection

575 LOQ: Limit of quantification

576 EF: Enhancement factor

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601 Table 3. Application results of the IL-UA-DLLME method to fruit and vegetable samples (n=5)

Samples	Spiked (ng mL <sup>-1</sup> )	Found (ng mL <sup>-1</sup> )	Matrix effect (%)	Recovery (%)	Found by independent method (ng mL <sup>-1</sup> )
Zucchini	-	25±1	4.8	-	24±2
	100	120±7		95±2	122±4
	300	314±15		96±3	311±12
Radish	-	32±3	6.3	-	34±2
	100	129±7		97±1	133±4
	300	328±20		96±2	325±13
Cauliflower	-	17±2	5.9	-	14±1
	100	110±6		93±4	112±4
	300	302±11		95±2	299±15
Gherkin	-	25±2	8.7	-	27±3
	100	119±7		94±3	123±6
	300	314±19		96±3	311±21
Collards	-	13±1	3.1	-	14±1
	100	108±4		95±4	105±3
	300	306±14		98±1	301±12
Watermelon	-	62±4	6.8	-	68±3
	100	153±8		91±5	151±7
	300	350±18		96±3	358±14
Melon	-	29±3	9.1	-	24±2
	100	126±7		97±2	130±6
	300	326±20		99±2	331±18

602 \* Mean ± standard deviation.

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604 Table 4. Comparison of the method with other approaches.

Analytical method	Extraction solvents	LOD	Linearity range	RSD (%)	Enrichment factor	Samples	References
<sup>1</sup> LC-TQMS	Water, acetonitriles and n-hexane	0.01 mg L <sup>-1</sup>	0.01-0.1 mg L <sup>-1</sup>	17	----	human blood serum	(López-Ruiz et al., 2020)
<sup>2</sup> IC-QOMA	Methanol and water	0.01 mg Kg <sup>-1</sup>	0.01–0.50 mg Kg <sup>-1</sup>	----	40	Fruits and Vegetables	(Rajski et al., 2018)
<sup>3</sup> MS-NEI	ASE Dionex and water	0.3 ng mL <sup>-1</sup>	1-700 ng mL <sup>-1</sup>	10	75	Particulate Matter	(Buiarelli et al., 2018)
<sup>4</sup> LC-TMS;	HOAc, Na <sub>2</sub> EDTA, MeOH/H <sub>2</sub> O	29 µg Kg <sup>-1</sup>	10-1000 µg Kg <sup>-1</sup>	17	----	Grapes	(Chamkasem, 2017)
<sup>5</sup> HI-LC-TMS	Water and acetonitrile	5 µg Kg <sup>-1</sup>	10–2000 µg Kg <sup>-1</sup>	6.2	114	Wheat	(Li et al., 2021)
<sup>7</sup> CFI-IP	Methanol, acetonitrile	0.0041 mmol L <sup>-1</sup>	0.005–1.8 mmol L <sup>-1</sup>	2.1	--	Commercial formula	(Sadiq and Hammood, 2022)
<sup>8</sup> IL-UA-DLLE	<sup>9</sup> IL	1.5 µg L <sup>-1</sup>	5-600 µg L <sup>-1</sup>	1.9-3.3	114	Fruits, vegetables	Present method

605 <sup>1</sup>LC-TQMS; Liquid chromatography coupled to triple quadrupole mass spectrometer, <sup>2</sup>IC-QOMA; Ion chromatography coupled to a quadrupole Orbitrap mass analyzer, <sup>3</sup>MS-  
606 NEI; mass spectrometry-negative electrospray ionization, <sup>4</sup>LC-TMS; Liquid Chromatography/Tandem Mass Spectrometry, <sup>5</sup>HI-LC-TMS; Hydrophilic interaction  
607 chromatography tandem mass spectrometry, <sup>6</sup>IC-TMS; Ion Chromatography–Tandem Mass Spectrometry, <sup>7</sup>CFI-IP; Continuous Flow Injection/Indirect Photometry, <sup>8</sup>IL-UA-  
608 DLLE; Ionic liquid based ultrasonic-assisted dispersive liquid-liquid micro-extraction, <sup>9</sup>IL; Ionic liquid (1-ethyl-3-methylimidazolium hydrogen sulfate).

