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

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

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27 Abstract

This manuscript presents a new method for selective extraction and determination of fosetyl-28 aluminum in fruits and vegetable samples based on ultrasonic-assisted dispersive liquid-liquid 29 microextraction method using ionic liquids (IL-UA-DLLME). А UV-Visible 30 spectrophotometer was used for detection and quantification. Plants used for sample 31 collection were grown under controlled conditions in a greenhouse. Cental composite design 32 33 (CCD)-response surface methodology (RSM) analysis was used for the optimization of significant factors (volume of IL, pH, ultrasonication time, and THF volume). Under optimal 34 conditions, the limit of detection and limit of quantification of the IL-UA-DLLME procedure 35 were 1.5 ng mL⁻¹ and 5.0 ng mL⁻¹ respectively with relative standard deviation 1.9-3.3%. The 36 developed IL-UA-DLLME procedure demonstrated linearity within the concentration range 37 of 5-600 ng mL⁻¹ with an R² value of 0.9914. The enrichment factor was 114 with a 38 recovery% of 94.2-98.6% (n=3) at optimal conditions. The IL-UA-DLLME assay was used 39 for the analysis of fosetyl-aluminum in a variety of food samples and was found highly 40 selective and efficient. 41

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

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50 **1. Introduction**

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

Fungicides, which belong to a large group of pesticides, are frequently used in high-yield agriculture to protect plants against the detrimental effects of phytopathogens and enhance crop production. These chemicals are utilized to combat a wide range of fungal diseases and prevent plant infections. It is applied to agricultural production for the preservation of root crops, vegetables, and fruits, or as a direct treatment for ornamental plants, trees, field crops, cereals, and grasses. In a study conducted by Kiselev et al. (2022), it was discovered that fungicides with long-lasting effects, developed for use on potato plants, are capable for effectively suppressing disease development and increasing potato yield. Additionally, these preparations gradually release pesticides into the soil during precipitation or irrigation, reducing the abrupt release of these chemicals. The researchers also noted that these new formulations enable the reduction of pesticide application rates, minimizing the risk of pesticide dispersion and accumulation in the biosphere (Pérez-Lucas, Vela et al. 2019, Tudi, Daniel Ruan et al. 2021).

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

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

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

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

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

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

141 **2.** Materials and methods

142 **2.1. Instrumentation**

A UV-Visible spectrophotometer (Shimadzu 1800 model, Kyoto, Japan) was used for
absorbance measurements. A cuvette (Fisher, Germany) made from quartz glass (volume 500
µL) was used as sample holder for spectrophotometric measurments. Microwave system
(Milestone Ethos, Italy) was used for the digestion of fruit and vegetable samples. Ultra-pure
water (18.2 MΩ) was obtained from Milli-Direct Q3 system (Millipore, Bedford, MA, USA).
An ultrasonic bath (SK5210LHC Kudos, Shanghai, China) was used for sonication. A pH
meter (model 630 Metrohm, Switzerland) with digital pH measuring input for the intelligent

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

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2.2. Chemicals and solutions

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

164 **2.3. Sampling**

This study was conducted in the greenhouse of Sivas Cumhuriyet University, 165 Department of Crop and Animal Production, using a plastic pots with a capacity of 3 kg. The 166 research followed a randomized plot design, with 3 replications. A soil samples were 167 collected from a depth of 0-20 cm and had a clay loam texture, calcareous (13.8%), salt-free 168 (0.026%), low organic matter content (1.77%), slightly alkaline pH (7.89), low phosphorus 169 content (48.8 kg ha⁻¹) and sufficient potassium content (1099.5 kg ha⁻¹). In the study, melon, 170 watermelon, collards, gherkin, cauliflower, radish, and zucchini were used as test plants. As 171 basic fertilization, nitrogen 150 mg Kg⁻¹ (CaNO₃.4H₂O), phosphorus 100 mg Kg⁻¹, and 172 potassium 125 mg Kg⁻¹ (KH₂PO₄) were applied for all plants with planting. After the plants 173

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

183 **2.4. Experimental design**

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

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

The experimental steps of the IL-UA-DLLME procedure are as follows. First, 10 mL of the digested samples were added to the conical tubes. After this step, the pH of the sample solution was adjusted to pH 5.2 using 0.1 M citrate buffer solution. In order to extract the fosetyl-aluminum in the sample solution, first 410 μ L of [P_{4,4,4,14}]Cl (as extraction solvent) and then 480 μ L of THF (as the dispersing solvent) were added to the obtained mixture. Conical tubes were placed in an ultrasonic bath and sonicated for 2.5 min at room temperature to effectively disperse the $[P_{4,4,4,14}]Cl$ in the sample solution. At this stage, the $[P_{4,4,4,14}]Cl$ phase containing fosetyl-aluminum was collected on top of the aqueous solution. The $[P_{4,4,4,14}]Cl$ phase was transferred to microcuvettes using a syringe and absorbance measurements were made using UV-spectrophotometer at 289 nm. All these studies were carried out in parallel with the sample blank and standard spiked samples.

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

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

208
$$Percent\ recovery = \frac{c_e}{c_a} \times 100$$
 (1)

In the above equation, C_e is the concentration experimentally determined in the model sample and C_a is the actual/expected concentration.

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

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

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

$$RSD(\%) = \frac{sy/x}{c_m} \times 100 \tag{3}$$

220 In the above equation, sy/x is the residual standard deviation and C_m is the 221 mean concentration in real samples.

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

$$225 EF = \frac{c_f}{c_i} (4)$$

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

$$LOD = \frac{3 \times sy/x}{m} \tag{5}$$

$$LOQ = \frac{10 \times sy/x}{m} \tag{6}$$

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

234 **3. Results and discussion**

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

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

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

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

265 Recovery (%) = +73.49 +0.2722A -5.74B +1.54C +5.88D -3.16AB -1.67AC -4.47AD 266 +3.14BC -1.34BD 2.77CD -4.28A² -7.23B² +8.32C² +7.02D²

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

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

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

3.2 Analytical parameter of the IL-UA-DLLME procedure

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

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

The matrix effect is an essential consideration in developing a new analytical method 308 and understanding its impact on the method can help in optimizing the method to provide 309 accurate and reliable results. In a new analytical method, it is crucial to assess the matrix 310 effect to ensure that the method can accurately measure the analytes of interest in the sample 311 matrix. In this method, the matrix effect of the most commonly existing 19 different types of 312 cations, anions, and organic compounds were studied. The tolerance limit was calculated as 313 "matrix species amount (ng mL⁻¹)/ fosetyl-aluminum amount (ng mL⁻¹). A tolerance limit test 314 is required for an analytical method to determine the method's ability to measure a specific 315 analyte accurately and precisely within a predefined range. The tolerance limit test helps in 316 assessing the method's ability to meet the acceptance criteria and the regulatory requirements 317 for the specific application. Tolerance limit for the selected ions Na⁺ (20000 ng mL⁻¹), Ca²⁺ 318 (20000 ng mL⁻¹), SO₄²⁻ (20000 ng mL⁻¹), CO₃²⁻ (15000 ng mL⁻¹), F⁻ (15000 ng mL⁻¹), C₂O₄²⁻ 319 $(10000 \text{ ng mL}^{-1})$, Mg²⁺ $(10000 \text{ ng mL}^{-1})$, Fe²⁺ $(10000 \text{ ng mL}^{-1})$, Cd²⁺ $(4000 \text{ ng mL}^{-1})$, and 320 Pb²⁺(1000 ng mL⁻¹) was quite high. In case of ions presence, the recovery was 96-99% with 321

RSD 1.7-2.6%. For other organic species like boscalid (1000 ng mL⁻¹), metconazole (500 ng mL⁻¹), tebuconazole (200 ng mL⁻¹), spiroxamine (200 ng mL⁻¹), cycloheximide (100 ng mL⁻¹), chlorothalonil (100 ng mL⁻¹), carbendazim (100 ng mL⁻¹), azoxystrobin (50 ng mL⁻¹), and triadimefon (50 ng mL⁻¹) the recovery was 94-97% with RSD 1.9-.2%. This study reveals that this method is highly selective and no considerable interference was observed. The summary of this study is given in Table S3.

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

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

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

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

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

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

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3.6. Comparison with previous studies

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

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

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

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instrumentation like a UV-Visible spectrophotometer and an easy extraction procedure. There 397 398 are no complicated steps and the least time is required for the extraction procedure. Furthermore, this method uses IL which is considered as a green solvent system. The method 399 has been applied to multiple fruit and vegetable samples and was found highly selective. 400 Furthermore, for sample preparation, plants and vegetables were grown under controlled 401 402 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 fosetyl-aluminum in food samples. 404

405 **4. Conclusions**

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

420 None.

421 Informed Consent

| 422 | Not applicable. |
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| 423 | References |
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Figure 1. Agreement between experimental data and CCD's prediction data



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

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557 Table 1. ANOVA for quadratic model

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| 550 | | | | | | |
|--|--------------------------------------|---------|---------|--------------------------|----------|-----------------|
| | Source Sum of Mean Square Squares | | F-value | p-value | | |
| | Model | 2550,60 | 182,19 | 672,76 | < 0.0001 | significant |
| | А | 1.33 | 1.33 | 4.93 | 0.0423 | |
| | В | 593.98 | 593.98 | 2193.38 | < 0.0001 | |
| | С | 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 ² | 47.40 | 47.40 | 175.03 | < 0.0001 | |
| | B ² | 135.33 | 135.33 | 499.73 | < 0.0001 | |
| | C ² | 179.47 | 179.47 | 662.73 | < 0.0001 | |
| | D^2 | 127.78 | 127.78 | 471.87 | < 0.0001 | |
| | Lack of Fit | 3.41 | 0.3407 | 2.60 | 0.1515 | not significant |
| | | | Fit St | tatistics | | C |
| | R ² | 0.9984 | | Predicted R ² | 0.9910 | |
| | Adjusted R ² | 0.9969 | | Adeq. Precision | 105.9189 | |
| 562 563 564 565 566 567 568 569 | | | | | | |
| 570 571 | | | | | | |

| | Parameters | Value | |
|--------------------------|---|------------|--|
| | Working range, ng mL ⁻⁺ Coefficient of determination (D^2) | 5-600 | |
| | LOD ng mI ⁻¹ (K^{-}) | 0.9914 | |
| | LOO, ng mL ⁻¹ | 1.3 5 0 | |
| | EF | 114 | |
| | *Recovery% | 94.2-98.6 | |
| 572 | *RSD% | 1.9-3.3 | |
| 574 575 576 577 | LOD: Limit of detection LOQ: Limit of quantification EF: Enhancement factor |). | |
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| 572 | Table 2. Analytical performance of the IL-UA-DLLME procedure |
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| Samples | Spiked | Found | Matrix | Recovery | Found by independent |
|-------------|----------------|----------------|--------|----------|----------------------|
| _ | $(ng mL^{-1})$ | $(ng mL^{-1})$ | effect | (%) | method |
| | - | - | (%) | | $(ng mL^{-1})$ |
| 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 |

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

602 * Mean ± standard deviation.

| Analytical method | Extraction solvents | LOD | Linearity range | RSD (%) | Enrichment factor | Samples | References |
|------------------------|-----------------------------|------------------------|------------------------------|---------|----------------------|--------------------|-----------------------|
| methou | | | | | 140101 | | |
| ¹ LC-TQMS | Water, acetonitriles and | 0.01 mg L ⁻ | 0.01-0.1 mg L ⁻¹ | 17 | | human blood | (López-Ruiz et al., |
| | n-hexane | 1 | | | | serum | 2020) |
| ² IC-QOMA | Methanol and water | 0.01 mg | 0.01–0.50 mg Kg ⁻ | | 40 | Fruits and | (Rajski et al., 2018) |
| | | Kg^{-1} | 1 | | | Vegetables | |
| ³ MS-NEI | ASE Dionex and water | 0. 3 ng | 1-700 ng mL ⁻¹ | 10 | 75 | Particulate Matter | (Buiarelli et al., |
| | | mL ⁻¹ | | | | | 2018) |
| ⁴ LC-TMS; | HOAc, Na ₂ EDTA, | 29 µg Kg ⁻¹ | 10-1000 µg Kg ⁻¹ | 17 | | Grapes | (Chamkasem, 2017) |
| | MeOH/H ₂ O | | | | | | |
| ⁵ HI-LC-TMS | Water and acetonitrile | 5 µg Kg ⁻¹ | 10–2000 µg Kg ⁻¹ | 6.2 | 114 | Wheat | (Li et al., 2021) |
| ⁷ CEI ID | Mathanal acatonitrila | 0.0041 | 0.005 ± 1.8 mmol | 2.1 | | Commercial | (Sadia and |
| CI-I-IF | Methanol, accionnine | 0.0041 | 0.005–1.8 111101 | 2.1 | | Commercial | (Sauly allu |
| | | mmol L^{-1} | L^{-1} | | | formula | Hammood, 2022) |
| ⁸ IL-UA- | ⁹ IL | $1.5 \ \mu g \ L^{-1}$ | 5-600 $\mu g L^{-1}$ | 1.9-3.3 | 114 | Fruits, vegetables | Present method |
| DLLE | | | | | | | |

Table 4. Comparison of the method with other approaches.

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