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

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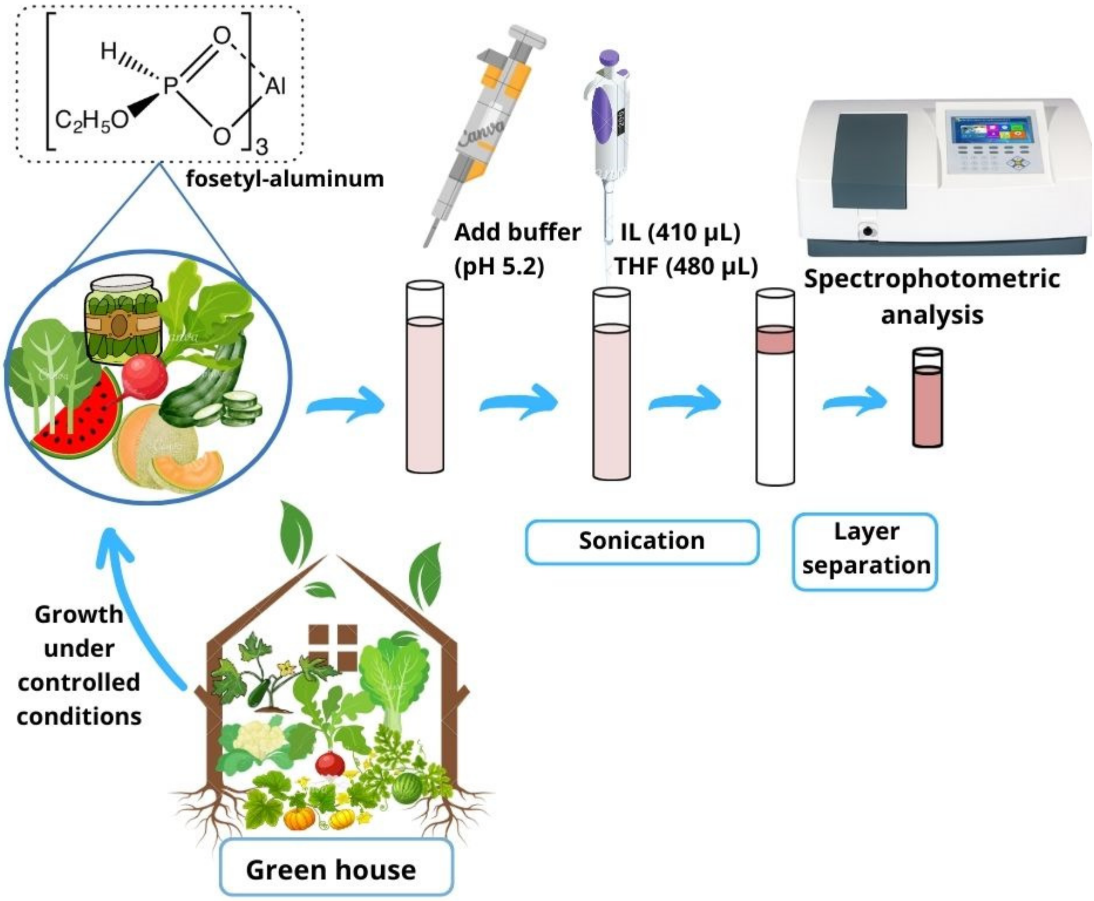
Graphical abstract



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

Abstract

This manuscript presents a new method for selective extraction and determination of fosetyl-aluminum in fruits and vegetable samples based on ultrasonic-assisted dispersive liquid-liquid microextraction method using ionic liquids (IL-UA-DLLME). A UV-Visible spectrophotometer was used for detection and quantification. Plants used for sample collection were grown under controlled conditions in a greenhouse. Cental composite design (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 conditions, the limit of detection and limit of quantification of the IL-UA-DLLME procedure were 1.5 ng mL^{-1} and 5.0 ng mL^{-1} respectively with relative standard deviation 1.9-3.3%. The developed IL-UA-DLLME procedure demonstrated linearity within the concentration range of 5-600 ng mL^{-1} with an R^2 value of 0.9914. The enrichment factor was 114 with a recovery% of 94.2-98.6% (n=3) at optimal conditions. The IL-UA-DLLME assay was used for the analysis of fosetyl-aluminum in a variety of food samples and was found highly selective and efficient.

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

1. Introduction

As the population continues to grow at a rapid pace, the issue of nutrition has emerged as a major concern in our era. To enhance the quality and quantity of crops, farmers are widely using pesticides to solve the issues related to presence of undesirable organisms (Tudi et al., 2021). Statistics indicate that herbicides account for 47.5% of all pesticides utilized globally, while insecticides make up 29.5%, fungicides 17.5%, and the remaining 5.5% represent other methods of pest control (Sharma et al., 2019). Fungal plant pathogens are capable of causing significant reductions in crop yield across all agricultural systems globally (Wan de Wouw et 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 living organisms, pollution of soil and water, and destruction of beneficial organisms are 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 fungicides essential for agriculture and ensuring food safety (Zubrod et al., 2019). To prevent risks related to phytopathogens and increase productivity, fungicides are extensively used (Shahid et al., 2020). Despite their beneficial effects on preventing damage caused by phytopathogens, the detrimental impact of fungicides on soil microbiota's composition and functions is a significant concern for both plant and human health along the food chain (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 diseases in plants, such as downy mildew, Phytophthora, and Pythium. It is a phosphonate-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 concerns about its potential environmental and health impacts. Some studies have suggested 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 irritation (Han et al., 2012, Rouabhi, 2010). Fosetyl-aluminum is generally considered to have 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 doses of fosetyl-aluminum can cause reproductive and developmental toxicity, including effects on fertility and fetal development. Additionally, there have been concerns raised about potential carcinogenic effects. Skin contact, inhalation of spray mists, or ingestion of contaminated food or water are potential routes of exposure. Short-term exposure may cause irritation to the skin, eyes, and respiratory system (Authority, Arena et al. 2018, Gormez, Golge et al. 2022).

Several analytical methods are available for fosetyl-aluminum analysis including HPLC, flow injection analysis (Sadiq and Hammood, 2022), ion chromatography (Rajski et al., 2018), liquid chromatography-triple quadrupole mass spectrometer (López-Ruiz et al., 2020), liquid chromatography-tandem mass spectrometry (Chamkasem, 2017). However, sample pretreatment/sample preparation is required before analysis. Sample preparation is an important step in analytical methods, where extraction is a commonly used procedure to enhance sensitivity and selectivity. This method involves isolating and concentrating analytes from complex sample matrices like food or biological fluids before analysis (Ullah et al., 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 effects of the sample matrix on the analysis and save time and resources by reducing the volume of the sample matrix (Haq et al., 2023).

Ionic liquids (ILs) are a particular type of salts that possess distinctive characteristics such as low volatility, high thermal stability, and adjustable polarity. Due to these characteristics, 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 compounds, making them an effective extractants for a diverse range of samples (Han et al., 2012). Unlike many traditional solvents, ILs are often less toxic, non-volatile, and non-flammable, making them more environmentally friendly. By altering the chemical structure of 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 et al., 2021). ILs have high thermal stability and do not undergo phase separation or degradation at high temperatures or in the presence of water or other polar solvents (Huddleston et al., 2001). ILs can be easily recovered and reused multiple times, making them a cost-effective and sustainable alternative to traditional solvents (Chiappe et al., 2016). ILs are compatible with many analytical instruments and do not require additional derivatization or extraction steps (Farajzadeh et al., 2020; Rykowska et al., 2018).

Central Composite Design (CCD) is an important experimental design technique widely used to optimize analytical methods (Rasheed et al., 2023). It allows for a systematic and efficient exploration of the design space by carefully selecting a limited number of experiments. By incorporating a balanced combination of factorial, axial, and center points, CCD ensures coverage of a broad range of factor settings, facilitating the identification of optimal operating conditions (Chen et al., 2020). CCD is especially useful for detecting and modeling nonlinear relationships between variables. It effectively captures curvature and interaction effects through the inclusion of axial points, resulting in a more precise representation of the response surface. This capability enhances the understanding of complex 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).

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.

2. Materials and methods

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.

2.2. Chemicals and solutions

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 further purification step was applied. The stock solution (500 mg L^{-1}) of fosetyl-aluminum 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 stock solution. Tributyl-tetradecylphosphonium chloride (Sigma, $[P_{4,4,4,14}]Cl$) ionic liquid was used as the extraction solvent. Tetrahydrofuran (THF) (Merck) was used to disperse IL in the sample solution. Citrate buffer solution (0.1 M pH 5.2) was prepared by dissolving appropriate amount of sodium citrate dihydrate and citric acid in water.

2.3. Sampling

This study was conducted in the greenhouse of Sivas Cumhuriyet University, Department of Crop and Animal Production, using a plastic pots with a capacity of 3 kg. The research followed a randomized plot design, with 3 replications. A soil samples were collected from a depth of 0-20 cm and had a clay loam texture, calcareous (13.8%), salt-free (0.026%), low organic matter content (1.77%), slightly alkaline pH (7.89), low phosphorus content (48.8 kg ha^{-1}) and sufficient potassium content (1099.5 kg ha^{-1}). In the study, melon, watermelon, collards, gherkin, cauliflower, radish, and zucchini were used as test plants. As basic fertilization, nitrogen 150 mg Kg^{-1} ($CaNO_3.4H_2O$), phosphorus 100 mg $Kg^{-1,}$ and potassium 125 mg Kg^{-1} (KH_2PO_4) were applied for all plants with planting. After the plants emerged and reached a certain size, the fungicide containing 80% Fosetyl-Al was added 3 times in total, at weekly intervals. 50 days after sowing, the plants were harvested by cutting them from the soil surface. The harvested plants included melons, watermelons, collards, gherkins, cauliflowers, radishes, and zucchinis, which were then left to dry in the shade at room temperature. The dried plants were subsequently grinded using a plant grinding mill, with 1 g of each plant weighed and combined with 10 mL of distilled water (at a ratio of 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 band filter paper, and the water was evaporated using a rotary evaporator at 40°C.

2.4. Experimental design

To optimize and design the experimental parameters and perform ANOVA analysis of the analytical data, trial version 11.0.3.0 of the Design-Expert® package from STATISTICA was used. A central composite design-response surface methodology approach was used for the optimization of extraction-affecting factors for fosetyl-aluminum. Four parameters including IL volume (300-900 µL), pH (4-8), ultrasound time (2-10 min), and THF volume (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 parameters, their units, symbols, and their lowest-highest limits are given in Table S1.

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 µL of $[P_{4,4,4,14}]Cl$ (as extraction solvent) and then 480 µL 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.

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.

$$Percent\ recovery = \frac{C_e}{C_a} \times 100 \qquad (1)$$

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.

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

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.

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

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

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.

$$EF = \frac{C_f}{C_i} \tag{4}$$

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.

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 optimizing analytical methods. Response surface methodology (RSM) is used to develop a mathematical model that describes the relationship between the response (i.e., the analytical 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 CCD comprises three categories of points: factorial, axial, and center points. Factorial points are ordinary experimental points utilized to ascertain the primary and interaction effects of independent variables. Axial points aid in estimating the curvature of the response surface, 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.

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 such as the coefficients of determination (R^2), adjusted R^2 and predicted R^2, the p-value, and the lack-of-fit (LOF) test. The effect of the optimized parameters on the extraction of fosetyl-aluminum was evaluated using ANOVA statistical analysis, and the results are presented in Table 1. The high values of R^2, adjusted R^2, and predicted R^2 suggest that the proposed model is well-suited to the experiment. The predicted R^2 value is in reasonable agreement with the adjusted R^2 value, with a difference of 0.0077, which indicates that the CCD methodology is 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 significant effect. The statistical analysis reveals that the proposed model is well-suited to the experiment, as evidenced by the R^2 (0.9984), adjusted R^2 (0.9969), and predicted R^2 (0.9910) values which are close to 1. The p-values for the model terms indicate that A, B, C, D, AB, AD, BC, BD, CD, A^2, B^2, C^2, and D^2 are all significant. Lack of Fit for the proposed method is not significant. The final equation in terms of coded factors

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

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 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 achieved, especially when the pH was less than 6. Interestingly, phase separation could not be achieved due to decreased activity of IL binding sites in the basic region. The effect of ultrasonic time versus IL volume on the recovery of fosetyl-aluminum was presented in 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. In particular, the recovery of fosetyl-aluminum was not quantitative at high ionic liquid 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 the recovery of fosetyl-aluminum was shown in Figure 2c. THF (as a dispersive solvent) 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 transferred to the IL phase. Due to this phenomenon, quantitative recoveries were obtained when THF volume and IL volume were in the range of 380-490 µL and 330-450 µL, respectively.

In the optimization step, CCD was applied to maximize the recovery of fosetyl-aluminum. According to the CCD, the maximum recovery was obtained using IL volume (410 µL), pH (5.2), ultrasonic time (2.5 min), and THF volume (480 µL). 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 optimized extracting conditions (IL volume 410 µL, pH 5.2, ultrasonic time 2.5, and THF volume 480 µL). The linearity of the method was observed within the concentration range of 5-600 ng mL^{-1} with a high coefficient of determination (R^2) of 0.9914. The limit of detection (LOD) and limit of quantification (LOQ) were calculated to be 1.5 ng mL^{-1} and 5.0 ng mL^{-1}, respectively. The percentage recovery for actual samples was between 94.2-98.6%, with an EF of 114. The RSD was between 1.9-3.3%. The analytical performance of the IL-UA-DLLME procedure is shown in the Table 2. The robustness of the method was tested for 10% changes in basic analytical parameters (IL volume, pH, ultrasonic time, THF volume), and an effective recovery (≥93.8%) was obtained.

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

The matrix effect is an essential consideration in developing a new analytical method and understanding its impact on the method can help in optimizing the method to provide accurate and reliable results. In a new analytical method, it is crucial to assess the matrix effect to ensure that the method can accurately measure the analytes of interest in the sample matrix. In this method, the matrix effect of the most commonly existing 19 different types of cations, anions, and organic compounds were studied. The tolerance limit was calculated as "matrix species amount (ng mL^{-1})/ fosetyl-aluminum amount (ng mL^{-1}). A tolerance limit test is required for an analytical method to determine the method's ability to measure a specific analyte accurately and precisely within a predefined range. The tolerance limit test helps in assessing the method's ability to meet the acceptance criteria and the regulatory requirements for the specific application. Tolerance limit for the selected ions Na^+ (20000 ng mL^{-1}), Ca^{2+} (20000 ng mL^{-1}), SO_4^{2-} (20000 ng mL^{-1}), CO_3^{2-} (15000 ng mL^{-1}), F^- (15000 ng mL^{-1}), $C_2O_4^{2-}$ (10000 ng mL^{-1}), Mg^{2+} (10000 ng mL^{-1}), Fe^{2+} (10000 ng mL^{-1}), Cd^{2+} (4000 ng mL^{-1}), and Pb^{2+}(1000 ng mL^{-1}) was quite high. In case of ions presence, the recovery was 96-99% with

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.

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

Precision in the context of analytical methods refers to the degree of agreement or reproducibility between repeated measurements of the same sample under identical experimental conditions. In other words, precision is a measure of how closely individual measurements of a sample agree with each other. Inter-day and intra-day experiments were 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 Table S4). The recover for intra-day (N=5) experiments was 94.8-97.8% with 2.5-3.8% of RSD. For inter-day precision, a total of 15 experiments were performed on three consecutive days (n=3x5). The recovery for inter-day experiments was 93.8-96.1% with 3.8-4.7% of RSD.

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, pH, or sample preparation. An analytical method can produce consistent and reliable results even when small changes in experimental conditions are introduced. Robustness is typically 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 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 THF volume±10% (see Table S5). The volume of IL was in the range of 400-450 µL. pH was 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 μL. 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.

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 analysis of fosetyl-aluminum in fruit and vegetable samples. Melon, watermelon, collards, 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 spiked with concentrations of 100 ng mL^{-1} and 300 ng mL^{-1} of fosetyl-aluminum. The IL-UA-DLLME procedure was then applied to these samples under optimized conditions. Five replicate samples were used for analysis to get reliable results. For the reliability of the results obtained, the same samples were also analyzed by independent method (Tóth et al., 2022). 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%), collards (95.5-97.6), watermelon (91.7-95.9), and melon (96.3-98.8%). Results reveal that the IL-UA-DLLME procedure is applicable for fruit and vegetable samples.

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 minutes) for sample preparation (López-Ruiz et al., 2020). Raski et al. developed a method based on ion chromatography for the analysis of fosetyl-aluminium in fruits and vegetables (Rajski et al., 2018). The method is simple and robust however less sensitive, applicable only at ppm level. RSD value is high (17%) and applicable within a limited range of concentration (0.01-0.1 mg L^{-1}). Buiarelli et al. has described a different analytical approach for the detection of fosetyl-aluminum in airborne particulate matter, which involves hydrophilic interaction liquid chromatography coupled with tandem mass spectrometry. This method required more time (60 minutes time) for sample preparation. This method required advanced instrumentation (Buiarelli et al., 2018). Chamkasem et al. introduced a liquid chromatography/tandem mass spectrometry approach for quantifying the presence of fosetyl-aluminum in grapes. This method required advanced instrumentation and a complicated 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 of extraction coupled with hydrophilic interaction liquid chromatography-tandem mass spectrometry to detect fosetyl-aluminum in wheat flour (Li et al., 2021). The method is applicable for a wide range of concentrations (10–2000 μg Kg^{-1}) with a preconcentration 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 employs continuous flow injection and indirect photometric detection for detecting fosetyl-aluminum in commercial formulations. However, this method applies to only commercial formulations and may not apply to complex matrices. Secondly, the method is more time-consuming and required advanced instrumentation (Sadiq and Hammood, 2022).

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 instrumentation like a UV-Visible spectrophotometer and an easy extraction procedure. There 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 has been applied to multiple fruit and vegetable samples and was found highly selective. Furthermore, for sample preparation, plants and vegetables were grown under controlled conditions in a greenhouse which is another addition to the sample preparation process. Based on the results obtained it was evidently proved that this method can be effectively used for fosetyl-aluminum in food samples.

4. Conclusions

In conclusion, the proposed method using IL-based UA-DLLME coupled with chemometric modeling was successfully applied for the analysis of fosetyl-aluminum in various fruit and vegetable samples. The use of this method demonstrated several advantages, including high selectivity and sensitivity, short analysis time, and low consumption of organic solvents. The results obtained for the optimized experimental conditions were in good 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 limit of detection (1.5 ng mL^{-1}) and limit of quantification (5.0 ng mL^{-1}), enrichment factor of 114, very good precision (RSD in the range of 1.9-3.3%) as well as robustness. Stable and effective extraction conditions were developed, ensuringhigh and repeatable recovery values (94.2-98.6%). Therefore, the IL-UA-DLLME method can be considered as a reliable and efficient alternative method for the determination of fosetyl-aluminum in fruit and vegetable samples possible to be applied in routine analysis.

Compliance with Ethical Standards

None.

Informed Consent

Not applicable.

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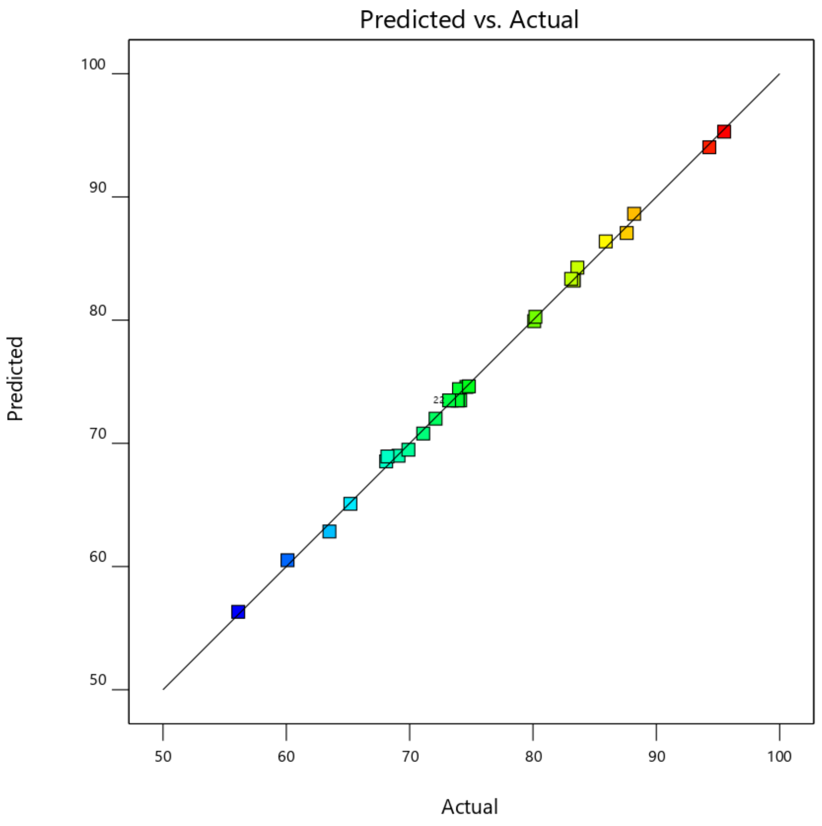
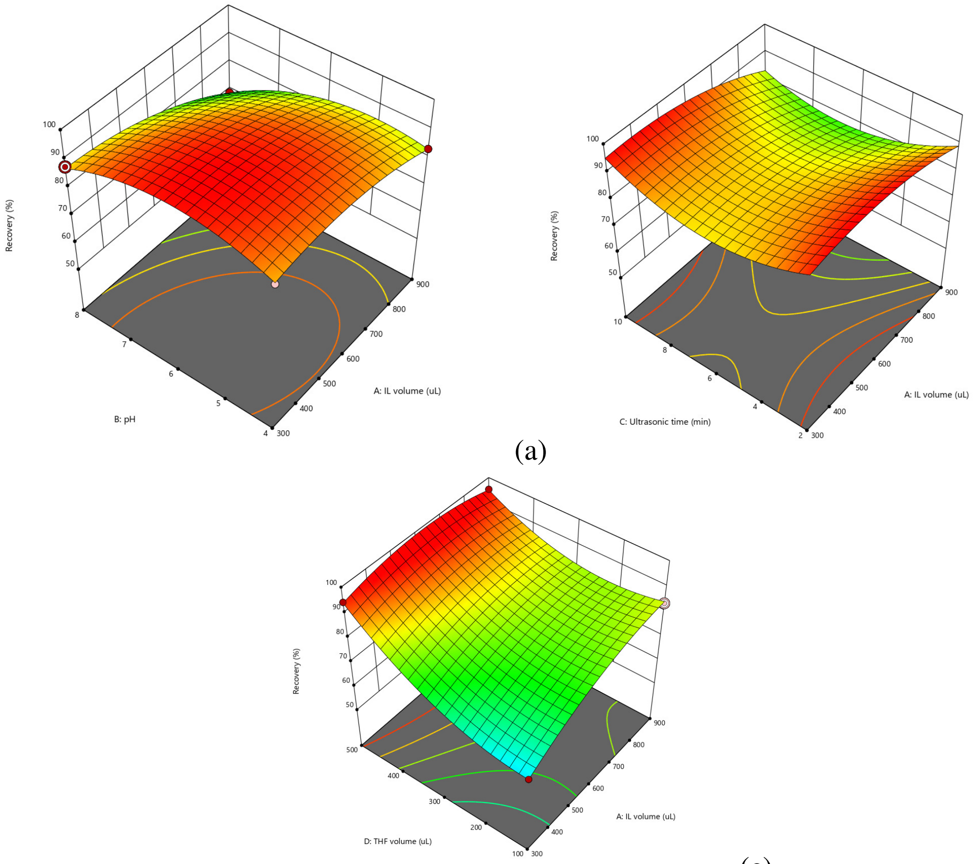


Figure 1. Agreement between experimental data and CCD's prediction data



(a) (b)

(c)

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

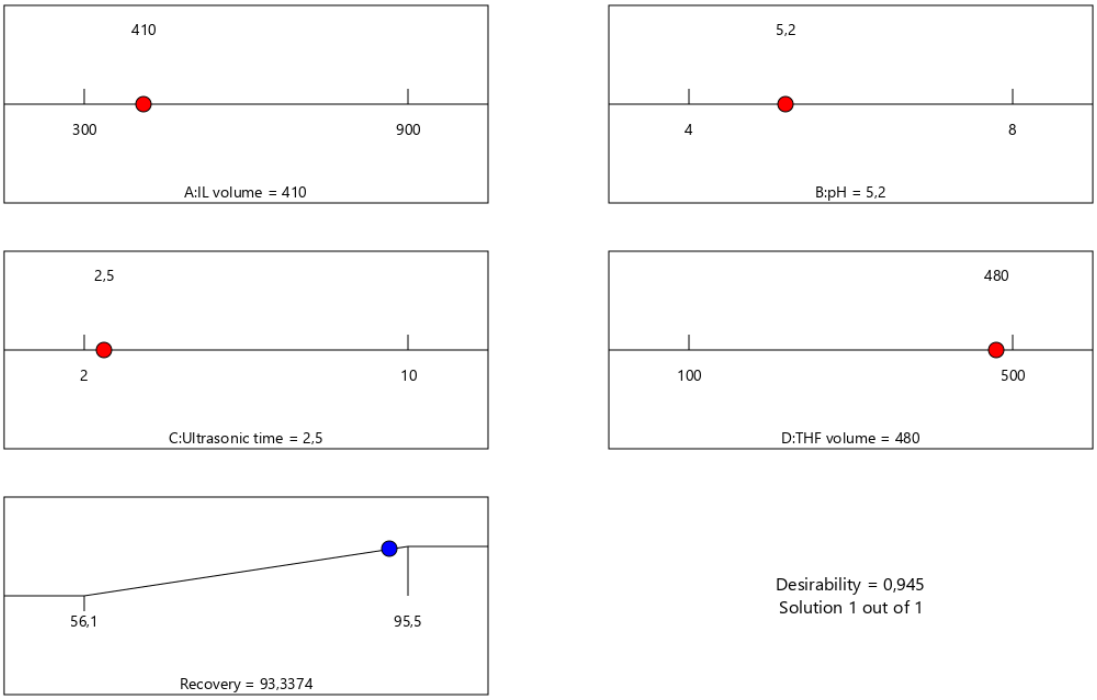


Figure 3. The optimum values predicted by the model for the variables

Table 1. ANOVA for quadratic model

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^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 Statistics					
R^2	0.9984		Predicted R^2	0.9910	
Adjusted R^2	0.9969		Adeq. Precision	105.9189	

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

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

* At concentrations of 10, 300 and 500 ng mL^{-1} of fosetyl-aluminum (n=3).

LOD: Limit of detection

LOQ: Limit of quantification

EF: Enhancement factor

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

Samples	Spiked (ng mL^{-1})	Found (ng mL^{-1})	Matrix effect (%)	Recovery (%)	Found by independent 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

* Mean ± standard deviation.

Table 4. Comparison of the method with other approaches.

Analytical method	Extraction solvents	LOD	Linearity range	RSD (%)	Enrichment factor	Samples	References
[1]LC-TQMS	Water, acetonitriles and n-hexane	0.01 mg L^{-1}	0.01-0.1 mg L^{-1}	17	----	human blood serum	(López-Ruiz et al., 2020)
[2]IC-QOMA	Methanol and water	0.01 mg Kg^{-1}	0.01–0.50 mg Kg^{-1}	----	40	Fruits and Vegetables	(Rajski et al., 2018)
[3]MS-NEI	ASE Dionex and water	0. 3 ng mL^{-1}	1-700 ng mL^{-1}	10	75	Particulate Matter	(Buiarelli et al., 2018)
[4]LC-TMS;	HOAc, Na_2EDTA, MeOH/H_2O	29 µg Kg^{-1}	10-1000 µg Kg^{-1}	17	----	Grapes	(Chamkasem, 2017)
[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]CFI-IP	Methanol, acetonitrile	0.0041 mmol L^{-1}	0.005–1.8 mmol L^{-1}	2.1	--	Commercial formula	(Sadiq and Hammood, 2022)
[8]IL-UA-DLLE	[9]IL	1.5 µg L^{-1}	5-600 µg L^{-1}	1.9-3.3	114	Fruits, vegetables	Present method

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