Plant and Soil Science

Journal homepage: https://agriculturalscience.com.ua/en
Plant and Soil Science, 16(3), 9-21
Received: 13.05.2025 Revised: 31.08.2025 Accepted: 23.09.2025

UDC 543.544:631.416.3:632.95

DOI: 10.31548/plant3.2025.09

Nataliia Tereshchenko*
PhD in Chemical Sciences, Senior Researcher
Bogomolets National Medical University
01601, 13 Shevchenko Blvd., Kyiv, Ukraine
https://orcid.org/0000-0003-0620-2888

Olena Khyzhan
PhD in Chemical Sciences, Associate Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0002-2986-3251

Kateryna Nesterova
Postgraduate Student
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0003-4081-4570

Methodological approaches to sample preparation for xenobiotic content analysis

Abstract. The aim of the article was to improve sample preparation protocols and chromatographic analysis conditions for the determination of selected xenobiotics in agricultural products. To achieve this goal, a method of artificial fortification of homogenised samples of sunflower seeds, corn kernels, lettuce leaves, and apples with model xenobiotics of different classes was used, followed by extraction with organic solvents using a modified QuEChERS approach and quantitative analysis. For the effective extraction of acetochlor and prometryn from lettuce leaves and apples, which are characterised by a high water content, an optimal raw material-to-extractant ratio of 1:3-1:5 was found. In the case of corn kernels, which have a structure containing a negligible amount of lipids, the extraction was performed with a ratio of 1:10. The greatest difficulties were observed during the processing of sunflower seeds, where a three-phase system formed due to the high fat content, complicating the mass transfer of analytes. Nevertheless, the use of acetonitrile as an extractant in a 1:17-1:20 ratio allowed for high extraction rates of the target xenobiotics to be achieved. For the extraction of benomyl and cyprodinil, a mixture of acetonitrile and methanol in a 4:1 ratio was used, which ensured effective transfer of the specified analytes in all studied matrices. Specifically, in lettuce and apple samples, the extraction rates for both substances exceeded 96%, which indicated good solubility in the mixture used and a low matrix effect. For diquat, a bipyridylium compound with a pronounced ionic nature, the best results were obtained using a solution of trifluoroacetic acid in methanol in a 9.5:0.5 ratio. This composition ensured stable extraction from all types of plant matrices, with maximum values in lettuce samples and minimum values in sunflower

Suggested Citation:
Tereshchenko, N., Khyzhan, O., & Nesterova, K. (2025). Methodological approaches to sample preparation for xenobiotic content analysis. *Plant and Soil Science,* 16(3), 9-21. doi: 10.31548/plant3.2025.09.

*Corresponding author

seeds (86%), which still meets modern requirements for analytical accuracy. In the case of corn kernel samples, the extraction level for all substances was 88-92%, which indicated satisfactory extractability in cereal matrices. The lowest extraction values were recorded for sunflower seeds; however, even under these conditions, the extraction remained within the 80-86% range, which is acceptable according to international standards for pesticide analysis quality control. The results of the chromatographic analysis confirmed the high reproducibility and sensitivity of the method

Keywords: high-performance liquid chromatography; gas chromatography with mass-selective detectors; extracts; pesticides; plant extracts

INTRODUCTION

The contamination of plant products with pesticides and other xenobiotics is a serious problem, as agrochemical residues in plant-based foods pose a potential threat to food safety, consumer health, and the natural environment. The detection of residual amounts of these substances requires high-precision analytics, particularly chromatographic methods, the effectiveness of which is highly dependent on correct sample preparation. Pesticides, which are widely used in modern agriculture to increase crop yields, can remain in food products in the form of xenobiotics that require strict control (Salnikova & Salnikov, 2021; Garud *et al*., 2024). In response to the need for effective monitoring, international and national regulations, including DSanPiN 8.8.1.2.3.4-000-2001 (2001) and Regulation (EC) No 396/2005 (2005), regulate the maximum permissible levels of such substances in food products.

One of the key areas in this field is the improvement of analytical control methods. As noted in the work of K. Pszczolińska *et al.* (2024), modern analytics is based on multi-residue approaches using gas chromatography in combination with mass spectrometry, which allows for the identification of dozens of pesticides in a plant matrix. The study by S. Kang *et al.* (2023) emphasises the importance of standardising methods for analysing pesticide residues in plants, taking into account their unique chemical composition. The work of J. Veiga-del-Baño *et al.* (2024) demonstrates the effectiveness of the QuEChERS method as a universal approach for the extraction and purification of plant-based samples, which significantly reduces preparation time and improves the quality of analytical results. The prospects for improving these approaches are also confirmed by the results of S. Perumal *et al.* (2024), who found the possibility of optimising salts and sorbents in the QuEChERS method to increase the recovery of target analytes in vegetable samples.

In the context of food safety, it is relevant to study the selectivity, effectiveness, and simplicity of the practical application of various sample preparation protocols for multi-residue analysis. The QuEChERS method, which combines the stages of homogenisation, extraction, purification, and analysis, is one of the leading technologies in the field of pesticide control in plant-based products. The aim of the presented study was to provide an experimental basis for the conditions of sample preparation and analysis of plant raw materials with varying water and lipid content to ensure the reliable quantitative determination of pesticide residues by chromatography. The practical value of the study lies in establishing a complex relationship between the properties of the matrix, the type of xenobiotic, and the extraction conditions, which allows the control method to be adapted to a wide range of plant products. The data obtained can be implemented in laboratory practice to improve the control of food product safety in accordance with modern requirements.

LITERATURE REVIEW

In the works of N. Casado *et al.* (2022) and N. Patel *et al.* (2024), the concept of the QuEChERS method and its evolution through various modifications that have expanded its scope of application were analysed. Particular attention has been paid to the use of QuEChERS for the determination of xenobiotics in food products, specifically pesticide residues in fruits, the control

of which is an important task for complying with regulatory requirements and ensuring their safety. For this purpose, QuEChERS is combined with liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), which is characterised by high sensitivity and specificity. The study by N. Patel *et al.* (2024) describes in detail the use of LC-MS/MS for the extraction, purification, and quantitative analysis of pesticide residues in fruit samples. The QuEChERS method involves the initial homogenisation of samples, the use of acetonitrile and salting out for effective separation, as well as subsequent dispersive solid-phase extraction (d-SPE) to remove matrix interferences. After purification, the extract is analysed using LC-MS/MS: liquid chromatography ensures the separation of pesticides, and tandem mass spectrometry provides their ionic fragmentation and identification. This approach allows for the simultaneous detection of trace amounts of numerous pesticides with high accuracy and reliability, which confirms the universality and practical significance of the QuEChERS method in controlling food product safety.

For the simultaneous determination of several pesticides using high-performance liquid chromatography (HPLC), a careful optimisation of analytical conditions for each active substance is necessary (Liu *et al*., 2023; Dong *et al*., 2023). One of the key approaches is the use of gradient elution, which facilitates the effective separation of compounds in combination with detection at a variable wavelength. This method is rarely used in procedures focused on the determination of individual pesticides. Most often, the effective separation of both individual and multiple pesticides is performed using: 1) reversed-phase chromatography, where a non-polar C18 adsorbent is used as the stationary phase, and the mobile phase is formed on the basis of water, acetonitrile and/or methanol with the possible addition of modifiers, particularly orthophosphoric acid; 2) detection in the ultraviolet range, and in some cases, by the fluorescent method (Korshun, 2024).

In the study by K. Pszczolińska *et al.* (2022), the proposed method was applied to the analysis of residual pesticides in vegetable samples. Pendimethalin was found to be the most common compound, with concentrations ranging from 0.007-0.065 mg/kg, while for difenoconazole, this value was 0.013-0.025 mg/kg. An important step of the method is the preparation of calibration curves specific to each product type, which complicates the conduct of a multi-component analysis of residual pesticides. However, the method demonstrates high effectiveness and involves the purification of plant extracts (6 ml) using QuEChERS, with 900 mg of anhydrous $MgSO_4$, 300 mg of C18, and 300 mg of ChloroFiltr at the d-SPE stage. Pesticides are then analysed by gas chromatography with tandem mass spectrometry, which allows for the detection of up to 164 compounds in vegetables with varying chlorophyll content. This approach allows calibration curves built for one matrix to be applied to the analysis of pesticides in products with different levels of chlorophyll, increasing the accuracy and reproducibility of the results.

The methodological aspects of multi-residue pesticide analysis are aimed at developing environmentally safe, effective, and reliable approaches to guarantee the quality, safety, and traceability of products. As noted in the study by A. Santana-Mayor *et al.* (2023), the QuEChERS method is characterised by high versatility, which allows for obtaining accurate results for a wide range of matrices and analytes. Due to the ability to vary the solvents, salts, salting-out buffers, and sorbents for purification, this approach provides significant flexibility. In addition, QuEChERS is an environmentally safe alternative to traditional sample preparation methods, corresponding to modern trends in sustainable development in analytical chemistry.

Apples are one of the most common crops grown by European producers. Before they enter the market, it is necessary to check the content of residual pesticides used to protect the harvest from pests, in order to guarantee the safety of the products for consumers. For this purpose, a new simple method for the simultaneous determination of captan, folpet, difenoconazole, and chlorpyrifos in apple samples was developed and verified (Velkoska-Markovska *et al.*, 2024). The method includes the extraction of residual pesticides with acetone, as well as liquid-liquid (LLE) and solid-phase (SPE) extraction. Reversed-phase high-performance liquid

chromatography (RP-HPLC) with a diode-array detector (UV-DAD) was used for the separation and quantitative analysis of the analytes. Optimal results were obtained using a LiChrospher 60 RP-select B analytical column (250 mm × × 4 mm, 5 µm) with isocratic elution, where the mobile phase was a mixture of acetonitrile and 0.1% acetic acid in water (70:30, V/V). The eluent flow was 1 ml/min, and detection was performed at wavelengths of 220 and 230 nm. The linearity of the method was confirmed in the concentration ranges of 1.50-3.60 mg/kg for captan and folpet, and 0.35-0.60 mg/kg for difenoconazole and chlorpyrifos. Recovery varied from 94.94% to 114.63%, and the relative standard deviation (RSD) was 0.09-9.25%. The developed method was successfully applied to the analysis of apple samples for residual pesticide content, which confirms its effectiveness and reliability.

The study by D. Yun *et al.* (2024) was aimed at developing a fast and accurate method for the simultaneous analysis of multi-residue pesticides, as well as monitoring their content in agricultural products. Within its framework, representative samples of brown rice, soybeans, potatoes, tangerines, and green peppers were selected. Gas chromatography with tandem mass spectrometry (GC-MS/MS) was used for the analysis of 272 pesticide residues. Sample extraction was performed using the QuEChERS-EN method with subsequent dispersive solid-phase extraction (d-SPE) using anhydrous $MgSO_4$ and primary-secondary amine (PSA) sorbents. The proposed approach demonstrated high effectiveness and accuracy, which made it possible to ensure reliable monitoring of residual pesticides in various types of agricultural products.

MATERIALS AND METHODS

The research was carried out during the spring and autumn of 2024 at the Ukrainian Laboratory for the Quality and Safety of Agricultural Products of the National University of Life and Environmental Sciences of Ukraine (NULES). The study used samples of plant products, including sunflower seeds (LG50479 SX hybrid from Limagrain), corn kernels (Rist SV hybrid), lettuce leaves (Iceberg), and apples (Golden Delicious). These were collected from a variety of sources: private farms in the Kyiv region that use integrated pest management or practice organic farming with minimal use of chemical agents, as well as the NULES research farms in Kyiv. The collection of seeds, kernels, leaves, and fruits was done when they reached full biological maturity, in line with the sampling procedure approved by the current Order of the Ministry of Agrarian Policy of Ukraine No. 289 (2018). All samples were transported to the laboratory within 12 hours of collection at a temperature of 4-6 °C, which meets the requirements for transporting food and biological materials. To prevent changes in their chemical composition and the breakdown of active biological substances, the samples were stored in sterile, light-protected containers made of an inert material. Before the analytical procedures began, all material was kept in refrigerators at +4 °C, except for samples intended for the study of volatile components or pesticides, which were stored at -18 °C according to the methodological guidelines of the European Food Safety Authority (EFSA, n.d.).

For each experiment, four parallel laboratory samples of 100 g each were prepared. Preparing the samples for analysis began with homogenisation, a standard step for different food groups. Homogenisation was done by grinding the samples in a glass laboratory grinder-homogeniser (LHM-1) at varying temperatures (from +4 °C to +25 °C) and a speed of 10,000 rpm. Due to the different chemical compositions of the raw materials, the resulting homogenised samples had different physical states: fine-grained, pasty, or liquid. Using the coning and quartering method, 5 g was taken from each homogenised sample for analysis, and the remaining 95 g was hermetically sealed and stored in a freezer at -18 °C until the end of the experiment.

To simulate contamination, three of the four samples were artificially fortified with solutions of analytical standards of the target xenobiotics produced by Sigma-Aldrich (with a purity of ≥ 98%). The range of concentrations of added xenobiotics was developed individually for each substance and corresponded to a level of 0.5 to 5 times the maximum permissible concentration (MPC). The control sample was not contaminated and was used to assess the background content of analytes and to validate the chromatographic method, specifically to evaluate matrix noise, detect and

quantify the background content of xenobiotics, and determine the extraction efficiency of the added analytical standards (recovery rate, %).

Organic solvents of "pure for analysis" ("p.f.a.") grade were used to obtain plant extracts. Organic solvents were used as individual extractants or in mixtures, in particular with deionised water. Buffering of the homogenised sample solution and purification of the plant extract were carried out using magnesium sulphate, sodium chloride, sodium citrate, calcium chloride and aluminium oxide ("p.f.a."). Extraction was performed in polytetrafluoroethylene plastic tubes, dark glass flasks, and polymethylpentene plastic containers, which were all protected from light with opaque covers. Mass transfer during analyte extraction was intensified by changing the ratio of raw material to extractant, regulating temperature, mechanical mixing, and using ultrasonic waves (37 kHz, Advantage Lab generator). The plant extract was purified using dispersive or liquid-liquid extraction methods with organic solvents and cartridges containing amine sorbents (Supelco). The purified extract was concentrated using an IKA rotary evaporator. Xenobiotics were analysed using High-Performance Liquid Chromatography with mass-spectrometric detectors (HPLC/MS/MS) and Gas Chromatography/Mass Spectrometry (GC/MS) on Dionex and Agilent instruments. Spectral data was processed using Cromeleon 6.0 software and the NIST 0.5 mass spectral library. The chromatographic analysis was performed with a Dionex Summit MSD-3200Q Trap instrument with a ChromSpher C18 column (3 µm, 4.6×5 mm). The mobile phase was a methanol/acetic acid gradient, transitioning from 10:90 to 90:10 over 0-5 minutes, with a reverse transition back to 10:90 over 12-15 minutes. Analytes were identified using a programmed screening method.

Based on data from M. Arnold *et al.* (2023) and Z. Li *et al.* (2024) regarding the nutritional value and chemical composition of the tested samples, the mass fractions (W, %) of the main chemical components of the matrix were calculated using formula (1).

$$w = \frac{m_{component}}{m_{homogenised\ sample}} \times 100\%. \quad (1)$$

Homogenised sunflower seeds were classified as a group of samples with a high lipid content (30-52%), indicating their belonging to a fatty raw material. Corn kernels contain a small percentage of lipids (6%). In contrast, homogenised samples of "Iceberg" lettuce leaves and apples (with skin) are characterised by a predominant water content of over 95% and 85% respectively. This composition places these samples in the category of high-moisture plant products, where the proportion of lipids, proteins, and other components is negligible. As the choice of extractants is determined by the physico-chemical properties of the matrix and the target xenobiotics, which influence their solubility and extraction, the study used polar (acetonitrile, methanol, deionised water) and non-polar (chloroform), protic (methanol, water, acetic, trifluoroacetic acid) and aprotic solvents (acetonitrile, chloroform), as well as their mixtures (acetonitrile + methanol, methanol + trifluoroacetic acid), which are common in laboratory practice. Additionally, organic and mineral acids were used to shift the equilibrium of the dissociation process of ionisable xenobiotics towards the formation of molecular forms, which facilitated their effective extraction from the homogenised raw material.

In conducting this study, the authors relied on data from the scientists S. Petrović *et al.* (2023) and V. Goel *et al.* (2025), whose work provides an in-depth analysis of the relationship between the physico-chemical properties of target analytes (including xenobiotics) and the characteristics of plant matrices, which directly affects the efficiency of the extraction process. For example, the article by S. Petrović *et al.* (2023) details the influence of polarity, acidity, hydrophobicity, and the degree of ionisation of substances on their extraction from different types of matrices (moist, fatty, fibrous, etc.). The authors also highlight the importance of selecting acids and salt components that allow for the optimisation of the medium's pH to achieve the maximum conversion of target compounds into their molecular form, which is critical for extraction. The work by V. Goel *et al.* (2025) presents a comprehensive comparative analysis of polar and non-polar solvents, their mixtures, and the role of organic acids in improving the extraction of ionised pesticides and other xenobiotics from matrices containing

high levels of moisture or lipids. These works are practically oriented, and their results were directly applied in this study during the development of the extraction protocol. In addition, the work by C. Poole (2023) provided a scientifically substantiated basis for the selection of solvents and medium modifiers in the analytical procedure used in the current study.

To establish optimal sample preparation conditions, the method of artificial fortification was applied to both homogenised and non-homogenised blank samples of the raw material with the xenobiotics acetochlor (a marker for chloroacetamide herbicides), prometryn (a marker for triazine herbicides), benomyl (a marker for benzimidazole fungicides), cyprodinil (a marker for pyrimidine fungicides), diquat (a marker for bipyridylium herbicides), 2.4-dichlorophenoxyacetic acid (2.4-D), and 4-chloro-2-methylphenoxyacetic acid (MCPA) (markers for phenoxy herbicides). Compounds belonging to different chemical groups of xenobiotics were used as markers. The optimal ratio of extractant to homogenised sample was determined on the basis of a visual analysis used to observe the hydrodynamic regime of the extraction process, as well as on the results of the quantitative analysis of the xenobiotic extracts. The optimal ratio of sample-to-extractant components was chosen experimentally by a stepwise increase in the amount of extractant in a ratio range from 1:1 to 1:20. Under optimal sample-to-extractant conditions, there is No formation of compaction zones in the material, and the material freely contacts the extractant, allowing for the most complete isolation of the xenobiotic within the extract. The chromatographic control of the analyte content in the plant extracts allowed for the evaluation of xenobiotic extraction efficiency under various hydrodynamic conditions.

Statistical processing of the experimental data was performed using the Microsoft Excel software package. The measurement error was calculated using the standard deviation (Sr, %), and the degree of extraction of xenobiotics from the artificially fortified laboratory samples was estimated in percentages (r, %). For quantitative analysis, calibration was performed and the following were established: the linearity of the method (coefficient of determination $R^2 > 0.99$), the limit of detection (LOD), and the limit of quantification (LOQ) for each of the xenobiotics.

RESULTS AND DISCUSSION

As shown in Figures 1 (a) and 1 (b), the level of extraction of xenobiotics such as the herbicide acetochlor (a marker for chloracetamides) and promethrin (a marker for triazines) depended on the amount of extractant added to 1 gram of homogenised sample. The studies showed that sunflower seeds and corn kernels required the most extractant in experiments with crop products – about 20 ml per 1 g of ground sample. Homogenised samples of oilseed crops were studied using different extractants. The most effective extraction of these xenobiotics was observed when using acetonitrile. The method of extracting pesticides from sunflower seeds and corn kernels using acetonitrile allowed the removal of up to 98% of artificially added pesticides at a sample-extractant ratio of 1:20 (Fig. 1 (a, b), curve 4).

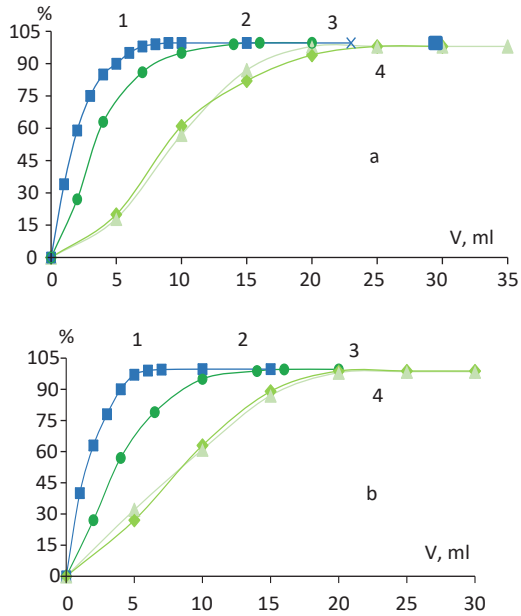

Figure 1. Percentage of extraction of acetochlor (a) and promethrin (b) from 1 g of artificially enriched homogenised samples into plant extract

Note: 1 – lettuce leaves; 2 – apples; 3 – sunflower seeds; 4 – corn kernels. Extraction agent – acetonitrile. Extraction duration – 5 min

Source: developed by the authors

For another group of xenobiotics, it was necessary to optimise the composition of the extractant. Taking into account the hydrophobic properties of xenobiotics, the peculiarities of their chemical structure, and based on the results of chromatographic analysis (Figs. 2, 3), extractant systems were identified that promote the effective transfer of analytes from the plant matrix to the organic phase of the extraction medium.

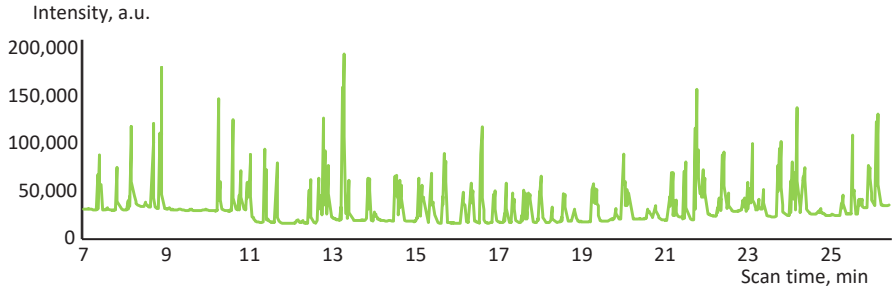

Figure 1. Gas chromatography-mass spectrometry (GC-MS) chromatogram of an extract from apples enriched with xenobiotics (Benomyl, Iprodione, Diquat)

Note: Chromatographic analysis of xenobiotic residues was performed for all studied matrices, including sunflower seeds, corn kernels, and lettuce leaves. All samples underwent procedures for artificial enrichment with the target compounds, extraction, and subsequent chromatographic determination. The chromatogram obtained from the homogenised apples was included in the main text of the work as a representative example because it has the most representative signal structure, clear peak separation, and reflects the effectiveness of the analytical method used

Source: developed by the authors

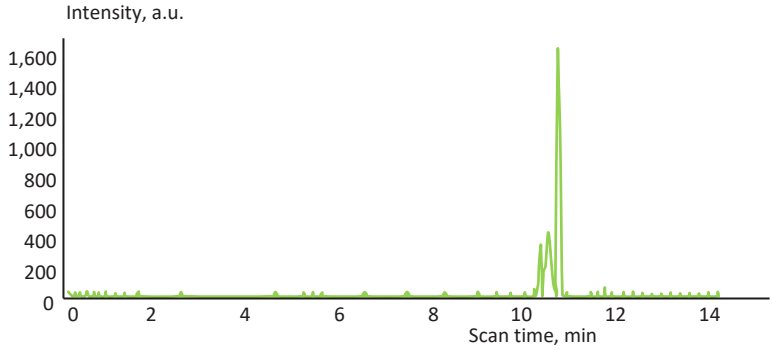

Figure 2. Liquid chromatography chromatogram with mass spectrometric detection (LC-MS) of plant extract from apple enriched with xenobiotics (2,4-D and MCPA)

Note: Chromatographic analysis of xenobiotic residues was performed for all matrices studied, including sunflower seeds, corn kernels, and lettuce leaves. All samples underwent artificial enrichment with target compounds, extraction, and subsequent chromatographic determination. The main text of the study included a chromatogram obtained from homogenised apples as an illustrative example, as it has the most representative signal structure, clear peak separation and reflects the effectiveness of the analytical method used

Source: developed by the authors

The peaks recorded on the chromatograms (Figs. 2, 3) were identified using a mass spectrum library and by comparing the retention times of the analytes and characteristic ions of the xenobiotics under study with the corresponding parameters of the analytical standards.

The chromatogram of the extract from homogenised apples, obtained by gas chromatography with mass spectrometry (GC/MS), recorded about 70 analytical signals (Fig. 2). From the total number of peaks, using standardised solutions of target analytes, peaks corresponding to the presence of the xenobiotics Benomyl, Cyprodinil and Diquat were identified. It should be noted that the use of liquid chromatography with mass spectrometric detection (LC-MS) allows obtaining chromatograms with minimal noise from matrix components (Fig. 3). The visual and analytical characteristics of such chromatograms differ significantly from chromatograms obtained using gas chromatography with mass spectrometry (GC-MS) (Fig. 2). Thus, Figure 3 shows a chromatogram of a plant extract obtained after alkaline hydrolysis of a sample in a 0.1% sulphuric acid solution, followed by extraction with a mixture of acetonitrile and methanol in a 4:1 ratio.

The results of the identification of target compounds are presented in Table 1. The analysis shows that the highest extraction rates of xenobiotics are observed in samples of plant products with a low content of lipid components in the matrix.

Table 1. Quantitative indicators of xenobiotic content in extracts from samples artificially enriched with target compounds

Xenobiotic	Benomyl	Cyprodinil	Diquat
Extractant	mixture of acetonitrile with methanol		solution of trifluoroacetic acid in methanol
	Sunflower seeds		
Added, μg/kg	0.50 ± 0.01	0.50 ± 0.01	0.50 ± 0.01
Determined, μg/kg	0.41 ± 0.03	0.40 ± 0.03	0.43 ± 0.03
Extracted, %	82.0 ± 6	80.0 ± 6	86.0 ± 6
	Lettuce leaves		
Added, μg/kg	0.50 ± 0.01	0.50 ± 0.01	0.50 ± 0.01
Determined, μg/kg	0.49 ± 0.01	0.49 ± 0.02	0.51 ± 0.03
Extracted, %	98.0 ± 2	98.0 ± 4	102.0 ± 6
	Apples		
Added, μg/kg	0.50 ± 0.01	0.50 ± 0.01	0.50 ± 0.01
Determined, μg/kg	0.48 ± 0.01	0.49 ± 0.02	0.47 ± 0.02
Extracted, %	96.0 ± 2	99.0 ± 4	94.0 ± 4
	Corn kernels		
Added, μg/kg	0.50 ± 0.01	0.50 ± 0.01	0.50 ± 0.01
Determined, μg/kg	0.45 ± 0.02	0.46 ± 0.01	0.44 ± 0.02
Extracted, %	90.0 ± 1	92.0 ± 3	88.0 ± 2

Source: developed by the authors

Based on the results of experimental studies that covered the process of extracting xenobiotics using different extractants and the subsequent quantitative analysis of the content of target compounds using chromatographic methods, optimal conditions for the extraction of compounds of different chemical natures were established. For the extraction of acetochlor and prometryn from samples with a water content of 90-96%, the optimal ratio of homogenised sample to extractant was 1:3-1:5 (Fig. 1 (a, b), curve 1). For samples with a water content of 75-85%, extraction was performed at a raw material-to-solvent ratio of 1:10 (Fig. 1 (a, b), curve 2). Sunflower seeds, which contain a large percentage of lipids, required a raw material-to-extractant ratio in the range of 1:17-1:20 (Fig. 1 (a, b), curve 3).

Pesticides belonging to the benzimidazole group are characterised by high lipophilicity and good solubility in organic solvents. In the presence of a buffer medium in the aqueous phase of the extraction system, benzimidazole-series compounds that do not have ionisable fragments are effectively extracted into the organic phase, for example, into acetonitrile. Many benzimidazole derivatives, such as benomyl, contain several functional groups,

which necessitates improving the composition of the extractant. To enhance the extraction of such compounds, especially those with a carboxyl group that can dissociate with the release of a proton, weak organic acids were added to the extraction mixture. A small amount of it, present in the acetonitrile extract, does not interfere with the chromatographic analysis and does not affect the operation of the mass spectrometric detectors. For the effective extraction of benzimidazole (Benomyl) and anilinopyrimidine (Cyprodinil) derivatives, a mixture of acetonitrile and methanol in a volume ratio of 4:1 is recommended. In turn, bipyridylium derivatives (Diquat) are best extracted using a methanolic solution of trifluoroacetic acid in a ratio of 9.5:0.5. As can be seen from Table 1, the highest indicators of xenobiotic extraction efficiency are observed in samples of lettuce leaves and apples, whose matrix is characterised by a low lipid content. In particular, the extraction from sunflower seeds presents the greatest difficulty in terms of sample preparation for analysis and, accordingly, the level of extraction of the target compounds. Despite the fact that the percentage of xenobiotics extracted from sunflower seeds is lower compared to the results obtained for samples of lettuce leaves and apples, the sample preparation process in this case is assessed as satisfactory. This is due to the fact that the average level of extraction of the added xenobiotic exceeds 80%, which corresponds to acceptable analytical criteria (Guidance document..., 2023; DSTU EN 15662:2023, 2024).

The most complete extraction of xenobiotics for sunflower seeds was achieved under the following conditions: 1 g of homogenised sample; 10 ml of acetonitrile; 9 ml of deionised water; 1 ml of acetic acid; 4 g of Na_2SO_4; 1 g of NaCl. Shaking was carried out for 3 minutes, and centrifugation of the extraction system took place at 7,000 rpm for 5 minutes. In addition, an important factor affecting the effectiveness of xenobiotic extraction from samples artificially fortified with analytes is the temperature regime and the duration of contact with the extractant. The most optimal extraction conditions were established in the temperature range from 4 °C to 25 °C with a duration of exposure to the extraction medium for 5-25 minutes.

In contrast to many previous works, this study was focused not only on optimising the pesticide extraction method, but also on a systematic analysis of the relationship between the physico-chemical characteristics of the plant matrix and the effectiveness of xenobiotic extraction. Specifically, K. Tong *et al.* (2022) applied a combination of the QuEChERS method with high-resolution gas and liquid chromatography for screening 237 pesticides in cottonseed husks. Similar to the approaches implemented in the current study, the authors noted the high effectiveness of acetonitrile as a universal extractant. At the same time, it is in this study that the dependence of extraction efficiency on the lipid composition of the matrix is emphasised, which was not considered in the work of K. Tong *et al.* (2022). X. Theurillat *et al.* (2021) also studied fatty food matrices and emphasised that high lipid content is a factor that complicates the extraction process. The results obtained in this work confirm these conclusions: sunflower seeds, which are characterised by high fat content, demonstrated a lower level of extraction (80-86%) even under optimised extraction conditions. A common feature of both studies is the need to modify the extraction protocol – in particular, by using acetonitrile with pH regulators or buffer components. P. Aralimarad *et al.* (2025) highlighted the importance of careful sample preparation for fatty matrices (e.g., peanut oil), taking into account the assessment of analytical uncertainty and risks. These principles were also implemented in the current study, which used standardised sample fortification and performed a comparative analysis of extraction for different plant matrices with different polarities. F. Lan *et al.* (2024) demonstrated the advantages of liquid chromatography with mass spectrometry (LC-MS) in detecting fungicide residues in fruits. Similar results were obtained in this study, where LC-MS provided high sensitivity, clear peak separation, and minimal background noise - especially for matrices with a high water content. In comparison with other modern studies, this study is more comprehensive: it covers the analysis of matrix effects, the optimisation of solvents depending on the type of raw material, the influence of the acid-base nature of xenobiotics, and also the hydrodynamic conditions of the extraction.

CONCLUSIONS

As a result of the completed research, optimal conditions were established for the preparation of plant product samples for the qualitative and quantitative determination of xenobiotics, specifically pesticide residues. The study confirmed the feasibility of applying the QuEChERS method in combination with High-Performance Liquid (HPLC) and Gas Chromatography (GC), especially for samples with high moisture and low lipid content, such as leafy vegetables and fruits. For such samples, the highest extraction rates were achieved, which indicates the effectiveness of the extraction procedure. The study established that the effectiveness of xenobiotic extraction is significantly dependent on the physico-chemical properties of the plant matrix. Samples with high water content, such as lettuce leaves (>95%) and apples (>85%), required a significantly smaller volume of extractant (a ratio of 1:3-1:5) compared to fatty sunflower seeds (up to 52% lipids), where a ratio of 1:17-1:20 was used to achieve high extraction efficiency.

For the extraction of benomyl and cyprodinil, a mixture of acetonitrile with methanol (4:1) proved effective, ensuring a high level of extraction in all matrix types, particularly over 96% in lettuce and apples, which points to good solubility and a low matrix effect. For diquat, the best results were obtained using a solution of trifluoroacetic acid in methanol (9.5:0.5), with maximum values in lettuce and acceptable values (86%) in sunflower seeds. In corn kernels, the extraction level was 88-92% for all compounds, which confirms the effectiveness of the applied methodology across different plant matrices. It was also established that optimal extraction conditions – a temperature within 4-25 °C and an extraction duration of 5-25 minutes – ensure stable mass transfer and minimise matrix interferences. The chosen method, which includes a modified QuEChERS system, demonstrated high accuracy and analytical reproducibility, underscoring its suitability for the routine control of pesticide residues in various types of plant products. The obtained results have practical significance for improving laboratory control of the safety of plant products, which is particularly relevant in the context of increased demands for food and environmental safety. Prospects for further research include expanding the methodology to new classes of xenobiotics, automating sample preparation, and studying the stability of analytes during storage.

ACKNOWLEDGEMENTS

None.

FUNDING

None.

CONFLICT OF INTEREST

None.

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Наталія Терещенко
Кандидат хімічних наук, старший дослідник
Національний медичний університет імені О. Богомольця
01601, бул. Шевченка, 13, м. Київ, Україна
https://orcid.org/0000-0003-0620-2888
Олена Хижан
Кандидат хімічних наук, доцент
Національний університет біоресурсів та природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0002-2986-3251
Катерина Нестерова
Аспірант
Національний університет біоресурсів та природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0003-4081-4570

Методологічні підходи до підготовки проб для аналізу вмісту ксенобіотиків

Анотація. Метою статті було вдосконалення протоколів підготовки зразків та умов хроматографічного аналізу для визначення вибраних ксенобіотиків у сільськогосподарській продукції. Для досягнення мети використовували методику штучного збагачення гомогенізованих зразків насіння соняшнику, зерна кукурудзи, листя салату та плодів яблук модельними ксенобіотиками різних класів, подальше екстрагування органічними розчинниками з використанням модифікованого підходу QuEChERS та кількісний аналіз. Для ефективного вилучення ацетохлору та прометрину з листя салату та яблук, які характеризуються високим вмістом води, оптимальним виявилося співвідношення сировини до екстрагенту на рівні 1:3-1:5. У разі зерна кукурудзи, структура якого містить незначну кількість ліпідів, екстракцію здійснювали зі співвідношенням 1:10. Найбільші труднощі спостерігалися під час обробки насіння соняшнику, де через високу жирність формувалася трифазна система, що ускладнювала масоперенос аналітів. Незважаючи на це, застосування ацетонітрилу як екстрагенту у співвідношенні 1:17-1:20 дозволило досягти високих показників вилучення цільових ксенобіотиків. Для екстракції беномілу та ципродинілу застосовувалась суміш ацетонітрилу та метанолу у співвідношенні 4:1, яка забезпечила ефективне перенесення зазначених аналітів у всіх досліджених матрицях. Зокрема, у зразках листя салату та яблук показники вилучення для обох речовин перевищували 96 %, що свідчило про добру розчинність у застосованій суміші та низький матричний вплив. Для диквату, який є представником біпіридилієвих сполук із вираженою іонною природою, найкращі результати були отримані при використанні розчину трифтороцтової кислоти в метанолі у співвідношенні 9,5:0,5. Такий склад забезпечував стабільне вилучення з усіх типів рослинних матриць, із максимальними значеннями у зразках салату і мінімальними – у соняшнику (86 %), що все одно відповідає сучасним вимогам до аналітичної точності. У випадку зразків зерна кукурудзи рівень вилучення для всіх речовин становив 88-92 %, що свідчило про задовільну екстрагованість у зернових матрицях. Найнижчі значення вилучення зафіксовано для насіння соняшнику, тим не менш, навіть у цих умовах вилучення перебувало в межах 80-86 %, що є прийнятним відповідно до міжнародних стандартів контролю якості пестицидного аналізу. Результати хроматографічного аналізу підтвердили високу відтворюваність та чутливість методу

Ключові слова: високоефективна рідинна хроматографія; газова хроматографія з мас-селективними детекторами; екстракти; пестициди; рослинні витяжки