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Optimization of QuEChERS sample preparation followed by gas chromatography–tandem mass spectrometry (GC–MS/MS) for simultaneous analysis of organochlorine pesticide residues in different agricultural products

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Abstract: QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) is widely applied as an effective sample preparation for determining pesticide residues. In this study, an analytical method based on QuEChERS followed by gas chromatography–tandem mass spectrometry (GC–MS/MS) was validated to determine 20 organochlorine pesticide residues in agricultural products. While the extraction step employed the acidified acetonitrile (ACN), this study focused on optimizing the subsequent steps, including clean-up dispersive solid phase extraction (d-SPE) and the additional liquid–liquid extraction (LLE) concentration step. The results indicated the most effective d-SPE mixture of $MgSO_4$ and primary secondary amine (PSA). The introduced LLE switched the sample solvent from ACN to *n*-hexane, subjected to GC injection, and created a "salting-out" effect using NaCl, resulting in further removal of matrix components and enhancing recoveries by a factor of ~1.5. Negligible matrix effect (ME) was observed (|ME ratio| < 20 %) for the spinach–a representative matrix with deep green color. The method showed quantification limits of 5–10 μg/kg, good linearity ($R^2 \geq 0.995$), and satisfactory precision for intra- (relative standard deviation of RSD_r = 1.1–9.4% and inter-day (RSD_R = 2.9–7.1 %). The method was applied to tomatoes, cucumbers, Swiss chard, sweet potato leaves, and sweet potatoes, and detected no background pesticides. Recovery tests were conducted for these matrices at 10 μg/kg and for spinach at 10–30 μg/kg spiked levels, yielding 80–103 % recoveries. Overall, the method demonstrated its reliability, sensitivity, and robustness for routine monitoring of organochlorine pesticide residues in agricultural commodities.

Keywords: salting-out, dispersive solid phase extraction, PSA, LLE, GC–MS/MS

1. Introduction

Pesticides are widely used in crop production to enhance agricultural productivity.[1] However, inappropriate or excessive application, as well as non-compliance with pre-harvest intervals, may result in residues

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exceeding established maximum residue limits (MRLs) in agricultural products.[2] Such violations can result in product rejection in international trade due to the strict food safety regulations of importing countries. Pesticide residues include parent active ingredients, their metabolites, degradation products, and other transformation species.[1,3] These residues may persist in crops, agricultural products, soils, and water for varying periods, potentially affecting human health, organisms, and environmental quality. Moreover, long-term or high-level pesticide exposure has been associated with adverse health impacts, for example, carcinogenicity, cardiovascular and respiratory disorders, and neurotoxicity.[4] Therefore, the pesticide residues are monitored at very low concentrations, i.e., several mg or mg per kg samples.[5-7]

Several regulatory parameters have been established to assess and ensure the safety of pesticide residues in food and agricultural products. Internationally, the Codex Alimentarius Commission sets MRLs for pesticide residues in various food commodities.[3] In the European Union, the European Food Safety Authority provides scientific risk assessments supporting the establishment of MRLs, while the European Commission enforces the relevant legislation.[8] Similarly, in the United States, the Environmental Protection Agency (EPA) establishes and regulates pesticide residue tolerances in food.[9] Many other countries have also implemented their own national regulations and management of pesticides.[10] While MRLs represent the highest legally permitted concentrations of pesticide residues in food (mg/kg),[3] the acceptable daily intake (ADI) as another regulatory parameter refers to the estimated amount of a pesticide that can be consumed daily over a lifetime without adverse health effects, expressed in mg per kg body weight per day.[11]

Pesticides can be classified based on their derivative groups, such as pyrethroids, organophosphates, carbamates, pyridines, phenoxy herbicides, triazines, benzimidazoles, dithiocarbamates, organochlorines, etc.[4] Among these, organochlorine pesticides are considered among the most hazardous and have been banned in many countries.[5,12] Organochlorine pesticides have been associated with various adverse health effects in humans, including headaches, nausea, respiratory distress, lung inflammation, nervous depression, convulsions, reproductive function impairment, and cancer.[12] Organochlorine pesticides also have the potential to accumulate in the environment and persist in the bodies of living organisms.[12] Moreover, they can also negatively impact biodiversity and lead to a decline the number of animal and plant species. Although alternative pesticides, such as pyrethroid, organophosphate, carbamate, and triazole derivatives are encouraged, their higher costs and sometimes lack of effectiveness still lead to the continuous use of organochlorine pesticides in some areas.[5]

Analytical methods have become increasingly important in determining pesticide residues in agricultural products. QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) is a widely used method as a sample preparation procedure for analyzing pesticide residues.[13-16] Overall, QuEChERS consists of serial steps: (i) extraction of analyte(s) from the sample matrix using organic solvent(s), for example, (acidified) acetonitrile (ACN), with the addition of a salt (mixture) to assist in the partitioning, followed by (ii) clean-up to remove the matrix components using dispersive solid phase extraction (d-SPE) with an appropriate sorbent (mixture).[14,15,17] The solution after d-SPE clean-up can be subjected to instrumental analyses or endured further purification. The first QuEChERS version was introduced in 2003 by Anastassiades et al., using a mixture of magnesium sulfate–sodium chloride ($MgSO_4$–NaCl) of 4:1 during the extraction with ACN.[18] The second version, presented in AOAC 2007.01, used acidified ACN (1 % acetic acid) and a mixture of magnesium sulfate–sodium acetate ($MgSO_4$–CH_3COONa) to create a buffer system (pH 4.5).[19] The European Committee for Standardization created a third version (EN 15662), using ACN and citrate buffer.[20] All three methods commonly employ $MgSO_4$ in combination with sorbents, such as primary secondary amine (PSA), C18, and graphitized carbon black (GCB) for the clean-up step.[14] In detail, PSA is generally used to remove polar matrix components, including organic acids and sugars; C18 is applied for the removal of non-polar interferences such as

lipids; and GCB is utilized to remove pigments, particularly chlorophyll and carotenoids. Numerous studies have successfully applied QuEChERS followed by chromatography–mass spectrometry techniques, particularly liquid chromatography, LC–MS/MS, and gas chromatography, GC–MS/MS, for multi-residue pesticide analysis in various matrices, demonstrating high extraction efficiency, good recoveries, low detection limits, and robust method performance.[13-15,17,21,22]

Although QuEChERS has been widely applied for pesticide residue analysis, continuous optimization is still required to maximize the analytical performance, for example, enhancing extraction efficiency, reducing matrix interference for specific sample matrices, as well as improving cost-effectiveness. Therefore, the objective of this study was to optimize, validate, and apply a QuEChERS sample preparation followed by GC–MS/MS for the simultaneous determination of 20 organochlorine pesticide residues in agricultural products. While acidified ACN was used for the extraction step (i), optimization focused on the clean-up step (ii), investigating three widely used sorbents, namely PSA, C18, and GCB. Here, we introduced an additional liquid–liquid extraction (LLE) concentration step (iii) to switch the solvent from ACN to *n*-hexane, directly subjected to GC–MS/MS analysis, with the addition of NaCl to create a "salting-out" effect. The final LLE step (iii) also serves as a post clean-up step to further remove the matrix components to enhance the method sensitivity and robustness. This refined QuEChERS ensured applicable coverage across different matrices, including tomatoes, cucumbers, Swiss chard, sweet potato leaves, and sweet potatoes, with varying background complexity.

2. Experimental

2.1. Chemicals and reagents

The EPA CLP Organochlorine Pesticide Mix solution (2000 mg/L, Sigma-Aldrich) was used to prepare the working standard solutions by appropriate dilution in acetonitrile (ACN, analytical grade, Merck, Germany). In this study, we focused on 20 organochlorine pesticides as shown in *Table* 1. Triphenylphosphate (TPP, 99.5 %, Dr. Ehrenstorfer) was used as the internal standard (IS) for GC–MS/MS analysis. The commercial QuEChERS salts (CHROMABOND) comprise of 6000 mg of anhydrous $MgSO_4$ and 1500 mg of anhydrous CH_3COONa. Bondesil–C18 (40 μm), PSA ethylenediamine–N–propyl (polymerically bonded, 40 μm), and GCB (40 μm) were obtained from Agilent. Other chemicals, including sodium chloride (NaCl), $MgSO_4$, *n*-hexane (C_6H_{14}), and acetic acid (CH_3COOH), were of analytical grade and obtained from Merck (Germany). Ultrapure water (18 MΩ-cm, UW) was used throughout this study.

2.2. Sample collection and pretreatment

A spinach sample (organic farming) with no organochlorine pesticide background was collected and used for optimizing QuEChERS sample preparation method throughout this study. The spinach matrix was spiked with organochlorine pesticide mixture (pesticide list shown in *Table* 1) at 20 μg/kg per each pesticide.

For further application, we purchased tomatoes, cucumbers, Swiss chard, sweet potato leaves, and sweet potatoes (five samples/each type) from the supermarkets in Ho Chi Minh City (Vietnam). These sample matrices were also spiked with organochlorine pesticide mixture at different levels of 10, 20, and 30 μg/kg to test the method recoveries.

The collected samples and spiked samples were homogenized and stored at -18 °C until further analysis.

2.3. QuEChERS sample preparation procedure

The QuEChERS sample preparation procedure used in this study includes three serial steps (with the introduction of an additional LLE concentration) and is described as below:

(i) Extraction of organochlorine pesticides from the matrix: The organochlorine pesticides were extracted from the sample matrix based on AOAC 2007.01.[19] Briefly, 10 g of the homogenized sample was weighed into a 50 mL polypropylene (PP) centrifuge tube. Then, 50 μL of 1000 μg/L TPP were added to the tube, and the mixture was vigorously vortexed for one minute and added 10 mL of 1 % w/v acetic acid

in ACN. The sample mixture was kept at -18 °C for 30 minutes. Subsequently, the tube was added QuEChERS salts (6000 mg of $MgSO_4$ and 1500 mg CH_3COONa) and vigorously vortexed for two minutes, then centrifuged at 4000 rpm for eight minutes to achieve phase separation.

(ii) Clean-up of the co-existing matrix components: The matrix components potentially co-extracted with the analytes were removed or minimized by d-SPE using 4 mL of the supernatant obtained from the extraction step (i) in a 15 mL PP centrifuge tube already containing the d-SPE mixture (or salt). The sample mixture was vortexed for one minute and centrifuged at 4000 rpm for five minutes. After that, 3 mL of the extract was transferred to a glass vial and evaporated under a gentle N_2 stream to around 1 mL remaining to proceed to the next step. In this study, three different d-SPE sorbent mixtures were investigated, including mixture 1 (600 mg $MgSO_4$ and 200 mg PSA), mixture 2 (600 mg $MgSO_4$ and 200 mg C18), and mixture 3 (600 mg $MgSO_4$ and 200 mg GCB). To compare the effectiveness among these investigated sorbents, 600 mg of $MgSO_4$ was used as the control d-SPE sorbent. The sorbent recipe followed the suggestion of AOAC 2007.01 for $MgSO_4$/PSA mixture of 3/1 w/w and 200 mg per 1 mL of the extract.[19]

(iii) LLE concentration of the organochlorine pesticides: In the concentration step, 1 mL of *n*-hexane and NaCl solution of 20 % w/v were added to the glass vial containing the extract achieved from step (ii). High concentration of NaCl was used to sufficiently generate a strong "salting-out" effect. The mixture was vortexed for 1 minute and centrifuged at 4000 rpm for 2 minutes. Ultimately, the top layer was transferred to a 1.5 mL glass vial for GC–MS/MS analysis. We investigated different volumes of 20 % w/v NaCl solution, including 1, 2, 5, and 10 mL, to reach the highest method sensitivity.

2.4. GC–MS/MS operation conditions

The organochlorine pesticides were separated by a TG–5MS column (0.25-μm film thickness, 0.25-mm i.d., 30-m length, Thermo Fisher Scientific) and measured by the GC–MS/MS (Thermo Fisher Scientific). The separation was conducted based on a temperature gradient program, which involved an increase from 60 °C to 140 °C (a rate of 10 °C per minute), followed by a rise to 200 °C (20 °C per minute), and an increase to 230 °C (5 °C per minute, keeping for 5 minutes). Finally, the temperature was further increased to 280 °C (15 °C/minute) and maintained for 5 minutes. The injection volume was 1 μL, and the GC inlet was set at 250 °C. The ion source and GC–MS interface were maintained at 230 °C and 280 °C, respectively.

2.5. Analytical method performance validation

The analytical method for organochlorine pesticide residues in agricultural products using QuEChERS, followed by GC–MS/MS measurement was validated before applying to real samples.

The method limits of detection (LOD) and quantification (LOQ) were estimated by simultaneously analyzing 10 spinach samples as organochlorine-pesticide-residue-free or blank samples, spiked with organochlorine pesticides at the estimated LOD. The applied equations are LOD = 3SD and LOQ = 10/3 LOD, in which SD is the standard deviation of the estimated concentrations analyzed from the above 10 separate (spiked) spinach samples. The calibration curves of organochlorine pesticides were established based on the linear relationship between the analyte concentrations (from 10 to 100 μg/L) and the respective peak areas normalized by the internal standard (IS, TPP at 50 μg/L).

The spinach samples spiked with two concentration levels of 10 and 20 μg/kg were used to evaluate the intra- and inter-day precision (repeatability and reproducibility) through the calculation of (i) relative standard deviation from six replicates within the same working day (RSD_r) and (ii) from three separate working days (six replicates per day, RSD_R) using one-way ANOVA at a significance level of 0.05, respectively.[23] Recovery tests were conducted for spiked spinach sample at 10, 20, and 30 μg/kg and other agricultural products at 10 μg/kg, using the following equation:

$$\text{Recovery}(\%) = \frac{(\text{measured concentration})}{(\text{spiked concentration})} \times 100\%$$

The matrix effect (ME) was evaluated by calculating the ME ratio (%) using the slopes of the calibration curves constructed in the organochlorine-pesticide-residue-free spinach matrix ($\text{slope}_{\text{matrix}}$), prepared using the optimal QuEChERS sample preparation procedure, and in the pure ACN solvent ($\text{slope}_{\text{solvent}}$):

$$\text{ME ratio}(\%) = \left(\frac{\text{slope}_{\text{matrix}}}{\text{slope}_{\text{solvent}}} - 1\right) \times 100\%$$

3. Results and Discussion

3.1. Optimization of GC–MS/MS operation parameters

In this study, a TG–5MS column was used to separate the organochlorine pesticides. The column contains 5 % phenyl and 95 % dimethylpolysiloxane in the stationary phase, resulting in moderate polarity and suitability for a wide range of analytes, including organochlorine pesticides.[24] By applying a temperature gradient program (presented in section 2.4), a favorable separation was achieved for 20 organochlorine pesticides within less than 30 minutes (*Table* 1). The GC column also allowed a high temperature period in the gradient program (280 °C and keeping for 5 minutes), ensuring the elimination of the matrix component residues out of the GC column to avoid the carryover between injections. Besides, such operation time is reasonable for routine analysis of various samples. Running period of less than 30 minutes was also reported in several publications regarding analyzing pesticide residues (≥ 20 species).[15,17,25]

By applying the MS scan mode in Selected Reaction Monitoring (SRM) in the software, the quantification ions and qualification/confirming ions (m/z) were selected (*Table* 1). The optimal collision energy (eV) was also achieved and applied for further analysis.

Table 1. The GC retention time, quantification and qualification/confirming ions (m/z), and optimal collision energy achieved from SRM mode

No.	Analytes	t_R (min)	Quantification ions			Confirming ions		
			Precursor (m/z)	Product (m/z)	Collision energy (eV)	Precursor (m/z)	Product (m/z)	Collision energy (eV)
1	4,4'–DDD	22.5	235	165	20	237	165	20
2	4,4'–DDT	24.35	235	165	20	237	165	20
3	4,4'–DDE	20.79	316	246	15	246	176	30
4	alpha-BHC	15.82	217	181	5	219	183	5
5	beta-BHC	13.93	219	183	5	219	183	5
6	gamma-BHC	13.93	219	183	5	181	145	15
7	delta-BHC	14.58	217	181	5	219	183	5
8	cis–chlordane	19.33	373	266	20	272	237	15
9	trans–chlordane	19.92	272	237	15	373	266	15
10	methoxychlor	28.10	227	169	25	227	141	40
11	heptachlor	15.82	272	237	15	274	239	15
12	heptachlor epoxide	18.43	353	263	15	355	265	15
13	aldrin	16.93	263	193	35	263	191	35
14	endrin	21.68	263	193	35	279	243	10
15	dieldrin	20.83	263	193	35	279	243	10
16	endrin ketone	26.79	281	245	12	281	209	24
17	endrin aldehyde	22.9	250	215	18	250	179	16
18	alpha–endosulfan	19.81	263	293	30	195	160	10
19	beta–endosulfan	22.06	241	206	25	207	172	15
20	endosulfan sulfate	24.07	272	237	15	274	239	15
21	TPP (internal standard)	25.62	326	325	10	326	233	10

3.2. Optimization of QuEChERS sample preparation

In this study, the spinach was selected as a representative sample matrix for QuEChERS sample preparation optimization due to its deep green color and relatively high content of co-extracted components, for example, sugar and chlorophyll, making it one of the most challenging matrices among the tested samples. Therefore, successful optimization using spinach was considered a practical basis for applying the method to other agricultural products. For the remaining matrices, recovery tests were conducted using the optimal conditions to confirm the efficiency and applicability of the optimized QuEChERS method.

In the literature, different solvents were widely investigated for the extraction step (step (i) as described in section 2.3) in the QuEChERS for pesticide analysis, for example, ethyl acetate, ACN, etc., in which ACN has been widely used for vegetables and fruits.[14,17,26] For instance, acidified ACN was used to extract pesticide residues for tea samples with complicated matrix components, showing favorable extraction effectiveness.[27] Therefore, in this study, we used acidified ACN (with 1 % acetic acid) in the extraction step (i) and did not test the effects of extraction solvents on method recoveries.

Besides the two serial main steps of (i) extraction and (ii) d-SPE clean-up, we introduced additional LLE as a concentration step (iii) with the assistance of the "salting-out" effect to maximize the method recoveries, targeting different agricultural product matrices. Subsequently, the study focused on optimizing the d-SPE clean-up step (ii) and the LLE concentration step (iii). The discussion highlighted the effects of the clean-up sorbents and NaCl-induced salting-out on the recoveries of individual organochlorine pesticides as shown in sections 3.2.1 and 3.2.2. Accordingly, matrix-spiked samples at known concentrations to mimic the real sample matrices were considered sufficient for optimizing these post-extraction steps via determining method recoveries.

3.2.1. Effect of different d-SPE sorbent mixtures on clean-up effectiveness

During QuEChERS, after finishing step (i) for the extraction of organochlorine pesticides from the sample matrix, the extract can contain other matrix components, possibly interfering with the method accuracy. The interested matrices in this study were agricultural products (e.g., vegetables and fruits), which are rich in vitamins, minerals, fibers, organic acids, (poly)phenols, carotenoids, and sugars.[28] Moreover, the use of acidified ACN as an extraction solvent could accelerate the release of (poly)phenolic compounds and pigments (e.g., chlorophyll, carotenoids), resulting in a complicated matrix cocktail in the extract (with organochlorine pesticides as our desired analytes). It is apparent from the spinach sample that its extract showed a very deep green color. The pigments can be retained or trapped in the injector liner or at the GC column inlet region. Their progressive deposition leads to the formation of active sites, increased background signal, peak distortion, and accelerated stationary phase degradation. Therefore, more frequent liner replacement, column trimming, and maintenance are required,[29] thereby shortening the effective operational lifetime of the GC system. In this study, d-SPE was employed as a clean-up step (ii) to remove or minimize the co-extracted interferences. Different sorbent mixtures (1, 2, and 3) were attempted, i.e., the combination of $MgSO_4$ with another sorbent as described in section 2.3. The d-SPE clean-up effectiveness was assessed through the calculation of method recoveries of each organochlorine pesticide (*Fig.* 1). Single $MgSO_4$ was used as the control sorbent in d-SPE, the method recoveries ranged from 42 % to 61 %, indicating the need of another sorbent as a combination. The use of $MgSO_4$ cannot be eliminated due to its role as a drying agent to remove the residual water in the extract (in step (i) extraction).[14] Removing the water is crucial because water is not compatible with GC–MS/MS analysis; besides, the presence of water can result in further interferences from water-soluble matrix components. The results show that the additional use of C18 (mixture 2) and of GCB (mixture 3) did not demonstrate significant

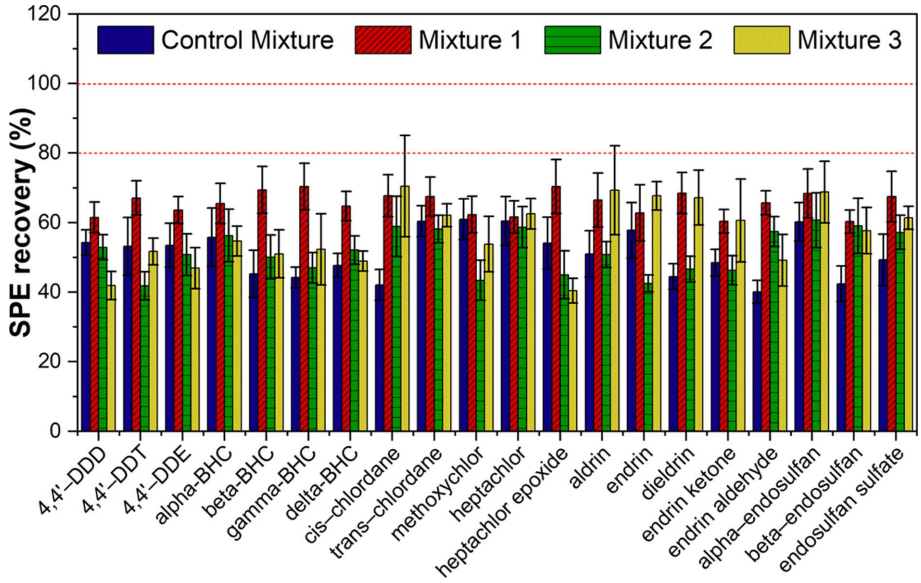

Fig. 1. The method recoveries of organochlorine pesticides obtained from different d-SPE sorbents. Spinach sample spiked with organochlorine pesticide mixture (20 compounds) at 20 µg/kg. Three tested d-SPE sorbents included mixture 1 (600 mg $MgSO_4$ and 200 mg PSA), mixture 2 (600 mg $MgSO_4$ and 200 mg C18), and mixture 3 (600 mg $MgSO_4$ and 200 mg GCB). 600 mg $MgSO_4$ was used as the control mixture condition. The method recovery (%) was calculated by considering the determined concentration and spiked concentration. The horizontal red lines depict the recoveries of 80 and 100 %. The bar heights represent the averages with error bars indicating standard deviations (SD) from triplicate experiments.

improvement in the method recoveries, for example, recoveries of 45 % to 61 % (mixture 2) and from 40 % to 71 % (for mixture 3). C_{18}-bonded silica was reported to be effective in removing non-polar interferences, such as lipids, but less effective in removing sugars and acids as polar components,[14] which are rich in our interested sample matrices in this study (spinach and other agricultural products). Mixture 3 (with GCB) increased the method recoveries for most analytes, but heptachlor epoxide, 4,4'–DDD, 4,4'–DDE, and BHC (delta isomer) showed the recoveries ≤ 50 % and low precision (RSDs of 5.3 up to 21 %) (*Fig.* 1). Overall, GCB was reported to demonstrate good removal of pigments (e.g., chlorophyll, carotenoids),[14] revealing the compatibility for our sample matrices. However, GCB is also a strong adsorbent, especially for planar geometry with one or more active groups in the chemical structures,[30] including pesticides, resulting in partial loss of analytes. For example, mixture 3 containing GCB showed the lowest recoveries for 4,4'-DDD (42 %), 4,4'-DDE (47 %), and heptachlor epoxide (40 %) and low precision (RSD, %) for cis-chlordane (21 %), aldrin (18 %), dieldrin (12 %), and endrin ketone (20 %). The results showed that mixture 1 (with PSA) indicated the most favorable method recoveries (~60–70 %) and precision for most organochlorine pesticides, especially guaranteed the higher recoveries than the control sorbent (single $MgSO_4$) for all analytes (*Fig.* 1), highlighting the need of combination of drying agent salt with another sorbent to effectively remove or minimize the interferences (as earlier mentioned).

Even though GCB and PSA sorbents have been reported to be used in d-SPE clean-up for pesticide residue analysis,[14,30] they exhibit certain differences. In details, GCB demonstrates the hydrophobicity and large surface area to adsorb a wide range of matrix compounds (for example, planar or aromatic ring-based structures as discussed earlier), while PSA with amine group is more selective for polar interferences, such as pigments, anthocyanins, and chlorophyll,[14] assisting in reducing the loss of pesticides.

Therefore, we defined mixture 1 (600 mg $MgSO_4$ and 200 mg PSA) as the optimal sorbent. However, the recoveries were still less than 70 % (*Fig.* 1), indicating the suppression from matrix effects. The hypothesis was supported by the fact that the extract after d-SPE clean-up still indicated a slight green color due to incomplete color removal. In this regard, we additionally introduced LLE concentration step (iii) to further reduce the matrix components as well as to improve the method recoveries.

3.2.2. Effect of NaCl concentrations on LLE effectiveness

After d-SPE clean-up step (ii), the LLE was applied as a concentration step (iii) to switch the solvent from ACN (over 1 mL) to *n*-hexane (1 mL), subjected to GC–MS/MS analysis. Moreover, this step (iii) can be considered an additional clean-up step to further remove the matrix components, covering different matrices, especially the pigments still existing in the solution after the clean-up step (ii), which were believed to result in low recoveries (*Fig.* 1). Experimentally, the slight green color disappeared after the LLE step (iii).

Organochlorine pesticides, characterized by moderate to low hydrophobicity ($logK_{ow}$ ~3–7),[5] can be efficiently extracted into *n*-hexane. Besides, NaCl was added to the extraction system to increase the ionic strength to increase the partitioning of the analytes to the *n*-hexane phase because the "salting-out" effect of NaCl could decrease the solubility of the targeted organochlorine pesticides in the ACN phase and increase their solubility in the *n*-hexane phase.[14,26] We can also observe the effect of added 20 % w/v solution on the recoveries, in which NaCl volumes of 5–10 mL demonstrated higher recoveries by factors of around 1.4 to 1.7, compared to 1–2 mL (*Fig.* 2). The poor recoveries obtained from 1–2 mL could be explained due to the effects of extraction ratio and insufficient dissolution/dispersion of ACN phase in NaCl solution. In this study, 5 mL of NaCl solution was used to ensure the recoveries of above 90 % for all compounds.

3.3. Matrix effect (ME) assessment

The ME assessment was conducted to quantitatively evaluate the effects of the matrix components on the

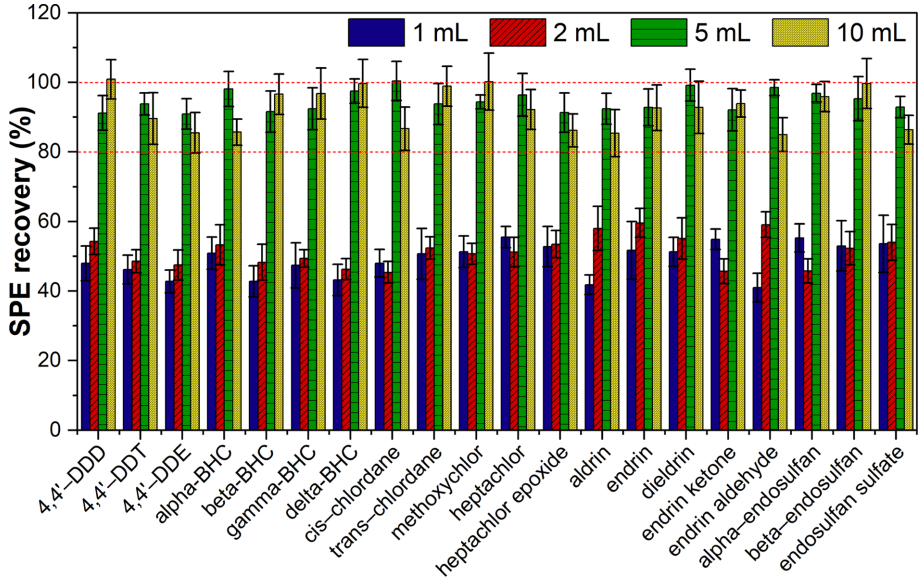

Fig. 2. The method recoveries of organochlorine pesticide residues obtained by adding different 20 % (w/v) NaCl volumes to the ACN extract from the clean-up step in the QuEChERS. Mixture 1 (600 mg $MgSO_4$ and 200 mg PSA) was used for d-SPE in the extraction step (i). The method recovery (%) was calculated by considering the determined concentration and spiked concentration. The horizontal red lines depict the recoveries of 80 and 100 %. The bar heights represent the averages with error bars indicating standard deviations (SD) from triplicate experiments.

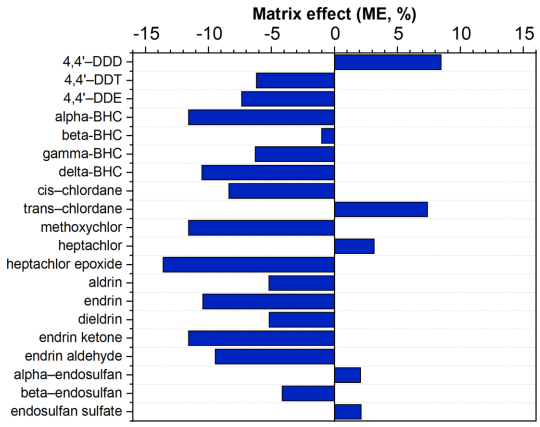

Fig. 3. The assessment of matrix effect (%ME).

overall analytical method accuracy, through the calculation of %ME values. Theoretically, there are ion suppression and ion enhancement matrix effects when %ME values less than 0 and higher than 0, respectively. There is no matrix effect when %ME = 0. However, practically, the matrix effect can be negligibly considered when %ME value is within ± 20 %.[31] *Fig.* 3 presents the results of matrix effect assessment for 20 pesticide compounds, which all showed %ME values within ± 20 %. Most %ME values were negative, except for 4,4'–DDD, trans–chlordane, heptachlor, alpha–endosulfan, and endosulfan sulfate. However, the amplitude of the enhancement effect was lower than suppression. The matrix suppression effect was also revealed from the recoveries of 60–70 % using the optimal sorbent mixture 1 (without post clean-up LLE, *Fig.* 1). The improved recoveries to over 90%, but still less than 100 %, achieved from the optimal QuEChERS was consistent with the negative matrix effect results in *Fig.* 3. Subsequently, the effectiveness in matrix removal achieved from the combination of d-SPE and additional post clean-up LLE was highlighted via ME calculation.

3.4. Method validation

3.4.1. Limit of detection and quantification (LOD and LOQ)

Table 2 shows the estimated LOD and LOQ of the overall analytical method, including QuEChERS followed by GC–MS/MS. *Table* 2 also indicates the

Table 2. Estimated limit of detection (LOD), limit of quantification (LOQ), and maximum residue limits (MRLs) of the organochlorine pesticides.[32]

No.	Compound	MLOD (mg/kg)	MLOQ (mg/kg)	MRL value (mg/kg)
1	4,4'–DDD	0.002	0.006	0.01
2	4,4'–DDT	0.003	0.008	0.01
3	4,4'–DDE	0.003	0.008	0.01
4	alpha-BHC	0.002	0.006	0.01
5	beta-BHC	0.002	0.005	0.01
6	gamma-BHC	0.002	0.007	0.05–0.2*
7	delta-BHC	0.003	0.010	0.01
8	cis–chlordane	0.002	0.005	0.01
9	trans–chlordane	0.003	0.008	0.01
10	methoxychlor	0.003	0.009	0.05–0.2*
11	heptachlor	0.002	0.007	0.01
12	heptachlor epoxide	0.003	0.009	0.01
13	aldrin	0.003	0.008	0.01
14	endrin	0.003	0.010	0.01
15	dieldrin	0.003	0.008	0.01
16	endrin ketone	0.003	0.010	0.01
17	endrin aldehyde	0.002	0.005	0.01
18	alpha–endosulfan	0.002	0.006	0.05–0.2*
19	beta–endosulfan	0.002	0.007	0.05–0.2*
20	endosulfan sulfate	0.002	0.007	0.05–0.2*

(*) 0.05 mg/kg for apples, pears, quinces, medlars, loquats; 0.2 mg/kg for strawberries, raspberries, blackberries, currants, gooseberries, cranberries, blueberries; 0.01 mg/kg for other vegetables and fruits.

MRLs of the organochlorine pesticides in different vegetable and fruit matrices, regulated by the European Union (EU).[32] Most organochlorine pesticides have MRLs of 0.01 mg/kg, except for lindane (gamma-BHC), methoxychlor, alpha–endosulfan, beta–endosulfan, and endosulfan sulfate, which have MRLs ranging from 0.05 to 0.2 mg/kg, depending on the specific matrices. The LOQs are in the range of 0.005 to 0.010 mg/kg, within the established MRL regarding the specific organochlorine pesticides. Therefore, this method was suitable for quantifying organochlorine pesticides in vegetable and fruit matrices.

3.4.2. Linearity

In this study, we constructed calibration curves with concentrations ranging from 10 to 100 μg/L for each organochlorine pesticide (*Table* 3). The calibration

Table 3. Analytical method performance for the organochlorine pesticides

No.	Compound	Linearity		[a]RSD_r (%)		[a]RSD_R (%)		[b]Recovery (%)		
		Equation	R^2	LOQ	2LOQ	LOQ	2LOQ	LOQ	2LOQ	3LOQ
1	4,4'–DDD	y = 0.0584x + 0.0046	0.9994	1.8	1.9	3.0	3.6	93.6–100	93.0–103	98.5–99.9
2	4,4'–DDT	y = 0.0244x – 0.0346	0.9988	3.6	1.7	3.9	3.6	86.3–89.7	88.5–88.6	89.3–91.1
3	4,4'–DDE	y = 0.0144x – 0.0088	0.9976	3.7	2.3	4.9	3.2	84.4–87.3	85.6–88.9	88.1–90.5
4	alpha-BHC	y = 0.0042x + 0.0134	0.9951	3.0	3.1	5.0	3.7	82.0–85.7	84.5–89.0	91.5–91.7
5	beta-BHC	y = 0.0115x + 0.0032	0.9972	2.2	1.5	5.2	3.3	89.6–90.0	90.5–90.6	90.0–90.8
6	gamma-BHC	y = 0.0096x – 0.0034	0.9987	5.4	1.4	6.8	3.7	88.1–88.9	87.2–90.6	86.1–87.7
7	delta-BHC	y = 0.0042x + 0.0134	0.9951	3.0	3.0	5.1	3.6	82.0–83.5	86.7–89.0	91.7–94.5
8	cis–chlordane	y = 0.0033x – 0.0035	0.9982	2.8	3.2	4.2	3.4	85.9–86.2	87.1–89.1	87.6–89.2
9	trans–chlordane	y = 0.005x + 0.018	0.9979	3.5	2.6	4.2	2.9	94.5–94.6	98.0–98.8	97.8–98.0
10	methoxychlor	y = 0.0293x – 0.0691	0.9984	3.5	4.0	5.7	4.3	81.5–88.2	85.2–91.5	87.2–88.4
11	heptachlor	y = 0.0163x – 0.0139	0.9977	4.4	4.2	4.7	5.0	82.9–83.9	85.6–89.1	89.4–90.2
12	heptachlor epoxide	y = 0.0025x – 0.0024	0.9971	2.1	3.3	3.4	3.9	80.1–80.4	81.8–85.6	85.7–87.5
13	aldrin	y = 0.0034x – 0.0012	0.9985	4.7	2.8	7.1	4.3	88.2–90.6	85.4–89.8	87.0–90.2
14	endrin	y = 0.0026x – 0.0036	0.9995	2.8	4.3	2.9	4.3	81.1–81.7	82.4–86.0	86.6–86.8
15	dieldrin	y = 0.0018x + 0.0005	0.9993	3.7	4.0	4.9	5.0	88.5–89.7	88.7–91.1	90.9–91.6
16	endrin ketone	y = 0.0012x – 0.0027	0.9969	5.1	3.8	5.4	4.5	80.2–83.4	81.2–81.7	86.5–88.0
17	endrin aldehyde	y = 0.0003x + 0.0038	0.9959	9.4	8.1	6.9	6.1	82.8–85.3	82.4–86.4	86.5–88.5
18	alpha–endosulfan	y = 0.0011x – 0.0002	0.9954	5.2	3.4	4.7	4.9	86.1–86.2	89.6–90.3	91.3–92.6
19	beta–endosulfan	y = 0.0011x – 0.002	0.9953	5.0	1.1	5.5	3.0	85.6–89.4	89.4–89.7	92.5–92.8
20	endosulfan sulfate	y = 0.0027x + 0.0033	0.9996	3.5	4.4	5.2	4.9	90.6–91.1	89.1–93.1	90.3–93.3

[a]RSD values were calculated from spinach matrix spiked with organochlorine mixture at 1× and 2 × LOQ (using LOQ of 10 μg/kg as the highest value among the organochlorine pesticides).
[b]Recoveries were determined for spinach matrix spiked with organochlorine mixture 1× and 2×, and 3 × LOQ (using LOQ of 10 μg/kg as described above).

curve equation was made by fitting the linearity between the pesticide concentrations and respective GC–MS/MS peak areas normalized by the TPP internal standard. The goodness of linearity was achieved ($R^2 \geq 0.995$).

3.4.3. Repeatability and reproducibility

Repeatability (%RSD_r) and reproducibility (%RSD_R) were evaluated at 10 and 20 μg/kg, corresponding to 1×LOQ and 2×LOQ levels, respectively. Repeatability experiments were conducted in six replicates within the same day, while reproducibility experiments were conducted in three separate days (six replicates per day). The results in *Table* 3 show 1.8 % to 9.4 % (%RSD_r) and 2.9 % to 7.1 % (%RSD_R) for 10 μg/kg. For 20 μg/kg, the values are 1.1 % to 8.1 % (%RSD_r) and 2.9 % to 6.1 % (%RSD_R). These values are in accordance with the Appendix F of AOAC (2016) for analytical method performance.[23]

3.4.4. Recovery

For analytical method validation, recovery test plays a crucial role in ensuring accuracy and determining the compatibility of the developed method with real samples. The recovery values were determined by comparing the practically determined concentrations and the spiked levels of the organochlorine pesticides. Spinach matrix was spiked with the organochlorine pesticide mixture at three levels of 10 μg/kg, 20 μg/kg, and 30 μg/kg, corresponding to the 1×LOQ, 2×LOQ, and 3×LOQ levels (using the highest LOQ value = 10 μg/kg among the organochlorine pesticides), respectively. The validation results in *Table* 3 show that all evaluated compounds achieved recovery values that met the requirements of Appendix F of AOAC (2016) for the concentration range from 10 to

100 μg/kg, typically 80–100 %, 82–103 %, and 86–100 % corresponding to the spiked levels of 1×LOQ, 2×LOQ, and 3×LOQ.[23]

3.5. Application of the QuEChERS followed by GC–MS/MS to the real samples

The validated analytical method, based on QuEChERS extraction followed by GC–MS/MS determination, was applied to various agricultural products, including tomatoes, cucumbers, Swiss chard, sweet potato leaves, and sweet potatoes (5 samples/each). The results showed that within the sensitivity of the developed method, no measurable organochlorine pesticide contamination was present in the tested products (all target organochlorine pesticides < LODs shown in *Table* 2). To further confirm the applicability of the method, recovery tests were conducted. Five samples from each matrix were combined and homogenized to generate one representative matrix for each product type, in which a mixture of organochlorine

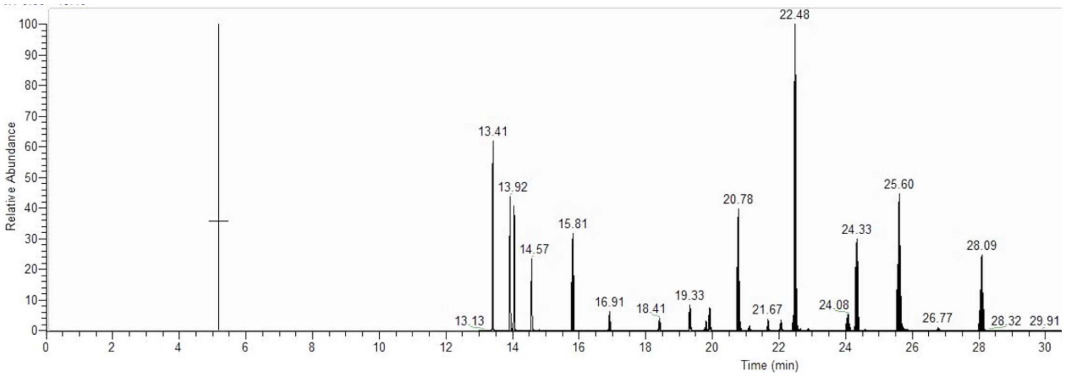

Fig. 4. A chromatogram obtained from sweet potato leaves spiked with 10 μg/kg organochlorine pesticide mixture.

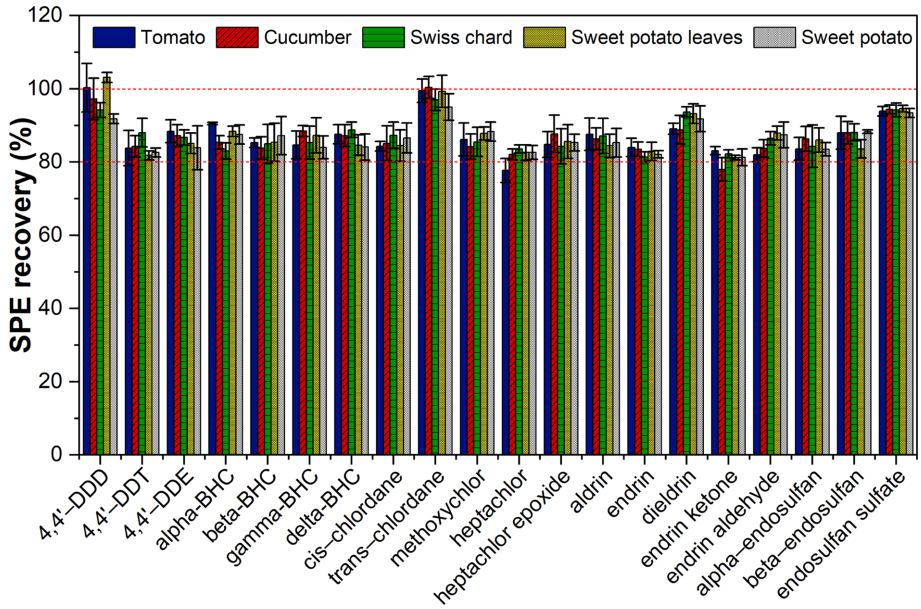

Fig. 5. Recovery of organochlorine pesticides in spiked samples. The pesticides were spiked in each sample matrix at 10 μg/kg. The recovery (%) was calculated by considering the determined concentration and spiked concentration. The horizontal red lines depict the recoveries of 80 and 100 %. The bar heights represent the averages with error bars indicating standard deviations (SD) from triplicate experiments.

pesticides was spiked at 10 μg/kg (1×LOQ level, using LOQ = 10 μg/kg as the highest value determined among the organochlorine pesticides). A representative chromatogram of the spiked sweet potato leaves is shown in *Fig.* 4, demonstrating well-resolved analyte peaks and a relatively clean baseline, which supported the selectivity and reliability of the optimized method.

The results in *Fig.* 5 showed the recoveries from 80 % to 103 % across all tested matrices, satisfying the Appendix F of AOAC (2016) regarding analytical method performance.[23] The method also demonstrated favorable repeatability, with RSD values ranging from 0.3 % to 7.1 %. These low RSDs confirmed good method precision across diverse agricultural product matrices. Collectively, the recovery and precision results demonstrate that the proposed method is reliable and suitable for routine monitoring of organochlorine pesticide residues.

4. Conclusions

In this study, an analytical method using QuEChERS extraction followed by GC–MS/MS analysis, based on AOAC 2007.01, was optimized and validated for the determination of 20 organochlorine pesticide residues in different agricultural products. Among the tested clean-up d-SPE conditions, a sorbent mixture containing $MgSO_4$ and PSA was identified as the most effective for matrix purification, providing efficient removal of co-extractive components while maintaining satisfactory recoveries and precision. An additional LLE step based on a “salting-out” effect was introduced after the d-SPE clean-up. This supplementary step proved the effectiveness in enhancing the method recoveries and further reducing matrix interferences potentially present in different agricultural products matrices, particularly pigments, thereby improving chromatographic performance and prolonging GC system stability. The optimized method achieved LOQ values ranging from 5 to 10 μg/kg, complying with the MRLs established by European Union regulations. Method validation demonstrated that repeatability, reproducibility, and recovery values met the performance criteria specified by AOAC at the corresponding concentration levels. The validated method was subsequently applied to various agricultural products, including tomatoes, cucumbers, Swiss chard, sweet potato leaves, and sweet potatoes. No detectable organochlorine pesticide residues were found in the analyzed samples, while spiked samples exhibited high recovery rates and good precision, confirming the robustness and suitability of the method for routine monitoring of organochlorine pesticides in agricultural products.

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References

1. Y. Wu, L. Zhou, L. Kang, H. Cheng, X. Wei, and C. Pan, *Environ. Pollut.*, **316**(P1), 120557 (2023). https://doi.org/10.1016/j.envpol.2022.120557
2. Maximum Residue Levels. European Commission. https://food.ec.europa.eu/plants/pesticides/maximum-residue-levels_en (last accessed 2026.03.01)
3. Human health risks–Dietary. https://www.fao.org/pesticide-registration-toolkit/registration-tools/registration-criteria/human-health-risks/dietary-risks/ru/ (last accessed 2026.03.01)
4. C. Shekhar, R. Khosya, K. Thakur, D. Mahajan, R. Kumar, S. Kumar, and A. K. Sharma, *Toxicol. Reports*, **13**, 101840 (2024). https://doi.org/10.1016/j.toxrep.2024.101840
5. E. N. Tzanetou and H. Karasali, *Agriculture*, **12**(5), 728 (2022). https://doi.org/10.3390/agriculture12050728
6. B. Manggala, C.Chaichana, W. N. H. Syahputra, and W,Wongwilai, *Nat. Life Sci. Commun.*, **22**(3), e2023049 (2023). https://doi.org/10.12982/NLSC.2023.049
7. X. Shu, N. Chu, X. Zhang, X. Yang, X. Meng, and J. Yang, *Int. J. Environ. Res. Public Health*, **19**, 12639 (2022). https://doi.org/10.3390/ijerph191912639
8. EU legislation on MRLs. European Commission. https://food.ec.europa.eu/plants/pesticides/maximum-residue-

levels/eu-legislation-mrls_en (last accessed 2026.03.01)
9. Setting tolerances for pesticide residues in foods. United States Environmental Protection Agency (EPA). https://www.epa.gov/pesticide-tolerances/setting-tolerances-pesticide-residues-foods (last accessed 2026.03.01)
10. RAP Publication, Advancement of pesticide regulatory management in Asia (2013).
11. Pesticide registration toolkit. Food and Agriculture Organization of the United Nations. https://www.fao.org/pesticide-registration-toolkit/registration-tools/assessment-methods/method-detail/en/c/1187112/ (last accessed 2026.03.01)
12. R. Jayaraj, P. Megha, and P. Sreedev, *Interdiscip Toxicol.,* **9**(3-4), 90-100 (2016). https://doi.org/10.1515/intox-2016-0012
13. P. Payá, M. Anastassiades, D. Mack, I. Sigalova, B. Tasdelen, J. Oliva, and A. Barba, *Anal. Bioanal. Chem.*, **389**, 1697-1714 (2007). https://doi.org/10.1007/s00216-007-1610-7
14. H. Musarurwa, L. Chimuka, V. Emmanuel, and N. Tawanda, *J. Food Compos. Anal.*, **84**, 103314 (2019). https://doi.org/10.1016/j.jfca.2019.103314
15. Ö. Görel, Ş. Dinç-zor, and G. Alpdo, *Microchem. J.*, **144**, 124-129 (2019). https://doi.org/10.1016/j.microc.2018.08.056
16. L.-T. Anh-Dao, D. Minh-Huy, V. Hong-Phong, and N. Cong-Hau, *Anal. Sci. Technol.*, **36**(5), 205-215 (2023). https://doi.org/10.5806/AST.2023.36.5.205
17. M. Tankiewicz and A. Berg, *Microchem. J.*, **181**, 107794 (2022). https://doi.org/10.1016/j.microc.2022.107794
18. M. Anastassiades, S. J. Lehotay, D. Štajnbaher, and F. J. Schenck, *J. AOAC Int.*, **86**(2), 412 (2003). https://doi.org/10.1093/jaoac/86.2.412
19. AOAC Official Method 2007.01. Pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate.
20. EN 15662:2008. Foods of plant origin–Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE–QuEChERS–method
21. S. Li, Y. Liu, D. Jiang, M. Liu, J. Ding, F. Zhao, Y. Liu, X. Hu, X. Mao, and Q. Zhao, *Food Anal. Methods*, **17**, 797-811 (2024). https://doi.org/10.1007/s12161-024-02602-z
22. S. Song, K. Zhu, L. Han, Y. Sapozhnikova, Z. Zhang, and W. Yao, *J. Agric. Food Chem.*, **66**, 5031-5038 (2018). https://doi.org/10.1021/acs.jafc.7b05339
23. Appendix F of AOAC (2016). Guidelines for standard method performance requirements.
24. TraceGOLD TG-5MS AMINE GC Columns. ThermoFisher Scientific. https://www.thermofisher.com/order/catalog/product/26097-1420 (last accessed 2026.03.01)
25. V. Tripathy, A. Saha, and J. Kumar, *J. Food Sci. Technol.*, **54**(2), 458-468 (2017). https://doi.org/10.1007/s13197-017-2487-x
26. P. Qi, J. Wang, Z. Liu, Z. Wang, H. Xu, S. Di, H. Zhao, and X. Wang, *J. Chromatogr. A*, **1659**, 462589 (2021). https://doi.org/10.1016/j.chroma.2021.462589
27. M. Selvaraj, M. Mathew, and S. R. Nambiar, *J. Food Compos. Anal.*, **148**, 108499 (2025). https://doi.org/10.1016/j.jfca.2025.108499
28. Y. Liu, J. Deng, T. Zhao, X. Yang, J. Zhang, and H. Yang, *Curr. Res. Food Sci.*, **8**, 100715 (2024). https://doi.org/10.1016/j.crfs.2024.100715
29. Ł. Rajski, A. Lozano, N. Belmonte-valles, A. Uclés, S. Uclés, M. Mezcua, and A. R. Fernandez-alba, *Analyst*, **138**, 921-931 (2013). https://doi.org/10.1039/c2an35995b
30. Y. Wang, J. Luo, Y. Huang, T. Ke, Y. Luo, and M. Yang, *Bioresour. Technol.*, **376**, 128918 (2023). https://doi.org/10.1016/j.biortech.2023.128918
31. F. Raposo and D.Barceló, *TrAC*, **134**, 116068 (2021). https://doi.org/10.1016/j.trac.2020.116068
32. Pesticide residue(s) and maximum residue levels (mg/kg). European Commision. https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/mrls/details?lg_code=EN&pest_res_id_list=65&product_id_list= (last accessed 2026.03.01)