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Research Article

Multiclass Pesticide Residue Analysis in Fruit and Vegetable Samples by Combining Acetone-Based Salting-Out Assisted Extraction with Dispersive Liquid-Liquid Microextraction

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Isolation and enrichment of multiclass pesticides' residue, namely, fungicides (benalaxyl), herbicides (atrazine), carbamate insecticides (carbofuran), organophosphate insecticides (chlorpyrifos), organochlorine insecticides (4,4′-DDT), and pyrethroid insecticides (bifenthrin), were made by combining acetone-based salting-out assisted extraction with the dispersive liquid-liquid microextraction (SADLLME) method, followed by high-performance liquid chromatography-diode array detection (HPLC-DAD). The effect of the type and volume of the extraction solvent in the pretreatment step, the volume of the disperser solvent (acetone extract), the type and volume of the extraction solvent, pH, and salt addition in the DLLME procedure was studied. Good coefficient of determination ($R^2 \geq 0.9964$) was obtained for all the target analytes. The limits of detection and quantification limits were between 2.1 and 4.5 and 5.7 and 12.9 μg/kg, respectively, with adequate enrichment factors ranging from 37.6 to 191. The recoveries of spiked blank tomato ranged from 86.8 to 109.5%. The limit of quantification of the proposed method was lower than the maximum residue limits set by the European Union. The repeatability and reproducibility of precisions ranged between 2.9 and 8.0 and 4.9 and 9.5%, respectively. The optimized and validated method was applied to quantify pesticides in tomato, pear, apple, and melon obtained from different markets. However, all target compounds studied in this work were not detected in any real samples applied. Overall, the work results revealed that the proposed method is useful for the sample extraction and preconcentration of the target analytes from fruits and vegetables.

1. Introduction

Vegetables and fruits are a significant source of vitamins and minerals. However, fruits and vegetables can also be sources of pollutants such as pesticides, which may be found on the peel or penetrate and translocate into plants via the xylem or phloem [1]. Pesticides are entered into agricultural products in different ways directly and/or indirectly from contaminated soils, surface, and ground water and lastly created a serious problem via the food chain on human health [2]. Owing to these, the European Union (EU) has established the maximum residue limits (MRLs) for several pesticides in food [3, 4].

Nowadays, people utilize enormous amounts of different types of pesticides to obtain good quality and high yield of fruits and vegetables. However, after applying these pesticides, their residue contaminates fruits and vegetables [5]. Pesticides are found in foods in low concentration and a wide range of polarities; therefore, accurate, reliable, and low-cost analytical methods should be employed to concentrate the target analytes [6]. Conventional sample preparation techniques such as liquid-liquid extraction (LLE) and solid-phase extraction (SPE) have been used to isolate the compound of interest from food samples [7, 8]. However, the aforementioned methods have some

drawbacks: long extraction time, consume large amounts of organic solvents, and are not green. Liquid-phase microextraction (LPME) is an alternative miniaturized sample preparation technique that overcomes many disadvantages of LLE. The recently developed methodology (LPME) has many advantages, such as it significantly reduced the volume of hazardous organic solvents, is environmentally benign, is inexpensive, and is fast for extraction/preconcentration of multiresidue pesticides from complex matrices compared with the conventional method [9–11].

Currently, microextraction techniques such as reversed-phase ultrasonic assisted liquid-liquid microextraction (RP-UALLME) [11], liquid-phase microextraction and the freezing of deep eutectic solvent (LPME-FDES) [12], vortex-assisted dispersive liquid-liquid microextraction based on the freezing of deep eutectic solvent (VADLLME–FDES) [13], and sonication and dispersive liquid-liquid microextraction based on the solidification of floating organic drop (SDLLME-SFO) [14] have been developed for the determination of organic and inorganic compounds in different matrices. Among different kinds of LPME, DLLME is the youngest one. Its extraction is carried out based on a ternary component solvent system including extraction solvent, disperser solvent, and an aqueous sample (water/disperser solvent/extraction solvent). The advantages of DLLME compared to other LPME are easy operation, rapidness, inexpensive, high extraction efficiency, and short extraction time (i.e., significantly large contact surface area between the extractant and the aqueous sample has to cause to the fast analytes' extraction) [15]. Since scholars' introduction of DLLME, various approaches have been made to this technique including the use of solvents with a density lower than that of water [16]; extraction without the need for centrifugation [17]; and the use of various ionic liquids as the extraction solvent [18].

DLLME method is predominately utilized for the preconcentration of species, which are nonpolar or moderately polar, in aqueous samples [15, 19]. Moreover, DLLME is not only an appropriate method for the extraction and cleanup of species from water samples but also combines with different sample preparation techniques during the extraction of pesticides from solid samples. Mixed-mode extraction techniques (DLLME with other techniques) are utilized to analyze target analytes from solid samples. Many methods have been employed to extract pesticide residues from solid samples for the following DLLME procedure, such as ultrasound-assisted extraction-dispersive liquid-liquid microextraction (UAE-DLLME) [6], acetonitrile-based extraction with dispersive liquid-liquid microextraction (MeCN-DLLME) [20], and quick, easy, cheap, effective, rugged, and safe and dispersive liquid-liquid microextraction (QuEChERS-DLLME) [21, 22].

High-performance liquid chromatography (HPLC) or/and gas chromatography (GC) has been used to analyze pesticides/contaminants in different matrices such as in environmental water [23], in food [24–26], and in biological [27] samples. Thus, a combination of acetone-based extraction with DLLME followed by high-performance liquid chromatography-diode array detection has been applied to investigate pesticides in tomato samples. To our knowledge, there is no published report on the simultaneous determination of pesticides, namely, fungicides (benalaxyl), herbicides (atrazine), carbamate insecticides (carbofuran), organophosphate insecticides (chlorpyrifos), organochlorine insecticides (4,4′-DDT), and pyrethroid insecticides (bifenthrin), from tomato samples using acetone-based extraction with DLLME followed by HPLC-DAD.

In the present work, a simple and fast acetone-based salting-out assisted extraction with DLLME has been developed. Parameters under the optimization of the sample pretreatment step (type and volume of the extraction solvent) and DLLME procedure (volume of the disperser solvent, type of the extraction solvent, the volume of the extraction solvent, pH, and salt addition) were optimized. Generally, due to the complexity of food matrices and low concentration of pesticides in them, direct extraction of target analytes using DLLME alone is challenging. Therefore, mixed-mode extraction techniques are required to isolate multiclass pesticides from food samples and to achieve high enrichment factors. Finally, to assess the proposed method's applicability, it was applied for the quantitative determination of target pesticides in apples, tomato, pear, and melon.

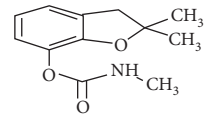
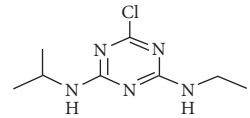
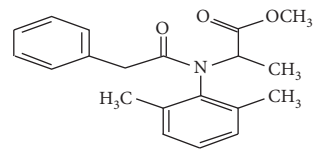
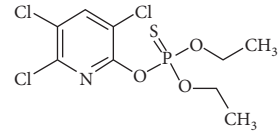
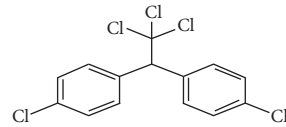
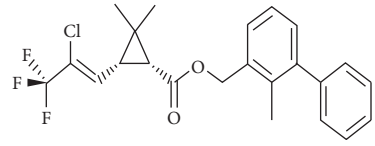
2. Materials and Methods

2.1. Chemicals and Reagents. Chromatographic-grade acetonitrile (CH_3CN), methanol (CH_3OH), and acetone (CH_3COCH_3) were obtained from Sigma-Aldrich (Steinheim, Germany). Pesticide standards used (carbofuran, atrazine, benalaxyl, chlorpyrifos, 4,4′-DDT, and bifenthrin) with purity >98% were obtained from Sigma-Aldrich (Seelze, Germany). Extractive solvents, chloroform (Chlo) ($CHCl_3$), chlorobenzene (C_6H_5Cl), dichloromethane (CH_2Cl_2), and tetrachloroethylene (TCE) (C_2Cl_4), were purchased from Sigma-Aldrich (Steinheim, Germany). Sodium hydroxide and hydrochloric acid were supplied by Merck (Darmstadt, Germany). Sodium chloride (NaCl) was purchased from Sigma-Aldrich (Steinheim, Germany). Ultrahigh purity (UHP) water of 18.2 MΩ cm resistivity was generated from the Milli-Q system, Millipore (Billerica, MA, USA). The chemical structures, solubility in water, and log K_{ow} of the target pesticides are given in Table 1.

2.2. Instrumentation. Chromatographic separation was accomplished using an Agilent 1260 high-performance liquid chromatography series (Agilent Technologies, Waldbronn, Germany) equipped with a UV-Vis diode array detector (DAD). ChemStation software (version 1.9.0) was used for data acquisition and processing. A vortex mixer (Velp Scientifica, Italy) and centrifuge (Thermo Electron Corporation, Massachusetts, USA) were used for sample preparation.

2.3. Chromatographic Conditions. The reversed-phase separation of selected pesticides was made on an XTerra MS C_{18} 3.5 μm 4.6 × 100 mm column. A binary mobile phase

Table 1: Chemical structure, solubility in water, and log K_{ow} of target analytes.

Substances	Chemical classes	Structure	Solubility in water at 20°C (mg/L)	Log K_{ow}
Carbofuran	Carbamate insecticide		322	1.8
Atrazine	Triazine herbicides		35	2.7
Benalaxyl	Acylamino acid fungicide		28.6	3.54
Chlorpyrifos	Organophosphate insecticides		1.05	4.7
4,4′-DDT	Organochlorine insecticides		0.006	6.2
Bifenthrin	Pyrethroid insecticides		0.001	6.6

containing solvent A (water) and solvent B (acetonitrile) with a gradient program consisting of 70 to 80% B (0-1 min), 80 to 90% B (1-2 min), and 90% B (2–4 min) was used during the course of the analysis. Ahead of each injection, the system was re-equilibrated at the initial conditions (70% B) from 4 to 6 min. Analyses were performed with a flow rate of 1.4 mL/min, column temperature of 40°C, and injection volume of 5 μL. The DAD was set at 210 and 235 nm.

2.4. Salting-Out Assisted Extraction and DLLME Procedure. Many of the studies that have been reported on DLLME show the determination of the organic and inorganic compounds in the water sample is not complicated. However, in complex matrices such as food, it is highly challenging because the target analytes should be extracted from the complex matrix before proceeding to DLLME. The proposed procedure is shown in Figure 1.

2.4.1. Sample Pretreatment (Acetone-Based Salting-Out Assisted Extraction). The core of apple, pear, and melon, except tomato, was separated from the edible part. Afterward, a representative sample of tomato (500 g) was cut into small pieces using a kitchen knife and blended thoroughly in a homogenizer. A subsample of 7.0 g homogenized tomato was accurately weighed into a 50 mL screw-cap conical-bottom polyethylene test tube. A mixture of 300 μg/kg of atrazine, benalaxyl, chlorpyrifos, DDT, and bifenthrin and 600 μg/kg of carbofuran was spiked and vortexed and then was allowed to stand for about 1200 s to establish equilibration. Subsequently, 5 mL of acetone was added, and the tube was vigorously shaken by hand for 30 s, followed by vortexing for 30 s. Then, a mixture of 2.8 g of anhydrous $MgSO_4$ (to remove water from the organic phase) and 0.7 g NaCl (to induce the separated layer) was added and shaken energetically (to avoid the formation of outsized $MgSO_4$ agglomerates) by hand for 30 s, followed by vortexing and centrifugation for 30 s at 1600 rpm and 600 s at 4000 rpm, respectively. At this stage, three phases were formed. The upper, middle, and lower phases were the extract analyte, tomato residue, and salt solution, respectively. Finally, one milliliter of the acetone extract (upper phase) was subjected to DLLME.

2.4.2. Dispersive Liquid-Liquid Microextraction Procedure. Five milliliters of sodium chloride aqueous solution (12%, w/v) was transferred into a 15 mL screw-cap polyethylene centrifuge tube with a conical bottom, and pH was adjusted to 7. A mixture of 1000 μL of the extract (acetone) and 70 μL of tetrachloroethene was rapidly injected. As a result, cloudy solution from the dispersion of fine droplets of the extraction solvent in the aqueous solution was produced. The tube was vortexed at 1200 rpm for 60 s to enhance the contact surface area between the droplet of the extraction solvent and the aqueous phase, and then it was centrifuged at

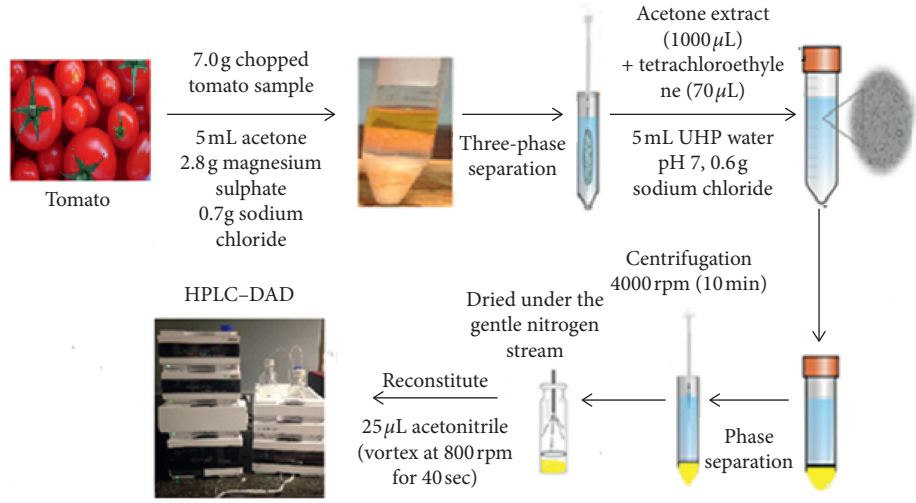


Figure 1: Schematic representation of the proposed acetone-based salting-out assisted extraction followed by the DLLME procedure.

4000 rpm for 600 s. The sediment phase was then collected with a 1 mL microsyringe and transferred into insert vials. The extract was evaporated to dryness under a moderate stream of nitrogen, and the residue was dissolved in 25 μL of acetonitrile. Finally, the reconstituted residue was vortexed for 40 s at 800 rpm before injecting into the HPLC for the separation and quantitative measurements. A schematic diagram of the whole experimental procedure is depicted in Figure 1.

3. Results and Discussion

Acetone-based salting-out assisted extraction combined with the DLLME method was used for the extraction and preconcentration of targeted multiclass pesticides from vegetable and fruit samples.

3.1. Optimization of the Sample Pretreatment Step. Parameters that affected acetone-based salting-out assisted extraction (liquid-solid extraction) at the sample pretreatment stage such as extraction solvent (acetone, acetonitrile, and methanol) and volume of the extraction solvent were investigated.

3.1.1. Effect of the Extraction Solvent on the Sample Pretreatment Step. Unlike water samples, complex matrices such as fruits, vegetables, and biological animal tissues require a sample pretreatment step prior to applying the DLLME. This step is essential to extract target analytes from the matrix to the extraction solvent. In the sample pretreatment step, key parameters that affected the recovery of the extractions (the type and volume of the extraction solvents) were studied. So, acetonitrile, acetone, and methanol were candidates to be used as the extraction solvent in liquid-solid extraction as well as the dispersive solvent in the DLLME procedure. When methanol was used as the extraction solvent, the layer between the organic and aqueous phase was not distinguished. Owing to this, methanol was excluded from the candidates. As can be seen in Figure 2, recoveries of target analytes were obtained in the range of 74.5–89.8% and 70.4–97.0% for acetonitrile and acetone, respectively. Therefore, the recoveries of both candidates were comparable. However, because of low cost and less impact on the environment, acetone was selected as the extraction solvent for the subsequent experiments.

3.1.2. Effect of Extraction Solvent Volume on the Sample Pretreatment Step. The effect of extraction solvent volume (acetone) was examined in the range of 5000–8000 μL. The recoveries of target compounds significantly decreased with the increase in the volume of acetone (5000–8000 μL) (Figure 3). The decrease in the recoveries could be due to the dilution effect since the volume of the supernatant increases with acetone volume. On the basis of the experimental results, 5000 μL was chosen as optimum volume of acetone for the subsequent experiments.

3.2. Optimization of DLLME Parameters Using the Acetone Extract as the Dispersive Solvent

3.2.1. Effect of Acetone Extract (Disperser Solvent) Volume. The dispersive solvent plays a crucial role in DLLME. To maximize its role, the disperser solvent should be miscible in the extraction solvent and water. It is also beneficial to disperse the extracting solvent into the aqueous phase so as to get the cloudy solution (water/acetone extract/tetrachloroethylene). The disperser solvent (acetone) has two roles: firstly, it carries the extracted analytes from the crude sample to water. Secondly, it acts like a disperser solvent to achieve the principle of DLLME (ternary system). The effect of the disperser solvent volume was examined by using different volumes of the acetone extract ranging from 500 to 2000 μL. Figure 4 reveals that the enrichment factor increased by increasing the volume of acetone initially and then decreased with a further increase of acetone extract volume. It is likely that, at a low volume of acetone (500 μL), a cloudy solution is not well formed, whereas at high volume, the solubility of target analytes in water increases [27]. Thus,

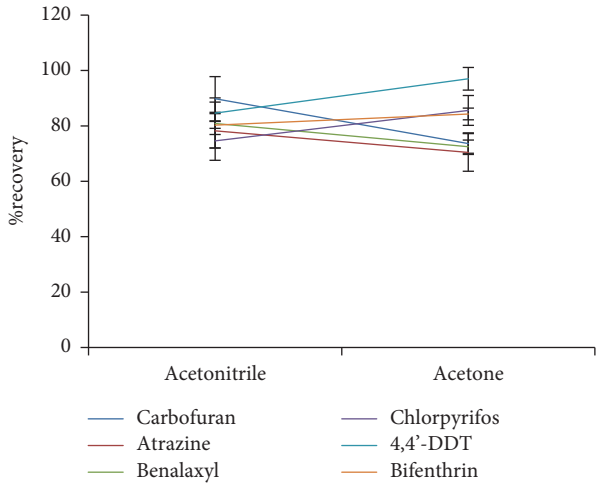


Figure 2: Effect of the extraction solvent on the %recovery of pesticides from the tomato sample. Experimental conditions: 7 g tomato sample; 5000 μL extraction solvent; 2.8 g anhydrous $MgSO_4$; 0.7 g sodium chloride; 300 μg/kg pesticide mixture spiked into 7 g tomato samples; $n = 5$.

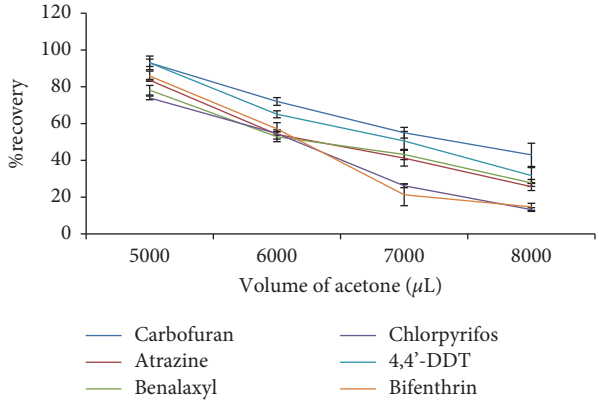


Figure 3: Effect of extraction solvent volume on the %recovery of pesticides from the tomato sample. Experimental conditions: 7 g tomato sample; acetone; 2.8 g anhydrous $MgSO_4$; 0.7 g sodium chloride; 300 μg/kg pesticide mixture spiked into 7 g tomato samples; $n = 5$.

the optimum volume of 1000 μL acetone extract was selected for subsequent experiments.

3.2.2. Effect of the Extraction Solvent. In this study, four common halogenated solvents, $CHCl_3$ (density: 1.49 g/L; water solubility: 8.1 g/L; vapour pressure: 26.2 kPa), C_6H_5Cl (density: 1.11 g/L; water solubility: 0.5 g/L, vapour pressure: 1.58 kPa), CH_2Cl_2 (density: 1.32 g/L; water solubility: 13.8 g/*L*; vapour pressure: 55 kPa), and C_2Cl_4 (density: 1.62 g/L; water solubility: 0.17 g/L; vapour pressure: 2.46 kPa), were investigated. Among the four candidates, residue was not observed after injecting a mixture of acetone extract and dichloromethane. This might have been due to higher solubility of dichloromethane in water and greater volatility than other investigated solvents [28]. As can be seen in Figure 5, the EF (2.8) of carbofuran using

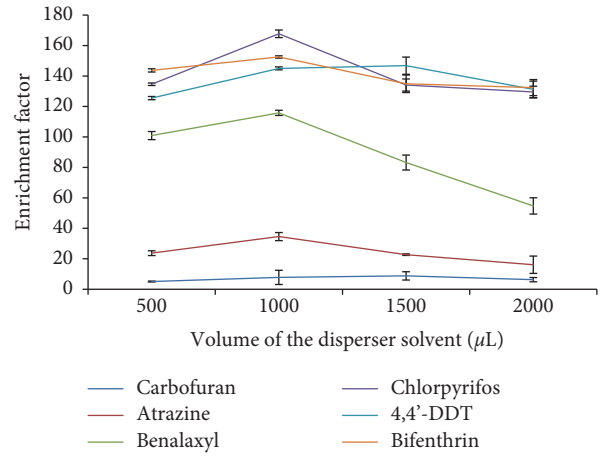


Figure 4: Effect of disperser solvent volume on the enrichment factors of pesticides from the tomato sample. Experimental conditions: 5 mL UHP water; 50 μL tetrachloroethylene; acetone extract (as the disperser solvent); centrifugation rate and time, 4000 rpm and 600 s, respectively; $n = 5$.

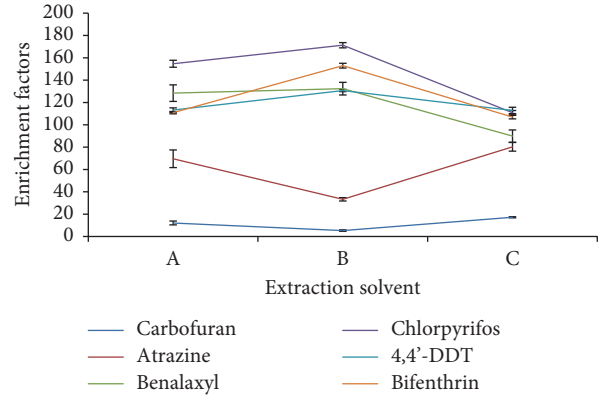


Figure 5: Effect of the extraction solvent on the enrichment factors of pesticides from the tomato sample. Experimental conditions: (A) chlorobenzene, (B) tetrachloroethylene, and (C) chloroform; 5 mL UHP water; 1000 μL acetone extract; 50 μL extraction solvent; centrifugation rate and time, 4000 rpm and 600 s, respectively; $n = 5$.

tetrachloroethylene was very low compared to other solvents which may be due to the higher polarity of carbofuran in comparison to other target analytes [29]. However, tetrachloroethylene gave the highest enrichment factor for other analytes when compared to chloroform and chlorobenzene. Hence, tetrachloroethylene was chosen for subsequent analysis.

3.2.3. Effect of the Extraction Solvent Volume. To study the effect of extraction solvent volume on the enrichment factors of the compounds of interest from the samples, a series of solutions comprising different volumes of tetrachloroethylene from 30 to 90 μL with a fixed volume of the disperser solvent (i.e., 1000 μL) were used. From the results in Figure 6, when the volume of tetrachloroethylene increased

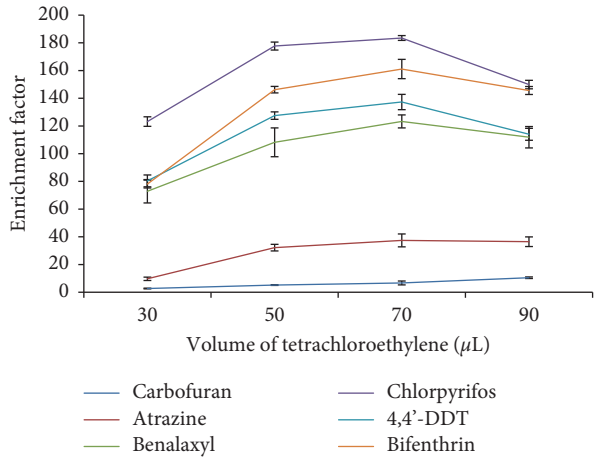


Figure 6: Effect of extraction solvent volume on the enrichment factors of pesticides from the tomato sample. Experimental conditions: 5 mL UHP water; 1000 μL acetone extract; tetrachloroethylene; centrifugation rate and time, 4000 rpm and 600 s, respectively; $n = 5$.

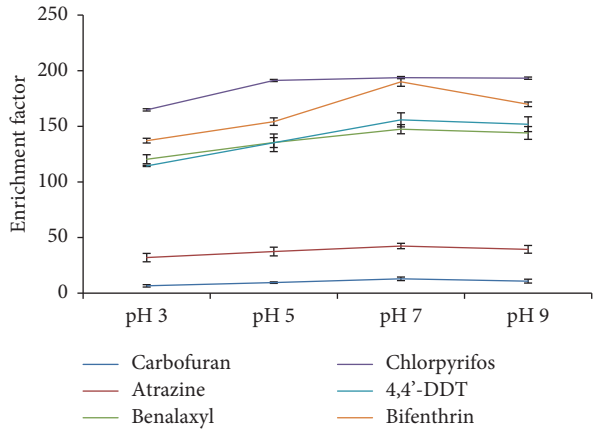


Figure 7: Effect of pH on the enrichment factors of pesticides from the tomato sample. Experimental conditions: 5 mL UHP water; 1000 μL acetone extract; 70 μL tetrachloroethylene; centrifugation rate and time, 4000 rpm and 600 s, respectively; $n = 5$.

from 30 to 70 μL, the EFs of target analytes also increased. However, volumes greater than 70 μL resulted in an insignificant decrease. A decrease of the enrichment factors was observed with higher volumes of tetrachloroethylene which may be associated to the dilution effect, ensuing from the higher volume of the sedimented phase that can be separated after extraction. Then again, at low volume (30 μL) of the extraction solvent, low enrichment factor was observed. This might be due to limited contact surface areas between target analytes and the extraction solvent [30]. Thus, 70 μL was chosen as the optimum volume of the extraction solvent for the subsequent experiments.

3.2.4. Effect of pH. pH is among the key parameters that affects the enrichment factors of analytes. Its effect was investigated by adjusting pH of the aqueous solution using 0.05 M NaOH or 0.05 M HCl. A series of experiments in the pH range of 3–9 were carried out. As shown in Figure 7, high EFs for all the compounds were obtained at pH 7.0. At lower pH, the EFs of target analytes were low, probably due to incomplete mass transfer of target analytes that do not exist in the neutral form to the extraction solvent. Similarly, at higher pH, lower EFs were observed, likely as a result of hydrolysis of the target compounds [31]. Thus, the optimum pH value for the subsequent experiments was 7.

3.2.5. Effect of Salt Addition. Addition of salt to the sample solution has various effects on the enrichment factors of analytes of interest. On the one hand, addition of an appropriate amount of salt to the sample solution increases the enrichment factor of target analytes by reducing the solubility of the analytes in the aqueous phase (the salting-out effect) [32]; on the other hand, when an excess amount of salt is added to the sample solution, the mass transfer of target analytes from the aqueous phase to the extraction solvent decreases because of the increasing viscosity of the solution. In this work, the effect of salt addition on the enrichment factor of compounds was evaluated by adding NaCl from 0.0 to 0.8 g in 0.2 g intervals into UHP water. As shown in Figure 8, when the amount of sodium chloride increased up to 0.6 g, the EFs of target analytes increased, probably due to the salting-out effect (i.e., the lower solubility of target analytes in the aqueous phase would lead to higher partitioning to the extraction solvent). However, the EFs of most target analytes were decreased when a large amount of NaCl (i.e., beyond 0.6 g) was added to the sample solution. This may be due to an increase in the viscosity of the sample solution which significantly affects the movement of pesticides, especially those which have high log K_{ow} (less polar compounds), from the aqueous phase to the extraction solvent [33, 34]. Thus, subsequent experiments were carried out with the addition of 0.6 g NaCl.

3.3. Analytical Features of the Proposed Method. The analytical performance of the proposed analytical method was carried out using fortified blank tomato samples. The main analytical parameters for the validation of the developed analytical method including linearity, limit of detection (LOD), limit of quantification (LOQ), precision (repeatability and reproducibility), and accuracy (recovery) were evaluated.

3.3.1. Calibration Curves and Analytical Performance Characteristics. Calibration curves were constructed using matrix-matched calibration standards, in which a series of ten levels in the concentration ranges of 5.7–1400 μg/kg were fortified into tomato samples, under optimum conditions. Each concentration level was extracted in six replicates. The calibration curve data are shown in Table 2, together with the

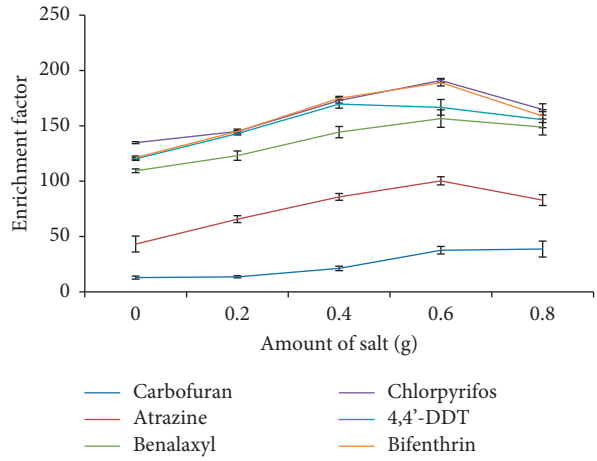


Figure 8: Effect of salt addition on the enrichment factors of pesticides from the tomato sample. Experimental conditions: 5 mL UHP water; 1000 μL acetone extract; 70 μL tetrachloroethylene; pH 7; centrifugation rate and time, 4000 rpm and 600 s, respectively; $n = 5$.

Table 2: Analytical performance characteristics of the proposed method.

Analyte	Calibration curve	Tomato LR (μg/kg)	R^2	LOD (μg/kg)	LOQ (μg/kg)	MRL (μg/kg)
Carbofuran	$Y = 0.1227x + 0.9182$	12.9–1400	0.9981	4.5	12.9	20
Atrazine	$Y = 0.6128x + 2.8833$	5.7–700	0.9979	2.1	5.7	250
Benalaxyl	$Y = 0.7221x - 1.7875$	6.7–700	0.9964	2.5	6.7	200
Chlorpyrifos	$Y = 0.2682x + 1.4994$	12.4–700	0.9985	4.3	12.4	1000
4,4′-DDT	$Y = 0.5503x - 0.2787$	9.2–700	0.9978	3.4	9.2	NA[a]
Bifenthrin	$Y = 0.3755x + 1.57$	6.5–700	0.9973	2.3	6.5	150

[a]Not available.

coefficient of determination (R^2), LODs, LOQs, and MRLs. The calibration curves gave good linearity, at various ranges, with the coefficient of determination equal to or better than 0.9964 for all target analytes. The LOD and LOQ were calculated based on the 3- and 10-time standard deviation of a blank tomato sample extract with the minimum analyte concentration, respectively. The LODs ranged from 2.1 to 4.5 μg/kg, whereas the LOQs ranged from 5.7 to 12.9 μg/kg. Based on the MRLs of tomato set by the European legislation, LOQs of the proposed method were below MRLs set by the EU MRL Database for Pesticides.

3.3.2. Precision Study. Repeatability and reproducibility analyses for the developed method were performed by spiking blank tomato samples at three concentration levels of 60, 200, and 500 μg/kg with five replicates. Repeatability (intraday precision or within a single laboratory) was carried out three times in eight-hour intervals within a day, while reproducibility (interday precision or with different laboratories) was accomplished similarly, except the day of the analysis, within ten days in two-day intervals. The relative standard deviation (%RSD, $n = 5$) of repeatability and reproducibility of precision was 2.9–8.0 and 4.9–9.5, respectively (Table 3). Thus, the %RSD indicated that the developed method is precise.

3.3.3. Recovery. A recovery study was conducted to observe the extraction efficiency of the proposed method. Blank tomato samples were spiked at the three concentration levels (i.e., 60, 200, and 500 μg/kg) in order to check the trueness of the proposed method. Each concentration level was extracted in five replicates. Results in Table 4 reveal that recoveries of the analytes were in the range of 86.8–109.5%, with the RSDs ≤7.6%. Based on the matrix-matched calibration curve, the concentration of each spiked analyte was calculated, and all target analytes were recovered from tomato samples within the acceptable recovery range of 70 to 120% [35].

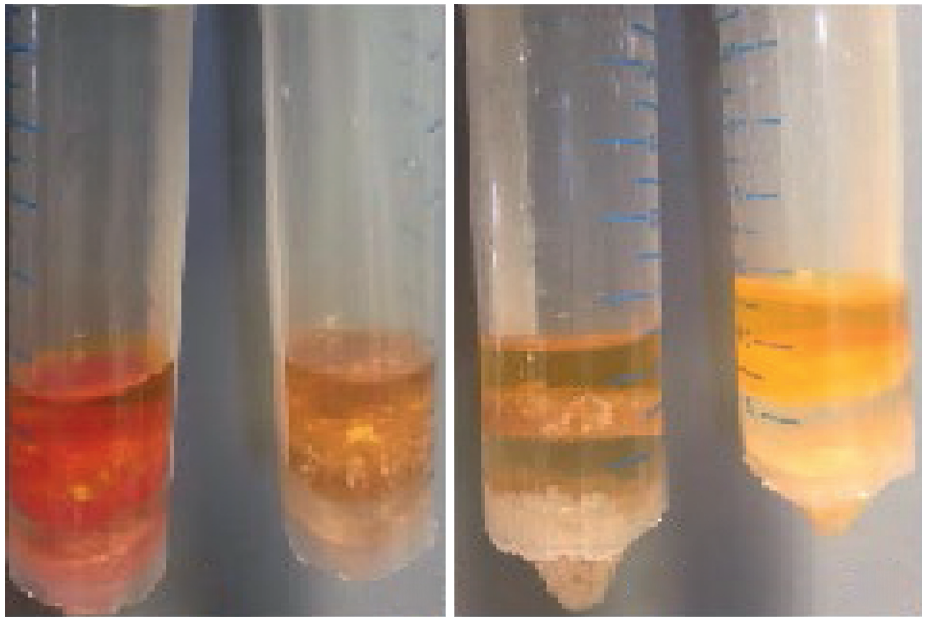
3.3.4. Application of the Method to Real Samples. To evaluate the effectiveness of the optimized and validated method, it was applied for the analysis of twenty samples, namely, fruiting vegetables (tomato) and fruits (apple, melon, and pears). As can be seen in Figure 9, the real samples were treated according to the proposed method of acetone-based salting-out assisted extraction followed by DLLME. The presence of target analytes was investigated by spiking a mixture of target analytes into the representative samples. Next, all the real samples were extracted and analysed at the optimum conditions of the proposed method. However, all of the target analytes were not detected in the studied fruits and vegetables. Probably, the concentrations of target analytes were found in the unquantifiable level.

3.3.5. Selectivity. The selectivity of the developed method was estimated by comparing the chromatograms of the

Table 3: Repeatability (intraday) and reproducibility (interday) of the developed method in spiked tomato samples.

	Carbofuran	Atrazine	Benalaxyl	Chlorpyrifos	4,4′-DDT	Bifenthrin
Tomato sample						
Intraday (%RSD, n = 15)						
Level 1	7.7	6.8	7.1	5.8	4.3	4.2
Level 2	6.8	6.2	2.9	7.3	6.7	7.1
Level 3	6.4	7.1	4.9	6.5	8.0	8.0
Interday (%RSD, n = 25)						
Level 1	7.7	5.9	6.5	7.3	9.0	7.4
Level 2	8.1	6.6	7.8	9.5	4.9	8.2
Level 3	6.9	6.3	7.6	5.7	8.6	5.0

Level 1: 60 μg/kg for atrazine, benalaxyl, chlorpyrifos, 4,4′-DDT, and bifenthrin and 120 μg/kg for carbofuran. Level 2: 200 μg/kg for atrazine, benalaxyl, chlorpyrifos, 4,4′-DDT, and bifenthrin and 400 μg/kg for carbofuran. Level 3: 500 μg/kg for atrazine, benalaxyl, chlorpyrifos, 4,4′-DDT, and bifenthrin and 1000 μg/kg for carbofuran.



(a) (b) (c) (d)

Figure 9: Three layers of extracted vegetable and fruit samples at the sample pretreatment stage. The organic phase at the upper layer of the centrifuge tube: tomato (a), apple (b), pear (c), and melon (d).

unspiked and spiked tomato samples at the concentration level of 400 μg/kg (carbofuran) and 200 μg/kg (atrazine, benalaxyl, chlorpyrifos, 4,4′-DDT, and bifenthrin). The absence of interference at the retention time of the target analytes confirms the selectivity of the proposed method. As can be seen in Figure 10, nontargeted peaks were observed at 210 nm (4,4′-DDT) and 235 nm (benalaxyl) with their corresponding retention time of 3.2 and 1.7 min, respectively. The analysis of the developed method was carried out simultaneously at different wavelengths to acquire ideal peaks, reduce the effect of interference, and quantify properly the compounds of interest.

3.3.6. Comparison of the Proposed Method with Other Methodologies. The most important validated parameters that expressed the performance of the current proposed method are comparable with or better than other works such as solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) [36], microwave-assisted extraction-dispersive solid-phase extraction-retention time locked-gas chromatography-mass spectrometry (MAE-d-SPE-RTL-GC-MS) [37], and acetonitrile-based extraction with dispersive liquid-liquid microextraction followed by gas chromatography-mass spectrometry applied to tomato [20]. Based on the results in Table 5, the current study has many good findings over the

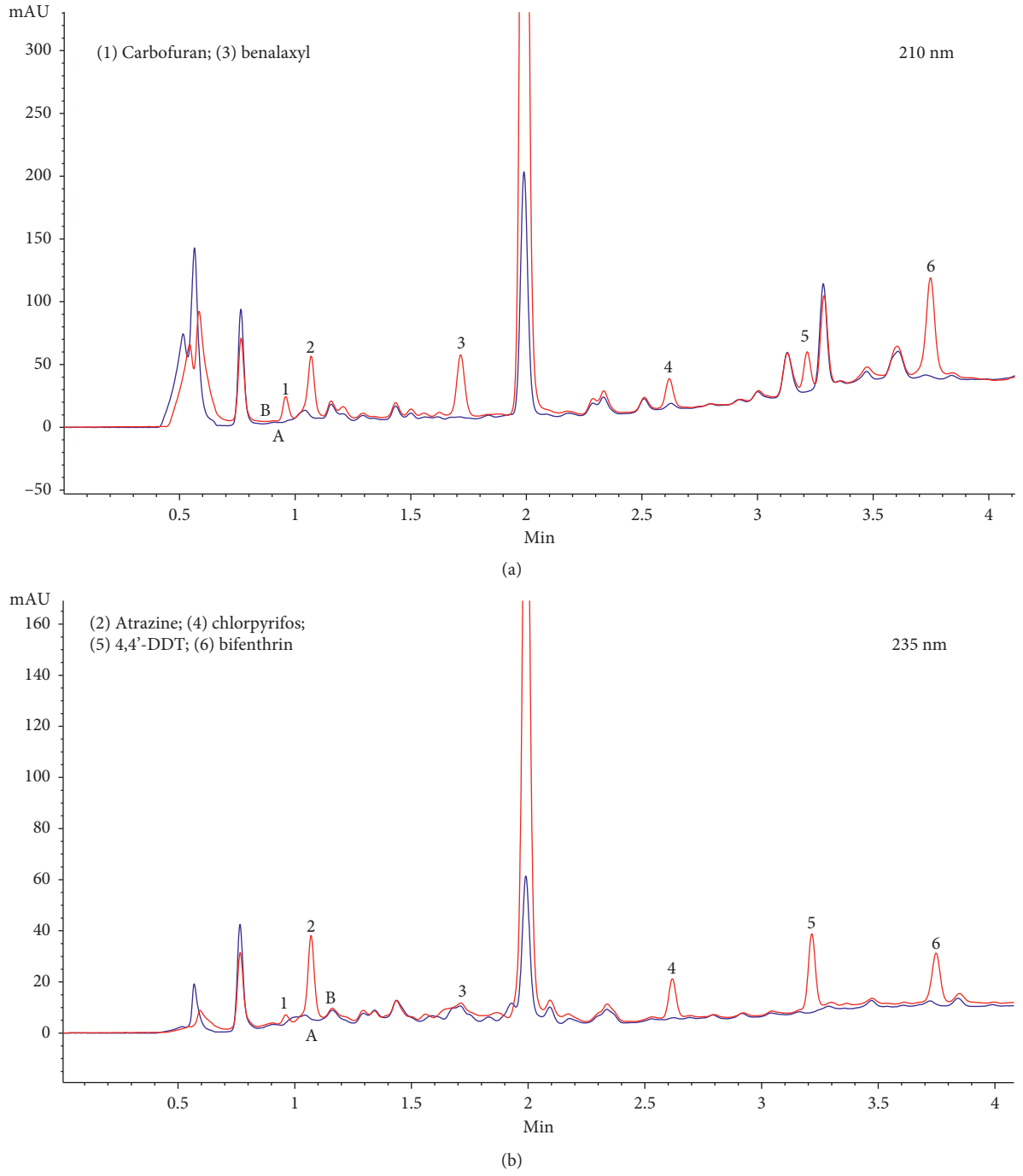


Figure 10: Typical chromatograms at 210 nm and 235 nm. Spiked concentrations were 200 μg/kg for atrazine, benalaxyl, chlorpyrifos, 4,4′-DDT, and bifenthrin and 400 μg/kg for carbofuran. At each wavelength, A and B stand for unspiked and spiked chromatograms, respectively. Peak identification: (1) carbofuran; (2) atrazine; (3) benalaxyl; (4) chlorpyrifos; (5) 4,4′-DDT; (6) bifenthrin.

others, such as LOD lower than the other stated techniques, shorter extraction time, simple extraction procedure, and insignificant difference in %RSD. Besides, it requires lower consumption of the extraction solvent (except the SPME-GC-MS method) and low-cost equipment. Thus, the sample pretreatment step of the current study is simple and fast compared with the reported ones. On the whole, acetone-based salting-out assisted extraction followed by DLLME is an alternative extraction and preconcentration method for the determination of pesticide residues in fruits and vegetables.

Table 5: Comparison of the proposed method with the other methods.

Methods	Analytes	LOD (μg/kg)	%RSD	Extraction time (min)	Extraction solvent volume (μL)	References
SPME-GC-MS	Bifenthrin	3	14	30	200	[36]
MAE-d-SPE-GC-MS	Carbofuran	6	10.9	9	15,000	[37]
Acetonitrile-based DLLME-GC-MS	Atrazine Bifenthrin Chlorpyrifos	6.2 2.6 150	2.2–9.0	NA[a]	10,100	[20]
Acetone-based DLLME-HPLC-DAD	Carbofuran Atrazine Bifenthrin Chlorpyrifos	4.7 2.1 2.3 4.3	4.2–9.5	3	5070	This work

[a]Not available.

4. Conclusions

The proposed method is cost effective, miniaturized, and simple apart from multiclass pesticides which were simultaneously determined (i.e., different polarities) with high enrichment factors (37.6–191) and acceptable precision (2.9–9.5%) in tomato. Generally, the recoveries (86.8–109.5%) and RSDs $\leq$ 7.6% of target analytes were not greatly affected by sample matrices. Therefore, the developed method could work as a quantitative determination of pesticides in tomato. The analysis of the developed method is also carried out using the instrument (HPLC) that is available in many laboratories and affordable to buy by developing countries compared to GC. Moreover, the proposed method also has many advantages: short extraction and run time and also utilized environmentally friendly solvent (acetone). Besides, the LOD of carbofuran, atrazine, bifenthrin, and chlorpyrifos is even lower than the LOD of the same analytes detected by MS (Table 5). The developed method is an efficient alternative that can be successfully applied for the monitoring of multiclass pesticides in vegetable and fruit samples for quality control.

Data Availability

The raw data required to reproduce these findings cannot be shared. The authors need the data to compare the extraction efficiency of the extraction solvent with an ongoing study.

Conflicts of Interest

All the authors declare that they have no conflicts of interest.

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