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Optimization and Efficiency Comparison of Dispersive and Cartridge Solid Phase Extraction Cleanup Techniques in the Analysis of Pesticide Residues in Some Vegetables Using Gas Chromatography-Mass Spectrometry

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Abstract:
This work was conducted to demonstrate the optimization procedures and results for a sample of preparation method combining Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction with cartridge solid phase extraction (c-SPE) cleanup utilized for the analysis of pesticides residues in some vegetables using gas chromatography-mass spectrometry (GC-MS). The method applied for the analysis of four pesticides of different classes; dimethoate (Organophosphorus), fenvalerate (Pyrethroid), difenoconazole (Triazole) and deltamethrin (Pyrethroid) on four types of vegetables (i.e. tomato, potato, cucumber, and carrot). The procedures simply involve the use of acetonitrile containing 1% acetic acid for the extraction, and for cleanup; a manually prepared solid-phase extraction cartridge containing primary secondary amine (PSA) and normal charcoal were used. The validated GC-MS analysis method for the pesticide residues in the selected vegetables has high linearity with R^2 ranged from 0.9965 to 0.9999. The precision of the method estimated as relative standard deviation (%RSD) was ≤ 9.4% for all target pesticides which were indicative of the high repeatability of the optimized method. The accuracy calculated as average recoveries (%R) was between 80.52% and 99.63%. LODs for target pesticides in spiked cucumber, tomato, carrot, and potato samples ranged between 0.0950 and 0.5590 ng/g. The combined sample preparation method is cost-effective and has shown good simplification, recovery and cleanup capacity and proved to be efficient and suitable for the proposed application.

Keywords: QuEChERS, d-SPE, c-SPE, Cleanup, Pesticides, GC-MS.

INTRODUCTION

Pesticides are artificially synthesized compounds produced to fight pests and diseases of plants to increase and improve agricultural products. Although their use has tremendously increased agricultural production in many parts of the world (Galani *et al.,* 2018; Osadebe *et al.,* 2018), their uses have been of concern due to their toxicity and adverse effects on human health. Thus, efforts have to be made to ensure that pesticide contaminations were kept at levels below the maximum residue levels (MRLs). Pesticides are classes of chemical substances either naturally or synthetically made to fight diseases affecting crops (Cabras, 2003; Mahmood *et al.*, 2016; Akhtar *et al.,* 2018). Pesticides are classified into different categories including target organisms, chemical structures, mode of action, and their environmental persistence and pathway of movement into the target organisms. WHO classified them into four classes: extremely dangerous, highly dangerous, moderately dangerous and slightly dangerous (Rajveer *et al.*, 2019). The detection and quantitation of the presence of different classes of pesticides particularly in trace levels in complex matrixes such as vegetables presenting a challenge for scientists (Kataoka *et al.,* 2000; Lambropoulou and Albanis, 2007).

The first step in pesticide analysis of vegetables is the preparation of the sample for analysis, which involves cutting, grinding and blending to form a homogeneous sample structure. Subsampling is then taken for further treatment. This step is important because the success of subsequent steps depends on obtaining a homogeneous sample. Extraction and cleanup are the two steps used to extract the pesticide residue of interest from the matrix and to remove interferences that could compromise pesticide detection and quantitation. As the matrix gets complicated, the cleanup procedure gets more involved to ensure that the instrument performance is not compromised (Huertas-Pérez *et at.,* 2019; Vaclavik *et al.,* 2018). Among sample preparation techniques that have been used in the pesticide cleanup step, some stand out, which include solid-phase extraction (SPE), matrix solid-phase dispersion (MSPD) and solid-phase micro extraction (SPME) which were developed with the aim of simplifying steps. Furthermore, stir bar sorptive extraction (SBSE) has been found to provide low detection limits, especially for hydrophobic analytes. Supercritical fluid extraction (SFE), accelerated solvent extraction (ASE) and microwave-assisted extraction (MAE) (Wilkowska and Biziuk, 2011; Lambropoulou and Albanis, 2007) are also of great value as tools for pesticides sample preparation. The ability of the QuEChERS method to extract various compounds of different chemical classes is a major advantage over traditional methods that are typically capable to extract only one analyte or multiple analytes of the same chemical class (Wilkowska and Biziuk, 2011). Furthermore, proficiency testing employing the QuEChERS method demonstrates that the method is highly robust, and successfully transferred between the participating laboratories (Kaczyński and Łozowicka 2017; Lee *et al.,* 2016). The first and the most significant modifications were developed to expand the method applicability to some pesticides that are ionized and/or degraded during the extraction, depending on the pH of the matrix (Gonzalez-Curbelo *et al.*, 2015). Thus, the first modification proposed for the QuEChERS method was the addition of a buffering step, where the buffering effect (pH 4.8) promoted the addition of sodium acetate and 1% acetic acid in acetonitrile MeCN. This method was adopted in 2007 by the Association of Official Analytical Chemists (AOAC) as an official method for the determination of pesticide residues (Gonzalez-Curbelo *et al.,* 2015; Lehotay *et al.,* 2005; Schenck and Hobbs, 2004; Wilkowska and Biziuk, 2011; Lehotay *et al.,* 2007; Lehotay *et al.*, 2010). To remove matrix components in the clean-up step, modifications of the original d-SPE step by used graphitized carbon black (GCB) and C18 sorbent. QuEChERS offers several advantages over most conventional techniques because it does

not require glassware or auxiliary equipment (e.g. vacuum manifolds), uses low volumes of solvent, generates little solvent waste and provides high recovery of analytes (Seccia *et al.,* 2011; Acebal *et al.,* 2016; Ma *et al.,* 2016).

The main disadvantage of QuEChERS (d-SPE cleanup) is that for 1g sample per milliliter of final extract, the obtained concentration of the extract is usually lower than the concentration that could be obtained by the use of most traditional procedures such as (LLE). Thus, the final extract must be concentrated to a greater extent to furnish the necessary sensitivity and to achieve the desired limits of quantification (LOQ). Despite this drawback, the quantitative results obtained from a large number of pesticides indicate that combination of QuEChERS as a (d-SPE) with hyphenated methods of detection (GC-MS, GC-MS/MS, LC-MS) provides scientists with the capability to achieve efficient and effective monitoring of pesticide residues in food (Lambropoulou and Albanis, 2007).

SPE could be used for different purposes including a sample cleaning and enrichment where the sample passes through adsorbent loaded in a cartridge. The analyte of interest could be adsorbed on the surface of the adsorbent (and is eluted later on) and interferences pass through or vise visa. Various adsorbing materials are commonly available including C18, normal-phase aminopropyl (-NH_2) and primary secondary amine (PSA), anion-exchanger three-methyl ammonium (SAX) and adsorbents such as graphitized carbon black (GCB). The efficiency and selectivity of these adsorbents vary depending on their nature and thus, the physicochemical properties of the analyte under investigation will give guidance to select the appropriate adsorbent (Lambropoulou and Albanis, 2007). In some cases, a user prefers using her/his own adsorbent to suit the intended application. SPE has some attractive features such as the cost-effectiveness in which only a small amount of solvent is needed, and it is easy to use and to authorize. Numerous methods have been published on the analysis of several hundreds of pesticide residues of different types of food and environmental samples using various analytical systems. Each of the published methods has some advantages and limitations which make it successful with few types of commodities and fail with others. This reason drives the continuous development of the sample preparation procedures as the core and the most important step in any analysis method.

The aim of this work was to optimize, validate the sample preparation and apply the gas chromatographic method for the detection and quantitation of pesticide residues in some vegetables.

MATERIALS AND METHODS

All pesticide standard: Dimethoate, 99.6%, Fenvalerate, 98.3%, Difenoconazole, 99.3%, Deltamethrin, 98%) were from Sigma-Aldrich and Fluka/ (Zwijndrecht, The Netherlands). Individual pesticides standard solutions (1000 µg/mL) for all target pesticides were prepared in hexane-acetone (9:1) and kept at (-4 °C) until use (Bozena *et al.,* 2015; Bozena *et al.*, 2016)

All used solvents were HPLC grade. Primary Secondary Amin (PSA 40 mm particle size, Agilent, USA), activated charcoal 15-30 mesh size (Merck, Germany) and C18 (Supelco, USA) were also used as adsorbents.

Gas Chromatography-Mass Spectrometry (GC-MS): GCMS-QP2010 (Shimadzu, Kyoto, Japan) was used in electron ionization (EI) mode. Analytes were separated in a fused silica capillary column DB 5MS (5% phenyl polysiloxane as polar stationary phase), (0.25 mm x 30 m, 0.25 µm film thickness, supplied by Agilent, Palo Alto, CA, USA). GC–MS was equipped with a split/splitless injector and the splitless mode at 250 °C was used. The oven temperature was set initially at 85 °C (2 minutes), and raised to 280 °C at 15 °C min^{-1}

and hold at 280 °C for 10 minutes. The total run time was 25 minutes. The temperatures of the mass detector interface and ion source were set at 280 °C and 200 °C, respectively. Helium gas (99.999%) was used as the carrier gas with a flow rate of 1.29 mL. min^{-1}. The solvent cut of time was set at 4 minutes. Selected ion monitoring (SIM) mode was used in the quantitation step. The optimization of the retention times and chromatographic resolution were done in the scan mode from m/z 50 to 550 at 0.5 sec. per scan.

Blank and Spiked Samples:

Blank vegetable samples of cucumber, tomato, carrot, and potato were collected from organic cultivation sources and used for method development, calibration, and recovery studies. They were first analyzed to ensure the absence of the target pesticide residues. Vegetable samples were chopped into small pieces before mixer blending then homogenized and spiked with suitable amounts of pesticide mixture to levels of 0.01, 0.05, 0.1, 0.5, 1.5 and 2 µg/g and used for calibration and validations study. The spiked samples were properly homogenized and kept overnight before the extraction and cleanup procedures.

Preparation of Solid Phase Extraction Cartridges (c-SPE):

10 mL medical syringes were packed with suitable weights of PSA, activated charcoal and 1 g of anhydrous sodium sulfate was added to the top of each cartridge. Thin discs made up of pre-cleaned medical cotton were inserted at the bottom, top, and between the sorbents layers.

QuEChERS Extraction:

(AOAC Official Method 2007.01) The QuEChERS method was used for sample extraction (AOAC, 2011). 5 g of grounded vegetable and 5mL of H_2O for carrot and potato, and for cucumber and tomato, 10 g of a grounded sample was taken to 50 mL extraction tube. 10 mL of acetonitrile containing 1% acetic acid was added to each wet sample. After a one-minute shake, buffering extraction salts 4 g anhydrous magnesium sulfate, 1 g anhydrous sodium acetate was added. Following another two-minute shake, the sample was centrifuged for 5 minutes at 5000 rpm. Finally, the acetonitrile layer was separated and used for the cleanup procedures.

Cleanup of d-SPE and c-SPE

Dispersive Solid Phase Extraction Cleanup (d-SPE)

8 ml of the supernatant (acetonitrile layer) was transferred to a 15 mL PTFE tube, 500 mg anhydrous magnesium sulfate, suitable amounts of PSA and activated charcoal was added. The extract was shaken for 2 minutes and then centrifuged at 5000 rpm again for 5 minutes. The supernatant layer was filtered through a 0.45 µm syringe filter before analysis.

Cartridge Solid Phase Extraction Cleanup (c-SPE): 8.0 mL of the acetonitrile layer was transferred into an SPE cartridge packed with PSA in the bottom, activated charcoal as a middle layer and anhydrous sodium sulfate on the top, which was formerly conditioned with 5 mL of acetonitrile: toluene (3:1), the conditioned solvent mixture was discarded. After elution with 20 mL of acetonitrile: toluene (3:1), the collected eluents were then evaporated using a rotary evaporator near to dryness before reconstituted to 2 mL using acetone: hexane (1:9).

The resulting final extracts for all matrixes with cleanup by either a d-SPE or c-SPE procedures were analyzed by GC-MS.

Optimization and Efficiency Comparison of the Cleanup Methods

Optimization of Dispersive Solid Phase Cleanup (d-SPE) and (c-SPE) Procedures

For (d-SPE) and (c-SPE) cleanup optimization, the amount of each sorbent was studied to find the optimum amounts of PSA,

charcoal, and C18 for the cleanup of vegetable extracts.

Comparison of Sample Preparation Efficiency Using d-SPE and c-SPE in Vegetable Samples

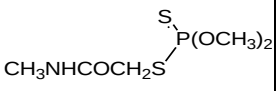
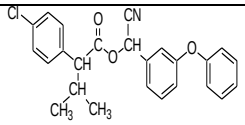
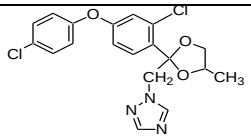
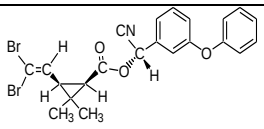
After the optimization of the d-SPE and c-SPE cleanup procedures for selected vegetables, the method of efficiency for the analysis of pesticides residues in the selected types of vegetables using d-SPE and c-SPE was comprised (Maciej, 2019; Michelle *et al.*, 2013; Tomás *et al.*, 2018).

Quantitation

Pesticides were identified according to the retention times, the quantification and three confirmation ions with the assistance of the National Institute for Standards and Testing (NIST,s) and Wily,s libraries (El Shoubaky and Salem, 2014; Lincy *et al.*, 2015). The quantitation was based on the Total Ion Chromatogram (TIC) of peak areas of pesticides. Table 1 summarized the selected pesticides with their quantification and confirmation ions used in SIM mode to analyze dimethoate, fenvalerate, difenoconazole and deltamethrin in cucumber, tomato, carrot, and potato.

RESULTS AND DISCUSSION

Table 1. Name, Chemical Class, Molecular Weight, Elemental Composition, Quantification Confirmation Ions for SIM Conditions and Chemical Structure of the Selected Pesticides.

Pesticide	Class	M. Wt.	Elemental Composition	Quantification Ion(m/z)	Confirmation Ions (m/z)	Chemical Structure
Dimethoate	OPP	229.26	$C_5H_{12}NO_3PS_2$	87	93, 125, 143	
Fenvelarate	Pyrethroid	419.9	$C_{25}H_{22}ClNO_3$	167	169, 181, 225	
Difenoconazole	Triazol	406.3	$C_{19}H_{17}Cl_2N_3O_3$	265	267, 323, 325	
Deltmethrin	Pyrothroid	505.2	$C_{22}H_{19}Br_2NO_3$	251	181, 253, 255	

RT= Retention Time; M. Wt.= Molecular Weight, OPP= Organophosphorouse Pesticide

Optimization of Dispersive Solid Phase Cleanup (d-SPE) Procedures:

The amounts of PSA, C18 and charcoal sorbents were studied to find out the optimum weights that offer the best cleanup efficiency without affecting the method recovery. PSA was used in the amount of 50 mg/mL of the extract according to the (AOAC 2007.01) method (AOAC, 2011), higher amounts of PSA examined (i.e 75 &100 mg per 1mL vegetable extract) did not enhance the cleanup efficiency which could be due to the low contents of carbohydrates, sugars, fatty acids and organic acids in the extract which sufficiently removed by the 50mg of PSA (Nho-Eul *et al.*, 2019). Similarly, the addition of C18 to the cleanup of the tube in 50 mg/ml of the extract was studied. The chromatogram (Figure 1) clearly shows that no improvement in the cleanup efficiency was achieved. This could be due to the low levels of fatty compounds, sterols and non-polar compounds in the tested samples (Abul Kasem *et al.*, 2019) or that interferences could be already removed by PSA.

The amount of carbon-based adsorbent is matrix dependent to obtain its optimum amount per unit volume of each matrix is very important. The use of insufficient quantity will fail in removing the pigments and related interferences which will lead to poor sensitivity and affects the analysis system performance due to the contamination (Beatriz *et al.*, 2001). On the other hand, excessive quantities mostly lead to poor recovery (Sivanandha and Subba, 2017). For the above reason, to realize the optimum amount of charcoal for the cleanup of vegetables extracts with various masses (50, 75, 100, 150 and 200 mg) of charcoal were added to separated cleanup tubes containing 8 mL of 1ppm spiked cucumber extracts, 1200 mg of anhydrous magnesium sulfate and 400 mg of PSA. The tubes were then shaken for 2 minutes and centrifuged for 5 minutes, followed by filtration through a 0.45 µm filter before the injection to the GC-MS system.

As expected, the pigments color intensity was decreased when increasing the charcoal amount as shown in Photo 1. When 50 or 75 mg of charcoal were used, the decreasing of the green color intensity was observed which indicates that the amounts used were not sufficient to remove some of the interferences which could compromise pesticide detection with quantitation as well as the instrument performance (Chai, 2008). However, complete removal of the green pigment was only achieved by using a higher amount of charcoal ≥ 100 mg.

Figure 1 showed that the peak area, and consequently the recovery of the spiked pesticides were highly decreased when using 200 mg of charcoal. This could possibly happen due to the adsorption of some amounts of the pesticides on the charcoal surface. The results also showed that optimum recoveries were obtained when using 100 mg of charcoal for cleanup procedures. Comparable results were obtained when optimizing the charcoal amount needed for the cleanup of tomato, carrot and potato extracts.

Optimization of Cartridge Solid-phase Cleanup (c-SPE) Procedures

In the c-SPE optimization experiment, a similar quantity of PSA per 1mL vegetable extract (i.e 50 mg) was used for d-SPE. Preliminary trials using higher amounts of PSA (i.e 75 and 100 mg per 1mL vegetable extract) were made and consequently, no enhancement in the cleanup efficiency was observed. The 50 mg PSA/1 mL extract was found to be adequate. The optimization of charcoal was carried out to produce the best possible cleanup efficiency without affecting the method recovery. To achieve that, with adjusted flow rate, different amounts of charcoal (100, 200, 300 and 400 mg) and different types of eluting solvents (acetonitrile, toluene, acetonitrile: toluene (1:1), acetonitrile: toluene (3:1)) and volumes (5, 10, 15 and 20 mL) were inspected.

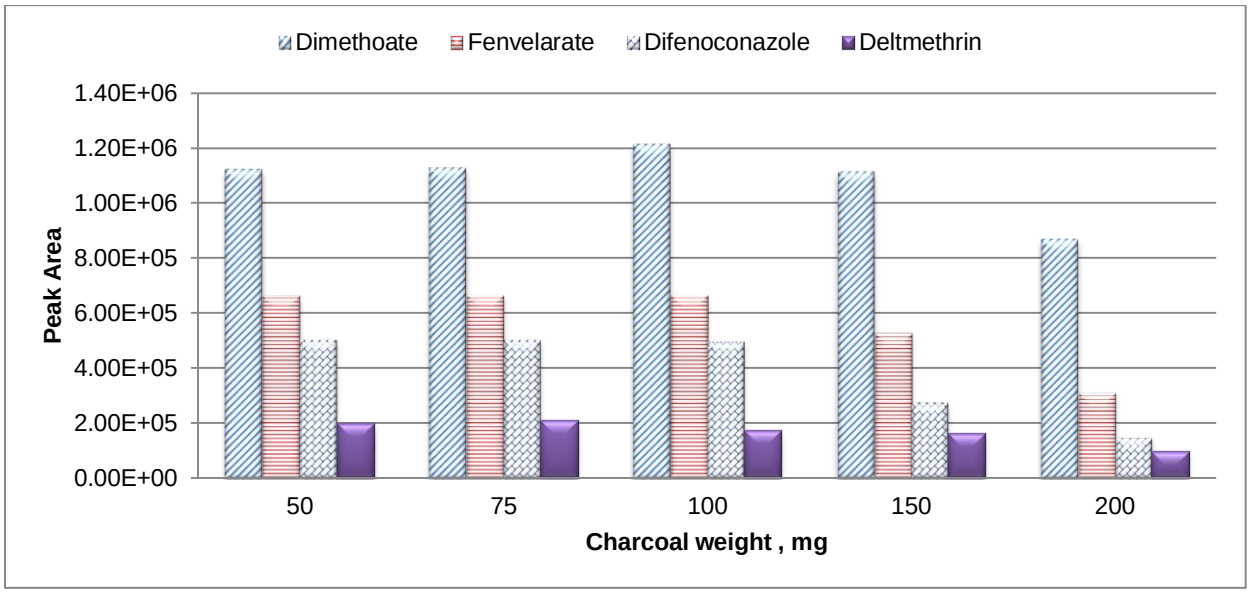


Fig. 1. Effect of Charcoal Weight Used in d-SPE on the Cleanup of 1 ppm Spiked Cucumber Samples.

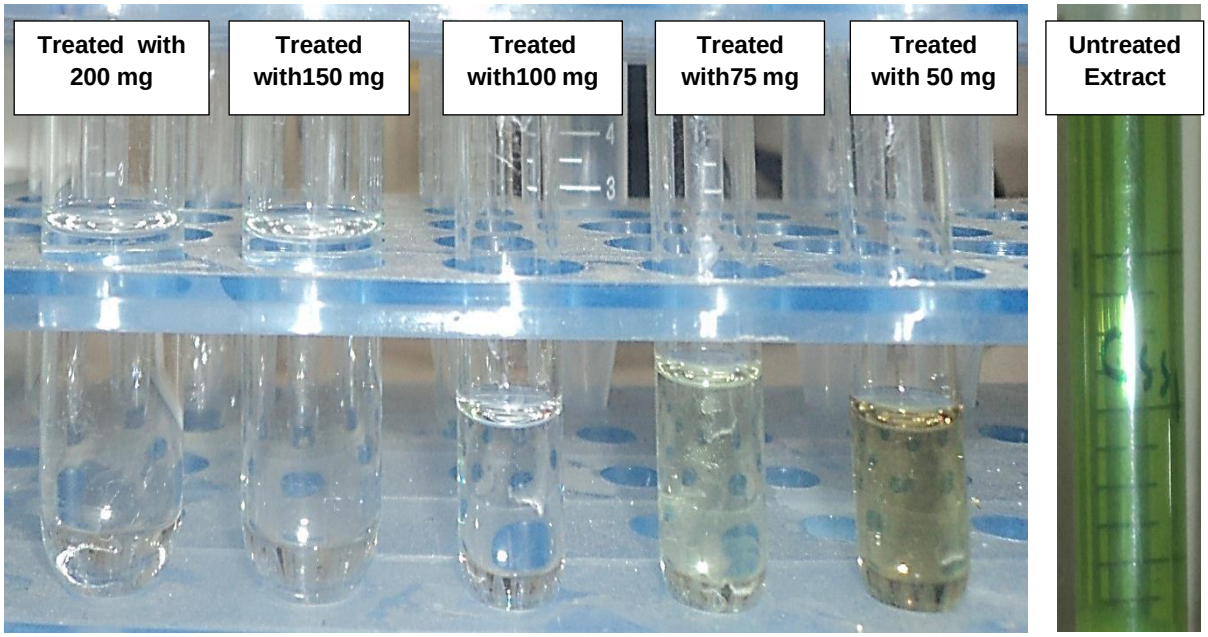


Photo 1. Effect of Charcoal Weights on Pigments Removal from Cucumber Sample Using d-SPE Cleanup. The Masses of Charcoal Varied from 50 mg/8 mL Extract to 200 mg/8 mL Extract.

The cartridges were used to trap the interferences and; hence, the extract after passing through the cartridges were collected along with eluting solvent and together were used in the next step of procedures (i.e. evaporation). The best results were obtained when using 50 mg/mL (i.e 400 mg/8 mL) of charcoal and 20 mL of acetonitrile: toluene (3:1)

as eluting solvent at a flow rate (3 mL/min). The extract was colorless when injected into the GC-MS system (Michelle *et al.*, 2013).

To study the effect of C18 in the enhancement of the cleanup efficiency, a syringe cartridge contained 300 mg of C18 was prepared and conditioned. The cleaned extract obtained from the previous PSA/charcoal cartridge was passed through the prepared C18 cartridge. After concentration and GC-MS analysis, the chromatogram was compared with one cleaned up without C18 cartridge, (Figure 2), the chromatogram clearly showed that no enhancement in the cleanup efficiency was obtained using C18. Therefore, C18 was not used in further experiments. Moreover, for the tested matrixes, the elimination of the C18 use has beneficiary of reducing the overall cost of the cleanup procedures.

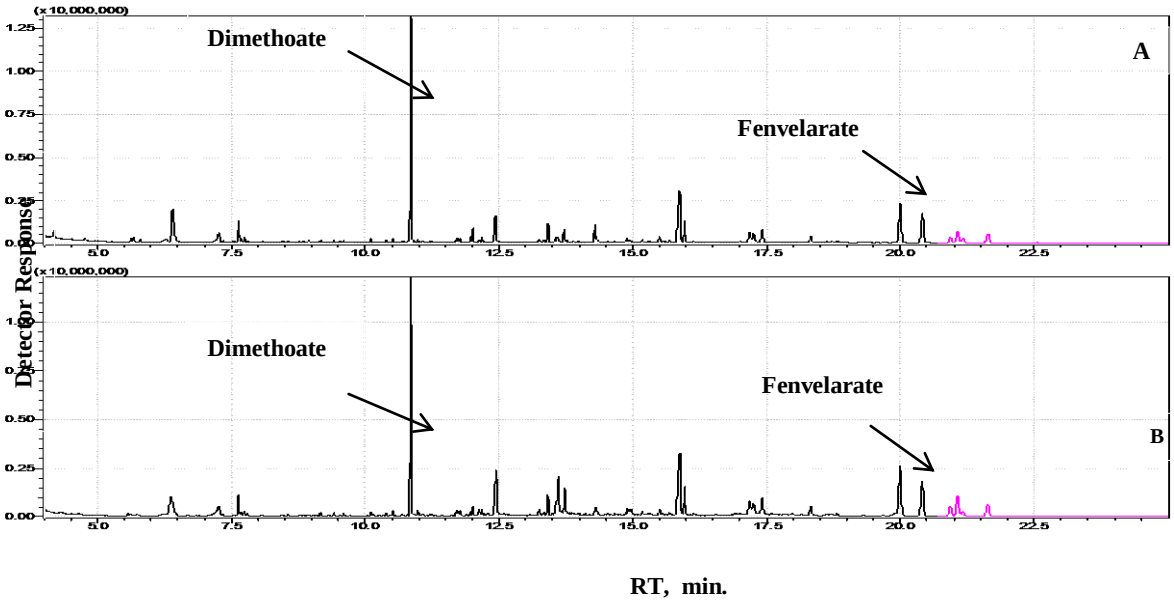


Fig. 2. Chromatogram Showing the Effect of Addition of C18 Cartridge Used as a Sorbent for Cleaning Extract of Cucumber Spiked Sample, A: Without C18, B: With C18.

Optimized Procedures for Cleanup Using (c-SPE) Pressures

As a result of the optimization of the cleanup procedures using c-SPE, the following quantities and steps were used. A 10 mL of the medical syringe was well packed with 400 mg of PSA followed with 400 mg of activated charcoal and finally with 1 g of anhydrous sodium sulfate. The three layers were separated with a thin layer of pre-cleaned and dried cotton as shown in Photo 2. The prepared cartridge was then conditioned with 5 mL of acetonitrile: toluene (3:1) before use. A volume of 8 mL of the sample extract was transferred to the prepared conditioned cartridge followed with 20 mL of acetonitrile: toluene (3:1). The collected eluent was evaporated using a rotary evaporator near to dryness before reconstituted to 2 mL using acetone: hexane (1:9). Then 1 µL of the final clean extract was injected into the GC-MS system.

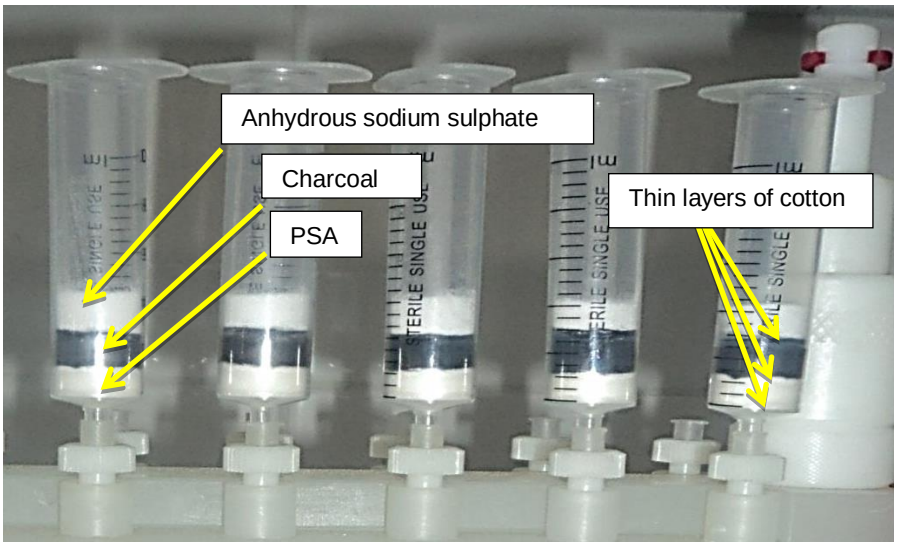


Photo 2. The Components of the Prepared Cleanup SPE Cartridges.

Comparison of Sample Preparation Efficiency Using d-SPE and c-SPE in vegetable Samples

Cleanup efficiency using d-SPE and c-SPE for 1 ppm spiked cucumber, tomato, carrot, and potato samples with pesticide mixture containing dimethoate, fenvalerate, deltamethrin, and difenoconazole were studied.

A volume of 8 mL of each vegetable extract was cleaned up using the optimized d-SPE and optimized c-SPE. The cleaned extracts were then injected into the GC-MS system.

Figure 3 showed the chromatograms of cucumber extract cleaned up using d-SPE and c-SPE. The chromatograms showed that the cleanup efficiency was better in the case of c-SPE as the noises and co-extracts peaks were highly reduced to that of d-SPE. Moreover, the chromatograms indicated that the recovery method would be higher in the case of c-SPE due to the higher peaks of target pesticides observed in the chromatograms.

Figures 4 and 5 showed the comparison of peak areas and the recoveries of both d-SPE and c-SPE for the four pesticides, respectively. The figures clearly show that the peak areas in the case of c-SPE are higher (about 4-times) than that in the case of d-SPE, although the recoveries of both are comparable (Georgakopoulos *et al.,* 2011). This is due to the concentration step in c-SPE as the extract volume was decreased from 8 mL to 2 mL and could also decrease to 0.5 mL which increases the concentration to 16 folds. This cannot be achieved using d-SPE as the intense color appeared when the purified extract concentrated. The use of c-SPE enables the use of higher quantities of adsorbent materials without the fear of losing some of analyte due to the advantages of using suitable solvent mixture that elutes selectively the target analytes and preserve the recovery, sensitivity and accuracy of the method at high values (Tayeb *et al.*, 2015).

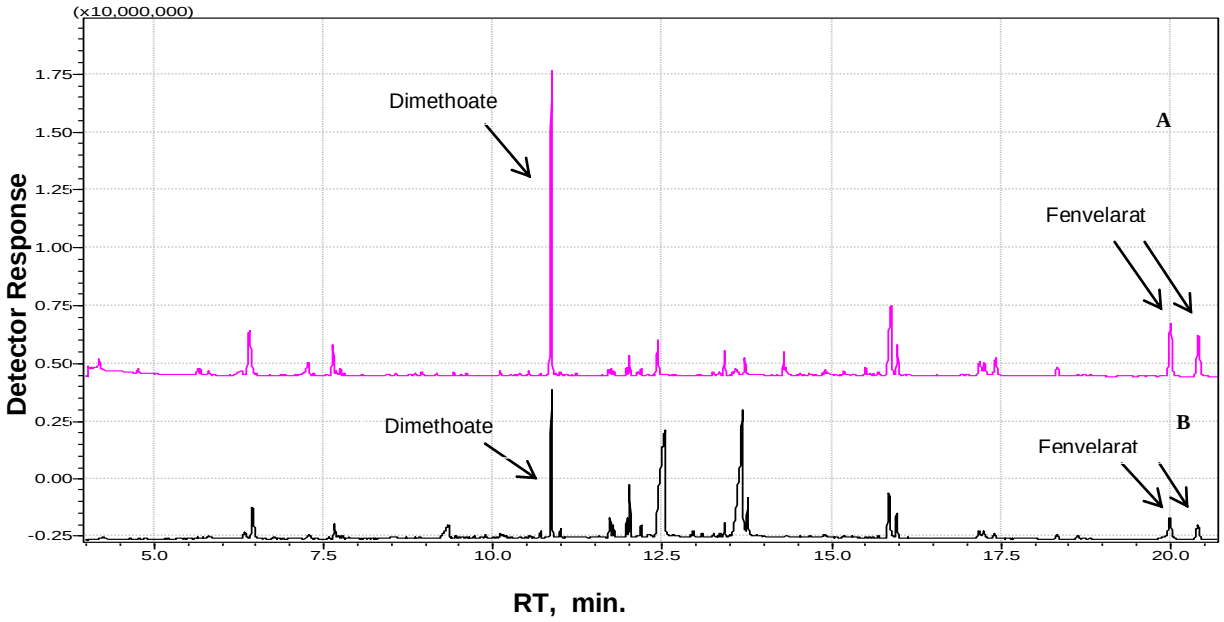


Fig. 3. GC-MS Chromatogram of Cucumber Extract: A: Extract spiked with 1 ppm pesticides mixture. Extract was cleaned via c-SPE procedure using 400 mg PSA, 400mg charcoal, B: extract spiked with 1 ppm pesticides mixture. Extract was cleaned via d-SPE using 100 mg charcoal, 400 mg PSA, eluted with Acetonitrile :Toluene (3:1), Reconstitute in acetone: hexane(1:9).Chromatogram were made in the same scale. Injected volume: 1 µl for c-SPE and d-SPE.

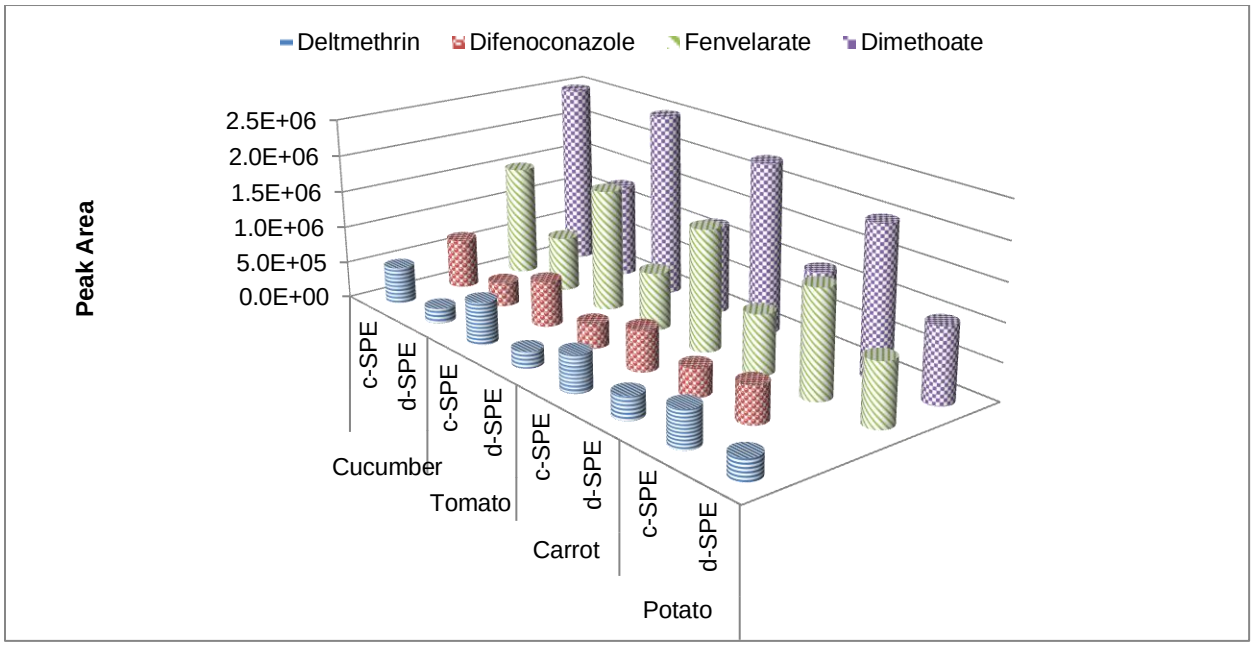


Fig. 4. Sample Preparation Efficiency Using d-SPE and c-SPE for Selected Pesticides in Vegetables Samples.

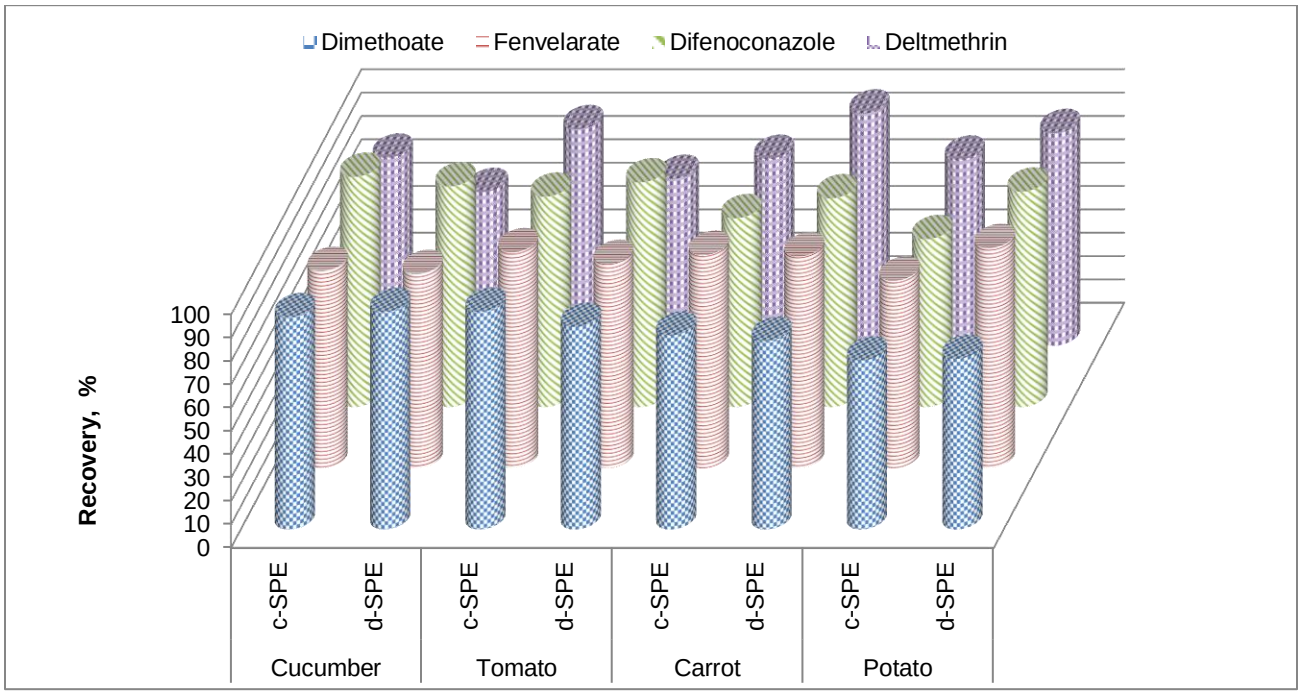


Fig. 5. The Recovery Using d-SPE and c-SPE for Selected Pesticides in Vegetables Samples.

Validation Method

Validation experiments that assess linearity, accuracy, precision and LODs were investigated.

Precision (Repeatability) and Accuracy (Recovery):

The results obtained of repeatability, as % RSD were ≤ 9.4% for all the target pesticides which are indicative of the high repeatability of the developed method and the calculated average recoveries ranged from 80.52 to 99.63% in accordance with the SANTE validation requirements (OECD, 2007) (Table 2).

Linearity:

The blank vegetable samples were spiked with five different concentrations for each pesticide. The detector response was linear over the studied range and the least squares regression analysis of the data provided excellent correlation for all compounds. The R2 values ranged from 0.9964 to 0.9999 for the four vegetables. The results of the correlation coefficient along with the linear regression equation for each pesticide are shown in (Tables 3 and 4).

Limits of Detection (LODs):

The limits of detections for the analyzed pesticides of vegetable samples were calculated from the quantification ion chromatogram of the matrix matching standard as the concentration yields signal to noise (S/N) ratios of 3. LODs for target pesticides in the spiked four vegetable samples ranged between 0.0950 and 0.5590 ng/g. The results are shown in (Table 5).

Table 2. Recoveries (%R) and Relative Standard Deviations (%RSD) for Each Pesticide in Fortified Samples (spiked level 0.5 ppm), n=3

Pesticide	Cucumber		Tomato		Carrot		Potato	
	%R	%RSD	%R	%RSD	%R	%RSD	%R	%RSD
Dimethoate	91.16	6.10	93.51	7.4	89.09	3.62	87.12	6.71
Fenvelarate	84.66	4.30	92.60	5.2	98.73	7.14	88.03	8.22
Difenoconazole	98.62	3.70	90.13	2.2	89.78	5.11	88.97	9.40
Deltamethrin	80.52	6.40	92.72	8.5	86.29	1.70	99.63	5.00

Table 3. Linearity, Calibration Equation and the Correlation Coefficients for Selected Pesticides in Spiked Cucumber and Tomato Matrixes.

Pesticide	Calibration Rang ug/g	Cucumber		Tomato	
		Calibration Equation	R^2	Calibration Equation	R^2
Dimethoate	0.01-1	y = 1.25E+07x + 2.57E+05	0.9994	y = 1.35E+07x - 1.50E+05	0.9997
Fenvelarate	0.01-1	y=7.54E+06x + 4.17E+04	0.9993	y = 7.23E+06x - 3.42E+04	0.9981
Difenoconazole	0.01-1	y = 3.71E+06x - 6.95E+04	0.9999	y = 3.21E+06x - 6.58E+04	0.9994
Deltamethrin	0.01-1	y = 2.53E+06x + 2.35E+03	0.9997	y = 2.74E+06x +4.19E+03	0.9993

Table 4. Linearity, Calibration Equation and the Correlation Coefficients for Selected Pesticides in Spiked Carrot and Potato Matrixes.

Pesticide	Calibration Rang ug/g	Carrot		Potato	
		Calibration Equation	R^2	Calibration Equation	R^2
Dimethoate	0.02-2	y = 1.10E+07x + 3.90E+05	0.9976	y = 1.15E+07x - 8.01E+04	0.9981
Fenvelarate	0.02-2	y = 7.41E+06x - 1.94E+05	0.9979	y = 7.45E+06x + 7.59E+03	0.9973
Difenoconazole	0.02-2	y = 3.90E+06x - 2.09E+0	0.9991	y = 3.58E+06x + 3.42E+04	0.9985
Deltamethrin	0.02-2	y = 2.66E+06x - 8.58E+04	0.9965	y = 2.87E+06x - 5.94E+04	0.9980

Table 5. LODs for Each Pesticide in Fortified Samples (spiked level 0.1 ppm), n=3.

Pesticide	Quantification Ion	LOD ng/g			
		Cucumberer	Tomato	Carrot	Potato
Dimethoate	87	0.4330	0.5250	0.3370	0.3460
Fenvelarate	181	0.2940	0.3208	0.2260	0.1520
Difenoconazole	265	0.3910	0.5590	0.3340	0.0980
Deltamethrin	251	0.1220	0.1514	0.0950	0.1020

CONCLUSION

The authors demonstrated that an optimized QuEChERS extraction with cartridge SPE cleanup procedures in combination with GC-MS of vegetable samples resulted in good recoveries of the four pesticides. The use of c-SPE enables the use of higher quantities of adsorbent materials without the fear of losing some of the analytes due to the advantages of using a suitable solvent mixture that selectively elutes the target analytes and preserves the recovery, sensitivity, and accuracy of the method at high values. The high cleanup efficiency permits further reduction of the eluent volume to enhance the method of sensitivity without affecting the analysis system performance. In addition, the use of c-SPE eliminates the filtration step that was needed to remove the fine particles of the adsorbent materials or contamination as c-SPE possesses built-in filtration features.

Validation of this multi-residues analytical method for the chosen pesticides in the four types of studied vegetables was successfully achieved. The pesticides were selected from different classes that possess a wide range of physicochemical properties to ensure that, the proposed method is valid for application in the analysis of multiclass pesticides.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

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