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ORIGINAL ARTICLE

Trace level determination of insecticide using gas chromatography, and the application for residual monitoring in local Syrian vegetables

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Abstract We have developed a new extraction and purification method for high sensitive determination of four pesticides, Demethoate (Di), Chlorpyrifos-ethyl (CPE), Deltamethrin (Del) and Cypermethrin (Cyp) from vegetables. The method involves the extraction of samples with acetone and Ethylacetate:Hexane (95:5,v/v) mixture, purification using Florisil cartridges at optimum eluting ratio of 5% acetone in hexane, then followed by gas chromatography using electron capture detection (ECD). Under the optimized condition, the recovery of the pesticides from vegetables reach the range of (80–112%) with RSD% $\leqslant$ 5% ($n = 3$), the limit of detection for Di, CPE, Cyp, and Del, were 1, 0.96, 1.3, and 1.9 ng mL^{-1}, and the limit of quantification was 3.3, 2.9, 3.9, and 5.8 ng mL^{-1}, respectively.

This analytical procedure was characterized with high accuracy and acceptable sensitivity to meet the requirements for monitoring pesticides in vegetables.

The method was applied successfully for the determination of pesticides in some local vegetable contamination.

1. Introduction

One of the major challenges for chemist is the development of faster and easier methodologies for characterization and quantification of trace compounds in mixture. A special attention is given to the substances that can compromise food safety, such as pesticide (CODEX ALIMENTARIUS COMMISSION, 1998). In general, the analytical method involves several steps, such as sampling, sample preparation, separation, detection and data analysis. More than 80% of the analysis time is spent on sampling and sample preparation steps that include homogenization of samples, extraction and concentrating the analyte by liquid–liquid partitioning (LLE), followed by clean up of the final extract and then determination using appropriate methodology (Goto et al., 2003). Therefore, it is not an exaggeration to say that the choice of an appropriate sample preparation method greatly influences the reliable and accurate analysis of food.

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Table 1 Molecular formula, molecular weight, and chemical class of the selected pesticides.

Name	Molecular formula	Molecular weight	Chemical class
Dimethoate	$C_5H_{12}NO_3PS_2$	229.30	Phosphorous
Chlorpyrifos-ethyl	$C_9H_{11}Cl_3NO_3PS$	350.62	Phosphorous
Cypermethrin	$C_{22}H_{19}Cl_2NO_3$	416.35	Pyrethroid
Deltamethrin	$C_{22}H_{19}Br_2NO_3$	502.97	Pyrethroid

Some of these methods, (Lee et al., 1991), involve the use of solid-phase extraction cartridges (SPEC), with acetonitrile for the extraction of pesticide residues from fruits and vegetables. Leoni and his group (Leoni et al., 1992) have determined twenty-eight phosphorous (OP) insecticides utilizing a gas chromatography analysis technique (GC) after acetone and benzene mixture extraction and silica catridges cleaning up. In other work (Yamazaki and Ninomiya, 1999), forty-eight OP insecticides, including chlorpyrifos, methidathion and methyl parathion, were extracted with Methanol:Dichloromethane (1:9), followed by cleaned up step using solid phase excretion (SPE) with gel permeation chromatography and silica gel mini columns. Riediker et al. (2002), described a simultaneous analytical method of pesticides, by direct injection of food extract into an online SPE using a strong cation-exchange resin.

The two main methods which have been employed recently for the determination of polar and less-polar pesticides in non-fatty food samples are the European Norm DIN 12393 (1993) method (EN 12393, 1998), and the QuEChеRS method (Anastassiades and Lehotay, 2003; Díez et al., 2006; Lesueur et al., 2007). The main steps involved in these two methods are crops extraction: LLE, SPE followed by instrumental detection. The LLE step involved the implementation of various kinds of solvent extraction with recovery in both methods not exceeding 80% in some crops.

The present work reports a simplified, sensitive and accurate method for extraction, LLE, SPE and then GC determination for four pesticides. The pesticides chosen belonged to two groups of phosphorous and pyrethroid. Namely, the pesticides are Demethoate (Di), Chlorpyrifos-ethyl (CPE), Deltamethrin (Del) and Cypermethrin (Cyp), that were selected as the model compounds due to their residues found in the local vegetables. Table 1 shows the properties of the selected pesticides. The developed procedure is applied for the analysis of vegetable samples taken locally such as Cucumber, Tomato, Squash, and Eggplant.

2. Experimental

2.1. Apparatus

A Shimadzu GC version 2010 gas chromatography with an electron capture detector (ECD) was used. A trb-1, 30 m × 0.32 mm i.d. capillary column with a 0.25 μm film thickness was used. The temperature program: initial temperature of 120 °C held for 1 min, 8 °C/min ramp to the final temperature at 250 °C, held for 2.5 min. The injector temperature was at 250 °C and the detector temperature was maintained at 300 °C. Nitrogen gas (99.999%) was used as the carrier gas with a flow rate of 6 ml/min at a pressure of 105 kPa., the injection volume of 1 μl was used in a splitless mode.

2.2. Reagents and chemicals

Reagent-grade chemicals were of the highest purity available from their sources. Methanol, acetone, ethyl acetate, acetonitrile, hexane, dichloromethane and diethyl ether were purchased from the Merck Company. Anhydrous sodium sulfate and sodium chloride were purchased from J. T. Baker. The pesticide standards of Dimethoate (Di), Chlorpyrifos-ethyl (CPE), Cypermethrin (Cyp), and Deltamethrin (Del), were of 90.0–99.5% purity and purchased from Accu Standard Inc. New Haven, CT, USA.

A stock solution pesticide of 1000 μg mL^{-1} was prepared in acetone. Working solutions of each pesticide were prepared daily by diluting the stock solution of corresponding pesticide

Table 2 Average recovery of the pesticides Di, CPE, Cyp, and Del, from the vegetables with the concentration of 0.05, 0.1, and 1 ppm ($n = 3$), using the: Acetone as the extraction solvent.

Substance	Concentration (ppm)	Average recovery% ± RSD			
		Di	CPE	Cyp	Del
Cucumber					
	0.05	87 ± 3	80 ± 5	93 ± 5	91 ± 4
	0.1	90 ± 2	84 ± 4	89 ± 2	90 ± 2
	1	92 ± 4	82 ± 3	91 ± 4	87 ± 5
Tomato					
	0.05	94 ± 3	85 ± 3	94 ± 3	83 ± 5
	0.1	92 ± 4	88 ± 2	97 ± 4	85 ± 3
	1	96 ± 5	87 ± 4	91 ± 2	87 ± 2
Squash					
	0.05	95 ± 5	84 ± 5	92 ± 1	86 ± 5
	0.1	92 ± 3	82 ± 4	87 ± 4	84 ± 4
	1	93 ± 4	86 ± 2	89 ± 3	85 ± 3
Eggplant					
	0.05	90 ± 4	80 ± 3	82 ± 4	84 ± 3
	0.1	92 ± 5	82 ± 3	84 ± 5	82 ± 4
	1	94 ± 2	84 ± 4	81 ± 1	81 ± 5

Table 3 Average recovery of the pesticides Di, CPE, Cyp, and Del from the vegetables with the concentration of 0.05, 0.1, and 1 ppm ($n = 3$), using the acetonitrile as the extraction solvent.

Substance	Concentration (ppm)	Average recovery% ± RSD ($n = 3$)			
		Di	CPE	Cyp	Del
Cucumber					
	0.05	62 ± 6	64 ± 9	60 ± 5	62 ± 4
	0.1	65 ± 11	56 ± 3	66 ± 9	59 ± 7
	1	68 ± 3	62 ± 4	70 ± 3	61 ± 9
Tomato					
	0.05	61 ± 3	56 ± 7	68 ± 5	63 ± 3
	0.1	66 ± 8	64 ± 3	70 ± 4	64 ± 5
	1	71 ± 6	66 ± 8	72 ± 3	66 ± 1
Squash					
	0.05	66 ± 12	62 ± 5	69 ± 9	60 ± 6
	0.1	67 ± 9	65 ± 8	65 ± 3	64 ± 4
	1	70 ± 6	68 ± 3	71 ± 6	67 ± 8
Eggplant					
	0.05	61 ± 8	60 ± 8	66 ± 8	56 ± 4
	0.1	68 ± 5	69 ± 5	58 ± 4	60 ± 9
	1	70 ± 7	65 ± 7	62 ± 6	62 ± 8

to give a concentration within the range of 0.01–0.5 µg mL^{-1}. An internal standard of 1-Chloro-4-fluorobenzene (1 mg/kg) was used.

2.3. *Pesticide extraction from vegetable sample*

A preliminary study was carried out to optimize the extraction procedures, by trying three different solvents: Acetone, acetonitrile, and ethyl acetate (Seddik et al., 2010, 2012).

Vegetables of cucumber, tomato, squash, and eggplant were collected from Maraet Alnouman areas (Edlab, Syria), that are known to be contaminated with the studied pesticides. The same vegetables were provided from local market for analytical comparison. Vegetable sample of 1 kg weight was cut into pieces and mixed properly for solvent extraction. The extraction process was made on 10 g of sample with 100 mL extracting solvent. A proper quantity of standard solutions of indicated pesticide was spiked to give 0.0 (blank), 0.05, 0.10 and 0.50 mg/kg for each compound, the mixture was then stirred to homogenize for 5 min. The resulting mixture was then filtrated using 5A (541) filter paper. The extraction and filtration processes were repeated on the residue with 50 mL of extraction solvent. The resulting filtrates were then mixed and evaporated using rotary evaporator with a temperature

Table 4 Average recovery of the pesticides Di, CPE, Cyp, and Del, from the vegetables with the concentration of 0.05, 0.1 and 1 ppm ($n = 3$), using the ethylacetate as the extraction solvent.

Substance	Concentration (ppm)	Average recovery% ± RSD ($n = 3$)			
		Di	CPE	Cyp	Del
Cucumber					
	0.05	74 ± 5	68 ± 8	83 ± 8	69 ± 11
	0.1	76 ± 8	65 ± 4	85 ± 6	76 ± 8
	1	82 ± 6	72 ± 7	81 ± 5	75 ± 3
Tomato					
	0.05	79 ± 7	78 ± 5	81 ± 3	77 ± 10
	0.1	81 ± 9	72 ± 8	82 ± 8	81 ± 7
	1	83 ± 8	77 ± 6	84 ± 4	79 ± 8
Squash					
	0.05	79 ± 8	71 ± 3	81 ± 6	73 ± 6
	0.1	80 ± 4	74 ± 5	81 ± 4	69 ± 8
	1	83 ± 7	76 ± 9	84 ± 9	75 ± 4
Eggplant					
	0.05	82 ± 3	72 ± 5	84 ± 5	69 ± 6
	0.1	78 ± 6	72 ± 8	79 ± 8	73 ± 4
	1	80 ± 9	74 ± 7	81 ± 3	71 ± 8

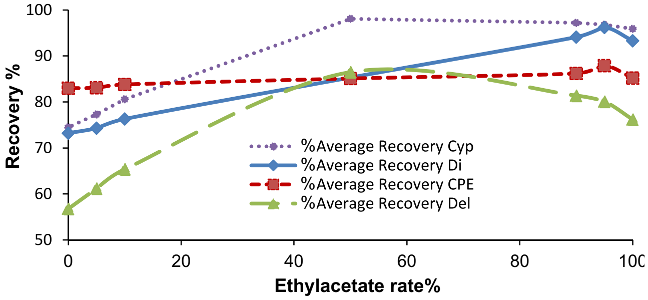

Figure 1 Effect of the mixture ratio (Ethylacetate:Hexane) used at the LLE process at the recovery ratio of the pesticides (Di, CPE, Cyp, Del) in Tomato samples extraction by acetone.

less than 40 °C to 50 mL volume. The resulting mixture was then mixed with 100 mL NaCl 10% (w/v) and subjected to purification extraction process with 20 mL × 5 batches (100 mL the final volume) of various Ethylacetate:Hexane ratio. The organic extracted phase was then filtered on 5A (541) filter paper after moisture absorption using 10 g of anhydrous sodium sulfate. The filtrate was then evaporated to dryness and the residue dissolved again with acetone using Pasteur pipe and the volume was made to 10 mL using the same solvent to give the Pesticide Extract (PE).

2.4. Solid-phase extraction (SPE) and cleanup

Solid-phase extraction (SPE) was performed using an LC-Silica Gel (100–200 mesh), or LC-Florisil (magnesium silicate, 100–200 mesh) provided from Supelco. The extraction columns were washed with 6 ml of ethyl acetate and conditioned by passing 2 ml of methanol, followed by 2 ml of deionised water. The cartage sorbent was never allowed to dry during the conditioning and sample loading steps. Sample loading was 5 mL of the PE volume, and performed under a vacuum in a flow rate of 5 ml/min. The pesticides were eluted from cartage with three × 2 ml portions of the eluting solvent. The eluates were collected in a 15 ml tube under gravity flow. The eluate evaporated to dryness, the residue dissolved with acetone, spiked with 100 μL internal standard, the final volume was made to 5 mL, and 1 μL of this solution was injected into the GC–ECD for analysis.

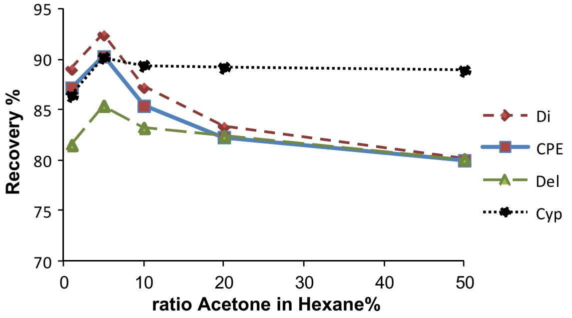

Figure 2 Effect of acetone ratio in hexane as an eluting solvent in SPE process for the recovery average of the pesticides (Di, CPE, Cyp, Del) from Tomato samples.

2.5. GC calibration curve

Proper volumes of the target pesticides' working solution were transferred to the volumetric flask to cover a concentration range of 0.01–0.50 mg L^{-1}. Three standard extractions were made for each concentration levels of the mixture solution. The calibration graph was plotted by the ratio (S/Sa) of the peak area of the analyte (S) to the peak area of the internal standard (Sa); versus the concentration of the analyte. Calibra-

Table 5 Average recovery and RSD% of pesticides from Tomato samples using different purification methods for Di, CPE, Cyp, and Del at various concentrations (n = 3).

Substance	Concentration (ppm)	Average recovery% ± RSD (n = 3)		
		No purification	Florisil	Silica
Di				
	0.5	96 ± 3	88 ± 5	84 ± 5
	0.1	98 ± 3	86 ± 2	82 ± 4
	0.05	96 ± 4	85 ± 4	83 ± 3
CPE				
	0.5	87 ± 5	80 ± 4	78 ± 5
	0.1	86 ± 5	78 ± 3	77 ± 2
	0.05	84 ± 3	82 ± 2	76 ± 4
Cyp				
	0.5	95 ± 2	87 ± 2	83 ± 5
	0.1	96 ± 3	89 ± 3	85 ± 3
	0.05	94 ± 4	86 ± 5	81 ± 5
Del				
	0.5	87 ± 3	81 ± 2	79 ± 3
	0.1	88 ± 2	82 ± 3	81 ± 3
	0.05	86 ± 5	79 ± 4	76 ± 5

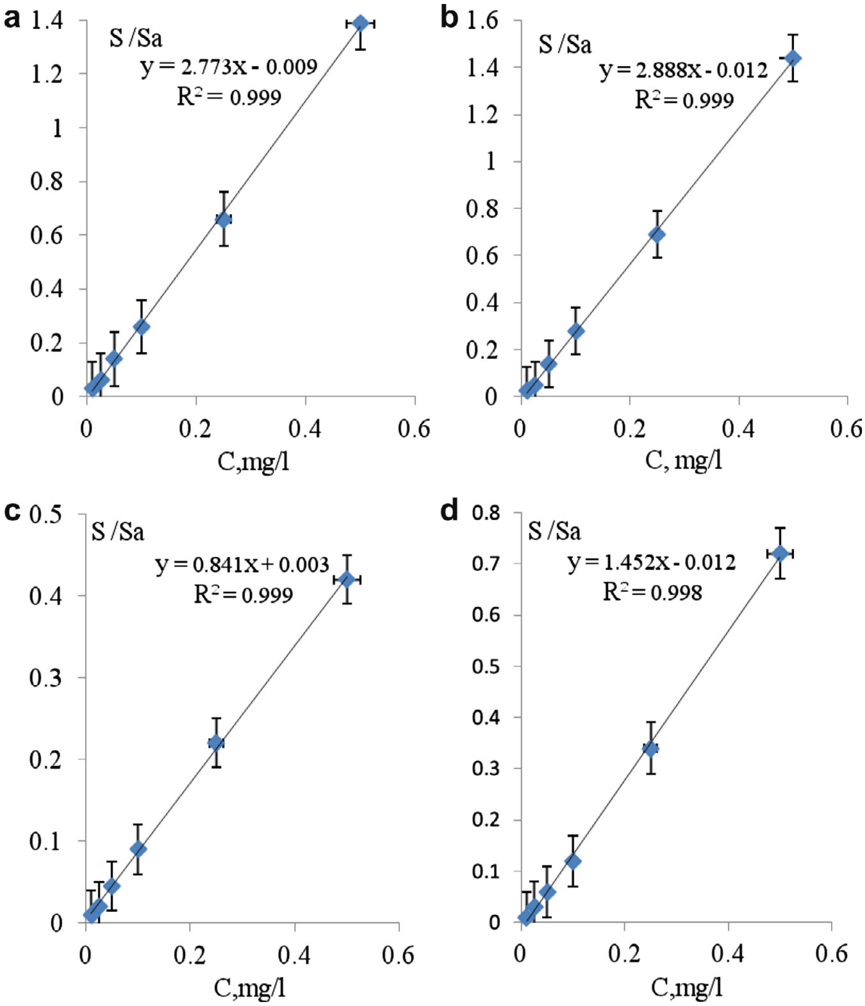

Figure 3 The constructed calibration curves for (a) Dimethoate, (b) Chlorpyrifos-ethyl, (c) Deltamethrin, and (d) Cypermethrin in tomato using 1-Chloro-4-fluorobenzene as an internal standard.

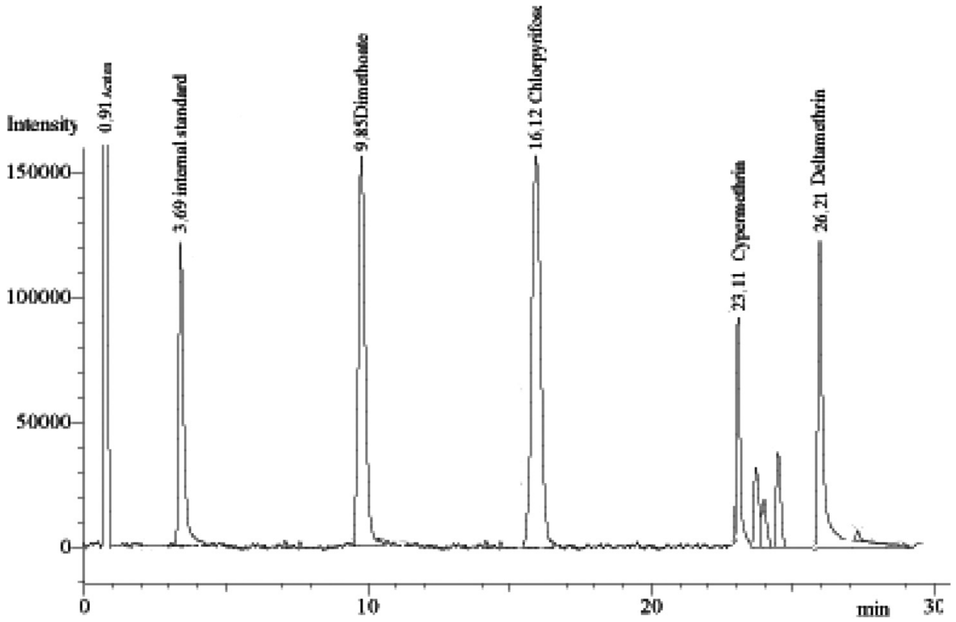

Figure 4 Chromatogram on the recovery of tomato spiked at 0.05 mg/kg of (1) internal standard, (2) Di, (3) CPE, (4) Cyp, and (5) Del.

Table 6 Residual effect of the pesticides Di, CPE, Cyp, and Del on samples (obtained) from bazaars and farms of the Maaret Numan area.

Sample	Number of analyst samples	Number of analyst samples	Residual effect of the pesticides (ppm)	(MRL)
Exported from Jordan				
Cucumber	5	1	Del 0.37	Del 0.5
Tomato	8	2	Di 0.07	Di 0.5
Squash	6	3	Di[a] 1.55	Di 0.5
			Del[a] 1.01,1.23	Del 0.5
Potato	2	1	Cyp[a] 0.83	0.05
Local from Dir shargi				
Cucumber	5	1	CPE[a] 0.13	0
Tomato	9	2	Di[a] 0.73, Del 0.43	Di 0.5 Del 0.5
Squash	7	2	Di 0.31, Del[a] 0.91	Di 0.5 Del 0.5
Eggplant	3	1	Di[a] 0.81	Di 0.5
Paper	5	0	—	—
Spinach	2	1	Del[a] 0.61	Del 0.1
Cabbage	1	0	—	—
Potato	3	1	Cyp 0.028	Cyp 0.05
Local from Babela				
Cucumber	2	0	—	—
Tomato	5	1	CPE[a] 1.12	0
Squash	2	1	Del 0.017	Del 0.05
Eggplant	2	1	Di[a] 0.97	Di 0.5
Spinach	3	1	Del 0.037	Del 0.1
Potato	2	0	—	—
Local from Maarshimaren				
Squash	2	2	Di 0.22, Del 0.041	Di 0.5 Del 0.5
Spinach	1	1	Del 0.039	Del 0.1
Cabbage	1	0	—	—
Cucumber	1	1	Di 0.41	Di 0.5
Pepper	1	0	—	—
Local from Masaran				
Cucumber	2	2	Di 0.36	Di 0.5
Tomato	3	1	Di 0.36	Di 0.5
Squash	1	0	—	—
Eggplant	2	1	Di 0.18	Di 0.5
Spinach	2	1	Cyp[a] 0.68	Cyp 0.1
Cabbage	2	0	—	—
Potato	2	1	Del 0.011	Del 0.05
Local from Jarjanaz				
Cucumber	6	2	Cyp 0.42,CPE[a] 0.42	Cyp 0.5 CPE 0
Tomato	8	0	—	—
Squash	2	0	—	—
Spinach	2	1	Del 0.048	Del 0.1

— The samples free of pesticides (under the limits of detection 0.001 ppm).

* Higher than acceptable limits.

tion lines were used for quantification in subsequent experiments.

3. Results and discussion

3.1. The choice of extraction solvent

Solvents such as Acetone, Acetonitrile, and Ethylacetate were used to prepare pesticide extract (PE) directly from vegetables. Tables 2–4 summarize the average recovery of four pesticides studied (Di, CPE, Cyp, and Del) from Acetone, Acetonitrile, and Ethylacetate, respectively. The results indicate that the average recovery using acetonitrile was not exceeding 72 ± 3 with Cyp from tomato. Better recovery was shown with Ethylacetate with ratio not more than 85 ± 6 with Cyp from Cucumber, but some recovery rate was still less than 80%. Whereas, using acetone the recovery was within the range of 80–97%, that indicating the best solvent used in this work for PE process was Acetone.

3.2. Purification extraction process

It was found, Fig. 1, that using hexane alone as a solvent for the extraction process resulted in low recovery with less than 80% for Di, Cyp, and Del. On the other side, using Ethylacetate alone shows increases with recovery to values exceeding 80% for Di, CPE, and Cyp. Varying the mixing ratio of Ethylacetate:Hexane, indicates that the ratio of 95:5 shows recovery exceeding 80% for the four pesticides from tomato.

The solvent mixture Ethylacetate:Hexane ratio of 95:5 (v:v) was used as the extraction solvent for further finding in this work.

3.3. Solid-phase extraction (SPE) and cleanup

The cleanup process has been done by using the solid phase by cartridges filled with either Florisil or silicagel, and determining the recovery average of each pesticide (Di, CPE, Del, Cyp) from Tomato samples. Table 5 shows that the cleanup process may result in a decrease of the recovery average for all of the studied pesticides. Despite that, the cleanup procedure was employed using, preferably, the Florisil cartage, only if required to inhibit the destruction of GC column.

Varying the eluting solvent mixture using Hexane:Acetone, Fig. 2, resulted in higher recovery values at 95:5 (v:v). As a try, replacing the acetone with more polar solvents, using Hexane:Methanol mixture resulted in high GC peaks interference (chromatographic resolution between peaks less than 1.2). Whereas, decreasing the polarity using Hexane:Ether, resulted in a less recovery ratio, that was not exceeding 80% with the four pesticides.

3.4. Method validation

The method shows linearity for the four pesticides in the same mixture, with no interference. Linear equations are presented in Fig. 3, showing high correlation coefficient with values more than 0.998 ($n = 3$). The limit of detection for Di, CPE, Cyp, and Del, were 1, 0.96, 1, 3, and 1.9 ng mL^{-1}, respectively. Calculating the quantification limit for the pesticides, that was calculated as three times the standard deviation of the blank divided by the slope of the respective calibration graph (Miller and Miller, 1993), indicating values not exceeding 3 ng mL^{-1}.

The precision was assessed according to the IUPAC recommendations by analyzing 0.01, 0.025, 0.05, 0.1, 0.25, and 0.5 μg mL^{-1}, the recovery of the pesticides from vegetables was within the range (80–112%) with RSD% $\leqslant$ 5% ($n = 3$). The Student's t-test values (the tabulated t-value for the 95% confidence level and $n = 3$ is 4.303 (Miller and Miller, 1993)). The t-test could not detect any systematic error and proved accuracy of the proposed method.

The chromatogram on the recovery of tomato spiked at 0.05 mg/kg is indicated in Fig. 4.

4. Application

4.1. The study of residual effect to the pesticides

The analytical results for the determination of the trace level of Di, CPE, Del, and Cyp pesticide residuals in 110 various vegetable samples that were obtained from bazaars, or known farms of the Maraet Al noman area (Idlb, Syria) are summarized in Table 6. The indicated results show that high percentage (29%) of total vegetable samples was polluted with one or two of the studied pesticides. From these polluted samples there were thirty samples contaminated above the maximum regulated level (MRL), of which three samples contain CPE, four samples contain Di, two samples contain Cyp, and four samples contain Del. The contaminated samples indicate high levels of various pesticides as pollutants, which demand more attention in the region.

5. Conclusion

The use of gas chromatography in the existence of specific detector ECD for determining the studied pesticides was a good choice. The extraction method for Di, CPE, Del, and Cyp was also suitable to implement due to the simplicity, ease, safe, and low cost method. The method involved three steps that include acetone extraction from vegetable, purification extraction process, followed by solid phase clean up process. The method validation proved the accuracy and precision for the routine application of low level pesticides determination.

The high concentration level of pesticides found in vegetables that have been analyzed, also emphasizes the environmental awareness in the region, lowering the impact on human health and environment.

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