RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Modification of QuEChERS for extraction 15 pesticides in Egyptian fish and their analysis using GC/MS

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Received on: 20. 10. 2020
Revised on: 23. 11. 2020
Accepted on: 29. 11. 2020

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Abstract

A Quick, Easy, Cheap, Effective, Rugged and Safe method (QuEChERS) was modified to extract 15 pesticides, which are the most used in Egypt, in fish. Optimization was performed by Experimental Design expert 7.1 to get the best conditions. A new Gas chromatography coupled with a mass spectrometer (GC-MS) has been utilized for pesticides separation and quantification. In this study, the use of 200mg Primary secondary amine, 150mg C18 and 2ml chloroform were found to be the best conditions giving higher recoveries for extraction of the selected pesticides. The optimized QuEChERS method was applied to real samples of fish collected from different places of drainage water in Egypt in which residues of pesticides were detected.

Keywords: GC-MS;QuEChERS; Pesticides; Fish.

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1. Introduction

Contamination of food by pesticides is a serious concern because of the extensive use of pesticides in agriculture in the past. Agricultural activities induced a pesticide contamination of many aquatic ecosystems (Loos,2009, Devault,2009). Many pesticides have been found in samples from coastal environments (Arienzo,2013). Pesticide residues in the environment can concentrate and diffuse by the effect of biological enrichment and appear in food products (X. Sun,2011). Because of this widespread contamination, pesticides have been identified in fish muscle tissues because they uptake contaminants directly from water and diet. Pesticides metabolized in fish moderately; Thus, contaminants in fish reflect the state of pollution in surrounding environments (. Belenguer,2014). Pesticides have a great effect on fish health (Bony, 2010; Marchand,2006).

Fish is considered to be an important component of a balanced human diet. In the last few decades, fish consumption has increased worldwide (Kalachova,2013; Nácher-Mestre, 2010). Fish are an excellent source of iodine, selenium, vitamins A, D and also lipids because of their high content of the long chain n-3 polyunsaturated fatty acids, which are useful for the cardiovascular system (Molina-Ruiz,2015). Humans are still exposed to pesticides through consuming contaminated seafood (Zhou,2012 ; Moon,2009).

Fish contaminated with pesticides have a greater risk for consumers (Hu,2010; John,2003).In recent years, there has been a great concern about fish consumption risk to human health due to the presence of persistent organic pollutants as pesticides (Sun,2006; Greco,,2010).

Long-term exposure to pesticides residues which have negative effects on human and animal health as a cause of cancer, kidney failure, liver and fetal abnormalities because of accumulation in adipose tissue may trigger endocrine disruptions, neurotoxicity, cancer, and other adverse health effects (Sánchez-Avila,2011) so the analysis of pesticides in environmental samples is an essential part of monitoring and managing the risks posed in the environment pesticides have a potential for bioaccumulation in the food by low polarity, low aqueous solubility and high lipid solubility (lipophilicity) (Afful,,2010)

Regulation (EC) No 396/2005 (Regulation EC/396/2005 2008), brought into force on the 1 September 2008, defines a new full a set of rules for pesticide residues; The default maximum pesticide residue level in foodstuffs is 0.01 mg $kg^{-1}$is applicable in all cases wherean MRL has not been specifically set for a product (Molina-Ruiz,2015). Catfish (siluriformes) is considered t most species found in drainage water in Egypt.

The chosen pesticides were from different chemical classes as organochlorines, organophosphate, chlorinated cyclodiene, pyrethroids,chloroacetanilides.

Selected pesticides include insecticides (Dimethoate, Diazinon, Chloropyrifos ethyl, Malathion, Primiphos ethyl, 4, 4-Dichlorodiphenyldichloro-ethane (4,4DDD), o,p-Dichlorodiphenyltrichloro-ethane (o,p-DDT), Tetradifon), fungicides (Chlorothalonil, Vinclozolin, cyprodinil), acaricide (Parathion ethyl, alpha-Endosulfan, beta-Endosulfan) and rodenticide (Endrin).

In food control analysis, isolation of pesticides from matrices containing a high content of fat requires complicated sample treatment procedures as efficient extraction and clean-up (Su,2011, Covaci,2007). Biological samples are complex, so it usually involves many steps in preparing biological samples for OCP analysis (Murthy,2013). Despite several methods developed in the last years for the analysis of pesticides in different matrices, only a few were developed for fish matrix. Liquid extraction was sometimes used, but concerned generally one family of the compound with similar properties. Pressurized liquid extraction was also applied (Blasco,2005).I have applied recently modified QuEChERS methodto fatty complex matrices such as fish and fish feed (Lazartigues,2011).GC-MS and LC-MS have been the main analytical tools in most pesticide monitoring laboratories to meet world standards.

In this work we look forward to modify the QuEChERS extraction procedure for the simultaneous analysis of a list of 15 pesticides in Catfish (Siluriformes) and to apply gas chromatography/mass spectrometer to separate selected pesticides and determine them in real samples.

2. Experimental

2.1. Chemicals and materials

Acetonitrile, Acetone and N-Hexane HPLC grade were purchased from Merck (Darmstadt, Germany). Acetic acid, citric acid and NaCl were purchased from Carlo-ErbaReagenti SPA. The dispersive solid phase extraction (D-SPE) sorbents including primary secondary amine (PSA), octadecylsilyl silica (C18) were purchased from Sigma-Aldrich and

aluminum neutral (Al-N) purchased from Carlo-Erba. Pesticides reference standards were purchased from Dr. Ehrenstorfer Laboratories (Augsburg, Germany). Anhydrous sodium acetate (Anhydrous NaAc) and anhydrous magnesium sulfate (Anhydrous $MgSO_4$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Extraction QuEChERS kits (6gm $MgSO_4$+1.5gm NaAc) and D-SPE clean-up kits (150 mg Anhydrous. $MgSO_4$+50 mg PSA) was purchased from Waters Corporation (Milford, U.S.A.)

2.2. Equipment

Chromatographic analyses were performed using Gas Chromatography (Agilent 7890B) with G456A autosampler coupled with Mass Spectrometry-electron impact ionization instrument (5977A) (Agilent Technologies, Santa Clara, CA) pulsed split-less inlet and HP-5MS (30 m × 250 µm) capillary column coated with 0.25 µm of 5% phenyl and 95% methyl poly siloxane film (Agilent J & W Scientific, Folsom, CA). The injection temperature was 250 ˚C with 1 ml volume. Carrier gas used helium gas 99.999% with a flow rate (0.9ml/min). The GC oven was operated with the following temperature program: an initial temperature of 122℃ (for 1 min). Then increase with 30˚C per minute to reach 190˚C stands for 2 min. Then increase with 5˚C per minute to reach 255˚C stands for 2 min. MS condition was operated at selective ion method (SIM) mode with source temp 325˚C, quadruple temp 150˚C and transfer line temp 300˚C. The solvent delay was 10 min. The total analysis time was 25 min.

2.3. Standard solution preparation:

Stock standard solutions of each pesticide were prepared at a concentration of 100µg/ml in the solvent (Hexane: acetone (9:1)), after that, they were used to prepare intermediate standard solutions by serial dilution with (Hexane: acetone (9:1)) to yield a final concentration of10µg/ml and stored in amber glass vials at −20 ˚C. The intermediate standard solutions were used to prepare a mixture solution in N-hexane: acetone (9:1). A working standard pesticides mixture solution containing all pesticides was prepared at 1µg/ml each. This mixture also served as the spiking solution in recovery experiments, Matrix-matched calibration standards at concentrations of (10, 20, 50, 100, 200 ng/ml) were prepared in (N-hexane: acetone (9:1)) by diluting the standard mixture solution.

2.4. Sample preparation method

The QuEChERS extraction procedure was used. Fish samples were taken from different sources of drainage water in Egypt. The muscle tissues of the fish samples were ground in a blender to get a homogenous composite. Five grams of each homogenate were weighed into a 50 ml centrifuge tube. Tenml of water and15ml of acetonitrile (containing 1% acetic acid) were added. After that, 1.5g anhydrous NaAC, 6g anhydrous $MgSO_4$ and 1g NaCl were added, then each sample was shaken vigorously for 7 minutes and centrifuged for 5 min at 4000 rpm. For dispersive-SPE clean-up 1 ml of the supernatant was transferred into a polypropylene 15mlcentrifuge tube containing 200 mg PSA, 150 mg C_{18}, 2 ml chloroform and 150 mg Anhydrous MgSO4. The tube was shaken for 30 s and then placed into centrifuge for 5 min at 4000 rpm. At the last stage of the procedure, freezing was also evaluated as a practical way to reduce the amount of co extracts then; the extract was dried by evaporation and then dissolved in N-Hexane for GC/MS analysis into a vial.

Reference samples with no pesticides detected previously were used for recovery studies and for preparing matrix-matched standards for calibration.

2.5. Experimental design

For fish samples, α-Endosulfan, β-Endosulfan, DDD, DDT and Chloropyrifosonly gave low recoveries (<50%) with the conventional method. Optimization of the QuEChERS method was made using a central composite design (CCD). The effects of

PSA, C18, and chloroform amounts. Twenty experiments were carried out.

2.6. Statistical tool

Work on experimental design, data analysis, response surfaces and graphs were performed by Design Expert Version 7.1 (Stat Ease Inc., Minneapolis, MN, USA)**.**

2.5 .Sample stability

Spiked fish samples were used to determine sample stability. Samples were spiked with a mixture of all pesticides. Some were analyzed directly, and the results represented the day-0 storage period. Others were prepared and analyzed after storing at −20°C for different periods during six months. The recoveries were used to evaluate the sample stability**.**

2.7. Matrix effect

Suppression or enhancement of (0–20%) is negligible as a soft matrix effect. To avoid matrix effects in GC/MS, matrix-matched calibration standards can be used) (Rajski, 2013).

3. Results and discussion:

3.1. Optimization of QuEChERS method:

3.1.1. Primary secondary amine (PSA) amount:

PSA removes matrix co-extracts better thanNH2, because PSA has both primary and secondary amine(Ru-zhen,2011). Central composite design was used to reach the best amount which will give best recoveries. Adding 200mg PSA to fish samples during clean up provided higher recoveries for all 15 studied pesticides.

3.1.2. C_{18} amount:

C18 is the most hydrophobic sorbent, because of its extreme retentive nature for non-polar compounds such as fat (Molina-Ruiz,2015).The central composite design was used to reach the best amount which will give best recoveries. Adding 150mg C18 to fish samples during clean up provided higher recoveries for all 15 studied pesticides.

3.1.3. Chloroform amount:

The addition of chloroform was included to drive water from the acetonitrile phase and thus effectively remove both the salts and the very polar matrix components of the extract(Liu,2011).The central composite design was used to reach the best amount which will give best recoveries. Adding 2ml chloroform to fish samples during clean up provided higher recoveries for all 15 studied pesticides.

3.1.4. Optimization by central composite design (CCD):

CCD was performed to determine the optimal conditions for QuEChERS method. ANOVA was applied to evaluate selected factors and their effects and to determine if the multiple regression is significant or non-significant (Rizzetti, 2016). Independent factor had a significant effect on a response when it had a p value < 0.05.Three independent variables were selected: Primary secondary amine (PSA) amount, C18 amount and adding chloroform (**Table 1**). Central composite design was applied on fish samples for the three parameters. **Table 2** summarizes the conducted experiments and responses. Statistical parameters obtained from ANOVA were given in **Table 3** which showed that Primary secondary amine (PSA) amount, C18 amount had the most significant effects on the selected responses where p-values for these two factors is smaller than 0.05. The amount of chloroform had no significant effect on all the selected responses (p-values > 0.05). R^2adj was greater than 0.8 good fit of experimental data (Candioti, 2014). Response surfaces are shown in **Figure** 1 (a, b, c, d and e) as interaction effects of Primary secondary amine (PSA) amount and C18 amount are illustrated on the selected responses.

Derringer's desirability function D was used to estimate the optimum conditions of extraction(Sivakumar,2007) .

$$D = ¼ [d_1^{p1} \times d_2^{p2} \times .. \times d_n^{pn}]^{1/n} \quad (1)$$

Where di is the individual desirability function of each response, pi is the weight of the response and n is the number of responses. The scale of the desirability function varieties as a completely undesired response di = 0 and a fully desired response di = 1. Derringer's desirability function D can take values from 0 to 1. When D close to 1, response values are

near the target value. The constraints in this study that were imposed on the responses included maximizing recovery of all selected pesticides with low recoveries. **Figure 2** shows the response surface for the desirability function for fish.

Therefore, the following conditions were considered optimal conditions for extraction of studied 15 pesticides in fish samples 200mg of PSA, 150mg of $C_{18}$and 2ml chloroform. The response surface obtained for the desirability function is presented in **Figure 2**.

3.2. GC–MS conditions Optimization:

Several flow rates 0.2ml/min, 0.5ml/min, 0.8 ml/min were examined, and the flow rate of 0.2 ml/min resulted in overlapping of some peaks especially (Diazinon and Chlorothalonil). The flow rate of 0.5 ml/min also resulted in overlapping of DDT and Tetradifon. We selected a flow rate of 0.8ml/min as giving the best results.The method was optimized to increase the signal for each pesticide. The sufficient conditioning time of a column produced reproducible results (2 hours).

This system was provided with HP-5MS capillary column (30 m × 250 μm) covered by 0.25 μm of 5% phenyl and 95% methyl polysiloxane film. The mass spectrometer was functioning with an electron impact ionization source in the SIM mode. Mass spectrometry was existing in the SIM mode, with the selection of four m/z values for each pesticide. We used the SIM mode peak location to execute the analysis and to match GC/MS retention times. Retention times of the 15 selected pesticides were shown in **Table 4.**

Figure 3 shows a GC/MS chromatogram of the spiked 15 pesticides in fish matrix, where they were well separated.

3.3. Method Validation:

3.3.1. Selectivity:

Analysis of blank fish samples extracted by the optimized QuEChERS method and the corresponding spiked sample with pesticides was used to assess Selectivity of the method. No peaks interfered in the chromatographic range of interest as shown in **Figure 3**.

3.3.2. Linearity, LOD, LOQ

Regression and calculation of the squared correlation coefficient (R^2) was used to measure Linearity of the method. Plot of calibration was made in triplicate (n =3) for analysis of blank fish samples fortified by the addition of standard solutions of the pesticides, at levels of 10, 20, 50, 100, and 200ng/ml. All R^2 values were equal or higher than 0.99 (**Table 5**).Limit of detection (LOD) and limit of quantification (LOQ) were estimated based on SD using the formula (LOD=3.75 × SD)(Donkor,2015) . The limits of quantitation (LOQ) defined as 3 times the LOD. The results obtained were shown in **Table 6**.

3.3.3. Accuracy and precision

To test the intra-day precision and accuracy, we analyzed five replicates at three concentration levels (10, 100, 200 ng/ml) in the same day. Inter-day precision and accuracy were evaluated by examining the three sample concentrations on five consecutive days. The relative error (RE %) was used to show the inter-day and intra-day accuracy, while relative standard deviation (RSD %) was used to show the precision. The results obtained were shown in **Table 7**.

3.3.4. Storage stability

Storage condition of −20 ˚C for 6 months was found to achieve stable results for recoveries of fish samples.

3.3.5. Matrix effects

Values of ME% are present in **Table 6**. Matrix matched calibrated chromatogram was used for more accuracy.

3.4. Application to real samples

Fifteen fish samples collected from different drainage water areas in Egypt and analyzed for pesticides following the adjusted conditions to show the utility of the method. Many pesticides were detected in different samples as DDT, DDD and Diazinon. DDT was detected in two samples (14 and17 ng/ml). DDD was detected in one sample (16 ng/ml). Diazinon was detected in one sample (13ng/ml). All found pesticides were shown in **Table 8**. The chromatograms of fish samples having pesticides were shown in **Figure 4**.

Table (1): Factors examined for fish in central composite design.

Independent factor	levels				
	-α	(-1)	center	(+1)	+α
PSA(mg)	50	100	150	200	251
C18(mg)	83	100	125	150	167
Chloroform(ml)	0	1	2	3	4

Table (2):Central composite design for QuEChERS factors optimization in fish and percentage recoveries of selected responses.

					Percentage recoveries of				
runs	Std.	A-PSA	B-C18	C-Chloroform	Chloropyrifos	α-Endosulfan	β-Endosulfan	DDT	DDD
1	11	100	83	2	70	68	75	76	**74**
2	10	251	125	2	80	78	84	82	83
3	3	50	150	1	80	82	88	85	85
4	13	150	125	0	73	70	75	75	77
5	18	100	125	2	75	70	80	78	78
6	8	200	150	3	105	112	106	101	95
7	20	150	125	2	75	72	80	78	79
8	16	100	125	2	76	71	79	77	79
9	15	100	125	2	75	70	79	78	78
10	2	200	100	1	77	80	90	80	87
11	12	150	167	2	95	88	100	99	90
12	5	50	100	3	75	73	80	85	78
13	1	50	100	1	72	69	78	79	76
14	14	100	125	4	77	71	82	77	76
15	6	200	100	3	78	81	92	82	89
16	7	50	150	3	70	79	91	87	87
17	19	150	125	2	77	72	81	77	77
18	9	50	125	2	77	70	83	80	80
19	4	200	150	1	103	110	105	100	94
20	17	150	125	2	75	71	81	78	78

Table (3): Anova results of CCD fish. A 5% level of significance was desired. Insignificant interaction effects were excluded.

	Chloropyrifos		α-Endosulfan		β-Endosulfan		DDD		DDT	
	F	P[a]	F	P[a]	F	P[a]	F	P[a]	F	P[a]
Model	7.37	0.0022	266.66	<0.0001	144.55	<0.0001	8.98	0.0010	95.19	<0.0001
A-PSA	14.97	0.0031	265.07	<0.0001	81.00	0.0003	2.65	0.1349	80.99	0.0003
B-C18	28.84	0.0003	250.00	<0.0001	390.62	<0.0001	39.11	<0.0001	225.88	<0.0001
C-Chloroform	0.022	0.8842	0.63	0.4650	30.62	0.0026	1.10	0.3191	0.88	0.3907
AB	12.81	0.0050	275.63	<0.0001	10.00	0.0250	8.74	0.0144	5.51	0.0657
AC	0.51	0.4906	0.63	0.4650	0.62	0.4650	0.23	0.6437	0.22	0.6584
BC	0.74	0.4105	5.63	0.0638	0.0	1.000	0.23	0.6437	0.22	0.6584
A^2	0.16	0.6993	733.31	<0.0001	394.12	<0.0001	4.76	0.0540	369.82	<0.0001
B^2	6.37	0.0302	91.88	0.0002	105.47	0.0002	21.53	0.0009	38.90	0.0016
C^2	0.23	0.6437	0.47	0.5240	4.22	0.0952	0.21	0.6562	7.35	0.0422
ABC	--	--	10.00	0.0250	0.62	0.4650	--	--	0.22	0.6584
A^2B	--	--	68.07	0.0004	5.79	0.0611	--	--	4.55	0.0861
A^2C	--	--	0.17	0.6970	4.84	0.0791	--	--	**8.04**	0.0365
AB^2	--	--	10.21	0.0241	0.65	0.4565	--	--	0.65	0.4561
R^2	0.8689		0.9987		0.9975		0.8899		0.9963	

[a] p-value should be less than 0.05 to be statistically significant

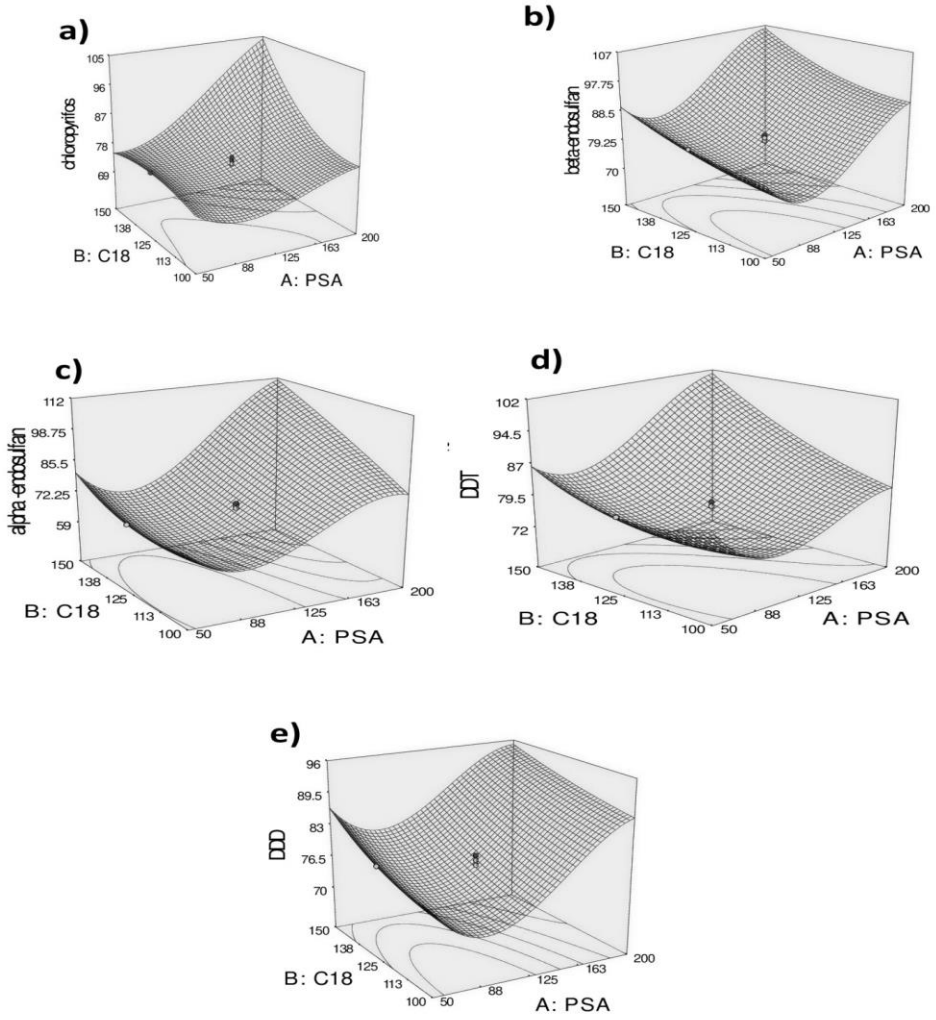

Fig.1:Response surfaces related to PSA and C18 amount for: a) Chloropyrifos, b) Beta-Endosulfan, c) Alpha-Endosulfan, d) DDT and e) DDD.

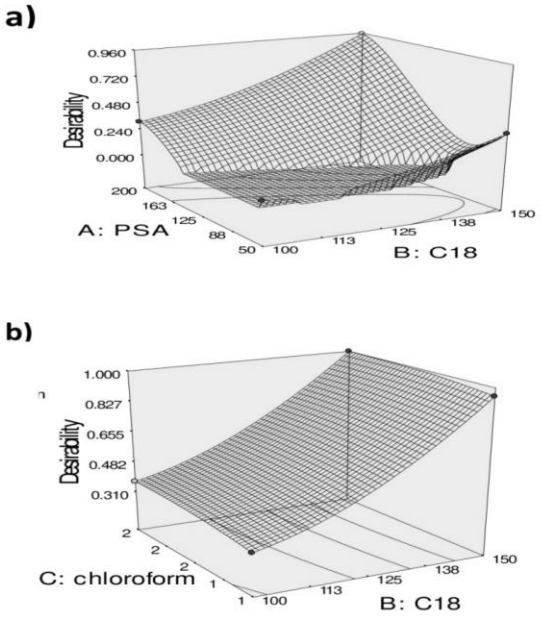

Fig.2: The response surface obtained for the desirability function for fish samples.

Table (4) Retention times of the selected pesticides, quantification ions and qualification ions of the pesticides.

no	Pesticides	Retention time (min)	Quantification ions (m/z)	Qualification ions (m/z)
1	Dimethoate	10.201	125,93	143,229
2	Diazinon	10.810	152,137	197,304
3	Chlorothalonil	11.613	266,264	268,270
4	Vinclozolin	12.501	285,212	187,198
5	Malathion	14.102	127,125	158,173
6	Chloropyrifos	14.521	199,197	257,316
7	Parathion ethyl	15.105	235,155	291,293
8	Primiphos ethyl	16.312	304,168	318,333
9	Cyprodinil	17.151	224,210	225,226
10	alpha-Endosulfan	19.012	237,207	239,339
11	Endrin	20.942	281,263	317,345
12	beta-Endosulfan	22.271	207,195	237,339
13	4,4 DDD	23.107	235,165	237,239
14	op- DDT	24.723	165,235	320,354
15	Tetradifon	24.967	229,159	354,356

Table (5): Regression equation and linearity for pesticides found in fish samples.

No	Analyte	R^2	Regression equation
1	Dimethoate	0.996	Y= 9058.97 X+2925.62
2	Diazinon	0.996	Y= 3814.09 X+2260.67
3	Chlorothalonil	0.994	Y= 3555.32 X+1355.48
4	Vinclozolin	0.999	Y= 5761.38X+3263.65
5	Malathion	0.999	Y= 27425.81 X+3467.43
6	Chloropyrifos ethyl	0.990	Y= 5333.10 X+1324.65
7	Parathion ethyl	0.998	Y= 4225.04 X+2366.32
8	Primiphos ethyl	0.994	Y= 7786.44 X+6108.50
9	Cyprodinil	0.997	Y= 12511.12 X+4359.69
10	Alpha-Endosulfan	0.996	Y= 27729.82 X+2370.35
11	Endrin	0.999	Y= 422.18 X+2188.69
12	Beta-Endosulfan	0.996	Y= 11460.09 X+3360.01
13	4,4 DDD	0.998	Y= 2323.46 X+2776.61
14	op DDT	0.998	Y= 4331.49 X+3253.24
15	Tetradifon	0.999	Y= 1615.23 X+1932.04

Y: Peak area.

X: Concentration of pesticides in ng/ml.

Table (6): Standard deviation of response (SD), limit of detection (LOD), limit of **quantification (LOQ) and Matrix effect % of selected pesticides in fish.**

No	Analyte	SD	LOD (ng/ml)	LOQ (ng/ml)	ME%
1	Dimethoate	1.47	5.52	16.56	4.11
2	Diazinon	0.82	3.06	9.19	9.10
3	Chlorothalonil	1.03	3.87	11.62	8.50
4	Vinclozolin	0.75	2.82	8.47	3.35
5	Malathion	0.63	2.37	7.12	5.23
6	Chloropyrifos ethyl	0.89	3.35	10.06	1.34
7	Parathion ethyl	1.17	4.38	13.15	5.61
8	Primiphos ethyl	1.17	4.38	13.15	6.84
9	Cyprodinil	0.82	3.06	9.19	9.12
10	Alpha-Endosulfan	1.37	5.12	15.37	5.76
11	Endrin	0.89	3.35	10.06	8.56
12	Aeta-Endosulfan	0.75	2.82	8.47	8.34
13	4,4 DDD	1.17	4.38	13.15	6.56
14	op.DDT	0.89	3.35	10.06	8.96
15	Tetradifon	0.89	3.35	10.06	7.65

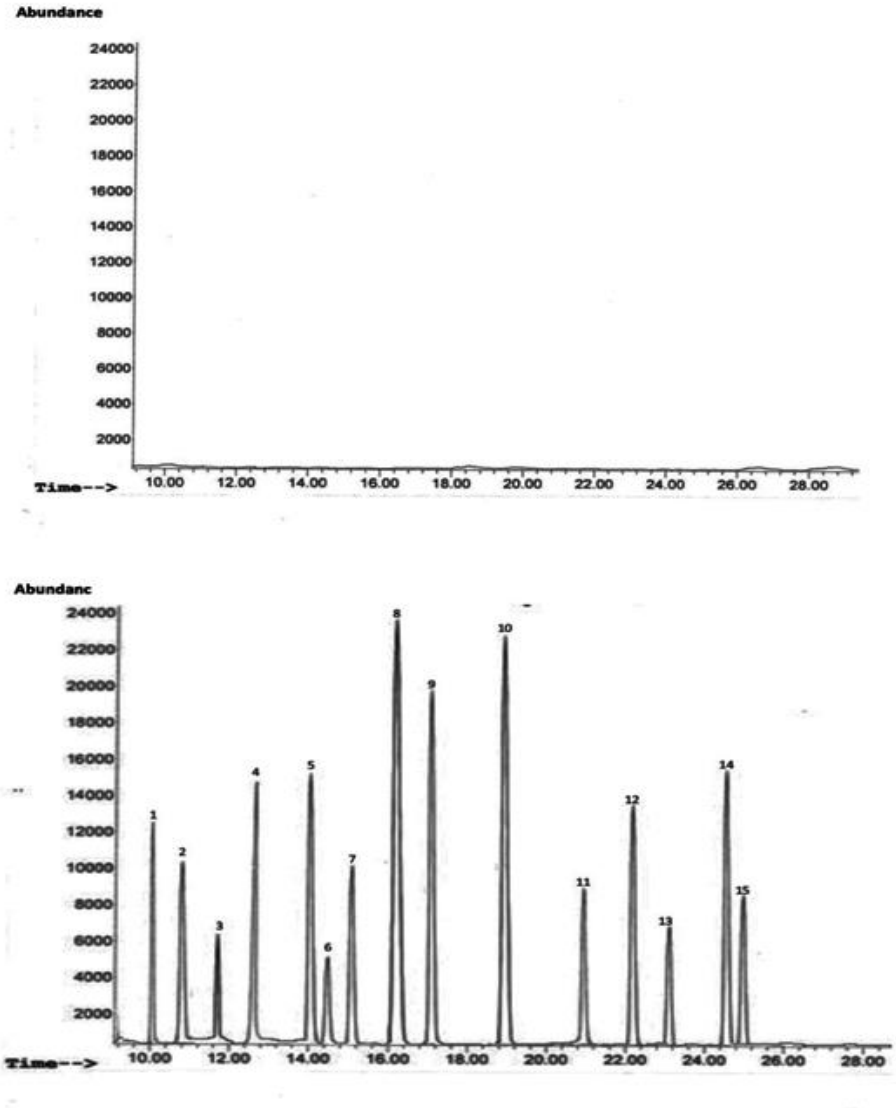

Fig.3:GC/MS Chromatograms from analysis of: a) Blank fish sample and b) Fish sample spiked with concentration of 50 ng/ml of: 1) Dimethoate, 2) Diazinon, 3) Chlorothalonil, 4) Vinclozolin, 5) Malathion, 6) Chloropyrifos Ethyl, 7) Parathion Ethyl 8) Primiphos Ethyl 9) Cyprodinil 10) Alpha-Endosulfan, 11) Endrin, 12) Beta-Endosulfan, 13) 4,4 DDD, 14) O, P DDT,and 15) Tetradifon

Table (7): Relative standard deviation percentage and relative error percentage for intraday and interday accuracy and precision of the selected pesticides in fish.

	Analyte	RSD%						RE%					
		intra-day			inter-day			intra-day			inter-day		
		RSD%1	RSD%2	RSD%3	RSD%1	RSD%2	RSD%3	RE%1	RE%2	RE%3	RE%1	RE%2	RE%3
1	Dimethoate	0.72	0.83	4.39	0.56	1.59	5.85	-4.25	-9.83	-14.17	-6.25	-13.50	-11.67
2	Diazinon	0.62	0.96	4.14	0.44	1.70	4.45	-6.08	-14.67	-9.17	-6.17	-17.00	-8.33
3	Chlorothalonil	0.39	1.13	2.67	0.62	1.13	2.96	-4.58	-8.33	-3.33	-2.17	-8.67	-7.50
4	Vinclozolin	0.50	0.73	3.01	0.58	1.34	5.61	-0.92	3.17	5.00	0.58	2.33	4.17
5	Malathion	0.44	0.70	4.37	0.68	1.28	6.09	-6.17	-9.00	-6.67	-2.58	-8.83	-10.00
6	Chloropyrifos ethyl	0.96	0.91	4.88	1.07	1.79	4.07	0.92	-2.00	7.50	1.33	-3.83	10.00
7	Parathion ethyl	0.45	1.33	4.37	0.52	1.98	3.51	-1.00	-12.17	-6.67	-1.17	-13.17	-10.00
8	Primiphos ethyl	0.39	1.25	2.17	0.45	1.43	2.96	-2.92	-6.17	-5.83	-1.00	-4.67	-7.50
9	Cyprodinil	0.28	0.83	4.47	0.83	1.51	5.25	-1.25	-1.67	0.00	-1.25	-2.83	-1.67
10	Alpha-Endosulfan	0.44	1.21	5.59	0.50	1.22	5.68	2.50	12.67	8.33	3.67	11.67	6.67
11	Endrin	0.40	1.00	2.82	0.40	0.46	4.14	-6.08	-11.00	-8.33	-5.92	-12.17	-9.17
12	Beta-Endosulfan	0.39	0.72	1.96	0.50	0.99	4.26	4.17	5.17	4.17	4.25	5.50	5.00
13	4,4 DDD	0.38	1.23	2.82	0.38	0.79	4.22	-1.08	-5.17	-8.33	-0.92	-4.83	-10.83
14	op.DDT	0.52	0.89	4.22	0.59	1.05	5.25	-0.67	1.00	-3.33	-0.92	-1.67	-1.67
15	Tetradifon	0.52	0.85	2.02	0.51	1.43	3.56	1.75	5.00	0.83	0.67	5.33	5.83

Table 8: Pesticide residues found in real fish samples and their concentrations (ng/ml).

Pesticides found	**no of samples**	**Conc. found** (ng/ml)
DDT	2	14,17
DDD	1	16
Diazinon	1	13

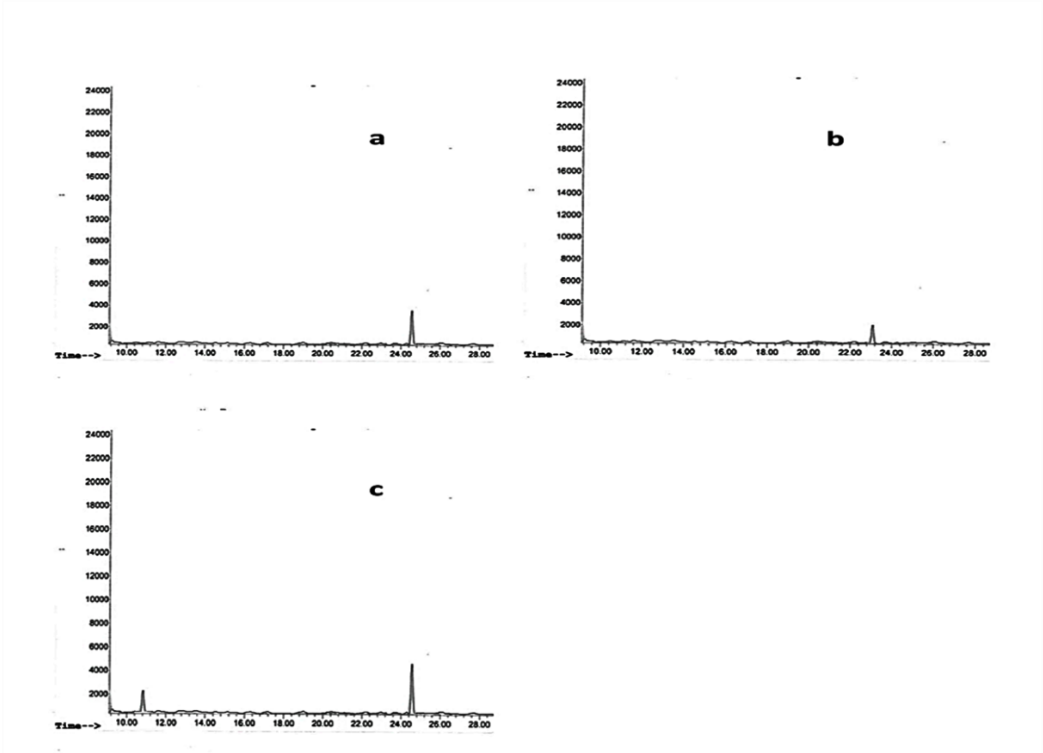

Fig.4: Real fish sample containing a) 14 ng/ml of DDT, b) 16 ng/ml of DDD and c) 17 ng/ml of DDT and 13 ng/ml of Diazinon

4. Conclusion

The Experimental Design was used to optimize The QuEChERS method to give higher recoveries for some selected pesticides with low recoveries (<50%) in fish. For fish samples (200mg of PSA, 150mg of C18 and 2ml chloroform) were used According to experimental design application to give higher recoveries for the selected pesticides. Relatively good analytical results regarding good repeatability and recovery for the investigated pesticides in fish were obtained in the experiment. Fifteen real fish samples collected from different agricultural fields in Egypt were successfully tested using the optimized QuEChERS methods.

Conflict of interest statement

The authors have declared no conflict of interest.

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