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Recent Techniques Applied for Pesticides Identification and Determination in Natural Products and Its Impact to Human Health Risk

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1. Introduction

Based on the compilation of the British Crop Protection Council, approximately 860 active substances are formulated in pesticide products currently (Tomlin, 2003). These substances belong to more than 100 substance classes. Benzoylureas, carbamates, organophosphorous compounds, pyrethroids, sulfonylureas, or triazines are the most important groups. The chemical and physical properties of these pesticides may differ considerably. There are several acidic pesticides; others are neutral or basic and some compounds contain halogens, others phosphorous, sulfur, or nitrogen. These heteroatoms may have relevance for the detection of pesticides in natural products. Pesticides such as polychlorinated biphenyls PCB'S organochlorines and organophosphates are found in various parts of the environment in quite small concentrations, but they accumulate and thus become a threat to human health and life. Maximum residue levels (or tolerances) have been established for pesticides in foodstuffs and drinking water in most countries to avoid any adverse impact on public health, and to insist on good agricultural practice. For these reasons a large number of researchers are involved in the surveillance of maximum residue levels or in the identification and quantification of pesticide residues in environmental matrices. A lot of these pesticides were registered in Egypt or most frequently detected in fruits and vegetables in Egyptian market as well as in Europe and USA. To control local, imported and exported food, multi-residue analytical methods are preferred to reduce the workload. In this study, simple and reliable multi-residue method of analysis for determination of pesticide residues in different agricultural products was developed. In this method different pesticide groups, e.g. organophosphates, moderately polar organochlorines, benzimidazoles, N-methylcarbamates and phenoxy acids could be analyzed in one multiresidue method using Liquid Chromatography tandem mass spectrometry (LC-MS/MS) and fulfill the Codex and EU regulations. Grape, green beans even vegetable samples were extracted by shaking with acetonitrile .Phase separation was induced by shaking with buffer—salt mixture consisting of magnesium sulfate, sodium chloride, disodium hydrogen citrate sesquihydrate and trisodium citrate dihydrate .The sample was centrifuged and an aliquot of the clear solution dried by shaking with magnesium sulphate . The extract was centrifuged and an aliquot of the clear solution evaporated, re-dissolved in methanol/water buffer solution and injected into LC-system (Afify, 2010) .Quantitation and

identity confirmation was attained by using atmospheric pressure electrospray positive ionization LC-MS/MS in multiple reactions monitoring MRM mode .The recoveries of pesticides at three different concentration levels 0.01, 0.05 and 0.1 mg/kg ranged from 70 to 110 % . The repeatability expressed as relative standard deviation RSDr (was l-25) % n = 6 . Matrix matched standards were used to compensate for the matrix effect.
The present chapter will concern extensively with Multiresidue method for determination of 150 pesticides in grapes and green beans by validating and using Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction method followed by liquid chromatography tandem mass spectrometry (LC-MS/MS). In addition GC systems with three different detectors (GC-ECD, GC-NPD and GC-MSD) were used. Compare between GC (ECD, NPD and MSD) and LC-MS/MS for its efficiency and sensitivity were carried out . The mass spectrometric parameters were optimized to give the best sensitivity, two MRM's were chosen for quantification and conformation of pesticides. The selected MRM's based on the optimized declustring potential and collision energy were used which help pesticides selectivity and justification. Protein binding of Serum Transferrin and Albumin with pesticides during transportation in the living cells will be studied including three pesticides (Trichlorphenol, Fenvalerate and α-Endosulphan). Impact of pesticides contamination to human risk through studying pesticides contamination in milk as well as potatoes tuber were investigated.

2. Materials and methods

2.1 Materials

Polyethylene or PFTE 15 ml and 50 ml with screw cap tubes .Centrifuge Heraeus up to 4000 rcf .(LC–MS/MS was performed with an Agilent 1200 Series HPLC instrument coupled to an API 4000 Q-trap MS/MS from Applied Biosystems with electrospray ionization ESI interface.

2.1.1 Pesticides

Reference standards for 150 pesticides were obtained from Dr. Ehrensdorfer (Augsburg, Germany), Purity was >95%. The common names, KOW log, field of use and chemical class of the tested pesticides are shown in Table (1) (British crop protection council 2002).

2.1.2 Samples

Different types of agricultural products (e.g. green beans and grapes) were purchased from local markets in Egypt. Samples were grinded with high speed grinder 2 litter capacities jar with lid and stored at -20 ±2 ^{0}C till sample analysis according to Codex Alimentarius (2003).

2.2 Methods

2.2.1 Reagents

Acetonitrile from Lab-scan HPLC, assay >99(%, Methanol, 99.9 %HPLC grade Merck. Formic Acid, 98-100) % Riedel–de Haen , Ammonia solution, 33 % Riedel–de Haen , Sodium chloride, 99 % Riedel–de Haen , Disodium hydrogencitrate sesquihydrate , Fluka, Trisodium citrate dihydrate ,Fluka, Sodium chloride and anhydrous magnesium sulphate Merck, De-ionized water was produced by Milli-Q unit Millipore.
Buffer-salt-mixture for second extraction and partitioning was prepared by weighing 4±0.2 g of anhydrous magnesium sulphate, 1±0.05 g of sodium chloride, 1±0.05 g of trisodium

citrate dehydrate, 3.16 and 0.5±0.03 g of disodium hydrogencitrate, sesquihydrate into 25 ml glass tube. LC mobile phase was 10 mM ammonium format solution in methanol-water (1:9), pH 4±0.1 . Sample dilution buffer was 10 mM ammonium format solution in methanol/water (1:1),pH 4±0.1 .Pesticide reference standards purity >95 %were from Dr . Ehrensdorfer ,Augsburg, Germany.

2.3 Standard preparations

2.3.1 Stock solution

Reference standard solutions (1000 µg/ml) of all the analyzed pesticides were prepared in methanol. Stock solution was kept at -20 ± 2 °C. (Banerjee et al., 2007).

2.3.2 Intermediate mixture solution

Mixture 10 µg/ml from all compounds was prepared as intermediate stock solution in methanol and used as spiking mixture and kept at -20 ± 2 °C (Banerjee et al., 2007).

2.3.3 Calibration mixture solution

Calibration mixtures of concentration levels 0.005, 0.01, 0.05, 0.1 and 0.5 µg/ml were prepared in methanol: ammonium format buffer 10 mM pH 4 (1:1) kept at -20 ± 2 °C.

2.4 Pesticide stability mixtures

The calibration mixture was prepared in 5 different pH (3,4,5,6 and 7) in the same solution (methanol : ammonium format buffer 10 mM, 1:1) and concentration (0.5 ppm) to check pesticides stability for 2 weeks then injected 4 different calibration mixture solution after storage at -20 ± 2 °C for two weeks and calibration mixture 0.5 ppm in methanol as reference standard solution fresh prepared.

2.5 Extraction procedure

Extraction procedures used in our studied for analysis of 150 pesticides in grapes as well as in green beans were described as follows:

2.5 Extraction procedures

2.5.1 QuEChERS method as described by Anastassiades, et al., (2008)

Green beans sample (10g) was add in Polyethylene (PFTE) 50 ml tube then 10 ml acetonitrile was add and shacked vigorously for one minute, then buffer-salt-mixture (4g±0.2g of magnesium sulfate anhydrous, 1g±0.05 g of sodium chloride, 1g±0.05 g of trisodium citrate dehydrate and 0.5g±0.03g of disodium hydrogencitrate sesquihydrate) added and shakes immediately for one minute. Centrifugation carried out at 4000 rpm for 5 minutes. Supernatant (4 ml) of the clear solution was transferred to 50 ml round-bottomed flask and evaporated with rotary evaporator at 40 °C. Residues were redissolved in 4 ml (Methanol: ammonium format buffer 10 mM pH 4 (1:1). Injection of 25 µl of the sample into LC-MS/MS system was carried out.

2.5.2 Luke et al., (1975) method

Green beans sample (50g) was add with 100 ml acetone and blended for 2 min at medium speed, homogenized sample is filtered through Buchner funnel containing filter paper

(Whatman no.1) fitted on Buchner flask, the blender jar is rinsed with 50 ml acetone and filtered again on the same funnel, the extracted volume is recovered.
A 40 ml sample extract is transferred to 500 ml separator funnel, 50 ml petroleum ether and 50 ml dichloromethane are added and shake vigorously for 2 min, transfer the lower aqueous layer to graduated cylinder, the upper organic layer is transferred by passing through anhydrous sodium sulphate supported on washed cotton in funnel on receiving flask, about 2g sodium chloride is added to the aqueous phase and shake vigorously for 1 min until most sodium chloride dissolved, transfer it to the same separator funnel, 50ml dichloromethane is added and shake for 1 min, lower dichloromethane layer is filtered through sodium sulphate, the water layer is taken and the last dichloromethane partitioning step is repeated, sodium sulphate is rinsed with 25ml dichloromethane, the received solution is evaporated using rotary evaporator to about 2ml at 35-40 °C, continued evaporation by air just to dryness, the residue was re-dissolved in 10 ml [Methanol: ammonium format buffer 10 mM pH 4 (1:1) and filtered through 0.45 µm syringe filter, the clear filtrate was injected directly into LC/MS/MS system.

2.5.3 Ethyl acetate method by Banerjee et al., (2007)

Green beans sample (50g) was add with 10 ml ethyl acetate in 50 ml PTFE centrifuge tube and blended for 1 min. An aliquot of 4 ml was evaporated using rotary evaporator at 40 ^{o}C just to dryness. The residue was re-dissolved in 4 ml [Methanol: ammonium format buffer 10 mM pH 4 (1:1) and filtered through 0.45 µm syringe filter. The clear filtrate was injected directly into LC-MS/MS system.

2.6 Choosing of pesticides

The 150 chosen pesticides used in this investigation were collected and identified with type of pesticides, chemical class, Field of use and KOW logP as shown in the following table:

Pesticides	KOW logP	Field of use	Chemical class
1-Abamectin	4.4	Insecticide, acaricide	Bio Pesticide
2-Acephate	-0.89	Insecticide	organophosphorus
3-Acetamiprid	0.8	Insecticide	neonicotinoid
4-Aldicarb	1.359	Insecticide, nematicide	carbamate
5-Aldicarb Sulfoxide	0.97	Insecticide, nematicide	carbamate
6-Aldicarb Sulphone	1.13	Insecticide, nematicide	carbamate
7-Ametryn	2.63	Herbicide	triazine
8-Aminocarb	1.73	Insecticide	carbamate
9-Anilofos	3.81	Herbicide	organophosphorus
10-Atrazine	2.5	Herbicide	triazine
11-Azinophos-ethyl	3.18	Insecticide	organophosphorus
12-Azinphos-methyl	2.96	Insecticide	organophosphorus

Pesticides	KOW logP	Field of use	Chemical class
13-Azoxystrobin	2.5	Fungicide	methoxyacrylate
14-Benalaxyl	3.54	Fungicide	phenylamide
15-Bendiocarb	1.72	Insecticide	carbamate
16-Bensulfuron-Me	2.45	Herbicide	sulfonylurea
17-Bromuconazole	3.24	Fungicide	triazole
18-Bupirimate	3.9	Fungicide	pyrimidinol
19-Buprofezin	4.3	Insecticide, acaricide	thiadiazines
20-Butachlor	4.5	Herbicide	chloroacetamide
21-Butralin	4.93	Herbicide	dinitroaniline
22-Carbaryl	1.85	Insecticide	carbamate
23-Carbendazim	1.38	Fungicide	benzimidazole
24-Carbofuran	1.52	Insecticide, nematicide	carbamate
25-Carbofuran-3OH	1.1	Insecticide, nematicide	carbamate
26-Carboxin	2.2	Fungicide	oxathiin
27-Chlorfluazuron	5.8	Insecticide	benzoylurea
28-Chlorpyrifos	4.7	Insecticide	organophosphorus
29-Chlorpyrifos-methyl	4.24	Insecticide	organophosphorus
30-Clodinafop-propargyl	3.9	Herbicide	aryloxyphenoxypropionate
31-Clothianidin	5	Insecticide	neonicotinoid
32-Cyanophos	2.65	Insecticide	organophosphorus
33-Cyhalothrin-L	6.9	Insecticide	pyrethroid
34-Cymoxanil	0.59	Fungicide	Unclassified
35-Cyprodinil	3.9	Fungicide	anilinopyrimidine
36-Deltamethrin	4.6	Insecticide	pyrethroid
37-Demeton-S-methylsulphon	-0.47	Insecticide	organophosphorus
38-Diafenthiuron	5.76	Insecticide, acaricide	thiourea
39-Diazinon	3.3	Insecticide, acaricide	organophosphorus
40-Dichlofuanid	3.7	Fungicide	sulphamide
41-Diclorvos	1.16	Insecticide	organophosphorus
42-Difenoconazole	4.2	Fungicide	triazole

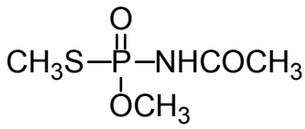
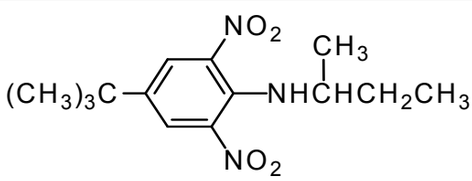
Pesticides	KOW logP	Field of use	Chemical class
43-Diflufenican	4.9	Herbicide	pyridinecarboxamide
44-Dimethoate	0.704	Insecticide, acaricide	organophosphorus
45-Dimethomorph	2.63	Fungicide	cinnamic acid
46-Diniconazole	4.3	Fungicide	triazole
47-Diuron	2.85	Herbicide	urea
48-Edifenphos	3.83	Fungicide	phosphorothiolate
49-Ethion	4.28	Acaricide, insecticide	organophosphorus
50-Ethoprophos	3.59	Nematicide, insecticide	organophosphorus
51-Famoxadone	4.65	Fungicide	oxazolidinedione
52-Fenamiphos	3.3	Nematicide	organophosphorus
53-Fenarimol	3.69	Fungicide	pyrimidine
54-Fenhexamid	3.51	Fungicide	hydroxyanilide
55-Fenoxaprop-P-ethyl	1.83	Herbicide	Aryloxyphenoxy-propionate
56-Fenpropathrin	6	Acaricide, insecticide	pyrethroid
57-Fenpyroximate	5.01	Acaricide	pyrazole
58-Fenthion	4.84	Insecticide	organophosphorus
59-Fipronil	4	Insecticide	phenylpyrazole
60-Flamprop	3.09	Herbicide	Arylalanine
61-Flufenoxuron	4	Insecticide, acaricide	benzoylurea
62-Flumetsulam	0.68	Herbicide	triazolopyrimidine
63-Fluroxypyr	-1.24	Herbicide	pyridinecarboxylic acid
64-Flusilazole	3.74	Fungicide	triazole
65-Flutolanil	3.7	Fungicide	oxathiin
66-Hexaconazole	3.9	Fungicide	triazole
67-Hexythiazox	2.53	Acaricide	thiazolidinone
68-Imazalil	3.82	Fungicide	imidazole
69-Imazamethabenz-methyl	1.54	Herbicide	imidazolinone
70-Imidacloprid	0.57	Insecticide	neonicotinoid
71-Indoxacarb	4.65	Insecticide	oxadiazine
72-Isoprothiolane	3.3	Fungicide	phosphorothiolate

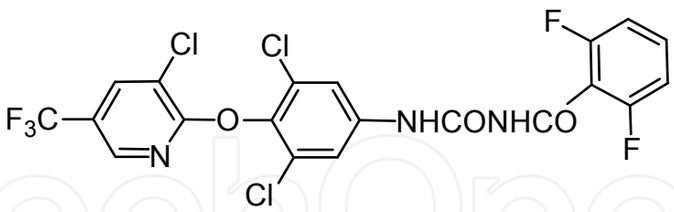
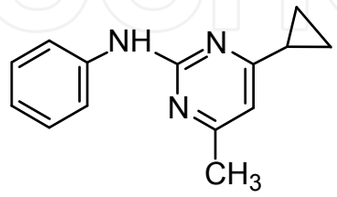
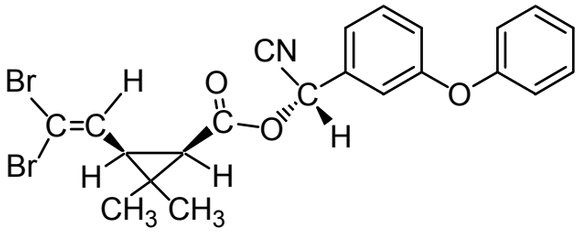
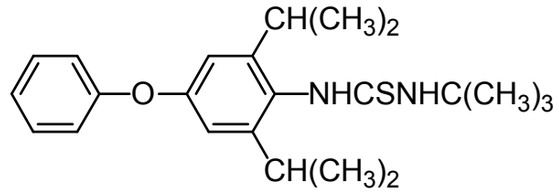
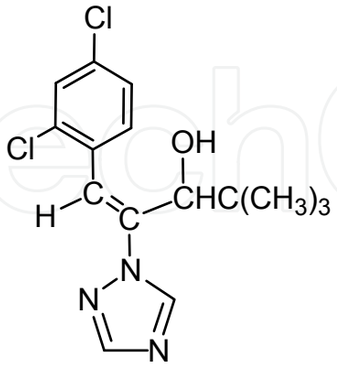
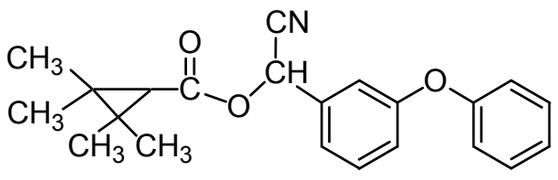
Pesticides	KOW logP	Field of use	Chemical class
73-Isoproturon	2.5	Herbicide	phenyl-urea
74-Linuron	3	Herbicide	phenyl-urea
75-Lufenuron	5.12	Insecticide, acaricide	benzoylurea
76-Malaoxon	2.89	Insecticide, acaricide	organophosphorus
77-Malathion	2.75	Insecticide, acaricide	organophosphorus
78-Metamitron	0.83	Herbicide	triazinone
79-Methamidophos	-0.8	Insecticide, acaricide	organophosphorus
80-Methiocarb	3.08	Molluscicide, insecticide	carbamate
81-Methiocarb Sulfoxid	2.87	Molluscicide, insecticide	carbamate
82-Methiocarb Sulphon	2.95	Molluscicide, insecticide	carbamate
83-Methomyl	0.093	Insecticide, acaricide	carbamate
84-Methoxyfenozide	3.7	Insecticide	diacylhydrazine
85-Metosulam	0.9778	Herbicide	triazolopyrimidine
86-Metribuzin	1.6	Herbicide	triazinone
87-Metsulfuron-methyl	-1.74	Herbicide	sulfonylurea
88-Monocrotophos	-0.22	Insecticide, acaricide	organophosphorus
89-Myclobutanil	2.94	Fungicide	triazole
90-Nuarimol	3.18	Fungicide	pyrimidine
91-Omethoate	-0.74	nsecticide, acaricide	organophosphorus
92-Oxadiargyl	3.95	Herbicide	oxadiazole
93-Oxadiazon	4.91	Herbicide	oxadiazole
94-Oxamyl	-0.44	Insecticide, acaricide	carbamate
95-Oxycarboxin	0.772	Fungicide	oxathiin
96-Oxydemeton-methyl	-0.74	Insecticide	organophosphorus
97-Paraoxon-ethyl	1.98	Insecticide, acaricide	organophosphorus
98-Parathion-ethyl	3.83	Insecticide, acaricide	organophosphorus
99-Penconazole	3.72	Fungicide	triazole
100-Pencycuron	4.68	Fungicide	phenylurea
101-Pendimethalin	5.18	Herbicide	dinitroaniline

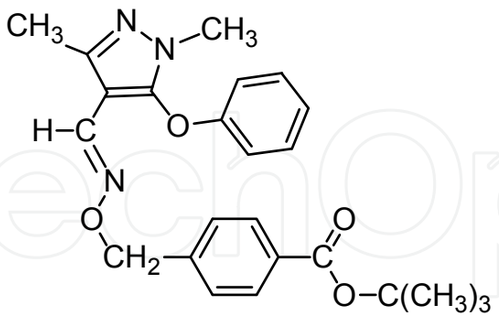
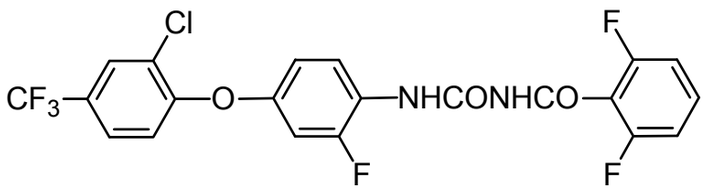
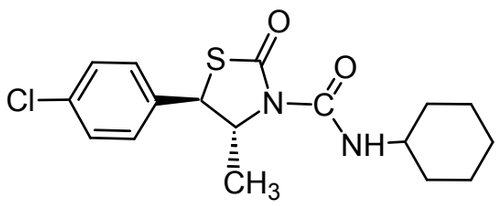
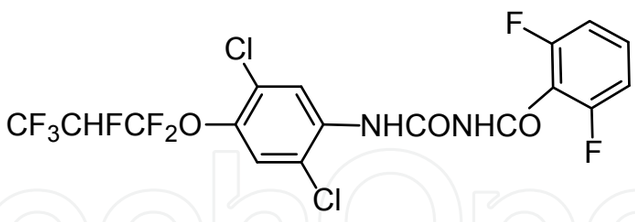
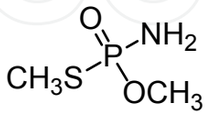
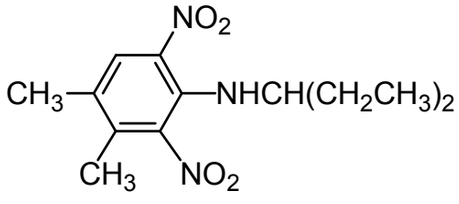
Pesticides	KOW logP	Field of use	Chemical class
102-Phenmedipham	3.59	Herbicide	carbamate
103-Phenthoate	3.69	Insecticide, acaricide	organophosphorus
104-Phosalone	4.01	Insecticide, acaricide	organophosphorus
105-Phosphamidon	0.79	Insecticide, acaricide	organophosphorus
106-Piperonyl butoxide	4.75	Insecticide	hydrocarbone
107-Pirimicarb	1.7	Insecticide	carbamate
108-Pirimiphos-ethyl	5	Insecticide	organophosphorus
109-Pirimiphos-methyl	4.2	Insecticide, acaricide	organophosphorus
110-Prochloraz	4.12	Fungicide	imidazole
111-Profenofos	4.44	Insecticide, acaricide	organophosphorus
112-Promecarb	3.189	Insecticide	carbamate
113-Prometryn	3.1	Herbicide	triazine
114-Propamocarb-HCl	-2.6	Fungicide	carbamate
115-Propargite	3.73	Acaricide	organosulfite
116-Propiconazole	3.72	Fungicide	triazole
117-Propoxur	1.56	Insecticide	carbamate
118-Pymetrozine	-0.18	Insecticide	pyridine
119-Pyrazophos	3.8	Fungicide	phosphorothiolate
120-Pyrazosulfuron-ethyl	1.3	Herbicide	sulfonylurea
121-Pyrethrins	5.9	Insecticide, acaricide	pyrethrin
122-Pyrifenox	3.4	Fungicide	pyridine
123-Pyrimethanil	2.84	Fungicide	anilinopyrimidine
124-Pyriproxyfen	5.37	Insecticide	Unclassified
125-Quizalofop-Et	4.28	Herbicide	Aryloxyphenoxy propionic acid
126-Spinosad-A	2.8	Insecticide	Spinosyns
127-Spinosad-D	3.2	Insecticide	Spinosyns
128-Tebuconazole	3.7	Fungicide	triazole
129-Tebufenozide	4.25	Insecticide	diacylhydrazine
130-Terbuthylazine	3.21	Herbicide	triazine
131-Tetraconazole	3.56	Fungicide	triazole

Pesticides	KOW logP	Field of use	Chemical class
132-Thiabendazole	2.39	Fungicide	benzimidazole
133-Thiacloprid	1.26	Insecticide	neonicotinoid
134-Thiamethoxam	-0.13	Insecticide	neonicotinoid
135-Thifensulfuron-methyl	0.2	Herbicide	sulfonylurea
136-Thiobencarb	3.42	Herbicide	thiocarbamate
137-Thiocyclam-OH	-0.07	Insecticide	Nereistoxin analogues
138-Thiodicarb	1.62	Insecticide, molluscicide	oxime carbamate
139-Thiometon	3.15	Insecticide, acaricide	organophosphorus
140-Thiophanate-methyl	1.5	Fungicide	benzimidazole
141-Tolclofos-methyl	4.56	Fungicide	aromatic hydrocarbon
142-Tolylfluanid	3.9	Fungicide	sulphamide
143-Triadimefon	3.11	Fungicide	triazole
144-Triadimenol	3.08	Fungicide	triazole
145-Triazophos	3.34	Insecticide, acaricide	organophosphorus
146-Triclopyr-butotyl	0.42	Herbicide	pyridinecarboxylic acid
147-Trifloxystrobin	4.5	Fungicide	oximinoacetate
148-Triflumizole	5.06	Fungicide	imidazole
149-Triforine	2.2	Fungicide	piperazine
150-Triticonazole	3.29	Fungicide	triazole

Table 1. Tested pesticides with their KOW logP, field of use and chemical class (British crop protection council 2002).

Pesticides	Structure
1-Acephate KOW logP = -0.89	
2-Butralin less soluble in water	

Pesticides	**Structure**
3-Chlorfluazuron	
4-Cyprodinil	
5-Deltamethrin less soluble in water	
6-Diafenthiuron less soluble in water	
7-Diniconazole less soluble in water	
8-Fenpropathrin KOW logP = 6 less soluble in water	

Pesticides	**Structure**
9-Fenpyroximate	
10-Flufenoxuron Solubility In water 7 10-11 g/l	
11-Hexythiazox	
12-Lufenuron	
13-Methamidophos Solubility In water >200 g/l KOW logP = -0.8	
14-Pendimethalin	

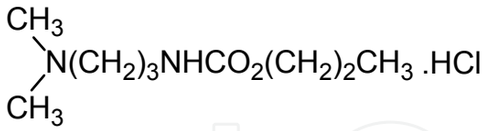
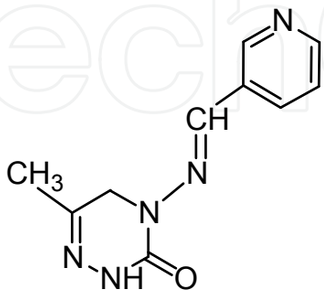
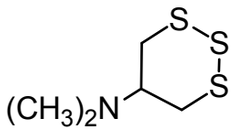
Pesticides	Structure
15-Propamocarb-HCl KOW logP = -2.6	
16- Pymetrozine	
17-Thiocyclam-HO KOW logP = -0.07	

Table 2. Chemical structure of seventeen selected pesticides:

2.8 Risk / safety assessment

The insecticide residues concentrations found in the analyzed potatoes were compared with the tolerance limits established by Codex Alimentarius Commission and the Egyptian Organization for Standardization and Quality Control (EOS), respectively. The dietary intake of insecticides was estimated and compared with the WHO-ADIs, (Tomlin, 2004) as cited by Mansour et al., (2009) as follow:

Estimated dose (mg/kg) = Residues (mg/kg) Food item x daily potato consumption (kg) / Body weight (kg)

3. Analysis methods of pesticides residues

3.1 LC-MS/MS

3.1.1 LC-MS/MS analysis

Separation was performed on a C18 column ZORBAX Eclipse XDB-C18 4.6 mm x 150 mm, 5 µm particle size .The injection volume was 25 µl .A gradient elution program was at 0.3 ml/min flow, in which one reservoir contained 10 mM ammonium format solution in methanol-water 1:9 and the other contained methanol .The ESI source was used in the positive mode, and N2 nebulizer, curtain, and other gas settings were optimized according to recommendations made by the manufacturer; source temperature was 300^{o}C, ion spray potential 5500 V, decluster potential and collision energy were optimized using A Harvard Apparatus syringe pump by introducing individual pesticide solutions into the MS instrument to allow optimization of the MS/MS conditions. The Multiple Reaction monitoring mode MRM was used in which one MRM was used for quantitation and other was used for confirmation .

3.2 GC - measurements with different detectors

3.2.1 GC-NPD parameter

GC-NPD analyses were run on HP 6890 series gas chromatograph equipped with nitrogen phosphorous detector (NPD). Data acquisition, processing, and instrumental control were performed by the Agilent ChemStation software. A split/split less (S/Sl) inlet was used with 1.8 mm id liner. Analytes were separated in an Agilent HP-Pass 5 capillary column, 25 m length ,0.32 mm id, 0.52 μm film thickness. The inlet operating temperature is 225 °C, injection volume 1 μL. The nitrogen carrier gas flow was maintained at a constant flow of 1.3 ml/minute. N_2 make up gas flow rate 8 ml/minute for the NPD and H_2 with flow rate of 4.5 ml/minute. The oven temperature program was 90 °C for 2 minute, programmed to 150 °C at 20 °C/minute, and then to 270 °C at 6 °C/minute, it was kept at this temperature for 15 minute. Detector temperature was maintained at 280 °C with H_2 flow of 3.5 ± 0.1 ml/minute and air flow of 100-120 ml/minute.

3.2.2 GC-ECD parameter

GC-ECD analyses were run on HP 6890 series gas chromatograph equipped with electron capture detector (ECD). Data acquisition, processing, and instrumental control were performed by the Agilent ChemStation software. A split/split-less (S/Sl) inlet was used with 1.8 mm id liner. Analyte samples were separated in an Agilent HP-Pass 5 capillary column, 25 m length, 0.32 mm id, and 0.52 μm film thicknesses. The inlet operating temperature is 225 °C, injection volume 1 μl. The nitrogen carrier gas flow was maintained at a constant flow of 1.3 ml/minute. The oven temperature program was 90 °C for 2 minute, programmed to 150 °C at 20 °C/minute, and then to 270 °C at 6 °C/minute, it was kept at this temperature for 15 minute. Detector temperature was maintained at 300 °C.

3.2.3 GC-MSD parameter

GC-MSD analyses were run on an Agilent 7890 series gas chromatograph (Agilent Technologies, Santa Clara, CA) interfaced to an Agilent 5975 mass selective detector (MSD). Data acquisition, processing, and instrumental control were performed by the Agilent MSD ChemStation software (E.0200.493 version). A split/split less (S/Sl) inlet was used with 1.8 mm id liner. Analyte samples were separated in an Agilent HP-5MS capillary column (5% biphenyl/95% dimethylsiloxane), 30 m, 0.25 mm id, 0.25 μm film thickness. The inlet operating conditions were injection volume, 1 μl, flow rate 1.3 ml/minute; the temperature program was set at 79 °C for 0.25 minute, programmed to 300 °C at 10 °C/minute, and kept at this temperature for 2 minute. The helium carrier gas flow was maintained at a constant pressure of 17.296 psi. The oven temperature program was 70 °C for 1 minute, programmed to 150 °C at 50 °C/minute, then to 200 °C at 6 °C/min, and finally to 280 °C at 16 °C/minute; it was kept at this temperature for 5 minute. Electron impact mass spectra in the full-scan mode were obtained at 70 eV; the monitoring was from m/z 50 to 400. The ion source and quadrupole analyzer temperatures were fixed at 230 and 150 °C, respectively.

4. Results and discussion

4.1 Recovery tests on grapes

The method recoveries for 150 pesticides were tested by performing 6 replicates of spike grapes at different concentration levels; 0.01, 0.05 and 0.1 mg/kg. The average recoveries and relative standard deviation on each level were calculated (Table 3). The precursor ion,

product ion (1) and product ion (2) and retention time will be included in the tables. The injection of 25 µl of acetonitrile into LC system leads to non-symmetrical peak shapes, so that acetonitrile was evaporated and re-dissolved in methanol-water solution. This step improved the pesticide peak shapes and lowered the matrix effect due to precipitation of some insoluble substances. The recovery of most pesticides (143 pesticides) is in the range 70%-110%. The recoveries of 7 pesticides (Chlorfluazuron, L- Cyhalothrin, Deltamethrin, Diafenthiuron, Flufenoxuron, Lufenuron and Pymetrozine) are lower than 70% due to the evaporation of acetonitrile and re- dissolving in methanol-water solution as reported by Afify et al., (2010). The conclusions stated that the proposed method using acetonitrile extraction followed by LC-MS/MS determination is simple, rapid and reliable satisfactory recoveries and repeatability observed .The described method requires little amount of solvents and sample and could be used in controlling levels of pesticides from different classes in natural products samples.

						0.01 mg/kg		0.05 mg/kg		0.1mg/kg	
No.	**Pesticide**	**RT**	**Precursor ion**	**Product ion (1)**	**Product ion (2)**	**Mean Rec.%**	**CV%**	**Mean Rec.%**	**CV%**	**Mean Rec.%**	**CV%**
1	Abamectin	27.5	890.5	305.3	143.0	94%	13%	76%	25%	85%	17%
2	Acephate	10.8	184.0	143.0	126.0	85%	4%	91%	12%	70%	10%
3	Acetamiprid	16.5	223.2	126.0	86.2	97%	5%	95%	7%	83%	3%
4	Aldicarb	12.3	208.2	116.0	186.0	86%	7%	90%	5%	68%	11%
5	Aldicarb Sulfoxide	12.8	207.3	132.0	163.1	88%	6%	97%	10%	81%	5%
6	Aldicarb Sulphone	18.6	223.1	86.2	116.0	66%	27%	77%	18%	85%	14%
7	Ametryn	22.2	228.0	186.0	152.1	93%	4%	99%	8%	84%	3%
8	Aminocarb	16.3	209.0	152.1	198.9	87%	2%	85%	8%	74%	3%
9	Anilofos	23.3	367.9	198.9	174.0	84%	1%	98%	6%	86%	4%
10	Atrazine	21.2	216.1	174.0	132.0	88%	4%	98%	13%	87%	3%
11	Azinophos Ethyl	22.8	346.3	132.0	132.0	85%	6%	94%	18%	70%	8%
12	Azinphos Methyl	21.5	318.0	132.0	372.0	78%	9%	92%	17%	85%	12%
13	Azoxystrobin	21.2	404.0	372.0	148.1	100%	6%	98%	9%	89%	4%
14	Benalaxyl	23.7	326.3	148.1	167.0	98%	3%	107%	9%	79%	4%
15	Bendiocarb	19.5	224.0	167.0	149.0	96%	3%	96%	9%	92%	4%
16	Bensulfuron Methyl	21.2	411.0	149.0	159.0	89%	2%	95%	14%	90%	7%
17	Bromuconazole	22.7	378.0	159.0	166.2	91%	2%	101%	9%	87%	4%
18	Bupirimate	23.2	317.0	166.2	201.0	93%	5%	93%	8%	87%	3%
19	Buprofezine	25.1	306.2	201.0	238.0	95%	7%	76%	13%	82%	6%
20	Butachlor	25.5	312.3	238.0	240.1	73%	8%	89%	8%	69%	7%
21	Butralin	26.7	296.0	240.1	145.1	72%	3%	71%	5%	74%	6%
22	Carbaryl	20.0	202.1	145.1	160.1	99%	3%	99%	10%	91%	5%
23	Carbendazim	17.1	192.1	160.1	165.0	87%	2%	85%	8%	74%	3%
24	Carbofuran	19.6	222.1	165.0	142.9	96%	2%	94%	11%	88%	4%
25	Carbofuran-3OH	16.7	238.3	163.1	209.2	90%	3%	98%	4%	86%	6%
26	Carboxin	20.1	236.0	142.9	158.0	102%	5%	92%	11%	88%	8%
27	Chlorfluazuron	25.7	540.0	158.0	197.9	52%	10%	34%	11%	58%	11%
28	Chlorpyrifos	26.0	349.9	197.9	124.9	86%	3%	82%	5%	79%	5%
29	Chlorpyrifos Methyl	24.6	322.0	124.9	105.0	99%	6%	84%	5%	74%	6%

						0.01 mg/kg		0.05 mg/kg		0.1mg/kg	
No.	**Pesticide**	**RT**	**Precursor ion**	**Product ion (1)**	**Product ion (2)**	**Mean Rec.%**	**CV%**	**Mean Rec.%**	**CV%**	**Mean Rec.%**	**CV%**
30	Clodinafop propargyl	23.1	350.0	266.0	169.0	89%	2%	92%	8%	88%	3%
31	Clothianidin	15.9	250.0	169.0	124.9	95%	5%	92%	8%	90%	5%
32	Cyanophos	21.5	261.2	124.9	128.0	91%	7%	106%	11%	87%	7%
33	Cyhalothrin-L	25.5	467.2	225.0	141.0	68%	11%	36%	23%	65%	10%
34	Cymoxanil	17.5	199.2	128.0	93.0	88%	2%	94%	10%	82%	4%
35	Cyprodinil	24.0	226.0	93.0	169.1	88%	3%	88%	2%	80%	2%
36	Deltamethrin	26.3	523.2	281.1	181.1	46%	13%	29%	22%	65%	8%
37	Demeton-S-ethylsulphon	14.1	263.0	169.1	329.1	89%	3%	90%	10%	86%	5%
38	Diafenthiuron	26.0	385.0	329.1	169.1	26%	21%	17%	63%	38%	25%
39	Diazinon	23.8	305.1	169.1	109.0	86%	4%	108%	7%	81%	4%
40	Dichlofuanid	22.6	350.1	224.0	123.0	83%	6%	90%	9%	86%	4%
41	Diclorovs	19.5	221.0	109.0	251.0	90%	8%	96%	13%	84%	5%
42	Difenoconazole	24.0	406.0	251.0	266.0	90%	5%	98%	7%	76%	6%
43	Diflufenican	24.0	395.1	266.0	199.0	86%	3%	76%	6%	83%	2%
44	Dimethoate	16.8	230.0	199.0	301.0	96%	2%	94%	9%	86%	3%
45	Dimethomorph	22.1	388.0	301.0	70.0	90%	5%	95%	10%	95%	5%
46	Diniconazole	24.2	326.1	70.0	72.0	88%	3%	92%	7%	80%	3%
47	Diuron	21.3	233.2	72.0	283.0	92%	2%	95%	9%	88%	5%
48	Edifenophos	23.5	311.0	283.0	171.0	88%	2%	99%	8%	85%	5%
49	Ethion	25.2	385.0	171.0	131.0	86%	4%	89%	6%	80%	5%
50	Ethoprophos	23.1	243.1	131.0	141.0	94%	4%	99%	8%	85%	5%
51	Famoxadone	23.2	392.0	331.0	217.0	99%	10%	91%	7%	84%	1%
52	Fenamiphos	23.0	304.1	217.0	268.1	92%	5%	95%	10%	88%	4%
53	Fenarimol	22.8	331.1	268.1	97.0	94%	3%	93%	10%	87%	5%
54	Fenhexamid	22.6	302.0	97.0	288.1	93%	2%	98%	11%	86%	8%
55	Fenoxap-p-ethyl	24.7	362.0	288.1	366.0	96%	12%	86%	2%	81%	3%
56	Fenpropathrin	25.9	350.2	125.1	97.0	76%	6%	67%	5%	76%	6%
57	Fenpyroximate	26.6	422.0	366.0	368.0	78%	7%	70%	7%	70%	11%
58	Fenthion	23.7	279.2	247.1	169.0	91%	7%	100%	6%	81%	7%
59	Fipronil	22.6	454.0	368.0	105.1	94%	9%	102%	12%	85%	5%
60	Flamprop	21.0	321.9	105.1	129.0	87%	6%	90%	14%	83%	6%
61	Flufenoxuron	24.9	489.1	158.0	129.0	64%	8%	40%	14%	57%	9%
62	Flumetesulam	15.8	326.2	129.0	208.9	95%	4%	100%	8%	90%	6%
63	Fluroxypyr	17.4	255.2	208.9	165.1	92%	8%	106%	19%	84%	8%
64	Flusilazole	22.8	316.1	165.1	262.0	89%	4%	97%	9%	87%	4%
65	Flutolanil	21.9	324.0	262.0	70.0	89%	2%	98%	9%	80%	4%
66	Hexaconazole	23.8	314.0	70.0	228.0	91%	4%	90%	9%	79%	5%
67	Hexythiazox	25.9	353.1	228.0	144.2	88%	3%	85%	3%	72%	6%
68	Imazalil	22.1	297.0	159.0	201.0	85%	4%	92%	6%	83%	5%
69	Imazamethabenz Methyl	19.7	289.0	144.2	99.0	92%	2%	94%	8%	85%	5%
70	Imidacloprid	15.8	256.2	209.2	203.1	97%	3%	96%	5%	80%	6%

						0.01 mg/kg		0.05 mg/kg		0.1mg/kg	
No.	Pesticide	RT	Precursor ion	Product ion (1)	Product ion (2)	Mean Rec.%	CV%	Mean Rec.%	CV%	Mean Rec.%	CV%
71	Indoxacarb	23.7	528.0	203.1	189.0	90%	4%	92%	13%	95%	9%
72	Isoprothiolane	22.5	291.0	189.0	72.0	95%	2%	96%	6%	86%	2%
73	Isoproturon	21.3	207.3	72.0	182.1	94%	6%	97%	7%	86%	3%
74	Linuron	22.2	249.1	182.1	158.0	94%	3%	95%	7%	80%	2%
75	Lufenuron	24.3	511.0	158.0	99.0	83%	11%	45%	22%	61%	10%
76	Malaoxon	19.6	315.1	99.0	220.2	95%	3%	100%	5%	87%	3%
77	Malathion	22.2	331.0	99.0	185.0	92%	4%	105%	9%	91%	4%
78	Metamitron	17.1	203.1	175.1	94.0	90%	10%	101%	7%	78%	4%
79	Methamidophos	9.8	142.2	94.0	122.0	96%	18%	83%	3%	66%	5%
80	Methiocarb	15.9	243.0	169.1	88.0	91%	2%	93%	10%	88%	4%
81	Methiocarb Sulfoxid	16.8	242.1	185.0	122.0	91%	3%	99%	3%	85%	3%
82	Methiocarb Sulphon	22.2	275.1	122.0	169.1	91%	4%	91%	4%	70%	8%
83	Methomyl	14.2	163.2	88.0	149.0	90%	5%	90%	2%	82%	4%
84	Methoxyfenozide	22.2	369.0	149.0	175.0	92%	5%	97%	9%	80%	3%
85	Metosulam	19.4	418.0	175.0	187.1	96%	7%	92%	5%	89%	5%
86	Metribuzin	20.1	215.2	187.1	167.2	94%	4%	96%	5%	86%	3%
87	Metsulfuron Methyl	18.8	382.3	167.2	127.0	96%	4%	100%	7%	90%	7%
88	Monocrotophos	14.7	224.0	127.0	70.0	89%	2%	97%	2%	84%	3%
89	Myclobutanil	22.4	289.0	70.0	252.0	94%	2%	95%	7%	88%	3%
90	Nuarimol	22.0	315.0	252.0	183.0	93%	4%	102%	7%	80%	4%
91	Omethoate	11.8	214.0	183.0	223.1	85%	3%	94%	5%	76%	4%
92	Oxadiargyl	23.7	340.8	223.1	303.0	91%	3%	90%	8%	91%	6%
93	Oxadiazon	25.4	345.3	303.0	175.0	86%	7%	94%	15%	82%	7%
94	Oxamyl	13.0	237.0	72.0	88.1	96%	3%	95%	11%	85%	8%
95	Oxycarboxin	17.5	268.0	175.0	169.0	94%	1%	99%	5%	87%	4%
96	Oxydemeton Methyl	13.8	247.0	169.0	219.9	85%	2%	96%	6%	79%	5%
97	Paraoxon Ethyl	20.7	276.2	219.9	235.9	96%	2%	100%	6%	84%	3%
98	Parathion Ethyl	23.4	292.2	235.9	159.0	109%	14%	82%	19%	90%	16%
99	Penconazole	23.7	284.0	159.0	125.0	91%	5%	92%	8%	89%	5%
100	Pencycuron	24.1	329.1	125.0	168.1	91%	3%	90%	7%	90%	5%
101	Pendimethalin	26.5	282.1	212.0	194.0	83%	4%	76%	17%	82%	5%
102	Phenmedipham	21.2	301.3	168.1	247.2	95%	5%	90%	8%	90%	3%
103	Phenthoate	23.4	321.0	247.2	182.0	90%	4%	95%	10%	86%	2%
104	Phosalone	23.9	368.0	182.0	174.0	93%	2%	95%	10%	90%	5%
105	Phosphamidon	18.9	300.1	174.0	177.0	96%	3%	98%	7%	82%	2%
106	Piperonyl butoxid	25.9	356.0	177.0	182.3	92%	2%	95%	11%	78%	17%
107	Pirimicarb	21.0	239.2	182.3	198.0	94%	2%	98%	3%	81%	4%
108	Pirimiphos Ethyl	25.6	334.1	198.0	164.0	94%	5%	96%	13%	80%	12%
109	Pirimiphos Methyl	24.4	306.2	164.0	308.0	94%	4%	109%	12%	80%	11%
110	Prochloraz	24.1	376.0	308.0	302.9	92%	5%	92%	11%	88%	8%

						0.01 mg/kg		0.05 mg/kg		0.1mg/kg	
No.	**Pesticide**	**RT**	**Precursor ion**	**Product ion (1)**	**Product ion (2)**	**Mean Rec.%**	**CV%**	**Mean Rec.%**	**CV%**	**Mean Rec.%**	**CV%**
111	Profenofos	25.1	373.0	302.9	109.1	103%	15%	98%	13%	76%	21%
112	Promecarb	22.4	208.0	109.1	158.0	98%	3%	97%	5%	82%	3%
113	Prometryn	23.2	242.0	158.0	102.0	93%	2%	94%	8%	85%	3%
114	Propamocarb	12.2	189.0	102.0	175.1	82%	3%	88%	4%	71%	7%
115	Propargite	26.0	368.1	175.1	159.0	85%	3%	76%	17%	82%	13%
116	Propiconazole	23.8	342.1	159.0	111.1	95%	3%	92%	9%	83%	6%
117	Propoxur	19.7	210.1	111.1	105.1	90%	2%	103%	6%	84%	3%
118	Pymetrozine	13.6	218.2	105.1	222.0	40%	2%	56%	4%	30%	12%
119	Pyrazophos	24.2	374.0	222.0	182.1	99%	3%	86%	10%	89%	5%
120	Pyrazosulfroun Ethyl	22.5	415.3	182.1	161.1	91%	2%	79%	22%	88%	14%
121	Pyrethrins	26.4	329.1	161.1	93.0	84%	2%	84%	20%	82%	6%
122	Pyrifenox	23.4	295.0	93.0	107.2	90%	3%	92%	6%	78%	6%
123	Pyrimethanil	22.7	200.1	107.2	96.0	89%	3%	95%	7%	86%	3%
124	Pyriproxyfen	25.9	322.2	96.0	299.0	84%	4%	79%	15%	81%	16%
125	Quizalofop Ethyl	25.2	373.0	299.0	178.4	88%	9%	111%	14%	78%	19%
126	Spinosad-A	23.4	732.0	142.0	142.0	96%	2%	88%	11%	83%	4%
127	Spinosad-D	24.1	746.0	142.0	70.1	87%	10%	97%	10%	78%	5%
128	Tebuconazole	23.6	308.0	70.1	133.0	90%	2%	91%	11%	91%	6%
129	Tebufenozide	23.1	353.0	133.0	174.0	92%	5%	95%	13%	90%	7%
130	Terbuthialzine	22.5	230.0	174.0	159.0	92%	2%	96%	8%	87%	3%
131	Tetraconazole	22.6	372.0	159.0	175.0	97%	4%	96%	10%	83%	2%
132	Thiabendazole	18.5	202.1	175.0	126.0	79%	4%	101%	1%	75%	5%
133	Thiacloprid	17.4	253.2	126.0	211.0	93%	6%	96%	6%	84%	4%
134	Thiamethoxam	14.4	292.0	211.0	167.0	94%	4%	98%	4%	78%	5%
135	Thifensulfuron Methyl	18.6	388.2	167.0	125.0	95%	1%	105%	10%	87%	4%
136	Thiobencarb	24.5	258.3	125.0	137.1	87%	3%	101%	10%	83%	8%
137	Thiocyclam	11.1	182.0	137.1	151.0	73%	7%	73%	5%	50%	5%
138	Thiodicarb	19.8	355.1	88.1	108.0	79%	24%	96%	12%	91%	6%
139	Thiometon	23.3	247.1	88.9	169.0	85%	25%	103%	22%	88%	14%
140	Thiophanate Methyl	19.4	343.0	151.0	175.0	80%	13%	99%	8%	82%	5%
141	Tolclophos Methyl	24.3	301.1	175.0	238.0	89%	7%	91%	13%	88%	10%
142	Tolylfluanid	23.4	364.0	238.0	197.0	88%	4%	95%	13%	90%	6%
143	Triadimifon	22.6	294.1	197.0	162.0	98%	3%	94%	8%	85%	1%
144	Triadiminol	22.6	296.1	70.0	277.0	87%	3%	98%	13%	88%	7%
145	Triazophos	22.6	314.2	162.0	155.0	93%	3%	94%	7%	88%	2%
146	Triclopyr Butatyl	25.5	356.2	237.7	186.0	89%	3%	84%	15%	83%	9%
147	Trifloxystrobin	24.1	409.0	186.0	278.0	93%	2%	94%	8%	89%	5%
148	Triflumizole	24.4	346.3	278.0	387.8	90%	5%	95%	12%	76%	17%
149	Triforine	21.3	432.4	387.8	70.0	103%	8%	92%	8%	87%	6%
150	Triticonazole	23.0	318.3	70.0	567.4	89%	3%	92%	7%	83%	5%

Table 3. Recovery tests at different concentration levels on grapes sample

4.2 Recovery tests on green beans

The optimized LC-MS/MS parameters and the best extraction procedures (QuEChERS) were used to study the method performance by carrying out recovery tests of pesticides at different levels on green beans samples. Six replicates of recovery tests were done at concentration levels 0.01 mg/kg, 0.05 mg/kg and 0.1 mg/kg on grapes and green beans, Table(4).

Pesticides	Green beans			Pesticides	Green beans		
	0.01 mg/kg (%)	0.05 mg/kg (%)	0.1 mg/kg (%)		0.01 mg/kg (%)	0.05 mg/kg (%)	0.1 mg/kg (%)
Abamectin	74 ±20	104 ±11	68 ±13	Malaoxon	89 ±15	93 ±5	87 ±4
Acephate	94 ±19	75 ±6	78 ±5	Malathion	95 ±18	97 ±3	81 ±6
Acetamiprid	91 ±12	92 ±4	78 ±3	Metamitron	85 ±14	92 ±6	84 ±3
Aldicarb	90 ±14	91 ±4	77 ±6	Methamidophos	73 ±18	78 ±3	77 ±2
Aldicarb Sulfoxide	81 ±31	84 ±6	83 ±3	Methiocarb	80 ±15	98 ±9	91 ±7
Aldicarb Sulphone	98 ±14	90 ±4	83 ±3	Methiocarb Sulfoxid	93 ±12	90 ±3	77 ±3
Ametryn	96 ±13	96 ±2	78 ±4	Methiocarb Sulphon	89 ±16	91 ±5	86 ±3
Aminocarb	86 ±9	85 ±4	73 ±4	Methomyl	95 ±22	90 ±5	89 ±3
Anilofos	84 ±12	92 ±1	77 ±4	Methoxyfenozide	82 ±21	102 ±1	83 ±4
Atrazine	91 ±15	92 ±4	80 ±3	Metosulam	87 ±12	93 ±4	86 ±4
Azinophos-ethyl	85 ±16	97 ±10	82 ±6	Metribuzin	98 ±25	86 ±6	81 ±4
Azinphos-methyl	97 ±10	106 ±9	79 ±8	Metsulfuron-methyl	85 ±20	104 ±3	87 ±4
Azoxystrobin	98 ±14	96 ±4	78 ±2	Monocrotophos	89 ±22	87 ±5	87 ±4
Benalaxyl	95 ±14	93 ±3	76 ±4	Myclobutanil	92 ±17	95 ±5	85 ±4
Bendiocarb	93 ±11	91 ±3	78 ±2	Nuarimol	78 ±20	89 ±4	81 ±2
Bensulfuron-Me	105 ±14	92 ±4	79 ±3	Omethoate	82 ±24	81 ±5	84 ±2
Bromuconazole	87 ±12	90 ±6	81 ±4	Oxadiargyl	75 ±16	84 ±4	77 ±7
Bupirimate	85 ±11	93 ±4	75 ±4	Oxadiazon	71 ±17	85 ±5	73 ±10
Buprofezin	98 ±18	94 ±4	74 ±6	Oxamyl	99 ±16	89 ±5	80 ±4
Butachlor	81 ±17	110 ±5	66 ±13	Oxycarboxin	87 ±17	92 ±5	87 ±2
Butralin	63 ±19	68 ±7	79 ±6	Oxydemeton-methyl	91 ±24	83 ±3	86 ±4
Carbaryl	93 ±13	93 ±2	79 ±2	Paraoxon-ethyl	89 ±15	91 ±3	86 ±4
Carbendazim	89 ±11	90 ±3	74 ±3	Parathion-ethyl	88 ±36	118 ±12	80 ±20
Carbofuran	99 ±16	93 ±2	80 ±2	Penconazole	78 ±16	89 ±3	85 ±5
Carbofuran-3OH	90 ±15	87 ±5	87 ±4	Pencycuron	71 ±16	84 ±2	81 ±7
Carboxin	76 ±15	67 ±18	73 ±5	Pendimethalin	61 ±19	74 ±1	62 ±11
Chlorfluazuron	24 ±17	30 ±5	24 ±16	Phenmedipham	88 ±18	88 ±2	86 ±3
Chlorpyrifos	65 ±15	76 ±6	68 ±4	Phenthoate	87 ±19	91 ±4	86 ±5
Chlorpyrifos-methyl	80 ±16	82 ±10	77 ±3	Phosalone	77 ±19	82 ±3	80 ±9

Pesticides	Green beans			Pesticides	Green beans		
	0.01 mg/kg (%)	0.05 mg/kg (%)	0.1 mg/kg (%)		0.01 mg/kg (%)	0.05 mg/kg (%)	0.1 mg/kg (%)
Clodinafop-propargyl	82 ±13	90 ±3	68 ±4	Phosphamidon	90 ±15	94 ±5	93 ±7
Clothianidin	93 ±12	85 ±4	82 ±3	Piperonyl butoxide	77 ±23	90 ±2	85 ±4
Cyanophos	95 ±11	94 ±8	72 ±6	Pirimicarb	91 ±18	97 ±3	89 ±1
Cyhalothrin-L	25 ±27	32 ±12	31 ±17	Pirimiphos-ethyl	84 ±18	99 ±3	87 ±4
Cymoxanil	97 ±12	88 ±4	79 ±4	Pirimiphos-methyl	83 ±19	113 ±8	88 ±6
Cyprodinil	80 ±10	89 ±3	68 ±4	Prochloraz	86 ±18	87 ±2	72 ±11
Deltamethrin	23 ±22	29 ±5	24 ±14	Profenofos	75 ±19	85 ±4	82 ±5
Demeton-S-methylsulphon	95 ±15	94 ±4	83 ±5	Promecarb	88 ±20	95 ±3	87 ±4
Diafenthiuron	11 ±30	6 ±35	42 ±64	Prometryn	83 ±16	91 ±3	83 ±4
Diazinon	96 ±15	118 ±4	82 ±4	Propamocarb-HCl	81 ±23	73 ±5	81 ±3
Dichlofuanid	87 ±16	69 ±12	53 ±4	Propargite	78 ±19	71 ±4	64 ±12
Diclorvos	113 ±19	69 ±16	79 ±6	Propiconazole	78 ±25	89 ±3	86 ±4
Difenoconazole	86 ±17	86 ±4	74 ±4	Propoxur	87 ±15	92 ±4	86 ±3
Diflufenican	64 ±12	79 ±4	68 ±5	Pymetrozine	59 ±28	68 ±8	56 ±5
Dimethoate	96 ±14	91 ±4	77 ±3	Pyrazophos	73 ±20	91 ±3	83 ±5
Dimethomorph	99 ±13	94 ±4	85 ±24	Pyrazosulfuron-ethyl	82 ±32	96 ±2	85 ±14
Diniconazole	92 ±14	90 ±3	80 ±7	Pyrethrins	62 ±20	86 ±4	61 ±15
Diuron	100 ±10	95 ±3	77 ±2	Pyrifenox	96 ±9	95 ±4	78 ±8
Edifenphos	82 ±11	92 ±3	77 ±4	Pyrimethanil	87 ±17	87 ±3	81 ±4
Ethion	64 ±16	79 ±5	68 ±4	Pyriproxyfen	65 ±17	80 ±1	71 ±8
Ethoprophos	86 ±31	91 ±6	80 ±7	Quizalofop-Et	72 ±17	85 ±3	74 ±7
Famoxadone	68 ±14	79 ±6	69 ±6	Spinosad-A	78 ±17	84 ±5	61 ±13
Fenamiphos	81 ±9	86 ±5	72 ±3	Spinosad-D	87 ±6	92 ±8	69 ±11
Fenarimol	78 ±14	90 ±5	77 ±4	Tebuconazole	76 ±21	95 ±13	84 ±9
Fenhexamid	88 ±7	88 ±7	58 ±5	Tebufenozide	88 ±17	89 ±6	90 ±7
Fenoxaprop-P-ethyl	112 ±20	81 ±6	117 ±17	Terbuthylazine	82 ±21	93 ±2	86 ±4
Fenpropathrin	48 ±17	60 ±7	52 ±7	Tetraconazole	92 ±20	93 ±3	87 ±5
Fenpyroximate	66 ±19	65 ±6	60 ±8	Thiabendazole	86 ±16	92 ±6	81 ±3
Fenthion	82 ±16	88 ±9	67 ±4	Thiacloprid	84 ±16	90 ±4	82 ±3
Fipronil	99 ±13	87 ±6	85 ±7	Thiamethoxam	87 ±21	84 ±4	91 ±4
Flamprop	86 ±13	84 ±5	76 ±3	Thifensulfuron-methyl	86 ±20	99 ±3	89 ±2
Flufenoxuron	29 ±17	39 ±7	34 ±15	Thiobencarb	77 ±20	86 ±3	87 ±4
Flumetsulam	105 ±14	91 ±4	78 ±5	Thiocyclam-OH	75 ±23	66 ±4	68 ±4

Pesticides	Green beans			Pesticides	Green beans		
	0.01 mg/kg (%)	0.05 mg/kg (%)	0.1 mg/kg (%)		0.01 mg/kg (%)	0.05 mg/kg (%)	0.1 mg/kg (%)
Fluroxypyr	104 ±20	88 ±9	77 ±5	Thiodicarb	95 ±12	96 ±2	73 ±3
Flusilazole	92 ±14	92 ±6	75 ±4	Thiometon	115 ±35	90 ±19	86 ±12
Flutolanil	94 ±12	99 ±4	76 ±3	Thiophanate-methyl	71 ±35	83 ±19	79 ±30
Hexaconazole	97 ±16	91 ±4	78 ±4	Tolclofos-methyl	84 ±18	82 ±6	76 ±8
Hexythiazox	68 ±16	74 ±5	79 ±5	Tolylfluanid	76 ±21	89 ±6	80 ±9
Imazalil	113 ±20	98 ±4	74 ±3	Triadimefon	84 ±19	89 ±3	87 ±3
Imazamethabenz-methyl	98 ±14	90 ±2	78 ±3	Triadimenol	97 ±23	87 ±8	80 ±4
Imidacloprid	85 ±16	93 ±5	85 ±4	Triazophos	88 ±19	92 ±2	89 ±2
Indoxacarb	70 ±22	90 ±3	74 ±10	Triclopyr-butotyl	68 ±16	84 ±2	77 ±8
Isoprothiolane	86 ±18	94 ±2	90 ±3	Trifloxystrobin	73 ±19	86 ±2	85 ±6
Isoproturon	90 ±17	90 ±3	90 ±2	Triflumizole	93 ±21	85 ±4	76 ±8
Linuron	77 ±16	94 ±3	88 ±4	Triforine	53 ±18	87 ±10	76 ±39
Lufenuron	43 ±18	55 ±18	42 ±18	Triticonazole	85 ±19	89 ±3	84 ±4

Table 4. Recovery tests on green beans samples at 0.01 mg/kg, 0.05 mg/kg and 0.1 mg/kg.

The results in Table (4) showed that the 150 pesticides could be determined at concentration 0.01 mg/kg with accepted recovery and precision. The recovery of most pesticides (143 pesticides) is in the range 60%-120%, as cited for grapes. The recoveries of the same 7 pesticides (Chlorfluazuron, L-Cyhalothrin, Deltamethrin, Diafenthiuron, Flufenoxuron, Lufenuron and Pymetrozine) are lower than 60% due to the evaporation of acetonitrile and re-dissolving in methanol-water solution mixture as approved in the recovery tests of pesticides in grapes(Afify et al ., 2010) .0n the other hand recovery test of some pesticides exceeds 100 at concentration at 0.01 mg/kg (Flumetsulam, Fluroxypyr Imazalil), at concentration of 0.05 mg/kg (Butachlor, Pirimiphos-methyl, Metsulfuron-methyl, Parathion-ethyl, Methoxyfenozide) and at concentration of 0.1 mg/kg (Fenoxaprop-P-ethyl).

5. Optimization of sample extraction

Different types of extraction procedures were tested as described in materials and methods using three method (e.g. Luke, QuEChERS and ethyl acetate according to Luke et al. (1975), Anastassiades et al., (2008): and Banerjee et al., (2007). Extraction was done on green beans sample at spiking level of 0.5 mg/kg .

Blank samples, standard in solvent and standard in matrix were injected in parallel to spike samples and in the same run. Due to suppression effect of these types of matrices (decreasing in signal intensity) standard prepared in matrix were used for recovery calculations, the results of recovery tests on green beans samples using the different three methods were discussed by the compound with recovery less than 60% Table (5).

Pesticides	Luke	Ethyl-acetate	QuEChERS
Acephate	57%	a	a
Butralin	54%	41%	a
Chlorfluazuron	34%	25%	26%
Cyhalothrin-L	31%	23%	29%
Cyprodinil	a	58%	a
Deltamethrin	19%	24%	25%
Diafenthiuron	12%	17%	20%
Diniconazole	54%	a	a
Fenpropathrin	48%	55%	53%
Fenpyroximate	50%	41%	a
Flufenoxuron	37%	30%	34%
Hexythiazox	56%	a	a
Lufenuron	a	41%	47%
Methamidophos	31%	a	a
Pendimethalin	56%	51%	a
Propamocarb-HCl	11%	1%	a
Pymetrozine	57%	58%	a
Thiocyclam-HO	46%	35%	a
Total	16	14	7

a = Accepted recovery of pesticide at ≥ 60%.

Table 5. Recovery tests on green beans samples using different extraction methods for pesticides < 60%.

As shown in Table (5) propamocarb-HCl is an example for high polar pesticides which had a low recovery in the extraction by ethyl acetate (1 %) and not completely recovered in the partitioning step in Luke method (11%) .On the other hand the solubility of the pesticides in the different methods are different depending on its polarity which could seen in the results of the recovery test of the three methods such as Pymetrozine and Fenpropathrin pesticides The same results were observed by D´ıez et al. (2006) that Luke was significantly more effective for the extraction of non-polar and medium-polar compounds, but the best recoveries for polar compounds were achieved by QuEChERS and ethyl acetate methods. QuEChERS was the only method that provided an overall recovery value of 60–70% for none, medium and polar compounds, also Kruve *et al*. (2008) reported in his comparison between Luke method and matrix solid-phase dispersion (MSPD) that the best recoveries were obtained with the QuEChERS method.
Therefore the QuEChERS extraction method was found to be better than Luke method and ethyl acetate method because of higher recovery, less solvent and short time of analysis were observed.

6. Comparison of pesticides chromatograms using GC-NPD, ECD, MSD and LC-MS/MS

The chromatograms of the 150 pesticides injected into GC systems with three different detectors ECD, NPD and MSD (Fig. 4.a,b,c) were used to compare between GC efficiency and LC-MS/MS (Fig. 5.a) in separation and sensitivity.

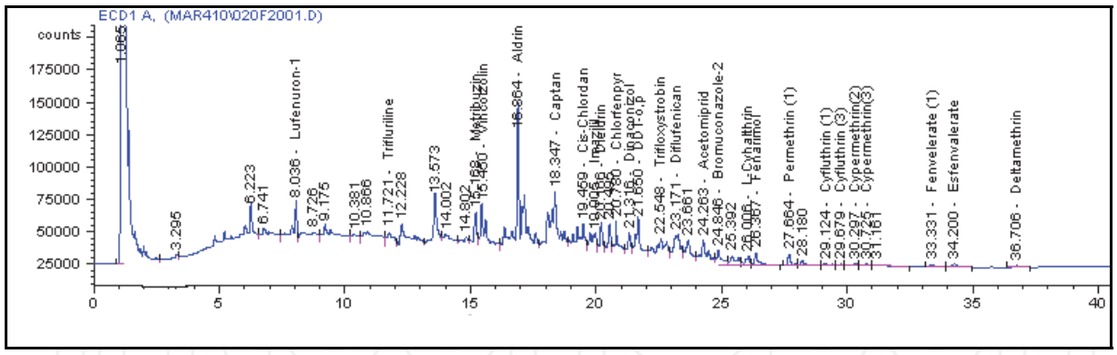


Fig. 4. a. All tested pesticides chromatogram detected by GC-ECD.

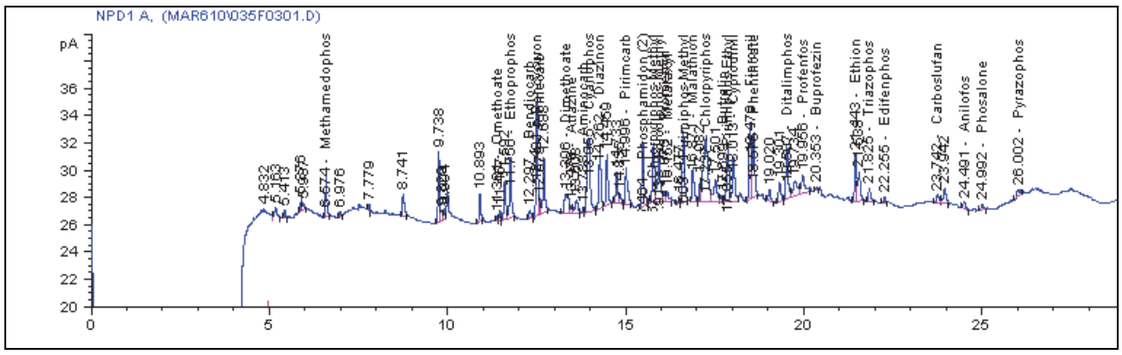


Fig. 4. b. All tested pesticides chromatogram detected by GC-NPD.

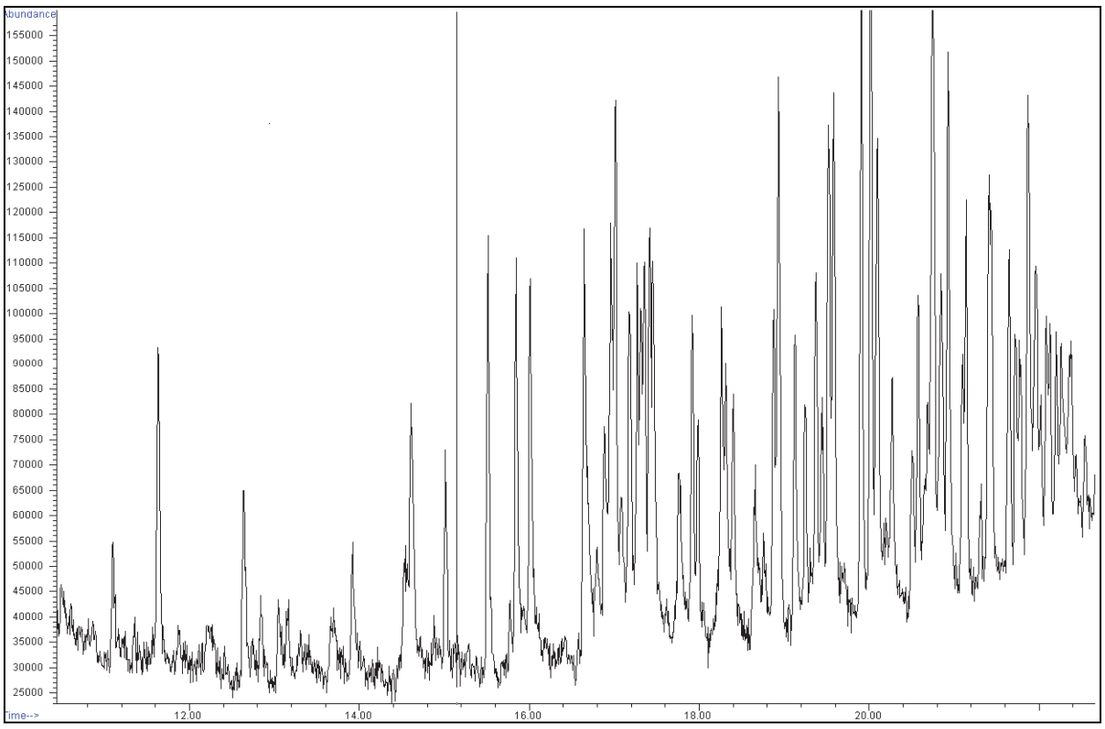


Fig. 4. c. All tested pesticides chromatogram detected by GC-MSD.

The total ion chromatogram for the 150 pesticides injected into LC-MSMS system illustrate in (Fig. 5a). It looks that the pesticide peaks are not resolved but in fact due to the high selectivity of the MS/MS system the peaks can be resolved easily (Fig. 5 b, c,d).

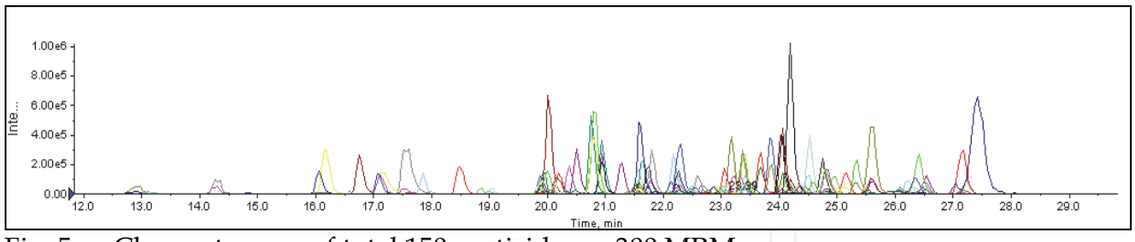


Fig. 5. a. Chromatogram of total 150 pesticides as 300 MRM.

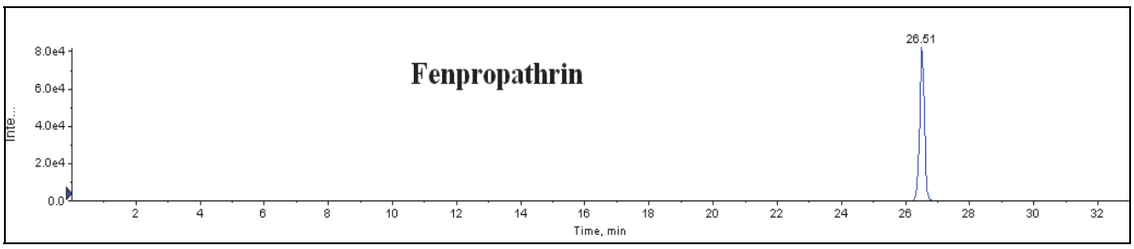


Fig. 5. b. Chromatogram of selected MRM for fenpropathrin.

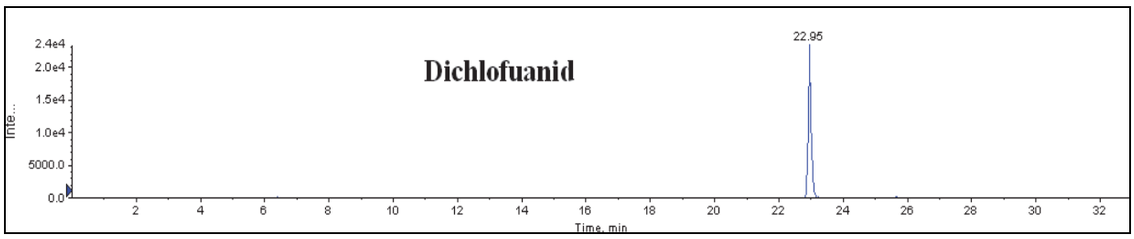


Fig. 5. c. Chromatogram of selected MRM for dichlofuanid.

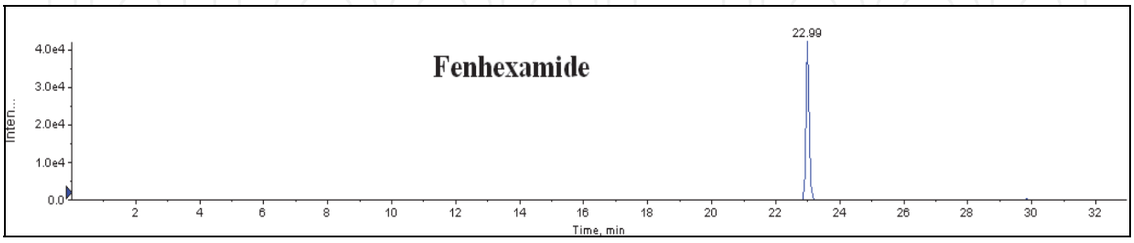


Fig. 5. d. Chromatogram of selected MRM for fenhexamide

It is observed that the pesticide peaks showed in (Fig. 4.a,b,c) by using ECD,NPD and MSD are not resolved and have very low sensitivity while in (Fig. 5a) separation of the 150 pesticides could be analyzed by single chromatographic run of 33 minutes and each MRM could be separated as single peak in a chromatogram by LC-MS/MS system as shown in

(Fig 5, a, b, c and d) for fenpropathrin, dichlofuanid and fenhexamide pesticides as studied by El-Gammal (2010).

It is clear that although dichlofuanid (Fig. 5.c) has the same molecular weight of fenpropathrin (Fig. 5b) (absence of cross talk) and has the same retention time of fenhexamide (Fig. 5d), but it is easily resolved from both compounds. These results were supported by Applied Biosystems (2004) (Application Note: Mass Spectrometry) for fenoxycarb 302/88 and methomyl 163/88 that are measured using the same product ions (but with different precursor ions) they are completely separated, (Publication 114AP30-01).

7. Optimization of mobile phase

A modified multi-residue method for analysis of 150 pesticide residues in green beans using liquid chromatography-tandem mass spectrometry by using three methods as described in material and methods ;QuEChERS as described by Pya, et al., (2008), Luke et al., (1975) method and Ethyl acetate method by Banerjee et al., (2007). The extracts solution of three methods were re-dissolved in methanol ,buffer solution (1:1) 10 mM in pH 4 as modification to increased injection volume to 25 µl without losing our good peak shape. Stabilities of tested pesticides in five different calibration mixture pH for two weeks were studied. Quantitation and identity confirmation was attained by using atmospheric pressure electrospray positive ionization LC-MS/MS in multiple reactions monitoring (MRM) mode. The signal intensity in LC-MS/MS can be influenced by the mobile phase composition. In order to optimize the signal intensity, standard mixtures in methanol were injected into the LC-MS/MS, using different mobile phase compositions. Four different buffer constituents were tested: ammonium format (0.1, 1, 5, and 10 mM) at three pH (3, 3.5 and 4). Evaluation was done by recording the MS/MS signal for each pesticide with a calculation based on 5 mM, pH 4. The mobile phase during this test was composed of 50% buffer constituent in water and 50% methanol.

Generally results showed that there is no variation in signal more than 4% between all of tested mobile phase except the 10 mM in pH 4 which had increasing in 26 compounds more than 15% as shown in the following table(6) .

Pesticides	SE	Pesticides	SE	Pesticides	SE
Pyrazophos	15%	Cyanophos	20%	Carboxin	24%
Thifensulfuron_Me	15%	Flamprop	20%	Thiocyclam HO	27%
Pyrimethanil	15%	Chlorpyrifos-Me	20%	Bensulfuron-Me	28%
Methoxyfenozide	16%	Carbofuran-3OH	21%	Aldicarb	29%
Thiobencarb	17%	Diafenthiuron	21%	Myclobutanil	30%
Triadimifon	17%	Tolylfluanid	21%	Nuarimol	32%
Butralin	19%	Isoproturon	21%	Diuron	33%
Pyrifenox	19%	Linuron	22%	Tetraconazole	38%
Prochloraz	19%	Phenthoate	23%	-	-

Table 6. Comparison between pesticides sensitivity using 10 mM buffer compared to 5 mM buffer.

SE: Signal enhancement in 10 mM buffer compare by 5 mM buffer.
Pesticides which had high matrix effect suppress its standard signals in the compounds with intensity increased up to 38 % (Tetraconazole) . However, when analyzing different samples, which themselves can influence the signal by altering the mobile phase composition, it is important to use a buffer with a sufficient buffering capacity to stabilize the system. Therefore, higher ionic strength contributes to a more stable system, both for retention and signal. By using ammonium format buffer 10 mM with pH 4, the results of 26 pesticides out of 150 pesticides compounds has increased in its sensitivity. These results approved by Jansson et al. (2004) reported that the best signal response was obtained with pH ranging from 4.0 to 4.2 and that the buffer strength of 10 mM was chosen as a compromise on 57 pesticides. Finally the use of ammonium format mobile phase 10 mM in pH 4 represented the most suitable condition for the separation and sensitivity of tested pesticides, which should be considered during determination of pesticides residues.

7.1 Effect of pH on tested pesticides stability

Standard solution of the 150 pesticides was prepared at concentration 0.5 µg/ml and kept in freezer for 15 days at -20± 2 °C and compared to fresh prepared standard solution, the stability of these pesticides at different pH showed by storage recovery in (Fig. 6) and Table (7) also the degradation of pesticides with decreasing more than 10% in different pH were measured Table (8).

Pesticides Recovery	pH 3	pH 4	pH 5	pH 6	pH 7
90-110%	133	150	148	145	142
80-90%	10	0	1	4	4
70-80%	4	0	0	0	2
<70%	3	0	1	1	2

Table 7. Effect of pH on stability of 150 pesticides standard solution.

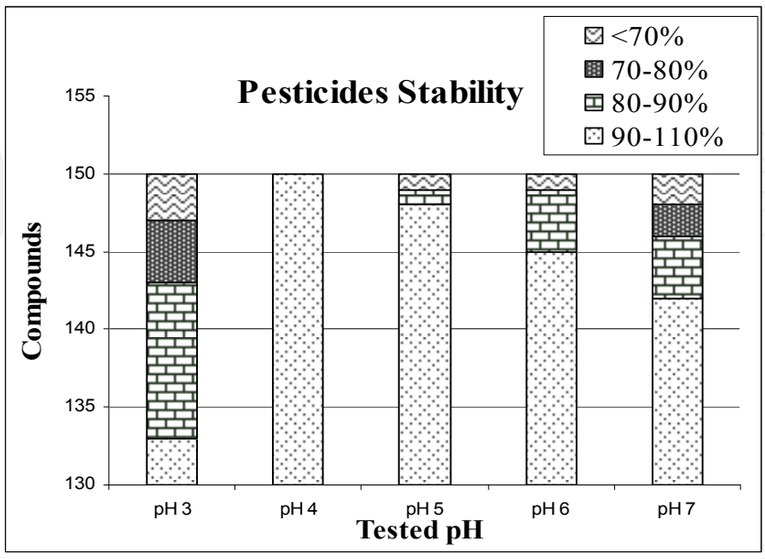


Fig. 6. Pesticides stability in pH 3, 4, 5, 6 and 7.

Pesticides	pH 3	pH 5	pH 6	pH 7
Triflumizole	56%	a	a	a
Fenoxaprop-ethyl	50%	a	a	a
Aldicarb Sulfoxide	30%	a	a	a
Thiophanate-methyl	22%	a	a	a
Metribuzin	20%	a	a	a
Propamocarb-HCl	20%	a	a	a
Thiocyclam-HO	18%	a	a	a
Acephate	16%	a	a	a
Diazinon	14%	a	a	a
Metosulam	14%	a	a	a
Diclorovs	13%	a	a	a
Omethoate	12%	a	a	a
Edifenophos	12%	a	a	a
Butachlor	12%	a	a	a
Pymetrozine	11%	a	a	a
Diafenthiuron	25%	43%	45%	37%
Diniconazole	12%	12%	11%	18%
Parathion-ethyl	a	a	24%	28%
Flamprop	a	a	17%	17%
Piperonyl-butoxide	a	a	13%	14%
Dimethomorph	a	a	a	35%
Thiobencarb	a	a	a	12%
Diflufenican	a	a	a	24%

a = Accepted stability of pesticide at tested pH (>90%).

Table 8. Degradation of pesticides at different pH 3,5,6 and 7.

The pesticides which had lost more than 10% of their concentration were showed by degradable percentage in Table (8), for example triflumizole which showed a degradation of 56% followed by 50 % for Fenoxaprop-ethyl at pH 3 . The result was in agreement with the US- EPA (EPA Pesticide Fact Sheet 10/91) studies on triflumizole which showed that hydrolysis studies of phenyl-labeled Carbon 14 triflumizole (radiochemical purity greater than 99%), at 5 ppm, degraded in sterile aqueous 0.01 M buffered solutions with half-lives of 7 to 15 days at pH 5, greater than 30 days at pH 7 and pH 3 to 17 days at pH 9 when incubated in the dark at 25± 2 ^{o}C. Fenoxaprop-ethyl showed degradation of 50% which is in agreement with the study done by Zablotowicz et al. (2000) stated that stability was pH sensitive in acidic buffered solutions; that is, below pH 4.6, rapid nonenzymatic hydrolysis of the benzoxazolyl-oxy-phenoxy ether linkage occurred, forming 6-chloro-2,3-dihydro-benzoxazol-2-one (CDHB) and ethyl 4-hydroxyphenoxypropanoate or 4-hydroxyphenoxypropanoate. Due to high sensitivity, high duty cycle and simple cleaning of the interface of the API 4000 QT, method development and recovery tests were done using methanol/buffer in pH 4 as calibration mixture solution, using this instrument.

7.2 Optimization of MS/MS

7.2.1 Optimization for precursor ion (parent) and product ion (daughter)

Pesticide standard solutions were prepared in methanol/ ammonium format buffer (1/1) at concentration level of 0.1-0.5 µg/ml and injected individually to optimize for parent ion (MS1 scanning & MS2 static) by scanning at different declustering potential (DP). The optimum DP, which gave the highest sensitivity, was used and changing the collision energy (CE) to optimize for the daughter ion (MS1 static & MS2 scanning). The standard solutions were injected directly into LC/MS/MS system without analytical column, the protonated ions were chosen in ESI+ (MW+1) mode. The compounds which gave accepted intensity with the optimized DP and CE were divided into 3 mixtures and injected into LC/MS/MS system in presence of analytical column using Multiple Reaction Monitoring mode (MRM, MS1 scanning, MS2 scanning) at the optimum DP and CE were used.

Optimization of six pesticides will be discussed as an example. In this chapter we will discuss the optimization of Acetamiprid pesticide and the detailed results of the five remaining pesticides (Lambada-Cyhalothrin, Malathion, Methomyl Propargite and Tetraconazole) were described by El-Gammal (2010).

7.2.1.1 Acetamiprid optimization

7.2.1.2 Calculation of isotopic distribution

The analyst software is used to calculate the isotope distribution, the expected nominal molecular weight of 222.1 for the parent compound also isotopic mass of 224.1 of 33% abundance due to the presence of one chlorine atom (37Cl) (Fig. 7).

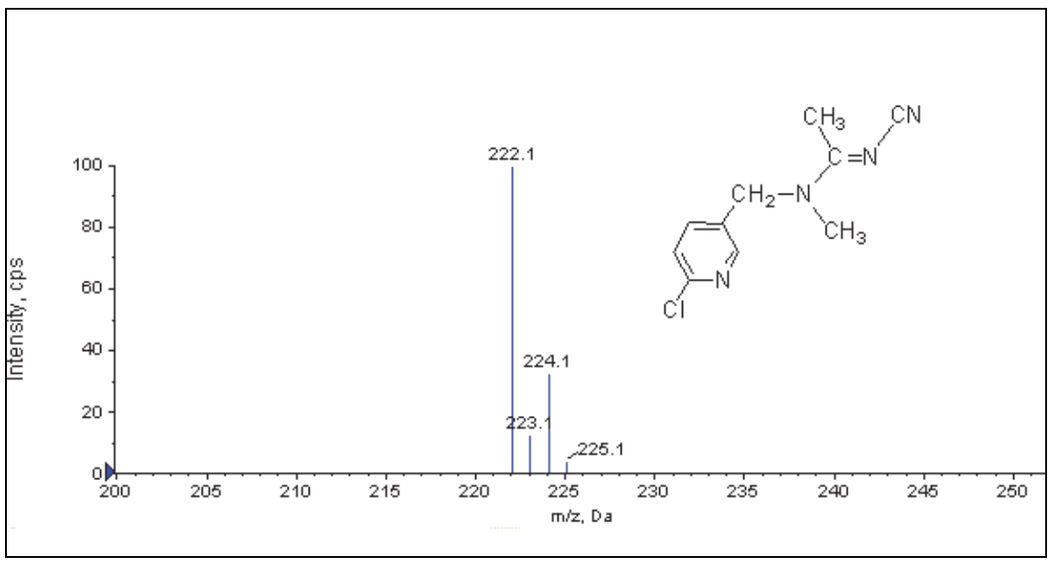


Fig. 7. Expected isotopic distribution of acetamiprid as calculated by the Analyst software.

7.2.1.3 Optimization of the precursor ions

The injection of individual standard of acetamiprid showed in (Fig. 8) and running Q1 scan (MS1 scanning & MS2 static). It is clear that the parent compound has gained a proton to give molecular ion mass at 223 (M+1), also isotopic molecular ion mass at 225 of 33% abundance due to the presence of one chlorine atom (37Cl).

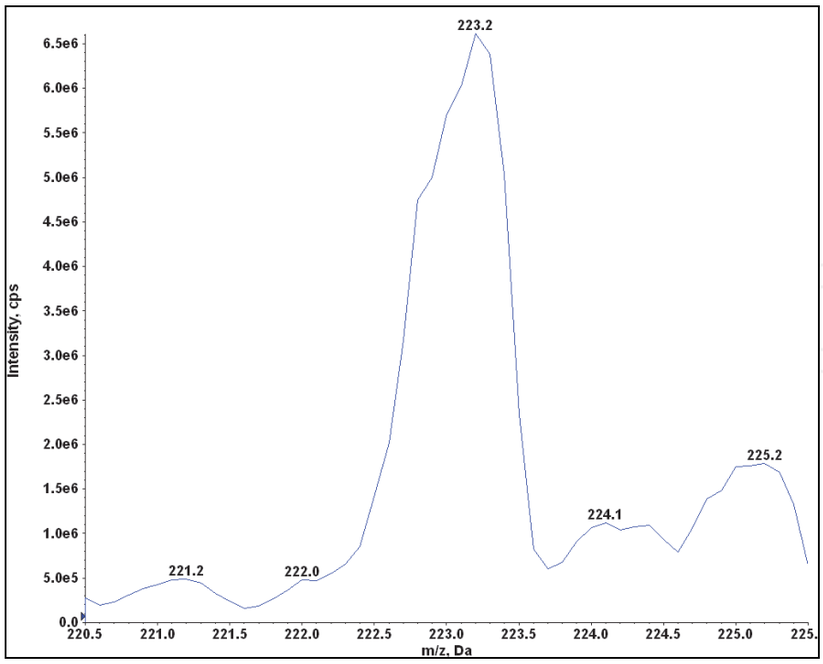


Fig. 8. Injection of individual standard of acetamiprid and running Q1 scan.

7.2.1.4 Optimization of the declustering potential

Q1 scanning (MS1 scanning & MS2 static) of acetamiprid while changing the declustering potential from 0 to 240 volts to get the optimum DP. It is clear that the optimum DP for acetamiprid is 49 volts (Fig. 9).

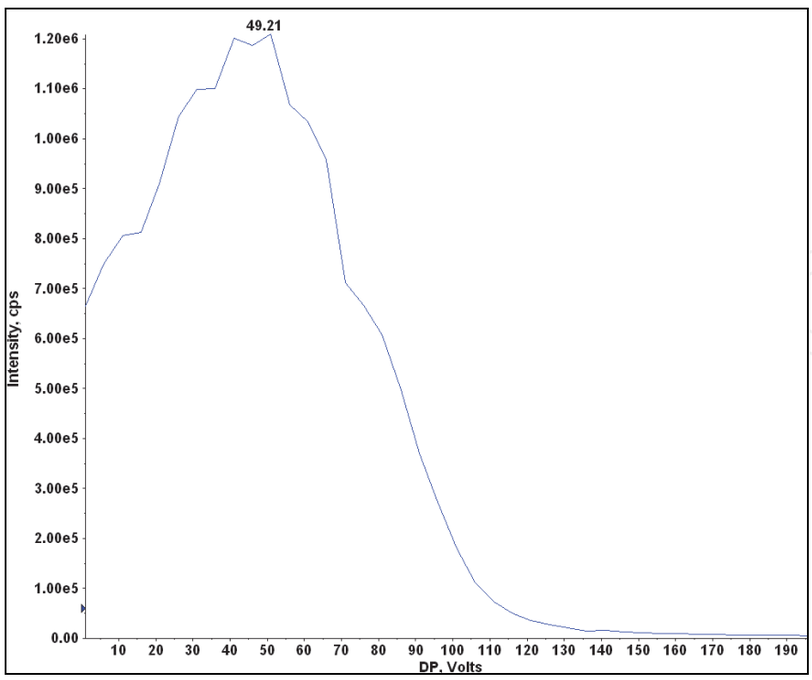


Fig. 9. Optimization of declustering potential (DP).

7.2.1.5 Optimization of the daughter ions

The fragmentation of acetamiprid in the collision cell and the Quadra poles Q1 scanning and Q3 scanning (MS1 scanning & MS2 scanning) (Figs 10, 11).

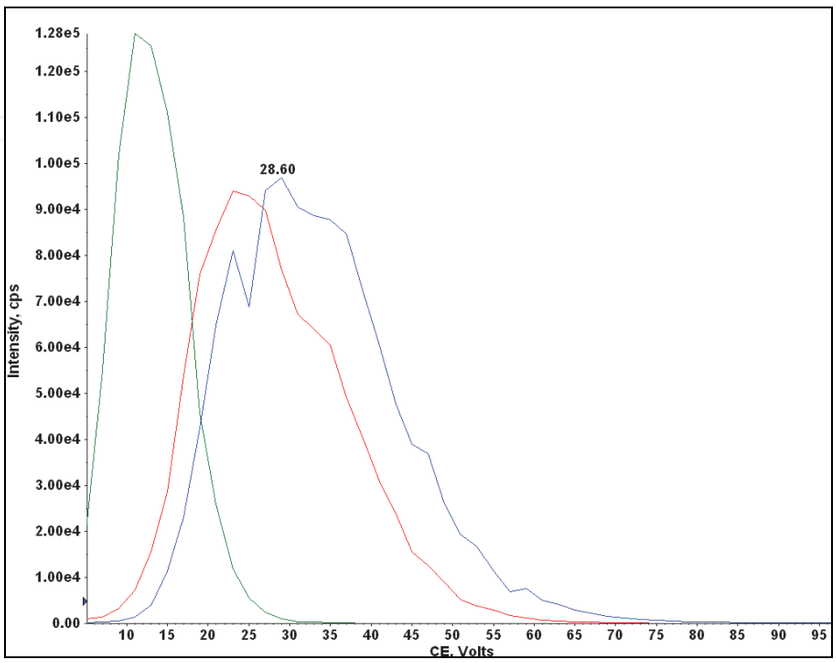


Fig. 10. Optimization of collision energy (CE)

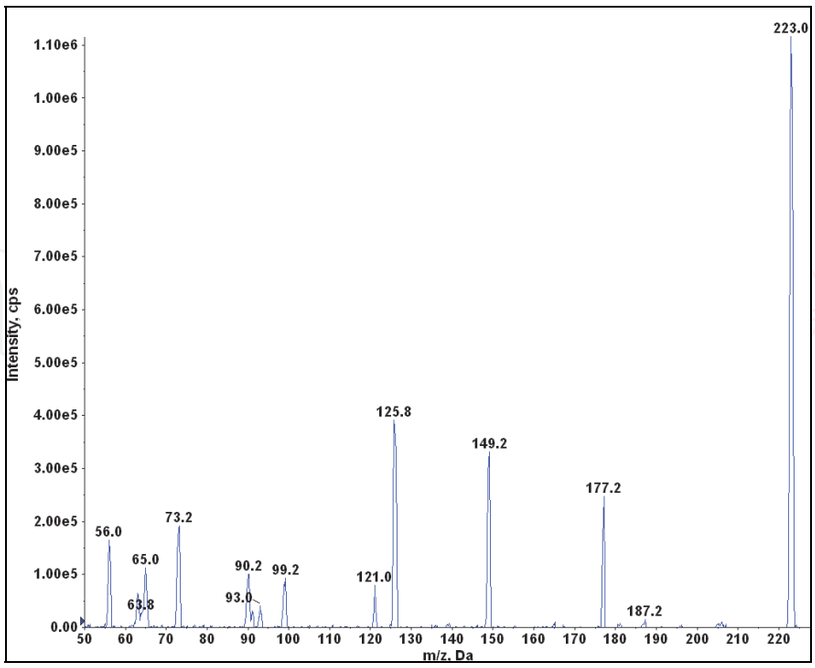


Fig. 11. LC-MS/MS spectrum of acetamiprid.

The following table(9) showed the molecular weight, the calculated molecular weight related to isotopic distribution, isotopic elements of six pesticides compound with their masses, declustring potential (DP) which was very important for the tuning of parent ion and collision energy (CE) necessary for fragmentation .

	Pesticide	Molecular weight/Da	Nominal molecular weight /Da	Isotopic element (Mass)	DP (volt)	CE (volt)
1	Acetamiprid	222.7	222.1, 223.1, 224.1	Cl(35,37) , N(14,15)	49.2	28.6
2-1	Cyhalothrin-L	449.9	449.1, 450.1, 451.1	Cl(35,37) , N(14,15) , O(16,17,18)	–	–
2-2	Cyhalothrin-L-NH4	467.9	467.1, 468.1, 469.1	Cl(35,37) , N(14,15) , O(16,17,18)	52.1	21
3	Malathion	330.4	330, 331, 332	S(32,33,34) , O(16,17,18)	66.1	31.6
4	Methomyl	162.2	162, 163, 164	S(32,33,34) , O(16,17,18) , N(14,15)	41.2	39
5	Propagiter	350.5	350.2, 351.2, 352.2	S(32,33,34) , O(16,17,18)	65.5	24.8
6	Tetraconazole	372.1	371, 372, 373	Cl(35,37) , N(14,15) , O(16,17,18)	56.6	54.4

Da = Dalton.

Table 9. Molecular weight and nominal molecular weight related to isotope distribution.

The conclusion from Table (9) showed that every pesticide compound needs this tuning to get the best conditions for highest sensitivity. It is clear also that each pesticide has different DP and CE to get the best sensitivity; these parameters have been collected to build up the acquisition method for the 150 pesticides

8. Risk assessment based pesticides contamination

8.1 Impact of pesticides contamination to human risk

Human milk the major source of infant food have been studied in detailed about the distribution of pesticides residues in all over 26 Governorate of Egypt . Different types of pesticides have been identifies in milk and the results described as follows:

8.1.1 Chlorinated insecticides levels in human milk

The data in Table 10 shows that the main detected organochlorine insecticides and their metabolites were DDE and lindane .DDT and endosulfan I residues were also detected in some milk samples .Endrin was only detected in one of the milk samples in New valley, while aldrin was not detected in any of the milk samples .However, from the 60 human milk samples, 51% of the samples were free from any detectable DDT level a fact which may suggest that there were no recent sources of pollution by intact DDT (Saleh et al., 1996a,b , 1999).

8.1.2 Hexachlorocyclohexanes HCH isomers

δ-HCH lindane was detected in 95 %of the analyzes human milk samples .The lowest levels were found in governorates between Cairo and Assiut and in Suez 0.00-10.00 ppb while the higher levels 10.00-33.00 ppb (were found in the Delta area and in Alexandria .

The higher levels could be a reflection of the use of lindane in agriculture and in the control of cattle ecto-parasites .Also, this might be due to the human consumption of large quantities of polluted fatty fish (table 10). Kucinski, (1986) have pointed out the presence of organochlorine residues including lindane in different food stuffs meat, dairy products, grain and drinks. Residues of some organochlorine pesticides OCPs, such as HCB and heptachlor as well as some organophosphorus pesticides OPPs, such as methamidophos,thiometon, profenofos, phorate and pirimiphos-methyl were found in a number of potatoes samples produced under different condition (convention, C; organic, O) at concentration levels exceeding their MRLs as reported by Mansour et al., (2009).
The results in table (10) proved that pesticides residue in human milk product depends mainly on the regional area .Pesticides residues do its effect and shows its impact factor through creation a lot of diseases as described by the transportation of the pesticides in biological system to reach its biological function .Transportation of pesticides were carried out through protein binding with the major protein in serum like serum albumin as well as other protein exists in liver such as of α-Synuclein Fibril protein Formation and other organs as described by Afify et al., (2000) and Afify (2010) .Parkinson's disease involves intracellular deposits of α-synuclein in the form of Lewy bodies and Lewy neurites .The etiology of the disease is unknown; however, several epidemiological studies have implicated environmental factors, especially pesticides .Here we show that several

	Lindane		Endosulfane I		4,4'-DDE		4,4- DDT	
Governorate*	Average	Range	Average	Range	Average	Range	Average	Range
Greater Cairo								
Cairo (7)	5.05	2.72-8.98	0.00	0.00 -0.00	11.30	1.40-19.7	0.95	0.00-2.85
Giza (8)	4.96	2.69-6.59	0.69	0.00-2.08	12.02	1.96-19.7	0.00	0.00-0.00
Kaliobia (6)	13.87	1.21-33.20	0.00	0.00-0.00	20.62	8.95-27.3	2.04	0.00-3.87
Delta Region								
Sharkia (5)	6.57	4.15-9.78	0.00	0.00-0.00	54.50	19.7-83.3	2.43	1.7-3.64
Gharbia (1)	4.63	3.47-6.49	10.5	0.00-18.90	25.82	4.06-53.3	1.68	0.00-2.53
Behera (3)	12.82	4.47-22.80	29.98	7.34-57.90	50.95	5.74-117.0	5.51	0.00-10.6
Dakahlia (2)	19.53	13.40-24.70	6.00	0.00-18.00	41.70	7.3-67.2	4.12	0.00-9.38
Menoufia (4)	1.52	4.15-9.78	0.00	0.00-0.00	7.93	2.4-10.9	2.59	0.00-7.76
Upper Egypt								
Fayoum (9)	3.77	0.68-7.82	0.00	0.00-0.00	20.06	4059-37.4	1.84	0.00-4.61
Beny Sweif (10)	2.04	0.95-2.90	3.25	0.00-5.81	27.26	9.17-46.7	1.59	0.00-3.41
Minia (11)	3.11	0.81-6.30	8.03	0.00-20.30	16.36	6.88-21.7	4.83	0.00-14.5
Assuit (12)	10.95	0.78-31.00	1.63	0.00-4.90	30.89	3.47-71.3	5.12	3.51-7.02
Sohag (13)	12.88	6.75-21.30	4.77	0.00-14.3	29.42	2.97-77.5	5.47	0.00-13.6
Aswan (14)	12.84	4.47-28.20	7.02	2.41-12.7	8.95	3.8-18.5	2.46	0.00-7.38
Costal Areas								
Alexandria (17)	12.46	2.37-20.50	0.00	0.00-0.00	9.35	7.32-12.1	0.00	0.00-0.00
Matrouh (16)	11.30	6.41-15.00	25.83	0.00-61.8	15.11	7.04-23.0	0.00	0.00-0.00
North Sinai (18)	11.81	9.92-12.9	0.00	0.00-0.00	8.84	5.17-13.7	14.52	4.77-32.9
Ismailia (19)	3.71	1.36-5.43	0.00	0.00-0.00	19.17	14.2-25.4	0.00	0.00-0.00
Suez (20)	1.44	0.00-2.21	0.00	0.00-0.00	11.35	5.78-20.3	2.39	0.00-7.16
Desert								
New Vallery (15)	13.08	8.93-18.20	0.00	0.00-0,00	5.83	4.13-8.54	1.04	0.00-3.13
Average in Egypt	8.42	0.00-31.00	4.84	0.00-61.8	1.37	1.4-117	2.93	0.00-32.9

*No .of collection samples

Table 10. Distribution of the main organochlorine insecticide residues in Egyptian Mother's milk

pesticides, including rotenone, dieldrin and paraquat, induce a conformational change in α-synuclein and significantly accelerate the rate of formation of -α synuclein fibrils in vitro . They propose that the relatively hydrophobic pesticides preferentially bind to a partially folded intermediate conformation of α -synuclein, accounting for the observed conformational changes and leading to association and subsequent fibrillation .These observations suggest one possible underlying molecular basis for Parkinson's disease .α-Synuclein, a relatively abundant brain protein of 140 amino acids and of unknown function, was first identified in association with synaptic vesicles Maroteaux *et al*., 1988. α-Synuclein belongs to the class of proteins known as natively unfolded; i.e., the purified protein at neutral pH is substantially disordered (Uversky et al., 2001a,b) .

8.2 Impact of pesticides contamination in potatoes

Table (11) presents a survey for the numbers and percentages of contaminated samples, as well as the violated ones. In case of (C) potatoes contaminated samples with HCB accounted to 41.7%, compared to 16.7%, for (O) potatoes, while 33.3% and 16.7%, of the total samples exceeded the MRL of HCB, for (C) and (O) potatoes, respectively. The highest percentage of insecticide contamination of (C) potatoes reached 58.3% with methamidophos in case of (O) potatoes the highest percentage reached 25% with heptachlor.

Insecticide	Contaminated samples with each insecticide				Violated samples			
	C		O		C		O	
	n	%	n	%	n	%	n	%
HCB	15	41.7	6	16.7	12	33.3	6	16.7
lindane	18	50.0	6	16.7	0	0.0	0	0.0
heptachlor	12	33.3	9	25.0	12	33.3	9	25.0
aldrin	nd	-	3	8.3	-	-	0	0.0
dieldrin	12	33.3	3	8.3	4	11.1	0	0.0
o,p-DDD	6	16.7	3	8.3	-	-	-	-
p,p-DDD	21	58.3	15	41.7	-	-	-	-
o,p-DDT	3	8.3	3	8.3	0	0.0	0	0.0
p,p-DDT	6	16.7	9	25.0	1	2.8	0	0.0
chlorpyrifos	3	8.3	nd	-	0	0.0	-	-
chlorpyrifosmethyl	6	16.7	nd	-	0	0.0	-	-
fenthion	3	8.3	nd	-	0	0.0	-	-
malathion	6	16.7	6	16.7	3	8.3	0	0.0
methamidophos	21	58.3	18	50.0	4	11.1	3	8.3
phorate	3	8.3	9	25.0	3	8.3	3	8.3
pirimiphos-methyl	9	25.0	3	8.3	5	13.9	0	0.0
profenofos	6	16.7	nd	-	4	11.1	-	-
thiometon	6	16.7	6	16.7	6	16.7	6	16.7

Table 11. Numbers and percentages of contaminated and violated samples of different types of potato tubers collected from the Egyptian local markets during 2006/2007 with respect to detected insecticides in the analyzed samples

nd :not detected; na :not available . A Total number of analyzed samples for each type of potatoes =36, B Maximum Residue Limits (MRLs) refer to (Codex 2006a) for potatoes.

Regarding potatoes, risk assessment based on their contamination levels from pesticides presented in Tables 12 and 13 a daily potato consumption of 0.06 kg for an adult person of 60 kg body weight (WHO, 2003) yielded the estimates .
Comparing the estimated dietary doses for the studied pesticides with their Acceptable Daily Intake-ADI; JMPR (Tomlin, 2004), revealed that only phorate residues either in (C) potato (0.001mg/kg b.w/d) or in organic potato, (0.0013 mg/kg b.w/d) pose risks to human health due to consumption of such potatoes since the estimated dietary doses accounted to 2.22 and 2.68 times the WHO-ADI for this pesticide (0.0005mg/kg b.w/d), respectively table (12)(Mansour et al.,2009).

Insecticide	WHO-ADI (mg/kg bw/d)	Estimated dose (mg kg bw/d)		Hazard Index		Risk	
		C	O	C	O	C	O
lindane	0.005	0.0004	0.0002	0.09	0.04	No	No
HCB	-	0.0014	0.0010	-	-	?	?
p,p-DDT	-	0.0002	0.00006	-	-	?	?
chlorpyrifos-methyl	0.01	0.00003	nd	0.003	nd	No	No
fenthion	0.007	0.000004	nd	0.0006	nd	No	No
malathaion	0.30	0.001	0.0002	0.003	0.001	No	No
methamidophos	0.004	0.001	0.0003	0.30	0.09	No	No
phorate	0.0005	0.0011	0.0013	2.22	2.68	Yes	Yes
pirimiphos-methyl	0.03	0.001	0.00001	0.03	0.0004	No	No
profenofos	0.01	0.00007	nd	0.007	nd	No	No
thiometon	0.003	0.0001	0.0003	0.03	0.08	No	No

Table 12. Calculated health risks for systemic effects associated with dietary intake of insecticide residues from potato tubers insecticide WHO-ADI (mg/ kg bw/d) estimated dose (mg kg bw/d) Hazard Index Risk.
Acceptable Daily Intake (ADI) (JMPR), cited from Tomlin (2004). Estimated dose = Residues (mg/kg) Food item / Body weight; where the following was considered in calculations: Residues = the highest mean value for each insecticide over12 months, daily potato consumption = 0.06 kg and body weight = 60 kg (WHO, 2003).Hazard Indices are resulted from dividing estimated doses by ADIs; indices <1 mean no risk and vice versa for indices >1. C: conventionally-farmed potatoes; O: organically-farmed potatoes; nd: not detected; no data available.

9. Pesticides binding to individual proteins

In vitro Binding of three pesticides Trichlorphenol, Fenvalerate and α-Endosulphan to Rat Serum Transferrin and Albumin for Bio-monitoring of Pesticides Pollution were carried out according to Afify et al., (2000). The results of the electrophoresis separation of the protein subunits of rat serum treated with different pesticides concentration 5, 10, 15 and 20 PPm (Table 13) showed that these pesticides have high affinity to albumin as well as high molecular weight proteins .The increase in the intensity of transferring protein was occurred with trichlorophenol and α-endosulphan .On the other hand, the intensity of the

albumin fraction was decreased with fenvalerate, while it is markedly increased with trichlorophenol and α-endosulphan .The individual incubation of each pesticide with transferrin,, albumin or prealbumin showed that trichlorophenol and α -endosulphan was found to cause aggregation of transferring by 49.l and 43.9%, respectively, while fenvalerate was found to cause marked disintegration of transferrin as compared to controls .The albumin fraction was significantly decreased with the three pesticides .The Pre-albumin was found to markedly increased in its Intensity by 44.8 and 57.3 %with Trichlorophenol 5 ppm and α-endosulphan 15 ppm, respectively. The results concluded that several proteins have responded to pesticides treatment including the known serum proteins, transferrin, albumin, pre-albumin and small molecular weight proteins (Table 14) .However, some of the small molecular weights proteins have been identified as results of pesticides binding which require further characterization .Therefore, the detection of serum proteins after electrophoresis is considered a very good diagnostic parameter for bio-monitoring of pesticides pollution as studies by Saleh et al., (1996b); Afify et al., (1997).

	Rat serum proteins MW (kDa)												
Groups	300	200	160	100	76	70	67	55	52	45	37	35	30
Control	0.1	0.7	0.6	0.8	5.2	5.2	40.1	15.2	14.3	3.4	3.9	5.9	4.6
Trichlorophenol													
5 ppm	2.2	2.9	1.5	1.8	8.9	4.2	58.2	8.5	7.5	2.7	1.6		
10 ppm	2.8	3.3	1.3	1.5	9.6	2.3	56.7	7.9	9.5	2.2	2.9		
15 ppm	1.4	2.9	2.5	4.5	10.6	2.5	56.8	5.7	6.6	4.2	2.3		
20 ppm	2.4	3.1	2.3	2.2	15.2	3.5	56.2	4.5	6.4	5.3	2.2		
Fenvalerate													
5 ppm	0.1	0.3	0.3	0.7	12.5	21.9	38.5	10.9		3.6	3.8	4.2	3.2
10 ppm				1.2	1.4	0.6	45.9	18.1		5.8	4.8	5.5	4.3
15 ppm					10.7		45.3	26.2		6.4	4.2	3.1	4.1
20 ppm							30.6	22.1		25.9	2.4	1.8	17.2
α-Endosulphan													
5 ppm	3.5	2.8	7.2	3.1	7.5	2.9	52.8	16.5	5.6	1.1			
10 ppm	1.5	4.9	8.3	3.4	8.1	3.6	48.3	12.7	5.4	3.8			
15 ppm	2.1	4.4	8.6	3.2	8.3	3.9	52.2	9.2	4.6	3.1			
20 ppm	2.1	6.8	7.1	2.5	8.5	3.8	55.5	9.5	4.5	3.7			

Table 13. Scanning of electrophoretic pattern of rat serum protein subunits treated with different concentration of trichlorophenol, fenvalerate and α-endosulphan pesticides

	Transferrin MW (kDa)									Albumin MW (kDa)						
Groups	76	60	55	52	48	45	35	30	67	55	52	48	45	40	35	30
Control	38.8	22.1	18.1	9.4	6.9	3.4	0.7	0.6	64.1	21.2	4.1	4.2	6.4			
Trichlorophenol																
5 ppm	48.7	13.6	12.6	6.9	13.4	4.8			37.6	44.8	4.3	6.8	6.5			
10 ppm	49.1	13.3	12	6.1	13.8	5.7			49.5	26.1	11.2	6.9	6.3			
15 ppm	46.2	14.4	18.1	7.5	10.1	3.7			49.8	18.4	15.5	9.3	7			
Fenvalerate																
5 ppm	34.8	16.7	16.6	6.6	12.6	7.3	2.3	3.1	41.6	30.6	9.2	12	6.6			
10 ppm	36.5	15.6	15.2	8.9	15.6	8.2			39.8	33.4	12.5	7.8	6.5			
15 ppm	34.1	14.1	16	8.4	17.8	9.6			49.3	30.5	5.8	2.2	12.2			
α-Endosulphan																
5 ppm	42.5	14.2	8.6	8.5	10.9	10.1	2.8	2.4	31.1	29.6	13.4	2.9	7.1	1.6	4.2	10.1
10 ppm	43.9	12.4	8.5	8.5	11.8	7.7	2.3	4.9	30.2	52.1	9.4	3.5	4.8			
15 ppm	43.6	12.6	8.6	8.4	9.1	6.6	5.7	5.4	26.9	57.3	8.2	2.8	3.5	1.3		

Table 14. Showed that scanning of electrophoretic profiles of Transferring and albumin incubated with different concentration of pesticides 5, 10 and 15 ppm.

Investigation was carried out to determine if there are any changes among serum proteins which could be used as a biomarker for pesticides pollution .In addition, during the transport of the pesticides with carrier proteins in blood throughout the organs, do complex cause destruction in macromolecules .The data in table (14) of the present study revealed that the incubated pesticides have, high affinity to the proteins binding sites (Saleh et al., 1996b; Afify et al., 2000). Similar, observations have been recorded for particle mediated uptake of chlorinated pesticides by human, rat and insect lipoprotein (Shalsky and Guthrie, 1975; Larsen et al., 1994) and by serum albumin and α-globulin in rat and rabbit (Shakoori et al., 1996) . The binding of pesticides to proteins is correlated to the binding of DNA .DNA was considered the most important leader of the genetic code in human (Hemminki, 1986)which may induce genetic, risks) (Ehrenberg *et al*., 1974.). Therefore, the binding of pesticides to the macromolecules of rat serum protein could be serve as biomarker in the monitoring of pesticide (Hemminki, 1986). Pahler et al .(1999) showed that the accumulation of some proteins such as alpha 2 macro-globulin has been implicated in the tumorigenicity of many nongenotoxic chemicals to the kidney of the male rat .These chemicals have been shown to bind to alpha 2 macro-globulin and this binding was found to impair the renal degradation of the protein, resulting in lysosome overload, cell death, increased cell proliferation and, presumably renal tumor formation .The present study proved that the major proteins transferrin and albumin are the main sites for the three studied pesticides .The data of incubation of the three pesticides with transferrin and albumin were showed that the destruction of transferrin and albumin with the three pesticides produced a similar but not identical protein profile and the prealbumin was found to represent the major one as recorded by Altland et al .*(*1981). Dissociation into small MW proteins has been demonstrated in case of in vitro incubation with the tested pesticides .These results are in agreement with the results obtained by prolonged exposure of proteins to pesticides)Nilsson *et al*., 1975) .The changes in the binding of serum acute phase proteins such as transferrin and albumin with some chemicals has been used to detect or identify human breast cancer (Heys *et al*., 1998). Insecticides have been shown to bind to blood protein especially organochlorine compounds which are extensively bound to blood lipoproteins (Shalsky &Guthrie, 1975, 1977) .Dutta et al .(1992) revealed that malathion an organophosphorus pesticide has profound effect on serum protein as other parameters .Therefore, the detection of the prealbumin as well as small MW proteins after electrophoresis is considered a very good diagnostic marker for pesticide pollution .In conclusion the induced destructed proteins by pesticides in-vivo and in vitro may be utilized as biomarkers reliable for pesticides monitoring (Saleh et al., 1996b; Afify et al., 2000).

10. Conclusion

To improve agricultural productivity and control pesticide residues in food and environment; three different methods of extraction for pesticides were applied and methods based on chromatographic separation HPLC with mass spectrometric detection(LC-MS/MS tandem spectroscopy) considered useful methods for determination of pesticide residues in natural products under different types of farming production . Therefore this chapter evaluates the capabilities of mass spectrometry (MS) in combination with liquid chromatography (LC) for the determination of multi-residue pesticides extracted with three different methods. LC–MS/MS using electrospray ionization (ESI) are identified as techniques most often applied in multi-residue methods for pesticides at present in most

labs . Therefore, applicability and sensitivity obtained by LC-MS/MS is evaluated for each of the selected pesticides. A modified multi-residue method for analysis of 150 pesticide residues in green beans and grapes using liquid chromatography-tandem mass spectrometry were evaluated and compared for a wide range of physicochemical properties followed by LC-MS/MS detection. GC systems with three different detectors GC-ECD, GC-NPD and GC-MSD were used to compare between its efficiency and LC-MS/MS in separation and sensitivity.

Multi-residue method of determination of 150 pesticides is developed at 0.01 mg/kg limit of determination which fulfills the EU MRLs for organic agricultural products and baby foods. Grapes and green beans were selected not only for their wide consumption in the local market but also because they are promising exporting products to the international markets. The mass spectrometric parameters were optimized to give the best sensitivity, two MRM's were chosen for quantification and conformation of pesticides. The selected MRM's were based on the optimized declustring potential and collision energy which help improve pesticides selectivity and justification.

Risk associated with consumption of foods contaminated by pesticides has stimulated research to find out their impact to human health risk .Therefore Human milk samples were analyzed for pesticides residues along 26 of Egypt Governorates as well as pesticides residues in potatoes produced under different farming condition. In vitro binding of three pesticides e.g. Trichlorphenol, Fenvalerate and α-Endosulphan to rat serum proteins were studied to evaluate their binding and predict biomarker molecules.

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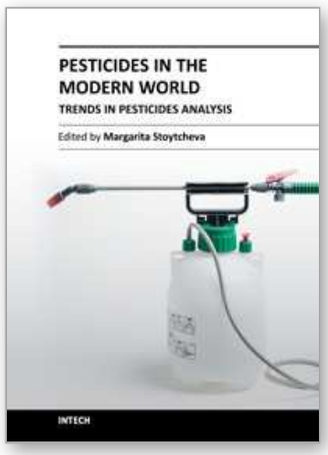

Pesticides in the Modern World - Trends in Pesticides Analysis
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The book offers a professional look on the recent achievements and emerging trends in pesticides analysis, including pesticides identification and characterization. The 20 chapters are organized in three sections. The first book section addresses issues associated with pesticides classification, pesticides properties and environmental risks, and pesticides safe management, and provides a general overview on the advanced chromatographic and sensors- and biosensors-based methods for pesticides determination. The second book section is specially devoted to the chromatographic pesticides quantification, including sample preparation. The basic principles of the modern extraction techniques, such as: accelerated solvent extraction, supercritical fluid extraction, microwave assisted extraction, solid phase extraction, solid phase microextraction, matrix solid phase dispersion extraction, cloud point extraction, and QuEChERS are comprehensively described and critically evaluated. The third book section describes some alternative analytical approaches to the conventional methods of pesticides determination. These include voltammetric techniques making use of electrochemical sensors and biosensors, and solid-phase spectrometry combined with flow-injection analysis applying flow-based optosensors.

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