

Validation of Multiresidue Method for Determination of 200 Pesticide Residues in Fresh Pepper using GC–MSMS

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ABSTRACT

The Egyptian exports of fresh peppers has suffered from EU border rejections during the last few years due to violations of the EU pesticide maximum residue limits (MRLs), The EU Rapid Alert System for Food and Feed (RASFF) has reported 10 border rejections of fresh peppers imported from Egypt during 2015/2016. The EU has included Egyptian exports of pepper to increased level of control (Regulation (EC) No 1021/2014).

In the current study a simple and rapid method for simultaneous determination of 200 GC-amenable pesticides in fresh pepper was validated using the quick, easy, cheap, effective, rugged and safe extraction method (QuEChERS) followed by gas chromatography with tandem mass spectrometry (GC–MSMS).

Samples were extracted with acetonitrile, followed by a liquid-liquid partitioning step and phase separation by adding buffer–salt mixture consisting of magnesium sulfate, sodium chloride, disodium hydrogen citrate sesquihydrate and trisodium citrate dihydrate.

Sample extract was centrifuged and an aliquot of the clear solution was evaporated, reconstituted in hexane/acetone and injected into the GC-MSMS system. Qualitative determination of the analytes was based on characteristic ion pairs and retention time using Electron Impact (EI) ionization GC-MS/MS in multiple reactions monitoring (MRM) mode. The standard curves were used for quantification, the calibration curves were in the range of 0.01–0.5 µg/ml. Due to GC-MSMS signal enhancement, determination of pesticide residues was based on matrix-matched standard calculations.

Recoveries at three different concentration levels (0.01, 0.05 and 0.1 mg/kg) for most of tested pesticides were ranged from 70 to 120% and reproducibility expressed as relative standard deviation (RSD_{WR} %) was less than 20%.

Keywords: Pepper, pesticide residue, EU regulation, method validation, GC-MSMS

Introduction

Pesticide residues are critical factor for allowing agriculture products consignments to enter the importing countries, especially EU countries. As from 1 September 2008, Regulation (EC) No 396/2005 of the European Parliament and of the Council on maximum residue levels (MRLs) of pesticides in products of plant and animal origin defines a new fully harmonised set of rules for pesticide residues (EU regulation No 396/2005).

The Egyptian exports of fresh peppers has suffered from EU border rejections during the last few years due to violations of the EU pesticide maximum residue limits (MRLs), The EU Rapid Alert System for Food and Feed (RASFF) has reported 10 border rejections of fresh peppers imported from Egypt during 2015/2016. The EU has included Egyptian exports of pepper to increased level of control (Regulation (EC) No 1021/2014).

The use of multiclass, multiresidue methods was found to be the most efficient approach to pesticide residue analysis in terms of analysis costs and turnaround time. Many multiresidue methods had been developed for determination of pesticide residue in fruits and vegetables.

Steve Schachterle and Carl Feigel (1996) used GC-MSMS to analyze for multiple pesticide residues in fresh fruits and vegetables. Nineteen pesticides, including fungicides, herbicides, organophosphorus insecticides and chlorinated insecticides were spiked into seven different fruit and vegetable matrices. The specificity of GC-MSMS provided for low detection limits (ranging from 1–5 ppb) and unambiguous

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spectral confirmation for the target compounds in these complex matrices. Good precision was obtained (RSD values ranged from 1 to 9%), without extensive sample clean-up steps.

A new analytical method was devised using gas chromatography with tandem mass spectrometry (GC-MSMS) for the routine analysis of 31 multi-class pesticide residues. Extraction of the pesticides with dichloromethane was carried out. The optimal ionization mode, either electron impact or chemical ionization, was selected for each pesticide in the same run. The average recoveries in cucumber obtained for each pesticide ranged between 71 and 119% at two different fortification levels ($n=10$ each) that ranged between 7 and 300 ng g⁻¹ (depending on the pesticide). The relative standard deviation was lower than 19% for all compounds tested (Martinez *et al.*, 2002).

Anastassiades *et al.* (2003) recently introduced the so-called quick, easy, cheap, effective, rugged, and safe (QuEChERS) method of pesticide residue analysis. In a follow-up study, Lehotay *et al.* (2005) demonstrated its effectiveness for >200 pesticides in lettuce, orange, and several other matrixes using gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS).

Arrebola *et al.* (2003) has proposed analytical method to determine 81 multiclass pesticide residues in vegetables. The method was based on a fast extraction of the pesticides with dichloromethane and a further analysis of the extract by GC-MSMS. The method was validated in order to be applied to real samples. Recoveries in cucumber at two different fortification levels were evaluated and ranged between 73% and 108% for all pesticides. The relative standard deviation (RSD%) was lower than 22% in all cases.

The quantitation of pesticides can be adversely affected by a phenomenon known as the matrix-induced chromatographic response enhancement effect. This effect is noted by improved chromatographic peak intensity and shape when affected compounds are injected in the presence of a complex matrix. Matrix components mask potentially active sites in the GC flow path, leading to fewer of these sites being available to interact with analytes, and thus fewer losses of analytes and better peak shapes. One approach to compensate for the effect of matrix is to use analyte protectants which bind to potential active sites in the GC flow path and inhibit their interference in the analysis. Anastassiades *et al.* (2003) have proposed several approaches to minimize peak distortion phenomena and compensate for matrix-induced effects. The protecting agents are added to extracts and matrix-free standards alike to provide the chromatographic enhancement effect even for the most susceptible analytes in a very dirty GC system. In this study, 93 different compounds were evaluated to find the most suitable ones for improving chromatographic quality of the signal. Because hydrogen bonding has been shown to be an important factor in analyte interactions with active sites, dramatic peak enhancements were achieved using compounds containing multiple hydroxy groups, such as sugars and sugar derivatives, and gulonolactone appears to be the most effective protecting agent for the most pesticides that were tested.

Stanisław Walorczyk (2008) has validated a multi-residue method for the determination of pesticides in cereals and dry animal feed using GC-MSMS. The pesticides were extracted using buffered QuEChERS method and then cleaned up using dispersive solid-phase extraction with Bondesil PSA and C18 sorbents, and optionally by a freezing-out clean-up step. The final extracts were analyzed in a single injection GC-MS/MS acquisition method. A high degree of confidence was achieved by entering two multiple reaction monitoring transitions per compound. Quantification of analytical results and unequivocal identification of pesticide residues in compliance with the recent European Union criteria could be done in a single analysis.

A rapid, sensitive, accurate and reliable multiresidue method for quantification and confirmation of 121 common agricultural pesticides in fruits and vegetables by GC-MSMS was developed, validated. Prior to instrumental analysis an extraction procedure, based on a sample extraction of multi-class analytes according to QuEChERS method with some modifications was used. Mass spectrometric conditions were individually optimized for each analyte to achieve maximum sensitivity in the Selected Reaction Monitoring (SRM) mode. Recovery and precision were evaluated. The mean recoveries ranged from 80% to 116%, with relative standard deviations lower than 20% for all components. The method was accredited according to UNE-EN ISO/IEC 17025:2005 international standard (Camino-Sánchez *et al.*, 2011).

A simple and easy method was established for the simultaneous determination of 73 pesticide residues in vegetables and fruits by GC-MSMS. After samples were ultrasonically extracted with hexane and centrifuged, the analytes in sample solution were separated with DB-5MS column. Quantitation and identity confirmation was attained by using GC-MSMS Dan Lu *et al.* (2013).

Yan-Fei Li *et al.* (2014) established a new method for the determination of multiple pesticides in vegetables and fruits based on a modified QuEChERS sample preparation method with Fe₃O₄ magnetic nanoparticles (MNPs) as the adsorbing material and GC-MSMS determination in multiple reaction

monitoring (MRM) mode. Recoveries were evaluated in four representative matrices (tomato, cucumber, orange and apple) with the spiked concentrations of 10 µg/kg, 50 µg/kg and 200 µg/kg in all cases. The results showed that the recovery of 101 pesticides ranged between 71.5 and 111.7%, and the relative standard deviation was less than 10.5%.

The current study covers 200 GC-amenable pesticides, this study is complementary to our previous study of the analysis of 340 pesticides using LC-MSMS (Emad Attallah, 2016). Considering that there are 120 pesticides which are detected by both LC-MSMS and GC-MSMS, the validated method using LC-MSMS and GC-MSMS covers the analysis of 420 pesticide residues in fresh pepper and includes as many pesticides registered in Egypt as possible in one multiresidue method.

Materials and Methods

Apparatus:

Agilent 7980 GC with 7000B Quadrupole equipped with EI source. Ultra-Turrax® T25 Digital. PTFE or polyethylene 15 ml and 50 ml with screw cap tubes. Centrifuge (Heraeus up to 4000 rpm).

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). De-ionized water was produced by a mille Q unit (Mille Pore).

Ready-made QuECHERS kits from Agilent Technologies; reagent packet contains 4g of anhydrous magnesium sulfate, 1g of sodium chloride, 1g of trisodium citrate dehydrate and 0.5g of disodium hydrogen citrate sesquihydrate.

Standard preparation: Stock solution: 1000 µg/ml reference standard solution of each pesticide was prepared in toluene.

Intermediate solutions: Mixture of standards of 5 µg/ml of each pesticide was prepared by diluting stock solution in toluene.

Calibration solutions: Calibration mixtures of concentration levels 0.01, 0.05, 0.1, 0.5 µg/ml for GCMS/MS were prepared in n-hexane/acetone (9:1) containing blank green beans extract as analyte protectants. Calibration solutions were stored in refrigerator at 4 ±2 °C. The preparation of multiple standards covering a broad concentration range will allow the construction of a linear calibration curve.

Injection standard solution:

Working standard of Aldrin with concentration 0.1 µg/ml was prepared in n-hexane/ acetone (9:1) solution used as injection standard for GCMS/MS.

Extraction Procedure:

Ten grams (W) of sample were weighed in 50 ml PTFE tube, 10 ml Acetonitrile was mixed with the sample by mechanical shaking for one minute. The buffer-salt-mixture was added and shaken immediately for one minute. The sample was centrifuged at 4000 rpm for 5 minutes. Portion of Acetonitrile layer was evaporated and redissolved in injection standard solution, filtered using syringe filter and directly injected into GC-MS/MS system.

GC-MS/MS analysis:

Agilent 7980 GC with 7000B Quadrupole equipped with Electron Impact (EI) ionization source was used for pesticide identification and quantitation. Separation was attained by injecting of 1 µl of the sample to Agilent DB-35ms Ultra Inert GC columns (35%-Phenyl)-methylpolysiloxane with dimensions 20 m x 0.18mm x 0.18 µm.

GC oven conditions: Initial oven temperature of 70°C for 2 min, heating from 70 to 135°C at 50°C /min, holding for 0 min at 135°C, heating from 135 to 200°C at 6°C/ min, holding for 0 min at 200°C, heating from 200 to 310°C at 16°C /min, holding for 8.2 minutes. The total run time is 30 minutes.

MS conditions: MS source: EI -70eV; Quadrupole temperature: 180 °C; Transfer line temperature: 320 °C, gain: 40, Acquisition mode: MRM; Dwell time: 10 ms; Solvent delay time: 5 minutes.

Quantification of the pesticides was performed by comparing the peak areas of the pesticides to a calibration curve of the standards, and multitude point calibration was used. Matrix-matched standards were used to correct for matrix effect.

Results and Discussion

Method validation:

The selected parameters for in-house validation were mainly taken from Eurachem guideline on method validation (Eurachem Guide, 2014). The acceptance criteria were taken from guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. (DG Document N° SANTE/11945/2015). The acceptance criteria for method performance: recovery range 70-120%, repeatability relative standard deviation (RSD_r) <20% and within laboratory reproducibility (RSD_{wR}) <20% (DG SANTE/11945/2015).

The performance of the QuEChERS method was tested by performing six replicates of spiked blank pepper samples at three different concentration levels (0.01, 0.05 and 0.1 mg/kg) for 200 pesticides using GC/MSMS. There were 198 pesticides showed accepted average recovery and precision, while 2 pesticides; biphenyl (53%) and dichlobenil (64%) had shown unacceptable average recovery (Table 1).

#	Pesticide Name	0.01 mg/kg		0.05 mg/kg		0.1 mg/kg		All levels	
		Mean Recovery %	CV%	Mean Recovery %	CV%	Mean Recovery %	CV%	Mean Recovery %	CV%
1	Acrinathrin	97%	6%	92%	17%	90%	7%	93%	4%
2	Alachlor	94%	3%	110%	7%	97%	3%	101%	8%
3	Ametryn	106%	13%	108%	7%	91%	4%	102%	9%
4	Amitraz	77%	5%	75%	6%	64%	10%	72%	9%
5	Atraton	124%	14%	101%	10%	86%	9%	104%	19%
6	Atrazine	99%	4%	111%	8%	105%	8%	105%	6%
7	Azinphos-ethyl	98%	3%	112%	6%	95%	16%	102%	9%
8	Azinphos-methyl	105%	8%	117%	10%	96%	24%	106%	10%
9	Benalaxyl	97%	8%	115%	5%	97%	3%	103%	10%
10	Bendiocarb	88%	12%	113%	8%	97%	10%	100%	13%
11	Bifenthrin	92%	6%	111%	5%	97%	4%	100%	10%
12	Bifinazate	91%	7%	102%	5%	87%	11%	93%	9%
13	Biphenyl	38%	21%	66%	14%	54%	22%	53%	27%
14	Bitertanol	94%	4%	109%	5%	86%	12%	96%	12%
15	Boscalid	100%	8%	121%	7%	94%	21%	105%	13%
16	Bromophos-ethyl	93%	7%	105%	4%	95%	4%	98%	7%
17	Bromophos-methyl	93%	6%	102%	7%	97%	6%	97%	5%
18	Bromopropylate	91%	4%	112%	5%	95%	4%	99%	11%
19	Bromuconazole	92%	7%	106%	6%	90%	9%	96%	9%
20	Bupirimate	106%	5%	110%	5%	98%	3%	105%	6%
21	Buprofezin	85%	11%	116%	5%	99%	4%	100%	15%
22	Butachlor	96%	5%	115%	9%	101%	3%	104%	10%
23	Butralin	98%	3%	112%	4%	96%	1%	102%	9%
24	Cadusafos	100%	3%	107%	5%	96%	3%	101%	5%
25	Captan	89%	21%	91%	20%	89%	8%	90%	1%
26	Carbofuran	89%	13%	101%	13%	99%	11%	96%	6%
27	Carbosulfan	97%	3%	66%	11%	66%	13%	76%	24%

#	Pesticide Name	0.01 mg/kg		0.05 mg/kg		0.1 mg/kg		All levels	
		Mean Recovery %	CV%	Mean Recovery %	CV%	Mean Recovery %	CV%	Mean Recovery %	CV%
28	Carboxin	83%	9%	66%	17%	65%	24%	71%	14%
29	Chlordane cis-	97%	6%	108%	5%	100%	4%	102%	5%
30	Chlordane trans-	106%	9%	98%	6%	98%	2%	101%	4%
31	Chlorfenapyr	115%	12%	110%	4%	103%	4%	109%	5%
32	Chlorfenvinphos	99%	4%	110%	7%	98%	5%	103%	6%
33	Chlorobenzilate	95%	4%	109%	6%	98%	4%	101%	7%
34	Chlorothalonil	82%	7%	79%	12%	66%	10%	76%	11%
35	Chlorpropham	87%	12%	96%	9%	98%	9%	94%	6%
36	Chlorpyrifos	88%	6%	103%	3%	98%	5%	96%	8%
37	Chlorpyrifos-methyl	89%	8%	107%	6%	99%	5%	98%	9%
38	Chlorthal-dimethyl	100%	10%	110%	6%	98%	3%	103%	6%
39	Chlorthiophos	95%	2%	111%	5%	98%	5%	101%	8%
40	Chlozolate	105%	8%	102%	6%	98%	4%	102%	3%
41	Clodinafop-propargyl ester	101%	3%	98%	9%	94%	7%	98%	4%
42	Coumaphos	122%	6%	107%	13%	97%	15%	109%	12%
43	Cyanophos	92%	7%	99%	9%	93%	6%	95%	4%
44	Cyfluthrin	104%	7%	116%	5%	94%	11%	105%	11%
45	Cyhalothrin lambda-	90%	4%	109%	7%	95%	5%	98%	10%
46	Cypermethrin	97%	4%	114%	3%	86%	12%	99%	14%
47	Cyproconazole	100%	5%	103%	6%	96%	7%	100%	3%
48	Cyprodinil	92%	3%	105%	5%	93%	5%	97%	8%
49	Cyromazine	ND	ND	82%	9%	85%	7%	84%	2%
50	DDD op`-	93%	4%	108%	4%	99%	3%	100%	7%
51	DDD pp`-	98%	3%	109%	5%	99%	3%	102%	6%
52	DDE pp`-	88%	4%	102%	3%	97%	2%	95%	7%
53	DDT op`-	98%	3%	109%	5%	99%	3%	102%	6%
54	Deltamethrin	107%	15%	119%	8%	89%	10%	105%	14%
55	Diazinon	85%	4%	102%	5%	99%	4%	95%	10%
56	Dichlobenil	68%	12%	67%	13%	57%	16%	64%	10%
57	Dichlofenthion	96%	4%	98%	5%	97%	4%	97%	1%
58	Dichlofluanid	99%	1%	94%	5%	89%	5%	94%	5%
59	Diclofop-methyl	95%	7%	108%	6%	95%	5%	99%	8%
60	Dicloran	111%	9%	80%	19%	71%	18%	87%	24%
61	Dicofol	104%	9%	129%	8%	103%	6%	112%	13%
62	Dieldrin	98%	9%	108%	3%	95%	4%	100%	7%
63	Dimethachlor	89%	7%	108%	6%	97%	4%	98%	10%
64	Dimethoate	84%	12%	96%	9%	87%	7%	89%	7%
65	Diniconazole	91%	4%	105%	7%	92%	6%	96%	8%
66	Diphenylamine	95%	2%	97%	3%	94%	9%	95%	1%
67	Disulfoton	91%	5%	93%	4%	89%	6%	91%	2%
68	Ditalimfos	85%	6%	90%	6%	84%	4%	86%	4%
69	Endosulfan alpha-	80%	12%	115%	5%	97%	3%	97%	18%

#	Pesticide Name	0.01 mg/kg		0.05 mg/kg		0.1 mg/kg		All levels	
		Mean Recovery %	CV%	Mean Recovery %	CV%	Mean Recovery %	CV%	Mean Recovery %	CV%
70	Endosulfan beta-	96%	9%	107%	4%	112%	5%	105%	8%
71	Endosulfan-sulfate	101%	2%	109%	5%	96%	3%	102%	6%
72	Endrin	100%	6%	107%	6%	101%	3%	103%	4%
73	EPN	95%	3%	112%	5%	96%	6%	101%	9%
74	Epoxiconazole	89%	8%	101%	7%	86%	11%	92%	9%
75	Ethion	99%	3%	109%	6%	98%	5%	102%	6%
76	Ethofumesate	89%	7%	110%	2%	104%	2%	101%	11%
77	Ethoprophos	96%	3%	102%	5%	94%	5%	97%	4%
78	Ethoxyquin	81%	5%	88%	4%	81%	6%	83%	5%
79	Etofenprox	92%	3%	112%	4%	90%	11%	98%	12%
80	Etoxazole	91%	6%	112%	5%	97%	5%	100%	11%
81	Etridiazole	101%	4%	96%	11%	85%	13%	94%	9%
82	Fenarimol	93%	1%	94%	16%	88%	11%	92%	3%
83	Fenazaquin	94%	4%	109%	5%	93%	4%	99%	9%
84	Fenbuconazole	101%	5%	113%	5%	90%	17%	102%	11%
85	Fenitrothion	88%	11%	103%	8%	97%	7%	96%	8%
86	Fenoxaprop-P-ethyl	94%	8%	113%	9%	85%	15%	97%	14%
87	Fenoxycarb	83%	9%	118%	9%	87%	17%	96%	20%
88	Fenpropathrin	86%	4%	108%	6%	101%	5%	98%	12%
89	Fenpropidin	91%	6%	101%	7%	86%	5%	93%	8%
90	Fenpropimorph	84%	6%	108%	5%	96%	4%	96%	12%
91	Fenvalerate	100%	2%	100%	3%	90%	6%	97%	6%
92	Fluazifop-P-butyl	96%	11%	108%	4%	100%	5%	101%	6%
93	Flucythrinate	96%	3%	114%	2%	93%	9%	101%	12%
94	Fludioxonil	96%	4%	109%	8%	97%	13%	101%	7%
95	Fluquinconazole	105%	2%	112%	6%	86%	9%	101%	13%
96	Flusilazole	95%	5%	112%	5%	95%	5%	101%	10%
97	Fluvalinate tau-	ND	ND	121%	20%	93%	19%	107%	19%
98	Formothion	95%	3%	89%	11%	110%	9%	98%	11%
99	HCH alpha-	94%	3%	102%	4%	93%	2%	97%	5%
100	HCH beta-	95%	4%	102%	3%	100%	2%	99%	4%
101	HCH delta-	102%	6%	104%	6%	96%	4%	101%	4%
102	HCH gamma-	97%	1%	99%	4%	95%	4%	97%	2%
103	Heptachlor	98%	2%	103%	6%	101%	4%	100%	2%
104	Heptachlor-endo-Epoxide	115%	10%	110%	5%	99%	5%	108%	8%
105	Heptachlor-exo-Epoxide	95%	6%	107%	9%	94%	3%	99%	7%
106	Heptenophos	92%	4%	99%	5%	91%	3%	94%	4%
107	Hexachlorobenzene	80%	4%	88%	6%	84%	4%	84%	5%
108	Hexaconazole	92%	7%	108%	6%	99%	7%	99%	8%
109	Hexazinone	93%	5%	107%	5%	87%	13%	96%	11%
110	Imazalil	81%	14%	98%	7%	82%	13%	87%	11%
111	Iprodione	89%	3%	110%	6%	93%	11%	97%	12%

#	Pesticide Name	0.01 mg/kg		0.05 mg/kg		0.1 mg/kg		All levels	
		Mean Recovery %	CV%	Mean Recovery %	CV%	Mean Recovery %	CV%	Mean Recovery %	CV%
112	Isufenphos	96%	7%	109%	7%	97%	6%	100%	7%
113	Isufenphos-methyl	99%	5%	108%	8%	98%	6%	101%	5%
114	Isoprothiolane	115%	5%	106%	4%	101%	6%	107%	7%
115	Kresoxim-methyl	93%	5%	113%	6%	102%	4%	103%	10%
116	Linuron	78%	12%	113%	7%	95%	4%	95%	18%
117	Malathion	93%	3%	114%	7%	99%	5%	102%	10%
118	Mecarbam	101%	10%	113%	11%	102%	5%	106%	6%
119	Mefenacet	98%	5%	114%	6%	88%	20%	100%	13%
120	Mefenpyr-diethyl	96%	6%	114%	5%	95%	3%	102%	10%
121	Mepronil	96%	9%	114%	8%	89%	9%	100%	13%
122	Metalaxyl	97%	16%	108%	8%	94%	8%	100%	7%
123	Metazachlor	88%	9%	110%	6%	95%	4%	98%	12%
124	Methacrifos	93%	5%	97%	5%	87%	5%	92%	6%
125	Methidathion	99%	3%	105%	7%	98%	6%	100%	4%
126	Methoxychlor	100%	3%	115%	5%	103%	4%	106%	8%
127	Metribuzin	96%	3%	100%	5%	93%	5%	96%	4%
128	Mevinphos	109%	13%	103%	6%	89%	7%	100%	10%
129	Mirex	86%	4%	102%	4%	92%	2%	93%	9%
130	Monolinuron	92%	8%	100%	14%	97%	17%	96%	4%
131	Myclobutanil	92%	7%	112%	4%	93%	3%	99%	11%
132	Napropamide	91%	3%	111%	6%	95%	4%	99%	11%
133	Nuarimol	93%	5%	108%	6%	93%	4%	98%	9%
134	Oxadiazyl	91%	11%	97%	4%	90%	5%	93%	4%
135	Oxadiazon	95%	5%	110%	4%	100%	4%	102%	7%
136	Oxyfluorfen	96%	4%	104%	5%	98%	5%	99%	4%
137	Parathion-ethyl	100%	1%	104%	6%	99%	6%	101%	3%
138	Parathion-methyl	97%	3%	98%	6%	98%	6%	97%	1%
139	PCB 028	72%	21%	120%	5%	86%	11%	93%	27%
140	PCB 052	87%	7%	101%	4%	95%	2%	94%	8%
141	PCB 101	78%	5%	99%	4%	93%	2%	90%	12%
142	PCB 138	73%	11%	101%	3%	90%	4%	88%	16%
143	PCB 153	75%	10%	97%	3%	92%	3%	88%	13%
144	PCB 180	59%	10%	87%	2%	87%	2%	78%	21%
145	Penconazole	89%	6%	109%	5%	94%	4%	97%	11%
146	Pendimethalin	107%	8%	104%	10%	96%	8%	102%	6%
147	Pentachlorobenzene	80%	11%	78%	5%	73%	7%	77%	4%
148	Permethrin	94%	4%	109%	6%	95%	8%	99%	8%
149	Phenthoate	92%	7%	109%	8%	100%	6%	101%	9%
150	Phenylphenol ortho- (OPP)	85%	8%	98%	9%	90%	5%	91%	7%
151	Phorate	92%	2%	99%	3%	90%	4%	93%	5%
152	Phosalone	101%	2%	116%	4%	95%	8%	104%	10%
153	Piperonyl-butoxide	94%	7%	114%	6%	98%	4%	102%	11%

#	Pesticide Name	0.01 mg/kg		0.05 mg/kg		0.1 mg/kg		All levels	
		Mean Recovery %	CV%	Mean Recovery %	CV%	Mean Recovery %	CV%	Mean Recovery %	CV%
154	Pirimicarb	91%	10%	100%	7%	97%	3%	96%	5%
155	Pirimiphos-ethyl	96%	3%	106%	8%	97%	4%	100%	6%
156	Pirimiphos-methyl	101%	6%	109%	6%	99%	5%	103%	6%
157	Prochloraz	108%	11%	113%	10%	94%	12%	105%	10%
158	Procymidone	102%	4%	108%	4%	98%	4%	103%	5%
159	Profenofos	93%	7%	100%	7%	98%	5%	97%	4%
160	Profluralin	97%	4%	108%	4%	102%	4%	102%	5%
161	Promecarb	96%	4%	103%	7%	101%	4%	100%	3%
162	Prometon	89%	4%	108%	5%	97%	4%	98%	10%
163	Prometryn	102%	6%	110%	6%	98%	5%	104%	6%
164	Propachlor	97%	3%	101%	4%	91%	4%	96%	5%
165	Propazine	102%	2%	104%	4%	97%	3%	101%	4%
166	Propiconazol	94%	12%	113%	9%	91%	9%	99%	12%
167	Prothiofos	93%	4%	110%	7%	99%	5%	101%	9%
168	Pyrazofos	99%	2%	112%	5%	93%	10%	101%	9%
169	Pyridaphenthion	110%	2%	109%	3%	93%	13%	104%	9%
170	PyrifenoX	91%	12%	101%	8%	94%	5%	95%	5%
171	Pyrimethanil	91%	9%	100%	16%	98%	9%	97%	5%
172	Pyriproxyfen	94%	3%	111%	6%	93%	7%	99%	10%
173	Quinalphos	97%	4%	108%	6%	96%	4%	100%	6%
174	Quintozene	105%	6%	101%	6%	92%	4%	100%	7%
175	Simazine	105%	13%	92%	4%	97%	8%	98%	7%
176	Spiromesifen	89%	20%	100%	10%	114%	4%	101%	12%
177	Spiroxamine	90%	2%	105%	3%	96%	4%	97%	8%
178	Sulfotep	100%	4%	103%	4%	95%	4%	99%	4%
179	Tebuconazole	86%	6%	106%	9%	92%	7%	95%	11%
180	Tebufenpyrad	91%	3%	108%	6%	93%	4%	98%	10%
181	Tecnazene	88%	7%	97%	2%	92%	4%	93%	5%
182	Tefluthrin	98%	5%	108%	3%	99%	3%	102%	6%
183	Terbufos	98%	3%	103%	2%	95%	4%	99%	4%
184	Terbumeton	93%	4%	101%	3%	94%	2%	96%	5%
185	Terbuthylazine	99%	3%	108%	4%	96%	4%	101%	6%
186	Terbutryn	95%	7%	106%	4%	97%	4%	99%	6%
187	Tetraconazole	95%	7%	110%	5%	96%	4%	100%	8%
188	Tetradifon	96%	4%	101%	4%	89%	4%	95%	6%
189	Tetramethrin	112%	32%	113%	5%	97%	5%	107%	8%
190	Thiobencarb	92%	7%	106%	4%	95%	4%	98%	7%
191	Thiometon	87%	7%	89%	5%	85%	7%	87%	2%
192	Tolclofos-methyl	99%	4%	109%	5%	99%	4%	102%	6%
193	Tolyfluanid	98%	1%	108%	9%	95%	5%	100%	7%
194	Triadimefon	100%	1%	106%	6%	101%	4%	102%	3%
195	Triadimenol	92%	8%	101%	4%	93%	5%	95%	5%

#	Pesticide Name	0.01 mg/kg		0.05 mg/kg		0.1 mg/kg		All levels	
		Mean Recovery %	CV%	Mean Recovery %	CV%	Mean Recovery %	CV%	Mean Recovery %	CV%
196	Triazophos	94%	2%	109%	6%	96%	7%	100%	8%
197	Trifloxystrobin	101%	3%	111%	5%	99%	4%	104%	6%
198	Triflumizole	92%	7%	103%	7%	94%	5%	96%	6%
199	Trifluralin	96%	2%	106%	4%	97%	3%	100%	6%
200	Vinclozolin	104%	9%	109%	6%	95%	6%	103%	7%

The average recovery at the limit of quantitation (LOQ) level (0.01mg/kg) ranged between 72%-120% for 195 pesticides, Four pesticides showed unaccepted recoveries; Biphenyl (38%), Dichlobenil (68%), PCB 180 (59%), Atraton (124%) and Coumpos (122%). The repeatability $RSD_r\%$ ranged between 1%-20% for 196 pesticides. While four pesticides showed $RSD_r\% > 20\%$; Biphenyl (21%), PCB 028 (21%), Captan (21%) and tetramethrin (32%).

The LOQ for all pesticides was 0.01 mg/kg which fulfill the EU MRLs for almost all agricultural products. Only two pesticides couldn't be detected at the 0.01 mg/kg which were cyromazine and fluvalenate and their LOQs were considered to be 0.05 mg/kg. However, the LOQ of 0.05 mg/kg will fairly fulfill the high EU MRLs of 1.5 mg/kg for cyromazine. While the EU MRL for fluvalenate is 0.01 mg/kg on pepper, so it is better to quantify this pesticide using LC-MSMS with LOQ of 0.01 mg/kg according to our recent publication (Emad Attallah, 2016).

The average recovery at 0.05mg/kg concentration level ranged between 75%-120% for 195 pesticides, Four pesticides showed unaccepted recoveries; Biphenyl (66%), Carbosulfan (66%), carboxin (66%), Dichlobenil (67%), Fluvalenate (121%), Boscalid (121%) and Dicofol (129%). The repeatability $RSD_r\%$ ranged between 2%-20% for all pesticides.

The average recovery at 0.1mg/kg concentration level ranged between 71%-114% for 194 pesticides, Six pesticides showed unaccepted recoveries; Biphenyl (54%), Amitraz (64%), carboxin (65%), Carbosulfan (66%) Dichlobenil (57%), and Chlorothalonil (66%). The repeatability $RSD_r\%$ ranged between 1%-20% for 196 pesticides. While four pesticides showed $RSD_r\% > 20\%$; Biphenyl (22%), Boscalid (21%), Carboxin (24%) and Azinophos methyl (24%).

The mean recovery for all levels was ranged between 71%-112% for 198 pesticides and reproducibility expressed as CV% for all level ranged between 1%-20% for 195 pesticides which fulfill the requirements of the DG-SANTE for recovery (70%-120%) and reproducibility (CV < 20%). However, there were 2 pesticides had shown unacceptable average recovery; biphenyl (38%) and dichlobenil (68%). While there are 5 pesticides showed unaccepted reproducibility (21%-27%); PCB 180 (21%), Carbosulfan (24%), Dicloran (24%), Biphenyl (27%) and PCB 028 (27%).

Conclusion

In the last few years, the Egyptian exports of pepper were considered of great concern, due to the international market demand.

In the present study, multiresidue method of determination of 200 pesticides using GC-MSMS was validated at three different concentration levels, the limit of quantitation of 0.01 mg/kg which fulfills the EU MRLs for organic agricultural products and baby foods.

The QuEChERS method followed by GC-MSMS was found to be the best combination for determination of the 200 GC-amenable pesticides in terms of accepted recovery, short time of analysis, low cost and safety.

The combination of both GC-MSMS and LC-MSMS enable the analysis of 420 pesticides in one multiresidue method. This method could be used to control the contamination levels of pesticide residues in fresh pepper to fulfill the EU and international of pesticide maximum residue limits (MRLs) for conventional and organic products.

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