Taylor & Francis
Taylor & Francis Group

Journal of Environmental Science and Health, Part B
Pesticides, Food Contaminants, and Agricultural Wastes

ISSN: 0360-1234 (Print) 1532-4109 (Online) Journal homepage: http://www.tandfonline.com/loi/lesb20

Validation of QuEChERS method for the determination of 36 pesticide residues in fruits and vegetables from Ghana, using gas chromatography with electron capture and pulsed flame photometric detectors

Augustine Donkor, Paul Osei-Fosu, Stephen Nyarko, Robert Kingsford-Adaboh, Brajesh Dubey & Isaac Asante

To cite this article: Augustine Donkor, Paul Osei-Fosu, Stephen Nyarko, Robert Kingsford-Adaboh, Brajesh Dubey & Isaac Asante (2015) Validation of QuEChERS method for the determination of 36 pesticide residues in fruits and vegetables from Ghana, using gas chromatography with electron capture and pulsed flame photometric detectors, Journal of Environmental Science and Health, Part B, 50:8, 560-570, DOI: 10.1080/03601234.2015.1028833

To link to this article: https://doi.org/10.1080/03601234.2015.1028833

Published online: 11 Jun 2015.

Journal of Environmental Science and Health, Part B (2015) **50**, 560–570

ISSN: 0360-1234 (Print); 1532-4109 (Online)
DOI: 10.1080/03601234.2015.1028833

Validation of QuEChERS method for the determination of 36 pesticide residues in fruits and vegetables from Ghana, using gas chromatography with electron capture and pulsed flame photometric detectors

AUGUSTINE DONKOR[1], PAUL OSEI-FOSU[1,2], STEPHEN NYARKO[1], ROBERT KINGSFORD-ADABOH[1], BRAJESH DUBEY[3] and ISAAC ASANTE[4]

[1]*Department of Chemistry, University of Ghana, Legon, Accra, Ghana*
[2]*Pesticide Residue Laboratory, Ghana Standards Authority, Accra, Ghana*
[3]*Environmental Engineering, School of Engineering, University of Guelph, Guelph, Canada*
[4]*Department of Botany, University of Ghana, Legon, Accra, Ghana*

In this study, "Quick, Easy, Cheap, Effective, Rugged and Safe" 'QuEChERS' method was modified for the determination of 36 pesticides fortified at (0.01–1.0) mg kg^{-1} in three vegetables and a fruit (lettuce, carrot, tomatoes and pineapples respectively) from Ghana. The method involved extraction with acetonitrile, phase separation with primary secondary amine and magnesium sulfate; the final injection solution was reconstituted in ethyl acetate. Organochlorine and synthetic pyrethroids residues were detected with electron capture detector whereas organophosphorus, pulsed flame photometric detector was used. The recoveries at different concentration levels (0.01, 0.1 and 1.0 mg kg^{-1}) were in the range of 83% and 93% with relative standard deviation ranging from 2% to 10% ($n = 5$) and the coefficient of determination (R^2) was greater than 0.99 for all the 36 pesticides. The method was successfully tested on 120 real samples from Accra markets and this proved to be useful for monitoring purposes particularly in laboratories that have no gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry.

Keywords: Validation, pesticides, ethyl acetate, gas chromatography-electron capture detector, gas chromatography-pulsed flame photometric detector.

Introduction

Multi-residue pesticide analysis in fruits and vegetables typically involves so many possible analyte/matrix pairs and concentration levels that it is impractical for a method to be fully validated for each combination. Thus, representative pesticides and commodities are typically selected to assess the laboratory performance of the method. Chemical and physical properties of common pesticides differ considerably, and this diversity causes challenges in the development of a universal analytical method for residues, which usually need to have the widest scope possible.[1,2]

Anastassiades et al.[3] published the "quick, easy, cheap, effective, rugged and safe" (QuEChERS) method for the monitoring of pesticide residues in fruits and vegetables. Acetonitrile (MeCN) was used for the extraction followed liquid-liquid partitioning with anhydrous magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl). The dehydration and cleanup step, known as the dispersive solid-phase extraction (d-SPE) was achieved with primary secondary amine (PSA) sorbent (which effectively removes many polar matrix components common in food matrices, such as organic acids, fatty acids, sugars and some polar pigments) and anhydrous $MgSO_4$. Quantitative and qualitative analysis were evaluated only with gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS). Thus this method being very simple has seen various modifications successfully applied to the extraction of pesticides in different foods in several laboratories and in inter-laboratory trials for some time and now has become an Official Method of AOAC International.[4] Additionally, many studies have

Address correspondence to Augustine Donkor, Department of Chemistry, University of Ghana, Legon, Accra, Ghana; E-mail: adonkor@ug.edu.gh or augdonkor@gmail.com.
Received December 8, 2014.
Color versions of one or more figures in this article can be found online at www.tandfonline.com/lesb.

also demonstrated that the method also works well with flame photometric detector (FPD) and electrolytic conductivity detector in GC and LC with post-column fluorescence derivatization and liquid chromatography-tandem mass spectroscopy.[3,5–9]

In most of the modified QuEChERS methods developed, the final solvent for injection into GC/MS or LC/MS has been MeCN. MeCN has also been used for GC analysis.[4] In recent times, studies with hexane, toluene and isooctane as exchange solvents have also been reported.[10] Nonetheless, in the GC system, the solvent expansion volume is very essential, thus according to Maštovská and Lehotay[10] the GC system must be little in size so as to permit high injection volumes without risk of liner overflow which provides high sensitivity without inlet contamination, sample discrimination and/or reduction of reproducibility. In this regard, ethyl acetate (EtOAc) has been found to be a better solvent than MeCN as it is less polar, provides a smaller liquid-to-gas expansion volume and greater stability to certain pesticides, for example, chlorothalonil.[5] Isooctane, hexane and toluene are also known to have bigger expansion volume than EtOAc. Likewise acetone and hexane have been noted for their low boiling points and volatilities compared to EtOAc which would prevent long-term storage of the final extract. Although toluene is one of the best solvent for GC injection process; however, it is more toxic than EtOAc and affects human health.[10]

Additionally, one critical advantage in the use of EtOAc over MeCN is that, the nitrogen-phosphorus detector (NPD), which is a common GC selective detector for organophosphorus (OP) is not usable with MeCN as injection solvent unless the instrument is equipped with a solvent bypass valve for the detector. The MeCN solvent front also produces a high response in the sensitive NPD, and re-equilibration in ionization would only be reached after many hours, depending on the condition of the detector's bead.[4]

The main purpose of this study was to validate the QuEChERS method for the determination of pesticide residues in fruits and vegetables from Ghana using ethyl acetate as the final solvent for injection, GC with pulsed flame photometric detector (PFPD) for OP and electron capture detector (ECD) for organochlorines and synthetic pyrethroid pesticides. These detectors are mostly available in laboratories that are not equipped with GC-MS and LC-MS.

Materials and methods

Chemicals and reagents

Sodium chloride (NaCl) (high purity), disodium hydrogen citrate sesquihydrate, trisodium citrate dihydrate, anhydrous magnesium sulphate ($MgSO_4$) (all analytical grade) and formic acid (pesticide grade) were obtained from BDH Laboratory Supplies, England. The anhydrous $MgSO_4$ ($\geq$ 98% purity) was baked at 130°C for 12 h in an oven to remove phthalates. Bondesil-PSA sorbent of 40 μm particle size was obtained from Varian (Harbor City, CA, USA). All organic solvents used in the study were pesticide grade. The 36 pesticides reference standards (Methaminphos, Ethoprophos, Phorate, Diazinon, Fonofos, Dimethoate, Pirimiphos-methyl, Chlorpyrifos, Malathion, Fenitrothion, Parathion-ethyl, Chlorfenvinphos, Profenofos, Beta-HCH, Gamma-HCH, Delta-HCH, Heptachlor, Aldrin, Allethrin, Gamma-chlordane, Alpha-endosulfan, p.p′-DDE, Dieldrin, Eldrin, Beta-endosulfan, p,p′-DDT, p,p′-DDD, Endosulfan sulfate, Bifenthrin, Methoxychlor, Lambda-cyhalothrin, Permethrin, Cyfluthrin, Cypermethrin, Fenvalerate and Deltamethrin) about 100% certified purity were all obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Standard mixture stock solutions were prepared in ethyl acetate at 1,000 μg g^{-1} for each pesticide. This solution was used as spiking solution and also to prepare the standard solutions to obtain the calibration curves, by dilution with ethyl acetate.

Apparatus

The chromatographic system consisted of a Varian CP 3800 GC (Varian Associates Inc, Palo Alto, CA, USA), equipped with CTC Analytics CombiPAL, ECD and PFPD. Chromatographic separations for organochlorine and synthetic pyrethroid residues were performed with a capillary column coated with VF-5 ms (30 m + 10 m EZ Guard, 0.25 mm, 0.25 μm film thickness) and carrier and make-up gases were nitrogen at a flow rate of 1.0 and 29 mL min^{-1} respectively, while that of the organophosphorous were performed with capillary column coated with VF-1701 ms (30 m, 0.25 mm, 0.25 μm film thickness). The carrier gas was nitrogen at a flow rate of 2.0 mL min^{-1} with Air 1, Air 2 and hydrogen flow rates of 17, 10 and 14 mL min^{-1} respectively.

Organochlorine and synthetic pyrethroid residues were detected with ^{63}Ni ECD. The temperature of injector operating in splitless mode was held at 270°C, and the ECD was set at 300°C. The column oven temperature was programmed as follows; 70°C for 2 min. and increased steadily at a rate of 25°C min^{-1} to 180°C and increased at 5°C min^{-1} up to 300°C. The injection volume of the GC was 1.0 μL.

The OP residues were detected with PFPD. The corresponding temperature of injector operating in splitless mode was held at 270°C and the PFPD was set at 280°C. The column oven temperature was programmed as follows: 70°C for 2 min and increased steadily at a rate of 25°C min^{-1} to 200°C and increased at 20°C min^{-1} up to 250°C. The injection volume of the GC was 2.0 μL. For instrument control based on the results obtained from the

calibration of each pesticides type, data acquisition and processing were done with the Star Workstation software.

Other equipment used in the study included a Foss Homogenizer to comminute fruit and vegetable samples; an IKA Ultra Turrax with T25 head to homogenize samples during extraction; a Jouan CR3i multifunction centrifuge to centrifuge the extracts in 50 mL Teflon and 10 mL polypropylene (PP) centrifuge tubes with screw caps: a Thermolyne (Maxi Mix-Plus vortex) to shake the tubes; Mettler Toledo XS 105 and PG 1003-S analytical balances to weigh standards, salts and samples; and a R-210 Buchi Ratovapor to concentrate extracts. For extraction, 50 mL Teflon centrifuge tubes were used and 10 mL PP centrifuge tubes were used for d-SPE cleanup.

Sample collection

Blank samples of pineapples, carrots, lettuces and tomatoes were purchased from the local market and checked for absence of pesticides before used in the validation studies. These pesticides/crops were chosen based on current pesticides registry in Ghana for plant protection (which has more pesticides registered for vegetable application than fruits) as well as other properties. Carrot is root and tuber vegetable with high water content; pineapple has high acid content; tomato is fruiting-vegetable with high water content and lettuce which has high water and chlorophyll contents. These were used in the fortification experiments. Fruit and vegetable samples of 1–2 kg were comminuted using the Foss homogenizer and homogenous samples were stored in plastic bags in a deep-freezer (−18°C), or subsamples were quickly extracted.

Extraction and clean-up

Pineapple, carrots, lettuces and tomatoes were used as representative matrices in the validation experiments. Each commodity was spiked with the 36 pesticides solution at 0.01, 0.1 and 1 mg kg^{-1} respectively; 5 replicates at each level (i.e. 4 matrices at 3 levels/matrix and 5 replicates/level = 60 spiked samples). For each batch, a matrix blank, method blank and solvent blank were analyzed in addition.

An amount of 10.0 g of the comminuted homogenous sample was weighed into a 50 mL centrifuge tube. The samples were fortified with the proper pesticide solution to give 1.0, 0.1 or 0.01 mg kg^{-1} concentration. The fortified samples were made to stand for 20 min for the pesticides to interact with the matrix. An amount of 10 mL of acetonitrile was added and vortex for 1 min. After that a mixture of 4 g $MgSO_4$, 1 g NaCl, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogen citrate sesquihydrate were added and immediately vortex for 1 min and centrifuged for 5 min at 3,000 rpm.

An amount of 6.0 mL of aliquot of the extract was transferred into a 15 mL PP centrifugation tube which contains 150 mg PSA and 900 mg $MgSO_4$. The tube was closed and shaken vigorously and vortex for 30 s and centrifuged for 5 min at 3,000 rpm. 4.0 mL of the cleaned extract was transferred into a round bottom flask and adjusted quickly to a pH 5 by adding 40 μL of 5% formic acid solution in acetonitrile (v/v). The filtrate was concentrated to just dryness on a pressure reduced rotary evaporator with a water bath temperature below 40°C.

The extract was re-dissolved by adding 1 mL of ethyl acetate and was transferred into a 1.5 mL standard opening vial for quantitation with GC-ECD and GC-PFPD.

Confirmatory analysis

Confirmation was performed in all instances for each pesticide involved in the validation process by the use of different polar capillary columns. 30 m × 0.25 mm i.d. × 0.25 μm film thickness, VF-5 Pesticides (5% diphenyl 95% dimethylsiloxane) capillary column was used for the confirmation of the pesticides for the GC-PFPD, whereas 30 m × 0.25 mm i.d. × 0.25 μm film thickness, VF-1701 Pesticides (14% cyanopropyl/phenyl 86% methpolysiloxane) capillary column was utilized on the GC-ECD. Operating conditions were the same as described above.

Quality control and quality assurance

Solvent blanks, method blanks and matrix blanks were analyzed alongside samples. Results from the solvent, method and matrix blank were subtracted from the calculated results of each sample. These were used as a quality control check. Certified reference materials (lettuce and tomato) from Food Analysis Performance Assessment Scheme, (FAPAS) were analyzed as a quality assurance parameter.

Method validation

In all, 36 pesticides were analyzed by GC–ECD/PFPD; 13 pesticides with the GC-PFPD and 23 pesticides with GC-ECD.

Linearity study, limit of detection and limit of quantification determination

The method was validated at three residue concentrations within the range of 0.01–1.0 mg kg^{-1}. The calibration mixtures were prepared for ECD and PFPD detection at seven concentration levels in ethyl acetate (0.005, 0.01, 0.02, 0.05, 0.10, 0.5 and 1.0 ppm). Calculations were performed for the average peak areas, relative standard deviations (RSD%) and calibration curve equations, correlation

coefficients (R^2) and linear ranges. From the calibration curves, linearity data and the repeatability (RSD%) at the lowest concentration levels, of each one of the 36 pesticides studied, the limit of detection (LOD) and limit of quantification (LOQ) were estimated respectively. The LODs were calculated for each pesticide using the formula (LOD = 3.75 × standard deviation)[11] on the five sets of 0.01 mg kg^{-1} spike recovery data. The corresponding LOQ = 3 × LOD.

Analysis of real samples

Analysis of real samples was made up of 120 different fruit and vegetables (watermelons, aubergines, green peppers and okra) purchased from five (5) markets in the Accra Metropolitan City. These samples were collected during the first quarter of 2010. All samples were stored in plastic bags in a refrigerator (±5°C) until analysis. Preparation and analysis of the samples were as described above.

Statistical analysis

One way ANOVA was used to compare the average recoveries of the pesticides found. The Cochran test was also used to check if any of the variances obtained at one spiking level for all representative analytes were significantly different whereas Dixon test was applied to establish the outliers. A confidence interval of 95% was utilized for all the statistical evaluation.

Results and discussion

Selection of pesticides and representative compounds

For the validation of the method, 36 pesticides and 4 representative commodities (carrots, tomatoes, pineapples and lettuces) were used to determine the typical characteristics. The pesticides were chosen based on a wide range of physico-chemical properties (water solubility from 0.0002 mg

Table 1. Calculated LODs (mg kg^{-1}) and LOQs (mg kg^{-1}) data for fortified samples.

	Carrots		*Tomatoes*		*Pineapple*		*Lettuce*	
Pesticides	*LOD*	*LOQ*	*LOD*	*LOQ*	*LOD*	*LOQ*	*LOD*	*LOQ*
Methamidophos	0.003	0.010	0.004	0.011	0.002	0.006	0.002	0.006
Ethoprophos	0.004	0.012	0.003	0.009	0.002	0.007	0.003	0.010
Phorate	0.004	0.012	0.003	0.010	0.002	0.007	0.003	0.008
Diazinon	0.005	0.015	0.004	0.011	0.003	0.009	0.004	0.011
Fonofos	0.004	0.013	0.004	0.013	0.003	0.008	0.001	0.003
Dimethoate	0.002	0.006	0.002	0.007	0.001	0.003	0.003	0.008
Pirimiphos-methyl	0.005	0.015	0.003	0.008	0.002	0.007	0.004	0.013
Chlorpyrifos	0.006	0.017	0.001	0.003	0.002	0.006	0.002	0.006
Malathion	0.005	0.014	0.001	0.004	0.003	0.010	0.004	0.013
Fenitrothion	0.005	0.015	0.002	0.006	0.005	0.014	0.003	0.009
Parathion-ethyl	0.003	0.010	0.002	0.005	0.001	0.003	0.003	0.010
Chlorfenvinphos	0.006	0.017	0.004	0.012	0.003	0.010	0.003	0.008
Profenofos	0.003	0.008	0.002	0.006	0.004	0.012	0.001	0.004
Beta-HCH	0.002	0.006	0.003	0.008	0.001	0.002	0.002	0.007
Gamma-HCH	0.001	0.004	0.003	0.008	0.002	0.007	0.001	0.003
Delta-HCH	0.003	0.010	0.002	0.007	0.001	0.004	0.001	0.004
Heptachlor	0.003	0.008	0.002	0.006	0.001	0.004	0.002	0.007
Aldrin	0.003	0.009	0.002	0.005	0.002	0.007	0.001	0.003
Gamma Chlordane	0.003	0.008	0.002	0.006	0.001	0.003	0.002	0.007
Alpha-Endosulfan	0.002	0.005	0.003	0.008	0.003	0.009	0.001	0.003
p,p'-DDE	0.002	0.005	0.004	0.013	0.003	0.010	0.003	0.010
Dieldrin	0.005	0.015	0.002	0.005	0.001	0.002	0.001	0.003
Endrin	0.003	0.010	0.002	0.005	0.003	0.008	0.002	0.007
p,p'-DDT	0.001	0.004	0.003	0.010	0.002	0.005	0.003	0.009
p,p'-DDD	0.001	0.003	0.002	0.005	0.002	0.007	0.004	0.011
Endosulfan Sulphate	0.002	0.007	0.002	0.007	0.003	0.010	0.002	0.007
Methoxychlor	0.004	0.011	0.003	0.009	0.004	0.013	0.003	0.010
Allethrin	0.004	0.013	0.002	0.006	0.001	0.003	0.001	0.004
Bifenthrin	0.002	0.006	0.001	0.003	0.003	0.009	0.004	0.012
Lambda-cyhalothrin	0.003	0.008	0.001	0.004	0.002	0.006	0.001	0.004
Permethrin	0.005	0.014	0.004	0.011	0.003	0.008	0.005	0.015
Cyfluthrin	0.002	0.005	0.001	0.004	0.001	0.003	0.003	0.010
Cypermethrin	0.003	0.010	0.003	0.008	0.005	0.014	0.006	0.019
Fenvalerate	0.005	0.015	0.004	0.011	0.002	0.006	0.001	0.004
Deltamethrin	0.002	0.006	0.004	0.013	0.002	0.005	0.005	0.016

Table 2. Summary of recovery (Q%) coefficient of variation (CV_A) data in carrot, pineapple, lettuce and tomato.

	Fortification level (mg kg^{-1})						
	0.01		*0.10*		*1.0*		
	Q	***CV$_A$***	***Q***	***CV$_A$***	***Q***	***CV$_A$***	***Q$_A$***
				Carrot			
Organophosphorus	92.05	12.38	92.31	10.42	105.33	3.65	96.56
Organochlorines	91.61	7.51	93.93	4.96	89.16	2.42	91.57
Synthetic pyrethroids	88.50	8.49	93.50	4.57	85.25	2.48	89.08
Average	**90.72**		**93.25**		**93.25**		**92.41**
CV$_{typ}$		**9.46**		**6.65**		**2.85**	
				Pineapple			
Organophosphorus	90.22	7.86	87.08	9.20	100.89	4.98	92.73
Organochlorines	89.89	6.43	90.97	5.46	92.29	3.86	91.05
Synthetic pyrethroids	81.74	7.45	88.24	5.96	95.97	3.10	88.65
Average	**87.28**		**88.76**		**96.38**		**90.81**
CV$_{typ}$		**7.25**		**6.87**		**3.98**	
				Lettuce			
Organophosphorus	84.66	9.03	95.68	5.78	91.45	3.43	90.59
Organochlorines	88.96	6.47	86.13	5.73	85.11	3.71	86.73
Synthetic pyrethroids	93.25	10.20	90.19	7.17	78.40	4.68	87.28
Average	**88.96**		**90.67**		**84.99**		**88.20**
CV$_{typ}$		**8.57**		**6.23**		**3.94**	
				Tomato			
Organophosphorus	82.31	8.88	96.12	4.66	91.76	3.62	90.07
Organochlorines	87.98	7.38	91.31	6.54	85.98	5.30	88.42
Synthetic pyrethroids	81.38	8.26	88.24	4.91	85.91	6.12	85.18
Average	**83.89**		**91.89**		**87.88**		**87.89**
CV$_{typ}$		**8.17**		**5.37**		**5.01**	

Q is the average of replicate recovery tests at one level.
Q$_A$ is the average of recoveries at all fortification levels.
CV$_A$ is the coefficient of variation at one level.

L^{-1} to 200 g L^{-1}; $LogP_{ow}$, from −0.79 to 6.91; vapour pressure from 1.24×10^{-5} to 112 mPa and hydrolytic stability from 1 to 1130 days at pH 7)[12] and their current use in fruit and vegetables cultivation in Ghana.

Method validation and performance—analytical curves, linearity, LOD and LOQ

The various basic analytical parameters studied, precision, LOD, LOQ, recovery and linearity were to be certain that this modified method would be appropriate for real samples. Calibration curves obtained for all the 36 pesticides showed excellent linearity and the correlation coefficient (R^2) of $R^2 \geq 0.99$; the (R^2) for the OP pesticides ranged from 0.9976 to 0.9998, organochlorines pesticides from 0.9903 to 0.9981 while that of the synthetic pyrethroids pesticides ranged from 0.9925 to 0.9998. Deltamethrin, chlorfenvinphos, profenofos, dimethoate and methamidophos had the highest R^2 of 0.9998. The linear range was between 0.005 and 1.0 ppm. Figures A1 and A2 show the chromatograms for OP and OC/SP mixed standards, blank pineapple and tomato and spiked pineapple and tomato samples respectively.

Table 1 shows the estimated LOD and LOQ of each pesticide in its respective matrix. The LOD and LOQ for carrots matrix ranged from (0.001–0.006) mg kg^{-1}; (0.003–0.017) mg kg^{-1} respectively, tomatoes (0.001–0.004) mg kg^{-1}; (0.004–0.013) mg kg^{-1} respectively, pineapple (0.001–0.005) mg kg^{-1}; (0.003–0.014) mg kg^{-1} and lettuce (0.001–0.006) mg kg^{-1}; and (0.003–0.019) mg kg^{-1} respectively.

The LOQs with very low values below 0.01 mg kg^{-1} would generally be set at 0.01 mg kg^{-1} for reporting purposes. From the results, cypermethrin in lettuce had the highest LOQ value of 0.019 mg kg^{-1}.

Accuracy and precision of the method

The accuracy and precision of the method were determined by the analysis of five replicate analytical portions of each commodity spiked at 0.01, 0.1 and 1.0 mg kg^{-1}. The spiking solutions contained all the 36 compounds which were determined together.

Table 3. Overview of method validation performance characteristics for the different matrices.

		Accuracy		*Precision*	
Matrix	*Spiking level (mg kg^{-1})*	*Average recovery (%)*	*Codex acceptable range*	*Repeatability of recoveries RSD (%RSD)*	*Codex acceptable limit*
Tomato	0.01	83.89	70–120	8.17	30
	0.1	91.89	70–110	5.37	20
	1.0	87.88	70–110	5.01	10
Pineapple	0.01	87.28	70–120	7.25	30
	0.1	88.76	70–110	6.87	20
	1.0	96.38	70–110	3.98	10
Lettuce	0.01	88.96	70–120	8.57	30
	0.1	90.67	70–110	6.23	20
	1.0	84.99	70–110	3.94	10
Carrots	0.01	90.72	70–120	9.46	30
	0.1	93.25	70–110	6.65	20
	1.0	93.25	70–110	2.85	10

Validation results obtained are given in Table 2. The results indicated that the performance of the method with the commodities and representative pesticides meets the requirements of a quantitative method (mean recovery 70–120%, reproducibility CV_A 23–32%) specified in the Guideline for Single-laboratory Validation of Analytical Methods for Trace-level Concentration of Organic Chemicals (AOAC/FAO/IAEA/IUPAC Expert Consultation) and Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed.[13]

The recovery at all fortification levels for the carrots, pineapple and lettuce were within the acceptable range of 70–120%; however, the recovery at the fortification level of 0.01 mg kg^{-1} was the lowest and had the highest CV of 9.46 (carrot), 7.25 (pineapple) and 3.94 (lettuce) Table 2.

Likewise, the recovery at all fortification levels for the tomatoes were within the acceptable range of 70–120%; however, the recovery at the fortification level of 0.01 mg kg^{-1} was the lowest and had the highest CV of 8.17, Table 2.

Statistical tests for the evaluation of the recovery data

The differences between average recoveries, coefficient of variation and variances of recoveries for the pesticides in all the matrices and levels were not found to be statistically significant. These were calculated with the use of Dixon outlier test, *t* test, Cochran tests[14] and one way ANOVA. This is demonstrated in the average results in Tables 3 and 4 for the pesticides from the combined results for each matrix and/or level. For all the pesticides, four matrices and three levels, overall recovery of the method was 90% with RSD of 6.19%.

Analysis of real samples

The analysis of 120 real samples was to confirm the applicability of the method for the analysis of fruits and vegetables. No pesticides were detected in 12.5% of the samples and 87.5% of the samples gave positive detection (Table 5), suggesting they contained at least one pesticide with the concentration below or above the EU MRLs. In these positive samples, 30.2% had only one or two compounds, while the rest had three or more. The results proved that all the recoveries were relatively good. Further, confirmation of the identity of the pesticides in the real samples (using only watermelon) was carried out by the use of different polar capillary columns, Figure A3. Thus this demonstrates that the proposed method has a wide applicability towards samples of fruits and vegetables.

Table 4. Overview of method validation performance characteristics of the method at different levels independent of the matrix.

	Accuracy		*Precision*	
Spiking level (mg kg^{-1})	*Average recovery (%)*	*Codex acceptable range*	*Repeatability of recoveries RSD (%RSD)*	*Codex acceptable limit*
0.01	87.71	70–120	8.36	30
0.1	91.14	70–110	6.28	20
1.0	90.13	70–110	3.94	10
Overall	**90**		**6.19**	

Table 5. Summary of pesticide residue analysis in real fruits and vegetables.

Commodity	*No. of samples analysed*	*Residues level, range (mg kg^{-1})*	*No. of samples with residues not detected*	*%*	*No. of samples with residues detected*	*%*
Watermelon	30	(0.01–0.09)	6	20.0	24	80.0
Aubergine	30	(0.01–0.12)	2	6.7	28	93.3
Green pepper	30	(0.01–0.34)	3	10.0	27	90.0
Okra	30	(0.01–0.11)	4	13.3	26	86.7
Total	120		15	12.5	105	87.5

Conclusion

The QuEChERS method with the final injection solvent as ethyl acetate and using GC-ECD and GC-PFPD for multi-residue analysis was successfully validated for 36 pesticides in four matrices and three spiking levels. LODs and LOQs were <0.02 mg kg^{-1} and typically ranged between 0.005 and 0.02 mg kg^{-1} for the pesticides in the different matrices. The method developed was successfully tested on 120 vegetable and fruit samples which revealed its suitability for routine multi-residue analysis of OP, organochlorines and synthetic pyrethroids for monitoring purposes. Therefore, this method could be very practicable for the analysis of pesticides in fruits and vegetables, particularly in developing countries where laboratories are not equipped with MS instrumentation.

Acknowledgments

The authors thank Chemistry Department, University of Ghana and Pesticide Residues Laboratory, Ghana Standards Authority, Accra for technical support.

Funding

The authors are very grateful to anonymous friends for financial support for this study.

References

[1] Alder, L.; Greulich, K.; Kempe, G.; Vieth, B. Residue analysis of 500 high priority pesticides: better by GC-MS or LC-MS? Mass Spectrom. Rev. **2006**, *25*(6), 838–865.

[2] Food and Agriculture Organization/World Health Organization FAO/WHO. *Guidelines on Good Laboratory Practice in Residue Analysis*, CAC/GL-40, Rome, Italy, 1993.

[3] Anastassiades, M.; Lehotay, S.J.; Štajnbaher, D.; Schenck, F.J. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid phase extraction" for the determination of pesticide residues in produce. J. AOAC Int. **2003**, *86*(2), 412–431.

[4] Anastassiades, M. http://www.quechers.com/, (Accessed on 13 September 2013) Chemisches und Veterinä runtersuchungSant Stuttgart, Germany.

[5] Aysal, P; Ambrus, A; Lehotay, S.J.; Cannavan, A. Validation of an efficient method for the determination of pesticide residue in fruits and vegetables using ethyl acetate for extraction. J. Environ. Sci. and Health Part B. **2007**, *42*, 481–490.

[6] Lehotay, S.J. Determination of Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate: *Collaborative Study*. J. AOAC Int. **2007**, *90*, 485–520.

[7] Lehotay, S.J.; de Kok, A.; Hiemstra, M.; Van Bodegraven, P. Validation of a fast and easy method for the determination of 229 pesticides in fruits and vegetables using gas and liquid chromatography and mass spectrometric detection. J. AOAC Int. **2005**, *88*(2), 595–614.

[8] Schenck, F.J.; Hobbs, J.E. Evaluation of the quick, easy, cheap, effective, rugged and safe (QuEChERS) approach to pesticide residue analysis. Bull. Environ. Contam. Toxcol. **2004**, *73*(1), 24–30.

[9] Schenck, F.J., Callery, P.S., Gannett, P.M., Daft, J., Lehotay, S.J. "How Dry is dry? Removal of Water from Pesticide Extracts of Foods After Salting Out". J AOAC Int. **2002**, *85*: 1177–1180.

[10] Maštovská, K.; Lehotay, S.J. Evaluation of common organic solvents for gas chromatographic analysis and stability of multiclass pesticide residues. J. Chromatogr. A. **2004**, *1040*, 259–272

[11] US Environmental Protection Agency; Appendix B to 40 CFR Part 136 – *Definition and procedure for the determination of the method of detection limits*, Revision 1.11., 2012.

[12] Pesticide properties database-University of Hertfordshire, United Kingdom. Available at http://sitem.herts.ac.uk (accessed Oct 2014).

[13] SANCO/12571/2013. Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed. 2014. Available at http://ec.europa.eu/food/plant/plant_protection_products/guidance_documents/docs/qualcontrol_en.pdf (accessed Jun 2014).

[14] Horwitz W. Protocol for the design, conduct and interpretation of collaborative studies. Pure and Applied Chem. **1988**, *60*, 855.

Appendix

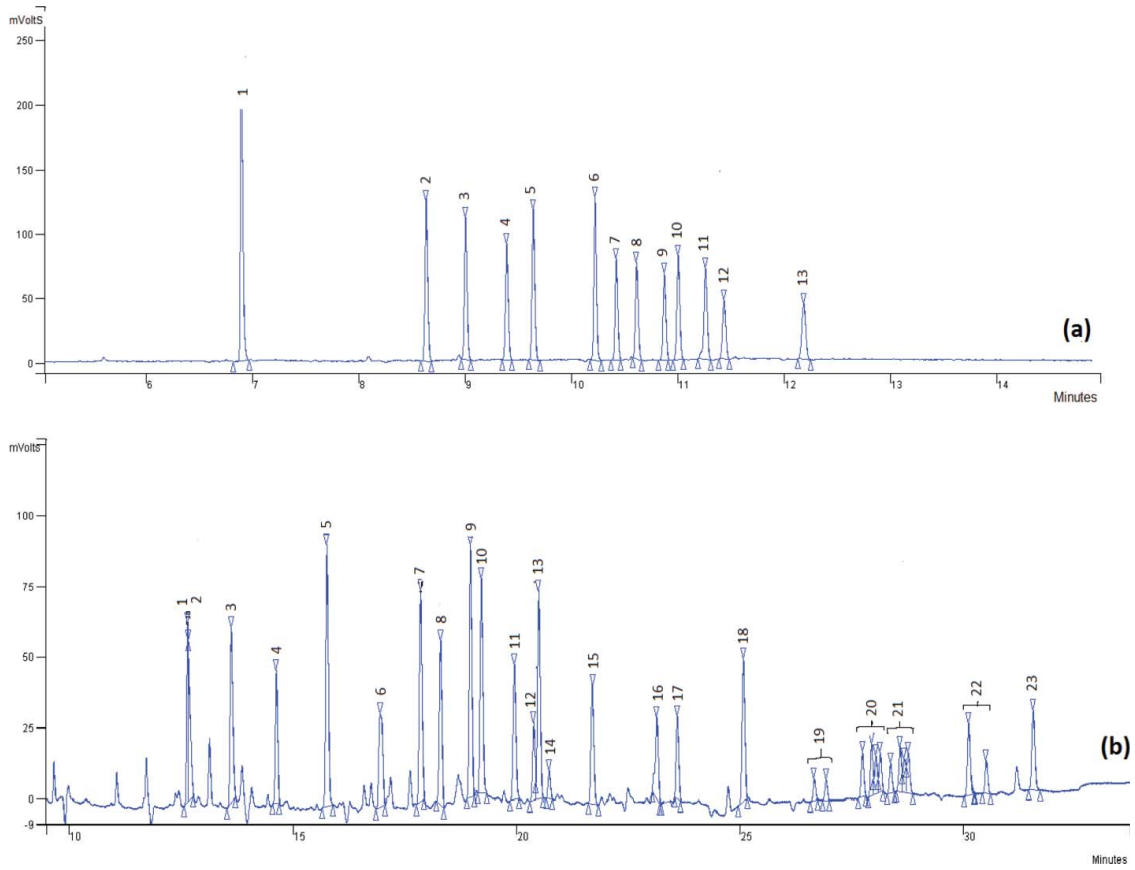

Fig. A1. GC/PFPD chromatograms of pesticide standards mixture (1.0 μg mL^{-1}) for OP (a) Peaks: 1: Methaminphos, 2: Ethoprophos, 3: Phorate, 4: Diazinon, 5: Fonofos, 6: Dimethoate, 7: Pirimiphos-methyl, 8: Chlorpyrifos, 9: Malathion, 10: Fenitrothion, 11: Parathion-ethyl, 12: Chlorfenvinphos, 13: Profenofos and GC/ECD chromatograms of pesticide standards mixture (0.10 μg mL^{-1}) for OC/SP (b) Peaks: 1: Beta-HCH, 2: Gamma-HCH, 3: Delta-HCH, 4: Heptachlor, 5: Aldrin, 6: Allethrin, 7: Gamma-chlordane, 8: Alpha-endosulfan, 9: p.p'-DDE, 10: Dieldrin, 11: Eldrin, 12: Beta-endosulfan, 13: p,p'-DDT, 14: p,p'-DDD, 15: Endosulfan sulfate, 16: Bifenthrin, 17: Methoxychlor, 18: Lambda-cyhalothrin, 19: Permethrin, 20: Cyfluthrin, 21: Cypermethrin, 22: Fenvalerate, 23: Deltamethrin.

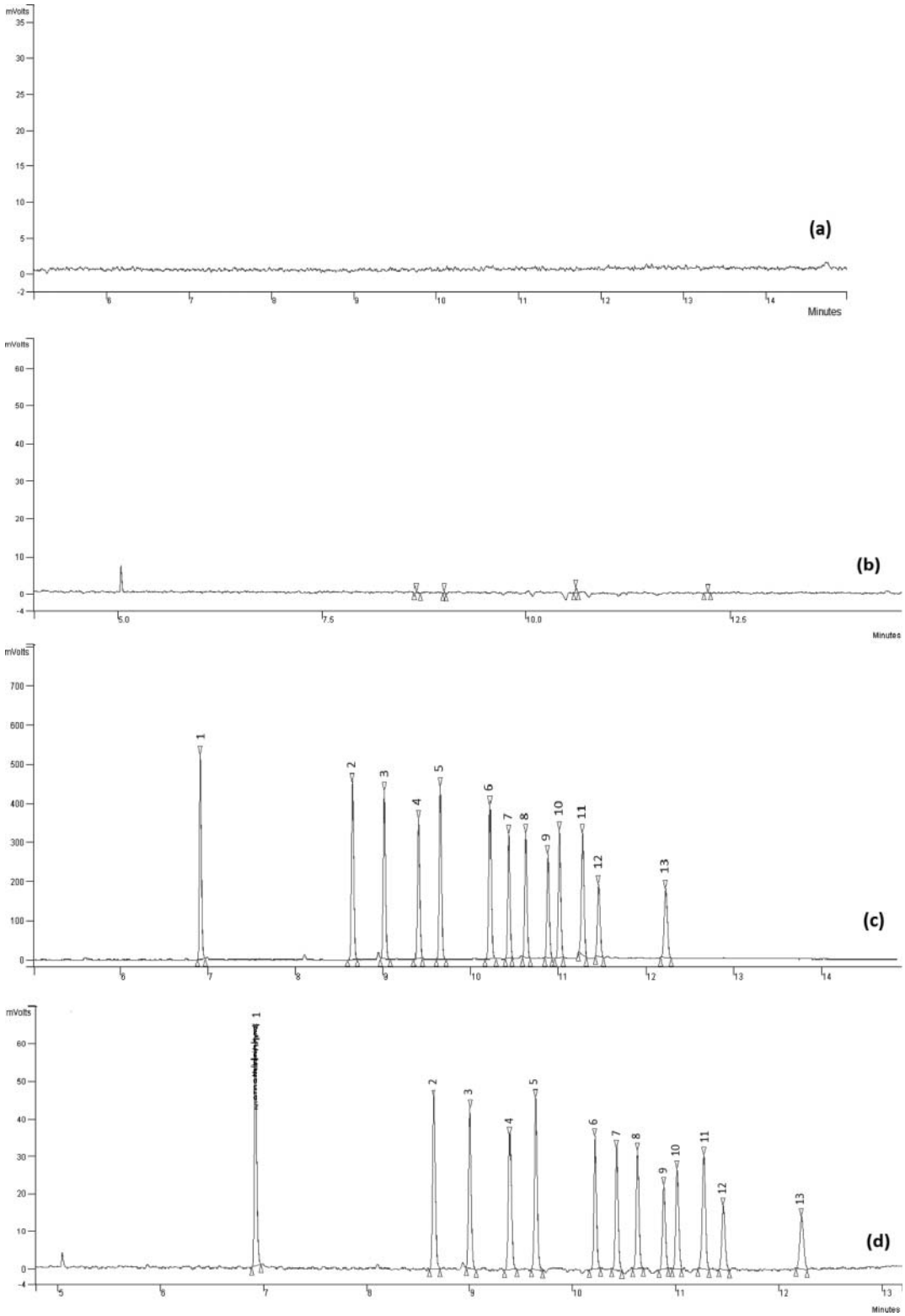

Fig. A2. Chromatograms of (a) extract of blank pineapple samples, (b) extract of blank tomato samples, (c) extract of pineapple and (d) tomato samples fortified at 1.0 mg kg^{-1} by OP pesticides. Peaks: 1: Methaminphos, 2: Ethoprophos, 3: Phorate, 4: Diazinon, 5: Fonofos, 6: Dimethoate, 7: Pirimiphos-methyl, 8: Chlorpyrifos, 9: Malathion, 10: Fenitrothion, 11: Parathion-ethyl, 12: Chlorfenvinphos, 13: Profenofos and chromatograms of (e) extract of blank pineapple samples, (f) extract of blank tomato samples, (g) extract of pineapple and (h) tomato samples fortified at 0.10 mg kg^{-1} by OC/SP pesticides. Peaks: 1: Beta-HCH, 2: Gamma-HCH, 3: Delta-HCH, 4: Heptachlor, 5: Aldrin, 6: Allethrin, 7: Gamma-chlordane, 8: Alpha-endosulfan, 9: p.p'-DDE, 10: Dieldrin, 11: Eldrin, 12: Beta-endosulfan, 13: p,p'-DDT, 14: p,p'-DDD, 15: Endosulfan sulfate, 16: Bifenthrin, 17: Methoxychlor, 18: Lambda-cyhalothrin, 19: Permethrin, 20: Cyfluthrin, 21: Cypermethrin, 22: Fenvalerate, 23: Deltamethrin.

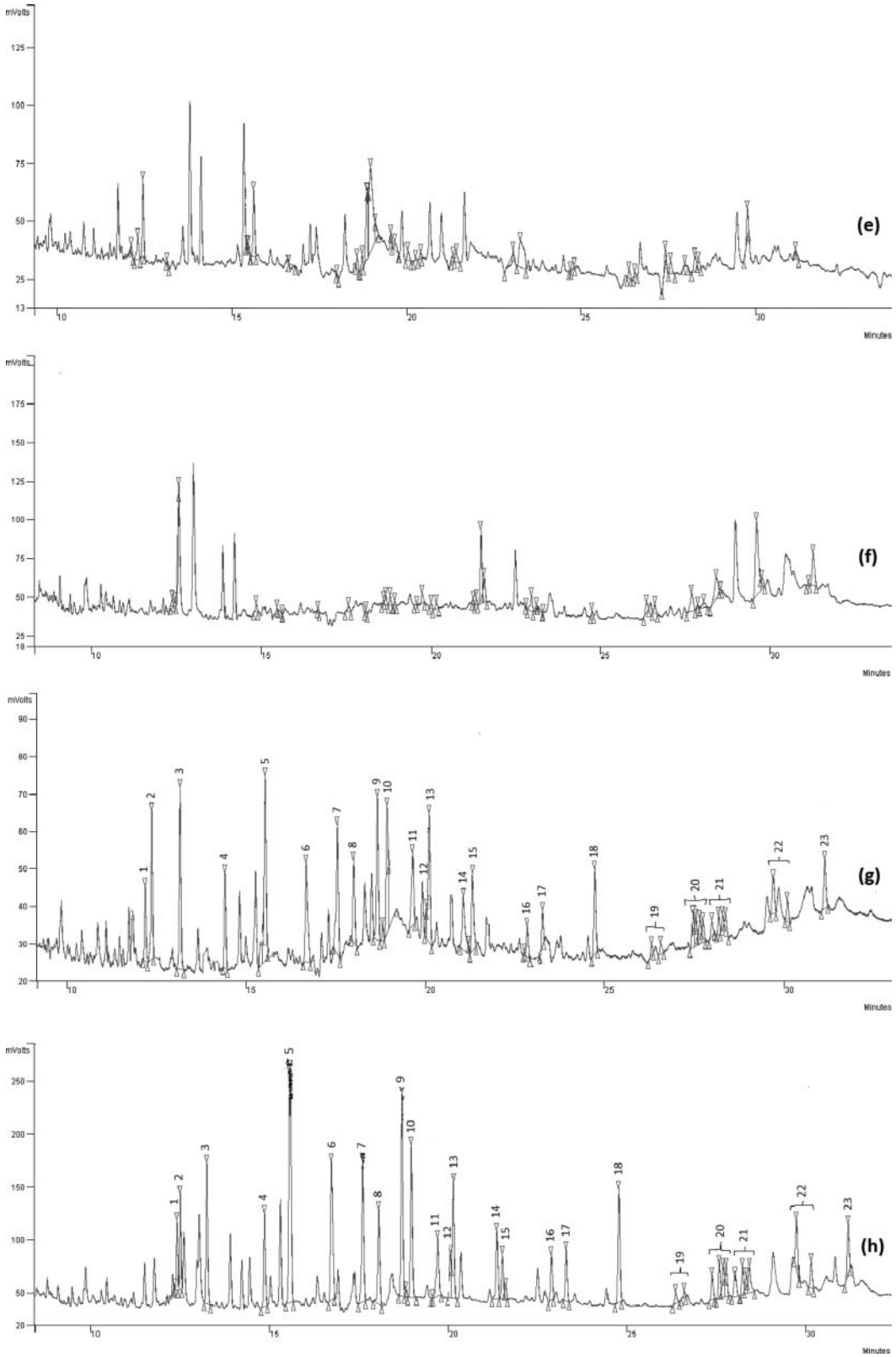

Fig. A2. Contiued

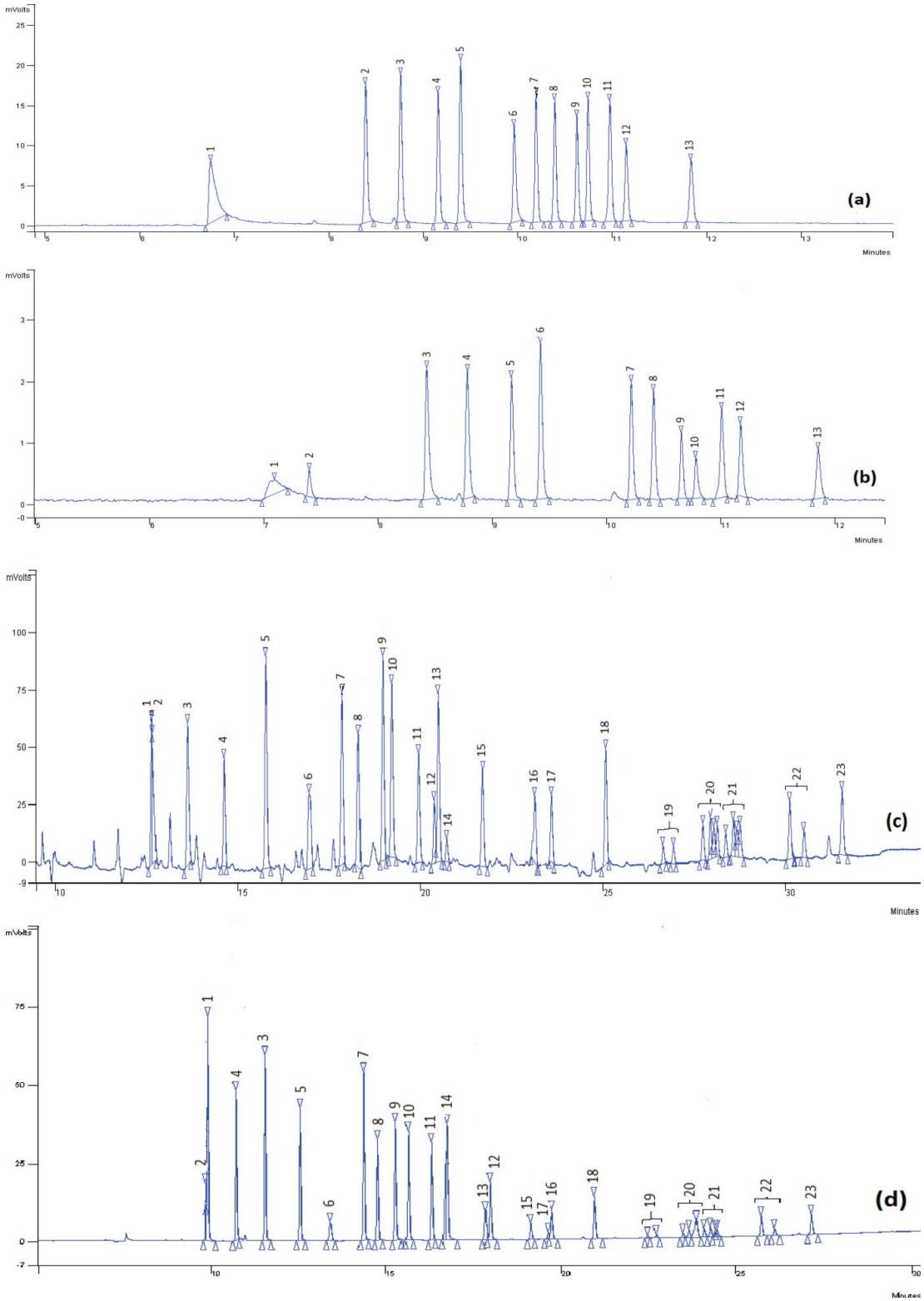

Fig. A3. Chromatograms of (a) for watermelon fortified by OP standards using VF-1701 Pesticides on GC-PFPD, (b) for watermelon fortified by OP standards using VF-5 Pesticides on GC-PFPD. Peaks: 1: Methaminphos, 2: Ethoprophos, 3: Phorate, 4: Diazinon, 5: Fonofos, 6: Dimethoate, 7: Pirimiphos-methyl, 8: Chlorpyrifos, 9: Malathion, 10: Fenitrothion, 11: Parathion-ethyl, 12: Chlorfenvinphos, 13: Profenofos and chromatograms of (c) for watermelon fortified by OC/SP standards using VF-5 Pesticides on GC-ECD, (d) for watermelon fortified by OC/SP standards using VF-1701 Pesticides on GC-ECD. Peaks: 1: Beta-HCH, 2: Gamma-HCH, 3: Delta-HCH, 4: Heptachlor, 5: Aldrin, 6: Allethrin, 7: Gamma-chlordane, 8: Alpha-endosulfan, 9: p.p'-DDE, 10: Dieldrin, 11: Eldrin, 12: Beta-endosulfan, 13: p,p'-DDT, 14: p,p'-DDD, 15: Endosulfan sulfate, 16: Bifenthrin, 17: Methoxychlor, 18: Lambda-cyhalothrin, 19: Permethrin, 20: Cyfluthrin, 21: Cypermethrin, 22: Fenvalerate, 23: Deltamethrin.