Journal of Food and Drug Analysis, Vol. 17, No. 3, 2009, Pages 163-177 藥物食品分析 第十七卷 第三期

Multiresidue Analysis of 176 Pesticides and Metabolites in Pre-harvested Fruits and Vegetables for Ensuring Food Safety by Gas Chromatography and High Performance Liquid Chromatography

SHU-JEN TUAN, HSIU-MEI TSAI, SHANG-MEI HSU, HONG-PING LI*

Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Taichung, Taiwan (R.O.C.)

(Received: January 7, 2008; Accepted: March 17, 2009)

ABSTRACT

A broad-range, sensitive and reliable multiresidue analytical method is presented for identifying and inspecting 176 pesticide residues, including organophosphate, organochlorine, synthetic pyrethroid, organonitrogen and carbamate, in pre-harvested fruits and vegetables. Pesticide residues were extracted from samples with acetone, followed by liquid-liquid partition and solid-phase extraction. Seventy one and 83 pesticides were determined, respectively, by gas chromatography with a flame photometric detector and an electron capture detector. Nineteen carbamate pesticides and several metabolites were measured by high-performance liquid chromatography (HPLC) with a post-column derivation system and a fluorescence detector; and three additional pesticides, i.e., carbendazim, thiabendazole, and imidacloprid, were detected by HPLC using an ultraviolet detector at 280 nm. The proposed method was validated for 14 fruits and 8 vegetables using samples spiked with pesticide standards (0.1- 5.0 μg/g) in triplicate. Good sensitivity and repeatability were obtained with detection limits of 0.001- 0.03 μg/g, and all limits of detection were lower than one-fifth of their specific maximum residue levels. The recovery rates for most pesticides in various fruits and vegetables were 60-120% with relative standard deviations < 20%. With more than 4,300 samples collected from the field examined to assess the performance, it is proposed that this method be applied for routine monitoring, legislation implementation and farmer education programs.

Key words: pesticide residues, multiresidue analysis, gas chromatography, high-performance liquid chromatography, vegetables, fruits

INTRODUCTION

Farmers in Taiwan depend highly upon chemicals to manage the serious year-round pest problems which arise from the subtropical climate and intensive agricultural practice in this island. Hundreds of insecticides, fungicides, herbicides, and acaricides have been recommended for pest and disease control in order to improve the yield and quality of agro-products. Pesticide application patterns vary from area to area according to in farm size and the diversity of crops grown in Taiwan. How to minimize the use of pesticides and produce safe agro-products is becoming increasingly important as customer awareness rises. In Taiwan the Council of Agriculture provides plant protection manuals for appropriate use of pesticides by farmers[1] to ensure the availability of sufficient food and Department of Health (DOH) sets maximum residue levels (MRLs) to ensure the safety of food[2-4]. Gas chromatography (GC) and high-performance liquid chromatograph (HPLC) are commonly used in the inspection of pesticide residues in food commodities. For routine monitoring, the spectrum of surveillance should be as complete as possible in order to encompass the versatile pesticides currently employed in farms. Liquid-liquid partition (LLP) was frequently used with solvents such as acetone, acetonitrile, hexane, ethyl acetate, and dichloromethane-acetone mixture[5-11]. Solid-phase extraction cartridge had been utilized as an effective tool for purification, clean-up and concentration protocol[11-15]. In addition, the tandem solid phase extraction (SPE) technique and a macroporous diatomaceous earth column have been employed as environment-friendly alternatives to reduce the usage of organic solvents[9,16]. In recent years, many methodologies, such as GC-mass spectrometry (GC-MS) and LC-tandem mass spectrometry (LC-MS/MS), have been adapted to improve the reliability and sensitivity of identification and quantification analysis[11,14,15,17].

* Author for correspondence. Tel: +886-4-23302101 ext 401; Fax: +886-4-23324738; E-mail address: hplee@tactri.gov.tw

However, conventional GC and HPLC combined with a simple technique for determining pesticide residues might be more practical for routine monitoring program than the sophisticated, high-priced GC-MS and LC-MS/MS. This work presents a modified multiresidue method combining conventional GC and HPLC for determining residue of 176 pesticides and metabolites, mostly recommended plus several banned ones in Taiwan. Over 4,300 real samples of 22 vegetables and fruits that belong to 13 crop groups classified by DOH[2-4] were analyzed to assess the efficacy of the proposed method.

MATERIALS AND METHODS

I. *Pesticide Standards and Preparation of Stock Solutions*

Table 1 lists the pesticides tested in this study. In total, 176 pesticides were divided into 14 groups (Table 1) according to the analytical conditions of instruments and retention time determined by GC and HPLC. Pesticide standards of 88-99.5% purity were purchased from Dr. Ehrenstorfer (Augsburg, Germany), Merck (Darmstadt, Germany) or Riedel-deHaen (Germany) and Chem

Table 1. The targeted 176 pesticides/metabolites in this study and their grouping for multiresidue determination.

Analytical instrument		Pesticide or metabolite
GC/FPD Total: 71 (in acetone)	Group 1	Acephate, Bromophos-methyl, Chlorpyrifos, Dichlorvos, EPN, Ethion, Ethoprophos, Isoxathion, Methamidophos, Mevinphos, Monocrotophos, Parathion, Parathion-methyl, Phorate, Phosalone, Profenophos, Prothiophos, Tokuoxon (Prothiophos-meta)
	Group 2	Carbophenothion, Demeton-s-methyl, Diazinon, Dimethoate, Fenthion, Fonofos, Malathion, Mephosfolan, Methidathion, Omethoate, Phenthoate, Phosmet, Pirimiphos-methyl, Pyridaphenthion, Quinalphos, Terbufos, Triazophos, Trichlorofon
	Group 3	Cadusafos, Chlorfenvinphos, Cyanofenphos, Dialifos, Dicrotophos, Disulfoton, Ditalimfos, Formothion, Heptenophos, Iprobenfos, Isazofos, Leptophos, Naled, Phosdiphen, Propaphos, Pyraclofos, Tetrachlorvinphos, Vamidothion
	Group 4	Azinphos-methyl, Bromophos-ethyl, Chlorthiophos, Coumaphos, Salithion, Edifenphos, Etrimfos, Fenamiphos, Fenchlorphos, Fenitrothion, Fensulfothion, Fosthiazate, Mecarbam, Phosphamidon, Pirimiphos-ethyl, Thiometon, Tolclofos-methyl
GC/ECD Total: 83 (in *n*-hexane)	Group 5	Bifenthrin, Captafol, Captan, Chinomethionat, Chlorothalonil, Cypermethrin, Dicofol, Fenvalerate, Fluvalinate, Permethrin, Procymidone
	Group 6	Alphacypermethrin, Cyfluthrin, Cyhalothrin, Deltamethrin, Endosulfan, Esfenvalerate, Fenpropathrin, Iprodione, Triadimefon, Vinclozolin
	Group 7	Alachlor, Aldrin, Butachlor, Dichlofluanid, Dicloran, Dieldrin, Endrin, Heptachlor, Heptachlor Epoxide, Methoxychlor, Metolachlor, *o,p′*-DDD, *o,p′*-DDE, *o,p′*-DDT, *p,p′*-DDT, Tetradifon, Trifluralin, α-HCH, γ-HCH, δ-HCH
	Group 8	Bifenox, Bupirimate, Chloropropylate, Fenarimol, Flufenoxuron, Haloxyfop-methyl, Isoprothiolane, Nuarimol, Oxyfluorfen, *p,p′*-DDD, Pyridaben, Pyrifenox
	Group 9	Bromopropylate, Bromuconazole, Chlorfenapyr, Diniconazole, Epoxiconazole, Hexaconazole, Kresoxim-methyl, Penconazole, Pendimethalin, Prochloraz, Propiconazole, Tetraconazole, Uniconazole
	Group 10	Acrinathrin, Allethrin, Cyphenothrin, Flucythrinate, Halfenprox, Myclobutanil, Tefluthrin, Tetramethrin, Tralomethrin
	Group 11	Bitertanol, Cyproconazole, Difenoconazole, Fipronil, Flutolanil, Flutriafol, Paclobutrazol, Triadimenol
HPLC/FLD Total: 19 (in acetonitril)	Group 12	1-Naphthol, 3-keto carbofuran, 3-OH Carbofuran, Aldicarb Sulfone, Aldicarb Sulfoxide, Carbaryl, Carbofuran, Methiocarb, Methomyl, Thiodicarb
	Group 13	Aldicarb, Butocarboxim, Fenobucarb, Isoprocarb, MTMC, Oxamyl, Promecarb, Propoxur, XMC
HPLC/UV Total: 3 (in methanol)	Group 14	Carbendazim, Imidacloprid, Thiabendazole

Journal of Food and Drug Analysis, Vol. 17, No. 3, 2009

Service (West Chester, PA, USA). Stock solutions of 1 mg/mL (1000 μg/mL) for organochlorine, organophosphate, synthetic pyrethroid, and carbamate pesticides were prepared individually with *n*-hexane, acetone, *n*-hexane, and acetonitrile or methanol according to their polarity and solubility. Working solutions were mixed well and then serially diluted with the appropriate solvent. All standard solutions were stored in the dark at 4°C.

II. *Reagents and Chemicals*

Organic solvents, i.e., *n*-hexane (GC grade), acetone (LC grade), petroleum-ether (GC grade), acetonitrile (LC grade), methanol (LC grade), and glacial acetate (GR for analysis), sodium chloride (extra pure, USP), sodium hydrogen carbonate (GR for analysis) and anhydrate sodium sulfate (extra pure, USP), were purchased from Merck (Darmstadt, Germany). Dichloromethane (LC grade) was purchased from Mallinckrodt Baker (USA). Post-column derivative reagents, *o*-phthaladehyde (OPA), thiofluor, and OPA diluent (CB 910), and hydrolysis reagent C47 (CB130), all of chromatographic grade, were purchased from Pickering Laboratories (alifornia, USA). The cleanup-functional solid-phase extraction cartridge, florisil (1000 mg / 6 mL), was purchased from J&T Baker (Phillipsburg, USA). Water was purified using an apparatus from Millipore (Billerica, USA). The Nitrogen used for evaporation was of 5N purity.

III. *Sample Selection and Collection*

Twenty-two crops belonging to 13 crop groups classified by DOH, i.e., strawberry, grape, carambola, guava, papaya, pineapple, banana, orange, lemon, pear, apple, litchi, mango, melon, ching-geeng, cabbage, green pepper, cucumber, kidney bean, taro, coba, and mushroom, with various level of pH and Brix degree (Table 2), were selected for recovery test. All samples were collected from farms 3-14 days prior to harvest during Jan.-Dec. in 2006, and analyzed. Residues detected in samples were assessed according to MRLs, established by DOH, in March, July and September in 2006[2-4].

IV. *Sample Preparation*

All samples were minced without pretreatment, except for pineapple, which was peeled and litchi and mango whose seeds were removed, and stored at -20°C. To measure the pH and Brix, samples were homogenized for 1 min, frozen at -18°C for 30 min, and allowed to stand at room temperature for 15 min. The upper layer of the supernatant was analyzed using a "Pocket" refractometer (Tokyo, Japan) and pH meter (from WTW Weilheim, Germany). Blank samples were spiked with the appropriate concentrations of pesticides (Table 1). Fortified samples were evaporated with nitrogen for 20 min at room temperature before extraction.

(I) *Extraction, Partition, and Salting Out*

Figure 1 presents a flow diagram for the entire analytical procedures. Fresh fruit and vegetable samples were thoroughly chopped, and a 20 g portion was homogenized with 80 mL acetone for 1 min by polytron. The homogenate was filtered through Advantec No. 1 filter paper (11 μm aperture) via vacuum suction and made up to 160 mL with acetone. Forty mL of filtrate was condensed to 3-5 mL in a round-bottom bottle, and after the addition of 1.5 g NaCl, it was transferred to a separation funnel for three liquid-liquid partitions. First, the sample solution was extracted with 50 mL of petroleum ether: dichloromethane (1:2, v/v) for 1 min. The organic phase was collected into a 300 mL flask, and the water layer was extracted as once more above. Then, 1 mL of 12% $NaHCO_3$ solution and 5 mL of 30% NaCl solution were added to the water layer

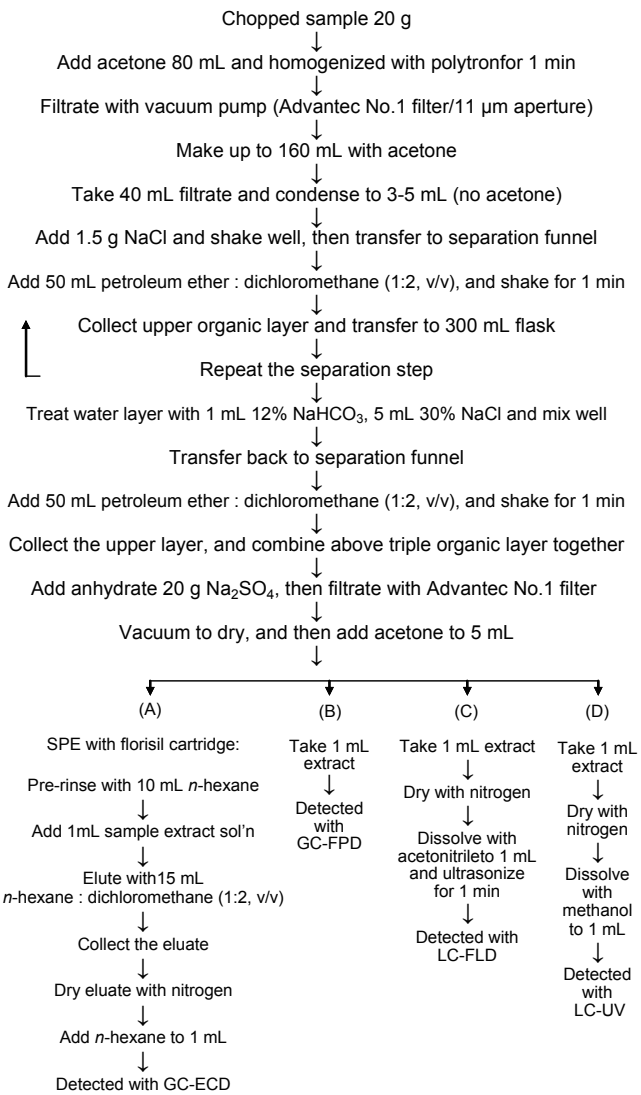

Figure 1. Analytical procedure for 176 pesticide and metabolite residues in vegetables and fruits.

before repeating the third partition step. The combined organic layers were added 20 g anhydrate Na_2SO_4, then filtered with Advantec No. 1 filter and the filtrate was evaporated to dryness at 40°C using a rotary evaporator. The residue was dissolved in 5 mL acetone.

(II) *Clean-up and Quantification*

One mL of the sample extract was analyzed for organophosphorus pesticide by GC-flame photometric detector (FPD). Another 1 mL portion of the sample extract was loaded onto a florisil cartridge pre-rinsed with 10 mL of *n*-hexane, followed by eluting with 10 mL *n*-hexane : dichloromethane (1:2, v/v). The collected eluent was evaporated with nitrogen (5N purity) and quantified to 1 mL with n-hexane for GC-electron capture detector (ECD) analysis of organochlorine pesticides and pyrethroids. The third 1 mL portion of acetone residue was evaporated to dryness with nitrogen and dissolved in 1 mL acetonitrile for HPLC-fluorescence detector (FLD) analysis of carbamates and some metabolites. Then the fourth 1 mL portion was evaporated to dryness and dissolved in 1 mL methanol for HPLC-ultraviolet detector (UVD) analysis of carbendazim, thiabendazole and imidocloprid.

(III) *GC-ECD Analysis*

The HP6890 GC system (california, USA), equipped with a ^{63}Ni-ECD, was utilized for analyzing organochlorine, synthetic pyrethroids, and pesticides containing nitrogen. The column was a J&W DB-608 (agilent, california, USA; 30 m × 0.53 mm, 0.83 μm film thickness), with nitrogen as the carrier gas and make-up gas at a flow rate of 9.5 and 60 mL/min, respectively. Injector and detector temperatures were set at 250°C and 300°C, respectively. Oven temperature was programmed as follows: 170°C for 2 min, 3°C/min up to 230°C and held for 5 min, 10°C/min up to 260°C and held for 20 min, 20°C/min up to 270°C and held for 10 min.

(IV) *GC-FPD Analysis*

The HP6890 GC system, equipped with a FPD, was used for analyzing organophosphate pesticides. The column and oven temperature settings were the same as those for the GC-ECD. The injector and detector temperatures were set at 240°C and 250°C, respectively. The carrier gas and make-up gas were nitrogen supplied at flow rate of 4.7 and 60 mL/min, respectively; H_2 flow rate was 150 mL/min, and air flow rate was 110 mL/min.

(V) *HPLC-Fluorescence Analysis*

The Agilent 1100 HPLC system (california, USA), equipped with a C18 (250 × 4.6 mm) analytical column, a post-column derivatizer (Pickering Laboratories, california, USA) PCX 5200 and a fluoresence detector, was employed for analyzing carbamates and metabolites. Injection volume was 20 μL. The mobile phase consisted of water (solvent A) and acetonitrile (solvent B) with a gradient program as follows: A/B= 70/30 at time 0 to A/B= 60/40 at 5 min, then to A/B= 50/50 at 10 min, and to A/B= 40/60 at 15 min, and then equilibrated at initial conditions for 3 min; flow rate was 1 mL/min. The C18 column temperature was 40°C and the catalytic reactor temperature was 100°C; and the OPA-reagent flow rate of derivatization was 0.3 mL/min. Excitation and emission wavelengths of detection were 330 and 466 nm, respectively.

(VI) *HPLC-UV Analysis*

The Agilent 1100 HPLC system, equipped with a photodiode array UV detector (280 nm) and a Merck RP-select B column (250 × 4 mm, 5 μm), was utilized for analyzing imidachlorprid, carbendazim, and thiabendazole residues. Injection volume was 5 μL. The mobile phase, 5% acetic acid (A) and acetonitrile (B) was

Table 2. °Brix and pH values of 14 fruits and 8 vegetables.

Crop	°Brix	pH
Grape	14.7	3.62
Strawberry	9.8	3.44
Carambola	8.4	4.92
Guava	6.6	3.98
Papaya	9.0	5.47
Pineapple	11.9	3.80
Banana	3.3	5.48
Orange	9.0	4.51
Lemon	7.5	2.76
Pear	11.2	4.65
Apple	11.8	4.10
Litchi	12.7	4.73
Mango	4.7	3.50
Muskmelon	8.6	5.83
Ching-Geeng	2.7	5.59
Cabbage	6.6	5.37
Green pepper	3.4	5.33
Cucumber	3.4	6.22
Kidney Bean	4.7	5.74
Taro	4.0	5.80
Coba	5.5	6.15
Shiitake	7.1	6.30

Journal of Food and Drug Analysis, Vol. 17, No. 3, 2009

programmed with a gradient of A/B= 80/20 and a flow rate of 1 mL/min.

V. *Recovery Test , Limit of Detection (LOD) and Method Validation*

Recovery tests were performed by spiking 8 vegetables and 14 fruits (Table 2), in triplicates, with the given concentrations of 176 pesticides (Table 3). Blank samples were also prepared to identify the matrix effect. Fortified samples were blown with pure nitrogen gas for 15 min at room temperature to evaporate solvent residues before extraction, and then analyzed by GC and HPLC as described above.

Table 3. Average recoveries and relative standard deviations (RSD, %) from vegetables and fruits materials fortified with 176 pesticides at various concentrations, and limits of detection

Detector	Pesticides	Spike level (μg/g)	Ching-Geeng	Green pepper	Mango	Muskmelon	Apple	
			R (%)	R (%)	R (%)	R (%)	R (%)	LOD (μg/g)
ECD								
	Acrinathrin	1.0	87.2[a] (1.7)[b]	80.8 (5.4)	85.2 (0.7)	85.6 (2.1)	78.8 (8.9)	0.002
	Alachlor	0.5	86.2 (5.1)	83.8 (2.6)	88.4 (0.2)	88.9 (1.2)	83.9 (1.9)	0.004
	Aldrin	0.5	75.5 (2.9)	75.1 (2.6)	60.3 (3.9)	69.9 (1.8)	56.2 (4.5)	0.002
	Allethrin	0.2	82.3 (3.4)	89.5 (3.8)	84.8 (1.4)	85.6 (3.1)	80.2 (9.1)	0.001
	Alphacypermethrin	1.0	90.8 (3.3)	91.1 (2.0)	79.9 (1.3)	83.5 (3.9)	81.5 (1.9)	0.010
	Bifenox	0.1	83.4 (3.6)	89.0 (1.6)	84.1 (3.1)	89.5 (4.8)	88.0 (1.8)	0.001
	Bifenthrin	0.1	92.3 (2.5)	75.9 (9.9)	78.4 (2.1)	121.6 (9.9)	96.5 (9.5)	0.002
	Bitertanol	5.0	63.3 (5.7)	69.9 (4.5)	72.6 (2.2)	51.9 (2.5)	94.9 (3.6)	0.022
	Bromopropylate	0.5	71.6 (8.9)	73.3 (1.9)	80.2 (3.0)	87.6 (3.5)	79.9 (3.8)	0.001
	Bromuconazole	0.5	77.9 (3.7)	88.9 (0.5)	73.8 (2.4)	86.9 (4.2)	69.0 (7.5)	0.001
	Bupirimate	0.2	78.2 (6.1)	91.3 (2.4)	79.3 (2.6)	87.5 (4.1)	85.9 (1.7)	0.003
	Butachlor	1.0	86.4 (3.6)	84.4 (6.3)	88.2 (0.5)	91.6 (6.2)	85.9 (1.0)	0.005
	Captafol	0.5	82.2 (10.5)	77.1 (23.4)	76.1 (8.3)	65.4 (24.2)	81.7 (2.6)	0.001
	Captan	0.3	94.0 (8.4)	75.8 (19.4)	69.7 (2.8)	74.3 (15.9)	83.7 (3.3)	0.001
	Chinomethionat	0.2	94.7 (0.8)	78.5 (21.3)	72.6 (0.8)	90.0 (2.5)	80.0 (0.7)	0.001
	Chlorfenapyr	0.2	79.5 (8.1)	102.6 (15.3)	83.1 (3.5)	89.4 (2.8)	87.3 (4.7)	0.001
	Chloropropylate	0.5	75.6 (1.6)	124.3 (8.7)	80.5 (3.0)	86.4 (3.9)	82.7 (4.8)	0.001
	Chlorothalonil	0.2	72.3 (8.1)	77.5 (12.5)	70.4 (1.3)	83.8 (3.5)	78.1 (0.9)	0.001
	Cyfluthrin	1.0	91.3 (3.2)	90.8 (1.9)	82.9 (0.9)	82.9 (3.6)	82.3 (2.8)	0.002
	Cyhalothrin	0.5	91.9 (3.1)	90.7 (1.6)	82.9 (0.9)	83.1 (3.8)	82.8 (2.4)	0.002
	Cypermethrin	1.0	94.9 (0.9)	81.0 (22.6)	79.7 (1.1)	91.2 (2.5)	85.7 (1.2)	0.002
	Cyphenothrin	1.0	87.7 (2.0)	83.1 (4.8)	86.7 (0.9)	83.3 (1.9)	78.1 (9.5)	0.002
	Cyproconazole	3.0	63.2 (0.7)	63.2 (1.0)	71.4 (1.8)	67.5 (0.1)	81.8 (5.5)	0.014
	Deltamethrin	1.0	92.9 (3.4)	90.5 (1.5)	83.1 (0.7)	84.3 (3.9)	81.7 (1.8)	0.002
	Dichlofluanid	0.2	63.0 (8.4)	62.3 (4.0)	79.3 (0.1)	20.4 (18.2)	83.4 (0.4)	0.002
	Dicloran	0.1	117.0 (22.6)	83.6 (3.5)	79.3 (0.4)	96.2 (1.5)	76.9 (3.6)	0.001
	Dicofol	2.0	92.0 (3.7)	81.2 (7.1)	81.9 (5.3)	66.2 (11.6)	79.2 (5.0)	0.001
	Dieldrin	0.1	89.4 (2.4)	90.0 (6.3)	83.4 (0.7)	84.8 (2.2)	81.0 (7.7)	0.002
	Difenoconazole	0.5	61.4 (9.8)	58.8 (3.5)	73.3 (2.7)	62.3 (3.2)	80.6 (5.2)	0.002
	Diniconazole	0.2	68.9 (2.5)	84.8 (0.7)	74.4 (4.0)	82.3 (4.7)	65.8 (12.2)	0.001

Table 3. Continued.

Detector	Pesticides	Spike level (μg/g)	Ching-Geeng	Green pepper	Mango	Muskmelon	Apple	
			R (%)	R (%)	R (%)	R (%)	R (%)	LOD (μg/g)
ECD								
	Endosulfan	0.5	90.3 (3.6)	88.9 (1.5)	79.7 (2.6)	82.1 (2.4)	81.6 (1.6)	0.002
	Endrin	0.1	86.2 (1.1)	87.7 (5.1)	84.3 (0.2)	84.3 (0.9)	85.7 (1.8)	0.002
	Epoxiconazole	0.5	71.2 (2.4)	89.6 (0.5)	74.0 (3.2)	82.5 (4.4)	69.3 (6.7)	0.002
	Esfenvalerate	1.0	91.8 (3.4)	90.5 (1.7)	82.5 (1.5)	83.5 (3.7)	81.8 (1.8)	0.014
	Fenarimol	0.1	68.0 (2.9)	94.8 (4.9)	69.8 (2.6)	76.4 (4.1)	74.2 (5.4)	0.001
	Fenpropathrin	1.0	92.7 (1.8)	90.7 (1.5)	81.4 (1.7)	84.9 (3.4)	82.1 (1.9)	0.005
	Fenvalerate	1.0	94.2 (1.1)	82.6 (22.5)	78.1 (1.2)	92.2 (2.5)	86.6 (2.1)	0.001
	Fipronil	0.1	85.0 (4.2)	82.3 (2.9)	87. 9 (1.5)	82.3 (0.6)	100.3 (3.3)	0.001
	Flucythrinate	1.0	88.3 (3.6)	81.9 (4.1)	87.0 (1.7)	87.3 (3.0)	78.5 (9.1)	0.002
	Flufenoxuron	0.5	60.7 (2.3)	89.5 (3.8)	71.0 (4.1)	86.9 (2.7)	65.6 (11.2)	0.002
	Flutolanil	1.0	110.3 (7.4)	84. 4 (4.1)	87.2 (4.3)	88.7 (9.6)	105.5 (19.3)	0.006
	Flutriafol	5.0	58.7 (5.0)	62.4 (2.0)	66.6 (5.4)	68.8 (11.6)	75.3 (5.9)	0.033
	Fluvalinate	1.0	94.9 (0.5)	81.4 (22.4)	78.7 (0.1)	92.2 (2.7)	84.9 (1.5)	0.001
	Halfenprox	1.0	90.6 (2.4)	84.0 (3.3)	88.1 (1.0)	–[c]	79.9 (8.2)	0.002
	Haloxyfop-methyl	0.1	101.6 (4.8)	82.7 (2.4)	83.6 (2.6)	93.8 (6.5)	89.3 (1.3)	0.002
	Heptachlor	0.1	73.6 (2.9)	71.1 (4.5)	57.3 (2.5)	67.6 (2.2)	52.5 (2.5)	0.002
	Heptachlor Epoxide	0.2	84.0 (0.7)	83.7 (3.5)	80.7 (1.0)	83.8 (2.9)	80.8 (1.1)	0.002
	Hexaconazole	0.2	54.4 (2.8)	63.7 (1.0)	66.0 (3.7)	72.9 (5.3)	66.8 (18.8)	0.001
	Iprodione	2.0	90.8 (4.2)	90.4 (2.0)	84.2 (25.3)	82.4 (4.3)	88.8 (3.0)	0.002
	Isoprothiolane	0.2	77.8 (4.9)	89.8 (2.8)	81.8 (3.0)	84.0 (5.4)	81.5 (1.0)	0.001
	Kresoxim-methyl	0.5	84.7 (8.6)	88.6 (5.8)	81.2 (4.6)	98.2 (1.5)	86.0 (4.2)	0.002
	Methoxychlor	0.2	85.5 (1.1)	86.2 (5.0)	79.8 (2.7)	83.3 (3.0)	88.5 (1.7)	0.001
	Metolachlor	1.0	85.4 (6.7)	83.9 (4.3)	85.8 (0.1)	86.7 (0.2)	84.3 (0.5)	0.011
	Myclobutanil	2.0	88.7 (2.0)	82.9 (5.5)	79.6 (2.1)	70.4 (2.0)	62.7 (11.6)	0.004
	Nuarimol	0.1	54.3 (12.2)	95.0 (5.2)	75.6 (10.6)	76.8 (3.3)	69.9 (8.0)	0.002
	o,p'-DDD	0.2	89.1 (1.7)	100.3 (13.8)	89.0 (0.8)	90.4 (1.2)	86.6 (1.0)	0.001
	o,p'-DDE	0.2	87.1 (1.0)	84.0 (5.7)	82.2 (1.5)	84.5 (1.1)	82.7 (0.9)	0.001
	o,p'-DDT	0.1	87.4 (0.6)	87.7 (5.2)	84.3 (0.1)	86.4 (2.7)	86.1 (1.2)	0.002
	Oxyfluorfen	0.1	75.5 (3.4)	86.9 (2.7)	75.7 (2.4)	89.5 (3.6)	88.8 (1.4)	0.001
	p,p'-DDD	0.2	82.8 (2.8)	86.6 (0.5)	81.7 (2.8)	89.0 (4.9)	85.8 (0.5)	0.001
	p,p'-DDT	0.1	86.7 (1.8)	86.9 (5.3)	74.2 (5.8)	83.9 (2.7)	86.9 (2.2)	0.002
	Paclobutrazol	3.0	74.6 (0.3)	74.5 (1.7)	79.6 (1.0)	78.9 (0.4)	85.3 (5.1)	0.015
	Penconazole	0.2	69.4 (5.2)	74.5 (0.5)	70.2 (3.3)	77.4 (6.5)	63.9 (3.6)	0.001
	Pendimethalin	0.5	78.1 (7.7)	87.7 (4.6)	79.0 (2.9)	86.3 (3.4)	89.0 (5.0)	0.002
	Permethrin	5.0	94.2 (0.9)	83.7 (21.0)	80.0 (0.8)	92.3 (2.1)	88.7 (0.8)	0.005
	Prochloraz	0.5	60.6 (5.9)	90.0 (6.2)	64.4 (3.8)	76.0 (7.8)	56.9 (14.8)	0.002
	Procymidone	2.0	93.2 (1.1)	83.0 (19.1)	80.4 (1.7)	93.1 (1.9)	87.9 (1.7)	0.002

Table 3. Continued.

Detector	Pesticides	Spike level (μg/g)	Ching-Geeng	Green pepper	Mango	Muskmelon	Apple	
			R (%)	R (%)	R (%)	R (%)	R (%)	LOD (μg/g)
ECD								
	Propiconazole	0.5	70.3 (4.4)	85.3 (1.3)	74.5 (3.3)	77.3 (4.1)	69.8 (4.7)	0.002
	Pyridaben	0.5	84.5 (3.0)	89.6 (1.7)	85.2 (0.3)	93.9 (6.0)	92.8 (1.4)	0.002
	Pyrifenox	0.2	71.3 (9.2)	79.8 (3.4)	71.6 (3.7)	78.0 (1.9)	67.5 (9.9)	0.002
	Tefluthrin	0.2	75.3 (3.9)	78.1 (6.0)	77.8 (4.7)	80.8 (2.9)	71.8 (7.3)	0.001
	Tetraconazole	0.2	76.1 (3.3)	105.2 (15.8)	75.5 (3.5)	81.8 (4.2)	68.9 (16.8)	0.001
	Tetradifon	0.1	87.7 (1.1)	87.5 (5.8)	89.0 (0.1)	87.3 (1.6)	89.2 (1.5)	0.002
	Tetramethrin	1.0	82.4 (4.0)	82.1 (4.4)	84.6 (6.1)	85.6 (2.7)	76.5 (9.3)	0.003
	Tralomethrin	1.0	86.4 (3.9)	83.4 (5.3)	82.1 (1.7)	87.0 (6.2)	77.0 (9.0)	0.001
	Triadimefon	0.5	85.2 (3.3)	83.3 (2.0)	76.4 (1.0)	78.3 (4.7)	81.5 (3.2)	0.005
	Triadimenol	2.0	73.2 (2.6)	69.1 (1.6)	74.7 (1.7)	62.7 (0.4)	86.8 (8.8)	0.022
	Trifluralin	0.1	78.8 (3.4)	72.0 (2.9)	66.6 (0.5)	77.7 (2.6)	61.1 (0.2)	0.002
	Uniconazole	2.0	73.0 (2.2)	91.5 (0.2)	76.6 (3.7)	86.9 (2.1)	64.6 (16.8)	0.002
	Vinclozolin	0.3	82.5 (4.9)	89.7 (0.8)	75.9 (2.9)	82.4 (4.0)	79.0 (4.3)	0.002
	α-HCH	0.1	75.7 (1.9)	65.8 (11.9)	60.2 (2.1)	73.0 (2.0)	52.8 (5.6)	0.001
	γ-HCH	0.1	85.0 (3.4)	77.9 (5.2)	70.7 (0.7)	81.9 (3.6)	67.5 (0.2)	0.001
	δ-HCH	0.1	89.2 (3.0)	89.5 (3.1)	84.0 (1.1)	92.3 (0.3)	85.5 (0.1)	0.001
FLD								
	1-Naphthol	1.0	65.1 (3.6)	58.3 (10.8)	73.9 (9.2)	75.7 (2.6)	20.4 (8.2)	0.037
	3-keto carbofuran	1.0	88.7 (0.8)	99.4 (3.5)	82.6 (6.1)	91.4 (2.7)	89.8 (2.4)	0.003
	3-OH Carbofuran	1.0	90. 0 (1.8)	97.3 (2.6)	86.3 (16.4)	86.2 (1.5)	90.9 (2.0)	0.002
	Aldicarb	1.0	87.9 (3.2)	77.3 (13.3)	71.1 (0.6)	83.5 (2.3)	81.0 (4.3)	0.001
	Aldicarb Sulfone	1.0	88.2 (1.2)	93.0 (2.4)	77.1 (2.5)	87.7 (3.2)	90.5 (2.2)	0.002
	Aldicarb Sulfoxide	1.0	86.6 (1.8)	95.8 (3.6)	70.2 (1.8)	86.8 (2.6)	89.1 (2.6)	0.002
	Butocarboxim	1.0	87.5 (4.0)	86.7 (14.2)	68.6 (1.2)	80.4 (5.0)	82.1 (3.6)	0.002
	Carbaryl	1.0	89.5 (0.3)	98.2 (3.4)	81.9 (6.6)	86.9 (2.7)	89.8 (2.6)	0.003
	Carbofuran	1.0	87.9 (0.6)	93.6 (3.7)	81.0 (6.6)	83.8 (3.0)	88.6 (2.9)	0.004
	Fenobucarb	1.0	89.3 (2.4)	92.8 (11.4)	77.6 (0.7)	87.4 (2.2)	80.9 (1.3)	0.002
	Isoprocarb	1.0	87.0 (2.8)	89.7 (8.1)	75.7 (0.5)	88.2 (4.6)	78.2 (3.8)	0.002
	Methiocarb	1.0	88.7 (0.7)	97.5 (3.5)	80.9 (7.1)	84.9 (3.1)	91.8 (5.3)	0.003
	Methomyl	1.0	97.3 (3.2)	97.2 (3.2)	81.4 (5.0)	88.8 (3.4)	87.2 (2.4)	0.002
	MTMC	1.0	82.2 (3.0)	81. 0 (9.6)	71.7 (1.4)	86.6 (3.3)	71.7 (5.0)	0.001
	Oxamyl	1.0	94.3 (1.9)	90.9 (2.9)	71.6 (2.7)	90.9 (2.5)	88.6 (6.7)	0.002
	Promecarb	1.0	91.5 (2.3)	90.0 (4.1)	81.4 (1.6)	89.9 (4.4)	84.2 (4.6)	0.002
	Propoxur	1.0	91.2 (3.1)	97.4 (14.4)	79.2 (1.5)	89.6 (1.7)	85.1 (5.1)	0.001
	Thiodicarb	1.0	70.3 (4.5)	93.8 (2.3)	72.0 (3.2)	76.5 (4.8)	89.2 (2.2)	0.003
	XMC	1.0	88.9 (2.6)	86.5 (4.3)	77.1 (0.7)	89.3 (2.6)	82.5 (3.8)	0.001
	Acephate	0.5	82.2 (3.3)	83.4 (3.2)	80.5 (4.6)	77.1 (1.7)	64.2 (1.3)	0.002
	Azinphos-methyl	1.0	93.2 (1.3)	92.8 (8.4)	97.7 (5.2)	83.8 (5.9)	78.7 (2.0)	0.017

Table 3. Continued.

Detector	Pesticides	Spike level (μg/g)	Ching-Geeng	Green pepper	Mango	Muskmelon	Apple	
			R (%)	R (%)	R (%)	R (%)	R (%)	LOD (μg/g)
FPD								
	Bromophos-ethyl	1.0	87.8 (1.9)	92.5 (6.8)	85.6 (4.0)	84.1 (4.0)	76.1 (1.8)	0.012
	Bromophos-methyl	0.5	88.6 (2.5)	91.7 (2.4)	84.5 (4.2)	87.1 (1.6)	71.8 (2.9)	0.011
	Cadusafos	0.5	86.3 (1.1)	60.8 (2.7)	78.1 (10.7)	83.3 (5.0)	73.1 (4.4)	0.007
	Carbophenothion	0.5	90.2 (2.1)	89.2 (2.7)	82.1 (0.6)	86.4 (3.1)	88.0 (0.1)	0.012
	Chlorfenvinphos	1.0	87.3 (1.1)	77.3 (3.9)	85.4 (8.3)	85.0 (3.1)	78.2 (5.2)	0.019
	Chlorpyrifos	0.5	87.5 (2.5)	90.6 (2.6)	85.7 (3.6)	86.9 (1.3)	72.7 (1.1)	0.009
	Chlorthiophos	1.0	86.8 (2.0)	92.1 (7.1)	83.9 (3.7)	83.2 (3.6)	77.0 (2.8)	0.017
	Coumaphos	1.0	86.6 (2.7)	91.6 (7.0)	81.0 (14.6)	86.3 (6.2)	74.9 (3.9)	0.023
	Cyanofenphos	0.5	89.0 (2.6)	75.3 (1.7)	84.2 (9.1)	84.7 (3.8)	78.0 (6.0)	0.010
	Demeton-s-methyl	0.5	92.1 (1.4)	75.6 (4.1)	65.8 (1.6)	73.4 (2.1)	62.2 (6.0)	0.009
	Dialifos	2.0	90.4 (1.1)	76.8 (4.4)	81.4 (13.1)	126.7 (16.2)	83.5 (7.7)	0.292
	Diazinon	0.5	81.7 (0.8)	87.0 (3.7)	75.0 (2.3)	85.2 (3.6)	84.9 (1.8)	0.007
	Dichlorvos	0.5	76. 7 (6.5)	59.6 (10.1)	34.2 (12.8)	63.5 (3.8)	34.1 (14.1)	0.012
	Dicrotophos	0.5	96.0 (1.5)	80.8 (4.3)	90.2 (7.1)	83.8 (4.4)	82.8 (2.2)	0.015
	Dimethoate	0.5	91.9 (0.5)	90.7 (2.6)	84.7 (3.4)	89.9 (3.8)	93.0 (0.6)	0.006
	Disulfoton	0.5	81.2 (0.7)	53.2 (6.2)	75.6 (6.5)	72.3 (1.6)	60.9 (5.6)	0.012
	Ditalimfos	0.5	88.3 (0.8)	74.3 (0.4)	83.1 (10.6)	84.1 (3.8)	79.5 (2.8)	0.012
	Edifenphos	1.0	85.2 (1.9)	92.7 (6.7)	103.1 (2.6)	83.7 (4.5)	76.4 (2.5)	0.008
	EPN	0.5	88.2 (2.7)	89.8 (2.2)	87.4 (2.6)	86.5 (1.4)	74.3 (3.4)	0.011
	Ethion	0.5	88.8 (2.2)	90.4 (2.4)	86.9 (4.3)	87.1 (1.7)	76.5 (1.8)	0.013
	Ethoprophos	0.5	86.3 (3.3)	87.7 (3.0)	82.4 (2.8)	83.1 (3.4)	75.1 (3.0)	0.005
	Etrimfos	0.5	84.3 (2.2)	91.0 (6.5)	82.3 (4.1)	84.5 (3.9)	73.5 (1.0)	0.012
	Fenamiphos	1.0	86.1 (1.8)	89.2 (7.6)	82.4 (3.5)	82.1 (3.9)	71.9 (3.3)	0.011
	Fenchlorphos	0.5	86.4 (1.7)	93.2 (7.0)	84.3 (3.6)	84.1 (3.6)	74.0 (1.6)	0.009
	Fenitrothion	0.5	87.4 (1.8)	92.9 (7.2)	86.3 (2.0)	84.2 (4.3)	76.5 (1.7)	0.008
	Fensulfothion	1.0	93.1 (2.3)	93.9 (7.1)	86.4 (3.1)	83.9 (3.5)	78.2 (1.5)	0.009
	Fenthion	0.5	86.0 (2.4)	85.9 (3.1)	78.4 (1.5)	81.4 (3.0)	82.0 (1.6)	0.008
	Fonofos	0.5	76.0 (2.1)	–	77.4 (0.9)	83.2 (3.3)	63.7 (1.0)	0.008
	Formothion	0.5	94.3 (0.7)	73.8 (1.8)	86.3 (4.6)	85.9 (3.0)	78.7 (1.7)	0.007
	Fosthiazate	1.0	95.0 (2.9)	93.6 (7.1)	98.9 (5.0)	85.3 (5.3)	78.3 (1.5)	0.017
	Heptenophos	1.0	91.3 (0.6)	71.2 (8.1)	82.0 (6.2)	83.2 (4.1)	76.6 (2.4)	0.011
	Iprobenfos	0.5	87.9 (1.1)	77.7 (0.3)	83.4 (9.6)	84.3 (3.5)	80.1 (3.2)	0.008
	Isazofos	0.5	86.5 (1.1)	70.9 (1.1)	82.8 (12.0)	84.1 (3.7)	76.1 (4.2)	0.009
	Isoxathion	0.5	90.8 (2.8)	99.6 (9.5)	85.9 (2.7)	83.9 (18.5)	74.6 (3.1)	0.014
	Leptophos	1.0	89.9 (0.5)	77.8 (0.4)	85.5 (7.2)	84.8 (3.1)	78.8 (3.8)	0.021
	Malathion	0.5	89.0 (3.2)	89.1 (2.6)	81.0 (1.7)	84.8 (3.2)	88.1 (0.5)	0.011
	Mecarbam	0.5	84.4 (1.8)	92.2 (7.3)	85.6 (4.2)	83.3 (3.8)	76.0 (0.7)	0.008

Table 3. Continued.

Detector	Pesticides	Spike level (μg/g)	Ching-Geeng	Green pepper	Mango	Muskmelon	Apple	
			R (%)	R (%)	R (%)	R (%)	R (%)	LOD (μg/g)
FPD								
	Mephosfolan	0.5	92.0 (1.5)	90.9 (2.5)	81.8 (4.6)	90.2 (3.6)	91.7 (1.0)	0.012
	Methamidophos	0.3	78.3 (5.8)	74.2 (4.5)	70.6 (4.9)	71.8 (2.8)	58.8 (1.5)	0.002
	Methidathion	0.5	92.2 (2.0)	90.8 (2.9)	81.3 (1.3)	87.6 (3.4)	91.7 (0.9)	0.011
	Mevinphos	0.5	88.3 (5.7)	86.0 (3.7)	77.4 (3.2)	80.2 (1.9)	63.6 (2.4)	0.007
	Monocrotophos	0.5	88.4 (0.1)	91.3 (3.9)	95.4 (2.8)	87.3 (2.1)	74.7 (1.8)	0.007
	Naled	1.0	53.9 (1.3)	26.9 (28.7)	127.5 (17.2)	69.7 (15.3)	96.5 (3.0)	0.084
	Omethoate	0.5	76.9 (5.4)	92.4 (2.9)	82.8 (0.7)	86.7 (3.5)	94.4 (2.5)	0.005
	Parathion	0.5	87.1 (3.0)	90.1 (2.6)	84.9 (3.0)	86.8 (1.1)	72.7 (2.0)	0.008
	Parathion-methyl	0.5	87.9 (2.6)	90. 6 (2.6)	85.1 (3.3)	87.0 (1.6)	72.5 (2.9)	0.005
	Phenthoate	0.5	88.4 (2.3)	88.8 (2.6)	82.3 (5.0)	85.9 (2.9)	87.3 (0.5)	0.008
	Phorate	0.5	70.3 (5.3)	75.1 (5.9)	67.0 (5.8)	76.6 (1.7)	54.1 (3.8)	0.008
	Phosalone	1.0	88.0 (2.4)	91.0 (1.8)	86.9 (1.6)	87.2 (1.5)	75.4 (2.8)	0.015
	Phosdiphen	1.0	88.6 (0.9)	76.3 (1.7)	81.3 (12.8)	85.9 (4.5)	79.8 (4.0)	0.023
	Phosmet	0.5	91.6 (2.7)	90.8 (3.3)	83.7 (2.4)	86.1 (3.2)	91.2 (0.6)	0.014
	Phosphamidon	1.0	94.5 (2.5)	93.4 (6.3)	92.4 (1.1)	81.4 (7.7)	79.3 (3.2)	0.020
	Pirimiphos ethyl	0.5	86.1 (2.4)	92.0 (7.0)	84.3 (2.8)	84.6 (4.0)	76.0 (2.9)	0.009
	Pirimiphos-methyl	0.5	88.6 (2.2)	88.5 (3.0)	82.3 (2.0)	85.8 (3.3)	87.9 (1.3)	0.009
	Profenophos	0.5	89.6 (2.3)	91.2 (2.6)	86.8 (1.1)	87.1 (1.6)	73.6 (2.9)	0.010
	Propaphos	1.0	86.0 (1.8)	71.8 (1.9)	82.3 (10.0)	80.1 (2.9)	73.9 (3.4)	0.016
	Prothiophos	0.5	88.1 (2.2)	90.7 (2.9)	87.1 (2.3)	87.4 (1.8)	73.9 (2.0)	0.011
	Pyraclofos	1.0	89.3 (3.2)	89.9 (3.0)	82.7 (1.7)	85.3 (3.2)	88.4 (0.8)	0.020
	Pyridaphenthion	0.5	88.4 (3.2)	89.7 (2.9)	84.5 (2.7)	85.2 (3.3)	87.8 (0.9)	0.013
	Quinalphos	0.5	89.3 (3.0)	90.2 (2.6)	83.7 (5.0)	86.1 (3.3)	88.0 (0.7)	0.010
	Salithion	0.5	91.8 (1.7)	89.1 (6.6)	78.9 (7.6)	81.0 (3.8)	70.0 (2.3)	0.005
	Terbufos	0.5	83.8 (3.6)	81.2 (4.6)	73.3 (1.7)	80.7 (3.8)	71.5 (4.0)	0.009
	Tetrachlorvinphos	1.0	87.7 (1.0)	78.1 (2.4)	83.8 (7.0)	84.8 (3.7)	79.7 (2.3)	0.020
	Thiometon	0.5	82.6 (0.7)	70.5 (6.3)	67.5 (6.4)	74.0 (3.3)	50.0 (8.2)	0.008
	Tokuoxon	0.5	89. 3 (2.6)	91.0 (2.1)	87.1 (1.3)	86.9 (1.9)	73.6 (2.1)	0.012
	Tolclofos-methyl	0.5	84.8 (1.8)	91.5 (6.7)	84.3 (3.9)	84.0 (6.2)	73.9 (1.7)	0.008
	Triazophos	0.5	88.8 (3.2)	90.2 (2.8)	82.5 (3.3)	85.9 (3.2)	88.9 (0.2)	0.008
	Trichlrofon	0.5	81.7 (0.8)	–	84.0 (1.3)	86.3 (4.4)	–	–
	Vamidothion	1.0	94.1 (0.5)	82.4 (0.6)	79.3 (19.7)	83.7 (2.8)	82.2 (4.2)	0.014
UV								
	Carbendazim	1.0	97. 7 (5.2)	93.7 (9.4)	99.7 (7.4)	92.3 (2.2)	93.6 (7.6)	0.004
	Imidacloprid	2.0	103.9 (1.8)	85.2 (5.6)	93.7 (3.0)	90.8 (1.7)	98.4 (1.5)	0.012
	Thiabendazole	2.0	98.1 (2.2)	97.0 (8.9)	85.7 (14.2)	94.9 (1.7)	95.8 (5.6)	0.006

[a] Average recoveries from each crop in triplicate
[b] Value in the parenthesis is relative standard deviation.
[c] non-determined.

The proposed procedures were validated by recovering pesticides from fortified samples. Average recovery of each pesticide for each crop was utilized to calculate mean recovery and inter-replicate repeatability (expressed as the relative standard deviation RSD%). The LOD was set at a signal-to-noise ratio (S/N ratio) ≥ 3 by chromatography for individual pesticides in crops.

RESULTS AND DISCUSSION

I. *Method Development*

This multiresidue method is modified from methods recommended by DOH in 2001[7] and Ministry of Economic Affairs (MOEA) in 2000[8]. Those two methods were combined as one analytical method for fruit and vegetable samples in order to minimize the complexity of pretreatment procedures. Based on the various physical and chemical properties of pesticides, acetone was selected as the solvent for extraction due to its effectiveness for both polar and non-polar pesticides from diverse matrices. Acetone, a solvent of low toxicity and cost, completely water miscible and readily evaporated, is an excellent extractant, compared with some solvents popularly used in LLP, such as acetonitrile and ethyl acetate[18]. Besides, the non-polar co-solvents (petroleum ether : dichloromethane in 1:2, v/v) used in the current method induce a well-defined organic phase separation with the aqueous phase. The addition of 1 mL 12% $NaHCO_3$ and 5 mL 30% NaCl could neutralilze the sample extracts which tend to be acidic (Table 2). As a clean-up process was necessary prior to GC/ECD determination, the commercial florisil SPE cartridge was applied to effectively eliminate matrix interferences of most crops. The final identification and quantification of 176 pesticides and several metabolites were achieved by the conventional gas chromatography and high performance chromatography with or without post-column derivatization. GC/FPD was employed for detecting organophosphate pesticides, GC/ECD for halogenated pesticides, synthetic pyrethroids and other pesticides. A liquid chromatography with a post-column derivatization system and fluorescence detector was employed for detecting carbamate residues, and LC/UVD was used for detecting imidachlorprid, carbendazim and thiabendazole residues. In total, 176 pesticides and metabolites were divided into four main groups and 14 subgroups based on the use of analytical equipment and retention time, respectively (Table 1).

II. *Method Validation*

Performance of the proposed method was assessed by evaluating quality parameters, such as recovery, repeatability, matrix interference and LOD. Experimental data demonstrate that the recovery and reproducibility for this multiresidue method were satisfactory. Over 80% of the pesticides were well recovered by the proposed method from the spiked samples of most crops (Figure 2), with relative standard deviations for the recoveries in all crops generally < 20%.

The high recoveries and low RSDs were especially satisfactory for some crops which are of regulatory importance, including strawberry, carambola, guava, orange, mango, apple, banana, muskmelon, Ching-Geeng, green pepper and taro (Figure 3). However, approximately 15-30% of the pesticides could not be satisfactorily recovered from crops with high water content, such as pear, pineapple, cucumber, and cabbage, as well as from coba, kidney bean, shiitake, and papaya (data not shown). Matrix interferences of lemon, grape and litchi resulted in poor recoveries and high RSD values for pesticides spiked in these crops (Figure 3). The strong acidity (pH 2.7 for lemon) and high sugar content (Brix degree of 12.7-14.7 for grape and litchi) may be factors accounting for large variations in recoveries. Slight to moderate emulsification that occurred in the first liquid-liquid partition for grape and litchi samples was removed by the second partition. The average recoveries and RSDs of the 176 pesticides and metabolites from Ching-Geeng, green pepper, mango, muskmelon and apple are listed in Table 3. The majority of recoveries obtained by GC/ECD, GC/FPD, HPLC/FLD, and HPLC/UVD were 60-120%, 70-110%, 80-100%, and 85-105%, respectively. The low recoveries of a few pesticides, such as aldrin, captan, captafol, and hexaconazole, may result from the loss during the additional florisil clean-up procedure. Naled and trichlorfon, which are easily transformed into dichlorvos, were barely recovered by the proposed method in some crops, such as carambola, cabbage, and green pepper. Generally, the stability and polarity of pesticides are two critical factors affecting recovery. The challenge lies in achieving satisfactory recovery of very polar pesticides (commonly with log K_{ow}< 0), taking into consideration of previous reports[14,16,17] that the recoveries of these very polar pesticides generally ranged from 30 to 60%. The method in this study appears to be superior for a wide range of pesticides, and the recoveries of acephate (log K_{ow}= -0.89), methamidophos (log K_{ow}= -0.80), monocrotophos (log K_{ow}= -0.22), and omethoate (log K_{ow}= -0.74) were between 60 and 95% (Table 3). The recoveries of isoxathion, prothiofos, aldrin, chinomethionat, cyfluthrin, difenoconazole, hexaconalzole, permethrin, pyrifenox, and aldicarb sulfoxide detected from spiked carambola and orange mainly range from 60% to 100% (Table 3), significantly better than the results of a recently proposed multiresidue method, namely, 20% to 70%[16]. The recoveries obtained from Ching-Geeng, mango and apple spiked with carbendazim, imidacloprid and thiabendazole, commonly used on these crops, were 85.7-103.9% by HPLC/UV (Table 3). Both carbendazim and thiabendazole are popular fungicides for protecting crops in the field and post-harvest treatments. High recoveries of these two fungicides in the present study

Journal of Food and Drug Analysis, Vol. 17, No. 3, 2009

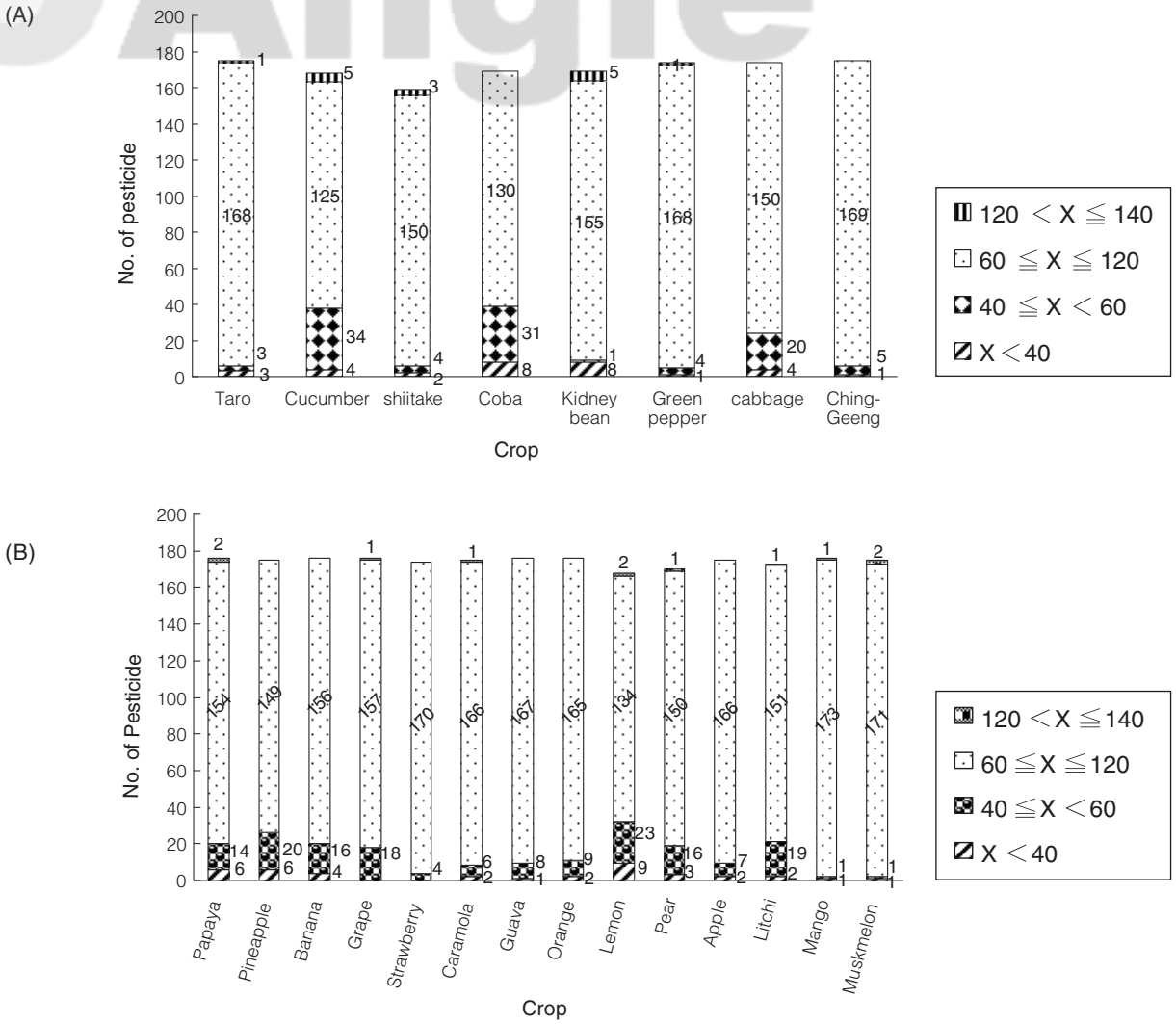

Figure 2. (A) Recovery of 176 pesticides and metabolites fortified in various vegetable crops. X represents the recovery (%) of pesticides and metabolites. (B) Recovery of 176 pesticides and metabolites fortified in various fruit crops. X represents the recovery (%) of pesticides and metabolites.

would assist in regulating residues by accurate detection regardless the matrix effect, which was suggested by Tseng *et al*. (2007) and thus to have significant influence on their detection .

III. *Determination of Pesticide Residues in Samples Collected in the Field*

A total of 4,305 pre-harvested vegetables and fruits from farms and orchards were analyzed by the proposed multiresidue method and Table 4 summarizes the analytical results. Fifty two percent of the samples examined had at least one pesticide residue. No pesticide residues were detected in banana and coba samples; however, some crops, such as lemon, mango, grape, litchi, orange and strawberry, had more residues than other crops, with a detection rate higher than 80%. Even though the total detection rate of samples was 52%, only 9% of the crops contained pesticide residues that exceeded the MRLs or no MRLs issued by DOH. Eighty-seven pesticides were detected in collected samples. The top ten detected pesticides are carbendazim, methomyl, fenvalerate, chlorpyrifos, deltamethrin, kresoxim-methyl, ethion, fenthion, cypermethrin, and omethoate, with detection rates of 9.87%, 8.66%, 6.53%, 6.39%, 6.20%, 5.62%, 5.62%, 5.37%, 5.32%, and 4.07%, respectively (Table 5). Non-compliant samples contained mainly residues of deltamethrin, chlorpyrifos, and omethoate over maximum residue limit. Some unregistered pesticides were detected on fruits, such as methomyl on papaya, chlorpyrifos on mango, ethion on mango and litchi, omethoate on mango, pineapple and litchi. While these unregistered pesticides were not recommended, farmers still frequently used them for the control of leaf-hoppers, leaf-miners,

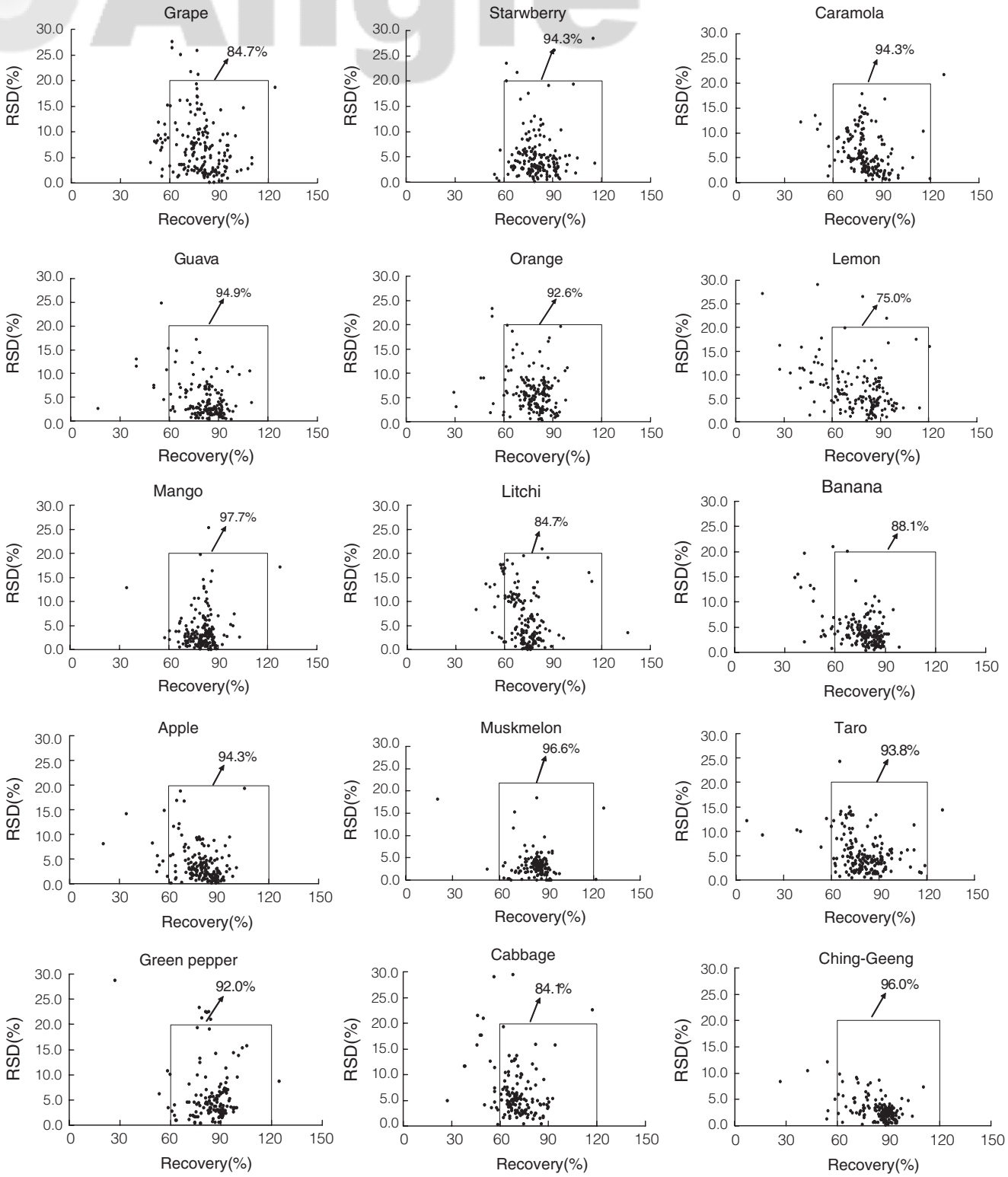

Figure 3. Recoveries vs relative standard deviations of 176 pesticides spiked in fruits and vegetables. Pesticides with recoveries between 60 and 120% and with RSD < 20% are framed.

ahpids, and thrips. Mango had the highest violation rate at 36.7%, with 43 pesticides detected (Table 4). The major reason for this high detection rate may be the timing of sampling, i.e. these samples were acquired from orchards growing the fruits for export 14 days before harvesting. In addition, there are no harmonized MRLs between/

Journal of Food and Drug Analysis, Vol. 17, No. 3, 2009

among countries for the same agro-product. Although COA has made considerable efforts to reduce pesticide residues in agro-products, much more work remains to be done to improve food safety.

CONCLUSION

A high-performance and eco-friendly analytical method is essential to monitoring programs and for implementing pesticide regulations. An effective multi-residue method should be specific and sensitive, with little or no matrix interference from various crops. Trace-level identification of a broad range of polar and nonpolar pesticides in complex matrices is a difficult task. The proposed method is suitable for use in routine inspection of pesticide residues in fruits and vegetables before harvesting, and data thus obtained can be used to educate farmers on proper usage of pesticides. Recently, a uniform limit for pesticides without MRLs was set at 0.01 mg/kg and issued by the Japanese government and EU for food safety concern. To effectively and efficiently determine such low levels of multiple analytes, advanced instrumental techniques must be applied for the identification and quantification. The main drawback of this method is time- and solvent- consuming. A further goal of this study would be the reduction of solvent consumption during LLP steps, especially dichloromethane, and to develop an alternative method of solvent extraction.

Table 4. Analysis of 176 pesticides from vegetables and fruits sampled in pre-harvest field during 2006

Crop	No. of samples	Residue detected rate (%)	Percentage of samples with residue ≤ MRL	Percentage of violated samples (%)	Violation rate (%)		No. of pesticide detected
					Over MRL	No MRL	
Grape	450	86.2	79.8	6.4	3.3	4.0	43
Strawberry	252	79.8	73.0	6.7	2.8	4.8	39
Carambola	102	68.6	63.7	4.9	3.9	2.0	17
Guava	227	19.4	18.5	3.5	0.0	3.5	21
Papaya	268	39.4	21.6	16.8	0.0	16.8	17
Pineapple	361	5.5	0.0	5.5	0.0	5.5	5
Banana	43	0.0	0.0	0.0	0.0	0.0	0
Orange	495	81.8	78.6	3.2	0.2	3.2	33
Lemon	38	86.8	84.2	2.6	0.0	2.6	15
Pear	443	43.1	42.4	0.7	0.7	0.0	24
Apple	11	36.4	18.2	18.2	0.0	9.1	5
Litchi	295	83.4	62.4	21.0	19.0	5.1	31
Mango	442	86.0	49.3	36.7	8.4	31.4	43
Muskmelon	219	12.8	10.5	2.3	0.0	2.7	15
Ching-Geeng	136	49.3	45.6	3.7	1.5	2.9	22
Cabbage	103	20.4	20.4	0.0	0.0	0.0	8
Green pepper	80	21.3	11.3	10.0	7.5	8.8	25
Cucumber	43	14.0	11.6	2.3	0.0	4.7	5
Kidney Bean	20	10.0	10.0	0.0	0.0	0.0	3
Taro	41	14.6	4.9	9.8	0.0	9.8	4
Coba	201	0.0	0.0	0.0	0.0	0.0	0
Shiitake	35	8.6	8.6	0.0	0.0	0.0	1
Total	4,305	52.0	42.9	9.1	3.0	7.0	87

Table 5. The top ten pesticides detected in 4,305 samples collected from pre-harvest field during 2006

Pesticide	Detection (%)	Violation (%)	Samples with residue over MRL	Samples with residue unregistered	Residue range (ppm)	Major crops with residue detected
Carbendazim	9.87	0.09	4	0	0.01-2.87	Mango, Grape, Orange, Litchi
Methomyl	8.66	0.81	4	31	0.01-3.18	Grape, Starfruit, Mango, Papaya
Fenvalerate	6.53	0.05	2	0	0.01-2.64	Orange, Mango, Litchi
Chlorpyrifos	6.39	1.44	16	46	0.01-3.26	Litchi, Orange, Mango, Grape, Pear
Deltamethrin	6.20	0.88	38	0	0.01-0.56	Litchi, Grape, Strawberry
Kresoxim-methyl	5.62	0.05	1	1	0.01-2.12	Grape, Mango
Ethion	5.62	0.28	1	11	0.01-4.46	Orange, Lemon, Mango, Litchi
Fenthion	5.37	0.02	1	0	0.01-1.16	Mango, Litchi
Cypermethrin	5.32	0.14	6	0	0.01-0.72	Litchi, Mango, Orange, Grape
Omethoate	4.07	2.02	14	73	0.01-2.81	Pear, Mango, Orange, Grape, Pineapple, Litchi

ACKNOWLEDGEMENTS

We would like to thank the Council of Agriculture of the Republic of China, Taiwan, for financial supporting under the project Monitoring and Regulation of Pesticide Residue in Agro-products. All technical staff in the laboratory at the Division of Residue Control in TACTRI is appreciated.

REFERENCES

1. Agricultural Chemicals and Toxic Substances Research Institute, Council of Agriculture, Executive Yuan. 2004. Plant Protection Manual. ISBN: 957-01-8877-4. Taiwan, R.O.C.
2. Department of Health, Executive Yuan. 2006. Tolerances for Residues of Pesticides. Announcement No. 0950401408. Taiwan, R.O.C.
3. Department of Health, Executive Yuan. 2006. Tolerances for Residues of Pesticides. Announcement No. 0950405513. Taiwan, R.O.C.
4. Department of Health, Executive Yuan. 2006. Tolerances for Residues of Pesticides. Announcement No. 0950407589. Taiwan, R.O.C.
5. Luke, M. A., Froberg, J. E., Dosse, G. M. and Masumoto, H. T. 1981. Improved multiresidue method gas chromatographic determination of organophosphorus, organonitrogen and organohalogen pesticides in produce, using flame photometric and electrolytic conductivity detectors. J. Assoc. Off. Anal. Chem. 64: 1187-1195.
6. Luke, M. A., Forberg, J. E. and Masumoto, H. T. 1975. Extraction and cleanup of organochlorine, organophosphate, organonitrogen, and hydrocarbon pesticides in produce for determination by gas-liquid-chromatography. J. Assoc. Off. Agri. Chem. 58: 1020-1026.
7. Department of Health, Executive Yuan. 2001. Method of Test for Pesticide Residues in Foods - Multi-residue Analysis (II). Announcement No. 0900025537. Taiwan, R.O.C.
8. Bureau of Standard, Metrology and Inspection, MOEA. 2000. Method of Test for Pesticide Residues in Foods - Multi-residue Analysis (III). CNS-13570-3.
9. Department of Health, Executive Yuan. 2005. Method of Test for Pesticide Residues in Foods - Multi-residue Analysis (III). Announcement No. 0949424750. Taiwan, R.O.C.
10. Otero, R. R., Grande, B. C. and Gandara, J. S. 2003. Multiresidue method for fourteen fungicides in white grapes by liquid-liquid and solid-phase extraction followed by liquid chromatography-diode array detection. J. Chromatogr. A 992: 121-131.
11. Ortelli. D., Edder, P. and Corvi, C. 2004. Multiresidue analysis of 74 pesticides in fruits and vegetables by liquid chromatography-electrospray-tandem mass spectrometry. Analytica Chimica Acta 520: 33-45.
12. Niessner, G., Buchberger, W. and Echerstorfer. 1999. Multiresidue screening methods for the determinaton of pesticides in plant materials. J. Chromatogr. A 846: 341-348.
13. Muccio, A. D., Girolimetti, S., Barbini, D. A., Pelosi, P., Generali, T., Vergori, L., Merulis, G. D., Leonelli, A. and Stefanelli, P. 1999. Selective clean-up application to a aqueous acetone extracts for the determination of carbendazim and thiabendazole in fruits and

vegetables by high-performance liquid chromatography with UV detection. J. Chromatogr. A 833: 61-65.
14. Stajnbaher, D. and Zupancic-Kralj, L. 2003. Multiresidue method for determination of 90 pesticides in fresh fruits and vegetables using solid-phase extraction and gas chromatography-mass spectrometry. J. Chromatogr. A 1015: 185-198.
15. Balinova, A., Mladenova, R. and Shtereva, D. 2007. Solid-phase extraction on sorbents of different retention mechanisms followed by determination by gas chromatography-mass spectrometric and gas chromatography-electron capture detection of pesticide residues in crops. J. Chromatogr. A 1150: 136-144.
16. Tseng, S. H., Lin, Y. J., Lee, H. F., Su, S. C., Chou, S. S. and Hwang, D. F. 2007. A multiresidue method for determining 136 pesticides and metabolites in fruits and vegetables: application of macroporous diatomaceous earth column. J. Food Drug Anal. 15: 316-324.
17. Hiemstra, M. and de Kok, A. 2007. Comprehensive multi-residue method for the target analysis of pesticides in crops using liquid chromatography–tandem mass spectrometry. J. Chromatogr. A 1154: 3-25.
18. Fenoll, J., Hellin, P., Martinez, C. M., Miguel, M. and Flores, P. 2007. Multiresidue method for analysis of pesticides in pepper and tomato by gas chromatography with nitrogen-phosphorus detection. Food Chemistry 105: 711-719.