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Analysis of 81 Pesticides and Metabolite Residues in Fruits and Vegetables by Diatomaceous Earth Column Extraction and LC/MS/MS Determination

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ABSTRACT

A multi-residue method for determining 81 pesticides and metabolite residues in vegetables and fruits by liquid chromatography/tandem mass spectrometry (LC/MS/MS) with electrospray ionization was developed. Pesticide residues were extracted from samples with acetone. Macroporous diatomaceous earth column was used instead of the separatory funnel for liquid/liquid extraction. Ethyl acetate was used as eluting solvent for diatomaceous earth column. This sample preparation technique by diatomaceous earth column was easy, fast and environment-friendly. It can reduce the sample preparation time and solvent consumption as well as eliminate the emulsion problem. Eighty-one analytes of different chemical families of insecticides, acaricides, fungicides, herbicides, plant growth regulators and 4 pesticide metabolites were determined in a single 25 min LC/MS/MS run. The pesticides were separated on an Atlantis T3 column using a gradient elution. Data acquisition under MS/MS for every pesticide or metabolite was achieved by applying multiple reaction monitoring (MRM) of two fragment ion transitions to provide high sensitivity and selectivity for both quantification and confirmation. A total of 162 MRM transitions were monitored. The standard addition was employed to compensate for the matrix effects to achieve the maximum accuracy of the LC/MS/MS method. Vegetable (bok choy) and fruit samples (grape or orange) were fortified with pesticides of low (0.05 or 0.1 ppm) and high (0.5 ppm) levels, and the triplicate results showed satisfactory recoveries and repeatability. The recoveries for most pesticides ranged from 70 to 120% and the coefficients of variation of all pesticides were below 25% in all matrices. The developed method, compared with traditional GC or LC method, showed less time-consumption and higher sensitivity. The proposed method is considered satisfactory for routine monitoring of pesticide residues in fruits and vegetables.

Key words: vegetable, fruit, pesticide, macroporous diatomaceous earth column, multiresidue analysis, LC/MS/MS

INTRODUCTION

The most convenient and economical way to increase production and reduce cost for farmers is applying pesticides on crops. However, because of the potential hazard effect on public health, Department of Health (DOH) in Taiwan set up the "Tolerances for the Residues of Pesticides in Crops" in 1976, which is revised often to ensure the food safety and protect consumers' health. Currently, maximum residue levels (MRLs) of more than 300 pesticides in various crops have been established and enforced by DOH[1]. Monitoring programs for pesticide residues in fruits and vegetables have been the routine work of food safety related authorities. For this type of target analysis, multi-residue analytical methods are preferred to reduce workload and costs[2]. A fast and easy multi-residue method using the macroporous diatomaceous earth (MDE) column for determining 135 pesticide residues in fruits and vegetables was announced as Taiwan's official method [Method of Test for Pesticide Residues in Foods-Multi-residue Analysis (3)] in 2005[3] based on our previous study[4]. The MDE column was used instead of a separatory funnel for liquid/liquid partition in sample preparation, thereby significantly reduced the preparation time and solvent consumption[4]. Traditional GC-FPD, GC-ECD, HPLC-FLD and HPLC-UV are used in this official method, which strongly limit the screening number of pesticides and further confirmation is needed. In the present study, an official MDE sample preparation method was also used, but followed by liquid

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chromatography with tandem mass spectrometry (LC/MS/MS) for determining non GC-amenable pesticides, including carbamates and benzimidazole pesticides, and some new generation of pesticides, such as acetamiprid, azoxystrobin, and indoxacarb. Twenty-one pesticides, which have already been listed in the official multiresidue method (3) and determined by laborious and time-consuming LC systems, including LC-FLD with post column derivatization system for determining carbamate pesticides and the LC-UV system for determining benzimedazole pesticides (carbendazim and thiobendazole), were included in the present study. The developed LC/MS/MS method for determining 81 pesticide residues was validated in terms of recovery, precision, and sensitivity. In addition, a small-scale survey of marketed vegetables and fruits was also conducted to evaluate suitability of the developed method for routine monitoring work.

MATERIALS AND METHODS

I. *Materials*

Bok choy, grape, and orange samples were purchased from supermarkets. MDE column (Varian Chem Elut^{TM}, 20 mL) with luer stopcock (PN. 12131005) was made by Varian (CA, USA). Membrane filter (Nylon, 13 mm, 0.22 μm) was purchased from Amchro (Hattersheim, Germany).

II. *Reagents*

Acetone was of residual grade. Methanol and ethyl acetate were of LC grade. Ammonium acetate was of analytical grade. Pesticide standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany), ChemService (West Chester, PA, USA), AccuStandard (New Haven, USA), and Riedel-de Haen AG (Hannover, Germany). The purities of pesticide standards were higher than 95% except for etrimfos (68.5%).

III. *Instruments and Analytical Conditions*

The LC/ESI (electrospray ionization)-MS/MS system used was Alliance® 2695 HPLC coupled with Micromass Premier^{TM} mass spectrometer with electrospray interface and MassLynx 4.1 software (Waters, MA, USA). For the LC separation, a Waters T3 guard column (10 mm × 2.1 mm, 3 μm) and a Waters Atlantis T3 analytical column (100 mm × 2.1 mm, 3 μm) were employed. Mobile phases were methanol/water (10/90, v/v) with 5 mM ammonium acetate (solvent A) and methanol/water (90/10, v/v) with 5 mM ammonium acetate (solvent B). The flow rate was 0.25 mL/min, and the injection volume was 10 μL. The mobile phase composition was changed during a run as follows. Starting with 0% B, the percentage of mobile phase B was increased linearly to 100% over 10 min and then kept constant for another 10 min. The composition was then changed back to 100% A in 0.1 min and the column was re-equilibrated for 4.9 min before the next injection. The total run time was 25 min for one injection.

MS parameters were set as follows. Ionization mode, electrospray positive ion mode; capillary voltage, 3.2 kV; source temperature, 100°C; desolvation temperature, 350°C; cone gas flow, 50 L/hr; desolvation flow, 700 L/hr; collision gas argon pressure, 2.5×10^{-3} mbar. The cone voltage, collision energy, and MRM transitions for each pesticide are listed in Table 1. The dwell time for every MRM transition was set at 5 ms.

IV. *Methods*

(I) *Preparation of Standard Solutions*

Each pesticide standard (50 mg) was accurately weighed into a 50 mL volumetric flask and methanol was then added up to the volume to make the stock standard solution (ca. 1000 μg/mL) individually. Stock solutions were stored at -18°C. They were kept for 2 hr at ambient temperature prior to use. Working standard mixtures, containing 10 μg/mL for each pesticide and diluted to 1 μg/mL, were prepared by mixing and diluting the stock solutions with methanol.

(II) *Preparation of Sample Solutions*

The fruit and vegetable samples were homogenized and 20 g of which were then sampled and extracted with 70 mL of acetone for 3 min. The extraction solution was then filtered under suction. The residues were extracted again with another 30 mL of acetone, which was then filtered. The filtrates were combined into an evaporation bottle and evaporated at 35°C under vacuum until no acetone left. The aqueous concentrate (ca. 18 mL) was applied onto a MDE column and kept standing for 10 min allowing the concentrates to evenly disperse in MDE column. The concentrate in MDE column was eluted with 80 mL of ethyl acetate at the flow rate of about 3~5 mL/min. The eluant was evaporated to dryness, dissolved in 5 mL of methanol, and filtered through a Nylon membrane filter as sample extract. The sample extract was ready to be diluted (five times) for screening or standard addition for accurate quantification by LC/MS/MS.

(III) *Evaluation of Matrix Effects*

Matrix effects were calculated as follows[5]:

$$\% \text{ Matrix Effects} = \left(\frac{\text{Response of post-spiked sample}}{\text{Response of standard}} - 1\right) \times 100\%$$

Where "Response of post-spiked sample" is the

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Table 1. The optimized LC/MS/MS MRM acquisition parameters of targeted pesticides

No.	Analyte	Retention time (min)	Quantification			Qualification		
			MRM transition (*m/z*)	Cone voltage (V)	Collision energy (eV)	MRM transition (*m/z*)	Cone voltage (V)	Collision energy (eV)
1	3-keto carbofuran	10.52	236 > 208	25	10	236 > 151	25	10
2	3-OH carbofuran	9.31	238 > 181	20	10	238 > 163	20	10
3	Acetamiprid	9.51	223 > 56	20	15	223 > 126	20	15
4	Alachlor	14.23	270 > 162	10	10	270 > 238	10	10
5	Aldicarb	10.55	208 > 116	10	8	208 > 89	10	8
6	Aldicarb sulfone	6.48	223 > 76	20	5	223 > 148	20	5
7	Aldicarb sulfoxide	5.82	207 > 89	16	10	207 > 132	16	10
8	Allethrin	16.08	320 > 135	15	15	320 > 93	15	15
9	Azoxystrobin	13.24	404 > 372	25	15	404 > 344	25	35
10	Bendiocarb	11.47	224 > 109	20	20	224 > 81	20	20
11	Benfuracarb	15.65	411 > 190	10	10	411 > 252	10	10
12	Bitertanol	14.88	338 > 269	15	10	338 > 99	15	10
13	Butachlor	16.16	312 > 238	15	15	312 > 162	15	15
14	Butocarboxim	10.46	213 > 75	35	15	213 > 116	35	15
15	Carbaryl	11.88	202 > 145	20	20	202 > 127	20	20
16	Carbendazim	10.21	192 > 160	30	30	192 > 132	30	30
17	Carbofuran	11.48	222 > 165	20	10	222 > 123	20	10
18	Carbosulfan	19.09	381 > 160	20	15	381 > 118	20	15
19	Clothianidin	8.97	250 > 169	20	20	250 > 132	20	30
20	Cyazofamid	14.16	325 > 108	15	15	325 > 261	15	9
21	Cyproconazole	13.92	292 > 70	20	25	292 > 125	20	25
22	Dicrotophos	8.47	238 > 112	20	10	238 > 193	20	10
23	Dimethomorph[a]	13.37;13.62	388 > 301	25	25	388 > 165	25	40
24	Diphenamid	12.88	240 > 134	25	25	240 > 167	25	35
25	Edifenphos	14.63	311 > 111	20	20	311 > 173	20	20
26	Etrimfos	14.73	293 > 265	25	20	293 > 125	25	20
27	Fenazaquin	18.46	307 > 161	20	20	307 > 57	20	20
28	Fenobucarb	13.22	208 > 95	20	10	208 > 152	20	10
29	Fenpyroximate	17.51	422 > 366	20	25	422 > 135	20	25
30	Fipronil	14.20	437 > 290	30	30	437 > 255	30	30
31	Flufenoxuron	16.66	489 > 158	25	30	489 > 141	25	30
32	Flusilazole	14.26	316 > 165	25	25	316 > 247	25	25
33	Flutolanil	13.54	324 > 262	25	20	324 > 242	25	20
34	Flutriafol	12.42	302 > 70	20	25	302 > 123	20	25

Table 1. Continued

No.	Analyte	Retention time (min)	Quantification			Qualification		
			MRM transition (*m/z*)	Cone voltage (V)	Collision energy (eV)	MRM transition (*m/z*)	Cone voltage (V)	Collision energy (eV)
35	Halfenprox	21.02	496 > 183	25	20	496 > 461	25	10
36	Haloxyfop-methyl	15.16	376 > 316	25	20	376 > 91	25	20
37	Heptenophos	12.77	251 > 127	20	25	251 > 109	20	25
38	Hexaflumuron	15.29	461 > 158	25	25	461 > 141	25	25
39	Hexythiazox	16.35	353 > 228	20	20	353 > 168	20	20
40	Imibenconazole	15.98	413 > 344	25	15	413 > 125	25	15
41	Imidacloprid	8.83	256 > 209	25	20	256 > 175	25	20
42	Indoxacarb	15.08	528 > 150	20	30	528 > 293	20	20
43	Isazofos	13.87	314 > 162	20	20	314 > 120	20	20
44	Isofenphos	14.91	346 > 287	10	10	346 > 245	10	10
45	Isoprocarb	12.46	194 > 95	20	10	194 > 137	20	10
46	Kresoxim-methyl	14.46	314 > 116	15	15	314 > 131	15	15
47	Mefenacet	13.87	299 > 148	15	20	299 > 120	15	20
48	Mepronil	13.71	228 > 119	35	30	228 > 91	35	30
49	Methiocarb	13.46	226 > 121	20	15	226 > 169	20	15
50	Methomyl	7.40	163 > 88	10	10	163 > 106	10	10
51	Metolachlor	14.26	284 > 252	20	20	284 > 176	20	20
52	Metolcarb	10.98	166 > 109	15	25	166 > 94	15	35
53	Metribuzin	11.19	215 > 187	25	20	215 > 84	25	20
54	Molinate	13.89	188 > 126	20	15	188 > 98	20	30
55	Napropamide	14.13	272 > 129	20	20	272 > 171	20	20
56	Nuarimol	13.33	315 > 81	25	25	315 > 252	25	25
57	Oxadiazon	16.03	345 > 303	25	15	345 > 220	25	15
58	Oxamyl	6.86	237 > 72	11	13	237 > 90	11	13
59	Oxycarboxin	9.84	268 > 175	20	25	268 > 147	20	30
60	Paclobutrazol	13.54	294 > 70	25	40	294 > 125	25	40
61	Pencycuron	15.04	329 > 125	20	15	329 > 218	20	15
62	Pendimethalin	16.63	282 > 212	20	10	282 > 194	20	20
63	Pirimicarb	12.40	239 > 72	20	15	239 > 182	20	15
64	Promecarb	13.60	208 > 151	15	10	208 > 109	15	10
65	Propanil	13.46	218 > 162	20	20	218 > 127	20	20
66	Propaphos	14.58	305 > 263	20	10	305 > 221	20	10
67	Propoxur	11.39	210 > 111	12	20	210 > 93	12	20
68	Pyriproxyfen	16.22	322 > 96	20	15	322 > 227	20	15

Table 1. Continued

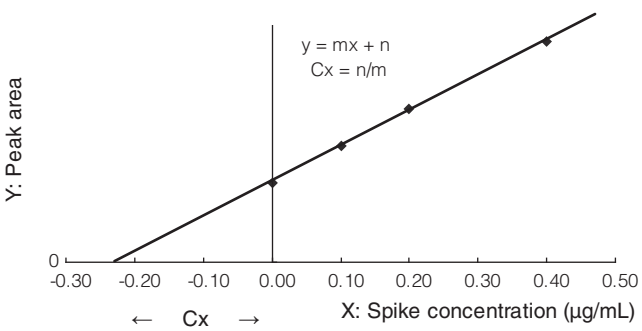
No.	Analyte	Retention time (min)	Quantification			Qualification		
			MRM transition (*m/z*)	Cone voltage (V)	Collision energy (eV)	MRM transition (*m/z*)	Cone voltage (V)	Collision energy (eV)
69	Pyroquilon	11.40	174 > 132	20	25	174 > 117	20	25
70	Quizalofop-ethyl	15.72	373 > 299	25	25	373 > 181	25	50
71	Tebuconazole	14.56	308 > 70	25	25	308 > 125	25	25
72	Tetraconazole	13.97	372 > 159	25	25	372 > 70	25	25
73	Tetramethrin	15.88	332 > 135	15	20	332 > 164	15	20
74	Thiabendazole	11.20	202 > 175	30	30	202 > 131	30	30
75	Thiamethoxam	8.68	292 > 211	20	15	292 > 181	20	25
76	Thiobencarb	15.01	258 > 125	20	15	258 > 100	20	30
77	Thiodicarb	12.37	355 > 88	25	15	355 > 108	25	15
78	Triadimenol	13.84	296 > 70	15	15	296 > 99	20	15
79	Trifloxystrobin	15.18	409 > 186	15	15	409 > 206	15	15
80	Triflumizole	15.45	346 > 278	15	15	346 > 250	20	15
81	XMC	12.15	180 > 123	12	20	180 > 95	12	20

[a] Two peaks, corresponding to geometric isomers.

average area count for the analyte, spiked into extracted matrix after the extraction procedure and "Response of standard" is the average area count for the same concentration of analyte in neat solution. The neat solution should be the same solvent composition as the reconstitution solution for the post-spiked sample. A negative result indicates suppression, and a positive result indicates enhancement of the analyte signal.

(IV) *Standard Addition for Quantification*

The sample preparation was the same as described above. After extraction, four portions, 200 μL (a) each, of the sample extract were transferred into four separate LC vials. 0, 100, 200, and 400 μL of analyte standard solution (1 μg/mL) were added to the vials and the samples were made up to 1000 μL (b) with methanol. The added concentrations of pesticide in four vials were 0, 0.1, 0.2, and 0.4 μg/mL, respectively. A linear regression plot of added concentrations vs. their responses was constructed to obtain the slope *m* and *y*-intercept *n* (y = *m*x + *n*). The pesticide content in sample (μg/g, ppm) was calculated as (C×V×F)/M, where C is the concentration of sample solution (calculated by *n*/*m*, μg/mL); V is the make up volume (mL) of sample extract; M is the sample weight (g); F = b/a.



(V) *Validation Study*

The method was tested to assess mean recovery (as measure of trueness), precision, and sensitivity. This requires performing recovery experiments with spiked blank samples to estimate accuracy of the method. Blank samples were tested in advance to ensure they were free of the 81 pesticides. Mean recovery and precision (repeatability, expressed as coefficient of variation in %) were determined by analyzing spiked vegetable (bok choy) and fruit (citrus or grape) samples in triplicate at low (0.05 or 0.1 μg/g.) and high spiking level (0.5 μg/g) each. The spiked samples were then kept in a hood for 30 min to evaporate the solvent residues.

The limit of quantification (LOQ) of the developed method was defined as the amount of each analyte in sample that would produce a signal/noise of at least 10/1 (S/N ≥ 10). The relative ion intensity (% of base peak) should meet the EU requirement of confirmation (SANCO/2007/3131)[6].

RESULTS AND DISCUSSION

I. *Optimization of Parameters for LC/MS/MS*

Each analyte was tuned individually in order to achieve a stable and high abundance of precursor ions, select 2 suitable mass transitions, and optimize the yield of product ions[2]. The transitions in the MRM of the tandem mass spectrometer were selected and tuned by using solutions of individual analytes in mobile phase with 5 mM ammonium acetate (mobile phase A:B = 1:1, v/v) at a concentration of 200 ng/mL. These solutions were introduced into the mass spectrometer via a syringe pump at a flow rate of 20 μL/min. Ammonium acetate was used as a modifier in the LC mobile phase so as to generate abundant ammonium adducts in the electrospray ion (ESI) source. The presence of ammonium adducts suppressed the formation of sodium adducts, and thereafter, pesticides formed $[M]^+$, $[M+H]^+$, and/or $[M+NH_4]^+$, which showed high sensitivity and constant responses[7].

The analyte-dependent parameters, such as cone voltage (CV) and collision energy (CE), were optimized in this study. $[M+H]^+$ was chosen as precursor ion for most pesticides. $[M]^+$ and $[M+NH_4]^+$ were chosen as precursor ions for some pesticides because of their higher ionization yield compared with that of the $[M+H]^+$. $[M+NH_4]^+$ was chosen as precursor ion for oxamyl, butocarboxim, aldicarb, allethrin, and halfenprox. The optimized MRM acquisition parameters for determining 81 pesticides and a total of 162 MRM transitions are summarized in Table 1. According to the European guidelines EC/657/2002[8], each analyte can earn 4 identification points (IPs) in this study based on determination of 1 precursor (1 IP) and 2 product ions (1.5 × 2 = 3 IPs) by LC/MS/MS technique. The developed mass spectrometric conditions met the EU confirmation requirement[6].

A reverse LC system has been commonly used for determining pesticide residues in vegetables and fruits by LC/MS/MS. The columns previously used include XTerra MS C18[9-10], Luna C18 and Aqua C18[2], and Zorbax RX C8[11]. An Atlantis T3 column, suitable for retention and separation of polar and non-polar compound, was chosen in this study. Pesticides were separated on an Atlantis T3 column under the given mobile phase gradient conditions within 25 min. The TIC (total ion chromatogram) of 81 pesticide mixtures (in solvent) and overlapping 162 MRM chromatograms are shown in Figure 1. The total run time for one injection was 25 min, which was shorter than that in previous LC/MS/MS papers for pesticide determination[2,11]. Addition of 5~10 mM ammonium acetate or 0.01% formic acid in LC/MS/MS mobile phase could enhance sensitivity[9,12]. A concentration of 5 mM ammonium acetate in mobile phase was prepared in this study and led to a satisfactory sensitivity. Repeatability of retention time and peak area of each analyte was qualified.

II. *Sample Pretreatment*

A fast and easy multi-residue method using the macroporous diatomaceous earth (MDE) column for determining 135 pesticide residues in fruits and vegetables was announced as the official method in Taiwan in 2005[3]. The MDE column is a polypropylene (PP) cartridge packed with highly pure and inert MDE, which was used instead of the separatory funnel for liquid/liquid partition in sample preparation. In this study, an official MDE sample preparation procedure was used and followed by LC/MS/MS determination. A solid phase extraction (SPE) clean-up procedure was not needed in this method. The advantages of using MDE column include simple device applied, simultaneous processing of multiple samples, elimination of emulsion problem, and no need for dehydration of the eluant by anhydrous sodium sulfate[13].

III. *Matrix Effects*

Matrix effects resulted from co-eluting matrix components that impact ionization of the target analyte, causing ion suppression or ion enhancement[5]. Matrix effects can be highly variable and difficult to control or predict[5]. In order to compensate the matrix effects, isotopically labeled internal standard[11], matrix matched calibration curve[12] or standard addition method[7] were used in published papers. Generally, it is very

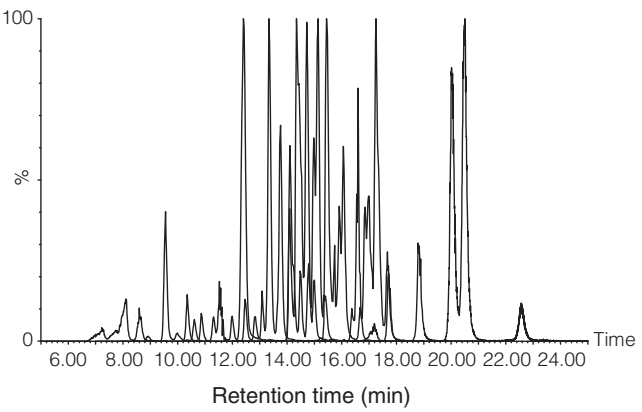


Figure 1. Total ion chromatogram of 81 mix standards in methanol at level of 0.05 g/mL in a single 25 minute run.

Table 2. Matrix effects of bok choy and grape on target pesticides

Pesticide	Spiking Level	Matrix Effect (%)	
	(μg/mL)	Bok choy	Grape
Azoxystrobin	0.05	-5.4	-7.2
	0.5	-4.6	-2.8
Cyazofamid	0.05	-3.4	-12.0
	0.5	-1.4	-7.7
Dimethomorph	0.05	-3.9	-12.0
	0.5	-2.3	-5.0
Indoxacarb	0.05	-3.6	-6.8
	0.5	-4.1	-3.2
Oxycarboxin	0.05	7.0	6.7
	0.5	4.5	5.9
Quizalofop-ethyl	0.05	-3.3	-5.5
	0.5	-5.9	-2.3
Thiamethoxam	0.05	-9.8	-17.8
	0.5	-5.6	-1.4
Trifloxystrobin	0.05	-3.3	-3.8
	0.5	-0.9	-0.1
Triflumizole	0.05	-2.7	-5.3
	0.5	-1.7	-2.4

difficult and expensive to obtain every single isotopically labeled standard for each individual pesticide of interest. In contrast, standard addition is a relatively cheap and practical quantification technique when no blank matrix is available[7]. Matrix effects of bok choy and grape for determining 9 target pesticides at 0.05 and 0.5 μg/mL spiking levels were calculated using equation as described above in Methods (Table 2). Our results showed that bok choy and grape matrices exhibited inhibition effects (-0.1 – -17.8%) on 8 pesticides and enhancement effects on oxycarboxin (+4.5 – +7.0%).

IV. *Method Validation and Performance*

The performance of this developed method was evaluated in terms of recovery, repeatability, and limit of quantification (LOQ). The accuracy of the method was estimated by means of recovery experiments at low spiking level (0.05 or 0.1 μg/g) and high spiking level (0.5 μg/g). Triplicate experiments were carried out at each level (Table 3). The test matrix of fruit was citrus or grape and that of vegetable was bok choy. The triplicate results showed satisfactory recoveries and repeatability. The recoveries for most pesticides ranged from 70 to 120% and the coefficients of variation of all pesticides were below 25% in all matrices (Table 3). However, the recoveries of some pesticides (12 in fruit matrix and 30 in vegetable matrix) were below 70% due to poor stability or higher polarity. Recoveries of aldicarb sulfoxide were lower than 50% due to loss of MDE

Table 3. Validation data of this developed LC/MS/MS method

Analyte	Recovery[a] (%)				LOQ (μg/g)
	Spiking level (μg/g)	Fruit Matrix (citrus or grape)		Vegetable matrix (bok choy)	
3-keto carbofuran	0.5	97.4 (20.4)[b]	Citrus	117.9 (6.7)	0.01
	0.05	116.1 (12.0)		120.2 (0.5)	
3-OH carbofuran	0.5	95.0 (22.2)	Citrus	104.5 (10.0)	0.001
	0.05	101.1 (29.1)		100.6 (10.3)	
Acetamiprid	0.5	95.6 (14.7)	Grape	104.8 (20.0)	0.0005
	0.1	74.3 (4.9)		93.8 (18.0)	
Alachlor	0.5	114.8 (3.8)	Grape	80.7 (10.6)	0.005
	0.1	81.3 (17.1)		61.3 (12.7)	
Aldicarb	0.5	73.3 (16.8)	Citrus	89.4 (8.7)	0.001
	0.05	83.7 (6.4)		90.4 (1.5)	
Aldicarb sulfoxide	0.5	47.4 (12.7)	Citrus	41.6 (10.6)	0.005
	0.05	43.2 (19.5)		36.0 (9.4)	
Aldicarb sulfone	0.5	97.3 (23.7)	Citrus	97.3 (12.0)	0.01
	0.05	109.4 (19.3)		102.5 (14.7)	

Table 3. Continued

Analyte	Recovery[a] (%)			LOQ (μg/g)
	Spiking level (μg/g)	Fruit Matrix (citrus or grape)	Vegetable matrix (bok choy)	
Allethrin	0.5	57.8 (11.0) Grape	53.1 (11.5)	0.01
	0.1	44.4 (7.6)	41.5 (5.7)	
Azoxystrobin	0.5	109.2 (0.8) Grape	95.2 (0.9)	0.002
	0.1	124.9 (4.2)	86.4 (2.2)	
Bendiocarb	0.5	88.3 (22.5) Citrus	97.0 (9.7)	0.001
	0.05	87.9 (21.8)	100.5 (4.7)	
Benfuracarb	0.5	55.5 (11.1) Grape	50.9 (2.1)	0.001
	0.1	50.8 (2.9)	50.1 (4.1)	
Bitertanol	0.5	96.1 (12.8) Grape	60.0 (7.9)	0.05
	0.1	59.3 (11.2)	53.7 (10.5)	
Butachlor	0.5	96.4 (19.1) Grape	44.5 (3.0)	0.01
	0.1	64.6 (7.9)	56.0 (13.9)	
Butocarboxim	0.5	76.5 (16.8) Citrus	74.7 (6.0)	0.001
	0.05	63.4 (17.4)	60.2 (11.1)	
Carbaryl	0.5	86.8 (24.7) Citrus	91.0 (7.6)	0.001
	0.05	82.4 (15.8)	84.9 (6.9)	
Carbendazim	0.5	100.9 (24.1) Citrus	60.4 (22.1)	0.01
	0.05	111.2 (10.9)	35.2 (8.7)	
Carbofuran	0.5	84.5 (14.9) Citrus	93.6 (11.3)	0.001
	0.05	86.5 (20.1)	92.5 (5.4)	
Carbosulfan	0.5	48.4 (7.5) Grape	52.7 (1.8)	0.002
	0.1	41.8 (3.7)	41.7 (4.9)	
Clothianidin	0.5	103.8 (11.9) Grape	93.6 (13.4)	0.01
	0.1	88.1 (8.4)	85.6 (9.2)	
Cyazofamid	0.5	109.1 (1.6) Grape	65.0 (7.9)	0.01
	0.1	70.7 (4.5)	78.6 (4.4)	
Cyproconazole	0.5	103.0 (10.4) Grape	81.5 (21.8)	0.01
	0.1	70.0 (14.6)	61.0 (10.9)	
Dicrotophos	0.5	107.5 (15.1) Grape	94.3 (16.5)	0.0005
	0.1	95.3 (9.8)	58.4 (13.5)	
Dimethomorph	0.5	99.9 (14.2) Grape	84.9 (0.7)	0.002
	0.1	102.7 (0.7)	75.7 (3.1)	
Diphenamid	0.5	115.1 (5.3) Grape	97.5 (15.6)	0.0005
	0.1	98.0 (7.0)	67.4 (11.7)	
Edifenphos	0.5	116.5 (2.7) Grape	80.7 (16.0)	0.001
	0.1	100.3 (14.1)	62.4 (11.5)	
Etrimfos	0.5	77.4 (4.8) Grape	58.3 (3.5)	0.05
	0.1	92.2 (21.8)	53.1 (7.7)	

Table 3. Continued

Analyte	Recovery[a] (%)				LOQ (μg/g)
	Spiking level (μg/g)	Fruit Matrix (citrus or grape)		Vegetable matrix (bok choy)	
Fenazaquin	0.5	60.1 (16.3)	Grape	52.2 (3.2)	0.01
	0.1	66.5 (5.9)		51.0 (5.7)	
Fenobucarb	0.5	86.6 (23.7)	Citrus	89.8 (17.1)	0.001
	0.05	84.0 (15.6)		87.8 (5.1)	
Fenpyroximate	0.5	76.6 (6.1)	Grape	46.9 (3.5)	0.01
	0.1	73.4 (12.6)		48.7 (4.2)	
Fipronil	0.5	63.9 (15.6)	Grape	56.6 (5.5)	0.05
	0.1	46.3 (7.25)		42.5 (10.1)	
Flufenoxuron	0.5	94.8 (9.8)	Grape	52.6 (4.7)	0.005
	0.1	59.3 (4.5)		43.6 (8.7)	
Flusilazole	0.5	108.8 (8.1)	Grape	57.6 (4.0)	0.005
	0.1	86.3 (22.0)		54.3 (6.1)	
Flutolanil	0.5	99.6 (22.1)	Grape	54.0 (5.4)	0.01
	0.1	63.0 (5.6)		47.1 (9.4)	
Flutriafol	0.5	102.7 (9.6)	Grape	87.4 (16.5)	0.01
	0.1	99.4 (9.1)		63.2 (13.9)	
Halfenprox	0.5	88.1 (19.5)	Grape	51.6 (12.7)	0.05
	0.1	62.3 (5.7)		52.4 (9.7)	
Haloxyfop-methyl	0.5	102.6 (13.8)	Grape	55.0 (8.3)	0.01
	0.1	58.0 (3.8)		70.7 (16.8)	
Heptenophos	0.5	113.5 (6.9)	Grape	93.6 (20.1)	0.002
	0.1	103.9 (12.1)		68.6 (16.2)	
Hexaflumuron	0.5	61.5 (5.1)	Grape	55.8 (1.0)	0.05
	0.1	63.0 (4.5)		55.8 (9.7)	
Hexythiazox	0.5	84.2 (18.3)	Grape	53.9 (4.9)	0.05
	0.1	79.2 (2.9)		48.8 (2.4)	
Imibenconazole	0.5	66.7 (10.3)	Grape	57.7 (3.2)	0.05
	0.1	51.4 (2.4)		51.6 (11.8)	
Imidacloprid	0.5	102.8 (12.9)	Grape	98.8 (17.3)	0.005
	0.1	103.5 (10.3)		77.8 (16.4)	
Indoxacarb	0.5	86.4 (3.1)	Grape	76.6 (1.5)	0.002
	0.1	75.2 (5.9)		81.8 (1.1)	
Isazofos	0.5	110.8 (3.1)	Grape	71.5 (18.6)	0.0005
	0.1	82.1 (23.5)		60.8 (14.2)	
Isofenphos	0.5	88.5 (4.8)	Grape	56.3 (7.8)	0.002
	0.1	72.4 (3.3)		51.7 (10.8)	
Isoprocarb	0.5	80.3 (16.1)	Citrus	88.7 (10.4)	0.001
	0.05	80.5 (19.9)		86.1 (6.0)	

Table 3. Continued

Analyte	Recovery[a] (%)				LOQ (μg/g)
	Spiking level (μg/g)	Fruit Matrix (citrus or grape)		Vegetable matrix (bok choy)	
Kresoxim-methyl	0.5	109.2 (6.1)	Grape	58.6 (6.2)	0.005
	0.1	68.2 (5.8)		62.4 (6.8)	
Mefenacet	0.5	107.1 (7.6)	Grape	82.3 (19.0)	0.0005
	0.1	107.2 (9.2)		63.7 (8.5)	
Mepronil	0.5	115.5 (3.4)	Grape	73.0 (20.3)	0.005
	0.1	72.0 (5.6)		57.4 (7.3)	
Methiocarb	0.5	84.5 (18.0)	Citrus	90.4 (12.5)	0.001
	0.05	82.8 (10.6)		93.1 (3.4)	
Methomyl	0.5	84.8 (14.9)	Citrus	101.1 (9.4)	0.002
	0.05	96.8 (15.1)		98.5 (1.5)	
Metolachlor	0.5	111.0 (9.6)	Grape	76.3 (18.1)	0.001
	0.1	107.9 (16.5)		60.6 (7.3)	
Metolcarb	0.5	84.3 (21.6)	Citrus	88.5 (17.0)	0.01
	0.05	69.8 (4.9)		84.9 (15.5)	
Metribuzin	0.5	75.9 (19.0)	Grape	82.7 (14.2)	0.05
	0.1	94.8 (11.2)		80.0 (18.0)	
Molinate	0.5	103.2 (18.9)	Grape	69.1 (21.6)	0.01
	0.1	72.7 (3.5)		74.3 (20.0)	
Napropamide	0.5	113.9 (8.6)	Grape	85.5 (18.0)	0.002
	0.1	77.7 (4.4)		61.0 (4.5)	
Nuarimol	0.5	113.9 (8.6)	Grape	85.5 (18.0)	0.05
	0.1	77.7 (4.4)		61.0 (4.5)	
Oxadiazon	0.5	90.4 (15.9)	Grape	80.5 (23.0)	0.05
	0.1	68.7 (3.7)		51.2 (5.4)	
Oxamyl	0.5	77.4 (13.6)	Citrus	85.9 (20.0)	0.002
	0.05	95.6 (3.9)		98.0 (5.8)	
Oxycarboxine	0.5	96.0 (2.2)	Grape	98.1 (2.0)	0.002
	0.1	101.5 (8.3)		90.8 (4.8)	
Paclobutrazol	0.5	93.0 (4.6)	Grape	77.6 (21.8)	0.05
	0.1	66.0 (3.6)		52.2 (7.0)	
Pencycuron	0.5	101.9 (5.3)	Grape	51.8 (5.3)	0.005
	0.1	75.4 (5.3)		59.8 (5.4)	
Pedimethalin	0.5	58.4 (22.0)	Grape	53.3 (6.1)	0.05
	0.1	49.6 (2.5)		42.5 (5.4)	
Pirimicarb	0.5	109.0 (13.3)	Grape	97.2 (11.2)	0.0005
	0.1	107.3 (5.8)		63.5 (5.6)	
Promecarb	0.5	76.7 (12.2)	Citrus	85.7 (14.6)	0.001
	0.05	83.8 (17.6)		87.0 (7.1)	

Table 3. Continued

Analyte	Recovery[a] (%)				LOQ (μg/g)
	Spiking level (μg/g)	Fruit Matrix (citrus or grape)		Vegetable matrix (bok choy)	
Propanil	0.5	112.0 (3.3)	Grape	86.6 (21.6)	0.005
	0.1	78.5 (1.8)		79.3 (21.7)	
Propaphos	0.5	53.1 (7.1)	Grape	70.0 (19.3)	0.01
	0.1	53.0 (9.5)		61.0 (8.8)	
Propoxur	0.5	79.0 (14.2)	Citrus	102.9 (10.6)	0.002
	0.05	93.5 (10.1)		104.5 (3.1)	
Pyriproxyfen	0.5	86.0 (8.8)	Grape	53.7 (5.3)	0.001
	0.1	81.4 (9.3)		51.7 (9.7)	
Pyroquilon	0.5	106.5 (10.6)	Grape	90.6 (19.3)	0.0005
	0.1	98.3 (6.8)		70.9 (23.5)	
Quizalopfop-ethyl	0.5	79.1 (12.1)	Grape	68.6 (2.0)	0.002
	0.1	70.4 (11.2)		62.4 (3.5)	
Tebuconazole	0.5	104.5 (7.7)	Grape	67.9 (11.3)	0.01
	0.1	73.0 (13.8)		58.4 (15.2)	
Tetraconazole	0.5	96.0 (18.0)	Grape	52.6 (3.6)	0.005
	0.1	105.7 (13.0)		53.3 (7.0)	
Tetramethrin	0.5	53.6 (5.3)	Grape	56.0 (5.5)	0.002
	0.1	45.3 (7.6)		50.6 (10.5)	
Thiabendazole	0.5	88.6 (27.7)	Citrus	91.7 (17.1)	0.01
	0.05	70.9 (22.5)		80.4 (8.8)	
Thiamethoxam	0.5	107.8 (16.0)	Grape	89.3 (11.3)	0.01
	0.1	111.6 (2.5)		87.3 (3.3)	
Thiobencarb	0.5	100.7 (7.8)	Grape	50.5 (6.6)	0.01
	0.1	80.3 (21.9)		52.9 (5.6)	
Thiodicarb	0.5	47.7 (17.6)	Citrus	92.0 (8.3)	0.001
	0.05	28.6 (10.1)		92.3 (6.1)	
Triadimenol	0.5	98.8 (16.6)	Grape	82.4 (22.6)	0.05
	0.1	76.4 (12.3)		71.5 (10.3)	
Trifloxystrobin	0.5	91.8 (7.4)	Grape	83.9 (2.3)	0.002
	0.1	83.2 (1.6)		91.8 (1.0)	
Triflumizole	0.5	79.2 (12.1)	Grape	76.6 (3.2)	0.05
	0.1	64.5 (14.3)		67.0 (1.5)	
XMC	0.5	78.4 (12.5)	Citrus	89.9 (9.9)	0.001
	0.05	83.8 (25.2)		90.4 (4.9)	

[a] average of triplicate.

[b] value in the parenthesis is coefficient of variation (CV, %).

liquid-liquid partition because of higher polarity of this compound. In some unstable pesticides, such as carbosulfan, benfuracarb, thiodicarb, decomposition might have happened during the analytical process. Specifically, carbosulfan and benfuracarb were easily decomposed to carbofuran under normal extraction condition, and performed low recovery (41.7–55.5%) in this study. Thiodicarb, an acid-labile pesticide, might be decomposed to methomyl during the preparation of citrus samples and performed low recovery (28.6–47.7%). We also found that acidity of sample was a critical factor influencing recoveries of some pesticides. That is the reason why grape and citrus fruits of high acid content showed significantly better recoveries than bok choy in some cases, e.g. carbendazim, edifenphos, fenpyroximate, flutriafol, isofenphos, pencycuron, pyriproxyfen, tetraconazole, and thiobencarb. The adjustment of pH value of extraction solvents (acidified and buffered solvents) might minimize this phenomenon. For the accurate quantification of pesticides with recoveries of < 60%, the use of calibration curve spiking standards at the beginning of extraction or individual methods may offer

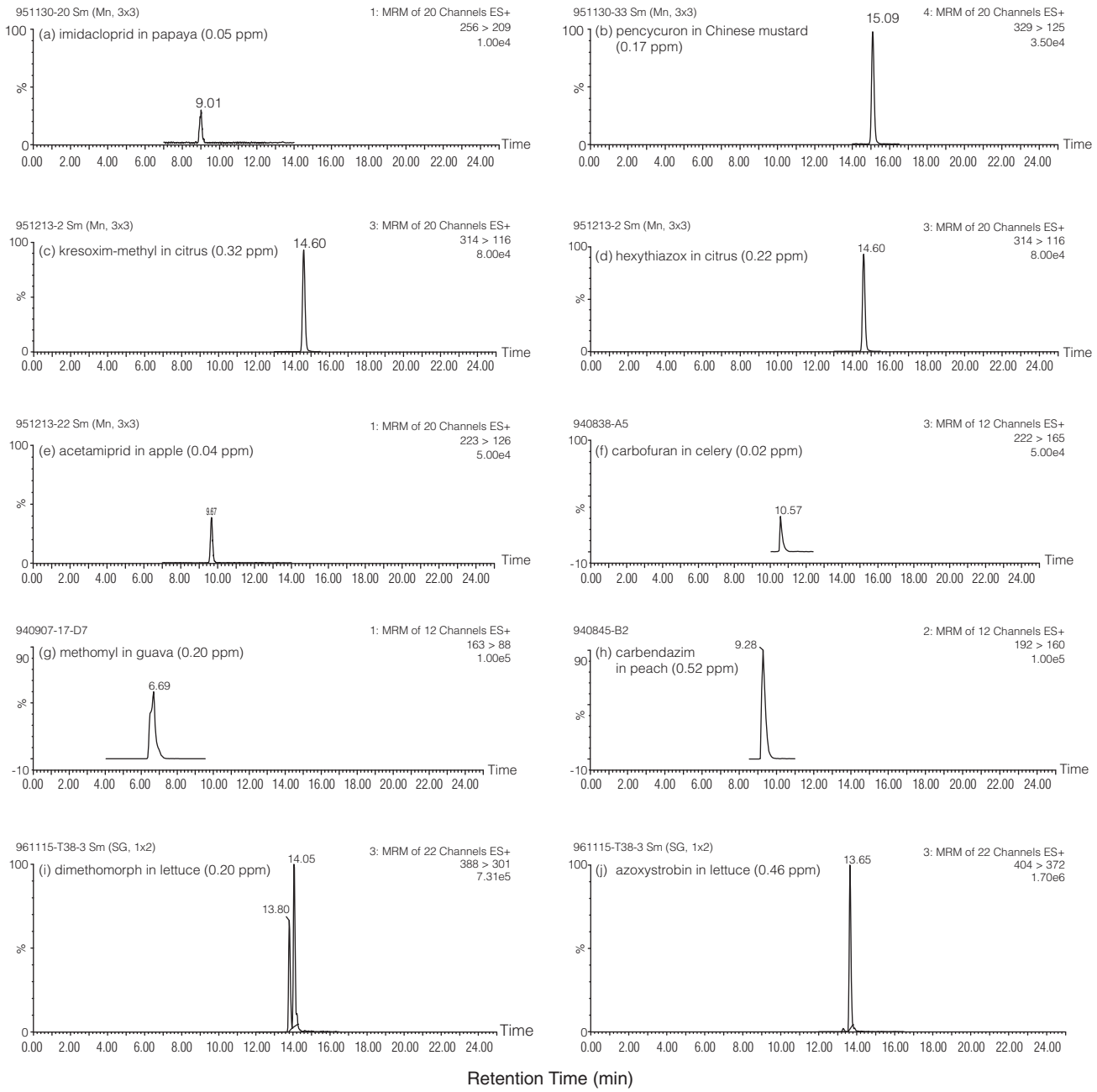


Figure 2. MRM chromatograms of pesticide residues identified in analyzed samples.

alternatives in the further study.

The limit of quantification (LOQ) of the developed method was defined as the amount of each analyte in sample that would produce a signal/noise of at least 10/1 (S/N ≥ 10), and the relative ion intensity (% of base peak) should meet the EU requirement of confirmation[6]. LOQs of 81 pesticides in this study ranged from 0.0005 to 0.05 ppm (Table 3). This method showed high sensitivity and suitability for muti-residue screening. The LOQ of fipronil determined by ESI (+) mode was 0.05 ppm in this study. However, our further study showed ESI (-) mode could reach higher sensitivity for fipronil, and the LOQ could reach about 0.001 ppm.

V. *Survey Results of Marketed Vegetables and Fruits*

The optimized analytical procedure was used to analyze 47 marketed various vegetable and fruit samples. Eighteen samples (approximately 40%) contained detectable pesticide residues, with 8 of which containing two to four pesticides. Selected MRM chromatograms of pesticide residues identified in analyzed samples are shown in Figure 2.

CONCLUSIONS

This method, compared with traditional GC or LC methods, is less time-consuming and has higher sensitivity. This study has shown that LC/MS/MS is a powerful analytical technique for simultaneous quantification and qualification of these 81 pesticide residues in fruits and vegetables. This developed method was already announced as Taiwan's official method [Method of Test for Pesticide Residues in Foods—Multi-residue Analysis (4)] in 2008[14]. Based on the same extraction procedure of official multi-residue method (3) and (4), a combined multi-residue analysis procedure for determining 195 pesticide residues in fruits and vegetables is available and suitable for routine monitoring study (Figure 3).

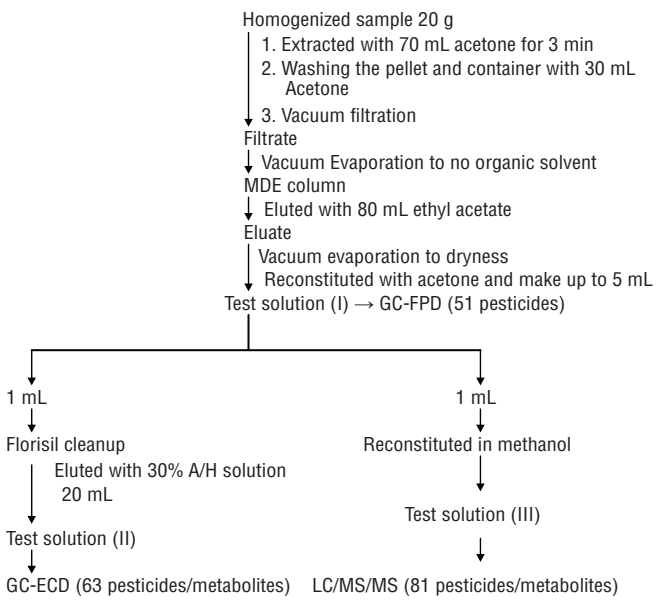


Figure 3. Analytical procedure for determining 195 pesticide residues in fruits and vegetables.

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