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Analysis of popular pesticide residues in Mekong Delta vegetables using a modified QuEChERS method and UPLC-MS/MS

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Abstract

Pesticide residues in vegetables (watercress, mustard green, choy sum, daikon, okra, and yam) from Mekong Delta, Vietnam were analysed by liquid chromatography-tandem mass spectroscopy (LC-MS/MS). QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) extraction, matrix-matched calibration, and dynamic multiple reaction monitoring methods were used. The linear range used was 5 - 200 ng/mL, resulting in $R^2 > 0.99$. The recovery was satisfactory with values within 74.47 - 116.93%, and the RSD was < 15% for most compounds. The percentage of samples with residues above the maximum residue levels (MRLs) was 59%. Pesticide residues were detected above their MRLs in samples of watercress (47%), mustard green (80%), choy sum (83%), daikon (87%), and yam (60%). The results indicated the prevalence of pesticide residues in commonly consumed vegetables in Vietnam, and emphasised the urgent need to develop comprehensive intervention measures to reduce the potential health risk to consumers.

Introduction

Fresh vegetables constitute an important part of a healthy diet. However, vegetables have been recognised as a food group that contains higher pesticide residue levels when compared with the other food groups (Nishina *et al.*, 2010; Hoi *et al.*, 2016). In agriculture, pesticides are widely used to protect crops, thus increasing the crop yields. Although these compounds provide unquestionable benefits that increase agricultural production, they are detrimental to health, and have been associated with dermatological, gastrointestinal, neurological, carcinogenic, respiratory, reproductive, and endocrine effects (WHO, 1990; Sanborn *et al.*, 2007; Alewu and Nosiri, 2011; Mnif *et al.*, 2011). Farmers tend to apply pesticides too close to harvest due to the lack of adequate knowledge regarding the safe and judicious use of pesticides (Nishina *et al.*, 2010; Hoi *et al.*, 2016). Therefore, quality control is highly demanded to protect the health and safety of consumers. To ensure food safety for consumers, and to protect human health, many organisations and countries around the world have established maximum residue limits (MRLs) for pesticides in food commodities. Although regulations on MRLs in food commodities exist in Vietnam, such as those set by the Codex Alimentarius Commission (Codex), these regulations are often not fully enforced. Due to the extensive use of pesticides in the Vietnam agriculture industry, systematic investigations are necessary to verify the presence of pesticide residues in different agricultural produce. Therefore, multi-residue methodologies capable of simultaneously determining different types of popular pesticides are urgently required.

Multi-residue liquid chromatography-tandem mass spectroscopy (LC-MS/MS) methods are widely recognised as ideal, highly specific, and sensitive for testing food products. The high selectivity provided by LC-MS/MS allows for the determination of many pesticides belonging to different chemical families in a single run (Stachniuk *et al.*, 2017). Liquid chromatography-mass spectrometry (LC-MS) and LC-MS/MS using either electrospray ionisation (ESI) or atmospheric pressure chemical ionisation (APCI) are powerful tools for the trace determination of more complex pesticide matrices, since the sensitivity of

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such methods is higher than that of liquid chromatography (LC) with conventional detectors. Moreover, the selectivity of such instruments can be improved by the selection of specific ionic fragments (Ferrer and Thurman, 2005; Hernández *et al.*, 2006; Botitsi *et al.*, 2007; Kmellár *et al.*, 2008). The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) method, as an alternative to classical extraction techniques, has been proven to be useful in food analyses (Anastassiades *et al.*, 2003). Unlike many methods previously developed for traditional chromatographic detection systems, the QuEChERS approach utilises the wide analytical scope by LC coupled with MS that provides high degrees of selectivity and sensitivity for detection. LC-MS/MS methods have become the main analytical tools in most pesticide monitoring laboratories to meet the world standards. Therefore, the streamlined features, practical benefits, and excellent results provided by the QuEChERS sample preparation approach combined with LC-MS/MS have contributed to the great popularity of QuEChERS.

Based on a survey of 120 respondents on pesticide residue analysis, the present work selected ten popular pesticides used in Mekong Delta, Vietnam. The present work was carried out in order to evaluate a modified QuEChERS method which was developed and validated for the multi-residue analysis of vegetables using LC-MS/MS. This method was then applied to 180 actual vegetable samples to assess the pesticide residue levels in commonly consumed vegetables in Vietnam, thereby providing a reference for future monitoring.

Materials and methods

Chemicals and reagents

Pesticide reference standards (purity ≥ 95%), internal standards (ISs), triphenyl phosphate (TPP), and D10-chlorpyrifos (CPR-d10) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Acetonitrile (ACN), methanol (MeOH), and water (H_2O) were of HPLC grade. Acetic acid (CH_3COOH), anhydrous magnesium sulphate ($MgSO_4$), sodium acetate trihydrate ($CH_3COONa{\cdot}3H_2O$), trisodium citrate dihydrate ($Na_3C_6H_5O_7{\cdot}2H_2O$), disodium citrate sesquihydrate ($Na_2HC_6H_5O_7{\cdot}1.5H_2O$), and sodium chloride were purchased from Merck (Darmstadt, Germany). Primary secondary amine (PSA), graphite carbon black (GCB), and octadecylsilane (C_{18}) sorbents were obtained from Agilent Technologies (Canada).

Sample collection

A total of 180 samples of six different kinds of vegetables (30 samples each of watercress, mustard green, choy sum, daikon, okra, and yam) were immediately collected after being harvested from fields in Mekong Delta for the pesticide residue analysis. The sampling was performed in accordance with the general principles and methods of the European Commission (EC) directive 2002/63/EC (EU, 2008) for establishing MRLs in food commodities. Each representative vegetable was collected when the harvest was prepared for sale, and samples grown during periods of intense sun or rain were avoided. All samples (1.5 - 2.1 kg each) were placed in sterile polythene bags, sealed with dark nylon, labelled, immediately transported to the laboratory for processing, stored at 4°C to avoid contamination and deterioration, and analysed within 12 h. As expected, all the farmers interviewed in the present work reported using various chemical pesticides. Based on the information gathered from the interview, ten pesticides widely used by the farmers were selected to be analysed in the present work including abamectin, alpha-cypermethrin, acetamiprid, chlorpyrifos-ethyl, chlorpyrifos-methyl, fenobucarb, fipronil, thiamethoxam, and trichlorfon classified in class II as moderately hazardous, and chlorantraniliprole classified in class III as slightly hazardous.

Preparation of standard solutions

The individual standard stock solutions (1 mg/mL) were dissolved in acetonitrile, and stored at -18°C in the dark. The intermediate standard solutions in acetonitrile were prepared by mixing appropriate quantities of the individual standard stock solutions, and also stored at -18°C in the dark. A series of working standard solutions in the range of 0.2 - 8 µg/mL were prepared in acetonitrile by mixing and diluting the intermediate standard solutions in acetonitrile. The working standard solutions were used to prepare matrix-matched calibration and solvent calibration standards for the validation study. The ISs were prepared as described earlier. The working standard solutions and ISs were stored at -20°C until further analysis.

Extraction

A modified QuEChERS method was utilised to extract pesticides from the vegetable samples. Following homogenisation, 10 g of each sample was placed in a 50-mL centrifuge tube. The ISs (TPP and CPR-d10) were added to the centrifuge tube, thus yielding a sample concentration of 20 µg/kg. Then, 10 mL of 1% acetic acid in acetonitrile was added, and the samples were vortexed for 1 min. Then, 6.0 g of anhydrous $MgSO_4$ and 1.5 g of CH_3COONa were gradually added to the sample tubes. The centrifuge tubes were then tightly capped, vortexed for 1 min, and centrifuged at 6,000 rpm for 5 min. For the clean-up procedure, 6 mL of the supernatant was transferred to a 15-mL centrifuge tube containing 900 mg of anhydrous $MgSO_4$, 150 mg of PSA, and 45 mg of GCB. The centrifuge tube was vortexed for 1 min, and centrifuged at 12,000 rpm for 1 min. Next, 4 mL aliquot of the supernatant was dried under a nitrogen stream at 40°C. The residue was then reconstituted with 1 mL of ACN:H_2O (40:60), and the extract was filtered through a 0.22 µm PTFE filter. Finally, the filtrate was transferred into a vial.

UPLC-MS/MS

The analysis was performed using an LC-MS/MS system consisting of an Acquity UPLC I-Class (Waters, USA) coupled with a Xevo TQ-S micro triple quadrupole mass spectrometer (Waters, USA) equipped with an electrospray ionisation (ESI) source, working simultaneously in the positive (ESI^+) mode for nine of the analytes, and in the negative (ESI^-) mode for fipronil. The source settings were as follows: capillary voltage, 3.0 kV in the positive mode or -1.0 kV in the negative mode; source temperature, 150°C; desolvation temperature, 400°C; and desolvation gas flow rate, 900 L/h. Argon was used as the collision gas. The mass transitions, settings for the cone voltages, and the collision energies for the hormones are listed in Table 1.

Table 1. Retention time (RT) and MS/MS parameters of the selected pesticide.

No.	Analyte	Precursor ion (*m/z*)	Cone voltage (V)	Collision energy (eV)	Quantifier ion	Collision energy (eV)	Qualifier ion	Retention time (min)
1	Abamectin	890.55	20	30	305.30	10	567.40	6.26
2	Alpha-cypermethrin	433.25	13	15	190.95	9	416.16	4.15
3	Acetamiprid	223.10	37	19	126.05	14	56.05	3.25
4	Chlorpyrifos-ethyl	349.95	23	33	96.9	23	197.90	4.09
5	Chlorpyrifos-methyl	321.9	26	21	124.95	16	289.85	3.93
6	Chlorantranilliprole	483.83	19	20	452.82	20	285.81	3.57
7	Fenobucarb	208.1	29	14	95.10	7	152.10	3.64
8	Fipronil (ESI-)	434.89	-45	15	329.8	25	249.8	3.47
9	Thiamethoxam	292.0	25	10	211.0	20	132.0	3.08
10	Trichlorfon	256.95	23	20	108.95	10	220.95	3.26
11	TPP (IS)	327.10	30	22	77.0	15	152.0	3.81
12	CPR-d10	362.05	30	30	99.0	19	201.0	4.08

For the chromatographic conditions, a Waters Acquity BHE C_{18} column (1.7 µm; 50 × 2.1 mm) was used and maintained at 25°C. The flow rate and injection volumes were 0.25 mL/min and 10 µL, respectively. The mobile phases consisted of (A) 5 mM ammonium acetate/0.1% formic acid in methanol, and (B) 5 mM ammonium acetate/0.1% formic acid in water. The gradient elution program was as follows: 5% A (0 - 1 min), 100% A (2 - 3 min), 100% A (4 - 6 min), and 5% A (until 7 min). The total chromatography run time was 7 min. The data acquisition in the multiple reaction monitoring (MRM) mode was optimised after direct infusion into the detector. Two ion transitions were selected for each compound; the quantifier and qualifier MRM, and the analytical method were validated according to Association of Official Analytical Chemists (AOAC) guidelines (AOAC, 2016).

Validation study

The optimised method was validated according to AOAC guidelines to assess the selectivity,

linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, and matrix effect. In total, nine pesticides were analysed by LC-MS/MS in the ESI (+) positive mode, and fipronil in the ESI (-) negative mode in a single chromatographic run.

The selectivity was evaluated based on the two signals of two product ions of each pesticide, and the ion ratios in the samples and in the standards were compared. The calibration curves of each pesticide were constructed in accordance with the European Commission guidelines (EU, 2008). Matrix-matched calibration standards were prepared in vegetable-blank acetonitrile extracts using the multi-residue working solutions to yield concentrations ranging from 5 to 200 µg/L. The ISs (CPR-d10 and TPP) were added for the LC-MS/MS analysis. The sensitivity was evaluated by determining the LOD and LOQ using the signal-to-noise ratios (S/N) of 3:1 and 10:1, respectively. The accuracy was evaluated using a blank spike recovery experiment of the ten pesticides. The spiking levels were 5, 10, and 20 ng/mL, and three parallel samples were used for each spiking level.

Matrix effect evaluation

An assessment of the matrix was carried out by comparing the detector responses (peak areas) of the standard pesticide solutions in acetonitrile/water with the detector response of the analyte in a matrix extract. To avoid matrix effects, matrix-matched calibration standards were used.

Results and discussion

Optimization of QuEChERS conditions and clean-up method

The QuEChERS method was first introduced by Anastassiades *et al*. (2003). Since then, this method has undergone various modifications and enhancements to ensure efficient extraction of pH-dependent compounds, minimise degradation of susceptible compounds, and expand the spectrum of food matrices, for a quick and effective extraction, and also to yield a clean extract. In previous studies (Lehotay *et al.*, 2005; Rejczak and Tuzimski, 2015), most pesticides, except for the pH-sensitive ones, produced excellent results when extracted with three different buffer salts. In the present work, the recovery and matrix effect were chosen as criteria to estimate the effectiveness of the method. Three versions of the public QuEChERS method, namely (1) the original QuEChERS methodology, (2) the CEN QuEChERS methodology (EN 15662), and (3) the AOAC QuEChERS methodology, have been extensively evaluated in several laboratories for a wide range of pesticides in various fruits and vegetables. Such methods were tested and compared at a spiking concentration of 50 mg/kg.

The original QuEChERS method is the unbuffered method, and the citrate buffer version (CEN) utilises a citrate buffer of weaker strength and slightly higher pH (5 - 5.5). The acetate buffer version (AOAC) using strong buffering at pH 4.8 often yields higher and more consistent recoveries for pH-dependent pesticides than methods (1) and (2). In the present work, because fenobucarb and alpha-cypermethrin are unstable in alkaline media, it was easier to obtain higher recoveries for such samples in a buffer salt system, with the pH of the matrix maintained between 4.5 and 5.0 throughout the experiment. As such, the recovery rates of most of the pesticides when the acetate buffer version (AOAC) was used were between 70 and 120%. Such recovery rates were higher than those when the citrate buffer version (CEN) was used. When the original version was used, the recovery rates of alpha-cypermethrin and abamectin were under 70%. Furthermore, according to previous studies (Lehotay, 2007; Lehotay *et al.*, 2010), the ionisation efficiency of acetonitrile, and the ability of the matrix to interfere with the acidity and alkalinity are enhanced in the acetate buffer version. Consequently, the pesticide extraction in the present work was performed by shaking the samples with 1% acetic acid in acetonitrile, and salting extraction out with magnesium sulphate and sodium acetate. Acetic acid was added to stabilise the pesticides during the extraction as some pesticides are unstable in acetonitrile which has a higher pH (Kmellár *et al.*, 2008). In summary, for all analytes, the AOAC (acetate buffer version) yielded higher recoveries for the pH-dependent pesticides than the CEN (citrate buffer version), thus confirming the results obtained in the present work. It can thus be concluded that the acetate buffer version yielded higher and more consistent recoveries for pH-dependent pesticides in vegetable matrices (Figure 1).

Clean-up procedure

Continuously, the extract was decanted into a

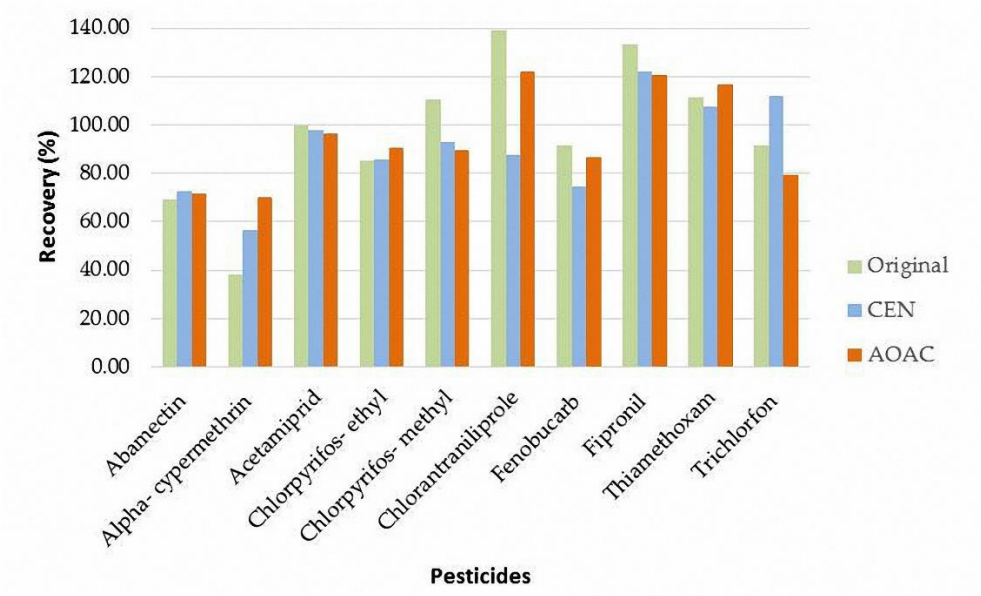

Figure 1. Comparison of three QuEChERS methods (the original, CEN, and AOAC method) for the extraction of ten pesticide residues in vegetable.

tube containing 150 mg of PSA sorbent, 900 mg of anhydrous $MgSO_4$, and 45 mg of GCB. This step corresponded to a clean-up procedure called dispersive solid-phase extraction. PSA was used because weak anion exchangers can remove organic acids, some sugars, and fatty acids. GCB has been reported to be a highly effective sorbent for sample clean-up (Koesukwiwat *et al*., 2008). GCB is a non-porous reversed-phase sorbent that can remove planar molecules such as natural pigments (*e.g.*, chlorophyll, haemoglobin, and carotenoids), sterols, and non-polar interferences (Zhang *et al.*, 2006). However, using high amounts of GCB (> 10 mg per 1 mL of acetonitrile extract) may lead to undesirable losses of some planar pesticides (EU, 2008). In the present work, 150 mg of PSA sorbent, 900 mg of anhydrous $MgSO_4$, and 45 mg of GCB were used for 6 mL aliquots of the extract, which corresponded to 25 mg of PSA and 7.5 mg of GCB per 1 mL of extract. In a previous study, Hou *et al*. (2013) tested the effectiveness of different amounts of PSA (25 - 150 mg/mL extract), and concluded that 75 mg PSA/mL extract was the most effective in reducing the content of fatty acids in the extract. Therefore, 375 mg of PSA was used for the extraction procedure.

In conclusion, there are some considerable advantages to the method discussed herein, namely the time and simplicity of sample extraction. Based on the analyses of the six different matrices, watercress, mustard green, and choy sum were abundant in chlorophyll; daikon and yam were abundant in protein and starch; okra was abundant in proteins, starch, minerals, and fibres along with phytonutrients. We have successfully developed a simple extraction procedure with high recoveries within the range of 74.47 - 116.93% and LOQs (0.1 - 5 ng/mL) below MRLs (10 ng/mL or 0.01 mg/kg according to Codex).

Optimization of LC-MS/MS

The electrospray ionisation ESI (+) or ESI (-) mode were used for the precursor ion selection. The ionisation modes were confirmed by the direct infusion of 1,000 ng/mL standard solutions; based on the precursor ions, which were fragmented in the collision cell, the ions with the most intense signals were selected (Table 1). The transitions from the precursor ion to product ions 1 and 2 were employed for the detection of the analytes, and the transition from the precursor ion to product ion 1 was employed for quantification.

To obtain better resolution and sensitivity, considering the analyte ionisation efficiency in the MS/MS system, we used acetonitrile, methanol, formic acid, acetic acid, and ammonium formate as mobile phases. The best peak symmetry and resolution were obtained with acetonitrile as the organic phase with formic acid and ammonium formate. The mobile phase was optimised in a gradient mode with different percentages of 5 mM ammonium acetate/0.1% formic acid in methanol and

5 mM ammonium acetate/0.1% formic acid in water. The analyte separation was completed after 7 min.

Method validation

Selectivity

Table 1 lists the product ions selected for each pesticide. The relative ion intensities were ensured to meet the criteria established by the European Commission in Decision 2002/657/EC (EU, 2008).

Linearity, LOD, and LOQ

The results of the validation of the method discussed herein indicated that good linearity and reproducibility of the calibration curves were achieved (R^2 > 0.99), as listed in Table 2. The LODs for the pesticides ranged from 0.03 to 1 ng/mL, and the LOQs ranged from 0.1 to 5 ng/mL. All LODs and LOQs of the pesticides were lower than the default maximum residue level (MRL = 10 ng/mL).

Table 2. Matrix-effect, linearity, LOD, and LOQ of selected pesticides in vegetable samples.

No.	Pesticide	ME (%)	Calibration curve	(R^2)	LOD (ng/mL)	LOQ (ng/mL)
1	Abamectin	35.75	y = 0.3535x + 1.5822	0.9970	0.6	2
2	Alpha-cypermethrin	-27.37	y = 0.2393x + 0.3059	0.9998	1	3
3	Acetamiprid	-26.05	y = 2.2461x + 0.7248	0.9998	0.03	0.1
4	Chlorpyrifos-ethyl	-5.60	y = 0.8743x + 1.8620	0.9979	0.5	3
5	Chlorpyrifos-methyl	16.92	y = 0.5272x + 0.1100	0.9942	0.3	1
6	Chlorantraniliprole	-21.32	y = 0.3010x + 0.1638	0.9997	0.5	2.0
7	Fenobucarb	-86.94	y = 1.992x + 1.7130	0.9976	0.1	0.5
8	Fipronil	85.45	y = 0.0905x + 0.2051	0.9996	0.1	0.4
9	Thiamethoxam	-4.91	y = 1.2803x + 1.9489	0.9997	0.1	1
10	Trichlorfon	-48.59	y = 0.3384x - 0.3586	0.9991	1	5

Table 3. Recovery and precisions of selected pesticides in vegetable samples.

No.	Pesticide	Recovery (%)			Intra-day precision (%RSD)			Inter-day precision (%RSD)		
		5 ng/mL	10 ng/mL	20 ng/mL	5 ng/mL	10 ng/mL	20 ng/mL	5 ng/mL	10 ng/mL	20 ng/mL
1	Abamectin	97.63	110.50	95.03	6.47	3.71	1.79	7.23	4.16	4.65
2	Alpha-cypermethrin	82.10	105.30	92.77	6.23	11.62	10.83	3.27	9.63	7.32
3	Acetamiprid	96.20	82.93	88.03	1.04	9.45	3.01	3.98	5.85	5.14
4	Chlorpyrifos-ethyl	91.50	100.57	103.33	5.22	3.35	0.69	4.91	2.50	3.7
5	Chlorpyrifos-methyl	113.33	108.03	116.93	1.87	8.43	0.97	1.38	9.11	2.8
6	Chlorantraniliprole	83.93	83.93	77.8	4.92	4.92	1.74	5.96	9.81	6.42
7	Fenobucarb	97.60	99.60	113.27	5.15	12.53	2.07	4.27	7.76	7.93
8	Fipronil	102.90	79.83	80.1	4.76	12.04	2.97	5.68	8.67	5.05
9	Thiamethoxam	76.77	83.10	87.13	2.41	6.20	4.4	2.32	6.19	4.10
10	Trichlorfon	102.00	76.67	83.17	2.55	2.48	5.83	10.23	6.03	5.77

Accuracy and precision

The accuracy of this method was investigated by a blank spike recovery experiment of the ten pesticides. The spiking levels were 5, 10, and 20 ng/mL, and three parallel samples were used for each spiking level. For the three spiking levels of most of the pesticides, the recoveries (R%) were between 74.47 and 116.93% (Table 3). To ensure the accuracy and reproducibility of the results, a better recovery rate can be obtained to better align with the AOAC 2007.01 and EN 15662 standards. All samples were analysed on the same day, and an acceptable accuracy was indicated by the RSD values which were below

15.0% for the three spiking levels. Figure 2 shows the MRM chromatograms of select pesticides in 20 ng/mL spiked sample.

Matrix effect

The matrix effect was calculated by comparing the slopes of the calibration curves with respect to those of the matrix and solvent. In the present work, 5, 10, 20, 50, 100, and 200 µg/L concentrations were used for the calibration curves for the matrix and solvent. The matrix effect was evaluated using Eq. 1:

$$ME(\%) = \frac{slope\ of\ matrix\ matched\ curve}{slope\ of\ sovent\ curve} \times 100 \qquad \text{(Eq. 1)}$$

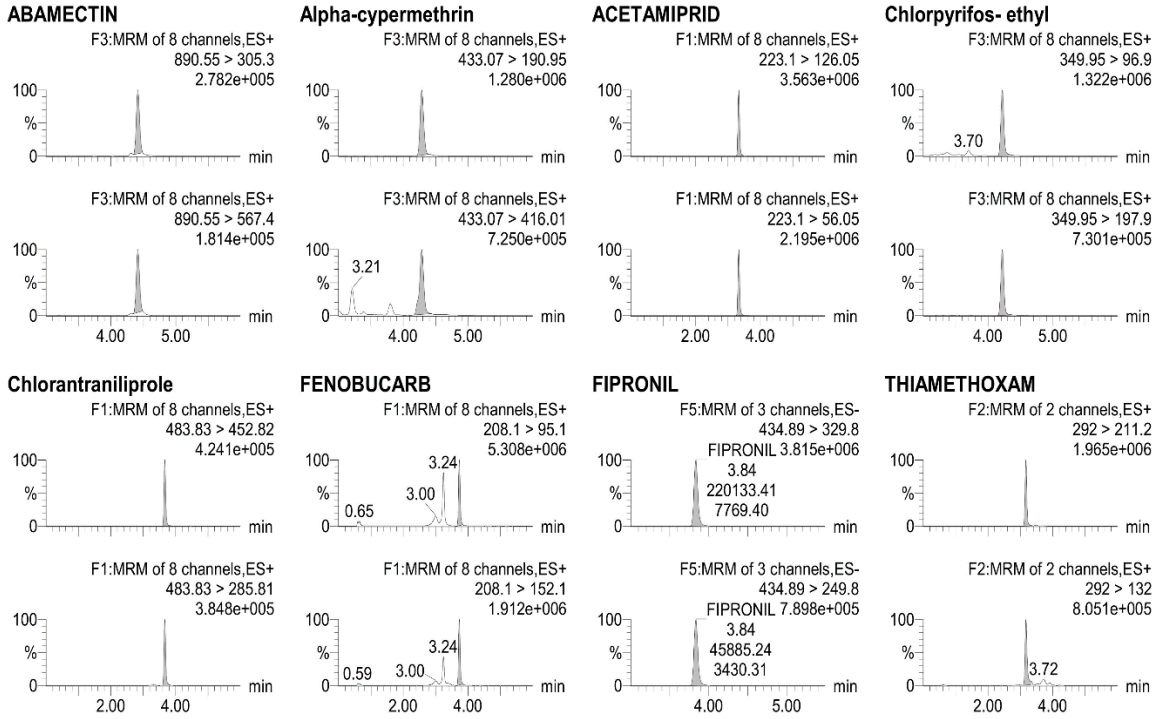

Figure 2. MRM chromatograms of vegetable samples at the validation level of 20 µg/kg.

Matrix effect is a drawback of the QuEChERS method, especially since the complex matrices of vegetables yield very high matrix effect due to the presence of many pigments. This modified QuEChERS method eliminated most of the pigments, thus minimising the matrix effect. Table 2 shows the matrix effect on the analysed pesticides. Most pesticides had lower signal intensities in the matrix than in the solvents. However, the matrix effects on all analysed pesticides were within ± 20%. To efficiently eliminate the effects of the matrix, matrix-matched calibration was used. For the analysis of the ten pesticides; three pesticides had low matrix effects ranging from -20 to +20%; five pesticides had low matrix effects ranging from -50 to +50%; fenobucarb and fipronil had strong matrix effects.

Pesticide residues in commercial samples

Following method validation, the proposed method was applied to determine the pesticide residues in 180 vegetable samples from the fields around Mekong Delta. Surprisingly, the results listed in Table 4 showed that all the samples contained at least one detectable pesticide residue.

Table 4. Frequency of vegetable samples with pesticide residue below and above the MRL.

Produce	Number of sample	With residue < MRL	With residue > MRL
Watercress	30	16 (53%)	14 (47%)
Mustard green	30	6 (20%)	24 (80%)
Choy sum	30	5 (17%)	25 (83%)
Daikon	30	4 (13%)	26 (87%)
Okra	30	30 (100%)	0 (0%)
Yam	30	12 (40%)	18 (60%)
Total	180	73 (41%)	107 (59%)

The percentages of samples which contained more than one exceeding MRLs pesticide was

38.34% (69 samples). The most frequent combination of two pesticides was alpha-cypermethrin and fipronil in mustard green and choysum samples; and alpha-cypermethrin and chlorpyriphos-ethyl in daikon. Besides, the frequent combination of three pesticides was abamectin, fenobucarb, and fipronil in daikon. Moreover, 107 of the 180 samples (59%) contained pesticide residues above the MRLs established by Codex. These percentages were significantly higher than that of vegetables from Thailand, of which 77.7% had detectable residues and 22.3% had residues exceeding the MRLs (Suntudrob *et al.*, 2018). Aside from okra, all of the vegetables had high percentages of pesticides exceeding the MRLs (> 40%). All okra samples contained pesticide residues below the MRLs, whereas mustard green (80%), choy sum (83%), and daikon (87%) had the highest number of samples with pesticide residues above the MRLs. Of the 107 samples containing residues exceeding the MRLs, the leafy vegetables (watercress, mustard green, and choy sum) accounted for a higher percentage (59%) than the root vegetables (daikon and yam).

Conclusion

The proposed multi-residue method was simple, straightforward, and made it possible to extract, determine, and confirm the contents of ten analytes in a single run using LC-MS/MS. This method yielded excellent results, and could be recommended as an alternative multi-residue method for the analysis of pesticide residues in vegetables. The present work also investigated the levels of pesticide residues in commonly consumed vegetables in Vietnam. Results indicated that a majority of the vegetable samples were contaminated with pesticide residues, with some samples having pesticide concentrations exceeding their respective MRLs (59%). These results emphasised the need to develop comprehensive intervention measures to reduce the potential health risks to consumers.

References

Alewu, B. and Nosiri, C. 2011. Pesticides and human health. In Stoytcheva, M. (ed). Pesticides in the Modern World - Effects of Pesticides Exposure, p. 31-50. United Kingdom: IntechOpen.

Anastassiades, M., Lehotay, S. J., Stajnbaher, D. and Schenck, F. J. 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. Journal of AOAC International 86: 412-431.

Association of Official Agricultural Chemists (AOAC). 2016. Official methods of analysis of AOAC international. United States: AOAC.

Botitsi, H., Economou, A. and Tsipi, D. 2007. Development and validation of a multiresidue method for the determination of pesticides in processed fruits and vegetables using liquid chromatography-electrospray ionization tandem mass spectrometry. Analytical and Bioanalytical Chemistry 389: 1685-1695.

European Union (EU). 2008. Commission decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Official Journal of the European Communities L221: 8-36.

Ferrer, I. and Thurman, E. M. 2005. Multi-residue pesticide analysis in fruits and vegetables by liquid chromatography-time-of-flight mass spectrometry. Journal of Chromatography A 1082(1): 81-90.

Hernández, F., Pozo, O. J., Sancho, J. V., Bijlsma, L., Barreda, M. and Pitarch, E. 2006. Multiresidue liquid chromatography tandem mass spectrometry determination of 52 non gas chromatography-amenable pesticides and metabolites in different food commodities. Journal of Chromatography A 1109(2): 242-252.

Hoi, P. V., Mol, A. P. J., Oosterveer, P., van den Brink, P. J. and Huong, P. T. M. 2016. Pesticide use in Vietnamese vegetable production: a 10-year study. International Journal of Agricultural Sustainability 14: 325-338.

Hou, X., Lei, S., Qiu, S., Guo, L., Yi, S. and Liu, W. 2013. A multi-residue method for the determination of 124 pesticides in rice by modified QuEChERS extraction and gas chromatography-tandem mass spectrometry. Food Chemistry 138: 1198-1205.

Kmellár, B., Fodor, P., Pareja, L., Ferrer, C., Martínez-Uroz, M. A., Valverde, A. and Fernandez-Alba, A. R. 2008. Validation and

uncertainty study of a comprehensive list of 160 pesticide residues in multi-class vegetables by liquid chromatography-tandem mass spectrometry. Journal of Chromatography A 1215(1-2): 37-50.

Koesukwiwat, U., Sanguankaew, K. and Leepipatpiboon, N. 2008. Rapid determination of phenoxy acid residues in rice by modified QuEChERS extraction and liquid chromatography-tandem mass spectrometry. Analytica Chimica Acta 626: 10-20.

Lehotay, S. J. 2007. Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate: collaborative study. Journal of AOAC International 90: 485-520.

Lehotay, S. J., Mastovska, K. and Lightfield, A. R. 2005. Use of buffering and other means to improve results of problematic pesticides in a fast and easy method for residue analysis of fruits and vegetables. Journal of AOAC International 88: 615-629.

Lehotay, S. J., Son, K. A., Kwon, H., Koesukwiwat, U., Fu, W., Mastovska, K., ... and Leepipatpiboon, N. 2010. Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables. Journal of Chromatography A 1217: 2548-2560.

Mnif, W., Hassine, A. I. H., Bouaziz, A., Bartegi, A., Thomas, O. and Roig, B. 2011. Effect of endocrine disruptor pesticides: a review. International Journal of Environmental Research and Public Health 8: 2265-2203.

Nishina, T., Kien, C. N., Noi, N. V., Ngoc, H. M., Kim, C. S., Tanaka, S. and Iwasaki, K. 2010. Pesticide residues in soils, sediments, and vegetables in the Red River Delta, northern Vietnam. Environment Monitoring Assessment 169: 285-297.

Rejczak, T. and Tuzimski, T. 2015. A review of recent developments and trends in the QuEChERS sample preparation approach. Open Chemistry Journal 13: 980-1010.

Sanborn, M., Kerr, K. J., Sanin, L. H., Cole, D. C., Bassil, K. L. and Vakil, C. 2007. Non-cancer health effects of pesticides. Systematic review and implications for family doctors. Canadian Family Physician 53: 1712-1720.

Stachniuk, A., Szmagara, A., Czeczko, R. and Fornal, E. 2017. LC-MS/MS determination of pesticide residues in fruits and vegetables. Journal of Environmental Science and Health - Part B 52(7): 446-457.

Suntudrob, J., Jongmevasna, W., Payanan, T., Srikote, R. and Wittayanan, W. 2018. Monitoring of pesticide residues in domestic vegetables in Thailand during 2015. Asia-Pacific Journal of Science and Technology 23(4): 1-8.

World Health Organization (WHO). 1990. Public health impact of pesticides used in agriculture. Switzerland: WHO.

Zhang, W.-G., Chu, X.-G., Cai, H.-X., An, J. and Li, C.-J. 2006. Simultaneous determination of 109 pesticides in unpolished rice by a combination of gel permeation chromatography and Florisil column purification, and gas chromatography/mass spectrometry. Rapid Communications in Mass Spectrometry 20: 609-617.