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Analysis of Pesticide Residues in Fruits and Vegetables Using Modified QuEChERS Method

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Abstract : The QuEChERS method which means 'quick, easy, cheap, efficient, rugged, safe' has been used to analyse temperate vegetables and fruits. This method is simple, rapid and use small amounts of solvents and chemicals. The pesticides were extracted by mixing the extracts with acetronitrile and cleanup was performed by dispersive solid phase extraction. In this study, the QuEChERS method was evaluated and modified to analyse organophosphorus (OP), organochlorine (OC) and pyrethroid (PY) pesticides in local fruits and vegetables, namely cucumber, green mustard, rock melon and local oranges. The results showed that the primary secondary amine (PSA) which was used in the original method as dispersive solid phase extraction (SPE) cleanup was not effective in removing the co-extractives especially the chlorophyll/coloured compounds. The extract obtained was not suitable for the analysis of OC and PY pesticides using electron capture detector (ECD) due to massive matrix interferences. In our modified method, the use of PSA was omitted. The matrix interferences resulting from high chlorophyll/coloured compounds were removed by cleanup with mini silica gel column. The purified extract was cleaner than those obtained using the conventional method. The recoveries for OP pesticides in vegetables and fruits fortified at 0.1, 0.5 and 1.0 mg kg^{-1} were 83.1-123.5%. For OC pesticides, recoveries of 70.1-107.2% were obtained for fortification at 0.1 and 0.05 mg kg^{-1}. Recoveries of 75.2-108.9% were obtained for PY pesticides fortified at 1.0, and 0.5 mg kg^{-1}. The standard deviations obtained were less than 14.8%. The limit of detection was 0.01 mg kg^{-1}. The amount of a chemical used in the new method was reduced by 82 % with shorter analysis time as compared to the conventional method.

Keywords : Organophosphorus pesticides, organochlorine pesticides, pyrethroid pesticides, silica gel cleanup, primary secondary amine cleanup

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Introduction

The major drawbacks of the current analysis methods today are that they require much labour and time, use large amounts of hazardous solvents and generated large amount of waste. For laboratories in the tropical regions, many types of vegetables and fruits with different matrix interferences need to be analysed. Some vegetables such as green mustard (*Brassica chinensis*) and Chinese kale (*Brassica alboglabra*) which contain high chlorophyll content could give rise to massive matrix interferences if efficient cleanup is not performed prior to gas chromatography (GC) determination. It has been reported that QuEChERS (Quick, easy, cheap, efficient, rugged, safe) extraction and cleanup procedures using dispersive SPE could provide excellent recoveries for many pesticides in various crops. The QuEChERS method was first developed by Anastassiades *et al*. (2003) for the extraction of pesticide residues from vegetables and fruits. The pesticides were extracted by mixing the extracts with acetronitrile and water was removed from the extract by salting out with sodium chloride and magnesium sulfate. Cleanup was performed by using the dispersive SPE, which involved mixing the extract on a mixer with SPE sorbent instead of passing it through an SPE column. The method was subsequently evaluated, modified and extended for the analysis of pesticide residues in other food matrixes such as rice (Koesukwiwat *et al*., 2008; Nguyen *et al*., 2008a), barley (Diez *et al*., 2006), cabbage and radish (Nguyen *et al*., 2008b), tomato, pear and lettuce (Schenck *et al*., 2008), milk, eggs, and avocado (Lehotay *et al*., 2005), grape, tomato, lemon and onion (Lesueur *et al*. 2008), oranges, red wine, red grapes, raisins and wheat flour (Paya *et al*., 2007). The method was also evaluated and modified for the analysis of herbicides (Diez *et al*., 2006; Koesukwiwat *et al*., 2008). However, it has been reported that the dispersive SPE cleanup method was not efficient compared to the conventional cleanup method using column chromatography or SPE (Schenck *et al*., 2008). In this study, the QuEChERs method was evaluated and modified to analyse OP, OC and PY pesticides in local fruits and vegetables, namely cucumber (*cucumis sativus*), green mustard (*brassica chinensis*), rock melon (*cucumis melo*) and local oranges (*citrus sinensis*).

Materials and Methods

Reagents and chemicals

Pesticides standards (purity 98 % and above) were obtained from Ehrenstorfer, Germany. Analytical and residue grades of acetronitrile, sodium chloride, dichloromethane, hexane, and anhydrous magnesium sulphate were purchased from J.T. Baker, USA. Silica gel and PSA were purchased from Merck, Germany and Varian, Australia, respectively. Pesticide stock solutions (500 mg L^{-1}) were prepared by dissolving the pesticide standards in residue grade acetone (OP pesticides) or hexane (OC and PY pesticides). Appropriate aliquots of the stock solutions were diluted with residue grade acetone or hexane to make solutions that contained 10 mg L^{-1}, 1.0 mg L^{-1}, 0.1 mg L^{-1} and 0.01 mg L^{-1} of the pesticides.

Apparatus and Instrumentation

A Robot Coupe food chopper was used to homogenize the vegetable samples. A Thermo Jouan model B4i centrifuge was used to centrifuge the vegetable extract. An Agilent Model 6890 GC equipped with a Flame Photometric Detector (FPD) was used for the determination of OP pesticides. A non-polar fused-silica capillary column, HP5, 15 m x 0.53 mm x 1.5 μm i.d. obtained from J & W Scientific, U.S.A. was used with nitrogen as carrier gas at a flow rate of 4.0 mL min^{-1}. The column temperature was maintained at 120°C for 1.0 min then programmed at 30°C min^{-1} to 150°C followed by another temperature ramp of 5°C min^{-1} to 270°C and held at 270^{o}C for 10 min. The injector and detector temperatures were maintained at 260°C and 250°C, respectively. The air and hydrogen gas flow were set at 80 mL min^{-1} and 67 mL min^{-1}, respectively. An Agilent Model 6890 GC equipped with ECD was used for the analysis of the OC and PY pesticides. A non polar fused-silica capillary column, Ultra 1, 25 m x 0.32 mm and 0.5 μm i.d., from J & W Scientific, U.S.A. was employed with nitrogen as a carrier gas at a flow rate of 1.2 mL min^{-1}. The column temperature was maintained at 120°C for 0.5 min then programmed at 10°C min^{-1} to 180°C followed by another temperature ramp of 6°C min^{-1} to 240°C and subsequently 10°C min^{-1} to 280°C and held at 280^{0}C for 12 min. The injector and detector temperatures were maintained at 260°C and 300°C, respectively.

Method development and validation

For recovery studies, fresh vegetable and fruit samples were fortified with known amounts of pesticide standards. Green mustard, cucumber, rock melon and oranges were chosen for this study to represent the wide diverse types of vegetable and fruit samples analysed by our laboratory. Notably, some vegetable samples especially the *Brassica* family such as green mustard, Chinese kale and Indian mustard contain high amount of chlorophyll. Appropriate amount of pesticide standards were spiked onto the vegetable and fruit samples to obtain the recoveries at 1.0, 0.5, 0.1, 0.05 mg kg^{-1}. The samples were mixed homogenously and left for one hour to allow the solvent to evaporate and the pesticide to get in contact with the vegetable samples. Each sample was prepared in three replicates. For the determination of OP and OC and PY pesticides, 2.0 μL of the final extract was injected into GC-FPD and GC-ECD, respectively. In this study, the limit of detection (LOD) is defined as the analyte concentration giving a signal equal three times the standard deviation of the blank.

Analysis of pesticides

Five hundred gram of vegetable and fruit samples was homogenized in a food chopper. Ten gram of homogenized samples was weighed into a 50 mL Teflon centrifuge tube. Fifteen mL of acetonitrile containing 1 % acetic acid was added to the samples and shaken vigorously for 1 min using a vortex mixer. Six gram of anhydrous magnesium sulphate and 1.5 g sodium chloride were added and vortexed vigorously for 1 min. The extracts were later centrifuged at 3000 rpm for 1 min. Two mL of the extracts were taken and shaken with 1.8 g of anhydrous magnesium sulphate. The extracts were centrifuged again at 3000 rpm for 1 min prior to OP pesticides determination using GC-FPD. For OC and PY pesticides, 2.0 mL of extract was transferred into a chromatography column packed with 3 g silica gel for cleanup. The column was eluted with a mixture of 30 mL of dichloromethane and hexane (1:1). The eluate was then analysed by GC-ECD.

Results

Recoveries of OP pesticides in vegetables and fruits at 0.1, 0.5 and 1.0 mg kg^{-1} were shown in Tables 1 and 2, respectively. The recoveries for almost all OP pesticides in fruits and vegetables were found within the acceptable recovery ranges of 70-120 %. For vegetables, the recoveries for green mustard ranged from 85.2 to 121.5 % with standard deviation (SD) of less than 9.1 %. Recoveries of 85.8 - 120.7 % were obtained for cucumber with SD of less than 9.7 %. For fruits, recoveries of 83.1 - 123.5 % and 86.0 - 145.0 % were obtained for rock melon and oranges, respectively with SD of less than 13.7%. As selective detector FPD was used, there was no interfering matrix peaks observed in the chromatographic determination.

Table 1 : Percentage recovery of OP pesticides in vegetables

	Green mustard			Cucumber		
	1.0 mg kg^{-1}	0.5 mg kg^{-1}	0.1 mg kg^{-1}	1.0 mg kg^{-1}	0.5 mg kg^{-1}	0.1 mg kg^{-1}
Methamidophos	95.6 ± 5.4	85.2±4.2	91.1±1.6	102.5±2.5	107.8±4.5	85.8±7.0
Acephate	116.3±3.4	121.0±8.5	93.3±5.9	118.9±8.6	110.9±3.9	105.7±3.5
Diazinon	98.8±5.5	120.9±2.9	99.8±6.0	96.8±1.1	119.2±9.1	99.2±1.6
Dimethoate	101.5±3.3	102.8±1.9	104.5±4.4	117.3±4.3	109.3±6.0	112.2±4.2
Malathion	123.2±2.1	103.0±5.0	119.9±2.2	114.6±1.5	107.1±5.9	99.2±6.3
Chlorpyrifos	94.9±3.1	120.4±0.5	95.4±1.3	92.3±1.5	120.7±8.2	105.5±5.8
Phenthoate	112.3±2.4	110.1±7.6	101.4±7.4	106.4±1.1	113.3±7.3	121.5±3.5
Prothiophos	98.2±3.0	98.6±9.1	112.8±3.5	95.1±2.4	103.0±9.7	100.6±6.1
Triazophos	111.0±2.6	103.0±2.8	122.9±2.7	105.9±3.3	95.7±1.5	103.5±8.8

Table 2 : Percentage recovery of OP pesticides in fruits

	Rock melon			Oranges		
	1.0 mg kg^{-1}	0.5 mg kg^{-1}	0.1 mg kg^{-1}	1.0 mg kg^{-1}	0.5 mg kg^{-1}	0.1 mg kg^{-1}
Methamidophos	99.5±5.9	102.3±3.3	93.0±14.8	117.9±9.4	110.3±5.8	110.9±9.5
Acephate	97.0±6.1	119.5±10.3	99.5±4.7	113.2±0.1	114.7±13.7	123.1±4.7
Diazinon	86.2±9.6	112.4±3.5	93.3±7.4	91.1±6.1	114.4±6.8	100.2±3.4
Dimethoate	101.4±10.1	107.5±2.4	123.5±0.1	120.5±10.1	101.8±9.3	93.2±5.2
Malathion	92.7±7.0	108.1±5.2	120.7±0.9	114.2±2.8	96.7±1.6	115.0±4.7
Chlorpyrifos	93.5±3.0	112.7±5.7	96.0±9.0	86.0±6.6	111.3±2.4	97.0±2.6
Phenthoate	83.1±11.1	113.1±6.1	108.5±10.8	97.3±10.5	104.1±1.7	104.9±13.5
Prothiophos	94.1±2.1	109.0±2.9	88.1±10.7	88.1±5.3	96.0±6.5	92.9±3.0
Triazophos	96.3±9.8	112.5±2.8	103.9±9.1	107.4±3.4	105.7±5.2	115.9±1.5

The recoveries of OC pesticides at 0.1 and 0.05 mg kg^{-1} in fruits and vegetables are shown in Table 3. For vegetables, the recoveries for OC pesticides in green mustard and cucumber were 78.1 - 107.2 % and 70.1 - 98.9 %, respectively with SD of less than 12.7 %. Recoveries for OC pesticides in rock melon and oranges were 75.9 - 91.0 % and 70.1 - 98.9 % respectively with SD of less than 12.8 %.

Table 3 : Percentage recovery of OC pesticides in vegetables and fruits

	Green mustard		Cucumber		Rock melon		Oranges	
	0.1 mg kg^{-1}	0.05 mg kg^{-1}	0.1 mg kg^{-1}	0.05 mg kg^{-1}	0.1 mg kg^{-1}	0.05 mg kg^{-1}	0.1 mg kg^{-1}	0.05 mg kg^{-1}
Alpha-Endosulfan	88.5±7.6	107.2±3.7	82.3±4.4	92.4±12.7	76.1±12.8	76.7±7.5	82.7±1.3	98.9±7.6
Endosulfan sulphate	82.4±7.3	96.8±4.7	78.1±1.4	97.7±4.8	75.9±6.2	91.0±11.0	85.5±6.8	70.1±5.6
Beta-Endosulfan	85.9±8.7	104.3±9.5	78.5±2.1	98.1±7.7	76.5±2.5	78.1±4.5	89.5±9.5	96.1±7.5

Table 4 : Percentage recovery of PY pesticides in vegetables and fruits

	Green mustard		Cucumber		Rock melon		Oranges	
	1.0 mg kg^{-1}	0.5 mg kg^{-1}	1.0 mg kg^{-1}	0.5 mg kg^{-1}	1.0 mg kg^{-1}	0.5 mg kg^{-1}	1.0 mg kg^{-1}	0.5 mg kg^{-1}
α-cyhalothrin	83.8±7.5	100.7±10.3	81.9±4.6	107.0±3.5	79.8±6.3	87.6±5.1	94.8±4.3	98.4±3.5
Permethrin	86.9±8.0	93.8±8.9	82.4±6.5	98.3±5.8	89.4±11.4	90.9±3.5	97.7±2.0	100.4±4.0
Cyfluthrin	79.1±6.3	90.5±8.4	77.4±2.8	106.1±3.9	77.2±4.0	86.6±5.2	88.1±9.8	99.2±2.7
Cypermethrin	100.8±13.1	86.8±8.2	93.1±2.7	83.6±3.2	92.5±3.5	88.5±2.9	94.6±4.0	104.6±8.6
Fenvelerate	77.9±8.4	101.5±13.5	78.1±2.8	108.9±6.7	75.2±0.9	86.6±4.6	91.3±9.3	98.8±2.1
Deltamethrin	80.5±7.3	92.2±11.3	80.0±3.3	106.4±3.7	74.2±5.7	87.2±5.1	92.1±8.2	101.0±1.3

The recoveries of PY pesticides in fruits and vegetables at 1.0 and 0.5 mg kg^{-1} fortifications are shown in Table 4. For vegetables, the recoveries for PY pesticides in green mustard and cucumber were 77.9 - 101.5 % and 77.4 – 108.9 %, respectively with SD of less than 13.5 %. For fruits, the recoveries for PY pesticides in rock melon and oranges were 75.2 - 92.5 % and 88.1 - 104.6 % respectively with SD of less than 11.4 %.

Discussion

The overall recoveries obtained for OP, OC and PY pesticides using the modified QuEChERS methods were within the accepted ranges. The results obtained were comparable to those reported earlier for tomato, pear and lettuce (Schenck *et al*., 2008), cabbage and radish (Nguyen *et al*., 2008b), grape, tomato, lemon and onion (Lesueur *et al*. 2008), cabbage, carrot, apple, orange, and cucumber (Liu *et al*., 2007). The QuEChERS method was efficient, quick and easy as the samples could be process quickly as compared to the more laborious conventional methods currently used in the laboratory such as the Steinwandter method (1985). This method was relatively cheap as minimum solvents and chemicals were used. In addition, this method was relatively safer as it has omitted the use of dichloromethane which was used in conventional method. The results showed that the PSA which was used in the original method as dispersive SPE cleanup was only suitable for OP pesticides analysis using selective detector, FPD. The final extract was found to contain less co-extractive as compared to the conventional methods, partly due to smaller sample size.

However, the dispersive PSA SPE cleanup advocated in the QuEChERS method was not suitable for OC and PY pesticides analysis using non-selective detector such as ECD due to massive matrix interference in the chromatographic determination. The final extracts prior to GC determination were found to contain more co-extractives and higher colour intensity as compared to the conventional method. This was due the facts that the PSA in the dispersive form was not effective in removing the co-extractives and/or coloured compounds especially for vegetables having high chlorophyll/coloured compounds such as green mustard. It has been reported that dispersive PSA did not provide sufficient cleanup for carbamate pesticides using liquid chromatograph fluorescence detector (Schenck *et al*., 2008). To overcome this, a mini column chromatography using 3 g of silica gel was used for cleanup before OC and PY pesticides determination. This has resulted in cleaner extract prior to ECD determination. Good recoveries were obtained by this new cleanup method. It was noted that the purified extract was better than those obtained using the conventional silica gel column chromatography cleanup method. As the PSA did not effectively remove the co-extractives and coloured compounds, it was omitted in our modified method. The amount of the chemicals used in the new method was reduced by 82 % with shorter analysis time as compared to the conventional method. The limit of detection was 0.01 mg kg^{-1} for OP, OC and PY pesticides.

Conclusion

A simple, fast and low cost modified QuEChERS method showed high sensitivity and good recoveries in the determination of OP, OC and PY pesticides in fruits and vegetables. The modified QuEChERS method resulted in 82 % reduction in solvent usage when compared with the conventional method currently used in our laboratory. The volumes of hazardous waste generated were also greatly reduced. Substituting PSA with mini silica gel column resulted in much cleaner extracts for the determination of OC and PY pesticides. The new method is useful for routine analysis due to its simplicity and speed.

References

1. Anastassiades, M., Lehotay, S.J., Stajnbaher, D. and Schenck, F.J. (2003) Fast and easy multi-residue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce, *J. AOAC Intern.* **86**, 412-431.
2. Diez, C., Traag, W.A., Zommer, P., Marinero, P. and Atienza, J. (2006) Comparison of an acetonitrile extraction, partitioning and dispersive solid-phase extraction method with classical multi-residue methods for the extraction of herbicide residues in barley samples, *J. Chrom. A.* **1131**, 11-23.
3. Frenich, A.G., Vidal, J.L.M., Montoro, E.P. and Gonzalez, R.R. (2008) High throughput determination of pesticide residues in food commodities by use of ultra-performance liquid chromatography-tandem mass spectrometry, *Anal. Bioanal. Chem.* **390**, 947-959.
4. Koesukwiwat, U., Sanguankaew, K. and Leepipatpiboon (2008) Rapid determination of phenoxy acid residue in rice by modified QuEChERS extraction and liquid chromatography-tandem mass spectrometry, *Anal. Chim. Acta*, **626**, 10-20.
5. Lehotay S.J., De Kok A., Hiemstra, M. and van Bodegraven P. (2005) Evaluation of two fast and easy methods for pesticide residue analysis in fatty food matrixes. *J. AOAC Intern.* **88**, 630-638.
6. Lesueur, C., Knittl, P., Gartner, M., Mentler, A. and Fuerhacker, M. (2008) Analysis of 140 pesticides from conventional farming foodstuff samples after extraction with the modified QuEChERS method, *Food Control*, **19**, 906-914.
7. Liu, B.L., Hashi, Y., Qin, Y.P., Zhou, H.X. and Lin, J.M. (2007) Development of automated online gel permeation chromatography-gas chromatograph mass spectrometry for measuring multiresidual pesticides in agricultural products, *J. Chrom. B*, **845**, 61-68.
8. Nguyen T.D., Han, M.E., Seo, M.S., Kim, S.R., Yun, M.Y., Lee, D.M. and Lee, G.H. (2008) A multi-residue method for the determination of 203 pesticides in rice paddies using gas chromatography mass spectrometry, *Anal. Chim. Acta*, **619**, 67-74.
9. Nguyen T.D., Yu, J.E., Lee, D.M. and Lee, G.H. (2008), A multi-residue method for the determination of 107 pesticides in cabbage and radish QuEChERS sample preparation method and gas chromatography mass spectrometry, *Food Chemistry*, **110**, 2007-213.
10. Paya, P., Anastassiades, M., Mack, D., Siglova, I., Tasdelen, B., Oliva, J. and Barba, A. (2007) Analysis of pesticide residues using the QuEChERS pesticide multi-residue method in combination with gas and liquid chromatography and tandem mass spectrometric detection, *Anal. Bioanal. Chem.* **389**, 1697-1714.
11. Schenck, F.J., Brown, A.N., Podhorniak, L.V., Parker, A., Reliford, M. and Wong, J.W. (2008) A rapid multi-residue method for determination of pesticides in fruits and vegetables by using acetonitrile extraction, partitioning and solid-phase extraction column cleanup, *J. AOAC Intern.* **91**, 422-437.
12. Steinwander, H. (1985) Online methods for the determination of pesticide residues, Fres. *Anal. Chem.* **322**, 752–754.
13. Wang, S., Xu, Y., Pan, C. Jiang, S., and Liu, F. (2007) Application of matrix solid-phase dispersion and liquid chromatography-mass spectrometry to fungicide residue analysis in fruits and vegetables. *Anal. Bioanal. Chem.* **387**, 673-685.

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