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Multiresidue method for determination of 20 organochlorine pesticide residues in fruits and vegetables using modified QuEChERS and GC-ECD/GC-MSD

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Abstract

A rapid multiresidue method has been developed for the analysis of 20 organochlorine pesticides (OCs) in fruits and vegetables. The method is based on a modified QuEChERS procedure employing acetonitrile for extraction and partition. Dispersive solid-phase extraction (dSPE), containing primary and secondary amine (PSA) and graphitized carbon black (GCB), was used for cleans-up. Analytical determinations of the 20 OCs were carried out by gas chromatography, using electron capture detector (GC-ECD) and confirmed by mass spectrometry detector (GC-MSD). Recovery studies were performed at 0.05, 0.15 and 1.5 mg kg^{-1} fortification levels, and the results of validation parameters were acceptable (accuracy ranged from 61.2% to 117.6%, precision expressed as %RSD were less than 20 and measurement uncertainty was lower than 50%). The correlation coefficients (r^2) obtained for linearity of working range test were higher than 0.995, showing that the method is applicable to routine sample. The limit of detection (LOD) and the limit of quantitation (LOQ) for the OCs tested in apple and lettuce were 0.02 and 0.05 mg kg^{-1} respectively. The developed method was applied to determine OCs in imported fruits and local vegetables. No residual OCs was detected in any vegetable sample. However, detectable pesticides residues were found in 0.4% (3 apple samples) of the fruit samples. All of the positive samples were contaminated with endosulfan sulfate, which is endosulfan metabolite. None of the samples had contamination higher than the maximum residue limit (MRL) set by Codex.

Introduction

Pesticides are applied worldwide in agriculture to ensure high crop yields and improve the food production (Rashi *et al.*, 2012). Their applications are especially recommended during production and post-harvest treatment of agricultural commodities (Levitt *et al.*, 2001), and also in the public health sector for disease vector control (Crentsil *et al.*, 2011). In Thailand, farmers use insecticides widely in fruit and vegetable production, probably due to their perception that no better alternatives are available, and the investment on pesticides application is good enough for higher income (Hardy, 1995). However, the pesticide residues persist in fruits and vegetables and result in environmental contamination. Furthermore, because fruits and vegetables are mainly consumed raw or semi-cooked, it is expected that they contain higher pesticide residues compared to other foodstuffs (Claeys *et al.*, 2011). Human health can be affected by these residues if these commodities are consumed (Solecki *et al.*, 2005) and the use of pest-control substances is not well executed (Bhanti *et al.*, 2007).

Organochlorine pesticides (OCs) were extensively used by farmers because of their cost effectiveness and their broad spectrum activity. This group of chemicals is very stable and was one of the most used in the past until the Stockholm convention on persistent organic pollutants (POPs) was applied in 2005. Thailand is one of the signatory countries of the convention and exempted the use, importation and exportation of POPs for a decade. The chemicals generally persist in the environment and move from contaminated soil to plants (Taha *et al.*, 2013). Because of their high persistence, they can be accumulated in the human body by regular consumption of plant products (Dipo *et al.*, 1999). In many countries, including Thailand, OCs in food are monitored to ensure that public health is not endangered by residues taken daily in excess of the recommended tolerance levels (Herrera *et al.*, 1996). The total dietary intake of pesticide residues that remain on agricultural commodities are known as carcinogenic, teratogenic, mutagenic and neuro-toxic substances. Therefore, it is desirable to reduce these residues presented in food (Zawiyah *et al.*, 2007).

In pesticides analysis, some strategies have been applied in the method of development of

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high moisture contents fruit, vegetable and high chlorophyll contents vegetable analysis. Recently, there have been many crops with pesticide residue. Analysis methods were developed, especially using gas chromatography (GC) and liquid chromatography (LC) instruments (Ochiai *et al*., 2005). Twenty GC-amenable OCs pesticides were selected, which were parents and metabolite species. In this paper, we used a rapid multiresidue method of analysis based on a QuEChERS AOAC official method. The method has been modified to be detected by ECD. The procedure involved acidic acetonitrile for extraction, and PSA and GCB for purification and using spiked calibration curve to simultaneously determine pesticides residues in vegetables.

The objective of the present research study was firstly to develop the most suitable method to determine qualitatively and quantitatively organochlorine pesticide residues in high moisture contents of fruits and high chlorophyll contents of vegetables, and secondly, to assess the concentration of OCs residues found in raw commodities in Thailand. The results of the survey should generate awareness about the lethal effect on human beings. This paper presents the results of the residue levels of OCs in imported fruits in 2014 to 2015 for consumer protection. In addition, because green vegetables are one of the most important components of the Asian diet (Odhiambo *et al.,* 2009), and are consumed sometimes without processing or transformation, a preliminary survey of OCs in high chlorophyll contents vegetables produced in Thailand was carried out in 2015. These make information on residue levels of pesticides in vegetables very important for the protection of human health.

Materials and Methods

Chemicals

Acetonitrile (PR), acetone (HPLC), ethyl acetate (HPLC) and glacial acetic acid (AR) were purchased from J.T. Baker, USA. N-hexane (PR) was supplied by RCILabscan, Thailand. Anhydrous sodium sulfate (AR), anhydrous magnesium sulfate (AR) and sodium acetate (AR) were obtained from Fisher Scientific, UK. A mixture of 150 mg MgSO4, 50 mg PSA and 50 mg GCB was from Unitedchem, USA. Reverse osmosis water was generated by Millipore Milli-Q system, USA. Organochlorine compound standards were aldrin, α-BHC, β-BHC, α-chlordane, β-chlordane, oxy-chlordane, p, p'-DDE, p, p'-TDE, p, p'-DDT, dicofol, dieldrin, endrin, α-endosulfan, β-endosulfan, endosulfan sulfate, heptachlor, trans-heptachlor epoxide, hexachlorobenzene, methoxychlor and tetradifon. All OCs standards were of high purity grade (>94.0±0.5%) and were purchased from Dr. Ehrenstorfer GmbH, Germany. Individual stock solutions were prepared at about 200 $\mu g\ mL^{-1}$ in n-hexane and stored at $\leq$ -10^{o}C in freezer. A mix of intermediate (2 and 10 $\mu g\ mL^{-1}$) and working (0.02, 0.05, 0.2, 0.8, and 1.5 $\mu g\ mL^{-1}$) standard solutions were prepared by serial dilution of stock solution to the appropriate concentrations. The residue concentrations were calculated using the calibration curve generated from the peak area versus the working solution concentrations.

Sample

For the method validation, apple and lettuce were selected to be the representatives of fruits and vegetables respectively. The imported blank apple was purchased from a supermarket, and the blank lettuce was acquired by organic agriculture. Both were tested for the non-pesticides contaminant before experimental studies. In 2014 and 2015, 677 samples of fresh fruits were collected by the Food and Drugs Administration of Thailand (Thai-FDA) and brought to the Department of Medical Sciences (DMSc) for pesticide residues screening before permission of importation to the country. Samples of fruits tested for food security were apple (n = 365), grape (n = 119), pear (n = 56), orange (n = 43), dragon fruit (n = 35), kiwi (n = 17), strawberry (n = 14), pomegranate (n = 12), berry (n = 11), persimmon (n = 2), peach (n = 1), cantaloupe (n = 1) and water melon (n = 1). Furthermore, during 2015, at each of the provinces (74 provinces), one kilogram of the four vegetables namely, Chinese kale, ivy ground, yard long bean and morning glory were collected from local markets (total of 934 samples). The vegetable samples were collected in clean polyethylene bags, labelled and transported to the Pesticides and Veterinary Drugs Residue Laboratory (PVRL) of the Bureau of Quality and Safety of Food (BQSF) and preserved in a refrigerator at 4^{o}C, pending extraction. The Codex guideline (Codex, 2000) for sampling and sample preparation was used, and the samples were blended by homogenizer before weighing 10 g for an analytical portion and other 10 g for QC sample. The rest of the blended sample portion was kept in a walk-in freezer at lower than -15^{o}C. For the analysis, only the edible portions were included, whereas bruised or rotten parts were removed.

Sample preparation

A representative portion of sample (10 g) was weighed in a 50 mL PTFE centrifuge tube and spiked with the proper amount of working standard solutions of pesticides. Then, 10 mL of 1% (V/V) acetic acid in acetonitrile were added, and the mixture was shaken vigorously for one minute. The mixture was added by 4 g anhydrous magnesium sulfate and 1 g sodium acetate and was shaken manually for a further one minute. The sample contained in the tube was centrifuged for five minutes at 3500-4000 rpm. The supernatant (5 mL) was transferred to a 15 mL PTFE centrifuge tube and a mixture of 150 mg MgSO4, 50 mg PSA and 50 mg GCB were added, and the tube was shaken in a vortex for 30 seconds. The system was centrifuged for 10 minutes at the same rpm. Finally, an aliquot of 2 mL of supernatant was transferred to an amber vial, was evaporated under a gentle stream of nitrogen, and was reconstituted for GC-ECD determination with 2 mL of n-hexane: ethyl acetate (75:25 v/v). With this treatment, 1 mL of sample extract represents 1 g of sample.

Instrumentation

For the analysis of organochlorine compounds, the instrument used was a GC-ECD: Agilent Technologies 6890N with ECD equipped with a DB-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The oven temperature was held at 80°C for one minute, then increased to 180°C by a rate of 15°C min^{-1} and then increased to 205°C by a rate of 3°C min^{-1} and held for five minutes. By using a rate of 40°C min^{-1}, the oven condition was increased to a final temperature at 260°C and was held for 20 minutes. The injection port and detector temperatures were maintained at 200°C and 300°C, respectively. The injection volume was 1 µL with splitless mode, and nitrogen make up gas was flowed at 60 mL min^{-1}.

For the confirmation of organochlorine compounds, the equipment was a GC-MSD: Agilent Technology 6890N-5973 inert MSD system chromatograph equipped with a DB-35ms capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness), and the oven temperature system was the same as GC-ECD. The temperatures of the injection port and detector were 250°C and 280°C, respectively, and the ion source temperature was 230°C with electron impact (EI) ionization mode.

Method validation

Method validation assessed the parameters: selectivity and specificity, LOD and LOQ, linearity of calibration curve and working range, accuracy and precision. The experiment was performed using blank apple and lettuce spiked with organochlorine compounds (OC) concentrations of 0.02, 0.05, 0.2, 0.8, 1.5 and 2 mg kg^{-1} or suitable concentrations to determine the parameters. Three random injections of matrix match calibration (MMC) were performed to ensure the linearity of calibration curves. Six replications were done for the test of LOD, LOQ, accuracy and precision, and for the linearity of working range were tested in three replicates according to the sample preparation described in the sample preparation section.

The LOD and LOQ were calculated from the standard deviation (SD) of the determinations (LOD = 3SD and LOQ = 10SD). Precision was calculated by dividing the SD of the instrument responses (peak area) by their average. The accuracy was expressed by recovery percentage (%), which was set as the peak area obtained for the spiked sample, divided by the peak area of the standard solution. Descriptive statistics (mean, SD, median, interval) and analyses of variance (ANOVA) were conducted using Excel software.

The estimation of measurement uncertainty (MU) was evaluated by considering the following factors as contribution sources: weight in analytical scale, volume measured in volumetric flasks and micropipettes, analytical curves linearity, standard solution of OCs and methodology accuracy and precision.

Results and Discussion

Method validation

Analysis was performed by GC-ECD. The chromatogram of 20 OCs is shown in Figure 1. To obtain this, chromatographic peaks related to each compound, blank apple was spiked with all analytes at 0.15 mg kg^{-1}. Pesticides were identified according to their retention times, and the quantitation was based on the peak area of the target pesticides to that of external standard calibration. The concentrations of pesticides were determined by interpolation of the relative peak areas for each pesticide to external standard peak areas in the sample on the spiked calibration curve. A chromatogram of 20 pesticides, analyzed in spiked apple and lettuce, is shown in Figure 1. All pesticides showed acceptable linearity of calibration curve. Linear spiked calibration curves for all the relevant pesticides were obtained with correlation factors (r^2) >0.995. LODs and LOQs of the proposed method were measured in spiked samples, and calculated by considering a value 3 and 10 times of background noise, which, for all pesticides, were 0.02 and 0.05 mg kg^{-1}, respectively. For the specificity of the method, there is no interference between the components of solvent used for preparation of standard and blank extract from the matrix. Table 1. presents the accuracy (% of recovery) and precision

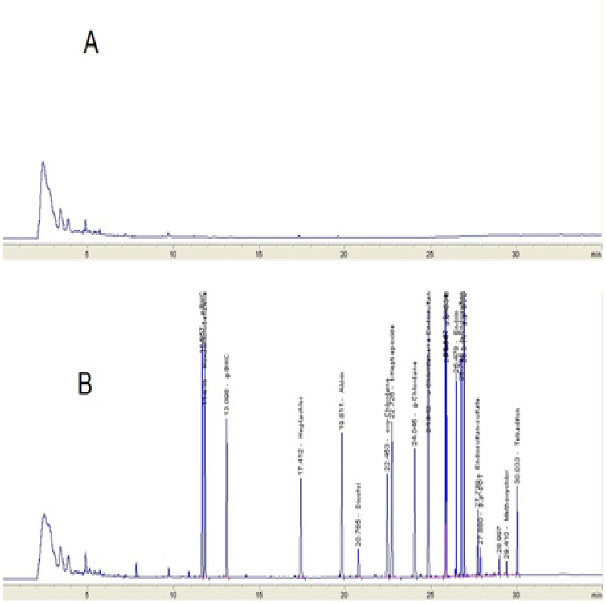

Figure 1. A. GC-ECD chromatogram of blank apple sample. B. Representative chromatogram obtained for the 20 pesticides: aldrin, α-BHC, α-BHC, α-chlordane, γ-chlordane, oxy-chlordane, p p’-DDE, p p’-TDE, p p’-DDT, dicofol, dieldrin, endrin, α-endosulfan, α-endosulfan, endosulfan sulfate, heptachlor, trans-heptachlor epoxide, hexachlorobenzene, methoxychlor and tetradifon spiked apple sample at 0.15 mg kg^{-1}.

(RSD) for three concentration levels (0.05, 0.15 and 1.5 mg kg^{-1}) of pesticides. The recovery of pesticides at three concentration levels (n=6 replicates per level) was in the acceptable range (60-120%). In terms of repeatability, the majority of the pesticides gave RSD < 20%. The recoveries and repeatabilities are in accordance with the criteria set by SANCO Guideline (Albero *et al*., 2005).

Comparing to the QuEChERS method introduced by Anastassiad *et al*., (2003), the modified method replaced MSD detector by ECD detector. This detector provides generaly higher sensitivity (intrumental LOD), better linearity, and stability than MSD even in SIM mode for the analysis of organochlorine pesticides. In GC-ECD, the LOD and LOQ values are below or close to the values of MRLs, making easier the identification and determination of possible contaminations. However, the validation results showed that the LOQs of some OCs of the method were higher than GC-MSD due to the lake of repeatibility of the extraction step. Despite the high sensitivity of GC-ECD for organochlorine pesticides, the identification of the compounds obtained by comparison with standards, makes the quantification of the pesticides not very reliable due to matrix interferences. The limits of detection and quantification, repeatability and recovery showed that GC-ECD is better than GC-MSD-SIM for the analysis of all pesticides tested. These results suggest that GC-MSD-SIM could be used as a screening method for the identification of the pesticide and GC-ECD as quantitative method. On the other hand, GC-MS/MS on MRM mode provides excellent sensitivity and satisfactory quantification limits, allowing the identification and quantification of pesticides at low levels.

Table 1. Analytical parameters of twenty organochlorine (OCs) pesticides in apple and lettuce

Organochlorine compounds	Average recovery, % (precision, RSD)					
	Apple (mg kg^{-1})			Lettuce (mg kg^{-1})		
	0.05	0.15	1.5	0.05	0.15	1.5
aldrin	86.8(3.9)	81.1(2.5)	83.6(2.0)	94.7(10.9)	62.9(5.2)	83.3(3.9)
α-BHC	85.9(9.5)	76.6(5.6)	85.9(4.5)	97.1(8.1)	87.8(1.6)	87.4(2.7)
β-BHC	75.0(5.6)	81.1(4.9)	91.6(0.5)	95.1(3.3)	107.9(1.5)	75.7(11.1)
α-chlordane	85.4(4.4)	82.5(3.4)	93.7(4.8)	88.9(14.8)	69.1(5.7)	85.0(1.4)
γ-chlordane	80.2(5.7)	83.6(2.4)	93.5(4.7)	96.8(9.3)	62.7(4.6)	78.8(2.0)
oxy-chlordane	68.2(2.7)	85.9(3.4)	97.5(4.0)	94.5(9.2)	64.0(0.1)	67.0(1.7)
p,p’-DDE	89.1(3.5)	97.4(0.3)	107.8(2.4)	97.3(15.4)	75.3(1.8)	86.0(3.3)
p,p’-DDD	94.5(8.0)	75.7(6.4)	102.0(2.1)	101.7(8.7)	71.1(13.3)	100.1(6.4)
p,p’-DDT	66.1(13.0)	88.9(3.3)	92.9(6.0)	93.4(12.4)	72.4(1.10	77.4(12.7)
dicofol	86.0(9.7)	110.1(4.6)	99.3(4.2)	95.2(6.2)	81.6(1.2)	82.6(15.6)
dieldrin	117.6(12.6)	87.4(3.5)	91.9(5.4)	93.4(15.2)	67.8(1.1)	110.3(5.9)
endrin	61.2(13.8)	96.9(5.2)	96.4(7.7)	89.1(15.3)	78.9(2.1)	65.4(5.8)
α-endosulfan	95.8(4.0)	80.9(5.2)	85.9(1.6)	89.3(15.0)	87.8(6.9)	94.0(2.5)
β-endosulfan	113.3(10.7)	83.9(1.7)	90.8(5.6)	97.5(11.7)	109.1(0.9)	114.4(3.3)
endosulfan sulfate	83.4(6.3)	73.7(6.2)	86.6(5.0)	92.9(2.5)	92.2(3.4)	93.4(11.7)
heptachlor	92.0(6.5)	104.7(0.2)	89.6(3.8)	106.0(4.3)	70.4(4.8)	94.7(3.7)
trans-hept epoxide	82.2(2.8)	82.4(3.4)	98.3(8.4)	92.6(2.6)	87.8(1.2)	79.2(4.6)
hexachlorobenzene	66.5(6.2)	97.4(8.6)	107.4(4.5)	90.5(14.1)	85.6(0.9)	66.7(1.1)
methoxychlor	110.0(10.7)	101.6(3.1)	101.6(5.5)	110.7(6.0)	93.6(19.6)	110.6(3.5)
tetradifon	103.3(2.5)	98.1(11.0)	105.0(4.4)	91.9(7.6)	98.4(1.0)	104.8(5.0)

The original QuEChERS method consists of two steps, a salting out extraction and dispersive solid-phase extraction (dSPE) for clean up. However, fruits and vegetables represented by apple and lettuce required different purification. Since lettuce is a high chlorophyll contents vegetable, and pigment presented in vegetables showed interference peaks in ECD detector, GCB needs to be used in this case. On the other hand, apple is a clean metrix face to ECD, so the use of PSA without GCB is sufficent. Then, QuEChERS procedure was used for extraction of pesticides in fruits and vegetables, but some modifications were made. Because the ECD detector is very sensitive to acetonitril, the solvent needs to be changed into hexane and ethyl acetate (3:1). The

Table 2. The retention time, diagnostic ions and selected quantification ion for endosulfan sulfate.

Compound	Diagnostic ion	Quantification ion	Retention time (min)
endosulfan sulfate	272, 274, 229	272	27.73

Table 3. Pesticides residue and their concentrations in apple samples

Sample	Source of apple	Pesticide	Concentration (mg kg^{-1})	LOQ (mg kg^{-1})	MRL (mg kg^{-1})
1	USA	endosulfan sulfate	<LOQ	0.05	5
2	USA	endosulfan sulfate	<LOQ	0.05	5
3	USA	endosulfan sulfate	0.07	0.05	5

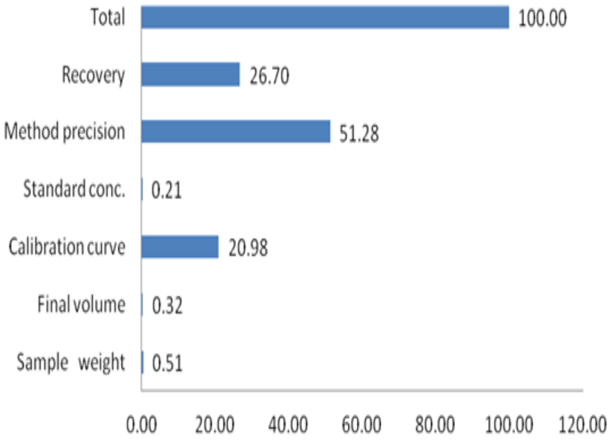

Figure 2. The main sources of the uncertainty for the method.

modified method was in-house validation to comply with the requirements of ISO/IEC 17025:2005 standard.

Liquid-liquid extraction method are used would wide as a multiresidue method for multiclass pesticides analysis. Luke *et al.* (1999) added a combination of fructose, MgSO$_4$, and NaCl to separate water from acetone in the initial extract without using nonpolar solvents. The Luke method became very popular and was widely accepted by many pesticide screening laboratories because it expanded the analytical range and improved the recoveries of polar analytes. However the method has many shortcomings. The sample preparation is complicated and are time consuming. The method required large amount of solvent, lengthy evaporation steps and extensive use of glassware. The developed modified QuEChERS method achieved a fast and easy extraction while still maintaining high recoveries for a wide range of analytes and providing the selectivity and repeatability for reliable procedure.

The MU was based on bottom up methodologies described in the EURACHEM guide. The main sources of the uncertainty for the method were described in Figure 2., and were composed of: 1. the mass measurements of the standards for the preparation of solution; 2. Dilution of standard solutions; 3. the measurements of volume of the extraction solution; 4. calibration curves and 5. intermediate precision. The MU calculated for each pesticide showed values below 50%, which accorded with the acceptable criteria established in the SANCO document.

Sample analysis

The validated method was applied to detect and quantify the OCs residues in fruits and vegetables available in Thailand. In order to ensure the quality of the results, internal and external quality controls (IQC and EQC) were carried out. In every batch of samples (or at least 5%), method blanks, sample blanks, spiked samples and duplicate analysis were included in the batch. The participation with the proficiency testing (PT) programme provided by international and national organizations had satisfactory results. The GC-MS spectrum for the confirmation of the results was the injection of positive samples, and compared with individual standard solutions and the instrumental library. The total ion current of GC-MS showed in table 2. the primary and secondary ions were exactly endosulfan sulfate.

Results of the survey in Table 3. had shown that imported fruits in Thailand are comparatively free from residues of pesticides or so much lower than established MRL. This presented that pesticides may not be used or the current period is spent. Analyses of 677 samples in 2014 and 2015 showed the presence of endosulfan sulfate in the endosulfan group in 0.4% (3 apple samples) of the samples with residues ranged from <0.05–0.07 mg kg^{-1}. These contaminated samples were lower than MRL established by international regulation (Codex). Thailand does not set the MRL of endosulfan in fruit and vegetables, because of the total ban, and the residue causing contamination in foodstuff is prohibited. The intensive use of endosulfan in agriculture results in the high concentration in surface water in the area and in excess of the criteria recommended for protecting aquatic ecosystems. Endosulfan reaches concentrations, which are lethal to important species of native fish and macroinvertebrated. Even so, endosulfan is an off-patent pesticide and was being phased out globally, the endosulfan positive samples were still found and all samples were the products of the United States of America. The USA had already banned the substance since 2010. According to US-

EPA, it has extensively been used in apples, and 626 thousand kilograms of endosulfan were used annually from 1987 to 1997.

The results of the analysis of pesticide residues indicated that none of the monitored vegetable samples had residues of other relevant pesticides. These results are not surprising since most of OCs pesticides were banned or their use severely restricted in Thailand. The introduction of laws and regulations, designed to control environmental contamination, results in the level of most OCs pesticides in food, showing a decrease from 1980 to now.

Conclusion

An accurate, precise and reliable method was developed to determine 20 OCs pesticide residues in fruits and vegetables; a main commodity in the Thai food basket. The method, which consists of a modified QuEChERS sample preparation and GC-ECD analysis confirmed with GC-MSD, showed a high sensitivity and confirmatory power necessary for the determination of pesticides residue at low levels. The excellent results of method validation show the applied strategy for determination and quantification of pesticides residue in food is reliable, useful and acceptable. The applicability of the method was demonstrated by analysis of 677 fruits and 934 vegetable samples. The contamination of 0.4% of the analyzed fruit samples with endosulfan sulfate calls for concern by regulatory bodies in Thailand for the imported products. However, none of the vegetable samples was OCs detectable, and showed that vegetables grown and consumed in Thailand are generally safe from OCs contamination. It can be concluded that there was a good observance of limitations in agricultural application of chlorinated pesticides.

Reference

Albero, B., Brunete, C. S. and Tadeo, J. L. 2005. Multiresidue determination of pesticides in juice by solid-phase extraction and gas chromatography–mass spectrometry. Talanta 66: 917-924.

Anastassiades, M., Lehotay, S.J., Štajnbaher, 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.

Bempah, C. K., Buah-Kwofie, A., Denutsui, D., Asomaning, J. and Tutu, A. O. 2011. Monitoring of Pesticide Residues in Fruits and Vegetables and Related Health Risk Assessment in Kumasi Metropolis, Ghana. Research Journal of Environmental and Earth Sciences 3(6): 761-771.

Bhanti, M. and Taneja, A. 2007. Contamination of vegetables of different seasons with organophosphorous pesticides and related health risk assessment in northern India. Chemosphere 69(1): 63-8.

Claeys, W. L., Jean-Francois, S., Bragard, C., Maghuin-Rogister, G., Luc, P. and Schiffers, B. 2011. Exposure of several Belgian consumer groups to pesticide residues through fresh fruit and vegetable consumption. Food Control 22: 508-516.

Codex [Codex Alimentarius Commision]. 2000. Food Standards Programme. Pesticide Residues in Food. Methods of Analysis and Sampling. Volume 2A Part 1. Rome: World Health Organization.

Diop, Y. M., Diouf, A., Fall, M., Thiam, A., Ndiaye, B., Ciss, M. and Ba, D. 1999. Pesticide bioaccumulation: measurement and levels of organochlorine residues in products of vegetable origin. Dakar Médical 44(2): 153.

Hardy, J. 1995. Apolipoprotein E in the genetics and epidemiology of Alzheimer's diseases: Vascular risk and beta-amyloid metabolism. American Journal of Medical Genetics 9: 456-460.

Herrera, A., Arino, A., Conchello, P., Lazaro, R., Bayarri, S., Perez-Arquillue, C., Garrido, M. D., Jordal, M. and Pozo, R. 1996. Estimates of mean daily intakes of persistent organochlorine pesticides from Spanish fatty foodstuffs. Bulletin of Environmental Contamination and Toxicology 56(2): 173-177.

Levitt, J. A. and Wehr, H. M. 2001. The importance of international activities to the work of the Food and Drug Administration's Center for Food Safety and Applied Nutrition. Food and Drug Law Journal 56: 1-9.

Luke, M., Cassias, I., and Yee, S. 1999. Laboratory Information Bulletin. No.4178, Office of Regulatory Affairs, U.S. Food and Drug Administration, Rockville, MD

Ochiai, N., Sasamoto, K., Kanda, H., Yamagami, T., David, F., Tienpont, B. and Snadra, P. 2005. Optimization of a multi-residue screening method for the determination of 85 pesticides in selected food matrices by stir bar sorptive extraction and thermal desorption GC-MS. Journal of Separation Science 28(9-10): 1083-1092.

Odhiambo, J. O., Shihua, Q., Xing, X., Zhang, Y. and Muhayimana, A. S. 2009. Residues of Organochlorine Pesticides in Vegetables from Deyang and Yanting Areas of the Chengdu Economic Region, Sichuan Province, China. Journal of American Science 5(4): 91-100.

Rashi, B., Ashwini, K. R., Ajai, K., Kuruba, A. and Sadhana, P. 2012. Organochlorine pesticide residues in vegetables of three major markets in Uttar Pradesh, India. Acta Biologica Indica 1(1): 77-80.

Solecki, R., Davies, L., Dellarco, V., Dewhurst, I., van Raaij, M. and Tritscher, A. 2005. Guidance on setting of acute reference dose (ARfD) for pesticides. Food and Chemical Toxicology 43: 1569–1593.

Taha, M. E. M., El-Zorgani, G. A., El-Hassan, A. M. and Salghi, R. 2013. Pesticide Residues of Organochlorine in Some Common Foods Cultivated in South Gezira Valley (Sudan). Journal of Materials and Environmental Science 4(5): 621-624.

Zawiyah, S., Che Man, Y. B., Nazimah, S. A. H., Chin, C. K., Tsukamoto, I., Hamanyza, A. H. and Norhaizan, I. 2007. Determination of organochlorine and pyrethroid pesticides in fruit and vegetables using SAX/PSA clean-up column. Food Chemistry 102: 98-103.