Application of Octadecylsiloxane-Coated Fiber in Solid-Phase Microextraction for Determination of Organophosphorus Pesticide Residues in Vegetables

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ABSTRACT

Analysis of organophosphorus (OPPs) insecticide residues in vegetables by using solvent extraction and chromatography is well accepted as an international standard method. However this method requires a lot of organic solvents and leaves much hazardous waste to the environment. Recently, solid-phase microextraction (SPME) and gas chromatography (GC) has been recognized as another suitable method which can solve all the mentioned problems. In this study, SPME was prepared by hydrolysis and polycondensation with octadecylsiloxane (ODS) on fused silica fiber. The efficicncy of extraction by ODS SPME was compared with available commercial SPME fibers and the standard GC-FPD analytical method. Results showed that the optimum conditions for analysis of OPPs using SPME/ GC with DB-1 capillary column at 30 m x 0.32 mm I.D., 1 μm film thickness, the optimum temperature program started with 80°C for 30 sec then increased to 300°C after injection at the rate of 30°C/min, the temperatures of GC injector port and PFPD detector were 250°C and 300°C. One gram of blended vegetable sample in 200 ml of ultrapure water had been shaken for 2 hrs and centrifuged for 2 min, then using ODS fiber to immerse in 1 ml of the aqueous extract for 30 min at room temperature. The analytes were desorbed for 4 mins at GC injector port. The optimum conditions were then used for determining the following insecticides: chlorpyriphos, methylparathion, profenofos and prothiofos standard mixtures spiked in vegetable samples. The percentage recoveries were 99.80%, 101.51%, 104.03% and 106.52% with RSD 9.42%, 6.46%, 7.73% and 3.96%, respectively. The detection limits were 5.00, 40.00, 5.00 and 50.00 μg/l, respectively. The precision of the insecticide determination using ODS was not significantly different from using commercial coated fibers. The ODS SPME developed in this study was found to be as effective as polyacralate(PAC) (1.43-5.56 %RSD).

Analysis of mevinphos, methylparathion, prothiofos and profenofos spiked in tomato samples using ODS SPME /GC-PFPD compared with using AOAC, was not found to be statistically different in percentage of recoveries.

It can be concluded that the development of ODS SPME/GC-PFPD in this study was suitable for analysis of organophosphorus insecticides; the method is simple, uses less time, no complicate extraction and without using organic solvent.

Key word: Organophosphorus insecticide residues (OPPs), Solid-phase microextraction (SPME), Gas Chromatograph with Pulsed-Flame Photometric Detector (GC-PFPD)

INTRODUCTION

Pesticides are useful for controlling insect pests of plant and animal. However, these chemicals are harmful to human, animal and environment. Pesticides today include insecticides, miticides, fungicides, herbicides, defoliants and rodenticides. Insecticides kill insects by ingestion, by contact or by inhalation. The insect pests cause a huge damage to human by destroying or damaging food, materials and crops (Pogacnik and Franko, 1999). The major chemical categories of insecticides are as follow: organophosphorus, synthetic pyrethroids, carbamate and organochlorine (Nollet, 1992). Most insecticides are in the organophosphorus group. In Thailand, organochlorine insecticides are now rarely used. They have been replaced by carbamate and organophosphorus insecticides such as chlorpyrifos, dichlorvos, prothiofos and methylmalathion. Organophosphorus insecticides are used in agriculture to control pests, weeds and plant diseases of cotton, rice, groundnut, vegetables, fruits etc. Organophosphorus pesticides were developed for their effects on insect which are similar to their effects on human. Some are very poisonous and have even been used in World War II as nerve agents. These insecticides were commonly used in the past but at present, many have been replaced due to their hazard to health, their effects and stability in the environment. Compounds of dangerously-high mammalian toxicity are included in this group, reflecting the interest of using them to make chemical weapons during the war years. Most organophosphorus compounds are toxic to the nervous system by disrupting an enzyme which regulates acetylcholine, a neurotransmitter. The action of the organophosphorus insecticides depends upon inhibition of the enzyme acetylcholinesterase. The neurotransmitter substance, acetylcholine, functions in various parts of the nervous system in both insect and mammal. Inhibition of the enzyme, this reaction, in contrast to the normal acetylating by acetylcholine, is less readily reversible to regenerate the active enzyme. Toxicity can cause illness or death. Neurotoxicity is restricted to the nervous system and teratology to embryonic stages (Hill and Wright, 1978). Royal Project Foundation (RPF) has been trying to persuade the highland farmers to minimize use of pesticides in agriculture by introducing IPM (Integrated Pest Management) to them. However, selected effective pesticides are still recommended to them when other methods cannot control the pest. Royal Project's vegetables and fruits are routinely checked at planting plots 1-2 days before harvesting. The produces are randomly rechecked at the local packing house and once more at the analytical laboratory of Plant Protection Center, RPF, using GT pesticide test kit (personal communication). Such test kit can detect only some pesticide residues in carbamate and organophosphorus group but not in quantitative amount. The test kit can be used in the laboratory with inextensive training. The test may result in false positive and negative readings. The strong-colored vegetables may interfere with reading. The results cannot tell the amount of each pesticide found as the high-performance liquid chromatograph (HPLC)

and gas chromatograph (GC) can. Analysis of vegetables for pesticide residue is complex because of the matrix. In order to obtain accurate result, be efficient in analyzing pesticide residue, several types of sample preparation have to be made. The qualitative and quantitative determination of pesticide in aqueous samples is usually performed by liquid- liquid extraction (LLE) and solid-phase extraction (SPE) method. Both procedures require several steps for sample preparation where some amounts of solvent have to be used (Horwitz, 1970; Helrich, 1990 ; Nollet, 1992 ; Cunniff, 1997 ; Sun et al., 2003). Solid-phase microextraction (SPME) is considered to be a fast, selective and solvent-free with direct extraction of the analytical technique which can combine both extraction and pre-concentration in one step (Ulrich, 2000; Mester et al., 2001).

Purpose of the study

To develop SPME fiber for analysis of organophosphorus insecticide residues in vegetable by using SPME/GC-PFPD.

MATERIALS AND METHODS

Chemicals

All chemicals, e.g., prothiofos, methylparathion, chlorpyriphos, methamidophos, dicrotophos, profenofos and mevinphos were purchased from Riedel-de Haen (Seelze Germany). The ultrapure water was obtained from a NANO-pure ultrapure water system (Dubuque, IA USA). The commercial SPME device and fibers were purchased from Supelco (Bellefonte, USA). Prothiofos, methylparathion, chlorpyriphos, methamidophos, dicrotophos, profenofos and mevinphos stock standard mixture solutions in acetone (1 mg/mL) were prepared by measuring the desired amount of the compounds and dissolved in a certain amount of acetone. Solutions of 0.1, 0.01, 0.001 and 0.0001 mg/mL pesticides were prepared by subsequently diluting the stock standard solutions with ultrapure water. All solutions were stored at 4°C and used after dilution with water. Working solutions were prepared every 2 weeks by dilution in ultrapure water to the required concentration.

Analysis of organophosphorus pesticides spiked in vegetable sample using ODS SPME/ GC-PFPD.

Analysis of OPPs started with preparation of vegetable by using 1 kg of vegetable, cut in halves and 500 g of the sample were cut into small pieces and then blended to fine particles. One gram of the blended vegetable was put in a 500 ml erlenmeyer flask, 200 ml of ultrapure water was poured into the flask. The mixture was shaken for 2 hrs, then centrifuged for 2 mins. The aqueous extract solution was collected and 1 ml of the extract was pipetted into a 2 ml vial and capped with silicone septum. The extract was analyzed for OPPs, using SPME/GC-PFPD.

GC Conditions

Instruments: A Varian 3600 CX GC fitted with pulsed-flame photometric detector (PFPD) and a Varian 8200 AutoSampler modified for SPME with agitation were used. Varian 8200 AutoSampler was controlled by the Varian Star version 5 software through a PC. The software allows the operator to select absorption times and desorption times, headspace

or direct sampling and the number of vials to be automatically analyzed.

Capillary column: The column is a 30 m x 0.32 mm fused silica coated with 1 μm DB-1 (J&W, Folsom, CA, USA).

Column program: Initial temperature at 80°C was held for 0.5 min, then ramped to 300°C at the rate of increase at 40°C/min and held at 8.50 mins for a total run time of 15 mins. Nitrogen was used as a carrier gas at a linear velocity of 2 cm/min at 25°C.

Fiber: The SPME fiber holder (Supelco, Bellefonte, PA) was used for automated SPME investigation. The 65 μm polydimethylsiloxane-divinylbenzene (PDMS-DVB), 100 μm polydimethylsiloxane (PDMS), polyacrylate (PAC), carbowax-divinylbenzene (CW-DVB) and carboxen- polydimethylsiloxane (CAR-PDMS) fibers, used in this study, were purchased from Supelco , Bellefonte, PA, USA. Each fiber was conditioned before use by inserting it into a GC injector and immediately used to prevent contamination.

Injector: The injection port was set at 250°C.

Detector: The detector was set at 300°C

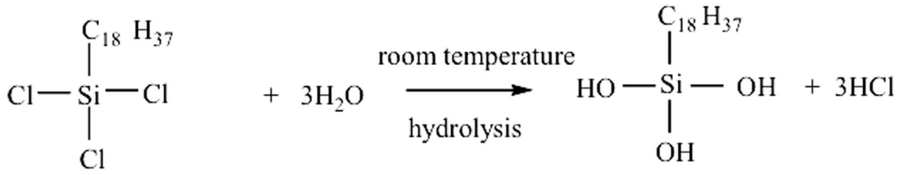
Preparation of ODS SPME fiber (octadecylsiloxane-coated SPME fiber with octadecyltrichlorosilane) Preliminary test

A study of technique on coating fiber with silica gel. The silica gel of 60 g for preparing TLC was used for coating. The gel was mixed with octadecyltrichlorosilane(ODS) and water at concentration of 99% (1% water). The mixture was left at room temperature for 10 mins and then rinsed with water. The coated silica gel was left air-dried overnight and kept in the oven at 250°C for 2 hrs before use.

A test on absorption and desorption of the coated silica gel. One gram of the coated silica gel was added in a mixed OPP standard solution which had 1 mg/l of dicrotophos, prothiofos, methylparathion, chlorpyriphos, mevinphos, profenofos and methamidophos and left at room temperature for 10 mins. The coated silica gel with insecticides was transferred to the screw-top vial, then heated on the hotplate at 250°C for 5 mins before using a syringe to take 2 μl of the gas in the vial. The gas containing insecticides was then injected to the GC at injection port. After the separated peaks had appeared on the chromatogram, it was considered to be suitable for using the coated fiber for the next experiments.

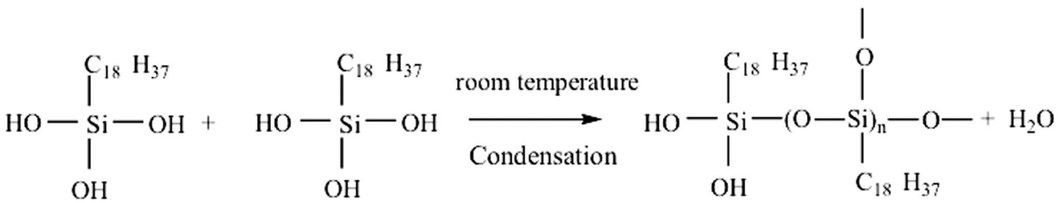
Preparation of octadecylsiloxane-coated fiber. The fibers used for preparation was the 100 μm-thick fibers coated with polydimethylsiloxane. The fibers had been treated with 98% sulfuric acid, then washed thoroughly with methanol and the bare fused-silica fiber was left dried (Xiao et al., 2001) overnight at room temperature. At this time, the fibers were free of polydimethylsiloxane and ready to be coated with ODS. The bare fused-silica fibers, obtained by chemical polymerization method, were dipped into the ODS in water at concentration of 99% (1% water), taken out and left for 1 min, then dipped in ultrapure water once again, taken out and left air- dried overnight. This process was repeated 3 times to increase the film thickness of the fiber. The ODS fibers were conditioned in the GC injection port at 250°C for 60 mins.

Chemical reaction to prepare polymer coating for SPME fiber



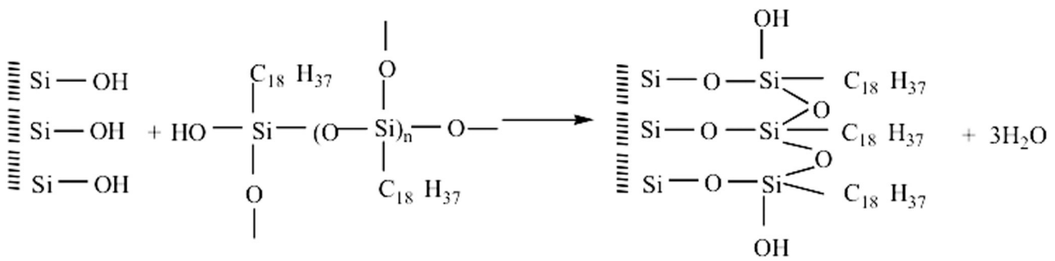
Octadecyltrichlorosilane — Octadecyltrihydroxylsilane

Scheme 1. Hydrolysis of the octadecyltrichlorosiliane.



Polyoctadecylsiloxane

Scheme 2. Polycondensation of the hydrolyzed products.

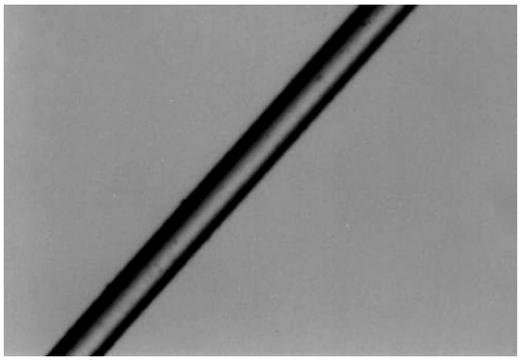


Surface of fused-silica fiber — Surface of coated fiber

Scheme 3. Chemical bonding of polymer onto the fiber surface.

RESULTS AND DISCUSSION

Preparation of ODS SPME. After the fiber had been coated with ODS 3 times, the surface of coated fiber was investigated under a stereomicroscope at 40x and a comparison with uncoated fiber was made. The surface of uncoated fiber was smooth and thin [Fig. 1-1] while the surface of coated fiber was rough and thick [Fig. 1-2]. The coated fiber was scanned with SEM (scanning electron microscope) at 5 kx and it was found that there were lumps of ODS lying along the surface of the fiber [Fig. 1-3].



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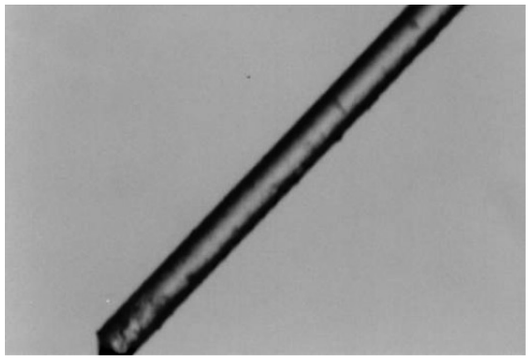

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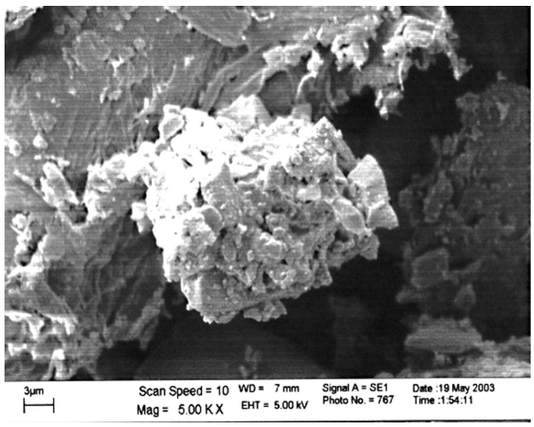

[3]

Figure 1. Comparison of SPME fiber before and after coating: [1] A bare fused-silica SPME fiber under a stereomicroscope (40x) [2] An octadecylsiloxane-coated SPME fiber under a stereomicroscope(40x) [3] An octadecylsiloxane-coated SPME fiber under SEM.

The gas chromatograph with pulsed-flame photometric detector was used to determine the optimum SPME methodology conditions. The SPME procedure is based on an equilibrium between the analyte in the sample and in the solid-phase of fiber coating. Herein, the amount of an analyte extracted depended on the mass transfer of an analyte through the aqueous phase and the extraction time. An PFPD chromatogram of 4 organophosphorus pesticides standard solution under chromatographic conditions described, is shown in Figure 2. The fiber coated with octadecylsiloxane was used for optimization of extraction condition of organophosphorus pesticides. An aqueous solution of 0.01 mg/l of each mixed standard solution was prepared by dilution of working standard with deionized water.

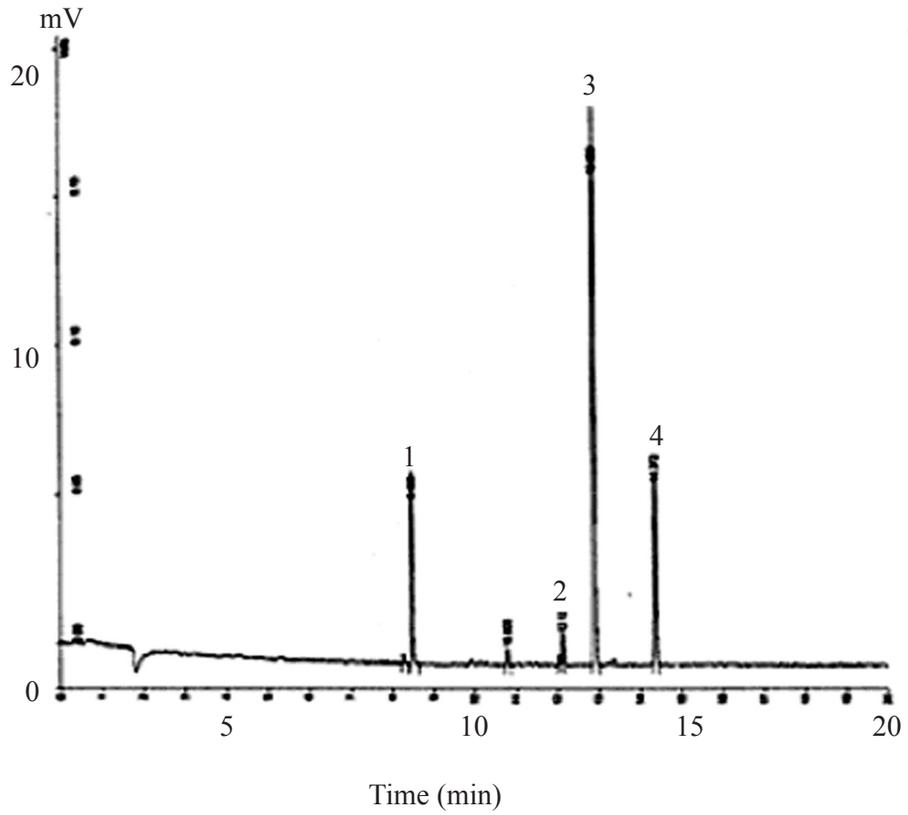

Figure 2. PFPD chromatogram of mixed OPPs standard solution was obtained by direct ODS -SPME sampling under optimum conditions of SPME -GC–PFPD identified peaks are: 1 Prothiophos, 2 Methylparathion, 3 Chlorpyriphos, 4 Profenofos.

Optimized solid-phase microextraction conditions. A time profile for analysis of OPP spiked in vegetable sample was determined, using ODS/GC-PFPD analytical method (n=3). Results are shown in Figure 3. It can be seen that as the signal increased with time up to 60 mins, the peak areas of chlorpyriphos and methylparathion still increased and no equilibrium could be found. However, considering the peak areas of all OPPs and the time used, it was decided to use 30 mins in all experiments.

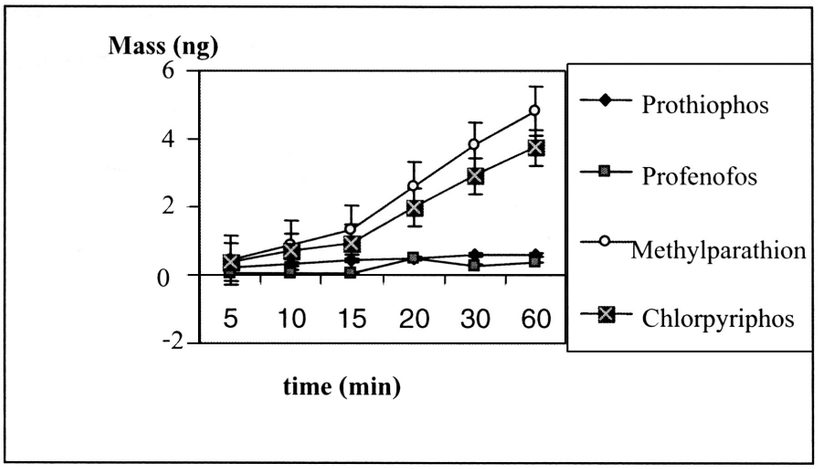

Figure 3. Absorption time profile of OPPs in vegetable using ODS SPME GC-PFPD analytical method (n=3).

The amounts of OPPs desorbed from the trapped fiber depended on the desorption temperature and the time that the fiber was in the GC injector port. The optimum desorption conditions were also studied. OPP working standards were analyzed by ODS SPME, using PFPD detection with the sampling periods of 30 minutes and desorption temperature at 250°C. Results are shown in Figure 4. Considering the peak areas of the OPP obtained at the time after 3 mins, there was not much change in the peak areas, so it was decided to use 4 mins for desorption time at 250°C in all experiments with ODS SPME /GC-PFPD analytical method.

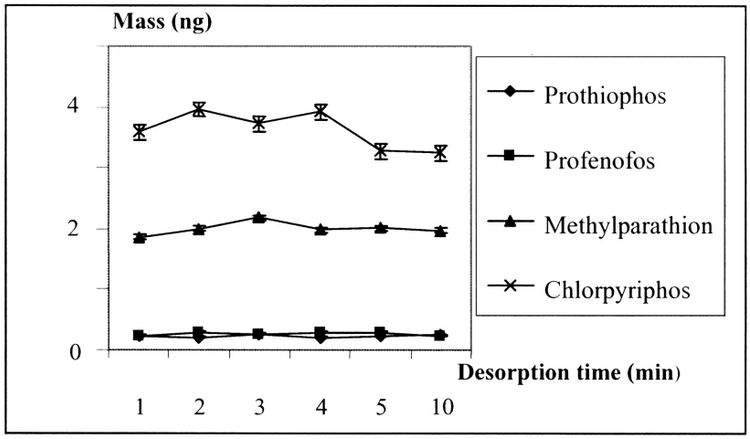

Figure 4. Desorption time profile for OPPs in vegetable (onion), using ODS/GC-PFPD analytical method (n=3).

Conditions for analysis of organophosphorus insecticide residues in vegetables using ODS SPME/GC PFPD analytical method for solid-phase microextraction of OPPs, as previously detailed in this paper, are summarized in Table 1.

Table 1. Optimized solid-phase microextraction conditions for analysis of some OPPs in vegetable samples, using ODS SPME/GC PFPD analytical method.

Operation	Optimal extraction conditions
1. extraction sampling mode	direct immersion
2. vial size	2 ml
3. sample volume	1 ml
4. sample preparation	1 g vegetable was added to 200 ml of ultrapure water
5. extraction temperature	ambient (25°C)
6. extraction time	30 minutes
7. desorption time	4 minutes
8. desorption temperature	250°C

Comparing the efficacy of fibers coated with different materials. The OPP standard solutions diluted with water were extracted, using PDMS, ODS, PAC, CW/DVB, CAR/PDMS and PDMS/DVB solid- phase microextraction fiber together with separation and quantification by DB-1 capillary column gas chromatography and pulsed-flame photometric detection. All components of OPP were resolved and the chromatogram was obtained from direct SPME sampling, and the results are shown in Table 2.

A suitable method for analysis of some organophosphorus pesticides was obtained in this study. The study began with developing ODS SPME fiber to replace commercial SPME fiber for extraction and using GC-PFPD method for analysis of methylparathion, chlorpyriphos, dicrotophos, prothiophos and profenofos. By using SPME to extract the analytes from sample solutions, the analytes are present in both fiber and solution. The distribution of analytes between both fiber and sample solution depends on the affinity of analyte for fiber and water. There are several reasons why a fiber coating was chosen. In the initial development of the SPME for determination of OPPs in the sample that has different matrix complexity such as water, soil and food, most papers described the use of 100 μm PDMS and PAC fiber coatings (Pawliszyn, 1999 ; Beltran et al., 2000). The precision of the OPP mixed standard determination, using ODS, was not significantly different from using commercial coated fibers. The ODS developed in this study was found to be as effective as PAC.

Table 2. Comparison of extraction capability of different coated fibers on the mean, standard deviation (S.D.) and the relative standard deviation, using aqueous solution of the OPP mixed standard of 1.00 mg/l of dicrotophos, chlorpyriphos, methamidophos, mevinphos, methylparathion, profenofos and prothiophos for analysis (n=3).

	Mean ± S.D. (mg/l) [% RSD]						
Prothiophos Type of fiber	Dicrotophos	Chlorpyriphos	Methamidophos	Mevinphos	Methylparathion	Profenofos	Prothiophos
PDMS	-	-	-	1.07 ± 0.48 [44.70]	0.90 ± 0.28 [30.39]	1.03 ± 0.05 [5.17]	0.94 ± 0.05 [5.31]
PAC	1.05 ± 0.63 [62.70]	0.64 ± 0.30 [47.20]	-	-	0.73 ± 0.37 [50.41]	-	0.87 ± 0.12 [13.60]
CW/DVB	-	-	1.06 ± 0.13 [12.50]	1.02 ± 0.03 [2.84]	1.09 ± 0.09 [8.46]	0.74 ± 0.23 [31.04]	0.56 ± 0.37 [66.67]
CAR/ PDMS	-	-	-	1.19 ± 0.18 [15.31]	-	1.03 ± 0.067 [6.44]	1.26 ± 0.33 [25.92]
PDMS/ DVB	-	-	-	1.24 ± 0.24 [19.72]	1.28 ± 0.01 [1.06]	1.22 ± 0.20 [17.16]	1.30 ± 0.40 [30.6]
ODS	0.99 ± 0.06 [5.56]	0.99 ± 0.04 [4.56]	-	-	1.00 ± 0.44 [4.42]	0.98 ± 0.01 [1.43]	0.99 ± 0.06 [5.56]

- no absorption

Standard solutions containing 1.00 μg/l, 5.00 μg/l, 0.01 mg/l and 1.00 mg/l of prothiophos, methylparathion, chlorpyriphos, mevinphos, methamidophos, profenofos and dicrotophos were added to sample solution. The solutions were analyzed (six replicates) by ODS/GC-PFPD analytical method, using the same procedure and conditions. The peak area appeared, it was graph-plotted with concentrations of the insecticide standards (10-50 μg/l, 0-1,000 μg/l, 0-0.40 mg/l). The graph that has R2 close to 1 was chosen to be linearity. Each working insecticide standard of the OPP was diluted and analyzed, using ODS/GC-PFPD. Dilutions were made in a series until no peak appeared. Detection limits (LOD) were determined by comparing the signal-to-noise (S/N) ratio of a minimum 3:1 to the lowest-detectable concentration. Results were then calculated to find percentage of relative standard deviation (%RSD). Precision, linearity and detection limits are shown in Table 3.

The linear concentration ranges are listed in Table 3. The relationships between the compound peak areas and OPP concentrations were linear with correlation coefficient (R^2), ranging from 0.950 to 0.999. Linearity extended in the range of 0-1,000 μg/l for chlorpyriphos, 0- 0.4 mg/l dicrotophos, 0- 0.4 mg/l methylparathion, 0-0.5 mg/l prothiophos and 10-50 μg/l profenophos. The limits of detection were determined based on a signal-to-noise ratio of 3,

ranging from 5 to 50 μg/l. The precision of the method, expressed as the relative standard deviation (RSD) of ODS SPME fiber were at concentrations ranging from about 5 μg/l to 1 μg/l OPPs from 5.0 to 10.8 %.

Table 3. Summarization of precision, linear range, detection limits of analysis of the OPP, using ODS SPME/GC-PFPD analytical method (n=6).

Compound	Retention time (min)	Regression equation	Linear range	R^2 between concentration OPP and peak area	Detection Limits (LOD) (μg/l)	Precision (%RSD)
Prothiophos	8.4	Y=25408x+513	0-0.5mg/l	0.996	50	5.2**
Profenofos	14.2	Y=76x+620	10-50μg/l	0.950	5	10.2*
Methylparathion	12.1	Y=134198x+5503	0-0.4 mg/l	0.967	40	10.8**
Chlorpyriphos	12.8	Y=39.76x+294	0-1,000μg/l	0.999	5	9.0*
Dicrotophos	14.2	Y=205229x+4102	0-0.4 mg/l	0.973	5	5.0*

*5 μg/l ,(n=6) time30 min and desorption time 4 min
**1 μg/ml ,(n=6) time5 min and desorption time 5 min

Table 4 shows mean recoveries of spiked OPP in vegetables, using ODS SPME fiber and GC-PFPD analysis. The ODS SPME fiber method for analyzing prothiofos, methylparathion, chlorpyriphos and profenofos in vegetables shows high mean recoveries. The recoveries of all compounds ranged from 99.80% to 106.52%. These recoveries are good enough for quantification of prothiofos, methylparathion, chlorpyriphos and profenofos in vegetables.

In comparing the percentage of recovery and percentage of standard deviation (%RSD) through analysis of OPPs using ODS, PDMS GC-PFPD with GC-FPD analytical method by applying t- test to analyzed the samples by $t_{calculated} < t_{table}$ at 95% confidence level, the results were not significantly different.

Table 4. Mean recoveries of spiked OPP in onion using ODS SPME fiber and GC-PFPD analysis (n=6).

Pesticide	Mean recoveries (%) onion
Methylparathion	101.51 ± 6.46
Chlorpyriphos	99.80 ± 9.40
Prothiophos	106.52 ± 3.96
Profenofos	104.03 ± 7.73

CONCLUSION

No report on using ODS for coating SPME fiber has been found but ODS- coated column has been reported to be used for analysis in the reversed phase of HPLC technique (Kohler

and Kirkland, 1987 ; Cazes, 2001). Moreover, there was no report on using SPME fiber with GC-PFPD.

In general, LLE, SPE and SCF are used for OPP analysis, of which require instrument and much solvent (Barnabas et al.,1994 ; Cunniff, 1997 ; Obana et al., 1997). At the Royal Project Foundation (RPF), GT pesticide test kit has been used for a routine check of pesticide residues in vegetables and fruits. This test kit is now used by most people who are involved with purchase of agricultural produces. Any random samples that were found to be of high chemical residue by this test kit would be analyzed further to find out what kind (name) of pesticide it was, using ODS/GCPFPD analytical method. The samples are extracted with organic solvent and each analysis consumes a lot of organic solvent. This ODS SPME is considered to be able to replace the organic solvent extraction. If so, it will help reduce the time for analysis; reduce the cost of investment and keep the environment clean and avoid hazard from the solvent to the analyst. Although, this study did not include analysis of carbamate and other pesticide group, there is a hope for using the ODS SPME to extract other pesticides. One thing that still cannot be done is making the bare fiber for coating . In this study, commercial coated fibers were used and the fibers had to be washed off the coated materials before coating with ODS. However, there is an advantage of using expired fiber of any kind which can no longer be reused by coating with ODS.

It is expected that this ODS SPME can be used to extract the pesticide residue directly from some fruits and vegetables by inserting the needle into them and allow the SPME fiber to absorb the pesticide directly from the fruit. If this expectation is correct, it would be very useful for the analytical work in the future because there is no need to blend the sample for extraction.

ACKNOWLEDGEMENTS

This work was supported by the Faculty of Science and Technology, Petchabun Rajabhat University, the analytical laboratory of Plant Protection Center, Royal Project Foundation and the Faculty of Pharmacy, Chiang Mai University.

REFERENCES

Barnabas, I.J., J.R. Dean, S.M. Hitchen, and S.P. Owen. 1994. Selective extraction of organochlorine and organophosphorus pesticides using a combined solid-phase extraction-supercritical fluid extraction approach. Anal. Chim. Acta., 261-267.

Beltran, J., F.J. Lopez, and F. Hernandez. 2000. Solid-phase microextraction in pesticide residue analysis. J. Chromatogr. A. 885: 389-404.

Cazes, J. 2001. Encyclopedia of chromatography. Marcel Dekker : New York.

Cunniff, P. 1997. Official Methods of Analysis of the AOAC International. 16th ed. Vol.1. AOAC International. : Virginia.

Helrich, K. 1990. Official Methods of Analysis of the Association of Official Analytical Chemists. 15th ed. Association of Official Analytical Chemist: Virginia.

Horwitz, W. 1970. Official Methods of Analysis of the Association of Official Analytical Chemists. 11th ed. Association of Official Analytical Chemist: Washington.

Hill, I.R., and S.T.L. Wright. 1978. Pesticide microbilology. J.W. Arrow smith : Bristol.

Kohler, J., and J.J. Kirkland. 1987 . Improved silica-based column packing for high-performance liquid chromatography. J. Chromatogr. 385 :125-150.

Mester, Z., R. Sturgeon, and J. Pawliszyn. 2001. Solid phase microextraction as a tool for trace element speciation. Spectrochimica Acta Part B. 56 : 233-260.

Nollet, L. M. L. 1992. Food analysis by HPLC. Marcel Dekker: New York.

Obana, H., K. Kikuchi, M.Okihashi, and S. Hori. 1997 . Determination of organophosphorus pesticides in foods using an accelerated solvent extraction system. Analyst 122 : 217 -220.

Pawliszyn, J. 1999. Applications of solid phase microextraction. Cornwall : MPG Books.

Pogacnik, L., and M. Franko. 1999. Determination of organophosphate and carbamate pesticides in spiked samples of tap water and fruit juices by a biosensor with photothermal detection, *Biosens.* Bioelectron 14: 569-578.

Sun, F., F. Lin, S. Wong, and G. Li. 2003. The screening of organophosphorus, organochlorine and synthetic pyrethroid pesticides residues in beef fat by tandem solid-phase extraction technique. J. Food and Drug Analysis. 11(3): 258-265.

Ulrich, S. 2000. Solid-phase microextraction in biomedical analysis. J.Chromatogr. A. 902: 167-194.

Xiao, C., H. Suqin, Z. Wang, J. Xing, and C. Wu. 2001. Application of the polysilicone fullerene coating for solid-phase microextraction in the determination of semi- volatile compounds. J. Chromatogr. A. 927: 121-130.