CHAPTER 4

RESULTS

4.1 The suitable GC-PFPD conditions for analysis of organophosphorus insecticide

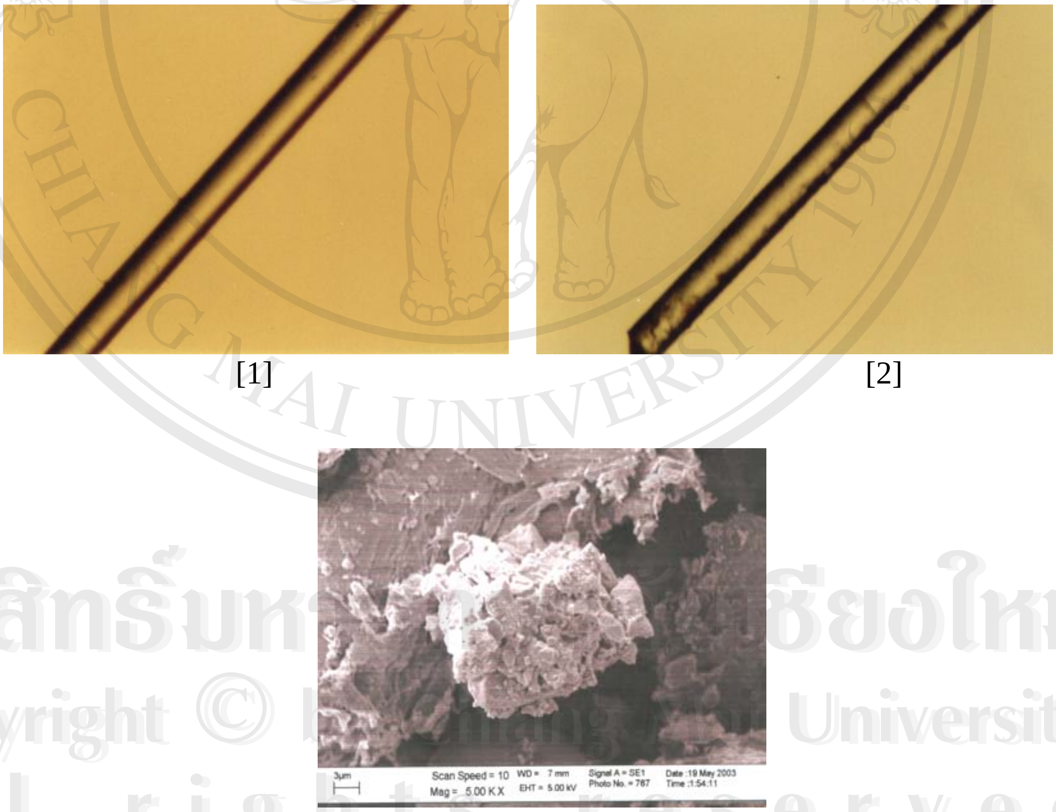
. The optimization of GC conditions was studied using the method shown in Table 3.2. Results form optimization of GC conditions for analysis of OPPs are shown in Table 4.1

Table 4.1 Conditions of GC PFPD employed

Operation	Conditions
1. Column	DB-1,30 m x 0.32 mm I.D.,1 μm film thickness
2. Temperature program	80ºC (0.5 min) –250 ºC (20 ºC /min) 250 ºC (1.75 min) – 300 ºC (20ºC /min) 300 ºC(6.75 min)
3. Injector temperature	250 ºC
4. Detector temperature	300 ºC
5. Injection	Splitless mode with SPME insert
6. Carrier gas	Nitrogen at 2.0 ml/min
7. Detector make up	Nitrogen at 45 ml/min
8. Hydrogen	14 ml /min
9. Air	27 ml /min

4.2 Coating SPME fibers with octadecylsiloxane

After the fiber had been coated with ODS 3 times, the surface of coated fiber was investigated under a stereomicroscope at 40x and made a comparison with uncoated fiber. The surface of uncoated fiber was smooth and thin [Fig. 4.1 -1] while the surface of coated fiber was rough and thick [Fig. 4.1 -2]. The coated fiber was scanned with SEM (scanning electron microscope) at 5 kx and found that there were lumps of ODS lie along the surface of fiber [Fig. 4.1-3].



[1] [2]

[3]

Figure 4.1 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 a SEM.

4.3 The suitable conditions of SPME/GC–PFPD for analysis of organophosphorus insecticide

4.3.1 Trying condition of SPME

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 chromatography was obtained from direct SPME sampling. Retention times of OPP are shown in Table 4.2, and six chromatograms of OPP are shown in Figures 4.2, 4.3 , 4.4 ,4.5, 4.6 and 4.7, respectively.

Table 4.2 Comparison of retention times (min) of OPP standard solution obtained from using various type coated fiber for analysis of OPPs insecticide.

Order of elution	Compound	Retention time (min)					
		PDMS	ODS	PAC	CW/ DVB	CAR/ PDMS	PDMS/ DVB
1	Mevinphos	8.67	-	-	8.60	8.58	8.58
2	Methylparathion	12.41	12.07	11.72	12.26	-	12.24
3	Profenofos	13.18	14.15	-	13.02	13.00	13.00
4	Prothiophos	14.66	8.39	9.58	14.50	14.48	14.48
5	Chlorpyriphos	18.00	12.78	12.48	-	-	-
6	Dicrotophos	-	14.15	13.80	-	-	-
7	Methamidophos	-	-	-	8.50	-	-

4.3.2 Fiber comparison

An autosampler SPME fiber was used to perform the experiments with fused silica fiber coated with different kinds of polymer i.e. polydimethylsiloxane (100 μm), polyaerylate (85 μm), polydimethylsiloxane/divinylbenxene (65 μm), carboxen ™/polydimethylsiloxane and carbowax®/divinylbenzene (65 μm) to compare with fiber coated with ODS developed in this study. Results from analysis of organophosphorus pesticides spiked in vegetable sample, using ODS SPME /GC-PFPD are shown in Table 4.3.

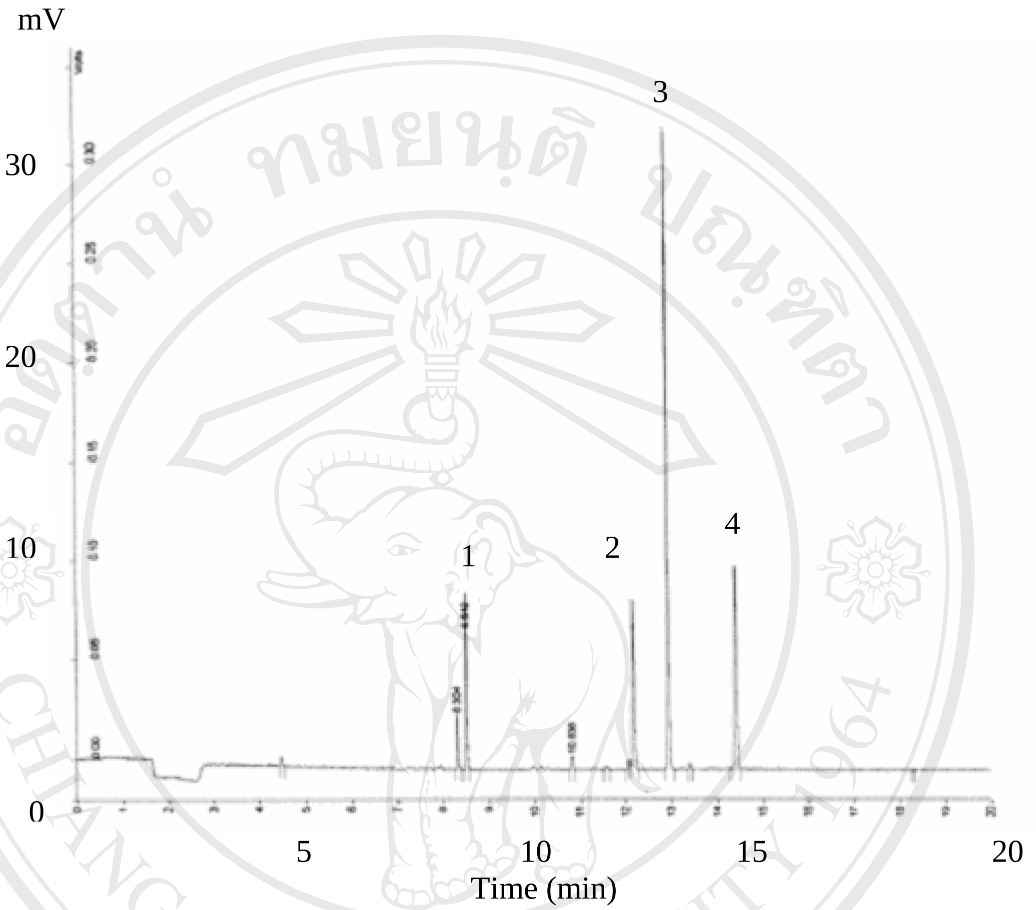


Figure 4.2 PFPD chromatogram of mixed OPPs standard solution was obtained by direct PDMS-SPME sampling under optimum conditions of SPME/GC–PFPD, using the conditions listed in Table 4.1. The identified peaks are mevinphos [1], methylparathion [2], profenofos [3], prothiophos [4].

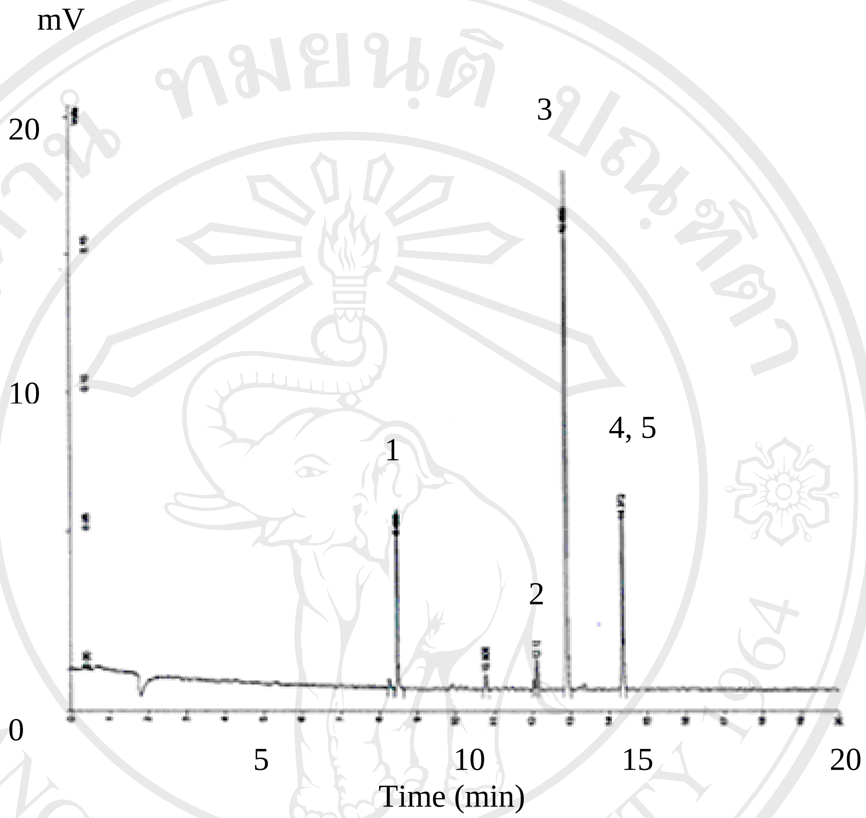


Figure 4.3 PFPD chromatogram of mixed OPPs standard solution was obtained by direct ODS-SPME sampling under optimum conditions of SPME/GC–PFPD, using the conditions listed in Table 4.1. The identified peaks are prothiophos [1], methylparathion [2], chlorpyriphos [3], profenofos [4], dicrotophos [5].

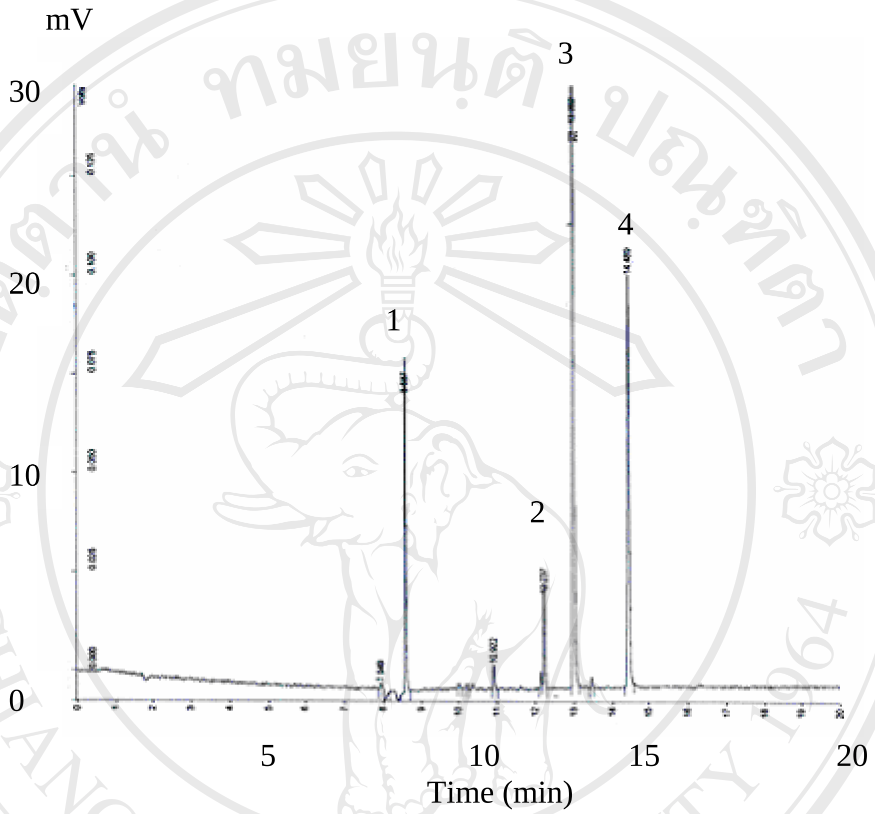


Figure 4.4 PFPD chromatogram of mixed OPPs standard solution was obtained by direct PAC-SPME sampling under optimum conditions of SPME/GC–PFPD, using the conditions listed in Table 4.1. The identified peaks are prothiophos [1], chlorpyriphos [2], methylparathion [3], dicrotophos [4].

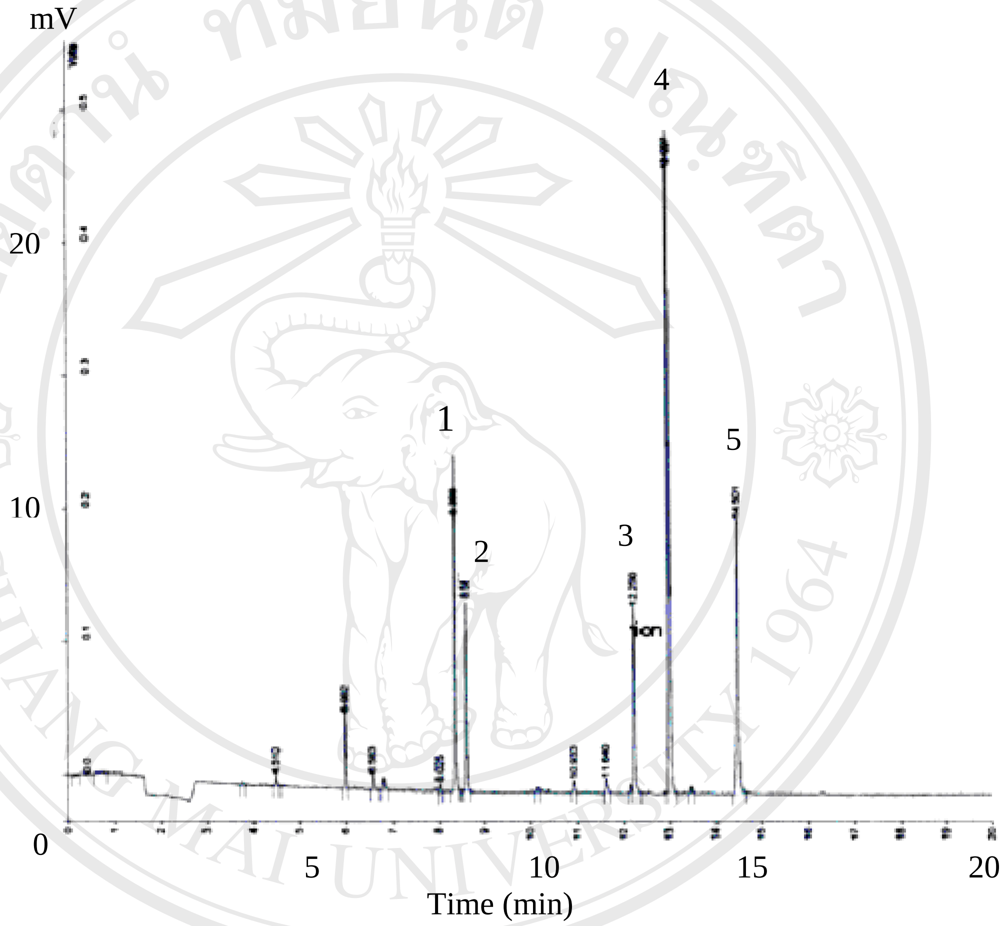


Figure 4.5 PFPD chromatogram of mixed OPPs standard solution was obtained by direct CW/DVB-SPME sampling under optimum conditions of SPME/GC–PFPD, using the conditions listed in Table 4.1. The identified peaks are methamidophos [1], mevinphos [2], methylparathion [3], profenofos [4], prothiofos [5].

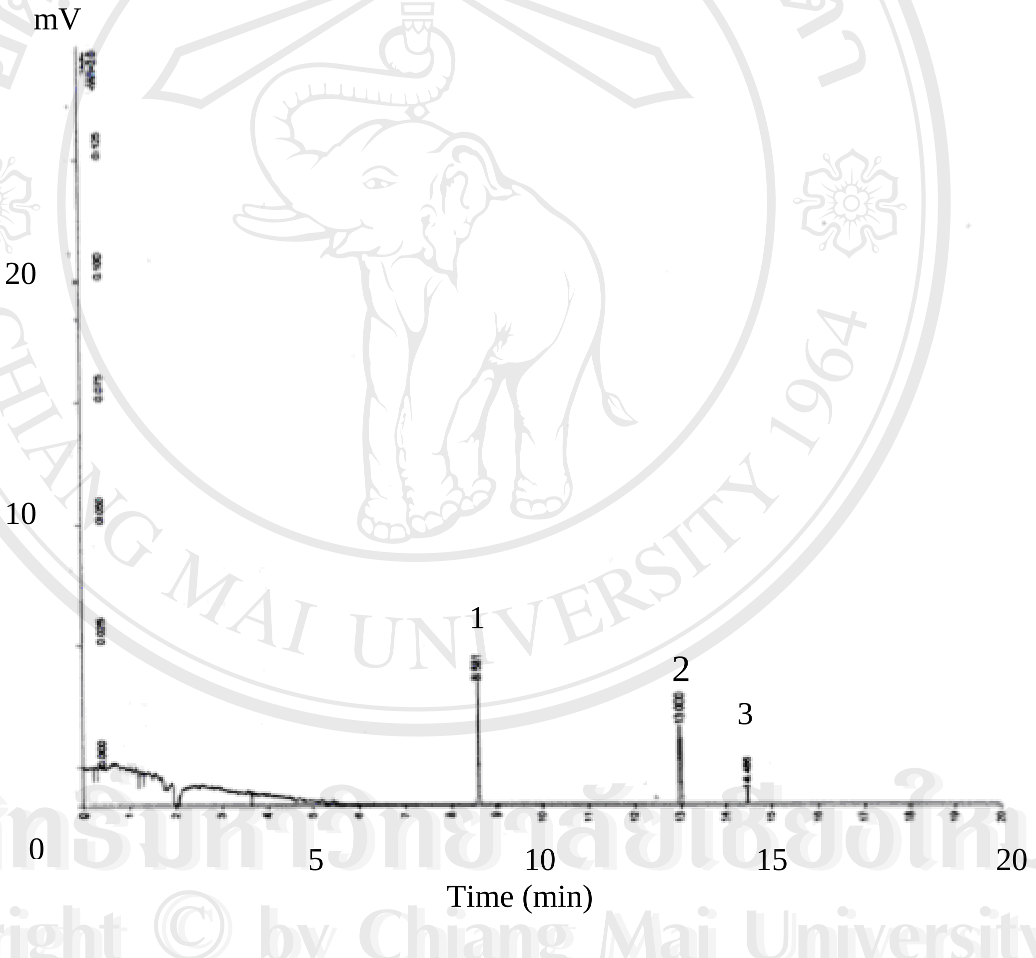


Figure 4.6 PFPD chromatogram of mixed OPPs standard solution was obtained by direct CAR/PDMS-SPME sampling under optimum conditions of SPME/GC–PFPD, using the conditions listed in Table 4.1. The identified peaks are mevinphos [1], profenofos [2], prothiophos [3].

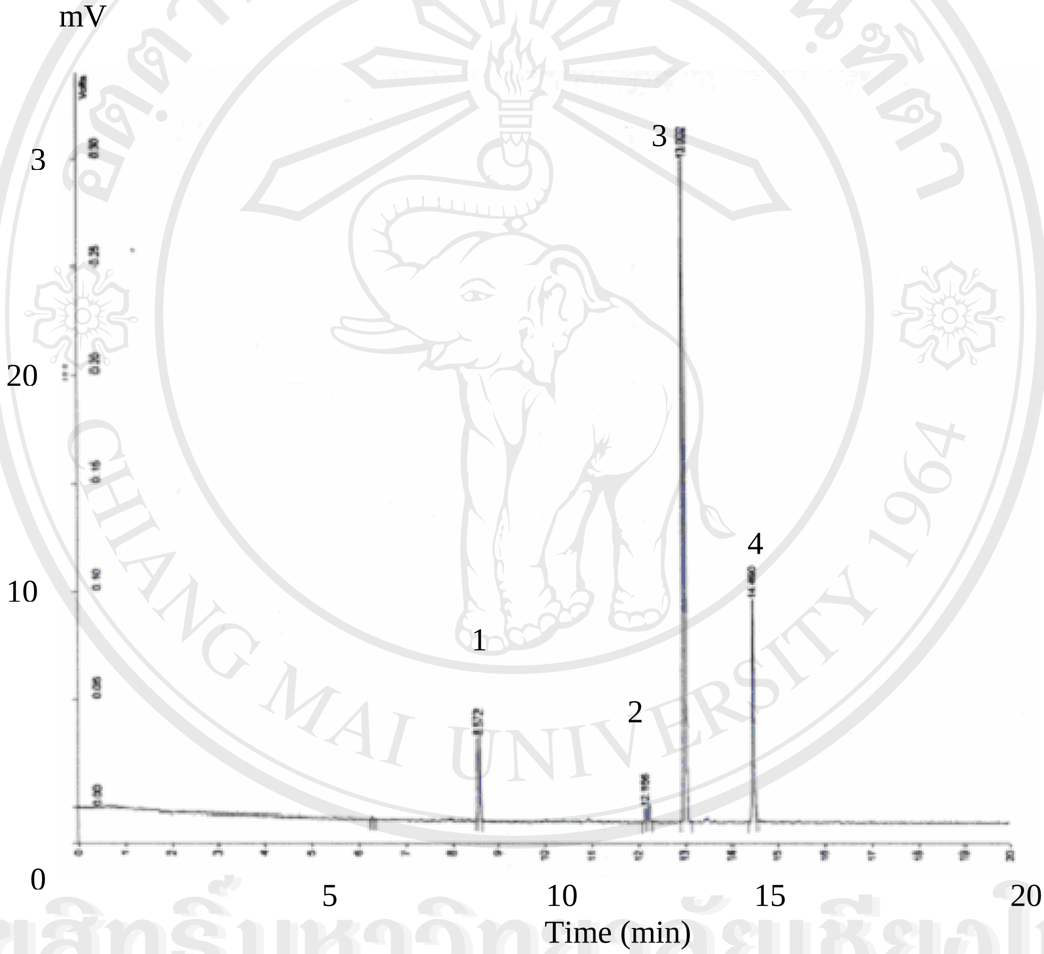


Figure 4.7 PFPD chromatogram of mixed OPPs standard solution was obtained by direct PDMS/DVB-SPME sampling under optimum conditions of SPME/GC–PFPD, using the conditions listed in Table 4.1. The identified peaks are mevinphos [1], methylparathion [2], profenofos [3], prothiophos [4].

Table 4.3 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]						
Type of fiber	Dicrotophos	Chlorpyriphos	Methamidophos	Mevinphos	Methylparathion	Profenofos	Prothiophos
PDMS	Not absorption	not absorption	not absorption	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]	not absorption	not absorption	0.73 ± 0.37 [50.41]	not absorption	0.87 ± 0.12 [13.60]
CW/ DVB	not absorption	not absorption	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	not absorption	not absorption	not absorption	1.19 ± 0.18 [15.31]	not absorption	1.03± 0.067 [6.44]	1.26 ± 0.33 [25.92]
PDMS /DVB	not absorption	not absorption	not absorption	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]	not absorption	not absorption	1.00 ± 0.44 [4.42]	0.98 ± 0.01 [1.43]	0.99 ± 0.06 [5.56]

The precision of the OPP mixed standard determination, using ODS SPME, was not significantly different from using commercial coated fibers. The ODS SPME developed in this study was found as effective as polyacralate (PAC) SPME.

4.3.3 Selection of the sampling mode

Three sampling modes of SPME were compared; direct immersion, headspace and agitation. Results of the peak areas of OPP obtained from comparison of agitation and direct immersion ODS procedures and their precision are shown in Table 4.4 and Table 4.5. Histograms of these results are shown in figure 4.9

Table 4.4 Peak areas of OPP obtained by ODS/GC-PFPD (n=3).

Compound	Peak area (pA) $\bar{X}$ ± S.D.		
	Direct immersion	Agitation	Headspace
Chlorpyriphos	75337 ± 3428	1318367± 218297	67543 ± 6653
Prothiophos	27354 ± 1520	46418 ± 8635	22202 ± 2469
Profenofos	26557 ± 379	594003 ± 326059	27518 ± 2063
Methylparathion	5769 ± 255	87948 ± 15850	5619 ± 15

Table 4.5 Comparison of the precision obtained by three techniques of extraction of the OPP aqueous solution, using ODS/GC-PFPD.

Extraction method	% RSD			
	Prothiophos	Methylparathion	Chlorpyriphos	Profenofos
Direct immersion	5.56	4.42	4.56	1.43
Agitation	18.60	18.02	16.56	54.89
Headspace	11.12	0.28	9.80	7.50

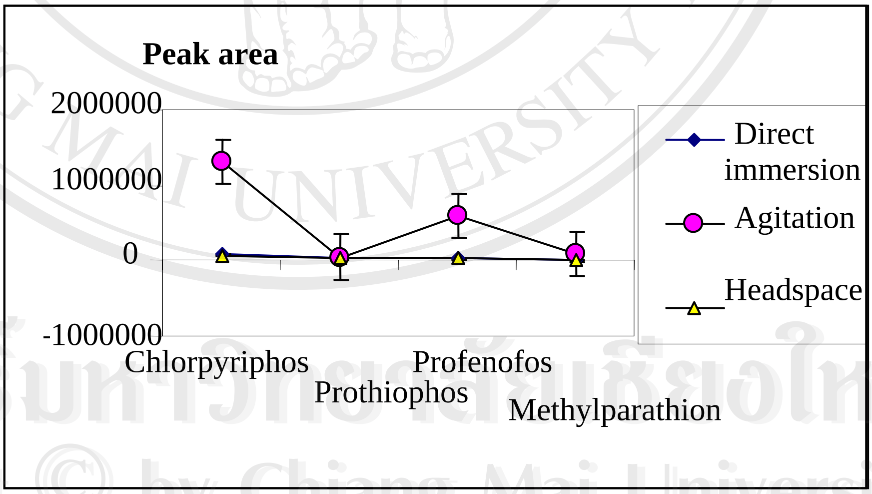


Figure 4.8 Peak areas of OPP obtained by three techniques using SPME/GC-PFPD (n=3)

The use of ODS- SPME for the OPP extraction, direct immersion gave best precision among the three techniques. Since the OPP compounds are not volatile so direct immersion can absorp the analytes more than the others. The ODS fiber was directly exposed to the aqueous solution at room temperature (25°C). So the sample temperature was measured at 25 ±2°C.

4.3.4 Determination of absorption time

A time profile for absorption into the ODS fiber of some organophosphorus was determined in order to assess the optimum SPME sampling period. Results are shown in Table 4.6 and Figure 4.9

Table 4.6 Determination of absorption-time profile for OPP absorption with an ODS fiber by GC-PFPD analysis (n=3).

Absorption time (min)	Peak area			
	Prothiophos	Methylparathion	Chlorpyriphos	Profenofos
1	5,222	0	16,313	11,227
2	7,613	0	36,187	38,495
3	11,241	2,104	44,449	31,374
4	14,662	28,94	88,088	70,862
5	12,321	4,471	92,955	104,830
10	17,136	7,318	169,711	143,542
15	32,563	24,920	342,899	244,997
20	22,718	30,680	387,831	341,920
25	26,323	58,095	451,736	360,577
30	26,676	60,021	455,431	370,579

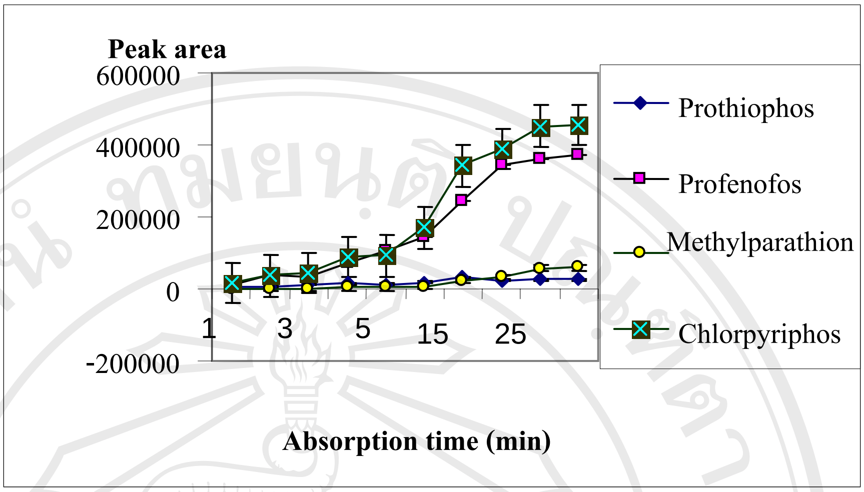


Figure 4.9 Absorption time profile of OPP into an ODS fiber, using GC-PFPD analysis (n=3).

A time profile for absorption of OPP spiked in vegetable sample was determined, using ODS/GC-PFPD analytical method (n=3). Result are shown in Table 4.7 and Figure 4.10.

Table 4.7 Determination of absorption-time profile for OPP spiked in vegetable of using ODS SPME /GC-PFPD analytical method (n=3).

Absorption time (min)	Peak area			
	Prothiphos	Profenofos	Methylparathion	Chlorpyriphos
5	10,589	1,563	21,264	19,585
10	17,742	2,642	45,163	35,053
15	22,185	3,588	66,111	48,385
20	25,056	23,736	131,814	99,569
30	29,553	15,019	190,523	146,088
60	30,424	18,748	242,505	187,504

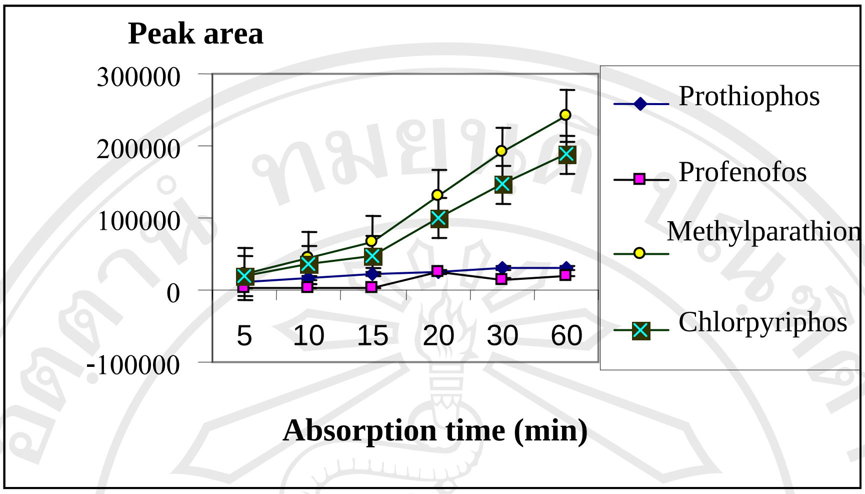


Figure 4.10 Absorption time profile of OPPs in vegetable using ODS SPME/GC-PFPD analytical method (n=3).

It can be seen that the signal increased with time up to 60 mins of absorption, the peak areas of chlorpyriphos and methylparathion still increased, no equilibrium could be found. However, considering the peak areas of all OPPs and the time used, it was decided to use 30 mins for absorption in all experiments.

A time profile for OPP absorption into the PDMS fiber was determined in order to assess the optimum SPME sampling period, using PDMS/GC-PFPD. Results are shown in Table 4.8 and Figure 4.11

Table 4.8 Determination of absorption time for OPP mixture solution with direct immersion, using PDMS SPME/GC-PFPD analytical method (n=3).

Absorption time (min)	Peak area			
	Mevinphos	Methylparathion	Profenofos	Prothiophos
5	-	-	17,367	3,443
10	2,798	-	35,682	5,522
20	5,500	-	72,441	13,802
30	6,614	2,320	104,978	24,578
40	7,361	2,451	118,424	27,609
50	9,980	4,735	179,085	39,020
60	10,039	5,438	163,636	34,952

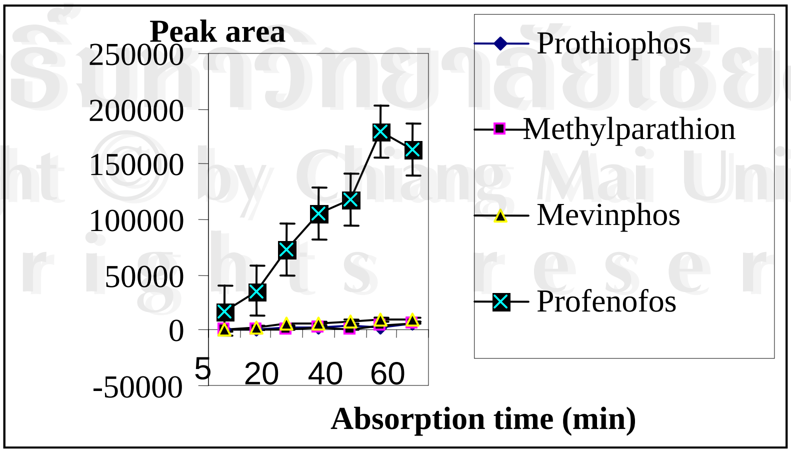


Figure 4.11 Absorption time profile of OPP mixture solution, using PDMS SPME/GC-PFPD analytical method (n=3)

A time profile for absorption of OPPs in vegetable sample was determined using PDMS SPME/GC-PFPD analytical method (n=3). Results are shown in Table 4.9 and Figure 4.12.

Table 4.9 Comparison of absorption periods on peak areas of OPP spiked in vegetable (onion) extract using direct immersion technique and PDMS SPME/GC- PFPD analytical method (n=3).

Absorption time (min)	Peak area			
	Mevinphos	Methylparathion	Profenofos	Prothiophos
5	-	-	13,456	-
10	2,131	-	36,113	-
20	3,100	-	39,834	1,530
30	4,962	1,588	78,926	3,025
45	6,121	2,013	102,346	4,223
60	6,631	2,112	54,142	2,981
90	8,982	6,600	179,005	6,772

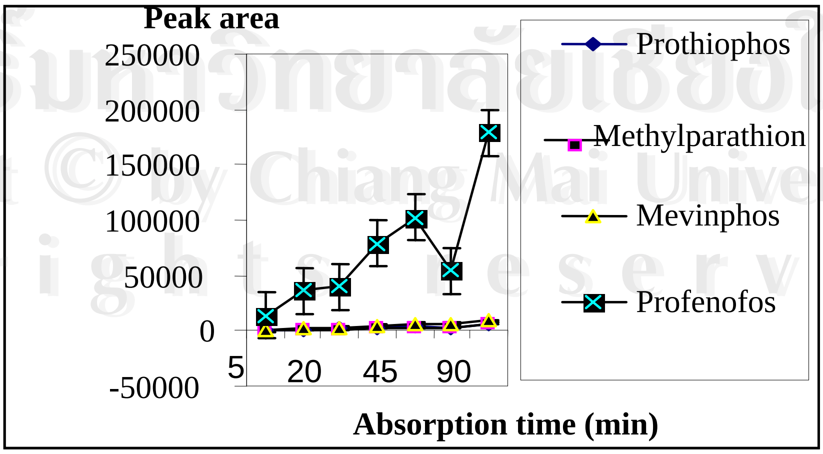


Figure 4.12 Absorption time profile for OPPs in vegetable (onion) extract using PDMS SPME/GC - PFPD analytical method (n=3).

No equilibrium could be observed up to 90 mins of absorption time on analysis of OPP in onion sample, using PDMS SPME/GC - PFPD analytical method. Considering the peak areas of all OPP and the time used, it was decided to use 30 mins for absorption.

4.3.5 Determination of desorption temperature

The temperature range from 210 – 250 °C were tested on desorption of the OPP. It was found that at 250 °C showed highest peak area of chlorpyriphos and prothiophos as shown in Table 4.10 and Figure 4.13

Table 4.10 Comparison of temperature (injection port temperature) on OPPs desorption from the ODS fiber, using SPME/GC-PFPD analytical method (n=2)

Temperature (°C)	Peak area	
	Chlorpyriphos	Prothiophos
210	14,205	5,114
220	97,781	37,908
230	83,124	18,619
240	42,449	13,491
250	123,303	41,207

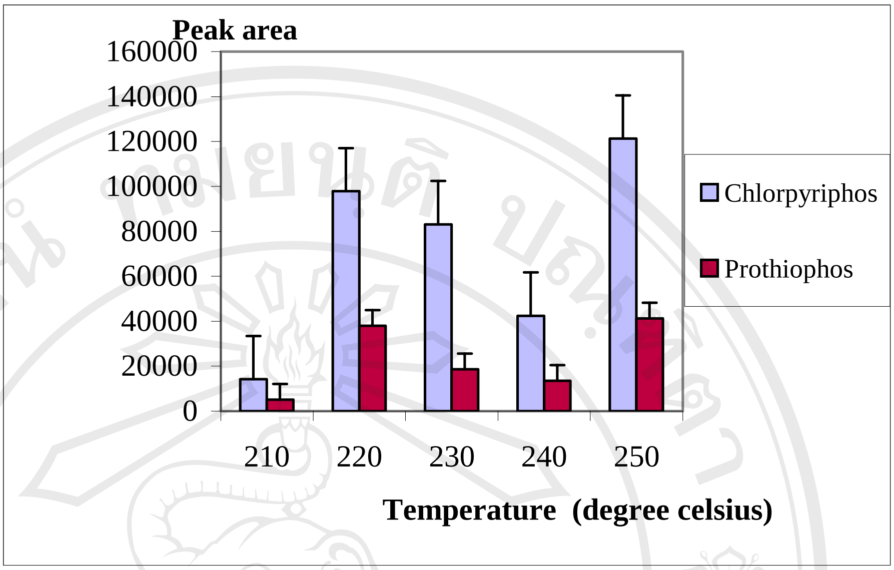


Figure 4.13 Desorption temperature (injection port temperature) of the OPP from ODS fiber, using SPME/GC-PFPD analytical method (n=2)

In order to allow all the OPP from the ODS fiber entirely desorped, it was decided to use 250°C for desorption temperature throughout all experiments.

4.3.6 Determination of desorption time profile

OPP working standards were analyzed by ODS SPME using PFPD detection with the sampling period of 30 minutes and desorption temperature at 250 ºC. Results are shown in Table 4.11- 4.13 and Figure 4.14- 4.16

Table 4.11 Comparison of time profile for desorption of OPP using ODS SPME/GC-PFPD analytical method (n=3).

Desorption time (min)	Peak area			
	Prothiophos	Methylparathion	Chlorpyriphos	Profenofos
1	14,370	8,102	132,407	87,067
2	15,536	6,595	124,024	92,345
3	14,687	9,951	120,990	89,685
4	13,646	6,239	107,689	94,588
5	12,661	4,521	88,798	72,450
10	14,597	5,854	142,413	92,398
15	14,478	5,345	116,637	87,138
20	15,033	4,640	117,167	97,488

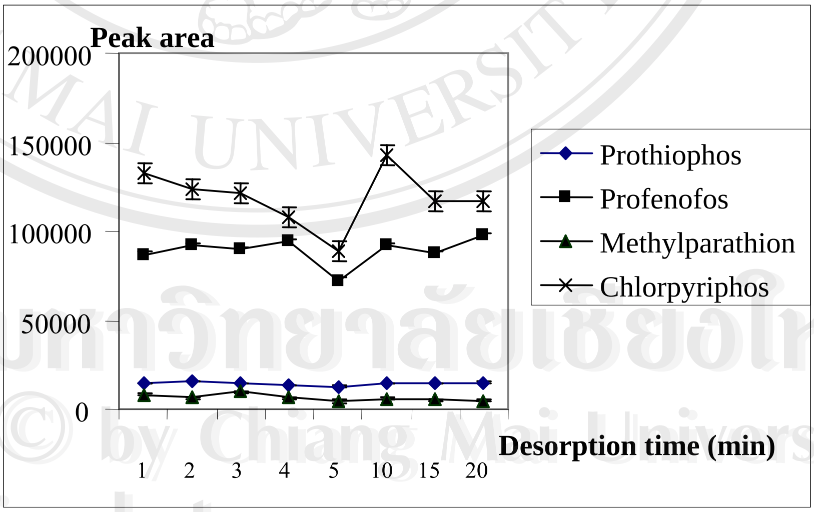


Figure 4.14 Desorption time profile for OPP using ODS SPME/GC-PFPD analytical method (n=3).

Table 4.12 Comparison of time profile for desorption of the OPPs in vegetable (onion), using ODS SPME/GC-PFPD analytical method (n=3).

Desorption time (min)	Peak area			
	Methylparathion	Chlorpyriphos	Profenofos	Prothiophos
1	93,296	179,634	10,669	11,226
2	100,298	198,005	14,256	10,268
3	109,372	186,648	12,897	12,362
4	99,094	196,028	15,590	10,467
5	94,145	164,185	13,438	7,726
10	98,536	162,676	11,847	10,701
15	83,097	132,872	8,729	11,870
20	111,999	176,725	12,894	12,116

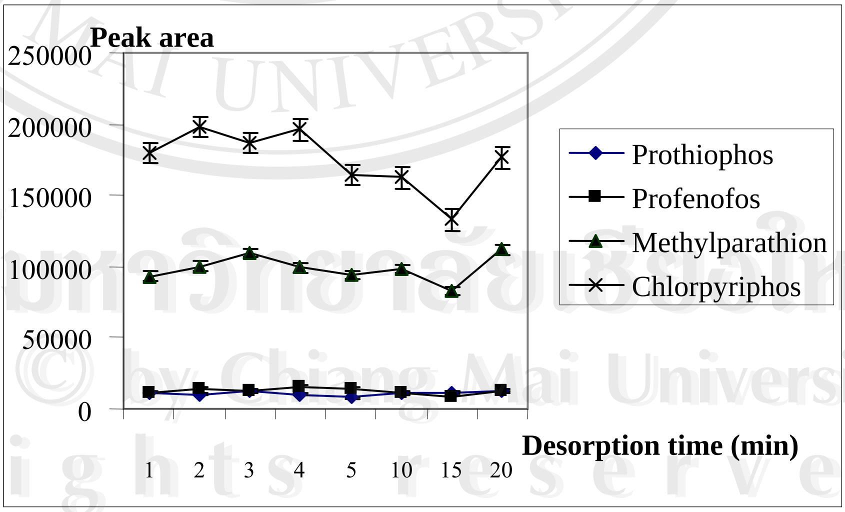


Figure 4.15 Desorption time profile for OPPs in vegetable (onion), using ODS SPME/GC-PFPD analytical method (n=3).

Table 4.13 Comparison of time profile for desorption of OPPs in vegetable (onion), using PDMS SPME/GC-PFPD analytical method (n=2).

Absorption time (min)	Peak area			
	Mevinphos	Methylparathion	Profenofos	Prothiophos
3	4,273	2,263	72,860	2,689
5	4,833	3,403	81,047	3,066
10	3,815	2,351	80,139	2,582
15	4,566	2,328	77,462	2,722
20	4,502	2,043	67,478	2,286

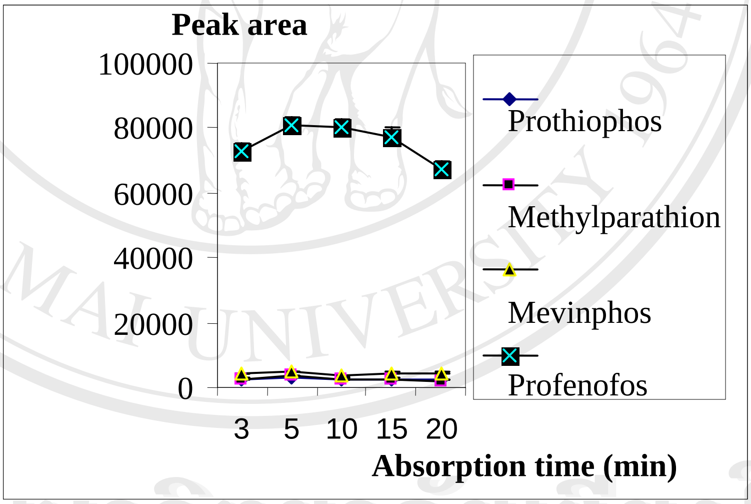


Figure 4.16 Desorption time profile for OPPs in vegetable(onion), using PDMS SPME/GC-PFPD analytical method (n=2).

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 PDMS/GC-PFPD analytical method.

4.3.7 Determination of ODS fiber capacity on absorption of the OPP at different concentration.

The ODS fiber was tested on its capacity on desorption of the OPP at different concentrations by dipping it in the OPP mixed standard solution at concentrations of 0.1, 0.2, 0.5 and 1.0 mg/l. This was determined by using SPME/GC-PFPD analytical method. Result are shown in Table 4.14 and Figure 4.17

Table 4.14 Comparison of capacity of ODS fiber on absorption of the OPPs in the onion extracts at different concentrations, using ODS SPME/GC-PFPD analytical method (n=2)

Concentration (ppm)	Peak area			
	Mevinphos	Methylparathion	Profenofos	Prothiophos
0.1	4,273	2,263	72,860	2,689
0.2	4,833	3,403	81,047	3,066
0.5	3,815	2,351	80,139	2,582
1.0	4,566	2,328	77,462	2,722

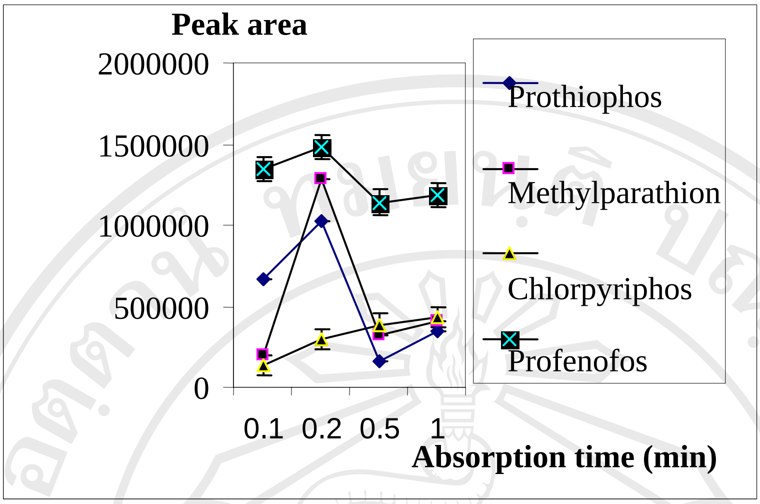


Figure 4.17 Capacity of ODS fiber for absorption of OPPs in onion extract, using ODS SPME/GC-PFPD analytical method (n=2)

Considering the peak areas of the OPP, it was decided to use the vegetable extract at concentration of <0.2 ppm (mg/l) for next experiment.

4.3.8 Determination of suitable conditions for analysis of organophosphorus insecticide residues in vegetable using ODS SPME/GC PFPD analytical method.

All suitable conditions for solid-phase microextraction of OPPs from vegetable previously described in this paper can be summarized in table 4.15

Table 4.15 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	1. direct immersion
2 Vial size	2. 2 ml
3 Sample volume	3. 1 ml
4 Sample preparation	4. 1 g vegetable in 200 ml of ultrapure water
5. Extraction temperature	5. at ambient (24-25°C)
6. Absorption time	6. 30 mins
7. Desorption time	7. 4 mins
8. Desorption temperature	8. 250 °C

4.3.9 Identification of prothiophos, methyl parathion, chlorpyriphos, mevinphos, methamidophos, profenofos and dicrotophos residues in vegetable sample Identification of OPP by ODS SPME/GC-PFPD

The optimum conditions for analysis of the OPPs summarized in 4.3.8 were used for running analysis of the OPP standard solution dissolved in ultrapure water, using ODS SPME/GC- PFPD analytical method. Results showed that such conditions produced good peaks of all five OPP compounds and retention time which can confirm the results of optimization as shown in Figure 4.18.

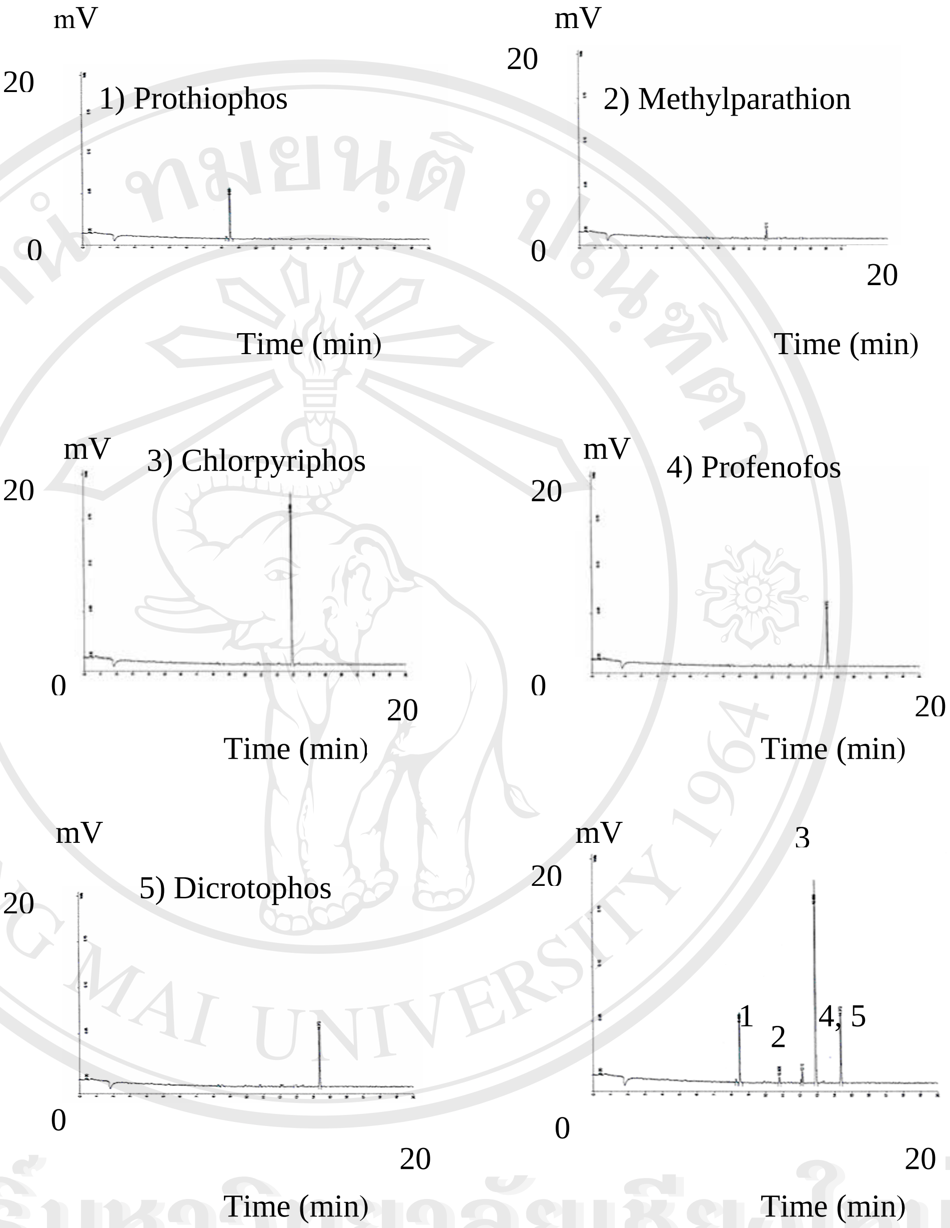


Figure 4.18 Chromatograms of 5 organophosphorus insecticides and the retention time required for each insecticide, using the ODS SPME/GC-PFPD

A analysis of the organophosphorus insecticides in vegetables under optimum conditions of ODS SPME/GC–PFPD listed in Table 4.15, and two chromatograms are shown in Figures 4.19 and 4.20

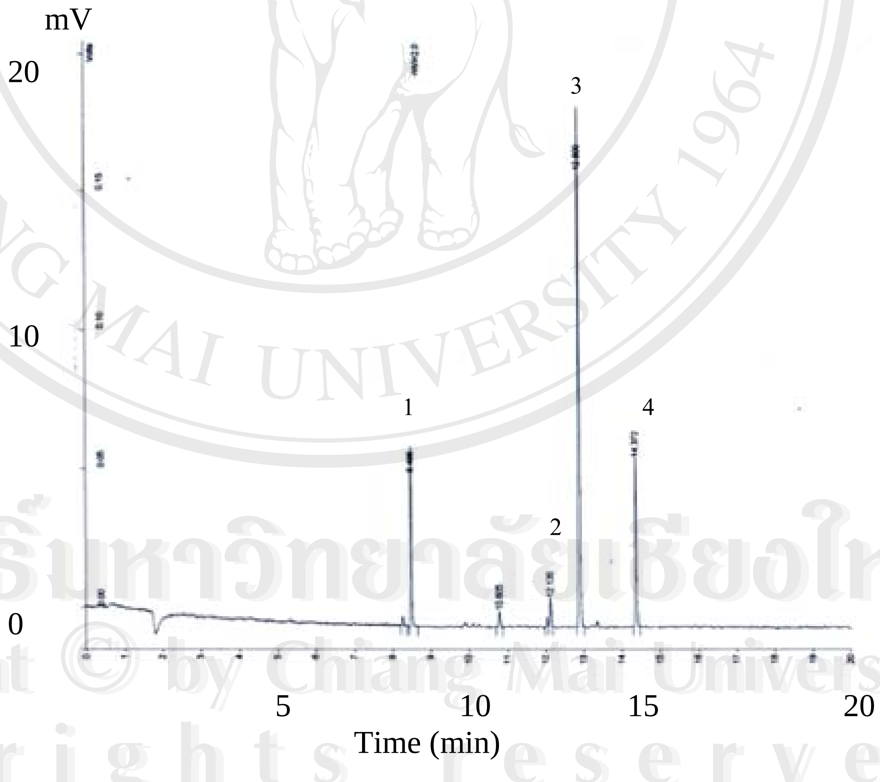


Figure 4.19 PFPD chromatogram water spiked with mixed OPP standard solution obtained from ODS SPME /GC – PFPD analytical method, using the conditions listed in Table 4.15. The identified peaks are prothiophos [1], methylparathion [2], chlorpyriphos [3], profenofos [4]

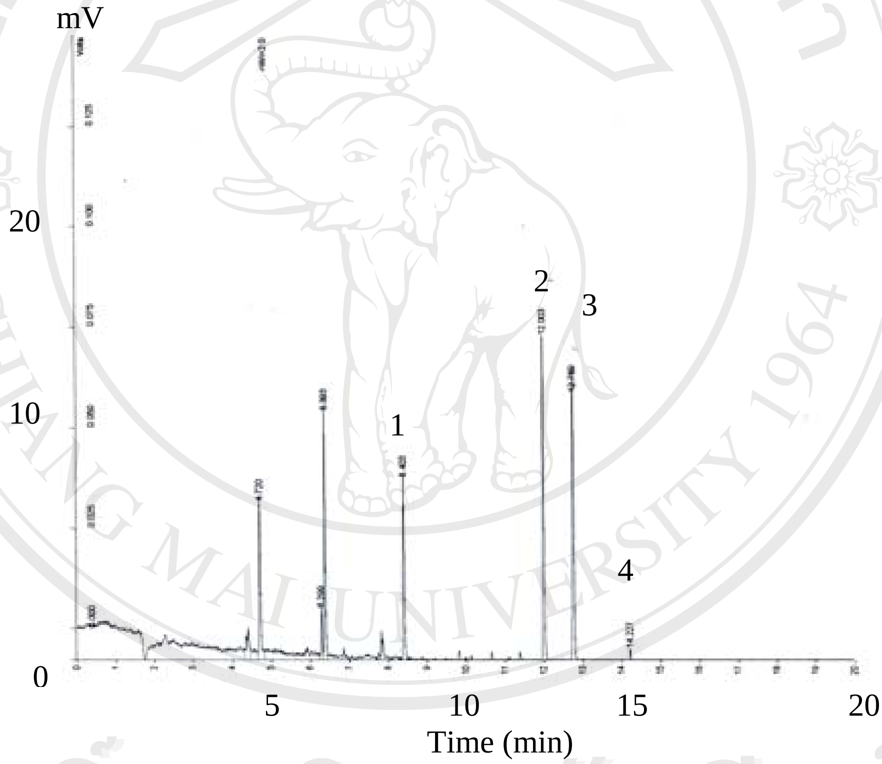


Figure 4.20 PFPD Chromatogram of onion extract spiked with mixed OPPs standard solution obtained from ODS SPME/GC–PFPD, using the conditions listed in Table 4.15. The identified peaks are prothiophos [1], methylparathion [2], chlorpyriphos [3], profenofos [4]

4.4 Validation of the analytical method using SPME /GC-PFPD.

4.4.1 Determination of linearity

4.4.1.1 Using PDMS SPME/GC-PFPD analytical method

After injection of each working insecticide standard into the GC. The peak area appeared, it was graph plotted with the insecticide standards at concentrations of 10-50 ppb, 0-1000 ppb, 0-0.40 ppm. Figure 4.21, 4.22, 4.23, 4.24 and 4.25 show linearity of organophosphorus standard, using PDMS SPME/GC-PFPD analytical method.

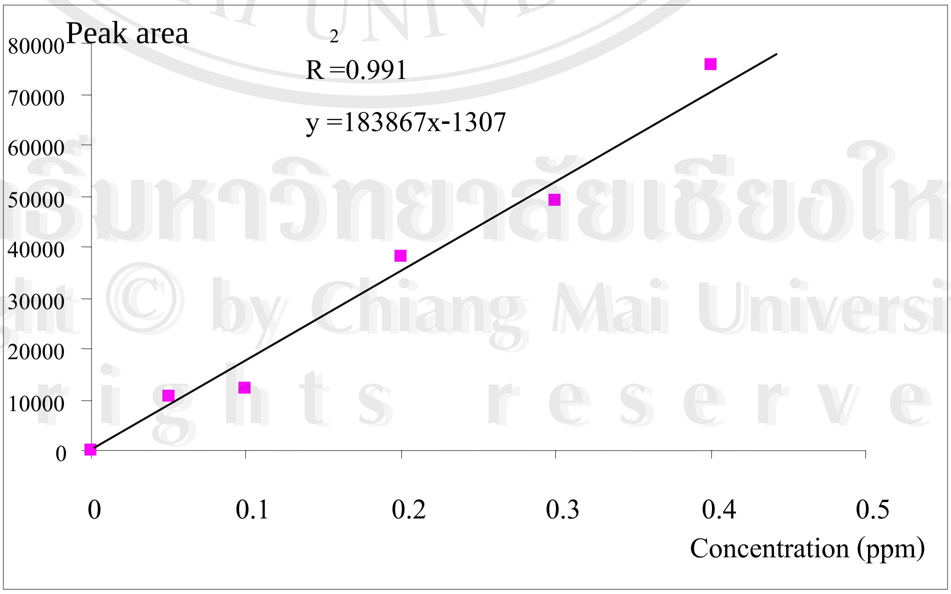


Figure 4.21 Linearity of peak area plotted with concentrations of mevinphos at 0-0.4 ppm, using PDMS SPME/GC-PFPD analytical method.

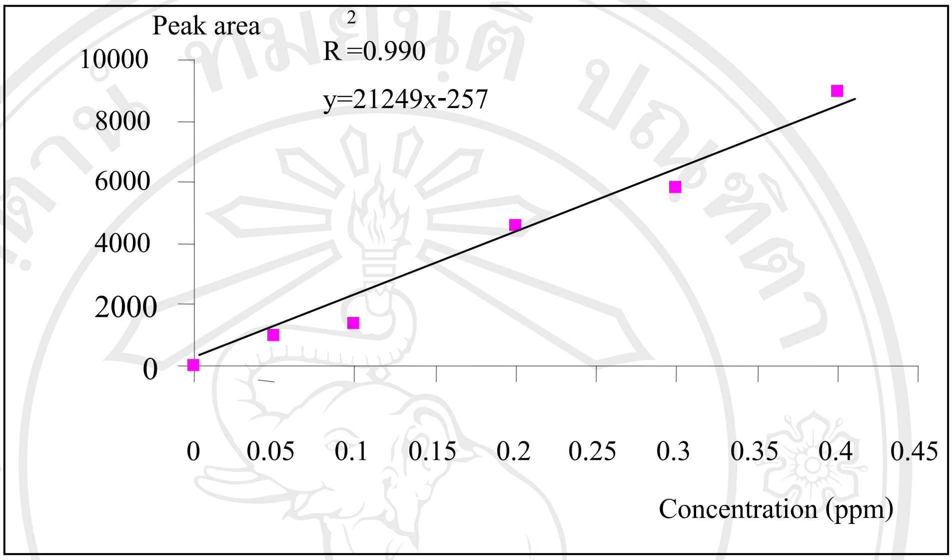


Figure 4.22 Linearity of peak area plotted with concentrations of methyl parathion at 0-0.4 ppm, using PDMS SPME/GC-PFPD analytical method.

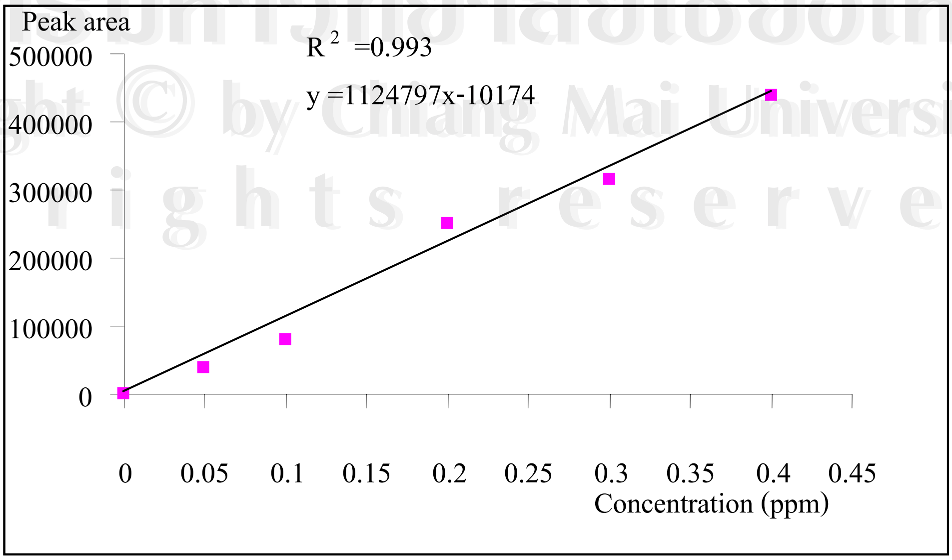


Figure 4.23 Linearity of peak area plotted with concentrations of profenofos at 0-0.4 ppm, using PDMS SPME/GC-PFPD analytical method.

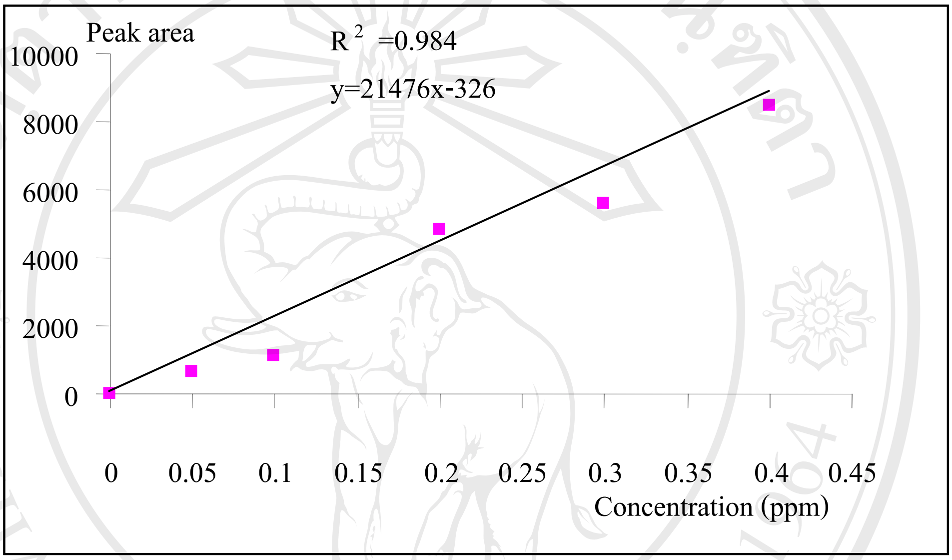


Figure 4.24 Linearity of peak area plotted with concentrations of prothiophos at 0-0.4 ppm, using PDMS SPME/GC-PFPD analytical method.

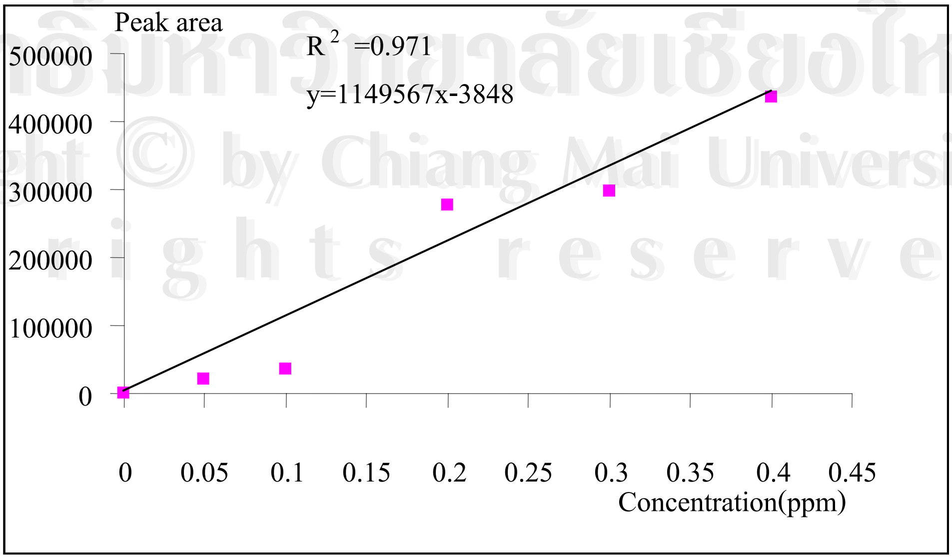


Figure 4.25 Linearity of peak area plotted with concentrations of chlorpyriphos at 0-

0.4 ppm, using PDMS SPME/GC-PFPD analytical method.

4.4.1.2 Using ODS SPME/GC-PFPD

After injection of each working organophosphorus standard into the GC. The peak area appeared, it was graph plotted with the insecticide standards at concentrations 10-50 ppb, 0-1000 ppb and 0-0.40 ppm. Figure 4.26, 4.27, 4.28 , 4.29 and 4.30 show that linearity of organophosphorus using ODS SPME/GC-PFPD analytical method.

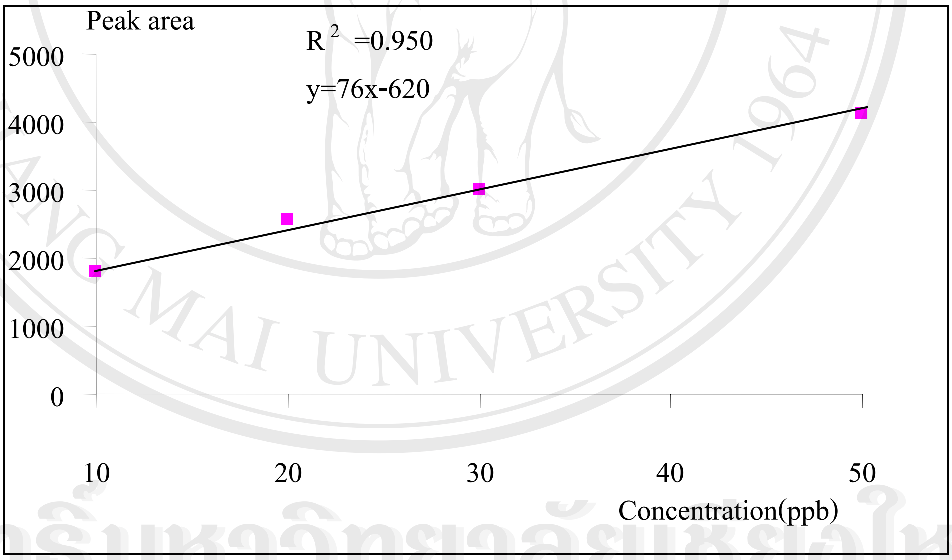


Figure 4.26 Linearity of peak area plotted with concentrations of profenofos at 10-50 ppb, using ODS SPME/GC-PFPD analytical method.

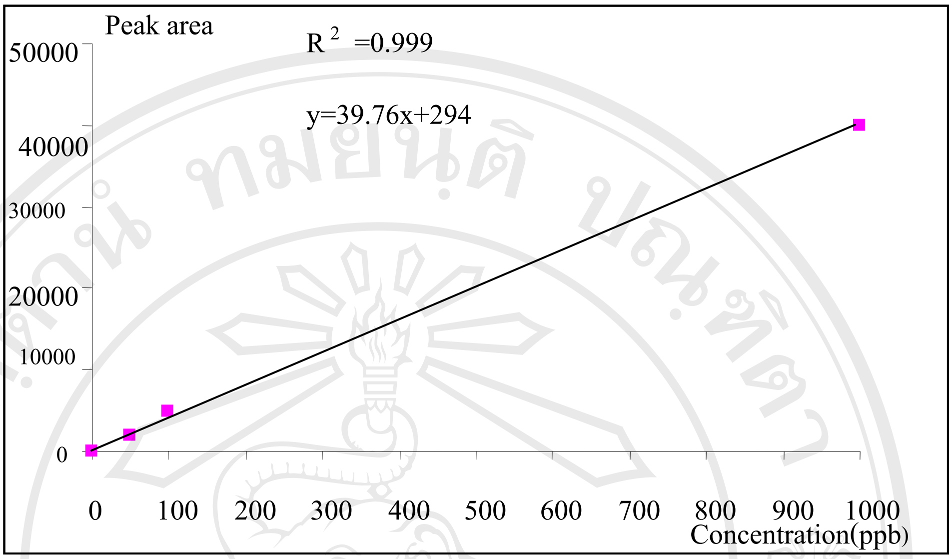


Figure 4.27 Linearity plot of peak area plotted with concentrations of chlorpyriphos at 0-1000 ppb, using ODS/GC-PFPD analytical method.

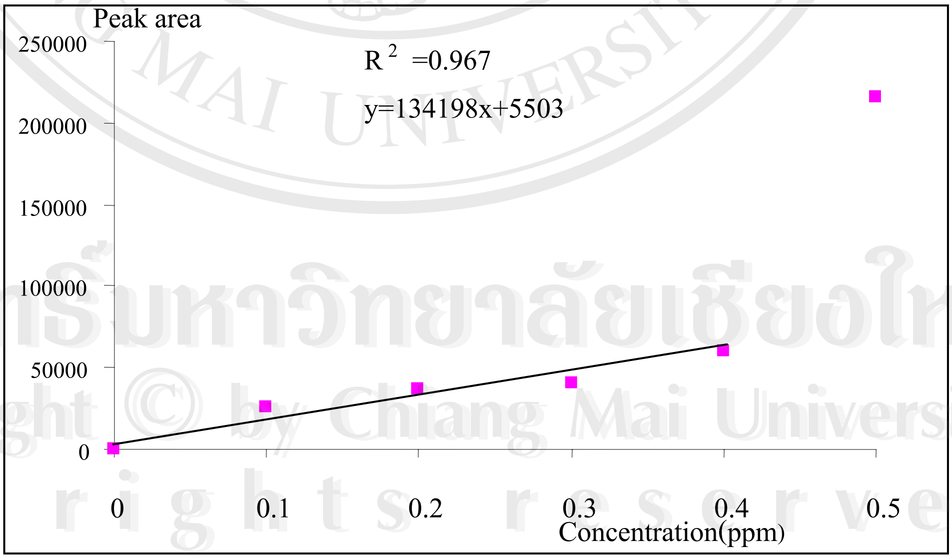


Figure 4.28 Linearity of peak area plotted with concentrations of methyl parathion at 0- 0.4 ppm , using ODS SPME/GC-PFPD analytical method.

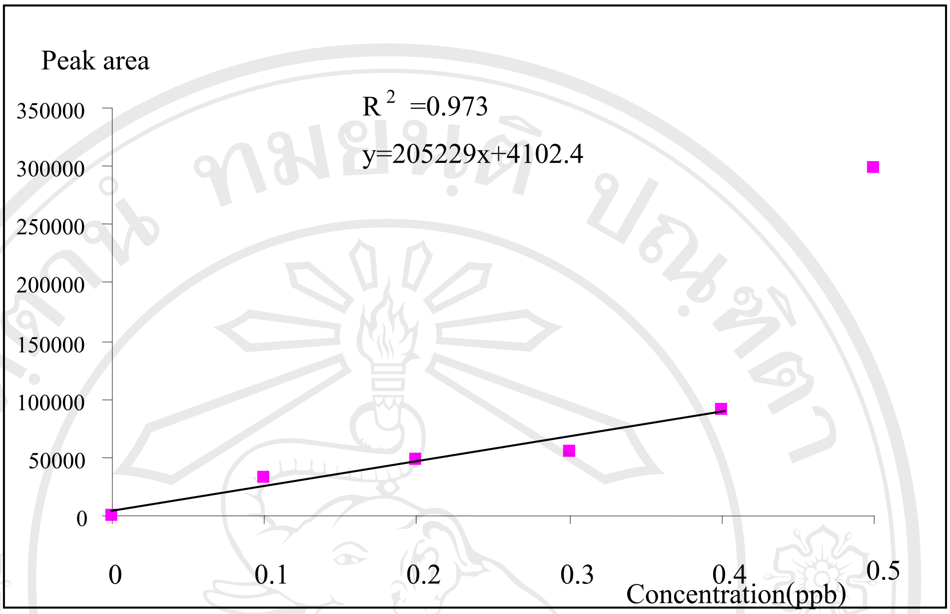


Figure 4.29 Linearity of peak area plotted with concentrations of dicrotophos at 0-0.4 ppm, using ODS SPME/GC-PFPD analytical method.

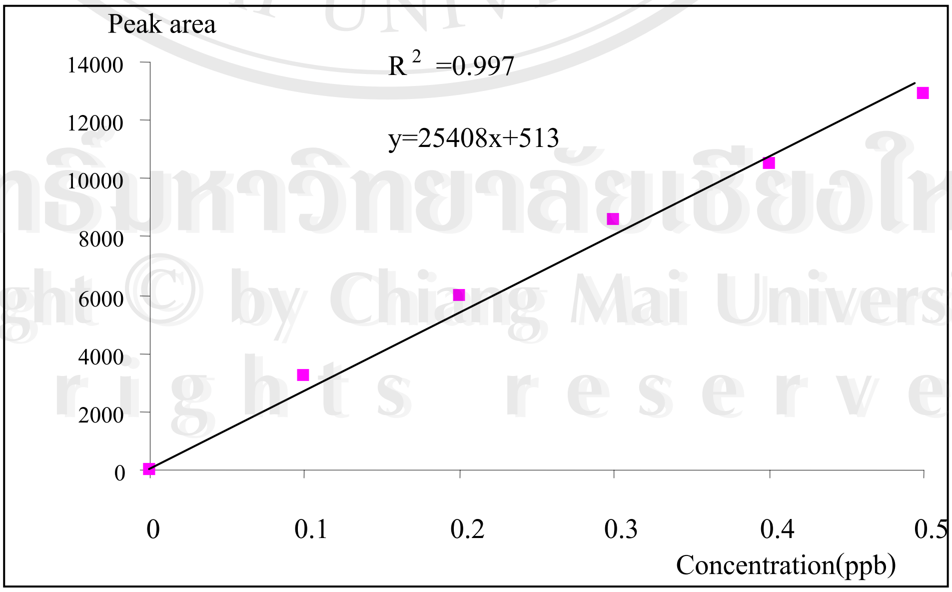


Figure 4.30 Linearity of peak area plotted with concentrations of prothiophos at 0-0.5 ppb, using ODS SPME/GC-PFPD analytical method.

The use of ODS SPME/GC-PFPD analytical method for analysis of the OPP compounds i.e. methylparathion, chloropyriphos, profenofos and prothiophos ; the linearity plotted between peak areas and concentrations can be summarized in Table 4.16.

Table 4.16 Summary of correlation coefficient (R^2) between concentration of OPP and peak area, using ODS SPME/GC-PFPD analytical method.

Linearity plot concentration (ppm)	R^2			
	Methylparathion	Chloropyriphos	Profenofos	Prothiophos
0.01-0.04	-	0.958	0.999	-
0.05-0.2	0.999	0.999	0.999	0.987
0.1-0.4	0.959	0.906	0.887	0.998
0.3-0.5	-	-	0.935	0.998
0.05-0.5	-	0.872	0.836	0.997

4.4.2 Detection limits

Each working standard of the OPP insecticides was determined to find detection limits. Results from the determination are listed in Table 4.17

Table 4.17 Detection limits of analysis of the OPPs, using ODS and SPME/GC-PFPD analytical method.

Compound	LOD (μg/l)	
	ODS	PDMS
Prothiophos	50	1
Profenofos	5	1
Methylparathion	40	1
Chlorpyriphos	5	100
Dicrotophos	5	-
Mevinphos	-	10

4.4.3 Precision

Standard solutions of the OPP at concentration of 1.00 μg/l, 5.00 μg/l, 0.01 mg/l and 1.00 mg/l were determined on precision using PDMS SPME/GC-PFPD and ODS SPME/GC-PFPD analytical method. Data were then calculated to find percentage of relative standard deviation (%RSD). Precision are shown in Table 4.18.

Reproducibility is the precision obtained when all measurements were made by the same analyst during a single period of laboratory work, using the same solutions and equipment. Standard solutions containing 0.1 mg/l of OPP added to the sample solutions. The solutions were analyzed (six replicates) by PDMS/GC-PFPD and ODS/GC-PFPD analytical method, using the same procedure and conditions. Results were then calculated to find percentage of relative standard deviation (%RSD). The reproducibility is precision obtained under the conditions determined, based on using 6 analysis with in a week. Reproducibility is shown in Table 4.19

Table 4.18 Comparison of precision on analysis of the OPPs, using ODS and PDMS SPME/GC-PFPD analytical method (n=6).

Compound	Precision (%R.S.D.)	
	ODS	PDMS
Prothiophos	5.2 **	5.6
Profenofos	10.2*	8.7
Methylparathion	10.8**	0.9
Chlorpyriphos	9.0*	4.8
Dicrotophos	5*	-
Mevinphos	-	5.6

[*] 5µg/l ,(n=6) absorption time at 30 mins and desorption time at 4 mins.

[**] 1µg/ml ,(n=6) absorption time at 5 mins and desorption time at 5 mins

Table 4.19 Comparison of precision of reproducibility on analysis of the OPPs (0.1 mg/l), using ODS and PDMS SPME/GC- PFPD analytical method (n=6).

Compound	Precision (%R.S.D.)	
	ODS	PDMS
Prothiophos	12.0	16.0
Profenofos	16.8	17.5
Methylparathion	26.7	30.0
Chlorpyriphos	22.6	11.9
Dicrotophos	10.7	-
Mevinphos	-	18.0

Table 4.20 Summarization of precision, linear range, detection limits of analysis of the OPP, using PDMS SPME/GC-PFPD analytical method (n=6).

Compound	Retention time(min)	Regression equation	Linear range	R^2 between concentration OPPs and peak area	Detection Limits (LOD)(ppb)	Precision (%R.S.D.)
Prothiophos	14.7	Y=21476x-326	0-0.4ppm	0.984	1	5.6
Profenofos	13.2	Y=1124797x-10174	0-0.4ppm	0.993	1	5.2
Methylparathion	12.4	Y=21249x-257	0-0.4ppm	0.990	1	0.9
Mevinphos	8.7	Y=183867x-1307	0-0.4ppm	0.991	10	5.6
Chlorpyriphos	18.0	Y=1149567x-3848	0-0.4ppm	0.971	100	4.8

Table 4.21 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 OPPs and peak area	Detection Limits (LOD) (μg/l)	Precision (%R.S.D.)
Prothiophos	8.4	Y=25408x+513	0-0.5ppm	0.996	50	5.2^{**}
Profenofos	14.2	Y=76x+620	10-50ppb	0.950	5	10.2^{*}
Methylparathion	12.1	Y=134198x+5503	0-0.4ppm	0.967	40	10.8^{**}
Chlorpyriphos	12.8	Y=39.76x+294	0-1000ppb	0.999	5	9.0^{*}
Dicrotophos	14.2	Y=205229x+4102	0-0.4ppm	0.973	5	5.0^{*}

*5μg/l ,(n=6) absorption time at 30 mins and desorption time at 4 mins

**1μg/ml ,(n=6) absorption time at 5 mins and desorption time at 5 mins

4.4.4 Recoveries

Standard solutions containing 5.00 μg/l, 10.00 μg/l, 0.01 mg/l 0. 10 mg/l, 0.20 mg/l, 0.30 mg/l, 0.40 mg/l and 0.50 mg/l of prothiophos, methylparathion, chlorpyriphos, mevinphos, methamidophos, profenofos and dicrotophos were added in 1 g of the sample and then followed the process of extraction as described in Fig. 3.1. Data were calculated based on the external calibration curves and peak areas. The percent recoveries are shown in Table 4.22-4.27.

Table 4.22 Percent recovery of the analysis of chlorpyriphos from spiked in onion sample, using ODS SPME/GC- PFPD analytical method (n=6).

Concentration of chlorpyriphos added	Chlorpyriphos found (mean ± S.D.)	Recovery (%)	%RSD
5 .00 (μg/l)	4.99 ± 0.47 (μg/l)	99.80 ± 9.40	9.42
10.00 (μg/l)	9.06 ± 1.28 (μg/l)	90.60 ± 18.80	20.47
0.50 (mg/l)	0.45 ± 0.03 (mg/l)	89.28 ± 6.33	7.25

Table 4.23 Percent recovery of the analysis of profenophos from spiked in onion sample, using ODS SPME/GC- PFPD analytical method (n=6).

Concentration of profenophos added	Profenophos found (mean ± S.D.)	Recovery (%)	%RSD
5 .00 (μg/l)	5.00 ± 0.94 (μg/l)	99.96 ± 18.80	17.17
10.00 (μg/l)	10.52 ± 2.25 (μg/l)	105.19 ± 22.48	19.52
0.50 (mg/l)	0.44 ± 0.04 (mg/l)	87.27 ± 9.06	10.38
1.00 (mg/l)	1.04 ± 0.08 (mg/l)	104.03 ± 7.73	7.43

Table 4.24 Percent recovery of the analysis of prothiofos from spiked in onion sample, using ODS SPME/GC- PFPD analytical method (n=6).

Concentration of prothiofos added	Prothiofos found (mean ± S.D.)	Recovery (%)	%RSD
0.50 (mg/l)	0.53 ± 0.04 (μg/l)	106.52 ± 3.96	7.44
1.00 (mg/l)	0.99 ± 0.12 (mg/l)	99.07 ± 12.04	12.15

Table 4.25 Percent recovery of the analysis of methyl parathion from spiked in onion sample, using ODS SPME/GC- PFPD analytical method (n=6).

Concentration of methylparathion added	Methylparathion found (mean ± S.D.)	Recovery (%)	%RSD
0.50 (mg/l)	0.51 ± 0.03 (μg/l)	101.51 ± 6.46	6.36
1.00 (mg/l)	1.09 ± 0.23 (mg/l)	109.48 ± 23.46	21.40

Table 4.26 Average percent recovery of the analysis of profenofos and prothiophos from spiked in vegetable sample (0.01mg/l) using ODS SPME/GC-PFPD analytical method (n=2)

Sample	Average recoveries (%)	
	Profenofos	Prothiophos
Cabbage	75.50	84.00
Garden pea (young pod)	61.85	57.30
Carrot	76.70	59.00
Cucumber	109.45	119.15
Spring cucumber (young leaves)	73.00	52.60
Sweet bell pepper	99.05	107.60
Tomato	110.50	94.40
Yard long bean (green)	69.65	55.60
Broccoli	75.10	62.95
Cauliflower	95.40	76.90
Chinese kale	62.85	51.80

Table 4.27 Average percent recovery of the analysis of the OPPs from spiked vegetables (0.1mg/l), using SPME/GC- PFPD analytical method (n=2).

Vegetable spiked with OPP	Recovery (%)			
	Mevinphos	Methylparathion	Profenofos	Prothiophos
Sweet bell pepper	89.80	45.35	60.85	64.15
Yard long beans (green)	74.35	104.60	56.30	50.85
Chinese kale	62.25	89.85	61.80	57.30
Cauliflower	80.85	45.20	60.05	49.05
Garden pea ,young pod	77.55	35.05	55.35	49.40
Cucumber	70.80	47.70	51.15	49.70
Carrot	73.75	77.95	50.80	41.25
Spring cucumber (young leaves)	83.75	59.90	44.55	38.85
Broccoli	72.75	4.35	40.60	31.50
Tomato	82.95	34.25	93.05	81.75
Cabbage	76.45	52.05	55.40	58.00

4.4.5 Analysis of pesticide residues from vegetables bought from the market

The optimum conditions for analysis of pesticide residues using ODS and PDMS/GC-PFPD developed in this study were used for analysis of OPPs from 11 vegetables

Table 4.28 Analysis of organophosphorus pesticide residues in vegetables, using SPME/GC- PFPD analytical method.

Vegetable	Residues (mg/kg)			
	Mevinphos	Methylpa-rathion	Profenofos	Prothiophos
Sweet bell pepper	ND	ND	ND	ND
Yardlong beans(green)	ND	ND	ND	ND
Chinese kale	ND	ND	ND	ND
Cauliflower	0.32	ND	ND	ND
Garden pea ,young pod	0.32	ND	ND	ND
Cucumber	0.36	ND	ND	ND
Carrot	0.82	ND	ND	ND
Spring cucumber(young leaves)	ND	ND	ND	ND
Broccoli	ND	ND	ND	ND
Tomato	ND	ND	ND	ND
Cabbage	0.16	ND	0.10	58.00

ND = not detectable

4.4.6 Method to compare ODS, PDMS GC-PFPD and GC-FPD

Results from analysis of organophosphorus pesticide spiked in tomato sample using GC-FPD are shown in Table 4.29 with percent recovery and percent RSD.

Table 4.29 Analysis of organophosphorus pesticide spiked in tomato sample using GC-FPD (n=6).

n	Type of Pesticide (mg/l)					
	Methamidophos	Mevinphos	Chlorpyrifos	Methyl-parathion	Prothiophos	Profenofos
1	2.74	0.99	1.59	2.12	0.60	1.79
2	2.94	1.04	1.54	2.08	0.78	1.61
3	2.73	1.03	1.50	2.03	0.69	1.73
4	2.89	1.08	1.59	2.19	0.64	1.76
5	2.96	1.05	1.46	2.07	0.68	1.66
6	2.72	1.01	1.60	2.15	0.77	2.06
x	2.83	1.03	1.55	2.11	0.69	1.77
S.D	0.11	0.03	0.06	0.06	0.07	0.16
Average %Recovery	115.50	42.16	63.10	85.71	28.16	72.1
% RSD	3.95	3.00	3.70	2.76	10.10	8.89

Results from using GC-FPD method were compared with result from using ODS SPME/GC-PFPD method. The comparison was made, using t- test and it was found that there was no statistical difference between the two methods as shown blow:

$t_{\text{calculated}} = \bar{d}\,[n]^{0.5} \div s_d$ ------- 1

$s_d = [\sum (d_i - \bar{d})^2 \div (n-1)]^{0.5}$ -------2

$\bar{d}$ = the average difference between method A and B
n = the number of pair of data

Table 4.30 Comparison of two method for measuring profenofos

Spiked vegetable sample	percentage recovery of Profenofos		
	ODS/ GC-PFPD analytical method	GC-FPD RPF’ method	Difference (d_i)
1	75.00	73.60	1.40
2	62.00	65.71	-3.71
3	76.00	70.60	5.40
4	73.00	71.84	1.16
5	70.00	67.76	2.24
6	75.00	84.08	-9.08
$\bar{d}$			-0.43

For the data in Table 4.30, s_d is

$s_d = 4.91$

$t_{\text{calculated}} = -0.43 \times [6]^{0.5} \div 4.91$

$t_{\text{calculated}} = 0.21$

$t_{\text{table}} = 2.447$

$t_{\text{calculated}} < t_{\text{table}}$ at 95% confidence level, two result are not significantly different.