CHAPTER 3

RESULTS AND DISCUSSION

The experimental results of optimum HPLC conditions obtained for the separation and determination of methomyl, carbofuran and carboxin for real samples are discussed further.

3.1 Investigation of Optimum Detection wavelength for Methomyl, Carbofuran and Carboxin

The single standard solutions of 1 ppm of methomyl, 5 ppm of carbofuran, and carboxin dissolved in methanol were measured at different wavelengths in the range of 190- 300 nm by using UV-VIS spectrometer. The relationship of detection wavelengths among methomyl, carbofuran and carboxin is shown in Figure 3.1.

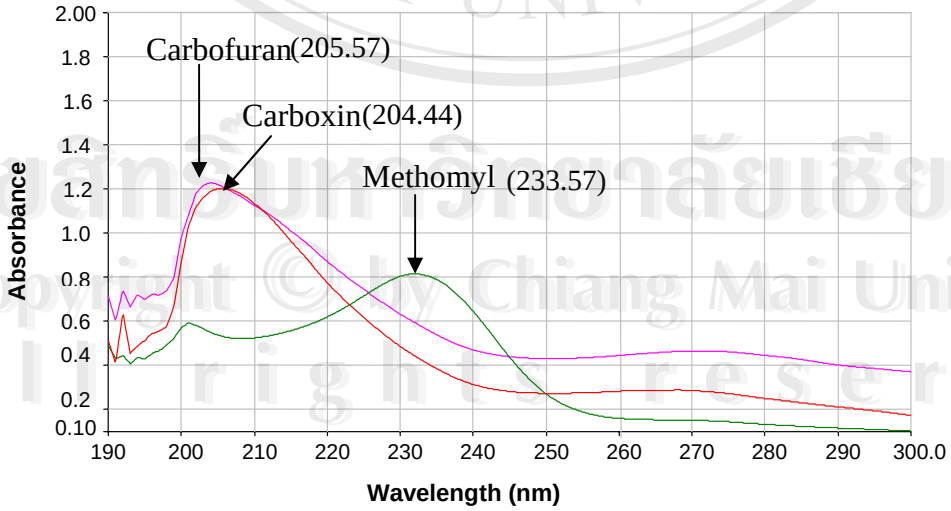

Figure 3.1 Spectra of 1 ppm of methomyl, 5 ppm of carbofuran and carboxin detected by UV-VIS Spectrometer

Detection wavelength is the first parameter to obtain the maximum sensitivity of each analyte. After finding out the maximum wavelength of each analyte, the single standards were mixed and run in the HPLC using 30% ACN in water with the flow rate at 1 ml/ min. The chromatographic conditions are shown in the Figure 3.2 and the optimum wavelength for analytes was selected.

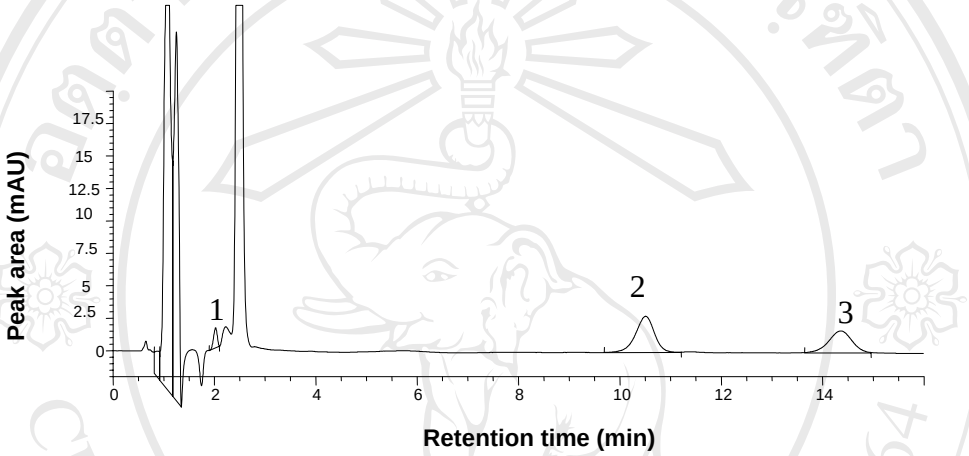

Figure 3.2 Chromatogram of 1 µg/ml mixed pesticide std detected at 205 nm

Peak no.1 = Methomyl, 2 = Carbofuran, 3 = Carboxin

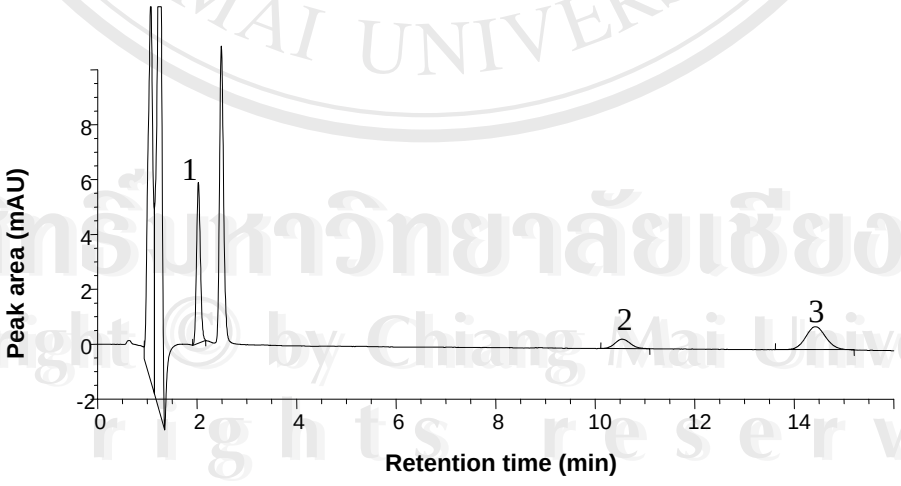

Figure 3.3 Chromatogram of 1 µg/ml mixed pesticide std detected at 233 nm

Peak no.1 = Methomyl, 2 = Carbofuran, 3 = Carboxin

Chromatograms Figures.3.2 and 3.3 showed that polar pesticide methomyl eluted first closely to the solvent peaks and less polar pesticides carbofuran and carboxin eluted later. There was some difficulty to a certain extent for adjusting the maximum wavelengths for all analytes. The chosen detection wavelength was 233 nm for all analytes.

3.2 Optimization of HPLC Conditions

The analytes, methomyl, carbofuran and carboxin were separated by using C_{18} reversed phase HPLC with UV detection isocratic mode. The HPLC systems were optimized by varying mobile phase composition and flow rate.

3.2.1 Optimization of mobile phase composition

Mobile phase compositions were varied by different ratios of acetonitrile in water such as 20%, 30%, 40%, and 50% whereas the flow rate was fixed at 1 ml/min. The results showing the retention time and peak area at different ratio of mobile phase compositions are illustrated in Table 3.1.

Table 3.1 Effect of retention time and peak area observed by different ratio of mobile phase composition.

Mobile Phase composition ACN in H_2O	Methomyl		Carbofuran		Carboxin	
	t_R (min)	Peak Area (mAU)	t_R (min)	Peak Area (mAU)	t_R (min)	Peak Area (mAU)
20%	2.25	1.65	37.55	32.24	49.78	90.13
30%	1.95	32.76	9.36	31.49	12.91	93.01
40%	1.59	34.98	4.58	34.81	6.02	96.68
50%	1.45	31.26	3.11	37.81	3.87	100.16

Figure 3.4 showed that the analysis times was decreased and the sensitivity was increased when the percentage of acetonitrile was increased .The mobile phase ratio of 20:80 (v/v) was not suitable because long analysis time and the sensitivity was poor for all analytes especially for methomyl. Even though the mobile phase ratio of 50:50 (v/v) resulted in short analysis times, the peak area of methomyl obtained was very close to the solvent peak. The mobile phase composition of 30:70 (v/v) showed that the suitable analysis time but the sensitivity is not significantly different from the mobile phase composition of 20:80 (v/v). The mobile phase composition of 40:60 (v/v) had provided a short analysis time, good sensitivity and resolution. Therefore, the optimum composition of acetonitrile in water at 40:60 (v/v) was selected for the further study.

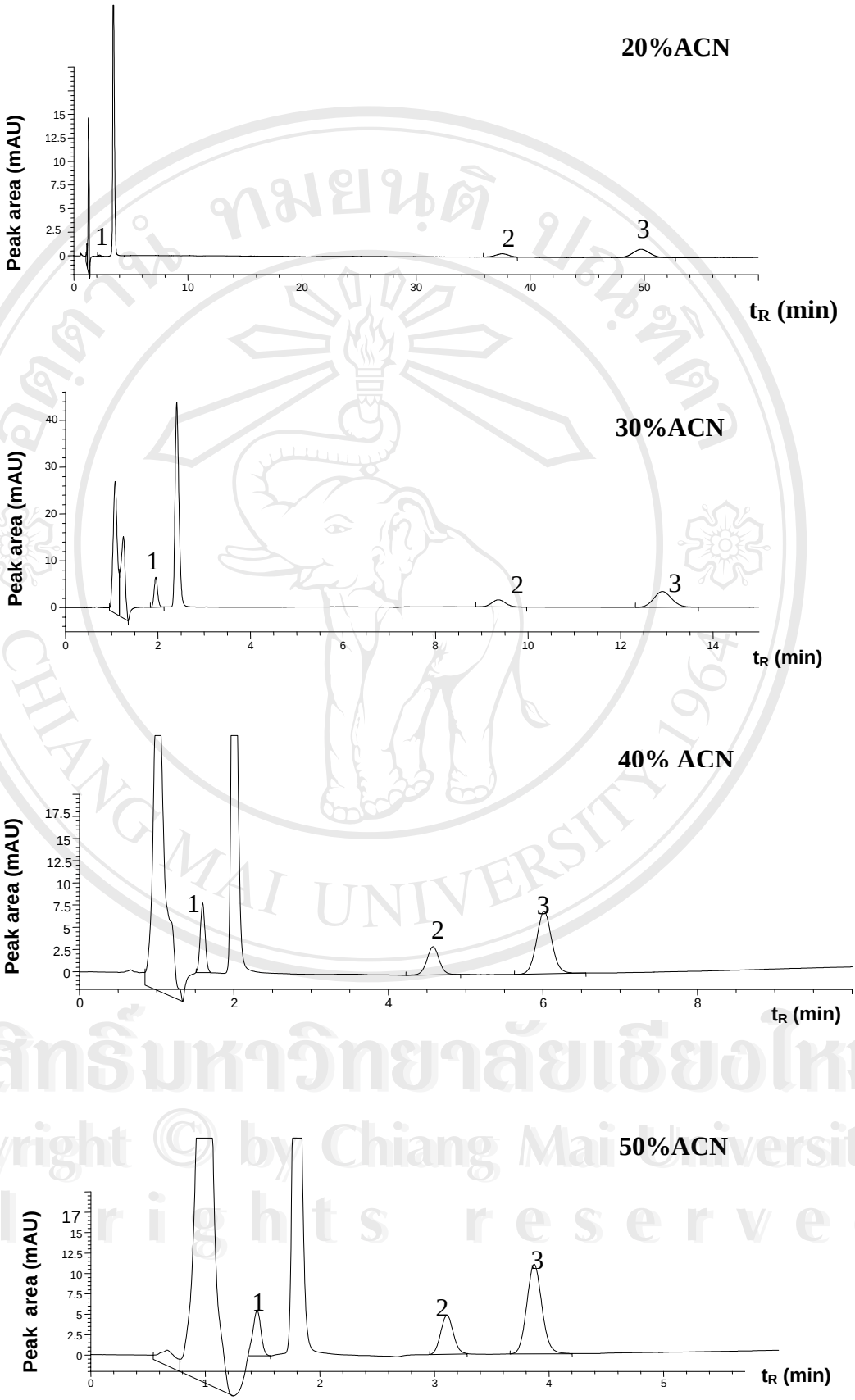

Figure 3.4 HPLC chromatograms of mixed standards showing the effect of mobile phase composition at the flow rate of 1.0 ml/min

3.2.2 Optimization of flow rate of mobile phase composition

The flow rate of mobile phase could effect the peak area and analysis time involved in the developed method. The flow rate was varied in the range of 0.6 to 1.2 ml/min. The results showing the effect of flow rate on the retention time and peak area are mentioned in the Table 3.2, Figures 3.5 and 3.6.

Table 3.2 Effect of flow rate of mobile phase composition related to retention time and peak area.

Flow rates (ml/min)	Analytes					
	Methomyl		Carbofuran		Carboxin	
	t_R (min)	Peak Area (mAU)	t_R (min)	Peak Area (mAU)	t_R (min)	Peak Area (mAU)
0.6	2.50	61.32	6.32	59.63	8.07	162.89
0.8	1.89	44.52	4.77	43.72	6.09	122.34
1.0	1.53	34.29	3.83	34.74	4.89	97.62
1.2	1.28	27.71	3.21	27.26	4.10	75.95

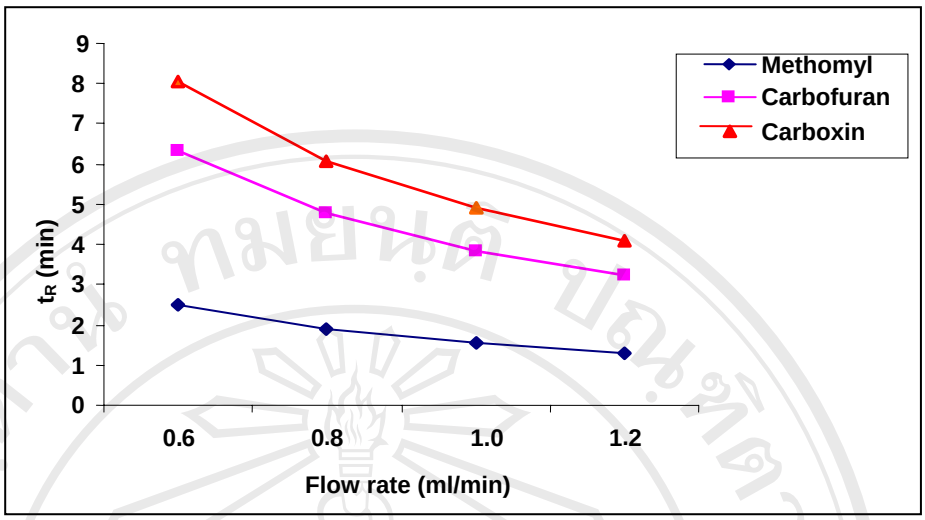

Figure 3.5 Effect of flow rate on retention time (t_R)

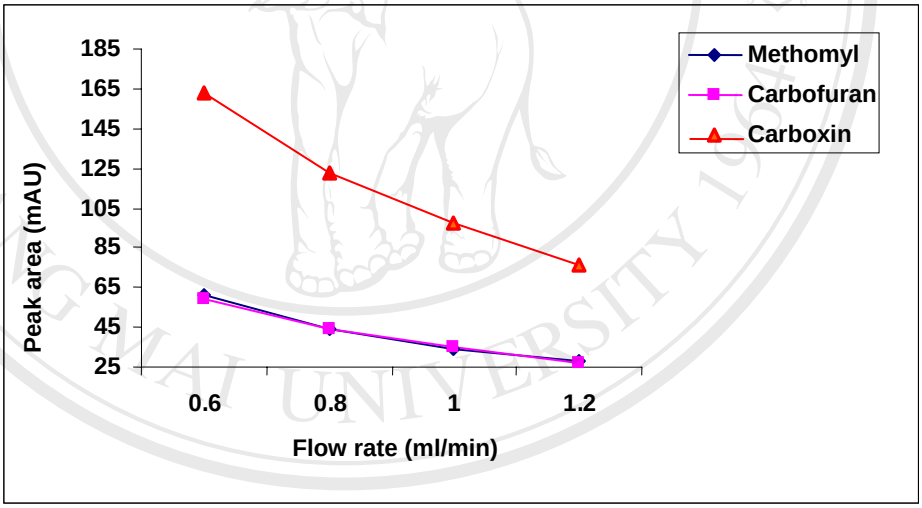

Figure 3.6 Effect of flow rate on peak area

Figures 3.5 and 3.6 showed the effect of flow rate on retention time and peak area. When the flow rate was increased, the analysis times were shorter and peak area obtained was decreased. The efficiency performance was increased by decreasing the flow rate. The flow rate of 0.8 ml/min could provide a suitable analysis time and peak area.

3.2.3 HPLC optimized conditions

The optimum HPLC conditions are summarized in Table 3.4 and chromatograms of mixed standards under the optimized condition are shown in Figure 3.7.

Table 3.3 Optimum conditions for HPLC

Analytical column	µBondapak C_{18} 4.0*125 mm 5 micron
Solvent	40% Acetonitrile/water (v/v)
Detection wavelength	233 nm
Flow Rate	0.8 ml/min
Run time	7 minutes
Injection volume	10µl

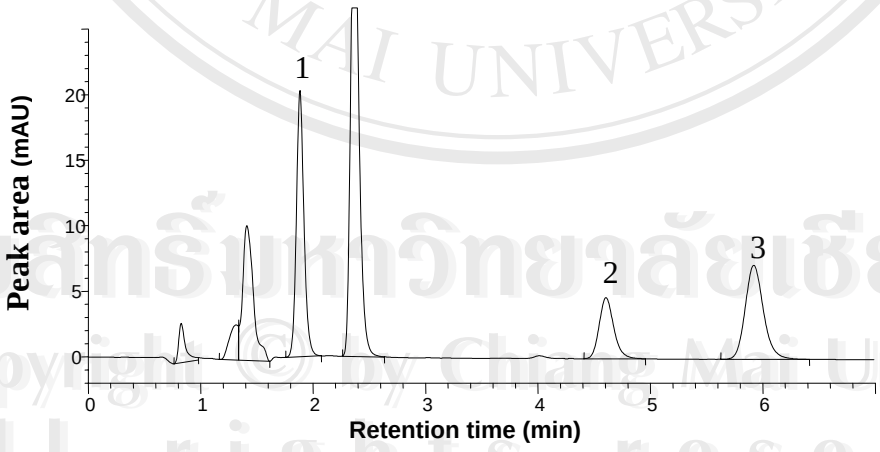

Figure 3.7 HPLC chromatograms of 2 µg/ml mixed standards under the optimized condition. Peak no.1 = Methomyl, 2 = Carbofuran, 3 = Carboxin

3.3 Validation of the Method

A validation of the method in terms of precision, linearity range, limit of detection and limit of quantitation were mentioned as follows.

3.3.1 Reproducibility test of HPLC system for precision

Inter-assay or reproducibility precision is expressed as in terms of relative standard deviation (RSD) as shown in Table 3.4. Satisfactory results were found that percent relative standard deviations of the retention time and peak area were in a range of 0.61 to 2.83% and 0.02 to 0.67% respectively.

Table 3.4 % RSD of retention time and peak area for reproducibility test (n = 10)

Day	Average Retention Time (min)			Average Peak Area (mAU)		
	Methomyl	**Carbofuran**	**Carboxin**	**Methomyl**	**Carbofuran**	**Carboxin**
1	1.91	4.83	6.20	46.08	44.77	123.77
2	1.89	4.62	5.91	45.55	45.27	123.82
3	1.89	4.62	5.91	45.55	45.27	123.82
Mean	**1.90**	**4.69**	**5.91**	**45.73**	**45.10**	**123.80**
SD	**0.01**	**0.12**	**0.17**	**0.31**	**0.29**	**0.03**
%RSD	**0.61**	**2.59**	**2.83**	**0.67**	**0.64**	**0.02**

3.3.2 Repeatability test of HPLC system for precision

The intra-assay or repeatability precision is described in terms of relative standard deviation (RSD) as shown in Table 3.5. Percent relative standard deviations

of the retention time and peak area were in ranges of 0.12 to 0.22% and 2.24 to 2.7%, respectively.

Table 3.5 % RSD of retention time and peak area for repeatability test (n =10)

Day	Retention Time (ml/min)			Peak Area (mAU)		
	Methomyl	**Carbofuran**	**Carboxin**	**Methomyl**	**Carbofuran**	**Carboxin**
1	1.91	4.83	6.20	49.12	45.12	125.90
2	1.91	4.83	6.20	46.12	42.20	116.84
3	1.91	4.83	6.20	47.39	44.09	125.35
4	1.91	4.83	6.20	45.17	44.45	121.70
5	1.91	4.83	6.20	45.46	45.00	122.86
6	1.90	4.82	6.19	45.70	45.38	124.96
7	1.91	4.83	6.20	45.59	45.23	124.36
8	1.90	4.83	6.20	45.33	45.28	125.96
9	1.91	4.82	6.20	45.45	45.54	124.76
10	1.91	4.84	6.22	45.44	45.39	124.97
Mean	**1.91**	**4.83**	**6.2**	**46.08**	**44.77**	**123.77**
SD	**0.004**	**0.006**	**0.007**	**1.244**	**1.009**	**2.772**
%RSD	**0.22**	**0.12**	**0.12**	**2.70**	**2.25**	**2.24**

3.3.3 Linearity range

An aliquot of mixed standard solutions was injected into HPLC system under the optimum conditions. Peak areas obtained were plotted against concentrations

resulted in linearity curves shown in Figures 3.7 and 3.8 for the linearity range of each analytes with high and low concentrations respectively. Good linearity of the response was found for all pesticides at concentration within tested interval. Linear determination coefficients (r^2) of every standard were in the range of 0.9962 to 0.9997 as shown in Table 3.6.

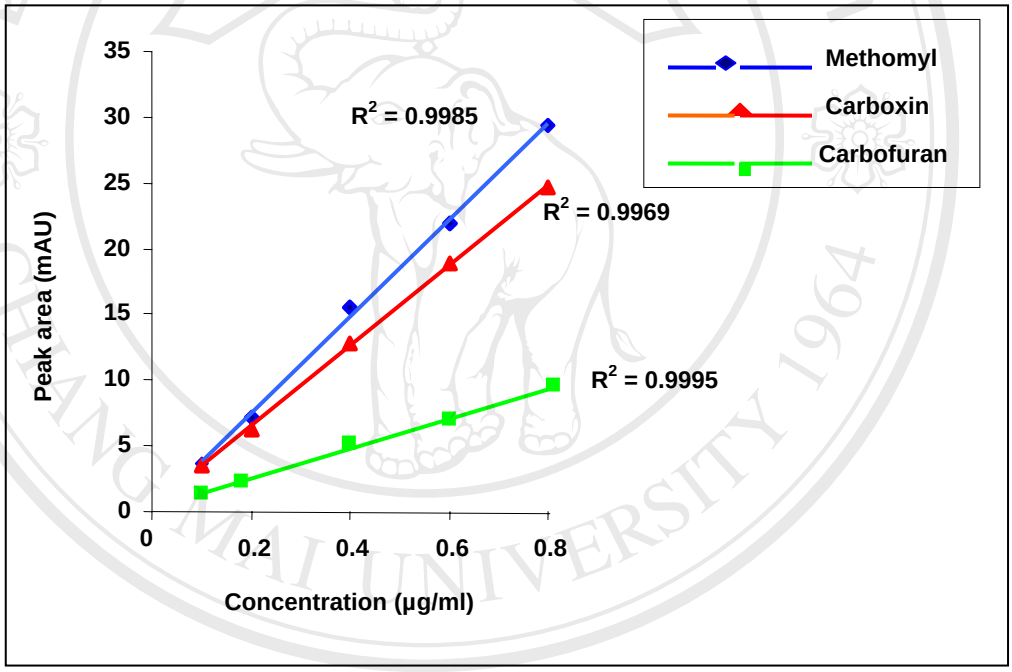

Figure 3.8 Linearity of analytes at low concentration (0.1-0.8 µg/ml) under the optimized condition.

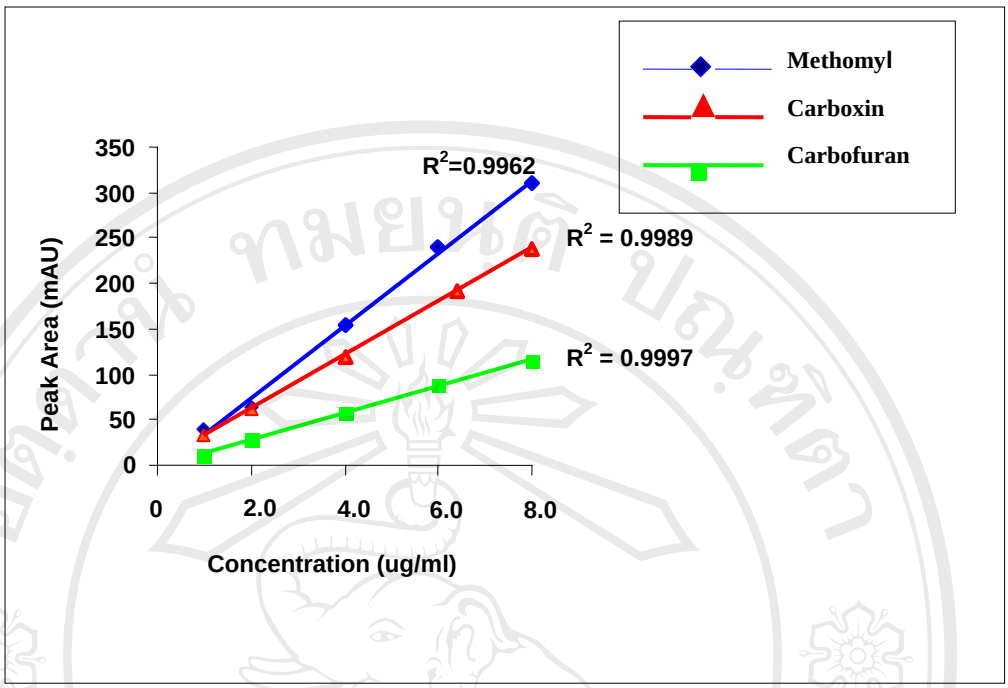

Figure 3.9 Linearity of analytes at high concentration (1- 8 µg/ml) under the optimized condition.

Table 3.6 Linearity of anlytes at low and high concentrations

Analytes	**Low concentration (0.1- 0.8 µg/ml)**		**High concentration (1- 8 µg/ml)**	
	Linear equation	**R^2**	**Linear equation**	**R^2**
Methomyl	y = 36.721x + 0.1033	0.9985	y = 39.951x - 6.4816	0.9962
Carbofuran	y = 11.274x + 0.307	0.9969	y = 14.75x – 1.2106	0.9989
Carboxin	y = 30.518x + 0.3946	0.9995	y = 29.564x + 2.5756	0.9997

3.3.4 Limit of detection and limit of quantification

Five concentrations of mix standards were injected into the HPLC system and linear regression was performed under the assumption that the y values are normally distributed around the regression line with a standard deviation Sy/x. The limits of detection (LOD) and limit of quantification (LOQ) are shown in Table 3.7. Example of calculation is shown in Appendix B.

Table 3.7 Limit of detection and limit of quantification for methomyl, carbofuran and carboxin

Anlytes	Linear regression	R^2	LOD (µg/ml)	LOQ (µg/ml)
Methomyl	y = 36.72x + 0.1033	0.9985	0.04	0.13
Carbofuran	y = 11.274x + 0.307	0.9995	0.06	0.18
Carboxin	y = 30.518x - 0.3946	0.9969	0.02	0.08

3.4 Optimization of Solid Phase Extraction

It is difficult to develop a simple and unique method for different polarities of the pesticides, especially if more than one compound is determined. The method used in this work for optimization of SPE is summarized as follows.

3.4.1 The composition of elution solvent

The composition of the eluting solvent was assessed by varying different concentrations of acetonitrile with water 40, 50, 60, 70 % using C_{18} SPE sorbent. In the elution step, the series of 1st fraction (3 ml), 2nd fraction (2 ml) and 3rd fraction

(2 ml) of elutes were injected into HPLC system. The peak area obtained for each fraction is shown in Table 3.8.

Table 3.8 Separation profiles of analytes by different concentrations of acetonitrile in water.

% ACN in water	Peak area of each fraction (mAU)											
	Fraction of Methomyl				Fraction of Carbofuran				Fraction of Carboxin			
	1st	2nd	3rd	Total	1st	2nd	3rd	Total	1st	2nd	3rd	Total
40	17.61	7.34	3.74	28.69	4.02	3.93	2.59	10.54	9.71	10.63	10.41	30.75
50	19.2	6.79	3.14	29.13	8.31	2.55	1.51	12.37	23.3	16.04	5.66	45.00
60	17.59	6.59	2.77	26.95	7.52	2.80	1.30	11.62	25.98	8.59	4.31	38.88
70	28.35	1.23	-	29.58	10.8	1.55	-	12.30	39.26	6.09	-	45.35

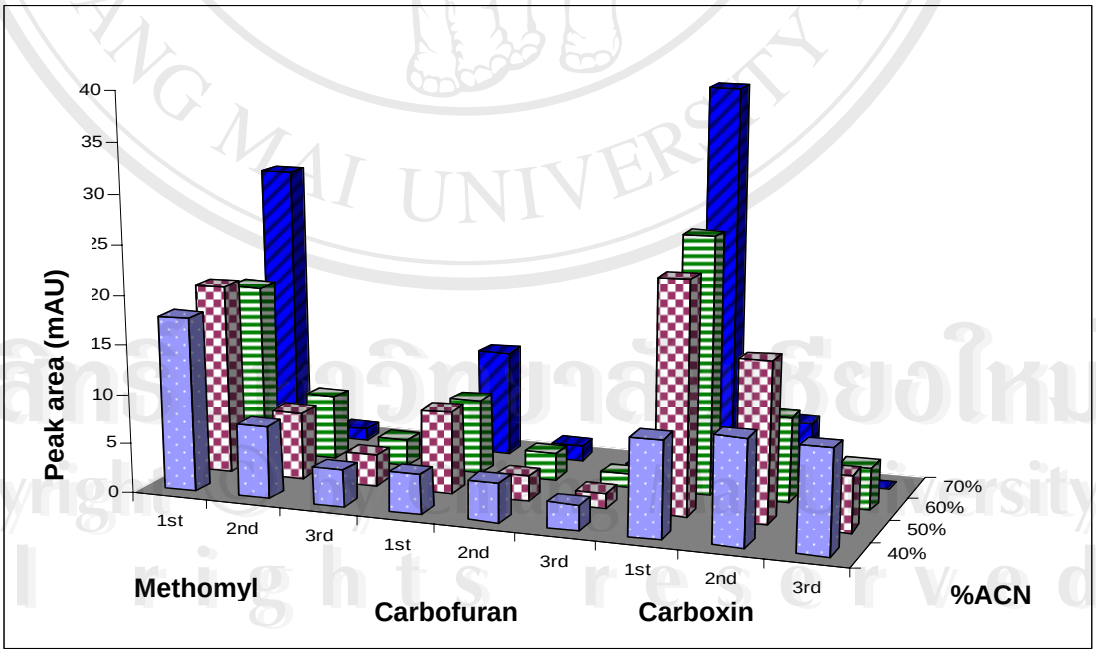

Figure 3.10 Separation profiles of analytes at different concentrations of acetonitrile in water

The results showed that the total peak area obtained by different composition of ACN in water was not significantly different from one another. The highest peak area was found in first fraction and decreased from second to third fraction. The eluting solvent of 40%, 50%, and 60% ACN in water showed similar patterns with all analytes in each fraction. 70% ACN in water eluted all of the analytes in 1st and 2nd fraction. The eluting strength of 70% ACN solvent is sufficiently enough to elute all analytes in the first two fractions and no more peak were found in 3rd fraction. Consequently, 70% ACN in water was chosen as the eluting solvent for the extraction of methomyl, carbofuran and carboxin by using C_{18} SPE sorbent.

3.4.2 Optimization to minimize volume of eluent (70% ACN/H_2O)

Elution volume of ACN/ water (70:30) as the eluent for the analytes was optimized. Each pesticide at 2µg/ml fortification level was used to evaluate the eluent optimum volume. After passing through the SPE cartridges, 1 ml eluent was collected in each seven fraction. Each fraction of peak areas is shown in Table 3.9 and elution profile is shown in Figure 3.11.

Table 3.9 Peak area of the analytes found in different fractions of eluent, (n=3)

Collected Fraction	Volume of eluent (ml)	Average Peak area of each fraction (mAU)		
		Methomyl	Carbofuran	Carboxin
1	0-1	149.41	44.38	129.49
2	1-2	25.59	9.80	34.43
3	2-3	3.74	-	5.18
4	3-4	2.86	-	-
5	4-5	2.06	-	-
6	5-6	1.08	-	-
7	6-7	-	-	-
SUM	**7**	**184.74**	**54.18**	**169.1**

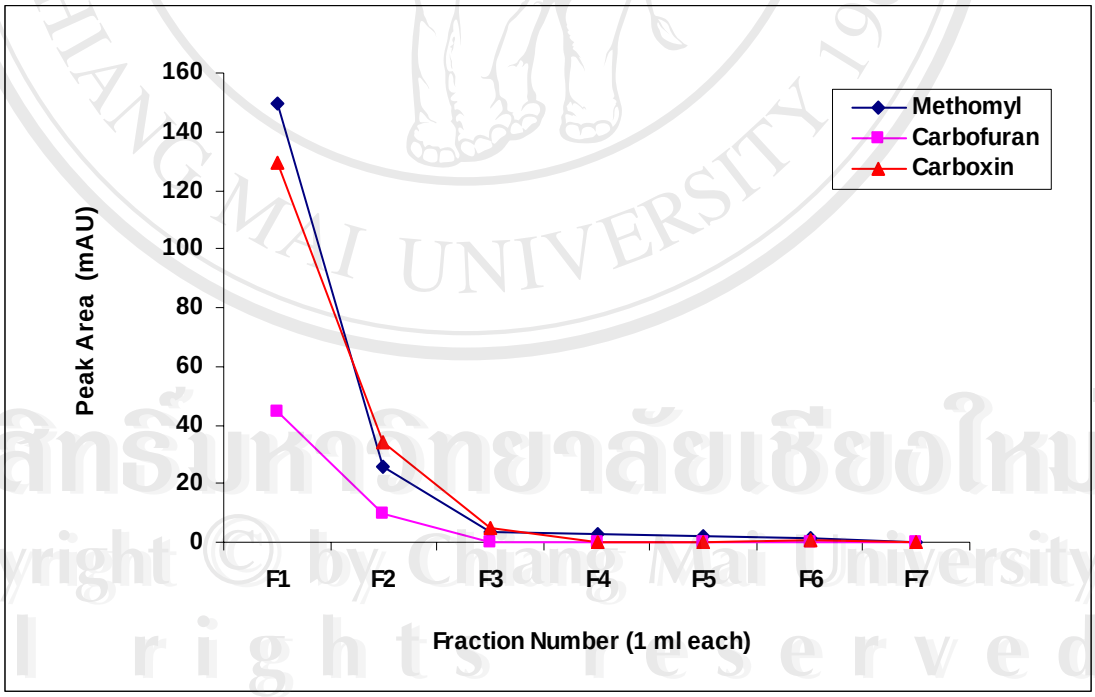

Figure 3.11 Elution profiles of methomyl, carbofuran, and carboxin eluted with 70% ACN/H_2O at 2 µg/ml fortification level

The analytes were found in the first fraction and became decreases in each later fraction. Eventhough the amount of methomyl was found until fraction no. 6, the amount obtained in fraction no. 4, 5, and 6 were quite low. The amount of carbofuran and carboxin were found until the fraction no. 2 and 3 respectively. Thus, the volume of 3 ml was chosen to be the optimum eluent volume.

3.4.3 Sorbent Selection

In order to obatain good recoveries, different sorbents of reverse phase C_{18} and normal phase florisil cartridges were studied. Recoveries were determined in triplicate at three concentration levels. The standard solutions were loaded after conditioning and recoveries were calculated from the chromatograms of the standard solutions before and after use of the SPE cartridges. Percent recoveries of analytes using different sorbents are described in Table 3.10 and Figure 3.12.

Table 3.10 Percent recoveries of analytes using different SPE sorbents

Pesticides	**% Recovery ± SD**					
	0.5 ppm		**1.0 ppm**		**2.0ppm**	
	Florisil	**C_{18}**	**Florisil**	**C_{18}**	**Florisil**	**C_{18}**
Methomyl	101.7 ± 2.1	100.4 ± 1.4	98.1 ±1.5	76.9 ±2.3	76.1 ± 1.1	36.5 ± 0.6
Carbofuran	101.8 ± 2.0	97.8 ± 0.9	99.6 ± 2.3	71.2 ± 3.3	57.4 ± 1.6	36.4 ± 1.3
Carboxin	106.4 ± 2.9	93.5 ± 1.6	87.1 ± 3.2	68.9 ± 0.9	60.3 ± 3.2	38.6 ± 0.50

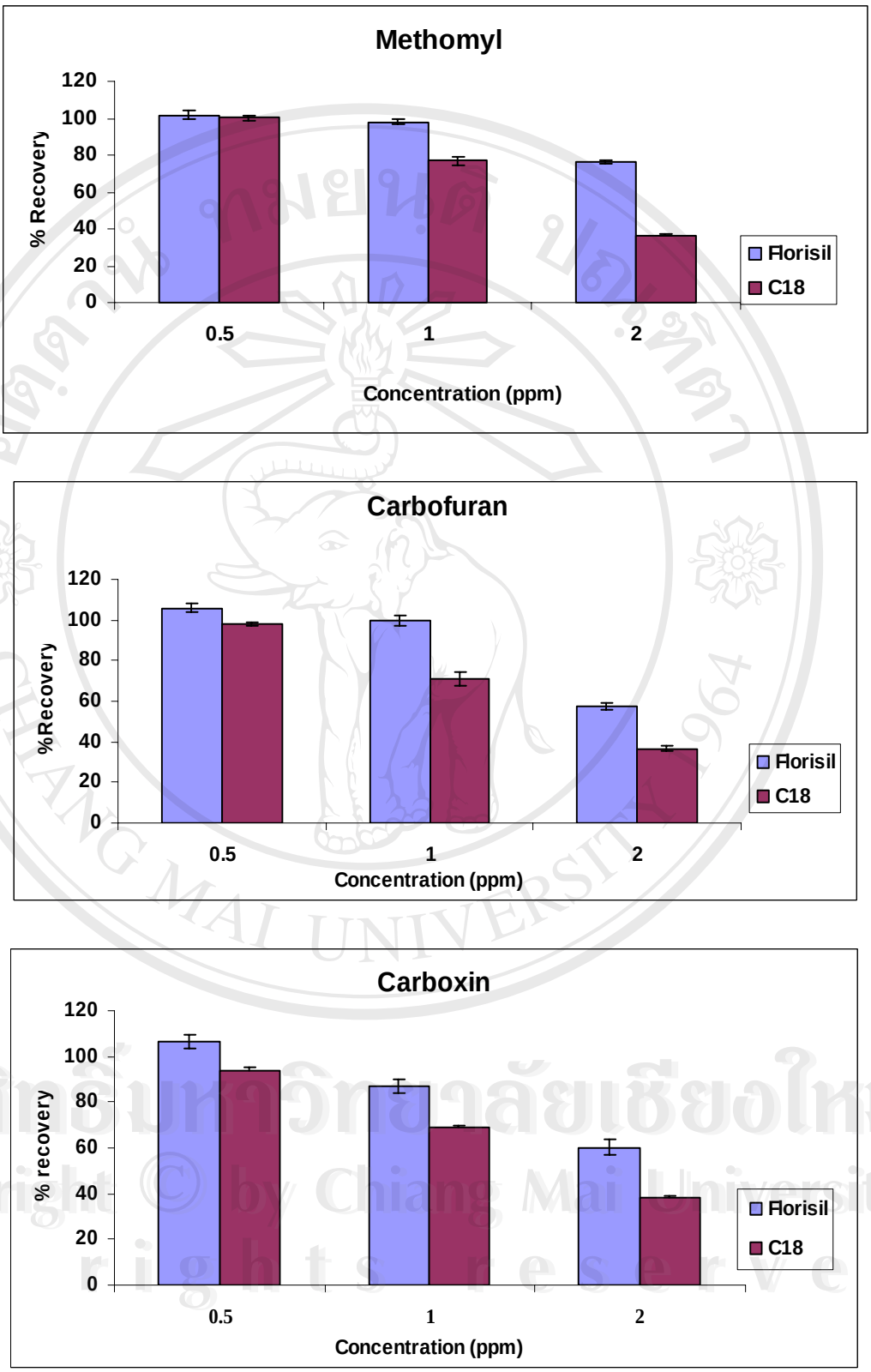

Figure 3.12 Percent recoveries of methomyl, carbofuran and carboxin using different SPE sorbents at different concentration levels

It was found that normal phase florisil SPE resulted in the higher recoveries than the reverse phase C_{18} for all concentrations of the analytes. Florisil SPE cartridges resulted adequate recoveries (87.1% – 106.4%) for all pesticides at 0.5, and 1.0 ppm fortification levels. C_{18} resulted adequate recoveries (93.5 – 100.4%) only at 0.5 ppm fortification level but low recoveries were obtained at higher concentration levels of 1.0 ppm and 2.0 ppm. Consequently, sorbent florisil sorbent was selected for the SPE clean up. It was found that there are many criteria that effect to the efficiency of the SPE. Retained capacities of the SPE, the concentration of the components, the characteristics and volume of the sample matrix have to be considered. It also found that even though, the SPE was able to retain some of the compounds or extracts; it was proposed that small amount of extract to be used for the clean up.

3.4.4 Summary of the optimum condition of the SPE procedures

The optimum conditions of the SPE procedure for sample clean up are shown in Table 3.11.

Table 3.11 Optimum conditions for SPE clean up procedure

Sorbent	Florisil
Conditioning solutions	10 ml of methanol followed by 10 ml of Mili Q water
Elution Solvent	70% ACN/water
Elution solvent volume	3 ml

3.5 Accuracy

The accuracy of the method was calculated through the recovery of each pesticide. For that sample was extracted followed by extraction procedure mentioned in Figure 2.1. The recovery rate of each pesticide at 0.5 ppm fortication level was evaluated in order to access the extraction efficiency of the proposed method. There was much interference in the recovery studies of the real samples. The sample contains co-extractives and co-eluted with the interest of pesticides. Relatively high background was encountered for the peak identification and quantitation and resulted in poor recoveries. Some background peaks emerged closely to the position of the interest of pesticides especially, polar pesticides methomyl. Due to this reasons, although the optimum condition for all selected pesticides was developed, polar pesticides methomyl could not be done for the peak identification and sample analysis. In addition, the further works for optimum condition were done to increase the adequate seperation and the percent recoveries of carbofuran and carboxin.

3.5.1 Further optimization of detection wavelength

In this study, the polar pesticides methomyl can not be identified because of the background of the sample polar matrix. In order to get the good separation for adequate recoveries, the optimum wavelength (205 nm) was selected instead of former optimum wavelength (233 nm).

3.5.2 Further optimization of mobile phase composition

After the wavelength was further optimized, the mobile phase composition was varied with 20%, 25%, 30 % acetonitrile in water. The sample extract spiked at 0.5 ppm fortification level was injected at the former optimum flow rate at 0.8 ml/ min. The results showing the retention time and peak area at different ratio of mobile phase composition are illustrated in Table 3.12.

Table 3.12 Effect of retention time and peak area of standard and spiked sample at 0.5 ppm fortification level by varying composition of mobile phase

Mobile phase composition (ACN/ H_2O)	Standard / Spike sample	Carbofuran		Carboxin	
		t_R (min)	PA (mAU)	t_R (min)	PA (mAU)
30%	Standard	11.37	46.62	15.63	49.25
	Spiked	11.34	44.44	15.58	28.91
25%	Standard	16.41	46.09	22.64	51.15
	Spiked	16.45	50.40	22.63	57.66
20%	Standard	26.93	53.89	36.55	41.44
	Spiked	26.92	40.47	36.63	53.51

Table 3.12 showed that mobile phase ratio of 30:70 (v/v) of ACN in H_2O resulted the proper retention times but carbofuran was co-eluted with co-extractives contained in the sample extracts and resulting low resolution. Even though long retention times were resulted in mobile phase ratio of 25:75 (v/v), higher peak area and good resolution compared with others was obtained. The mobile phase ratio of 20:80 (v/v) was not suitable because of long analysis times and carboxin was also

co-eluted with co-extractives and poor resolution was obtained. Consequently, satisfactory results were found in mobile phase composition 25:75 (v/v) acetonitrile in water selected for good separation and recoveries of carbofuran and carboxin.

3.5.3 Further optimization of flow rate of mobile phase composition

After the wavelength and mobile phase composition were optimized, flow rate was further optimized. The results at different flow rate of mobile phase composition are illustrated in Table 3.13

Table 3.13 Effect of retention time, peak area and % recovery at 0.5 ppm level of spiked sample by varying mobile phase flow rate

Flow Rate (ml/min)	Standard /Spiked sample	Carbofuran			Carboxin		
		t_R (min)	PA (mAU)	% Recovery	t_R (min)	PA (mAU)	% Recovery
0.6	Std	21.81	62.83		29.94	72.54	
	Spike	21.85	61.13	81.9	29.96	72.55	89.9
0.7	Std	18.70	53.36		25.71	61.16	
	Spike	18.73	53.47	84.3	25.69	70.41	96.9
0.8	Std	16.41	48.07		22.54	54.72	
	Spike	16.37	46.63	81.6	22.43	62.48	96.1
0.9	Std	14.52	42.23		19.96	47.65	
	Spike	14.55	39.75	79.2	19.92	52.41	92.5
1.0	Std	13.05	38.01		17.98	42.21	
	Spike	13.07	33.67	74.5	17.92	48.84	97.3

The results showed that the retention times and peak area decreased gradually with the increasing flow rate from 0.6 to 1.2 ml/min. The retention times, the resolution, peak area, and % recovery were evaluated. Recoveries of 74.5-84.3 %

and 89.9 - 97.3% for carbofuran and carboxin were obtained respectively. Flow rate at 0.8 ml/min was selected because high recoveries resulted 81.6% and 96.1% for carbofuran and carboxin, respectively. In addition, proper retention time and resolution were obtained. Chromatograms obtained from sample at 0.5 ppm fortification level before and after further optimization are shown in Figure 3.13.

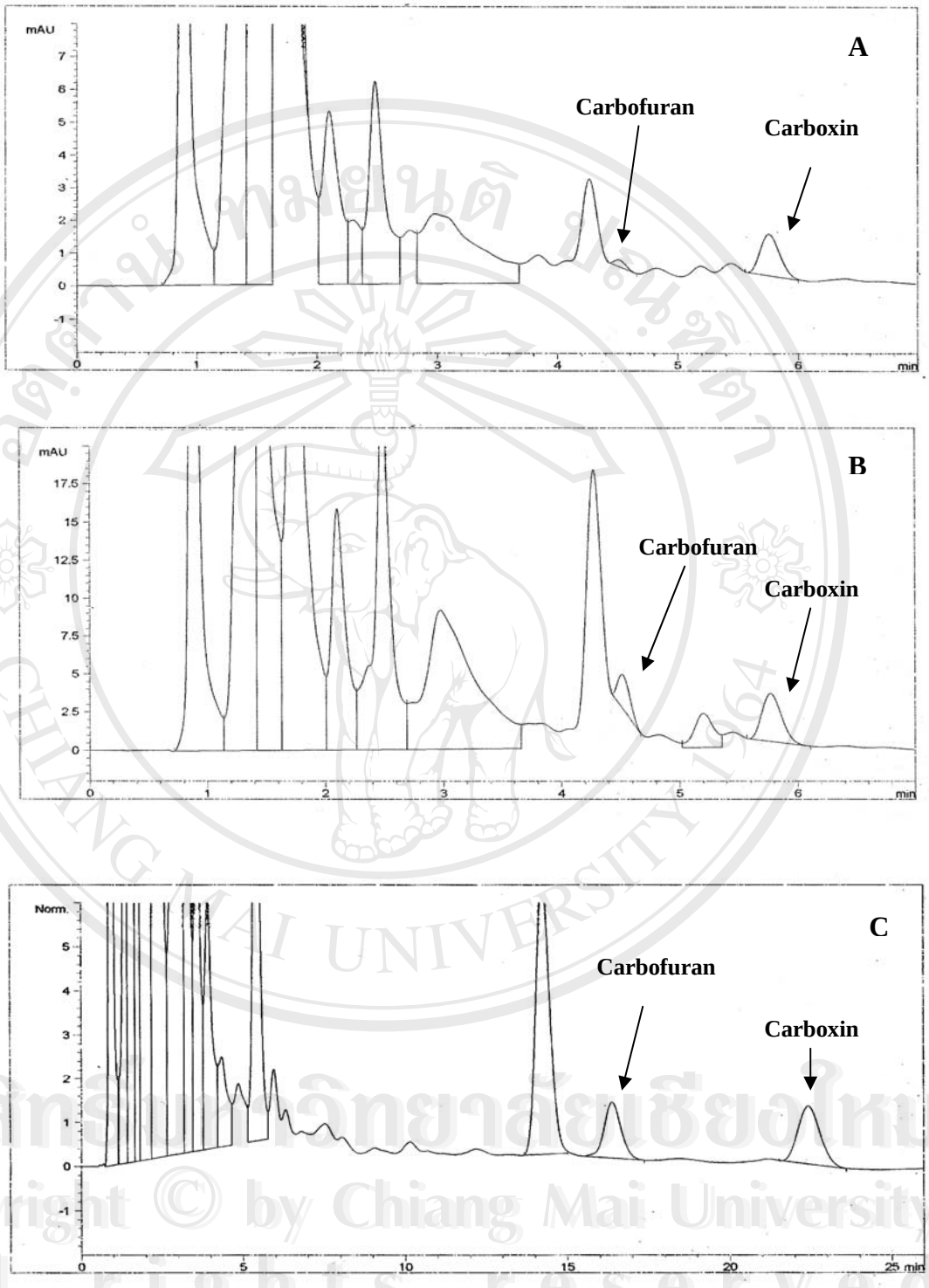

Figure 3.13 Chromatograms of spiked analytes obtained from the extracted cabbage sample at 0.5 ppm fortification level (A) Before further optimization (B) After further optimization (changing wavelength to 205 nm) (C) After further optimization (changing wavelength to 205 nm and mobile phase composition at 25% ACN in water)

Percent recoveries of carbofuran and carboxin at 0.5 ppm fortification level under further optimum condition are shown in Table 3.14.

Table 3.14 Percent Recoveries of carbofuran and carboxin at 0.5 ppm fortification level under further optimum condition, n = 3

	Carbofuran			**Carboxin**		
	PA (mAU)	**Volume (ml)**	**% Recovery**	**PA (mAU)**	**Volume (ml)**	**% Recovery**
Standard	54.35	3.1		34.21	3.1	
1	57.75	2.7	94.1	35.26	2.7	91.2
2	58.45	2.7	94.5	34.63	2.7	88.9
3	60.87	2.6	95.5	36.08	2.6	89.9
Mean	-		**94.7**	-		**90.0**
SD	-		**0.7**	-		**1.2**
%RSD	-		**0.8**	-		**1.3**

It was found that both the pesticides gave acceptable recoveries within the mentioned validation interval. The recoveries (94.1 - 95.5%) and % RSD 0.8 were obtained for carbofuran and recoveries (88.9-91.2%) and % RSD 1.3 were obtained for carboxin.

3.5.4 Development of HPLC conditions used for analysis of all sample cabbages in this experiment

In this research works, all the samples were analyzed by HPLC under the optimized conditions as shown in Table 3.15 and chromatograms of 8 µg/ml mixed standards obtained under the optimized condition are shown in Figure 3.14.

Table 3.15 HPLC operating conditions used for the analysis of all cabbage samples

Analytical column	µBondpak C_{18} 4.0*125 mm 5 micron
Solvent	25% Acetonitrile/water (v/v)
Detection Wavelength (nm)	205
Flow Rate	0.8 ml/min
Injection volume	10 µl
Run time	30 minutes

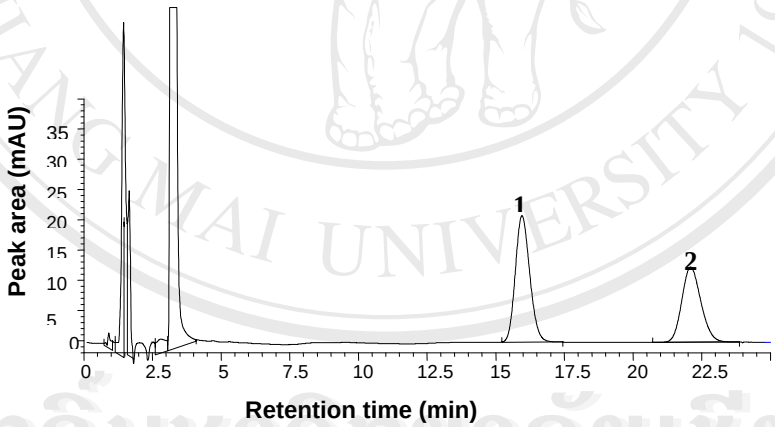

Figure 3.14 HPLC Chromatograms of 8 µg/ml mixed standards obtained under the optimized condition. Peak no.1 = Carbofuran, 2 = Carboxin

3.6 Amount of Carbofuran and Carboxin found in Cabbage Samples

The proposed method had been applied in the analysis of cabbage samples. The samples were pretreated with the optimum extraction as shown in section 2.8. Level of concentrations and frequency of carboxin residues found in real samples are mentioned in Tables 3.16 and 3.17. Example for the calculation of the concentration of the analyte in the sample is mentioned in Appendix D.

Table 3.16 Amount of carbofuran and carboxin detected in cabbage samples, n = 3 using HPLC -UV

No.	Sample code	Sample Description	Amount of Carbofuran (mg/kg) ± SD	Amount of Carboxin (mg/kg) ±
1	LF1	Local fresh market (without safety label)	ND	ND
2	SM1	Super market (without safety label)	ND	ND
3	SM3	Super market (with certified safety label)	ND	ND
4	MM1	Minimart (without safety label)	ND	ND
5	FS1	Farm shop (with safety label)	ND	ND
6	LF2	Local fresh market (without safety label)	ND	ND
7	SM2	Super market (without safety label)	ND	4.14 ± 0.74
8	SM4	Super market (with certified safety label)	ND	ND
9	MM2	Minimart (without safety label)	ND	0.65 ± 0.05
10	MM3	Minimart (with certified safety label)	ND	0.54 ± 0.14
11	FS2	Farm shop (with safety label)	ND	ND

Table 3.17 Frequency of carboxin residues detected in cabbage samples

Type of sample	No. of samples	Carbofuran		Carboxin	
		No. of positives	Percent positive	No. of positives	Percent positive
Without safety label	6	0	-	2	33.33
With safety label	5	0	-	1	20.00

In this research work, carbofuran was not detected in any samples. There are several factors affecting the level of residues in cabbage samples such as application technique, crop stage, formulation, dosage, replication and post harvest interval. In addition, environmental factors such as exposure to the light, temperature, volatilization, run off can be considered as the limiting factors in this study. On the other hand, it can be said that farmers applied the carbofuran pesticide in proper way. The results for carboxin showed that residues in the cabbage sample with safety level were lower than those without safety label. Table 3.17 showed the number and percent of samples of cabbages found to contain carboxin residues. The sample with the safety label is less likely to have detectable residues than sample without the safety label. The carboxin residues found in the sample with the safety label may be resulted from the treatment during transport or storage or mixing of treated and untreated produce somewhere between farm and retail as well as from possible mislabeling [41].

3.7 Confirmation for the analyte

Confirmation method was achieved on the basis of the comparison of the spiking the standards at 0.1 ppm fortification level in some extracted sample under the same optimum condition. The chromatograms of analytes with and without spiking standards compared are shown in Table 3.18 and Figures 3.15 and 3.16.

Table 3.18 Comparison of the extracted samples with and without standard spiking

Analyte	Without spiking		With spiking	
	t_R (min)	PA (mAU)	t_R (min)	PA (mAU)
Standard carboxin 0.1 µg/ml	-	-	22.24	5.80
Sample SM2 (Super market without safety level)	22.21	11.47	22.25	16.61

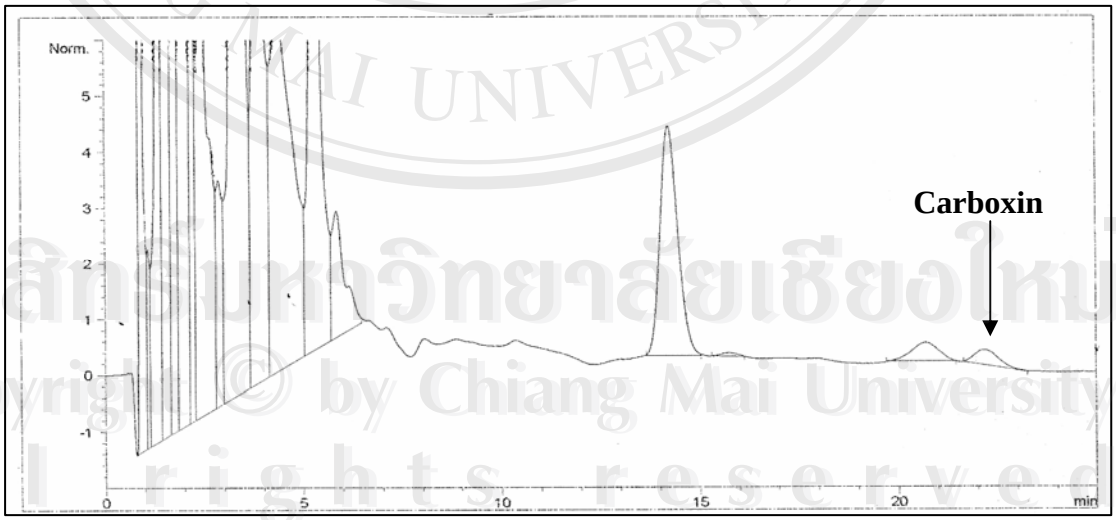

Figure 3.15 Chromatogram of sample SM2 (supermarket without safety label)

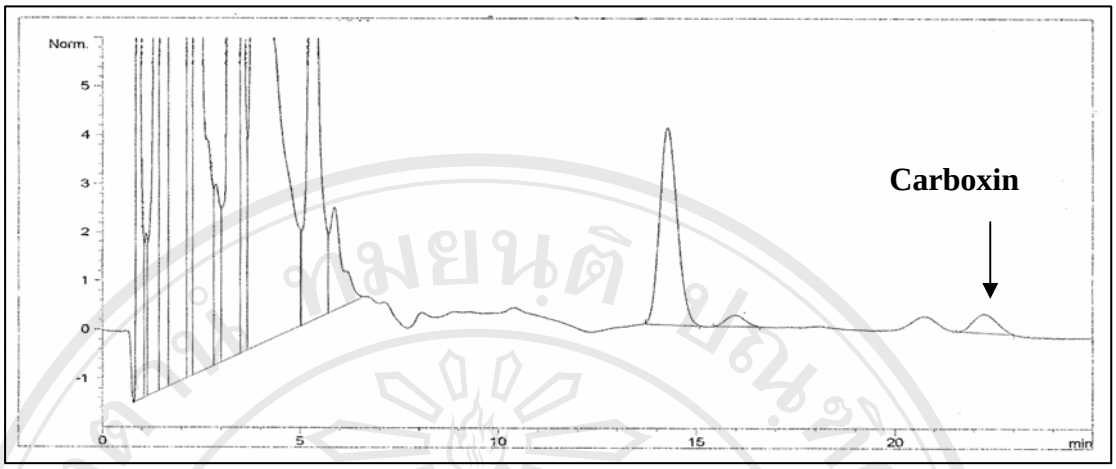

Figure 3.16 Chromatogram of sample SM2 (supermarket without safety label) with carboxin spiking

In addition, spiking the standard to the sample, some samples were also confirmed by the Laboratory Center for Food and Agricultural Products Co., Ltd (LCFA) in Chiang Mai. Carbofuran was not detected by HPLC equipped with fluorescence detector and whereas carboxin was detected by GC-MS see detail in Appendix C. The retention time and corrected area of standard carboxin and analyte found in GC-MS are shown in Table 3.19 and linearity of the calibration curve is shown in Figure 3.17.

Table 3.19 Retention time and corrected area of standard carboxin and analyte in the sample obtained from GC-MS system

Compounds	**t_R (min)**	**Corrected area**
Standard 0.1µg/ml	20.11	50492
Sample MM2 (Minimart without safety label)	20.17	11405

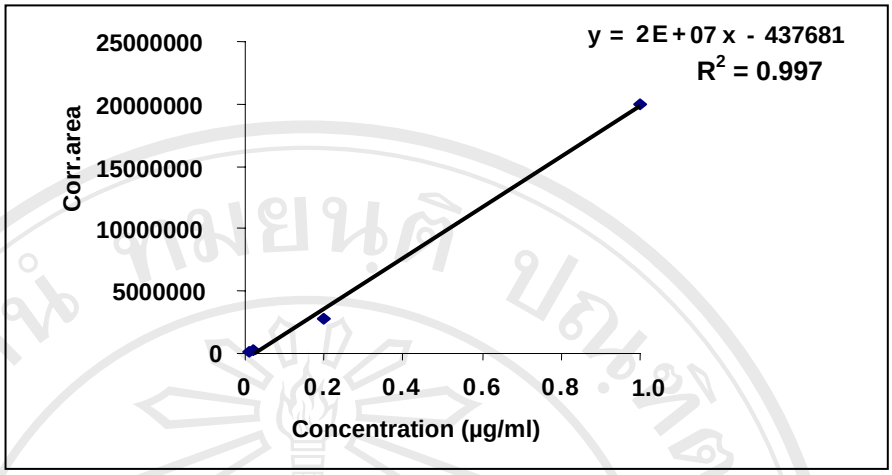

Figure 3.17 Calibration curve of carboxin by plotting the response of GC-MS in terms of corrected area against the concentration of standard injected (LCFA lab)

Table 3.20 Confirmation for the sample MM2 (Minimart 3 without safety label) by different methods

Method	Linear equation	Coefficient (R^2)	Corrected Area/Peak area (mAU)	Concentration (µg/ml)
HPLC-UV	y =74.402x+0.1132	0.997	1.71	0.02
GC-MS	y =2E+07x-437681	0.997	11405	0.02

Table 3.19 showed that retention time of analyte in sample was similar to those of standard. In addition, it was found that comparison of the methods for the confirmation of the sample was acceptable as shown in Table 3.20. Therefore, GC-MS could confirm the analyte compound carboxin.

In this research work, carbofuran was not detected in any samples. There are several factors affecting the level of residues in cabbage samples such as application

technique, crop stage, formulation, dosage, replication and post harvest interval. In addition, environmental factors such as exposure to the light, temperature, volatilization, run off can be considered as the limiting factors in this study. On the other hand, it can be said that farmers applied the carbofuran pesticide in proper way. The results for carboxin showed that residues in the cabbage sample with safety level were lower than those without safety label. In addition, sample with the safety label is less likely to have detectable residues than sample without the safety label. The carboxin residues found in the sample with the safety label may be resulted from the treatment during transport or storage or mixing of treated and untreated produce somewhere between farm and retail as well as from possible mislabeling [41].

3.8 Risk Assessment for Consumers

Human poisonings and illness are the highest price paid when pesticide reach non target areas. It is estimated that there are about 1 million accidental human pesticides poisoning each year in the world. Although the major source of exposure for human is probably residues in food, the other contaminated environmental media and routes of exposure should be considered [36]. Environmental contaminated media such as soil, water from which individuals are likely to be exposed to the media. In addition, relevant routes of exposure such as inhalation, ingestion, and dermal exposure and exposure to vegetables are necessary to be considered.

As a potential contaminated media point of view, methomyl has low persistence in the soil environment, with a reported half-life of approximately 14 days. Methomyl residues are not expected to be found in treated soil after the growing

season in which it is applied. The estimated aqueous half-life of the methomyl is 6 days in surface water and over 25 weeks in groundwater. Less than 3% methomyl remained in cabbage plants 1 week after they were given foliar treatment with the insecticide [13]. Methomyl degradation showed that it has unlikely to persist in the soil and surface water but there is potential contamination in the ground water. Methomyl is highly toxic via the oral route, with reported oral LD_{50} values of 17 to 24 mg/kg in rats 10 mg/kg in mice, and 15 mg/kg in guinea pigs Symptoms of methomyl exposure are similar to those caused by other carbamates and cholinesterase inhibitors. Methomyl is quickly absorbed through the skin, lungs, and gastrointestinal tract and are broken down in the liver. Breakdown products are readily excreted via respiration and urine. Although they do not appear to accumulate in any particular body tissue, they may alter many other enzymes besides the cholinesterase [13]. Due to the broad spectrum use of methomyl in agriculture protection and toxicity effects to humans, more data about contamination in the soil, water and food stuff are necessary to monitor to assess the potential risk to human health.

Carbofuran is soluble in water and is moderately persistent in soil. Its half-life is 30 to 120 days. The hydrolysis half-life of carbofuran in water at 25 ^{0}C is 690, 8.2, and 1.0 weeks at pH values of 6.0, 7.0, and 8.0, respectively. Carbofuran has a high potential for groundwater contamination. Half-life of carbofuran on crops is about 4 days when applied to roots, and longer than 4 days if applied to the leaves. Carbofuran is poorly absorbed through the skin. It is metabolized in the liver and eventually excreted in the urine. The half-life in the body is from 6 to 12 hours. Less than 1% of a dose will be excreted in a mother's milk. It does not accumulate in tissue

[13]. In this experiment, carbofuran pesticides are not detected in any sample cabbages resulted in no potential risk to consumers.

Carboxin is rapidly degraded to carboxin sulfoxide in soil and it has a low persistence, with a half-life of about 3 days in soil. In one study after 7 days, 95% of the parent was gone and the sulfoxide, a breakdown product, represented 31 to 45% of the amount applied**.** In water, carboxin oxidizes to the sulfoxide and sulfone within 7 days. Plants grown from treated seed had no carboxin present 6 weeks after emergence. Carboxin degradation in the soil, water and plant showed that this kind of pesticide could quickly decompose in the environment and thus belongs to the class of short lived pesticides. However, the half life of carboxin, like other pesticides, is generally independent to the original concentration. Although carboxin has possible acute and chronic toxic effects, it has no carcinogenic, mutagenic and reproductive effects. Illness as a result of carboxin poisoning involved vomiting and headache and irritating the eyes. Recovery is very rapid if the exposed individual is treated quickly. The compound does not acumulate in animal tissues. However, significant levels were found in milk a few days after exposure [13].

In this study, the relevant route of exposure through the carboxin contact of exposure to vegetables was assessed. In Thailand, vegetables are an important crop group. During 1994, annual per capita availability of vegetables at the farm level was about 53 kg (or 145 g/day). The latest available consumption survey suggests that Thais consume an average of 742 g/day of all foods, of which vegetables account for about 14%. Cereals, meat products and fruits account for 45%, 16% and 12%,

respectively. Cabbage is one of the major vegetable in Thailand and per capita consumption was about 9.67 g/day [37].

In this study, the highest concentration detected in cabbage sample was considered for human risk assessment. In order to evaluate this assessment, the amount of carboxin found in cabbage was compared with RfD. In terms of IUPAC Compendium of Chemical Terminology, RfD is an estimate of daily exposure to the human population that is likely to be without appreciable risk of deleterious effects during a lifetime [38]. The RfD of carboxin is 0.1 mg/kg/day. In general, daily human consumption of cabbage is 9.67 g/day. If the cabbage was contaminated by 4.14 mg/kg of fresh weight, a normal Thai man weighing about 60 kg would consume 0.04 mg of carboxin, corresponding to 0.0006 mg/kg body weight/day and this amount is much lower than the RfD value. Therefore, an average man could take this contaminated cabbage without causing human risk.

For the fruits and vegetables sold on markets, most of them could have some pesticlides remaining. However, it is clear that purchasing food with safety label is a reliable way to markedly reduce exposure to pesticides. Less exposure means greater margins of safety. In addition, it is highly recommended for the consumers to decrease the amount of pesticides remaining as much as possible such as thoroughly washing vegetables can substantially reduce exposures to contaminated pesticides.