CHAPTER 3

RESULTS

3.1 The suitable GC-ECD conditions for analysis of cypermethrin and fenvalerate insecticides

The optimization of GC-ECD conditions were studied using the parameters presented in 2.6.2. Results from optimization of GC-ECD conditions for analysis of cypermethrin and fenvalerate insecticide are shown in Table 3.1.

Table 3.1 Conditions of GC-ECD employed

Operation	Conditions
1. Oven	
initial temperature	100 °C (hold 2 min)
maximum temperature	320 °C
rate	100-180 °C, rate 8 °C/min (hold 3 min)
	180-260 °C, rate 10 °C/min (hold 3 min)
	260-300 °C, rate 4 °C/min (hold 3 min)
2. Detector	
type	ECD
temperature	300 °C
3. Injector	
volume	1.0 μL

Table 3.1 (continued)

Operation	Conditions
4. Column	
capillary column	HP-5 5% Phenyl Methyl Siloxane
	30.0 m × 320 μm I.D., 0.25 μm nominal
maximum temperature	325 °C
5. Front inlet	
mode	splitless
temperature	250 °C
6. Carrier gas	helium
mode	constant flow
initial flow	1.6 mL/min
7. Analysis time	42 minutes

3.2 The retention times of cypermethrin and fenvalerate insecticide standards

The retention time of cypermethrin and fenvalerate insecticide standards were measured under the current operating optimum conditions. Results are presented in Tables 3.2- 3.3 and Figures 3.1-3.3.

Table 3.2 Retention time of cypermethrin insecticide standard (n=10)

Day	Retention time (min)			
	Cypermethrin 1	**Cypermethrin 2**	**Cypermethrin 3**	**Cypermethrin 4**
1	28.188	28.383	28.533	28.607
2	28.187	28.383	28.531	28.607
3	28.185	28.379	28.527	28.601
4	28.181	28.375	28.523	28.598
5	28.191	28.384	28.534	28.607
6	28.187	28.382	28.530	28.604
7	28.192	28.384	28.535	28.606
8	28.181	28.376	28.526	28.601
9	28.182	28.376	28.528	28.598
10	28.182	28.375	28.526	28.599
Mean	**28.186**	**28.379**	**28.529**	**28.603**
SD	**0.0041**	**0.0038**	**0.0039**	**0.0038**
%RSD	**0.0144**	**0.0137**	**0.0138**	**0.0134**

Table 3.3 Retention time of fenvalerate insecticide standard (n=10)

Day	Retention time (min)	
	Fenvalerate 1	**Fenvalerate 2**
1	30.028	30.455
2	30.028	30.457
3	30.019	30.448
4	30.021	30.447
5	30.027	30.452
6	30.026	30.455
7	30.032	30.460
8	30.021	20.446
9	30.024	30.453
10	30.028	30.456
Mean	**30.025**	**30.453**
SD	**0.0041**	**0.0046**
%RSD	**0.0135**	**0.0152**

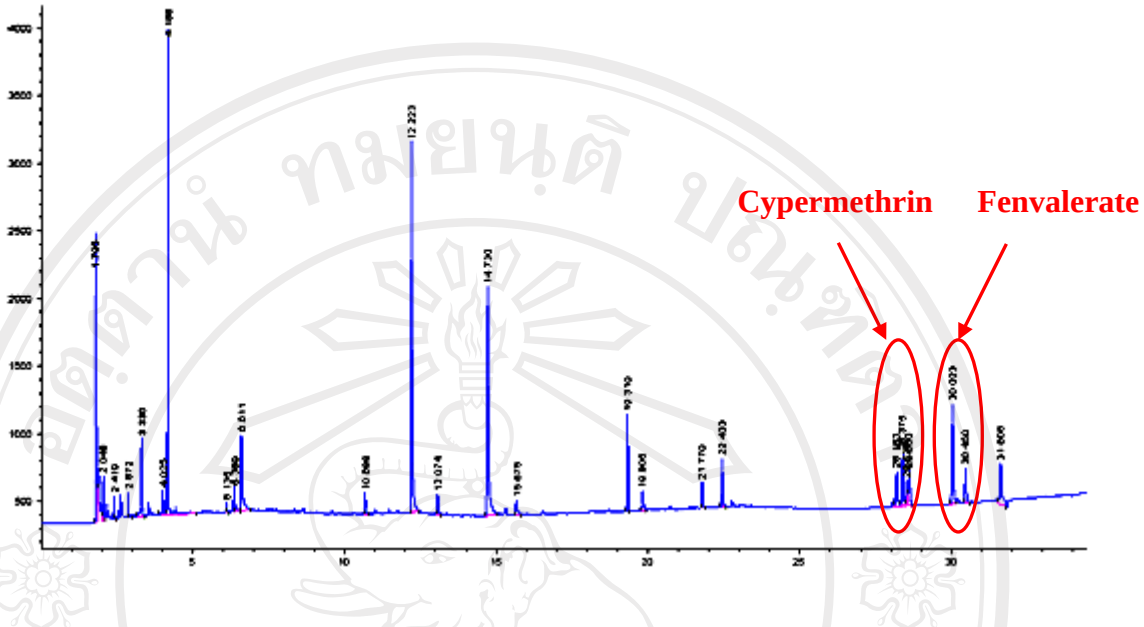

Figure 3.1 GC-ECD chromatogram of cabbage extract under optimum condition

(fortified with cypermethrin and fenvalerate, 3.0 mg/L)

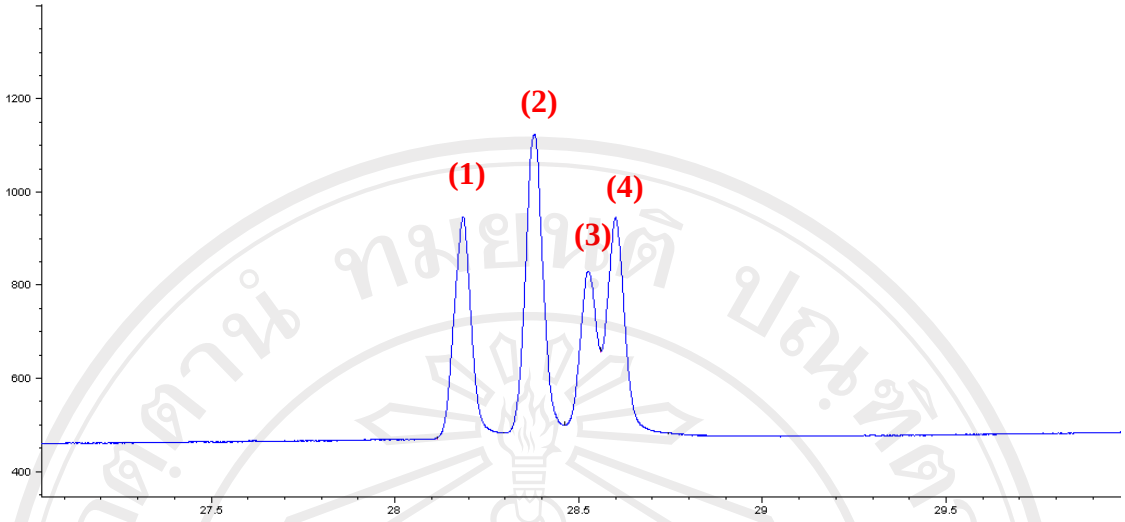

Figure 3.2 GC-ECD chromatogram of cypermethrin standard solution with 2 mg/L, **(1)** cypermethrin 1, **(2)** cypermethrin 2, **(3)** cypermethrin 3, **(4)** cypermethin 4.

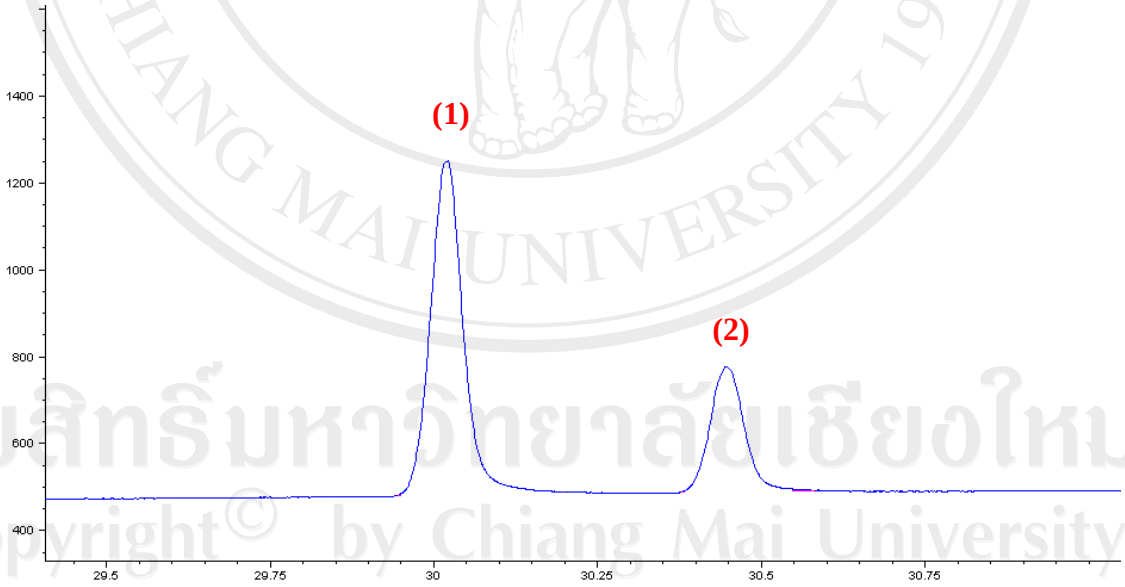

Figure 3.3 GC-ECD chromatogram of fenvalerate standard solution with 2 mg/L, **(1)** fenvalerate 1, **(2)** fenvalerate 2.

3.3 Investigation of suitable solvent for extraction of cypermethrin and fenvalerate insecticide residues from cabbage samples

The suitable ratios of solvent mixture between acetone, *n*-hexane and ethyl acetate for extraction of cypermethrin and fenvalerate insecticide residues from cabbage samples were investigated. Results are presented in Table 3.4.

Table 3.4 Comparison of SD, % RSD, % recovery and % error of cypermethrin and fenvalerate at each solvent mixture ratio (n=5)

The ratio of solvent mixture (A : H : E)*	Cypermethrin				Fenvalerate			
	SD	% RSD	% Recovery	% Error	SD	% RSD	% Recovery	% Error
100 : 75 (Acetone : CH_2Cl_2)**	0.06-0.27	10-15	82-89	11-18	0.06-0.29	11-16	78-85	15-22
90 : 5 : 5	0.08-0.14	41-14	33-38	62-67	0.05-0.30	21-34	30-46	54-70
80 : 10 : 10	0.06-0.32	24-28	40-45	55-60	0.06-0.31	27-29	35-48	52-65
70 : 15 : 15	0.05-0.45	24-36	41-47	53-59	0.04-0.44	18-36	41-46	54-59
60 : 20 : 20	0.05-0.47	19-36	43-48	52-57	0.04-0.33	14-19	52-58	42-48
50 : 25 : 25	0.05-0.28	18-19	50-53	47-50	0.04-0.21	11-15	58-62	38-42
40 : 30 : 30	0.05-0.41	15-17	71-82	18-29	0.08-0.41	17-22	70-79	21-30
33.33 : 33.33 : 33.33	0.05-0.19	6-10	93-103	3-7	0.05-0.36	12-13	83-91	9-17

Table 3.4 (continued)

The ratio of solvent mixture (A : H : E)*	Cypermethrin				Fenvalerate			
	SD	% RSD	% Recovery	% Error	SD	% RSD	% Recovery	% Error
5 : 90 : 5	0.06-0.20	16-39	33-42	58-67	0.05-0.25	22-30	33-37	63-67
10 : 80 : 10	0.05-0.40	23-27	46-48	51-54	0.04-0.30	18-20	43-49	51-57
15 : 70 : 15	0.06-0.30	15-21	56-67	24-33	0.06-0.29	16-24	50-62	38-50
20 : 60 : 20	0.07-0.27	13-21	63-70	30-37	0.05-0.43	17-20	65-70	30-35
25 : 50 : 25	0.05-0.42	15-18	73-79	21-27	0.06-0.34	15-18	69-76	24-31
30 : 40 : 30	0.05-0.24	9-11	86-91	9-14	0.07-0.31	12-17	77-85	15-23
5 : 5 : 90	0.05-0.34	20-24	39-55	45-61	0.06-0.62	27-34	42-61	39-58
15 : 15 : 70	0.04-0.17	11-19	45-49	51-55	0.05-0.08	6-16	45-63	37-55
25 : 25 : 50	0.04-0.06	4-9	54-80	20-46	0.05-0.08	4-14	63-74	26-36
30 : 30 : 40	0.05-0.08	4-12	64-83	17-36	0.05-0.17	8-12	70-85	15-30

(A : H : E)* = The ratio of acetone : *n*-hexane : ethyl acetate (by volume)

(Acetone : CH_2Cl_2)** = Solvent mixture for extraction method of the Department of Agriculture, Thailand

3.4 Validation of the method

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

1) Percent recovery

Standard solutions containing 0.5 mg/L and 3 mg/L of cypermethrin and fenvalerate were added in 10 g of sample and then followed by the process of extraction as described in Figure 2.1. The percent recoveries of the method are shown in Table 3.4. Example of the calculation is shown in Appendix B.

2) Detection limits

Sample blank was added to standard solution containing 0.1-1.0 mg/L of cypermethrin and fenvalerate insecticides and then analyzed by GC. The detection limit can be calculated from the linear calibration curves of each insecticide which were constructed by plotting the peak areas obtained from standard insecticides chromatogram against the corresponding concentration. Results of the determination are listed in Table 3.5.

Table 3.5 Detection limits of cypermethrin and fenvalerate insecticides, using GC-ECD analytical method

Analytes	Regression line	R^2	LOD (mg/L)
Cypermethrin	1191.5x + 161.4	0.9968	0.064
Fenvalerate	2724.4x – 80.932	0.9973	0.061

3) Precision

Standard solutions of cypermethrin and fenvalerate at concentration of 0.5 mg/L and 3.0 mg/L were determined using GC-ECD analytical method. The standard solutions were analyzed five replicates. Data were calculated to find the percentage of relative standard deviation (%RSD). Precisions are shown in Table 3.4 and example calculation is shown Appendix B.

4) Linearity

After injection of each working insecticide standard into GC, the peak area appeared. The peak areas are plotted against the insecticide standards at concentrations of 0.3, 0.5, 0.7, 1.0, 1.5, 2.0 and 3.0 mg/L. Results of linearity curves are presented in Tables 3.6- 3.7, and Figures 3.4-3.5.

Table 3.6 The ECD response (peak area) of cypermethrin with variation of concentration

Concentration (mg/L)	Peak area
0.3	924.22
0.5	1492.15
0.7	1883.59
1.0	3803.27
1.5	6000.53
2.0	8750.71
3.0	14490.37

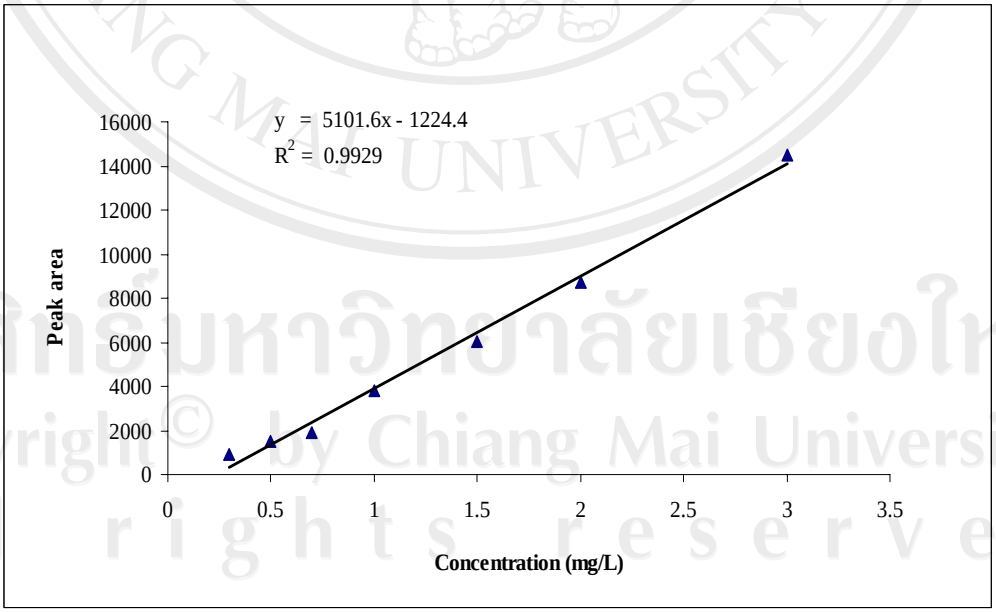

Figure 3.4 Linearity plot of peak area against concentration of cypermethrin under the optimized condition.

Table 3.7 The ECD response (peak area) of fenvalerate with variation of concentration

Concentration (mg/L)	Peak area
0.3	500.62
0.5	1011.31
0.7	1773.77
1.0	2877.23
1.5	5113.97
2.0	6833.73
3.0	10346.18

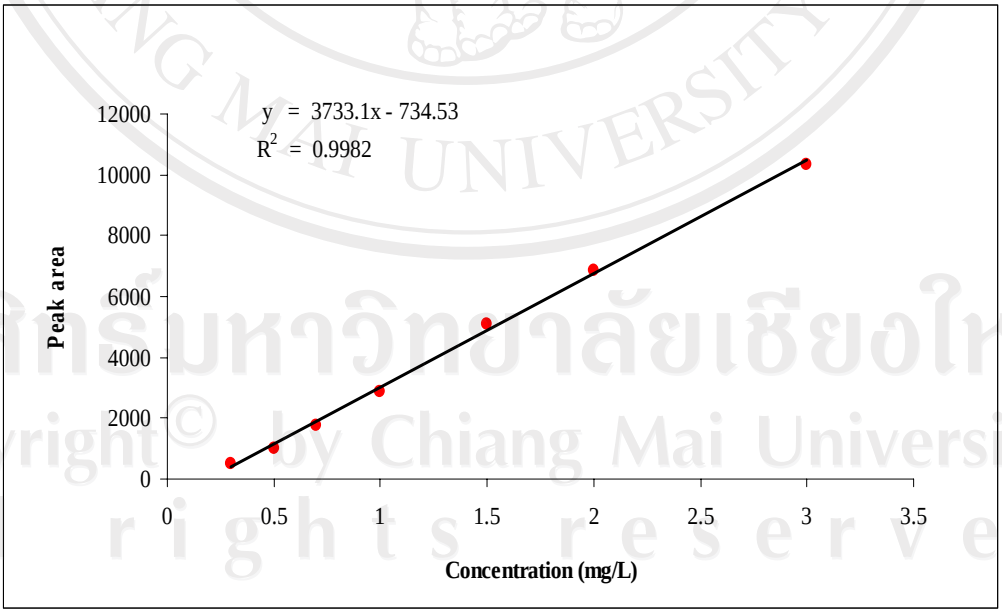

Figure 3.5 Linearity plot of peak area against concentration of fenvalerate under the optimized condition.

3.5 Determination of cypermethrin and fenvalerate insecticide residues in cabbages

The amounts of cypermethrin and fenvalerate insecticide residues in cabbage samples, using standard addition analytical method were determined. Results are presented in Table 3.8.

Table 3.8 Cypermethrin and fenvalerate insecticide residues in cabbage samples

Sample number	Residues (mg/kg)	
	Cypermethrin	Fenvalerate
Sample No.1	ND*	ND*
Sample No.2	ND*	ND*
Sample No.3	**0.221**	**0.201**
Sample No.4	ND*	0.110
Sample No.5	ND*	ND*
Sample No.6	ND*	0.093
Sample No.7	ND*	0.110
Sample No.8	0.086	0.185
Sample No.9	ND*	ND*
Sample No.10	ND*	ND*

ND* = not detectable

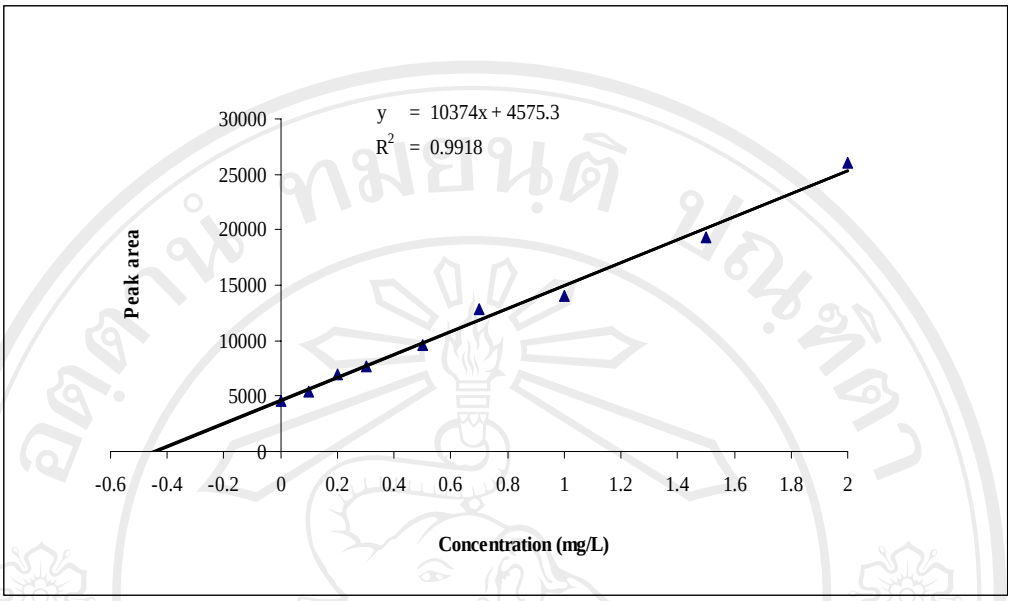

Figure 3.6 Relationship between peak area and concentration of cypermethrin insecticide in sample No. 3 by standard addition method

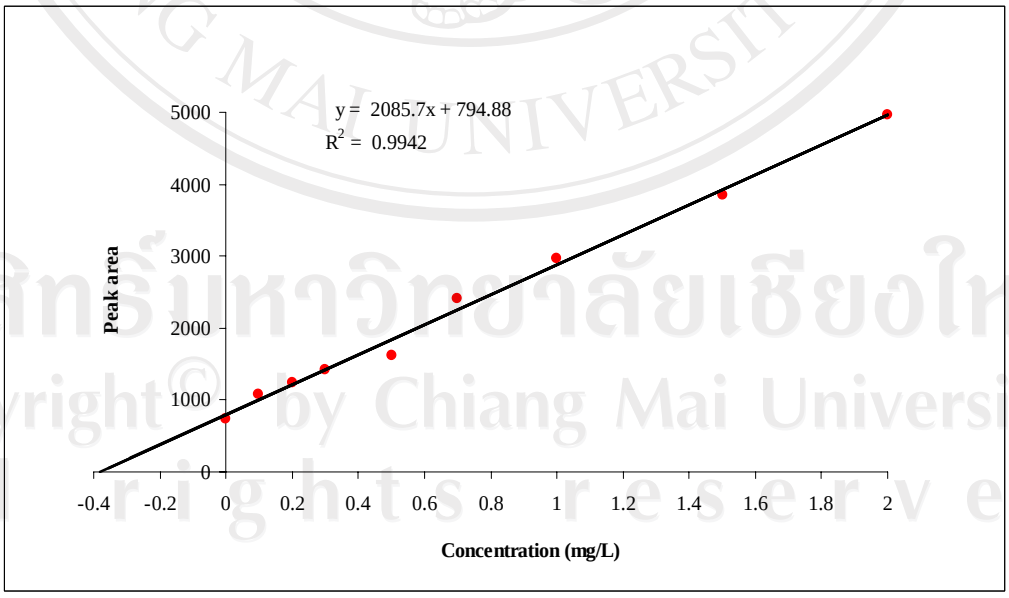

Figure 3.7 Relationship between peak area and concentration of fenvalerate insecticide in sample No. 3 by standard addition method

3.6 Desorption study

3.6.1) Desorption of cypermethrin at room temperature (28 °C)

A time profile for desorption of cypermethrin from cabbage in water and in washing liquid at room temperature was determined. Results are shown in Tables 3.9 and Figures 3.8.

Table 3.9 Comparison of time profile for desorption of cypermethrin from cabbage, in water and in washing liquid at room temperature (28 °C) *(initial amounts of cypermethrin in the cabbage samples were 17 and 16 mg for desorption in water, and in washing liquid, respectively)*

Desorption time h (min)	$h^{1/2}$ ($min^{1/2}$)	Amount of cypermethrin found			
		in water		in washing liquid	
		mg	%desorbed	mg	%desorbed
10	0.41	1.4	8.2	1.2	7.5
20	**0.58**	1.6	9.4	**2.0**	**12**
30	**0.71**	**1.8**	**11**	1.9	12
40	0.81	1.6	9.4	1.9	12
50	0.91	1.5	8.8	1.8	11
60	1.00	1.7	10	1.8	11
70	1.08	1.6	9.4	1.5	9.4
80	1.15	1.4	8.2	1.6	10
90	1.22	1.5	8.8	1.5	9.4
100	1.30	1.5	8.8	1.5	9.4
110	1.35	1.4	8.2	1.5	9.4
120	1.41	1.3	7.6	1.5	9.4

3.6.2) Desorption of cypermethrin at 90 °C

A time profile for desorption of cypermethrin from cabbage at 90 °C was determined, in water and in washing liquid. Results are shown in Table 3.10 and Figure 3.8.

Table 3.10 Comparison of time profile for desorption of cypermethrin from cabbage, in water and in washing liquid at 90 °C *(initial amounts of cypermethrin in the cabbage samples were 13 and 12 mg for desorption in water, and in washing liquid, respectively)*

Desorption time h (min)	**$h^{1/2}$ ($min^{1/2}$)**	**Amount of cypermethrin found**			
		in water		**in washing liquid**	
		mg	**%desorbed**	**mg**	**%desorbed**
10	**0.41**	**1.7**	**13**	**2.6**	**22**
20	0.58	1.6	12	1.3	11
30	0.71	1.4	11	2.1	18
40	0.81	1.1	8.5	1.7	14
50	0.91	1.4	11	1.1	9.2
60	1.00	1.2	9.2	1.5	13
70	1.08	1.1	8.5	1.4	12
80	1.15	1.1	8.5	1.2	10
90	1.22	1.0	9.0	1.2	10
100	1.30	1.1	8.5	1.3	11
110	1.35	1.0	9.0	1.2	10
120	1.41	1.0	9.0	1.2	10

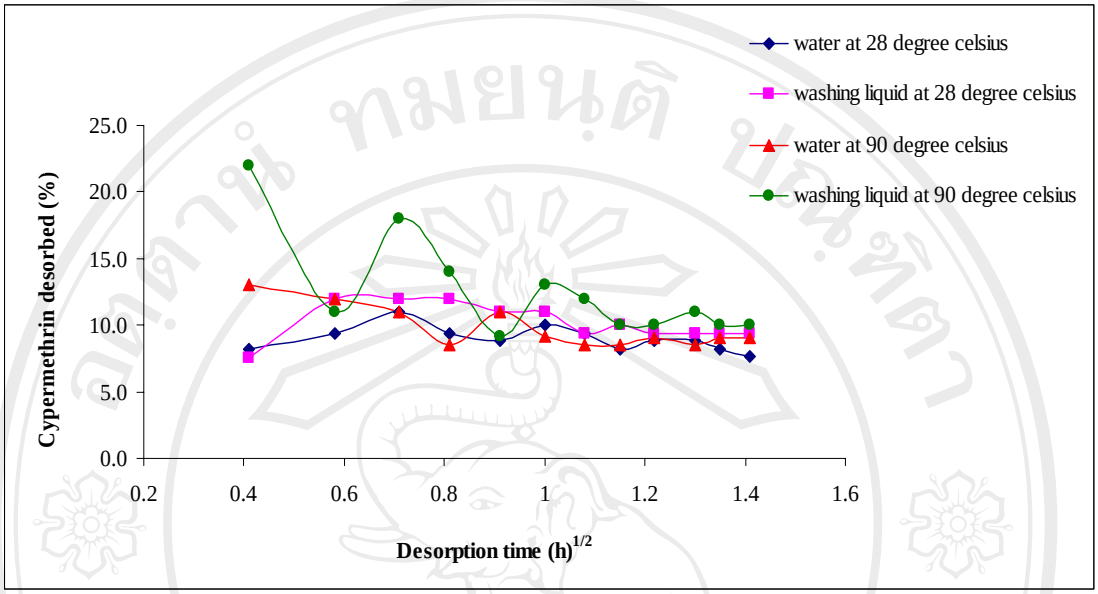

Figure 3.8 Desorption time profile of cypermethrin insecticide residue from cabbage (at 28 °C and 90 °C) in water and in washing liquid

3.6.3) Desorption of fenvalerate at room temperature (28 °C)

A time profile for desorption of fenvalerate from cabbage at room temperature was determined, in water and in washing liquid. Results are shown in Tables 3.11 and Figures 3.9.

Table 3.11 Comparison of time profile for desorption of fenvalerate from cabbage, in water and in washing liquid at room temperature (28 °C) *(initial amounts of fenvalerate in the cabbage samples were 7.4 and 12 mg for desorption in water, and in washing liquid, respectively)*

Desorption time h (min)	**$h^{1/2}$ ($min^{1/2}$)**	**Amount of fenvalerate found**			
		in water		**in washing liquid**	
		mg	**%desorbed**	**mg**	**%desorbed**
10	0.41	0.32	4.3	0.48	4.0
20	0.58	0.90	12	0.90	7.5
30	0.71	0.61	8.2	1.2	10
40	**0.81**	**1.8**	**24**	**2.4**	**20**
50	0.91	0.41	5.5	0.99	8.3
60	1.00	0.57	7.7	0.99	8.3
70	1.08	0.44	5.9	1.4	12
80	1.15	0.42	5.7	1.2	10
90	1.22	0.50	6.8	1.4	12
100	1.30	0.55	7.4	0.87	7.3
110	1.35	0.56	7.6	1.2	10
120	1.41	0.59	8.0	1.4	12

3.6.2) Desorption of fenvalerate at 90 °C

A time profile for desorption of fenvalerate from cabbage at 90 °C was determined, in water and in washing liquid. Results are shown in Table 3.12 and Figure 3.9.

Table 3.12 Comparison of time profile for desorption of fenvalerate from cabbage, in water and in washing liquid at 90 °C *(initial amounts of fenvaleratein the cabbage samples were 18 and 16 mg for desorption in water, and in washing liquid, respectively)*

Desorption time h (min)	**$h^{1/2}$ ($min^{1/2}$)**	**Amount of fenvalerate found**			
		in water		**in washing liquid**	
		mg	**%desorbed**	**mg**	**%desorbed**
10	0.41	1.8	10	2.3	14
20	**0.58**	2.1	12	**2.9**	**18**
30	0.71	2.9	16	2.3	14
40	0.81	2.3	13	2.6	16
50	0.91	2.5	14	2.7	17
60	1.00	2.5	14	2.7	17
70	**1.08**	**3.8**	**21**	2.9	18
80	1.15	2.6	14	2.6	16
90	1.22	2.5	14	2.5	16
100	1.30	2.6	14	2.5	16
110	1.35	2.3	13	2.5	16
120	1.41	2.5	14	2.3	14

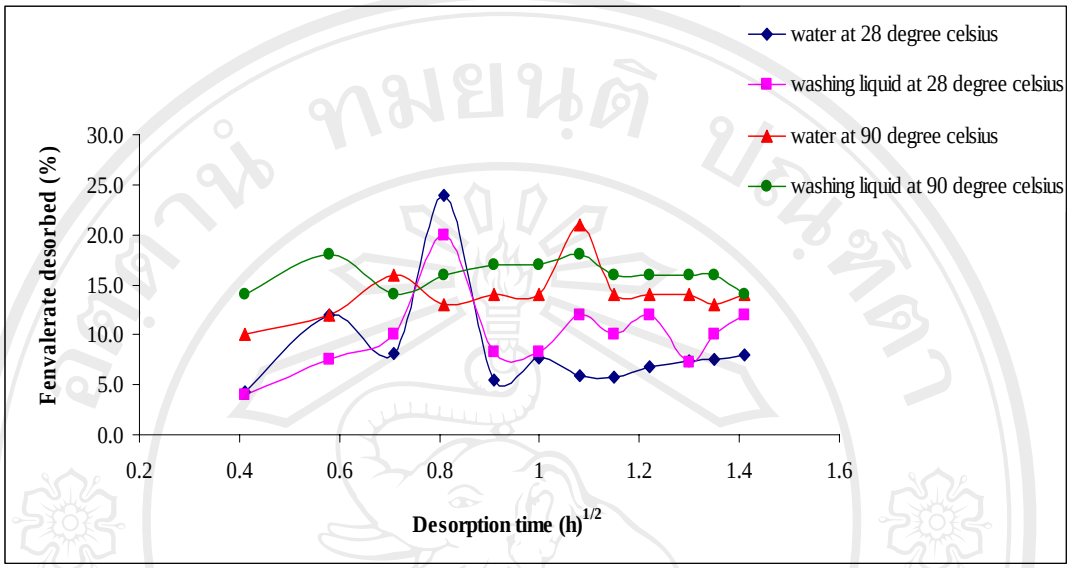

Figure 3.9 Desorption time profile of fenvalerate insecticide residue from cabbage (at 28 °C and at 90 °C) in water and in washing liquid