CHAPTER 2

MATERIALS AND METHODS

2.1 Apparatus and chemicals used in this study:

2.1.1 Apparatus

1) Gas chromatograph 6890N series (Agilent Technologies, U.S.A.) consists of
   a) Electron capture detector (ECD)
   b) Auto Sampler Agilent Technologies, 7683 series injector
   c) Capillary columns with HP-5, 30.0 m × 320 μm I.D., 0.25 μm nominal
2) Micropipette
   a) 20-200 μL, P series (Gilson Medical Electronics, France)
   b) 200-2000 μL, SL 2000 series (Rainin Instrument, LLC A Mettler Toledo, U.S.A.)
   c) 500-5000 μL, SL 5000 series (Rainin Instrument, LLC A Mettler Toledo, U.S.A.)
3) Rotary evaporator R-124 series (Buchi, Switzerland)
4) Shaker VRN-480 series (Gemmy Industrial)
5) Blender MX J210GN series (Panasonic, Japan)
6) Stirrer PMC series (Barnstead Thermolyne)
7) Analytical balance AB 204-S series (Mettler Toledo GmbH, Laboratory/Weighing Technologies)
8) Nylon syringe filter, 0.2 μm × 13 mm (Filtrex)

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9) Disposable syringe, 3 mL (Nipro, Thailand)

2.1.2 Chemicals

1) Acetone (AR grade, Lab-Scan)
2) Ethyl acetate (AR grade, Lab-Scan)
3) Dichloromethane (AR grade, BDH)
4) *n*-Hexane (AR grade, Fisher Scientific)
5) Cyclohexane (AR grade Merck, Germany)
6) Sodium chloride (AR grade Merck, Germany)
7) Anhydrous sodium sulfate (AR grade, Fisher Scientific)
8) Helium gas, 99.999% (UHP grade TIG, Thailand)
9) Nitrogen gas, 99.999% (UHP grade TIG, Thailand)

2.2 Standard insecticides

1) Cypermethrin standard (Rm, Riedel-deHaen, Germany), 98.8% (w/w)
2) Fenvalerate standard (Rm, GmbH, Germany), 98.0% (w/w)

2.3 Insecticide used in desorption study:

1) Cypermethrin
   - Brand : Tensho 35, manufactured by Sharp Formulators Co.,Ltd
   - Product : Cypermethrin (based on 35% w/v)
2) Fenvalerate
   - Brand : Fentom 10 ,manufactured by T.J.C. Chemical Co.,Ltd
   - Product : Fenvalerate (based on 10% w/v)

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2.4 Washing liquid for vegetables and fruits

- Brand : St. Andrews, manufactured by Lion (Thailand) Co.,Ltd

- Compound : Sodium Lauryl Ether Sulfate 7% (w/w)

2.5 Cabbage samples

The popular and commonly consumed vegetables in Thai food namely cabbage representing the brassicaceae families was selected for this study. Cabbage samples were purchased from Muang Mai market of Chiang Mai Province, Thiland. Details of the ten cabbage samples are presented in Table 2.1

Table 2.1 Details of the cabbage samples collected for the determination of cypermethrin and fenvalerate in this study

Sample number	Original source of the cabbage sample
Sample No.1	Mae Sareng district, Mae Hong Son province
Sample No.2	Mae Rim district, Chiang Mai province
Sample No.3	Mae Chaem district, Chiang Mai province
Sample No.4	Mae Sareng district, Mae Hong Son province
Sample No.5	Mae La Noi district, Mae Hong Son province
Sample No.6	Omkoi district, Chiang Mai province
Sample No.7	Mae Chaem district, Chiang Mai province
Sample No.8	Mae Taeng district, Chiang Mai province
Sample No.9	Samoeng district, Chiang Mai province
Sample No.10	Hot district, Chiang Mai province

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2.6 Methods

2.6.1 Preparation of standard solutions

1) Preparation stock solutions

Stock solutions of cypermethrin insecticide was prepared by dissolving 101.21 mg in 100 mL of solvent mixture of acetone, *n*-hexane and ethyl acetate (1:1:1, v/v/v) in a 100 mL volumetric flask, so the concentration of stock solution is 1000 mg/L. The stock solution of each insecticides were diluted to 100 mg/L with same solvent mixture and stored in amber bottles at 4 °C.

In case of the fenvalerate standard solution, the preparation was done similarly to cypermethrin standard solution and used was 102.04 mg.

2) Preparation of working standard solutions

A 10 mg/L of standard solutions were prepared from 100 mg/L of stock standard solution by dilution. Individual series working standard solutions were prepared by diluting 10 mg/L of standard solutions to produce a final concentration of 0.1, 0.2, 0.3, 0.5, 0.7, 1.0, 1.5 and 2.0 mg/L in the same solvent mixture as shown in Table 2.2.

For example, the preparation of 5 mL of 0.1 mg/L cypermethrin insecticide was done by pipetting cypermethrin insecticide 0.05 mL of 10 mg/L of cypermethrin standard solution into a 5 mL volumetric flask and diluting it near the mark with acetone, *n*-hexane and ethyl acetate (1:1:1, v/v/v). Then the solution was adjusted to concentration 0.1 mg/L and the solution was finally adjusted to 5 mL.

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Table 2.2 Composition of the standard solutions

Concentration of cypermethrin and fenvalerate insecticides (mg/L)	Volume of 10 mg/L cypermethrin and fenvalerate solutions
0.1	0.05 mL
0.2	0.10 mL
0.3	0.15 mL
0.5	0.25 mL
0.7	0.35 mL
1.0	0.50 mL
1.5	0.75 mL
2.0	1.0 mL

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2.6.2 Optimization of experimental parameter of GC-ECD

All the experiment parameters such as auto sampler, splitless injection mode, capillary columns, three multiramp temperature programming, the temperature of ECD detector and the temperature of injector port were constant, helium was carrier gas and nitrogen gas was used to make up a flow with a rate of 60.0 mL/min were optimized.

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2.6.3 Extraction

1) A method from the Department of Agriculture, Ministry of Agriculture and Cooperatives

The experiment was done by chopping a sample, A 50 g of sample was homogenized by a blender with 100 mL of acetone and 75 mL of dichloromethane, then 15 g sodium chloride was added. The mixture was mechanically shaken for 2 min and poured into flask which contained sodium sulfate. After that, the extract was filtered and evaporated using a rotary evaporator. Finally, the extract was eluted by acetone then determined with GC.

2) The extraction method used in this study

The extraction method started with preparation of cabbage using 1 kg of cabbage unwashed, cut in halves and the sample were cut into small pieces and homogenized in a blender. A 10 g of cabbage sample was put in a 250 mL erlenmeyer flask and 50 mL of solvent mixture (acetone + *n*-hexane + ethyl acetate, v/v/v) was mixed. The mixture was mechanically shaken for 10 min after adding 10 g of sodium chloride. The extract was filtered by filter paper. The filtrate was transferred to a 250 mL erlenmeyer flask and sodium sulfate anhydrous was added. Then, the mixture was mechanically shaken for 10 min and allowed to settle. The liquid phase was evaporated under vacuum using a rotary evaporator to dryness with a water bath at 40 °C. The obtained residue was re-dissolved in 5 mL of acetone. Finally, filtered by 0.2 μm membrane and was analyzed by GC-ECD. The schematic of sample extraction is shown in Figure 2.1

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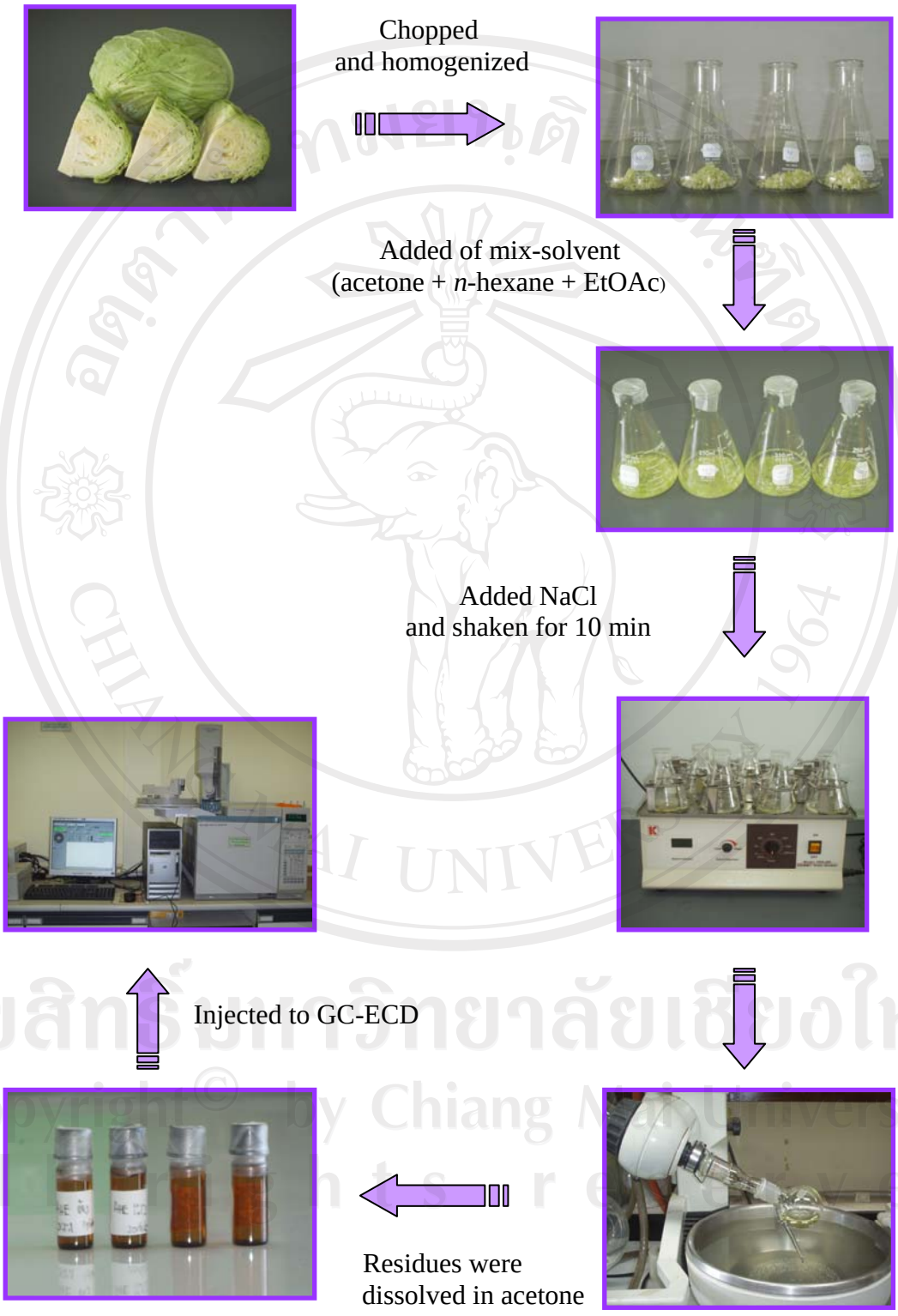

Figure 2.1 Schematic diagram of sample extraction

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2.6.4 Validation of method

After having the optimum condition for analysis of cypermethrin and fenvalerate using GC-ECD (Table 3.1), the same condition was repeated five times. Data were calculated and statistically compared. Validation of the method consists of percent recovery, detection limit, precision and linearity as described below.

1) Percent recovery

Fortification of cabbage samples were prepared by spiking 0.5 mg/L and 3 mg/L of standard solutions to 10 g of homogenized sample prior to extraction as described in Figure 2.1. The recovery was replicated five time and the results were calculated from the formula

$$\% \text{ Recovery} = \frac{\text{Peak area of the extract}}{\text{Peak area of standard solution}} \text{ X } 100$$

2) Detection limit

Standard insecticide solutions with proper concentrations were prepared and analyzed by GC-ECD. Detection limits (LOD) were obtained when the signal peak height was three times the noise or calculated using a signal-to-nose (S/N) ratio of 3.

3) Precision

Standard solution containing 0.5 mg/L and 3 mg/L of cypermethrin and fenvalerate were added to sample solution. The solutions were analyzed (five

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replicates) by GC-ECD using the same procedure and condition. Results were then calculated to find percent of relative standard deviation (% RSD).

4) Linearity

The linearity of insecticide standard curves were also studied. Two series of insecticide standard solution were prepared as followed : 0.1, 0.2, 0.3, 0.5, 0.7, 1, 1.5 and 2 mg/L of cypermethrin and fenvalerate. Each solution was analyzed by GC-ECD. The peak area appeared, and the area was plotted against concentration of the insecticide standards. The graph that has R^2 close to 1 was chosen to be linearity.

2.6.5 Determination of cypermethrin and fenvalerate insecticides in cabbage using standard addition method

The extract solution was obtained from extraction method as described in Figure 2.1, pipetted 2 mL of extract solution and standard insecticide solution at various concentrations were placed in a 5 mL volumetric flask. Then, volume is completed to mark using acetone and filtered by 0.2 μm membrane prior to analyzed by GC with ECD.

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2.6.6 Desorption of cypermethrin and fenvalerate insecticides in cabbage

1) Preparation of cypermethrin solution for desorption study

For the preparation of 350 mg/L of cypermethrin solution, pipetted 1 mL of cypermethrin insecticide (35%, w/v) into a 1000 mL volumetric flask and the volume is completed to mark with distilled water.

2) Preparation of fenvalerate solution for desorption study

For the preparation of 100 mg/L of fenvalerate solution, pipetted 1 mL of fenvalerate insecticide (10%, w/v) into a 1000 mL volumetric flask and the volume is completed to mark with distilled water.

2.6.6.1 Desorption study at room temperature (28 °C)

Two insecticides were sprayed on the cabbage sample. The experiments were conducted in three replicates and undisturbed for 1 h. The sample was cut into small pieces and then soaked in 1000 mL beaker which contained water and washing liquid solution. The mixtures were stirred constantly with magnetic bar. A 5 mL of water and washing liquid solution were collected at time intervals of 10, 20, 30,…,120 min and then proceeded to the process of extraction method as described in Figure 2.1. For desorption study at room temperature, the schematic diagram is shown in Figure 2.2

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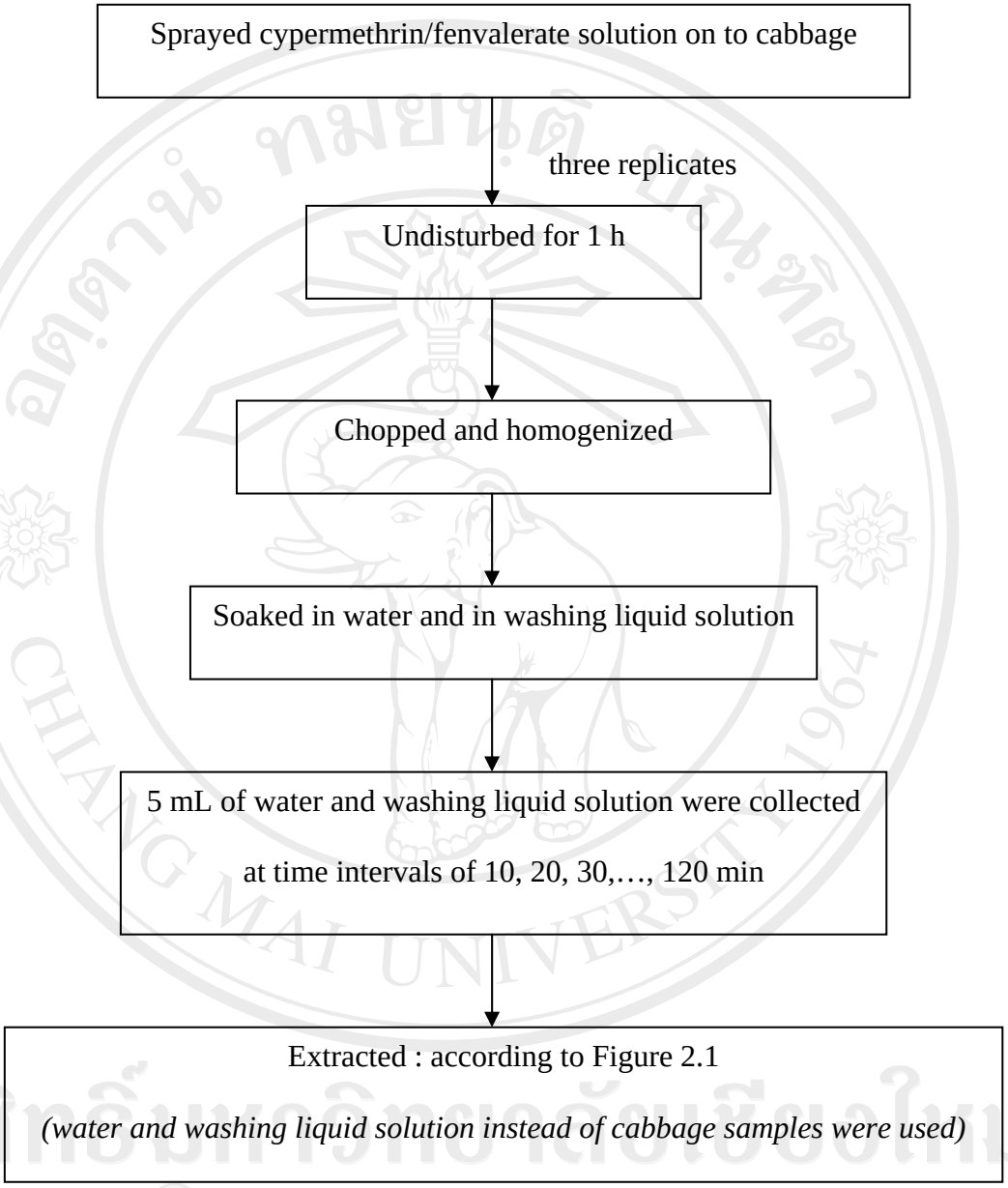

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Figture 2.2 Schematic diagram of desorption of cypermethrin and fenvalerate insecticides in cabbage at room temperature (28 °C)

2.6.6.2 Desorption of cypermethrin and fenvalerate insecticides in cabbage at 90 °C

The experiment was performed according to the desorption step in Figure 2.2. Temperature of water and washing liquid solution were kept constant at room temperature (28 °C) and at 90 °C.

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