CHAPTER III

MATERIALS AND METHODS

3.1 Chemicals used in this study

(1) Carbofuran Standard (Rm, Riedel-deHaen, Germanry)
(2) Parathion methyl Standard (Rm, Riedel-deHaen, Germanry)
(3) Cypermethrin Standard (Rm, Riedel-deHaen, Germanry)
(4) Sodium dodecyl sulfate (AR Grade Fluka, Germany)
(5) Sodium tetraborate (AR Grade Fluka, Germany)
(6) Boric acid (AR Grade Fluka, Germany)
(7) Sodium hydroxide (AR Grade Merk, Germany)
(8) Sodium chloride (AR Grade Merk, Germany)
9) Sodium sulphate (AR Grade Merk, Germany)
(10) Methanol (HPLC Grade, JT Baker)
(11) Dichloromethane (AR Grade, JT Baker)
(12) Acetone (AR Grade, JT Baker)

3.2 Instruments used in this study:

(1) Capillary Electrophoresis (MDQ , Beckman**)**

a) Fused silica column with Capillary diameter (ID) = 75 μm.

b) Total capillary length (L) = 50 cm. Effective capillary length (l) = 40.2 cm.

c) Column Temperature = 25 °C

d) Rinse column by 0.01N NaOH for 1 min.

e) Rinse buffer for 3 min.

f) Equilibrate column by buffer for 10 min.

g) Injection time set at 7 sec.

h) UV detector wavelength 190-800 nm

(2) High Performance Liquid Chromatograph

Conditions:

a) Mobile phase Water : Methanol : Acetonitrile

Table 3.1 Linear gradient program

Time (minute)	**Flow (ml/min)**	**%A (DI water)**	**%B (MeOH)**	**%C (ACN)**	**Curve**
Initial	1.50	88	12	0	0
4.00	1.50	88	12	0	1
4.10	1.50	68	16	16	3
16.10	1.50	30	35	35	10
19.00	1.50	88	12	0	9

b) Floe rate 1.5 ml/min

c) Column 39 x 150 mm Carbamate Analysis

d) Post Column OPA/NaOH 0.5 ml/min, 80 ^{o}C

e) Detector Excitation λ 339 nm, Emission λ 445 nm

(3)Gas Chromatograph (Agilent Technologies, 6890 N. Networt) consists of:

a) Auto Sample Agilent Technologies, 7683 series injector

b) Capillary Column with HP-1701, 30 m x 0.32 mm I.D., 0.15 μm film thickness

c) Temperature conditions

For detector 250 ^{o}C , for injector 220 ^{o}C

d) Oven Programme Temperature

Holding at 80 ^{o}C for 1 min, increased to 200 ^{o}C with 15 ^{o}C/min for 4 min, then changed to 220 ^{o}C with 3 ^{o}C/min for 5 min and finally raised up to 275 ^{o}C with 12 ^{o}C/min for 8 min

e) Flame Photometric Detector (FPD)

(4)Gas Chromatograph (Agilent Technologies, 6890 N. Networt) consists of:

a) Auto Sample Agilent Technologies, 7683 series injector

b) Capillary Column with HP-5, 30 m x 0.32 mm I.D., 0.25 μm film thickness

c) Temperature conditions

Detectore 300 ^{o}C , Injectore 250 ^{o}C

d) Oven Programme Temperature

Holding at 80 ^{o}C for 2 min, then increased up to 150 ^{o}C with 15 ^{o}C/min for 4 min, and to 250 ^{o}C with 3 ^{o}C/min until it reached 300 ^{o}C and remained at 12 ^{o}C/min for 5 min

e) Electron Capture Detector (ECD)

(5) Analytical Balance (AB204 , Mettler)

(6) Rotary Evaporator (EYELA SB 650, Tokyo Rikakikai)

(7) Homoginizer (T25, IKA Labortechnik)

(8) Blender, (Thomas Scientific, U.S.A)

(9) Autopipette (Brand Tech Scirntific Inc., U.S.A.)

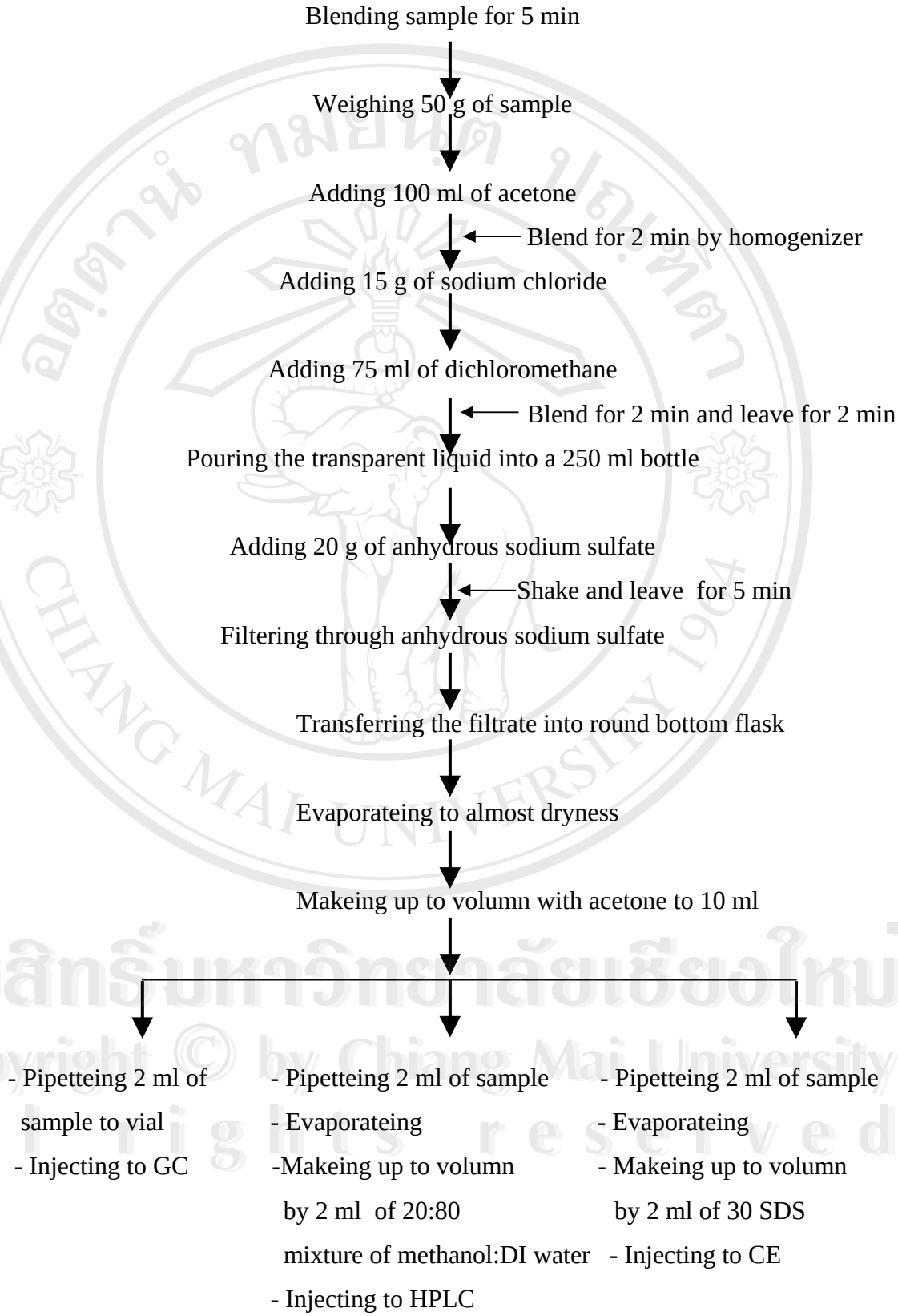
(10) micro pipette (P1000 , Gilson)

3.3 Method

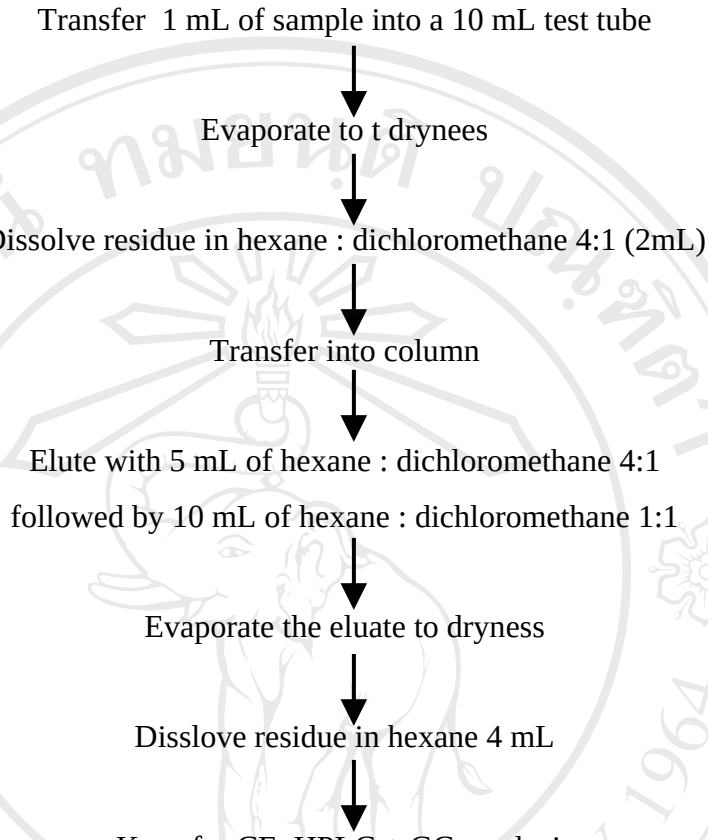
3.3.1. Preparation of strawberry samples

The experimental plots were set at a strawberry from which belongs to Mr. Vitaya at Baan Borkaew, Samoeng district, Chiang Mai province. Three insecticides were used to spray on the strawberry plants at fruiting stage: carbofuran (carbamate), parathion methyl (organophosphorus) and cypermethrin (synthetic pyrethroid). The experiment was statistically planned, using split plot design. There were 3 treatments (insecticides) with 3 replications each. The three insecticides were sprayed on the strawberry plants only once the plants sprayed with water served as control treatment. The fruits were harvested at 0 (1 hr.), 1, 3, 5, 7, 10 and 14 days after spraying. About 3 kilograms of each treatment were used for each analysis. This was then compared to the results from analyses with HPLC and GC.

3.3.2. Extraction of sample (18)



3.3.3. Clean up of sample (19)



3.3.4. Preparation of standard solutions

a) Stock standard solution

Stock solution of each compound at a concentration of 100 μg/mL was prepared, 0.0010 g of each standard was weighed accurately and poured into a 10 mL volumnetric flask, dissolved and diluted to volume with appropriate solvent

b) Intermediate standard solution

A 10 μg/mL of intermediate standard solution was prepared from stock standard solution by dilution by dilute to10

c) Mixed working standard solutions

Mixed working standard was prepared by pipetting appropriate volume of the intermediate standard solution of each compound in the same

group into the same volumetric flask and diluted to appropriate concentration. The mixed working standard was used for construction of calibration curve

3.3.5.Optimization of experimental parameters for CE

All the experimental parameter were optimized as follows **:**

1. pH: trail conditions at 7,7.5, 8, 8.5 and 9
2. Voltage trial conditions at 5, 10, 15, 20, 25 and 30 KV.
3. Wavelength trial conditions were scan from 190 nm to 800 nm
4. Total capillary length (L) = 50 cm and 70 cm. with effective capillary length (l) = 40.2 and 63 cm.
5. Fused silica column with capillary diameter (i.d.) = 50μm, 75 and 100 μm.

The optimal conditions for carbofuran, parathion methyl and cypermethrins are as follows:

1. Buffer: 5mM sodium tetraborate, containing 30mM SDS
2. Column Temperature = 25 °C
3. Rinse column by 0.01N NaOH for 3 min.
4. Rinse DI water for 3 min.
5. Equilibrate column by buffer for 10 min.
6. Injection time set at 7 sec.

3.3.6. Validation of the method

1. Accuracy

The reproducibility and accuracy of the proposed method were verified by analyzing the aliquots of sample solution in each plot spiked with various amounts of corresponding standard insecticide. The percentage recovery can be calculated from the formula

$$\% \text{ Recovery} = \frac{X_2 - X_1 \times 100}{C}$$

Where X1 = Stands for response of sample blank

X2 = Stands for response of fortified sample

C = Stands for standard solution added

2. Precision

The precision of the CE method was determined by analyzing five individual of appropriate concentration of each standard insecticide solution. The relative standard deviation (% RSD) can be calculated.

3. Limit of detection

Standard insecticide solution with proper concentration were prepared and analysed by CE. The limit of detection was obtained when the signal peak height was three times the noise or measuremend of the signal – to – noise peak height ratio of 3:1

4. Limit of Quantitation

The limit of quantitation of insecticide standard was achieved by measurement of the signal - to - noise peak height ratio 10:1

5. Linearity

The linearity ranges of standard curves were also studied. Three series of standard solution were prepared as follows : 0.5, 1, 2, 3 and 4 μg/ml of carbofuran, 0.5, 1, 2, 3 and 4 μg/ml of parathion-methyl and 0.5, 1, 2, 3 and 4 μg/ml of cypermethrin. Each solution was analyzed by CE. The peak areas were plotted against the insecticide concentrations using linear regression analysis.