CHAPTER 2

EXPERIMENTAL

2.1 Apparatus and Chemicals

2.1.1 Apparatus

1) Gas chromatograph 6890 series (Agilent Technologies, U.S.A.), consists of
   a) Flame photometric detector (FPD)
   b) Capillary columns with HP-5MS, 30.0 m × 0.25 mm i.d., 0.25 μm film thickness (Agilent, U.S.A.)
   c) Data processing system (HP chemstation)
2) Micropipette
   a) 0.5-10 μL, Vipro, Mechanical pipette
   b) 2-20 μL, Vipro, Mechanical pipette
   c) 20-200 μL, P series (Gilson Medical Electronics, France)
   d) 100-1000 μL, SL 1000 series (Rainin Instrument, LLC A Mettler Toledo, U.S.A.)
3) Super blender, model MX-T110PN (National, Taiwan)
4) Stirrer, model SP46920 (U.S.A.)
5) Analytical balance AB304-S series (Mettler Toledo, Switzerland)
6) pH meter model pH 744 (Metrohm, U.S.A.)

7) Microsyringe 10 μL (Agilent, U.S.A.)

8) Stopwatch

2.1.2 Chemicals

1) Toluene, 99.5% (AR grade, Merck, Germany)

2) Chlorocyclohexane, 98.0% (AR grade, Fluka, Italy)

3) Tetrachloroethylene,extra pure (AR grade, BDH, England)

4) *n*-Heptane, 99.5% (AR grade, BDH, England)

5) Octane, 95% (AR grade Fluka, Italy)

6) Isooctane, 99.5% (AR grade Lab-Scan, Ireland)

7) Methanol (HPLC grade, BDH, England)

8) Sodium chloride (AR grade Merck, Germany)

9) Helium gas, 99.999% (UHP grade TIG, Thailand)

10) Nitrogen gas, 99.999% (UHP grade TIG, Thailand)

11) Hydrogen (UHP grade TIG, Thailand)

12) Air zero (UHP grade TIG, Thailand)

2.1.3 Standard pesticides

1) Dimethoate standard, purity 99.0% (Dr. Erhenstorfer, Promochem, Wesel, Germany)

2) Malathion standard, purity 99.5% (Dr. Erhenstorfer, Promochem, Wesel, Germany)

3) Parathion methyl used as internal standard (IS), purity 99.0% (Dr. Erhenstorfer, Promochem, Wesel, Germany)

2.2 Preparation of the Solutions

2.2.1 Preparation of the stock standard solutions

Stock standard solutions of dimethoate, malathion and parathion methyl (IS) were prepared by dissolving 2 mg of each pesticides in methanol in a 2 mL volumetric flask, so the concentration of the stock solutions were 1000 mg/L. All the standard solutions were stored in a refrigerator prior to use.

2.2.2 Preparation of the working standard solutions

A 100 mg/L of standard solutions were prepared from 1000 mg/L of stock standard solution by dilution. Individual series working standard solutions were prepared by diluting 10 mg/L of each standard solution.

For example, the preparation of 10 mg/L of parathion methyl was dilution from stock standard solution into a 2 mL volumetric flask and diluting it to the mark with methanol.

2.3 Optimization of GC-FPD Conditions

All the experiment parameters such as splitless injection mode, capillary columns, temperature programming, the temperature of FPD detector and the temperature of injector port were optimized. The determination of dimethoate and malathion in vegetable and fruit samples by SDME–GC–FPD has been investigated. The experimental procedures for this technique are shown in **Figure 2.1**.

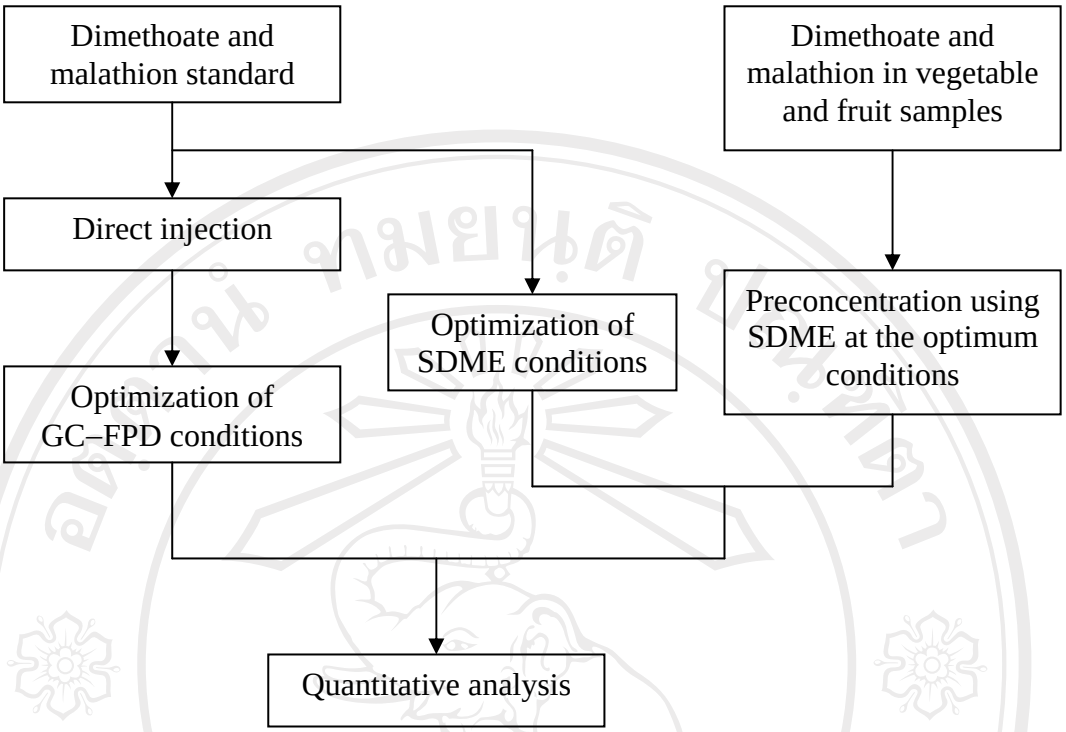

Figure 2.1 Summary of experimental procedures for the determination of dimethoate and malathion by SDME–GC–FPD.

2.4 Optimization of the SDME Conditions [22]

The different parameters that influence the partition of analytes between the organic drop and the solution were optimized. In order to perform the microextraction of dimethoate and malathion from aqueous solution efficiently, several parameters that influence on the extraction efficiency should be studied and optimized. Such factors included solvent type and drop volume, stirring rate, sample pH, salt concentration and extraction time. To obtain optimized extraction conditions, the ratio of peak area analyte and that of internal standard (parathion methyl) was used in GC–FPD analysis of the extracts. The determination of dimethoate and malathion in

vegetable and fruit samples by SDME method has been investigated. The experimental procedures for extraction are shown in **Figure 2.2**.

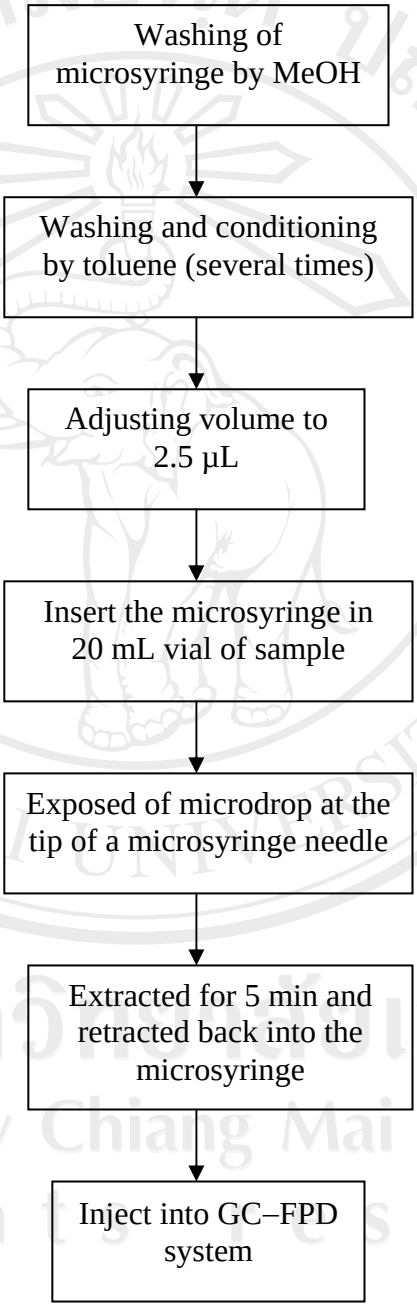

Figure 2.2 Summary of SDME procedures for the determination of dimethoate and malathion.

2.5 Validation of Method

After having the optimum condition for analysis of ditmethoate and malathion using SDME–GC–FPD (**Table 3.1**). Data were calculated and statistically compared. Validation of the method consists of percent recovery, limits of detection, linearity and precision (inter and intra day repeatability) as described below.

2.5.1 Percentage recovery

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy was calculated as the percentage of recovery by the assay of the known added amount of analyte in the sample. The percent recovery of spiked samples was calculated based on internal calibration curve and the area ratio. Fortifications of orange samples was prepared by spiking 20 µg/L and 100 µg/L of each standard solutions to 20 mL vial of sample prior to extraction as described in **Figure 2.1**. The recovery was replicated three times and the results were calculated from the formula.

$$\%\,\text{Recovery} = \frac{(\text{Area ratio of standard added in sample} - \text{Area ratio of sample})}{\text{Area ratio of standard solution}} \text{x}\,100$$

2.5.2 Limits of detection [21]

The limit of detection (LOD) is a characteristic of limit tests. It is the minimum concentration of analyte that can be determined with a given analytical method. The standard solution of pesticides were prepared and injected into the GC column under the optimum conditions. In this work, the limit of detection for each

analyte was calculated from the decrease concentration. LOD were obtained when the signal peak height was three times the noise or calculated using a signal-to-noise (S/N) ratio of 3.

2.5.3 Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. The standard mixture containing 40, 80 and 120 µg/L for dimethoate and 5, 50 and 100 µg/L for malathion were employed, the same condition was repeated five times. The intra–day repeatability was investigated by repeatedly analyzing of the standard mixture for five replicate in the same day and the inter–day repeatability was determined in the different days under optimum conditions. The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation of a series of measurements. Results were then calculated to find percent of relative standard deviation (% R.S.D.).

2.5.4 Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. The linearity of pesticide standard curves was also studied. Two series of pesticide standard solution were prepared as followed: 10, 20, 40, 60, 80, 100, 200, 400, 800 and 1000 µg/L of dimethoate and malathion. Each solution was analyzed by GC–FPD. The peak

appeared, and the area ratio was plotted against concentration of the pesticide standards.

2.6 Determination of Dimethoate and Malathion in Vegetable and Fruit Samples

Vegetable and fruit samples were selected for the determined of dimethoate and malathion was carried out by using the optimum conditions of SDME and GC–FPD system. For example, the steps of analysis are summarized as follow:

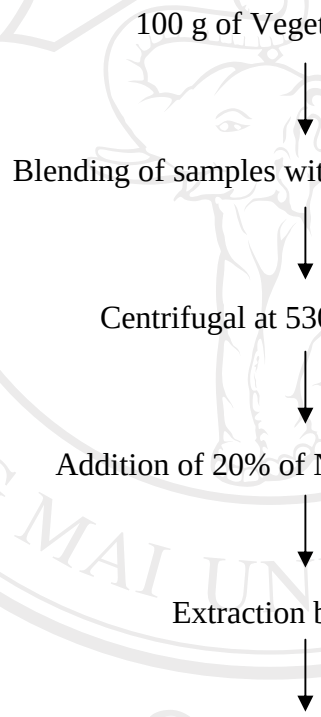

Figure 2.3 Summary of procedure of vegetable samples for the determination of dimethoate and malathion.

In addition, the procedure of fruit sample was prepared the same as the vegetable sample, but without blending (the second step).

2.7 Detail of Samples

The vegetable and fruit samples were collected from various sites in Chiang Mai, Thailand as described in **Table 2.1**.

Table 2.1 Detail of samples

Sample	Type of sample	Sampling site
V1	Collard	Muangmai market
V2	Cabbage	Muangmai market
V3	Cauliflower	Muangmai market
O1-1*	Fresh orange juice	Suthep market (Tonpayom)
O1-2**	Fresh orange juice	Suthep market (Tonpayom)
O1-3***	Orange peel	Suthep market (Tonpayom)
O2-1*	Fresh orange juice	Muangmai market
O2-2**	Fresh orange juice	Muangmai market
O2-3***	Orange peel	Muangmai market
O3-1*	Fresh orange juice	Thanathorn orange brand
O3-2**	Fresh orange juice	Thanathorn orange brand
O3-3***	Orange peel	Thanathorn orange brand
O4-1*	Fresh orange juice	J. Jalern orange brand
O4-2**	Fresh orange juice	J. Jalern orange brand
O4-3***	Orange peel	J. Jalern orange brand
O5-1*	Fresh orange juice	Saithong orange brand
O5-2**	Fresh orange juice	Saithong orange brand

Table 2.1 (continued)

Sample	Type of sample	Sampling site
O5-3***	Orange peel	Saithong orange brand
J1-1	Orange juice	Suthep market (Tonpayom)
J1-2	Orange juice	Suthep market (Tonpayom)
J1-3	Orange juice	Suthep market (Tonpayom)
J1-4	Orange juice	Food Center, Biology, Chiang Mai University
J1-5	Orange juice	Suthep market (Tonpayom)
J1-6	Orange juice	Suthep market (Tonpayom)
J2	Guava juice	Suthep market (Tonpayom)
J3	Lemon juice	Suthep market (Tonpayom)
J4	Tiger herbal juice	Suthep market (Tonpayom)

* = The orange juice was crush without peel

** = The orange juice was crush with peel

*** = Orange peel