

## CHAPTER III

### Materials and Methods

#### 3.1 SPE Optimum conditions

##### 3.1.1 Materials

Acetonitrile(ACN) and methanol (MeOH) were HPLC grade from J.T. Baker, U.S.A. Water was doubly distilled and filtered through 0.45  $\mu\text{m}$  membrane. The organophosphorus insecticides, Malathion, Methyl parathion and Chlorpyrifos were purchased from Soekawa Chemical Co.Ltd., Japan, with a certified purity of 99.0% and Profenofos was obtained from Ciba-Geigy (Thailand) with certified purity of 99.4%. The structure of the four OPs studied are shown in Figure 3.1. Disposable column C18 SPE cartridge were obtained from J.T. Baker, U.S.A. and Visiprep SPE Vacuum Manifold 5-7030 was obtained from Supelco.

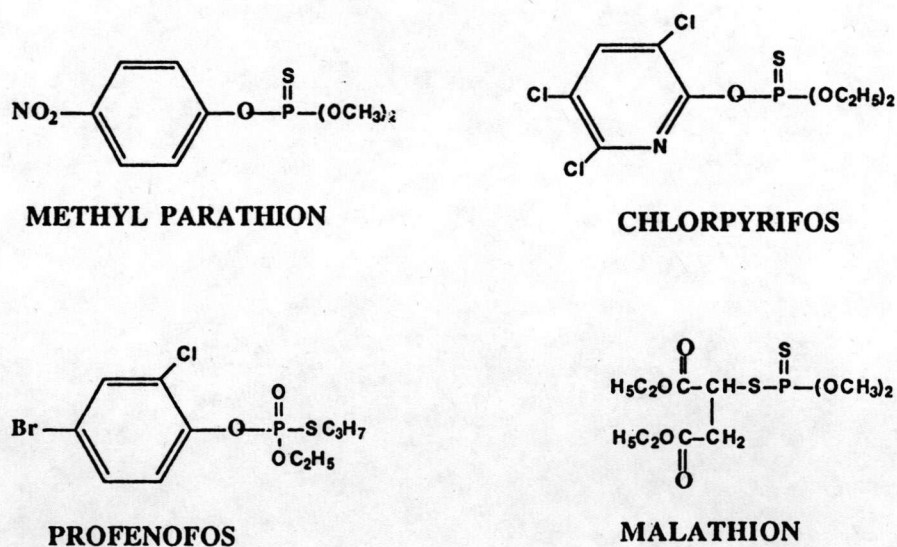


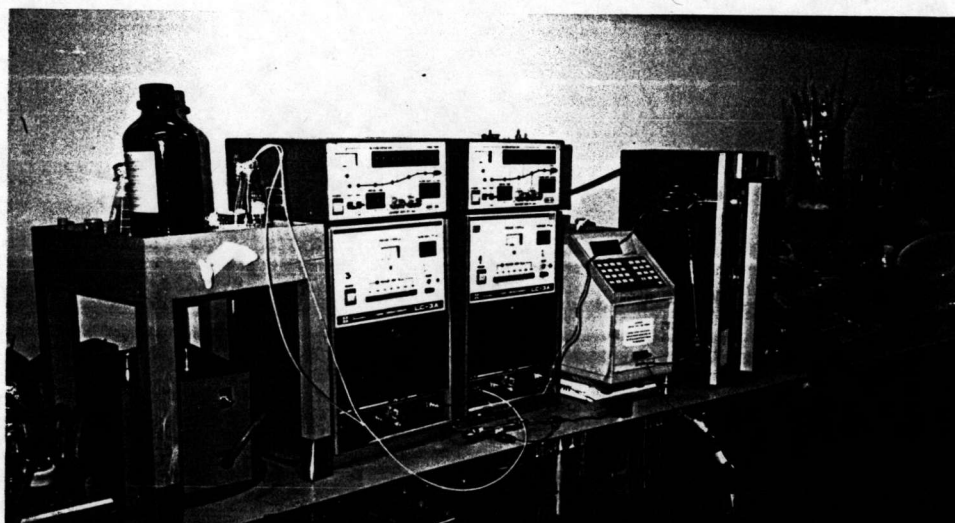
FIGURE 3.1 The chemical structures of the four selected pesticides

### 3.1.2 Preparation of the standard solutions

Stock standard solutions (1000  $\mu\text{g/ml}$ ) were prepared in acetonitrile and stored at  $-20^{\circ}\text{C}$ . The working standard solutions were serially diluted to 5.0, 10.0, 20.0, 30.0 and 40.0  $\mu\text{g/ml}$ .

### 3.1.3 High Performance Liquid Chromatography (HPLC)

The HPLC system consists of the LDC 4100 equipped with UV detector, Shimadzu integrator and LC-3A pump (Figure 3.2). The operating conditions were as follows : wavelength, 220 nm ; flow rate, 1.0 ml/min ; chart speed, 2 cm/min ; detector sensitivity, 0.05 AUFS (absorption unit full scale) ; attenuation, 2 ; column, ODS (Octadecyl bond silica) 12.5 cm  $\times$  4 mm ID ; temperature, ambient ; injection volume, 20  $\mu\text{l}$  ; injection interval, 15 min.



**FIGURE 3.2** The HPLC system used in this experiment

### 3.1.4 Solid Phase Extraction

The optimum condition of solid phase extraction was determined. The various parameters including  $C_{18}$  bulk packing mass, volume of sample, elution solvents (types and volumes) and pressure of SPE vacuum pump were evaluated. Table 3.1 was the protocol of different SPE parameter experiments. The general system of SPE attached to the HPLC analysis was illustrated in Figure 3.3. The 1 ml and 3 ml polypropylene SPE cartridges attached with 10-mm glassfiber prefilter were activated with 3 ml methanol and 3 ml of doubly distilled water through the Visiprep SPE vacuum Manifold 5-7030 Supelco applying 10 in. Hg pressure. Figure 3.4 shows the full system of SPE system used in this experiment. The relationships between the percent recovery of the mixed standard OPs and the optimum parameters were analysed.

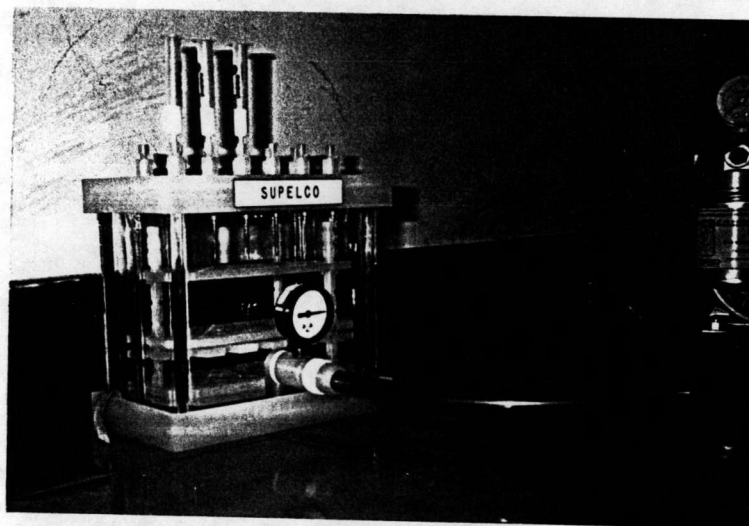


FIGURE 3.3 The general system of SPE

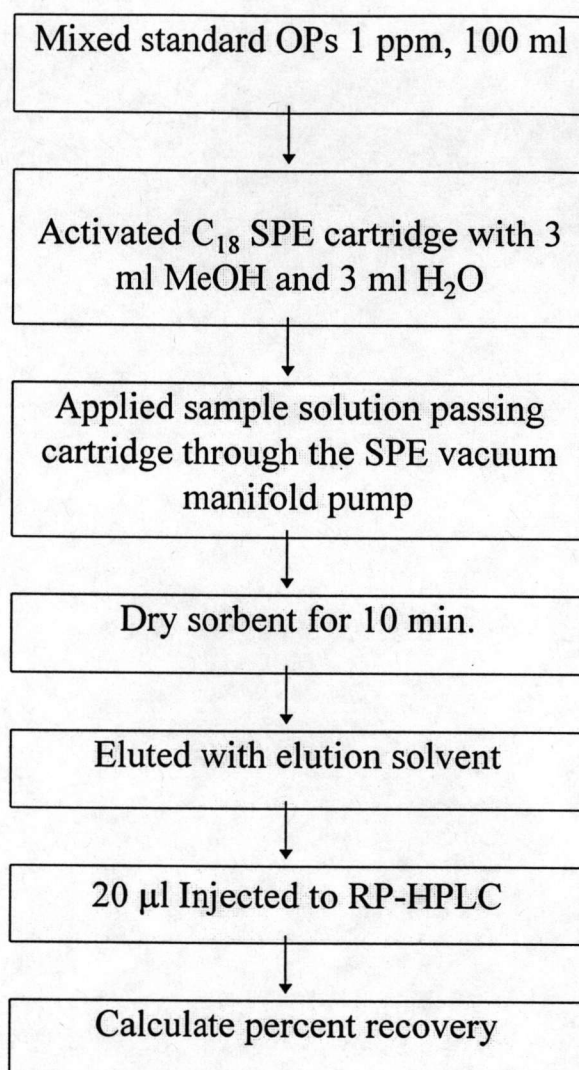


FIGURE 3.4 The full system of SPE using in the experiments

### 3.1.5 Quantitation

The percent recoveries were determined from external calibration using five points 5.0, 10.0, 20.0, 30.0 and 40.0  $\mu\text{g/ml}$  of standard OPs. The calibration graph for the OPs was obtained by plotting the concentration against peak area of the analytes. The sample OPs extract from water samples also were quantitated from the same calibration curve. A blank test was performed with using 100 ml of distilled water and there were not any peaks corresponding to the OPs in the chromatogram.

**TABLE 3.1** The protocol various SPE parameters  
( Each experiment was performed in replication )

Exp.No.	40 $\mu\text{m}$ C <sub>18</sub> Sorbent mass (mg)	Volume of sample (ml)	Elution solvent (ACN/H <sub>2</sub> O)	Eluent volume (ml)	Pressure of SPE pump (in.Hg)
I Sorbent mass	100	100	50/50	5	10
	200	↓	↓	↓	↓
	300	↓	↓	↓	↓
	400	↓	↓	↓	↓
	500	↓	↓	↓	↓
II Volume of sample	100	100	50/50	5	10
	↓	200	↓	↓	↓
	↓	300	↓	↓	↓
	↓	400	↓	↓	↓
	500	100	↓	↓	↓
III Elution solvent	↓	200	↓	↓	↓
	↓	300	↓	↓	↓
	↓	400	↓	↓	↓
	↓	500	↓	↓	↓
	100	100	%ACN,%MeOH	5	10
	↓	↓	10, 10	↓	↓
	↓	↓	20, 20	↓	↓
	↓	↓	30, 30	↓	↓
	↓	↓	40, 40	↓	↓
	↓	↓	50, 50	↓	↓
↓	↓	60, 60	↓	↓	
↓	↓	70, 70	↓	↓	
↓	↓	80, 80	↓	↓	
↓	↓	90, 90	↓	↓	

TABLE 3.1 (continue)

Exp.No.	40 $\mu\text{m}$ C <sub>18</sub> Sorbent mass (mg)	Volume of sample (ml)	Elution solvent (ACN/H <sub>2</sub> O)	Eluent volume (ml)	Pressure of SPE pump (in.Hg)
IV. Eluent volume	100	100	60% ACN	1	10
	↓	↓	↓	2	↓
				3	
				4	
				5	
				6	
				7	
				8	
				9	
				10	
V. SPE pump pressure	100	100	60% ACN	5	3.0
	↓	↓	↓	↓	5.0
					7.0
					10.0
					12.0
					15.0
					17.0
					20.0
					22.0

## 3.2 Analysis and Detection of some OPs in water samples

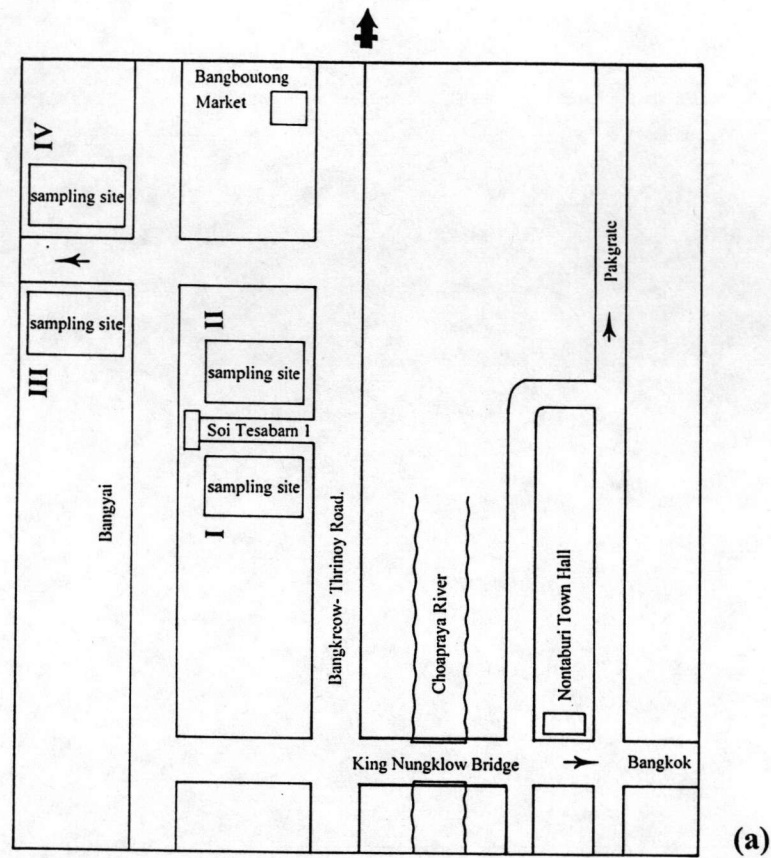
The use of organophosphorus pesticides on agriculture has become common and important. There is an increasing need for rapid reliable methods to measure pesticides concentrations in natural water to minimize risks associated with pesticides use. The analysis of pesticides in water samples is normally performed with either liquid-liquid extraction or solid-phase extraction (SPE) followed by gas chromatography (Capel *et al.*, 1995). Recent publications on this subject show that there is a tendency to use sophisticated techniques such as gas chromatography with mass spectrometry (Kobayashi *et al.*, 1993) and liquid chromatography with mass spectrometry (Molina *et al.*, 1994). for determining pesticide residues in water. However, pesticide monitoring of water is also possible with the use of less sophisticated instruments, reversed-phase HPLC is widely used in analysis of pesticides with high polarity, low volatility and thermal instability (Balnova, 1993). Solid-phase extraction has been accepted as a tool for extraction of water samples prior to analysis (Font *et al.*, 1993). In this experiment, we have developed a rapid and simple method for the determination of OPs in water using C<sub>18</sub> SPE and reversed-phase HPLC. The OPs structures were confirmed by mass spectrometry

### 3.2.1 Materials and Methods

The general chemicals using in this experiment were the same as chapter III section 3.1 and 3.3. The optimum conditions of SPE has been summarized in Table 4.1. The Electron Ionization (EI) mass spectra of OPs were obtained from GC/MS spectrometer JMS-DX 300 (JEOL) data system. The ionization voltage and the ion-source temperature were set at 70 eV, 150<sup>0</sup> C and direct probe.

### 3.2.2 Sampling

Agricultural drained water samples were collected at a site of vegetables farm at Bangbuotong district Patumtanee province, during the period of the crops application. The site location is shown in Figure 3.5 (a and b). Water was collected from the middle of furrow at 30-50 cm depths within 30 days intervals during the farming periods. Each two litre of collecting water was kept in a separated dark glass bottles and stored at 4<sup>0</sup> C before analysed within one day after sampling. Total number of water samples are thirty six.



**FIGURE 3.5** (a) Sampling site locations (b) vegetable farm, Bangboutong district Nontaburi province