CHAPTER IV

Results and Discussion

4.1 SPE Optimum Conditions

The optimum conditions of solid-phase extraction were evaluated using the calculated recovery efficiency of the overall performance of the SPE systems. The average percentage recoveries of organophosphorus insecticides were the mean value of the triplicate analysis. Following the procedure in Chapter III Table A-1 to A-10 are result in percent recoveries of OPs in different parameters which comparison of the percent recoveries from the various parameters, namely, sorbent mass, volume of sample, elution solvent, volume of elution solvent and pressure of SPE vacuum pump, respectively.

According to these results, the extraction of 100 ml aliquot fortified at 1.0 μg/ml was sufficient to obtain the satisfactory percentage recoveries and allowed quantitative analysis within the range of working calibration curve. The standard calibration curve for the four OPs were shown in Figure 4.9.

From the variety of sorbent mass; we found that the best percent recoveries of the four OPs occurred at 100 mg C_{18} cartridges which was the best value for the extraction of all pesticides in this study. The effect of solvent elution at pH 6.0, OPs concentration 1.0 ppm using 100 mg C_{18} SPE cartridge was optimised by using acetonitrile/water 60:40. The followed recoveries were obtained: malathion 100.00% ±1.13,methyl parathion 100.96% ± 1.51, profenofos 99.89% ± 1.92 and chlorpyrefos 84.81% ± 0.47, which was summarized in Table A-6 to A-7 and Figure 4.5.

The effect of the volume of eluent (1-10 ml) was investigated. The maximum recovery was obtained with 5.0 ml for all the pesticides. The results indicated that the ratio of eluent 60:40 acetonitrile in water 5.0 ml gives the best recovery in Table A-8 to A-9 and Figure 4.6. The optimum pressure of SPE vacuum pump effected on the percent recoveries of each OPs in solution at concentration 1.0 ppm, 100 mg C_{18} SPE cartridge, 5 ml of eluent and 100 ml aqueous solution was found at 17.0 in.Hg which

gave the maximum recoveries comparing with other pressure (Table A-10 and Figure 4.6)

Therefore, the optimum conditions for maximum recoveries of the four OPs were established as volume of aqueous solution used for the extraction 100 ml, the sorbent mass of C_{18} used for retaining the analyte 100 mg, the ratio of elution solvent 60:40 acetonitrile in water and using the volume of eluent 5.0 ml and the pressure of SPE vacuum pump when the aqueous solution passed through cartridge 17.0 in.Hg give the best optimum determination conditions shown in Table 4.1. Percent recovery for optimum experiments performed for methyl parathion, malathion, profenofos and chlorpyrifos were at 99.06 ± 0.67, 98.04 ± 0.41, 90.78 ± 0.75 and 71.51 ± 0.73, respectively.The method of detection limit of each OPs was detected by using the optimum condition of SPE which presented in Table A-11.

The optimum conditions for the solid-phase extraction of the four OPs, malathion methyl parathion, profenofos and chlorpyrefos in aqueous solution are summarized in Table 4.1

TABLE 4.1 The optimum SPE analysis conditions for determination each OPs in aqueous solution.

Parameter	Optimum value
Sorbent mass (C_{18})	100 mg
Volume of aqueous solution	100 ml
Ratio of elution solvent	60/40 (ACN/H_2O)
Volume of elution solvent	5.0 ml
Pressure of SPE vacuum pump	17.0 in.Hg

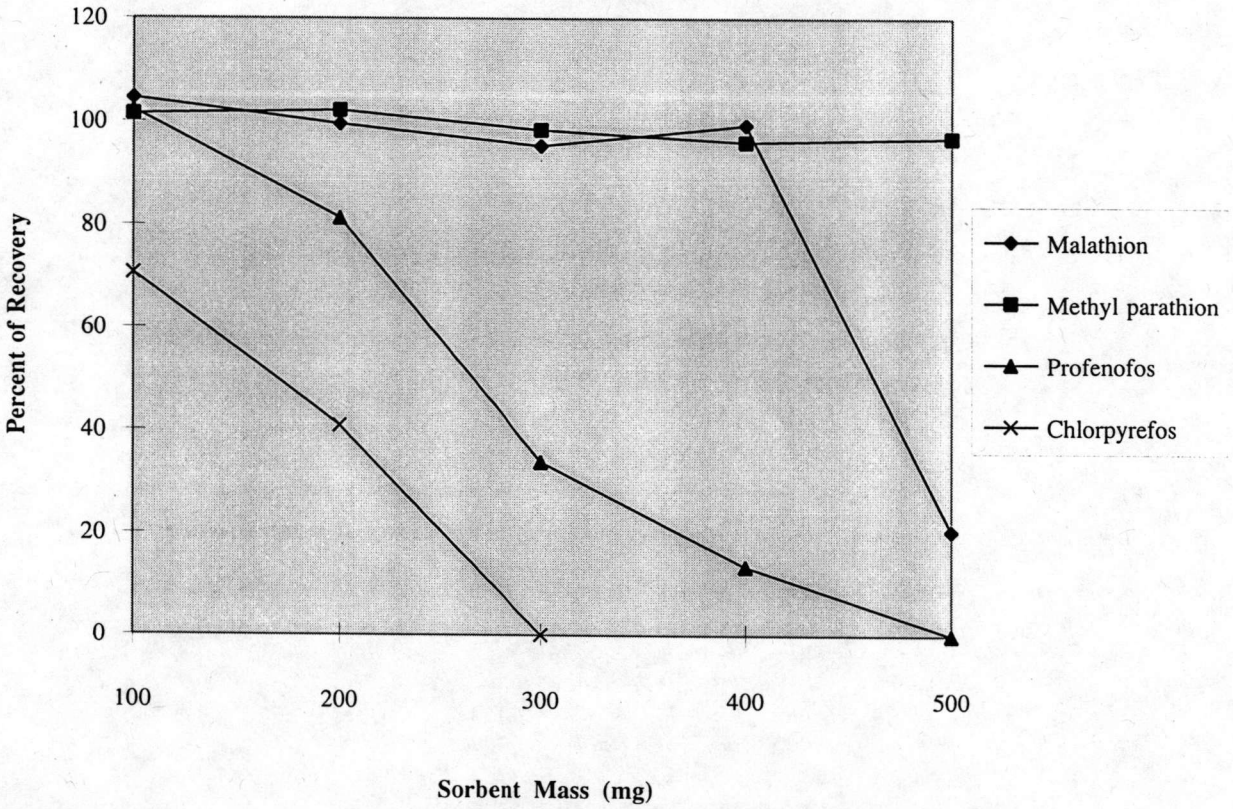

Figure 4.1 The effect of sorbent mass on the percent recovery for each OPs in mixture solutions

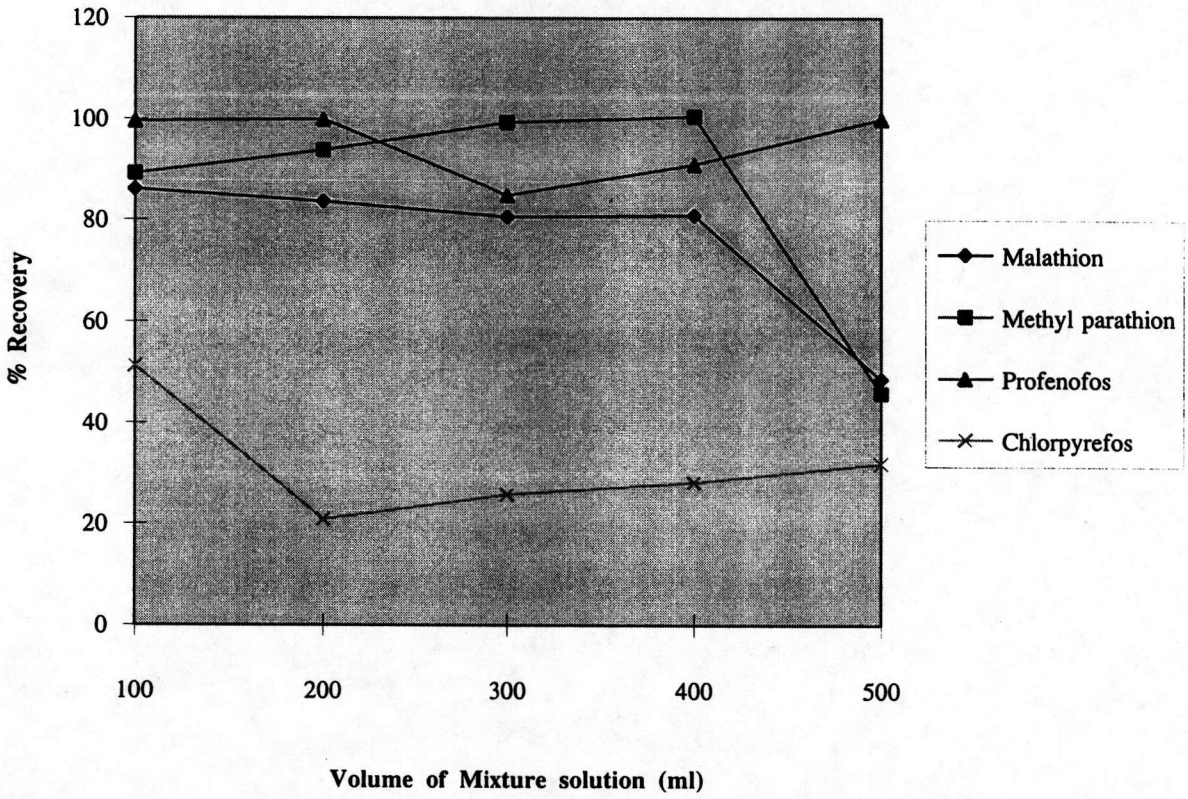

Figure 4.2 The effect of volume of mixture solutions on the percent recovery for each OPs using 100 mg SPE cartridge

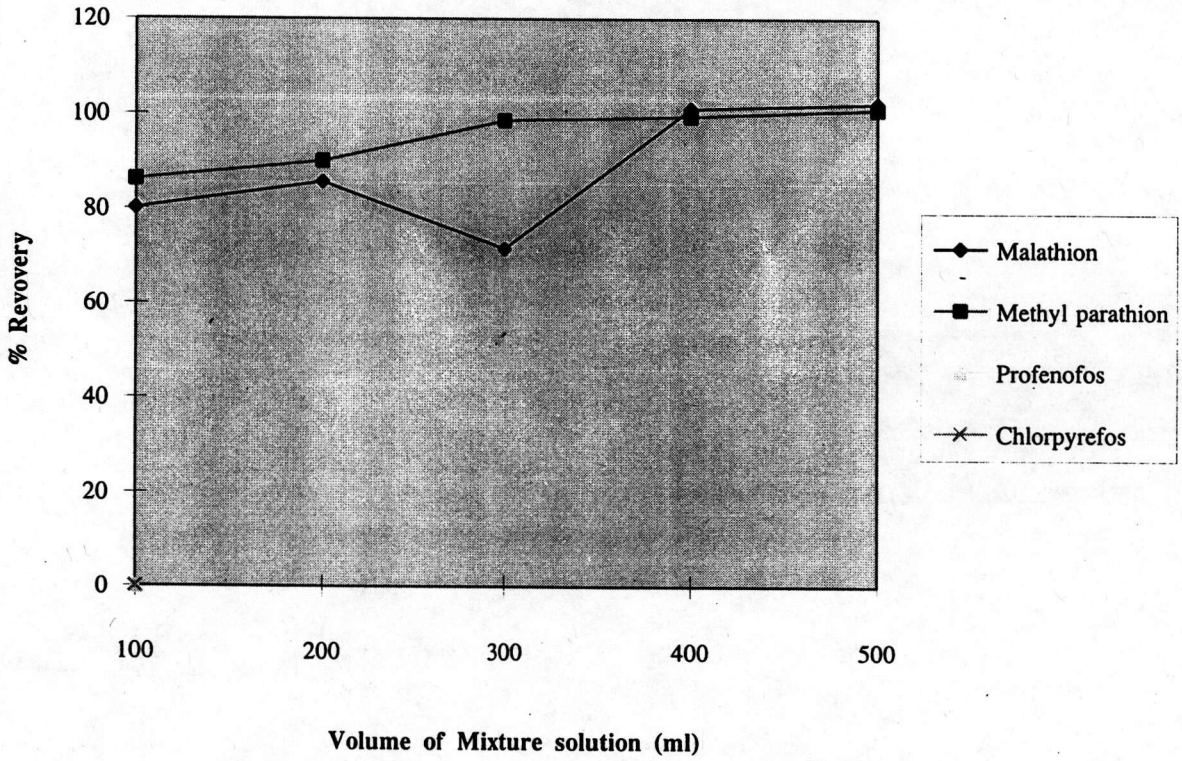

Figure 4.3 The effect of volume of mixture solutions on the percent recovery for each OPs using 500 mg SPE cartridge

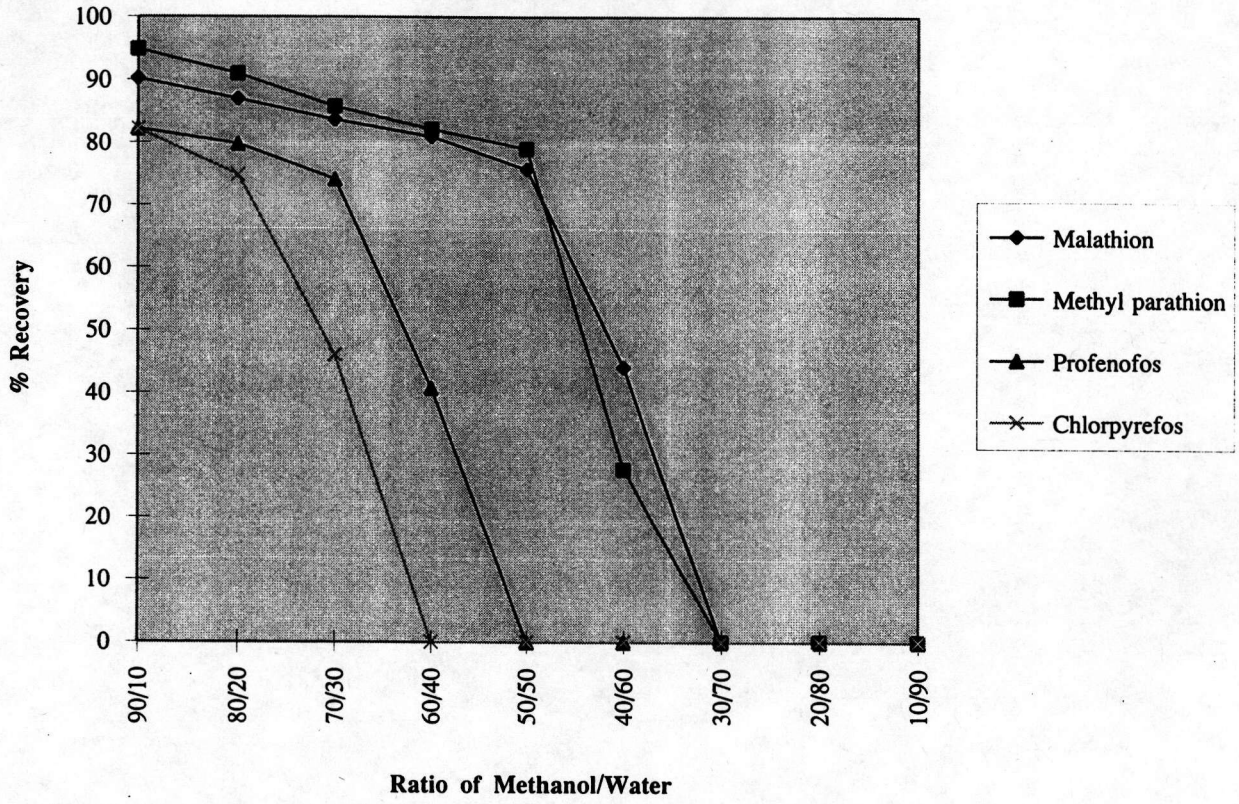

Figure 4.4 The effect of ratio of elution solvents (Methanol/water) on the percent recovery for each OPs in mixture solutions

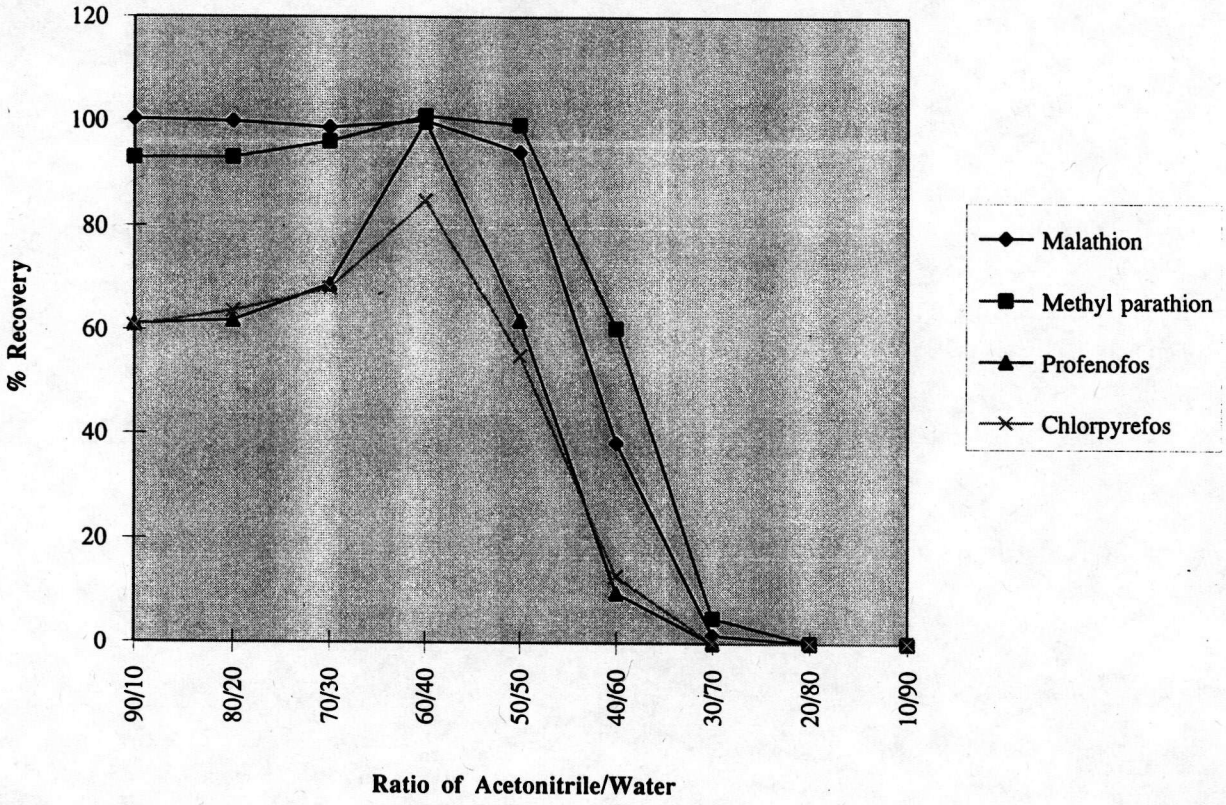

Figure 4.5 The effect of ratio of elution solvents (Acetonitrile/water) on the percent recovery for each OPs in mixture solutions

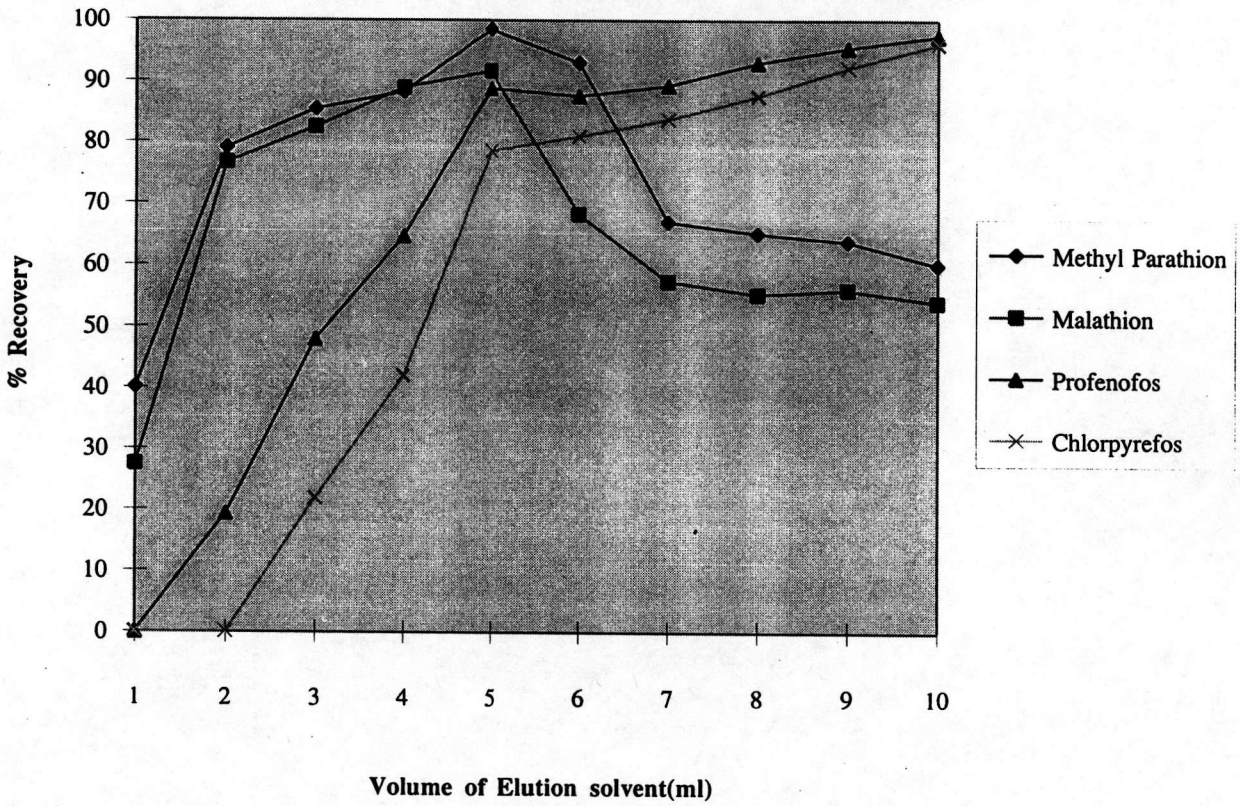

Figure 4.6 The effect of volume of elution solvent on the percent recovery for each OPs in mixture solutions

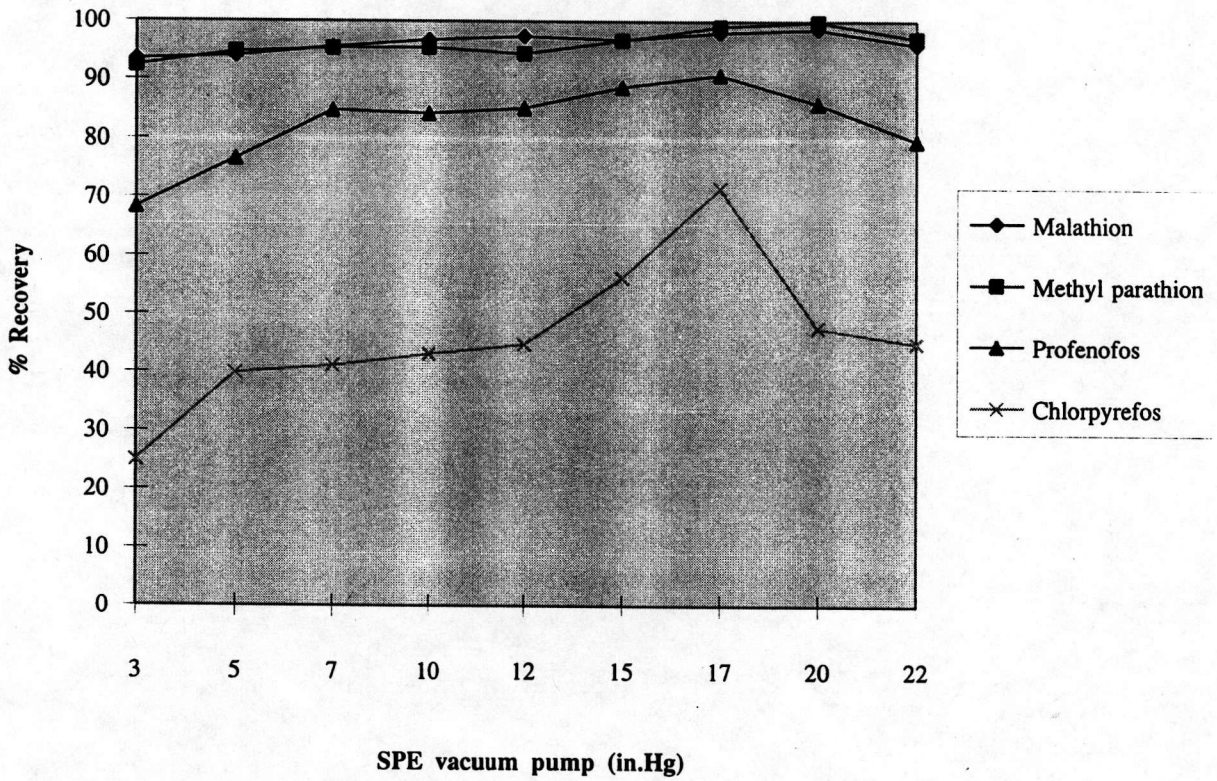

Figure 4.7 The effect of pressure of SPE vacuum pump on the percent recovery for each OPs in mixture solutions

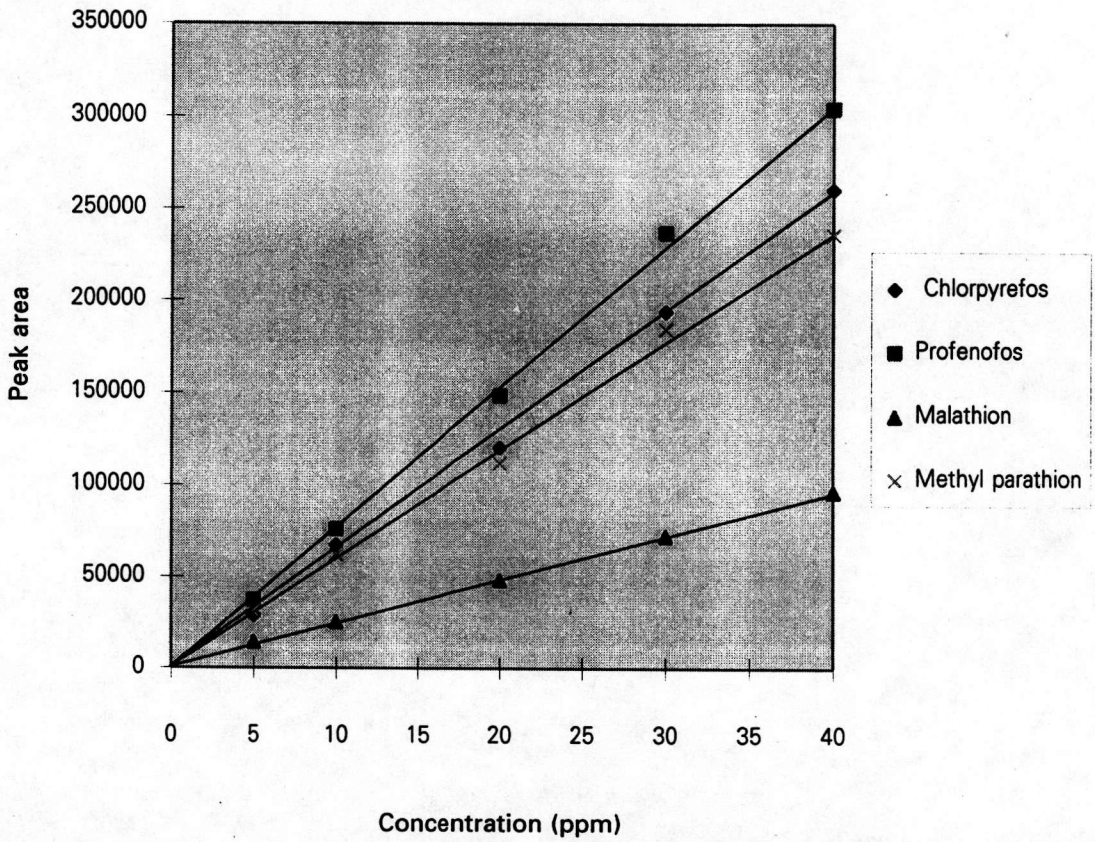

Figure 4.8 Standard calibration curves for the four OPs

4.2 Method Detection Limit (MDL)

The method detection limit is defined as the minimum concentration of compounds that can be identified or measured. The method detection limit of each OPs in aqueous solution was determined by using the optimum solid-phase extraction analysis condition in Table 4.1 and HPLC condition in section 3.3 Chapter III. The results of method detection limit are shown in Table 4.2

TABLE 4.2 The method detection limit of each OPs in aqueous solutions

Organophosphorus pesticides	**Method detection limit (ppb)**
Methyl parathion	25
Malathion	50
Profenofos	25
Chlorpyrifos	25

4.3 Analysis and Detection of some OPs in water samples

The structure of four high-use pesticides: malathion, methyl parathion, profenofos and chlorpyrifos are given in figure 3.1. The chemical different in these compounds indicate the polarity of phosphate moiety which should make them possible to be isolated from water. The optimum SPE conditions have been applied in the determination of OPs in water samples. 100 mL of agricultured drained water was transferred into 100 mg preactivated sep-pak C_{18} cartridge. Water samples were aspirated through the sep-pak C_{18} cartridge with vacuum at 17 in.Hg pressure. Trapped pesticides were eluted from the column with 5 mL of 60% acetonitrile in water and the analyst was evaporated to dryness with a stream of nitrogen. The 20 μL of the solution were injected into the RP-HPLC system. The HPLC analysis of residues in surface water from vegetable farms using our optimum SPE extract condition were well separated peaks.

From HPLC trace (Figure 4.10), there was the only one peak which relevanted to the retention time of the standard OPs (Figure 4.9) at 7.5 min, indicated the presence of profenofos. The other small peaks did not match with OPs standard therefore, they were not identified. This should imply that they may use different type of pesticides. The highest OPs residues which was significant to confirm the chemical structure was profenofos. MS analysis was used to confirm the pesticides' structures (section 4.3).

Table 4.3 is the quantitative results of profenofos residues from sampling site I, II, III and IV (Figure 3.5). Site I, II, and IV growed Chinese Brocory and site III growed Chinese celery. In sampling site I, the water samples wewe collected from the period of crop cycle for ten days duration within one months. The first sampling was found only profenofos residue concentration average 0.75 ± 0.06 ppm which vegetables were fully grown and ready for the harvest. The second and third sampling were found profenofos concentration 0.38 ±0.05 and 0.13 ± 0.05 ppm which were in the period of harvestation and the end of crop cycle, respectively. In sampling site II, the results were similar to the amount of profenofos in site I and also crop cycle was matched in the same period. However, profenofos in sampling site IV the first period was lower than in the second and third period. This was possible that the farm in site IV applied lower pesticide quantity than site I and II. There were also lower profenofos residue in other crop cycle period. Incontrast, site III which growed different vegetables from site I, II and IV, was not found any profenofos residue.

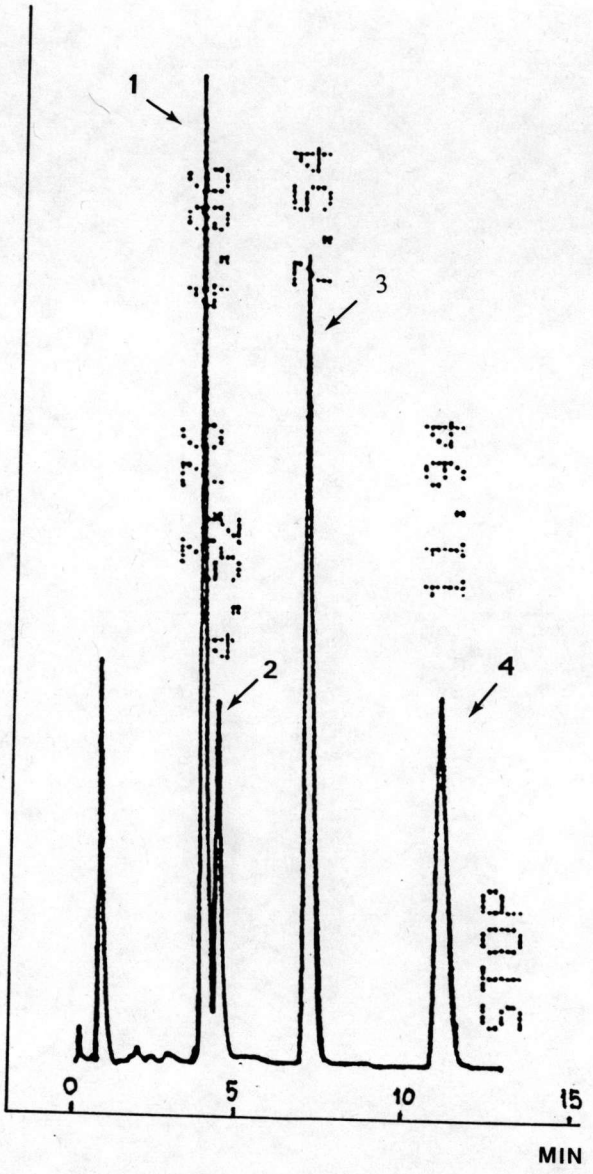

Figure 4.9 HPLC chromatograms obtained by injecting a mixed working standard solution 100 ng pesticides; peak numbering :
1= methyl paration, 2= malation, 3= profenofos and 4= chlorpyrefos

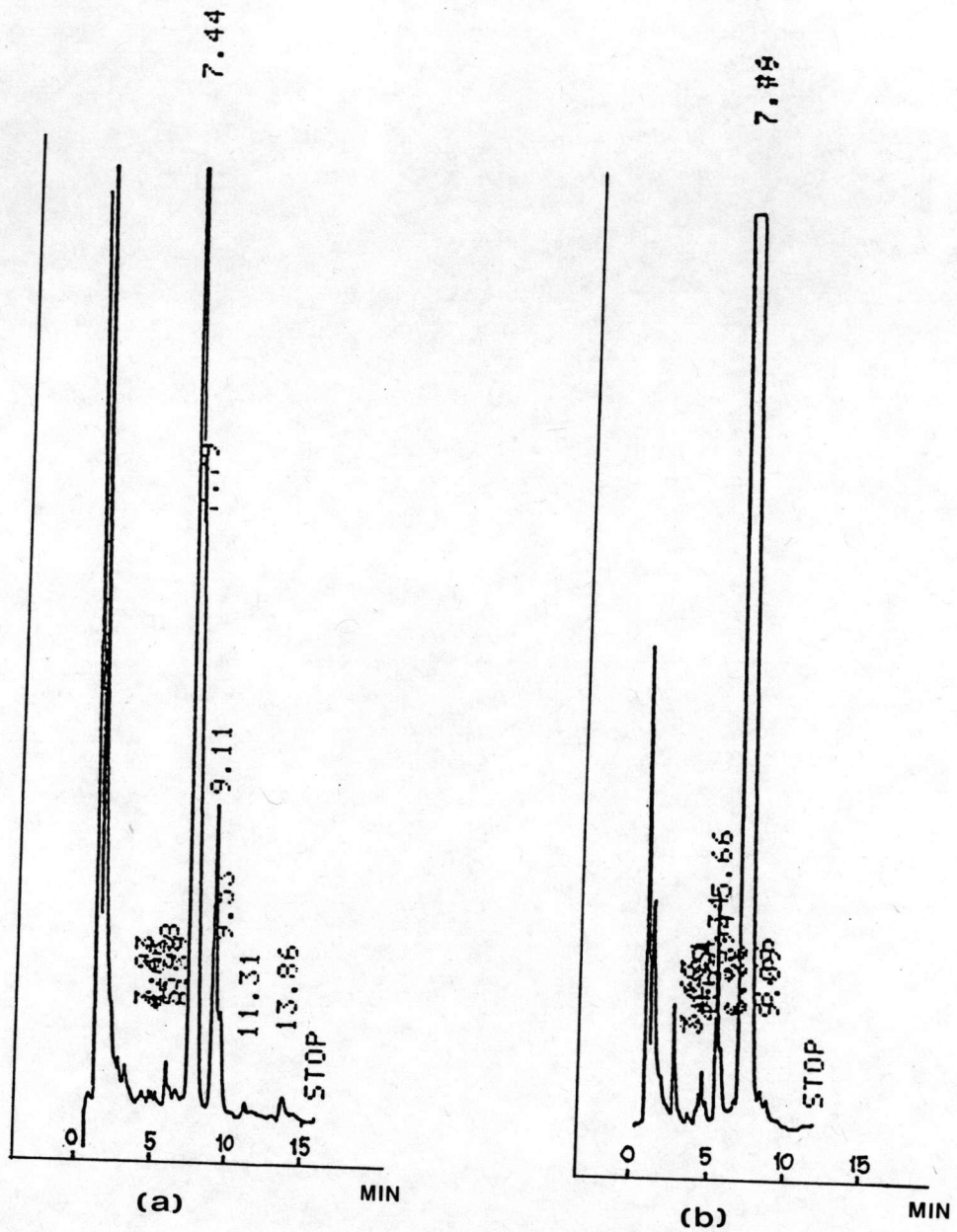

Figure 4.10 HPLC chromatograms for water sample extract from vegetable farm
(a) Farm I (b) Farm II

It would be possible that this farm may be use different type of pesticides which were not our standard pesticides studies.

TABLE 4.3 Contents of profenofos insecticide in water samples.

Date	Sampling site	Conc. (ppm) ± SD (n=3)
July 4, 96	I	0.75 ± 0.06
	II	0.25 ± 0.05
	III	ND
	IV	1.11 ± 0.25
July 14, 96	I	0.38 ± 0.05
	II	0.16 ± 0.03
	III	ND
	IV	0.64 ± 0.06
July 24, 96	I	0.13 ± 0.05
	II	0.11 ± 0.01
	III	ND
	IV	0.44 ± 0.07

Remark: ND = not detectable

4.4 Mass spectral analysis

The HPLC separated samples from section 4.2 were collected and confirmed structures by MS data using GC/MS spectrometer JMS-DX 300 (JEOL) data system at the Chemical Analysis Service Center, Hertfordshire University,UK. Figure 4.11-4.13 were some examples of MS spectral data of components at RT 7.5 min HPLC trace (section 4.2). Profenofos has been detected in all three samples, although the amount was very small (particularly in sample 2) but this was confirmed by examining the sample in duplicate. The spectra were consistent with profenofos although the standard profenofos was not examined.

The molecular ion cluster (m/z) 372, 374 and 376 were consistent with compound containing Cl and Br .The fragment ion m/z 206, 208 and 210 were Br containing ions. The halide containing ions were in the correct relative abundance

ratio. (McLafferty, 1980) The other fragment ions also consistently met the confirmation criteria and were summarized in Table 4.4.

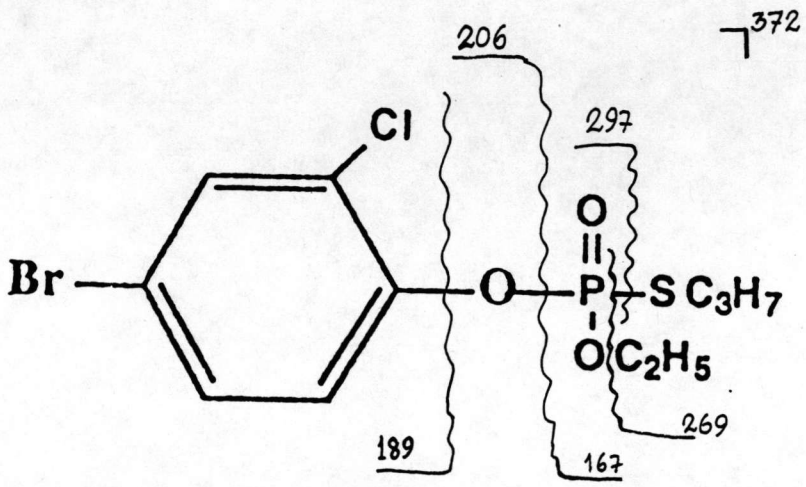

TABLE 4.4 Characteristic Mass Ions of Profenofos in real water sample I

m/z	characteristic ions	relative abundances
376	$[M+4]^{+\bullet}$	6.06
374	$[M+2]^{+\bullet}$	18.18
372	$[M]^{+\bullet}$	13.64
339	$[M+2] - Cl^{+}$	43.94
337	$[M- Cl]^{+\bullet}$	42.42
297	$[M - SC_3H_7]^{+\bullet}$	21.21
269	$[297 - (C_2H_5) + H]^{+\bullet}$	21.21
210	$[(M+4)- 167 + H]^{+\bullet}$	19.70
208	$[(M+2)- 167 + H]^{+\bullet}$	74.24
206	$[M - 167 + H]^{+\bullet}$	57.58
189	$[206 - OH]^{+\bullet}$	7.58
167	$[M - 206]^{+\bullet}$	25.76
139	$[167 - (C_2H_5)+ H]^{+\bullet}$	100.00
125	$[167 - (C_3H_7)+ H]^{+\bullet}$	43.94
97	$[139 - (C_3H_7)+ H]^{+\bullet}$	86.36

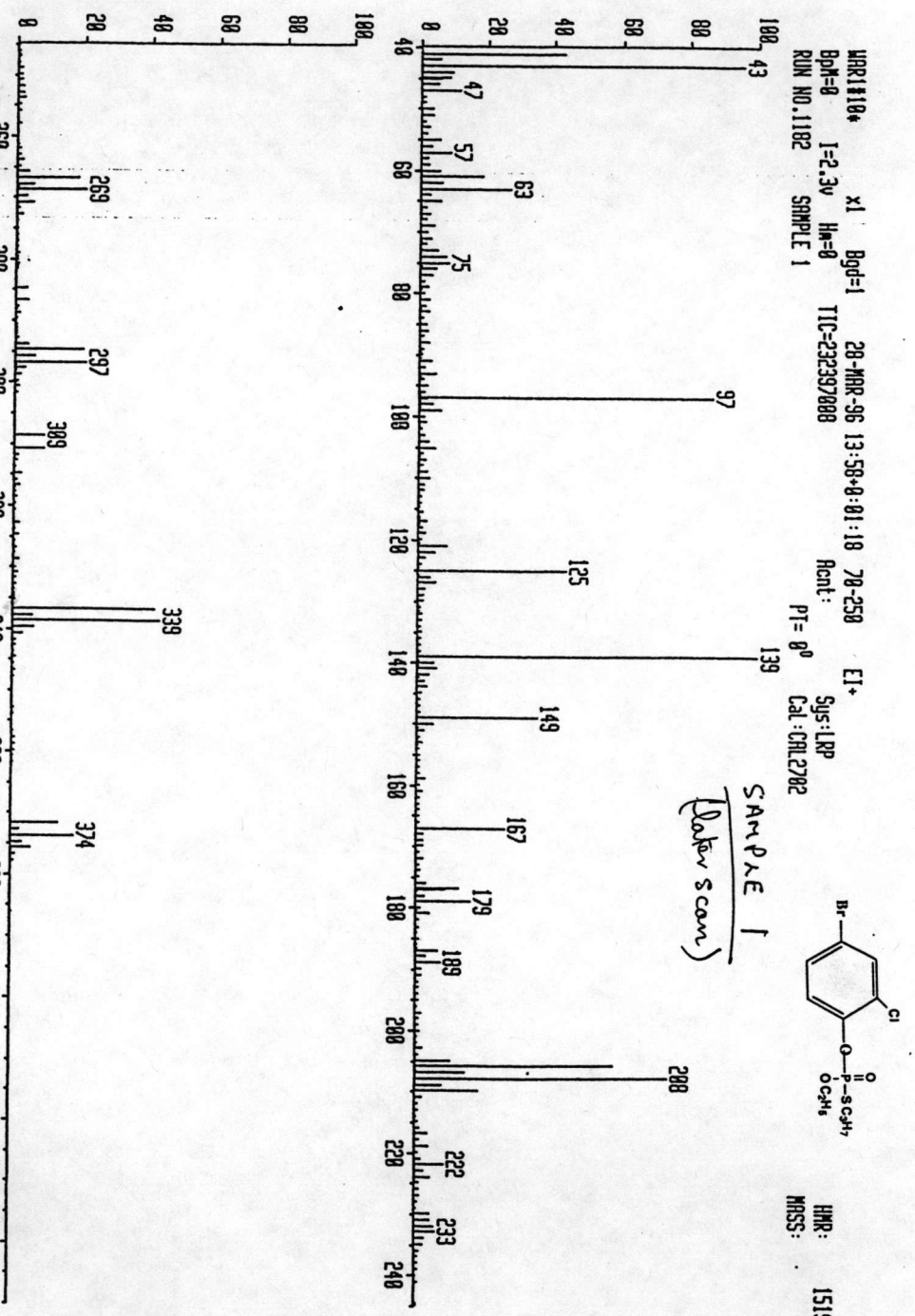

Figure 4.11 Mass spectrum of water extract from farm I

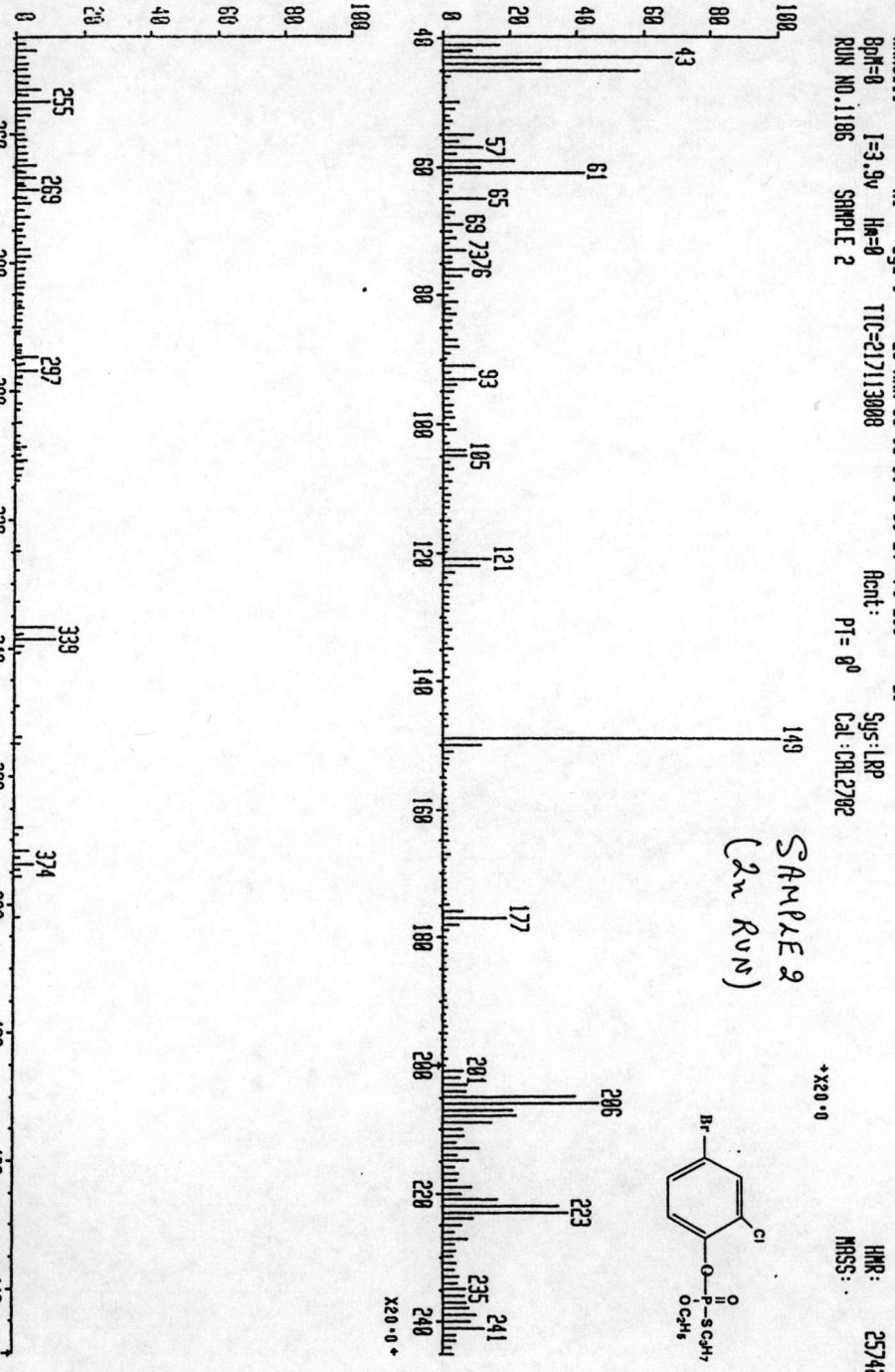

Figure 4.12 Mass spectrum of water extract from farm II

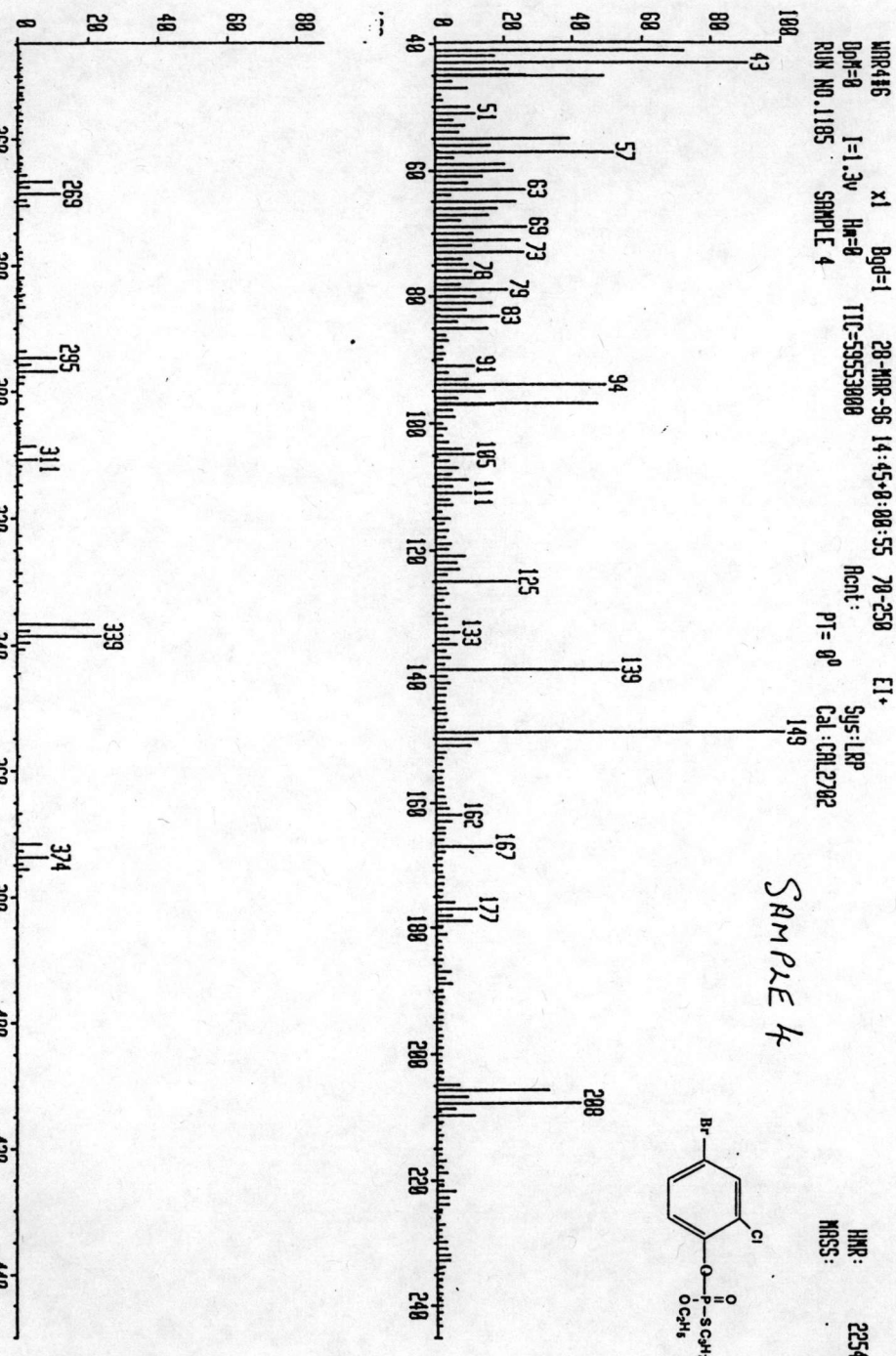

Figure 4.13 Mass spectrum of water extract from farm IV

Ions m/z 149 and 177 indicated the presence of diethyl phthalate. Figure 4.14 is the standard EI spectra of diethyl phthalate (McLafferty, 1980). Phthalates are some impurities usually found in mass spectra since they are the common components of plasticizers (tubing, cap liners, gaskets) and chromatographic column packings (Stenhagen *et al.*,1974).

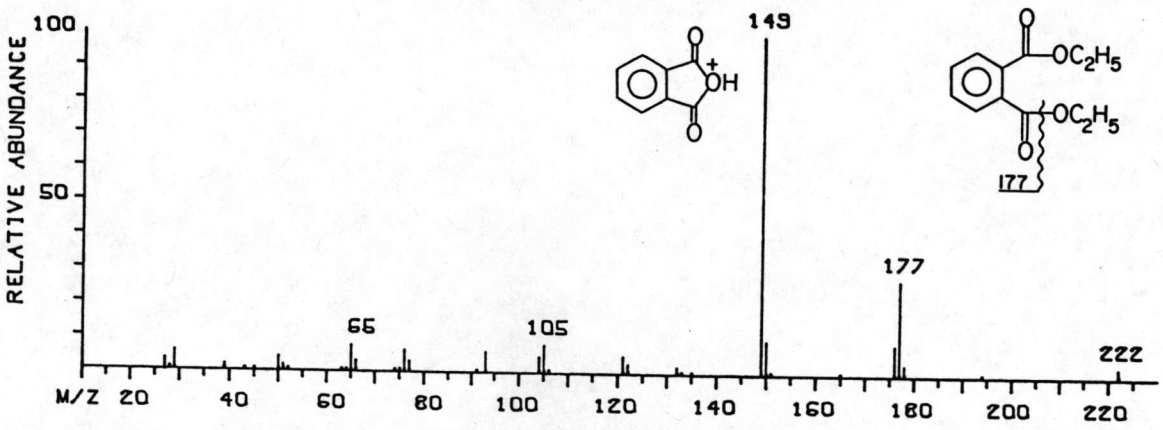

FIGURE 4.14 Mass spectrum of diethyl phthalate.

4.5 Comparative methods

There are several methods to determine OPs pesticides in water such as gas chromatography and liquid chromatography. The paper (Molto, 1991) describes a reliable, rapid and quantitative SPE method for the determination of OPs pesticides residues in water. Pesticide residues are extracted from water with acetonitrile and clean up on C_{18} SPE columns. OPs are determined by GC with nitrogen-phosphorus detection. The overall average recoveries were greater than 85% except dimethoate and trichlorfon. The method detection limit were 0.045, 0.089, 0.048, 0.007 μg/l for cumaphos, dimethoate, triazophos, trichlorfon and pyrazophos, respectively.

The paper (Brooks, 1990) determined the pesticides chlorpyrifos, isofenphos, carbaryl, iprodione and triadimefon in groundwater. The method involves the extraction of the pesticides on C_{18} columns and then elution with methylene chloride. After that solvent exchange to hexane and the extracts are analysed by gas chromatography using nitrogen-phosphorus detection. Recoveries average higher than 90% with a detection limit of 1 ppb for carbaryl, iprodione and triadimefon, and 0.1 ppb for chlorpyrifos and isofenphos.

The determination of pesticides in river water by gas chromatography-mass spectrometry (Kobayashi,1993) have been developed. The method is based on reversed-phase (C_{18}) solid-phase extraction. Recoveries at the 0.5-2.5 μg/l fortification level were between 79 and 98%. The detection limits were 0.05 μg/l for chlornitrofen and 0.01 μg/l for other pesticides.

Crespo (1994) developed method for determinating of group of pesticides in water by gas chromatography-mass spectrometry with electron impact ionization was developed. The preconcentration of 500 ml of water with C18 and styrene-divinylbenzene (SDB) at low μg/l levels. The use of SDB membrane extraction discs gave a large increase in the recovery of aldrin compared with the value obtained with C18 discs. The recoveries of SDB discs were > 85% for most compounds. The limits of detection were between 0.06 and 0.2 μg/l in th full scan mode.

In our study, we developed method for the determination of four organophosphorus pesticides, methyl parathion, malathion, profenofos and chlorpyrifos in agricultural drained water. Ops pesticides in water were analysed by solid-phase extraction and detected by HPLC. The optimized condition of RP-HPLC C18 SPE cartridge was evaluated from isolated parameter. Percent recoveries of optimum condition higher than 90% accepted chlorpyrifos 71%. The method detection limits were 25, 50, 25 and 25 μg/l , respectively. Profenofos was detected in water samples at the average level of 0.44 ± 0.07 μg/ml during the crop application period.

In general, gas chromatography analysis could be operated with lower detection limit than high performance liquid chromatography analysis organophosphorus pesticides but need more specific detector such as nitrogen-phosphorus detector (NPD) and flame photometric detector (FPD). Sometime it difficult to find these detector. The gas chromatography system consisted of vacuum under high pressure and high temperature. Therefore, the thermal labile and non volatile pesticides cannot be measured. Such case high performance liquid chromatography analysis become more useful. In our study, we illustrated the application of HPLC with UV detection in the analysis of pesticides which showed acceptable data. However, the standard analysis need to be improved for the lower limit of detection.