CHAPTER 3

RESULTS AND DISCUSSION

3.1 Results on Optimization of GC–FPD Conditions

The optimization of GC–FPD conditions were studied using the parameters presented in **Section 2.3**. Results from optimization of GC–FPD conditions for the analysis of dimethoate and malathion are shown in **Table 3.1**.

Table 3.1 Conditions of GC–FPD employed

Operation	Conditions
1. Injection port	
temperature	250 °C
mode	splitless
injection volume	2.5 µL
2. Oven	
initial temperature	200 °C
maximum temperature	250 °C
rate	200-250 °C, rate 20 °C/min (hold 3 min)

Table 3.1 (continued)

Operation	Conditions
3. Column	
capillary column	HP-5MS (5% Phenyl Methyl Siloxane)
	30.0 m × 0.25 mm i.d., 0.25 μm
	film thickness
maximum temperature	325 °C
4. Detector	
type	FPD
mode	phosphorus
temperature	200 °C
make-up gas (N_2)	60 mL/min
hydrogen	70 mL/min
air zero	100 mL/min
5. Carrier gas	
carrier gas	helium
mode	constant flow
flow rate	1.0 mL/min
6. Analysis time	5.5 minutes

3.2 Results on Optimization of SDME Conditions

Different SDME parameters were optimized in order to obtain the best sensitivity. The optimization of SDME conditions were studied using the parameters presented in **Section 2.4**. Results from optimization of SDME conditions for the analysis of dimethoate and malathion are described in the followings:

3.2.1 Selection of the extraction solvent [23]

The type of extraction used in SDME is an essential consideration for efficient preconcentration. To achieve good extraction, several solvents differing in polarity and water solubility were screened on the basis of the principle of "*like dissolves like*". As immersed SDME was concerned, the extractant has to meet several requirements: to have low solubility in water, to extract analytes well, to be separated from the chromatographic peaks of the analytes and to be less toxic. The final choice of solvent should be based on comparison of selectivity, extraction efficiency, incidence of drop loss, rate of drop dissolution (especially for faster stirring rates and extended extraction times). Several types of organic solvent including toluene, chlorocyclohexane, tetrachloroethylene, *n*-heptane, octane and isooctane were tested. Relative responses obtained for each pesticide with the six solvents shown in **Table 3.2** and **Figure 3.1** indicated that toluene had the highest extraction efficiency; therefore, toluene was employed for further experiments.

Table 3.2 Data of dimethoate and malathion for the extraction efficiency of different organic solvents by SDME.

Extraction solvent	Area ratio of dimethoate				Area ratio of malathion			
	1	**2**	**3**	**Average**	**1**	**2**	**3**	**Average**
Toluene	0.4699	0.4719	0.4740	**0.4719**	6.5617	6.5534	6.5427	**6.5526**
Chlorocyclohexane	0.3154	0.3084	0.3131	**0.3123**	5.9173	5.9125	5.9096	**5.9131**
Tetrachloroethylene	0.1074	0.0997	0.1068	**0.1046**	5.9403	5.9396	5.9407	**5.9402**
n-Heptane	n.d.	n.d.	n.d.	n.d.	6.1271	6.1301	6.1581	**6.1384**
Octane	n.d.	n.d.	n.d.	n.d.	5.9293	5.9129	5.9138	**5.9187**
Isooctane	n.d.	n.d.	n.d.	n.d.	5.6917	5.5925	5.7421	**5.6754**

n.d., not detectable

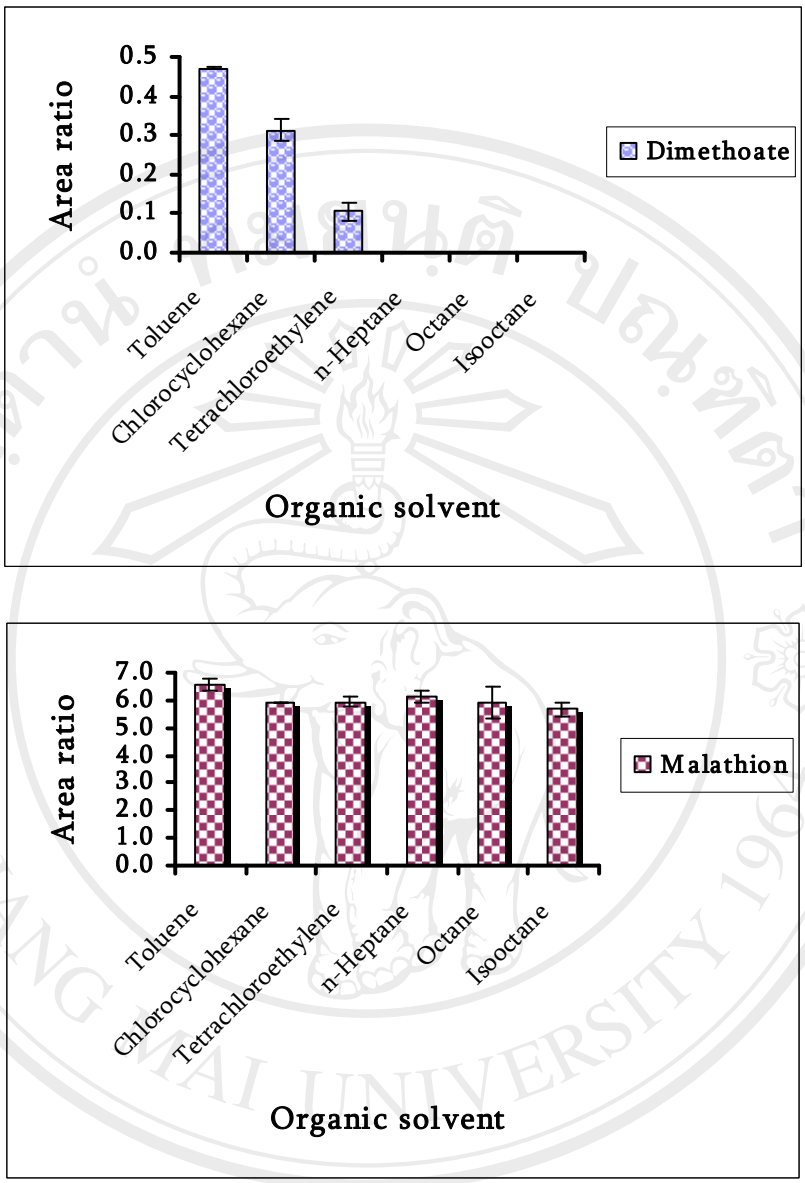


Figure 3.1 Extraction efficiency of dimethoate and malathion with different organic solvents.

3.2.2 Drop volume

The SDME theory reveals that the amount of analyte extracted by the drop is related to the volume of the drop, and the use of a large drop results in a enhancement of the analytical sensitivity and can also have an essential effect on the extraction efficiency. In these experiments, 20 ml aqueous solution containing 20% (w/v) NaCl, spiked at 100 µg/L for each analytes and stirred at 100 rpm were extracted for 5 min with organic drop volumes ranging from 1.0 to 3.0 µl, which were controlled by microsyringe. As it was expected, an increase in the drop volume (up to 2.5 µl) resulted in a sharp enhancement in the extraction efficiency of the system. However, at larger volume (i.e. >3 µl), the drop revealed a great tendency to fall down from the tip of the microsyringe. On the other hand, the larger volumes, after insertion into the chromatographic column, might cause peak tailing. Thus, a 2.5 µl drop volume was chosen for all subsequent extractions as shown in **Table 3.3** and **Figure 3.2**.

Table 3.3 Data of dimethoate and malathion for the extraction efficiency of drop volume by SDME.

Drop volume (µL)	Area ratio of dimethoate				Area ratio of malathion			
	1	2	3	Average	1	2	3	Average
1.0	0.3979	0.4021	0.4021	**0.4007**	6.1021	6.0094	5.9964	**6.0360**
1.5	0.4019	0.4029	0.3997	**0.4015**	6.2019	6.1897	6.2017	**6.1978**
2.0	0.4713	0.4783	0.4794	**0.4763**	6.3712	6.3535	6.3569	**6.3605**
2.5	0.5649	0.5586	0.5549	**0.5595**	6.6108	6.6002	6.6034	**6.6048**
3.0	0.5497	0.5685	0.5433	**0.5538**	6.4301	6.4559	6.4401	**6.4420**

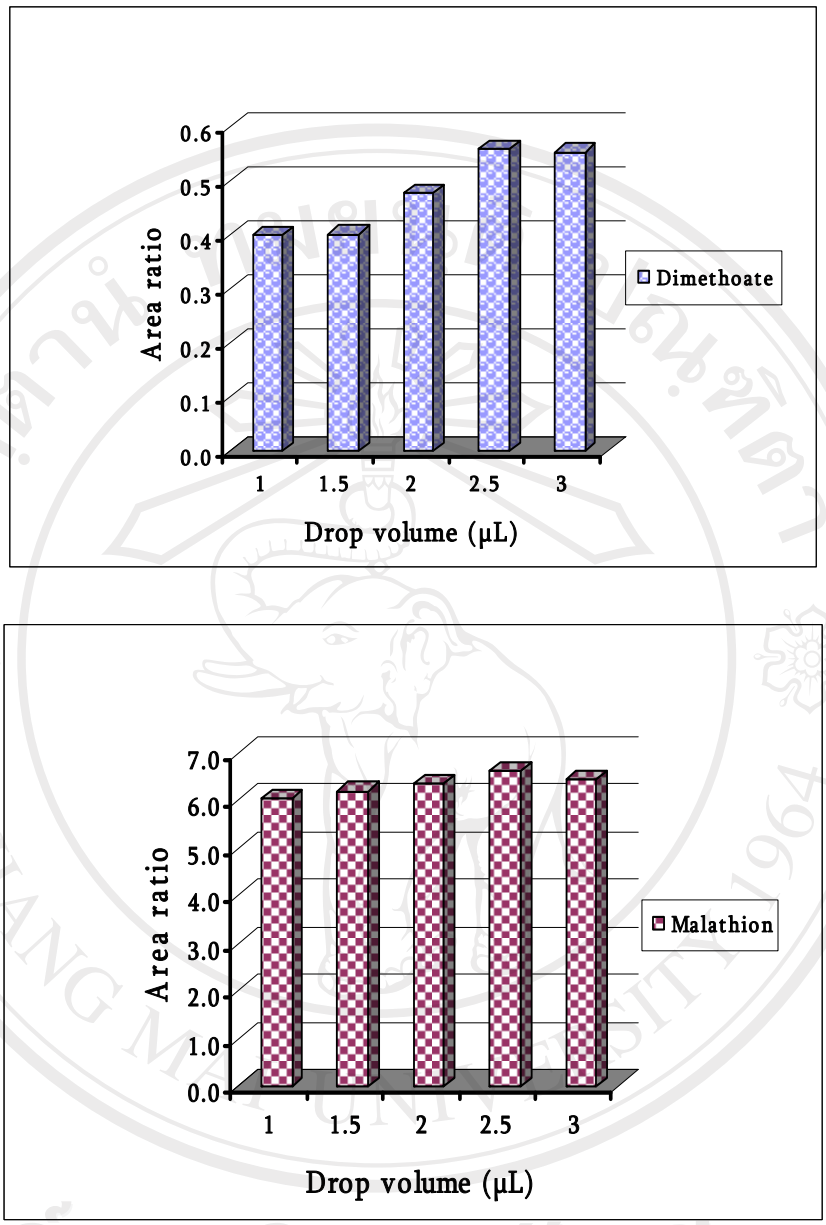


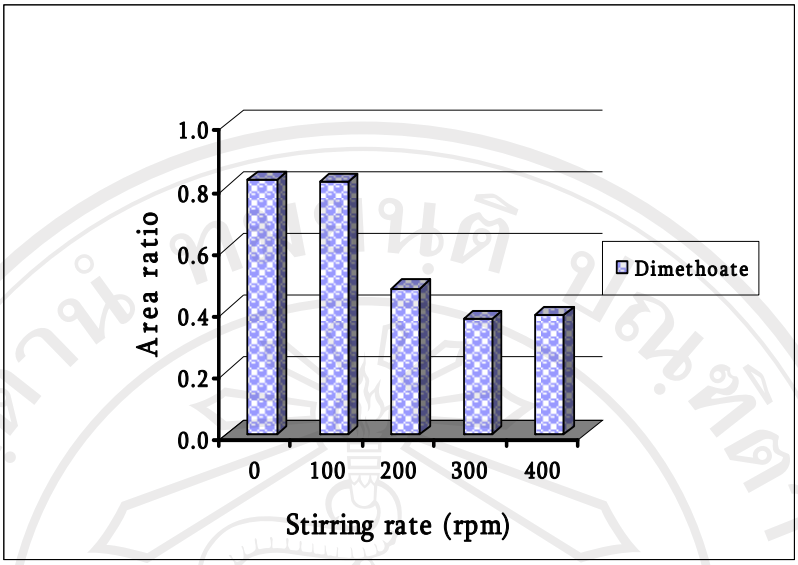
Figure 3.2 Effect of drop volume on the extraction efficiency of SDME for dimethoate and malathion.

3.2.3 Stirring rate

Extraction efficiency is increased with increasing sample stirring, while extraction time is reduced, because thermodynamic equilibrium between the aqueous and organic phase is established more rapidly. In this case of direct sampling, it is essential to obtain a linear relationship between the concentration of analytes in the water and in the organic phase. To evaluate the effect of sample stirring, aqueous sample (spiked at 100 µg/L with all analytes and internal standard) were extracted in extraction solvent for 5 min at different stirring rate (0-400 rpm). As expected, the results revealed that stirring dramatically enhanced extraction efficiency and according to **Table 3.4** and **Figure 3.3**, the amount of extracted analytes reached a maximum at 100 rpm. Based on these observations, it is not useful to use stirring rate which is higher than 100 because of spattering, therefore stirring of the sample at 100 rpm was found to be optimum, yielding thus acceptable results for all target analytes.

Table 3.4 Data of dimethoate and malathion for the extraction efficiency of stirring rate by SDME**.**

Stirring rate (rpm)	Area ratio of dimethoate				Area ratio of malathion			
	1	2	3	Average	1	2	3	Average
0	0.8189	0.8257	0.8246	**0.8231**	6.2053	6.1975	6.2191	**6.2073**
100	0.8071	0.8158	0.8231	**0.8153**	6.5705	6.5701	6.5704	**6.5703**
200	0.4714	0.4723	0.4708	**0.4715**	6.1790	6.1801	6.1815	**6.1802**
300	0.3709	0.3749	0.3698	**0.3719**	6.0908	6.0983	6.1083	**6.0991**
400	0.3872	0.3861	0.3853	**0.3862**	5.9324	5.9293	5.9472	**5.9363**



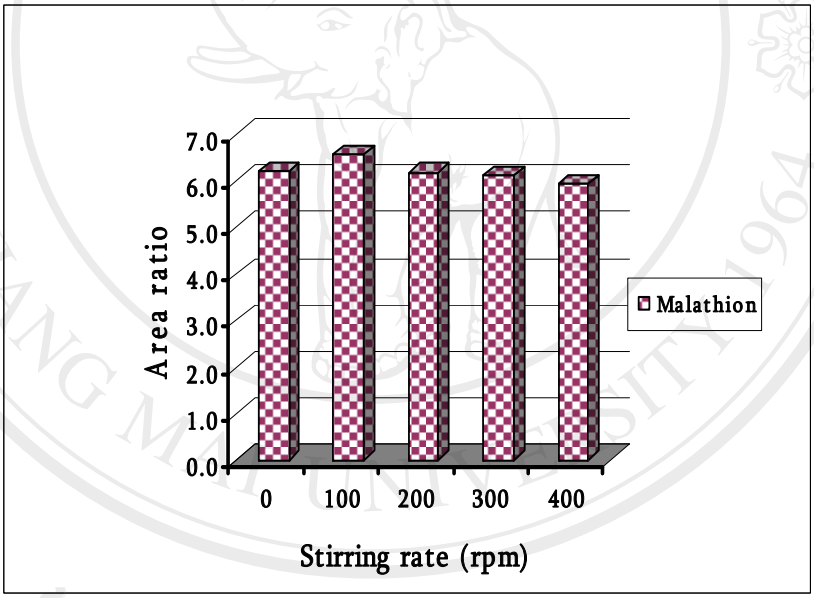


Figure 3.3 Effect of stirring rate on the extraction efficiency of SDME for dimethoate and malathion.

3.2.4 Salting-out effect

The effect of NaCl concentration (ranging from 0 to 30%) was investigated. For these experiments, 2.5 µl of toluene was immersed in 20 ml aqueous solution spiked with 100 µg/L for each analyte for 5 min at a stirring rate of 100 rpm. The results showed an initial increase in the extraction efficiency with an increase in salt concentration, with a maximum being reached at 20%, followed by a decrease in extraction efficiency with further increase in salt concentration. Extraction is usually enhanced with increasing salt concentration and increased polarity of the compound (salting-out effect).[24] In SDME, generally, there is an unexpected decrease in extraction efficiency with increased ionic strength (20% w/v) for the majority of analytes, which is more pronounced for the less polar analytes. When salt was added to the solution, water molecules could form hydration spheres around the ionic salt molecules. These hydration spheres reduced the amount of water available to dissolve analyte molecules. Thus, it drove additional analytes into the organic extractant.[23] As salt concentration increased further, salt molecules started to interact with analyte molecules. Thus, the initial increase in the amount of analytes extracted with salt addition was followed by a decrease, when salt concentration was increased further. A 20% (w/v) NaCl addition was selected since it provided the best extraction efficiency for all the analytes illustrate in **Table 3.5** and **Figure 3.4**.

Table 3.5 Data of dimethoate and malathion for the extraction efficiency of amount of NaCl addition by SDME**.**

Amount of NaCl (%)	Area ratio of dimethoate				Area ratio of malathion			
	1	**2**	**3**	**Average**	**1**	**2**	**3**	**Average**
0	0.3749	0.4011	0.3896	**0.3885**	5.4804	5.4294	5.4213	**5.4437**
5	0.4238	0.4469	0.4429	**0.4379**	5.5778	5.5605	5.5722	**5.5702**
10	1.0188	1.0319	0.9978	**1.0162**	5.7061	5.6852	5.6851	**5.6921**
15	1.7775	1.7855	1.8073	**1.7901**	5.9549	5.9479	5.9452	**5.9493**
20	3.4071	3.3975	3.3847	**3.3964**	5.9369	5.9379	5.9388	**5.9379**
25	3.2082	3.1804	3.1911	**3.1932**	5.2531	5.2391	5.2321	**5.2414**
30	3.0075	3.1275	3.0007	**3.0452**	5.1308	5.1401	5.1293	**5.1334**

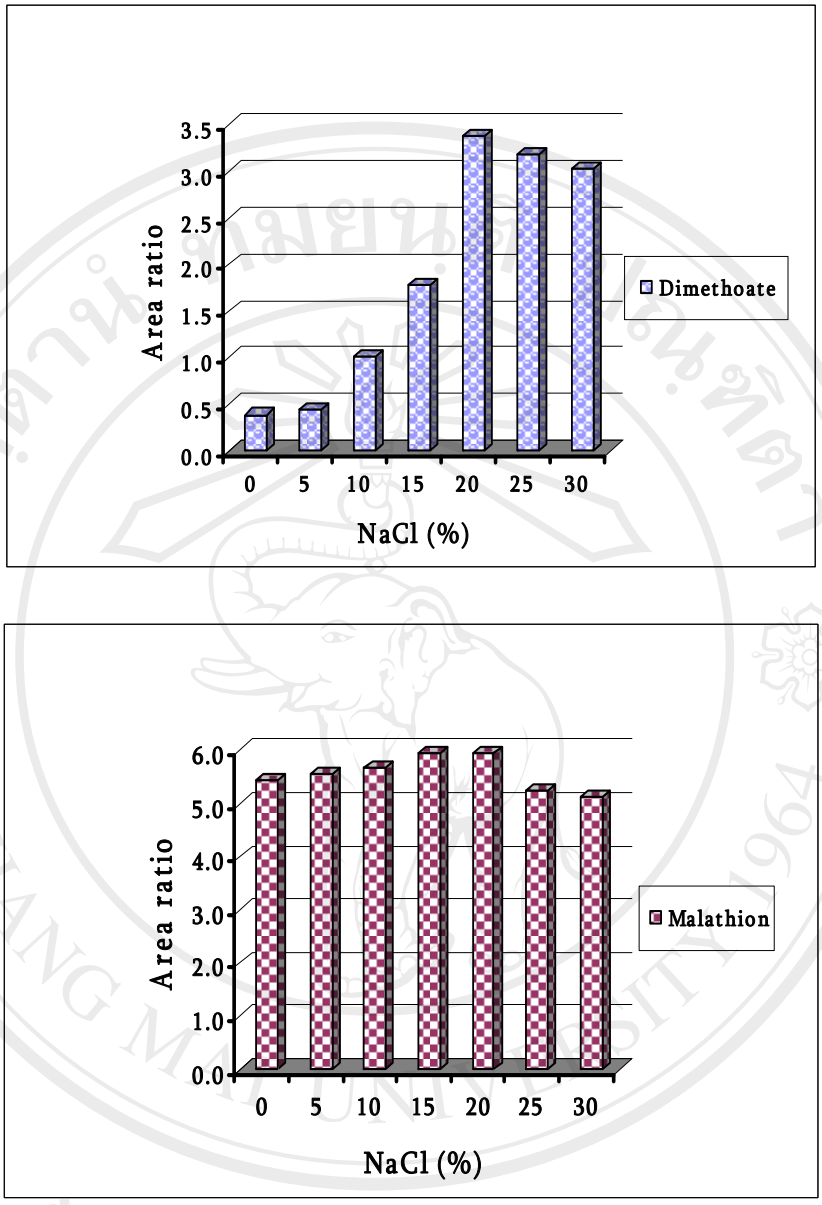


Figure 3.4 Effect of salt addition on the extraction efficiency of SDME for dimethoate and malathion.

3.2.5 Sample pH

The pH sample solution is an important factor which may have an effect on the extraction recovery of analytes from water. It is known to us that for a weak organic acid or base, the existence form of certain analytes will change with the change of solution pH and therefore the affect their water-solubility and extractability. When the pH is high, the acid/base equilibrium for the acidic pesticides shift significantly toward the neutral forms, which have greater affinities toward the non-polar solvent and the extraction efficiency is therefore increased.[25] So prior to extraction, sample solutions are often adjusted to appropriate pH value to maximize the extraction efficiency. In this work, a wide range of sample pH from 2 to 8 was evaluated. Based on results illustrate in **Table 3.6** and **Figure 3.5**, it was evident that the extraction efficiency at pH 6 were higher than for all the rest of analytes. This may have resulted from the hydrolysis of dimethoate and malathion under strongly acidic or basic aqueous environments. [23] Thus, sample solution was adjusted to pH value of 6 in the following experiments.

Table 3.6 Data of dimethoate and malathion for the extraction efficiency of sample pH by SDME**.**

Sample pH	Area ratio of dimethoate				Area ratio of malathion			
	1	2	3	Average	1	2	3	Average
2	0.5608	0.5658	0.5663	**0.5643**	5.9208	5.9203	5.9177	**5.9196**
3	0.6074	0.5876	0.5947	**0.5966**	6.3008	6.3016	6.2981	**6.3002**
4	0.5983	0.6092	0.6172	**0.6082**	6.3598	6.3591	6.3587	**6.3592**
6	0.6379	0.6341	0.6301	**0.6340**	6.5008	6.4997	6.4977	**6.4994**
8	0.6099	0.6167	0.6157	**0.6141**	6.0391	6.0574	5.9913	**6.0293**

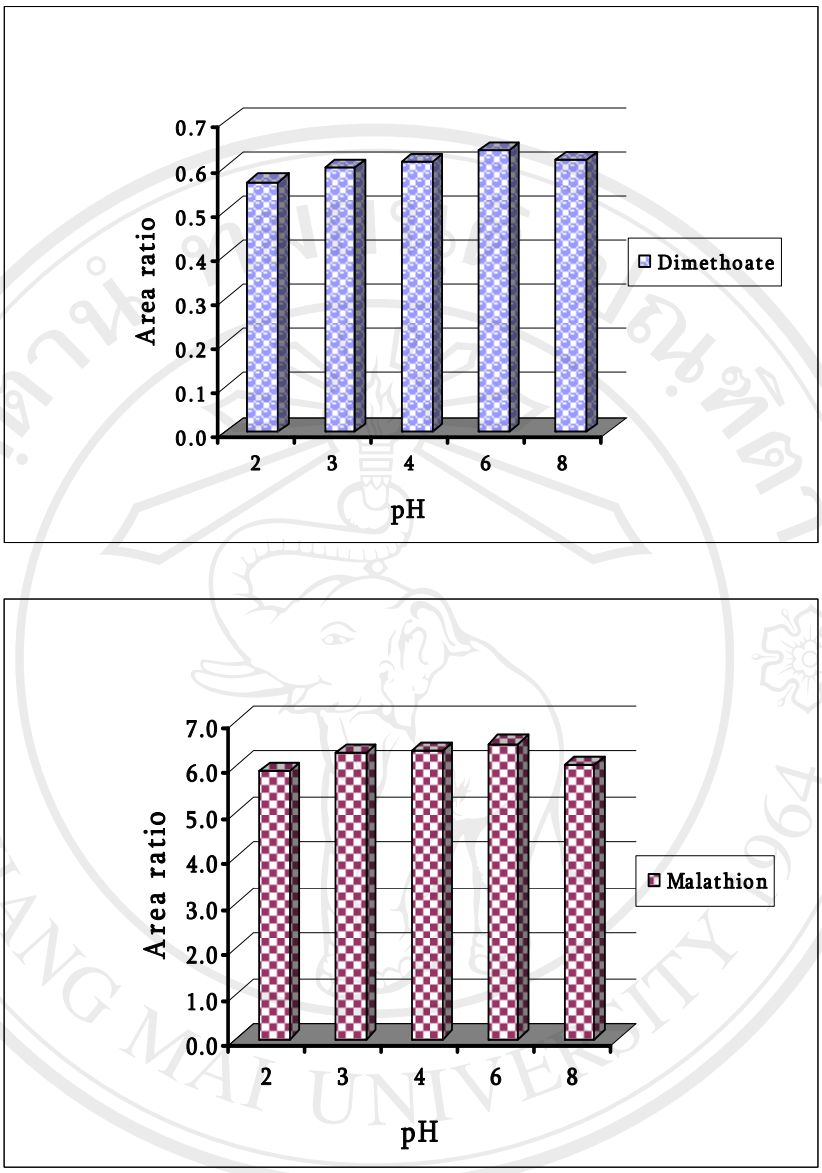


Figure 3.5 Effect of sample pH on the extraction efficiency of SDME for dimethoate and malathion.

3.2.6 Extraction time

A sufficient extraction time is necessary to attain equilibrium of analytes between the aqueous and organic drop, but a longer extraction time of microextraction to reach complete equilibrium may result in drop dissolution and have a high incidence of drop loss[26]. To extract the maximum amount of analytes the effect of sampling time in the yield of the microextraction was optimized. Based on this fact, extraction time was optimized in the range 5-30 min. As shown in **Table 3.7** and **Figure 3.6**, the extraction time profiles show that the equilibrium curves were attained in 5 min for all analytes. The amount extracted by SDME decreased rapidly with decreasing exposure from 5 to 30 min for dimethoate but remained nearly constant for malathion. Normally, the time for establishing equilibrium was selected as the extraction time. Therefore, an extraction time of 5 min was selected in order to make the SDME more quickly.

Table 3.7 Data of dimethoate and malathion for the extraction efficiency of extraction time by SDME**.**

Extraction time (min)	Area ratio of dimethoate				Area ratio of malathion			
	1	2	3	Average	1	2	3	Average
5	0.5315	0.5320	0.5311	**0.5315**	5.9652	5.9612	5.9614	**5.9626**
10	0.4809	0.4699	0.4705	**0.4738**	5.8011	5.7809	5.8036	**5.7952**
15	0.3033	0.2795	0.2803	**0.2877**	5.8767	5.8775	5.8874	**5.8805**
20	0.2901	0.2801	0.2814	**0.2839**	5.8615	5.8084	5.8851	**5.8517**
25	0.2197	0.2301	0.2313	**0.2270**	5.8301	5.8304	5.8101	**5.8235**
30	0.1257	0.1008	0.1105	**0.1123**	5.6936	5.7001	5.6434	**5.6790**

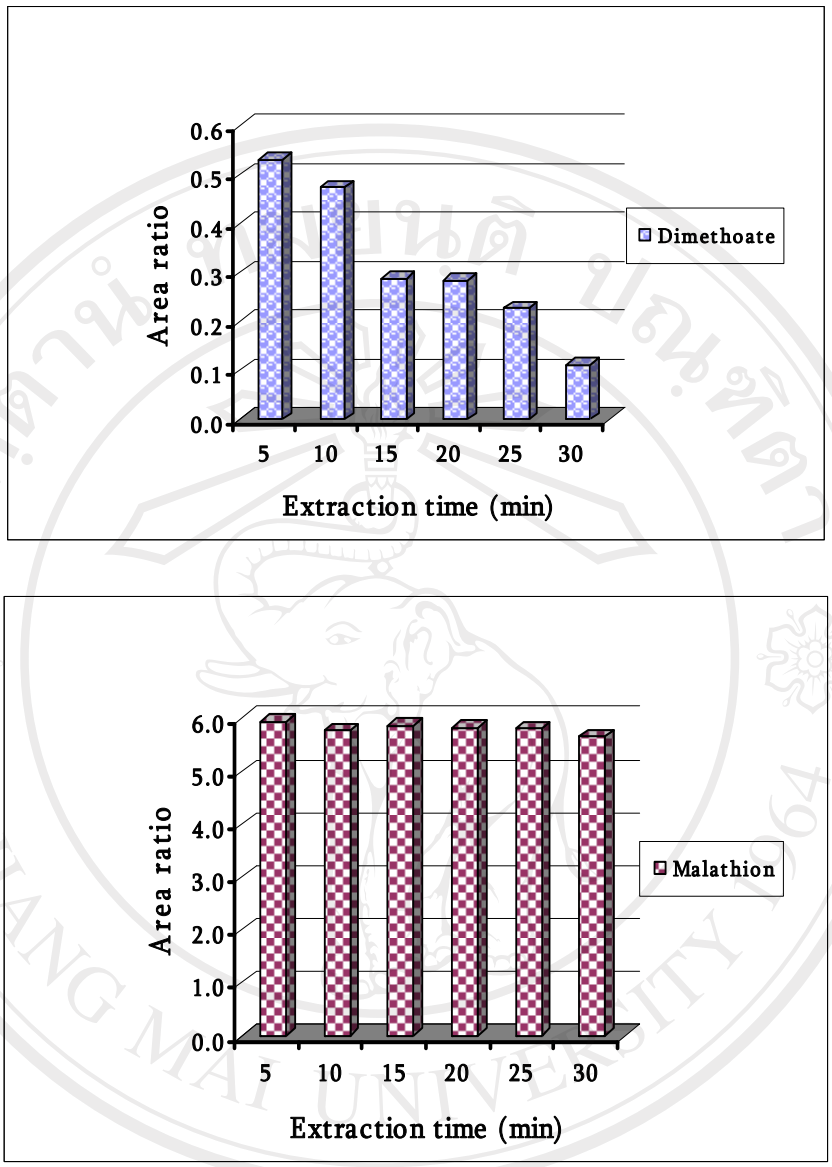


Figure 3.6 Effect of extraction time on the extraction efficiency of SDME for dimethoate and malathion.

Summary of the suitable conditions of SDME method for the analysis of dimethoate and malathion are shown in **Table 3.8**.

Table 3.8 Suitable conditions of SDME method

Parameters	**Optimum value**
Extraction solvent	Toluene
Drop volume	2.5 µL
Stirring rate	100 rpm
Salt addition	20 % (w/v)
Sample pH	6
Extraction time	5 minutes

The chromatogram obtained for the determination of dimethoate and malathion standard solutions under the optimized SDME–GC–FPD conditions are shown in **Figure 3.7**.

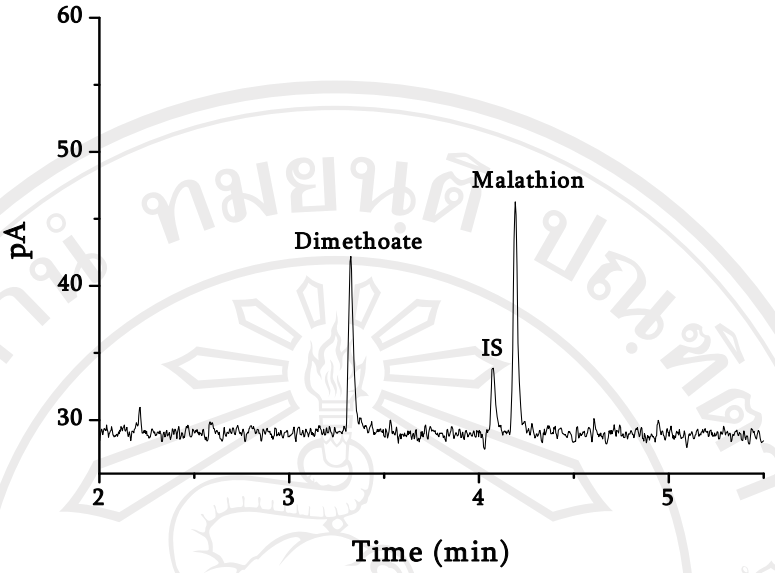


Figure 3.7 Optimum conditions of SDME-GC-FPD for the analysis of dimethoate and malathion.

3.3 Results on Validation of the Method

A validation of the method in terms of percentage recovery, limits of detection, precision (intra- and inter-day repeatability) and linearity were mentioned as follows:

3.3.1 Percentage recovery

The accuracy expressed in terms of percentage recovery was done by spiking the mixture of pesticide compounds in real samples. Triplicate extraction of orange sample was carried out by spiking in sample with 20 and 100 ppb of each pesticide. The percentage recoveries of this proposed method for dimethoate and malathion were in the range 87.6–103.4%. The results are shown in **Table 3.9**. Example of the calculation is shown in Appendix B.

Table 3.9 Limits of detection and recoveries at two spiked levels

Pesticide	**LOD (μg/L)**	**Mean recovery**	
		Added (μg/L)	**Recovery (%)**
Dimethoate	10	20	91.2
		100	103.4
Malathion	5	20	87.6
		100	102.0

3.3.2 Limits of detection

Investigation of limits of detection of pesticides analysis using a decreased concentration method was carried out for the concentration at S/N of 3 were 10 and 5 µg/L for dimethoate and malathion, respectively. The results of a standard solution of the analytes at concentration levels near their limits of detection are shown in **Table 3.9** and **Figure 3.8**.

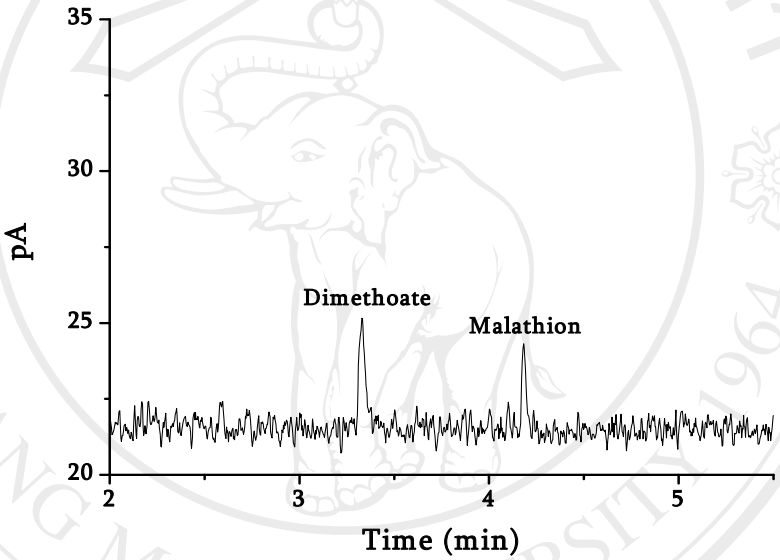


Figure 3.8 LOD value of dimethoate and malathion were obtained under the optimum conditions

3.3.3 Precision

The precision, intra- and inter-day repeatability give a measure of error in the development methodology and usually reported as a percentage of relative standard deviation (%R.S.D.). In this study, the intra-day repeatability was determined by seven injections of a standard mixture onto SDME–GC–FPD under the optimum

conditions in same day. The inter−day repeatability was determined by five injections in different day. Results are shown in **Table 3.10-3.11**. The intra−day repeatability of the average area ratio of each compounds expressed as % R.S.D. were found to be 1.4–4.0%. The inter−day repeatability of the average area ratio were obtained in the range 0.5–2.7%.

Table 3.10 Intra−day repeatability of dimethoate and malathion at three concentrations

Pesticide	**Concentration (μg/L)**	**Average area ratio**	**S.D.**	**R.S.D. (%)**
Dimethoate	40	0.1637	0.0065	4.0
	80	0.6472	0.0147	2.3
	120	0.8553	0.0120	1.4
Malathion	5	0.3852	0.0143	3.7
	50	3.3691	0.0612	1.8
	100	6.6311	0.1093	1.6

Table 3.11 Inter−day repeatability of dimethoate and malathion at three concentrations

Pesticide	**Concentration (µg/L)**	**Average area ratio**	**S.D.**	**R.S.D. (%)**
Dimethoate	40	0.2464	0.0048	1.9
	80	0.3550	0.0094	2.7
	120	0.7417	0.0085	1.1
Malathion	5	0.3240	0.0064	2.0
	50	2.9103	0.0594	2.0
	100	6.1078	0.0322	0.5

3.3.4 Linearity

Under the optimal conditions described above, after injection of each working standard solution into GC, the area ratio appeared. The area ratio is plotted against the standards at concentrations of 10, 20, 40, 60, 80, 100, 200, 400, 800 and 1000 µg/L. Each point of the calibration graph corresponded to the mean value from three replicate measurement. It was found that linearity of dimethoate and malathion are linear in the range 10-1000 µg/L. Results of linearity are presented in **Tables 3.12-3.13**, and **Figures 3.9-3.10**.

Table 3.12 The response (average area ratio) of dimethoate with variation of concentrations

Concentration (µg/L)	Average area ratio
10	0.2241
20	0.3859
40	0.8133
60	1.2866
80	1.7954
100	2.2672
200	5.5090
400	13.2135
800	29.9275
1000	38.2655

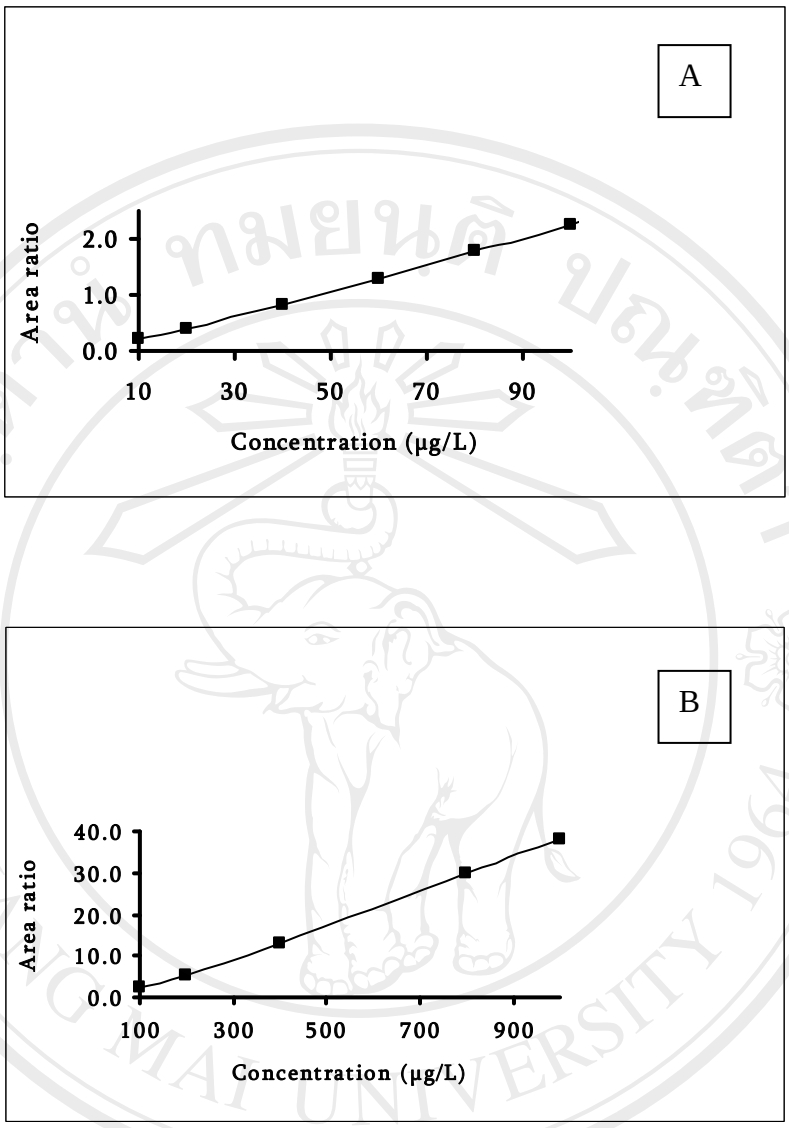


Figure 3.9 Linearity plot of average area ratio against concentration of dimethoate under the optimized conditions (A= concentration range 10-100 µg/L, B= concentration range 100-1000 µg/L).

Table 3.13 The response (average area ratio) of malathion with variation of concentration

Concentration (µg/L)	Average area ratio
10	0.5176
20	1.2374
40	2.4597
60	4.0429
80	5.3754
100	7.1751
200	16.5035
400	35.5615
800	73.0380
1000	91.7340

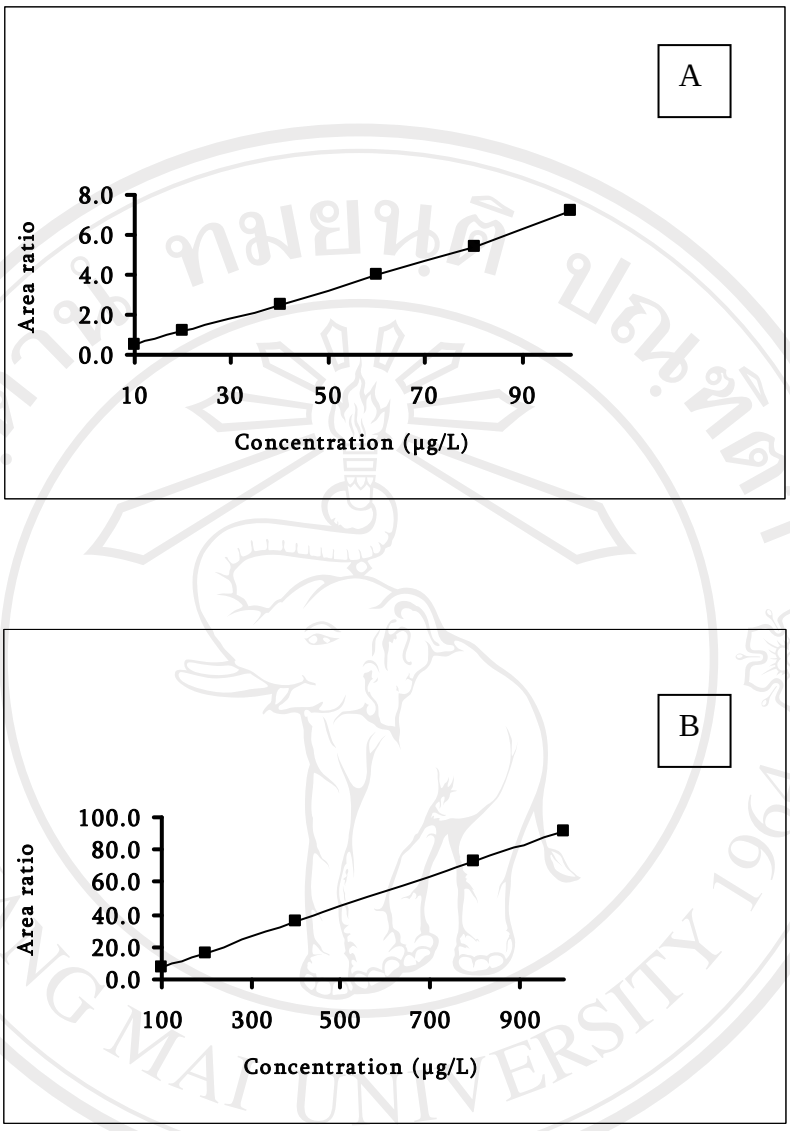


Figure 3.10 Linearity plot of average area ratio against concentration of malathion under the optimized conditions (A= concentration range 10-100 µg/L, B= concentration range 100-1000 µg/L).

3.4 Results on Retention Times of Dimethoate and Malathion Standards

The retention times of dimethoate and malathion standards were measured under the current operating optimum conditions. Results are presented in **Tables 3.14**.

Table 3.14 Retention times of dimethoate and malathion standards (n=10)

Day	Retention times (min)	
	Dimethoate	**Malathion**
1	3.331	4.187
2	3.330	4.188
3	3.332	4.191
4	3.334	4.195
5	3.325	4.190
6	3.327	4.191
7	3.327	4.195
8	3.326	4.189
9	3.322	4.190
10	3.329	4.196
Mean	**3.328**	**4.191**
S.D.	**0.0036**	**0.0027**
%R.S.D.	**0.1**	**0.1**

3.5 Results on Determination of Dimethoate and Malathion in Vegetable and Fruit Samples

In order to demonstrate the suitability of the proposed method in this study for quantification of dimethoate and malathion in real samples, several vegetable and fruit samples extracts were analysed. Calibration curves based on area ratio were linear (R^2 = 0.9991 for dimethoate and 0.9988 for malathion) for each pesticide compound as illustrated in **Figure 3.11-3.12**. Dimethoate was found to be the major compounds present in the entire fruit sample examined (**Figure 3.14-3.16**). Under the proposed condition, the concentrations of pesticide compounds in various samples determined using a SDME technique combination with the GC–FPD are presented in **Table 3.15**. Within the Thai agricultural commodity and food standard, maximum residue limits (MRLs) established for dimethoate and malathion in oranges are 5 and 7 mg/kg, respectively [31].

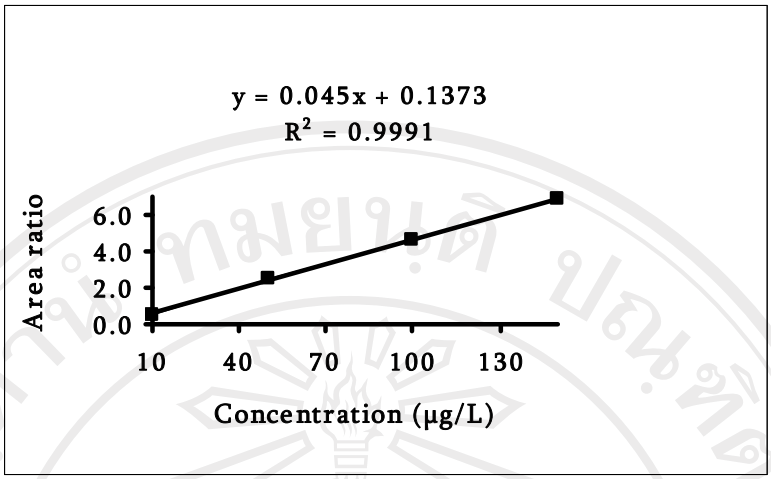


Figure 3.11 Calibration curve of dimethoate used for calculating concentration in vegetable and fruit samples.

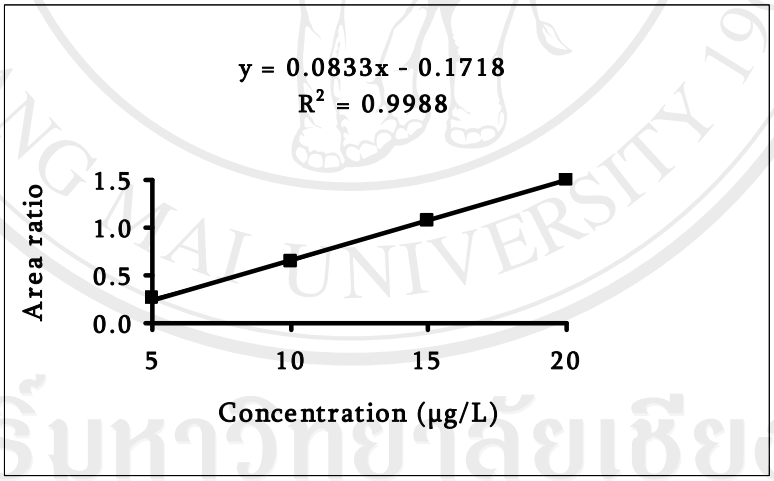


Figure 3.12 Calibration curve of malathion used for calculating concentration in vegetable and fruit samples.

Table 3.15 Amounts of dimethoate and malathion in vegetable and fruit samples (n=3)

Sample	Concentration (µg/L)	
	Dimethoate	**Malathion**
V1	n.d.	n.d.
V2	n.d.	n.d.
V3	n.d.	n.d.
O1-1	n.d.	n.d.
O1-2	**299**	n.d.
O1-3	**943**	n.d.
O2-1	n.d.	n.d.
O2-2	**327**	n.d.
O2-3	**144**	n.d.
O3-1	n.d.	n.d.
O3-2	n.d.	n.d.
O3-3	n.d.	n.d.
O4-1	n.d.	n.d.
O4-2	n.d.	n.d.
O4-3	n.d.	n.d.

Table 3.15 (continued)

Sample	Concentration (µg/L)	
	Dimethoate	**Malathion**
O5-1	n.d.	n.d.
O5-2	n.d.	n.d.
O5-3	n.d.	n.d.
J1-1	**109**	n.d.
J1-2	**64**	n.d.
J1-3	n.d.	n.d.
J1-4	n.d.	n.d.
J1-5	**90**	n.d.
J1-6	**59**	n.d.
J2	n.d.	n.d.
J3	n.d.	n.d.
J4	n.d.	n.d.

n.d., not detectable (or less than the detection limit value).

Several vegetable and fruit extracts were analysed in order to demonstrate that the method proposed in this work is suitable for the determination of dimethoate and malathion in real samples. Some chromatograms (**Figure 3.14-3.16**) showed the presence of dimethoate peak obtained in orange juice samples. However, the early eluting peak originating from sample impurities affected the peak shape of dimethoate and IS.

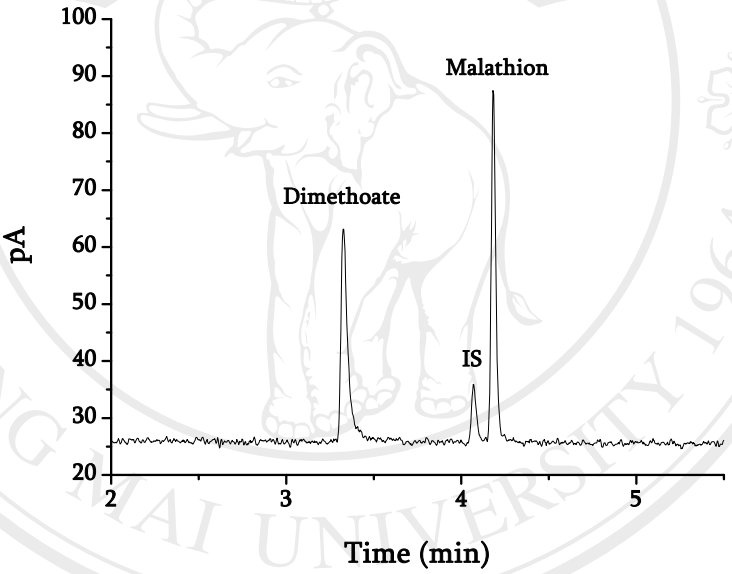


Figure 3.13 Chromatograms of dimethoate and malathion standard extracts under the proposed conditions.

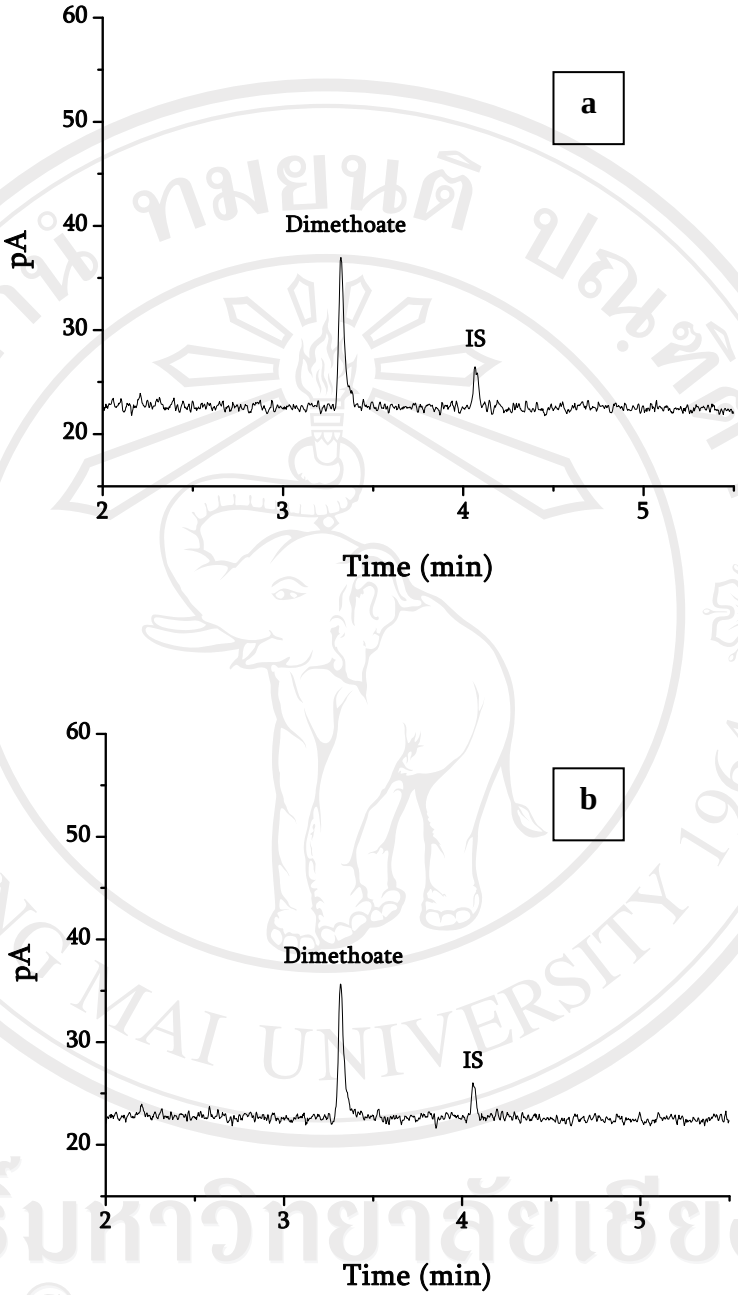


Figure 3.14 Chromatograms of dimethoate in orange juice extracts under the proposed conditions (a = O1-2 sample, b = O2-2 sample).

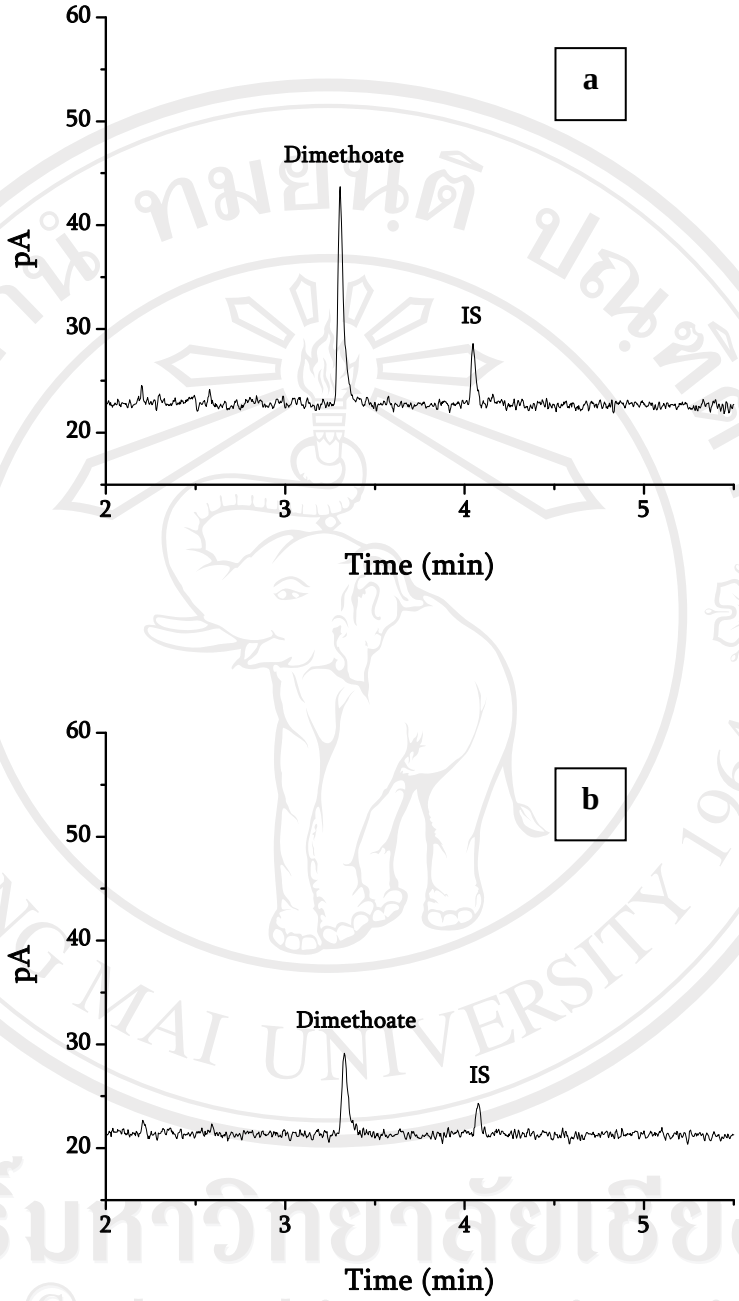


Figure 3.15 Chromatograms of dimethoate in orange juice extracts under the proposed conditions (a = O1-3 sample, b = J1-6 sample).

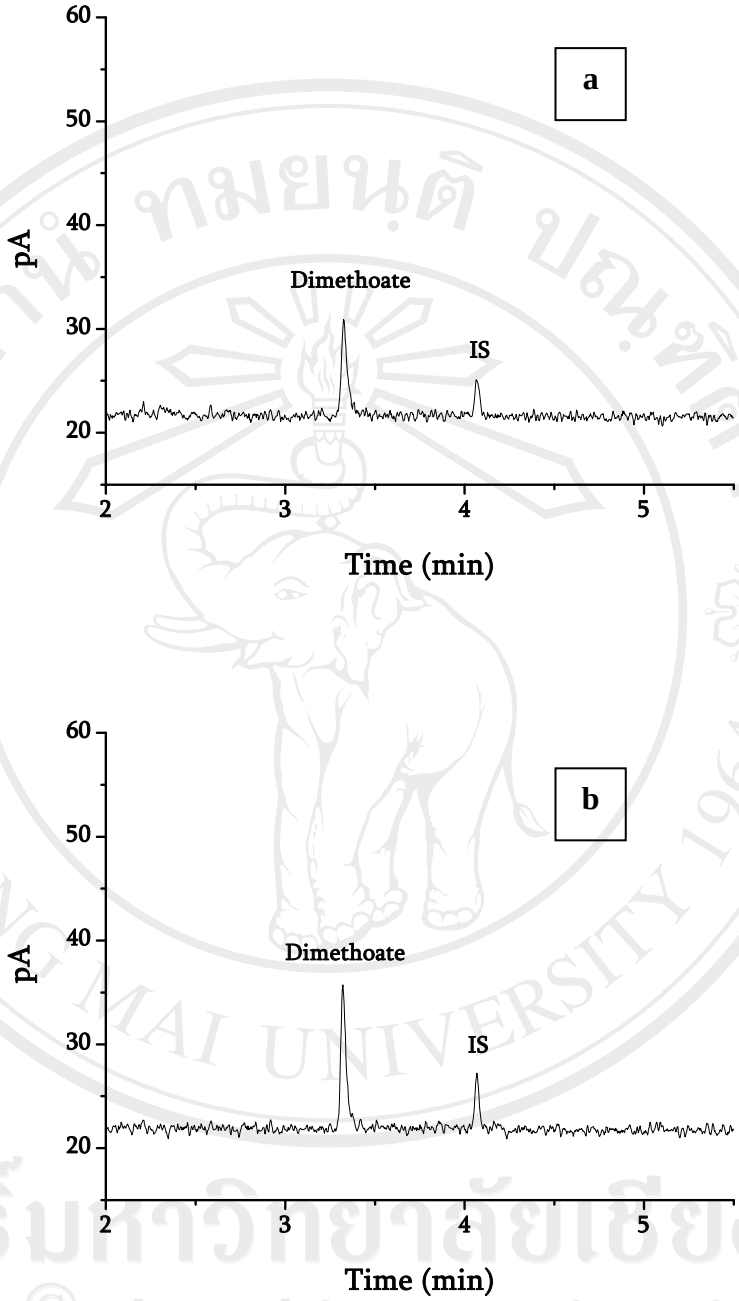


Figure 3.16 Chromatograms of dimethoate in orange juice extracts under the proposed conditions (a = J1-5 sample, b = J1-1 sample).