CHAPTER 3

RESULTS AND DISCUSSION

3.1 Investigation of extraction procedure for HPLC method

The selection of extracting solvent is very important to pesticide analysis, so various organic solvents were investigated.

3.1.1 Investigation of extracting solvent as diluent for standard solution

The extracting solvents including ethyl acetate, acetone, ethanol and their mixture (1:1:1) were investigated for their extraction efficiency of analytes. The dimethoate, carbaryl and fenvalerate were analyzed by HPLC (Table 3.1 and Figure 3.1). Dimethoate was also analyzed by GC (Table 3.2 and Figure 3.2). The peak area of dimethoate, carbaryl and fenvalerate in standard solution were calculated by the differentiation between standard solution and blank. From HPLC results obtained, acetone was the most appropriate diluent for use in simultaneous determination of these three pesticides. Therefore, it was further used as diluent in 3.1.2 and 3.1.3.

Table 3.1 HPLC peak data of dimethoate, carbaryl and fenvalerate in standard solution using different extracting solvents as diluent.

Solvent	Peak area of dimethoate (mAU*s)			Peak area of carbaryl (mAU*s)			Peak area of fenvalerate (mAU*s)		
	Std	Blank	P_A*	Std	Blank	P_A*	Std	Blank	P_A*
EtOAc	42	nd	39	6946	nd	6957	669	nd	674
	37	nd		6712	nd		650	nd	
	40	nd		7213	nd		704	nd	
Acetone	573	nd	592	6483	nd	6614	627	nd	643
	613	nd		6721	nd		648	nd	
	591	nd		6636	nd		654	nd	
EtOH	502	nd	515	5739	nd	5756	565	nd	564
	515	nd		5773	nd		566	nd	
	529	nd		5756	nd		562	nd	
1:1:1** (mL)	175	nd	177	6362	nd	6345	620	nd	611
	174	nd		6431	nd		626	nd	
	183	nd		6243	nd		587	nd	

*P_A = Peak area of triplicate results **1:1:1 = A ratio of EtOAc: Acetone: EtOH

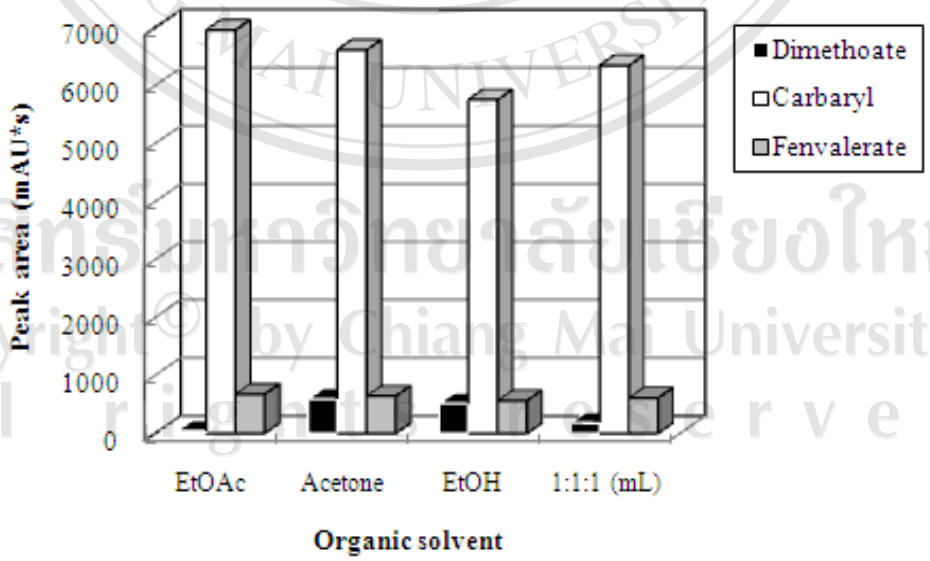

Figure 3.1 HPLC peak areas of dimethoate, carbaryl and fenvalerate in standard solution using different extracting solvents as diluent.

Table 3.2 GC peak data of dimethoate in standard solution using different extracting solvents as diluent.

Solvent	Peak area of dimethoate (pA)		
	Standard	**Blank**	**Average**
EtOAc	252	nd	256
	262	nd	
	253	nd	
Acetone	232	nd	240
	241	nd	
	248	nd	
EtOH	188	nd	189
	193	nd	
	185	nd	
1:1:1* (mL)	191	nd	198
	203	nd	
	200	nd	

*1:1:1 = A ratio of EtOAc: Acetone: EtOH

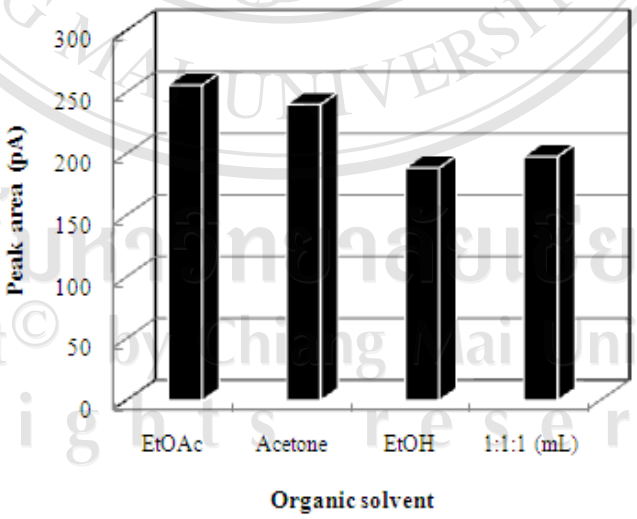

Figure 3.2 GC peak areas of dimethoate obtained in standard solution using different extracting solvents as diluent.

3.1.2 Investigation of extracting solvent using acetone as diluent for sample

The extracting solvents were investigated for its extraction efficiency of analytes using acetone as diluent. These solvents included ethyl acetate, acetone, ethanol and their mixture. The dimethoate, carbaryl and fenvalerate were analyzed by HPLC (Table 3.3 and Figure 3.3). Dimethoate was also analyzed by GC (Table 3.4 and Figure 3.4). The peak area of dimethoate, carbaryl and fenvalerate in sample were calculated by the differentiation between spiked sample and unspiked sample.

Table 3.3 HPLC peak data of dimethoate, carbaryl and fenvalerate in sample after extraction with different solvents using acetone as diluent.

Extracting solvent	Peak area of dimethoate (mAU*s)			Peak area of carbaryl (mAU*s)			Peak area of fenvalerate (mAU*s)		
	Spiked sample	Un-spiked sample	P_A*	Spiked sample	Un-spiked sample	P_A*	Spiked sample	Un-spiked sample	P_A*
EtOAc	nd	nd	3	2087	209	1903	243	nd	246
	nd	nd		2206	254		273	33	
	8	nd		2188	309		254	nd	
Acetone	21	nd	38	4164	788	3355	363	9	331
	43	nd		4161	776		326	8	
	50	nd		4095	790		333	12	
EtOH	nd	nd	0	4489	1006	3503	443	72	391
	nd	nd		4257	734		529	118	
	nd	nd		4623	1120		490	100	
1:1:1** (mL)	41	nd	28	4431	764	3720	493	109	389
	24	nd		4712	939		506	112	
	19	nd		4521	801		488	99	

*P_A = Peak area of triplicate results **1:1:1 = A ratio of EtOAc: Acetone: EtOH

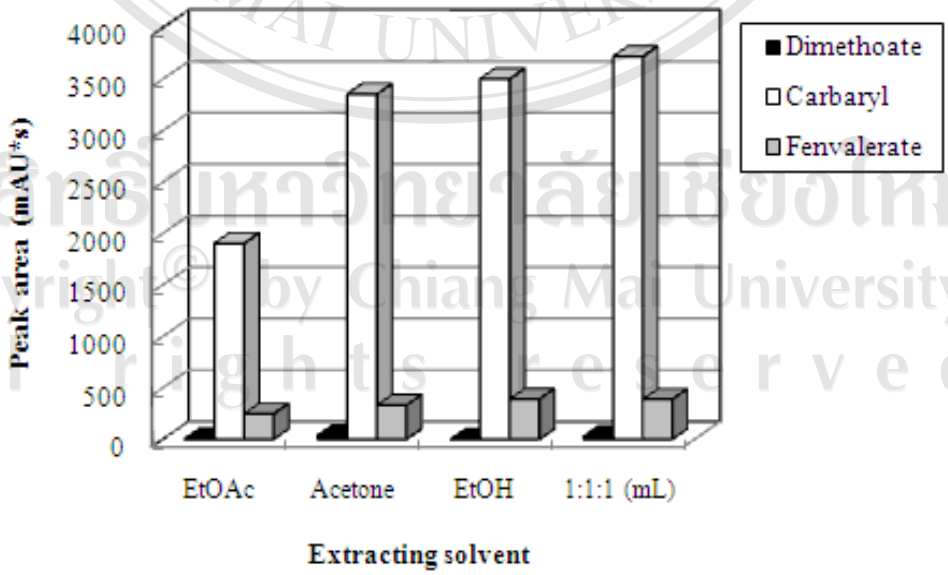

Figure 3.3 HPLC peak areas of dimethoate, carbaryl and fenvalerate in sample after extraction with different solvents using acetone as diluent.

Table 3.4 GC peak data of dimethoate in sample after extraction with different solvents using acetone as diluent.

Extracting solvent	Peak area of dimethoate (pA)		
	Spiked sample	Un-spiked sample	Average
EtOAc	278	nd	280
	286	nd	
	277	nd	
Acetone	176	nd	178
	176	nd	
	182	nd	
EtOH	175	nd	177
	176	nd	
	179	nd	
1:1:1* (mL)	116	nd	116
	120	nd	
	111	nd	

*1:1:1 = A ratio of EtOAc: Acetone: EtOH

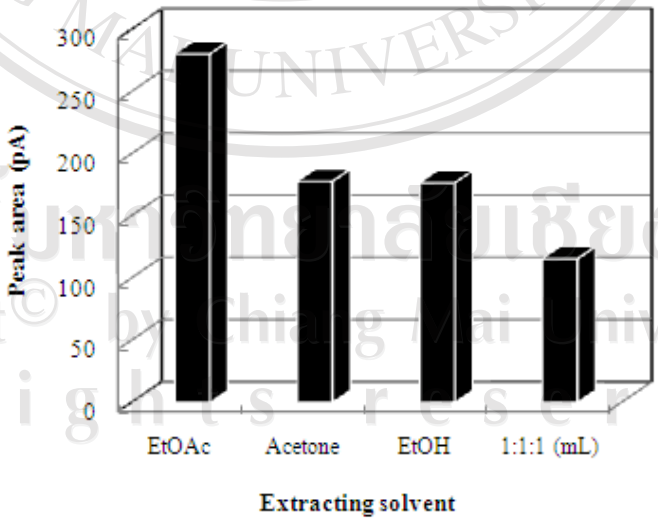

Figure 3.4 GC peak areas of dimethoate, carbaryl and fenvalerate in sample after extraction with different solvents using acetone as diluent.

It is well known that dimethoate and fenvalerate have always been detected by GC method while carbaryl has been detected by HPLC method. Since no derivatization is required for the thermalabile carbaryl in HPLC, so it was preferred over GC methods.

The type of interaction between the pesticides and the environmental (fruit, vegetable, soil, sediment, water etc.) depends on the biomass, organic matter content, pH, ionic strength capability, texture and hydrogeology [92]. Therefore the selection of extracting solvent is very important to pesticide analysis.

From the literatures review (Appendix D), the commonly extracting solvents used are dichloromethane (DCM), acetonitrile (ACN) and/or even in mixtures. As mentioned in Chapter 1, due to their environment hazard, a combination of ethyl acetate (EtOAc) and cyclohexane was employed to replace DCM [103], and since ACN has been banned by poisoning, thus acetone and ethyl acetate are considered to be used as extracting solvent. In addition P. Mayer *et al.* [104] recommended polar solvent like ethanol (EtOH) added to extraction solvent for increasing the signal detection of polar compounds. Thus organic solvents; ethyl acetate, acetone, ethanol and a mixture of ethyl acetate-acetone-ethanol (1:1:1, mL) were chosen to investigate.

From the experiment, peak areas of carbaryl and fenvalerate obtained in each organic solvent gave equivalent results except in the case of dimethoate in ethyl acetate presented the lowest in peak area due to UV cut-off point (Appendix E). In addition it can be noticed that peak areas of these pesticides in real sample are lower than in mixed standard solution because of co-extractives disturbing in HPLC and GC analysis.

In the sample extracts, the peak area of dimethoate obtained with acetone and a mixture of ethyl acetate-acetone-ethanol (1:1:1, mL) were similar whereas ethyl acetate and ethanol showed peak area lower than or approached to zero. Although ethyl acetate extract was clean, less color and less of polar matrix compounds than others but lipids and waxes were also co-extracted. Moreover ethyl acetate has less polar property; the dimethoate was not readily partition into ethyl acetate. In acetone extract both polar (dimethoate) and less polar (carbaryl and fenvalerate) pesticides could be extracted with acetone owing to the solubility property.

Increasing results in peak area of carbaryl with ethanol is supported by Mumma *et al.* hypothesis which reported that carbaryl interacting with surfactant such as phospholipids, sulfolipids and glycolipids from the matrix thus ethanol is needed for the extraction of carbaryl from the orange peels matrix [105]. Using ethanol extracting solvent made evaporation time much longer than others. Increasing in temperature might be reduce time in evaporation step but probable decomposition of carbaryl and led to low signal in peak area. Forcing the evaporation of ethanol extract took an extended period of time and led to resulting in the loss of volatile pesticides, particularly dimethoate.

The HPLC method is valid for carbaryl and fenvalerate except in the case of dimethoate lack of sensitivity at λmax: 220 nm detection. Due to lower in signal of dimethoate and to achieve reliable results and/or ensure the existence of dimethoate, the dimethoate is also determined with gas chromatography (GC) couple with flame photometric detection (FPD) in phosphorus mode (P-mode) which is a selective detector more than HPLC. From the experiment, it was found that the dimethoate results obtained from standard solution (Figure 3.1) and sample (Figure 3.3) by HPLC

were correlated and have a similar response by GC (Figure 3.2 and 3.4). The use of ethyl acetate as extracting solvent allowed a better in peak area of dimethoate by detection with GC (Appendix F) but worse in HPLC detection because of matrix compounds also likely absorbed UV at 220 nm. The GC-FPD is selective for compounds containing phosphorus or sulfur thus most of the sample matrix did not response [106]. In addition in acetone, ethanol and a mixture of ethyl acetate-acetone-ethanol (1:1:1, mL) extract, dimethoate produced similar in peak area.

Although peak area of dimethoate in a mixture of ethyl acetate-acetone-ethanol (1:1:1, mL) seem to lower than in acetone in both of HPLC and GC detection but presenting the maximum peak area in carbaryl and fenvalerate (Figure 3.3). According to extend the polarity range for extraction of different class of pesticides thus a mixture of ethyl acetate-acetone-ethanol (1:1:1, mL) is the most suitable extracting solvent.

3.1.3 Investigation of sonication time using acetone as diluent for sample

The sonication time was varied from 0 minute to 20 minutes. From the experiment, by using HPLC detection, peak areas of dimethoate fluctuated in the range of 5 to 10 minutes and obtained constant value at 15 minutes after that the peak areas decreased (Table 3.5). In addition, those peak areas was less and no different in significant (Figure 3.5). The maximum peak area was obtained at 15 minute by using GC detection (Table 3.6, Figure 3.6 and Appendix G). Due to GC-FPD is a selective detector so the different result obtained in HPLC. Increasing results in peak area of carbaryl and fenvalerate were obtained from 0 minute to 15 minutes after that the signal had a tendency to decrease.

Sonication of the sample in the presence of solvents is much more effective. The ultrasonic disrupted the cell walls of orange peels and accelerated the washing pesticides out of the cell contents. A longer period of extraction time, the pesticide residues inside the orange peels were gradually released cause of more cells were broken in the other word the pesticide residues released more and more as time expending. In addition, raised temperature caused by mechanical energy transfer to thermal energy also can profitably enhance the mass transfer [51]. Therefore, 15 minutes was reasonable sonication time.

Table 3.5 HPLC peak data of dimethoate, carbaryl and fenvalerate in sample using different sonication time.

Sonication time (min)	Peak area of dimethoate (mAU*s)			Peak area of carbaryl (mAU*s)			Peak area of fenvalerate (mAU*s)		
	Spiked sample	Un-spiked sample	P_A*	Spiked sample	Un-spiked sample	P_A*	Spiked sample	Un-spiked sample	P_A*
0	nd	nd	nd	1980	48	1867	142	nd	145
	nd	nd		1703	46		146	nd	
	nd	nd		2071	58		148	nd	
5	110	69	52	3280	nd	3363	202	nd	216
	80	nd		3330	nd		218	nd	
	36	nd		3479	nd		229	nd	
10	72	7	39	3610	nd	3778	197	nd	222
	30	nd		3906	nd		249	nd	
	22	nd		3820	nd		221	nd	
15	21	nd	20	3994	nd	3946	220	nd	437
	28	nd		3837	nd		533	nd	
	11	nd		4006	nd		557	nd	
20	6	nd	7	3645	7	3662	265	nd	265
	10	nd		3606	9		243	nd	
	6	nd		3757	6		286	nd	

*P_A = Peak area of triplicate results

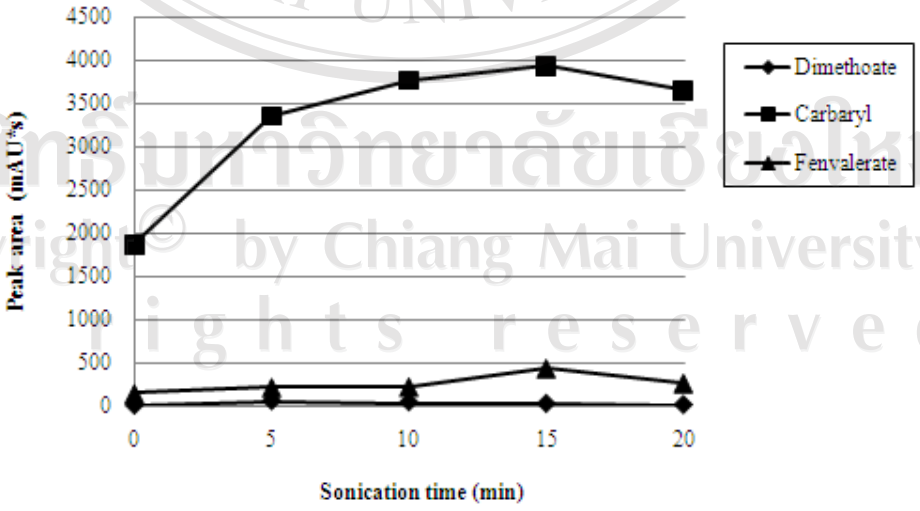

Figure 3.5 HPLC peak areas of dimethoate, carbaryl, fenvalerate in sample using different sonication time.

Table 3.6 GC peak data of dimethoate in sample using different sonication time.

Sonication time (minute)	Peak area of dimethoate (pA)			
	Spiked sample [X]	Un-spiked sample [Y]	X - Y	Average
0	215	8	207	196
	176	7	169	
	225	12	213	
5	345	17	328	333
	299	10	288	
	394	13	382	
10	444	15	428	446
	464	21	443	
	483	16	467	
15	512	33	479	466
	491	24	467	
	484	31	453	
20	51	3	48	50
	50	3	46	
	56	nd	56	

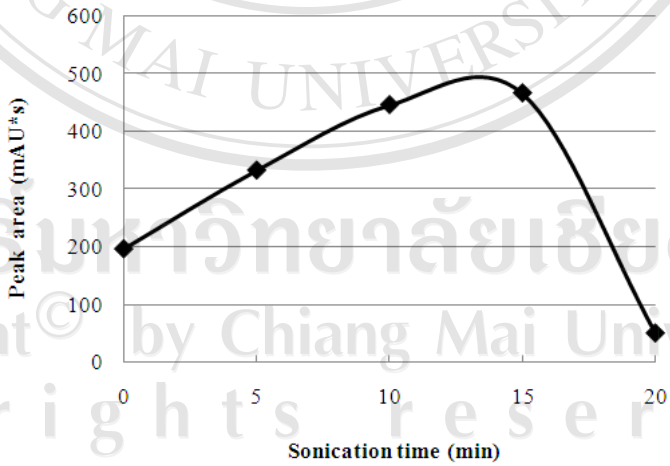

Figure 3.6 GC peak areas of dimethoate obtained in sample using different sonication time.

3.1.4 Investigation of solid phase extraction

3.1.4.1 Investigation of eluting solvent for standard solution

The eluting solvent was compared among a mixture of MeOH:H_2O (7:3, v/v), ACN:H_2O (7:3, v/v), acetone:H_2O (7:3, v/v) and ACN, respectively, using either acetone or deionized water as diluent. The dimethoate, carbaryl and fenvalerate were detected with HPLC (Tables 3.7-3.8 and Figures 3.7-3.8). The dimethoate was also detected with GC (Table 3.9 and Figure 3.9). The peak areas of dimethoate, carbaryl and fenvalerate in standard solution were calculated by the differentiation between standard solution and blank.

Table 3.7 HPLC peak data of dimethoate, carbaryl and fenvalerate in standard solution using different eluting solvent and acetone as diluent.

Eluting Solvent	Peak area of dimethoate (mAU*s)			Peak area of carbaryl (mAU*s)			Peak area of fenvalerate (mAU*s)		
	Std	Blank	P_A*	Std	Blank	P_A*	Std	Blank	P_A*
Un-adsorbed solution	45	nd	44	4149	nd	4087	7.38	nd	14
	45	nd		4100	nd		15.97	nd	
	42	nd		4012	nd		18.86	nd	
MeOH:H_2O (7:3, v/v)	12	nd	13	1151	nd	1178	6.36	nd	6
	14	nd		1187	nd		6.68	nd	
	13	nd		1196	nd		6.20	nd	
ACN:H_2O (7:3, v/v)	7	nd	8	1216	nd	1187	nd	nd	nd
	8	nd		1176	nd		nd	nd	
	9	nd		1168	nd		nd	nd	
acetone:H_2O (7:3, v/v)	11	nd	14	1189	nd	1178	319.49	nd	294
	15	nd		1142	nd		296.14	nd	
	15	nd		1202	nd		267.77	nd	
ACN	nd	nd	nd	1255	nd	1268	500.01	nd	495
	nd	nd		1265	nd		488.47	nd	
	nd	nd		1285	nd		495.48	nd	

*P_A = Peak area of triplicate results

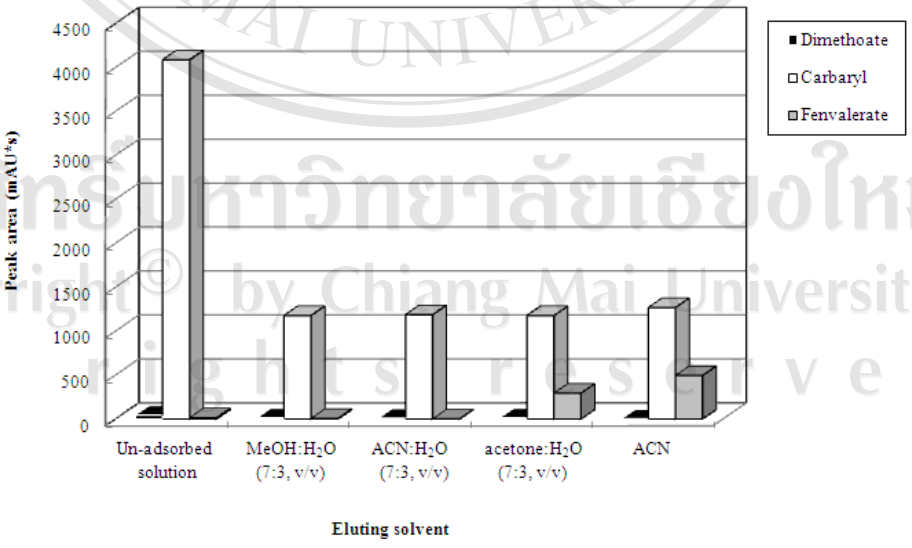

Figure 3.7 HPLC peak areas of dimethoate, carbaryl, and fenvalerate in standard solution using different eluting solvents and acetone as diluent.

Table 3.8 HPLC peak data of dimethoate, carbaryl and fenvalerate in standard solution using different eluting solvent and deionized water as diluent.

Eluting Solvent	Peak area of dimethoate (mAU*s)			Peak area of carbaryl (mAU*s)			Peak area of fenvalerate (mAU*s)		
	Std	Blank	P_A*	Std	Blank	P_A*	Std	Blank	P_A*
Un-adsorbed solution	382	nd	362	52	nd	47	13	nd	17
	369	nd		40	nd		16	nd	
	334	nd		50	nd		22	nd	
MeOH:H_2O (7:3, v/v)	85	nd	86	3379	nd	3422	30	nd	27
	92	nd		3533	nd		26	nd	
	82	nd		3352	nd		24	nd	
ACN:H_2O (7:3, v/v)	113	nd	103	3459	nd	3585	71	nd	77
	101	nd		3730	nd		77	nd	
	94	nd		3565	nd		83	nd	
acetone:H_2O (7:3, v/v)	82	nd	95	3985	nd	3804	102	nd	98
	111	nd		3737	nd		92	nd	
	92	nd		3689	nd		99	nd	
ACN	67	nd	57	3842	nd	3609	190	nd	194
	58	nd		3635	nd		177	nd	
	47	nd		3350	nd		217	nd	

*P_A = Peak area of triplicate results

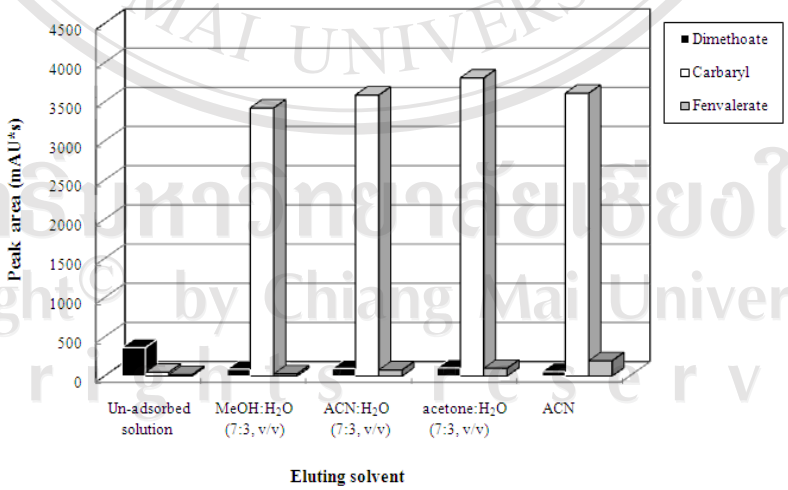

Figure 3.8 HPLC peak areas of dimethoate, carbaryl and fenvalerate in standard solution using different eluting solvent and deionized water as diluent.

Table 3.9 GC peak data of dimethoate in standard using different eluting solvent and acetone as diluent.

Eluting solvent	Peak area of dimethoate (pA)		
	Standard	Blank	Average
Un-adsorbed solution	139	nd	145
	133	nd	
	163	nd	
MeOH:H_2O (7:3, v/v)	20	nd	20
	23	nd	
	18	nd	
ACN:H_2O (7:3, v/v)	16	nd	16
	15	nd	
	18	nd	
acetone:H_2O (7:3, v/v)	36	nd	29
	29	nd	
	24	nd	
ACN	63	nd	41
	33	nd	
	28	nd	

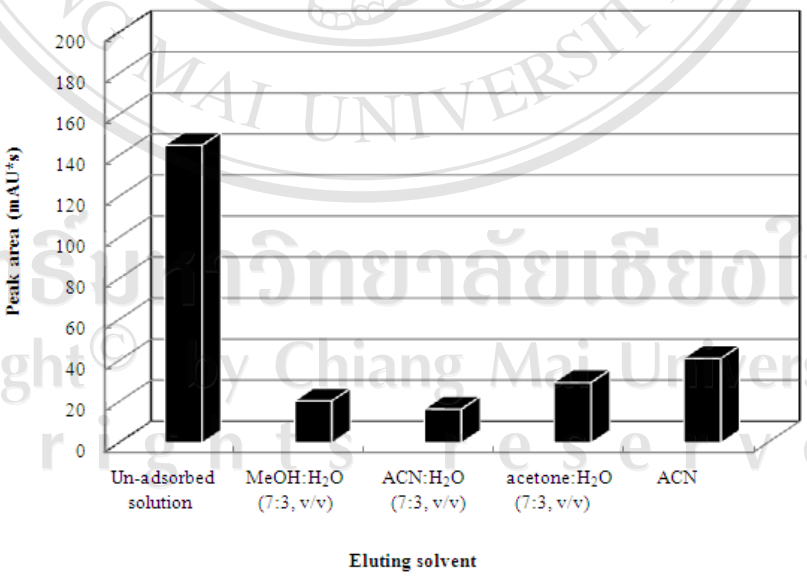

Figure 3.9 GC peak areas of dimethoate obtained in standard solution using different eluting solvent and acetone as diluent.

3.1.4.2 Investigation of eluting solvent for sample

The eluting solvent was compared among a mixture of MeOH:H_2O (7:3, v/v), ACN:H_2O (7:3, v/v), acetone:H_2O (7:3, v/v) and ACN, respectively, using either acetone or deionized water as diluent. The dimethoate, carbaryl and fenvalerate were detected with HPLC (Tables 3.10-3.11 and Figures 3.10-3.11). The dimethoate was also detected with GC (Table 3.12 and Figure 3.12). The peak area of dimethoate, carbaryl and fenvalerate in sample were calculated by the differentiation between spiked sample and unspiked sample.

Table 3.10 HPLC peak data of dimethoate, carbaryl and fenvalerate in sample using different eluting solvent and acetone as diluent.

Eluting Solvent	Peak area of dimethoate (mAU*s)			Peak area of carbaryl (mAU*s)			Peak area of fenvalerate (mAU*s)		
	Spiked sample	Un-spiked sample	P_A*	Spiked sample	Un-spiked sample	P_A*	Spiked sample	Un-spiked sample	P_A*
Un-adsorbed solution	nd	nd	nd	1484	nd	1653	nd	nd	nd
	nd	nd		1781	nd		nd	nd	
	nd	nd		1692	nd		nd	nd	
MeOH:H_2O (7:3, v/v)	nd	nd	nd	583	nd	527	nd	nd	nd
	nd	nd		536	nd		nd	nd	
	nd	nd		462	nd		nd	nd	
ACN:H_2O (7:3, v/v)	nd	nd	nd	539	nd	519	42	nd	34
	nd	nd		540	nd		33	nd	
	nd	nd		477	nd		27	nd	
acetone:H_2O (7:3, v/v)	nd	nd	nd	624	nd	552	87	nd	62
	nd	nd		477	nd		51	nd	
	nd	nd		555	nd		49	nd	
ACN	nd	nd	nd	730	nd	654	239	nd	229
	nd	nd		674	nd		237	nd	
	nd	nd		558	nd		209	nd	

*P_A = Peak area of triplicate results

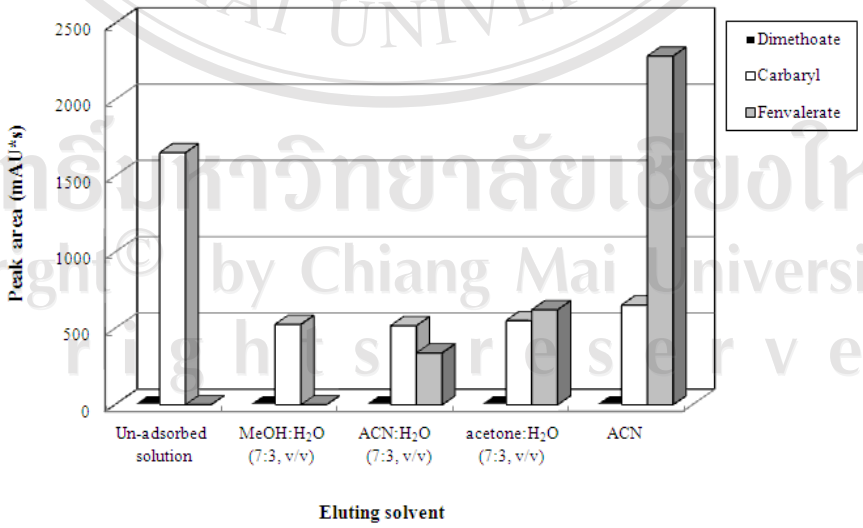

Figure 3.10 HPLC peak areas of dimethoate, carbaryl and fenvalerate in sample using different eluting solvent and acetone as diluent.

Table 3.11 HPLC peak data of dimethoate, carbaryl and fenvalerate in sample using different eluting solvent and deionized water as diluent.

Eluting Solvent	Peak area of dimethoate (mAU*s)			Peak area of carbaryl (mAU*s)			Peak area of fenvalerate (mAU*s)		
	Spiked sample	Un-spiked sample	P_A*	Spiked sample	Un-spiked sample	P_A*	Spiked sample	Un-spiked sample	P_A*
Un-adsorbed solution	11	nd	12	nd	nd	nd	nd	nd	nd
	11	nd		nd	nd		nd	nd	
	13	nd		nd	nd		nd	nd	
MeOH:H_2O (7:3, v/v)	nd	nd	nd	1489	nd	1721	nd	nd	nd
	nd	nd		1934	nd		nd	nd	
	nd	nd		1740	nd		nd	nd	
ACN:H_2O (7:3, v/v)	nd	nd	nd	1805	nd	1967	nd	nd	nd
	nd	nd		2220	nd		nd	nd	
	nd	nd		1877	nd		nd	nd	
acetone:H_2O (7:3, v/v)	nd	nd	nd	2223	nd	2085	99	nd	78
	nd	nd		1954	nd		65	nd	
	nd	nd		2191	nd		69	nd	
ACN	nd	nd	nd	1417	nd	1417	91	nd	90
	nd	nd		1340	nd		90	nd	
	nd	nd		1493	nd		88	nd	

*P_A = Peak area of triplicate results

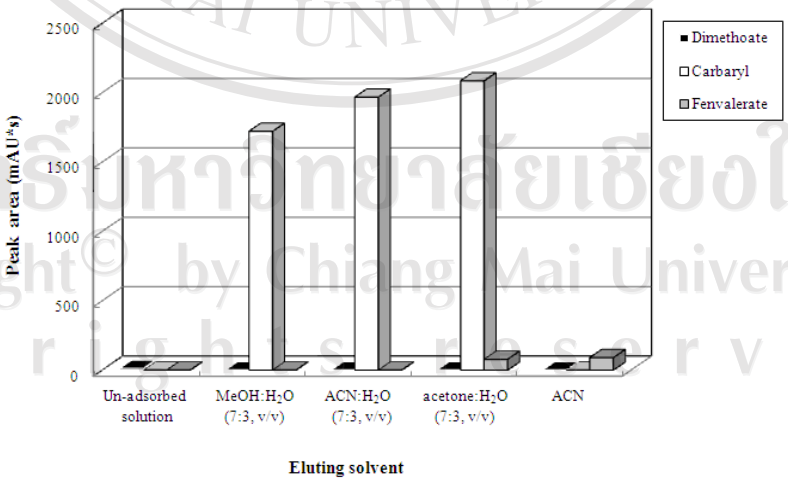

Figure 3.11 HPLC peak areas of dimethoate, carbaryl and fenvalerate in sample using different eluting solvent and deionized water as diluent.

Table 3.12 GC peak data of dimethoate in sample using different eluting solvent and acetone as diluent.

Eluting solvent	Peak area of dimethoate (pA)		
	Spiked sample	Un-spiked sample	Average
Un-adsorbed solution	27	nd	26
	30	nd	
	20	nd	
MeOH:H_2O (7:3, v/v)	11	nd	12
	13	nd	
	12	nd	
ACN:H_2O (7:3, v/v)	8	nd	8
	8	nd	
	7	nd	
acetone:H_2O (7:3, v/v)	11	nd	11
	12	nd	
	8	nd	
ACN	7	nd	8
	9	nd	
	9	nd	

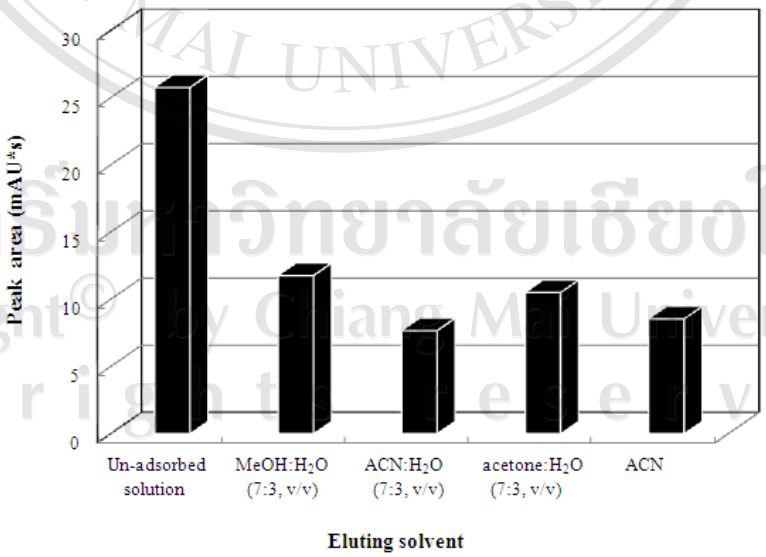

Figure 3.12 GC peak areas of dimethoate obtained in sample using different eluting solvent and acetone as diluent.

It is obvious that the direct injection of the crude extract produced unsatisfactory chromatograms, particularly dimethoate. This is because a mixture of ethyl acetate:acetone:ethanol (1:1:1, v/v) are capability to extract a wide range of compounds in orange peels including co-extractive compounds. It is not possible to analyze raw extracts by using HPLC-UV detection without clean-up step. Among the clean-up methods, SPE techniques have gained in popularity because detection limit is improved and advantage of the reduction of solvent consumption is offered with respect to classical extraction methods. Thus the additional SPE clean-up step is required in the extraction procedure in order to separate the analyte from the interfering co-extractives prior to determination by LC techniques. The criterion concerned to select the eluting solvent is ability to water miscible. Other solvents may have greater eluting power in reversed phase chromatography but are not water miscible. In this research work, the eluting solvents; MeOH:H_2O (7:3, v/v), ACN:H_2O (7:3, v/v), Acetone:H_2O (7:3, v/v) and acetonitrile were evaluated.

When acetone was used as dissolved solvent, it can be noticed that most of dimethoate and carbaryl was not retained on C18 sorbent or passed through cartridge together with redissolved solvent while as fenvalerate was retained in the cartridge. The appearing of dimethoate in unadsorbed solution was confirmed from GC results in both of standard solution and sample (Figures 3.9 and 3.12). The results obtained were different from using deionized water as dissolved solvent, which only dimethoate passed through cartridge while carbaryl and fenvalerate were retained. Caused by the favorable partition coefficient in acetone used as redissolved solvent, dimethoate likely passed

through the cartridge more than adsorbed on C18 sorbent. In addition a minimum of dimethoate was not eluted but retained by the residual silanol group or interact with the active site on C18 sorbent (Appendix H). The behavior of carbaryl is also similar to dimethoate, it expressed the maximum peak area in the filtrate or unadsorbed solution and produced equivalent signal when eluting with different organic solvents. The results obtained indicated that carbaryl was not completely retained on C18 sorbent because attractive force or van der Waals between C18 sorbent and carbaryl is less than dipolar attraction and/or hydrogen bonding between carbaryl and acetone.

The unadsorbed dimethoate in deionized water redissolution resulted from the hydrophilic structure or due to relatively polar, thus dimethoate was preferred to soluble in deionized water rather than to retain on C18 sorbent. The elution of carbaryl and fenvalerate adsorbed on C18 sorbent was maximum accomplished, due to polarity property, with a mixture of acetone:H_2O (7:3, v/v) and acetonitrile, respectively, while the co-extractives were retained by the sorbent.

Acetone has a wide range polarity to recover the pesticide from different class. G. S. Nunes *et al.* [107] presented acetone to concentrate the residue after extraction of OPPs in water with n-hexane and dichloromethane, evaporation and detection with GC-FPD technique. Therefore acetone was firstly used as dissolved solvent.

It is noteworthy that SPE clean-up step employing a solvent polarity gradient including type of dissolved solvent which is also significant in consideration. The chemical interactions between the matrix and C18 sorbent

allowed specific solvent elution of the interested pesticide [80]. Moreover to avoid many matrix which came out together with dimethoate in acetone more than in deionized water and allowed specific acetone:H_2O (7:3, v/v) and ACN elution of carbaryl and fenvalerate , thus deionized water is better choice than acetone to be used as diluent. In addition the deionized water is very compatible with reverse-phase HPLC than acetone and much more injected acetone to LC system did not recommend because it might risk C18 bleeding. Implicit acceptability that using deionized water as dissolved solvent, lower in peak area of fenvalerate is obtained.

The results obtained are related with R. J. Bushway *et al.* [88] that the C18 sorbent proved to be better for carbaryl and fenvalerate retention than dimethoate due to their hydrophobic characteristics which provided high affinity for either less polar or non-polar compounds. For polar pesticide, dimethoate, C18 sorbent is not necessarily the best choice. Lesage [108] and Chaput [109] obtained higher signal of polar pesticide on C8 than on C18 - bonded silica. This behavior may be explained by selective sorption of the polar pesticide on the free silanol groups of the silica, which are more accessible on the C8 than on C18 - bonded material.

According to the literatures review (Appendix I), the suitable wavelength was set at 220 nm which all of pesticides were detected by UV detection and the total analysis time was extended to 90 min because the target pesticides were belonging to different chemical classes (Figure 3.13). Although it seem to longer analysis time used but in many instance this time was needed to prevent interference. From this experiment, it was observed that dimethoate, carbaryl and fenvalerate in HPLC chromatogram which obtained before (Figures 3.14 – 3.15) and after SPE clean-up step (Figures 3.16 – 3.17) were still not well separated from the peak of co-extractive originating from the matrix such as pigments (e.g. chlorophyll), waxes, lipids etc. The presence of interferences appeared at a closer retention time of those target pesticides and disturbed signal in peak areas which might be leading to obtained higher peak area and/or percentage of recovery more than originated fortified in the sample. Therefore, by using HPLC detection, the extraction and clean-up conditions had to be carefully selected to achieve the highest recovery for the pesticides contained in orange peels while eliminating most of the interfering matrix components. The confirmations of dimethoate, carbaryl and fenvalerate pesticide in the sample were identified by comparison of retention time with standard solution and by using mass to charge ratios (m/z) with LC/MS technique.

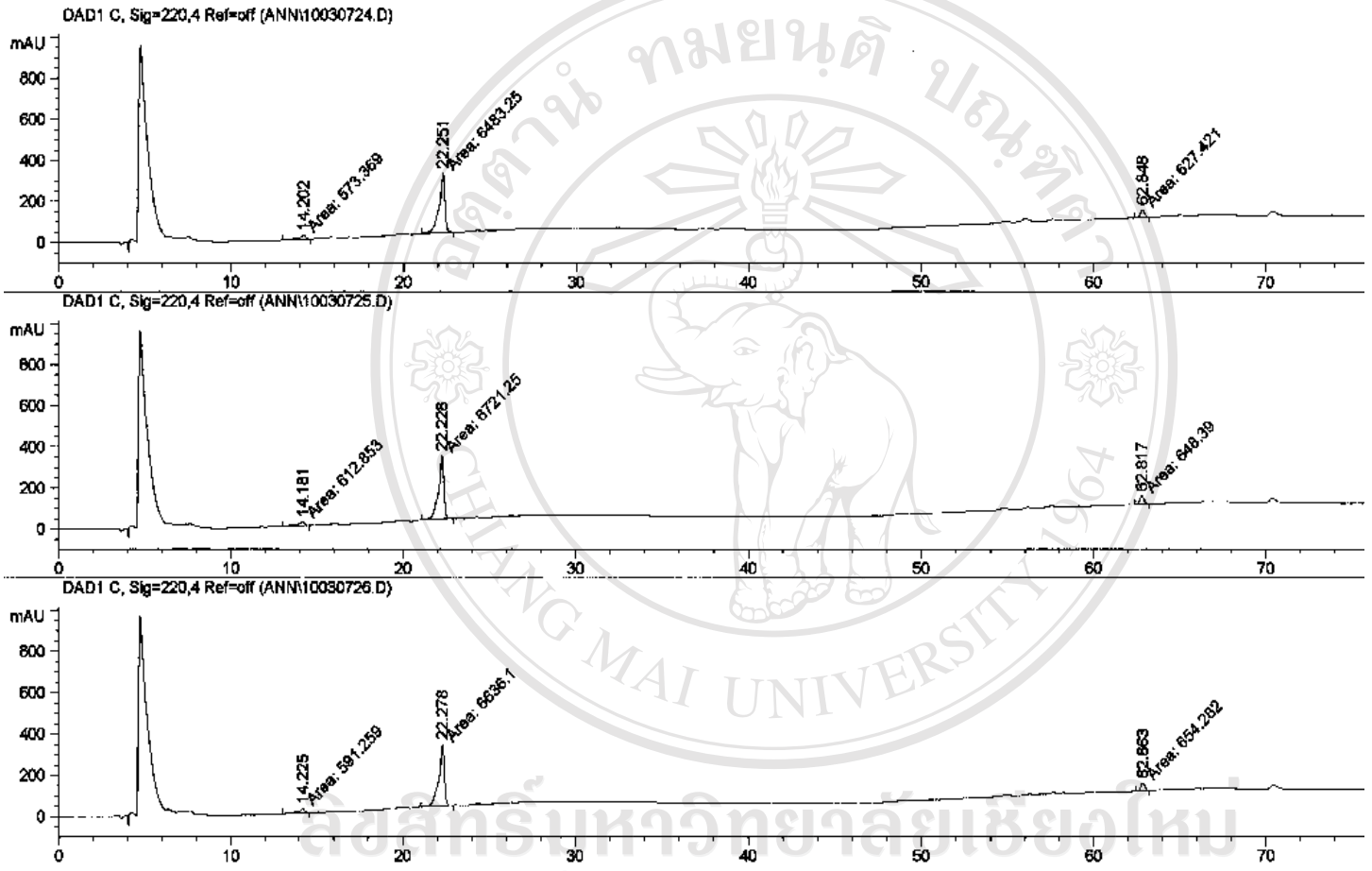

Figure 3.13 HPLC chromatograms of standard solutions of dimethoate, carbaryl and fenvalerate concentrations of 5.00, 1.50 and 2.00 mg L^{-1}, respectively. The retention time (t_R) of dimethoate, carbaryl and fenvalerate were approximately 14, 22 and 63 min, respectively.

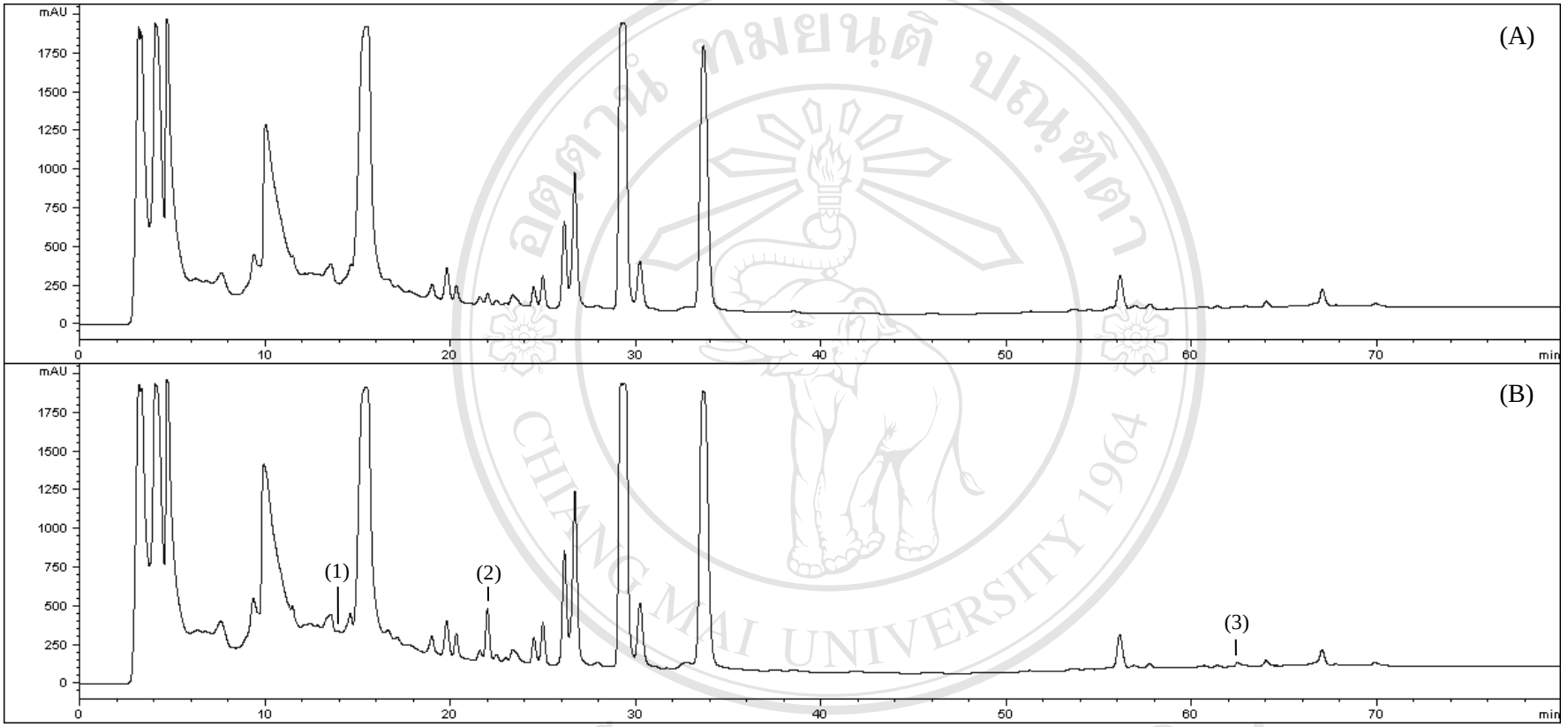

Figure 3.14 HPLC chromatograms of an orange sample peels extract before SPE clean-up step: unfortified orange peels (A) and fortified orange peels (B) with a mixture of dimethoate, carbaryl and fenvalerate at concentrations of 5.00, 1.50 and 2.00 mg L^{-1}, respectively. Peak identification: (1) dimethoate; (2) carbaryl; (3) fenvalerate.

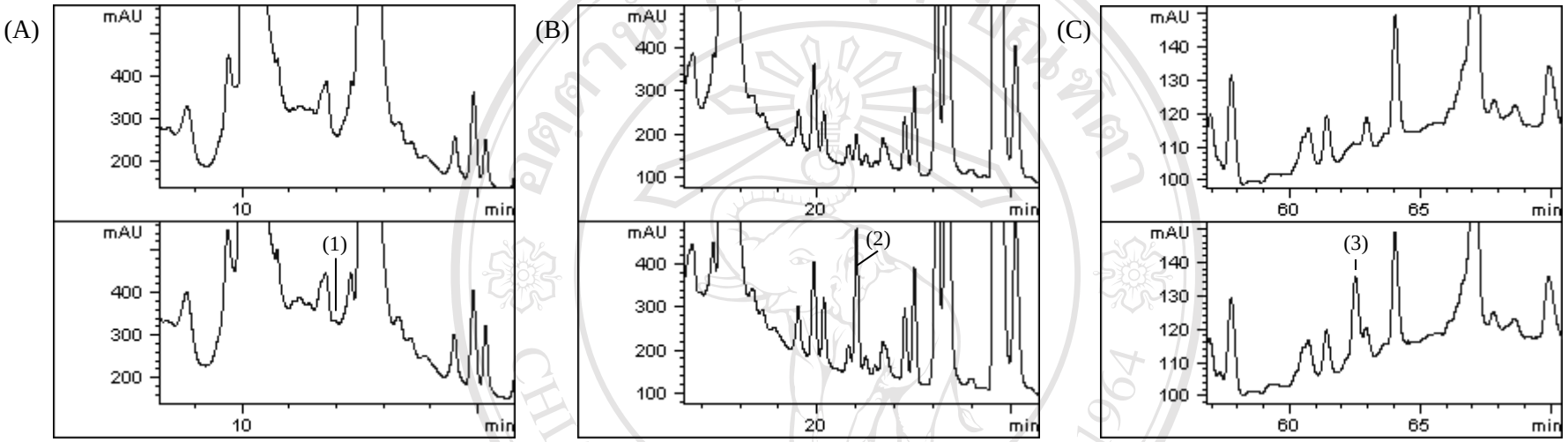

Figure 3.15 HPLC chromatograms of individual pesticide in the orange sample peels extract before SPE clean-up step: unfortified (above) and fortified orange peels (below) of (A) dimethoate, (B) carbaryl and (C) fenvalerate. Peak identification: (1) dimethoate; carbaryl; (3) fenvalerate.

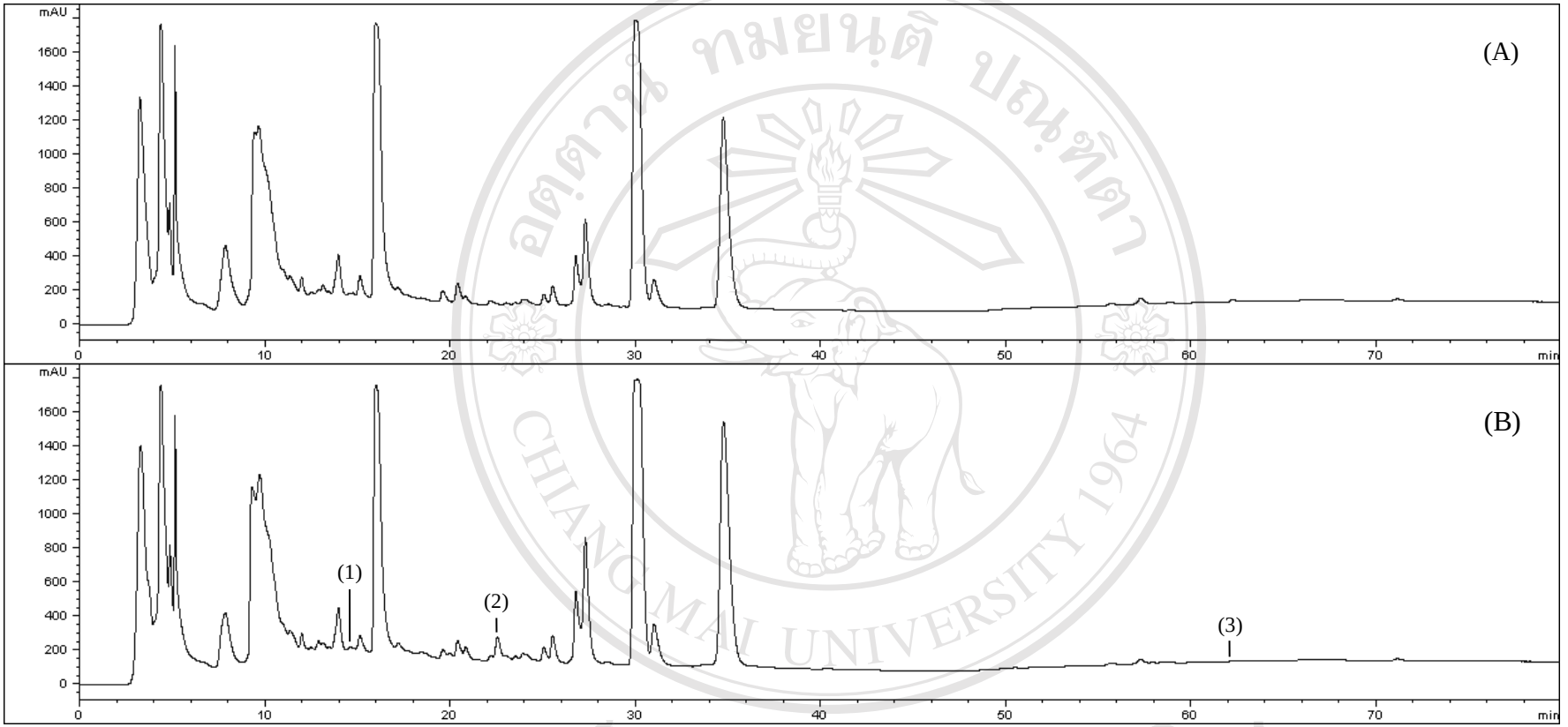

Figure 3.16 HPLC chromatograms of an orange sample peels extract after SPE clean-up step: unfortified orange peels (A) and fortified orange peels (B) with a mixture of dimethoate, carbaryl and fenvalerate at concentrations of 5.00, 1.50 and 2.00 mg L^{-1}, respectively. Peak identification: (1) dimethoate; (2) carbaryl; (3) fenvalerate.

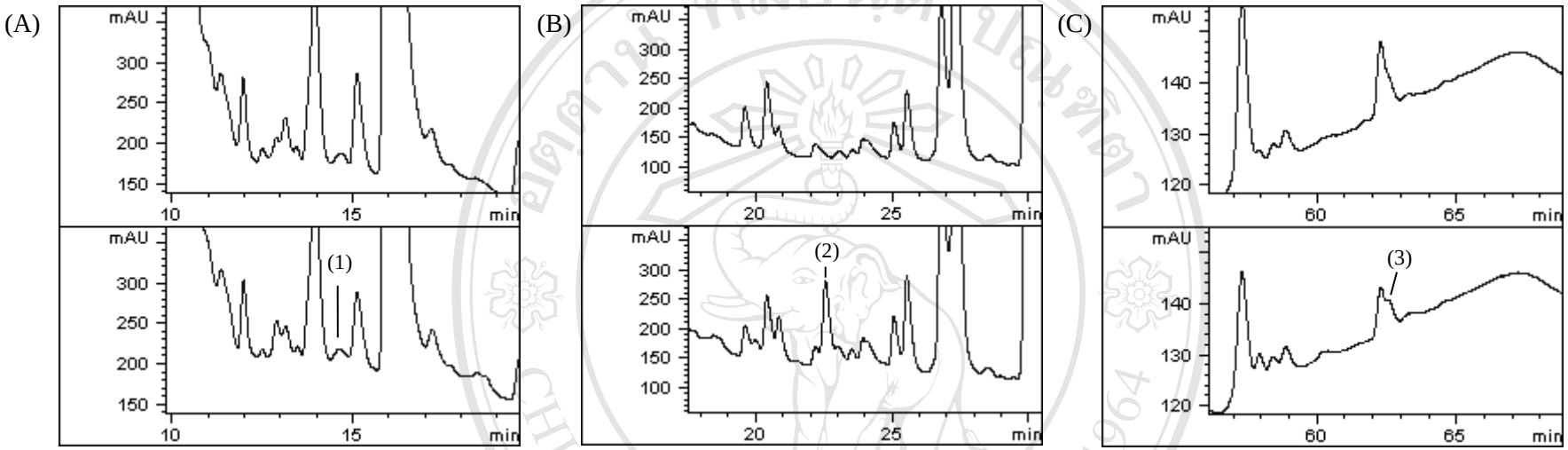

Figure 3.17 HPLC chromatograms of individual pesticide in the orange sample peels extract after SPE clean-up step: unfortified (above) and fortified orange peels (below) of (A) dimethoate, (B) carbaryl and (C) fenvalerate. Peak identification: (1) dimethoate; carbaryl; (3) fenvalerate.

3.2 Validation of HPLC method

3.2.1 Calibration curve

HPLC peak data of each pesticide at various concentrations for calibration curve are shown in Table 3.13. A graph is plotted between the peak area on the y-axis and the concentration on the x-axis (Figures 3.18 - 3.20).

Table 3.13 HPLC peak data of each pesticide at various concentrations for calibration curve.

Conc. (mg L^{-1})	Dimethoate		Conc. (mg L^{-1})	Carbaryl		Conc. (mg L^{-1})	Fenvalerate	
	P_A*	Average		P_A*	Average		P_A*	Average
0.13	5	5	0.040	125	133	0.050	7	10
	5			143			11	
	4			130			12	
0.27	26	27	0.080	511	506	0.11	66	42
	30			503			65	
	25			505			65	
0.50	67	67	0.15	529	535	0.20	42	65
	68			540			42	
	66			534			42	
0.80	130	125	0.24	790	766	0.32	79	97
	125			705			75	
	119			793			78	
1.0	150	149	0.30	1343	1033	0.40	131	131
	150			1322			129	
	148			1334			133	
2.0	165	176	0.60	2121	2238	0.80	237	234
	179			2305			229	
	184			2306			235	
3.0	230	238	0.90	3089	3250	1.2	337	339
	248			3400			343	
	235			3262			337	

*P_A = Peak area (mAU*s unit)

Table 3.13 (continued)

Conc. (mg L^{-1})	Dimethoate		Conc. (mg L^{-1})	Carbaryl		Conc. (mg L^{-1})	Fenvalerate	
	P_A*	Average		P_A*	Average		P_A*	Average
4.0	488	477	1.2	4490	4658	1.6	482	486
	475			4870			492	
	468			4614			485	

*P_A = Peak area (mAU*s unit)

From Table 3.13, the peak areas obtained were proportional to the concentration of standard solutions. Thus the calibration curves of dimethoate, carbaryl and fenvalerate were constructed in relationship between peak areas and concentrations of standard solutions in the ranges of 0.13 – 1.0, 0.24 – 1.2 and 0.20 – 1.2 mg L^{-1}, respectively (Figures 3.22 - 3.24).

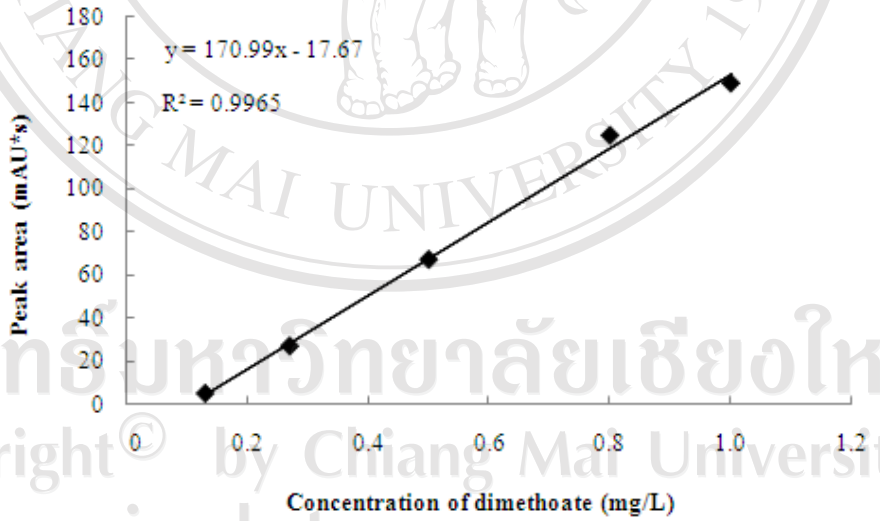

Figure 3.18 Calibration curve of dimethoate in the range of 0.13 - 1.0 mg L^{-1}.

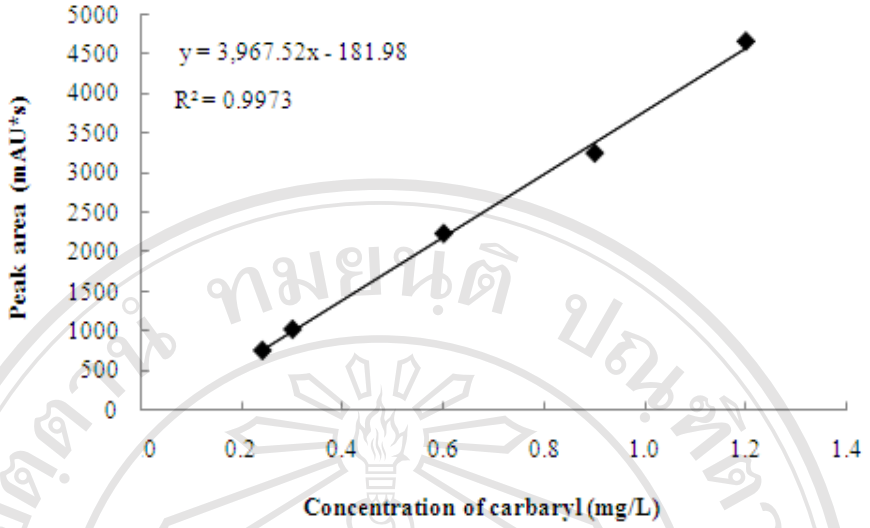

Figure 3.19 Calibration curve of carbaryl in the range of 0.24 - 1.2 mg L^{-1}.

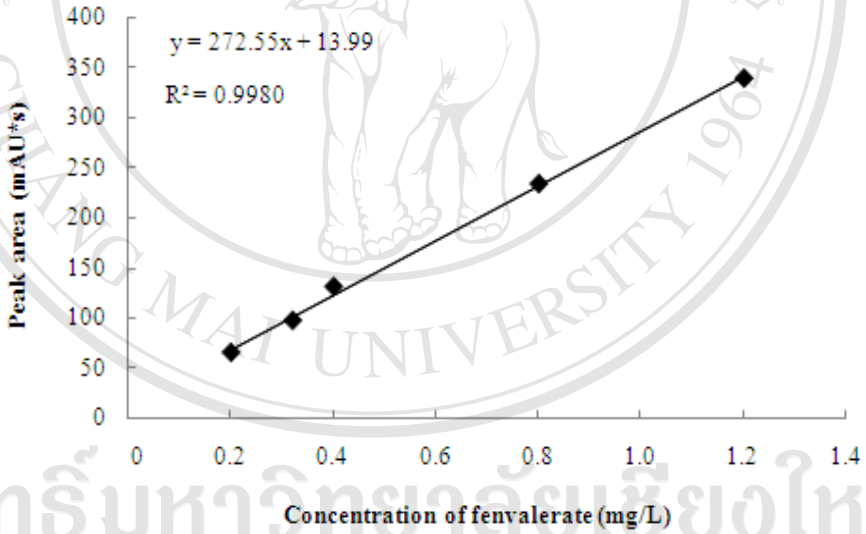

Figure 3.20 Calibration curve of fenvalerate in the range of 0.20 – 1.2 mg L^{-1}.

From Figures 3.18 - 3.20, peak area (y) and concentration (x) of each pesticide was subjected to regression analysis to calculate the linear regression equation (y = ax + b) and the correlation coefficients (R^2). The linear regression equations obtained were y = 170.99x - 17.67 with R^2 = 0.9965 for dimethoate

(Figure 3.18), y = 3967.52x - 181.98 with R^2 = 0.9973 for carbaryl (Figure 3.19) and y = 272.55x + 13.99 with R^2 = 0.9980 for fenvalerate (Figure 3.20).

3.2.2 Limit of detection

The limit of detection (LOD) was established as the lowest or minimum detectable concentration that provided the occurrence in peak area signal. Besides mentioned method, LOD was also determined by Miller - Miller method (Appendix J). The results are shown in Table 3.14.

Table 3.14 Minimum detectable concentration of each pesticide analyzed by HPLC.

Pesticide	Minimum detectable concentration (mg L^{-1})	LOD from Miller-Miller method (mg L^{-1})
Dimethoate	0.20	0.07
Carbaryl	0.0051	0.009
Fenvalerate	0.00020	0.02

From Table 3.14, LODs obtained by direct injection of minimum detectable concentration were not in agreement, even, not also in the same trend with Miller-Miller method owing to fluctuation in signal from each injection.

Chromatogram of dimethoate, carbaryl and fenvalerate pesticide at minimum detectable concentration are shown in Figures 3.21, 3.22 and 3.23, respectively.

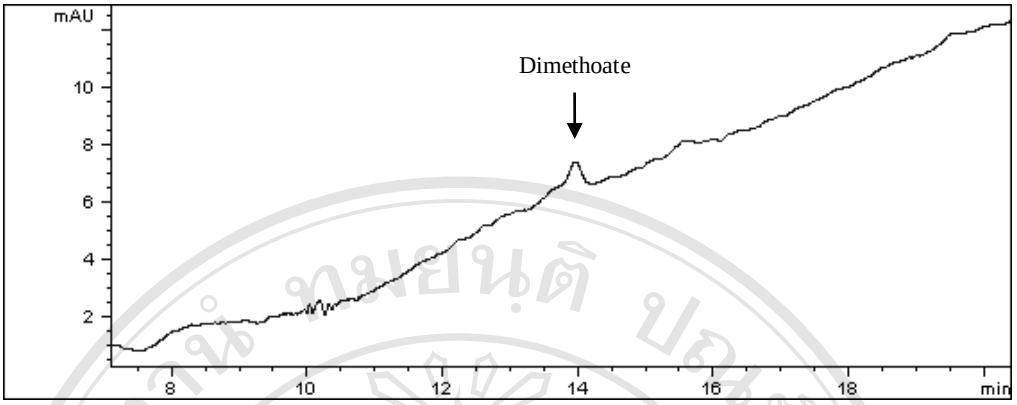

Figure 3.21 Minimum detectable concentration of dimethoate at 0.20 mg L^{-1}.

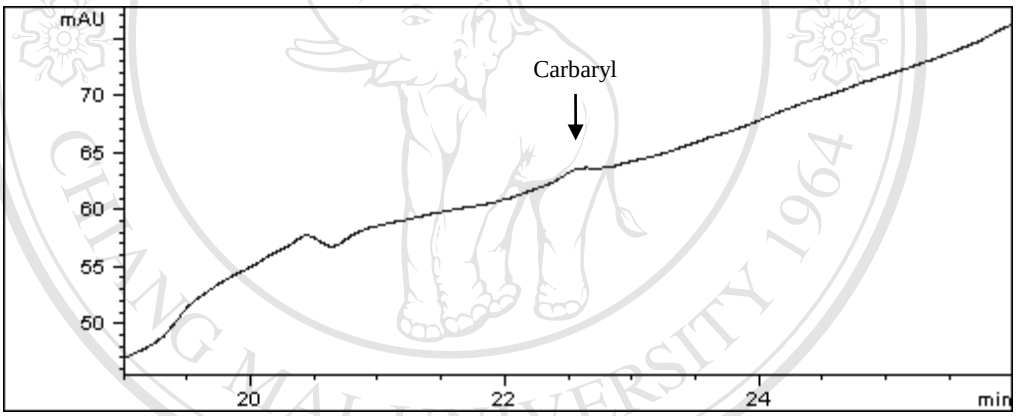

Figure 3.22 Minimum detectable concentration of carbaryl at 0.0051 mg L^{-1}.

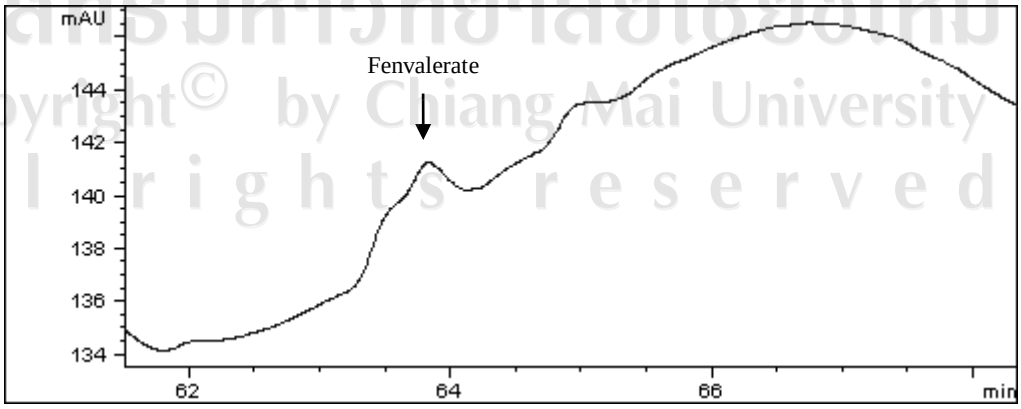

Figure 3.23 Minimum detectable concentration of fenvalerate at 0.00020 mg L^{-1}.

3.2.3 Accuracy

The accuracy was investigated in term of percentage of recovery. The equation for determination of percentage of recovery followed as:

$$\text{Percentage of recovery} = \frac{\text{Spiked sample response} - \text{Unspiked sample response}}{\text{Standard added response}} \times 100$$

Percentage of recovery was determined based on external calibration curve and the peak area obtained sample (Appendix K). The results are shown in Table 3.15.

Table 3.15 Percentages of recoveries of sample spiked with standard solution.

Pesticide	**Spiked (µg)**	**P_A of un-spiked sample (mAU*s)**	**P_A of spiked sample (mAU*s)**	**Average**	**Conc. (mg L^{-1})**	**Amount found (µg)**	**Recovery (%)**
Dimethoate	10.00	nd	98	101	0.70	2.80	28.0
		nd	107				
		nd	100				
Carbaryl	3.00	nd	1325	1442	0.41	1.64	54.7
		nd	1535				
		nd	1467				
Fenvalerate	4.00	nd	99	97	0.31	1.24	31.0
		nd	96				
		nd	96				

3.2.4 Precision

The precision was determined by injection the mixed standard solution at the concentrations of 6.00, 1.80 and 2.40 mg L^{-1} of dimethoate, carbaryl and fenvalerate, respectively, eight times in the same day (Table 3.16) and eight times for six day (Tables 3.17 - 3.22) for repeatability and reproducibility, respectively.

Table 3.16 Repeatability of retention time (min) and peak area of pesticide in standard solution analyzed by HPLC.

Run No.	Dimethoate		Carbaryl		Fenvalerate	
	t_R	P_A*	t_R	P_A*	t_R	P_A*
1	13.8	158	21.8	21956	62.5	2359
2	13.8	156	21.8	21984	62.5	2351
3	13.8	163	21.8	21853	62.5	2353
4	13.7	177	21.7	22089	62.5	2396
5	13.7	160	21.7	21739	62.5	2359
6	13.7	164	21.5	21780	62.5	2354
7	13.7	160	21.7	21861	62.5	2362
8	13.8	157	21.9	22120	62.6	2341
Average	13.8	162	21.7	21923	62.5	2359
SD	0.052	6.79	0.10	138.6	0.061	16.0
% R.S.D.	0.38	4.19	0.47	0.63	0.097	0.68

*P_A = Peak area (mAU*s unit)

From Table 3.16, average of the retention time (t_R) of dimethoate, carbaryl and fenvalerate were 13.8, 21.7 and 62.5 min, respectively. The repeatability or intra-day precision was determined on eight consecutive times (n = 8) with %R.S.D. values of retention time and peak area be in the range of 0.097 - 0.47% and 0.63 - 4.19%, respectively.

Table 3.17 Reproducibility of retention time (min) and peak area of pesticide in standard solution on the first day analyzed by HPLC.

Run No.	Dimethoate		Carbaryl		Fenvalerate	
	t_R	P_A^*	t_R	P_A^*	t_R	P_A^*
1	13.8	158	21.8	21956	62.5	2359
2	13.8	156	21.8	21984	62.5	2351
3	13.8	163	21.8	21853	62.5	2353
4	13.7	177	21.7	22089	62.5	2396
5	13.7	160	21.7	21739	62.5	2359
6	13.7	164	21.5	21780	62.5	2354
7	13.7	160	21.7	21861	62.5	2362
8	13.8	157	21.9	22120	62.6	2341
Average	13.8	162	21.7	21923	62.5	2359
SD	0.052	6.79	0.10	138.6	0.061	16.0
% R.S.D.	0.38	4.19	0.47	0.63	0.097	0.68

*P_A = Peak area (mAU*s unit)

Table 3.18 Reproducibility of retention time (min) and peak area of pesticide in standard solution on the second day analyzed by HPLC.

Run No.	Dimethoate		Carbaryl		Fenvalerate	
	t_R	P_A^*	t_R	P_A^*	t_R	P_A^*
1	13.9	170	21.9	21998	62.7	2344
2	13.9	179	21.9	22362	62.7	2396
3	13.9	168	22.0	22210	62.7	2364
4	14.0	158	22.0	22066	62.7	2368
5	13.9	174	22.0	22399	62.7	2376
6	13.8	181	21.9	22361	62.7	2397
7	13.8	161	21.8	22028	62.7	2365
8	13.9	166	21.8	22212	62.6	2353
Average	13.9	170	21.9	22205	62.7	2370
SD	0.065	8.24	0.079	160.3	0.052	18.6
% R.S.D.	0.47	4.86	0.36	0.72	0.083	0.78

*P_A = Peak area (mAU*s unit)

Table 3.19 Reproducibility of retention time (min) and peak area of pesticide in standard solution on the third day analyzed by HPLC.

Run No.	Dimethoate		Carbaryl		Fenvalerate	
	t_R	P_A*	t_R	P_A*	t_R	P_A*
1	13.9	165	21.9	22032	62.7	2372
2	13.8	159	21.8	22389	62.5	2363
3	13.9	174	21.9	22474	62.6	2404
4	13.8	181	21.8	22293	62.5	2408
5	13.7	180	21.7	22322	62.7	2404
6	13.8	169	21.8	22276	62.5	2381
7	13.8	180	21.9	22574	62.7	2408
8	13.8	181	21.8	22462	62.6	2366
Average	13.8	174	21.8	22353	62.6	2388
SD	0.047	8.51	0.069	165.0	0.063	19.8
% R.S.D.	0.34	4.90	0.32	0.74	0.10	0.83

*P_A = Peak area (mAU*s unit)

Table 3.20 Reproducibility of retention time (min) and peak area of pesticide in standard solution on the fourth day analyzed by HPLC.

Run No.	Dimethoate		Carbaryl		Fenvalerate	
	t_R	P_A*	t_R	P_A*	t_R	P_A*
1	13.7	162	21.6	22109	62.0	2372
2	13.6	175	21.5	22301	62.1	2407
3	13.6	168	21.5	22217	61.9	2401
4	13.6	166	21.5	22203	61.9	2392
5	13.6	165	21.4	22208	61.9	2394
6	13.7	159	21.7	22441	62.0	2403
7	13.6	176	21.5	22605	62.1	2444
8	13.6	159	21.5	22736	62.1	2419
Average	13.6	166	21.5	22352	62.0	2404
SD	0.056	6.73	0.073	221.1	0.086	21.1
% R.S.D.	0.41	4.05	0.34	0.99	0.14	0.88

*P_A = Peak area (mAU*s unit)

Table 3.21 Reproducibility of retention time (min) and peak area of pesticide in standard solution on the fifth day analyzed by HPLC.

Run No.	Dimethoate		Carbaryl		Fenvalerate	
	t_R	P_A*	t_R	P_A*	t_R	P_A*
1	13.6	176	21.5	22806	61.9	2465
2	13.6	165	21.4	23113	61.9	2444
3	13.7	162	21.6	23112	62.0	2439
4	13.6	172	21.6	23237	62.1	2447
5	13.6	182	21.5	23124	62.1	2477
6	13.6	177	21.6	23136	62.1	2465
7	13.7	174	21.5	22988	62.1	2460
8	13.7	179	21.5	23152	62.1	2484
Average	13.6	173	21.5	23083	62.0	2460
SD	0.049	6.93	0.057	131.2	0.11	15.8
% R.S.D.	0.36	4.00	0.27	0.57	0.17	0.64

*P_A = Peak area (mAU*s unit)

Table 3.22 Reproducibility of retention time (min) and peak area of pesticide in standard solution on the sixth day analyzed by HPLC.

Run No.	Dimethoate		Carbaryl		Fenvalerate	
	t_R	P_A*	t_R	P_A*	t_R	P_A*
1	13.6	165	21.5	22962	61.9	2455
2	13.6	179	21.5	22908	61.9	2482
3	13.6	164	21.4	23187	61.9	2462
4	13.6	164	21.4	23240	61.9	2474
5	13.6	168	21.5	23222	61.9	2470
6	13.6	171	21.4	23144	61.9	2491
7	13.7	160	21.6	23434	62.0	2495
8	13.6	178	21.5	23622	62.1	2534
Average	13.6	169	21.5	23215	61.9	2483
SD	0.055	6.91	0.069	232.6	0.078	24.8
% R.S.D.	0.40	4.10	0.32	1.00	0.13	1.00

*P_A = Peak area (mAU*s unit)

From Tables 3.17 - 3.22, %R.S.D. values of retention time and peak area are summarized Table 3.23.

Table 3.23 R.S.D. values of retention time (min) and peak area of each pesticide analyzed by HPLC.

Day	% R.S.D.					
	Dimethoate		Carbaryl		Fenvalerate	
	t_R	P_A^*	t_R	P_A^*	t_R	P_A^*
1	0.38	4.19	0.47	0.63	0.097	0.68
2	0.47	4.86	0.36	0.72	0.083	0.78
3	0.34	4.90	0.32	0.74	0.10	0.83
4	0.41	4.05	0.34	0.99	0.14	0.88
5	0.36	4.00	0.27	0.57	0.17	0.64
6	0.40	4.10	0.32	1.00	0.13	1.00

P_A^* = Peak area (mAU*s unit)

From Table 3.23, the reproducibility or the inter-day precision was determined on eight consecutive times in the six successive days (n = 8, 6 days). Average %R.S.D. values of retention time and peak area be in the range of 0.083 - 0.47% and 0.57 - 4.90%, respectively.

3.3 Investigation of extraction procedure for LC/MS method

3.3.1 Investigation of extracting solvent for sample

The extracting solvents were compared among ethyl acetate, acetone, ethanol and a mixture of ethyl acetate-acetone-ethanol (1:1:1, mL). The dimethoate, carbaryl and fenvalerate were detected with LC/MS. The peak areas of dimethoate, carbaryl and fenvalerate in sample were calculated by the differentiation between spiked sample and unspiked sample. The results are shown in Table 3.24 and Figure 3.24.

Table 3.24 LC/MS peak data of dimethoate, carbaryl and fenvalerate in sample using different extracting solvent.

Extracting solvent	Peak area of dimethoate (mAU*s)			Peak area of carbaryl (mAU*s)			Peak area of fenvalerate (mAU*s)		
	Spiked sample	Un-spiked sample	P_A*	Spiked sample	Un-spiked sample	P_A*	Spiked sample	Un-spiked sample	P_A*
EtOAc	329920	nd	350551	650544	nd	670277	47675	nd	59591
	377236	nd		711955	nd		66735	nd	
	344497	nd		648331	nd		64364	nd	
Acetone	184929	nd	179177	641150	nd	672715	139302	nd	153359
	176637	nd		697828	nd		152433	nd	
	175965	nd		679168	nd		168343	nd	
EtOH	183479	nd	164254	657091	nd	697199	122495	nd	149160
	149505	nd		741225	nd		163561	nd	
	159779	nd		693281	nd		161423	nd	
1:1:1** (mL)	151992	nd	195722	632465	nd	701884	155310	nd	163728
	228801	nd		743754	nd		174246	nd	
	206374	nd		729433	nd		161629	nd	

*P_A = Peak area of triplicate results

**1:1:1 = EtOAc: Acetone: EtOH

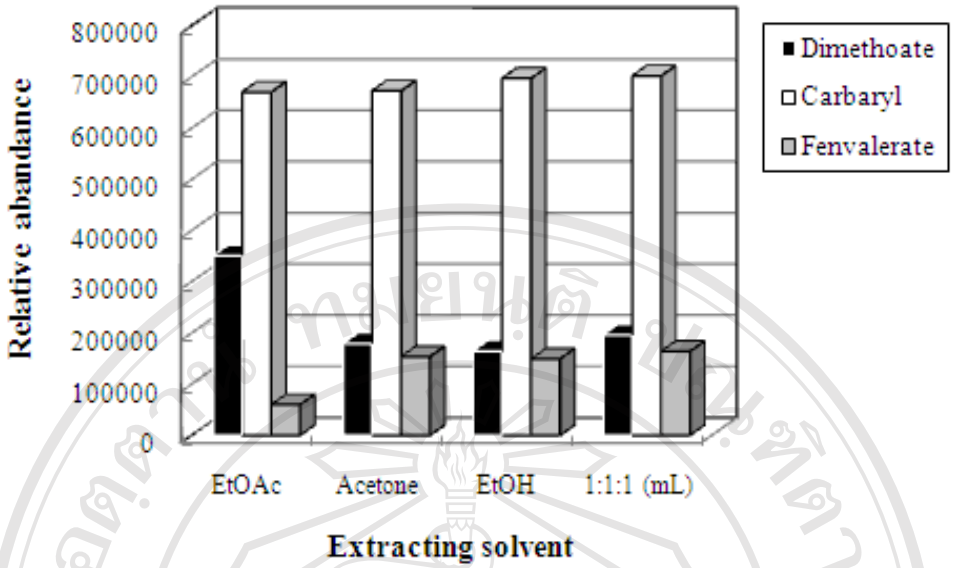

Figure 3.24 LC/MS peak areas of dimethoate, carbaryl and fenvalerate in sample using different extracting solvent.

In ethyl acetate extract, the peak area of dimethoate was higher than the others (Figure 3.24). The result obtained is opposite to HPLC results that showed the absence and lower of signal in ethyl acetate extract (Figure 3.3). As described above lipids and waxes were also co-extracted in ethyl acetate extraction thus these sample matrix influenced on ion formation processes, when a sample is introduced into MS without clean up. The occurrence of matrix presenting enhanced in signal and this effect strongly on the interface, especially electrospray ionization in positive mode (ESI+). In addition the obtained results are supported by K. Bester *et al.* [110] reported coeluting substances may cause quantification problems by compound specific suppression or enhancement. From the experiment, it was found that a mixture of ethyl acetate-acetone-ethanol (1:1:1, mL) is suitable extracting solvent to achieve simultaneous extraction of dimethoate, carbaryl and fenvalerate in sample.

3.3.2 Investigation of sonication time for sample

The sonication time was varied from 0 minute to 20 minute. The dimethoate, carbaryl and fenvalerate were detected with LC/MS (Table 3.25). The peak area of dimethoate, carbaryl and fenvalerate in sample were calculated by the differentiation between spiked sample and unspiked sample.

From the experiment, increasing results in peak area of dimethoate, carbaryl and fenvalerate were obtained from 0 minute to 15 minutes after that the peak areas decreased (Figure 3.25). The ultrasound radiation provokes molecules vibration and eases the diffusion of the solvent to the orange peels, favoring the contact between both phases. The mass transfer of pesticides from cellular orange sample to extracting solvent occurred by diffusion and/or osmosis. As described above, raised temperature caused by mechanical energy transfer to thermal energy also can profitably enhance the mass transfer. Based on the results obtained, 15 minutes was suitable sonication time for simultaneous determination of dimethoate, carbaryl and fenvalerate in orange sample peels.

Table 3.25 LC/MS peak data of dimethoate, carbaryl and fenvalerate in sample using different sonication time.

Sonication time (min)	Peak area of dimethoate (x10^6) (mAU*s)			Peak area of carbaryl (x10^6) (mAU*s)			Peak area of fenvalerate (x10^6) (mAU*s)		
	Spiked sample	Un-spiked sample	P_A*	Spiked sample	Un-spiked sample	P_A*	Spiked sample	Un-spiked sample	P_A*
0	1.414	1.107	0.260	0.889	nd	0.778	0.28	nd	0.256
	1.343	1.116		0.590	nd		0.17	nd	
	1.309	1.062		0.856	nd		0.32	nd	
5	2.434	1.976	0.780	0.988	nd	1.078	0.27	nd	0.273
	2.947	2.027		1.137	nd		0.30	nd	
	2.877	1.914		1.110	nd		0.28	nd	
10	3.017	1.994	1.081	1.011	nd	1.140	0.25	nd	0.276
	2.978	1.638		1.196	nd		0.30	nd	
	2.817	1.937		1.212	nd		0.28	nd	
15	2.226	1.517	1.126	1.101	nd	1.153	0.28	nd	0.279
	2.728	1.276		1.222	nd		0.30	nd	
	2.677	1.459		1.135	nd		0.25	nd	
20	4.091	3.406	0.568	1.142	nd	1.147	0.35	nd	0.279
	3.498	3.060		1.345	nd		0.26	nd	
	3.815	3.233		0.955	nd		0.22	nd	

*P_A = Peak area of triplicate results, calculation by (the differentiation between spiked and un-spiked sample)/3

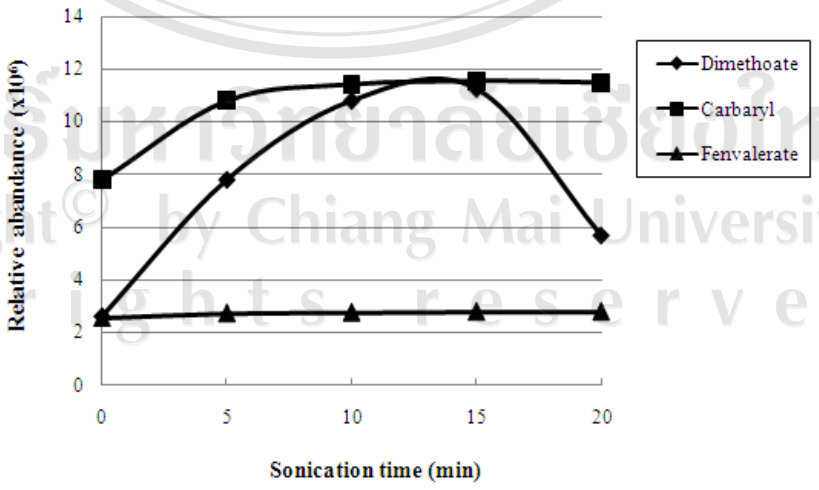

Figure 3.25 LC/MS peak areas of dimethoate, carbaryl and fenvalerate in the sample using different sonication time.

Typically either electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) is the most interface technique for residue analysis of different pesticides. From the injection of a mixture standard solution, the results demonstrated higher responses in ESI than APCI and provided higher responses in positive mode (ESI+) than negative mode. The result was supported by M. Liu *et al.* [95] presented the signal responses were 10 - 20 times higher by ESI than APCI for pesticide. Therefore, ESI+ was selected for real sample.

The mass spectrometer was operated in the positive ionization mode (ESI+) for measure pesticide residues in sample. The MS parameters such as capillary voltage, drying flow rate, nebulizer pressure, drying temperature and fragmentor voltage were optimized to provide the best possible sensitivity. From the experiment the optimum conditions are used of the capillary voltage 3.5 kV, nitrogen gas flow (N_2, 99.99% purity HP grade) at 10 L min^{-1}, nebulizer pressure 40 psi, drying gas temperature 300°C. The effect of these parameters did not affect significantly the signal of the analytes, except of the fragmentor voltage, which played an important role in both the sensitivity and fragmentation patterns. Because the fragmentor voltage provided valuable structural information or characteristic fragmentation for each pesticide making attainable the accurate mass of each characteristic fragment ion together with its elemental composition which can be used with the molecular ion for confident identification criteria [94]. As a compromise value between sensitivity for simultaneous quantitation of dimethoate, carbaryl and fenvalerate, a value of 60 V was chosen for fragmentor voltage.

In full scan mode or the total ion chromatogram (TIC) a great number of product ions were recorded across the range 50 to 1000 *m*/*z* but lack of detection sensitivity

for less concentrated residues in sample (Figure 3.26). Thus the extracted ion chromatography (EIC) is the reason why to be considerate (Figure 3.27). The m/z of each pesticide was extracted from EIC. Using the EIC, each pesticide enabled the selective and positive identification of dimethoate, carbaryl and fenvalerate by comparing the pattern of mass spectra in sample extracts with standard solutions.

In addition the MS signal for pesticides decreased by a factor of 5-10 when ACN-H_2O was compared to MeOH-H_2O [111]. This is most likely due to the fact that acetonitrile is a weaker proton donor than methanol. C. Crescenzi *et al.* [112] proposed methanol was suitable for obtaining high intensity of carbaryl since it is liable to provide hydrogen to the radical ion of carbaryl.

From the experiment, it was found that mass spectrums of the spiked sample were almost the same as the standard solution. For carbaryl, the ion used identify presented $[M+H]^+$ moreover 1-naphthol, thermal degradation of carbaryl, also demonstrated the fragmentation patterns. Therefore $[M+H]^+ = 202$ and $[M+H-57]^+ = 145$, equivalent to the molecular ion and 1-naphthol ion, respectively [7, 113] (Figure 3.28 (A)). The ion (m/z) used for dimethoate identify presented in the molecular ion or the protonated form of the molecule $[M+H]^+$. The ion used identify presented $[M+H]^+ = 230$ and $[M+H-31]^+ = 199$, equivalent to the molecular ion and $[M+H-31]^+$ ion, respectively (Figure 3.28 (B)).

It could be noticed that mass spectrum of fenvalerate was observed in the formation of strong ammonium adduct $[M+NH_4]^+$ signal. The presence of the $[M+NH_4]^+$ showed as base peak in sample and did not show the molecular ion $[M+H]^+$ [17] (Figure 3.28 (C)). According to fenvalerate containing chlorine atom in structure it can be seen the characteristic of chlorine isotopic pattern between Cl^{35}:Cl^{37}

in a ratio of 3:1 in height unit of $[M+NH_4]^+$ and $[M+H]^+$ in sample and standard solution, respectively.

Furthermore in advantage of using LC/MS, the presence of the co-extractives in the electrosprayed solution derived from the sample did not interfere significantly with the ionization process of the target analytes.

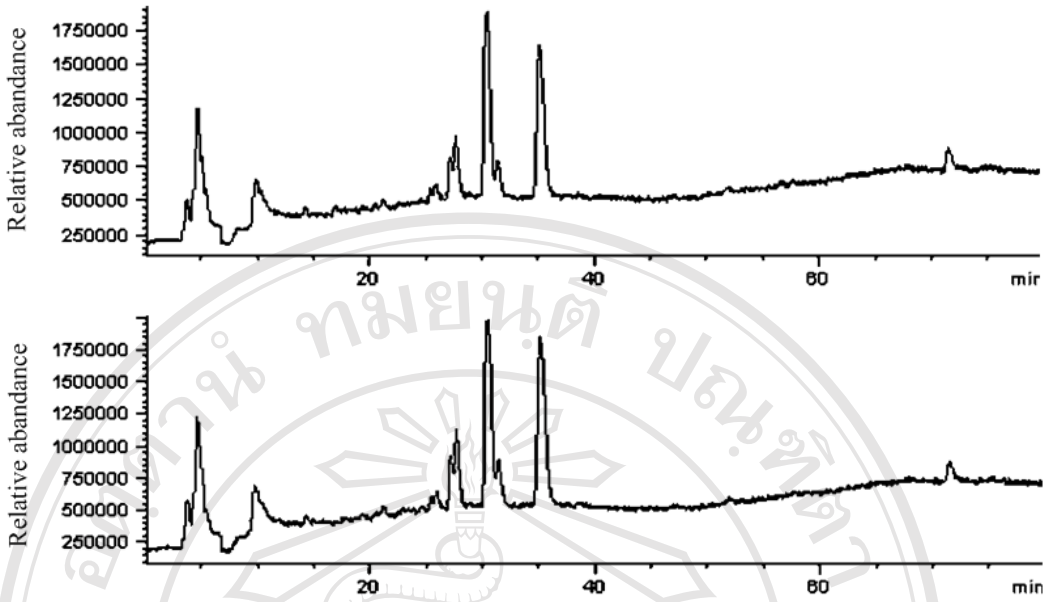

Figure 3.26 The total ion chromatogram (TIC) of an extract of an orange sample peels after SPE clean-up step. Unfortified (A) and fortified orange peels (B) with a mixture of dimethoate, carbaryl and fenvalerate at 5.00, 1.50 and 2.00 mg L^{-1}, respectively.

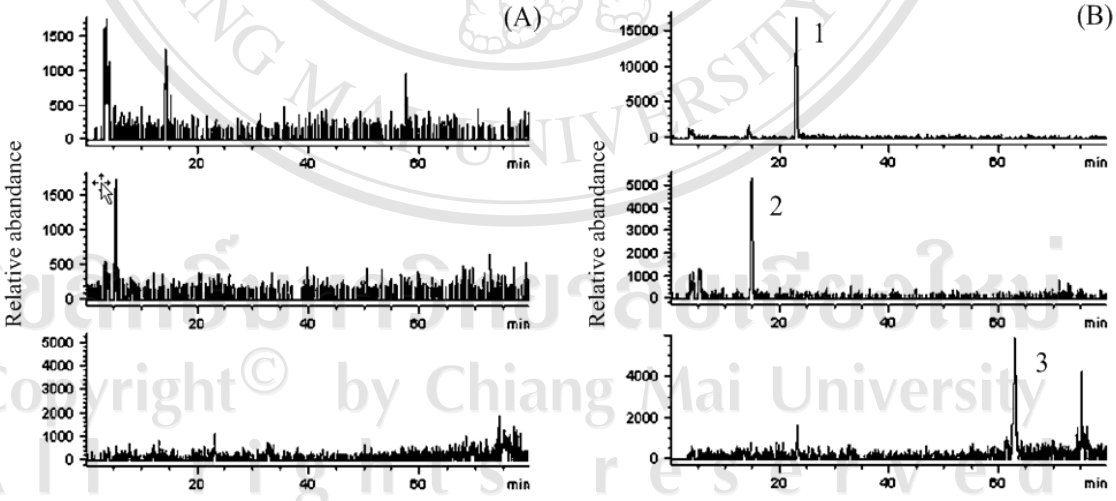

Figure 3.27 The extract ion chromatogram (EIC) of an extract of an orange sample peels after SPE clean-up step. Unfortified (A) and fortified orange peels (B) with a mixture of dimethoate, carbaryl and fenvalerate at 5.00, 1.50 and 2.00 mg L^{-1}, respectively. Peak identification: (1) carbaryl; (2) dimethoate; (3) fenvalerate.

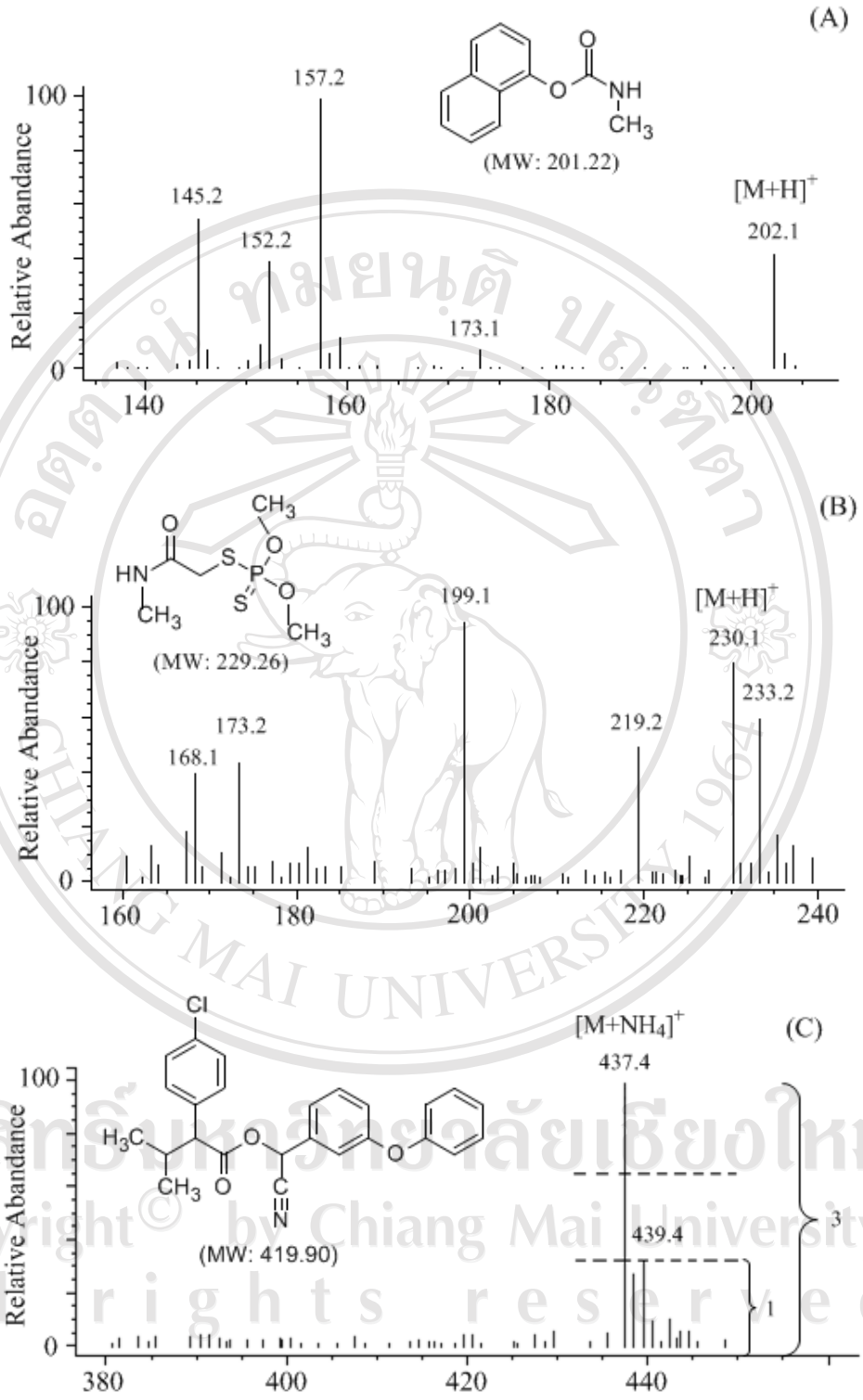

Figure 3.28 The mass spectras of (A) carbaryl (B) dimethoate and (C) fenvalerate. Assignment; $[M+H]^+$ = molecular ion and $[M+NH_4]^+$ = ammonium adducted ion with the chlorine isotopic pattern (Cl^{35}:Cl^{37} = 3:1).

3.4 Validation of LC/MS method

3.4.1 Calibration curve

The calibration curve is a linear range which obtains results directly proportional to the concentration of each analyte (Table 3.26). A graph is plotted between the peak area on the y-axis and the concentration on the x-axis.

Table 3.26 LC/MS peak data of each pesticide at various concentrations on calibration curve.

Conc. (mg L^{-1})	Dimethoate		Conc. (mg L^{-1})	Carbaryl		Conc. (mg L^{-1})	Fenvalerate	
	P_A*	Average		P_A*	Average		P_A*	Average
0.50	15304	15877	0.040	11567	11401	0.20	14066	13651
	15676			11404			13974	
	16652			11231			12915	
1.0	86081	87850	0.30	216584	204460	0.40	44896	47530
	91754			221136			57441	
	85715			175660			40254	
2.0	172930	163879	0.60	409587	397450	0.80	76432	81895
	176337			388475			86007	
	142369			394288			83247	
3.0	223005	228585	0.90	572993	581094	1.2	116902	122747
	232089			591883			130582	
	230661			578406			120757	
4.0	326107	306221	1.2	792071	812883	1.6	185107	160538
	296395			838462			168641	
	296161			808116			127867	
5.0	373495	373925	1.5	983329	987279	2.0	197235	199255
	375451			988978			212258	
	372828			989531			188272	

* P_A = Peak area (relative abundance unit)

Table 3.26 (continued)

Conc. (mg L^{-1})	Dimethoate		Conc. (mg L^{-1})	Carbaryl		Conc. (mg L^{-1})	Fenvalerate	
	P_A*	Average		P_A*	Average		P_A*	Average
6.0	434263 525711 360764	440246	1.8	1085277 1367490 1155008	1202592	2.4	222000 265631 242467	243366
7.0	610071 644369 512759	589066	2.1	1583190 1570290 1583730	1570290	2.8	336850 326478 325047	289458

*P_A = Peak area (relative abundance unit)

From data in Table 3.26, the peak areas were obtained proportional to the concentration of standard solutions. Thus the calibration curve of dimethoate, carbaryl and fenvalerate were constructed in a relationship between peak areas and concentration of standard solutions in the range of 1.0 – 5.0, 0.30 – 1.50 and 1.2 – 2.8 mg L^{-1}, respectively (Figures 3.29 – 3.31).

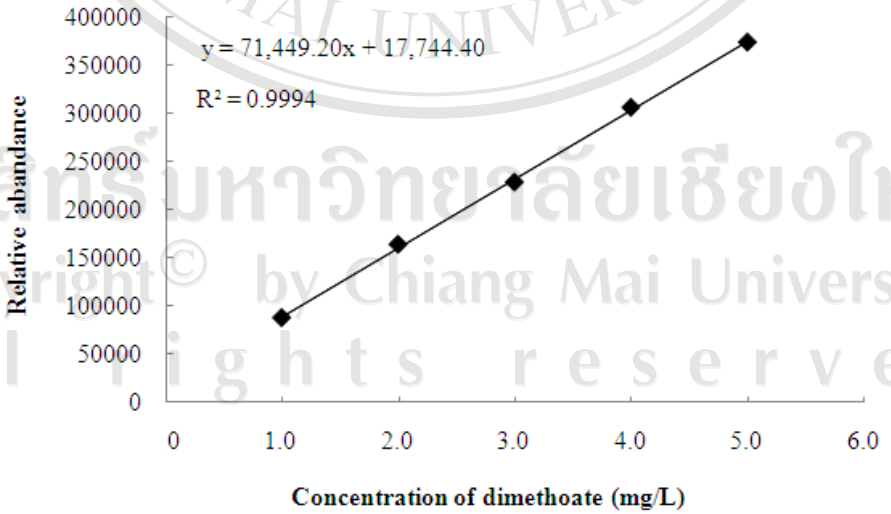

Figure 3.29 Calibration curve of dimethoate in the range of 1.0 – 5.0 mg L^{-1}.

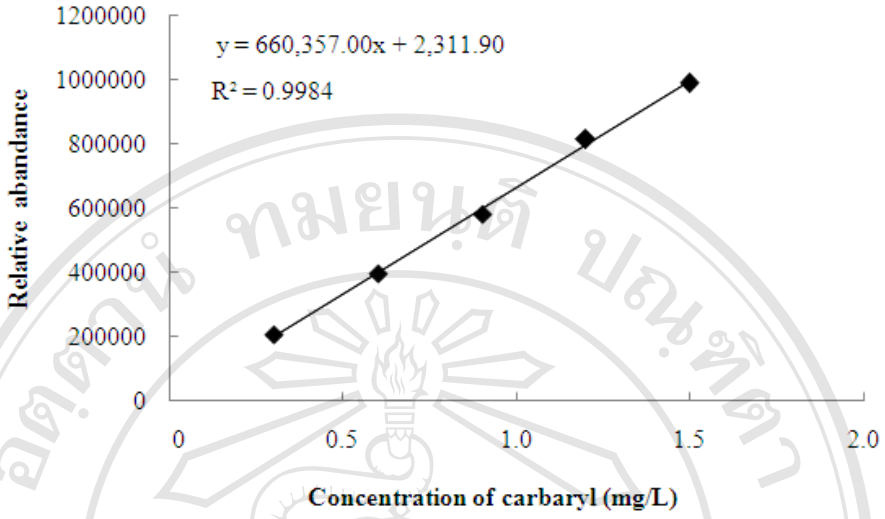

Figure 3.30 Calibration curve of carbaryl in the range of 0.30 – 1.5 mg L^{-1}.

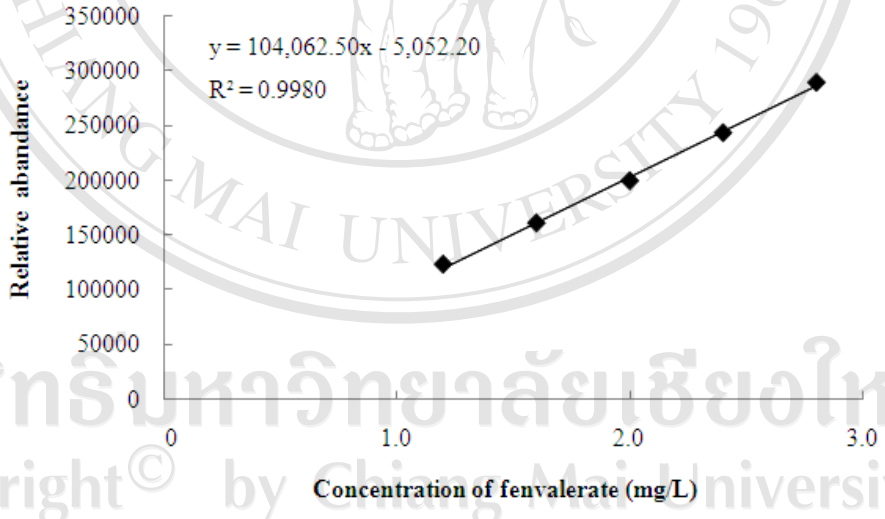

Figure 3.31 Calibration curve of fenvalerate in the range of 1.2 – 2.8 mg L^{-1}.

From Figures 3.29 - 3.31, peak area (y) and concentration (x) of each pesticide was subjected to regression analysis to calculate the linear regression equation (y = ax + b) and the correlation coefficients (R^2). The linear regression

equations obtained were y = 71,449.20x + 17,744.40 with R^2 = 0.9994 for dimethoate (Figure 3.29), y = 660,357.00x + 2311.90 with R^2 = 0.9984 for carbaryl (Figure 3.30) and y = 104,062.50x - 5,052.20 with R^2 = 0.9980 for fenvalerate (Figure 3.31).

3.4.2 Limit of detection

Limit of Detection (LOD) was established at a signal to noise ratio (S/N) of 3. LOD was experimentally verified by three injections of dimethoate, carbaryl and fenvalerate at the LOD concentration. Besides mentioned method, LOD was also determined by Miller - Miller method. The results are shown in Table 3.27.

Table 3.27 LOD of dimethoate, carbaryl and fenvalerate analyzed by LC/MS.

Pesticide	**Minimum detectable concentration (mg L^{-1})**	**LOD from Miller-Miller method (mg L^{-1})**
Dimethoate	0.50	0.001
Carbaryl	0.030	0.0003
Fenvalerate	0.20	0.002

From Table 3.27, the results obtained by both methods were not in agreement which the experimental LODs were higher than the theoretical LODs with Miller-Miller method owing to fluctuation in signal from each injection. The EICs of dimethoate, carbaryl and fenvalerate of LOD concentration are shown in Figures 3.32, 3.33 and 3.34, respectively.

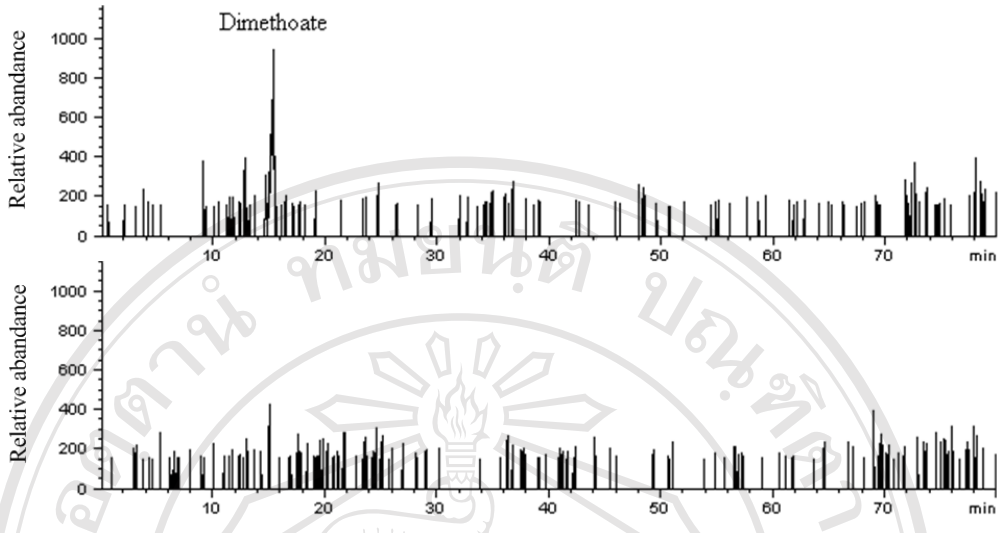

Figure 3.32 A signal of dimethoate to noise ratio (S/N) of 3.

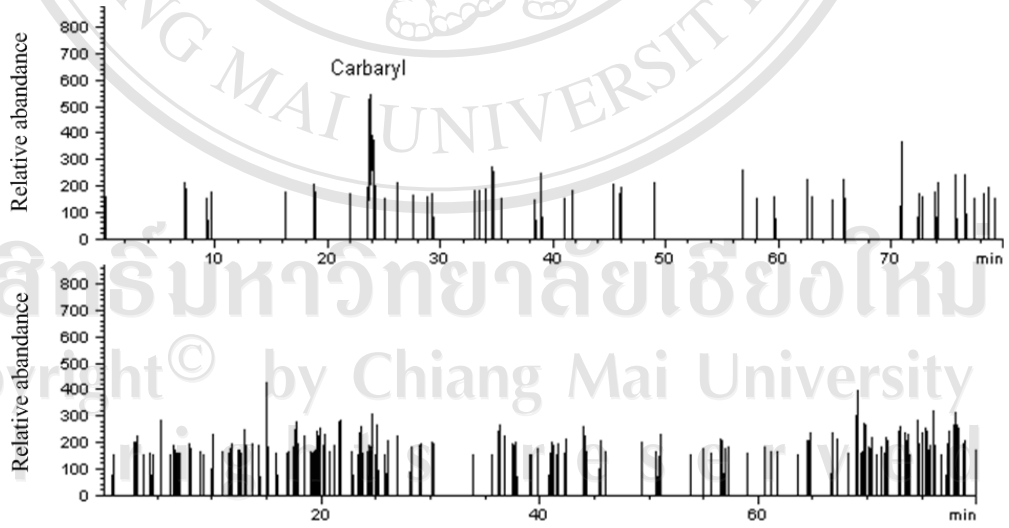

Figure 3.33 A signal of carbaryl to noise ratio (S/N) of 3.

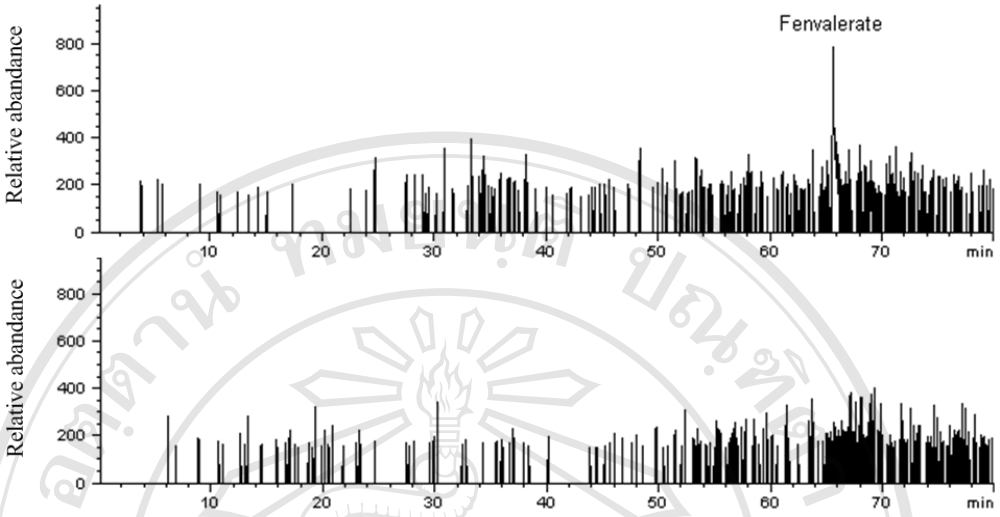

Figure 3.34 A signal of fenvalerate to noise ratio (S/N) of 3.

3.4.3 Accuracy

The accuracy was investigated in term of percentage of recovery. The equation for determination of percentage of recovery followed as:

$$\text{Percentage of recovery} = \frac{\text{Spiked sample response} - \text{Unspiked sample response}}{\text{Standard added response}} \times 100$$

Percentage of recovery was determined based on external calibration curve and the peak area obtained sample (Appendix L). The results are shown in Table 3.28.

Table 3.28 Percentages of recoveries obtained sample spiked with standard solution analyzed by LC/MS.

Pesticide	Spiked (µg)	P_A^* of un-spiked sample	P_A^* of spiked sample	Average	Conc. (mg L^{-1})	Amount found (µg)	Recovery (%)
Dimethoate	5.00	nd	254373	264472	3.45	3.45	69
		nd	278344				
		nd	260700				
Carbaryl	1.50	nd	729658	705229	1.06	1.06	71
		nd	707280				
		nd	678750				
Fenvalerate	2.00	nd	226182	212603	2.09	2.09	105
		nd	210881				
		nd	200745				

*P_A = Peak area (relative abundance unit)

3.4.4 Precision

The precision was determined by injection mixed standard solution at the concentration level 6.00, 1.80 and 2.40 mg L^{-1} of dimethoate, carbaryl and fenvalerate, respectively, eight times in the same day (Table 3.29) and eight times for two day (Tables 3.30 - 3.31) for repeatability and reproducibility, respectively.

Table 3.29 Repeatability of retention time (min) and peak area of pesticide in standard solution analyzed by LC/MS.

Run No.	Dimethoate		Carbaryl		Fenvalerate	
	t_R	P_A^* (x10^6)	t_R	P_A^* (x10^6)	t_R	P_A^* (x10^6)
1	14.6	2.22	22.8	4.93	63.8	0.99
2	14.5	2.21	22.7	5.03	63.7	0.99
3	14.6	2.18	22.7	5.05	63.7	0.95
4	14.6	2.38	22.7	5.47	63.7	1.07
5	14.5	2.39	22.7	5.22	63.6	0.95
6	14.5	2.31	22.6	5.00	63.6	0.97
7	14.5	2.29	22.6	5.38	63.6	1.05
8	14.5	2.22	22.6	5.39	63.6	1.04
Average	14.5	2.28	22.7	5.18	63.7	1.00
SD	0.049	0.080	0.070	0.21	0.086	0.046
% R.S.D.	0.34	3.52	0.31	4.02	0.14	4.60

*P_A = Peak area (relative abundance unit)

From Table 3.29, average of the retention time (t_R) of dimethoate, carbaryl and fenvalerate were 14.52, 22.67 and 63.66, respectively. The repeatability or intra-day precision was determined on eight consecutive times (n = 8) with R.S.D.

values of retention time and peak area were found to be in the ranges of 0.14 - 0.34% and 3.52 - 4.60%, respectively.

Table 3.30 Reproducibility of retention time (min) and peak area of pesticide in standard solution on the first day analyzed by LC/MS.

Run No.	Dimethoate		Carbaryl		Fenvalerate	
	t_R	P_A^* (x10^6)	t_R	P_A^* (x10^6)	t_R	P_A^* (x10^6)
1	14.6	2.22	22.8	4.93	63.8	0.99
2	14.5	2.21	22.7	5.03	63.7	0.99
3	14.6	2.18	22.7	5.05	63.7	0.95
4	14.6	2.38	22.7	5.47	63.7	1.07
5	14.5	2.39	22.7	5.22	63.6	0.95
6	14.5	2.31	22.6	5.00	63.6	0.97
7	14.5	2.29	22.6	5.38	63.6	1.05
8	14.5	2.22	22.6	5.39	63.6	1.04
Average	14.5	2.28	22.7	5.18	63.7	1.00
SD	0.049	0.080	0.070	0.21	0.086	0.046
% R.S.D.	0.34	3.52	0.31	4.02	0.14	4.60

*P_A = Peak area (relative abundance unit)

Table 3.31 Reproducibility of retention time (min) and peak area of pesticide in standard solution on the second day analyzed by LC/MS.

Run No.	Dimethoate		Carbaryl		Fenvalerate	
	t_R	P_A^* (x10^6)	t_R	P_A^* (x10^6)	t_R	P_A^* (x10^6)
1	14.5	2.17	22.7	5.45	63.6	0.99
2	14.5	2.20	22.6	5.45	63.6	0.95
3	14.4	2.25	22.6	5.15	63.6	0.96
4	14.4	2.24	22.6	5.03	63.5	0.96
5	14.4	2.18	22.5	5.19	63.5	0.98
6	14.4	2.17	22.6	5.25	63.5	1.00
7	14.4	2.25	22.6	5.12	63.5	0.95
8	14.4	2.20	22.6	5.24	63.5	0.99
Average	14.4	2.21	22.6	5.24	63.5	0.97
SD	0.036	0.035	0.045	0.15	0.038	0.020
% R.S.D.	0.25	1.55	0.20	2.85	0.060	2.22

*P_A = Peak area (relative abundance unit)

From Table 3.30 - 3.31, %R.S.D. values of retention time and peak area are summarized Table 3.32.

Table 3.32 R.S.D. values of retention time (min) and peak area of each pesticide analyzed by LC/MS.

Day	% R.S.D.					
	Dimethoate		Carbaryl		Fenvalerate	
	t_R	P_A^* (x10^6)	t_R	P_A^* (x10^6)	t_R	P_A^* (x10^6)
1	0.34	3.52	0.31	4.02	0.14	4.60
2	0.25	1.55	0.20	2.85	0.060	2.22

*P_A = Peak area (relative abundance unit)

From Table 3.32, the reproducibility or the inter-day precision was determined on eight consecutive times in the two successive days (n = 8, 2 days). Average R.S.D. values of retention time and peak area were found to be in the ranges of 0.060 - 0.34% and 1.55 - 4.60%, respectively.

3.5 The Comparison between HPLC and LC/MS method

The percentage of recoveries ≥ 70% were found for target pesticides by using both of HPLC and LC/MS technique. Unfortunately, the percentage of recovery as analyzed by HPLC presented less than 50% through the proposed method in the other word the amount found of target pesticides obtained were lower than originated fortified in sample due to interference disturbing and lost during extraction step. From the experiment, it can be seen LC/MS has a selectivity and sensitivity. The extracted ion chromatograms (EIC) of the spiked and un-spiked sample were very clean, no interference compounds were presented in the extract and, therefore, a clean-up step was not necessary. The results obtained were agreement with S. Jin *et al.* [114] that reported LC/MS need almost no sample pretreatment. Thus LC/MS technique is clearly preferable than HPLC for the determination of pesticides and also profitably in low concentration or trace level without interference disturbing.