

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Gas Chromatographic Conditions

The mixture of 17 standard organochlorine pesticide solutions, spiked sample and sample solutions were injected according to the GC conditions by the use of μ -ECD as a detector in Table 4.1. The chromatogram of mixed 17 standard organochlorine pesticides at 10 ng/mL, was shown in Figure 4.1.

First, my work was to optimise the GC by using GC- μ -ECD at Agilent Technologies. The column was PAS-1701 and the GC conditions were recorded as shown in Table 4.2. The PAS-1701 column could separate up to the total of 17 peaks. Later, it was found that working at the company was rather inconvenient, and the Department of Biology, Chulalongkorn University, allowed postgraduates to run their experiments at the department by using HP-5. Therefore, all of my subsequent experiments were conducted on campus. For HP-5, the mixed 17 standard organochlorine pesticide solutions in hexane 10 ng/mL were also separated by GC conditions as shown in Table 4.1. It could separate 16 peaks of pesticides from 17 organochlorine pesticides because dieldrin and P,P'-DDE had the same retention time, and couldn't be separately applied. The GC conditions in Table 4.2 could be used to separate all of the mixed 17 standard organochlorine pesticide solutions because the column PAS-1701 offers more selections for the analysis of organochlorine pesticides than that of HP-5.

Table 4.1 The gas chromatographic conditions using HP-5 column for the study of mixed 17 standard organochlorine pesticide solutions

GC Parameters	GC Conditions
Analytical Column	30 m x 0.32 mm x 0.25 μ m film thickness HP-5 (5% Phenyl Methyl Siloxane) capillary column
Temperature Program	50°C (1 min) rate A 25°C/min to 150°C 150°C rate B 3°C/min to 275°C
Injection Mode	Spiltless mode, purge time 0.75 min
Injection Temperature	250°C
Flow Rate of Carrier Gas (He)	1.0 mL/min
Flow Rate of Nitrogen Gas	60 mL/min
Detector	μ -Electron Capture Detector (μ -ECD)
Detector Temperature	325°C

Table 4.2 The gas chromatographic conditions using PAS-1701 column for the study of mixed 17 standard organochlorine pesticide solutions

GC Parameters	GC Conditions
Analytical Column	30 m x 0.32 m x 0.25 μ m film thickness PAS-1701 (14%-Cyanopropyl-phenyl)- methylpolysiloxane
Temperature Program	50°C (1 min) rate A 25°C/min to 150°C 150°C rate B 5°C/min to 275°C
Injection Mode	Spiltless mode, purge time 0.75 min
Injection Temperature	250°C
Flow Rate of Carrier Gas (He)	1.0 mL/min
Flow Rate of Nitrogen Gas	60 mL/min
Detector	μ -Electron Capture Detector (μ -ECD)
Detector Temperature	325°C

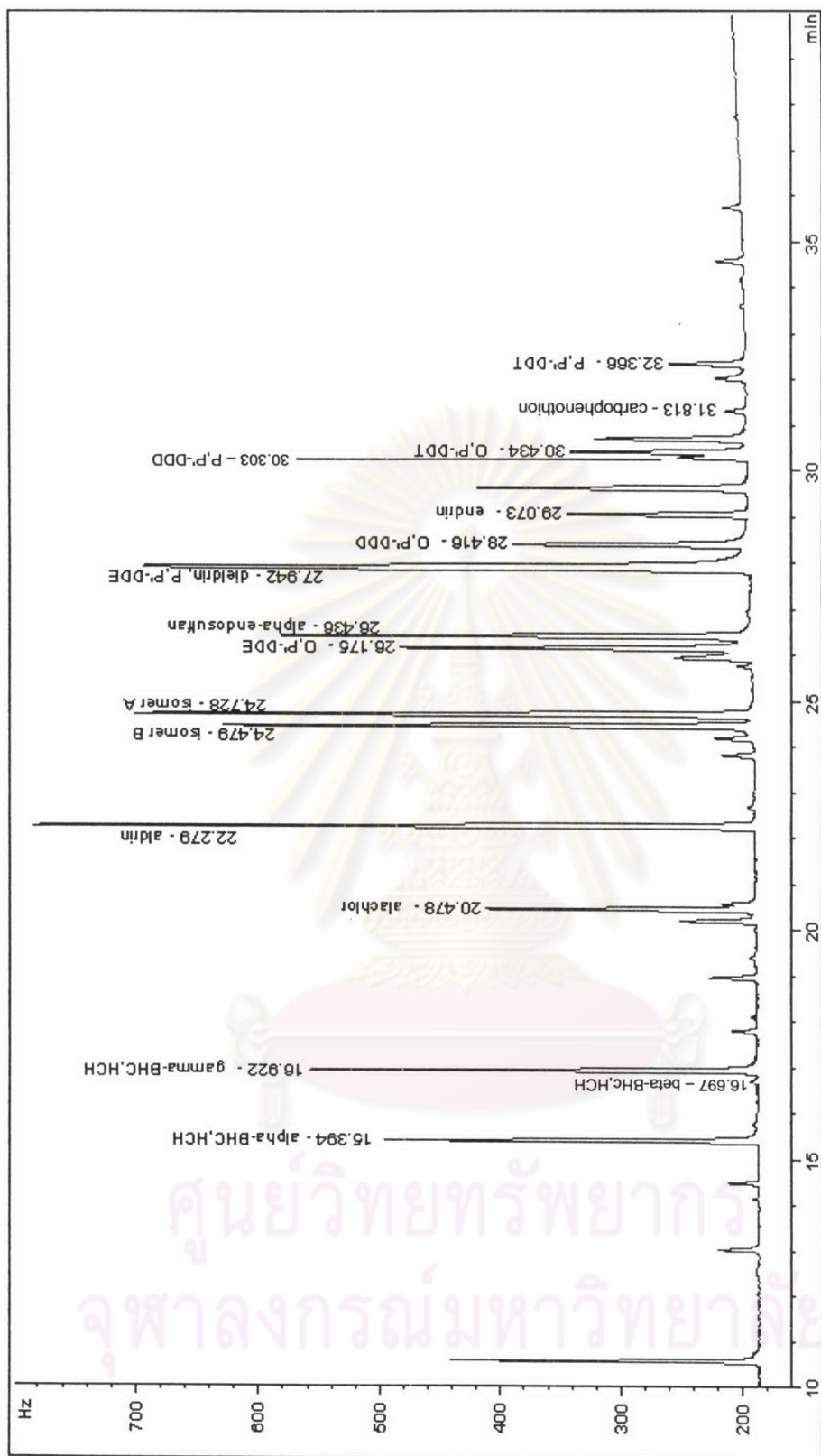


Figure 4.1 The chromatogram of mixed 17 standard organochlorine pesticides in hexane 10 ng/mL under GC conditions in Table 4.1

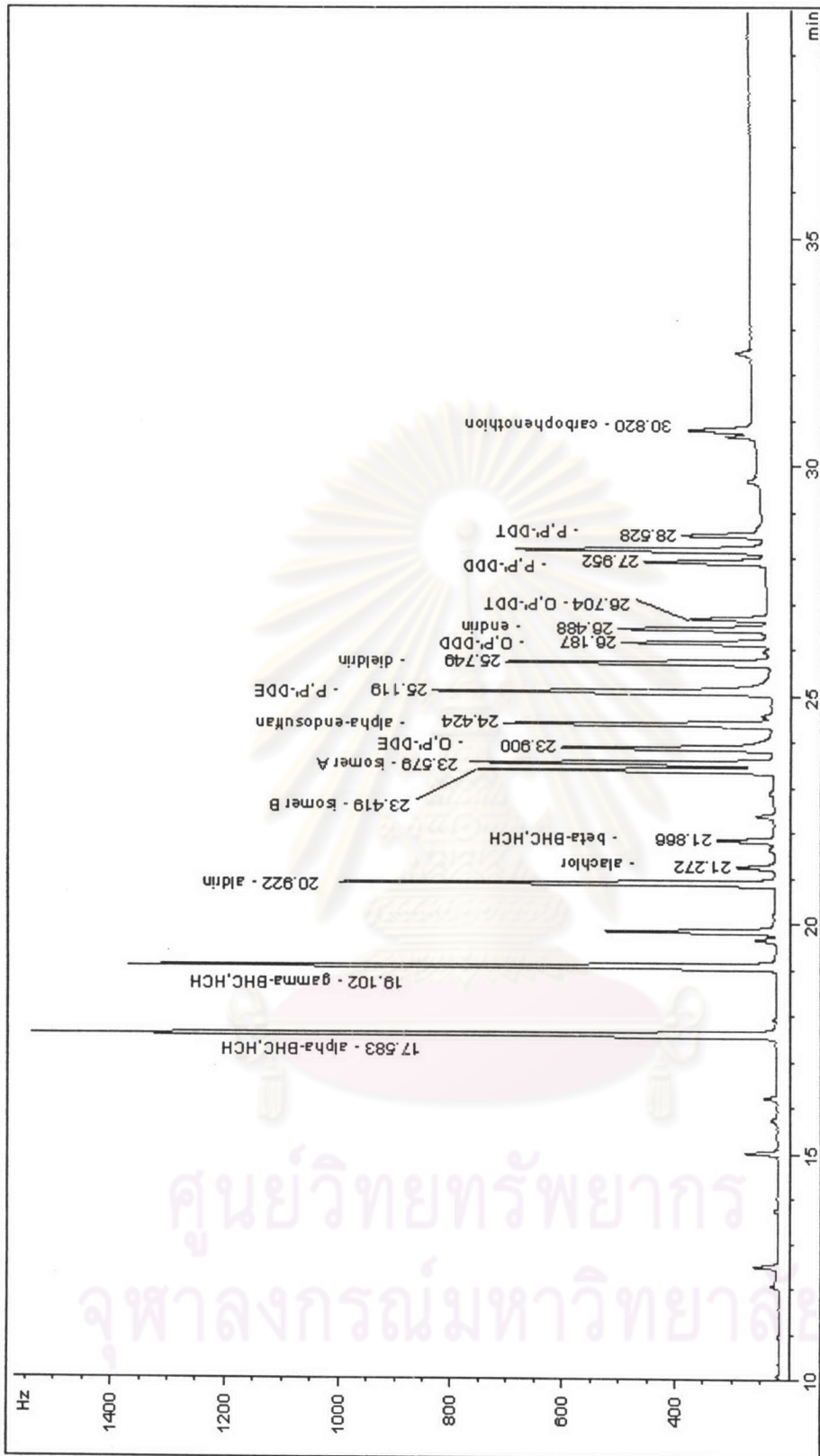


Figure 4.2 The chromatogram of mixed 17 standard organochlorine pesticides in hexane 10 ng/mL under GC conditions in Table 4.2

4.2 The Result of Selectivity of GC

Table 4.3 Retention time and resolution of 17 organochlorine pesticides under GC conditions in Table 4.1 (column HP-5).

No.	Pesticides	Retention time (min)	Resolution
1	α -BHC, HCH	15.394	-
2	β -BHC, HCH	16.697	10.24
3	γ -BHC, HCH	16.922	1.70
4	Alachlor	20.478	2.21
5	Aldrin	22.279	15.73
6	Heptachlor epoxide isomer B	24.479	18.80
7	Heptachlor epoxide isomer A	24.728	2.10
8	O,P'-DDE	26.175	11.66
9	α -endosulfan	26.436	2.06
10, 11	Dieldrin, P,P'-DDE	27.942	9.13
12	O,P'-DDD	28.416	2.85
13	Endrin	29.073	5.02
14	P,P'-DDD	30.303	4.71
15	O,P'-DDT	30.434	0.91
16	Carbophenothion	31.813	9.47
17	P,P'-DDT	32.366	2.23

The selectivity of GC conditions can be determined by retention time and resolution value of critical pairs (R_s) of each peak. From Table 4.3, dieldrin and P,P'-DDE are co-elution and there is no interference of the 16 peaks of organochlorine pesticides with other interfering peaks. Concerning the resolution, all of the peaks except O,P'-

DDT have a baseline resolution which is acceptable due to the fact that their resolution is greater than 1.5.



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Table 4.4 Retention time and resolution of 17 organochlorine pesticides under GC conditions in Table 4.2 (column PAS-1701).

No.	Pesticides	Retention time (min)	Resolution
1	α -BHC, HCH	17.583	-
2	β -BHC, HCH	21.866	12.03
3	γ -BHC, HCH	19.102	10.12
4	Alachlor	21.272	3.20
5	Aldrin	20.922	2.14
6	Heptachlor epoxide isomer B	23.419	9.68
7	Heptachlor epoxide isomer A	23.579	2.01
8	O,P'-DDE	23.900	1.86
9	α -endosulfan	24.424	1.65
10, 11	Dieldrin, P,P'-DDE	25.749, 25.119	1.71, 2.13
12	O,P'-DDD	26.187	2.05
13	Endrin	26.488	3.05
14	P,P'-DDD	27.952	3.25
15	O,P'-DDT	26.704	1.76
16	Carbophenothion	30.820	1.30
17	P,P'-DDT	28.528	8.45

The selectivity of GC conditions can be determined by retention time and resolution value of critical pairs (R_s) of each peak. From Table 4.4, all of interesting peaks can be separated and there is no interference of the 17 peaks of organochlorine pesticides with other peaks. Concerning the resolution, all of the peaks except carbophenothion have a baseline resolution which is acceptable due to the fact that their resolution is greater than 1.5.

4.3 The Result of Calibration Curve

The mixed 17 standard organochlorine pesticide solutions covering the concentration range of 1-500 ng/mL were plotted by peak height versus concentration. The 10 points calibration curve is shown in APPENDIX and the result of slope value summary, intercept and correlation coefficient are shown in Table 4.5.

From Table 4.5, the data of the 10 points calibration curve can be acceptable for quantitation because the correlative coefficient (R^2) was obtained at 0.9946 of heptachlor epoxide isomer A to 0.9986 of endrin, and that both have r^2 greater than 0.99. For the slope value, it implies that γ -BHC, HCH has the highest sensitivity while β -BHC, HCH has the lowest sensitivity.

For the linearity study, it can be proved that the range of concentration for calibration curve will be linearity (1 – 500 ng/mL). Similar to the 10 points of mixed standard calibration curve, the correlation coefficient was obtained at 0.9946 to 0.9986 and the results of linear were positive.



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Table 4.5 Summary of Value of Slope, Intercept and Correlation Coefficient (R^2) of each pesticide in mixed 17 standard organochlorine pesticides with the range of 1 – 500 ng/mL by the conditions in Table 4.1

No.	Pesticides	Slope (peak height units/ mg/kg)	Intercept	R^2
1	α -BHC, HCH	56.52	-64.0010	0.9980
2	β -BHC, HCH	0.64	0.5624	0.9952
3	γ -BHC, HCH	64.83	80.2900	0.9978
4	Alachlor	34.98	-65.0960	0.9977
5	Aldrin	53.02	-68.6010	0.9973
6	Heptachlor epoxide isomer B	48.87	-35.9030	0.9970
7	Heptachlor epoxide isomer A	58.26	-46.7490	0.9946
8	O,P'-DDE	29.76	-17.9560	0.9966
9	α -endosulfan	32.05	161.6800	0.9982
10, 11	Dieldrin, P,P'-DDE	64.06	-20.3870	0.9978
12	O,P'-DDD	23.37	-33.1960	0.9978
13	Endrin	27.92	-52.9600	0.9986
14	P,P'-DDD	12.70	11.4300	0.9978
15	O,P'-DDT	17.10	33.9813	0.9979
16	Carbophenothion	1.30	-4.1743	0.9969
17	P,P'-DDT	14.21	-22.2490	0.9977

4.4 The Result of Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of Detection (LOD) and Limit of Quantitation (LOQ) were defined as the peak height of analyte in matrix standard solutions that signalled significantly different from the peak height of noise equal 3 for LOD and 10 for LOQ of each compound. These are shown in Table 4.6

The limit of detection and limit of quantitation was 0.3 – 12.3 ng/mL and 1.1 – 41.0 ng/mL respectively.



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Table 4.6 The limit of detection and of quantitation of each pesticide in matrix standard solutions of organochlorine pesticides

No.	Pesticides	LOD (ng/mL)	LOQ (ng/mL)
1	α -BHC, HCH	0.7	2.3
2	β -BHC, HCH	12.3	41.0
3	γ -BHC, HCH	0.4	1.4
4	Alachlor	0.9	3.1
5	Aldrin	0.5	1.6
6	Heptachlor epoxide isomer B	0.7	2.3
7	Heptachlor epoxide isomer A	0.5	1.8
8	O,P'-DDE	0.9	3.0
9	α -endosulfan	1.0	3.4
10, 11	Dieldrin, P,P'-DDE	0.3	1.1
12	O,P'-DDD	1.1	3.6
13	Endrin	0.8	2.8
14	P,P'-DDD	1.2	3.8
15	O,P'-DDT	0.8	2.7
16	Carbophenothion	5.7	19.0
17	P,P'-DDT	0.6	1.7

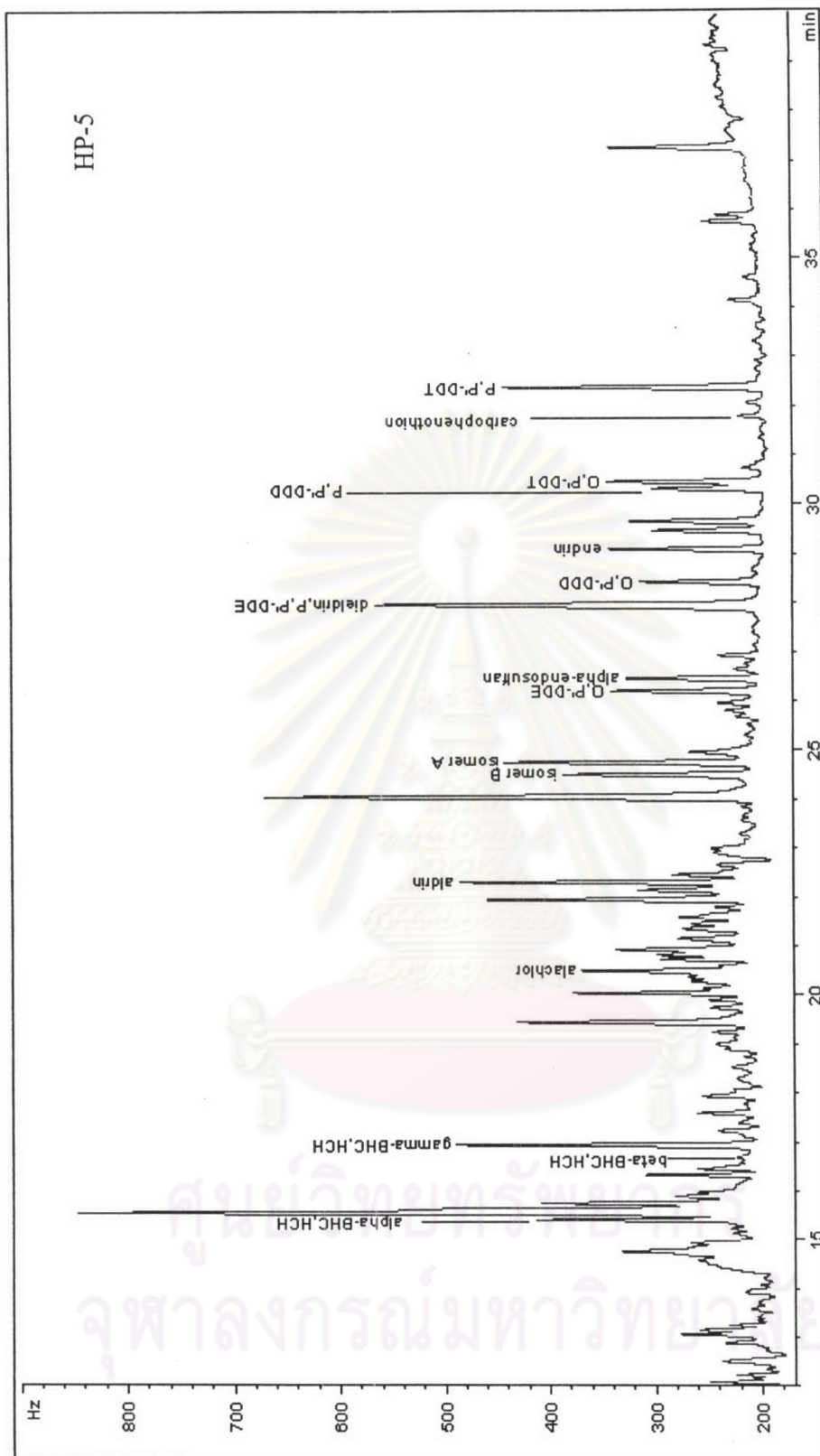


Figure 4.3 Chromatogram of matrix standard of organochlorine pesticides containing 1 ng/mL by GC conditions in Table4.1

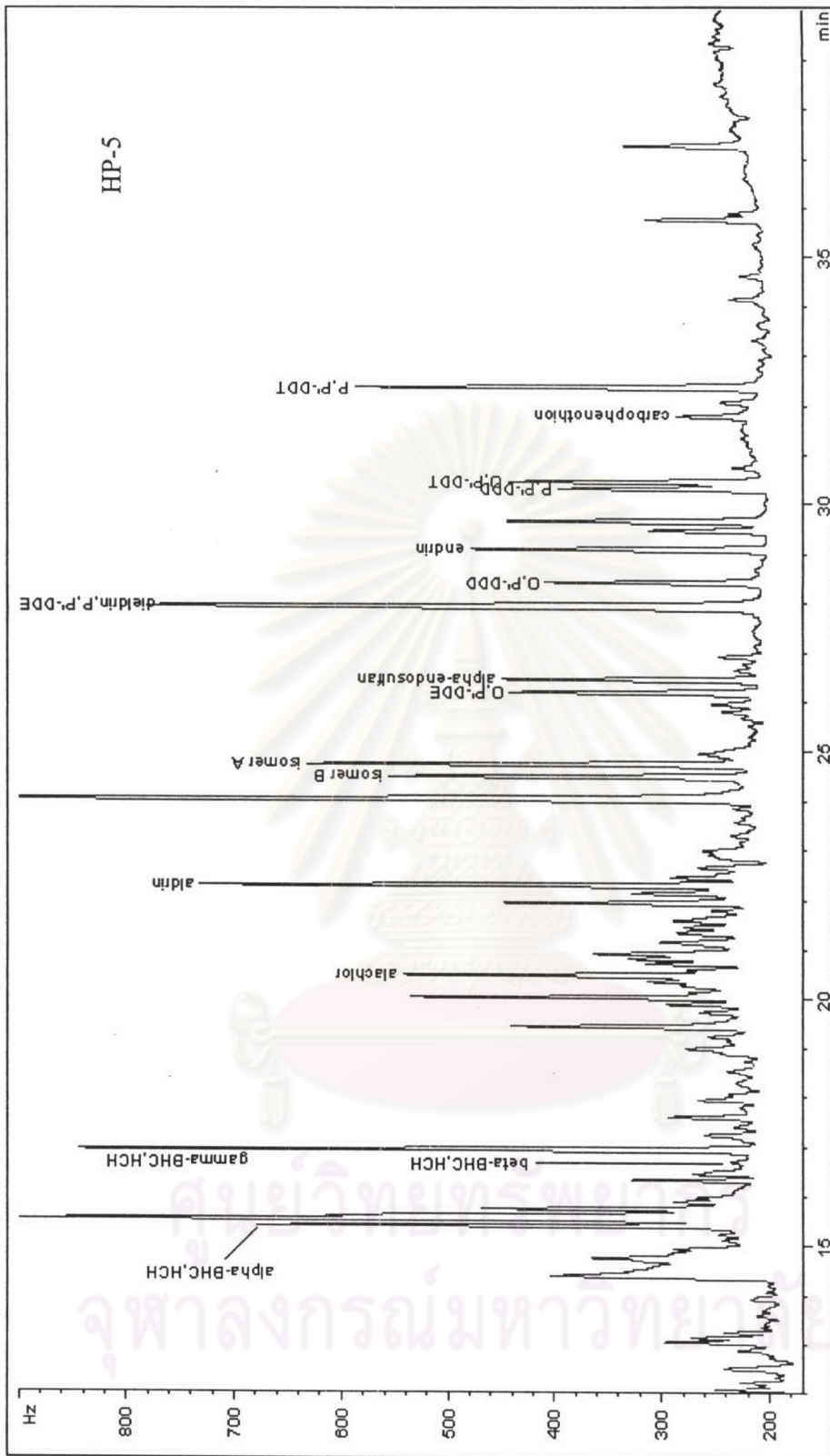


Figure 4.4 Chromatogram of matrix standard of organochlorine pesticides containing 10 ng/mL by GC conditions in Table4.1

4.5 The Result of Matrix Calibration Curve

The calibration curve of 17 standard organochlorine pesticides in matrix covering the concentration range of 1 to 500 ng/mL was plotted—peak height versus concentration at the GC conditions in Table 4.1. The matrix calibration curve is shown in APPENDIX. The summary of value of slope, intercept and correlation coefficient (R^2) are shown in Table 4.7.

Matrix calibration curve of 17 organochlorine pesticides in matrix covering 1 – 500 ng/mL is linear on this concentration range. It is acceptable for quantitation because the correlation coefficient was at 0.9948 of heptachlor epoxide isomer B to 0.9994 of α -BHC, HCH, and that both have R^2 greater than 0.99. The γ -BHC, HCH has the highest sensitivity while β -BHC, HCH contains the lowest sensitivity.



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Table 4.7 Summary of Value of Slope, Retention Time, Intercept and Correlation Coefficient (R^2) of each pesticide in 17 standard organochlorine pesticides in matrix under GC conditions in Table 4.1

No.	Pesticides	Slope (peak height Units/ mg/kg)	Intercept	R^2
1	α -BHC, HCH	63.62	92.9750	0.9994
2	β -BHC, HCH	0.52	12.6790	0.9959
3	γ -BHC, HCH	65.08	92.5090	0.9980
4	Alachlor	41.90	-93.3320	0.9961
5	Aldrin	64.31	-59.3290	0.9969
6	Heptachlor epoxide isomer B	43.10	-66.2430	0.9948
7	Heptachlor epoxide isomer A	53.83	-76.3020	0.9967
8	O,P'-DDE	26.18	1.4166	0.9976
9	α -endosulfan	32.70	-58.8500	0.9969
10, 11	Dieldrin, P,P'-DDE	60.94	66.0620	0.9966
12	O,P'-DDD	23.84	-9.9844	0.9977
13	Endrin	35.08	-22.6860	0.9975
14	P,P'-DDD	24.75	-44.7900	0.9964
15	O,P'-DDT	22.15	31.7770	0.9961
16	Carbophenothion	8.52	-14.3540	0.9971
17	P,P'-DDT	26.09	135.0800	0.9979

4.6 The Result of Matrix Effect

Since the method developed was to be used with turmeric powder samples available in most local markets in Thailand, a study of the matrix in all the samples was in order. It was to understand the effect that the matrix might have on the analysis and whether the result would be significant enough when the pair *t*-test was used in comparing it statistically to that of the standard matrix and solution.

The statistic analysis by pair *t*-test paired for finding the mean of the peak height from the same GC concentrations in Table 4.1 of standard calibration curve and matrix calibration curve as shown in Table 4.8

From Table 4.8, the effect of Turmeric matrix was calculated by using pair *t*-test with the mean of 95% confidential limit comparisons between standard calibration curve and calibration curve in matrix. From the results, the *t*-calculated of all organochlorine pesticides is less than *t*-critical (2.26) except alpha-endosulfan and O,P'-DDD. This means the sample in matrix is significantly different than that of the standard solutions. Therefore, the matrix calibration curve was used for the quantitation instead of standard solution in hexane.

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Table 4.8 The comparison of peak height of each concentration of 17 organochlorine pesticides between mixed standard solution and matrix standard solution and summary of pair *t*-test at 95% confidence level.

Concentration (ng/mL)	Peak height (Hz)					
	α -BHC,HCH		β -BHC,HCH		γ -BHC,HCH	
	mixed standard	matrix standard	mixed standard	matrix standard	mixed standard	matrix standard
1	112	189		11	120	132
3	204	237		13	250	264
5	389	497		14	467	481
10	550	444	6	18	625	641
20	1022	1194	14	22	1300	1313
30	1700	2032	23	32	1821	1869
50	2710	3213	38	43	3215	3305
100	5612	6666	63	68	7612	7552
300	15822	19762	175	156	18560	18630
500	28832	31530	328	280	32895	33050
Pairs <i>t</i> -test	2.05		0.45		2.03	

Concentration (ng/mL)	Peak height (Hz)					
	alachlor		aldrin		isomer B	
	mixed standard	matrix standard	mixed standard	matrix standard	mixed standard	matrix standard
1	85	132	223	263	170	174
3	148	154	292	318	216	196
5	284	301	524	563	410	368
10	326	273	501	516	451	331
20	652	749	1018	1215	955	812
30	978	1253	1527	1974	1428	1349
50	1630	1991	2565	3174	2504	2059
100	3260	3994	4698	6138	4705	4121
300	9782	11392	14870	17768	13525	11566
500	17852	21520	27123	33012	25075	22276
Pairs <i>t</i> -test	1.83		1.94		2.02	

Concentration (ng/mL)	Peak height (Hz)					
	isomer A		O,P'-DDE		alpha-endosulfan	
	mixed standard	matrix standard	mixed standard	matrix standard	mixed standard	matrix standard
1	196	231	107	132	147	123
3	262	242	133	143	183	134
5	498	453	260	257	366	261
10	820	400	259	229	383	235
20	1130	1011	579	518	767	589
30	1623	1649	898	840	1270	947
50	2567	2571	1347	1272	2052	1566
100	6023	5082	3098	2545	3391	3114
300	15650	14817	8185	7338	9257	9012
500	30125	27629	15275	13414	16458	16753
Pairs <i>t</i> -test	1.91		1.80		2.32	

Table 4.8 continued.

Concentration (ng/mL)	Peak height (Hz)					
	dieldrin		O,P'-DDD		endrin	
	mixed standard	matrix standard	mixed standard	matrix standard	mixed standard	matrix standard
1	184	361	75	110	58	143
3	261	393	115	119	115	162
5	513	631	210	220	194	305
10	583	571	180	198	227	273
20	1266	1280	460	466	494	669
30	1798	2019	720	738	761	1087
50	3362	3096	1082	1180	1435	1731
100	6430	6117	2138	2276	2507	3405
300	17923	16859	6577	6685	7981	9787
500	32750	31431	11927	12200	14152	17959
Pairs <i>t</i> -test	1.35		2.59		1.99	

Concentration (ng/mL)	Peak height (Hz)					
	P,P'-DDD		O,P'-DDT		carbophenothion	
	mixed standard	matrix standard	mixed standard	matrix standard	mixed standard	matrix standard
1	14	104	51	146		22
3	59	119	98	159	8	32
5	103	206	172	252	10	67
10	116	191	153	235	11	64
20	252	441	339	472	24	156
30	398	710	752	748	30	262
50	623	1163	817	1151	63	408
100	1426	2350	1749	2157	118	808
300	3587	6780	4892	6110	358	2355
500	6473	12705	8743	11460	662	4362
Pairs <i>t</i> -test	1.83		1.90		1.93	

Concentration (ng/mL)	Peak height (Hz)	
	P,P'-DDT	
	mixed standard	matrix standard
1	22	242
3	63	285
5	109	378
10	124	374
20	278	650
30	433	957
50	679	1436
100	1319	2650
300	3970	7481
500	7256	13485
Pairs <i>t</i> -test	2.18	

4.7 The Results of the Pretreatment with Vacuum Oven

According from the basis method, samples have to pretreatment with vacuum oven before used. In this section, considering the background noises of the chromatogram of spiked sample with and without the vacuum oven (PAS-1701 and GC conditions in Table 4.2), in Figure 4.6, there was non difference background noise between in the chromatogram as shown in Figure 4.5. Moreover, the spiked sample without the vacuum oven has lower interfering peaks than that with the vacuum oven except the interfering peaks at retention time 17 minutes.

Furthermore, from Table 4.9, almost all the %recoveries of the spiked sample without the vacuum oven are larger than those of the spiked sample with the vacuum oven. Using the vacuum oven could automatically eliminate the volatile substances, and thus, low %recoveries could be a result.

For spiking level of 50 ng/g, that less than MQL of β -BHC, HCH (51.2 ng/g) but more than MDL (15.4 ng/g). Therefore, it can be detected but we can't quantitation of this compound. Although, some %recoveries of the method with the vacuum oven yield a better result than those of the method without the vacuum oven, the method without the vacuum oven is much simpler and faster than the one with the vacuum oven.

From basis method;

$$\frac{2\text{gsample}}{25\text{mLsolution}} \times \frac{1\text{mLsolution}}{0.2\text{mLsolution}}$$

Concentration factor = 0.4

The ultimate concentration before the GC is injected is 0.4 times higher than the actual concentration.

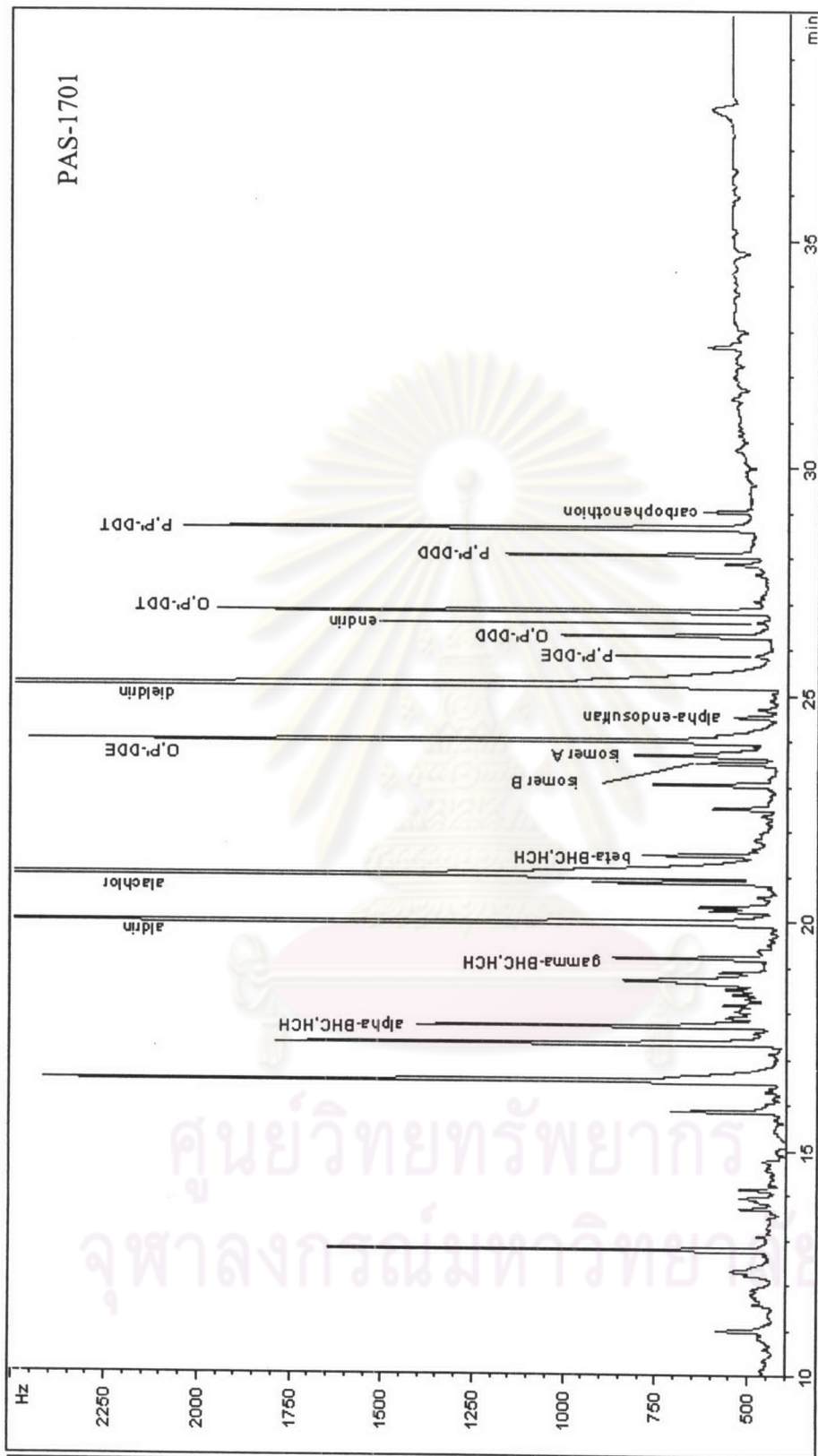


Figure 4.5 The chromatogram of spiked sample without vacuum oven at spiking level of 50 ng/g, concentration factor equal to 0.4 and the final concentration of 20 ng/g under GC condition in Table 4.2.

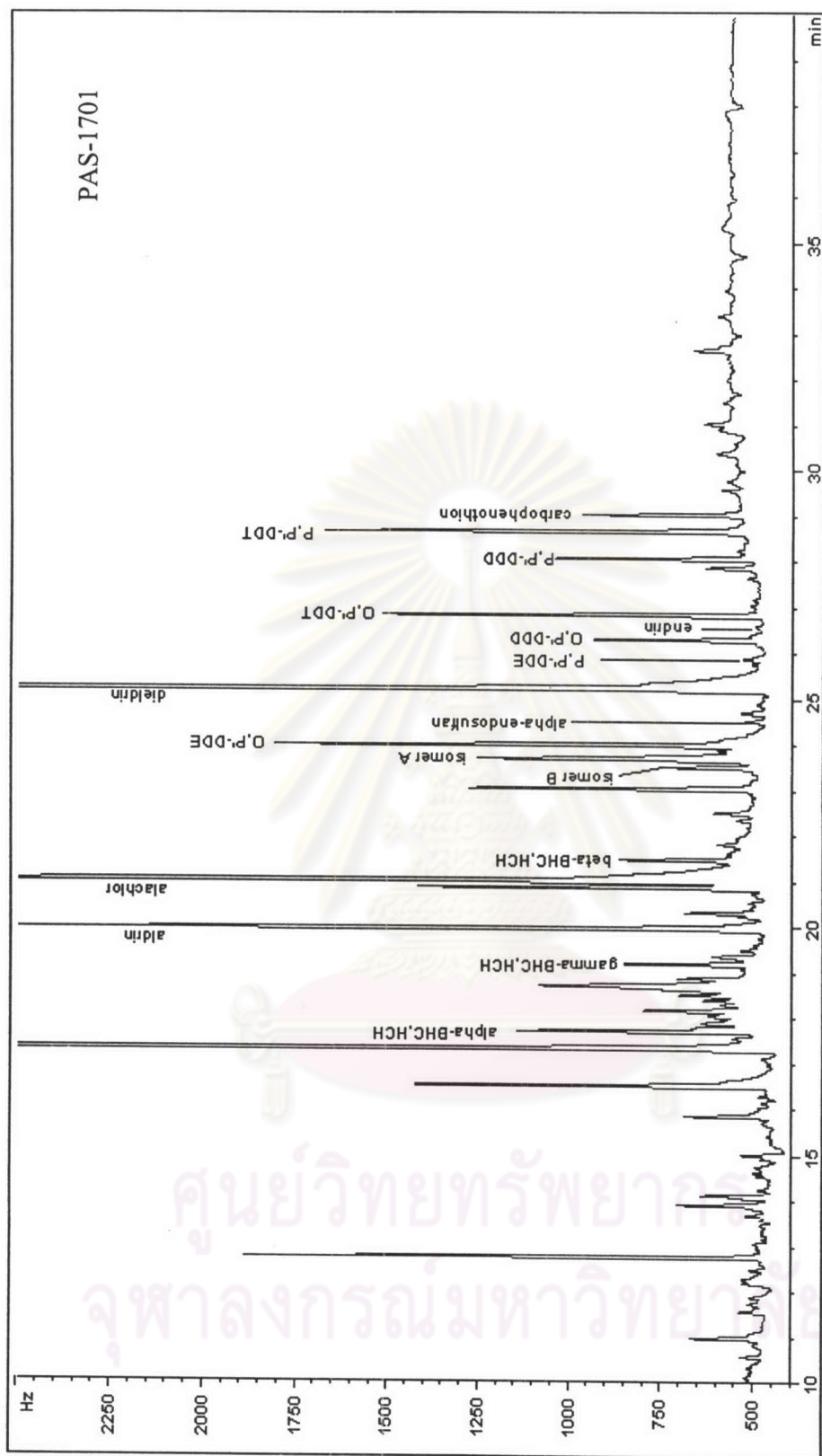


Figure 4.6 The chromatogram of spiked sample with vacuum oven at spiking level of 50 ng/g, concentration factor equal to 0.4 and the final concentration of 20 ng/g under GC condition in Table 4.2.

Table 4.9 %Recovery of spiked sample with and without vacuum oven at spiking level of 50 ng/g

No.	Pesticides	%Recovery of spiked sample	
		Without vacuum oven	With vacuum oven
1	α -BHC, HCH	43.4	44.6
2	β -BHC, HCH	_*	_*
3	γ -BHC, HCH	37.6	36.0
4	Alachlor	85.4	56.5
5	Aldrin	82.7	33.9
6	Heptachlor epoxide isomer B	37.3	34.7
7	Heptachlor epoxide isomer A	43.6	52.5
8	O,P'-DDE	67.9	51.2
9	α -endosulfan	44.5	39.6
10, 11	Dieldrin, P,P'-DDE	63.3, 1.7	48.3, 1.6
12	O,P'-DDD	24.5	21.1
13	Endrin	25.7	22.3
14	P,P'-DDD	27.7	25.0
15	O,P'-DDT	69.0	53.5
16	Carbophenothion	58.0	71.3
17	P,P'-DDT	65.2	62.1

*Spiking level < MQL

4.8 The Result of SPE Type

In this section, the use of SPE types in a mixed mode of SPE and the mixed mode SPE connected with ENVI-CARB were considered (PAS-1701 and GC conditions in Table 4.2). Comparing the background noise in the mixed mode of SPE to ENVI-CARB, it was found that background noise of the methods with SPE in Figure 4.7 was lower than that of the methods with the mixed mode SPE that was connected to ENVI-CARB. In Figure 4.8, there were more interfering peaks than those in Figure 4.7.

Moreover, the methods with mixed mode SPE that were connected with ENVI-CARB gave lower values of %recovery than the ones with mixed mode SPE except, γ -BHC, HCH and O,P'-DDD. It is possible that if more steps were taken in the analysis, they could cause the interested substances to be lost and, as a result, the %recovery was low. For %recovery of β -BHC, HCH as same results in Section 4.7. Regards to the data analysis, the method with the mixed mode SPE was chosen because this method was low-cost and required fewer steps than that with the mixed mode SPE connected to ENVI-CARB. Thus, the preferred method for extraction was the method that came with the mixed mode SPE and without the vacuum oven.

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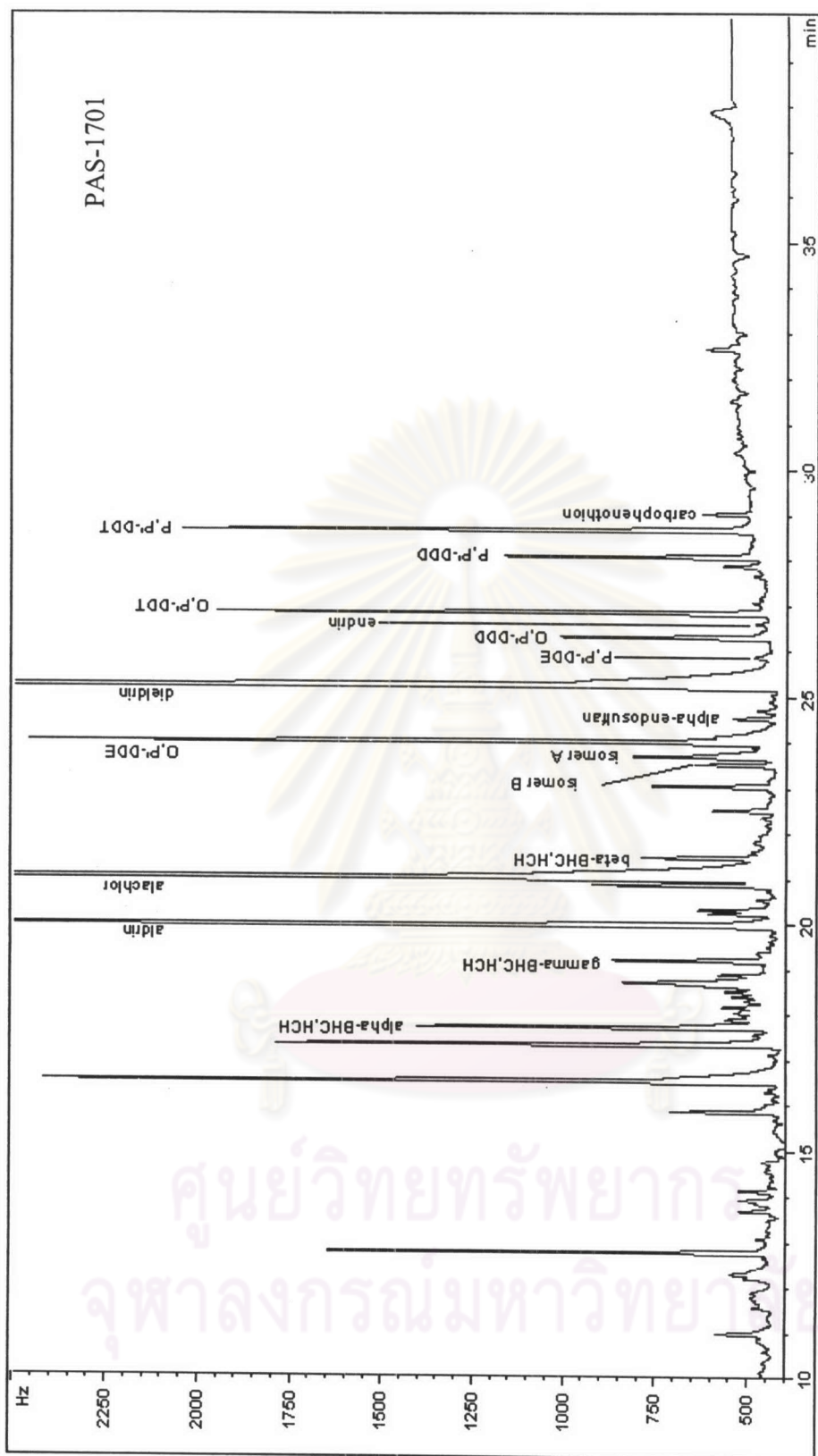


Figure 4.7 The chromatogram of spiked sample at spiking level of 50 ng/g, concentration factor equal to 0.4 and the final concentration of 20 ng/g that used mixed mode SPE and without vacuum oven

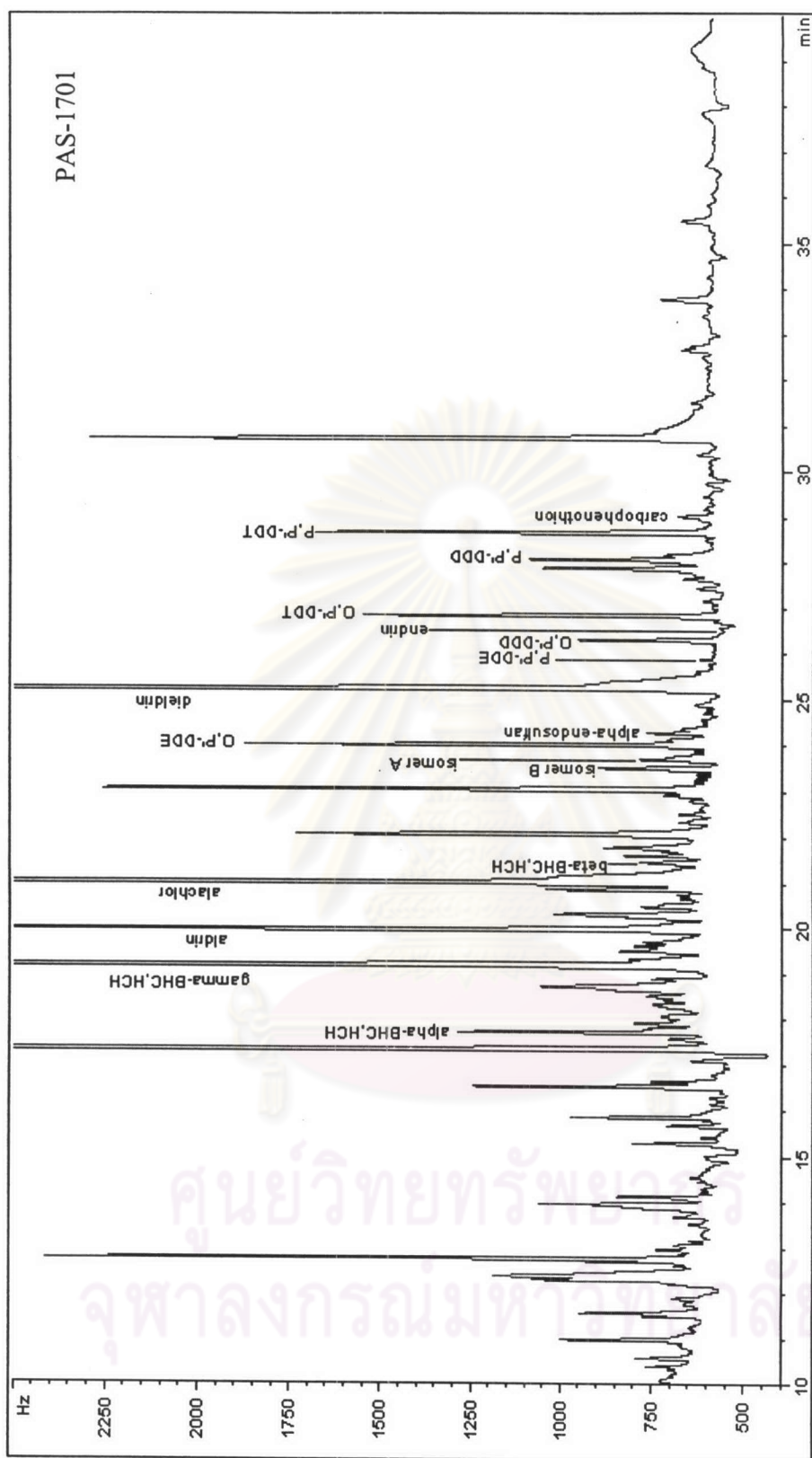


Figure 4.8 The chromatogram of spiked sample at spiking level of 50 ng/g, concentration factor equal to 0.4 and the final concentration of 20 ng/g that used mixed mode SPE and connected with ENVI-CARB without vacuum oven

Table 4.10 %Recovery of spiked sample at spiking level 50 ng/g with mixed mode SPE that without a vacuum oven and ENVI-CARB that without a vacuum oven

No.	Pesticides	%Recovery	
		Mixed mode SPE	Mixed mode SPE and ENVI-CARB
		Without vacuum oven	Without vacuum oven
1	α -BHC, HCH	43.4	40.3
2	β -BHC, HCH	_*	_*
3	γ -BHC, HCH	37.6	40.4
4	Alachlor	85.4	49.2
5	Aldrin	82.7	39.1
6	Heptachlor epoxide isomer B	37.3	30.1
7	Heptachlor epoxide isomer A	43.6	7.9
8	O,P'-DDE	67.9	42.2
9	α -endosulfan	44.5	21.1
10, 11	Dieldrin, P,P'-DDE	63.3, 1.7	48.8, 1.7
12	O,P'-DDD	24.5	25.0
13	Endrin	25.7	23.5
14	P,P'-DDD	27.7	26.7
15	O,P'-DDT	69.0	61.1
16	Carbophenothion	58.0	34.1
17	P,P'-DDT	65.2	43.1

*Spiking level < MQL

4.9 The Result of the Influence of Mixed Solvent Ratio for Extraction

From the results of previous section, extraction method without the vacuum oven and with the mixed mode SPE offered a better condition for extraction (HP-5 and GC conditions in Table 4.1). About mixed solvent of hexane and dichloromethane of the ratio 4:1 and 5:2, it was investigated and is illustrated in Figure 4.9 and Figure 4.10. Obviously, the peak heights of analyte of the method with mixed solvent contained the ratio of 5:2 which were higher than the peak heights of the method with mixed solvent, which contained the ratio of 4:1. This showed a better sensitivity of extraction with mixed solvent: the ratio of 5:2 than that of 4:1. Moreover, background noises in Figure 4.9 and 4.10 were not significantly different. To give additional information, the result from Table 4.11 shows better %recoveries and better detection ability of mixed solvent ratio 5:2. Hence, the ratio at 5:2 of hexane:dichloromethane could indeed increase the dissolving ability of the interested substances and lead to better %recoveries. Yet, certain substances could not be extracted satisfactorily. This stimulated a further look into other parameters and adjustments of the existing method so that the best results could be gained.

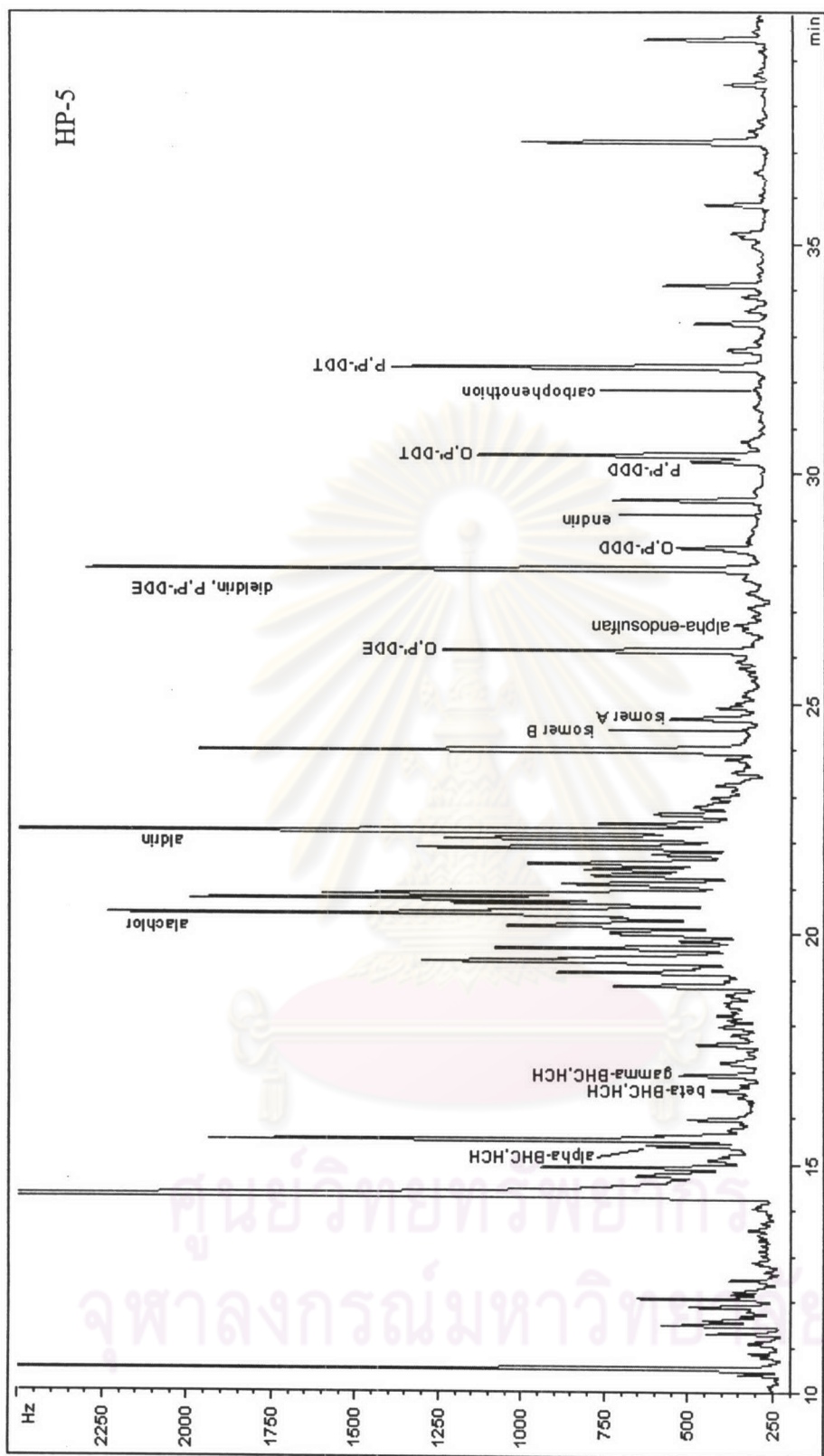


Figure 4.9 Chromatogram of spiked sample at spiking level of 100 ng/g, concentration factor equal to 0.4 and the final concentration of 40 ng/g with used mix solvent ratio 4:1

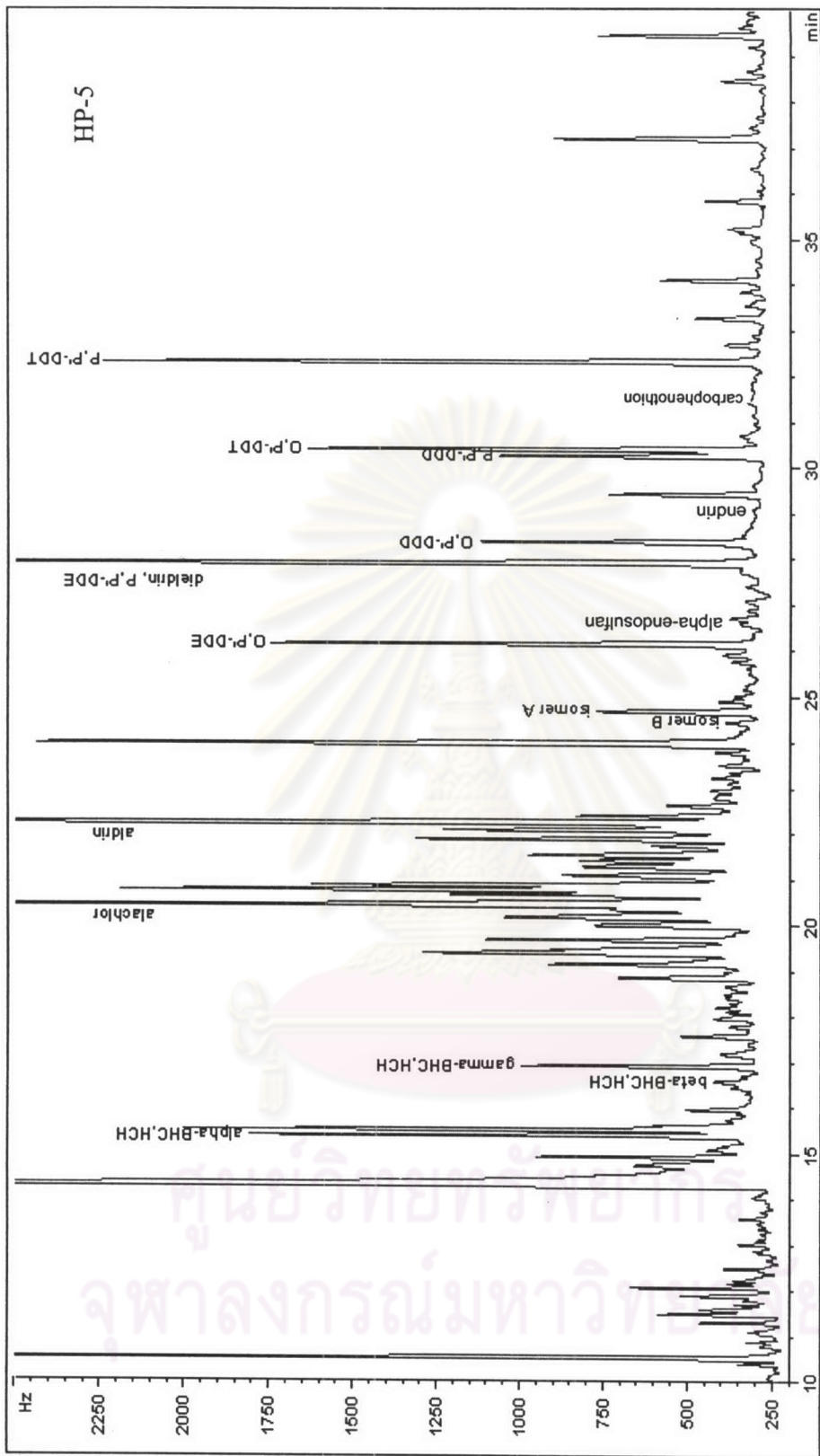


Figure 4.10 Chromatogram of spiked sample at spiking level of 100 ng/g, concentration factor equal to 0.4 and the final concentration of 40 ng/g with used mix solvent ratio 5:2

Table 4.11 %Recovery of spiked sample at spiking level of 100 ng/g concentration factor equal to 0.4, the final concentration of 40 ng/g with mixed solvent ratio of hexane:dichloromethane 4:1 and 5:2

No.	Pesticides	%Recovery of mixed solvent ratio	
		4:1	5:2
1	α -BHC, HCH	40.3	80.2
2	β -BHC, HCH	NA*	92.8
3	γ -BHC, HCH	33.3	77.7
4	Alachlor	83.9	110.6
5	Aldrin	85.8	100.2
6	Heptachlor epoxide isomer B	42.9	60.5
7	Heptachlor epoxide isomer A	46.4	76.0
8	O,P'-DDE	71.6	107.8
9	α -endosulfan	46.8	54.4
10, 11	Dieldrin, P,P'-DDE	65.0	85.7
12	O,P'-DDD	23.5	75.9
13	Endrin	27.1	27.4
14	P,P'-DDD	24.2	77.9
15	O,P'-DDT	73.8	109.4
16	Carbophenothion	60.9	45.0
17	P,P'-DDT	68.8	113.5

*NA; Non – Acceptable

4.10 The Result of the Influence of Elution Volumes of SPE

From the results of previous section, extraction method without the vacuum oven and using the mixed mode SPE at mixed solvent ratio 5:2 was a better condition for extraction (HP-5 and GC conditions in Table 4.1). The study of elution volumes of SPE between 5 mL and 8 mL were investigated. From Figure 4.11 (5 mL), Figure 4.12 (8 mL), and Table 4.12, it is obvious that the values of %recovery of the elute volume at 5 mL are better than the elute volume at 8 mL. However, some of the analyst peaks at the elute volume of 8 mL could contain more peak areas than those at the elute volume of 5 mL. This is because at 8 mL the interested substances would be satisfactorily eluted. At the same time, a lot of matrix would come off too, which was likely to cause a low %recovery. For %recovery of β -BHC, HCH as same results in Section 4.7. Therefore, the chosen extraction method was the one that had the elute volume at 5 mL.

With the procedures in preparing the sample method in mind, we are ready to launch a suitable extraction method for Turmeric. The summary of method and its procedures are shown in Figure 4.13

In the developed method, the final solution before being injected into GC had the concentration 0.8 times higher than that of the actual concentration of the original solution (shown in the diagram below), and when compared with the basis method it was found that the method was two times pre-concentrated.

$$\frac{2\text{gsample}}{25\text{mLsolution}} \times \frac{10\text{mLsolution}}{1\text{mLsolution}}$$

$$\text{Concentration factor} = 0.8$$

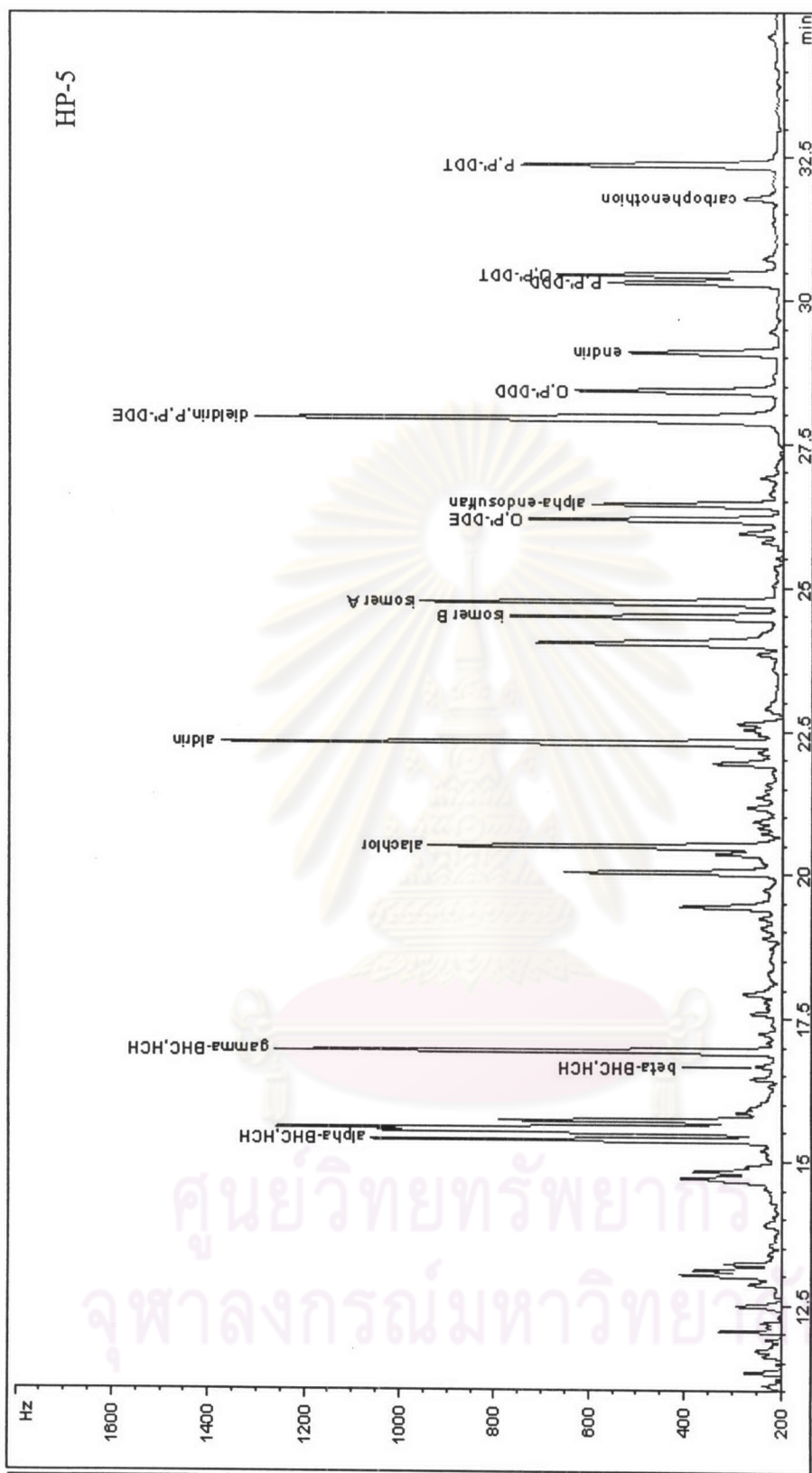


Figure 4.11 Chromatogram of spike sample at spiking level of 25 ng/g, concentration factor equal to 0.8, the final concentration of 20 ng/g with 5 mL elution volumes of SPE

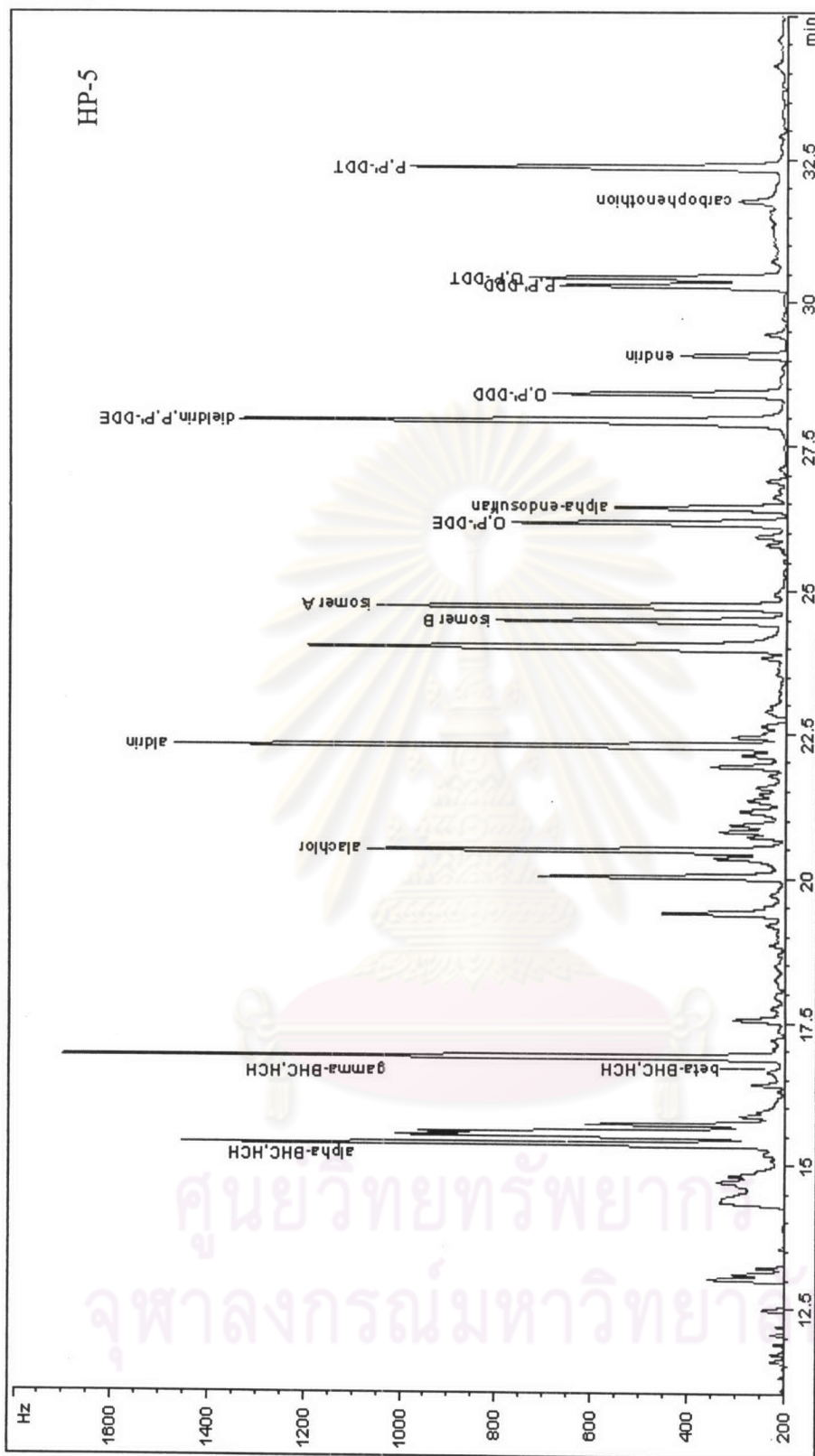


Figure 4.12 Chromatogram of spike sample at spiking level of 25 ng/g, concentration factor equal to 0.8, the final concentration of 20 ng/g with 8 mL elution volumes of SPE

Table 4.12 %Recovery of spiked sample at spiking level of 25 ng/g concentration factor equal to 0.8, the final concentration of 20 ng/g with 5 mL and 8 mL of elution volumes of SPE

No.	Pesticides	%Recovery of elution volumes	
		5 mL	8 mL
1	α -BHC, HCH	109.8	89.1
2	β -BHC, HCH	_*	_*
3	γ -BHC, HCH	110.7	81.5
4	Alachlor	129.9	102.4
5	Aldrin	110.9	99.8
6	Heptachlor epoxide isomer B	75.6	67.2
7	Heptachlor epoxide isomer A	83.3	79.9
8	O,P'-DDE	105.5	97.4
9	α -endosulfan	70.9	57.6
10, 11	Dieldrin, P,P'-DDE	84.1	73.4
12	O,P'-DDD	105.3	94.2
13	Endrin	58.5	30.5
14	P,P'-DDD	138.1	93.3
15	O,P'-DDT	113.3	87.9
16	Carbophenothion	178.0	48.3
17	P,P'-DDT	167.7	104.2

*Spiking level < MQL

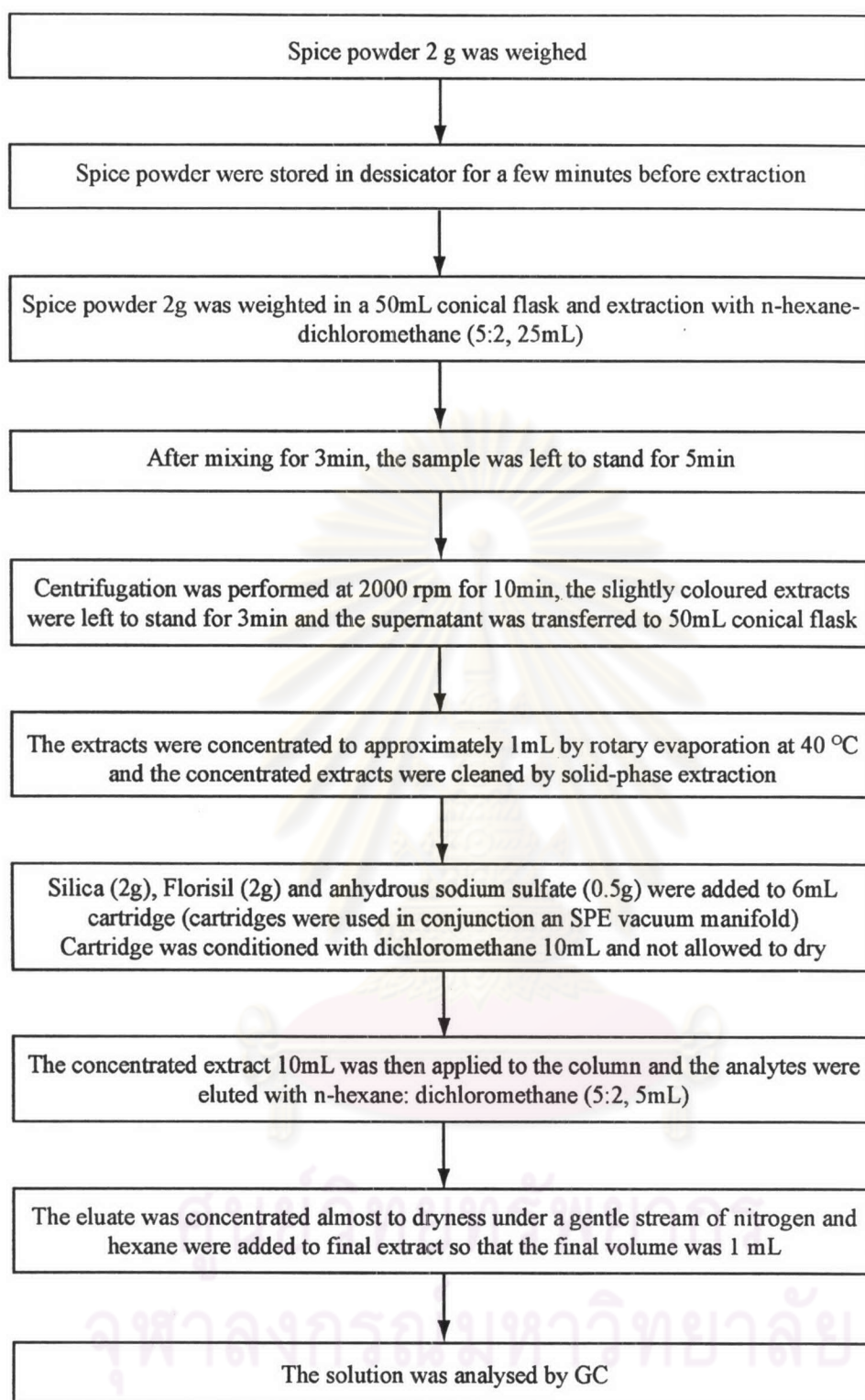


Figure 4.13 Schematic of procedure in developed method

4.11 The Result of Comparing to the Standard Method for Tobacco

In this section, the developed method with spiking level of 5 ng/g and the standard Tobacco method with spiking level of 100 ng/g were applied to Turmeric of which the chromatogram is shown in Figure 4.14 and Figure 4.15, respectively (HP-5 and GC conditions in Table 4.1). The peak heights of the developed method are higher than those of the Tobacco method unsurprisingly because the Tobacco method is developed for the extraction of pesticide compounds from leaves while the developed method in this thesis is designed specifically for Turmeric. Moreover, background noises of standard method for Tobacco are higher than those of the developed method. For %recovery of β -BHC, HCH as same results in Section 4.7. The results in Table 4.14. This has shown that the developed method can effectively work with organochlorine pesticides. The outstanding point is it was found that even though the pesticides were slight in amounts, the method could effectively eluted them and yield better %recoveries.



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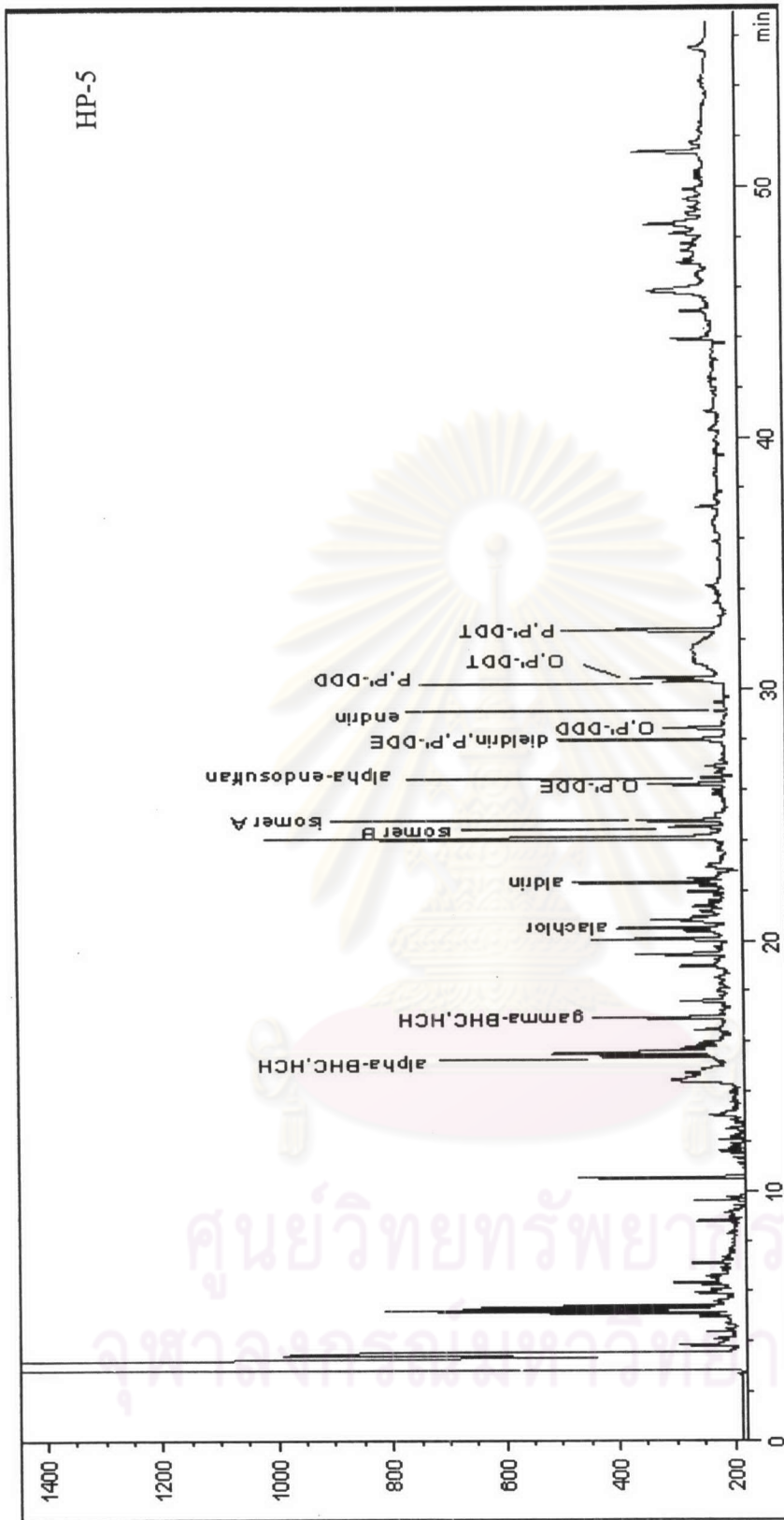


Figure 4.14 The chromatogram of spiked sample at spiking level of 5 ng/g, concentration factor equal to 0.8 and the final concentration of 4 ng/g that extraction by developed method

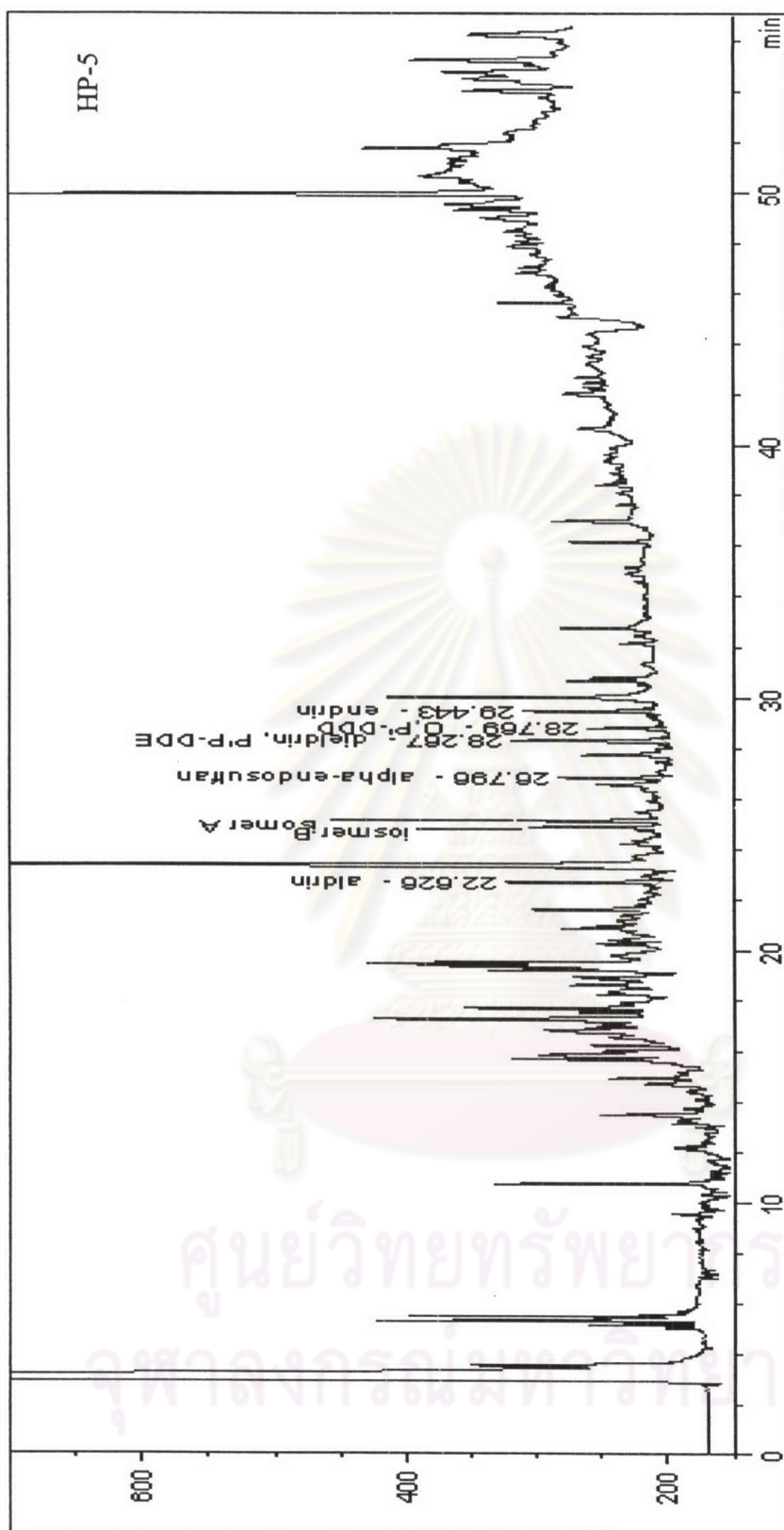


Figure 4.15 The chromatogram of spiked sample at spiking level of 100 ng/g, concentration factor equal to 0.06 and the final concentration of 6 ng/g that extraction by standard method for Tobacco

Table 4.13 %Recovery of spiked sample comparing developed method in Figure 4.13 with the standard method for Tobacco in Section 3.10.5.

No.	Pesticides	%Recovery	
		Developed method	Tobacco method
1	α -BHC, HCH	103.2	-
2	β -BHC, HCH	-*	-*
3	γ -BHC, HCH	93.0	-
4	Alachlor	127.5	-
5	Aldrin	103.9	0.1
6	Heptachlor epoxide isomer B	73.0	0.4
7	Heptachlor epoxide isomer A	79.8	0.2
8	O,P'-DDE	97.4	-
9	α -endosulfan	64.2	0.2
10, 11	Dieldrin, P,P'-DDE	89.5	0.9
12	O,P'-DDD	96.7	0.4
13	Endrin	50.0	0.7
14	P,P'-DDD	134.9	-
15	O,P'-DDT	118.9	-
16	Carbophenothion	-*	-
17	P,P'-DDT	128.9	-

*Spiking level < MQL

4.12 The Result of Method Detection Limit (MDL) and Method Quantification Limit (MQL)

Method Detection Limit (MDL) as the amount of analyte in spiked sample solution that gave signal-to-noise ratio equal to 3 and Method Quantification Limit (MQL) equal to 10. Table 4.14; showed the summary of MDL and MQL of 17 organochlorine pesticides in sample matrix and compared between MQL and MRLs from USP regulations. Obviously, MQL of the developed method has less than MRLs from USP regulations approximately more than 10 times.

From the developed method as described in Figure 4.13, it could be concentrated to 0.8-fold for extraction samples. Then the developed method can be used for quantitation analysis in trace level amounts of organochlorine pesticide in Turmeric samples. Method Detection Limit and Method Quantification Limit of the extraction method were 0.5 – 15.4 ng/g and 1.4 – 51.2 ng/g respectively. For regulation of USP, MRLs is in the range of 50 – 3000 ng/g. Thus, the developed method for this study has the MQL less than MRLs as suggested in the USP regulations.



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Table 4.14 The Method Detection Limit (MDL), Method Quantification Limit (MQL) of 17 organochlorine pesticides in matrix and MRLs from USP regulations (ng/g)

No.	Pesticides	MDL (ng/g)	MQL (ng/g)	MRLs from USP regulations (ng/g)
1	α -BHC, HCH	0.9	2.9	300
2	β -BHC, HCH	15.4	51.2	
3	γ -BHC, HCH	0.5	1.8	600
4	Alachlor	1.2	3.9	20
5	Heptachlor epoxide isomer B	0.9	2.9	50
6	Heptachlor epoxide isomer A	0.7	2.2	
7	α -endosulfan	1.3	4.3	3000
8	Endrin	1.0	3.5	50
9	Aldrin	0.6	2.0	50*
10, 11	Dieldrin, P,P'-DDE	0.4	1.4	
12	O,P'-DDE	1.1	3.8	1000**
13	O,P'-DDD	1.4	4.5	
14	P,P'-DDD	1.4	4.8	
15	O,P'-DDT	1.0	3.4	
16	P,P'-DDT	0.6	2.1	
17	Carbophenothion	7.1	23.8	-

* Excepted P,P'-DDE

**Concluded P,P'-DDE

4.13 The Result of Precision

The developed method for extraction as described in Figure 4.13 was used to determine %recovery and precision of this method at spiking level of 5, 25 and 125 ng/g.

4.13.1 The Result of %Recovery and Precision of Turmeric Sample at Spiking Level 5 ng/g

From Tables 4.15 – 4.17 as shown on the same day of extraction or intra-assay precision by developed method at the spiking level of 5 ng/g.

On the first day of extraction, β -BHC, HCH and carbophenothion could not be detected because both had MQL higher than 5 ng/g and on the other day the results remained the same as in the first day. %RSD of the first day is less than 15% that could be acceptable according to the regulations (AOAC regulations accept 15% RSD in ng/g level). Moreover, on the other day, %RSD was also less than 15%. From Table 4.18, with reference to intermediate precision, all of the pesticides contain less than 15% of RSD.

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Table 4.15 %Recovery organochlorine pesticides at spiking level of 5 ng/g of first extraction in Turmeric sample (n=6)

Pesticides	%Recovery						Mean \pm SD	%RSD
	1	2	3	4	5	6		
α -BHC, HCH	103.2	103.6	104.0	100.3	99.5	105.4	102.7 \pm 2.3	2.2
β -BHC, HCH	.*	.*	.*	.*	.*	.*	-	-
γ -BHC, HCH	93.0	94.1	93.6	90.1	89.8	94.4	92.5 \pm 2.0	2.2
Alachlor	127.5	127.3	130.0	129.3	133.7	130.3	129.7 \pm 2.3	1.8
Aldrin	103.9	105.1	105.1	106.6	107.9	106.6	105.9 \pm 1.5	1.4
Heptachlor epoxide isomer B	73.0	72.4	72.5	65.2	65.4	73.2	70.3 \pm 3.9	5.5
Heptachlor epoxide isomer A	79.8	79.8	80.7	77.1	76.8	81.9	79.4 \pm 2.0	2.6
O,P'-DDE	97.4	98.6	95.9	93.8	93.9	98.1	96.3 \pm 2.1	2.2
α -endosulfan	64.2	65.1	64.2	62.9	64.9	65.6	64.5 \pm 1.0	1.5
Dieldrin, P,P'-DDE	89.5	89.2	91.2	89.4	87.7	92.6	89.9 \pm 1.7	1.9
O,P'-DDD	96.7	96.7	97.4	94.1	93.8	98.9	96.3 \pm 2.0	2.1
Endrin	50.0	50.2	50.9	48.6	49.1	51.7	50.1 \pm 1.1	2.3
P,P'-DDD	134.9	136.3	131.3	136.4	136.2	132.6	134.6 \pm 2.2	1.6
O,P'-DDT	118.9	118.3	116.9	115.6	116.5	117.3	117.3 \pm 1.2	1.0
Carbophenot hion	.*	.*	.*	.*	.*	.*	-	-
P,P'-DDT	128.9	127.2	128.1	132.5	130.2	129.9	129.5 \pm 1.9	1.4

*Spiking level < MQL

Table 4.16 %Recovery organochlorine pesticides at spiking level of 5 ng/g of second extraction in Turmeric sample (n=6)

Pesticides	%Recovery						Mean \pm SD	%RSD
	1	2	3	4	5	6		
α -BHC, HCH	102.5	102.1	105.2	99.2	103.5	103.8	102.7 \pm 2.1	2.0
β -BHC, HCH	_*	_*	_*	_*	_*	_*	-	-
γ -BHC, HCH	95.8	95.1	89.1	93.5	94.9	92.8	93.5 \pm 2.4	2.6
Alachlor	126.4	126.5	131.6	124.0	125.3	127.6	126.9 \pm 2.6	2.1
Aldrin	106.7	113.8	108.8	110.0	104.1	105.9	108.2 \pm 3.5	3.2
Heptachlor epoxide isomer B	70.2	73.4	69.6	75.2	68.8	73.7	71.8 \pm 2.6	3.6
Heptachlor epoxide isomer A	81.6	84.1	81.9	81.3	80.7	78.9	81.4 \pm 1.7	2.1
O,P'-DDE	112.0	108.6	110.8	109.3	105.2	111.8	109.6 \pm 2.5	2.3
α -endosulfan	65.3	64.9	67.5	66.6	64.9	63.1	65.4 \pm 1.5	2.4
Dieldrin, P,P'-DDE	89.3	84.0	90.0	87.7	89.2	88.4	88.1 \pm 2.2	2.5
O,P'-DDD	95.5	93.5	97.4	96.9	94.6	94.3	95.4 \pm 1.5	1.6
Endrin	51.0	50.6	51.3	52.6	48.7	51.7	51.0 \pm 1.3	2.5
P,P'-DDD	132.8	130.8	133.8	136.5	131.9	132.1	133.0 \pm 2.0	1.5
O,P'-DDT	115.9	125.7	117.5	116.6	115.8	116.3	118.0 \pm 3.8	3.3
Carbophenot hion	_*	_*	_*	_*	_*	_*	-	-
P,P'-DDT	129.6	141.9	130.1	131.7	129.9	130.3	132.3 \pm 4.8	3.6

*Spiking level < MQL

Table 4.17 %Recovery organochlorine pesticides at spiking level of 5 ng/g of third extraction in Turmeric sample (n=6)

Pesticides	%Recovery						Mean \pm SD	%RSD
	1	2	3	4	5	6		
α -BHC, HCH	101.9	103.8	102.0	103.6	99.3	105.0	102.6 \pm 2.0	2.0
β -BHC, HCH	_*	_*	_*	_*	_*	_*	-	-
γ -BHC, HCH	97.9	95.8	92.4	93.7	89.7	94.5	93.2 \pm 2.1	2.2
Alachlor	126.3	129.3	127.1	127.3	124.5	133.1	127.9 \pm 3.0	2.3
Aldrin	106.3	107.4	107.2	108.6	105.4	110.6	107.6 \pm 1.8	1.7
Heptachlor epoxide isomer B	72.8	75.3	73.9	73.6	68.2	73.2	72.9 \pm 2.4	3.4
Heptachlor epoxide isomer A	80.8	83.2	82.0	78.3	79.9	83.6	81.3 \pm 2.0	2.5
O,P'-DDE	99.9	102.0	99.6	98.3	95.5	100.8	99.3 \pm 2.3	2.3
α -endosulfan	63.7	66.6	64.9	65.0	62.2	65.5	64.6 \pm 1.5	2.4
Dieldrin, P,P'-DDE	89.9	91.5	91.7	90.9	88.7	93.5	91.0 \pm 1.7	1.8
O,P'-DDD	94.3	97.7	95.6	94.6	92.8	96.5	95.3 \pm 1.7	1.8
Endrin	50.3	51.6	51.1	50.0	48.5	52.4	50.7 \pm 1.4	2.7
P,P'-DDD	133.7	135.2	135.9	136.2	131.3	136.5	134.8 \pm 2.0	1.5
O,P'-DDT	117.2	119.9	117.9	117.6	115.5	119.9	118.0 \pm 1.7	1.5
Carbophenot hion	_*	_*	_*	_*	_*	_*	-	-
P,P'-DDT	129.3	132.8	130.5	131.7	128.2	133.9	131.1 \pm 2.2	1.6

*Spiking level < MQL

Table 4.18 Mean of %recovery in 3 days of organochlorine pesticides (intermediate precision) at spiking level of 5 ng/g in Turmeric sample (n=3)

Pesticides	%Recovery			Mean	SD	%RSD
	1	2	3			
α -BHC, HCH	102.7	102.7	102.6	102.7	0.1	0.1
β -BHC, HCH	-*	-*	-*	-	-	-
γ -BHC, HCH	92.5	93.5	93.2	93.1	0.5	0.6
Alachlor	129.7	126.9	127.9	128.2	1.4	1.1
Aldrin	105.9	108.2	107.6	107.2	1.2	1.1
Heptachlor epoxide isomer B	70.3	71.8	72.8	71.6	1.3	1.8
Heptachlor epoxide isomer A	79.4	81.4	81.3	80.7	1.2	1.4
O,P'-DDE	96.3	109.6	99.3	101.7	7.0	6.9
α -endosulfan	64.5	65.4	64.6	64.8	0.5	0.7
Dieldrin, P,P'-DDE	89.9	88.1	91.0	89.7	1.5	1.7
O,P'-DDD	96.3	95.4	95.3	95.6	0.6	0.6
Endrin	50.1	51.0	50.7	50.6	0.5	0.9
P,P'-DDD	134.7	133.0	134.8	134.1	1.0	0.7
O,P'-DDT	117.3	118.0	118.0	117.7	0.4	0.4
Carbophenot hion	-*	-*	-*	-	-	-
P,P'-DDT	129.5	132.3	131.1	130.9	1.4	1.1

*Spiking level < MQL

4.13.2 The Result of %Recovery and Precision of Turmeric Sample at Spiking Level of 25 ng/g.

From Table 4.19 – 4.21, it shows %RSD on the same day of extraction or intra-assay precision by developed method at the spiking level of 25 ng/g. For β -BHC, HCH could not be detected because had MQL higher than 25 ng/g and on the other day the results remained the same as in the first day. On the first day of extraction, all of the 16 organochlorine pesticides had the %RSD less than 15% acceptable by the regulations (AOAC regulations accept 15% RSD in ng/g level). Moreover, on the other day, the %RSD was less than 15% too. From Table 4.22, with reference to intermediate precision, all of the pesticides contained %RSD less than 15%. Apart from this, it was obvious that when the value of the spiking level was high, the %RSD would be at the opposite end—low.



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Table 4.19 %Recovery of organochlorine pesticides at spiking level of 25 ng/g of first extraction in Turmeric sample (n=6)

Pesticides	%Recovery						Mean \pm SD	%RSD
	1	2	3	4	5	6		
α -BHC, HCH	109.7	110.6	110.7	106.6	111.8	113.9	110.6 \pm 2.4	2.2
β -BHC, HCH	_*	_*	_*	_*	_*	_*	-	-
γ -BHC, HCH	100.1	100.7	100.8	95.4	101.3	101.8	100.0 \pm 2.3	2.3
Alachlor	130.4	132.1	131.9	128.6	131.3	132.5	131.1 \pm 1.4	1.1
Aldrin	109.3	109.7	110.4	112.8	110.4	108.4	110.1 \pm 1.5	1.4
Heptachlor epoxide isomer B	74.3	75.0	74.7	72.6	70.7	77.5	74.1 \pm 2.3	3.1
Heptachlor epoxide isomer A	87.4	87.4	87.8	90.4	89.3	85.9	88.0 \pm 1.6	1.8
O,P'-DDE	103.7	102.8	103.9	108.2	104.4	100.9	104.0 \pm 2.4	2.3
α -endosulfan	69.1	69.2	69.1	72.5	67.3	71.0	69.7 \pm 1.8	2.6
Dieldrin, P,P'-DDE	77.8	77.3	76.2	75.5	80.9	76.6	77.4 \pm 1.9	2.5
O,P'-DDD	105.6	103.3	106.6	109.1	107.1	104.8	106.1 \pm 2.0	1.9
Endrin	59.8	59.8	59.7	57.6	56.8	57.4	58.5 \pm 1.4	2.4
P,P'-DDD	132.5	131.9	132.4	129.6	133.6	133.1	132.2 \pm 1.4	1.1
O,P'-DDT	110.3	110.6	113.0	110.5	110.7	110.7	111.0 \pm 1.0	0.9
Carbophenot hion	179.6	179.6	178.8	182.6	180.8	176.5	179.6 \pm 2.1	1.1
P,P'-DDT	124.5	125.4	126.0	129.4	126.5	128.3	126.7 \pm 1.8	1.5

*Spiking level < MQL

Table 4.20 %Recovery of organochlorine pesticides at spiking level of 25 ng/g of second extraction in Turmeric sample (n=6)

Pesticides	%Recovery						Mean \pm SD	%RSD
	1	2	3	4	5	6		
α -BHC, HCH	113.1	112.5	113.8	111.5	112.7	109.7	112.2 \pm 1.5	1.3
β -BHC, HCH	-*	-*	-*	-*	-*	-*	-	-
γ -BHC, HCH	99.2	100.8	98.7	102.7	100.2	99.8	100.2 \pm 1.4	1.4
Alachlor	132.4	131.2	131.5	130.7	133.4	134.5	132.3 \pm 1.4	1.1
Aldrin	105.7	107.0	106.8	109.9	107.8	106.2	107.2 \pm 1.5	1.4
Heptachlor epoxide isomer B	76.8	75.8	74.2	80.3	76.5	77.5	76.8 \pm 2.1	2.7
Heptachlor epoxide isomer A	84.7	82.9	86.8	85.6	84.3	85.9	85.0 \pm 1.4	1.6
O,P'-DDE	99.6	102.7	101.8	104.1	100.7	102.9	102.0 \pm 1.6	1.6
α -endosulfan	65.6	70.5	71.7	69.6	69.7	68.6	69.3 \pm 2.1	3.0
Dieldrin, P,P'-DDE	74.7	80.8	79.7	79.7	78.5	80.9	79.0 \pm 2.3	2.9
O,P'-DDD	102.5	105.6	103.4	103.9	102.7	104.5	103.8 \pm 1.2	1.1
Endrin	58.0	58.9	59.4	58.5	57.5	58.4	58.4 \pm 0.7	1.1
P,P'-DDD	134.3	133.7	134.5	135.8	134.1	131.6	134.0 \pm 1.4	1.0
O,P'-DDT	108.4	112.5	110.7	110.6	108.6	109.7	110.1 \pm 1.5	1.4
Carbophenot hion	179.1	178.5	181.4	177.8	179.7	176.4	178.8 \pm 1.7	1.0
P,P'-DDT	121.9	126.2	123.5	123.7	123.9	124.6	124.0 \pm 1.4	1.1

*Spiking level < MQL

Table 4.21 %Recovery organochlorine pesticides at spiking level of 25 ng/g of third extraction in Turmeric sample (n=6)

Pesticides	%Recovery						Mean \pm SD	%RSD
	1	2	3	4	5	6		
α -BHC, HCH	110.9	109.7	112.3	112.9	114.8	108.1	111.5 \pm 2.4	2.1
β -BHC, HCH	_*	_*	_*	_*	_*	_*	-	-
γ -BHC, HCH	101.1	100.5	102.4	103.4	105.8	99.7	102.1 \pm 2.2	2.2
Alachlor	129.1	130.9	131.1	131.8	133.7	129.6	131.0 \pm 1.6	1.2
Aldrin	108.7	107.7	109.6	110.9	111.3	107.5	109.3 \pm 1.6	1.5
Heptachlor epoxide isomer B	73.3	75.7	74.6	77.3	72.6	76.1	74.9 \pm 1.8	2.4
Heptachlor epoxide isomer A	86.3	85.8	87.2	90.4	85.6	88.6	87.3 \pm 1.9	2.1
O,P'-DDE	100.8	103.1	103.5	104.9	99.5	105.7	102.9 \pm 2.4	2.3
α -endosulfan	67.2	68.5	67.9	69.8	65.2	71.9	68.4 \pm 2.3	3.3
Dieldrin, P,P'-DDE	76.0	78.7	77.9	78.1	75.7	79.3	77.6 \pm 1.5	1.9
O,P'-DDD	105.6	103.3	106.6	109.1	107.1	105.0	106.1 \pm 2.0	1.9
Endrin	57.5	58.8	57.1	58.3	55.8	58.8	57.7 \pm 1.2	2.0
P,P'-DDD	133.5	132.1	132.7	132.0	131.2	134.9	132.7 \pm 1.3	1.0
O,P'-DDT	110.6	109.7	110.4	111.5	108.3	112.9	110.6 \pm 1.6	1.4
Carbophenot hion	177.3	178.4	178.8	179.5	176.9	182.7	178.9 \pm 2.1	1.2
P,P'-DDT	125.8	124.5	127.5	126.6	123.4	128.5	126.1 \pm 1.9	1.5

*Spiking level < MQL

Table 4.22 Mean of %recovery in 3 days of organochlorine pesticides (intermediate precision) at spiking level of 25 ng/g in Turmeric sample (n=3)

Pesticides	%Recovery			Mean	SD	%RSD
	1	2	3			
α -BHC, HCH	110.6	112.2	111.5	111.4	0.8	0.8
β -BHC, HCH	-*	-*	-*	-	-	-
γ -BHC, HCH	100.0	100.2	102.1	100.8	1.2	1.2
Alachlor	131.1	132.3	131.0	131.5	0.7	0.5
Aldrin	110.1	107.2	109.3	108.9	1.5	1.4
Heptachlor epoxide isomer B	74.1	76.8	74.9	75.3	1.4	1.9
Heptachlor epoxide isomer A	88.0	85.0	87.3	86.8	1.6	1.8
O,P'-DDE	104.0	102.0	102.9	102.9	1.0	1.0
α -endosulfan	69.7	69.3	68.4	69.1	0.6	0.9
Dieldrin, P,P'-DDE	77.4	79.0	77.6	78.0	0.9	1.2
O,P'-DDD	106.1	103.8	106.1	105.3	1.3	1.3
Endrin	58.5	58.4	57.7	58.2	0.5	0.8
P,P'-DDD	132.2	134.0	132.7	133.0	0.9	0.7
O,P'-DDT	111.0	110.1	110.6	110.5	0.5	0.4
Carbophenot hion	179.6	178.8	178.9	179.1	0.4	0.3
P,P'-DDT	126.7	124.0	126.1	125.6	1.4	1.1

*Spiking level < MQL

4.13.3 The Result of %Recovery and Precision of Turmeric Sample at Spiking Level of 125 ng/g.

From Table 4.23 – 4.25 it shows %RSD on the same day of extraction or intra-assay precision by developed method at the spiking level of 125 ng/g. On the first day of extraction, all of the 17 organochlorine pesticides had the %RSD less than 15% acceptable by the regulations (AOAC regulations accept 15% RSD in ng/g level). Moreover, on the other day, the %RSD was less than 15% too. From Table 4.22, with reference to intermediate precision, all of the pesticides contained %RSD less than 15%. At 25 ng/g as previously mentioned, when the value of the spiking level was high the %RSD was quite low.



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Table 4.23 %Recovery organochlorine pesticides at spiking level of 125 ng/g of first extraction in Turmeric sample (n=6)

Pesticides	%Recovery						Mean \pm SD	%RSD
	1	2	3	4	5	6		
α -BHC, HCH	102.0	102.2	99.3	104.1	101.9	102.1	101.9 \pm 1.5	1.5
β -BHC, HCH	181.0	179.6	182.1	184.9	178.7	182.8	181.5 \pm 2.2	1.2
γ -BHC, HCH	101.5	100.9	100.9	104.3	102.6	100.3	101.7 \pm 1.5	1.5
Alachlor	133.3	133.5	131.8	135.4	131.3	132.4	132.9 \pm 1.5	1.1
Aldrin	108.3	107.6	107.7	111.6	109.1	107.6	108.7 \pm 1.6	1.5
Heptachlor epoxide isomer B	69.9	69.5	69.3	71.7	73.5	70.5	70.8 \pm 1.6	2.3
Heptachlor epoxide isomer A	79.8	78.7	80.1	83.6	80.6	79.5	80.4 \pm 1.7	2.1
O,P'-DDE	109.0	109.8	108.5	112.3	108.8	110.2	109.8 \pm 1.4	1.3
α -endosulfan	68.1	67.1	68.1	70.8	69.3	68.5	68.7 \pm 1.3	1.9
Dieldrin, P,P'-DDE	73.9	74.0	73.5	75.7	76.7	74.4	74.7 \pm 1.2	1.7
O,P'-DDD	100.1	101.9	102.4	103.7	99.2	102.8	101.7 \pm 1.7	1.7
Endrin	59.3	59.3	59.2	62.4	60.5	58.2	59.8 \pm 1.4	2.4
P,P'-DDD	139.0	138.2	139.7	143.3	140.3	138.5	139.8 \pm 1.9	1.3
O,P'-DDT	116.0	113.0	113.1	116.4	114.1	112.4	114.2 \pm 1.7	1.5
Carbophenot hion	175.8	174.7	175.2	178.5	178.0	179.7	177.0 \pm 2.0	1.2
P,P'-DDT	123.4	124.5	123.4	126.6	125.9	122.6	124.4 \pm 1.6	1.3

Table 4.24 %Recovery organochlorine pesticides at spiking level of 125 ng/g of second extraction in Turmeric sample (n=6)

Pesticides	%Recovery						Mean \pm SD	%RSD
	1	2	3	4	5	6		
α -BHC, HCH	100.3	101.4	101.4	99.9	102.4	103.6	101.5 \pm 1.4	1.3
β -BHC, HCH	180.3	179.5	179.6	177.6	178.3	182.9	179.7 \pm 1.9	1.0
γ -BHC, HCH	102.8	101.2	102.4	100.7	101.3	105.5	102.3 \pm 1.8	1.7
Alachlor	135.7	130.8	133.2	131.8	131.6	134.2	132.9 \pm 1.8	1.4
Aldrin	106.5	107.8	106.1	106.0	109.6	110.5	107.7 \pm 1.9	1.8
Heptachlor epoxide isomer B	69.5	68.4	70.3	69.7	72.5	73.9	70.7 \pm 2.1	2.9
Heptachlor epoxide isomer A	81.9	79.6	81.7	79.0	83.2	83.9	81.6 \pm 1.9	2.4
O,P'-DDE	110.1	109.5	111.6	109.2	112.7	113.6	111.1 \pm 1.8	1.6
α -endosulfan	69.7	68.2	68.4	67.5	70.2	70.8	69.1 \pm 1.3	1.9
Dieldrin, P,P'-DDE	75.4	75.7	74.3	73.9	76.3	75.6	75.2 \pm 0.9	1.2
O,P'-DDD	101.5	99.7	100.8	101.2	102.4	103.5	101.5 \pm 1.3	1.3
Endrin	60.9	59.1	61.4	58.1	60.8	62.1	60.4 \pm 1.5	2.5
P,P'-DDD	135.9	136.8	137.2	135.9	139.6	138.8	137.4 \pm 1.5	1.1
O,P'-DDT	113.0	115.2	114.5	114.4	114.4	117.7	114.9 \pm 1.6	1.4
Carbophenot hion	176.4	177.6	175.1	174.8	178.2	179.8	177.0 \pm 1.9	1.1
P,P'-DDT	125.0	125.5	124.7	124.3	126.9	127.6	125.7 \pm 1.3	1.0

Table 4.25 %Recovery organochlorine pesticides at spiking level of 125 ng/g of third extraction in Turmeric sample (n=6)

Pesticides	%Recovery						Mean \pm SD	%RSD
	1	2	3	4	5	6		
α -BHC, HCH	101.5	101.8	103.7	100.2	102.2	104.9	102.4 \pm 1.7	1.6
β -BHC, HCH	179.3	180.7	181.9	177.9	181.7	182.8	180.7 \pm 1.8	1.0
γ -BHC, HCH	102.4	104.1	102.8	103.9	103.3	100.8	102.9 \pm 1.2	1.2
Alachlor	132.5	133.4	135.8	131.2	133.3	131.7	133.0 \pm 1.6	1.2
Aldrin	108.9	107.0	111.0	108.7	109.9	110.2	109.3 \pm 1.4	1.3
Heptachlor epoxide isomer B	70.6	71.3	73.4	68.8	72.4	73.6	71.7 \pm 1.8	2.6
Heptachlor epoxide isomer A	79.2	81.1	83.2	79.7	83.9	82.6	81.6 \pm 1.9	2.4
O,P'-DDE	111.5	110.0	112.6	110.3	112.2	113.3	111.6 \pm 1.3	1.2
α -endosulfan	68.8	68.4	71.3	67.8	70.3	70.5	69.5 \pm 1.4	2.0
Dieldrin, P,P'-DDE	74.0	73.4	74.1	72.8	75.1	74.5	74.0 \pm 0.8	1.1
O,P'-DDD	100.5	101.7	101.3	99.5	102.0	102.9	101.3 \pm 1.2	1.2
Endrin	60.6	58.9	61.4	59.9	62.5	61.3	60.8 \pm 1.3	2.1
P,P'-DDD	137.9	138.1	139.2	135.8	139.7	141.6	138.7 \pm 1.9	1.4
O,P'-DDT	113.2	114.9	114.2	112.2	114.9	116.6	114.3 \pm 1.5	1.3
Carbophenot hion	176.2	175.8	177.7	174.3	178.6	178.9	176.9 \pm 1.8	1.0
P,P'-DDT	124.5	125.8	125.8	124.1	127.7	126.9	125.8 \pm 1.4	1.1

Table 4.26 Mean of %recovery in 3 days of organochlorine pesticides (intermediate precision) at spiking level of 125 ng/g in Turmeric sample (n=3)

Pesticides	%Recovery			Mean	SD	%RSD
	1	2	3			
α -BHC, HCH	101.9	101.5	102.4	101.9	0.5	0.5
β -BHC, HCH	181.5	179.7	180.7	180.6	0.9	0.5
γ -BHC, HCH	101.7	102.3	102.9	102.3	0.6	0.6
Alachlor	132.9	132.9	133.0	132.9	0.0	0.0
Aldrin	108.7	107.7	109.3	108.6	0.8	0.7
Heptachlor epoxide isomer B	70.8	70.7	71.7	71.1	0.6	0.8
Heptachlor epoxide isomer A	80.4	81.6	81.6	81.2	0.7	0.9
O,P'-DDE	109.8	111.1	111.6	110.8	1.0	0.9
α -endosulfan	68.7	69.1	69.5	69.1	0.4	0.6
Dieldrin, P,P'-DDE	74.7	75.2	74.0	74.6	0.6	0.8
O,P'-DDD	101.7	101.5	101.3	101.5	0.2	0.2
Endrin	59.8	60.4	60.8	60.3	0.5	0.8
P,P'-DDD	139.8	137.4	138.7	138.6	1.2	0.9
O,P'-DDT	114.2	114.9	114.3	114.5	0.4	0.3
Carbophenot hion	177.0	177.0	176.9	177.0	0.0	0.0
P,P'-DDT	124.4	125.7	125.8	125.3	0.8	0.6

4.14 The Result of Accuracy

From Table 4.27, %recovery of all organochlorine pesticides in Turmeric sample at spiking level of 5 are in the range of 50.56 – 134.14%, at spiking level of 25 are in the range of 58.22 – 179.13% and at spiking level of 125 ng/g are in the range of 60.33 – 180.64%. This is within the acceptable range set forth by AOAC Regulations—%recovery 70 – 125 in ng/g level. This in turn shows that the developed method can yield high accuracy with samples of low concentration.



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Table 4.27 Accuracy of extraction method at spiking level of 5, 25 and 125 ng/g

No.	Pesticides	Mean of %Recovery		
		Spiking level of 5 ng/g	Spiking level of 25 ng/g	Spiking level of 125 ng/g
1	α -BHC, HCH	102.7 \pm 0.1	111.4 \pm 0.8	101.9 \pm 0.5
2	β -BHC, HCH	-*	-*	180.6 \pm 0.9
3	γ -BHC, HCH	93.1 \pm 0.5	100.8 \pm 1.2	102.3 \pm 0.6
4	Alachlor	128.2 \pm 1.4	131.5 \pm 0.7	132.9 \pm 0.1
5	Aldrin	107.2 \pm 1.2	108.9 \pm 1.5	108.6 \pm 0.8
6	Heptachlor epoxide isomer B	71.6 \pm 1.3	75.3 \pm 1.5	71.1 \pm 0.6
7	Heptachlor epoxide isomer A	80.7 \pm 1.2	86.8 \pm 1.6	81.2 \pm 0.7
8	O,P'-DDE	101.7 \pm 7.0	102.9 \pm 1.0	110.8 \pm 1.0
9	α -endosulfan	64.8 \pm 0.5	69.1 \pm 0.6	69.1 \pm 0.4
10, 11	Dieldrin, P,P'-DDE	89.7 \pm 1.5	78.0 \pm 0.9	74.6 \pm 0.6
12	O,P'-DDD	95.6 \pm 0.6	105.3 \pm 1.3	101.5 \pm 0.2
13	Endrin	50.6 \pm 0.5	58.2 \pm 0.5	60.3 \pm 0.5
14	P,P'-DDD	134.1 \pm 1.0	133.0 \pm 0.9	138.6 \pm 1.2
15	O,P'-DDT	117.7 \pm 0.4	110.5 \pm 0.5	114.5 \pm 0.4
16	Carbophenothion	-*	179.1 \pm 0.4	177.0 \pm 0.1
17	P,P'-DDT	130.9 \pm 1.4	125.6 \pm 1.4	125.3 \pm 0.8

*Spiking level < MQL

4.15 The Result of Stability

From Table 4.28, spiked sample of organochlorine pesticides in Turmeric sample at spiking level 125 ng/g was extracted with the developed method in Figure 4.13. These solutions were divided in 11 vials and stored in the same conditions. The results were calculated to find the %recovery and compare it with matrix standard solutions within the same injection date in each vial for 50 days. By using the average value of %recovery to find the precision at the spiking level of 125 ng/g \pm 2SD, the number of days that an interested substance can be kept is determined. Details are shown in Figure 4.20 to 4.35 and Table 4.29.

In Table 4.29, it is clear that the number of days that each substance can be kept is different. Most can be kept no longer than three days if no deviate effect is to be determined except beta-BHC,HCH (5 days) and O,P'-DDE (15 days).



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Table 4.28 The result of stability of extraction method at spiking level of 125 ng/g

Pesticides	%Recovery of storage days										
	1	2	3	10	15	17	22	30	35	40	50
α -BHC, HCH	102.0	100.3	101.5	89.9	87.8	85.5	84.4	79.1	77.2	75.1	74.3
β -BHC, HCH	181.0	180.3	179.3	177.9	176.9	174.7	174.2	172.5	169.1	167.1	166.2
γ -BHC, HCH	101.5	102.8	102.4	90.5	88.6	84.8	83.6	80.7	73.1	70.2	67.9
Alachlor	133.3	135.7	132.5	119.7	117.0	113.1	112.8	111.4	109.2	106.2	104.8
Aldrin	108.3	106.5	108.9	99.2	98.4	92.0	91.5	87.3	85.8	82.4	80.2
isomer B	69.9	69.5	70.6	63.6	62.7	55.7	55.5	53.1	51.5	48.2	47.1
isomer A	79.8	81.9	79.2	72.1	71.9	64.1	64.4	60.3	58.5	55.2	53.2
O,P'-DDE	109.0	110.1	111.5	108.2	109.0	100.5	100.9	94.2	93.1	89.2	87.7
α -endosulfan	68.1	69.7	68.8	63.4	61.9	57.9	57.9	54.2	52.1	49.2	47.2
Dieldrin, P,P'-DDE	73.9	75.4	74.0	62.2	61.6	57.4	57.6	55.3	52.9	50.6	46.9
O,P'-DDD	100.1	101.5	100.5	88.6	88.5	83.1	84.4	81.1	79.2	76.4	74.5
Endrin	59.3	60.9	60.6	50.6	46.9	46.2	44.6	42.2	41.5	39.4	37.2
P,P'-DDD	139.0	135.9	137.9	126.2	124.7	122.2	123.4	121.1	120.1	118.2	116.2
O,P'-DDT	116.0	113.0	113.2	100.0	101.3	95.1	96.5	92.9	87.1	85.5	82.2
Carbophenot hion	175.8	176.4	176.2	158.0	154.9	155.1	152.1	150.1	149.5	147.9	144.9
P,P'-DDT	123.4	125.0	124.5	108.6	108.8	105.0	103.2	100.2	98.1	96.2	94.6

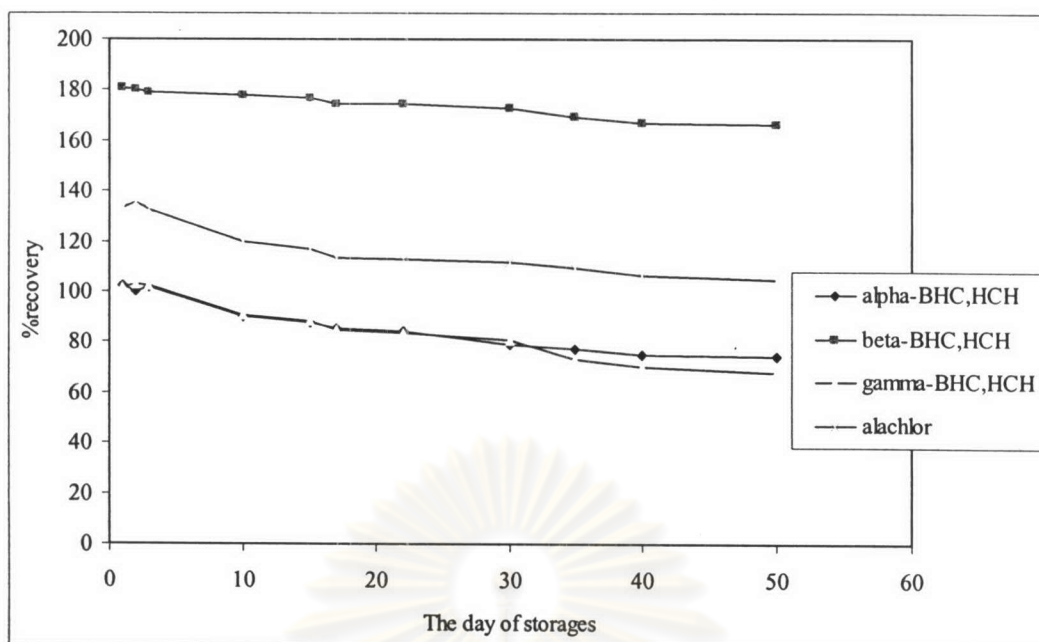


Figure 4.16 Stability of the α -BHC, HCH, β -BHC, HCH, γ -BHC, HCH and Alachlor in spiked sample at spiking level of 125 ng/g

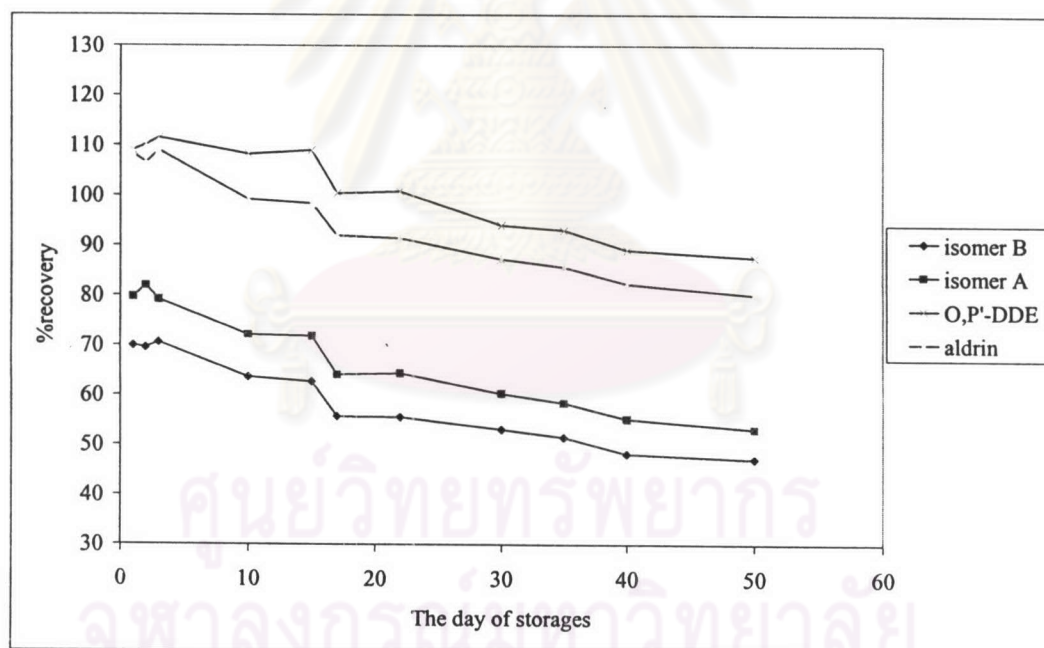


Figure 4.17 Stability of the Heptachlor epoxide isomer B, isomer A, O,P'-DDE and aldrin in spiked sample at spiking level of 125 ng/g

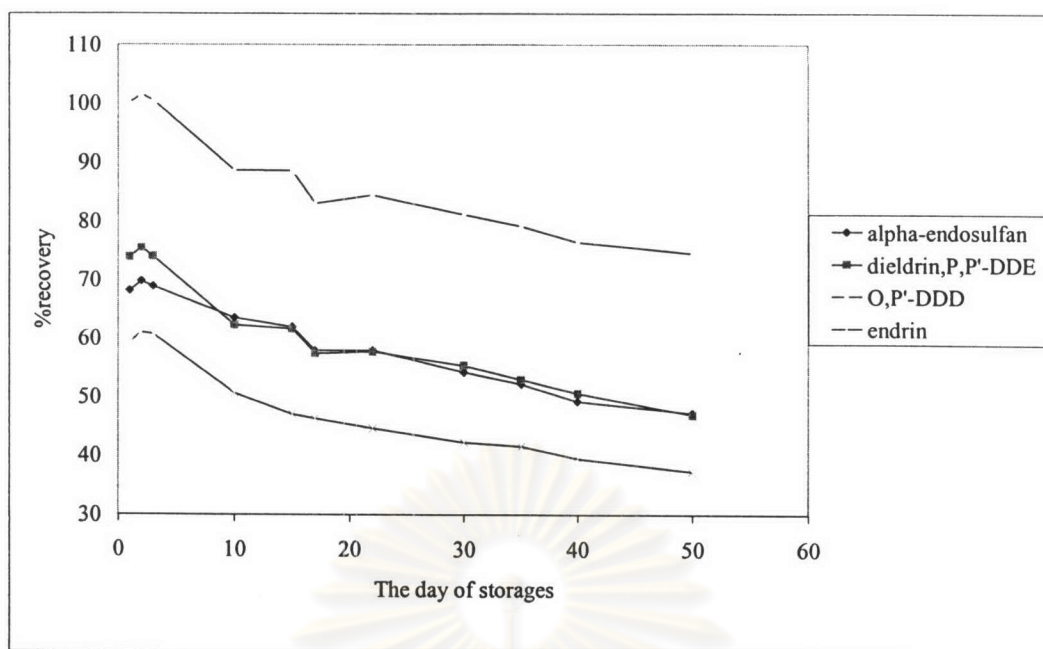


Figure 4.18 Stability of the_alpha-endosulfan, dieldrin, P,P'-DDE and endrin in spiked sample at spiking level of 125 ng/g

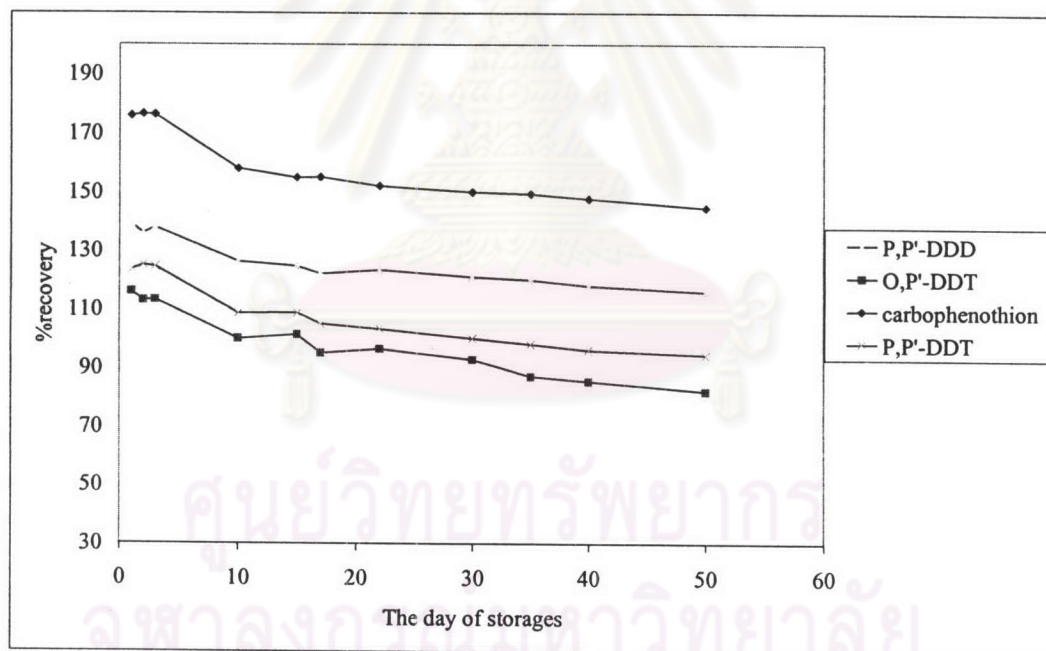


Figure 4.19 Stability of the_P,P'-DDD, O,P'-DDT, carbophenothion and P,P'-DDT in spiked sample at spiking level of 125 ng/g

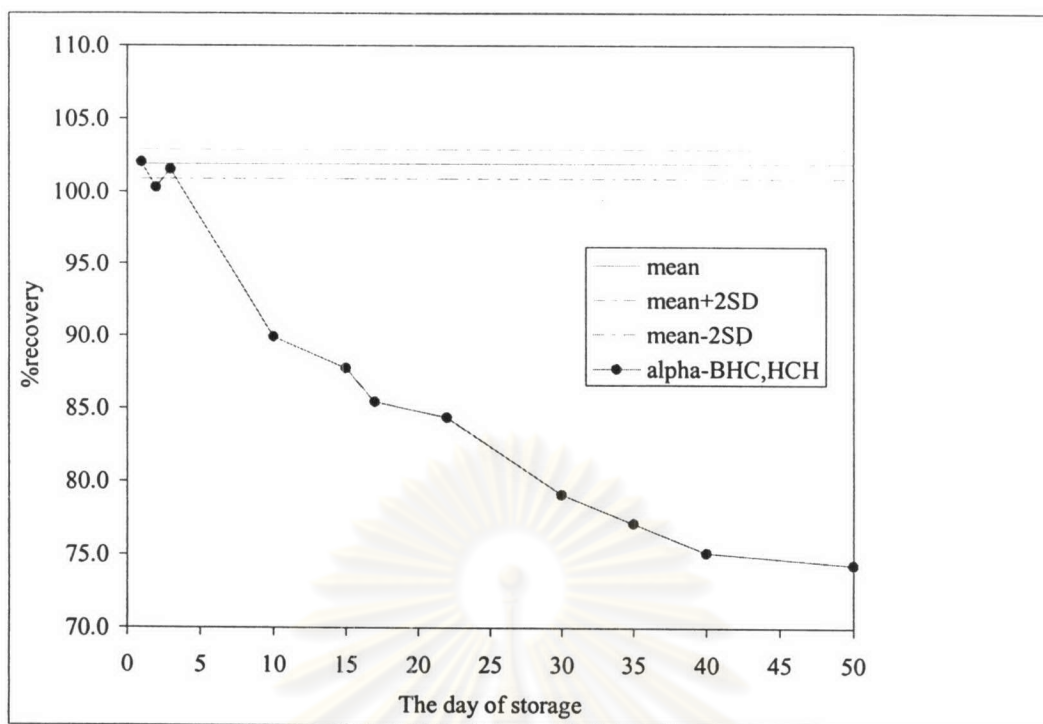


Figure 4.20 Control charge of alpha-BHC, HCH

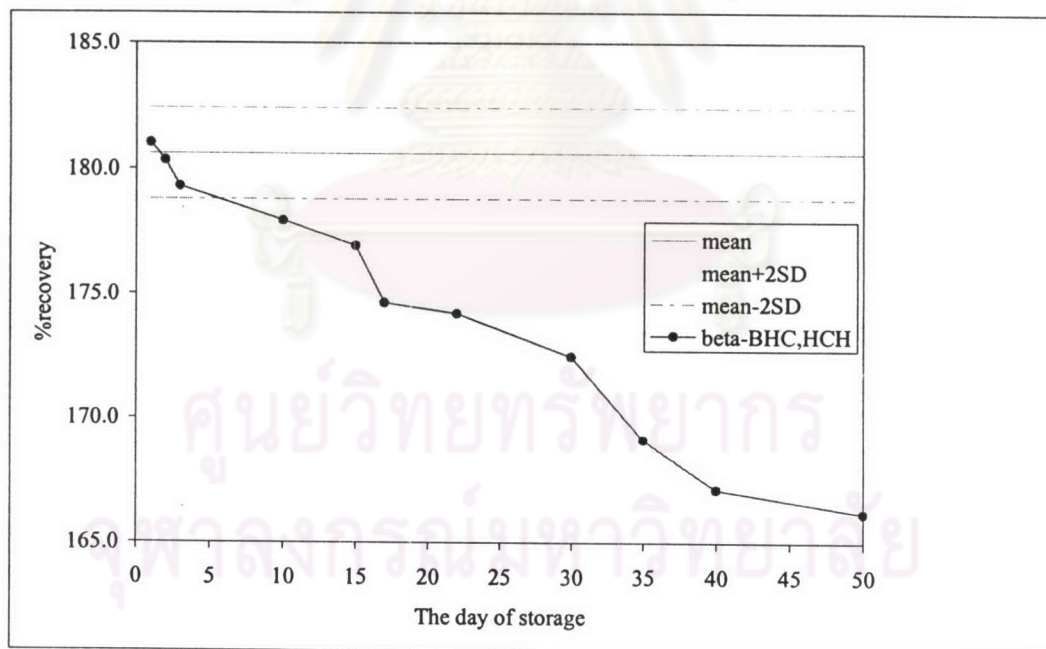


Figure 4.21 Control charge of beta-BHC, HCH

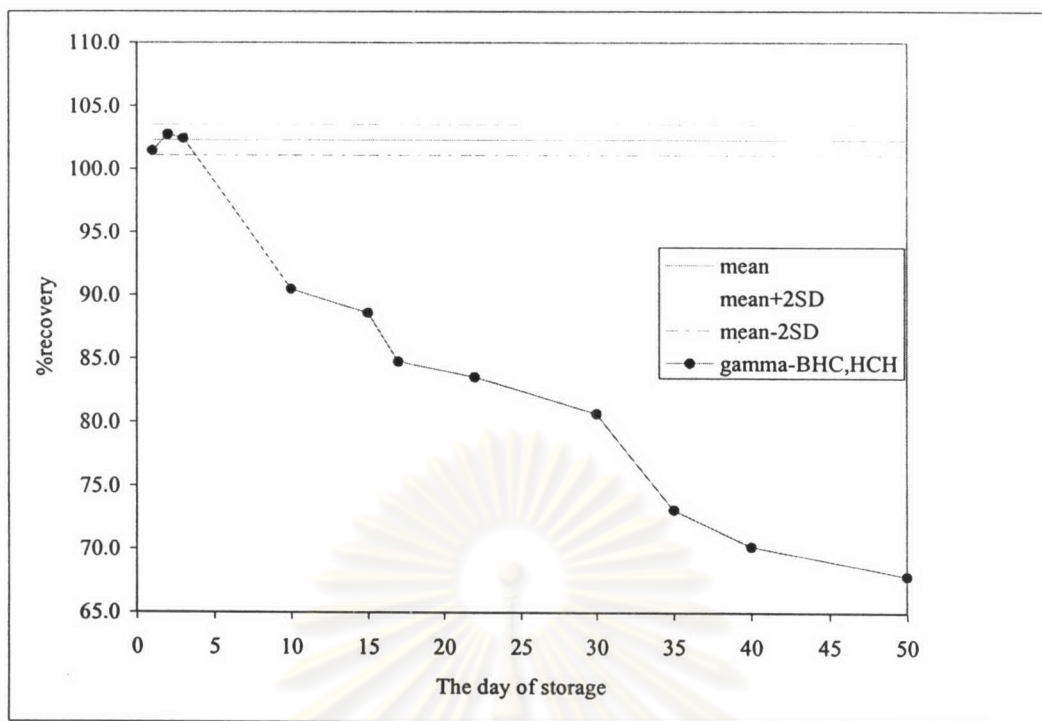


Figure 4.22 Control charge of gamma-BHC, HCH

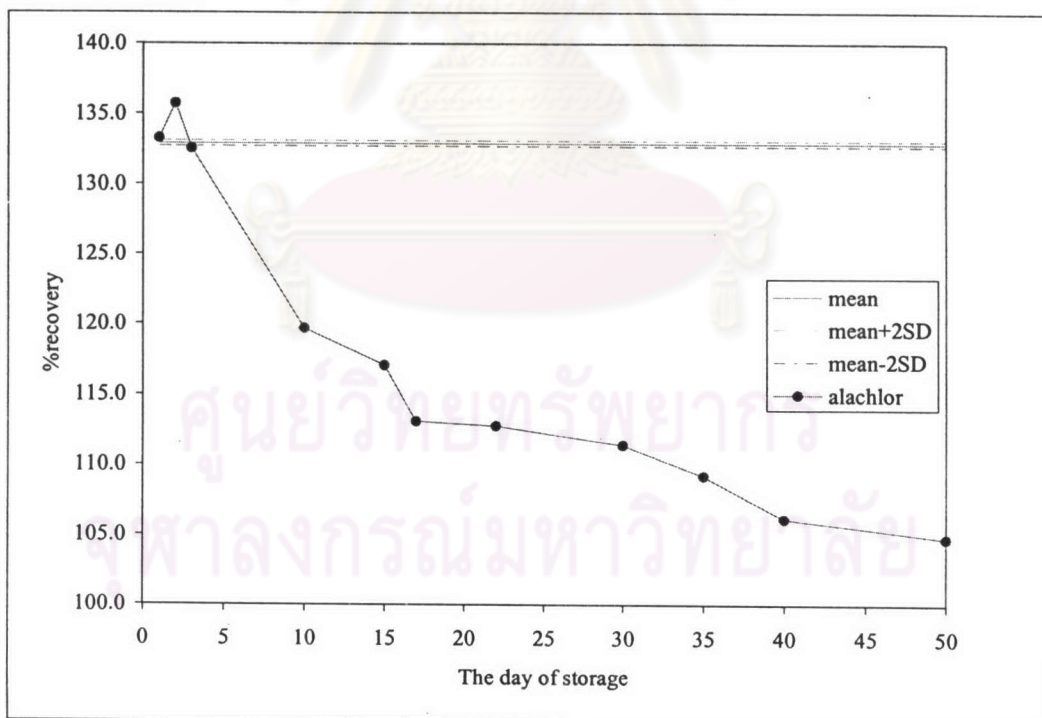


Figure 4.23 Control charge of Alachlor

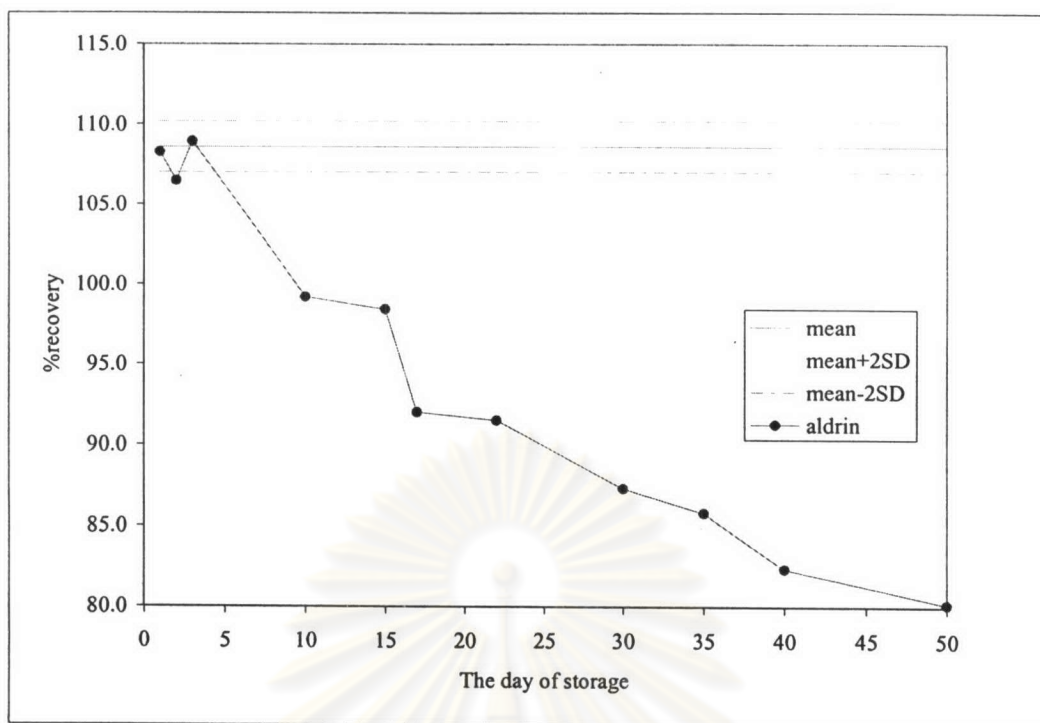


Figure 4.24 Control charge of Aldrin

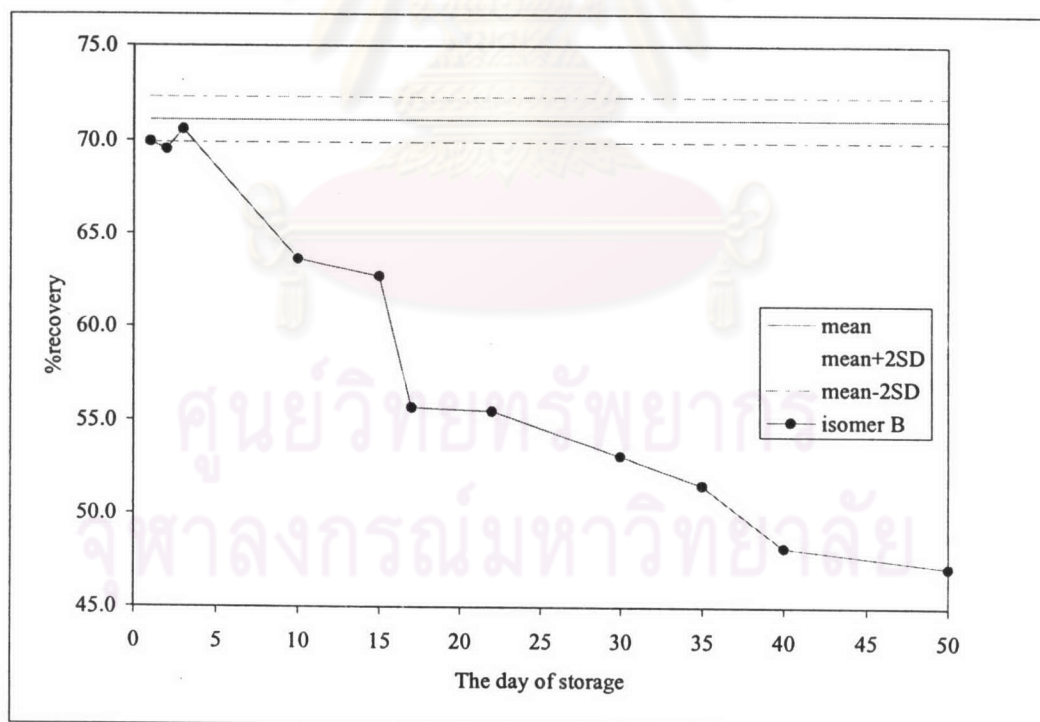


Figure 4.25 Control charge of isomer B

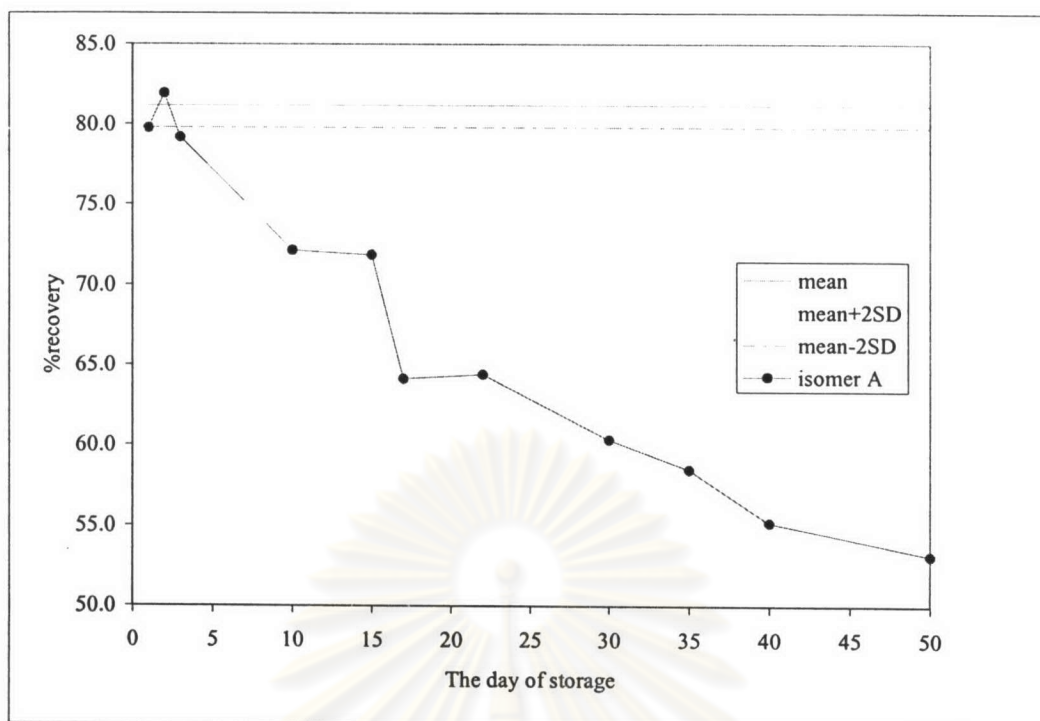


Figure 4.26 Control charge of isomer A

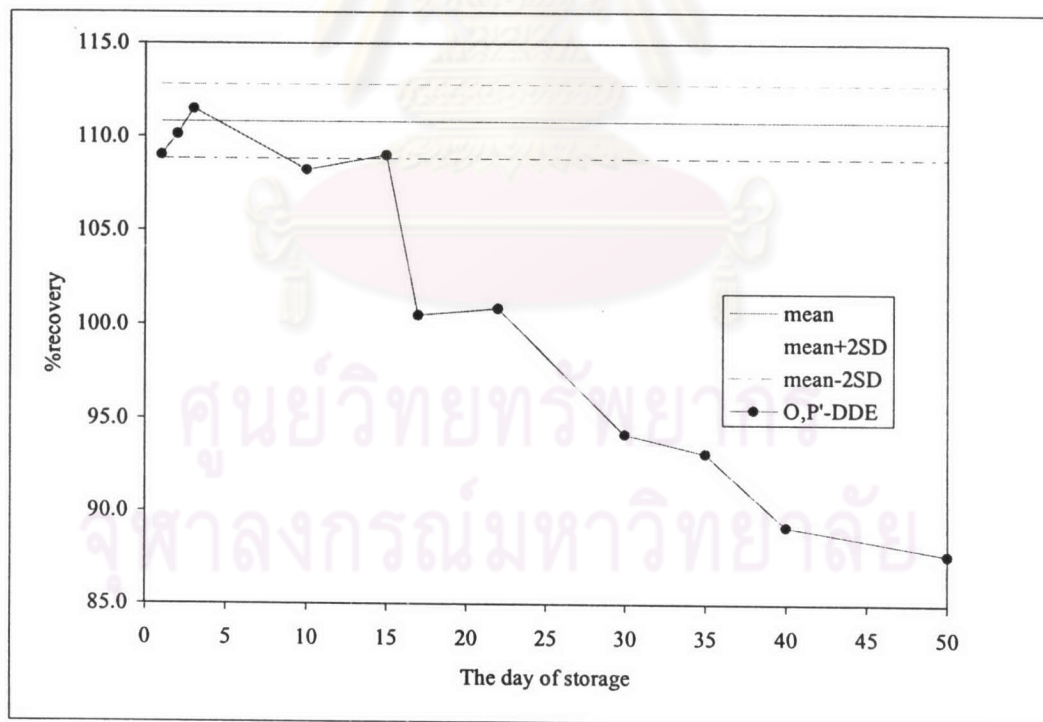


Figure 4.27 Control charge of O,P'-DDE

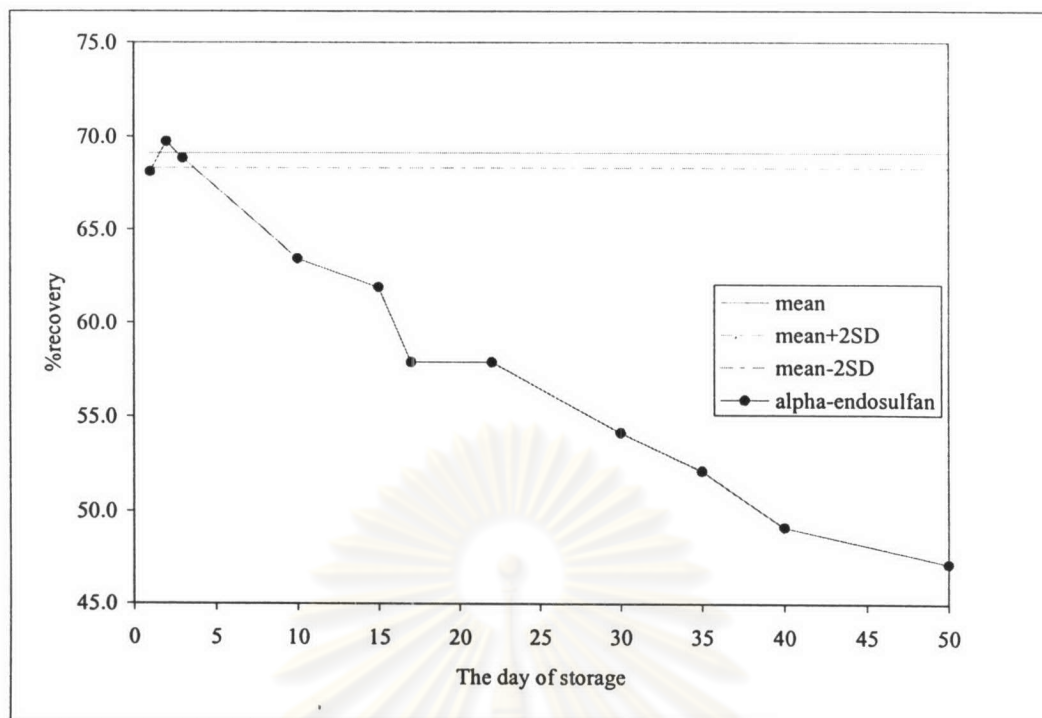


Figure 4.28 Control charge of alpha-endosulfan

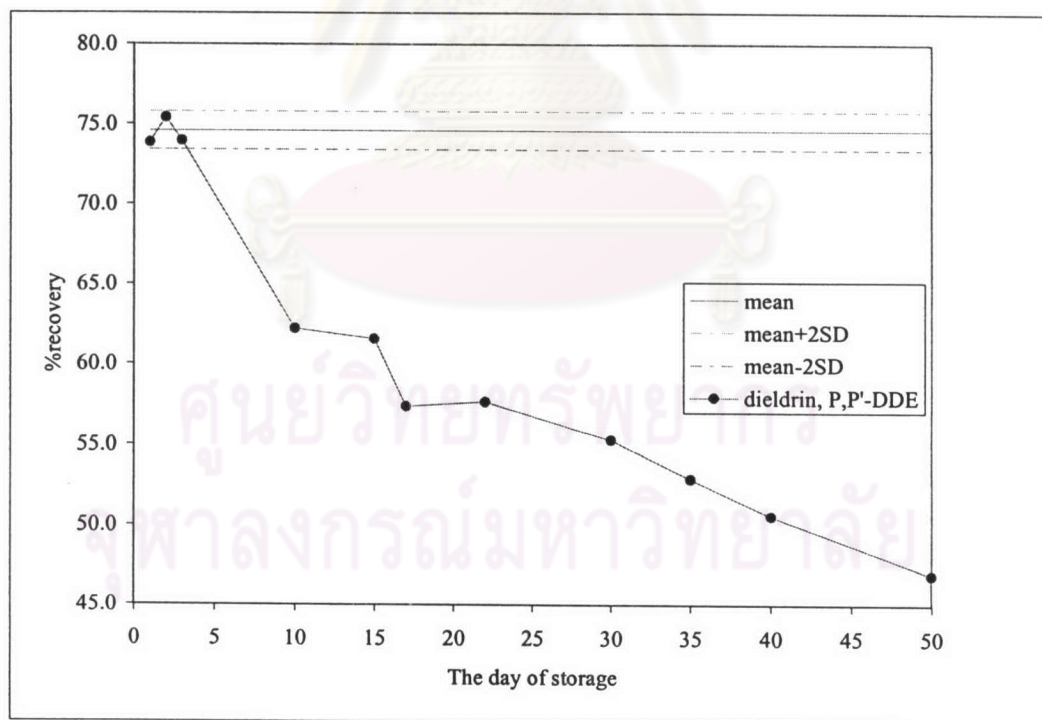


Figure 4.29 Control charge of Dieldrin, P,P'-DDE

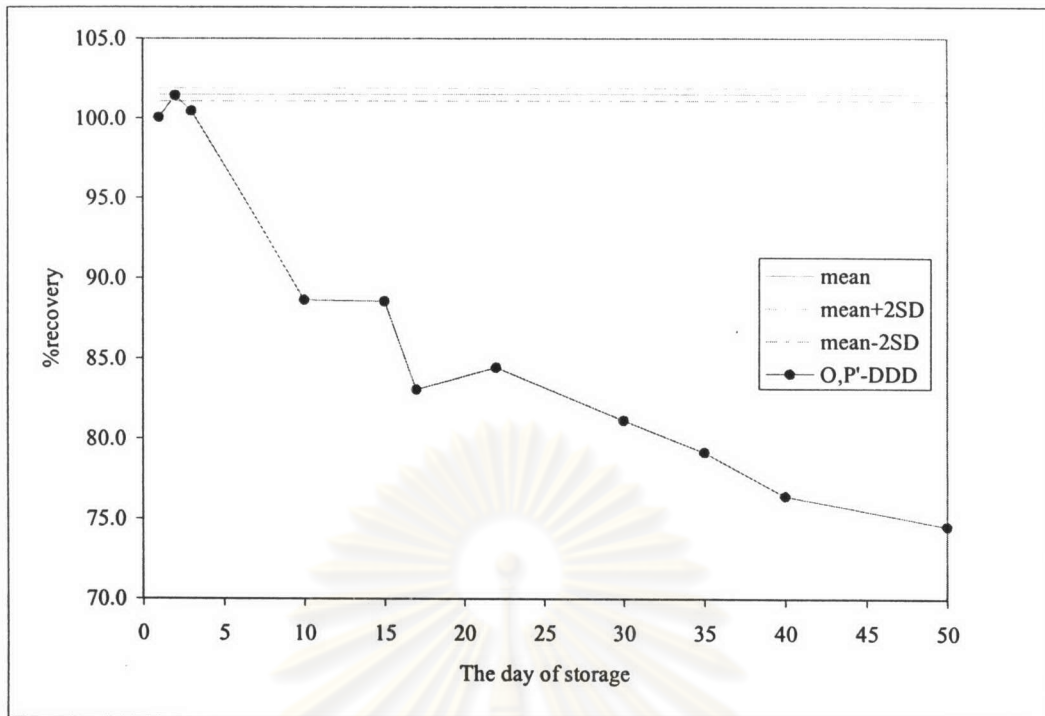


Figure 4.30 Control charge of O,P'-DDD

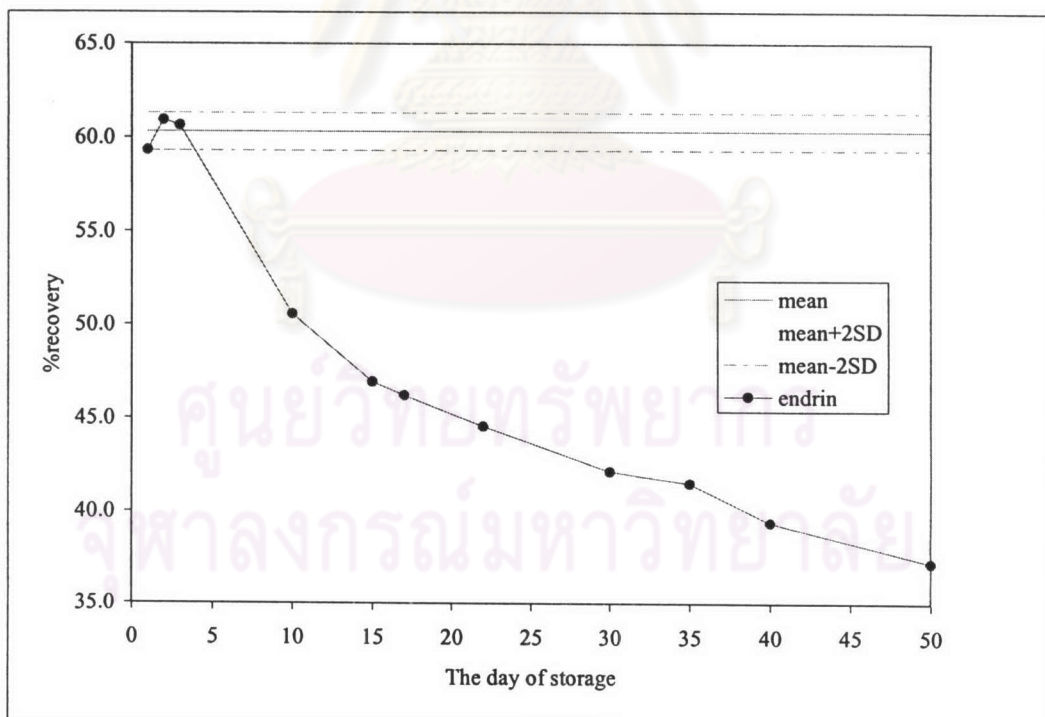


Figure 4.31 Control charge of Endrin

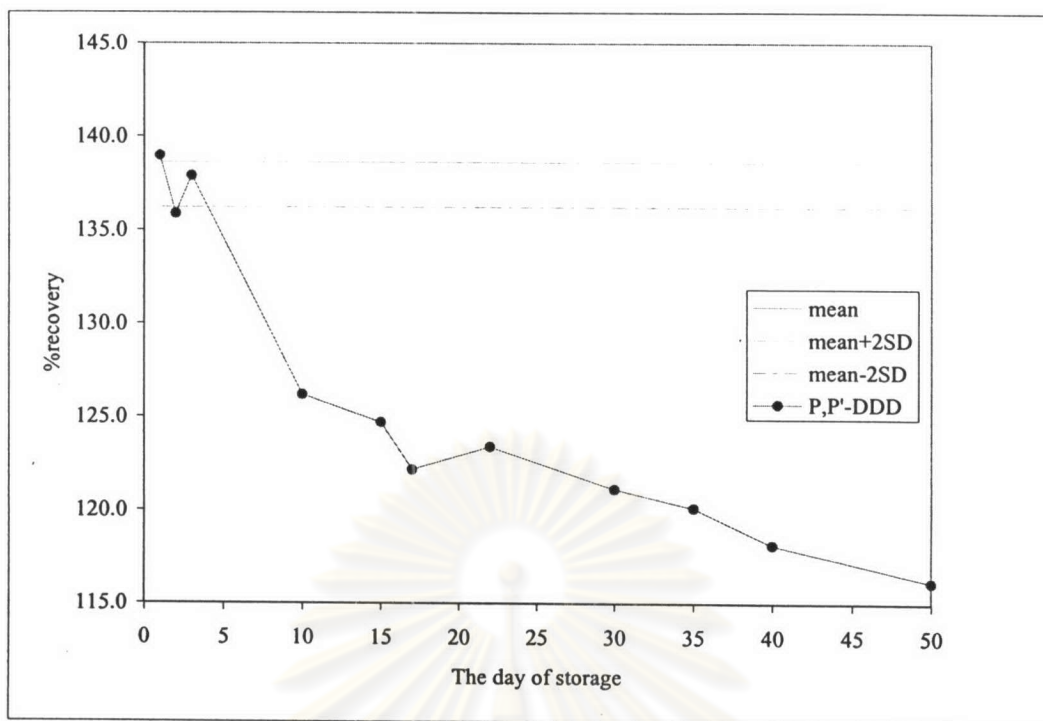


Figure 4.32 Control charge of P,P'-DDD

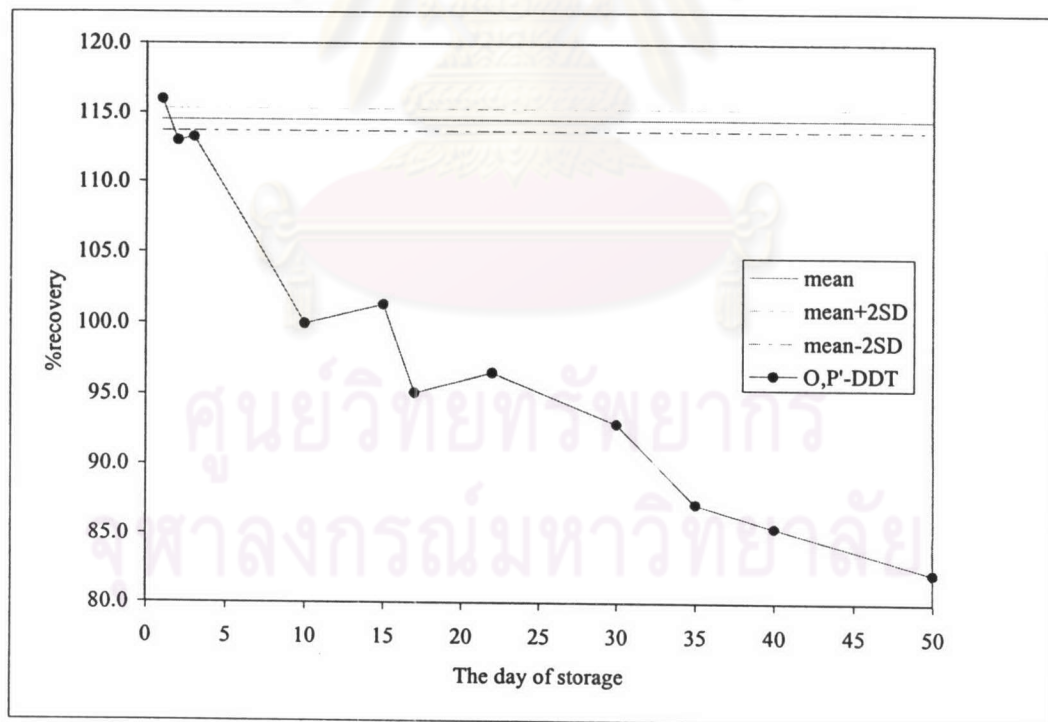


Figure 4.33 Control charge of O,P'-DDT

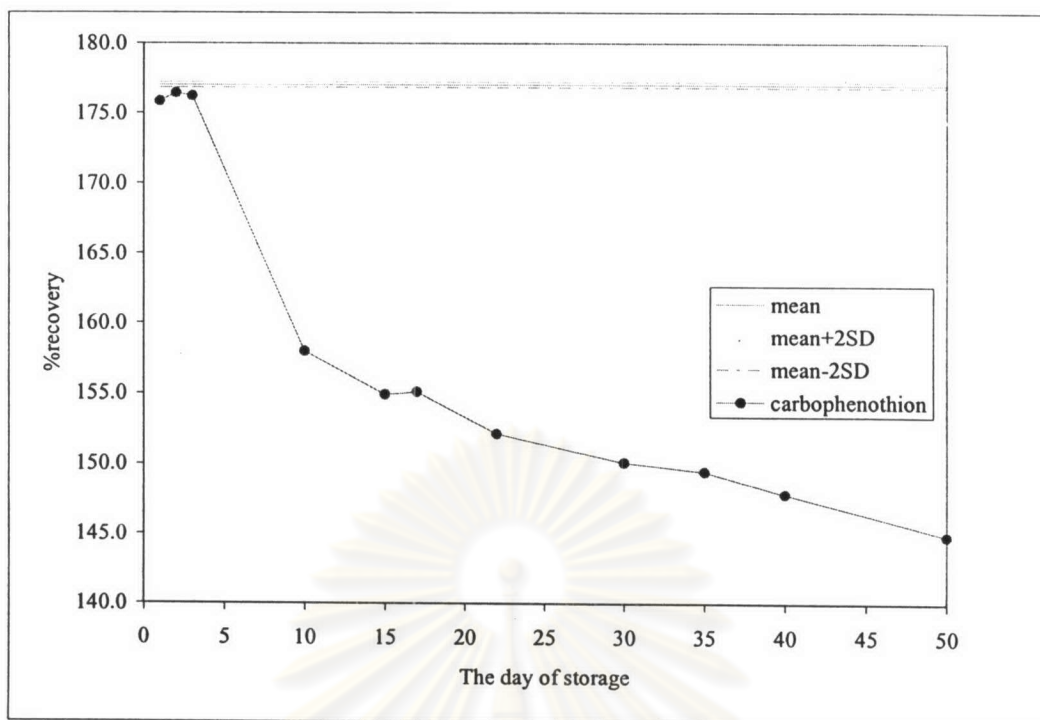


Figure 4.34 Control charge of Carbophenothion

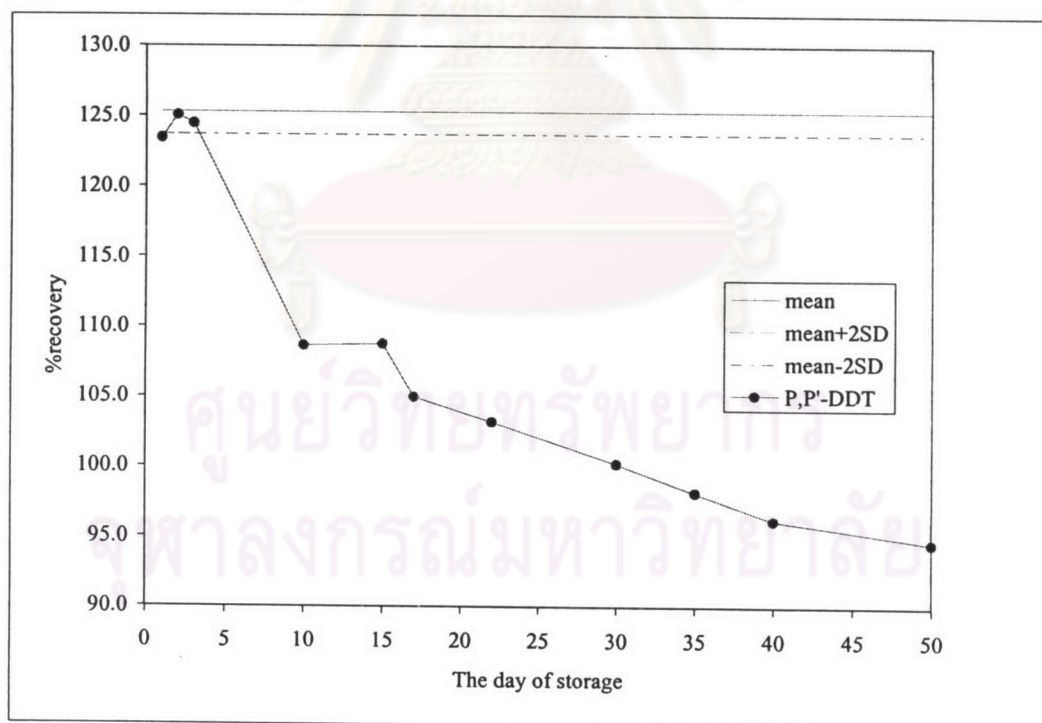


Figure 4.35 Control charge of P,P'-DDT

Table 4.29 The day of storage of 17 organochlorine pesticides in matrix

Pesticides	Mean of %recovery at spiking level 125 ng/g			Storage days
	Mean - 2SD	Mean + 2SD		
α -BHC, HCH	101.9	100.9	102.9	3
β -BHC, HCH	180.6	178.8	182.4	5
γ -BHC, HCH	102.3	101.1	103.5	3
Alachlor	132.9	132.7	133.1	3
Aldrin	108.6	107	110.2	3
Heptachlor epoxide isomer B	71.1	69.9	72.3	3
Heptachlor epoxide isomer A	81.2	79.8	82.6	3
O,P'-DDE	110.8	108.8	112.8	15
α -endosulfan	69.1	68.3	69.9	3
Dieldrin, P,P'-DDE	74.6	73.4	75.8	3
O,P'-DDD	101.5	101.1	101.9	3
Endrin	60.3	59.3	61.3	3
P,P'-DDD	138.6	136.2	141	3
O,P'-DDT	114.5	113.7	115.3	3
Carbophenothion	177.0	176.8	177.2	3
P,P'-DDT	125.3	123.7	126.9	3

4.16 The Result of Determination of Organochlorine Pesticides in Turmeric Products at 3 Thai Markets and 3 Commercially-packed Turmeric Powder

From Table 4.29, all of the six samples above have the concentration of DDT and metabolites of DDT (DDE and DDD) in low concentrations and under the limit of the USP regulations, 0.05 – 3.00 ppm (mg/kg). These are likely to be found in DDT and metabolite-of-DDT substances, and their concentration is also found to be low concentrations. Chromatogram of each sample is shown in APPENDIX.



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Table 4.30 Result of organochlorine pesticides in Turmeric powders at 3 Thai markets and 3 commercially-packed Turmeric powder (n=2)

Pesticides	Concentration of Organochlorine Pesticides in Turmeric Powders (ng/g)					
	Market A	Market B	Market C	Commercial A	Commercial B	Commercial C
α -BHC, HCH	ND	ND	ND	ND	ND	ND
β -BHC, HCH	ND	ND	ND	ND	ND	ND
γ -BHC, HCH	ND	ND	ND	ND	ND	ND
Alachlor	ND	ND	ND	ND	ND	ND
Aldrin	ND	ND	ND	ND	ND	ND
Heptachlor epoxide isomer B	ND	ND	ND	ND	ND	ND
Heptachlor epoxide isomer A	ND	ND	ND	ND	ND	ND
O,P'-DDE	ND	ND	ND	ND	ND	ND
α -endosulfan	ND	ND	ND	ND	ND	ND
Dieldrin, P,P'-DDE	3.69±0.03	ND	2.60±0.01	1.58±0.01	<1.40	<1.40
O,P'-DDD	ND	ND	ND	ND	ND	ND
Endrin	ND	ND	ND	ND	ND	ND
P,P'-DDD	< 4.80	ND	<4.80	<4.80	<4.80	ND
O,P'-DDT	< 3.40	ND	<3.40	<3.40	<3.40	<3.40
Carbophenothion	ND	ND	ND	ND	ND	ND
P,P'-DDT	6.65±0.13	5.17±0.11	7.10±0.03	3.32±0.49	2.53±0.04	2.77±0.03

ND; Non - Detectable