CHAPTER IV

RESULTS AND DISCUSSION

4.1 Carbofuran, parathion-methyl and cypermethrin in strawberries were determined by the proposed CE method.

4.1.1 The experimental conditions of the CE method for determining pesticide residues were studied results are presented as follows:

Optimum conditions for analysis of carbofuran

1. Rinse column by 0.01N NaOH for 3 min.
2. Rinse DI water for 3 min.
3. Equilibrate column with buffer solution for 10 min.
4. Injection time set at 7 sec.
5. Column temperature = 25 °C
6. Fused silica column with capillary diameter (ID) = 75 μm.
7. Retention time 5.2 min
8. Total capillary length (L) = 50 cm. Effective capillary length (l) = 40.2 cm
9. Buffer: 5mM sodium tetraborate, containing 30mM SDS, pH 8.5
10. Voltage = 10 KV.
11. Detection : UV detector at wavelength 254 nm

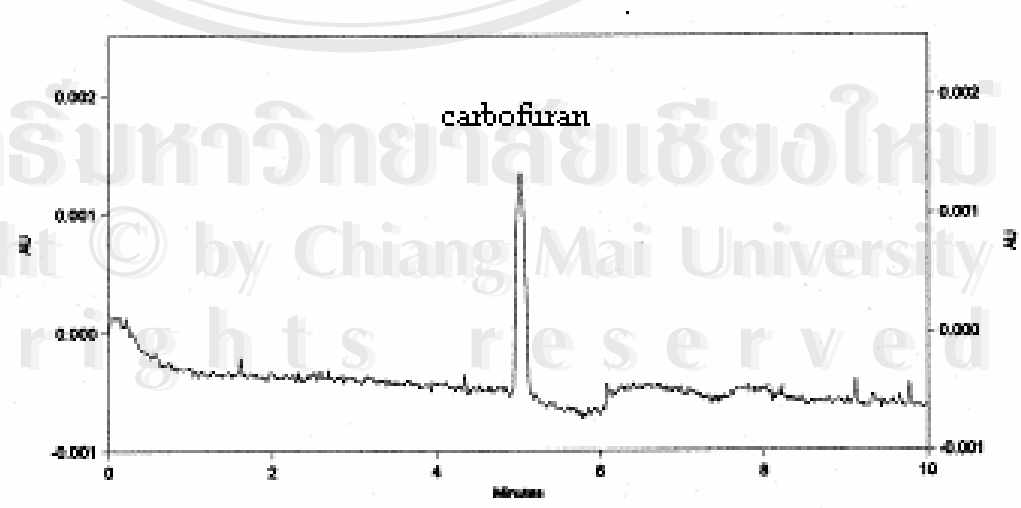

Figure 4.1 Electropherogram of carbofuran standard at the optimum condition

Table 4.1 Comparison of conditions used for CE analysis of carbofuran by Present study and Dechdamrongwut.

Column/ carbofuran	**Present study**	**Dechdamrongwut**
Internal diameter	75 μm	75 μm
Length	50 cm	50 cm
Length to detection point	40.2 cm	40.2 cm
Controlled temperature	25 ^{o}C	25 ^{o}C
UV detection	254 nm	205 nm
Buffer/carbofuran		
Sodium Tetraborate + SDS	5 mM + 30 mM	5 mM + 30 mM
pH	8.5	8
Electricity voltage	10 kV	20 kV

Optimum conditions for analysis of parathion-methyl

1. Rinse column by 0.01N NaOH for 3 min.
2. Rinse DI water for 3 min.
3. Equilibrate column with buffer solution for 10 min.
4 Injection time set at 7 sec.
5. Column temperature = 25 °C
6. Fused silica column with capillary diameter (ID) = 75 μm.
7. Retantion time 5.4 min
8. Total capillary length (L) = 50 cm. Effective capillary length (l) = 40.2 cm
9. Buffer: 5mM sodium tetraborate, containing 30mM SDS, pH 8.5
10. Voltage = 10 KV.
11. Detection : UV detector at wave length 254 nm

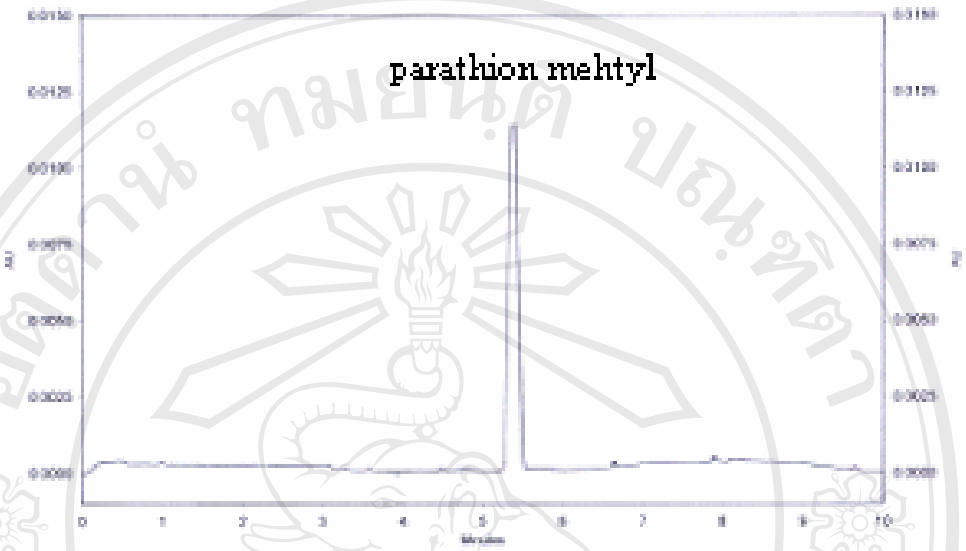

Figure 4.2 Electropherogram of parathion methyl standard at the optimum condition

Table 4.2 Comparison of conditions used for CE analysis of parathion methyl by Present study and Dechdamrongwut

Column/parathion methyl	**Present study**	**Dechdamrongwut**
Internal diameter	75 μm	75 μm
Length	50 cm	50 cm
Length to detection point	40.2 cm	40.2 cm
Controlled temperature	25 ^{o}C	25 ^{o}C
UV detection	254 nm	205 nm
Buffer/carbofuran		
Sodium Tetraborate + SDS	5 mM + 30 mM	5 mM + 30 mM
pH	8.5	8
Electricity voltage	10 kV	20 kV

Optimum conditions for analysis of cypermethrin:

1. Rinse column by 0.01N NaOH for 3 min.
2. Rinse DI water for 3 min.
3. Equilibrate column with buffer solution for 10 min.
4. Injection time set at 7 sec.
5. Column temperature = 25 °C
6. Fused silica column with capillary diameter (ID) = 75 μm.
7. Retention time 2 min
8. Total capillary length (L) = 50 cm. Effective capillary length (l) = 40.2 cm
9. Buffer: 5mM sodium tetraborate, containing 30mM SDS, pH 7.5
10. Voltage = 15 KV.
11. Detection : UV detector at wave length 205 nm

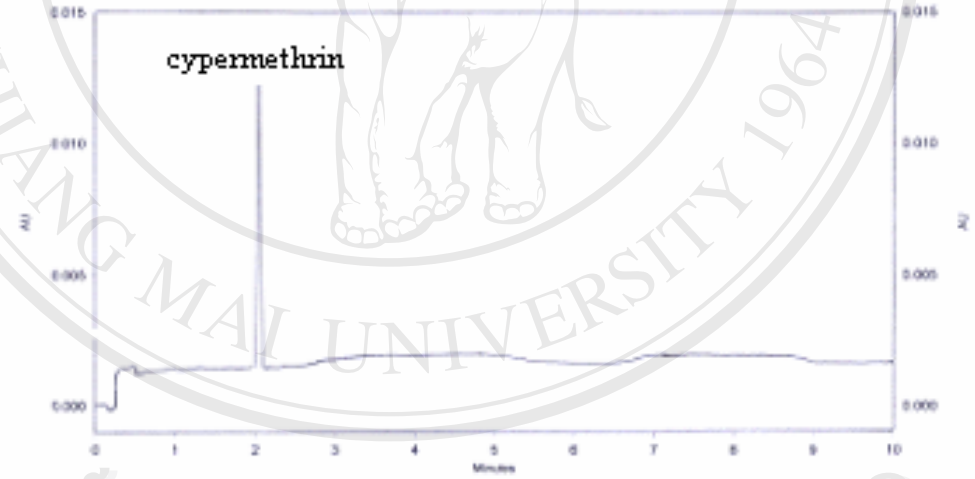

Figure 4.3 Electropherogram of cypermethrin standard at the optimum condition

4.2 Validation of the CE method

4.2.1. Precision

The standard solutions were analyzed 5 replicates by CE, using the procedure and conditions. CE method results are shown in Table 4.3 - 4.5. The reproducibility was obtained based on 3 analysis within a week.

Table 4.3 Precision for carbofuran

Trial No	Carbofuran at 1 ppm	
	Peak Area	Concentration (ppm)
1	82,358	0.970
2	81,249	0.958
3	83,762	0.985
4	83,204	0.979
5	82,247	0.969
Mean	82,564	-
SD	963.970	-
%RSD	0.011	-

Table 4.4 Precision for parathion-methyl

Trial No	Parathion methyl at 6 ppm	
	Peak Area	Concentration (ppm)
1	183,550	5.951
2	183,729	5.996
3	183,315	6.042
4	183,366	6.004
5	183,658	6.017
Mean	183,658	-
SD	179.792	-
%RSD	0.097	-

Table 4.5 Precision for cypermethrin

Trial No	Cypermethrin at 9 ppm	
	Peak Area	Concentration (ppm)
1	73,004	8.906
2	72,862	9.027
3	72,886	8.891
4	72,862	9.007
5	72,767	8.956
Mean	72,767	-
SD	84.789	-
%RSD	0. 116	-

Carbofuran:

$$\%\,RSD = \frac{963.970}{82,564} \times 100 = 0.011\%$$

Parathion methyl:

$$\%\,RSD = \frac{179.792}{183,658} \times 100 = 0.097\ \%$$

Cypermethrin

$$\%\,RSD = \frac{84.789}{72,768} \times 100 = 0.116\ \%$$

4.2.2. Recovery

The accuracy of the method was studied by adding known amounts of corresponding standard insecticide into known amounts of sample solution results are shown in Table 4.6-4.8

Table 4.6 Recovery assay of carbofuran

Fortified Sample with carbofuran (μg/mL)	***Amount of standard carbofuran found (μg/mL)**	**% Recovery**
0.2	0.216	108.25 ± 3.81
0.5	0.465	93.06±3.04
1	0.971	97.13±1.97
2	2.026	101.32±2.63
3	2.887	96.26±2.96

* Average from 3 determinations

Table 4.7 Recovery assay of parathion methyl

Fortified Sample with parathion methyl (μg/mL)	***Amount of standard parathion methyl found (μg/mL)**	**% Recovery**
0.5	0.519	103.96±3.99
1	0.977	97.77±6.30
2	1.882	94.11±1.83
3	2.961	98.73±2.79
4	3.898	97.45±4.68

Table 4.8 Recovery assay of cypermethrin

Fortified Sample with carbofuran (μg/mL)	***Amount of standard carbofuran found (μg/mL)**	**% Recovery**
0.5	0.475	95.26±4.80
1	1.011	101.13±3.05
2	1.936	96.83±5.68
3	2.838	94.61±3.34
4	3.954	98.85±2.16

The percentage recoveries of carbofuran, parathion methyl and cypermethrin were found to be 93.06-108.25 %, 94.11-103.96 % and 94.61- 101.13 %**,** respectively

4.2.3. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Sample blank was added to standard solution containing 0.2 μg/ml of carbofuran, 0.5 μg/ml of parathion methyl and 0.5 μg/ml of cypermethrin respectively and analysis by CE. The limits of quantification (LOQs) were determined considering a value 10 times of the background noise. The Quantitation limits were 0.076, 0.078 and 0.016 μg/mL for carbofuran, parathion methyl and cypermethrin respectively. Results are shown in Table 4.9.

Table 4.9 Limit of detection and quantitation for carbofuran, Parathion methyl and cypermethrin analysis by CE.

Trial No	Carbofuran 0.2 ppm		Parathion methyl 0.5 ppm		Cypermethrin 0.2 ppm	
	Area	Concentration (μg/mL)	Area	Concentration (μg/mL)	Area	Concentration (μg/mL)
1	11,762	0.206	44,376	0.499	7,114	0.199
2	11,668	0.205	43,893	0.495	7,096	0.196
3	10,479	0.192	43,971	0.496	6,987	0.195
4	10,893	0.196	43,432	0.490	7,028	0.197
5	10,147	0.189	42,441	0.479	7,123	0.199
Mean	10,990	0.198	43,623	0.4918	7,070	0.1973
SD	713.590	0.0076	740.712	0.0078	59.315	0.0016
LOD	2140.77	0.0228	2222.136	0.0234	177.945	0.0048
LOQ	1735.90	0.076	7407.12	0.078	593.15	0.016

Carbofuran:

The standard deviation (SD) = 0.0076

Limit of Detection (LOD) = 3 SD = 3 *0.0076 = 0.0228 ppm.

Limit of Quantiation (LOQ) = 10 SD = 10 * = 0.076 ppm.

Parathion methyl:

The standard deviation (SD) = 0.0078

Limit of Detection (LOD) = 3 SD = 3 * 0.0078= 0.0234 ppm.

Limit of Quantitation = 10 SD = 10 * 0.0080 = 0.078 ppm.

Cypermethrin

The standard deviation (SD) = 0.0016

Limit of Detection (LOD) = 3 SD = 3 * 0.0016 = 0.0048 ppm.

Limit of Quantitation = 10 SD = 10 * 0.0016 = 0.016 ppm.

4.2.4 Linearity

Three series of standard insecticide solutions containing 0.2, 0.5, 1, 2 and 3 μg/mL of carbofuran , 0.5, 1, 2, 3 and 4 μg/ml of parathion-methyl and 0.5, 1, 2, 3 and 4 μg/ml of cypermethrin were prepared and analysed by CE respectively. The peak areas were plotted against the concentration of each insecticide and the R square of each calibration curve was calculated by regression analysis. Results are presented in Fig. 4.4- 4.6 and Table 4.10 - 4.12.

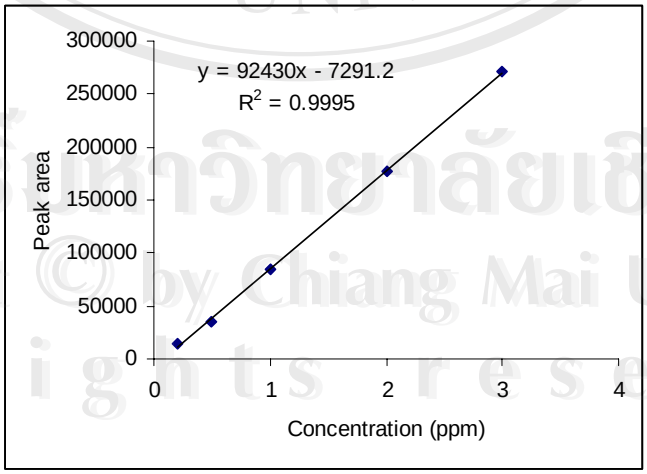

Figure 4.4 Linearity plot of peak area against concentrations of carbofuran 0.2, 0.5, 1, 2, 3 μg/mL.

Table 4.10 The regression analysis of carbofuran calibration curve Fig. 4.4.

Regression Statistics	
Multiple R	0.998537
R Square	0.997076
Adjusted R Square	0.747076
Standard Error	0.062309
Observations	5

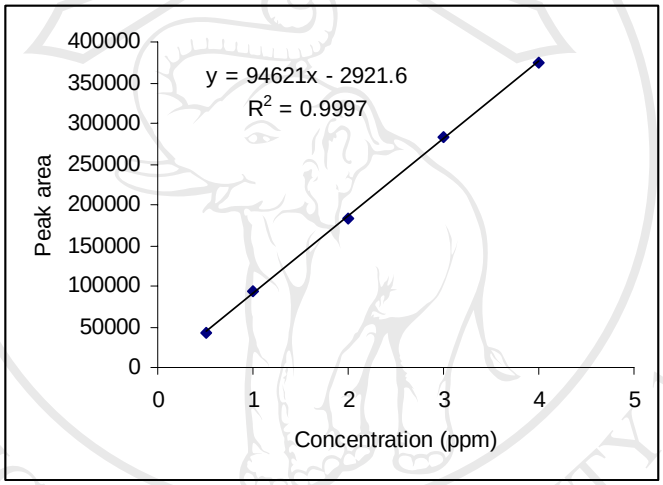

Figure 4.5 Linearity plot of peak area against concentrations of parathion-methyl 0.5, 1, 2, 3,4 μg/mL.

Table 4.11 The regression analysis of parathion-methyl calibration curve Fig. 4.5

Regression Statistics	
Multiple R	0.999748
R Square	0.999496
Adjusted R Square	0.749496
Standard Error	0.03213
Observations	5

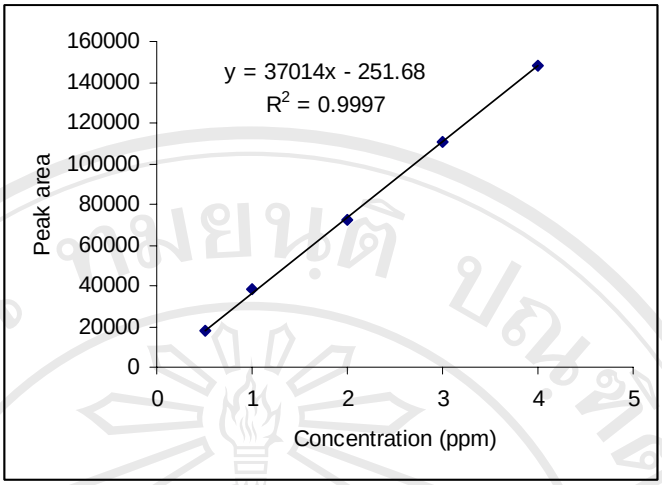

Figure 4.6 Linearity plot of peak area against concentrations of cypermethrin 0.5, 1, 2, 3,4 μg/mL

Table 4.12 The regression analysis of cypermethrin calibration curve Fig. 4.6

Regression Statistics	
Multiple R	0.999845
R Square	0.99969
Adjusted R Square	0.74969
Standard Error	0.025229
Observations	5

4.3 Determination of insecticide residues in strawberries by CE and comparison with HPLC and GC

Insecticide residues (carbofuran, parathion methyl and cypermethrin) in each plot of strawberries were determined by using the proposed CE method. Comparison was also made by using the HPLC and GC. Results are presented in Table 4.13, Table 4.15 and 4.17.

Table 4.13 Comparative determination of carbofuran residues in strawberries by CE and HPLC

Harvest Period after treatment with insecticide (day)	Amount of carbofuran found (μg/mL)	
	CE	HPLC
1 hr.	2.716	2.832
1 hr.	2.652	2.856
1 hr.	2.704	2.888
1	2.598	2.792
1	2.729	2.856
1	2.672	2.792
3	2.446	2.522
3	2.281	2.461
3	2.382	2.441
5	2.007	2.048
5	1.895	1.982
5	1.835	1.916
7	1.273	1.485
7	1.318	1.435
7	1.337	1.437
10	0.473	0.604
10	0.467	0.595
10	0.444	0.588
14	0.382	0.407
14	0.373	0.355
14	0.399	0.299

Table 4.14 Amount of carbofuran residues (mg/kg) detected from strawberry fruits at difference harvesting time (day) after spraying with carbofuran.

CE/carbofuran			HPLC/carbofuran		
Days	**Present study**	**D***	**Days**	**Present study**	**D***
0	2.691	1.018	0	2.859	1.031
1	2.666	0.721	1	2.813	0.733
3	2.370	0.696	3	2.475	0.701
5	1.912	0.601	5	1.982	0.609
7	1.309	ND	7	1.452	0.442
10	0.461	ND	10	0.596	0.263
14	0.385	ND	14	0.354	0.130

D* Dechdamrongwut

Two-sample T for HPLC vs CE

	N	Mean	SD	SE Mean
HPLC	21	1.795	0.963	0.210
CE	21	1.679	0.944	0.206

Difference = mu HPLC - mu CE

95% CI for difference: (0.09, 0.14)

T-Test of difference = 0 (vs not =): t-value = 0.39 p-value = 0.69 DF = 40

The p-value was higher than 0.05 at 95% confidential. This can be interpreted as HPLC and CE has no significant different CE in the result. see (Fig 4.7). Therefor there is no significant difference between CE and HPLC.

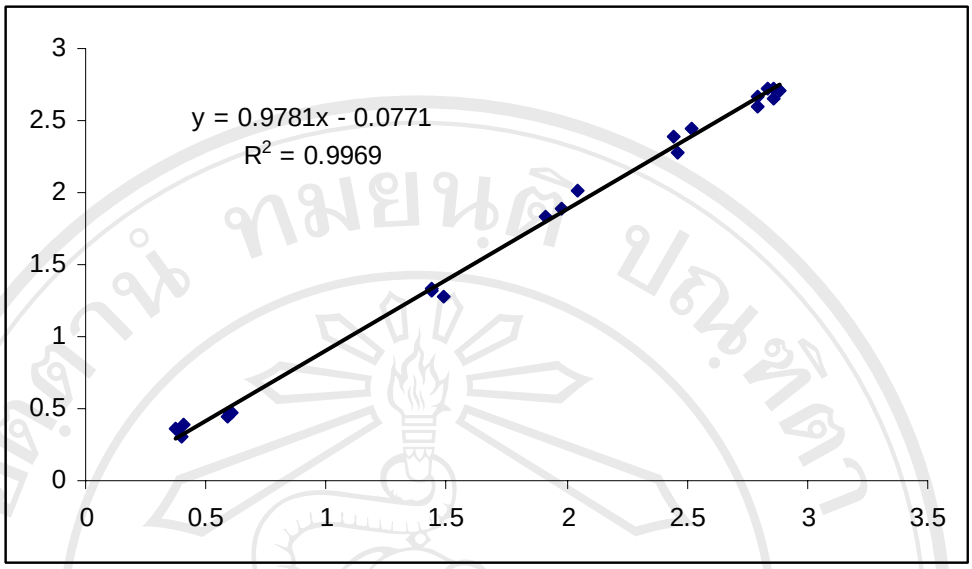

Figure 4.7 Correlation of the data obtained from using HPLC and CE for analysis of carbofuran.

The results from this study confirm the work previously done Dechdamrongwut analysis of carbofuran and parathion methyl but in this research the CE could detect higher amount of residues compared to the amounts reported by Dechdamrongwut (1). This might be resulted from the difference of optimum conditions used in both analysis.

It can be seen that differences between the optimum conditions for analysis of carbofuran, parathion methyl reported by Dechdamrongwut are the pH of buffer and the applied electricity voltage. The optimum condition of UV detection for CE analysis of parathion methyl found in this research was also different from the former report.

Table 4.15 Comparative determination of parathion methyl residues in strawberries by CE and GC-FPD.

Harvest Period after treatment with insecticide (day)	Amount of parathion methyl found (μg/mL)	
	CE	GC-FPD
1 hr.	11.233	11.440
1 hr.	9.395	9.680
1 hr.	9.801	9.820
1	6.331	6.591
1	6.127	6.344
1	5.718	5.722
3	2.355	2.543
3	2.662	2.745
3	2.560	2.723
5	1.940	2.144
5	2.200	2.256
5	2.048	2.224
7	1.433	1.725
7	1.638	1.832
7	1.536	1.853
10	0.921	1.112
10	1.024	1.201
10	1.126	1.298
14	0. 614	0.943
14	0.512	0.923
14	0.819	0.956

Table 4.16 Amount of parathion methyl residues (mg/kg)) detected from strawberry fruits at difference harvesting time (day) after spraying with parathion methyl

CE/parathion methyl			GC-FPD/parathion methyl		
Days	**Present study**	**D***	**Days**	**Present study**	**D***
0	10.143	5.745	0	10.313	5.898
1	6.059	0.755	1	6.219	0.782
3	2.526	0.241	3	2.670	0.284
5	2.063	ND	5	2.208	0.124
7	1.536	ND	7	1.803	0.079
10	1.024	ND	10	1.204	0.060
14	0.648	ND	14	0.941	0.030

D* Dechdamrongwut

Two-sample T for GC-FPD vs CE

	N	Mean	SD	SE Mean
GC-FPD	21	3.756	3.301	0.738
CE	21	3.569	3.318	0.742

Difference = mu GC-FPD - mu CE

95% CI for difference: (0.14, 0.23)

T-Test of difference = 0 (vs not =): t-value = 0.19 p-value = 0.84 DF = 40

The p-value was higher than 0.05 with 95% confidential. This can be interpreted as GC-FPD and CE has no significant different CE in the result. see (Fig 4.8). Therefor there is no significant difference between analyses of CE and GC-FPD.

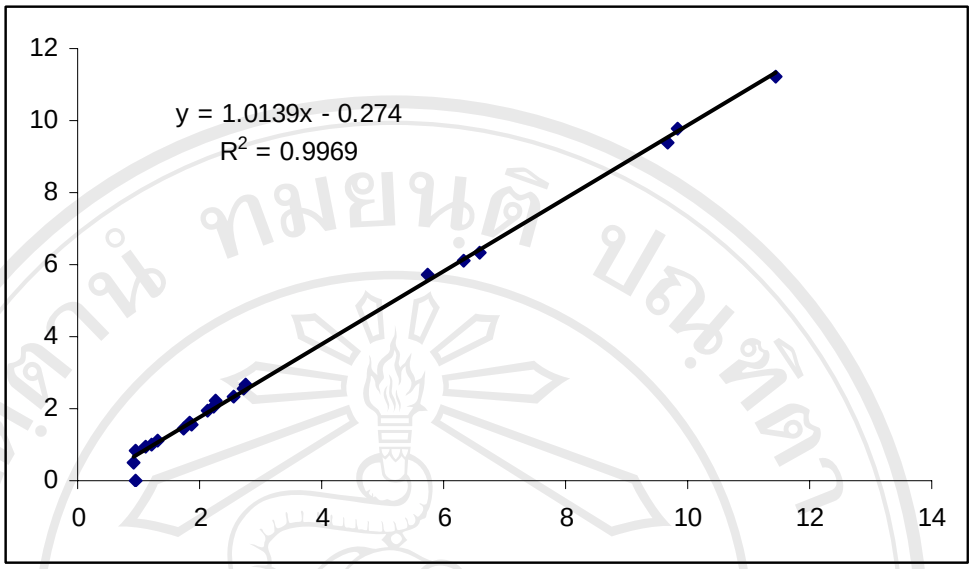

Figure 4.8 Correlation of the data obtained from using GC-FPD and CE for analysis of parathion methyl

Table 4.17 Comparative determination of cypermethrin residues in strawberries by CE and GC-ECD

Harvest Period after treatment with insecticide (day)	Amount of cypermethrin found (μg/mL)	
	CE	GC-ECD
1 hr.	14.618	14.800
1 hr.	14.792	15.000
1 hr.	15.094	15.400
1	13.081	13.280
1	12.679	12.960
1	13.786	14.080
3	7.044	7.392
3	7.245	7.504
3	7.547	7.728
5	7.044	7.120
5	5.735	6.080
5	5.853	6.160

Harvest Period after treatment with insecticide (day)	Amount of cypermethrin found (μg/mL)	
	CE	GC-ECD
7	4.226	4.644
7	4.629	4.716
7	4.830	5.004
10	3.998	4.300
10	4.730	2.958
10	5.031	5.366
14	3.924	4.192
14	4.025	4.288
14	3.622	3.752

Two-sample T for GC-ECD vs CE

	N	Mean	SD	SE Mean
GC-ECD	21	7.891	4.362	0.952
CE	21	7.787	4.216	0.920

Difference = mu GC-ECD - mu CE

95% CI for difference: (0.12, 0.33)

T-Test of difference = 0 (vs not =): t-value = 0.07 p-value = 0.93 DF = 40

The p-value was higher than 0.05 with 95% confidential. This can be interpreted as GC-ECD and CE has no significant different CE the in result. see (Fiq 4.9). Therefor there is no significant difference between analyses of CE and GC-ECD.

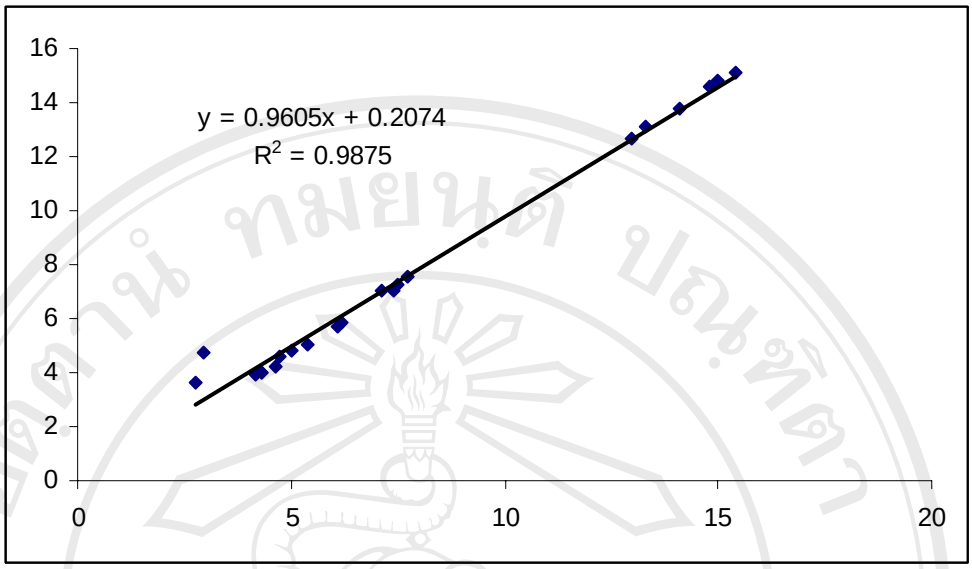

Figure 4.9 Correlation of the data obtained from using GC-ECD and CE for analysis of cypermethrin.