CHAPTER IV

RESULTS AND DISCUSSION

1. Praparation of BADGE.HCl

In the beginning of this research, BADGE.HCl standard reference material was not commercially available. The standard was synthesized in our laboratory. The synthesis method was adapted from M. Biedermann's method [7]. The synthesis condition of BADGE.HCl was slightly vigorous. The raw material was low molecular weight BADGE that contained mostly BADGE monomer and some oligomers. All compounds can react with HCl resulting in many chlorohydroxy derivatives. After the reaction finished, the reaction products were extracted into MTBE (organic phase) followed by the removal of excess HCl. After drying, viscous yellow liquid residues remained in the flask which is a mixture containing many chlorohydroxy derivatives of BADGE. Column chromatography with silica packing was selected to separate and purify BADGE.HCl from the mixture. The order of compounds eluted from the column was: BADGE, BADGE.HCl, BADGE.2HCl and other compounds. To further confirmed our result, BADGE.HCl fraction was analyzed by HPLC-FLD-DAD and confirmed the structure with HPLC-MS. The m/z peak at 399 was observed which corresponded to *m/z* $+Na^+$ fraction of BADGE.HCl (MW = 376). Chromatographic purity was determined to be 97%.

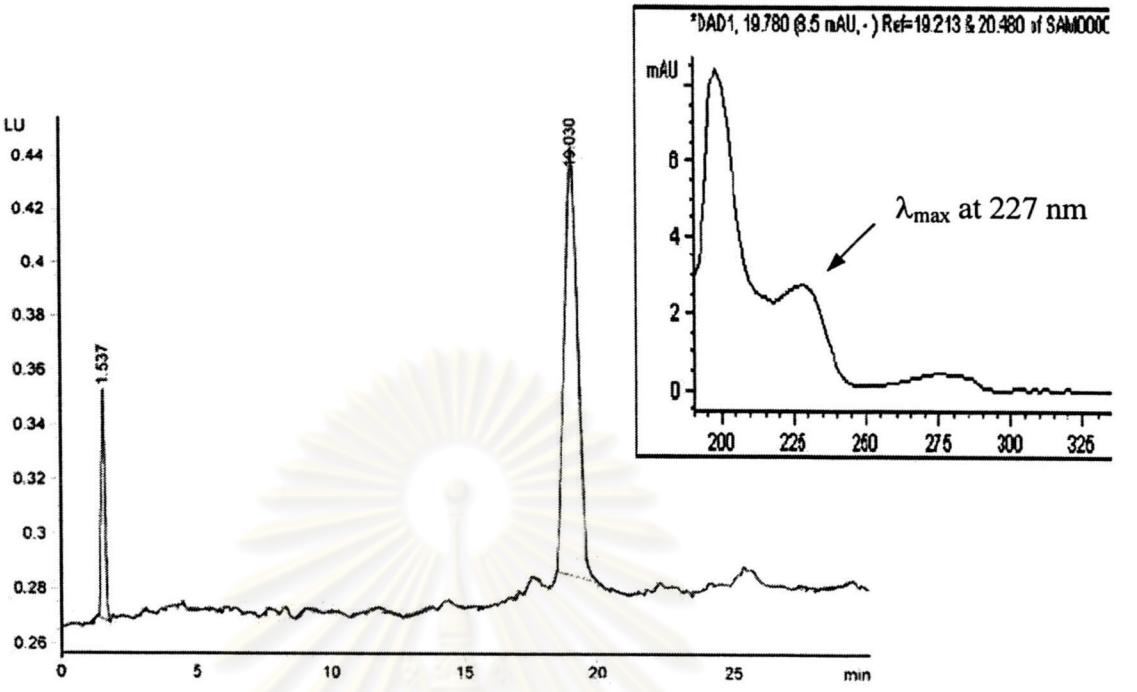

Figure 4-1. The chromatogram of BADGE.HCl fraction that separated on ODS Hypersil C18 column (4.0 x 150 mm, 5 μm) using a mobile phase of methanol-water (60:40) with fluorescence detection at wavelength ex 227/em 313 nm. The peak UV spectrum was obtained and showed λ_{max} at 227 nm that are characteristic of BADGE chromophore.

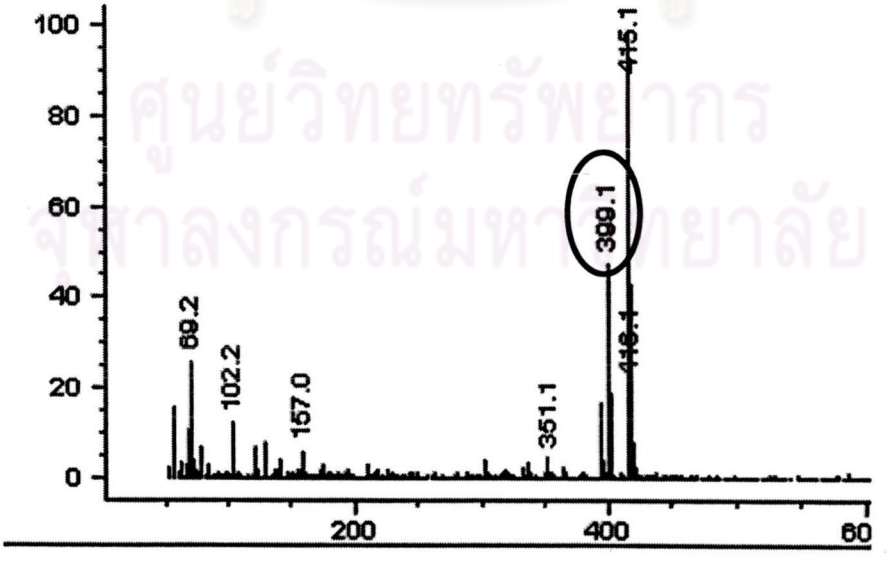

Figure 4-2. Mass spectrum of BADGE (*m/z* $+Na^+$ = 399.1).

2. The Optimization of HPLC Separation

Preliminary separation of BADGE, BADGE.HCl, BADGE.2HCl, BFDGE and BFDGE.2HCl were tested on Zorbax C18 (250 x 4.0 mm i.d., 5 μm). Mobile phase was water (A) and acetonitrile (B) using gradient elution at flow rate of 1 mL/min. Gradient profile was 0 min 40%B, 0-20 min 40-80 %B and equilibrated time was 10 minutes before the next analysis. The HPLC condition worked very well on standard solution. However, when using to analyze real sample there was interference from fish matrix with BFDGE.2HCl peak. In addition, the drifting of baseline was severed making peak area integration very difficult.

Different column (ODS Hypersil C18, 150 x 4.0 mm i.d., 5 μm) was tested. This column can separate BADGE, BADGE.HCl, BADGE.2HCl, BFDGE and BFDGE.2HCl under simple isocratic condition of water (A) and 60% methanol (B). In this condition, all 5 standards were separated with good resolution within acceptable length of time (30 minutes). Table 4-1. summarized the optimum HPLC condition that will be used throughout this work.

Table 4.1. Parameters of the optimal HPLC condition

Parameter	Condition
Column:	ODS Hypersil C18 (150 x 4.0 mm i.d., 5 μm) with guard column.
Mobile phase:	Isocratic elution at 60 percent methanol for 30 minutes. After sample analysis, flushed with 90 percent methanol for 15 minutes and re-equilibrated for 10 minutes.
Flow rate:	0.75 min/mL
Injection volume:	5 μL
Detector:	Fluorescence detector Excitation wavelength = 227 nm. Emission wavelength = 313 nm.
Temperature:	40 °C

Table 4.2. Chromatographic parameters obtained from for the optimum HPLC condition listed in Table 4.1. (t_m = 1.667 min)

Chromatographic parameter	Standard compound				
	BFDGE	BFDGE.2HCl	BADGE	BADGE.HCl	BADGE.2HCl
Retention time (min)	7.40, 8.17	12.59	14.25	18.89	25.22
Retention factor (k′)	3.44, 3.89	6.55	7.55	10.33	14.13
Band width ($W_{1/2}$, min)	0.256, 0.273	-	0.427	0.561	0.704
Column plate number (N)	5176, 5527	-	6847	6281	7110

The optimum sensitivity of fluorescence detector was determined to be at excitation wavelength of 227 nm and emission wavelength of 313 nm. It should be noted here that the response of BADGE and BFDGE parent compounds are higher than their derivatives. The chromatogram of standard mixture is shown in Figure 4-3.

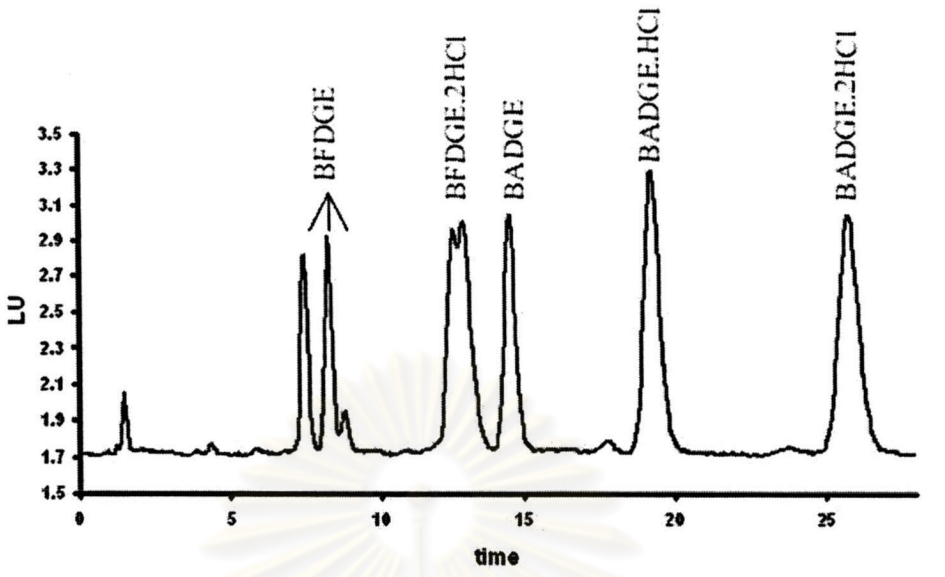

Figure 4-3. Chromatogram of 0.1 ppm standard mixture separated on ODS Hypersil C18 column (150x4.0 mm, 5 μm) using a mobile phase of methanol-water (60:40) with fluorescence detection at wavelength ex 227/em 313 nm.

From the optimum HPLC condition (Figure 3.1), the order of elution was BFDGE, BFDGE.2HCl, BADGE, BADGE.HCl and BADGE.2HCl respectively. The chromatogram showed 2 peaks for BFDGE and BFDGE.2HCl were partially separated. Because both BFDGE and BFDGE.2HCl exist as 3 isomers, multiple peaks are expected but baseline resolution between isomers are not necessary. Peaks of BADGE.HCl and BADGE.2HCl are broad because they have high affinity to the stationary phase and retain in the column too long.

This HPLC condition can separate all 5 analytes in fish matrixes very well with no observed interference and excellent resolution could be obtained for quantitative analysis. Figure 4-4 showed the chromatogram of standard mixture in fish matrix.

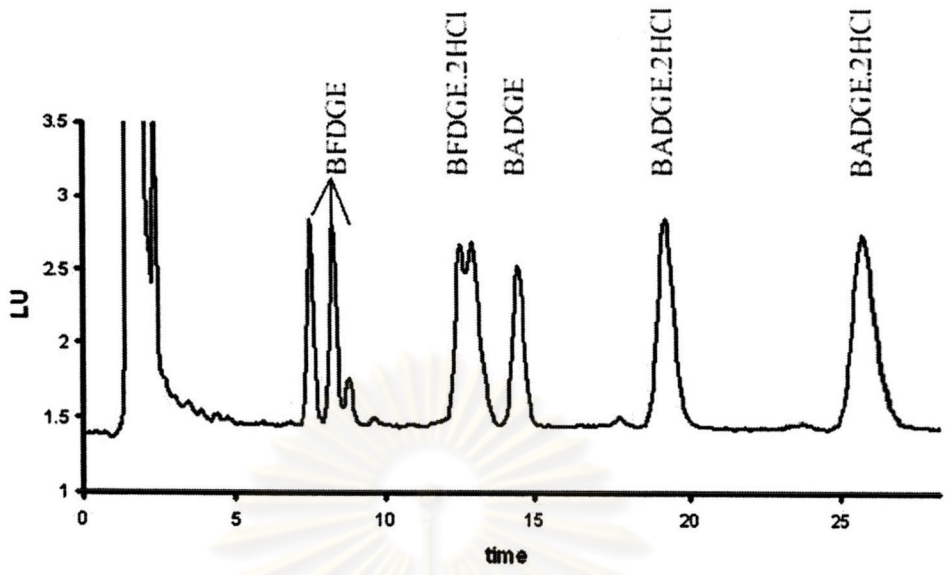

Figure 4-4. Chromatogram of matrix standard separated by the optimal condition.

3. The Specificity of Standards

Each peak in the chromatogram of standard mixture run under optimized HPLC condition was matched with individual standard. The retention times and UV spectra were compared. Chromatogram of each standard and its UV-spectrum are in appendix A. Due to their similarity in structures, all spectra are not significantly different. However, the spectra are useful for the confirmation of peaks in the chromatogram whether they are interference or analyte. Table 4.3 showed the retention time of all standards in the order of elution.

Table 4.3. Retention time (t_R, min) of all standards

Compound	Retention time (t_R, min)
BFDGE	7.40, 8.17
BFDGE.2HCl	12.59
BADGE	14.25
BADGE.HCl	18.89
BADGE.2HCl	25.22

4. The Study of Linearity

Linearity of the signal was tested between 0.035–5.000 ppm and found to be linear in this range. The regression data are summarized in Table 4.4. Regression plots of the relationship between concentration and peak area were shown in Figure 4-5 – 4-9.

Table 4.4. Linear regression results (10 level, n=2)

Compound	R^2	Intercept (LU·sec)	Slope (10^6·LU·sec·cm^3·g^{-1})
BADGE	0.9999	-2.814	203.22
BADGE.HCl	0.9999	-1.620	105.78
BADGE.2HCl	0.9999	-1.474	127.83
BFDGE	0.9999	-0.572	132.57
BFDGE.2HCl	0.9997	-2.137	136.23

Regression lines for BFDGE and BFDGE.HCl that exist as multiple peaks are calculated using total peak area of all isomers combined. Therefore, there is only one regression line for each compound. Linearity is normally measured as correlation coefficients (R^2) that approaches 1.0 when the relationship is perfectly positive linear. Regression data in Table 4.4 showed all R^2-values approach 1.0 indicating very high linear detector responses within this concentration range. Pearson's correlation coefficient test can be used to confirm the significant of this linear relationship. Since the degree of freedom for this experiment was 8. The Pearson's acceptable value at 95% confident limit is 0.632, which is lower than our calculated R^2-values. Therefore, it is safe to say that the detector response is perfectly linear from 0.035–5.000 ppm for all analytes.

The sensitivity of each standard could indicate by slope. The compound with steeper slope has higher detector sensitivity. Thus, BADGE has the highest sensitivity and BADGE.HCl has the lowest sensitivity.

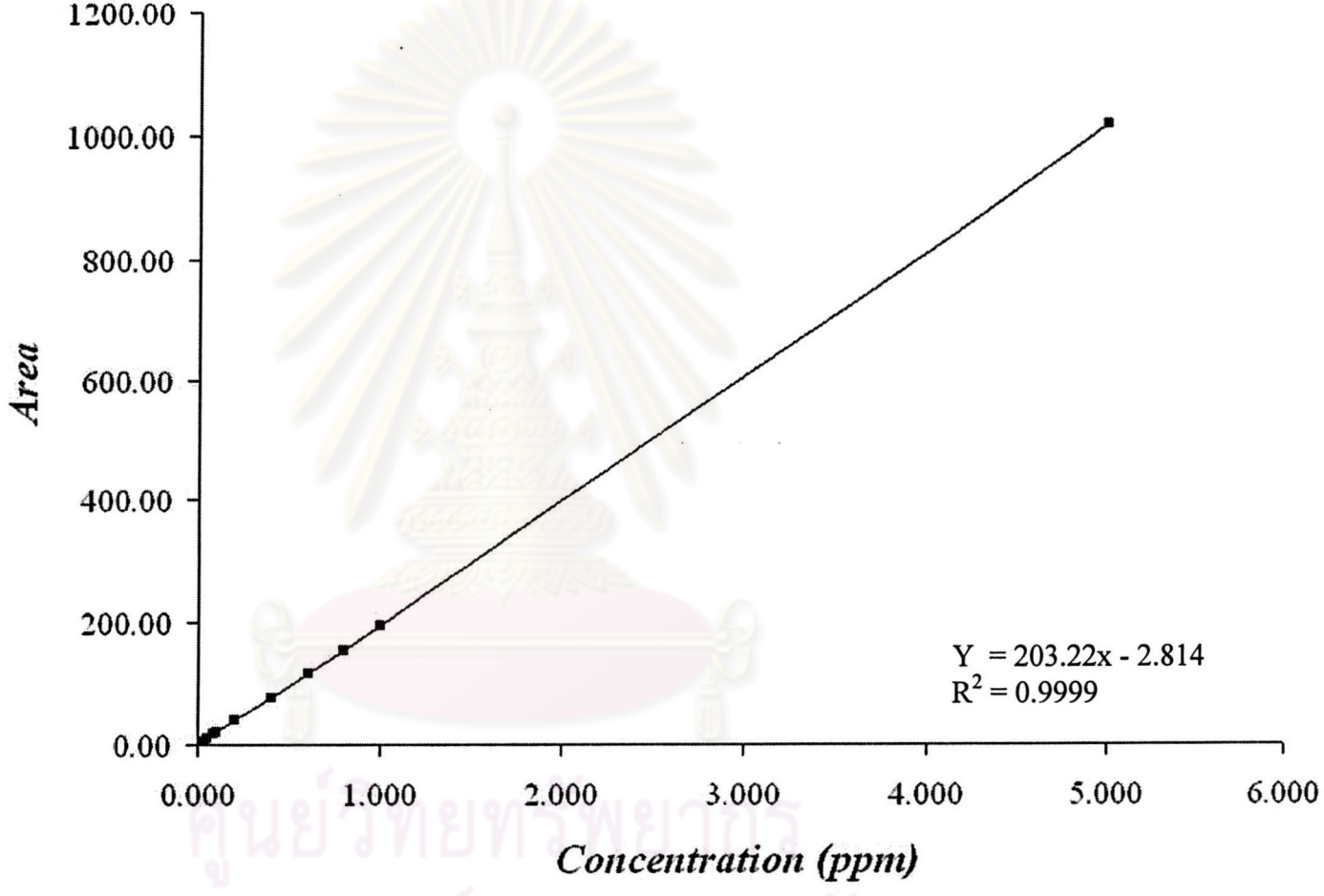

Figure. 4-5. Linear regression line of peak area vs. concentration of BADGE obtained from HPLC data.

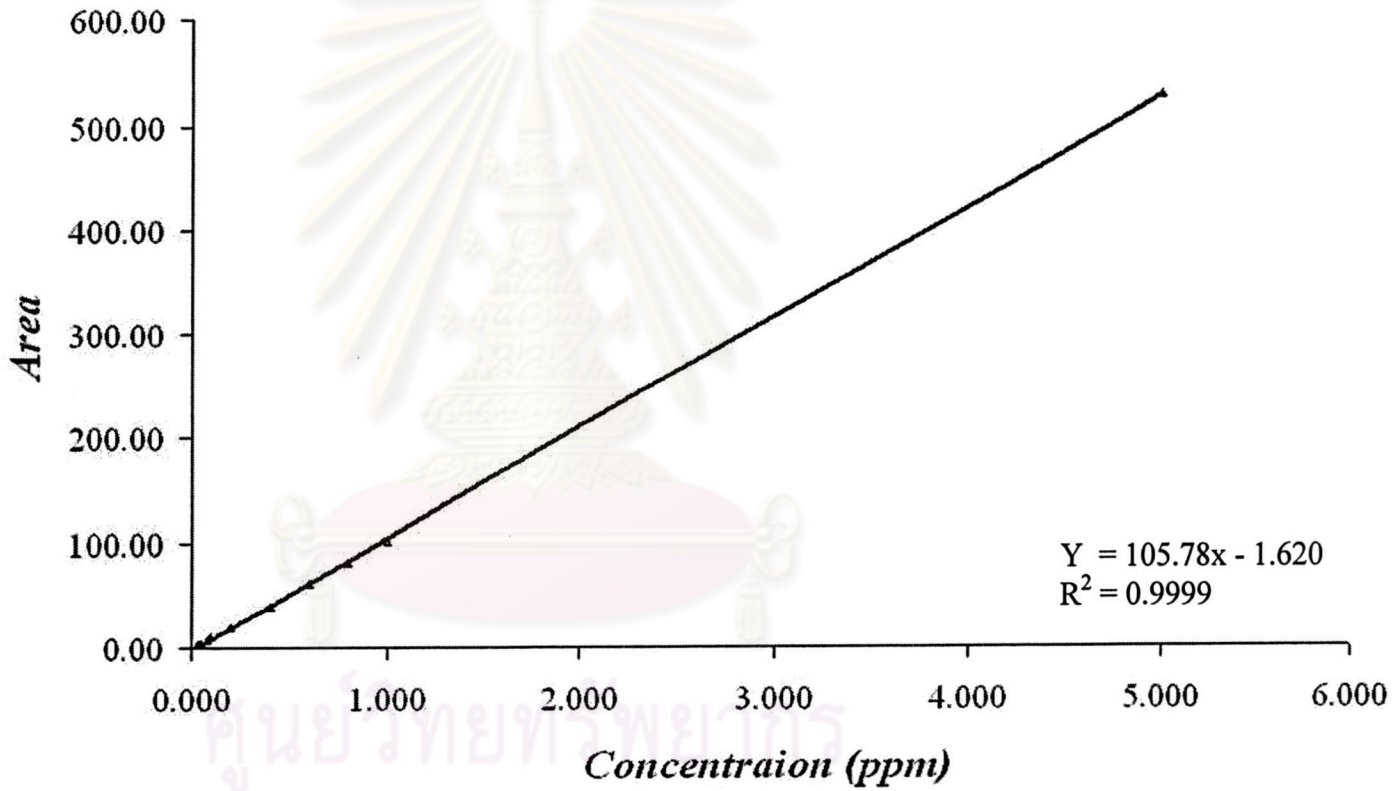

Figure. 4-6. Linear regression line of peak area vs. concentration of BADGE.HCl obtained from HPLC data.

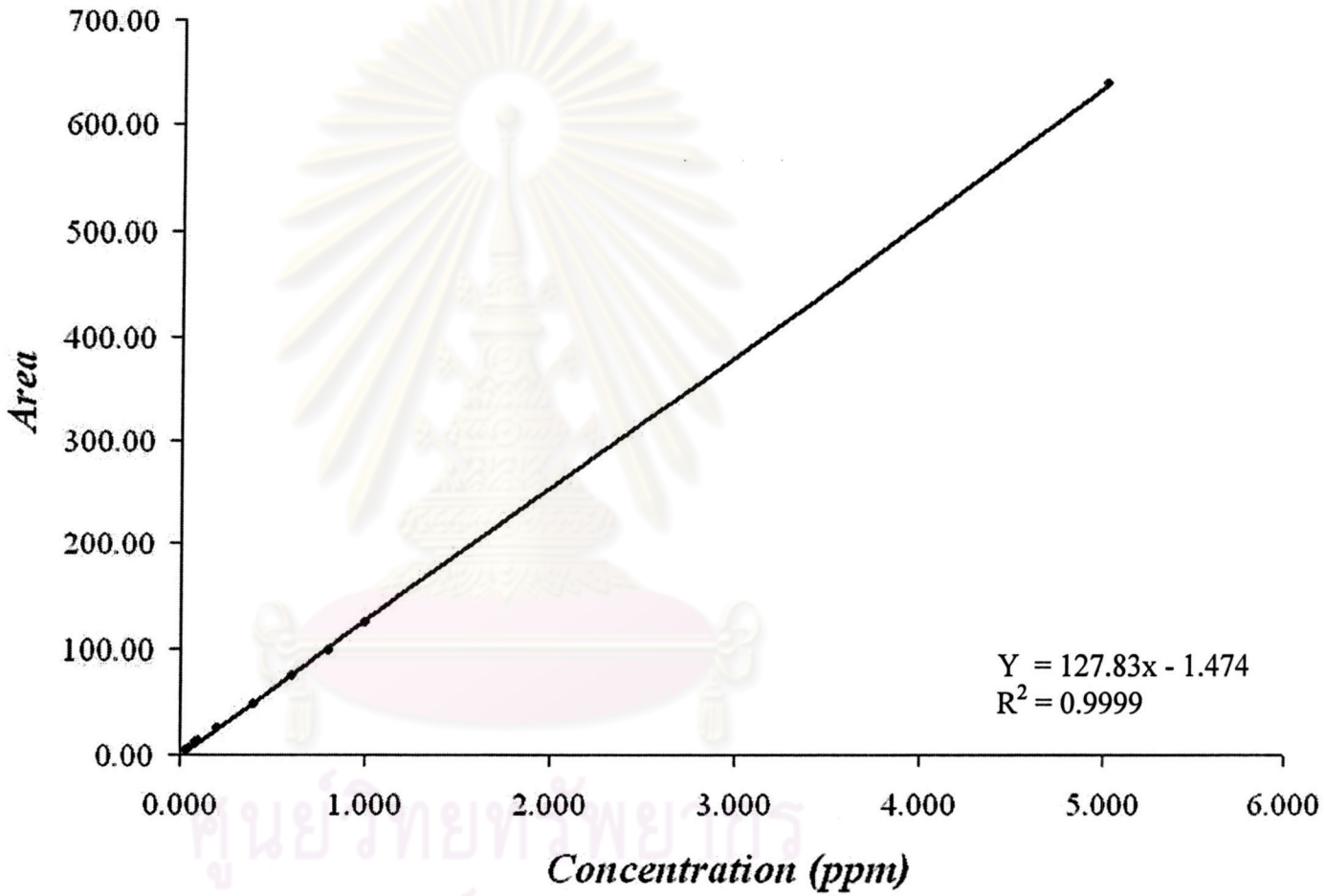

Figure. 4-7. Linear regression line of peak area vs. concentration of BADGE.2HCl obtained from HPLC data.

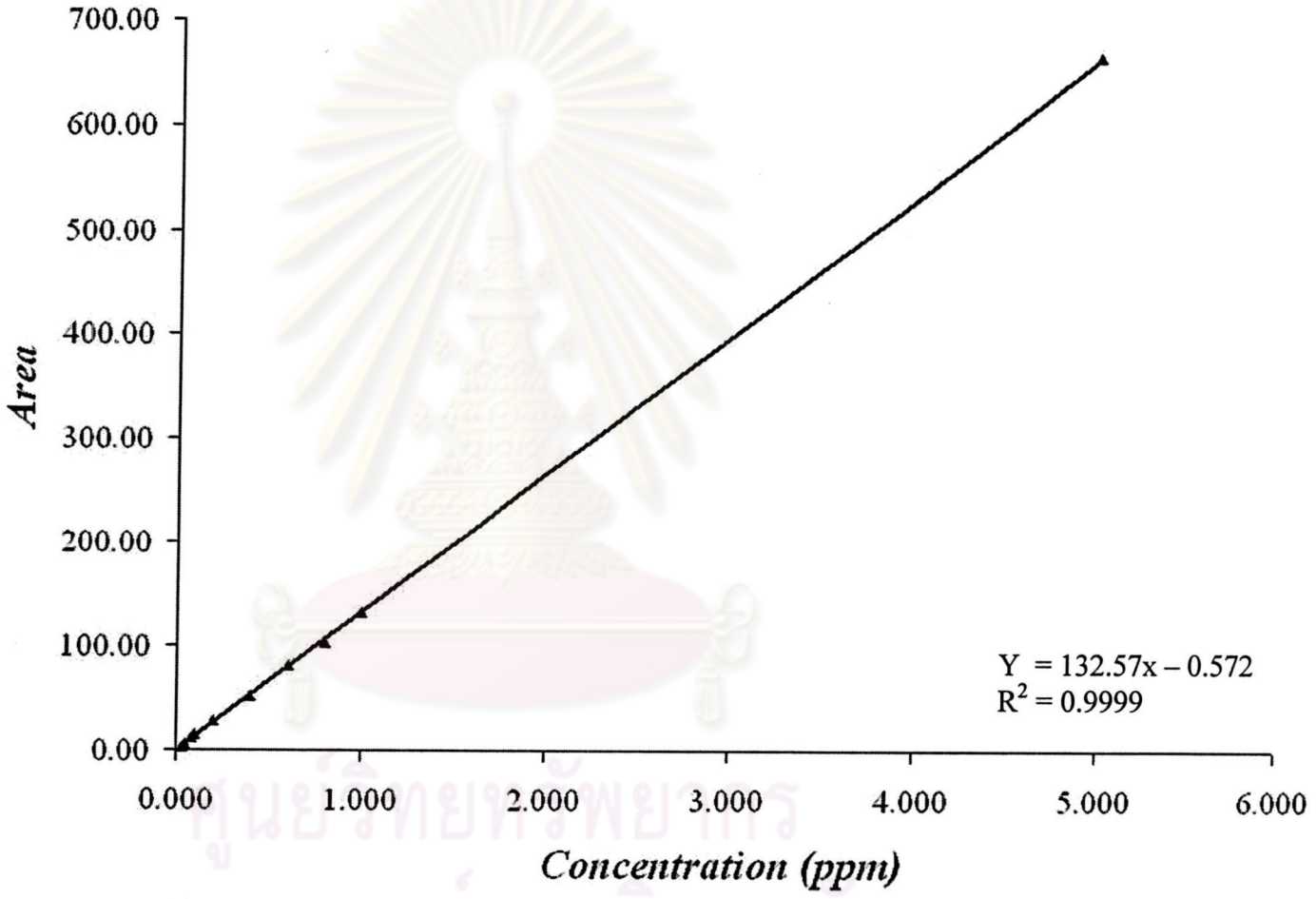

Figure. 4-8. Linear regression line of peak area vs. concentration of BFDGE obtained from HPLC data.

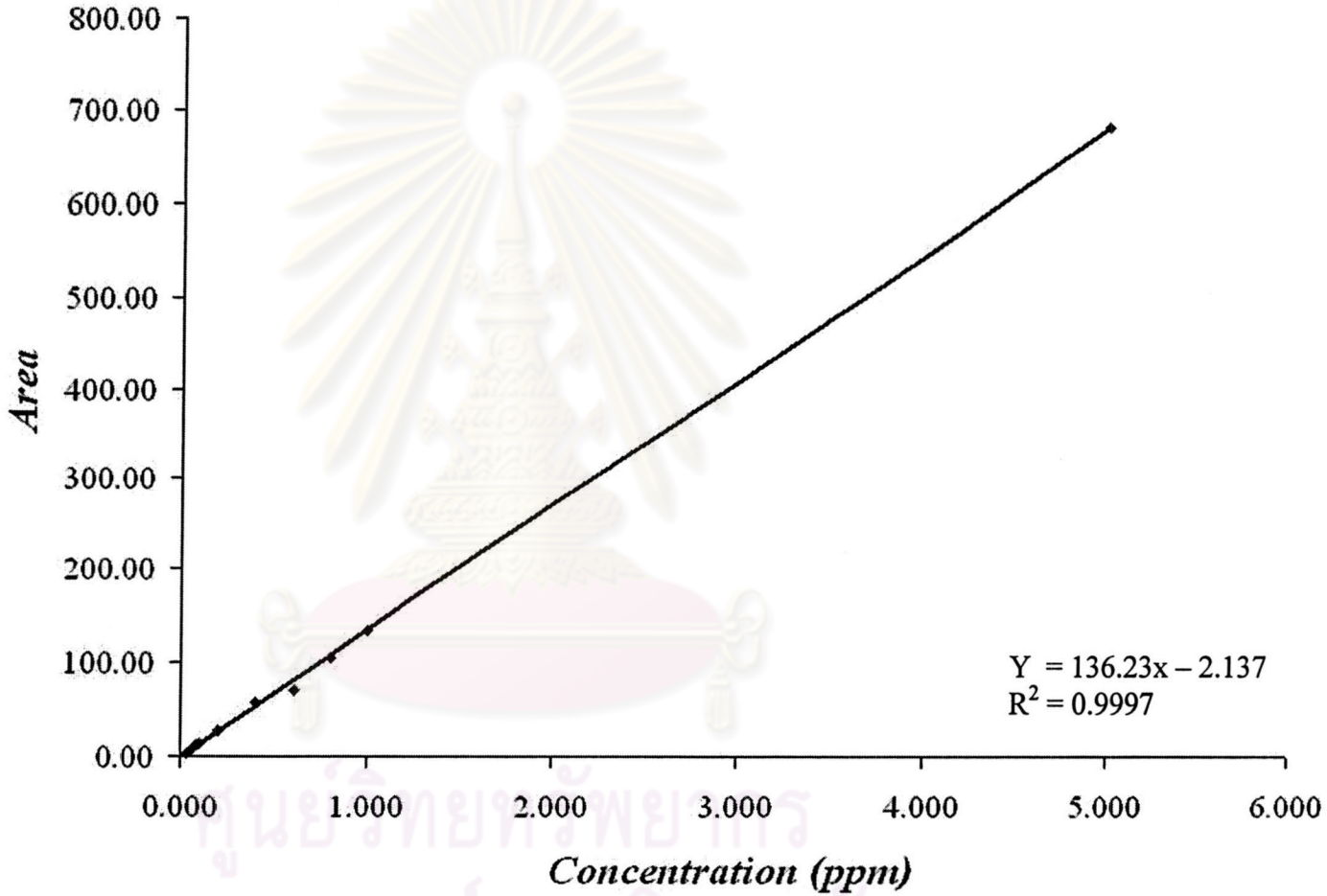

Figure. 4-9. Linear regression line of peak area vs. concentration of BFDGE.2HCl obtained from HPLC data.

5. The Construction of Standard Calibration Curves

Working calibration curves of all standards were constructed from 0.035-1.000 ppm, which is the maximum limit posed by the EU and also the expected range of contamination in real samples. The regression data are summarized in Table 4.6. Regression plots of relationships between concentration and peak area were shown in Figure 4-10 – 4-14.

Table 4.5. Linear regression results (9 level, n=9)

Compound	R^2	Intercept (LU·sec)	Slope (10^6·LU·sec·cm^3·g^{-1})
BADGE	0.9996	-0.162	195.37
BADGE.HCl	0.9999	-0.248	101.73
BADGE.2HCl	0.9997	0.131	123.08
BFDGE	0.9991	0.207	130.26
BFDGE.2HCl	0.9950	-0.376	131.01

Regression data in Table 4.6 showed all R^2-values approaching 1.0 indicating very high linear detector responses within this concentration range except for BFDGE.2HCl. Pearson's correlation coefficient test was used to confirm the significant of this linear relationship. Since the degree of freedom for this experiment was 7. The Pearson's acceptable value at 95% confident limit is 0.666, which is lower than our calculated R^2-values. Therefore, it is safe to say that the detector response is perfectly linear from 0.035–1.000 ppm for all analytes. As explained previously, regression lines for BFDGE and BFDGE.HCl existed as multiple peaks were calculated using total peak area of all isomers combined. Therefore, there was only one regression line for each compound. The slope of the regression line indicates sensitivity of detector responses; the higher the value, the higher the sensitivity. From Table 4.6, it is obvious that detector response of BADGE is the highest and BADGE.HCl is the lowest.

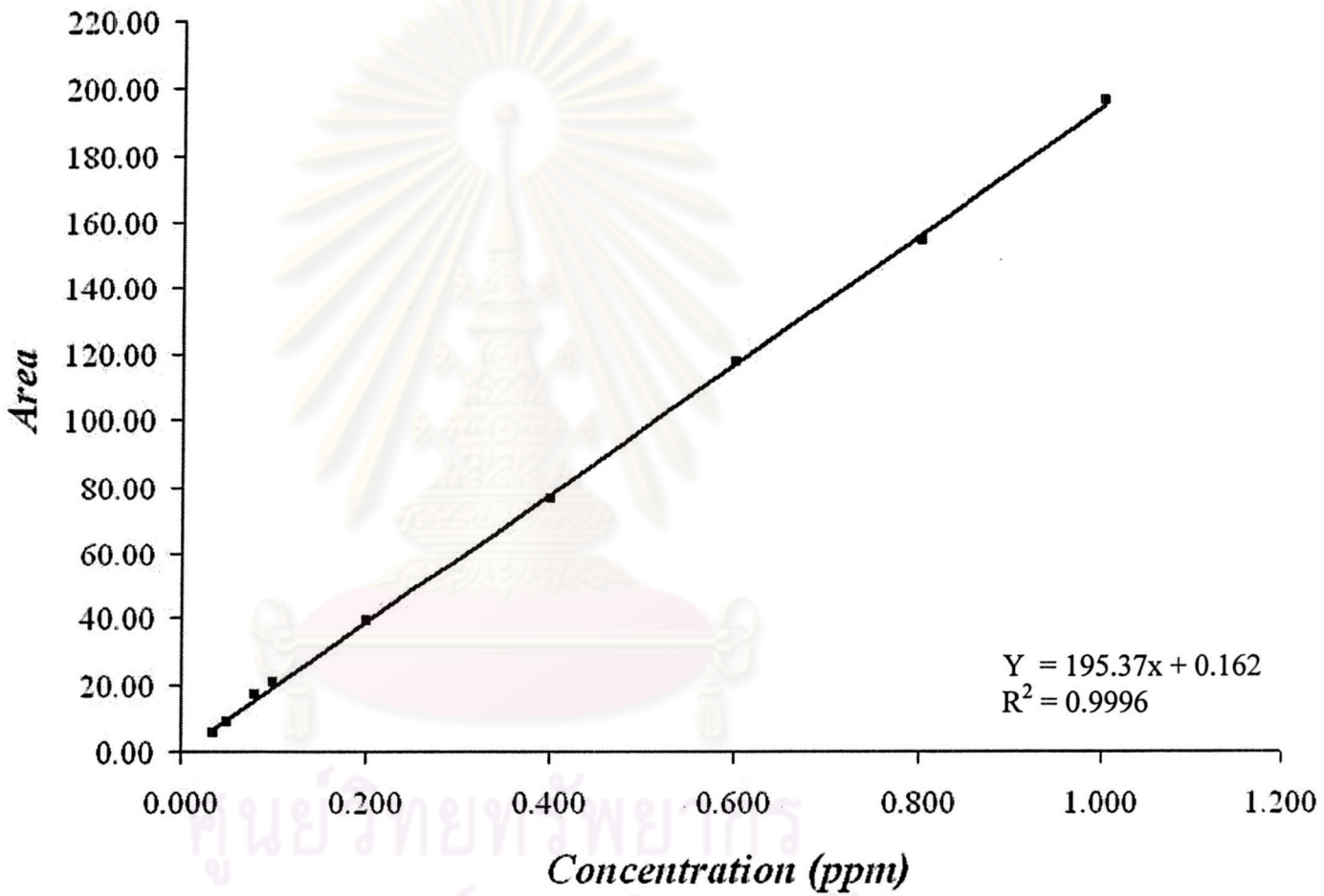

Figure. 4-10. Calibration curve of BADGE obtained from HPLC data.

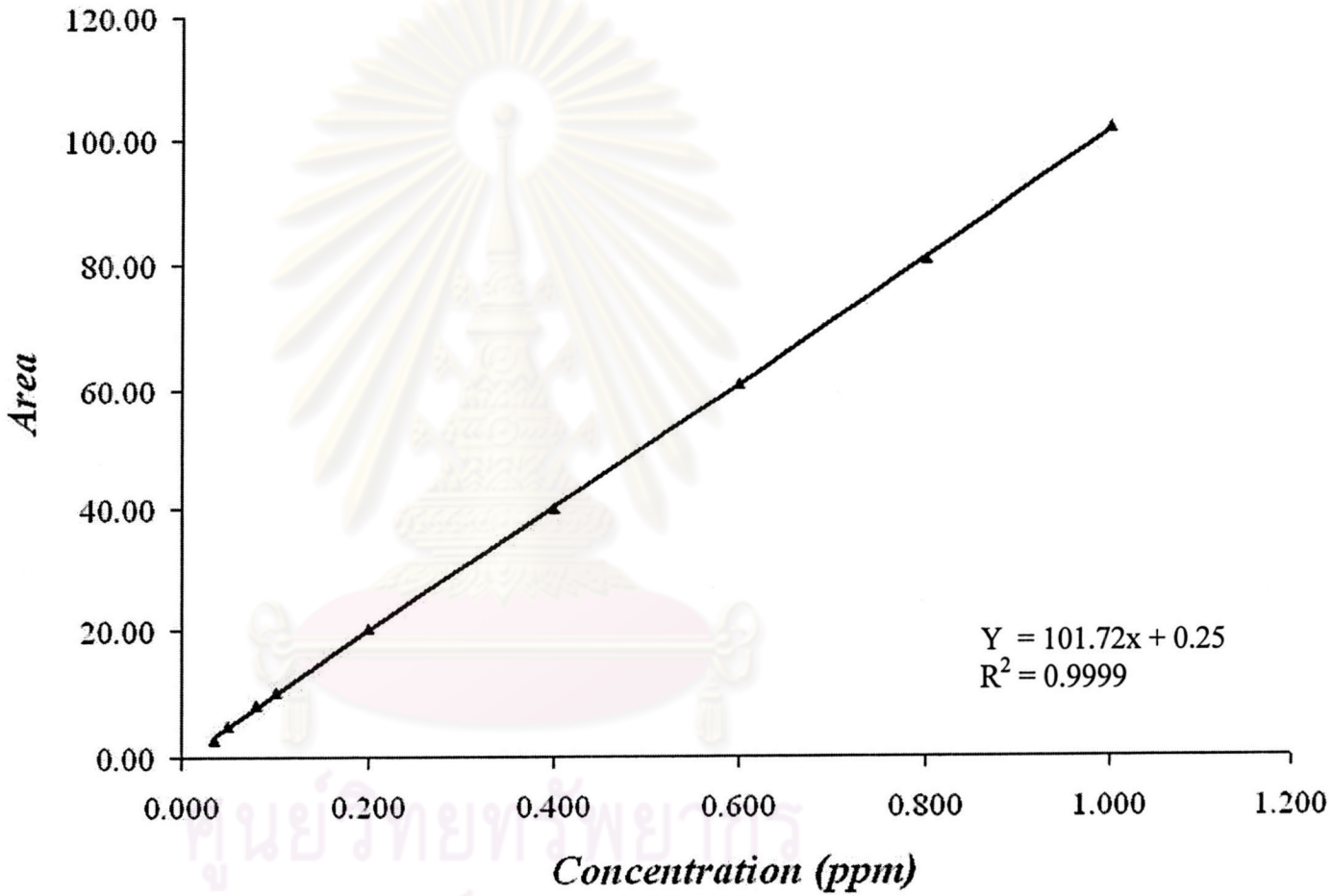

Figure. 4-11. Calibration curve of BADGE.HCl obtained from HPLC data.

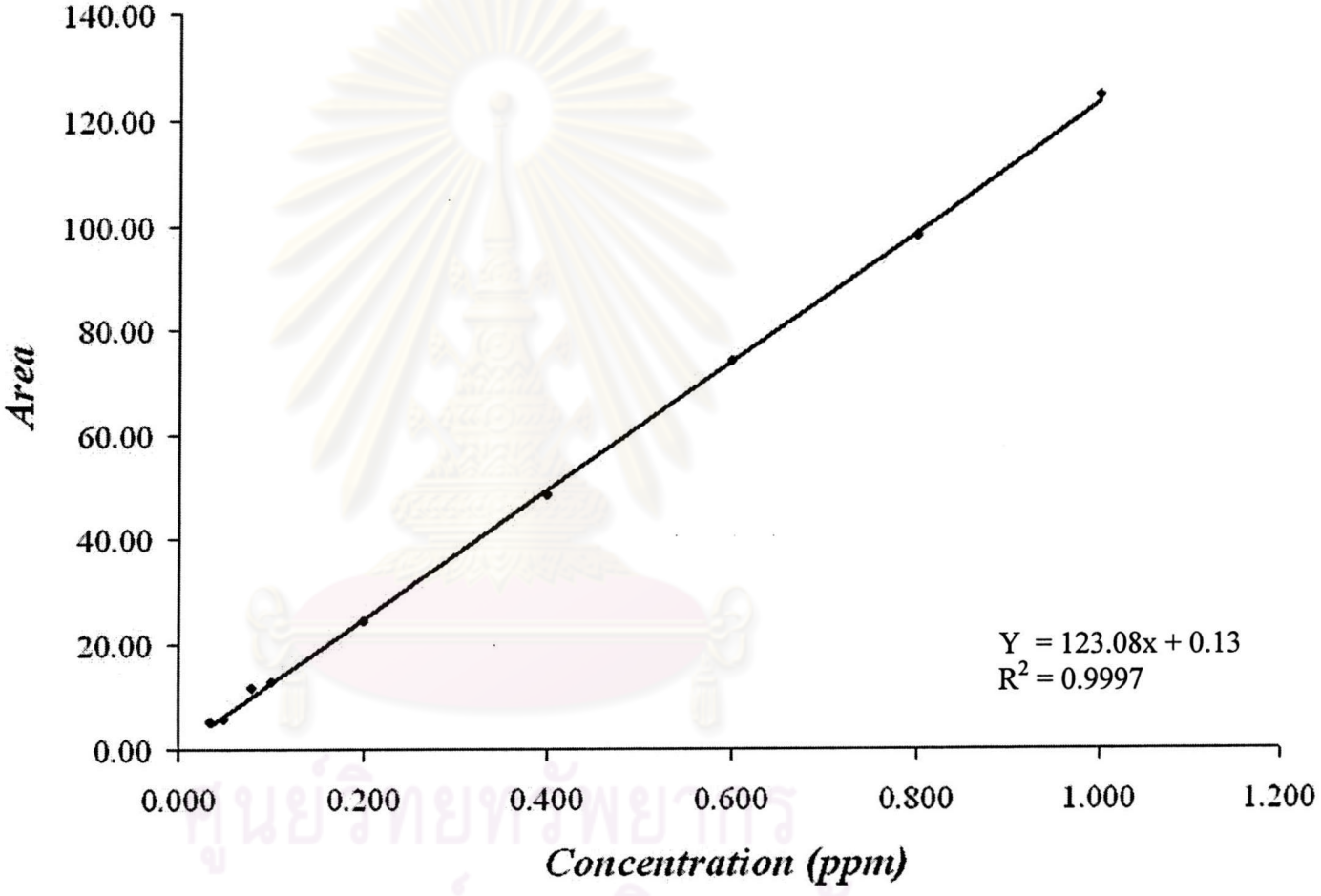

Figure. 4-12. Calibration curve of BADGE.2HCl obtained from HPLC data.

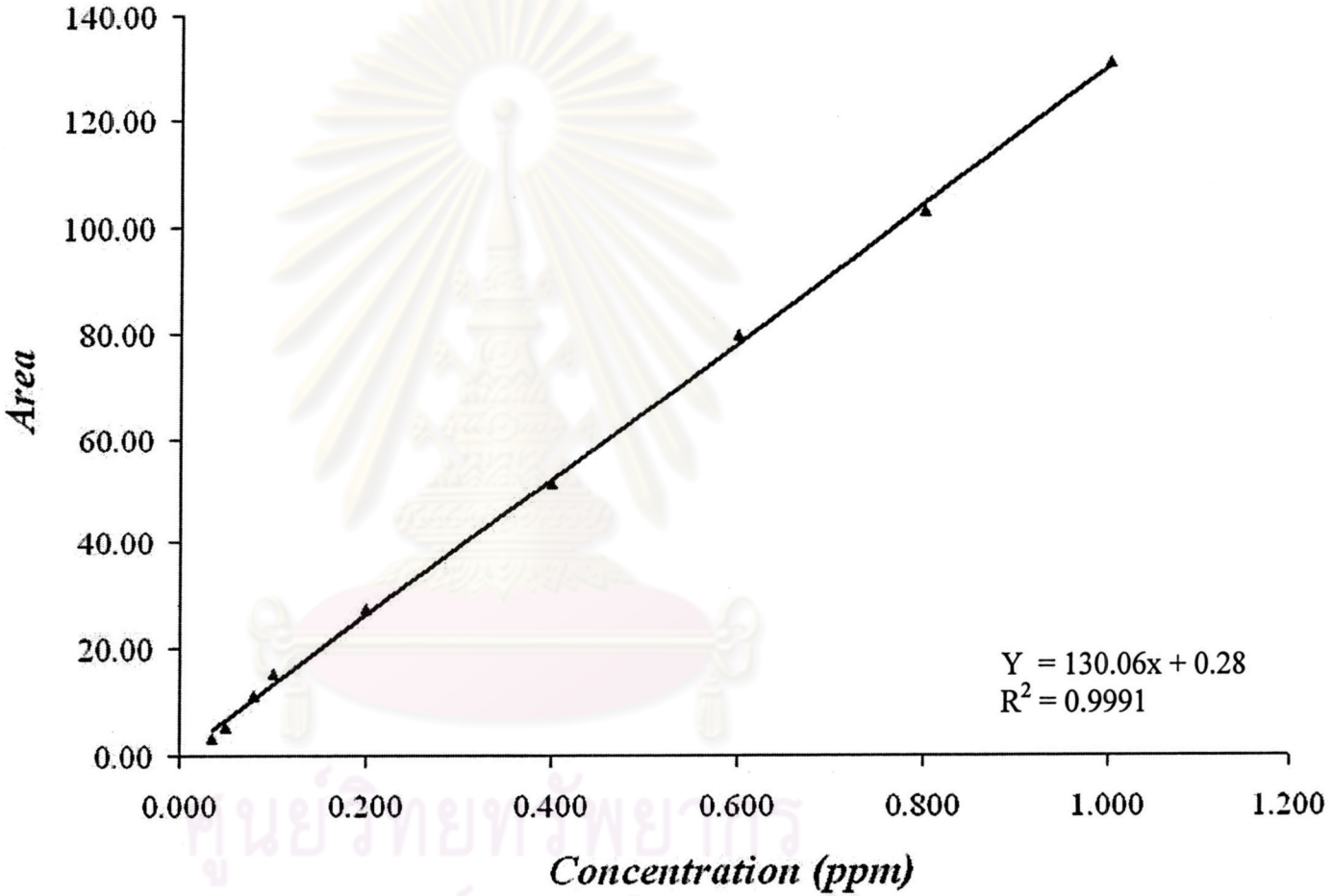

Figure. 4-13. Calibration curve of BFDGE obtained from HPLC data.

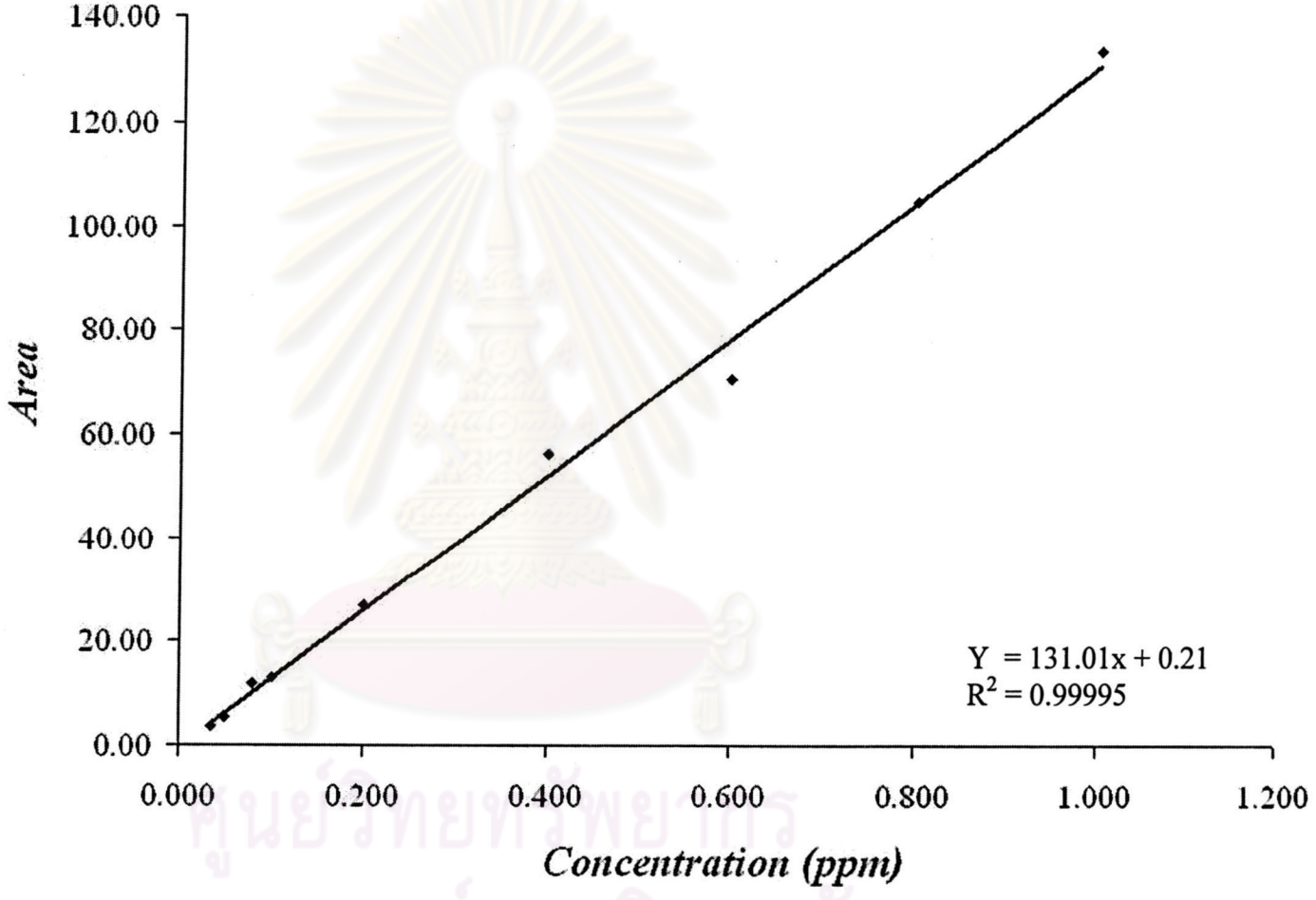

Figure. 4-14. Calibration curve of BFDGE.2HCl obtained from HPLC data.

6. The Determination of Limits of Detection (LoD)

Limit of detection (LoD) is defined as the amount of analyte in standard solution that yields a peak at signal-to-noise ratio equals to 3. Extrapolation of the standard calibration curve to intercept can estimate the limit of detection (LoD) concentration. The signal at LoD is equaled to the signal at y_B+3S_B, where y_B is the extrapolated blank signal at intercept and S_B is the standard deviation of blank signal. S_B is equal to $\sqrt{(\sum (y-y_i)^2/ n-2)}$, where y_i are the points on the regression line corresponding to the individual x value. Once the signal at LoD can be determined, the corresponded LoD concentration can be extrapolated from the calibration curve.

Table 4.6. Comparison of limit of detection (LoD) values

Compound	LoD (estimate, ppm)			LoD (experiment, ppm)
	y_B	S_B	LoD (ppm)	
BADGE	-0.162	1.416	0.022	0.012
BADGE.HCl	-0.248	0.351	0.010	0.034
BADGE.2HCl	0.131	0.851	0.021	0.031
BFDGE	0.207	1.467	0.034	0.020
BFDGE.2HCl	-0.376	3.579	0.082	0.030

LoD values obtained from both methods are significantly different. From the extrapolation method, LoD value depends on y-intercept and standard deviation of the regression line, therefore extrapolation of data set of higher variance will result in deviated LoD from true value. This is observed in the LoD values of BFDGE.2HCl (S_B = 3.579), BADGE (S_B = 1.416) and BFDGE (S_B= 1.467) are higher than the experimental values. Only LoD value of BADGE.HCl (S_B= 0.351) is lower than the experimental values.

The experimental LoD values obtained from taking ratio of S/N = 3 showed correlation with detector response. Since BADGE exhibits the highest detector

response, its detection is enhanced and lower LoD value is observed. On a contrary, BADGE.HCl has the lowest detector response, its LoD value was the highest. For BFDGE and BFDGE.2HCl, both compounds exist in multiple isomers that may coelute in the chromatogram. Therefore, the S/N ratio taken from only one peak does not represent the total signal from all isomers thus resulting in higher LoD. BADGE.2HCl eluted late and broad indicated low separation efficiency and thus results in lower peak height and higher LoD. The data in Table 4.6 suggests that the analysis of concentration lower than the LoD value will give signal that is insignificantly different from background. Therefore, sample reconcentration or higher concentration should be used to obtain meaningful result.

7. The Determination of Limits of Quantitation (LoQ).

Limit of quantitation (LoQ) is defined as the amount of analyte in standard solutions that yields a peak at signal-to-noise ratio equals to 10. Extrapolation of the standard calibration curve to intercept can estimate the limit of quantitation (LoQ) value. The signal at LoQ is equaled to y_B+10S_B, where y_B is the extrapolated blank signal that at intercept and S_B is the standard deviation of blank signal (same as Section 6 for LoD). Once the signal at LoQ can be determined, the corresponded LoQ concentration can be extrapolated from the calibration curve.

Table 4.7. Comparison of limit of quantitation (LoQ) values

Compound	LoQ (estimate, ppm)			LoQ (experiment, ppm)
	y_B	S_B	LoQ (ppm)	
BADGE	-0.162	1.416	0.072	0.041
BADGE.HCl	-0.248	0.351	0.035	0.113
BADGE.2HCl	0.131	0.851	0.069	0.103
BFDGE	0.207	1.467	0.113	0.066
BFDGE.2HCl	-0.376	3.579	0.273	0.100

Table 4.7 reports the lowest concentration for precise quantitative measurement of 5 analytes from 0.041-0.113 ppm. LoQ values obtained from both methods (extrapolation of the calibration curve and taking S/N ratio) are different and can be reasoned using the same logic as Section 6 for LoD. The HPLC method can detect lower concentration of analytes but it can not tell the exact amount present in sample if the concentration value is lower than LoQ.

8. The Determination of Method Precision and Percent Recovery

A precision of any method indicates the degree of control over every step of the analytical procedure from sample preparation to instrumental analysis. To ensure good reproducibility of the data, uncertainty of any analytical procedure within a laboratory must be estimated. Preliminary estimation of precision for a new method is normally performed over a short period of time such as within one day (repeatability).

Repeat extraction of spiked samples can be used to measure the efficiency of the analytical procedure especially the sample preparation step. Relative standard deviations from 10 repeat analyses at LoQ (0.041-0.113 ppm) and 5-fold LoQ (0.205-0.565 ppm) were used to estimate both percent recovery and method precision as shown in Table 4.8.

Table 4.8. Coefficients of variation (CV) and percent recovery data (n=10)

Compound	CV		% Recovery	
	LoQ level	5-fold LoQ level	LoQ level	5-fold LoQ level
BADGE	9.82	4.34	48.84 $\pm$ 4.63	64.40 $\pm$ 2.76
BADGE.HCl	11.17	6.20	74.17 $\pm$ 8.31	76.70 $\pm$ 4.76
BADGE.2HCl	12.84	5.93	86.61 $\pm$ 11.09	79.20 $\pm$ 4.69
BFDGE	12.35	5.56	77.33 $\pm$ 8.88	85.30 $\pm$ 4.74
BFDGE.2HCl	13.85	5.53	83.50 $\pm$ 11.56	78.00 $\pm$ 4.31

The CV values of repeat analysis (n=10) at LoQ level are approximately twice as high as the CV at 5-fold LoQ indicating consistency of all procedures e.g. sample preparation, and that system noises may be the culprit for higher variance at LoQ level. Percent recovery of analytes was obtained between 64.83-85.35%, which is low but acceptable for trace analysis in ppm range. The reason for low recovery maybe because the spiking standards dissolve in oil and fat very well. Since oil and fat were removed from sample to minimize interference during HPLC analysis, some of the dissolved standards may be lost.

Figure 4.15 and 4.16 are graphical presentations of percent recovery and method precision at LoQ and 5-fold LoQ respectively. It is obvious from the diagrams that the quantitative procedure can provide acceptable precision and fairly efficient.

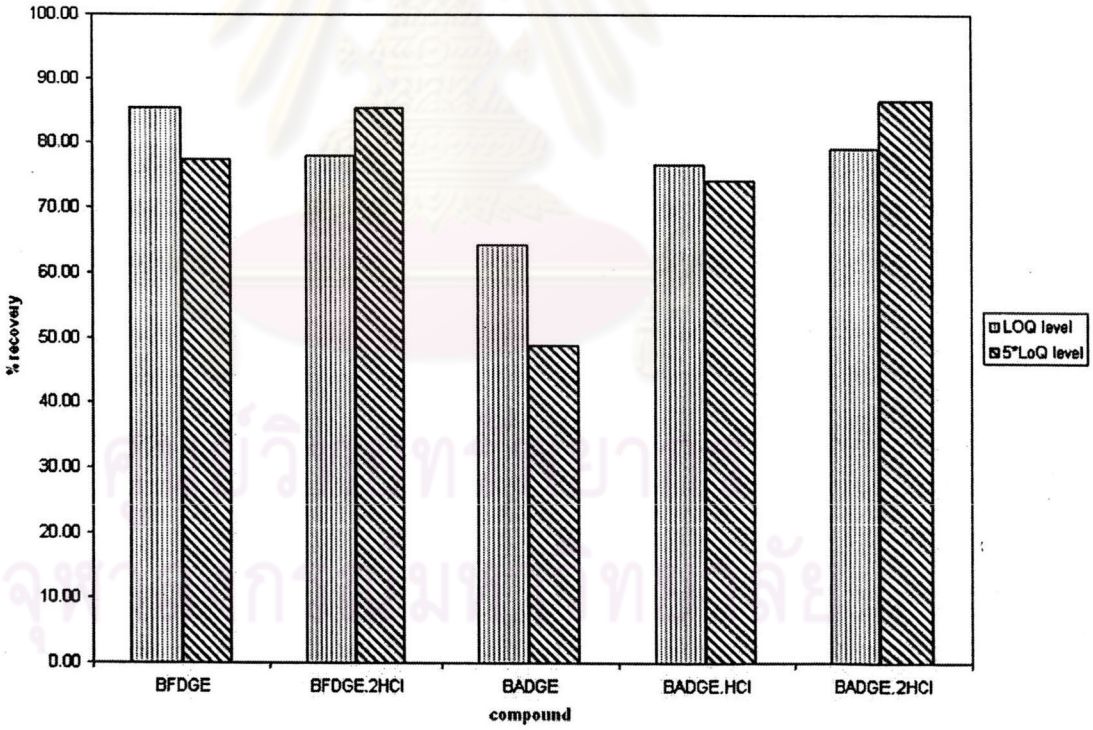

Figure 4-15. Graphical presentation of percent recovery at LoQ and 5-fold LoQ

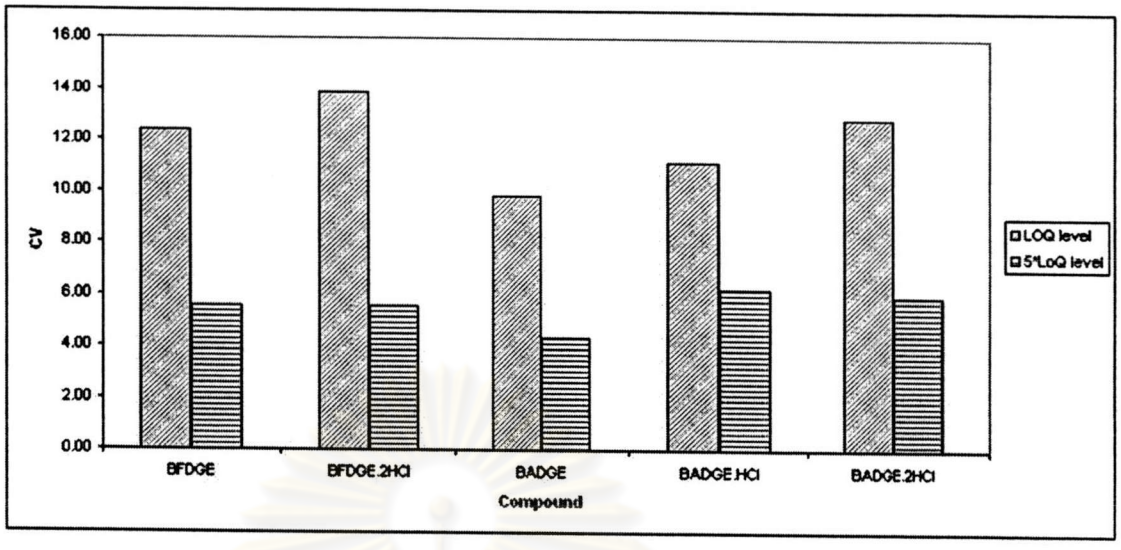

Figure 4-16. Graphical presentation of method precision at LoQ and 5-fold LoQ

9. The Study of Method Robustness

Many method development or validation protocols require that sensitivity to particular parameters be investigated directly. This is usually done by a preliminary robustness test, in which the effect of one or more parameter changed are observed. Robustness test data can provide information on the effect of important parameters.

The parameters selected to test for their effects on the analytical results were volume of extraction solvent and solvent evaporation temperature. Different volumes of acetronitrile used (20 mL vs. 30 mL) during extraction of spiked samples were compared. Also different heating temperature during solvent evaporation at 30 °C (normal) and 40 °C were compared. Precision data (coefficients of variation) and result of students *t*-test at 95% confident limit of 6 repeat analyses are compiled in Table 4.9.

Table 4.9. Coefficients of variation and student t-test data, (n=6)

Compound	CV		*t*-value	
	Method 1[a]	Method 2[b]	Method 1[a]	Method 2[b]
BADGE	9.28	12.60	1.034	0.564
BADGE.HCl	8.94	16.32	1.571	0.151
BADGE.2HCl	9.28	12.57	1.056	2.043
BFDGE	9.27	15.92	0.543	1.702
BFDGE.2HCl	9.30	12.05	0.189	0.619

[a] = *30* mL acetonitrile, 30 °C

[b] = 20 mL acetonitrile, *40* °C

A critical value of two-tailed *t*-test (n=5) is 2.57 at 95% confident limit.

The CV data indicating higher variance when temperature of the evaporation changed. Higher turbulance of the mixture during the evaporating step at temperature increased might be the cause of higher variation.

We can conclude that when volume of the extraction solvent changed up to 10 mL, there was no effect on the result. However, the change in temperature of the evaporation up 10 °C affected the result.

When the null hypothesis was the mean concentration of the normal method equal to the mean concentration of the new parameter for 5 degree of freedom. The critical *t*-value at 95% confidence level for comparing normal method and varied solvent extraction volume for 30 mL is less than the critical *t*-value. Then, the null hypothesis was accepted and can be concluded that there is no significant effect by changing the solvent extraction volume.

The observed *t*-value at 95% confidence level between normal method and varied evaporating temperature at 40 °C was also less than critical *t*-value, the null hypothesis was retained and not significantly different from normal method (evaporating temperature at 30 °C).

10. Analysis of Real Samples

Twenty oil-based canned foods from 7 companies were tested for contamination. All cans were 2 piece-cans with easy-open lids and came in 2 sizes. The standard cans had a dimension of 4.2 x 4.0 cm (radius x height) and internal surface area of 2.16 dm^2. The small size cans were 3.2 x 4.0 cm (radius x height) and internal surface area of 1.45 dm^2. Food contents selected for the test were from 2 categories: tuna in oil media (1) and fried foods (2). The general data of all sample tested are summarized in Table 4.10. Both cans and its food content were subjected to testing.

Beilstein's test was performed on all empty cans to indicate types of internal coating. Small amount of coating was put in the flame of a Bunsen burner. The flame color was observed. The positive result (green flame) indicated the presence of chlorine atoms in coating (Figure 4.17) and therefore the internal coating must be organosol. Negative results indicate other types of coating. Beilstein's test data are reported in Table 4.11. Data from Table 4.11 showed that lids of all cans were coated with organosol polymer (positive result). This is because great flexibility is required for easy-open type lid. Positive results also found with internal surfaces of sample number 1, 2, 3, 4, 5, 12, 13, 14, 15, 16, 17 and 18 indicating the presence of organosols. All of these samples except for number 16 and 17 are all fried foods.

Empty cans were extracted with acetonitrile that contacted with 50% of the internal can surfaces for 24 hrs and the solution was analyzed by HPLC. The data of extractable BADGE, BFDGE and their derivatives are illustrate in Table. 4.11. the range of extractable concentration are from 1.09 – 257 mg/dm^2. Extremely high contamination level were detected in sample 1, 2 and 4 which corresponded th high contamination level found in the can content as well. However, sample 3 (fried sardines) that was packed in cleaned can seems to have high contamination-suggested that food type may accelerate or force migration from coatings into food upon contract result in low extractable concentration. Thirty percent of can samples tested continued extractable components less than 10 mg/dm^2, 55% contained more than 10

mg/dm^2 but less than 50 mg/dm^2. Fifteen percent contained extremely high extractable components and should not be used as food contact materials.

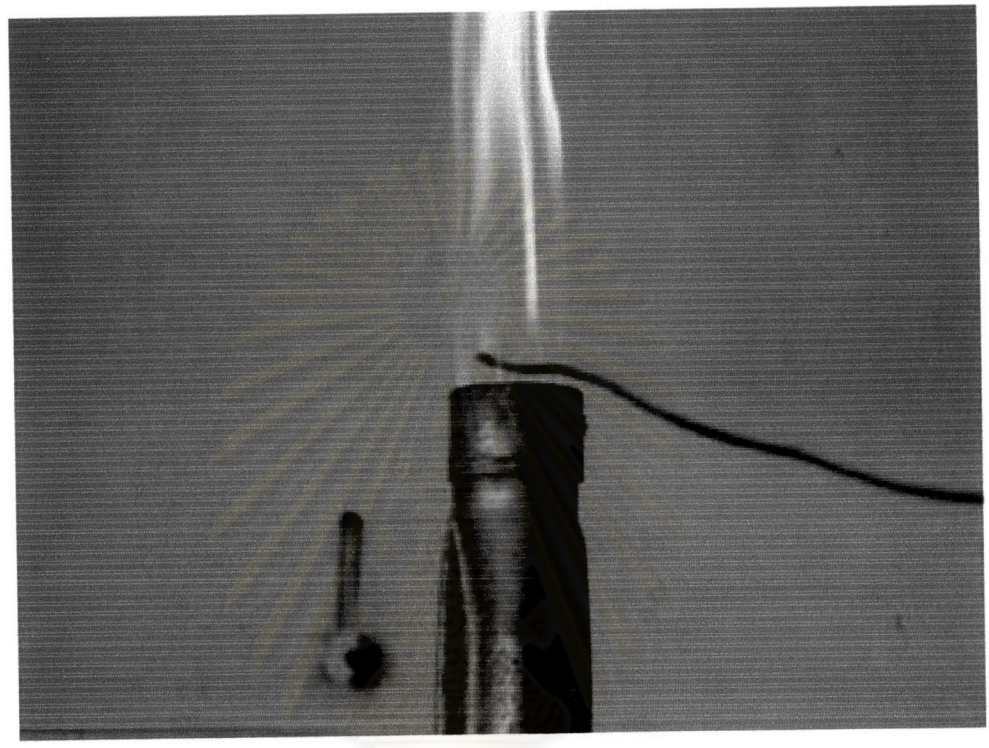

Figure 4.17. Green flame of Beilstein's test indicates positive result (organosol polymer presented).

Table 4.10. General data of food samples

No.	Company	Net weight (g/can)	Type	Size of can
1	A	40	Fried cockles	Small
2	A	30	Baby clam (dried)	Small
3	A	90	Fried sardines	Standard
4	A	40	Fried baby clam	Small
5	A	90	Fried catfish	Standard
6	B	185	Tuna steak	Standard
7	B	185	Tuna mayonnaise	Standard
8	B	185	Tuna sandwich	Standard
9	B	70	Fried baby clam	Standard
10	C	185	Tuna sandwich	Standard
11	C	180	Tuna mayonnaise	Standard
12	C	90	Fried sardines	Standard
13	C	40	Fried baby clam	Standard
14	D	185	Tuna sandwich	Standard
15	D	185	Tuna steak	Standard
16	E	185	Tuna sandwich	Standard
17	E	185	Tuna steak	Standard
18	F	25	Fried white scale fish	Standard
19	G	60	Fried chicken	Small
20	G	60	Fried pork	Small

Standard can = 4.2 x 4.0 cm (radius x height), surface area = 2.16 dm^2

Small can = 3.2 x 4.0 cm. (radius x height), surface area = 1.45 dm^2

Table 4.11. Beilstein's test results of empty cans and total contamination extracted from empty can (unit mg/dm^2)

No.	Company	Color		Beilstein's test		Contamination level (mg/dm^2)						
		Lid	Body	Lid	Body	A (1)	A.HCl (2)	A.2HCl (3)	F (4)	F.HCl (5)	F.2HCl (6)	Total
1	A	Silver	Gray	+	+	3.845	nd.	46.908	31.774	78.539	85.310	246.376
2	A	Silver	Gray	+	+	2.466	1.811	27.349	23.968	83.931	117.602	257.127
3	A	Silver	Gray	+	+	0.062	nd.	0.452	1.469	1.958	1.095	5.036
4	A	Silver	Gray	+	+	10.668	1.702	51.194	28.769	53.683	49.238	195.254
5	A	Silver	Gray	+	+	nd.	0.386	3.270	0.735	9.309	0.984	14.683
6	B	Gray	Gray	+	-	0.844	2.210	16.260	2.622	19.872	0.619	42.426
7	B	Gold	Gray	+	-	3.156	0.780	19.071	15.931	7.1154	1.117	47.180
8	B	Gray	Gray	+	-	0.520	1.096	10.878	2.812	14.640	0.716	30.661
9	B	Gold	Gray	+	-	3.503	2.156	11.442	2.410	10.120	0.927	30.559
10	C	Gold	Gray	+	-	nd.	0.467	5.029	0.967	4.983	0.169	11.615

(1) = BADGE, (2) = BADGE.HCl, (3) = BADGE.2HCl, (4) = BFDGE, (5) = BFDGE.HCl and (6) = BFDGE.2HCl.

nd = not detectable (BADGE < 0.012, BADGE.HCl < 0.034, BADGE.2HCl < 0.031, BFDGE < 0.020, BFDGE.HCl < 0.027 and BFDGE.2HCl < 0.030 ppm, respectively).

BFDGE.HCl result were calculated using data of Nongpanga Kulkaew and Suparee Pungboonlue [40].

Table 4.11 (continue). Beilstein's test results of empty cans and total contamination extracted from empty can (unit mg/dm^2)

No.	Company	Color		Beilstein's test		Contamination level (mg/dm^2)						
		Lid	Body	Lid	Body	A (1)	A.HCl (2)	A.2HCl (3)	F (4)	F.HCl (5)	F.2HCl (6)	Total
11	C	Gold	Gray	+	-	0.357	0.579	19.244	1.990	3.475	0.531	26.176
12	C	Gold	Gray	+	+	4.560	5.787	18.223	1.355	14.149	0.698	44.772
13	C	Gold	Gray	+	+	1.777	4.382	11.972	1.567	8.355	0.533	28.585
14	D	Gold	Gray	+	+	0.063	0.213	0.625	nd.	0.899	0.102	1.902
15	D	Gold	Gray	+	+	0.312	0.771	4.286	0.808	1.969	0.325	8.472
16	E	Gold	Gray	+	+	0.196	0.677	1.374	0.453	3.550	0.662	6.912
17	E	Gold	Gray	+	+	0.544	0.899	1.334	0.770	2.658	0.536	6.741
18	F	Gold	Silver	+	+	0.079	nd.	0.253	0.329	0.736	0.512	1.908
19	G	Gold	Gray	+	-	0.325	nd.	18.818	1.612	3.290	0.713	24.759
20	G	Gold	Gray	+	-	nd	0.977	19.306	1.695	3.562	0.498	26.038

(1) = BADGE, (2) = BADGE.HCl, (3) = BADGE.2HCl, (4) = BFDGE, (5) = BFDGE.HCl and (6) = BFDGE.2HCl.

nd = not detectable (BADGE < 0.012, BADGE.HCl < 0.034, BADGE.2HCl < 0.031, BFDGE < 0.020, BFDGE.HCl < 0.027 and BFDGE.2HCl < 0.030 ppm, respectively).

BFDGE.HCl result were calculated using data of Nongpanga Kulkaew and Suparee Pungboonlue [40].

Table 4.12 summarized total contamination detected in 20 oil-based canned foods. For foods contained oil, oil phase was also analyzes and the contamination was also reported. The current EU's regulation allows a total sum of BADGE, BFDGE, and their derivatives in canned foods tested are contaminated. Twenty five percent of samples were contaminated above the EU regulation and only one was only slightly tainted (sample 18, 0.079 mg/kg) sample 4 was badly contaminated with total contamination reached 4.289 mg/kg that is more than 4 times higher than the control limit.

Because migration of these contamination also depend on contact surface between food and internal can surface, food packs in different can size will have different contamination level due to different in contact surface area. Therefore, contamination can also report per surface area (mg/dm^2). The current EU's regulation allows maximum level of BADGE, BFDGE and their derivatives not to exceed 0.166 mg/dm^2. This is based on the assumption that we eat 1 kg of food per day and the food was packed in a cube of 1 dm x 1dm x 1dm, which give the surface area 6 dm^2 (appendix A and B). Table 4.13 summarized the contamination results of 20 oil-based canned foods available in Thailand. Our data indicate that 80% of samples tested exceeded the limit of this regulation, two out of 20 samples were contaminated more than 0.100 mg/dm^2 but not exceeded the limit, and 10% were contaminated less than 0.100 mg/dm^2. Sample number 4 was badly contaminated with total concentration reached 2.958 mg/dm^2, more than 18 times than the control value. The lowest contamination was detected at 0.036 mg/dm^2 (sample 18).

Table 4.12. Contamination data of food content and oil phase

No.	Contamination in meat (mg/kg)							Contamination in oil phase (mg/kg)							Contamination	
	A (1)	A.HCl (2)	A.2HCl (3)	F (4)	F.HCl (5)	F.2HCl (6)	Total	A (1)	A.HCl (2)	A.2HCl (3)	F (4)	F.HCl (5)	F.2HCl (6)	Total	(mg/kg)	(mg/can)
1	0.244	0.025	0.100	0.134	0.748	0.487	1.738	-	-	-	-	-	-	-	1.738	0.070
2	0.196	0.026	0.065	0.124	0.361	0.029	0.801	-	-	-	-	-	-	-	0.801	0.024
3	0.597	nd	0.064	0.163	1.260	0.288	2.372	-	-	-	-	-	-	-	2.372	0.231
4	1.166	nd	0.242	0.492	2.060	0.329	4.289	-	-	-	-	-	-	-	4.289	0.172
5	0.770	nd	0.121	0.358	2.086	0.415	3.750	-	-	-	-	-	-	-	3.750	0.338
6	nd	0.045	0.116	0.051	0.204	0.026	0.441	0.050	0.028	0.114	0.028	0.055	0.044	0.318	0.759	0.140
7	nd	nd	0.301	0.039	0.102	0.034	0.476	-	-	-	-	-	-	-	0.476	0.088
8	nd	nd	0.108	0.046	0.231	0.032	0.416	nd	nd	0.127	0.022	0.062	nd	0.211	0.627	0.116
9	0.160	0.035	0.045	0.084	0.345	0.022	0.692	-	-	-	-	-	-	-	0.692	0.048
10	nd	0.024	0.087	0.025	0.028	0.028	0.226	nd	nd	0.085	nd	0.404	0.022	0.177	0.403	0.075

(1) = BADGE, (2) = BADGE.HCl, (3) = BADGE.2HCl, (4) = BFDGE, (5) = BFDGE.HCl and (6) = BFDGE.2HCl.

nd = not detectable (BADGE < 0.012, BADGE.HCl < 0.034, BADGE.2HCl < 0.031, BFDGE < 0.020, BFDGE.HCl < 0.027 and BFDGE.2HCl < 0.030 ppm, respectively).

BFDGE.HCl result were calculated using data of Nongpanga Kulkaew and Suparee Pungboonlue [40].

Table 4.12 (continue). Contamination data of food content and oil phase

No.	Contamination in meat (mg/kg)							Contamination in oil phase (mg/kg)							Contamination	
	A (1)	A.HCl (2)	A.2HCl (3)	F (4)	F.HCl (5)	F.2HCl (6)	Total	A (1)	A.HCl (2)	A.2HCl (3)	F (4)	F.HCl (5)	F.2HCl (6)	Total	(mg/kg)	(mg/can)
11	nd	nd	0.112	0.020	0.068	nd	0.200	-	-	-	-	-	-	-	0.200	0.036
12	0.335	nd	0.093	0.184	1.193	0.028	1.832	-	-	-	-	-	-	-	1.832	0.165
13	0.356	nd	0.158	0.086	0.383	nd	0.983	-	-	-	-	-	-	-	0.983	0.039
14	nd	0.028	0.053	0.017	0.059	nd	0.165	nd	0.024	0.092	0.014	0.041	nd	0.171	0.327	0.061
15	nd	0.059	0.111	0.015	0.065	nd	0.250	0.029	0.058	0.192	0.057	nd	0.033	0.370	0.620	0.115
16	nd	nd	0.043	0.029	0.101	nd	0.172	0.012	0.037	0.092	0.014	0.077	nd	0.231	0.403	0.075
17	nd	nd	0.038	0.018	0.106	0.030	0.111	nd	nd	0.062	nd	0.056	nd	0.118	0.309	0.057
18	0.008	0.026	0.024	nd	0.010	0.020	0.089	-	-	-	-	-	-	-	0.079	0.002
19	0.158	nd	0.150	0.098	0.148	nd	0.554	-	-	-	-	-	-	-	0.554	0.033
20	0.283	nd	0.078	0.053	0.186	0.056	0.532	-	-	-	-	-	-	-	0.532	0.032

(1) = BADGE, (2) = BADGE.HCl, (3) = BADGE.2HCl, (4) = BFDGE, (5) = BFDGE.HCl and (6) = BFDGE.2HCl.

nd = not detectable (BADGE < 0.012, BADGE.HCl < 0.034, BADGE.2HCl < 0.031, BFDGE < 0.020, BFDGE.HCl < 0.027 and BFDGE.2HCl < 0.030 ppm, respectively).

BFDGE.HCl result were calculated using data of Nongpanga Kulkaew and Suparee Pungboonlue [40].

Table 4.13. Contamination level per contact surface area

No.	Concentration (mg/dm^2)						
	A (1)	A.HCl (2)	A.2HCl (3)	F (4)	F.HCl (5)	F.2HCl (6)	Total
1	0.168	0.017	0.069	0.093	0.516	0.336	1.199 ± 0.048
2	0.135	0.018	0.045	0.085	0.249	0.202	0.552 ± 0.049
3	0.276	nd	0.029	0.076	0.583	0.133	1.098 ± 0.024
4	0.804	nd	0.167	0.339	1.421	0.227	2.958 ± 0.189
5	0.356	nd	0.056	0.166	0.966	0.192	1.736 ± 0.002
6	0.023	0.034	0.106	0.036	0.119	0.032	0.351 ± 0.052
7	nd	nd	0.140	0.018	0.047	0.016	0.220 ± 0.051
8	nd	nd	0.109	0.031	0.136	0.015	0.290 ± 0.006
9	0.074	0.016	0.021	0.039	0.160	0.010	0.320 ± 0.023
10	nd	0.025	0.079	0.011	0.047	0.023	0.187 ± 0.017
11	nd	nd	0.052	0.009	0.031	nd	0.093 ± 0.007
12	0.158	nd	0.044	0.087	0.552	0.013	0.854 ± 0.091
13	0.065	nd	0.028	0.040	0.177	nd	0.455 ± 0.055
14	nd	0.024	0.067	0.014	0.047	nd	0.151 ± 0.012
15	0.014	0.054	0.140	0.033	0.030	0.015	0.287 ± 0.043
16	0.005	0.017	0.060	0.019	0.083	nd	0.187 ± 0.017
17	nd	nd	0.046	0.008	0.075	0.014	0.143 ± 0.016
18	0.004	0.012	0.011	nd	nd	0.009	0.036 ± 0.005
19	0.109	nd	0.103	0.036	0.102	0.039	0.382 ± 0.049
20	0.046	nd	0.054	0.087	0.043	0.013	0.367 ± 0.030

(1) = BADGE, (2) = BADGE.HCl, (3) = BADGE.2HCl, (4) = BFDGE, (5) = BFDGE.HCl and (6) = BFDGE.2HCl.

nd. = not detectable (BADGE < 0.012, BADGE.HCl < 0.034, BADGE.2HCl < 0.031, BFDGE < 0.020, BFDGE.HCl < 0.027 and BFDGE.2HCl < 0.030 ppm, respectively).

BFDGE.HCl result were calculated using data of Nongpanga Kulkaew and Suparee Pungboonlue [40].

Table 4.14. The contamination in samples from company A

Sample No.	Food type	Can coating		Concentration (mg/kg)
		Lid	Body	
1	Fried cockles	Organosol	Organosol	1.738
2	Baby clam (dried)	Organosol	Organosol	0.801
3	Fried sardines	Organosol	Organosol	2.372
4	Fried baby clam	Organosol	Organosol	4.289
5	Fried catfish	Organosol	Organosol	3.750

Samples from company A were fried foods and both parts of can were coated with organosol. The contamination levels detected were higher than the regulation except for sample 2 (0.0801 mg/kg). The data implied that organosol is unsuitable for coating the internal surface of food cans that required processing at high temperature such as fried foods.

Table 4.15. The contamination in samples from company B

Sample No.	Food type	Can coating		Concentration (mg/kg)
		Lid	Body	
6	Tuna steak	Organosol	Epoxy	0.759
7	Tuna mayonnaise	Organosol	Epoxy	0.476
8	Tuna sandwich	Organosol	Epoxy	0.627
9	Fried baby clam	Organosol	Epoxy	0.692

Two types of canned foods from company B were tested: tuna in oil media and fried foods. All can bodies were coated with epoxy resins and lids were all easy-open coated with organosols. The contamination level found in 4 food types tested were lower than the EU regulation.

Table 4.16. The contamination in samples from company C

Sample No.	Food type	Can coating		Concentration (mg/kg)
		Lid	Body	
10	Tuna sandwich	Organosol	Epoxy	0.403
11	Tuna mayonnaise	Organosol	Epoxy	0.200
12	Fried sardines	Organosol	Organosol	1.832
13	Fried baby clam	Organosol	Organosol	0.983

Fish in oil products from company C were packed in epoxy coated cans with easy open lids coated with organosols. The contamination level detected in this group of food met the EU regulation (sample 10 and sample 11). However, fried foods produced by this company were packed in organosol coated cans and the contamination detected increased several folds (sample 12 and sample 13). The data reconfirmed our previous observation that organosol resins are unsuitable for fried foods.

Table 4.17. The contamination in samples from company D and E

Sample No.	Food type	Can coating		Concentration (mg/kg)
		Lid	Body	
14D	Tuna sandwich	Organosol	Organosol	0.327
15D	Tuna steak	Organosol	Organosol	0.620
16E	Tuna sandwich	Organosol	Organosol	0.403
17E	Tuna steak	Organosol	Organosol	0.309

Samples from company D and E tested were tuna in oil. Both can bodies and lids were coated with organosol. The contaminations detected were below the EU regulation. The contaminations detected were comparable to fish-in-oil packed in epoxy coated cans (sample 10 and sample 11) implying that organosols can be used for oil-based foods packed by process with moderate heat.

Table 4.18. The contamination in samples from company F and G

Sample No.	Food type	Can coating		Concentration (mg/kg)
		Lid	Body	
18F	Fried white scale fish	Organosol	Organosol	0.079
19G	Fried chicken	Organosol	Epoxy	0.554
20G	Fried pork	Organosol	Epoxy	0.532

The contamination of fried foods packed in cans coated with organosol (sample 18) is very low indicating that it is possible to produce fairly clean organosol lacquers. Another reason may be because fried white scale fish were packed loosely and did not fully contact the internal can surface.

Sample 19 and 20 were packed in epoxy coated cans. Contamination level detected were lower than the EU limit. The result comfirmed our recommendation that organosol resins should not be used when sterilization at high heat is required. Epoxy resins should be the coating of choice when the process required high or prolong heat.