CHAPTER IV

RESULTS AND DISCUSSION

4.1 The Preparation of BFDGE.H_2O, BFDGE.HCl, and BFDGE.HCl.H_2O

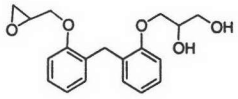
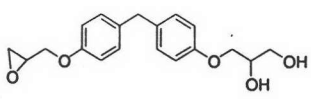
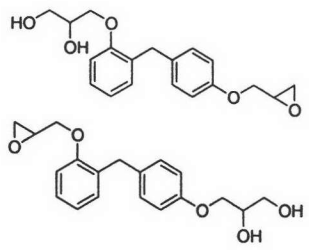
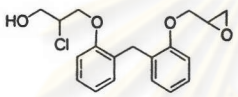
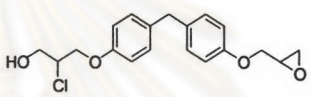
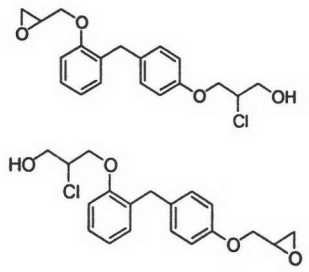
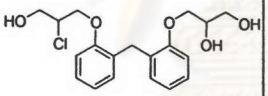
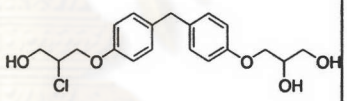
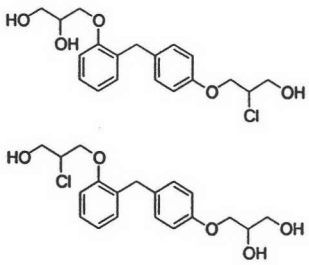
In this research, three derivatives of BFDGE namely BFDGE.H_2O, BFDGE.HCl, and BFDGE.HCl.H_2O were synthesized using reactions of two mole equivalents of BFDGE and one mole equivalent of reactants (Table 4.1). Separation and purification were achieved by mean of silica column chromatography obtaining light yellow syrup compounds with % yield and purity as shown in Table 4.1.

Table 4.1. Reactants, yield, and purity data of BFDGE.H_2O, BFDGE.HCl, and BFDGE.HCl.H_2O preparations.

Product	Reactants	Yield (%)	Purity (%)
BFDGE.H_2O	3% acetic acid	0.62	94
BFDGE.HCl	1.5% HCl in THF	6.77	91
BFDGE.HCl.H_2O	1.5% HCl	4.98	85

BFDGE exists in three forms: *o,o*-, *o,p*-, and *p,p*-isomers, and because the attack of the *o,p*- isomer can occur in 2 orientations, four possible products are produced as illustrated in Table 4.2.

Table 4.2. The four structures of BFDGE.H_2O, BFDGE.HCl, and BFDGE.HCl.H_2O.

Compound	*o,o*-isomers	*p,p*-isomers	*o,p*-isomers
BFDGE.H_2O			
BFDGE.HCl			
BFDGE.HCl.H_2O			

After purification, the compounds were tested for their chromatographic characteristics (Figure 4.1). It is observed that each derivative maintains the original isomeric characteristic and can be separated into respective peaks of *o,o*-, *o,p*-, and *p,p*-isomers. Our HPLC condition can detect all 4 isomers of BFDGE.H_2O and BFDGE.HCl.H_2O, but the resolution of BFDGE.HCl is not sufficient and peaks are co-elute, only 2 peaks are observed.

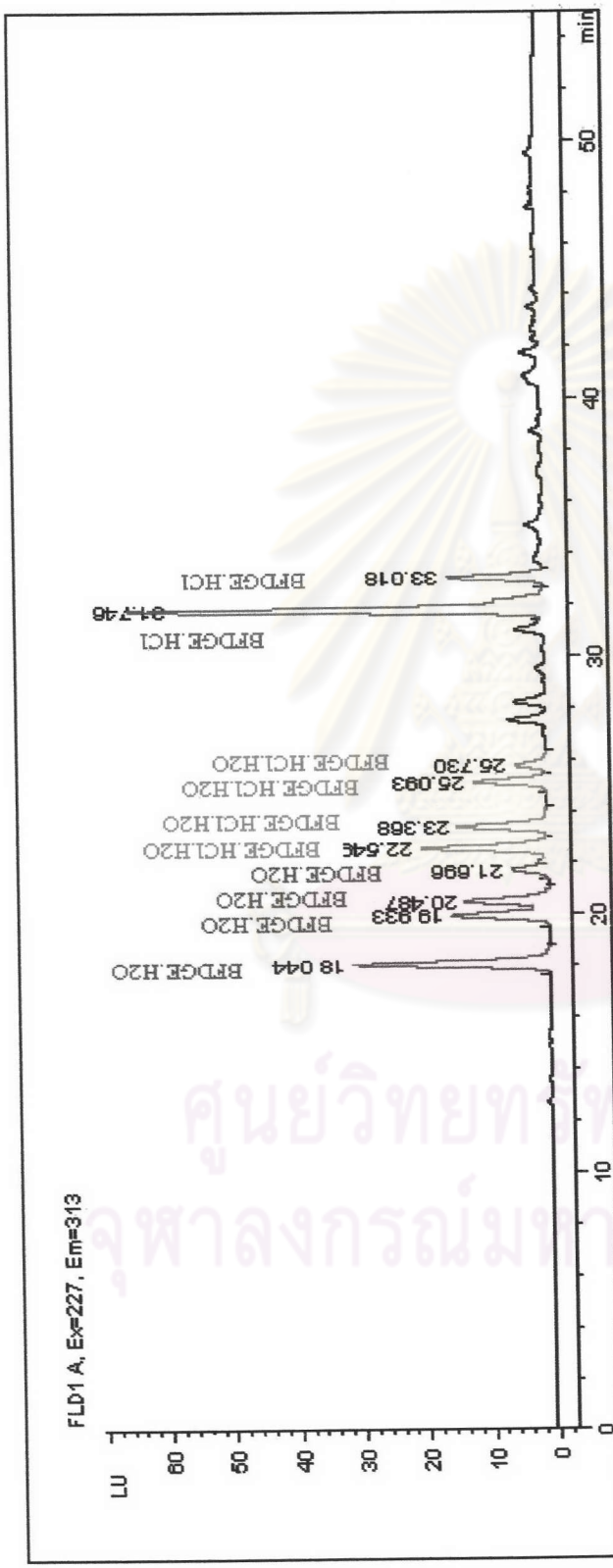


Figure 4.1. Chromatogram of three BFDGE monomeric derivatives by HPLC condition in Table 4.3.

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The characterization of these compounds were performed by UV and HPLC-MS with ESI interface to match the profiles of each compound. UV spectra of BFDGE.H_2O, BFDGE.HCl, and BFDGE.HCl.H_2O provide no useful differential information, but their mass-spectra allow for distinguishing by the mass spectrum pattern. The main ions observed on the ESI-MS spectra were the ammonium adduct ions $[M+NH_4]^+$, as illustrated on the ESI spectra of BFDGE.H_2O (m/z 348), BFDGE.HCl (m/z 366), and BFDGE.HCl.H_2O (m/z 384) in Figure 4.2-4.4.

The occurrence of one chlorine atom in 2 derivatives, BFDGE.HCl and BFDGE.HCl.H_2O, is clearly evidence by the relative abundance of the M^+ and $[M+2]^+$ ions reflect the abundance of ^{35}Cl and ^{37}Cl. As illustrated in Figure 4.2-4.4, there are m/z at 366 and 368 for BFDGE.HCl and m/z at 384 and 386 for BFDGE.HCl.H_2O.

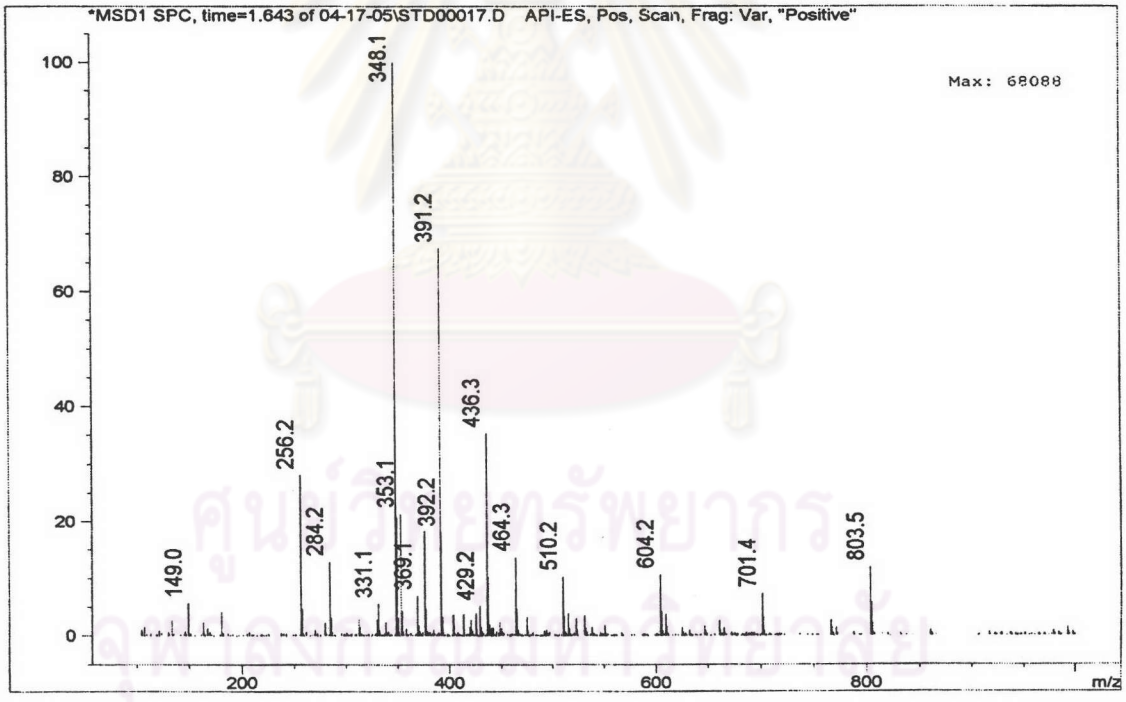

Figure 4.2. Mass spectrum of BFDGE.H_2O analyzed by LC-ESI-MS as described in Experimental Section 3.3.

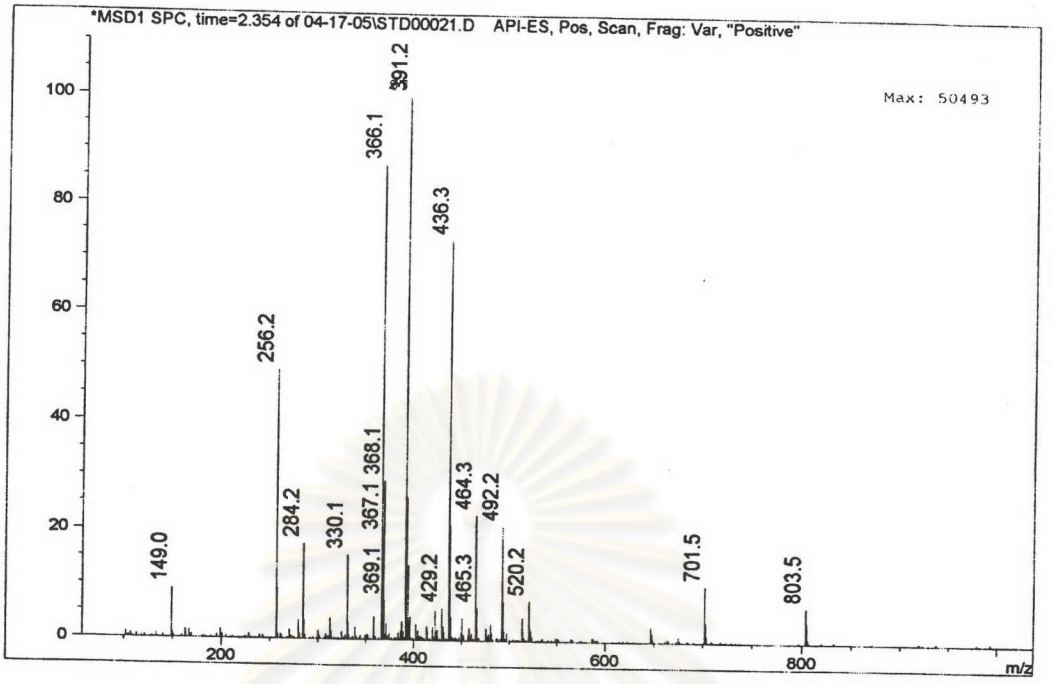

Figure 4.3. Mass spectrum of BFDGE.HCl analyzed by LC-ESI-MS as described in Experimental Section 3.3.

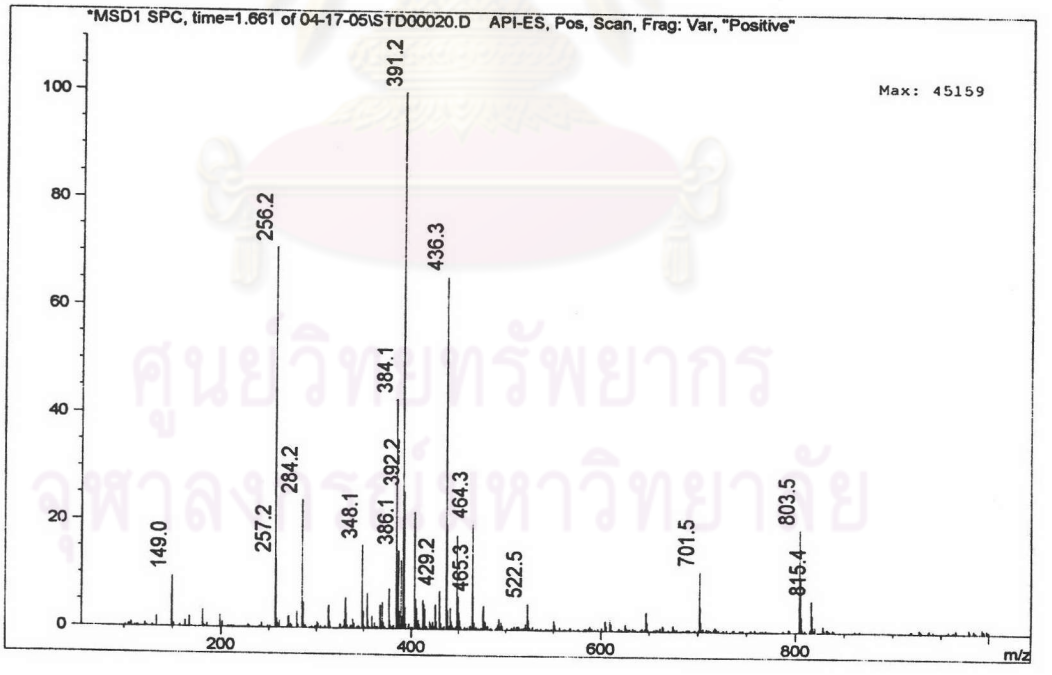

Figure 4.4. Mass spectrum of BFDGE.HCl.H_2O analyzed by LC-ESI-MS as described in Experimental Section 3.3.

4.2. HPLC Method Development

Injection volume of 5 μL was used for all samples. The HPLC method was developed and the optimum condition is reported in Table 4.3 The chromatogram of standard mixture solution is shown in Figure 4.5.

Table 4.3. High Performance Liquid Chromatographic conditions.

HPLC Parameter	HPLC Conditions	
Analytical column	4.0x250 mm, 5 μm Hypersil ODS	
Mobile phase	water and methanol	
Flow rate	0.70 mL/min	
Gradient program	Time	%Methanol
	0	40
	1	50
	15	55
	38	70
	45	90
	55	90
Column temperature	40°C	
Detector	Fluorescence detector	
	excitation and emission wavelengths of 227 and 313 nm	

Separation of 12 analytes was first tested on Hypersil ODS 4.0x125 mm, 5 μm column using water/acetonitrile as mobile phase but could not achieved baseline separation because the column was too short and therefore column efficiency was insufficient. Separations were improved on Hypersil ODS 4.0x250 mm, 5 μm column except for peaks of BADGE.H_2O and BADGE.HCl.H_2O. Adjustment of gradient elution or using different isocratic conditions did not improve the separation further but instead resulted in co-elution of other compounds. Therefore, a new tactic of changing the selectivity or varying the band spacing was tested. Result of changing organic mobile phase from acetonitrile to methanol was successful to achieve baseline resolutions of all 12 analytes. Figure 4.5 shows the separation of standard mixture

containing all 12 compounds using the HPLC condition in Table 4.3. Because of the difference in polarity of BADGE/BFDGE and their more polar reaction products, it is possible to use gradient RPLC to separate them. The chromatogram in Figure 4.5 showed 4 peaks of BFDGE.H_2O and BFDGE.HCl.H_2O (consist of 1 peak of *o,o*-, *p,p*-, and 2 peaks of *o,p*-isomers), 3 peaks of BFDGE (*o,o*-, *o,p*-, and *p,p*-), and 2 peaks of BFDGE.HCl (4 isomers co-elute). For quantification purposes, it is necessary to use separate calibration curve of each individual isomer because their isomeric distribution in the sample matrixes and in pure solvent are different.

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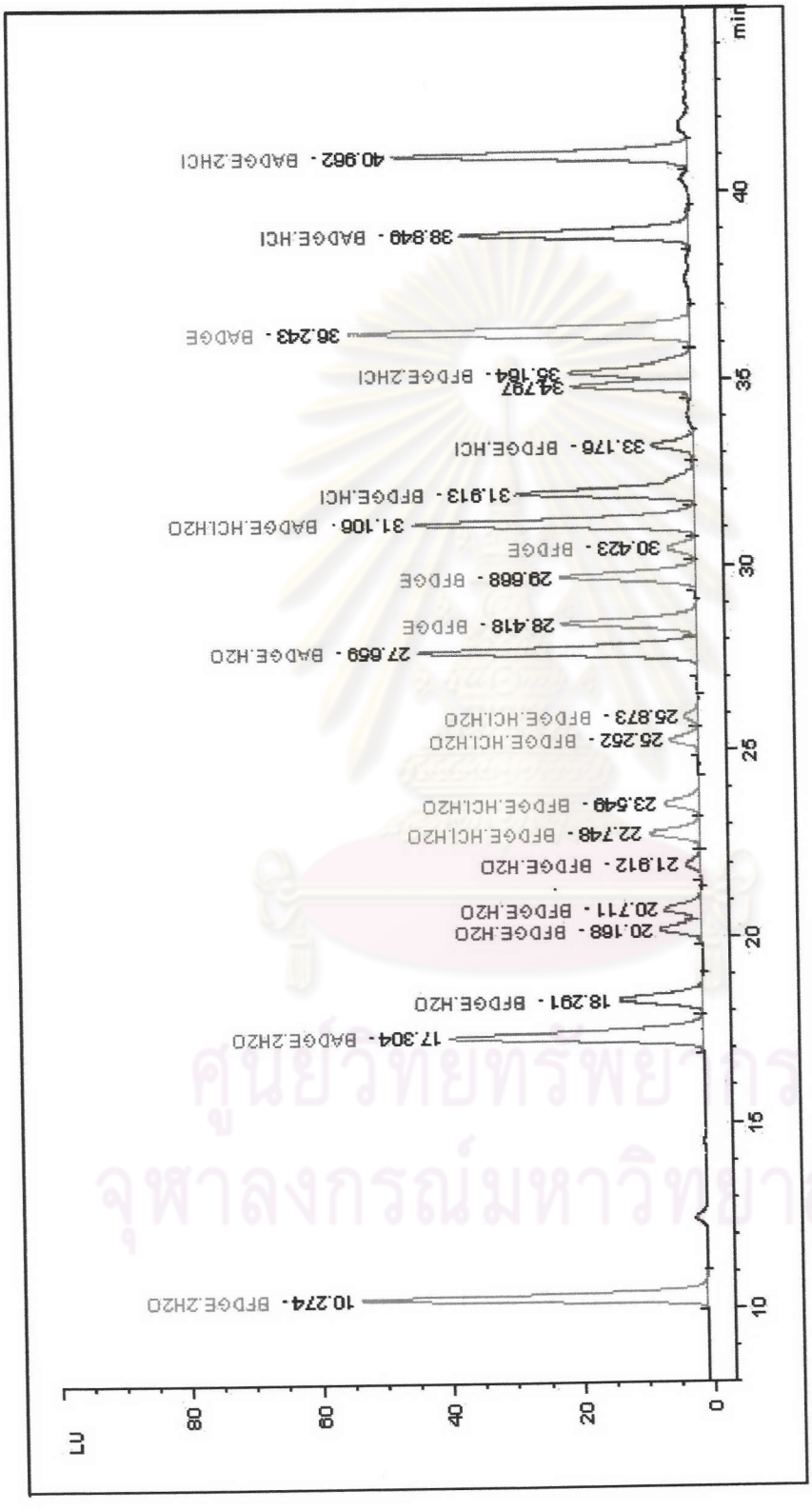

Figure 4.5. Chromatogram of a standard mixture by the HPLC conditions listed in Table 4.3.

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4.3 Result of Selectivity Evaluation

Because it is very important for the analytical method to have no (or minimal) interference from other species contained in the sample metrix, method selectivity must be proved. The selectivity of HPLC method was evaluated by peak retention time (t_R) matched with the value of standards and resolution values of critical pairs (R_s). Table 4.4. summarized the selectivity data. As shown in Figure 4.5, resolution of all critical pairs are at least 1.48 (baseline resolution) which is acceptable for quantitative analysis.

UV spectrum in APPENDIX A is the spectrum of BFDGE.$2H_2O$ representing all other compounds. Because of their similarity in structure, UV spectra of all analytes are the same and UV data were used to differentiate these compounds from interference only. Because fluorescence detection is very sensitive to phenolic and ether functional groups comparing to UV detection with no interference from background or matrix. Hence, it is recommended to use fluorescence for the detection of analyte in complex matrix.

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Table 4.4. Retention time and resolution of BADGE, BFDGE, and their derivatives by HPLC condition listed in Table 4.3 (n = 10).

No.	Compound	Retention time (min)	Resolution
1	BFDGE.2H_2O	10.20±0.04	-
2	BADGE.2H_2O	17.17±0.08	17.27
3	BFDGE.H_2O	18.15±0.08	2.07
		20.03±0.09	4.02
		20.57±0.10	1.22
		21.89±0.07	2.61
4	BFDGE.HCl.H_2O	22.62±0.09	1.75
		23.41±0.06	1.75
		25.26±0.08	3.79
		25.88±0.09	1.48
5	BADGE.H_2O	27.53±0.08	4.13
6	BFDGE	28.29±0.07	1.75
		29.54±0.08	2.83
		30.25±0.11	1.71
7	BADGE.HCl.H_2O	30.97±0.07	1.51
8	BFDGE.HCl	31.78±0.08	1.95
		33.05±0.11	2.97
9	BFDGE.2HCl	35.04±0.24	2.65
10	BADGE	36.11±0.07	1.48
11	BADGE.HCl	38.73±0.07	6.29
12	BADGE.2HCl	40.85±0.07	5.28

4.4 The Preparation of Standard Calibration Curves

The aim of this research is to determine the contamination level of 12 compounds of BADGE, BFDGE, and their derivatives regulated by the European Union that have combined SML equals 1.0 ppm. To determine the amount presents in the samples, linear calibration curves of pure standards were constructed for all 12 analytes from 0.0160-1.00 ppm, the expected contamination range, using the optimum HPLC condition listed in Table 4.3. The plots and regression lines of peak area and concentration are collected in APPENDIX B. Regression coefficients, obtained from least-square method, are summarized in Table 4.5.

From plots in APPENDIX B, it is obvious that the relationship are all linear. The corresponded least-squares regression correlation coefficients (R^2) in Table 4.5 are greater than 0.9900 for all major isomers, confirm strong linear relationships. The calibration curves were prepared at 7 concentration levels (duplicate analyses), all fit well with the linear model. Some of the R^2 values deviated from 1.00 but these are values of the less abundant isomers that exist only in very small amount in this range of study. The slope values are all different indicating different detector response of different analytes. The detector response of BADGE is the largest (slope = 178.57 10^6 LU.cm^3.g^{-1}) and the least for BFDGE.HCl.H_2O (slope = 6.20 10^6 LU.cm^3.g^{-1}).

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Table 4.5. Linear least-squares regression coefficients of BADGE, BFDGE, and their derivatives at a range of 0.0160-1.00 ppm, (7 points, duplicate analyses).

No.	Compound	Slope (10^6 LU.cm^3.g^{-1})	y-Intercept (LU)	R^2
1	BFDGE.2H_2O	147.53	0.1717	0.9976
2	BADGE.2H_2O	149.81	1.2057	0.9958
3	BFDGE.H_2O	50.53	-0.1469	0.9980
		25.98	-0.2367	0.9892
		20.90	-0.0808	0.9938
		12.39	-1.1604	0.9877
4	BFDGE.HCl.H_2O	30.37	-0.2968	0.9904
		20.49	-0.0813	0.9895
		15.63	0.5223	0.9853
		6.20	0.7393	0.9790
5	BADGE.H_2O	142.49	0.9192	0.9983
6	BFDGE	64.47	0.6845	0.9979
		70.95	1.0112	0.9975
		13.08	0.1776	0.9902
7	BADGE.HCl.H_2O	138.06	4.9862	0.9975
8	BFDGE.HCl	101.33	-1.5217	0.9959
		22.73	-0.4609	0.9920
9	BFDGE.2HCl	129.46	2.4109	0.9963
10	BADGE	178.57	0.5848	0.9977
11	BADGE.HCl	112.34	0.9607	0.9985
12	BADGE.2HCl	137.44	2.233	0.9978

4.5 The Linearity Range

From previous section, we are confident that the instrument response is linearly proportional to the analyte concentration from 0.0160-1.00 ppm. To be very useful, an analytical method should have a large magnitude of linear range. To further tested the linearity or dynamic range of the analytical method, concentration levels were extended to near the lowest concentration at which quantitative measurements can be made up to the concentration at which the instrument response departs from linearity or become saturated. We extended our study to 0.0160 to 10.0 ppm using linear least-square regression to predict best fit curves over this range. All plots are linear (see APPENDIX B) and regression coefficients data are reported in Table 4.6.

Excellent linear least-squares regression coefficients (>0.9900) were obtained for all compounds covering a large concentration range agreed well with our previous observations. And because excellent precision was observed over several concentration levels, we are confident that the analytical procedure can accurately determine amount of analytes up to 10.0 ppm, 10-fold over the EU limit.

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Table 4.6. Linear least-squares regression coefficients of BADGE, BFDGE, and their derivatives ranged from 0.0160-10.0 ppm, (11 points, duplicate analyses).

No.	Compound	Slope (10^6 LU.cm^3.g^{-1})	y-Intercept (LU)	R^2
1	BFDGE.2H_2O	145.81	-0.2849	0.9997
2	BADGE.2H_2O	149.17	-0.0272	0.9997
3	BFDGE.H_2O	49.41	-0.1167	0.9996
		25.32	0.1240	0.9997
		22.52	-1.6116	0.9986
		8.56	0.9801	0.9966
4	BFDGE.HCl.H_2O	28.48	0.8970	0.9997
		20.26	0.4962	0.9988
		15.48	1.1530	0.9982
		7.39	0.6184	0.9919
5	BADGE.H_2O	140.70	0.6589	0.9998
6	BFDGE	63.43	0.5984	0.9995
		70.44	0.9241	0.9998
		12.88	0.4723	0.9993
7	BADGE.HCl.H_2O	136.08	4.6492	0.9998
8	BFDGE.HCl	98.67	-0.9880	0.9998
		21.96	0.0811	0.9993
9	BFDGE.2HCl	126.97	2.8875	0.9999
10	BADGE	176.05	0.3301	0.9998
11	BADGE.HCl	111.97	1.5291	0.9998
12	BADGE.2HCl	134.75	2.7282	0.9998

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4.6 The Detection Limits and Quantitation Limits

The detection limits (LOD) and quantitation limits (LOQ) were defined as the amount of analyte in standard solutions that yielded an instrumental signal significantly different from the blank or background signal equal to 3 and 10, respectively. It is very important to establish the lower end of the practical operating range of a method to be certain that the result is accurate. LOD and LOQ data are summarized in Table 4.7.

As expected, compounds of higher sensitivity (larger slope) showed low LOD and LOQ values (BFDGE.2H_2O, BADGE.2H_2O, BADGE.H_2O, BADGE.HCl.H_2O, BADGE and BADGE.2HCl). On the contrary, compounds of lower sensitivity (smaller slope) showed higher LOD and LOQ values (BFDGE.H_2O, BFDGE.HCl.H_2O, BFDGE, BFDGE.HCl and BFDGE.2HCl). Because of BFDGE and their derivaitves exist in many forms (3 isomers for BFDGE and 4 isomers for BFDGE.H_2O, BFDGE.HCl.H_2O and BFDGE.HCl), the abundance of each peak is not the same. Therefore, reduced response is expected leading to higher LOD and LOQ values for these compounds. Because the gradient used in the HPLC method caused baseline drift and changes in system noises that can interfere with the analysis as can be seen with BADGE.HCl that regardless of its high sensitity has slightly higher LOD and LOQ values due to the interference caused by system peak and background.

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Table 4.7. Detection limits and the quantitation limits of BADGE, BFDGE, and their Derivatives.

No.	Compound	Detection limit (ppb)	Quantitation limit (ppb)
1	BFDGE.2H_2O	5	21
2	BADGE.2H_2O	5	28
3	BFDGE.H_2O	29	102
		60	210
		60	210
		130	400
4	BFDGE.HCl.H_2O	43	153
		55	200
		80	250
		120	350
5	BADGE.H_2O	7	22
6	BFDGE	13	45
		13	42
		60	180
7	BADGE.HCl.H_2O	4	16
8	BFDGE.HCl	14	44
		40	150
9	BFDGE.2HCl	18	53
10	BADGE	5	22
11	BADGE.HCl	9	34
12	BADGE.2HCl	5	22

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4.7 Result of Matrix Calibration Curve

Because the linearity of the response to pure standards may be different from real samples, it is crucial to construct the matrix calibration curves. All 12 matrix calibration curves ranged from 0.0160-1.00 ppm were constructed (see APPENDIX B) and the regression coefficients data are summarized in Table 4.8.

All plots are linear with good regression coefficient data indicating linear detector responses both in pure solution and in fish matrix. The results implied that the fish matrix does not interfere with signal and that linear instrument responses are observed from 0.0160-1.00 ppm.

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Table 4.8. Regression coefficients of matrix calibration curves for BADGE, BFDGE, and their derivatives (7 points, duplicate analyses).

No.	Compound	Slope (10^6 LU.cm^3.g^{-1})	y-Intercept (LU)	R^2
1	BFDGE.2H_2O	178.75	-2.7736	0.9966
2	BADGE.2H_2O	176.96	-5.1817	0.9979
3	BFDGE.H_2O	56.82	-1.8616	0.9975
		22.19	-0.4934	0.9937
		21.68	-0.8763	0.9921
		9.56	-0.3567	0.9912
4	BFDGE.HCl.H_2O	31.74	-0.8433	0.9884
		20.84	-0.2276	0.9838
		17.73	-0.5277	0.9924
		8.19	0.1241	0.9938
5	BADGE.H_2O	152.93	-3.4435	0.9978
6	BFDGE	76.64	-1.8217	0.9976
		69.64	-2.3381	0.9958
		15.94	1.5186	0.9855
7	BADGE.HCl.H_2O	146.00	0.4960	0.9958
8	BFDGE.HCl	106.86	-2.7611	0.9936
		26.64	-1.8209	0.9909
9	BFDGE.2HCl	139.90	-0.9620	0.9954
10	BADGE	202.23	-3.5205	0.9953
11	BADGE.HCl	124.72	-5.4114	0.9950
12	BADGE.2HCl	155.41	-0.072	0.9979

4.8 Result of The Matrix Effect Study

The effect of fish matrix on the separation of all 12 compounds was determined as described in Experimental Section 3.13. A suitable statistical tool for comparing results of 2 methods (two calibration curves) is the paired *t*-test. Taking the null hypothesis that there is **no** significant difference in the peak area given by pures standard solution (Section 4.4) and standard in fish matrixes (Section 4.7). The *t*-values are given in Table 4.9 compared with the critical values (P = 0.05) which are 2.57 (n=6) and 2.78 (n=5). Most *t*-calculated values are less than *t*-critical except for BFDGE.2H_2O, BFDGE.H_2O, BFDGE, and BADGE.2HCl. Therefore, the null hypothesis is ignored for these compounds and matrix effect should be countered for for the analysis of these compounds. Therefore, matrix calibration curves instead of methanol calibration curves of all compounds were used throughout this study.

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Table 4.9. *t*-calculated values of two tailed paired *t*-test at 95% confidence level.

No.	Compound	Concentration (ppm)	Peak area		pair *t*-test
			Standard solution	Standard in fish metrix	
1	BFDGE.2H_2O	0.100	15.48	16.24	3.03
		0.200	27.80	28.89	
		0.400	56.71	67.95	
		0.600	92.41	110.04	
		0.800	120.76	142.62	
		1.00	144.72	171.98	
2	BADGE.2H_2O	0.100	17.10	16.71	1.60
		0.200	28.92	27.20	
		0.400	58.56	64.58	
		0.600	94.92	102.64	
		0.800	125.62	132.96	
		1.00	146.46	184.51	
3	BFDGE.H_2O (1)	0.100	4.51	4.67	2.06
		0.200	9.71	9.14	
		0.400	20.11	20.86	
		0.600	31.15	32.90	
		0.800	40.96	44.37	
		1.00	49.32	54.02	
	BFDGE.H_2O (2)	0.100	2.61	1.80	3.77
		0.200	4.87	3.64	
		0.400	9.74	8.68	
		0.600	15.43	13.12	
		0.800	20.87	16.67	
		1.00	25.59	21.93	

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No.	Compound	Concentration (ppm)	Peak area		pair *t*-test
			Standard solution	Standard in fish metrix	
	BFDGE.H_2O (3)	0.100	2.22	1.63	2.26
		0.200	4.19	3.50	
		0.400	8.07	7.80	
		0.600	12.26	11.29	
		0.800	16.37	16.46	
		1.00	21.20	21.27	
	BFDGE.H_2O (4)	0.200	1.61	1.62	2.03
		0.400	3.70	3.31	
		0.600	5.68	5.50	
		0.800	9.06	7.28	
		1.00	11.32	9.20	
4	BFDGE.HCl.H_2O (1)	0.100	3.15	3.29	0.83
		0.200	5.60	5.46	
		0.400	11.25	11.43	
		0.600	18.75	19.17	
		0.800	23.04	25.73	
		1.00	30.59	29.75	
	BFDGE.HCl.H_2O (2)	0.100	1.72	1.68	0.38
		0.200	3.89	3.83	
		0.400	8.06	7.74	
		0.600	13.15	13.47	
		0.800	16.23	16.37	
		1.00	19.99	20.14	
	BFDGE.HCl.H_2O (3)	0.200	3.63	3.06	0.54
		0.400	6.41	6.68	
		0.600	10.37	10.11	
		0.800	13.22	13.16	
		1.00	15.85	17.55	

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No.	Compound	Concentration (ppm)	Peak area		pair *t*-test
			Standard solution	Standard in fish metrix	
	BFDGE.HCl.H_2O (4)	0.200	1.81	1.68	2.01
		0.400	3.42	3.52	
		0.600	4.55	5.13	
		0.800	5.61	6.43	
		1.00	6.91	8.42	
5	BADGE.H_2O	0.100	15.82	11.40	0.28
		0.200	27.57	22.70	
		0.400	55.62	57.06	
		0.600	89.34	90.69	
		0.800	116.74	118.25	
		1.00	141.39	149.69	
6	BFDGE (1)	0.100	6.66	4.69	0.47
		0.200	13.09	9.94	
		0.400	25.64	24.60	
		0.600	40.24	41.07	
		0.800	52.81	51.60	
		1.00	64.63	68.37	
	BFDGE (2)	0.100	8.08	5.75	2.04
		0.200	14.36	12.51	
		0.400	27.94	27.41	
		0.600	44.50	44.64	
		0.800	59.04	58.80	
		1.00	71.10	65.78	
	BFDGE (3)	0.200	2.71	4.35	7.02
		0.400	5.49	8.55	
		0.600	8.08	10.82	
		0.800	10.13	14.28	
		1.00	13.61	17.43	

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No.	Compound	Concentration (ppm)	Peak area		pair *t*-test
			Standard solution	Standard in fish metrix	
7	BADGE.HCl.H_2O	0.100	19.41	13.92	0.59
		0.200	32.92	25.69	
		0.400	60.26	59.80	
		0.600	91.04	91.94	
		0.800	117.34	121.00	
		1.00	139.49	141.76	
8	BFDGE.HCl (1)	0.100	8.653	5.892	1.21
		0.200	15.966	15.120	
		0.400	38.189	41.949	
		0.600	63.562	64.626	
		0.800	79.601	85.092	
		1.00	98.031	100.284	
	BFDGE.HCl (2)	0.200	3.78	2.73	1.60
		0.400	8.45	9.22	
		0.600	13.56	14.41	
		0.800	17.36	18.98	
		1.00	22.42	25.03	
9	BFDGE.2HCl	0.100	14.39	9.58	1.34
		0.200	27.84	28.83	
		0.400	53.98	56.96	
		0.600	85.67	87.82	
		0.800	105.16	111.78	
		1.00	129.50	134.67	
10	BADGE	0.100	18.99	14.31	2.11
		0.200	33.71	31.84	
		0.400	68.39	77.21	
		0.600	112.01	124.30	
		0.800	145.98	163.19	
		1.00	176.44	192.13	

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No.	Compound	Concentration (ppm)	Peak area		pair *t*-test
			Standard solution	Standard in fish metrix	
11	BADGE.HCl	0.100	12.40	8.53	0.15
		0.200	22.44	16.00	
		0.400	43.79	42.23	
		0.600	69.78	70.25	
		0.800	92.55	91.30	
		1.00	112.05	122.60	
12	BADGE.2HCl	0.100	14.90	15.03	2.78
		0.200	27.88	28.79	
		0.400	56.08	62.88	
		0.600	88.33	98.06	
		0.800	113.98	122.13	
		1.00	136.96	154.27	

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4.9 The Effect of Acidity in Aqueous-based Samples

Chromatograms in Figure 4.6-4.9 demonstrated the effect of solution acidity to %recovery in aqueous-based samples.

Chromatogram of lychee in syrup (sample blank, Figure 4.6) are not different from chromatogram of adjusted pH sample blank (pH 7.0, 1.0 M di-sodium hydrogen phosphate dihydrate) in Figure 4.7.

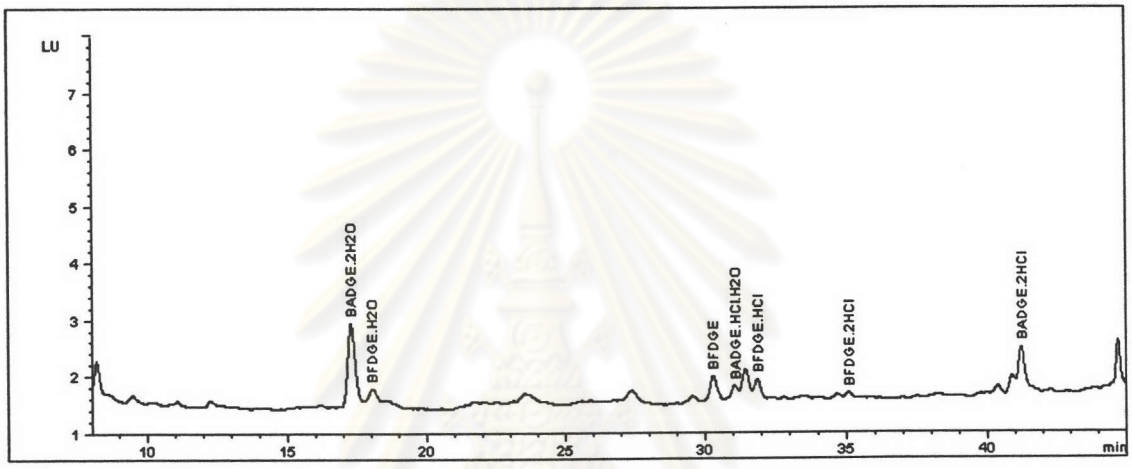

Figure 4.6. Chromatogram of lychee in syrup (sample blank).

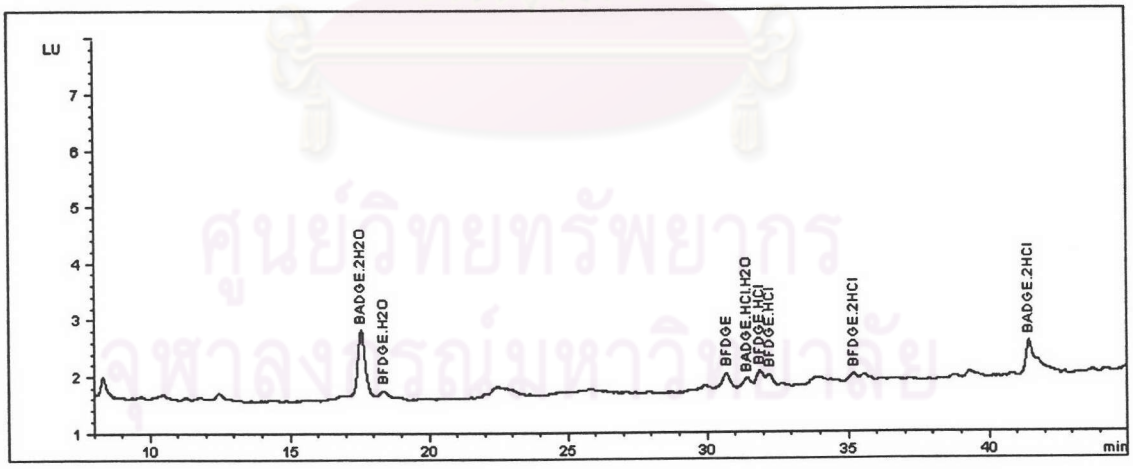

Figure 4.7. Chromatogram of lychee in syrup (sample blank) adjusted to pH 7.0 by 1.0 M di-sodium hydrogen phosphate dihydrate.

However, percent recovery of spiked lychee in syrup matrix at 10-fold MQL concentration (Figure 4.8) was unusually low for BFDGE.H_2O, BADGE.H_2O, BFDGE, BFDGE.HCl, BADGE, and BADGE.HCl; compounds with remaining epoxide ring(s). Because the epoxide ring can readily be opened and reacted with the components in acidic foods forming new derivatives. This is also true for spiked materials and as a result, percent recovery are unusually low leading to misinterpretation of the result. By adjusting the food pH to 7.0 prior to standard addition step, help lowering the activity of the epoxide rings and improve percent recovery of the extraction (Figure 4.9).

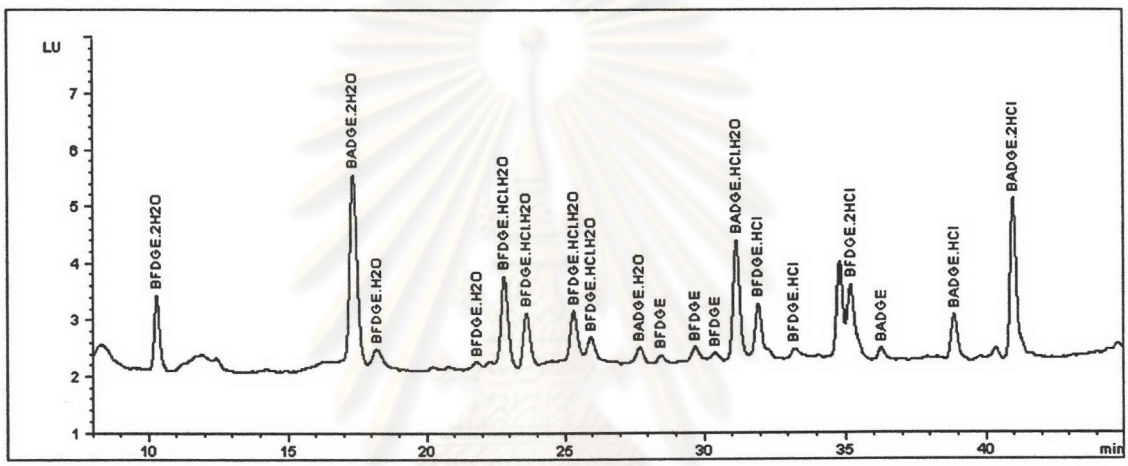

Figure 4.8. Chromatogram of lychee in syrup spiked with standard solution at 10-fold MQL without pH controlled.

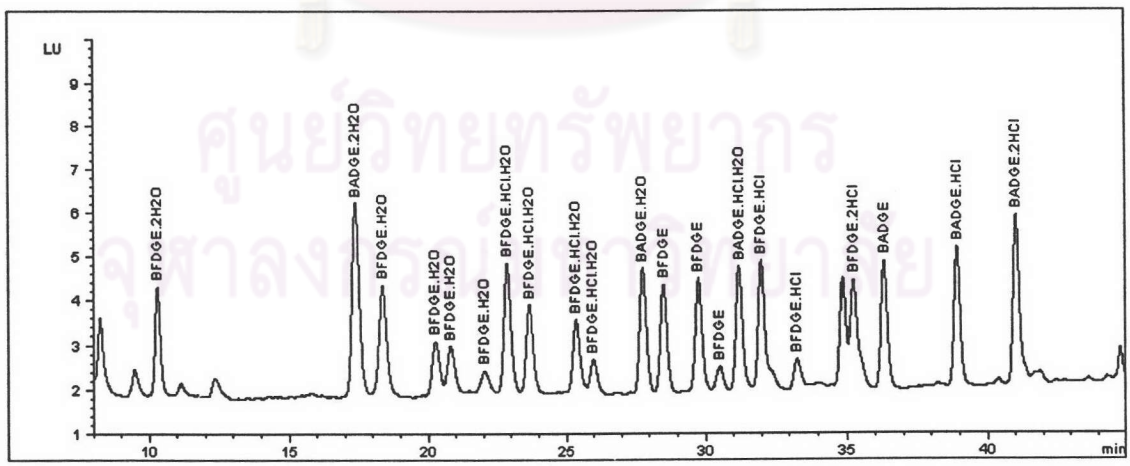

Figure 4.9. Chromatogram of lychee in syrup adjusted to pH 7.0 by 1.0 M di-sodium hydrogen phosphate dihydrate and spiked with standard solution at 10 fold MQL.

4.10 Method Detection Limits (MDL) and Method Quantitation Limits (MQL)

Method quantitation limit (MQL), is defined as the lowest amount of analytes that can be quantitated at S/N = 10 after passing through sample preparation steps. MQL is determined from corresponded LOQ and concentration factor. Similary, method detection limit (MDL) is defined resemblingly the MQL, but it is the lowest amount of analyte that the method can detectable at signal-to-noise ratio equal to 3. In this study, MDL values obtained by the calculation from the corresponded to MQL at signal-to-noise equal to 3. The MDL and MQL values determined in fish matrix are summarized in Table 4.10.

Because the spiked solutions were concentrated 6.67-fold during sample preparation process, then the MDL and MQL values would be lower than the previously established LOD and LOQ as show in Table 4.10 (comparison with Table 4.7). Hence, trace amounts of analytes in sample could be estimated from these concentration factors.

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Table 4.10. Method detection limits and method quantitation limits of BADGE, BFDGE, and their derivatives in fish matrixes.

No.	Compound	Method Detection Limit (ppb)	Method Quantitation Limit (ppb)
1	BFDGE.2H_2O	0.94	3.15
2	BADGE.2H_2O	1.26	4.20
3	BFDGE.H_2O	4.57	15.22
		9.45	31.50
		9.45	31.50
		18.00	60.00
4	BFDGE.HCl.H_2O	7.16	23.88
		9.00	30.00
		11.25	37.50
		15.75	52.50
5	BADGE.H_2O	0.97	3.22
6	BFDGE	2.00	6.68
		1.89	6.30
		8.10	27.00
7	BADGE.HCl.H_2O	0.72	2.40
8	BFDGE.HCl	1.96	6.52
		6.75	22.50
9	BFDGE.2HCl	2.39	7.95
10	BADGE	0.97	3.22
11	BADGE.HCl	1.53	5.10
12	BADGE.2HCl	0.99	3.30

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4.11 Method Precision

As mentioned before that detector responses may be different between pure solutions and sample matrixes either from matrix interferences or different distribution of isomers in real matrixes. Therefore, it is necessary to carry out method precision studies in real matrixes to evaluate the reliability of our procedure. Methods precision were studied at 2 concentration levels, Method Quantification Limit (MQL) and 5-fold MQL, as show in Table 4.11.

Table 4.11. Spiking level at MQL and 5-fold MQL concentration.

No.	Compound	Spiking level (ppb)	
		MQL-level	5-fold MQL level
1	BFDGE.2H_2O	3	15
2	BADGE.2H_2O	4	20
3	BFDGE.H_2O	15	75
4	BFDGE.HCl.H_2O	23	115
5	BADGE.H_2O	3	15
6	BFDGE	7	35
7	BADGE.HCl.H_2O	2	10
8	BFDGE.HCl	7	35
9	BFDGE.2HCl	8	40
10	BADGE	3	15
11	BADGE.HCl	5	25
12	BADGE.2HCl	3	15

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4.11.1 Method Precision for Spiked Fish Matrix at MQL Level

The method precision at MQL level was studied by repeated analyses on the same day (intra-assay precision) and on different days (intermediate precision). The results are summarized in Table 4.12-4.14.

The intra-assay precision at MQL level (2-23 ppb) was demonstrated as percent relative standard deviation (%RSD) which implied reproducibility of the method. %RSD data of the experiment performed for 3 consecutive days are summarized in Table 4.12-4.14. %RSD values ranging from 2.09-11.70% indicated that good intra-assay precision based on the AOAC standard can be achieved within the same day (AOAC method recommended %RSD values should not be greater than 15% at ppb-level). From the overall data in Table 4.15, excellent intra-assay precision and intermediate precision were observed ($\%RSD_{overall}$ = 0.98-8.90) at 2-23 ppb indicating that data obtained on different days are comparable.

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Table 4.12. %Recovery and %RSD of spiked fish matrix at MQL level (first day) n=6.

No.	Compound	%Recovery						Mean	%RSD
		1	2	3	4	5	6		
1	BFDGE.2H_2O	49.57	47.93	45.75	45.70	47.83	53.13	48.32 ± 2.78	5.75
2	BADGE.2H_2O	75.42	81.07	85.21	82.38	82.12	84.41	81.77 ± 3.47	4.24
3	BFDGE.H_2O	110.25	105.44	102.01	110.33	105.73	100.22	105.66 ± 4.14	3.92
4	BFDGE.HCl.H_2O	102.75	110.77	107.10	110.29	104.17	114.86	108.32 ± 4.53	4.18
5	BADGE.H_2O	95.46	93.31	94.25	100.44	99.84	89.57	95.48 ± 4.12	4.31
6	BFDGE	112.51	93.93	96.06	95.61	96.88	90.17	97.53 ± 7.72	7.91
7	BADGE.HCl.H_2O	87.98	113.71	113.98	98.15	100.80	118.77	105.56 ± 11.82	11.20
8	BFDGE.HCl	98.03	104.60	104.74	111.23	109.33	102.19	105.02 ± 4.78	4.55
9	BFDGE.2HCl	108.47	102.24	97.83	99.68	90.27	96.99	99.25 ± 6.03	6.08
10	BADGE	112.85	108.22	101.60	101.82	102.67	96.39	103.93 ± 5.77	5.55
11	BADGE.HCl	81.58	68.47	72.21	69.98	66.94	57.96	69.52 ± 7.67	11.04
12	BADGE.2HCl	126.65	129.91	115.21	124.71	115.40	100.05	118.66 ± 10.91	9.20

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Table 4.13. %Recovery and %RSD of spiked fish matrix at MQL level (second day) n=6.

No.	Compound	%Recovery						Mean	%RSD
		1	2	3	4	5	6		
1	BFDGE.2H_2O	48.03	47.20	49.70	51.14	46.34	51.44	48.98 ± 2.11	4.31
2	BADGE.2H_2O	70.76	76.37	68.28	70.93	67.36	67.48	70.19 ± 3.41	4.85
3	BFDGE.H_2O	101.87	95.46	95.49	97.46	90.35	91.73	95.39 ± 4.13	4.33
4	BFDGE.HCl.H_2O	101.28	104.68	102.36	101.02	99.16	97.18	100.95 ± 2.58	2.56
5	BADGE.H_2O	99.76	101.24	99.35	93.24	97.02	92.74	97.23 ± 3.55	3.66
6	BFDGE	93.09	102.84	97.07	93.17	96.06	88.35	95.10 ± 4.86	5.11
7	BADGE.HCl.H_2O	88.11	93.35	95.54	98.95	88.99	94.81	93.29 ± 4.12	4.41
8	BFDGE.HCl	95.42	113.59	115.54	110.90	94.76	98.83	104.84 ± 9.53	9.09
9	BFDGE.2HCl	69.54	93.13	88.41	98.10	93.55	82.90	87.60 ± 10.25	11.70
10	BADGE	98.78	105.89	98.94	92.17	96.11	95.34	97.87 ± 4.65	4.75
11	BADGE.HCl	69.18	61.53	60.95	70.91	66.80	76.01	67.56 ± 5.76	8.53
12	BADGE.2HCl	116.15	113.15	104.27	109.86	99.19	117.49	110.02 ± 7.12	6.47

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Table 4.14. %Recovery and %RSD of spiked fish matrix at MQL level (third day) n=6.

No.	Compound	%Recovery						Mean	%RSD
		1	2	3	4	5	6		
1	BFDGE.2H_2O	51.39	48.30	49.25	50.28	49.80	48.46	49.58 ± 1.17	2.36
2	BADGE.2H_2O	66.45	68.23	68.85	74.56	80.06	64.76	70.49 ± 5.75	8.15
3	BFDGE.H_2O	96.59	98.80	92.79	95.22	96.73	90.14	95.05 ± 3.12	3.28
4	BFDGE.HCl.H_2O	96.30	104.04	99.66	100.68	102.64	101.93	100.88 ± 2.71	2.69
5	BADGE.H_2O	96.56	100.99	93.79	96.91	98.64	94.99	96.98 ± 2.57	2.65
6	BFDGE	95.76	97.98	99.30	97.59	103.42	92.93	97.83 ± 3.52	3.59
7	BADGE.HCl.H_2O	91.52	84.68	110.19	103.25	97.96	90.85	96.41 ± 9.29	9.64
8	BFDGE.HCl	107.80	102.85	105.16	107.96	107.00	103.53	105.72 ± 2.21	2.09
9	BFDGE.2HCl	113.56	91.76	95.28	97.04	99.00	94.55	98.53 ± 7.75	7.87
10	BADGE	96.94	96.94	98.83	102.97	104.14	94.15	98.99 ± 3.85	3.89
11	BADGE.HCl	79.56	64.27	74.03	70.13	73.00	69.93	71.82 ± 5.09	7.09
12	BADGE.2HCl	93.77	101.10	104.35	105.44	100.44	101.27	101.06 ± 4.09	4.04

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Table 4.15. Overall %recovery and %RSD of spiked fish matrix at MQL level (n=3).

No.	Compound	%Recovery			Mean	%RSD
		1	2	3		
1	BFDGE.2H_2O	48.32	48.98	49.58	48.96 ± 0.63	1.29
2	BADGE.2H_2O	81.77	70.19	70.49	74.15 ± 6.60	8.90
3	BFDGE.H_2O	105.66	95.39	95.05	98.70 ± 6.03	6.11
4	BFDGE.HCl.H_2O	108.32	100.95	100.88	103.38 ± 4.28	4.14
5	BADGE.H_2O	95.48	97.23	96.98	96.56 ± 0.95	0.98
6	BFDGE	97.53	95.10	97.83	96.82 ± 1.50	1.55
7	BADGE.HCl.H_2O	105.56	93.29	96.41	98.42 ± 6.38	6.48
8	BFDGE.HCl	105.02	104.84	105.72	105.19 ± 0.46	0.44
9	BFDGE.2HCl	99.25	87.60	98.53	95.13 ± 6.52	6.86
10	BADGE	103.93	97.87	98.99	100.26 ± 3.22	3.21
11	BADGE.HCl	69.52	67.56	71.82	69.64 ± 2.13	3.06
12	BADGE.2HCl	118.66	110.02	101.06	109.91 ± 8.80	8.00

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4.11.2 Method Precision for Spiked Fish Matrix at 5-fold MQL Level

The method precision at 5-fold MQL were determined in a similar way to the previous study at MQL level. The results are summarized in Table 4.16-4.18.

The intra-assay precision at 5-fold MQL level was calculated from 6 extractions of fish matrix. Repeat analyses were performed for 3 consecutive days. Data are reported as percent relative standard deviation (%RSD) in Table 4.16-4.18.

%RSD are good for intra-assay precision (2.27 to 8.38%) as can be observed from individual day data in Table 4.16-4.18. The overall (3 days) %RSD are summarized in Table 4.19 with values range 1.10-7.17%. High intermediate precision also observed at spiking level of 10-115 ppb.

In conclusion, method precision at two spiking levels (MQL and 5-fold MQL) are very precised judging from the spread of %recovery data carried out in spiked fish matrix. As expected, results at higher concentration (5-fold MQL) are better than at lower concentration (MQL) because of less interference from background. Therefore, the sample preparation procedures and the HPLC method are reliable and can be used for the analyses of 12 analytes in oil-in-water matrixes with high precision over an extended concentration range.

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Table 4.16. %Recovery and %RSD of spiked fish matrix at 5-fold MQL level (first day) n=6.

No.	Compound	%Recovery						Mean	%RSD
		1	2	3	4	5	6		
1	BFDGE.2H_2O	47.38	49.73	54.27	55.08	53.31	53.69	52.24 ± 3.01	5.76
2	BADGE.2H_2O	67.33	69.73	70.82	72.52	71.82	69.14	70.23 ± 1.90	2.70
3	BFDGE.H_2O	79.39	86.46	89.09	81.69	82.90	82.43	83.66 ± 3.51	4.19
4	BFDGE.HCl.H_2O	100.38	97.14	95.56	105.08	104.03	100.41	100.43 ± 3.72	3.70
5	BADGE.H_2O	83.44	91.24	91.92	86.54	84.73	87.54	87.57 ± 3.42	3.91
6	BFDGE	74.94	91.34	86.73	82.03	75.29	80.95	81.88 ± 6.41	7.83
7	BADGE.HCl.H_2O	98.86	95.78	95.58	106.42	105.53	107.68	101.64 ± 5.53	5.45
8	BFDGE.HCl	104.79	102.76	100.93	106.26	109.10	105.11	104.83 ± 2.83	2.70
9	BFDGE.2HCl	103.23	101.69	102.39	107.50	106.36	105.83	104.50 ± 2.37	2.27
10	BADGE	82.23	90.36	92.05	86.37	83.95	81.81	86.13 ± 4.28	4.97
11	BADGE.HCl	97.77	99.04	100.04	104.85	104.55	100.23	101.08 ± 2.94	2.91
12	BADGE.2HCl	91.76	94.32	96.56	106.05	104.38	93.47	97.75 ± 6.01	6.14

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Table 4.17. %Recovery and %RSD of spiked fish matrix at 5-fold MQL level (second day) n=6.

No.	Compound	%Recovery						Mean	%RSD
		1	2	3	4	5	6		
1	BFDGE.2H_2O	51.72	48.17	46.74	53.03	53.66	53.80	51.19 ± 3.02	5.90
2	BADGE.2H_2O	67.21	65.76	63.34	66.62	68.04	70.61	66.93 ± 2.42	3.61
3	BFDGE.H_2O	85.24	79.84	83.45	89.44	89.13	86.02	85.52 ± 3.61	4.22
4	BFDGE.HCl.H_2O	96.03	99.10	89.42	95.53	100.85	98.75	96.62 ± 4.05	4.19
5	BADGE.H_2O	90.11	82.88	86.37	91.37	94.61	91.44	89.46 ± 4.18	4.67
6	BFDGE	84.91	81.15	85.74	90.01	93.62	102.13	89.59 ± 7.51	8.38
7	BADGE.HCl.H_2O	98.48	99.90	94.12	97.19	110.44	101.22	100.23 ± 5.57	5.55
8	BFDGE.HCl	99.58	100.86	97.18	103.40	111.30	105.82	103.02 ± 5.04	4.90
9	BFDGE.2HCl	101.84	103.08	97.49	107.30	106.25	103.71	103.28 ± 3.49	3.38
10	BADGE	91.39	81.34	87.67	93.33	92.34	92.53	89.77 ± 4.58	5.10
11	BADGE.HCl	96.46	100.33	99.70	101.46	109.24	102.34	101.59 ± 4.26	4.19
12	BADGE.2HCl	100.11	99.57	106.74	101.26	104.21	104.31	102.70 ± 2.82	2.75

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Table 4.18. %Recovery and %RSD of spiked fish matrix at 5-fold MQL level (third day) n = 6.

No.	Compound	%Recovery						Mean	%RSD
		1	2	3	4	5	6		
1	BFDGE.2H_2O	49.48	44.53	53.62	47.76	53.43	52.27	50.18 ± 3.61	7.19
2	BADGE.2H_2O	64.72	63.93	75.28	72.15	68.52	70.44	69.17 ± 4.37	6.32
3	BFDGE.H_2O	79.51	81.96	83.90	79.38	87.76	83.42	82.65 ± 3.14	3.80
4	BFDGE.HCl.H_2O	95.61	87.91	108.77	97.78	98.69	106.33	99.18 ± 7.55	7.61
5	BADGE.H_2O	85.98	84.42	88.09	84.85	91.42	86.93	86.95 ± 2.57	2.96
6	BFDGE	81.25	85.61	84.88	82.45	93.70	82.69	85.10 ± 4.52	5.31
7	BADGE.HCl.H_2O	121.14	113.46	119.71	113.60	112.89	129.76	118.43 ± 6.58	5.55
8	BFDGE.HCl	98.63	93.42	98.14	92.92	99.64	101.88	97.44 ± 3.55	3.64
9	BFDGE.2HCl	102.59	96.44	110.32	104.63	101.77	116.26	105.33 ± 6.99	6.63
10	BADGE	85.18	87.80	84.89	80.00	87.94	80.88	84.45 ± 3.37	3.99
11	BADGE.HCl	104.06	103.84	112.24	101.36	102.39	110.93	105.80 ± 4.61	4.35
12	BADGE.2HCl	110.36	105.26	111.41	103.74	106.54	111.48	108.13 ± 3.38	3.12

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Table 4.19. Overall %recovery and %RSD of spiked fish matrix at 5-fold MQL level (n=3).

No.	Compound	%Recovery			Mean	%RSD
		1	2	3		
1	BFDGE.2H_2O	52.70	51.55	50.64	51.63 ± 1.03	2.00
2	BADGE.2H_2O	71.62	66.65	68.51	68.93 ± 2.51	3.64
3	BFDGE.H_2O	84.45	86.72	83.91	85.03 ± 1.49	1.75
4	BFDGE.HCl.H_2O	102.31	96.66	97.94	98.97 ± 2.96	3.00
5	BADGE.H_2O	88.27	90.24	86.83	88.45 ± 1.71	1.93
6	BFDGE	82.56	86.07	86.25	84.96 ± 2.08	2.45
7	BADGE.HCl.H_2O	102.32	100.07	114.15	105.52 ± 7.56	7.17
8	BFDGE.HCl	106.40	103.12	103.95	104.49 ± 1.70	1.63
9	BFDGE.2HCl	105.46	103.21	104.04	104.24 ± 1.14	1.10
10	BADGE	87.68	90.56	88.20	88.81 ± 1.53	1.72
11	BADGE.HCl	101.71	101.46	106.50	103.22 ± 2.84	2.75
12	BADGE.2HCl	101.16	101.78	107.80	103.58 ± 3.67	3.54

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4.11.3 Method Precision for Spiked Lychee in Syrup Matrix at 5-fold MQL Level

To simulate the matrixes of fruits and vegetables, canned lychee in syrup was chosen as the representative of aqueous-based samples. The method precision was studied on the same day and different-days for 3 consecutive days at spiking level range 10-115 ppb. The result are summarized in Table 4.20-4.22.

Percent relative standard deviations of the analyses of aqueous-based samples on 3 consecutive days ranged 3.46 -15.09% implied acceptable intra-assay precision of the method. The overall %RSD (Table 4.23) ranged 2.41-13.84% also indicated acceptable intermediate precision based on the AOAC standard. The method can be used with confident that it will provide reliable data on different analyses.

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Table 4.20. %Recovery and %RSD of spiked lychee in syrup matrix at 5-fold MQL level (first day) n=6.

No.	Compound	%Recovery						Mean	%RSD
		1	2	3	4	5	6		
1	BFDGE.2H_2O	105.79	95.26	91.27	105.10	114.48	104.92	102.80 ± 8.31	8.08
2	BADGE.2H_2O	114.32	99.11	88.59	110.73	92.62	94.36	99.96 ± 10.37	10.37
3	BFDGE.H_2O	94.65	83.33	88.75	93.87	88.55	86.64	89.30 ± 4.32	4.83
4	BFDGE.HCl.H_2O	116.17	106.35	95.15	128.21	115.04	124.97	114.32 ± 12.18	10.65
5	BADGE.H_2O	78.63	79.46	80.83	85.84	79.30	77.12	80.20 ± 3.02	3.76
6	BFDGE	70.88	71.14	68.41	80.46	64.33	63.63	69.81 ± 6.11	8.75
7	BADGE.HCl.H_2O	109.61	101.70	87.55	100.53	98.82	94.48	98.78 ± 7.39	7.48
8	BFDGE.HCl	86.52	86.98	82.01	93.09	87.86	90.81	87.88 ± 3.82	4.34
9	BFDGE.2HCl	107.71	103.32	92.47	109.03	103.19	103.04	103.13 ± 5.82	5.64
10	BADGE	67.68	70.92	72.02	83.35	71.40	71.26	72.77 ± 5.40	7.43
11	BADGE.HCl	94.41	92.10	84.85	100.66	87.31	85.48	90.80 ± 6.13	6.75
12	BADGE.2HCl	104.22	103.11	87.67	101.35	91.02	67.58	92.49 ± 13.96	15.09

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Table 4.21. %Recovery and %RSD of spiked lychee in syrup matrix at 5-fold MQL level (second day) n=6.

No.	Compound	%Recovery						Mean	%RSD
		1	2	3	4	5	6		
1	BFDGE.2H_2O	112.71	90.61	98.35	100.22	107.70	108.30	102.98 ± 8.09	7.86
2	BADGE.2H_2O	112.30	99.13	103.64	102.41	111.47	122.79	108.63 ± 8.67	7.98
3	BFDGE.H_2O	94.03	69.79	88.58	85.18	93.86	94.72	87.69 ± 9.54	10.88
4	BFDGE.HCl.H_2O	108.99	94.58	108.49	110.76	109.20	115.19	107.87 ± 6.96	6.45
5	BADGE.H_2O	94.77	75.18	88.33	83.59	86.45	85.47	85.63 ± 6.40	7.47
6	BFDGE	88.73	67.75	75.66	67.50	73.13	79.23	75.34 ± 7.98	10.59
7	BADGE.HCl.H_2O	102.29	93.43	108.08	112.37	98.52	97.24	101.99 ± 7.11	6.97
8	BFDGE.HCl	97.97	89.67	103.25	99.77	93.77	125.63	101.68 ± 12.65	12.44
9	BFDGE.2HCl	105.73	93.41	109.74	109.87	101.74	115.19	105.95 ± 7.62	7.19
10	BADGE	92.00	72.39	80.95	71.27	78.61	78.18	78.90 ± 7.44	9.43
11	BADGE.HCl	103.32	87.38	97.93	97.03	93.30	103.85	97.13 ± 6.23	6.41
12	BADGE.2HCl	105.21	88.69	105.81	105.30	101.22	118.20	104.07 ± 9.49	9.12

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Table 4.22. %Recovery and %RSD of spiked lychee in syrup matrix at 5-fold MQL level (third day) n=6.

No.	Compound	%Recovery						Mean	%RSD
		1	2	3	4	5	6		
1	BFDGE.2H_2O	106.25	91.56	94.02	88.18	97.95	92.57	95.09 ± 6.33	6.66
2	BADGE.2H_2O	111.87	93.00	89.83	79.58	94.51	80.15	91.49 ± 11.83	12.93
3	BFDGE.H_2O	70.54	75.40	72.33	78.88	67.99	73.73	73.14 ± 3.80	5.20
4	BFDGE.HCl.H_2O	110.13	105.69	101.40	104.32	105.18	99.78	104.42 ± 3.62	3.46
5	BADGE.H_2O	70.88	70.46	64.93	68.23	68.63	76.91	70.00 ± 3.99	5.69
6	BFDGE	69.14	77.49	92.34	87.55	70.83	80.26	79.60 ± 9.13	11.47
7	BADGE.HCl.H_2O	108.58	94.61	96.94	92.38	98.97	92.29	97.29 ± 6.11	6.28
8	BFDGE.HCl	88.52	68.13	79.53	64.36	86.95	75.29	77.13 ± 9.79	12.70
9	BFDGE.2HCl	106.98	96.75	93.46	95.13	96.98	91.53	96.80 ± 5.39	5.57
10	BADGE	75.44	75.12	76.69	74.61	88.55	74.19	77.43 ± 5.51	7.12
11	BADGE.HCl	88.00	76.93	77.02	70.84	87.62	69.40	78.30 ± 7.99	10.20
12	BADGE.2HCl	109.50	93.32	92.77	92.07	99.63	91.69	96.50 ± 7.01	7.26

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Table 4.23. Overall %recovery and %RSD of spiked lychee in syrup matrix at 5-fold MQL concentration level (n=3).

No.	Compound	%Recovery			Mean	%RSD
		1	2	3		
1	BFDGE.2H_2O	102.80	102.98	95.09	100.29 ± 4.51	4.49
2	BADGE.2H_2O	99.96	108.63	91.49	100.02 ± 8.57	8.57
3	BFDGE.H_2O	89.30	87.69	73.14	83.38 ± 8.90	10.67
4	BFDGE.HCl.H_2O	114.32	107.87	104.42	108.87 ± 5.02	4.62
5	BADGE.H_2O	80.20	85.63	70.00	78.61 ± 7.93	10.09
6	BFDGE	69.81	75.34	79.60	74.91 ± 4.91	6.55
7	BADGE.HCl.H_2O	98.78	101.99	97.29	99.35 ± 2.40	2.41
8	BFDGE.HCl	87.88	101.68	77.13	88.90 ± 12.30	13.84
9	BFDGE.2HCl	103.13	105.95	96.80	101.96 ± 4.68	4.59
10	BADGE	72.77	78.90	77.43	76.37 ± 3.20	4.19
11	BADGE.HCl	90.80	97.13	78.30	88.75 ± 9.58	10.80
12	BADGE.2HCl	92.49	104.07	96.50	97.69 ± 5.88	6.02

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4.12 The Method Accuracy

Accuracy of the analytical method is indicated by the closeness of the measurements to its true or accepted value. In this research, back-calculations to determine the spiked concentration of standards at MQL and 5-fold MQL levels are chosen as means to determine method accuracy for oil-in-water samples and aqueous-based sample as described in Experimental Section 3.17. The accuracy of the method is evaluated by using the recovery of each compound as illustrated in Table 4.24.

Recovery data of spiked fish at MQL level (2-23 ppb) ranged 49 -110% and 52 -106% at 5-fold MQL level (10-115 ppb) met AOAC standards for method yielding recovery (70%-125%) at ppb level except for BADGE.$2H_2O$ and BFDGE.$2H_2O$ (<70%). Because these hydrolysis products contain two epoxide rings per structure, they have the highest polarity. Therefore, their solubilities in MTBE are low and extraction is incomplete. However, their presences are not regulated by the European Union because of their negligible toxicity.

For the spiked lychee matrix, the recovery at 5-fold MQL (10-115 ppb) ranged 75-109% which met the AOAC standard and implied acceptable accuracy of this method for the analysis ot these analytes in aqueous-based canned foods.

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Table 4.24. %Recovery of method at MQL and 5-fold MQL levels for oil-in-water and aqueous-based samples (n=3).

No.	Compound	Oil-in-water-based sample		Aqueous-based sample
		MQL level	5-fold MQL level	5-fold MQL level
1	BFDGE.2H_2O	48.96 ± 0.63	51.63 ± 1.03	100.29 ± 4.51
2	BADGE.2H_2O	74.15 ± 6.60	68.93 ± 2.51	100.02 ± 8.57
3	BFDGE.H_2O	98.70 ± 6.03	85.03 ± 1.49	83.38 ± 8.90
4	BFDGE.HCl.H_2O	103.38 ± 4.28	98.97 ± 2.96	108.87 ± 5.02
5	BADGE.H_2O	96.56 ± 0.95	88.45 ± 1.71	78.61 ± 7.93
6	BFDGE	96.82 ± 1.50	84.96 ± 2.08	74.91 ± 4.91
7	BADGE.HCl.H_2O	98.42 ± 6.38	105.52 ± 7.56	99.35 ± 2.40
8	BFDGE.HCl	105.19 ± 0.46	104.49 ± 1.70	88.90 ± 12.30
9	BFDGE.2HCl	95.13 ± 6.52	104.24 ± 1.14	101.96 ± 4.68
10	BADGE	100.26 ± 3.22	88.81 ± 1.53	76.37 ± 3.20
11	BADGE.HCl	69.64 ± 2.13	103.22 ± 2.84	88.75 ± 9.58
12	BADGE.2HCl	109.91 ± 8.80	103.58 ± 3.67	97.69 ± 5.88

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4.13 Comparison Between Two Sample Preparation Methods

As mentioned earlier, separated extraction methods were used for oil-in-water samples and aqueous-based samples. When compared chromatograms of sample blank in fish matrix with spiked fish at 5-fold MQL (Figure 4.10 and 4.11), the blank chromatogram showed rather clean baseline and excellent baseline resolutions were obtained in spiked oil-in-water matrix (uncanned fish). Previous sections already demonstrated acceptable precision and accuracy values.

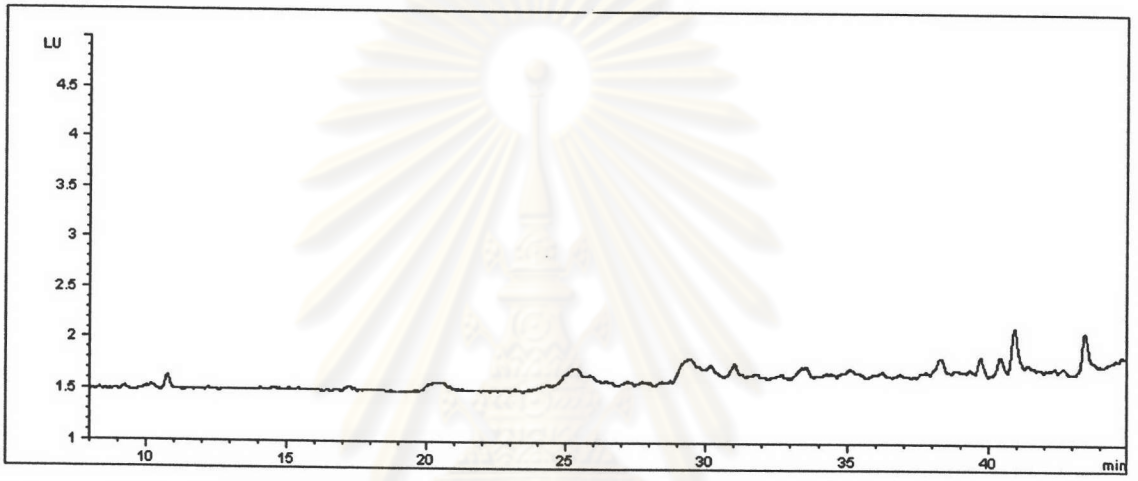

Figure 4.10. Chromatogram of fish matrix (sample blank).

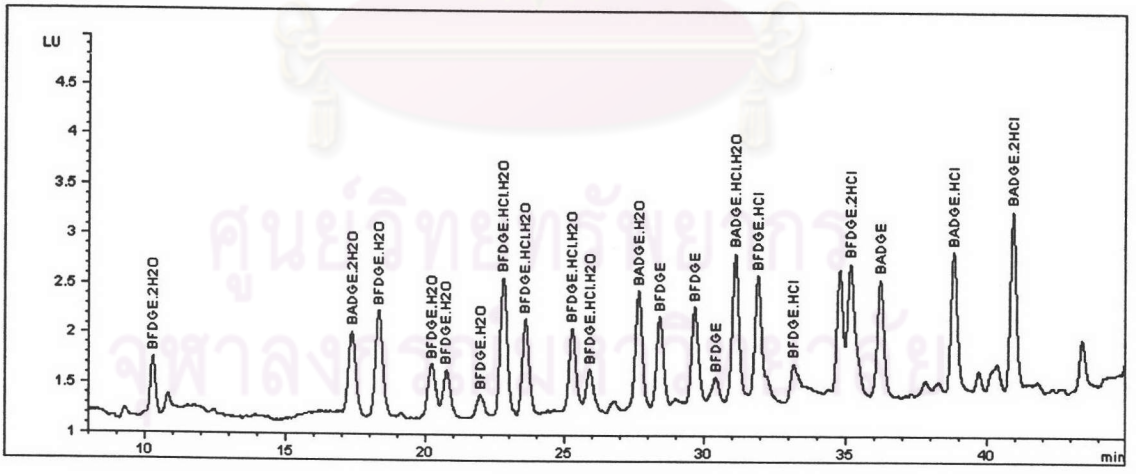

Figure 4.11. Chromatogram of spiked fish matrix at 5-fold MQL level.

The method for aqueous-based samples used lychee in syrup (from can) as blank. We were concerned that the matrix may be contaminated and may interfere with our study. However, the chromatogram of blank (Figure 4.12) showed only trace amount of BADGE and BFDGE derivatives but no interference from other constituent. Chromatogram of spiked lychee (Figure 4.13) showed excellent baseline resolution with no background interference.

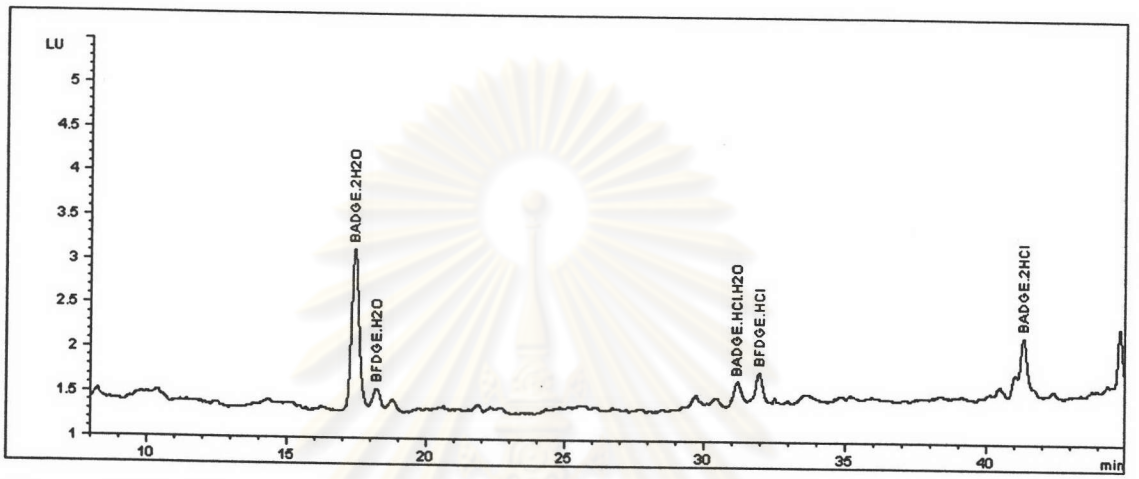

Figure 4.12. Chromatogram of lychee in syrup matrix (sample blank).

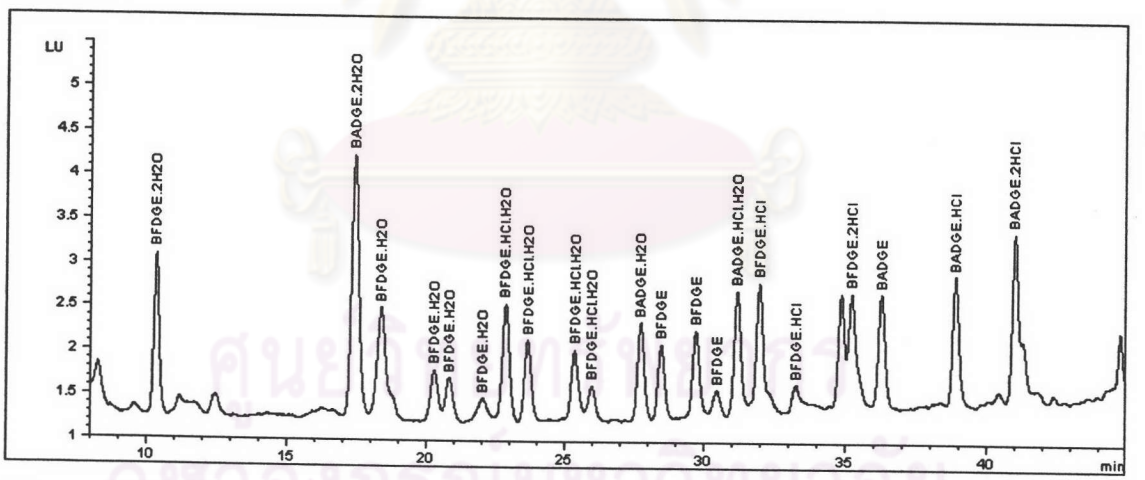

Figure 4.13. Chromatogram of spiked lychee in syrup matrix at 5-fold MQL level.

4.14 Sample Stability

The stability of analytes should be determined to see how long a sample can be stored without degradation. The sample stability was determined by extraction of spiked standard solutions in fish matrix as procedure in Experimental Section 3.10. A batch of sample was prepared and stored in the same condition up to 42 days. Samples were pull and analyzed at 8 different intervals using the same HPLC condition in Table 4.3. The results are summarized in form of %recovery in Table 4.25.

The replicate measurements at fixed time intervals, the mean, and acceptable range of replicate measurements are plotted using control chart. Control charts are used to monitoring the variability and to provide a graphical display of statistical control. In the control charts shown in Figure 4.14-4.25, replicate measurements are plotted as a function of time. A common approach is to use the average or expected value as the center line, and use a multiple of standard deviation to set the control limits. In this research, the 2 SD values set the upper and the lower control limits or the values within which the measurements must fall. Approximately 95% of the data should lie within the range $X \pm 2SD$. These are tracked to see if there is a trend or a systematic deviation from the center line. The recovery is plotted on the control chart. If the value falls outside the control limit, the samples must to be analyzed before that time.

The control charts of almost all analytes throughout 42 days were significantly deviated from the control limits except BADGE.$2H_2O$, BADGE.HCl.H_2O, BADGE.HCl, and BADGE.2HCl as illustrated in Table 4.25. Therefore, it is recommended to store sample no longer than 3 days before analysis.

Table 4.25. %Recovery of BADGE, BFDGE and their derivatives in fish matrix and storage up to 42 days

Compound	% Recovery calculated compared with linear equation of each day								X - 2SD	X + 2SD	Day of storage
	0	4	12	15	19	26	31	42			
BFDGE.2H_2O	50.55	49.92	53.69	42.06	37.31	35.45	33.09	35.64	49.57	53.69	5
BADGE.2H_2O	74.74	72.22	73.95	63.46	64.65	64.00	65.65	63.51	63.91	73.95	42
BFDGE.H_2O	88.7	85.5	88.01	88.44	88.89	85.01	84.59	83.91	82.05	88.01	14
BFDGE.HCl.H_2O	104.96	101.35	104.89	96.05	94.61	93.77	90.73	87.94	93.05	104.89	27
BADGE.H_2O	91.6	89.49	91.87	83.98	81.42	81.9	80.53	86.83	85.03	91.87	10
BFDGE	88.91	86.47	89.12	87.72	80.57	75.96	72.79	76.25	80.80	89.12	19
BADGE.HCl.H_2O	120.28	118.33	111.9	109.94	111.65	103.83	102.74	104.15	90.40	120.64	42
BFDGE.HCl	104.22	100.14	102.28	97.05	97.54	100.87	104.66	103.95	101.09	107.89	3
BFDGE.2HCl	103.66	107.41	101.77	95.02	89.68	88.25	85.03	84.04	101.96	106.52	3
BADGE	90.47	92.56	87.98	87.16	84.75	78.59	76.08	78.2	85.75	91.87	18
BADGE.HCl	101.07	100.65	97.66	98.28	101.61	97.47	98.15	106.5	97.54	108.90	42
BADGE.2HCl	110.2	106.59	100.22	100.17	99.87	97.38	95.76	97.8	96.24	110.92	42

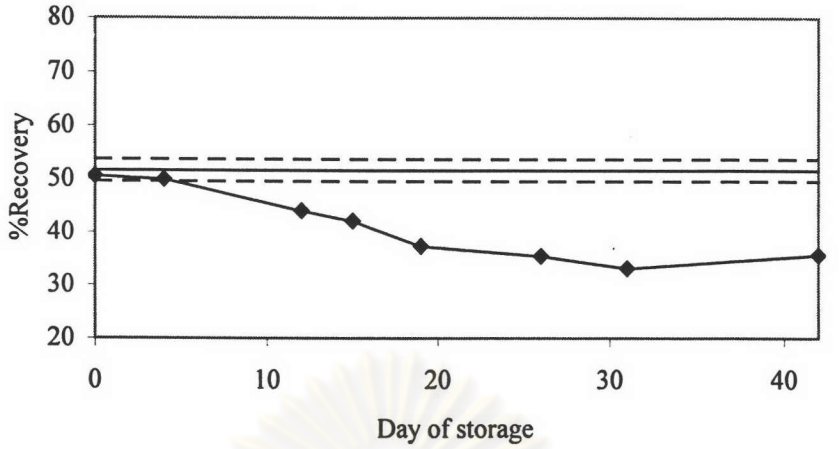

Figure 4.14. Control chart of BFDGE.$2H_2O$.

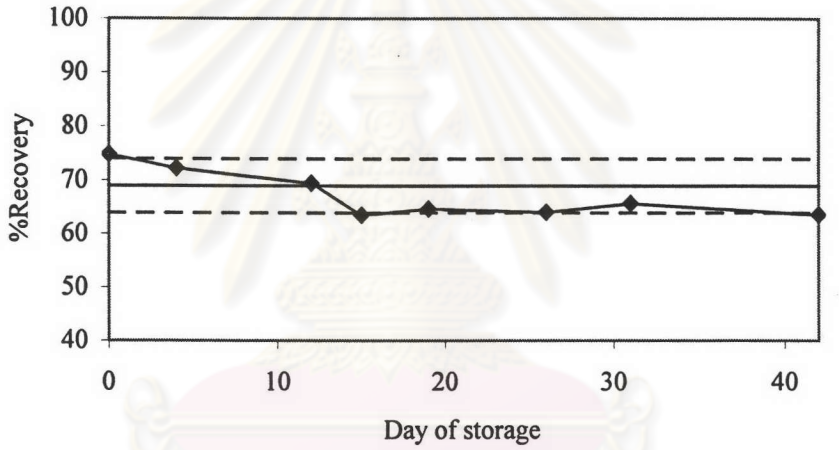

Figure 4.15. Control chart of BADGE.$2H_2O$.

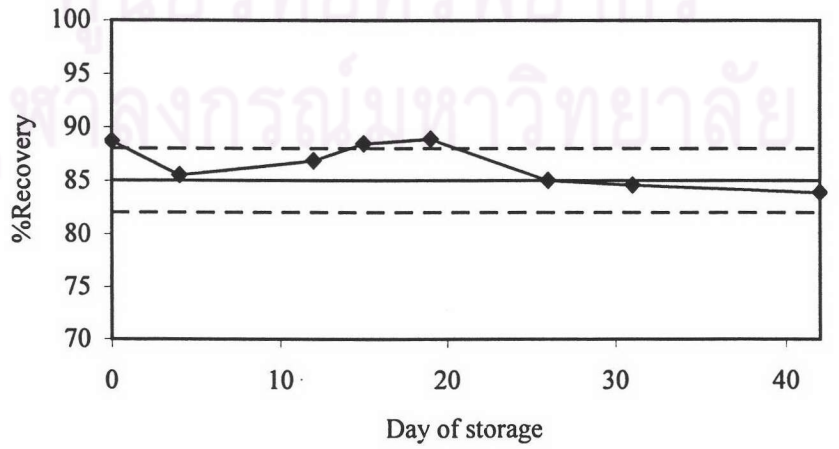

Figure 4.16. Control chart of BFDGE.H_2O.

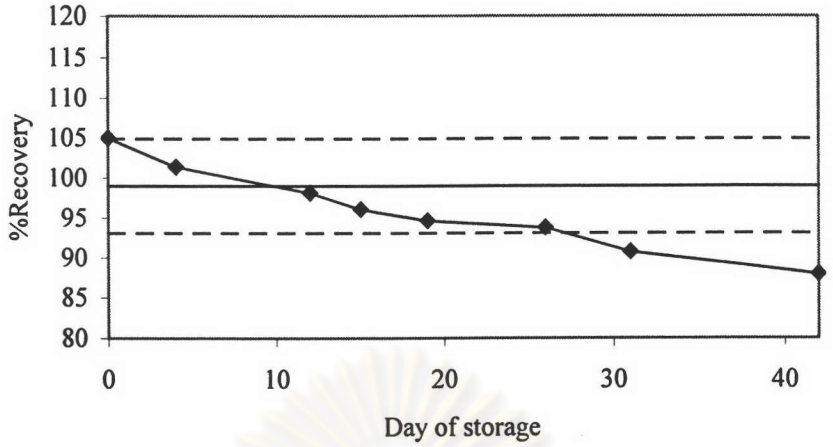

Figure 4.17. Control chart of BFDGE.HCl.H_2O.

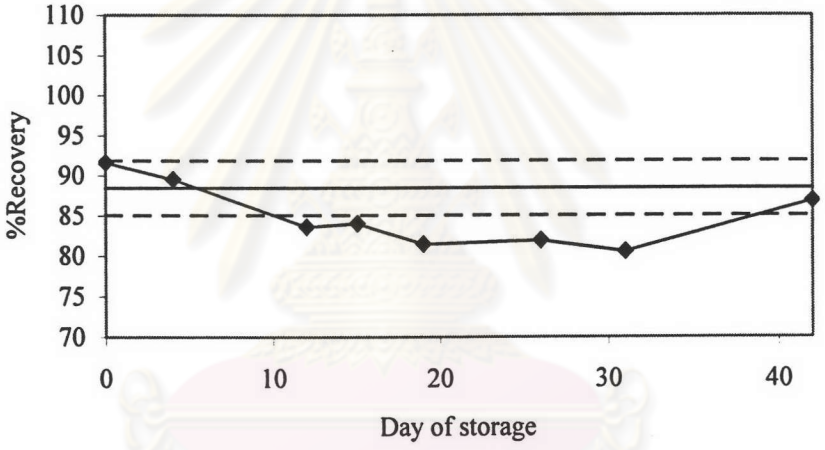

Figure 4.18. Control chart of BADGE.H_2O.

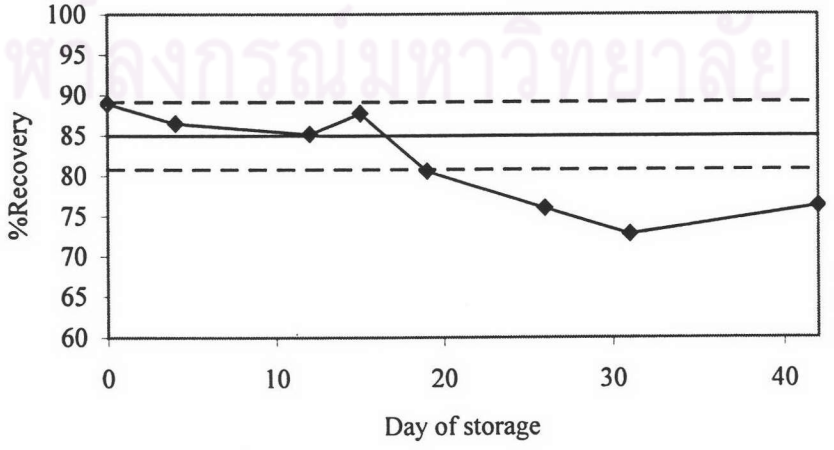

Figure 4.19. Control chart of BFDGE.

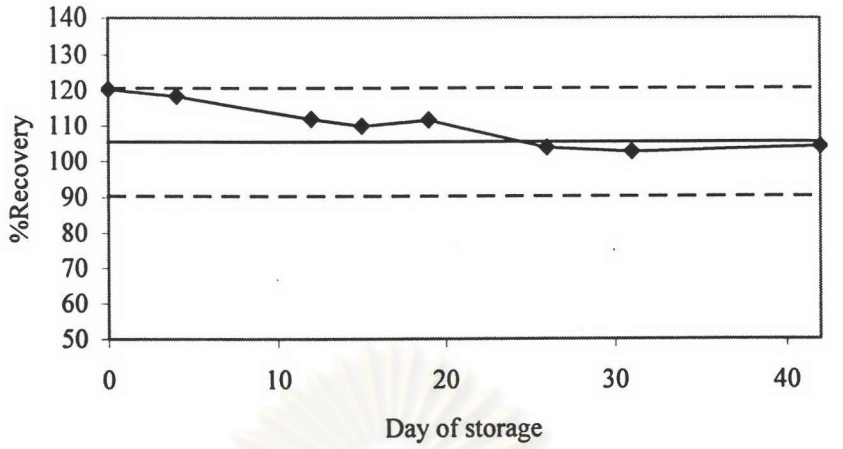

Figure 4.20. Control chart of BAGE.HCl.H_2O.

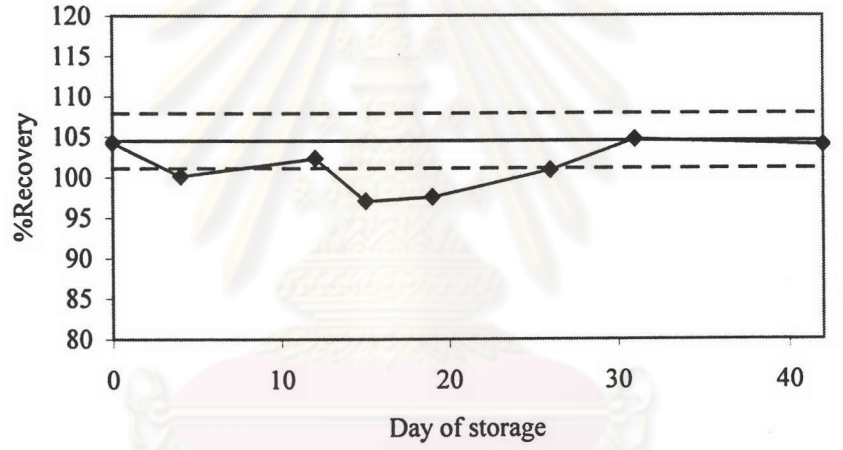

Figure 4.21. Control chart of BFGE.HCl.

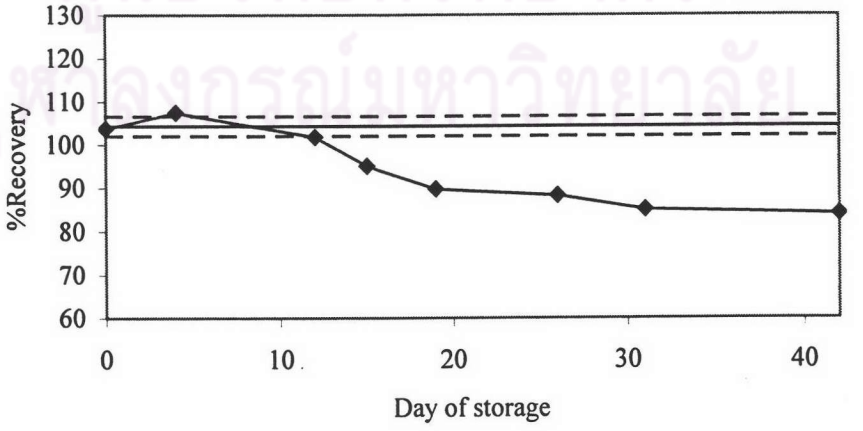

Figure 4.22. Control chart of BFGE.2HCl.

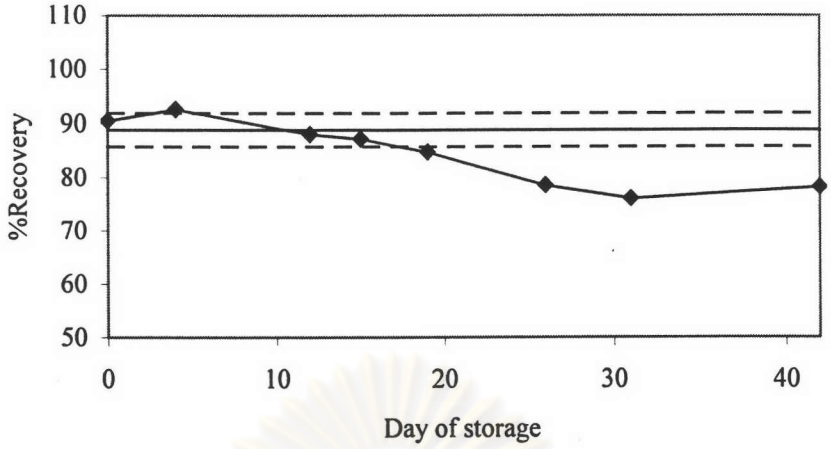

Figure 4.23. Control chart of BAGE.

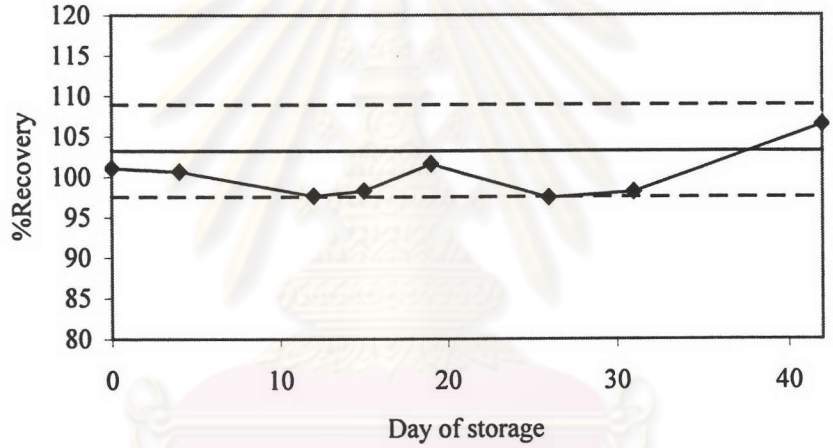

Figure 4.24. Control chart of BAGE.HCl.

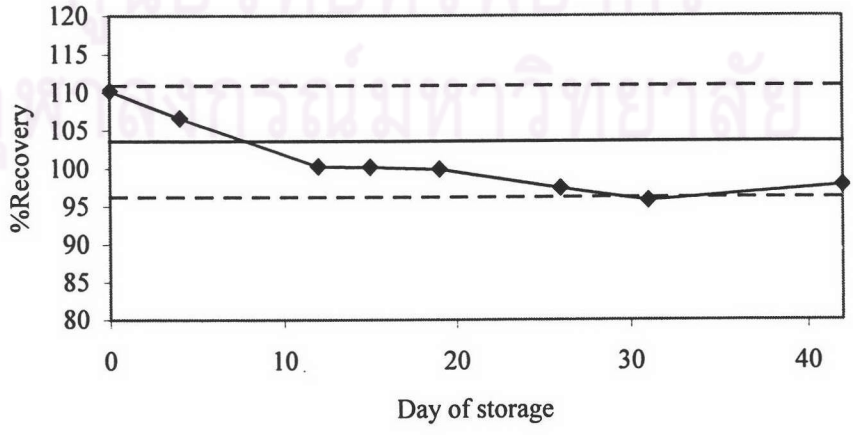

Figure 4.25. Control chart of BAGE.2HCl.

4.15 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 changes is observed. Robustness test provide information on the effect of important parameters to the analysis. Robustness of our analytical method was investigated by comparing a reference condition (A) with two varied conditions (B and C):

A is reference condition; using temperature of a rotary evaporator at 34 °C and extract with 15 mL of *tert*- methyl-butyl ether (MTBE) 2 times.

B is varied condition 1; using temperature of a rotary evaporator at 40 °C and extract with 15 mL MTBE 2 times.

C is varied condition 2; using temperature of a rotary evaporator at 34 °C and extract with 30 mL MTBE 1 time.

The recovery results of reference condition (**A**) and varied conditions (**B** and **C**) were illustrated in Table 4.27.

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Table 4.26. Comparisons of %recovery of spiked samples of extraction parameters (A, B, and C as described as below, n=2).

No.	Compound	% Recovery		
		A	B	C
1	BFDGE.2H_2O	65.59 ± 6.73	65.44 ± 2.65	73.22 ± 0.50
2	BADGE.2H_2O	71.47 ± 7.63	76.17 ± 0.77	80.55 ± 7.84
3	BFDGE.H_2O	106.10 ± 6.86	107.02 ± 0.91	101.62 ± 4.28
4	BFDGE.HCl.H_2O	88.44 ± 2.67	89.24 ± 2.95	82.43 ± 1.59
5	BADGE.H_2O	96.82 ± 2.30	97.14 ± 3.54	88.97 ± 1.91
6	BFDGE	103.00 ± 10.24	97.15 ± 3.44	86.39 ± 1.14
7	BADGE.HCl.H_2O	87.20 ± 2.88	82.03 ± 1.14	86.12 ± 7.54
8	BFDGE.HCl	111.32 ± 2.92	96.62 ± 7.41	103.12 ± 2.25
9	BFDGE.2HCl	107.26 ± 13.12	94.55 ± 4.21	87.06 ± 2.08
10	BADGE	109.00 ± 6.40	106.22 ± 7.30	96.36 ± 7.20
11	BADGE.HCl	103.80 ± 7.57	117.20 ± 3.17	103.27 ± 1.37
12	BADGE.2HCl	93.30 ± 2.46	93.73 ± 4.37	87.35 ± 4.02

A is reference condition; using the temperature of rotary evaporator at 34 °C and extract with 15 mL of *tert*- methyl-butyl ether (MTBE) 2 times.

B is varied condition; using the temperature of rotary evaporator at 40 °C and extract with 15 mL MTBE two times.

C is varied condition; using the temperature of rotary evaporator at 34 °C and extract with 30 mL MTBE one time.

The recovery results of reference condition (**A**) and varied conditions (**B** and **C**) were compared by two-tailed paired *t*-test and reported in Table 4.28.

Table 4.27. *t*-calculated values of two tailed paired *t*-test at 95% confidence level.

Parameter	Pair *t*-test	*t*-critical
Evaporation temperature	0.80	2.20
Number of extraction	2.22	

Taking the null hypothesis that there is **no** significant different in the %recovery given by reference condition and two varied conditions. For 11 degrees of freedom the critical value of $|t|$ is 2.20 (P = 0.05). The calculated value of $|t|$ at 95% confidence level for comparing reference condition using evaporating temperature at 34 °C (**A**) with varied condition at 40 °C (**B**) equal 0.80. Since the observed value of $|t|$ is less than the critical value, the null hypothesis is retained and can be conclude that there is no significant effect caused by changing the temperature of the rotary evaporator. Therefore, to reduce the time of sample preparation, evaporation temperature could be used at 40 °C.

The observed value of $|t|$ at 95% confidence level between conditions (**A**) and (**C**) was 2.22. Because it exceeded a critical value then the null hypothesis is rejected and the number of extraction with MTBE has great effect on the final result. Therefore, all sample preparation must be done by extraction twice with MTBE to improve the analysis efficiently.

4.16 The Determination of BADGE, BFDGE and Their Derivatives in Real Foods

Various types of oil-in-water and aqueous-based canned foods were randomly purchased from supermarkets in Bankok metropolitan area. Two cans of the same products manufactured on the same batch were selected. Types of samples chosen were sardines in tomato sauce, tuna in brine, orange juice, fruit in syrup, etc. Sample preparation was performed with procedure in Experimental Section 3.10 and 3.11 followed by HPLC analysis (Table 4.3). The final concentrations were calculated using linear regression equations. Total concentrations of BFDGE, BFDGE.H_2O, BFDGE.HCl.H_2O, and BFDGE.HCl were presented as the total sum of all present peaks. To identify sources of contamination, the internal coatings of the cans were tested by Beilstein's test method (Experimental Section 3.20.4). The results of extraction in foodstuff, internal coating and Beilstein's test were reported in Table 4.28-4.30 for oil-in-water-based canned foods and in Table 4.31-4.33 for aqueous-based canned foods. Related chromatograms are illustrated in APPENDIX D.

The result of duplicate analyses (2 cans) of each brand showed virtually comparable similar results except pork green curry. This might suggest the possibility of different coatings used. BADGE was detected in 38 out of 42 samples. In 11 samples (25%), BADGE alone exceeded 1 mg/kg limit, reaching up to 1.71 mg/kg. The hydrolysis products of BADGE were found in levels up to 2.08 and 3.92 mg/kg (BADGE.H_2O and BADGE.$2H_2O$). BADGE.$2H_2O$ was found in only 4 samples where BADGE was not detected. It is very likely that BADGE was totally hydrolyzed to BADGE.H_2O/ BADGE.$2H_2O$. The levels of chlorohydroxy products of BADGE in preserved foods were detected up to 1.67, 6.90, and 2.02 mg/kg for BADGE.HCl, BADGE.HCl.H_2O, and BADGE.2HCl, respectively.

The migration of BFDGE was found up to 8.21 mg/kg. Eleven samples showed the amount of BFDGE higher than 1 mg/kg. However, the amount of BFDGE.H_2O and BFDGE.$2H_2O$ derivatives were lower than the hydrolysis products of BADGE. For the chlorohydrins of BFDGE (BFDGE.HCl, BFDGE.HCl.H_2O, and BFDGE.2HCl) concentration reached 16.69, 5.44, and 0.26 mg/kg, respectively.

The data of extractable contaminants from internal can coatings are illustrated in Table 4.29. The emptied food cans were extracted with acetonitrile as mentioned in Section 3.20.3. Of 42 analyzed cans, 19 cases exceeded the sum of the EU regulation. The coatings of several cans contained higher derivative products than the starting materials. This could result from reactions with water and chloride in food matrixes. Or it could also come from the original coating. Furthermore, the Beilstein's test revealed the presence of chlorine in both the coatings of cans and lids (Table 4.30). Positive Beilstein's test suggested that the coating was organosol. Usually, easy-open cans used two types of lacquers: a Beilstein-negative one on the side wall and the bottom end and a Beilstein-positive one on the lid. On the other hand, classic cans were coated solely with Beilstein-negative polymers, presumably epoxy polymers. Examples of Beilstein test are shown in APPENDIX C.

Beilstein test results of tuna in brine (no.37 and 38) did not agree with HPLC data. We suspected that the chlorohydroxy derivatives in food were formed by the reactions of epoxy groups with chloride ions from salt.

Detectable levels of BADGE were found in 3 out of 38 samples of aqueous-base foodstuff, the level of BFDGE were the highest and were detected in 34 samples (Table 4.31). This is an indication that can manufactures in Thailand still use BFDGE based lacquers for food can coating. The hydrolysis products of BADGE (BADGE.$2H_2O$) was found in 36 samples, but not BADGE.H_2O. It is possible that BADGE was completely hydrolyzed to BADGE.$2H_2O$ in aqueous foods. Another group of detected reaction products were the chlorohydroxy compounds, which presented in 32 samples. Among these 32 samples, BADGE.HCl.H_2O and BADGE.2HCl derivatives were the dominating derivatives of BADGE. And BFDGE.HCl was found as the main product of BFDGE.

The Beilstein's test data in Table 4.33 were negative to young sweet corn in brine and champignons mushroom in brine (sample no.29-34). However, the chlorohydroxy derivatives were detected in foodstuffs. The presence of these compounds may result from the reactions of chloride from salt with the epoxy rings of BADGE or BFDGE.

Our data suggest that the contamination from can coating is in great distress. Contaminations were detected in all analysis, many at much higher than the EU's limit. Sixty-seven percent of oil-in-water based samples and 13% of aqueous-based samples contained more than 1 mg/kg of these compounds and the highest concentration reached 22.54 mg/kg. This suggested that the consumer may be ingest up to 3.5 mg/can of possible toxic substances. Amoung 80 samples of oil-in-water and aqueous-based foods tested during the course of this work, 41% were contaminated at levels higher than the EU regulation.

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Table 4.28. Concentration of BADGE, BFDGE, and their derivatives in oil-in-water based canned foods.

No.	Food content	Brands[a]	Concentration of BADGE and derivatives (mg/kg)[b]						Concentration of BFDGE and derivatives (mg/kg)						Total
			BADGE	.H_2O	.$2H_2O$	.HCl	.HCl.H_2O	.2HCl	BFDGE	.H_2O	.$2H_2O$	.HCl	.HCl.H_2O	.2HCl	
1	Sardines in tomato sauce	A1	0.08	ND	0.14	0.12	ND	0.12	0.73	0.27	0.01	0.14	0.78	0.16	2.55
2		A2	0.09	ND	0.09	0.14	ND	0.12	0.82	0.24	0.02	0.16	0.84	0.17	2.68
3		B1	ND	ND	0.21	ND	ND	ND	0.57	0.28	0.03	ND	0.04	ND	1.13
4		B2	ND	ND	0.10	ND	ND	0.01	0.72	0.13	ND	0.01	0.03	ND	1.00
5		C1	0.02	ND	0.16	0.01	ND	0.07	0.42	ND	ND	ND	ND	ND	0.67
6		C2	0.02	ND	0.02	0.01	ND	0.04	0.43	0.22	ND	ND	ND	ND	0.74
7		c3	0.02	ND	0.18	ND	ND	ND	0.48	0.04	0.03	ND	ND	ND	0.75
8		c4	ND	ND	0.15	ND	ND	ND	0.89	0.03	0.02	ND	ND	ND	1.09
9	Green curry fried sardines	D1	0.83	0.06	0.07	0.07	ND	0.07	4.12	0.03	0.02	ND	0.45	0.09	5.81
10		D2	0.52	0.05	0.08	0.09	ND	0.09	1.73	0.05	0.02	ND	0.41	0.12	3.14
11	Tom Yum sardines	A1	1.37	ND	0.14	0.12	2.24	0.11	1.10	ND	0.03	ND	0.67	0.26	6.05
12		A2	1.32	ND	0.20	0.11	1.35	0.13	1.05	0.35	0.02	0.04	0.53	0.19	5.29

No.	Food content	Brands	Concentration of BADGE and derivatives (mg/kg)						Concentration of BFDGE and derivatives (mg/kg)						Total
			BADGE	.H_2O	.$2H_2O$	.HCl	.HCl.H_2O	.2HCl	BFDGE	.H_2O	.$2H_2O$	.HCl	.HCl.H_2O	.2HCl	
13	Pork green curry	E1	0.64	ND	0.19	0.95	ND	0.02	0.45	0.12	ND	5.75	1.11	0.14	9.38
14		E2	0.68	ND	0.21	1.16	ND	0.03	0.47	0.17	ND	16.69	1.51	0.16	21.09
15	Tuna steak	C1	0.02	ND	0.07	ND	0.02	0.20	0.06	ND	ND	0.01	0.09	0.02	0.50
16		C2	0.03	0.01	0.09	ND	ND	0.21	0.38	ND	ND	0.07	ND	0.02	0.82
17	Tuna with ginger	C1	0.04	1.59	ND	ND	0.02	ND	ND	0.27	ND	0.06	3.84	ND	5.82
18		C2	ND	2.08	ND	ND	0.02	ND	ND	0.33	ND	0.09	5.44	ND	7.95
19	Seasoned vegetarian bamboo shoot with mushroom	E1	0.03	ND	0.12	0.03	0.01	ND	0.03	0.02	0.01	ND	ND	ND	0.26
20		E2	0.01	ND	0.13	0.01	0.01	ND	0.04	0.02	ND	ND	ND	ND	0.23
21	Chuchee sardines	D1	1.08	ND	0.07	0.15	2.05	0.18	0.85	0.02	0.05	0.49	0.54	0.18	5.66
22		D2	0.32	ND	0.07	0.07	0.84	0.07	0.32	0.03	0.03	0.23	0.39	0.11	2.49
23		F1	1.28	ND	0.35	ND	2.78	0.09	0.99	0.16	ND	ND	0.02	ND	5.68
24		F2	1.46	ND	0.14	ND	2.68	0.14	1.23	0.03	ND	ND	0.03	0.06	5.75

No.	Food content	Brands	Concentration of BADGE and derivatives (mg/kg)						Concentration of BFDGE and derivatives (mg/kg)						Total
			BADGE	.H_2O	.$2H_2O$	.HCl	.HCl.H_2O	.2HCl	BFDGE	.H_2O	.$2H_2O$	.HCl	.HCl.H_2O	.2HCl	
25	Vegetarian Palo soup	E1	0.02	ND	ND	ND	0.01	0.15	ND	ND	0.03	0.03	ND	ND	0.24
26		E2	0.02	ND	ND	ND	0.01	0.13	ND	ND	0.03	0.01	ND	ND	0.20
27	Seasoned Vegetarian Cabbage with mushroom	E1	0.01	ND	0.19	ND	ND	ND	0.17	0.03	0.02	ND	ND	0.01	0.43
28		E2	0.01	ND	0.22	ND	ND	ND	0.23	0.04	0.02	ND	ND	0.01	0.53
29	Chicken red thick curry	E1	0.31	ND	0.18	0.76	ND	ND	0.11	0.27	0.04	0.07	0.96	0.13	2.83
30		E2	0.31	ND	0.14	0.55	ND	ND	0.13	0.03	0.02	0.06	0.64	0.11	1.98
31	Numprik tuna	G1	1.71	ND	0.06	ND	5.22	0.04	8.21	0.05	0.04	0.08	0.11	ND	15.51
32		G2	1.69	ND	0.05	0.01	4.58	0.05	7.52	0.08	0.03	0.08	ND	0.01	14.09
33	Beef Masman curry	E1	0.30	1.27	3.92	1.67	ND	ND	0.29	0.12	ND	0.39	2.52	0.17	10.65
34		E2	0.27	1.16	2.51	1.27	ND	ND	ND	0.12	ND	0.16	2.09	0.14	7.73

No.	Food content	Brands	Concentration of BADGE and derivatives (mg/kg)						Concentration of BFDGE and derivatives (mg/kg)						Total
			BADGE	.H_2O	.2H_2O	.HCl	.HCl.H_2O	.2HCl	BFDGE	.H_2O	.2H_2O	.HCl	.HCl.H_2O	.2HCl	
35	Tuna steak in brine	G1	0.01	ND	0.24	0.02	0.10	ND	0.85	0.24	ND	ND	0.03	ND	1.50
36		G2	0.01	ND	0.21	ND	0.02	ND	ND	0.19	ND	ND	0.03	ND	0.46
37		h1	0.01	ND	0.01	0.01	0.01	ND	ND	0.03	0.01	0.02	ND	ND	0.10
38		h2	0.01	ND	0.01	0.01	0.06	ND	0.47	0.03	ND	0.01	ND	0.01	0.61
39	Tuna with chilli basil leaf	C1	1.70	ND	ND	0.09	6.90	2.02	6.90	0.33	ND	3.27	1.33	ND	22.54
40		C2	1.67	ND	ND	0.08	3.39	2.01	6.44	0.22	ND	0.11	1.39	ND	15.31
41		G1	2.17	ND	ND	ND	3.99	ND	1.84	ND	0.11	ND	0.08	ND	8.19
42		G2	2.58	0.01	ND	ND	5.04	ND	1.97	ND	0.07	ND	0.11	ND	9.79

[a]A1-A2 represent the same lot of the same brand
Capital letters: cans with easy-open
Small letters: classic cans

[b]ND: non detectable (<0.97 ppb for BADGE, BADGE.H_2O; <1.26 ppb for BADGE.2H_2O; <1.53 ppb for BADGE.HCl; <0.72 ppb for BADGE.HCl.H_2O; <0.99 ppb for BADGE.2HCl; <2.00, 1.89, 8.10 ppb for 3 isomers of BFDGE; <4.57, 9.45, 9.45, 18.00 ppb for 4 isomers of BFDGE.H_2O; <0.94 for BFDGE.2H_2O; 1.96, 6.75 ppb for 2 isomers of BFDGE.HCl; <7.16, 9.00, 11.25, 15.75 ppb for 4 isomers of BFDGE.HCl.H_2O; <2.39 ppb for BFDGE.2HCl

Table 4.29. Concentration of BADGE, BFDGE, and their derivatives extracted from empty cans of oil-in-water-type canned foods.

No.	Food content	Brands	Concentration of BADGE and derivatives (mg/dm^2)						Concentration of BFDGE and derivatives (mg/dm^2)						Total
			BADGE	$.H_2O$	$.2H_2O$	.HCl	$.HCl.H_2O$	.2HCl	BFDGE	$.H_2O$	$.2H_2O$	.HCl	$.HCl.H_2O$	.2HCl	
1	Sardines in tomato sauce	A1	1.51	0.37	0.34	2.13	0.46	1.28	2.76	ND	ND	2.72	1.98	0.82	14.37
2		A2	1.54	0.48	0.25	2.47	0.64	1.28	3.13	1.40	ND	3.32	2.56	1.12	18.18
3		B1	0.48	0.23	0.27	0.25	0.28	ND	ND	0.19	ND	ND	ND	ND	1.70
4		B2	0.87	0.30	ND	0.45	0.38	0.13	ND	ND	ND	ND	ND	ND	2.13
5		C1	0.16	0.22	0.53	0.11	0.19	0.19	ND	ND	ND	ND	ND	ND	1.40
6		C2	0.14	0.12	0.41	0.10	0.18	0.13	ND	ND	ND	ND	ND	ND	1.09
7		c3	0.11	ND	0.11	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.22
8		c4	0.11	ND	0.11	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.22
9	Green curry fried sardines	D1	1.38	0.33	0.20	1.37	0.74	ND	0.84	ND	ND	1.93	1.02	0.31	8.12
10		D2	4.19	0.86	0.27	3.48	1.06	ND	2.98	1.40	0.08	3.25	1.96	0.95	20.49
11	Tom Yum sardines	A1	2.04	ND	1.17	2.75	1.63	1.36	2.83	0.31	0.19	3.90	1.86	2.08	20.12
12		A2	2.80	ND	0.93	3.89	1.98	2.37	4.04	0.57	ND	6.06	2.83	3.88	29.34

No.	Food content	Brands	Concentration of BADGE and derivatives (mg/dm^2)						Concentration of BFDGE and derivatives (mg/dm^2)						Total
			BADGE	$.H_2O$	$.2H_2O$	.HCl	$.HCl.H_2O$	.2HCl	BFDGE	$.H_2O$	$.2H_2O$	.HCl	$.HCl.H_2O$	.2HCl	
13	Pork green curry	E1	0.19	ND	0.63	0.15	ND	ND	ND	ND	ND	0.86	ND	ND	1.84
14		E2	0.22	ND	0.65	0.19	ND	ND	ND	ND	ND	2.46	1.92	ND	5.45
15	Tuna steak	C1	0.05	0.06	0.18	ND	0.12	ND	ND	ND	ND	ND	ND	ND	0.41
16		C2	0.20	0.11	0.27	0.10	0.16	ND	ND	ND	ND	ND	ND	ND	0.84
17	Tuna with ginger	C1	0.09	0.22	0.14	0.08	0.13	ND	ND	0.26	ND	ND	1.07	ND	1.99
18		C2	ND	0.17	0.10	ND	0.09	ND	ND	ND	ND	ND	ND	ND	0.35
19	Seasoned vegetarian bamboo shoot with mushroom	E1	0.13	0.13	0.32	ND	0.11	ND	ND	ND	ND	ND	ND	ND	0.68
20		E2	ND	ND	0.33	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.33
21	Chuchee sardines	D1	0.42	0.19	0.21	1.08	0.41	0.71	0.43	ND	ND	1.30	0.47	0.43	5.67
22		D2	0.56	0.23	0.17	0.99	0.34	0.54	1.01	0.23	ND	1.63	0.86	0.33	6.90
23		F1	0.58	0.19	1.12	0.27	0.72	1.21	0.32	0.23	ND	ND	ND	ND	4.63
24		F2	0.33	0.11	0.26	0.17	0.48	0.06	0.25	ND	ND	ND	ND	ND	1.66

No.	Food content	Brands	Concentration of BADGE and derivatives (mg/dm^2)						Concentration of BFDGE and derivatives (mg/dm^2)						Total
			BADGE	$.H_2O$	$.2H_2O$	.HCl	$.HCl.H_2O$	.2HCl	BFDGE	$.H_2O$	$.2H_2O$	.HCl	$.HCl.H_2O$	.2HCl	
25	Vegetarian Palo soup	E1	0.12	0.13	0.33	ND	0.10	0.23	ND	ND	ND	6.35	ND	ND	7.25
26		E2	0.14	ND	ND	ND	0.09	0.26	ND	ND	ND	7.88	ND	ND	8.38
27	Seasoned Vegetarian Cabbage with mushroom	E1	ND	0.11	0.38	ND	0.10	ND	ND	ND	ND	ND	0.97	ND	1.56
28		E2	ND	0.14	0.42	ND	0.11	ND	0.14	ND	ND	ND	ND	ND	0.82
29	Chicken red thick curry	E1	0.13	ND	0.36	0.20	0.21	ND	ND	0.17	ND	ND	14.34	ND	15.41
30		E2	0.13	ND	0.26	0.20	0.24	ND	ND	ND	ND	ND	17.41	ND	18.25
31	Numprik tuna	G1	0.55	ND	ND	ND	1.13	0.10	0.40	ND	ND	0.20	ND	ND	2.38
32		G2	0.55	ND	ND	ND	1.01	0.08	0.38	ND	ND	0.17	ND	ND	2.20
33	Beef Masman curry	E1	0.13	ND	ND	0.25	ND	ND	ND	ND	ND	ND	ND	ND	0.38
34		E2	0.12	ND	ND	0.23	ND	ND	ND	ND	ND	ND	ND	ND	0.35

No.	Food content	Brands	Concentration of BADGE and derivatives (mg/dm^2)						Concentration of BFDGE and derivatives (mg/dm^2)						Total
			BADGE	$.H_2O$	$.2H_2O$	.HCl	$.HCl.H_2O$	.2HCl	BFDGE	$.H_2O$	$.2H_2O$	.HCl	$.HCl.H_2O$	.2HCl	
35	Tuna steak in brine	G1	0.13	ND	0.44	ND	0.13	ND	ND	ND	ND	ND	ND	ND	0.70
36		G2	0.12	0.15	0.46	ND	0.13	ND	ND	ND	ND	ND	ND	ND	0.85
37		h1	ND	0.11	0.14	ND	ND	ND	ND	0.22	ND	ND	ND	ND	0.46
38		h2	ND	0.10	0.14	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.25
39	Tuna with chilli basil leaf	C1	0.14	ND	ND	ND	0.19	0.08	0.12	ND	ND	ND	7.29	ND	7.81
40		C2	0.12	ND	ND	ND	0.21	0.07	ND	ND	ND	ND	17.42	ND	17.83
41		G1	0.23	0.13	0.36	ND	0.37	0.12	0.21	ND	ND	ND	ND	ND	1.42
42		G2	0.25	0.14	0.36	ND	0.44	0.09	0.24	ND	ND	ND	ND	ND	1.52

Table 4.30. Characterization of can coatings for cans of oil-in-water-type foods.

No.	Food content	Brands	Characterization of can			
			Type of can	Results of Beilstein test		
				Upper lid	Side wall	Bottom end
1	Sardines in tomato sauce	A1	3-p, e.o.	+	-	-
2		A2		+	-	-
3		B1		+	-	-
4		B2		+	-	-
5		C1		+	-	-
6		C2		+	-	-
7		c3	3-p, classic	-	-	-
8		c4		-	-	-
9	Green curry fried sardines	D1	3-p, e.o.	+	-	-
10		D2		+	-	-
11	Tom Yum sardines	A1	3-p, e.o.	+	-	-
12		A2		+	-	-
13	Pork green curry	E1	2-p, e.o.	+	-	-
14		E2		+	-	-
15	Tuna steak	C1	3-p, e.o.	+	-	-
16		C2		+	-	-
17	Tuna with ginger	C1	3-p, e.o.	+	-	-
18		C2		+	-	-
19	Seasoned vegetarian bamboo shoot with mushroom	E1	2-p, e.o.	+	-	-
20		E2		+	-	-
21	Chuchee sardines	D1	3-p, e.o.	+	-	-
22		D2		+	-	-
23		F1	2-p, e.o.	+	-	-
24		F2		+	-	-

No.	Food content	Brands	Characterization of can			
			Type of can	Results of Beilstein test		
				Upper lid	Side wall	Bottom end
25	Vegetarian Palo soup	E1	2-p, e.o.	+	-	-
26		E2		+	-	-
27	Seasoned Vegetarian Cabbage with mushroom	E1	2-p, e.o.	+	-	-
28		E2		+	-	-
29	Chicken red thick curry	E1	2-p, e.o.	+	-	-
30		E2		+	-	-
31	Numprik tuna	G1	3-p, e.o.	+	-	-
32		G2		+	-	-
33	Beef Masman curry	E1	2-p, e.o.	+	-	-
34		E2		+	-	-
35	Tuna steak in brine	G1	2-p, e.o.	+	-	-
36		G2		+	-	-
37		h1	2-p, classic	-	-	-
38		h2		-	-	-
39	Tuna with chilli basil leaf	C1	3-p, e.o.	+	-	-
40		C2		+	-	-
41		G1	2-p, e.o.	+	-	-
42		G2		+	-	-

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Table 4.31. Concentration of BADGE, BFDGE, and their derivatives in aqueous-based canned foods.

No.	Food content	Brands	Concentration of BADGE and derivatives (mg/kg)						Concentration of BFDGE and derivatives (mg/kg)						Total
			BADGE	.H_2O	.2H_2O	.HCl	.HCl.H_2O	.2HCl	BFDGE	.H_2O	.2H_2O	.HCl	.HCl.H_2O	.2HCl	
1	Orange juice	I1	0.02	0.36	4.61	0.04	0.52	0.02	1.24	0.34	0.01	0.09	0.22	ND	7.45
2		I2	0.03	0.04	4.13	0.05	0.45	0.01	0.42	0.37	0.01	0.08	0.28	ND	5.87
3		J1	ND	0.01	0.06	ND	ND	ND	0.09	ND	ND	ND	ND	ND	0.16
4		J2	ND	ND	0.04	ND	ND	ND	0.20	ND	ND	ND	ND	ND	0.24
5	Tomato juice	J1	ND	ND	0.04	ND	ND	0.01	0.27	ND	0.05	ND	0.97	0.11	1.46
6		J2	ND	ND	0.05	ND	0.02	0.01	0.08	ND	0.05	ND	1.06	0.11	1.37
7		I1	ND	ND	0.01	ND	0.01	ND	0.33	ND	ND	ND	ND	ND	0.35
8		I2	ND	ND	0.01	ND	0.04	ND	0.32	ND	ND	ND	ND	ND	0.37
9	Coffee	K1	ND	ND	0.69	ND	0.03	0.16	0.36	0.46	ND	ND	ND	0.02	1.72
10		K2	ND	ND	0.22	ND	ND	0.04	0.08	ND	ND	ND	ND	ND	0.33
11		L1	ND	ND	0.39	ND	ND	ND	0.22	0.27	ND	ND	ND	ND	0.88
12		L2	ND	ND	0.44	ND	ND	ND	0.28	0.24	ND	ND	ND	ND	0.96

No.	Food content	Brands	Concentration of BADGE and derivatives (mg/kg)						Concentration of BFDGE and derivatives (mg/kg)						Total
			BADGE	.H_2O	.2H_2O	.HCl	.HCl.H_2O	.2HCl	BFDGE	.H_2O	.2H_2O	.HCl	.HCl.H_2O	.2HCl	
13	Lychee in syrup	J1	ND	ND	0.07	ND	0.01	0.01	0.10	0.20	ND	0.01	ND	ND	0.40
14		J2	ND	ND	0.07	ND	0.01	0.01	0.03	0.24	ND	0.01	ND	ND	0.37
15		I1	ND	ND	0.04	ND	0.01	0.01	0.06	ND	ND	0.01	ND	ND	0.12
16		I2	ND	ND	0.04	ND	0.01	ND	0.12	ND	ND	0.01	ND	ND	0.17
17	Mustard green leaf	I1	ND	ND	0.10	ND	0.10	0.01	0.54	ND	ND	0.02	ND	0.02	0.80
18		I2	0.05	ND	0.11	ND	0.10	0.02	0.25	ND	ND	0.05	ND	ND	0.58
19		F1	ND	ND	0.03	ND	0.02	ND	0.14	0.12	ND	0.01	ND	ND	0.31
20		F2	ND	ND	0.03	ND	0.01	ND	0.12	0.10	ND	0.01	ND	ND	0.26
21	Rumbutan in syrup	M1	ND	ND	0.07	ND	0.02	0.02	0.20	ND	ND	ND	ND	ND	0.31
22		M2	ND	ND	0.09	ND	0.02	0.02	0.07	ND	0.01	ND	ND	ND	0.21
23		I1	ND	ND	0.02	ND	0.03	0.04	ND	ND	0.02	ND	0.29	0.06	0.46
24		I2	ND	ND	0.03	ND	0.03	0.04	0.06	ND	0.01	0.01	0.26	0.07	0.51

No.	Food content	Brands	Concentration of BADGE and derivatives (mg/kg)						Concentration of BFDGE and derivatives (mg/kg)						Total
			BADGE	.H_2O	.$2H_2O$	.HCl	.HCl.H_2O	.2HCl	BFDGE	.H_2O	.$2H_2O$	.HCl	.HCl.H_2O	.2HCl	
25	Carbonated beverages	N1	ND	ND	0.01	ND	ND	0.01	0.15	ND	ND	ND	ND	ND	0.17
26		N2	ND	ND	0.01	ND	ND	0.03	0.13	ND	ND	ND	ND	ND	0.16
27		O1	ND	ND	ND	ND	ND	0.01	0.10	ND	ND	ND	ND	ND	0.10
28		O2	ND	ND	ND	ND	ND	0.005	ND	ND	ND	ND	ND	ND	0.005
29	Young sweet corn in brine	i1	ND	ND	0.16	ND	0.01	0.02	0.23	ND	ND	0.03	ND	ND	0.45
30		i2	ND	ND	0.11	ND	0.01	0.01	0.12	ND	ND	0.02	ND	ND	0.27
31	Champignons mushrooms in brine	i1	ND	ND	0.15	ND	0.01	0.02	0.03	ND	ND	0.03	ND	ND	0.25
32		i2	ND	ND	0.02	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.02
33		m1	ND	ND	0.29	ND	ND	ND	0.02	ND	ND	ND	ND	ND	0.37
34		m2	ND	ND	0.30	ND	ND	ND	0.02	ND	ND	ND	ND	ND	0.40

No.	Food content	Brands	Concentration of BADGE and derivatives (mg/kg)						Concentration of BFDGE and derivatives (mg/kg)						Total
			BADGE	.H_2O	.$2H_2O$	.HCl	.HCl.H_2O	.2HCl	BFDGE	.H_2O	.$2H_2O$	.HCl	.HCl.H_2O	.2HCl	
35	Pineapple pieces in syrup	j1	ND	ND	0.03	ND	ND	ND	ND	0.09	ND	ND	ND	ND	0.12
36		j2	ND	ND	0.03	ND	ND	ND	0.10	0.12	ND	ND	ND	ND	0.25
37		p1	ND	ND	0.02	ND	ND	ND	0.23	ND	ND	ND	ND	ND	0.25
38		p2	ND	ND	0.02	ND	ND	ND	0.07	ND	ND	ND	ND	ND	0.09

Table 4.32. Concentration of BADGE, BFDGE, and their derivatives extracted from empty cans of aqueous-type canned foods.

No.	Food content	Brands	Concentration of BADGE and derivatives (mg/dm^2)						Concentration of BFDGE and derivatives (mg/dm^2)						Total
			BADGE	$.H_2O$	$.2H_2O$	.HCl	$.HCl.H_2O$	.2HCl	BFDGE	$.H_2O$	$.2H_2O$	.HCl	$.HCl.H_2O$	.2HCl	
1	Orange juice	I1	14.72	1.38	0.28	1.89	0.17	0.06	0.07	ND	ND	ND	ND	0.06	18.63
2		I2	18.54	1.68	0.35	2.27	0.21	ND	0.13	ND	ND	ND	ND	ND	23.18
3		J1	0.35	0.13	0.08	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.56
4		J2	0.32	0.12	0.07	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.51
5	Tomato juice	J1	0.16	0.07	0.14	0.07	0.10	0.67	3.83	1.13	ND	4.39	1.68	0.74	12.98
6		J2	0.09	0.06	0.17	0.07	0.08	0.62	3.65	1.15	ND	3.86	1.47	0.57	11.81
7		I1	0.06	0.04	0.20	0.09	0.05	0.08	ND	0.11	ND	0.10	ND	ND	0.72
8		I2	0.05	0.04	0.18	0.09	0.04	0.07	ND	ND	ND	0.09	ND	ND	0.57
9	Coffee	K1	0.54	0.16	0.22	ND	0.07	0.77	5.78	0.92	ND	5.73	0.93	1.38	16.51
10		K2	0.15	0.08	0.08	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.31
11		L1	1.82	0.28	0.31	ND	ND	ND	0.11	ND	ND	ND	ND	ND	2.52
12		L2	2.29	0.36	0.58	ND	ND	ND	0.26	ND	ND	ND	ND	ND	3.49

No.	Food content	Brands	Concentration of BADGE and derivatives (mg/dm^2)						Concentration of BFDGE and derivatives (mg/dm^2)						Total
			BADGE	$.H_2O$	$.2H_2O$	.HCl	$.HCl.H_2O$	.2HCl	BFDGE	$.H_2O$	$.2H_2O$	.HCl	$.HCl.H_2O$	.2HCl	
13	Lychee in syrup	J1	ND	ND	0.35	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.35
14		J2	ND	ND	0.41	ND	0.09	ND	0.13	ND	ND	0.13	ND	ND	0.76
15		I1	ND	0.10	0.49	ND	ND	ND	ND	ND	ND	0.13	ND	ND	0.72
16		I2	ND	0.10	0.45	ND	ND	ND	ND	ND	ND	0.14	ND	ND	0.69
17	Mustard green leaf	I1	ND	ND	1.59	ND	0.13	ND	0.24	1.78	ND	0.93	0.54	ND	5.21
18		I2	0.16	0.11	1.66	ND	0.19	ND	0.24	1.80	ND	1.09	0.49	ND	5.73
19		F1	0.81	0.47	0.26	0.18	ND	ND	ND	ND	ND	ND	ND	ND	1.72
20		F2	0.78	0.47	0.29	0.17	ND	ND	ND	ND	ND	ND	ND	ND	1.72
21	Rumbutan in syrup	M1	0.05	ND	0.04	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.09
22		M2	ND	ND	0.04	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.04
23		I1	0.46	0.08	ND	0.88	0.14	0.54	0.60	ND	ND	1.45	0.72	0.47	5.33
24		I2	0.16	0.07	ND	0.73	0.13	0.52	0.45	ND	ND	1.10	0.60	0.44	4.20

No.	Food content	Brands	Concentration of BADGE and derivatives (mg/dm^2)						Concentration of BFDGE and derivatives (mg/dm^2)						Total
			BADGE	$.H_2O$	$.2H_2O$	.HCl	$.HCl.H_2O$	.2HCl	BFDGE	$.H_2O$	$.2H_2O$	.HCl	$.HCl.H_2O$	.2HCl	
25	Carbonated beverages	N1	ND	ND	ND	0.08	0.04	0.32	ND	ND	ND	ND	ND	ND	0.45
26		N2	ND	ND	ND	0.11	0.05	0.75	ND	ND	ND	ND	ND	ND	0.91
27		O1	0.07	ND	0.08	0.44	0.08	0.66	ND	ND	ND	ND	ND	ND	1.33
28		O2	0.08	ND	0.09	0.50	0.09	0.67	ND	ND	ND	ND	ND	ND	1.43
29	Young sweet corn in brine	i1	ND	ND	0.07	0.06	ND	0.06	ND	ND	ND	0.04	ND	ND	0.23
30		i2	ND	ND	0.09	0.06	ND	0.08	ND	ND	ND	0.05	ND	ND	0.28
31	Champignons mushrooms in brine	i1	ND	ND	0.05	ND	ND	ND	ND	ND	ND	0.05	ND	ND	0.10
32		i2	ND	ND	0.06	ND	ND	ND	ND	ND	ND	0.05	ND	ND	0.11
33		m1	0.06	0.09	0.17	0.06	0.04	ND	ND	ND	ND	ND	ND	ND	0.42
34		m2	0.06	0.08	0.14	0.06	0.04	ND	ND	ND	ND	ND	ND	ND	0.38

No.	Food content	Brands	Concentration of BADGE and derivatives (mg/dm^2)						Concentration of BFDGE and derivatives (mg/dm^2)						Total
			BADGE	$.H_2O$	$.2H_2O$	.HCl	$.HCl.H_2O$	.2HCl	BFDGE	$.H_2O$	$.2H_2O$	.HCl	$.HCl.H_2O$	.2HCl	
35	Pineapple pieces in syrup	j1	ND	ND	0.07	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.07
36		j2	0.04	ND	0.08	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.12
37		p1	0.04	0.05	0.08	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.16
38		p2	ND	0.05	0.07	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.11

Table 4.33. Characterization of cans coatings for cans of aqueous-type foods.

No.	Food content	Brands	Characterization of can			
			Type of can	Results of Beilstein test		
				Upper lid	Side wall	Bottom end
1	Orange juice	I1	3-p, e.o.	+	-	-
2		I2		+	-	-
3		J1		-	-	-
4		J2		-	-	-
5	Tomato juice	J1	3-p, e.o.	+	-	-
6		J2		+	-	-
7		I1		+	-	-
8		I2		+	-	-
9	Coffee	K1	3-p, e.o.	+	-	-
10		K2		+	-	-
11		L1		-	-	-
12		L2		-	-	-
13	Lychee in syrup	J1	3-p, e.o.	+	-	-
14		J2		+	-	-
15		I1		+	-	-
16		I2		+	-	-
17	Mustard green leaf	I1	3-p, e.o.	+	-	-
18		I2		+	-	-
19		F1		+	-	-
20		F2		+	-	-
21	Rumbutan in syrup	M1	3-p, e.o.	+	-	-
22		M2		+	-	-
23		I1		+	-	-
24		I2		+	-	-

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No.	Food content	Brands	Charaterization of can			
			Type of can	Results of Beilstein test		
				Upper lid	Side wall	Bottom end
25	Carbonated beverages	N1	3-p, e.o.	+	-	-
26		N2		+	-	-
27		O1		+	-	-
28		O2		+	-	-
29	Young sweet corn in brine	i1	3-p, classic	-	-	-
30		i2		-	-	-
31	Champignons mushrooms in brine	i1	3-p, classic	-	-	-
32		i2		-	-	-
33		m1		-	-	-
34		m2		-	-	-
35	Pineapple pieces in syrup in brine	j1	3-p, classic	-	-	-
36		j2		-	-	-
37		p1		-	-	-
38		p2		-	-	-

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