CHAPTER V

CONCLUSIONS AND SUGGESTIONS FOR FURTHER STUDY

In this study, a new method for simultaneous identification and quantification of bisphenol-A-diglycidyl ether (BADGE), bisphenol-F-diglycidyl ethr (BFDGE), and 10 of their derivatives: BADGE.H_2O, BADGE.$2H_2O$, BADGE.HCl.H_2O, BADGE.HCl, BADGE.2HCl, BFDGE.H_2O, BFDGE.$2H_2O$, BFDGE.HCl.H_2O, BFDGE.HCl, and BFDGE.2HCl was developed. The analysis was carried out using reversed-phase gradient elution with fluorescence detection.

The work was also covered the preparation of 3 nonavailable standard reference materials: BFDGE.H_2O, BFDGE.HCl, and BFDGE.HCl.H_2O. Simple and reliable preparation methods for these three monomeric derivatives were proposed. Successful separations and purifications were performed by column chromatography. Light yellow syrup compounds were obtained with the purity of 94, 91, and 85%, respectively. After purification, the compounds were tested for their chromatographic characteristics and further confirmed by UV and LC-MS to match their profiles. The corresponded mass spectra showed profile of adduct ions $[M+NH_4]^+$ of BFDGE.H_2O (m/z 348), BFDGE.HCl (m/z 366), and BFDGE.HCl.H_2O (m/z 384).

The simultaneous analysis of targeted twelve compounds demonstrated baseline separation of all derivatives. The selectivity of the HPLC method was evaluated by resolution values of critical pairs (R_s) and matching peak retention time (t_R) with standards. In addition, UV spectra of all analytes were used to differentiate these compounds from interference superimposed with peaks of standards. The method is capable to isolate all isomers of BFDGE and its derivatives such as 4 peaks were observed for BFDGE.H_2O and BFDGE.HCl.H_2O, 3 peaks for BFDGE, 2 peaks (4 isomer are co-elute) of BFDGE.HCl, and 1 peak of BFDGE.$2H_2O$ and BFDGE.2HCl. The method showed good analytical characteristics, having linear relationships (R^2>0.9990) up to 10.0 ppm, 10-fold over the EU limit, as shown in

Table 5.1. The method detection limits and method quantitation limits were lower than the detection limit of the instrument 6.67-fold as described. Thus, the trace amount of analytes in canned food could be extrapolated.

Table 5.1. Characteristic validation data consists of retention time, resolution (R_s), coefficient correlation (R^2), method detection limit (MDL), and method quantitation limit (MQL) of each compound.

No.	Compounds	Retention time (min)	R_s	R^2	MDL (ppb)	MQL (ppb)
1	BFDGE.2H_2O	10.20±0.04	-	0.9997	0.94	3.15
2	BADGE.2H_2O	17.17±0.08	17.27	0.9997	1.26	4.20
3	BFDGE.H_2O	18.15±0.08	2.07	0.9996	4.57	15.22
		20.03±0.09	4.02	0.9997	9.45	31.50
		20.57±0.10	1.22	0.9986	9.45	31.50
		21.89±0.07	2.61	0.9966	18.00	60.00
4	BFDGE.HCl.H_2O	22.62±0.09	1.75	0.9997	7.16	23.88
		23.41±0.06	1.75	0.9988	9.00	30.00
		25.26±0.08	3.79	0.9982	11.25	37.50
		25.88±0.09	1.48	0.9919	15.75	52.50
5	BADGE.H_2O	27.53±0.08	4.13	0.9998	0.97	3.22
6	BFDGE	28.29±0.07	1.75	0.9995	2.00	6.68
		29.54±0.08	2.83	0.9998	1.89	6.30
		30.25±0.11	1.71	0.9993	8.10	27.00
7	BADGE.HCl.H_2O	30.97±0.07	1.51	0.9998	0.72	2.40
8	BFDGE.HCl	31.78±0.08	1.95	0.9998	1.96	6.52
		33.05±0.11	2.97	0.9993	6.75	22.50
9	BFDGE.2HCl	35.04±0.24	2.65	0.9999	2.39	7.95
10	BADGE	36.11±0.07	1.48	0.9998	0.97	3.22
11	BADGE.HCl	38.73±0.07	6.29	0.9998	1.53	5.10
12	BADGE.2HCl	40.85±0.07	5.28	0.9998	0.99	3.30

The method precision and accuracy were studied at 2 concentration levels [MQL (2-23 ppb) and 5-fold MQL (10-115 ppb)] and for 2 sample matrixes (oil-in-water and aqueous-based). In addition, the method precision were tested for both intra-assay precision and intermediate precision showing excellent precision with less than 15%RSD indicating high precision under the AOAC standard. Percent recovery values demonstrated excellent method accuracy that also met the AOAC standard in both matrixes. By comparison of sample blank with spiked samples of each matrix, it was found that there is no matrixes interference that disturbed the analysis. Thus, the develped method can be used to analyzed these compounds accurately and precisely.

The migrations of BADGE, BFDGE, and their derivatives into the canned foods manufactured in Thailand were determined. Among 80 cans analyzed, all were found to releasing detectable amounts of these compounds. In 42 samples of oil-in-water-base foodstuff, 67% were contaminated at a level higher than 1 mg/kg of the EU limit as show in Figure 5.1. The highest concentration reached 22.54 mg/kg suggesting that the consumer of this can will ingest 3.5 mg of these compounds. In aqueous-based foodstuff, maximum detected concentration was 7.54 mg/kg and 13% of sample tested were contaminated higher than the EU limit. Sources of chlorohydroxy derivatives contamination were further confirmed by Beilstein test which identified types of polymer containing chlorine e.g. organosols.

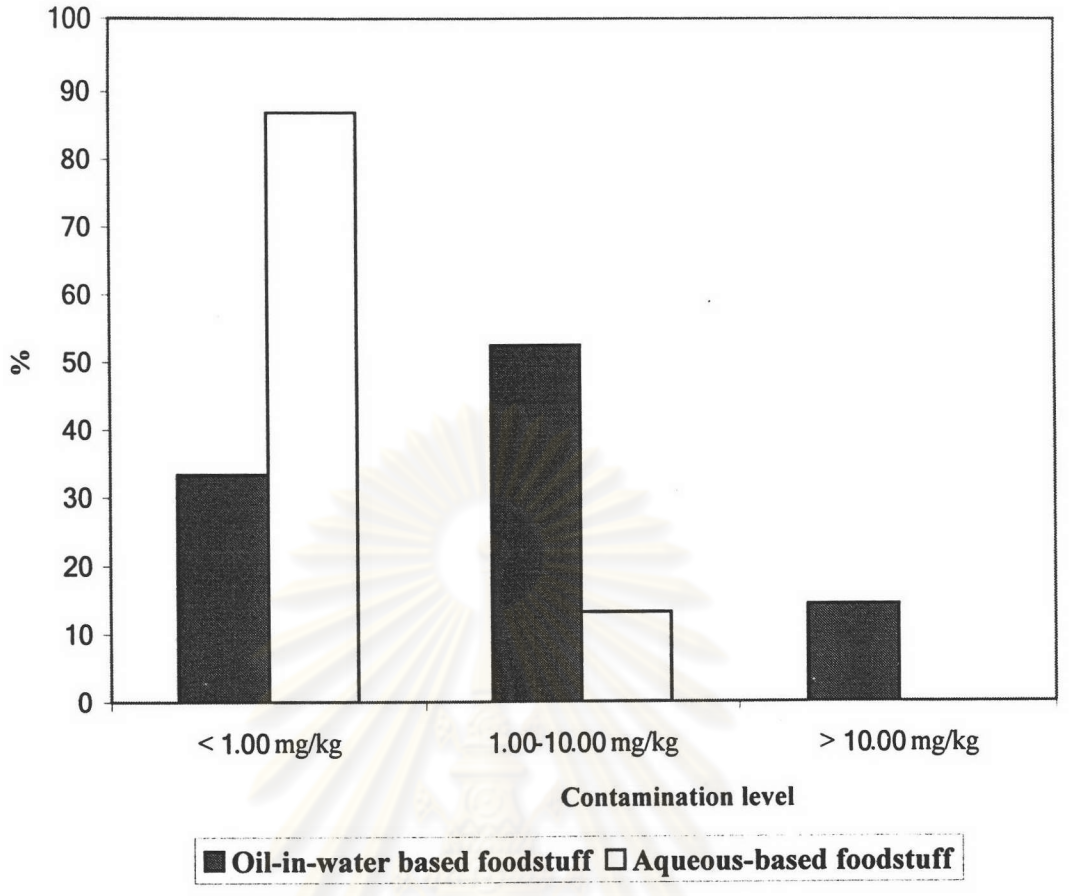

Figure 5.1. Results summary for the contamination in canned foods from Thai markets surveyed.

We want to emphasize here that it is very important to control the pH of food especially for aqueous acidic food such as vegetables, fruit, and juices to 7.0 before extraction to prevent opening of epoxide ring(s) as described in the previous section. The new developed method can be used to determine the contamination of BADGE, BFDGE, and their derivatives in all types of canned foods: oil, oil-in-water, and aqueous-based based on the current EU standard.

Further work should be focused on other, non-fluorescing compounds possibly released from can coatings as well and may have adverse health effects on consumers, such as catalysts, accelerators, epoxidized edible oils, amino resins, acrylic resins, esters, waxes, and lubricants.