CHAPTER V

CONCLUSIONS

Reversed-phase HPLC with fluorescence detection was chosen for the analysis of BADGE, BFDGE, and 3 derivatives (BADGE.HCl, BADGE.2HCl, BFDGE.2HCl) in oil-based canned foods. Acceptable resolutions of all targeted compounds were achieved. However, the method cannot be used to quantify individual isomers of BFDGE and its derivatives. Quantitative analysis of total contamination is based on the assumption that the emission energy of all isomers occur at the same wavelength and are additive. The optimal chromatographic conditions are:

Injection volume: 5 µL
Flow rate: 0.75 mL/min.
Mobile Phase: 60% methanol: 40% water
Column: ODS Hypersil C18, 150 x 4.0 mm, 5 µm
Detector: Fluorescence, λ_{ex} = 227 nm, λ_{em} = 313 nm

The response of each compound showed good linearity ($R^2 > 0.999$) from 0.035-5.000 ppm. All standard calibration curves revealed good linear relationships with $R^2 > 0.999$ from 0.035-1.000 ppm.

The limits of detection (LoD) and limits of quantitation (LoQ) were different for each compound and were from 0.012-0.034 ppm and from 0.041-0.113 ppm, for LoD and LoQ, respectively. The LoD and LoQ of BADGE were the lowest because it showed the highest detector response than other compounds.

Fluorescence detection was selected because of low molar absorptivities of the compounds and the complication of the food matrixes that interfered with the measurement of the different in absorbance in UV-vis technique. Moreover, the measurement of selected emission rays means zero baseline making fluorescence very suitable for trace analysis.

Standard addition method at 2 spiking levels (LoQ and 5-fold LoQ) was chosen to test the method precision and percent recovery of the extraction procedure. The coefficients of variation (CV) obtained at LoQ level (0.041-0.113 ppm) and at 5-fold LoQ (0.205-0.565 ppm) were from 9.82-13.85 and 4.34-6.20 respectively. The data implied good precision at ppm levels. Percent recovery from 48-87% and 64-85% were obtained at LoQ and 5-fold LoQ, respectively.

The method was found to be robust when minor changes were applied to some parameter (solvent extraction volume and heating bath temperature) in the extraction procedure (paired *t*-test values lower than *t*-table at n=5).

All validation parameters indicated that this analytical method is reliable with good linearity, high sensitivity, high precision, good percent recovery, and robust. Validation parameters are compiled in Table 5.1.

Because BFDGE.HCL was not available at the beginning of this work, it was not included in the HPLC procedure. However, it was later prepared and purified in our laboratory and was tested that this HPLC method could be used to analyzed it as well. The validation of BFDGE.HCl was completed by the work of Nongpanga Kulkaew and Suparee Pungboonlue [40] which proved that our developed method is fit for the analysis of all 6 compounds (BADGE, BADGE.HCl, BADGE.2HCl, BFDGE, BFDGE.HCl, BFDGE.2HCl) in oil-based canned foods.

The method was used to test 20 oil-based canned foods that could be classified in 3 categories as: tuna in oil, tuna in mayonnaise, and fried foods. All samples were packed in 2-piece cans with easy-open lids that required high flexibility and were coated with organosol resins. All 2-piece can bodies were coated with either epoxy or organosol resins.

The contamination level of tuna in oil and tuna in mayonnaise samples were found to be lower (0.1-1.0 mg/kg) than the EU regulation (1.0 mg/kg). The can bodies were coated with both epoxy and organosol resins and the level of contamination

detected between these types of resins are comparable indicating that both epoxy and organosol can be used for oil-based foods where sterilization process is moderate.

The contamination of fried-foods was all higher than tuna in oil media. The can bodies were coated with both epoxy resins and organosol resins. All samples packed in epoxy resin were acceptable based on the EU standard. Five out of 11 samples packed in organosol coated cans were badly contaminated with a maximum value reached 4.289 mg/kg, more than 4-times higher than the EU limit. However, there were samples packed in organosol coated cans that were acceptable. The data indicated that organosol resins are the major cause of contamination in fried-foods. However, it is possible to prevent extreme contamination if clean organosol resins are used and if the manufacturers are careful during the packaging process.

Table 5.1. The table showed all validation parameters of targeted compounds

Compound	R^2 (linearity)	R^2 (calibration)	LoD (ppm)	LoQ (ppm)	CV (precision)		%Recovery	
					LoQ level	5LoQ level	LoQ level	5LoQ level
BADGE	0.9999	0.9996	0.012	0.041	9.82	4.34	48.84 ± 4.63	64.40 ± 2.76
BADGE.HCl	0.9999	0.9999	0.034	0.113	11.17	6.20	74.17 ± 8.31	76.70 ± 4.76
BADGE.2HCl	0.9999	0.9997	0.031	0.103	12.84	5.93	86.61 ± 11.09	79.20 ± 4.69
BFDGE	0.9999	0.9991	0.020	0.066	12.35	5.56	77.33 ± 8.88	85.30 ± 4.74
BFDGE.HCl*	0.9999	0.9999	0.027	0.136	6.50	17.10	108.74 ± 7.07	82.30 ± 14.06
BFDGE.2HCl	0.9997	0.9950	0.030	0.100	13.85	5.53	83.50 ± 11.56	78.00 ± 4.31

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

Table 5.2. Summerized contamination data of oil-based canned food from Thai market

Food type	Can type	Coating		Number of sample (level of contamination, mg/kg)				
		Lid	Body	< 0.1	0.1-0.5	0.5-1.0	1.0-2.0	> 2.0
Tuna in oil	2p/eo	Organosol	Organosol	-	3	1	-	-
Tuna in oil	2p/eo	Organosol	epoxy	-	1	2	-	-
Tuna in mayonnaise	2p/eo	Organosol	epoxy	-	-	2	-	-
Fried food	2p/eo	Organosol	Organosol	1	-	2	2	3
Fried food	2p/eo	Organosol	epoxy	-	2	1	-	-

2p/eo = 2 piece can/easy open can

RECOMMENDATIONS

The HPLC method developed in this study used ODS Hypersil C18 column (150 x 4.0 mm, 5 μm) that separates all 6 compounds to baseline resolutions. However, it can not separate individual isomer of the chlorohydroxy derivatives of BFDGE namely BFDGE.HCl and BFDGE.2HCl that exists in 3 isomers each. Because each isomer may have slightly different detector responses, further work should attempt to separate these isomers apart to baseline resolution on new column such as a longer one or using different mobile phase system and condition. The linearity and sensitivity of the new method should be re-evaluated.

Because some food matrixes may affect the method sensitivity, matrix calibration curves should be prepared for each isomer and test for linearity and sensitivity as well.

The liquid-liquid extraction method used in this work is complicated and involved many steps leading to accumulation of errors. However, our data proved that consistent results are possible if the analyst is skilled and well trained. However, for routine analysis of better precision and accuracy, better sample preparation should be investigated such as solid phase extraction (SPE).

The qualitative analysis used in this work (Beilstein's test) to screen types of can coating has low sensitivity and selectivity. A more sensitive and selective method such as FTIR could be used to identify types of polymer.