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Simultaneous determination of bisphenol-A-diglycidyl ether, bisphenol-F-diglycidyl ether, and their derivatives in oil-in-water and aqueous-based canned foods by high-performance liquid chromatography with fluorescence detection

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Abstract

A gradient reversed-phase liquid chromatography with fluorescence detection method for simultaneous identification and quantification of bisphenol-A-diglycidyl ether (BADGE), bisphenol-F-diglycidyl ether (BFDGE), and their 10 derivatives in food matrixes was developed and validated for the analysis of oil-in-water- and aqueous-based foodstuffs. The method linearity range 0.016–10 ppm which are hundred-fold below and tenfold above the EU restriction at 1 ppm (mg/kg). The method detection limits range 0.72–4.20 ppb and the method quantitation limits range 2.40–14.85 ppb, respectively. The validation data indicate excellent precision, acceptable recovery, and good robustness, all supporting a good potential to further develop the method as a standard method for the determination of migrations from interior can coatings into foodstuffs.
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Keywords: Validation; Canned food; BADGE; BFDGE; HPLC-FD; Migration; Epoxy resin; Organosol resin

1. Introduction

At present, the predominant protective coatings for the interior of metal food cans are epoxy-resin based. Epoxy resins are commonly synthesized from bisphenol-A-diglycidyl ether (BADGE). Usually, epoxy resins require further crosslinking for optimum performance leading to the superior quality of many epoxy-based coatings. Other popular coatings for the interior of metal cans are PVC organosol resins. Additives such as BADGE and bisphenol-F-diglycidyl ether (BFDGE) are required during the synthesis of organosol resins in order to prevent thermal degradation of the polymer by surplus hydrochloric acid apparently formed during the curing process.

Both coating types contain residual BADGE and BFDGE monomers that can migrate into food upon contact. As previously mentioned, BADGE and BFDGE are used to remove hydrochloric acid in organosol resins. This can result in the formation of many chlorinated compounds such as BADGE·HCl, BADGE·2HCl, BFDGE·HCl, and BFDGE·2HCl. The remaining epoxy groups can be hydrolyzed via contact with aqueous and acidic foods resulting in the formation of mono- and di-hydrolyzed products such as BADGE·H_2O, BADGE·2H_2O, BADGE·HCl·H_2O, BFDGE·H_2O, BFDGE·2H_2O, and BFDGE·HCl·H_2O. Structures of BADGE, BFDGE, and their reaction products are illustrated in Fig. 1.

The key reason for studying food contamination caused by chemicals leached from packaging is the potential adverse health effects to the consumer when exposed to these compounds. Recently, epoxy compounds were reported as potential alkylating agents with possible specific cytotoxic actions in tissues affecting rates of cell division. The toxicity depends mainly upon fractional concentration of unreacted epoxy groups. Moreover, the chlorohydroxy derivatives

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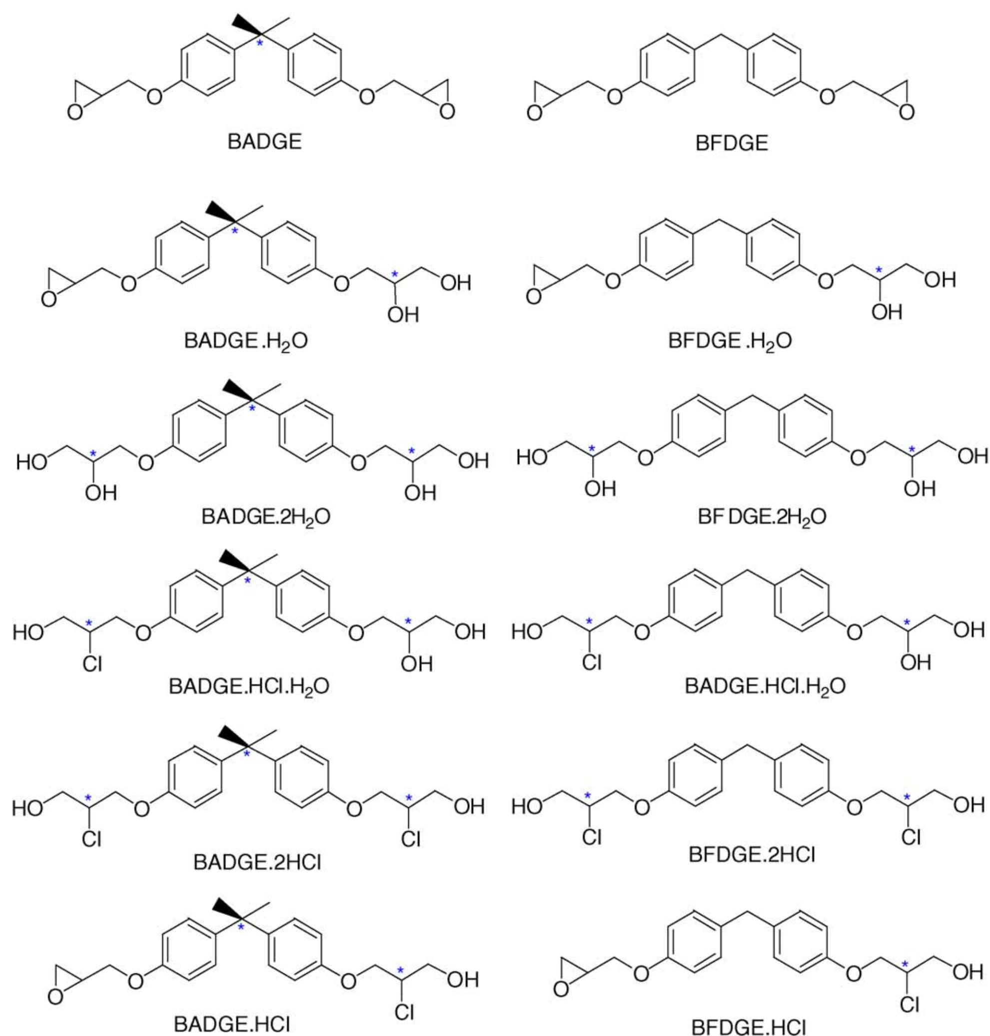

Fig. 1. Structures of BADGE, BFDGE and their derivatives. (*) Denotes chiral carbon designating possibility of many isomers.

are considered potentially toxic because of their structural analogy to the genotoxic monochloropropanediol and other chloropropanols [1].

Coatings that are in contact with food must compliance to a multitude of regulations on materials and articles intended to come into contact with foodstuffs. However, these rules are not equally applied throughout the world. The most important legislation in use today is issued by the European Union (EU) that limits the total migration levels of BADGE, BFDGE, and their hydroxy, and chlorohydroxy derivatives to 1 mg/kg in foodstuffs [2].

Several methods were described in the literatures for the analysis of BADGE, BFDGE, and their reaction products in food simulants by chromatographic techniques [3–5]. Most published methods used reversed-phase high-performance liquid chromatography (RPLC) on C18 or C8 silica gels with mixture of acetonitrile/water as mobile phase. Fluorescence detection (FD) was typically selected [3–6]. Mass spectrometry coupled with RPLC [7–9] and gas chromatography–mass spectrometry (GC–MS) [10] methods were also reported. RPLC–MS–MS was proposed for the analysis of BADGE in food after freeze-drying of food products [11]. When using RPLC determination, the fat or oil in samples must be removed prior to injection. Where as, normal phase HPLC (NPLC) allows for direct injection of oily samples with no sample preparation requirement [12–14].

Biedermann and co-workers presented a combination of three methods for a complete analysis of BADGE, BFDGE, and their derivatives in canned foods. Initially, RPLC-FD was utilized. If positive results were obtained, the results were further confirmed by acetylation and analysis using NPLC-FD. If both results were inconsistent, then NPLC fractions were collected and analyzed by GC–MS [15]. Adequate separations were achieved with ethanol as organic modifier, but resulted in co-elution of some derivatives. Lintschinger and Rauter [16] used a binary systems consisting of methanol/water and acetonitrile/water to separate BADGE, BFDGE, and their derivatives. However, their binary method could not sufficiently resolute BADGE·H_2O and BADGE·HCl·H_2O and a second isocratic

method of methanol/water was required to complete the separation.

To date, no analytical method capable of simultaneous determination of BADGE and BFDGE, along with the 10 regulated chlorinated and hydrolysis products as listed in Fig. 1 exists. Because of technical limitations, not all standard substances are commercially available. It was necessary for Lintschinger et al. to prepare a mixture of substances in order to simultaneously achieve identification of these compounds [16].

This study describes the development and validation of a straight forward one-shot analytical procedure capable of simultaneous analysis of all monomeric BADGE and BFDGE derivatives in oil-in-water and aqueous-based foods using RPLC. This method allows for simple monitoring of the migrations of contaminants from interior can coatings under surveillance by the EU.

2. Experimental

2.1. Materials and reagents

The chemicals used were all analytical grade. The extraction solvent, *tert*-butyl methyl ether (MTBE), was purchased by Fluka Chemika (Buchs, Switzerland). Di-sodium hydrogen phosphate dihydrate and sodium chloride were obtained from E. Merck (Darmstadt, Germany). Acetonitrile and anhydrous sodium sulfate were purchased from J.T. Baker Chemical Company (Deventer, Holland). Eluents for HPLC and standard solutions were prepared with high-purity water obtained using a Milli-Q Water system (Millipore, Billerica, Massachusetts, USA). Methanol of HPLC grade was supplied by J.T. Baker Chemical Company. Liquid chromatography solvents were filtered with 0.45 μm teflon and nylon membranes prior used. Purified samples were filtered through 13 mm, 0.45 μm nylon filters (Agilent Technologies, Palo Alto, California, USA).

2.2. Standards

Bisphenol-A-diglycidyl ether (BADGE, CAS no. 1675-54-3), bisphenol-A-(3-chloro-2-hydroxypropyl) glycidyl ether (BADGE·HCl, CAS no. 13836-48-1), bisphenol-A-bis(3-chloro-2-hydroxypropyl) ether (BADGE·2HCl, CAS no. 4809-35-2), bisphenol-A-(2,3-dihydroxypropyl) glycidyl ether (BADGE·H_2O, CAS no. 76002-91-0), bisphenol-A-bis(2,3-dihydroxypropyl) ether (BADGE·$2H_2O$, CAS no. 5581-32-8), bisphenol-A-(3-chloro-2-hydroxypropyl) (2,3-dihydroxypropyl) ether (BADGE·H_2O·HCl, CAS no. 227947-06-0), bisphenol-F-diglycidyl ether (BFDGE, CAS no. 2095-03-6), bisphenol-F-bis(3-chloro-2-hydroxypropyl) ether (BFDGE·2HCl), and bisphenol-F-bis(2,3-dihydroxypropyl) ether (BFDGE·$2H_2O$, CAS no. 72406-26-9) with percent purity of 95–99% were supplied by Fluka Chemika (Buchs, Switzerland). The non-symmetric derivatives of BFDGE: bisphenol-F-glycidyl-(2,3-dihydroxypropyl) ether (BFDGE·H_2O), bisphenol-F-glycidyl-(3-chloro-2-hydroxypropyl) ether (BFDGE·HCl), and bisphenol-F-(3-chloro-2-hydroxypropyl)-(2,3-dihydroxypropyl) ether (BFDGE·HCl·H_2O) were not commercially available and were prepared and purified in-house following a procedure described by Biedermann et al. [15].

2.3. Instrumentation and chromatographic conditions

All chromatographic analyses were performed on a Hewlett-Packard 1100 series equipped with an automatic degasser, binary pump, autosampler, column thermostat, diode-array detector, and fluorescence detector (Agilent Technologies).

The chromatographic separation was accomplished with gradient elution on an ODS Hypersil C18, 5 μm, 250 mm × 4.0 mm analytical column (Agilent Technologies). The gradient of water (A) and methanol (B) mobile phase was pumped at a flow rate of 0.7 mL/min. The gradient profile was: at 0 min–40% B, 1 min–50% B, 15 min–55% B, 38 min–70% B, 45–55 min–90% B. The fluorescence detector was set at excitation and emission wavelengths of 227 and 313 nm, respectively.

2.4. Food matrixes preparations

Because only trace amount of interested compounds are presented in complex food matrix that can heavily mask or interfere with the result, it is very important to develop the analytical method using clean food matrix. In our case, we chose uncanned pre-cooked tuna as the representative of oil-in-water based canned foods because of its global availability and popularity. Canned lychee in syrup was chosen to represent aqueous-based foods (mainly fruits and vegetables) because it is very popular and widely available in Thailand and Asia Pacific Region. Because the fruit is seasonal, uncanned pre-cooked samples were difficult to obtain. The canned cooked fruit was chosen due to its wide availability in the local market at a time and because it could represent blank matrix when existing contamination was accounted for and was subtracted from any determine value.

Uncanned pre-cooked tuna sample was homogenized and a 10 g ± 0.01 g aliquot of the homogenate was extracted with 15.0 mL of MTBE. Allow the layers to separate for about 5 min and the whole MTBE layer was transferred to a 50 mL round-bottom flask. The extraction process was repeated once, fractions combined, and 20.0 mL of MTBE layer was removed and evaporated by rotary evaporation to dryness at 34 °C. The residue was further extracted with 5.0 mL methanol thrice. The methanol fractions were then combined in a 50 mL round-bottom flask and the solvent was removed by rotary evaporation to dryness at 34 °C. The dried residue was re-dissolved to 1.00 mL with methanol and the solution was filtered through 0.45 μm filter

prior to HPLC analysis. Spiked samples were prepared by adding standard mixture solution to 10 g ± 0.01 g homogenized sample and extraction by means of the above procedure.

The whole can of commercially available canned lychee in syrup was homogenized and a 10 g ± 0.01 g aliquot of the homogenate was adjusted to pH 7.0 by 1.0 M $Na_2HPO_4 \cdot 2H_2O$. Extraction was achieved by introducing 15.0 mL of acetonitrile and 10 g NaCl. Acetonitrile layer was transferred into a 50 mL round-bottom flask. The extraction process was repeated once and the extract fractions were combined. Solvent was removed by rotary evaporation to dryness at 34 °C. The dried residue was re-dissolved to 1.00 mL with methanol. The solution was filtered through 0.45 μm filter prior to HPLC analysis. Spiked samples were prepared by adding standard mixture solution to 10 g ± 0.01 g homogenized sample and extraction by means of the above procedure.

2.5. Preparation of calibration curves and linearity

Individual stock solutions of 12 compounds containing 1000 ppm of each solute were prepared in acetonitrile. The working solutions were made by appropriately diluted the stock solutions with methanol. In order to construct the calibration curves and determine the linearity of the responses, sets of working standard solutions were formulated at several standard concentrations as described in Table 1.

3. Results and discussion

3.1. Selectivity

The selectivity of the HPLC method was evaluated by matching peaks retention times (t_R) with the values of the corresponding standards. Good resolution values (R_s) of all critical pairs were obtained (R_s = 1.5–17.3) supporting our method as suitable for quantitative analysis. Fig. 2 illustrates the chromatogram of 12-standard mixture. Some compounds showed multiple peaks of respective isomers. For example, BFDGE·H_2O and BFDGE·HCl·H_2O appeared as four peaks (one peak of *o*,*o*′-, *p*,*p*′-, and two peaks of *o*,*p*-isomers). BFDGE emerged as three peaks (*o*,*o*′-, *o*,*p*-, and *p*,*p*′-isomers). BFDGE·HCl appeared as two peaks (four isomers co-eluted). The developed HPLC method was not only separate all 12 standards but was also capable to separate isomers of these compounds.

3.2. Calibration curves and linearity

Because it was discovered that the isomeric distribution of some isomers in matrix differed their presence than in pure solvent, separate calibration curves were prepared for each individual isomer. Moreover, the calibration results implied that the detector responses were equal for all isomers. This assumption was supported by Biedermann et al. [15]. The calibration curves were constructed by plotting peak area versus analyte concentration assuming equal detector response for

Table 1
Chromatographic and linear regression parameters of BADGE, BFDGE, and their derivatives, range of 0.0160–1.00 ppm (7 points, duplicate analyses, standard solutions)

Compounds	t_R (min)	Slope (10^6 LU cm^3 g^{-1})	*y*-intercept (LU)	R^2
BFDGE·$2H_2O$	10.20 ± 0.04	147.53	0.1717	0.9976
BADGE·$2H_2O$	17.17 ± 0.08	149.81	1.2057	0.9958
BFDGE·H_2O	18.15 ± 0.08	109.80	−0.7857	0.9983
	20.03 ± 0.09	110.39	−0.4637	0.9985
	20.57 ± 0.10	110.23	−0.3725	0.9983
	21.89 ± 0.07	108.94	−0.1043	0.9980
BFDGE·HCl·H_2O	22.62 ± 0.09	75.81	−0.6318	0.9910
	23.41 ± 0.06	86.86	−0.2344	0.9940
	25.26 ± 0.08	72.74	0.1839	0.9907
	25.88 ± 0.09	71.72	0.1388	0.9889
BADGE·H_2O	27.53 ± 0.08	142.49	0.9192	0.9983
BFDGE	28.29 ± 0.07	150.06	0.3232	0.9983
	29.54 ± 0.08	149.99	0.3842	0.9983
	30.25 ± 0.11	149.52	0.0884	0.9983
BADGE·HCl·H_2O	30.97 ± 0.07	138.06	4.9862	0.9975
BFDGE·HCl	31.78 ± 0.08	124.82	−2.0662	0.9959
	33.05 ± 0.11	124.85	−0.4801	0.9958
BFDGE·2HCl	35.04 ± 0.24	129.46	2.4109	0.9963
BADGE	36.11 ± 0.07	178.57	0.5848	0.9977
BADGE·HCl	38.73 ± 0.07	112.34	0.9607	0.9985
BADGE·2HCl	40.85 ± 0.07	137.44	2.233	0.9978

Refer to HPLC condition in Section 2.3, preparation of standard solutions in Section 2.5.

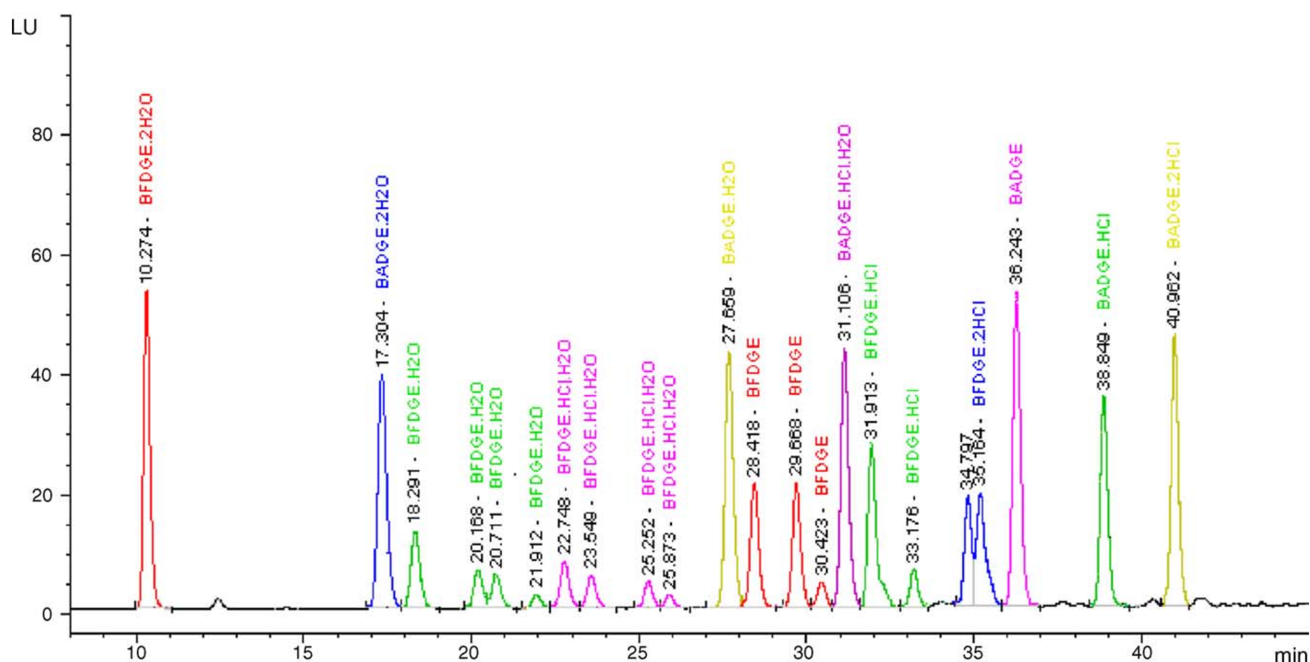

Fig. 2. The chromatogram of a standard mixture using HPLC conditions listed in Section 2.3.

all isomers. Linear regression parameters are summarized in Table 1.

Excellent linear regression coefficients (>0.9900) were obtained for all compounds covering a large concentration range. Since excellent precision was observed over a range of concentration levels, we are confident that the developed analytical procedure can accurately determine the presence of analytes hundred-fold below (0.01 ppm) and tenfold above (10.0 ppm) the EU Commission Directive 2002/16/EC that sets the limit at 1.0 mg/kg (ppm) [2].

3.3. Matrix effect

To test if the detector responses of pure standards were masked or interfered by the matrixes, individual matrix calibration curves were constructed for all 12 compounds. The detector responses of standards prepared in pure methanol were compared to the values obtained from standards prepared in selected matrixes using two tailed paired *t*-test at 95% confidence level. The matrix effects were discovered on BFDGE·2H_2O, BFDGE·H_2O, BFDGE, and BADGE·2HCl. Thus, matrix calibration curves were chosen in preference to methanol calibration curves.

3.4. The effect of acidity in aqueous-based samples

The epoxide ring can readily be opened followed by reacting with the components of acidic foods forming many new derivatives. The chromatogram in Fig. 3A shows a situation where unusually low recovery of BFDGE·H_2O, BADGE·H_2O, BFDGE, BFDGE·HCl, BADGE, and BADGE·HCl were observed in spiked lychee in syrup matrix. Adjusting the food pH to 7.0 by 1.0 M $Na_2HPO_4 \cdot 2H_2O$ prior to the standard addition step lowered the activity of the epoxide rings. As a result, improved percent recovery of the extraction process was obtained as illustrated in Fig. 3B.

3.5. Limit of detection and limit of quantitation

The detection limit (LOD) and quantitation limit (LOQ) are defined as the amount of analyte in standard solutions that yields an instrumental signal significantly different from the blank or background signal which equals to 3 and 10, respectively. Table 2 summarizes LOD and LOQ values of individual compounds and clearly indicates that the analytical method has excellent sensitivity.

3.6. Method detection limit and method quantitation limit

Because the sample enrichment factor is 6.67-fold (see Section 2.4), the LOD and LOQ values should also be 6.67-fold lower. It is a common practice for trace analysis to report method detection limit (MDL) and method quantitation limit (MQL) values instead of LOD and LOQ values. The rationale behind the concept is to incorporate the enrichment factor altogether. MDL and MQL are useful because they allow accurate interpretation of data to points many fold below the equipment detection limit (LOD and LOQ).

3.7. Method precision

The precision of the proposed method is reported as intra-assay and intermediate precision. Intra-assay precision

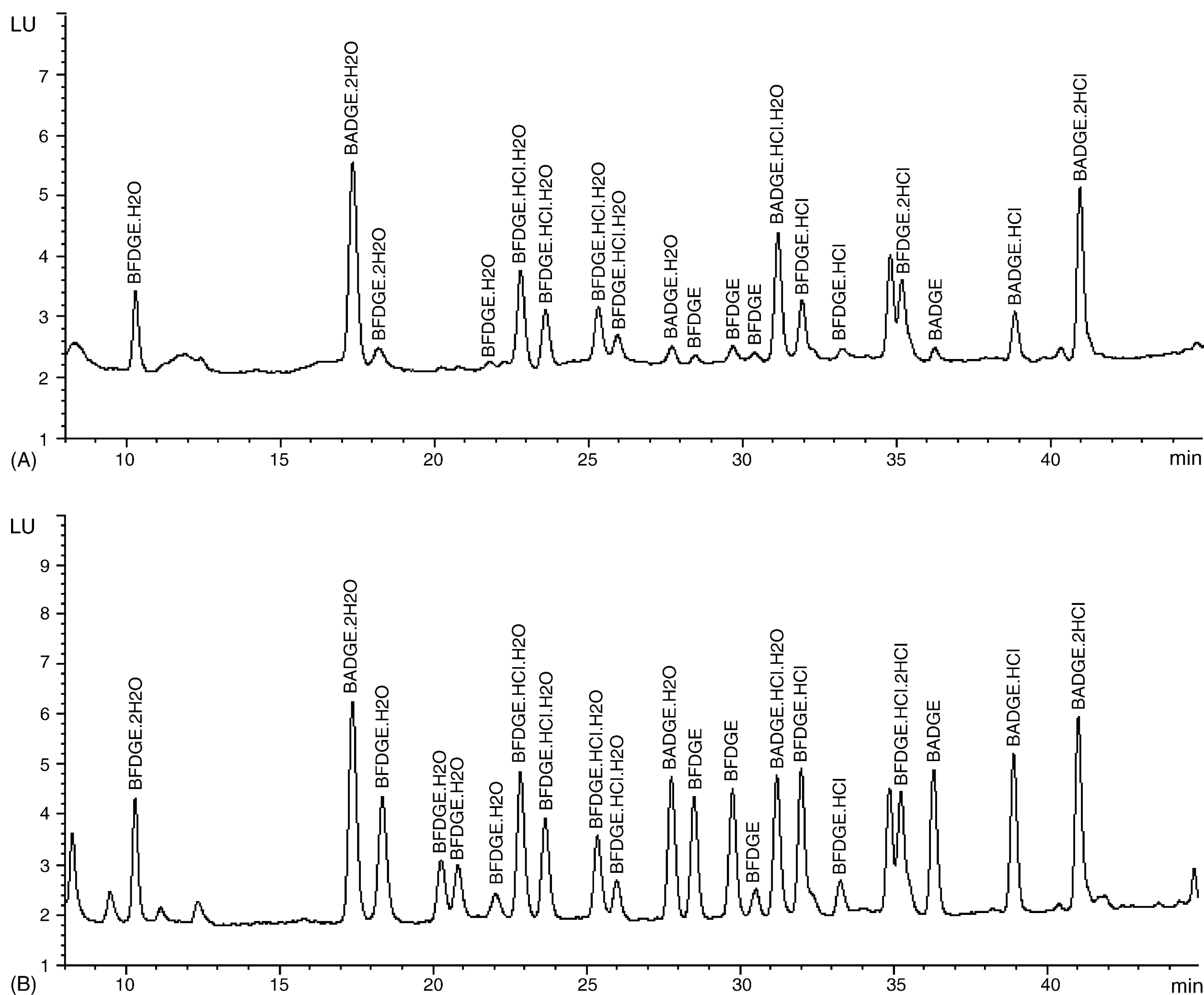

Fig. 3. Chromatogram of extracted lychee in syrup spiked with standard solution (A) no pH adjustment (B) extraction at pH 7.0.

and intermediate precision can be determined from relative standard deviations (R.S.D.) resulting from the analysis of spiked standard solutions at MQL and five-fold MQL, respectively. Intra-assay precision was determined by extractions at each level six times on the same day. The study was repeated on three successive days to determine the intermediate precision. Method precision studies were conducted on both precooked tuna and canned lychee in syrup matrixes.

3.8. Method accuracy

Method accuracy was determined by the mean recovery at two spiking levels for tuna matrix. However, because clean precanned lychee in syrup was unavailable at a time, commercially canned products of the same lots were used. Since contaminations were detected in this matrix, study at MQL level was not possible and only data of fivefold MQL were reported here. Values of intra-assay precision and intermediate precision are summarized in Tables 3 and 4. %R.S.D. values obtained for both types of samples in our study exceeded the AOAC standard that recommends better than 15% R.S.D. value for an analysis performs at ppb-level.

The mean recoveries were in the range of 70–110% for all compounds except for BADGE·2H_2O and BFDGE·2H_2O that exhibited much lower values. Because these hydrolysis products contained two hydroxyl groups per structure, they are more polar and become less soluble in MTBE. Therefore, MTBE can not efficiently extract both compounds from the food matrix results in lower recovery value. Our observation is in agreement with Lintschinger and Rauter [16] that also reported lower than expected recovery values for both BADGE·2H_2O and BFDGE·2H_2O. Then again, the presence of both derivatives should not be of great concern because of their negligible toxicity [2].

3.9. Stability of BADGE, BFDGE, and their derivatives in tuna matrix

The stabilities of analytes in spiked standard solutions added to tuna matrix were determined. A sample was

Table 2
LOD and LOQ values of standard solutions, and MDL and MQL values of matrix calibration curves for BADGE, BFDGE, and their derivatives

Compounds	Slope[a] (10^6 LU cm^3 g^{-1})	LOD[b] (ppb)	LOQ[b] (ppb)	MDL[a] (ppb)	MQL[a] (ppb)
BFDGE·2H_2O	178.75	5	21	0.94	3.15
BADGE·2H_2O	176.96	5	28	1.26	4.20
BFDGE·H_2O	110.62	14	49	2.10	7.35
	111.26	17	59	2.55	8.85
	111.15	14	50	2.10	7.50
	110.87	10	30	1.50	4.50
BFDGE·HCl·H_2O	81.13	28	99	4.20	14.85
	81.48	20	72	3.00	10.80
	77.79	20	61	3.00	9.15
	94.41	15	44	2.25	6.60
BADGE·H_2O	152.93	7	22	0.97	3.22
BFDGE	158.18	6	18	0.90	2.70
	160.48	7	21	1.05	3.15
	158.16	6	18	0.90	2.70
BADGE·HCl·H_2O	146.00	4	16	0.72	2.40
BFDGE·HCl	137.28	11	36	1.65	5.40
	131.81	8	30	1.20	4.50
BFDGE·2HCl	139.90	18	53	2.39	7.95
BADGE	202.23	5	22	0.97	3.22
BADGE·HCl	124.72	9	34	1.53	5.10
BADGE·2HCl	155.41	5	22	0.99	3.30

[a] Values obtained from the calibration curves of matrix solutions.
[b] Values obtained from the calibration curves of standard solutions.

prepared and stored under the same conditions (refrigerated) for 42 days (see Table 5). Samples were pulled and analyzed at eight time points and the data were compared using control chart plot. It was found that detector responses of all compounds except for BADGE·2H_2O, BADGE·HCl·H_2O, BADGE·HCl, and BADGE·2HCl reduced significantly at longer storage time. Therefore, we recommend that sample be analyzed within 3 days of extraction. Our observation is in agreement with Richard et al. [17] and Berger et al. [8].

3.10. Method robustness

Robustness is the capacity of a method to remain unaffected by small variations in method parameters. Robustness of the proposed method was investigated by applying minor changes to the sample preparation step (evaporation temperature and numbers of extraction). The data were compared with data obtained from normal conditions using two-tailed paired *t*-test at 95% confidence level. No significant effect was observed when changing evaporation temperature from 34 to

Table 3
The method precision expressed as %R.S.D. of (i) spiked tuna as oil-in-water-based samples, and (ii) lychee in syrup matrix as aqueous-based samples

Compounds	Oil-in-water based sample (%R.S.D.)								Aqueous-based sample (%R.S.D.)			
	MQL				Five-fold MQL				Five-fold MQL			
	Day 1[a]	Day 2[a]	Day 3[a]	Overall[b]	Day 1[a]	Day 2[a]	Day 3[a]	Overall[b]	Day 1[a]	Day 2[a]	Day 3[a]	Overall[b]
BFDGE·2H_2O	5.75	4.31	2.36	1.29	5.76	5.90	7.19	2.00	8.08	7.86	6.66	4.49
BADGE·2H_2O	4.24	4.85	8.15	8.90	2.70	3.61	6.32	3.64	10.37	7.98	12.93	8.57
BFDGE·H_2O	3.92	4.33	3.28	6.11	4.19	4.22	3.80	1.75	4.83	10.88	5.20	10.67
BFDGE·HCl·H_2O	4.18	2.56	2.69	4.14	3.70	4.19	7.61	3.00	10.65	6.45	3.46	4.62
BADGE·H_2O	4.31	3.66	2.65	0.98	3.91	4.67	2.96	1.93	3.76	7.47	5.69	10.09
BFDGE	7.91	5.11	3.59	1.55	7.83	8.38	5.31	2.45	8.75	10.59	11.47	6.55
BADGE·HCl·H_2O	11.20	4.41	9.64	6.48	5.45	5.55	5.55	7.17	7.48	6.97	6.28	2.41
BFDGE·HCl	4.55	9.09	2.09	0.44	2.70	4.90	3.64	1.63	4.34	12.44	12.70	13.84
BFDGE·2HCl	6.08	11.70	7.87	6.86	2.27	3.38	6.63	1.10	5.64	7.19	5.57	4.59
BADGE	5.55	4.75	3.89	3.21	4.97	5.10	3.99	1.72	7.43	9.43	7.12	4.19
BADGE·HCl	11.04	8.53	7.09	3.06	2.91	4.19	4.35	2.75	6.75	6.41	10.20	10.80
BADGE·2HCl	9.20	6.47	4.04	8.00	6.14	2.75	3.12	3.54	15.09	9.12	7.26	6.02

[a] Intra-assay precision of data analyzed within the same day ($n = 6$).
[b] Intermediate precision of data analyzed on different day ($n = 3$).

Table 4
The method accuracy expressed as % recovery ($n = 3$) of (i) spiked tuna as oil-in-water-based samples, and (ii) lychee in syrup matrix as aqueous-based samples

Compounds	Oil-in-water-based sample		Aqueous-based sample
	MQL level	Five-fold MQL level	Five-fold MQL level
BFDGE·2H_2O	48.96 ± 0.63	51.63 ± 1.03	100.29 ± 4.51
BADGE·2H_2O	74.15 ± 6.60	68.93 ± 2.51	100.02 ± 8.57
BFDGE·H_2O	98.70 ± 6.03	85.03 ± 1.49	83.38 ± 8.90
BFDGE·HCl·H_2O	103.38 ± 4.28	98.97 ± 2.96	108.87 ± 5.02
BADGE·H_2O	96.56 ± 0.95	88.45 ± 1.71	78.61 ± 7.93
BFDGE	96.82 ± 1.50	84.96 ± 2.08	74.91 ± 4.91
BADGE·HCl·H_2O	98.42 ± 6.38	105.52 ± 7.56	99.35 ± 2.40
BFDGE·HCl	105.19 ± 0.46	104.49 ± 1.70	88.90 ± 12.30
BFDGE·2HCl	95.13 ± 6.52	104.24 ± 1.14	101.96 ± 4.68
BADGE	100.26 ± 3.22	88.81 ± 1.53	76.37 ± 3.20
BADGE·HCl	69.64 ± 2.13	103.22 ± 2.84	88.75 ± 9.58
BADGE·2HCl	109.91 ± 8.80	103.58 ± 3.67	97.69 ± 5.88

Table 5
Stability of BADGE, BFDGE, and their derivatives spiked in tuna matrix and stored from 0 to 42 days

Compounds	% Recovery calculated compared by linear equation each day							
	0	4	12	15	19	26	31	42
BFDGE·2H_2O	50.55	49.92	43.97	42.06	37.31	35.45	33.09	35.64
BADGE·2H_2O	74.74	72.22	69.43	63.46	64.65	64.00	65.65	63.51
BFDGE·H_2O	88.70	85.50	86.85	88.44	88.89	85.01	84.59	83.91
BFDGE·HCl·H_2O	104.96	101.35	98.11	96.05	94.61	93.77	90.73	87.94
BADGE·H_2O	91.60	89.49	83.59	83.98	81.42	81.90	80.53	86.83
BFDGE	88.91	86.47	85.17	87.72	80.57	75.96	72.79	76.25
BADGE·HCl·H_2O	120.28	118.33	111.90	109.94	111.65	103.83	102.74	104.15
BFDGE·HCl	104.22	100.14	102.28	97.05	97.54	100.87	104.66	103.95
BFDGE·2HCl	103.66	107.41	101.77	95.02	89.68	88.25	85.03	84.04
BADGE	90.47	92.56	87.98	87.16	84.75	78.59	76.08	78.20
BADGE·HCl	101.07	100.65	97.66	98.28	101.61	97.47	98.15	106.50
BADGE·2HCl	110.20	106.59	100.22	100.17	99.87	97.38	95.76	97.80

40 °C. However, changes in numbers of extraction by MTBE caused major deviation. Therefore, all sample preparations were extracted twice with MTBE to improve the efficiency of the analysis.

4. Conclusions

We have described the development and validation procedures for an HPLC method for simultaneous determination of BADGE, BFDGE, and their derivatives in oil-in-water and aqueous-based foods using RPLC. The method is able to separate all 12 compounds currently regulated by the EU. Sample preparation steps can easily be performed with excellent precision and accuracy. Validation statistics show that this method is reliable and has great potential to develop into a standard method for the determination of migration from interior can coatings into foodstuffs. The method was tested and successfully employed in the survey of contamination level caused by can coatings in canned foods available in Thailand. The survey data will be reported in the near future.

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