Two RP-HPLC Sensitive Methods To Quantify and Identify Bisphenol A Diglycidyl Ether and Its Hydrolysis Products. 1. European Union Aqueous Food Simulants

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Two RP-HPLC with fluorescence detection alternative methods have been proposed to quantify and identify bisphenol A diglycidyl ether (BADGE), a monomer of epoxy resins used as a coating for food packaging materials, and its hydrolysis products (HPs) in three aqueous food liquid simulants: 15% (v/v) ethanol, 3% (w/v) acetic acid, and distilled water. Both isocratic and gradient chromatographic methods were developed to determine this monomer at the specific migration limit (SML) level (restriction proposed by European Union legislation) and also the two HPs in aqueous EEC food simulants. All calibration lines had correlation coefficients greater than 0.997 and detection limits of less than 2 μg of BADGE/L. Standard deviations (in percent) obtained in the precision calculation were less than 3% for BADGE. Furthermore, both methods were applied to analyze these compounds in three types of epoxy can coatings that contact food.

Keywords: *Bisphenol A diglycidyl ether; hydrolysis products; epoxy resins; aqueous food simulants; food can coatings; HPLC*

INTRODUCTION

Oxirane {2,2′-[(1-methylethylidene)bis(4,1-phenyleneoxymethylene)]bis-, CAS Component Name (CAS Registry Number, 1675-54-3); also known as bisphenol A diglycidyl ether (BADGE), common name; 4,4′-isopropylidenediphenol-epichlorohydrin epoxy resin, Federal Regulation Code (U.S. legislation); 2,2-bis(4-hydroxyphenyl)propane-bis(2,3-epoxypropyl)ether in European Directives (EU legislation} is the basic monomer in numerous epoxy resins destined for use as articles and/or components of articles intended for use in manufacturing, packaging, transporting, or holding food, such as laminate adhesives used for food contact but used mainly in paints and lacquers for coating foodstuff containers ranging from small cans for food to large metal or concrete vats or tanks for water, fruit juices, wine, oil, or other liquids. The purpose of these coatings is to reduce interaction between the container and the foodstuff it contains, but if they are badly formulated they can be a source of serious abiotic contamination due to the migration of chemicals from the coating to the food.

Updated toxicology information on BADGE is collected in the Registry of Toxic Effects of Chemical Substances (RTECS; National Institute for Occupational Safety and Health, 1996) and in Material Safety Data Sheets from Occupational Health Services, Inc. (MSDS-OHS) and shows the potential toxicity of this substance, classified as tumorigen, mutagen, and primary irritant. It does not have carcinogenic status [Occupational Safety and Health Administration (OSHA), National Toxicology Program (NTP), and International Agency on Research Cancer (IARC)], but there is limited animal evidence (IARC, Group 3), that related compounds have shown chromosomal aberrations (Rosenkranz *et al.*, 1990) and carcinogenic activities (Haseman and Clark, 1990; Zeiger *et al.*, 1990). Recently, studies of the estrogenic effect of bisphenol A (basic monomer of BADGE; Olea *et al.*, 1996) and food extracts obtained from lacquered cans containing BADGE have been published (Brotons *et al.*, 1995). Actually, in the EU, the SCF (Scientific Committee for Food) includes BADGE in List 4A (CEC, 1993), which contains substances for which an ADI (admissible daily intake) or TDI (tolerable daily intake) could not be established but which could be used if the substance migrating into food is not detectable by an agreed sensitive method. The European Community Commission Directive of February 23, 1990 (CEC, 1990), establishes that BADGE may be used for the manufacture of plastic materials and articles subject to the restriction $Q_m = 1$ mg/kg, where Q_m is the maximum permitted quantity of the residual substance in the material or article, or to an SML (specific migration limit in food or food simulant) that is not detectable (detection limit = 0.020 mg/kg; analytical tolerance included).

We discussed various aspects of the determination of BADGE in previous papers (Paseiro Losada *et al.*, 1991a,b, 1992, 1993; Simal Gándara *et al.*, 1992, 1993a,b). We proposed an HPLC method with fluorescence detection ($\lambda_{ex} = 275$ nm, $\lambda_{em} = 300$ nm) and a calibration range of 20−800 μg/L. Since the SML of BADGE is 20 μg/L, we changed the fluorescence conditions to $\lambda_{ex} = 225$ nm and $\lambda_{em} = 305$ nm to improve the sensitivity of the proposed method. The excitation spectrum clearly shows greater sensitivity at 225 nm than at 275 nm when emission is recorded at 305 nm, while the emission spectrum for excitation at 225 nm peaks at 305 nm.

In addition to the changes in detection conditions (listed above), we have also modified the chromatography protocol to minimize overall analysis time. We have

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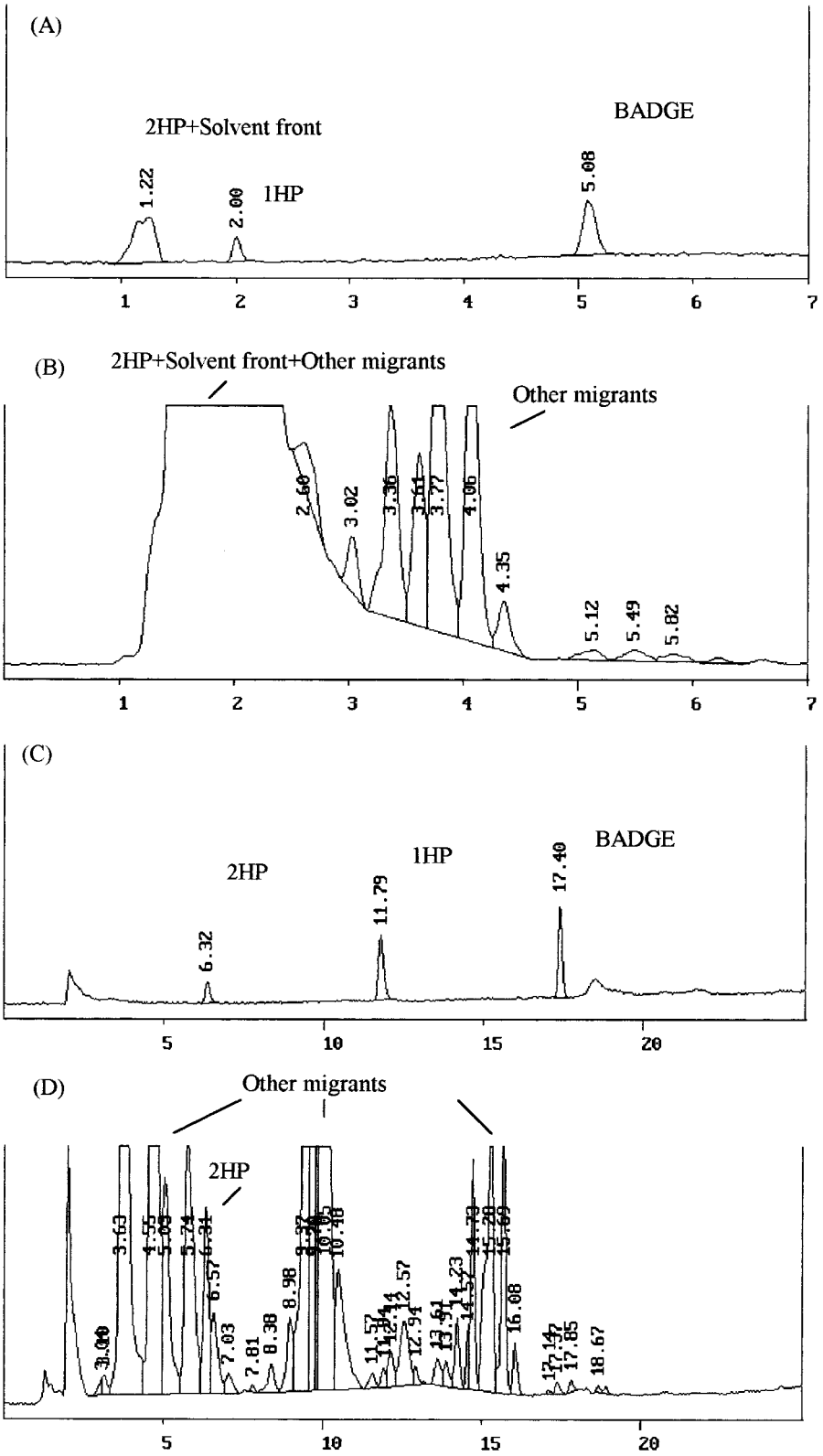


Figure 1. Distilled water chromatograms. (A and C) Solution with 40 μg of BADGE/L after 1.5 days at 40 °C, isocratic and gradient methods, respectively. (B and D) Can coating type 2 after test a (10 days at 40 °C), isocratic and gradient methods, respectively.

developed two methods designed for different sets of operational priorities: the gradient method and the isocratic method. The gradient method was optimized to separate BADGE and its hydrolysis products (HPs) from other migrants. The isocratic method was optimized to separate BADGE from its HPs and other migrants as well, but the HPs themselves are not well separated. Figure 1 shows chromatograms applying both the gradient and isocratic methods.

MATERIALS AND METHODS

Chemicals. BADGE was obtained as Epikote 828 from Shell and purified (>99%) by Gairesa Industry as described by Paz Abuín *et al.* (1990).

Solvents. Water was demineralized Milli-Q quality (Millipore Corporation). Acetic acid was 3% (w/v) (analytical quality). Ethanol was 15% (v/v) (analytical quality). Acetonitrile was HPLC supragradient grade (Scharlau Ref. Ac 331), and tetrahydrofuran was HPLC grade (Scharlau Ref. Te 225). *Note*! Tetrahydrofuran is a potent irritant and must be handled carefully.

Solutions. Stock solution of BADGE standard (1.0 mg/mL) was made in tetrahydrofuran. A 100 mg amount of BADGE was weighed to the nearest 0.1 mg into a 100 mL volumetric flask, and the flask was filled to the mark with tetrahydrofuran.

To dilute the stock solution of the BADGE standard, a 10 mL amount of the stock solution was transferred into a 100 mL volumetric flask and diluted to volume with tetrahydrofuran. Next, 10 mL of this solution was transferred into a 100 mL volumetric flask and diluted to volume with simulant; 10 mL of the resulting solution was then transferred into a 100 mL volumetric flask and diluted to volume with simulant. Finally, 1.0 μg/mL of BADGE solution was transferred to a 250 mL cylindrical flask and stored at −20 °C. The solutions should not be exposed to room temperature any more than a few minutes at a time.

Others. Sep-Pak Plus C18 cartridges were used (part no. WAT020515) only for third confirmation option.

Apparatus. A liquid chromatograph system consisting of binary pump (Spectra Physics (SP), P2000 LC) with helium degassing kit was used. The data-jet integrator was connected via Labnet to a personal computer with SP Winner for Windows software. The fluorescence detector was from Perkin Elmer (PE LS 40). The ultraviolet detector was from SP (UV2000), with scanning function on eluting peaks for the second and third confirmation options.

Chromatographic Conditions. An injection system with a 50 μL loop was used.

Fluorescence Detector. Settings: excitation wavelength, 225 nm, and emission wavelength, 305 nm.

Ultraviolet Detector. The wavelength was set at 225 nm (only for second confirmation option); scanning between 190 and 340 nm (third confirmation option).

Column. Length, 15 cm; internal diameter, 4.6 mm; packing, 5 μm Spherisorb ODS 2.

Elution Program for Gradient Method. Gradient elution consisted of a 2-min isocratic elution with acetonitrile−water (30:70), an 18-min linear gradient to 80% acetonitrile, a 3-min linear Gradient to 100% acetonitrile, and a 2-min isocratic elution at 100% acetonitrile. Typical retention times for BADGE and its first and second HP are 17.4, 11.9, and 6.3 min, respectively.

Elution Program for Isocratic Method. Isocratic elution with acetonitrile−water (65:35) (v/v). Typical retention time for BADGE is 5.3 min; flow rate, 1 mL/min.

RESULTS AND DISCUSSION

Calibration. *Preparation of Calibration Solutions.* Various amounts (0.5, 1, 2, 3, and 4 mL) of the 1 μg/mL BADGE standard was transferred to a series of 100 mL volumetric flasks which were filled to the marks with simulant to obtain 5, 10, 20, 30, and 40 μg/L BADGE solutions. These calibration solutions are not stable at room temperature and should be used rapidly. To prepare blank calibration solutions, 0.02 mL of THF was transferred to a 100 mL volumetric flask, which was filled to the mark with simulant.

Hydrolysis products of BADGE are not commercially available, so to quantify them it is necessary to use the BADGE calibration line.

For each of the three food simulants we prepared a set of calibration samples and ran 50 μL of each in triplicate in the chromatograph and determined the area of the BADGE peak in the resulting chromatograms.

Table 1. Parameters of Calibration Lines ($y = a + bx$) Obtained from Data for BADGE in the Range 5−40 μg/L

simulant	method	intercept (a)	slope (b)	coefficient correlation (r)
distilled water	gradient	579	1518	0.997
	isocratic	−196	1521	0.999
3% (w/v) acetic acid	gradient	−129	1526	0.998
	isocratic	−96	1491	1.000
15% (v/v) ethanol	gradient	1045	1284	0.997
	isocratic	-406	1325	0.998

The essential data and regression results of both the isocratic and gradient methods for the calibration are summarized in Table 1.

All calibration lines had correlation coefficients (r) greater than 0.997. For verification of effective detection limit calculated from the calibration lines, a 1.5 μg/L BADGE solution was run in triplicate. In all cases the signal-to-noise ratio of the BADGE peak was about 6, noise being measured as the maximum amplitude of the chromatogram of a blank between 4.8 and 5.8 min (isocratic method) or between 16.9 and 17.9 min (gradient method).

Experiments on the performance of the method for measurement of BADGE samples of concentrations greater than 40 μg/L showed that linearity was maintained.

Stability of Stock and Dilute Stock Solutions. BADGE stock solutions containing 1 mg of BADGE per mL of tetrahydrofuran were stored for 3 months at −20 °C, 0−5 °C, and room temperature, and their concentrations were checked by the gradient method after dilution to 40 μg/L with simulants. No change in BADGE concentration was detected in any of these solutions. BADGE dilute stock solutions, stored at the same conditions, with 1 μg of BADGE per mL of simulant are not stable at 0−5 °C or at room temperature and should be prepared immediately before the calibration solutions are run.

Precision. In view of the BADGE SML (20 μg/L), for each food simulant we prepared six BADGE samples at a concentration of approximately 20 μg of BADGE/L of simulant as described in the section "Preparation of Calibration Solutions". These samples and the corresponding blanks were run in duplicate; in all cases, standard deviations less than 3% were obtained.

Confirmation. The following three options could be used to confirm BADGE identity by mean isocratic and gradient methods and its hydrolysis products only by gradient method.

Calibration-type BADGE solutions with concentrations of 20, 30, and 40 μg/L or BADGE hydrolyzed solutions, containing its two HPs, were run in triplicate.

First Option. Under this option the identity of BADGE (or HPs) is confirmed by its fluorescence emission spectrum obtained [stopping the chromatography when the maximum of the BADGE (or HPs) peak is detected] from 255 to 410 nm, setting excitation wavelength to 225 nm.

Second Option. Under this option, chromatograms are obtained by both fluorescence and UV detection, and the identity of BADGE is confirmed by the ratio between the areas of its peaks in the two chromatograms (A_F/A_{UV}). In these experiments the UV detector was located in series and in front of the fluorescence detector.

In all cases, the mean A_F/A_{UV} ratio was close to 3.0; i.e., 3.2 for BADGE, 3.3 for 1HP, and 2.9 for 2HP, with a standard deviation less than 3% for BADGE with the

isocratic method and less than 17% for the three compounds with the gradient method. Thus this ratio of approximately 3 holds for BADGE concentrations of between 1 and 2 times the SML; at lower concentrations, UV detection is unreliable. Poorer precision of the gradient method is expected, since elution with a water/acetonitrile gradient makes for significant base line drift in the UV chromatogram recorded at 225 nm and hence reduces the accuracy with which the area of the BADGE peak is calculated.

The A_F/A_{UV} ratio should be established experimentally in each chromatographic system because it is dependent on experimental conditions (detector capability, output voltages, etc).

Third Option. Under this option the identity of BADGE (or HPs) is confirmed by its UV spectrum obtained [stopping the chromatography when the maximum of the BADGE (or HPs) peak is detected] from 190 to 340 nm. It is necessary concentrate the sample approximately 50-fold. A 25 mL amount of sample was passed through the C18 cartridge (previously activated as instructions), rinsed once with 3 mL of water, and pumped through 5 mL of air, and the eluates were discarded. Finally, 5 mL of methanol was passed through the cartridge, and the eluate was collected in a 10 mL vial. Under a stream of nitrogen, the solvent was evaporated to obtain approximately 0.5 mL. Similar spectra were found for BADGE and HPs with two maxima at 225 and 275 nm.

Comparison. The first option is simple but relatively error-prone. For the isocratic method, better reliability can be achieved by using the gradient method to confirm BADGE identity (the fourth option). The second option requires two detectors, but it is more reliable. The third option is the most complicated because it is necessary to concentrate the sample; besides, the spectra obtained are very similar to phenolic compounds related to BADGE in structure.

Procedure with Migration Samples. *Preparation of Samples.* The migration solution was filtered through the microfilter, and the first few milliliters of filtrate were discarded. To prepare blank solution, simulant was used instead of the migration solution.

BADGE in Simulants after 10 Days at 40 °C and 30 min at 121 °C. The stability of BADGE in the food simulants was investigated by determining BADGE in simulant that had been fortified with BADGE to a concentration of 20 μg/L and then stored for 10 days at 40 °C or for 30 min at 121 °C. No BADGE peak indicative of a concentration greater than detection limit (1.5 μg/L) was obtained with any of the aqueous food simulants.

BADGE Hydrolysis Kinetics. In view of the above results and our previous experience (Paseiro Losada *et al.*, 1992, 1993), we conclude that BADGE is not stable in the aqueous simulants, in which it is converted into two hydrolysis products (HPs) corresponding to the opening of one or both epoxy rings in accordance with the reaction: Scheme 1.

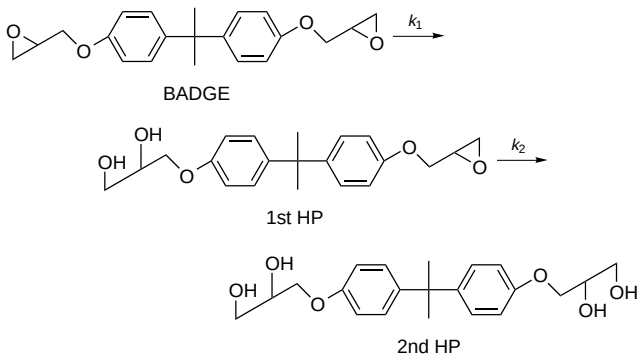
The corresponding rate equation for BADGE is

$$\mathrm{d[BADGE]}/\mathrm{d}t = k_1[\mathrm{BADGE}]$$

When it is assumed that initially only BADGE is present, at a concentration $[\mathrm{BADGE}]_0$, integration of the rate equation affords

$$[\mathrm{BADGE}] = [\mathrm{BADGE}]_0 \exp(-k_1 t) \qquad (1)$$

Scheme 1



where t indicates time. The value of k_1 can be calculated from experimental results either by nonlinear regression (as implemented in suitable statistical software) to fit the above integrated equation or by linear regression (with ln[BADGE] as the dependent variable and time as the independent variable) to fit the simpler transformed equation

$$\ln [\mathrm{BADGE}] = (\ln[\mathrm{BADGE}]_0) - k_1 t \qquad (2)$$

When the latter method is used, k_1 is the absolute value of the regression coefficient. The half-life of BADGE, $t_{1/2}$, is (ln 2)/k_1.

For these experiments, each of the three aqueous food simulants was fortified with BADGE at a concentration of 40 μg/L [procedure: same as for calibration samples, but using the simulant (previously brought to 40 °C) for the final dilution] and then stored in an oven at 40 °C. Samples (0.5 mL) were analyzed immediately after preparation of the fortified simulant and periodically thereafter (at intervals depending on the food simulant) for 20 days (by which time the degradation of BADGE was complete); the gradient method was used for these analyses. The kinetic curves are shown in Figure 2. BADGE concentrations of 0 μg/L or less cannot be used for fitting eq 2 above, though they could be used in fitting eq 1 by nonlinear regression analysis; the calculated value of k_1 would of course differ somewhat. BADGE half-lives expressed in days/hours are 1.09/26.0 for distilled water, 0.14/3.4 for 3% (w/v) acetic acid and 1.35/32.4 for 15% (v/v) ethanol.

In acetic acid the only substance remaining after 10 days was the second HP, whereas in distilled water and ethanol both HPs remained; remaining BADGE was hardly quantifiable and in any case was less than 3% of the initial concentration (40 μg/L). We found that $t_{1/2}$[in 15% (v/v) ethanol] > $t_{1/2}$[in distilled water] > $t_{1/2}$[in 3% (w/v) acetic acid]. The half-lives are all shorter than those we have reported previously because of differences in measurement criteria and subsequent calculations: in the preliminary work BADGE concentration was calculated relative to initial concentration as the ratio between the BADGE peak area and the sum of the areas of the BADGE and HPs peaks (since it is now clear that the HPs fluoresce 25−40% less intensely than BADGE, this criterion will have overestimated BADGE concentration); and the final value of k_1 was the mean of the values obtained by using nonlinear regression to fit the data with integrated equations for BADGE, the first HP, and the second HP (whereas here we only consider BADGE).

Migration from Epoxy Polymer Formulations. We tested three epoxy formulations for use as coatings on food cans as follows.

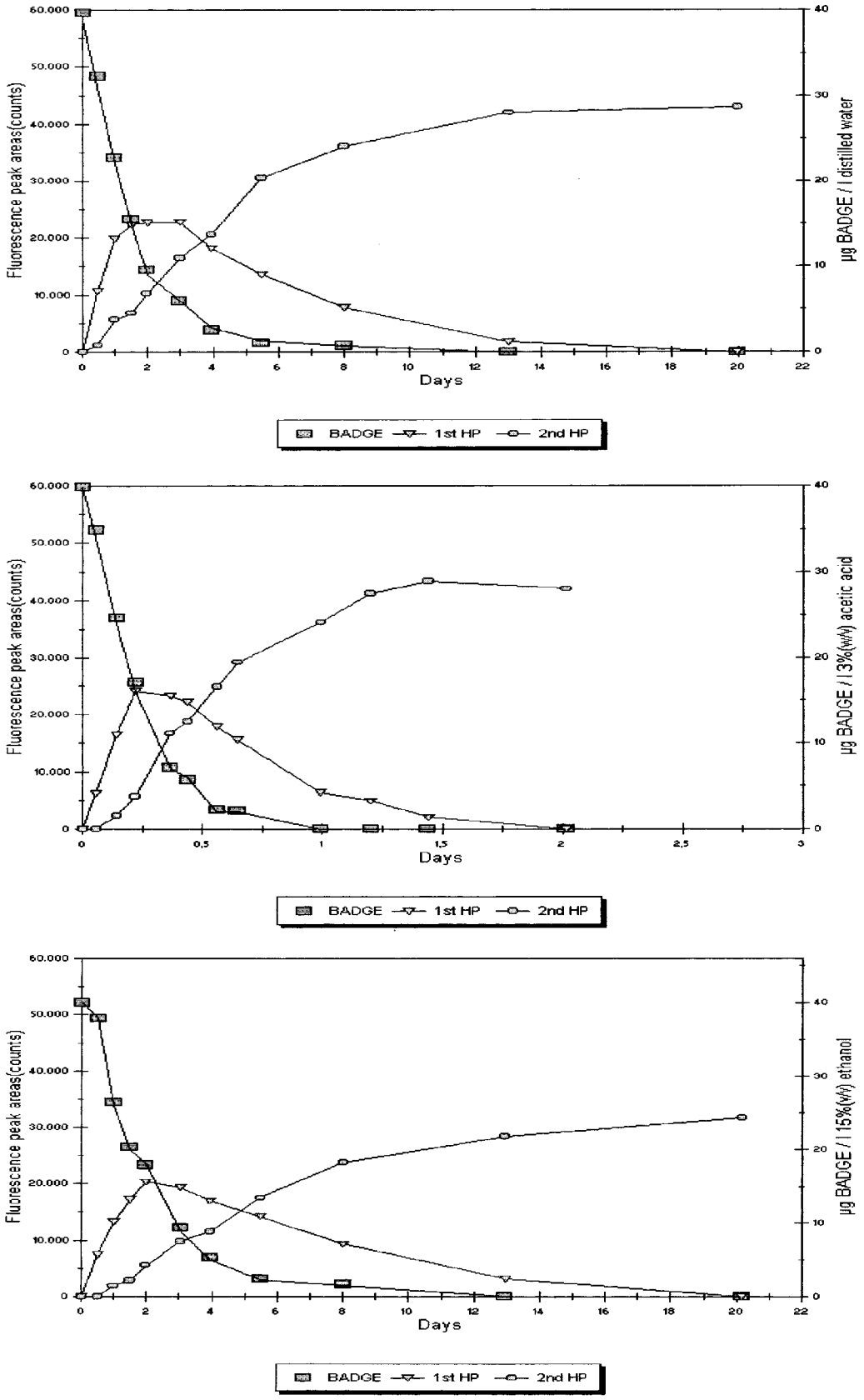


Figure 2. BADGE kinetic curves at 40 °C in the three aqueous simulants.

Formulation Type 1. This is a special coating for cans intended to contain sulfurous products. Epoxy-phenolic; drying, 15 min at 205 °C; surface density of dry film, 4−6 g/m^2.

Formulation Type 2. Epoxy-phenolic; drying, 15 min at 205 °C; surface density of dry film, 4−6 g/m^2.

Formulation Type 3. Epoxy; drying, 15 min at 195 °C; surface density of dry film, 14−18 g/m^2.

These formulations were tested as films in three-piece and two-piece cans. The cans were filled with simulant (inside surface/simulant volume ratio ~1 dm^2/100 mL), and the lid was sealed on mechanically. Since cans may

Table 2. Migration Results of BADGE from Three Types of Coating Obtained under Two Different Test Conditions in the Three Aqueous Food Simulants Using the Gradient Method

		coating[a]			coating[b]		
simulant	analyte	type 1	type 2	type3	type 1	type 2	type 3
distilled water	BADGE	<20	<20	<20	<20	<20	<20
	BADGE + HPs	<20	50	<20	257	285	362
	BADGE + HPs + other migrants[c]	92	1809	67	476	2247	914
3% (w/v) acetic acid	BADGE	<20	<20	<20	<20	<20	<20
	BADGE + HPs	25	127	<20	210	266	26
	BADGE + HPs + other migrants[c]	39	2289	46	636	3023	1134
15% (v/v) ethanol	BADGE	<20	<20	<20	<20	<20	<20
	BADGE + HPs	43	76	63	373	477	472
	BADGE + HPs + other migrants[c]	139	2394	118	1192	5033	1942

[a] 10 days at 40 °C (test a), concentrations measured in μg/L. [b] 30 min at 121 °C (test b), concentrations measured in μg/L. [c] The concentrations of "other migrants" are expressed in BADGE.

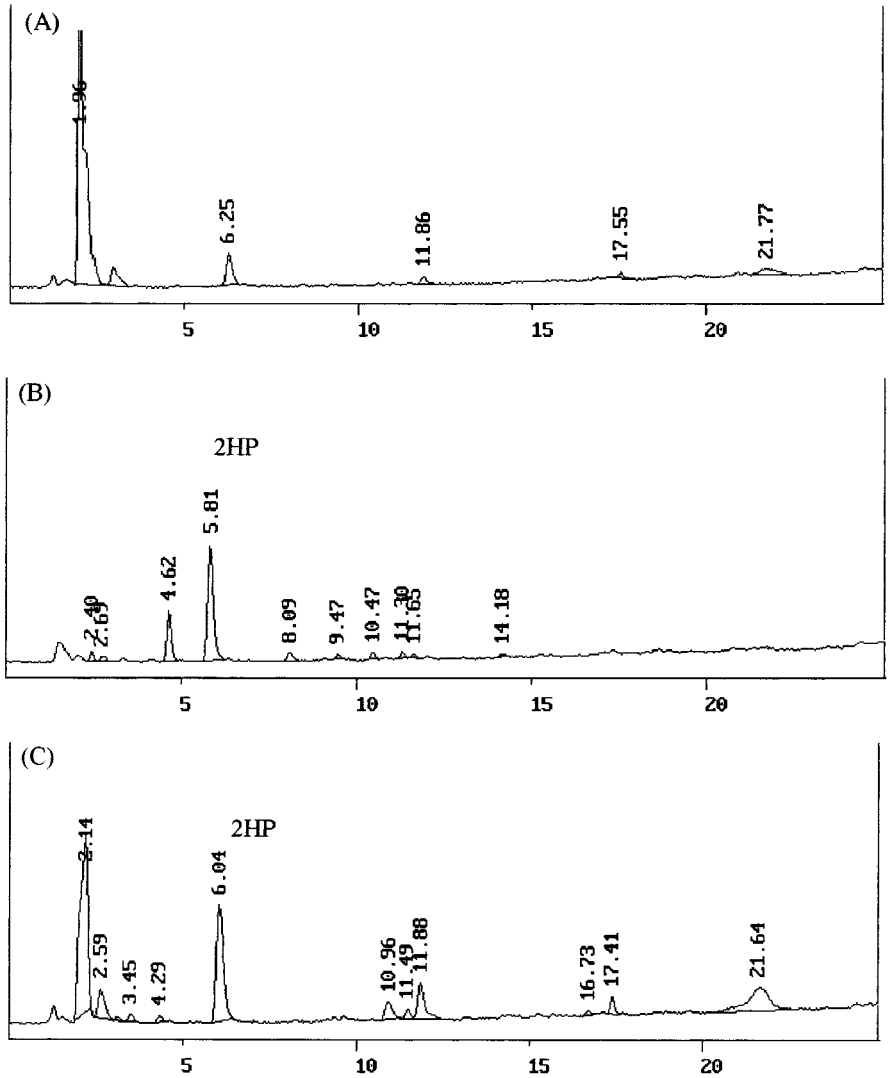


Figure 3. Chromatograms of type 1 can coatings after test a: (10 days at 40 °C). (A) Simulant is distilled water. (B) Simulant is 3% (w/v) acetic acid. (C) Simulant is 15% (v/v) ethanol.

be sterilized after being filled with foods, we carried out migration testing under two sets of conditions: (a) the standard 10 days at 40 °C and (b) 30 min at 121 °C.

The results of analyzing the migration solutions are listed in Table 2, typical chromatograms recorded after 10 days at 40 °C are shown in Figure 3, and typical chromatograms recorded after 30 min at 121 °C are shown in Figure 4. In neither test was any BADGE peak indicative of a concentration greater than 20 μg/L obtained for any of the aqueous food simulants used. The sum of BADGE content plus HPs content expressed as BADGE and determined by the gradient method is greater than 20 μg/L in formulation type 2 for water and in formulation types 1 and 2 for acetic acid and in the three formulation types for ethanol in test a. In test b the sum value is greater than 20 μg/L in all formulations and for all aqueous simulants, exceeding the SML upto 23-fold. The sum of BADGE, HPs, and other migrants contents are exceeding the SML between 2- and 250-fold.

The gradient method chromatogram shows many peaks corresponding to "other migrants" (unidentified

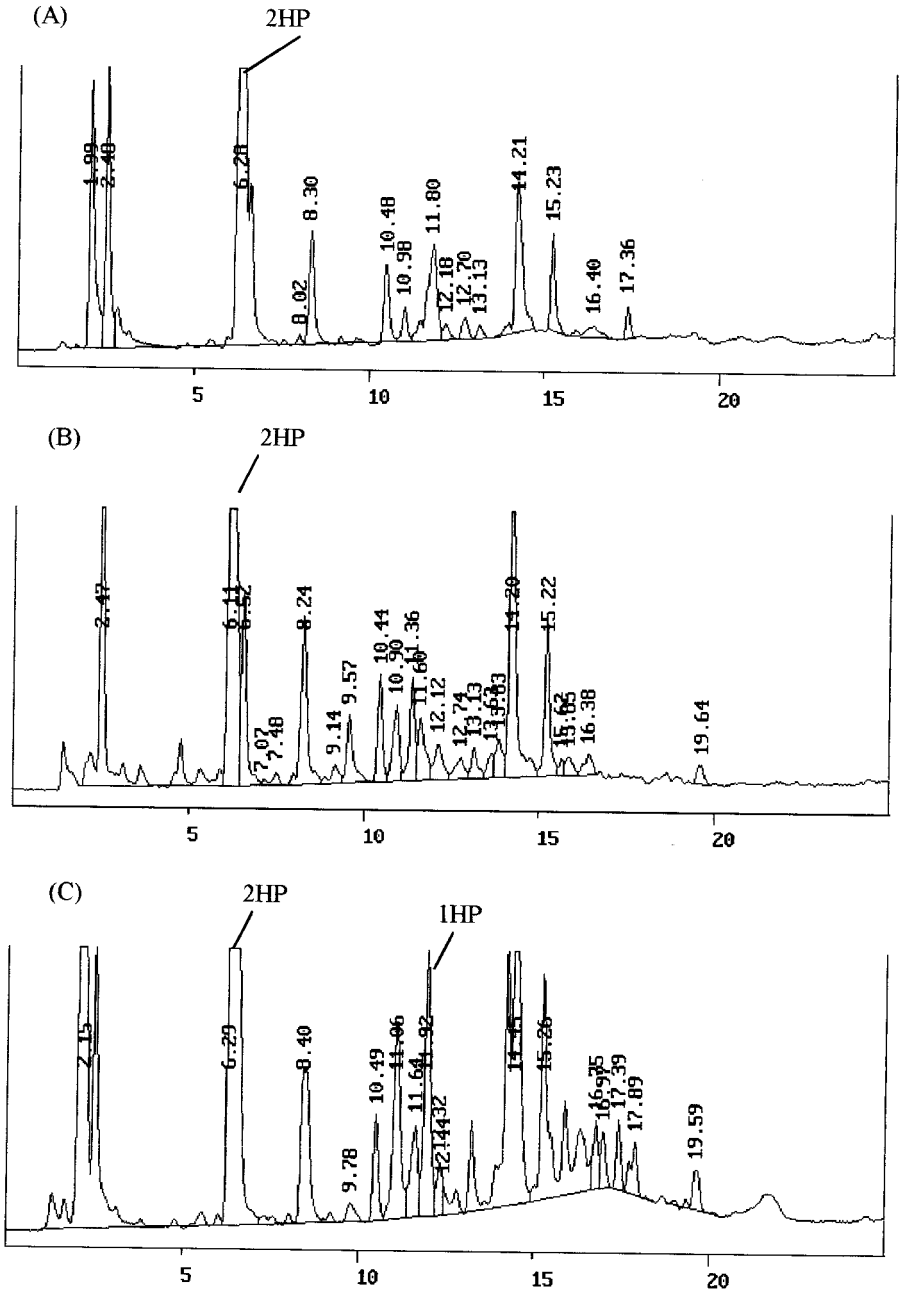


Figure 4. Chromatograms of type 1 can coatings after test b: (30 min at 121 °C). (A) Simulant is distilled water. (B) Simulant is 3% (w/v) acetic acid. (C) Simulant is 15% (v/v) ethanol.

compounds) plus BADGE hydrolysis products. In both test a and test b, the peaks of chromatograms obtained for distilled water were less numerous and smaller than for the other aqueous simulants [3% (w/v) acetic acid and 15% (v/v) ethanol].

CONCLUSIONS

(1) The isocratic method (7 min per injection) and the gradient method (25 min per injection) both perform well for quantitative determination of BADGE in the three aqueous food simulants. Both have good analytical quality characteristics (sensitivity, linearity, precision), and both separate BADGE from non-BADGE substances migrating from epoxy coatings into the simulants. The gradient method achieves better separation of BADGE from other migrants, separates BADGE from its hydrolysis products, allows the quantification of BADGE and the two hydrolysis products, and is less susceptible to interferences in real samples. Its drawback is that it is sensitive to the grade of acetonitrile used (high-purity acetonitrile must be used for satisfactory results). The isocratic method does not separate BADGE from other migrants as well as the gradient method, but it is a much simplier method.

To determine if the EU normative (LME = 20 μg/L) on BADGE has been surpassed, the isocratic method should be used.

(2) BADGE is unstable in aqueous simulants under test conditions, rapidly affording two hydrolysis products corresponding to the opening of one or both epoxy rings. This means that BADGE released by coatings (or plastic materials) into the simulants during the test will not be detected.

All coatings tested comply with the EU normative of 20 μg/L. Considering only hydrolysis products in the coatings tested at 121 °C for 30 min, the amounts of the second hydrolysis product, expressed as BADGE, are higher than ten or more times BADGE SML established

by law. In our opinion, the hydrolysis products from epoxidic coatings must not be ignored; therefore, future European legislation about them should take note of this circumstance. The results presented here show that the migration level of BADGE/hydrolysis products and other compounds is much higher than the actual SML (20 μg/kg). Unless BADGE hydrolysis products are quantified as well as BADGE, the current standard migration test is not a valid means of evaluating the quality of BADGE-based coatings.

(3) It is important to stress that some BADGE-based epoxy coatings release unidentified compounds which are neither BADGE nor its hydrolysis products (with chromatographic and spectroscopic properties very similar) in quantities that, if the specific fluorescence of these compounds is similar to BADGE, far exceed (hundreds times) the 20 μg/L SML established for BADGE. These compounds are related to the starting monomers of the epoxy formulations or their hydrolysis products. Since they may be very similar to BADGE and its hydrolysis products, consumer safety may require that they are assumed to be at least as toxic as BADGE. Though investigation of these non-BADGE compounds lies outside the scope of this work, public health authorities should be made aware of this complication of the epoxy coating problem and should respond by supporting studies of the toxicity, identity, and quantity of these compounds.

(4) The methods developed here could be useful to the companies working with or using these types of formulations as coatings in food cans to adjust the composition, the cure conditions (time and temperature), and to control (improve) the final formulation (under these conditions) of the amounts of BADGE and other epoxidic compounds susceptible to migrate into food and/or suffer a hydrolysis process to be as low as possible. Finally, food industries applying these materials to pack their products and public authorities looking after consumer health could check these formulations and in this way assure the quality of their products and consequently ensure the public health.

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