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Analysis of Reaction Products of Food Contaminants and Ingredients: Bisphenol A Diglycidyl Ether (BADGE) in Canned Foods

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Bisphenol A diglycidyl ether (BADGE) is an epoxide that is used as a starting substance in the manufacture of can coatings for food-contact applications. Following migration from the can coating into food, BADGE levels decay and new reaction products are formed by reaction with food ingredients. The significant decay of BADGE was demonstrated by liquid chromatographic (LC) analysis of foodstuffs, that is, tuna, apple puree, and beer, spiked with BADGE before processing and storage. Life-science inspired analytical approaches were successfully applied to study the reactions of BADGE with food ingredients, for example, amino acids and sugars. An improved mass balance of BADGE was achieved by selective detection of reaction products of BADGE with low molecular weight food components, using a successful combination of stable isotopes of BADGE and analysis by LC coupled to fluorescence detection (FLD) and high-resolution mass spectrometric (MS) detection. Furthermore, proteomics approaches showed that BADGE also reacts with peptides (from protein digests in model systems) and with proteins in foods. The predominant reaction center for amino acids, peptides, and proteins was cysteine.

KEYWORDS: Bisphenol A diglycidyl ether (BADGE); canned foods; food packaging; reaction products; food ingredients; stable isotopes

INTRODUCTION

Most food and beverage cans are internally coated to protect the foodstuff from the metal and, vice versa, to prevent metal corrosion occurring with aggressive food ingredients. The dominant type of coating for food and beverage cans is epoxy-phenolic based on bisphenol A and epichlorohydrin. These two starting substances are reacted together to form a low molecular weight prepolymerized resin, including bisphenol A diglycidyl ether (BADGE, **Figure 1**) and higher oligomers. The resin is then used to coat the metal, which is cured to form a hard polymeric protective coating.

The migration of BADGE from food contact materials has received a lot of attention in the past. The main focus then was on applications where BADGE was used not as a starting substance, for example, for epoxy-phenolic coatings, but was used as an additive (as a stabilizer) in certain PVC organosol can coatings. In response to these findings, a specific EU Directive on BADGE was prepared limiting the migration of BADGE and its derivatives (BADGE, BADGE·H_2O, BADGE·$2H_2O$, BADGE·HCl, BADGE·2HCl, and BADGE·HCl·H_2O) to 1 mg/kg food or food simulant (*1*). This was later updated in light of new toxicological data, to a restriction of 9 mg/kg for the sum of the migration of BADGE, BADGE·H_2O, and BADGE·$2H_2O$ and 1 mg/kg for the sum of the migration of BADGE·HCl, BADGE·2HCl, and BADGE·HCl·H_2O (*2*). The migration of BADGE and its derivatives is normally determined using so-called food simulants. These food simulants were introduced to mimic certain types of food; for example, olive oil represents fatty food (*3*). More than 50 papers have been published on this topic, mostly concerned with methods of analysis and surveillance, (see, e.g., ref *4*−*8*). Most often, reversed-phase liquid chromatography using C8 or C18 columns in combination with a mixture of acetonitrile/water as mobile phase and coupled to fluorescence detection (FLD) is used for the analysis of BADGE and related products. Basically, if the migration of BADGE and its known derivatives into food simulants is below the migration limit, the food contact material containing BADGE is in accordance with the current legislation. Although compliance is usually demonstrated by the analysis of food simulants exposed to the can coating, the concentrations of BADGE and its derivatives can also be measured in foodstuffs. From a practical point of view it is not obligatory to measure these substances in real food products. However, some studies on BADGE in food products have been carried out. The concentrations detected in the foodstuffs gave low recoveries for BADGE that could not be explained by the formation of its known derivatives (*9*, *10*). It was suggested that migrated BADGE reacted with food components. A few studies found evidence of the reactivity of BADGE toward food components such as amino acids and proteins (*10*−*12*). Recently,

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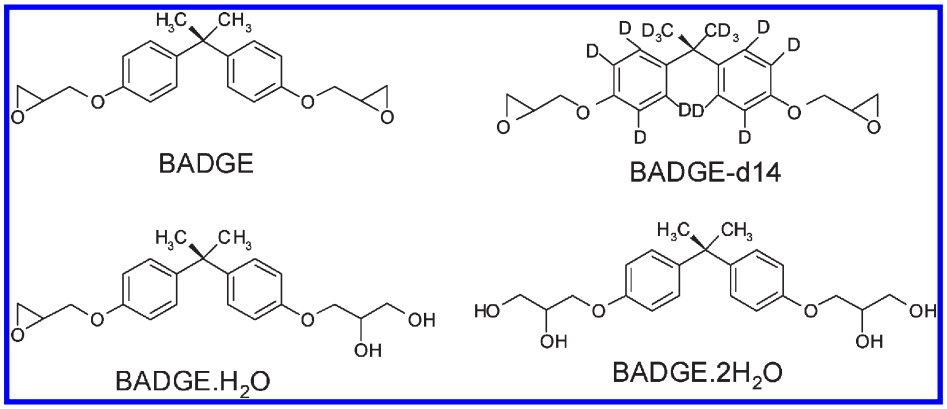

Figure 1. Structures of BADGE, BADGE·H_2O, BADGE·$2H_2O$, and BADGE-d14.

Petersen et al. (*10*) investigated the reaction of BADGE with proteins in more detail. The analysis of BADGE and possible reaction products in extracts of complex food matrices is not straightforward, leading to complex chromatograms in which it is difficult to identify the BADGE-related products (*13*).

To deal with the challenges of analyzing BADGE-related reaction products, which can differ widely in molecular weight, polarity, and chemical structure, in complex food matrices, sophisticated analytical approaches are necessary. In this study analytical technology inspired by life-science applications, such as the use of isotopically labeled compounds and peptide/protein analysis, is used to study the stability of BADGE and the formation of reaction products with food ingredients.

MATERIALS AND METHODS

Samples. Five food and beverage samples were selected for investigation. Two canned food samples, tuna in sunflower oil and apple puree, were prepared by a commercial food packer, and three beverage samples, an ale, a stout, and a lager, were purchased from retail outlets in northern England. For each foodstuff 10 control samples were prepared to which no BADGE was added and 50 samples were prepared and spiked with BADGE at a nominal concentration of 20 mg/kg. The same procedure was repeated at a later stage in the study in which both BADGE and deuterated BADGE were spiked into the food samples.

Canned Foods. Tuna in sunflower oil and apple puree were packed in metal cans lacquered internally with a standard epoxy-phenolic coating. For epoxy-phenolic coatings, BADGE is used as a starting substance, and it is intended to be used-up in the polymerization reaction. These samples were recanned, that is, they were obtained from cans, then "spiked" with BADGE, and then recanned. Control samples, canned water, and sunflower oil were also prepared. The canned samples were heated in a static steam sterilizer. For tuna, the heating time was 50 min at 122 °C steam temperature, and for apple puree, the low pH (pH 3.7) required a lower processing temperature, that is, a heating time of 38 min at 105 °C steam temperature. The samples were then stored at room temperature, and after 3 weeks, half of the cans were transferred to an oven set at 50 °C and the remainder were left at room temperature.

Bottled Beers. Three sets of beer samples were prepared. Bottled beers were opened in a carbon dioxide-filled glovebag (to maintain carbonation). The beers were spiked with BADGE and then recapped using fresh crown caps. The samples were stored at 40 °C.

Preparation of Deuterated BADGE. The protocol for the synthesis of labeled BADGE from bisphenol A-d16 was adapted from that of bisphenol F diglycidyl ether (BFDGE) (*14*). In short, 1 g of bisphenol A-d16 (98 atom % D, Sigma-Aldrich Chemie, Zwijndrecht, The Netherlands) was mixed with 0.65 g of KOH and 50 mL of ethanol. The mixture was evaporated to dryness using a rotary evaporator (60 °C, 150 mbar) followed by drying under vacuum at 60 °C overnight. Next, 30 mL of water-free acetonitrile (50 g of Na_2SO_4 + 200 mL of acetonitrile) and 9 mL of epibromohydrin (Sigma-Aldrich) were added to the dried potassium phenolate. The mixture was stirred for 30 min at room temperature and for 1 h at 50 °C and then refluxed for 10 min. Next, the mixture was cooled to room temperature and filtered. The precipitate was washed with 2 × 30 mL of water-free acetonitrile. The resulting solution was collected and dried at 60 °C and 150 mbar using a rotary evaporator. In this way BADGE-d14 (**Figure 1**) was synthesized with a yield of 85%.

Table 1. Recovery of BADGE and Its Hydrolysis Products BADGE·H_2O and BADGE·$2H_2O$ in Various Foods and Drinks after Spiking (20 mg/kg), Canning, and Storing

sample	storage	recovery (%)
tuna in oil	3 weeks at room temperature	25
	12 weeks at room temperature	0
	3 weeks at room temperature + 12 weeks at 50 °C	2
apple puree	3 weeks at room temperature	15
	12 weeks at room temperature	12
	3 weeks at room temperature + 12 weeks at 50 °C	17
ale	3 weeks at 40 °C	26
lager	3 weeks at 40 °C	5
stout	3 weeks at 40 °C	8

Analysis of (Spiked) Food Samples. The content of each can/bottle was homogenized, and duplicate portions (5 g) were taken. A further two 5 g portions were taken from each nonspiked food sample to determine analytical recovery and were fortified with BADGE, BADGE·H_2O, and BADGE·$2H_2O$ (Sigma-Aldrich) each at a concentration of 20 mg/kg. The samples were extracted into acetonitrile (5 mL) by shaking for 18 h at room temperature. Each sample was centrifuged and the supernatant passed through a 0.45 μm filter. Calibration solutions (0−25 mg/L) were prepared and run alongside the samples.

Sample extracts were analyzed by liquid chromatography with fluorescence detection (LC-FLD) and/or mass spectrometric detection (LC-MS). LC-FLD analyses were performed on a Hewlett-Packard 1100 series LC equipped with an automatic degasser, binary pump, autosampler, and fluorescence detector (Agilent Technologies, Palo Alto, CA). Chromatographic separation was carried out using a Sunfire C18 column with dimensions of 150 mm × 3.0 mm i.d., 3.5 μm (Waters, Milford, MA), with a mobile phase gradient from 80% water + 0.1% trifluoroacetic acid (A) to 100% acetonitrile + 0.1% trifluoroacetic acid (B) with a flow of 0.4 mL/min using a mobile phase gradient from 80 to 50% A in 22 min followed by 8 min at 50% A and rinsing of the column with 100% B for 5 min. Injection volume was 10 μL. The fluorescence detector was set at excitation and emission wavelengths of 275 and 305 nm, respectively. Quantification was carried out using solvent standards of BADGE, BADGE·H_2O, and BADGE·$2H_2O$ (0−25 mg/L).

LC-FT-MS analyses were performed on an LTQ Fourier Transform (FT) linear ion trap system consisting of a Surveyor AS autosampler and a Surveyor MS pump, equipped with an LTQ LT-1000 FT mass detector with an Opton ESI probe (Thermo Fisher Scientific, San Jose, CA).

Chromatographic separation for LC-MS was carried out using a Sunfire C18 column with dimensions of 150 mm × 3.0 mm, 3.5 μm (Waters), with a mobile phase gradient from 80% 5 mM ammonium acetate adjusted to pH 5 with acetic acid (A) to 50% 2.5 mM ammonium acetate in acetonitrile (B) in 22 min followed by 8 min at 50% A/50% B and rinsing of the column with

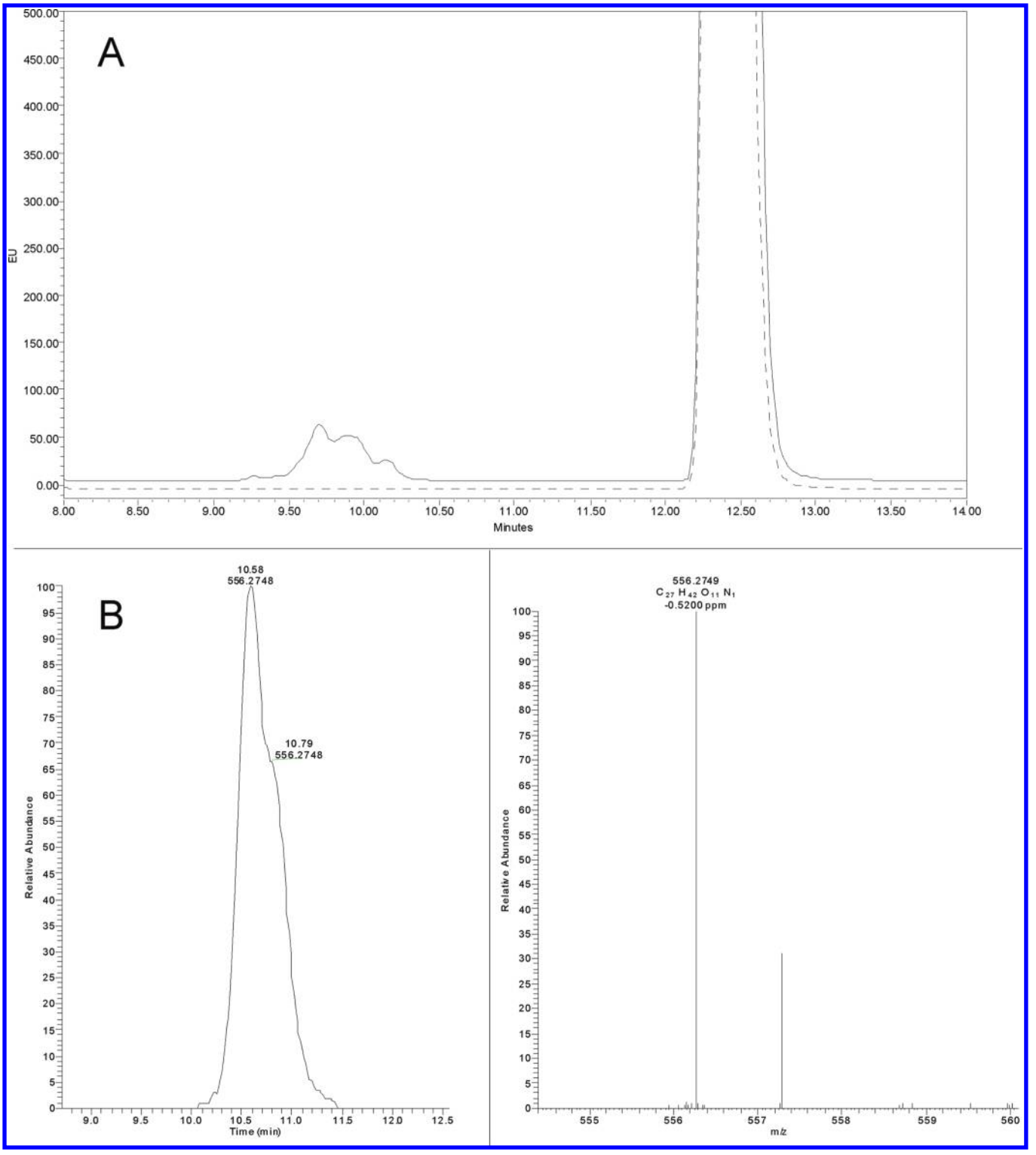

Figure 2. (**A**) LC-FLD chromatograms of BADGE (- - -) and a reaction mixture of BADGE and glucose after reflux for 2 h at 100 °C (—); (**B**) LC-FT-MS chromatogram of reaction product of BADGE and glucose with corresponding mass spectrum.

100% B for 5 min with a flow of 0.4 mL/min. Injection volume was 10 μL. Mass detection was carried out using electrospray ionization (ESI) in the positive mode (m/z 100−1200). Mass accuracy was generally better than 2 ppm. Calibration and optimization were carried out using solvent standards of BADGE, BADGE·H_2O, and BADGE·$2H_2O$.

BADGE−Monosaccharide Adducts. The reaction between BADGE and glucose was carried out by refluxing an aqueous solution containing 50 μg of BADGE, 65 μg of BADGE-d14, and 500 mg of glucose in 5 mL of water for 2 h at 100 °C. The reaction mixture was analyzed by LC-FLD and LC-MS.

BADGE−Amino Acid Adducts. BADGE (20 μg) was reacted with the 250 μg of the amino acid methionine, cysteine, histidine, lysine, or tyrosine in 25 mL of water for 30 min at 100 °C and also at room temperature. The reaction mixtures were analyzed by LC-FLD and LC-MS.

BADGE−Protein Adducts. Reaction of BADGE with insulin (Sigma-Aldrich) and bovine serum albumin (BSA, Sigma-Aldrich) was carried out for 30 min at 100 °C before and after digestion. To this purpose 100 μL of BADGE solution (~1 mg/mL) in acetonitrile was evaporated to dryness and redissolved in 100 μL of protein solutions (1 mg/mL) or

Table 2. Examples of Reaction Products of BADGE with Food Ingredients Identified by High-Resolution LC-FT-MS

reaction	observed *m/z*	elemental composition	elemental composition after subtraction of adducts and BADGE[a]	reaction product
BADGE + glucose	$[M + NH_4]^+$ = 556.2748	$C_{27}H_{42}O_{11}N_1$	$C_6H_{12}O_6$	BADGE·H_2O·glucose
BADGE + cysteine	$[M + H]^+$ = 480.2049	$C_{24}H_{34}O_7N_1S_1$	$C_3H_7NO_2S_1$	BADGE·H_2O·Cys
BADGE + cysteine	$[M + H]^+$ = 583.2141	$C_{27}H_{39}O_8N_2S_2$	$C_6H_{14}N_2O_4S_2$	BADGE·2Cys
BADGE + methionine	$[M + NH_4]^+$ = 424.2145	$C_{22}H_{34}O_5N_1S_1$	SCH_3	BADGE·H_2O·SCH_3
BADGE + methionine	$[M + NH_4]^+$ = 454.2117	$C_{23}H_{36}O_4N_1S_2$	$S_2C_2H_6$	BADGE·2SCH_3
BADGE + methionine	$[M + H]^+$ = 508.2359	$C_{26}H_{38}O_7N_1S_1$	$C_5H_{11}O_2N_1S_1$	BADGE·H_2O.Met
BADGE + lysine	$[M + H]^+$ = 505.2905	$C_{27}H_{41}O_7N_2$	$C_6H_{14}O_2N_2$	BADGE·H_2O·Lys

[a] BADGE = $C_{21}H_{24}O_4$, BADGE·H_2O = $C_{21}H_{26}O_5$, BADGE·2H_2O = $C_{21}H_{28}O_6$.

100 μL of digest solution. Insulin, a low molecular weight protein, was reacted with BADGE for 30 min at 100 °C and subsequently directly infused into the MS source.

Trypsin Digestion. To this purpose 100 μL of aqueous protein solutions (~10 mg/mL), 800 μL of 80 mM ammonium bicarbonate, 50 μL of 5 mM calcium chloride, and 20 μL of 2 mM dithiothreitol were mixed, and 20 μL of a trypsin solution (~1 mg/mL, Sigma-Aldrich) was added, resulting in an enzyme/protein ratio of 1:100. Digestion was carried out overnight at 37 °C.

Pronase Digestion. To this purpose 500 μL of aqueous protein solutions (~1 mg/mL), 500 μL of 100 mM ammonium bicarbonate, and 1.6 μL of 0.34 mM calcium chloride were mixed, and 100 μL of Pronase solution (~1 mg/mL, Sigma-Aldrich) was added, leading to an enzyme/protein ratio of 1:100. Digestion was carried out overnight at 37 °C.

Analysis of Proteins/Peptides. Tryptic peptides were analyzed by LC-MS/MS on an LTQ linear ion-trap system (Thermo Fisher Scientific) using an Inertsil ODS 2 column with dimensions of 100 mm × 3 mm (GL Sciences, Torrance, CA) and a mobile phase gradient from 95% water + 0.1% formic acid to 100% water + acetonitrile (20:80) + 0.1% formic acid in 20 min with a flow of 0.4 mL/min. Mass detection was carried out using ESI in the positive mode (*m/z* 150−2000). Mass spectra were collected in the full scan mode and in data-dependent mode.

RESULTS AND DISCUSSION

Levels of BADGE and Its Hydrolysis Products Detected in Foods. To confirm the disappearance of BADGE in canned foods and to determine the extent of its disappearance, the levels of BADGE, BADGE·H_2O, and BADGE·2H_2O (**Figure 1**) were determined in the foods stored under different conditions using LC-FLD (**Table 1**). All packaging materials contained epoxy-phenolic coatings and not PVC organosol; therefore, chlorohydrins could not be formed and were thus not analyzed. The precision of the method was generally better than 10%.

It can be clearly seen that for all food types and storage conditions low recoveries were found, varying from 0 to 26%. For tuna in sunflower oil the recovery was 25% after 3 weeks at room temperature, whereas after a longer storage time, that is, 12 weeks at room temperature or 50 °C, nearly all of the BADGE and its hydrolysis products have disappeared. The recovery in apple puree was about 15% and remained relatively constant over time. In the three beer samples the recovery of BADGE, BADGE·H_2O, and BADGE·2H_2O ranged from 5 to 26% after 3 weeks at 40 °C. The recoveries in water and sunflower oil were significantly higher, that is, >80%. These results prove that BADGE "disappears" in the different foods and that the loss of BADGE cannot be explained by the formation of the known derivatives, that is, hydrolysis products. The majority of BADGE disappears in the first 3 weeks of storage.

Reactivity of BADGE. Before the food samples were analyzed to determine where BADGE had gone, the reactivity of BADGE toward different food ingredients was investigated. To that purpose reactions were carried out between BADGE and monosaccharides and amino acids, which are compounds present in many types of foods. The resulting reaction solutions were analyzed by LC-FLD and high-resolution LC-FT-MS.

The reaction of BADGE with glucose resulted in the formation of an additional feature observed with LC-FLD (**Figure 2A**), and using high mass resolution LC-FT-MS, this feature could be well explained by a reaction product of BADGE and glucose, that is, BADGE·H_2O·glucose (**Figure 2B** and **Table 2**). Hence, it could be concluded that BADGE reacts with glucose and therefore it is expected that it will react with other monosaccharides in the same way. This is in contrast to the findings of Petersen et al. (*10*), who did not find reaction products of BADGE and glucose. These authors had similar reaction conditions and analytical methods, and hence the discrepancy with respect to the presence or absence of BADGE−glucose reaction products cannot be easily explained.

Reaction in model solutions was also carried out with amino acids. Reaction of BADGE with cysteine resulted in two main additional peaks observed with LC-FLD that could be identified by LC-FT-MS as BADGE·H_2O·Cys and BADGE·2Cys (**Figure 3** and **Table 2**). Similar reaction products were found for lysine and methionine (**Table 2**). Although different amino acids showed reaction products with BADGE, the number and intensity of the peaks differed widely between the different amino acids. The reaction of BADGE with amino acids increases, roughly, in the order Tyr < His < Met < Lys < Cys. For reaction with methionine some additional reaction products were observed that could be assigned to methylthio-BADGE reaction products, that is, BADGE·H_2O·SCH_3 and BADGE·2SCH_3 (**Table 2**). It has been shown that these methylthio derivatives can be formed from BADGE·H_2O·Met by cleavage and subsequent transfer of the methylthio group from methionine to BADGE (*10*). For all reactions all of the fluorescent response associated with the BADGE starting material was accounted for (assuming a 1:1 response). This indicates that the response factors of BADGE-containing products with LC-FLD are similar, indicating that the fluorescence response is largely determined by the BADGE backbone. It also indicates that, as expected, it is the epoxy groups of BADGE that are the reaction centers involved and that the fluorescent bisphenol A-like backbone of BADGE is not disrupted by the reactions.

Profiling of BADGE-Related Products in Foods Using Stable Isotopes. Following the confirmation that BADGE disappears in food, which could not be explained quantitatively by the formation of known hydrolysis products, and the observation that BADGE is able to react with food ingredients, an analytical approach was developed to find the reaction products of BADGE in the different foods. Straightforward analysis using either LC-FLD or LC-MS is not applicable as these detection methods were not sufficiently selective to distinguish BADGE reaction products from other compounds present in food. It was expected that many different types of compounds with nucleophilic moieties can react with BADGE, leading to the formation of reaction products with a wide range of chemical structures and polarities. It can be expected that the two epoxide groups in BADGE (**Figure 1**) are

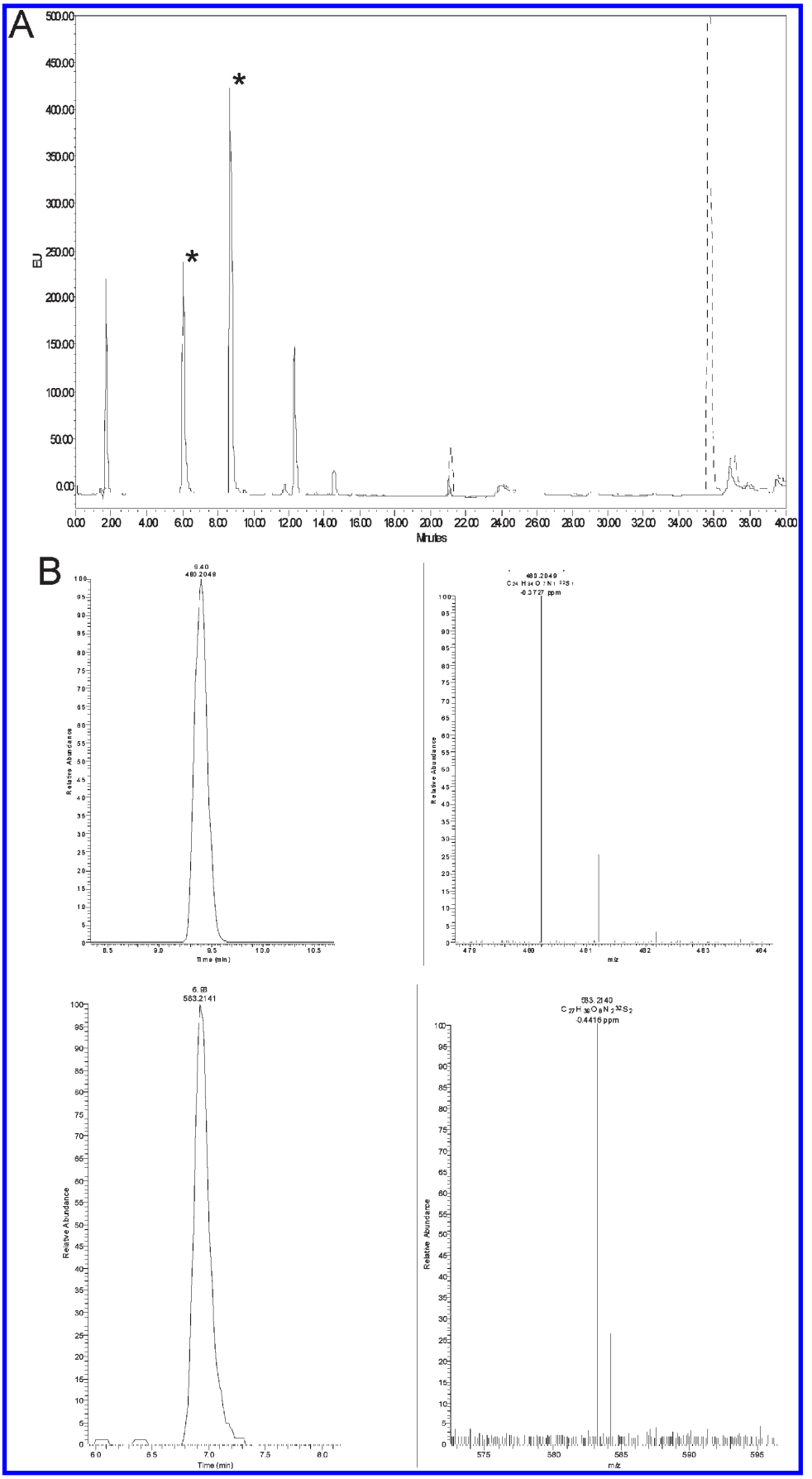

Figure 3. (**A**) LC-FLD chromatograms of BADGE (- - -) and a reaction mixture of BADGE and cysteine after storage at 100 °C (—); (**B**) LC-FT-MS chromatograms and corresponding mass spectra of two main reaction products of BADGE and cysteine.

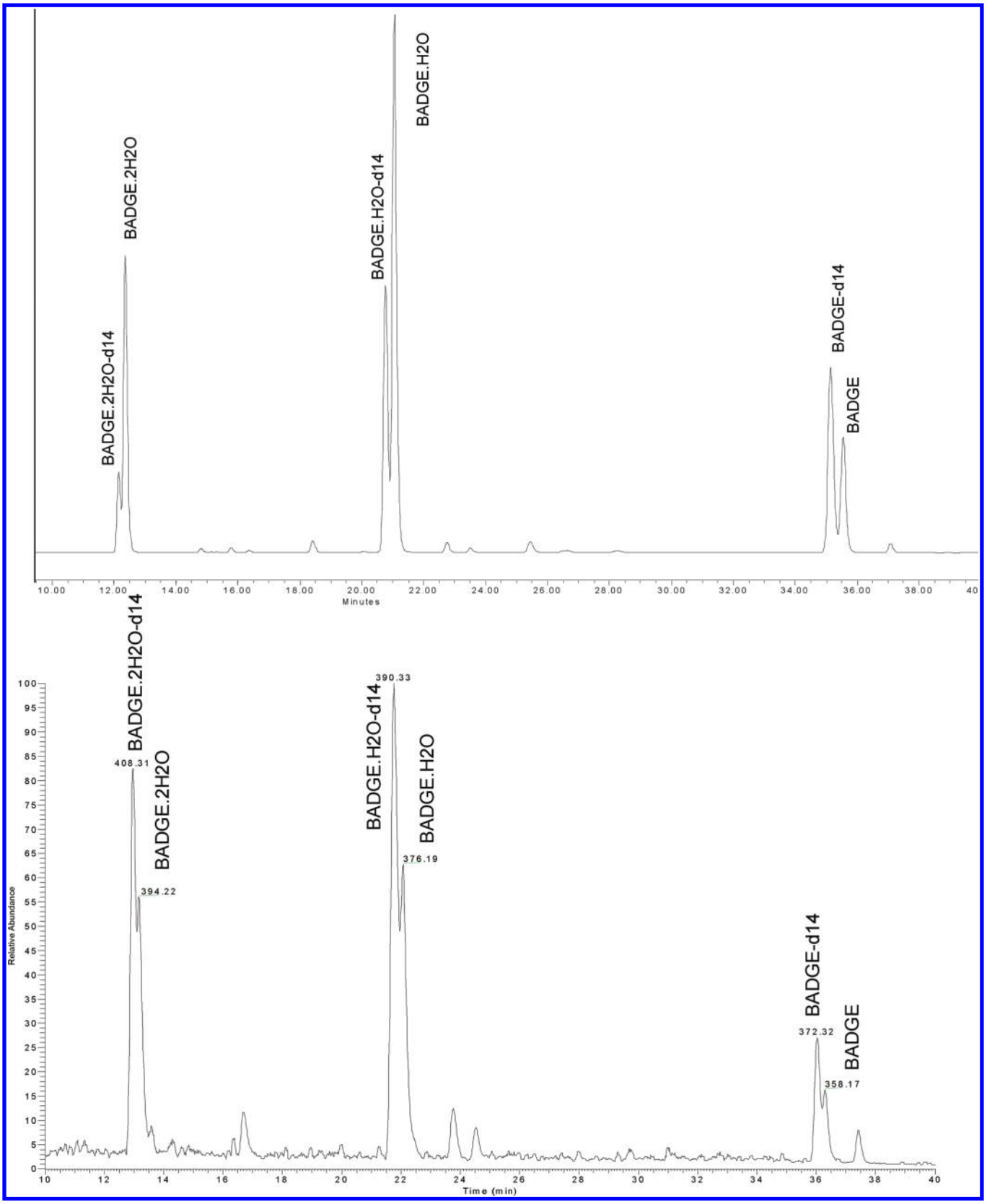

Figure 4. (**A**) LC-FLD and (**B**) LC-FT-MS chromatogram of a standard solution of BADGE and BADGE-d14 and their hydrolysis products.

the main centers of reactivity, resulting in the "addition" of new molecules to BADGE via the epoxide groups as was confirmed by the model studies with glucose and amino acids. Isotopically labeled BADGE, that is, BADGE-d14, was used together with nonlabeled BADGE. **Figure 4** shows LC-FLD and LC-MS chromatograms of BADGE, BADGE-d14, and their hydrolysis products in which clearly three doublets of peaks could be observed. The mass difference of m/z 14 due to the 14 deuterium atoms resulted in a partial chromatographic separation of the labeled and nonlabeled BADGE. With LC-MS it can be seen that the mass difference between the two peaks in the doublets is $\Delta m/z$ 14, thereby confirming the expected differences between BADGE

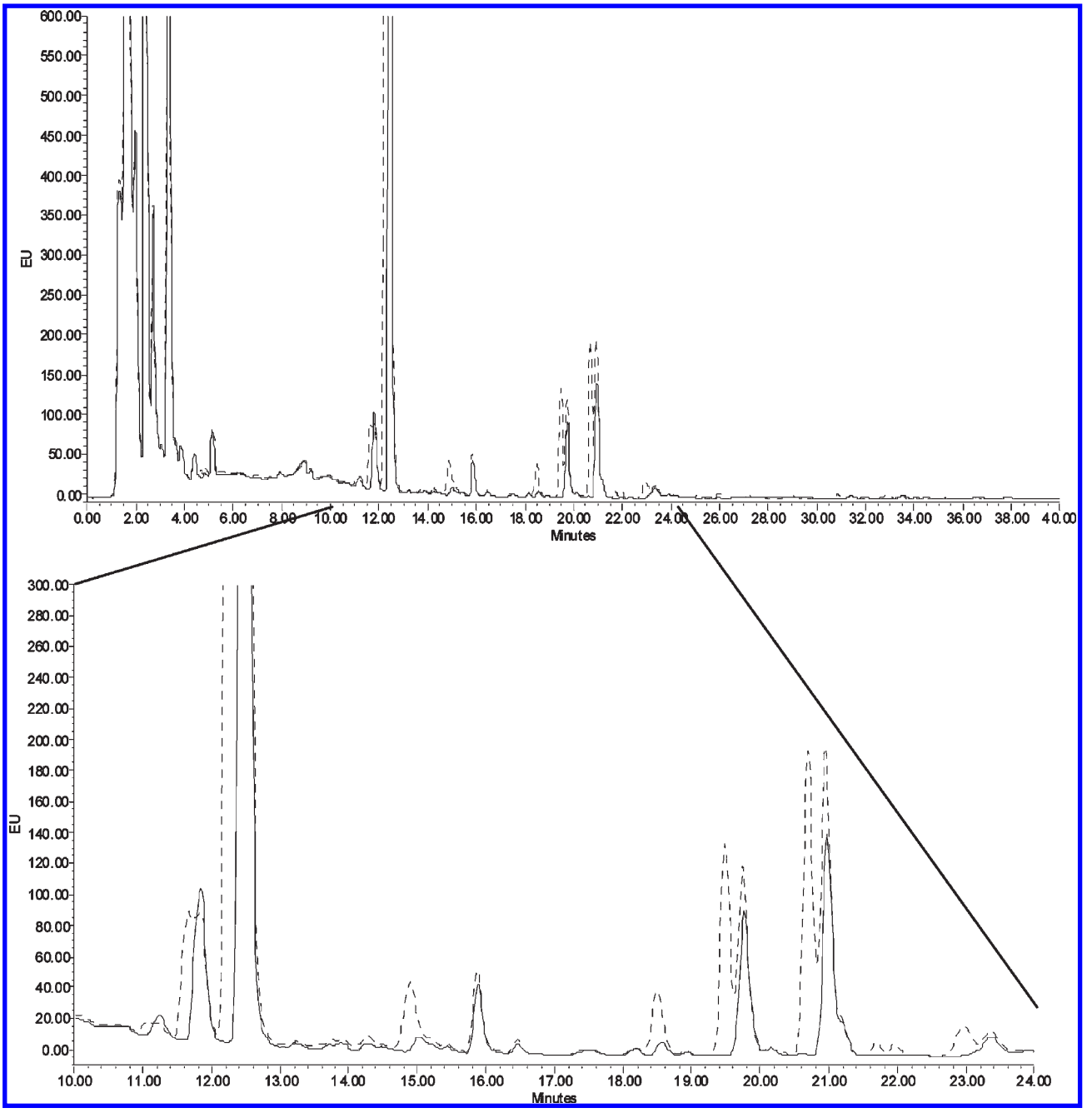

Figure 5. Overlay of LC-FLD chromatograms of direct analysis of stout spiked with BADGE (—) and with BADGE and BADGE-d14 (- - -).

and BADGE-d14. Further experiments showed that BADGE and BADGE-d14 had sensitivities similar to one another with both fluorescence and MS detection.

Mixtures of BADGE and BADGE-d14 were spiked into foods and were subsequently stored. Analysis of extracts of the food will result in numerous peaks that can be due to food ingredients themselves, contaminants introduced due to sample workup, or BADGE reaction products. However, BADGE and BADGE-d14 will react with the same food ingredients, and thus reaction products of BADGE were selectively profiled using the formation of doublets with LC-FLD and/or the mass difference of $\Delta m/z$ 14 with LC-MS (**Figures 5** and **6**).

Analysis of Foods by LC-FLD and LC-FT-MS: Mass Balance Studies and Identification of Reaction Products. The foods to which BADGE and BADGE-d14 were spiked prior to storage were analyzed by both LC-FLD and LC-FT-MS to screen for reaction products of BADGE with low molecular weight food ingredients. **Figures 5** and **6** show examples of LC-FLD and LC-MS chromatograms obtained by direct analysis of stout. The additional peaks in the sample spiked with BADGE and BADGE-d14 can be clearly seen in both figures. Moreover, the sample spiked with BADGE and BADGE-d14 shows the expected doublets with LC-FLD and the mass difference of m/z 14 with LC-MS. Using this approach, peaks in the LC-FLD chromatograms were identified as being reaction products of BADGE. As the exact identities of the additional peaks were not known, the levels present were quantified by comparing their peak areas with those derived from the analysis of standard solutions of BADGE, BADGE·H_2O, or BADGE·$2H_2O$. It was shown earlier that BADGE-related products have very similar fluorescent responses, more or less independent of the food ingredient. In this way the concentrations of peaks related to BADGE were added to calculate the recovery of BADGE (and related products) after storage (**Table 3**). The resulting recoveries

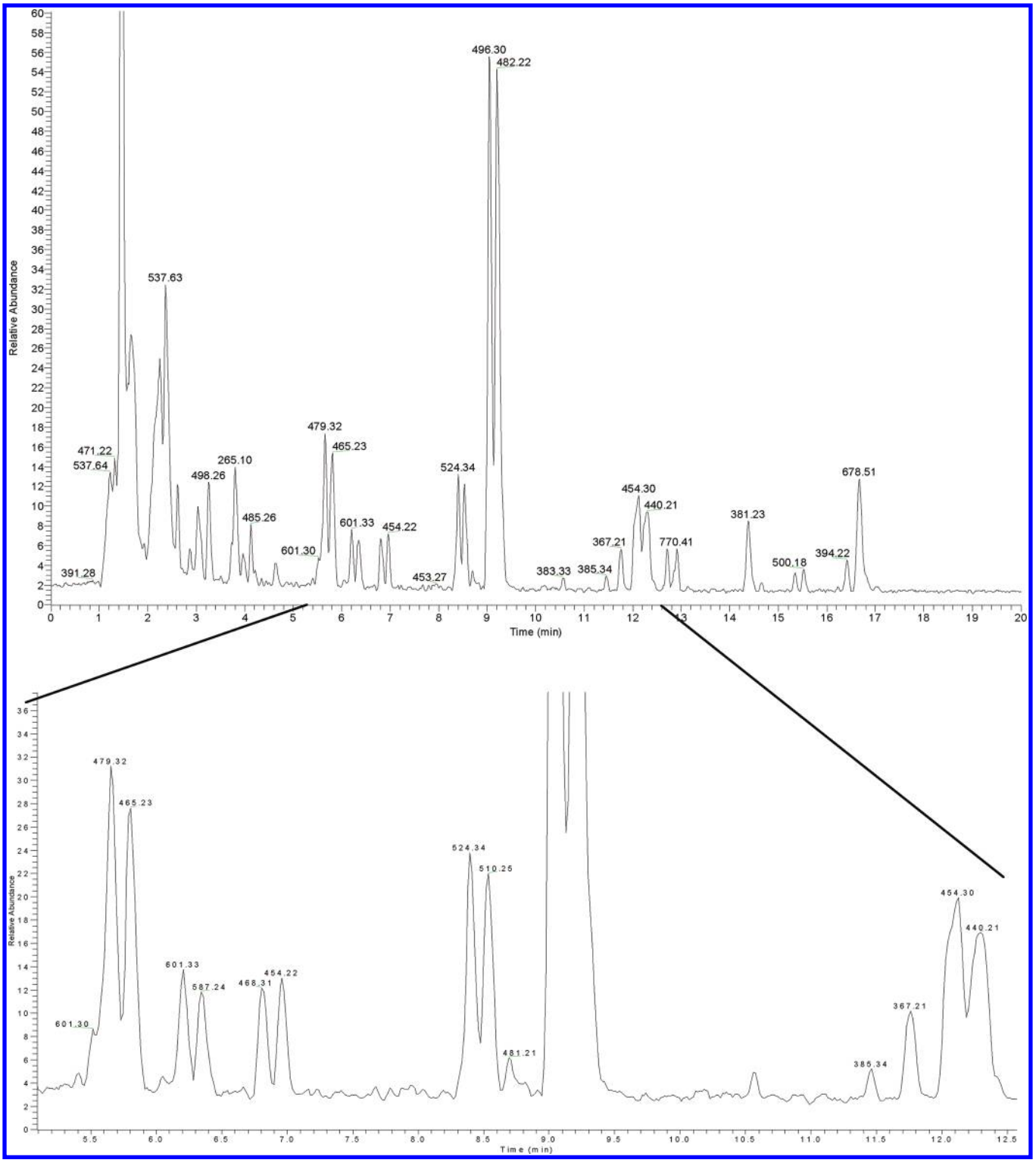

Figure 6. Base peak LC-FT-MS chromatograms of direct analysis of stout spiked with BADGE and BADGE-d14.

of BADGE were significantly higher, especially for lager, stout, and ale, compared to the recoveries observed earlier in **Table 1**. Therefore, it can be concluded that the approach described here, using isotopically labeled BADGE to identify BADGE-related products in food (extracts) and fluorescence detection to quantify, is successful in improving the mass balance of compounds in complex samples.

LC-FT-MS was also used to identify the reaction products observed for BADGE in the different foods. From the exact mass possible elemental compositions were obtained. The number of possible elemental compositions was reduced by making some assumptions and using isotope information. The calculated elemental compositions minus that of BADGE (or its hydrolysis products) was the input for a database search with the aim of identifying the BADGE reaction products. Some peaks could be tentatively identified with this approach, for example, reaction products of BADGE with ethanol and aminophenol in the beer samples. In apple puree, reaction products of BADGE with carbohydrates, for example, glucose and/or fructose, could be identified. This is contrast with Petersen et al. (*10*), who did not

Table 3. Recovery of BADGE and Its Reaction Products in the Different Foods and Drinks after Spiking (20 mg/kg), Canning, and Storing

sample	storage	recovery (%)
tuna in oil	13 weeks at 50 °C	9
apple puree	13 weeks at 50 °C	13
ale	2 months at 40 °C	46
lager	2 months at 40 °C	62
stout	2 months at 40 °C	36

Table 4. BADGE Adducts of Tryptic Peptides of Insulin Found by LC-MS/MS after Trypsin Digestion and Database Searching

m/z	origin
708	peptide 25−47 (β-chain) + BADGE (4+)
943	peptide 25−47 (β-chain) + BADGE (3+)
1415	peptide 25−47 (β-chain) + BADGE (2+)
894	peptide 85−105 (α-chain) + BADGE (3+)
1340	peptide 85−105 (α-chain) + BADGE (2+)
1510	peptide 85−105 (α-chain) + 2BADGE (2+)
1348	peptide 85−105 (α-chain) + BADGE·H_2O (2+)
1519	peptide 85−105 (α-chain) + BADGE·H_2O + BADGE (2+)

find any reaction products of BADGE with carbohydrates in canned peaches. Further analysis using, for example, MS^n, together with detailed knowledge of the composition of the different foods would be necessary for further identification.

Although the results in **Table 3** show that a significant improvement of the recovery has been achieved with the approach described here, still not all of the BADGE was accounted for, especially for tuna and apple puree. The most likely explanation for this is the fact that so far only low molecular weight compounds are taken into account. Apple puree consists mainly of carbohydrates, including polysaccharides, and fibres. These high molecular weight compounds cannot be detected by the methods applied here. It is likely that BADGE can react with these compounds because of its reactivity toward glucose. Furthermore, tuna consists largely of proteins; it has been demonstrated that BADGE reacts with amino acids, and it is likely that BADGE will also react with proteins, which has been described recently in the literature (*10*).

Reactivity of BADGE with Proteins. To verify whether BADGE reacts with proteins, both model studies and experiments with food samples were carried out. Reaction of BADGE with BSA and insulin was carried out using a proteomics approach, that is, digestion using trypsin followed by LC-MS/MS analysis and Mascot database searching. During database searching, BADGE was added as a post-translation modification. For insulin and BSA, tryptic peptides were found to which BADGE was attached (**Tables 4** and **5**). These peptides were confirmed by extracting the corresponding ions in the LC-MS measurements. For insulin, BADGE reacted with peptide 25−47 (β-chain) and peptide 85−105 (α-chain). The latter peptide also showed a reaction product with two BADGE molecules. From the experiments with amino acids, it was observed that cysteine showed the highest reactivity toward BADGE. The insulin peptides that reacted with BADGE both contain cysteine residues, that is, two for peptide 25−47 (β-chain) and four for peptide 85−105 (α-chain). For BSA 11 BADGE adducts of tryptic peptides were observed (**Table 5**). All of these tryptic peptides contained at least one cysteine residue. However, it was not possible from those experiments to determine exactly which amino acids in the tryptic peptides were attached to BADGE.

To that purpose digestion with Pronase was carried out, which usually results in single amino acids and small peptides. The resulting Pronase digests of proteins and BADGE were searched specifically for the two main reaction products of cysteine and BADGE, that is, BADGE·2Cys and BADGE·H_2O·Cys (**Table 2**). For both insulin and BSA both reaction products were observed. No other reaction products of BADGE and amino acids were found. These results confirm that BADGE reacts with proteins and that BADGE reacts mainly with the cysteine residues in proteins.

Table 5. BADGE Adducts of Tryptic Peptides of BSA Found by LC-MS/MS after Trypsin Digestion and Database Searching

m/z	origin
521	peptide 198−204 + BADGE (2+)
676	peptide 413−420 + BADGE (2+)
453	peptide 310−318 + BADGE (3+)
465	peptide 460−468 + BADGE (3+)
440	peptide 298−309 + BADGE (4+)
621	peptide 139−151 + BADGE (3+)
653	peptide 118−130 + BADGE (3+)
988	peptide 184−197 + BADGE (2+)
669	peptide 469−482 + BADGE (3+)
731	peptide 529−544 + BADGE (3+)
768	peptide 139−155 + BADGE (3+)

Finally, isolated proteins from tuna after reaction with BADGE were digested using trypsin and Pronase followed by LC-MS(/MS) analysis. Database searching of the tryptic peptides did not show any reaction products. This is likely due to the low abundance of these peptides as a result of the large difference between the amount of protein in tuna, that is, 26 wt % or 260 g/kg, and the amount of BADGE spiked to tuna, that is, 20 mg (or 0.02 g)/kg. However, in the Pronase digest of tuna the reaction product BADGE·H_2O·Cys was detected, showing that BADGE has reacted with proteins in tuna. If this approach can be made quantitative, the mass balance for BADGE could be improved further by taking into account reaction products with high molecular weight compounds such as proteins.

All of these findings have relevance in the future of migration testing for materials and articles intended for contact with foods and sold in the European Union. Although the example studied here involved the reaction products of the epoxy coating ingredient BADGE, reaction products may be formed with food for any reactive migrant. Although the unidentified reaction products are likely to be high molecular weight products of >1000 Da and therefore of no or lesser toxicological relevance, they may be broken down into smaller fragments in the stomach. These smaller fragments may be toxicologically important if they are sufficiently small to be absorbed from the gastrointestinal tract. Therefore, knowledge of the reactivity and fate of chemical migrants in food is important, and this work demonstrates some of the analytical tools available to study this aspect of food safety.

ABBREVIATIONS USED

BADGE, bisphenol A diglycidyl ether; LC, liquid chromatography; FLD, fluorescence detection; MS, mass spectrometry; FT, Fourier transform; FA, formic acid; TFA, trifluoroacetic acid; ESI, electrospray ionization; BSA, bovine serum albumin; BFDGE, bisphenol F diglycidyl ether; Cys, cysteine; Met, methionine; Lys, lysine; Tyr, tyrosine; His, histidine.

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