FOOD CHEMICAL CONTAMINANTS

Identification of Derivatives of Bisphenol A Diglycidyl Ether and Novolac Glycidyl Ether in Can Coatings by Liquid Chromatography/Ion Trap Mass Spectrometry

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A reversed-phase liquid chromatographic method combined with fluorescence and multiple mass spectrometric detection in series is presented for the separation and structure elucidation of bisphenol A diglycidyl ether (BADGE) and novolac glycidyl ether (NOGE) derivatives. Atmospheric pressure chemical ionization in the positive ion mode and collision induced fragmentation in the ion trap allowed identification of BADGE- and NOGE-related compounds originating from reactions of the glycidyl ethers with bisphenols, solvents, and chain stoppers. Two extracts from food-can coatings were investigated in detail. It was possible to elucidate the structures of many substances and consequently to draw conclusions about the production of the lacquers.

A variety of different materials are used for the internal coatings of food and beverage cans. Epoxy polymers are the types of lacquers most widely used in food preservation. Bisphenol A diglycidyl ether (BADGE) is a common starting material for such epoxy resins (Figure 1). BADGE and novolac glycidyl ether (NOGE, also known as epoxy novolac) are also used as additives (1) to polyesters and as scavengers for hydrochloric acid formed by the degradation of polyvinyl chloride (PVC)-based lacquers. NOGE, the technical reaction product of formaldehyde, phenol, and epichlorohydrin, contains a mixture of compounds with 2 or more aromatic rings. The 2-ring product of NOGE, bisphenol F diglycidyl ether (BFDGE), consists of the 3 isomers *p,p′*-, *o,p′*-, and *o,o′*-BFDGE. BADGE is synthesized correspondingly from acetone, phenol, and epichlorohydrin. It consists almost exclusively of the *p,p′*-isomer as well as further reaction products such as di-BADGE, tri-BADGE (linear or branched), etc. (Figure 1).

In a first polymerization step, the technical mixture of BADGE reacts with bisphenol A and a chain-terminating agent (usually a phenol) to form the epoxy resin. An applicable lacquer is obtained by dissolving the resin in an alcoholic or glycolic solvent together with curing agents. The catalyst, curing time, and temperature influence the degree of cross-linking in the coating (2, 3).

During this process, the glycidyl ethers may react with the hydroxyl groups of chain-terminating phenols, solvents (4, 5), or catalysts. The compounds formed are readily soluble in edible oils and food lipids, and also to a substantial extent in water during heat treatment of food (sterilization). Depending on the degree of cross-linking in the resin (3), these substances migrate into food (6–11), and even more reaction products with food components are formed (12). The complex mixture of substances generated by all possible reactions and the number of isomers formed make the structural identification of single components very difficult.

Reversed-phase (RP) or normal-phase (NP) liquid chromatography (LC) with fluorescence detection (FD) is the most frequently used technique for determining BADGE (6–10, 13–16), its hydrolysis and hydrochlorination products (7, 8, 15–17), as well as BFDGE (14, 16) and its H_2O- and HCl-adducts (16) in aqueous solvents, uncured epoxy resins, and various food matrixes. The diglycidyl ethers and their reaction products with relatively low molecular weights can also be determined by gas chromatography (GC) with detection by mass spectrometry (MS; 4, 6, 8–10, 12, 16, 18–21). Di- and tri-BADGE, 3-ring-NOGE, and substances with higher molecular weights can also be separated and detected by LC/FD (5, 7, 19) and size-exclusion chromatography/FD (1, 5). In standards and in extracts from lacquers, fish, and soup, BADGE and its hydrolysis products (20–23), di- and tri-BADGE (7, 22), and BFDGE and its H_2O-adducts (18) were also determined by RP–LC/MS techniques with electrospray, thermospray, or atmospheric pressure chemical ionization (APCI).

In 1990, the European Community restricted the residual level of BADGE in plastic materials in contact with food to 1 mg/kg. Furthermore, migration into food or food surrogates should not give detectable concentrations at a detection limit of 0.02 mg/kg (24). Because of the lack of evidence concerning the carcinogenic effects of BADGE in in vivo studies, the Scientific Committee for Food of the European Union provisionally increased the tolerated migration of the sum of BADGE and BADGE + H_2O to 1 mg/kg food in 1996. In 1999, a legal limit of 1 mg migrant/kg food was introduced, including

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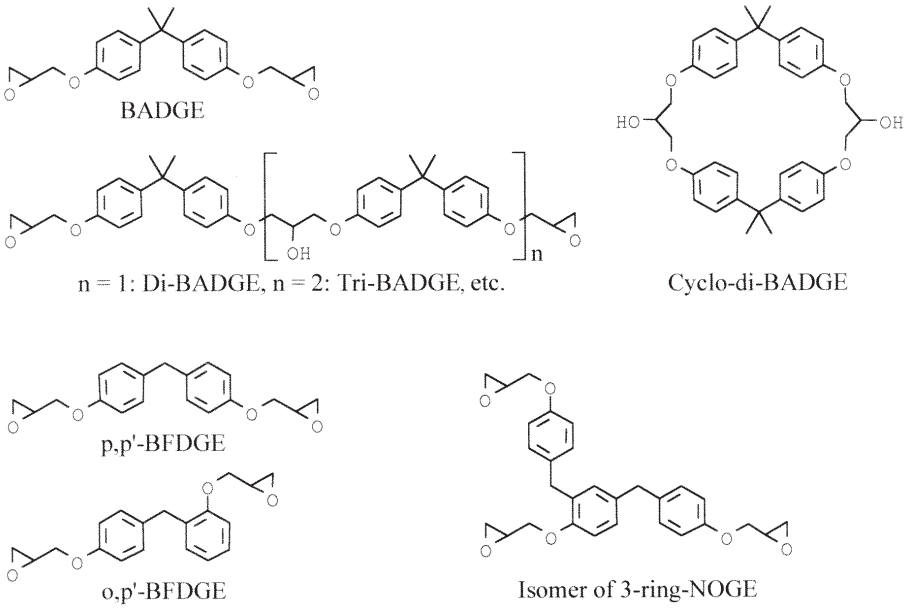

Figure 1. Structures of the technical reaction products of BADGE and NOGE.

the sum of BADGE, BADGE + H_2O, BADGE + HCl, BADGE + H_2O + HCl, and BADGE + 2HCl. Various surveys of BADGE have been performed in different European countries (8–11).

Although several working groups reported a "forest" of peaks in their chromatograms (15, 19, 25), little effort was focused on the separation, structure elucidation, and quantitation of compounds with a molecular weight of >500 u. This might have been because of the lack of structural information from LC/FD and the insufficient volatility of these substances that precluded GC/MS analysis. However, BADGE usually represents only a small fraction of the material migrating into food, and nothing is known about the toxicity of all the other derivatives. In general, epoxides and chlorinated molecules may represent a health hazard.

The purpose of this study was to develop an LC method that combines the high selectivity of FD for bisphenol derivatives with the ability of multiple mass spectrometry (MS^n) to obtain structural information. The MS^n technique allows multiple trapping of daughter ions and their consecutive fragmentation, enabling the online structure elucidation of unidentified substances. Solvent extracts of food-can coatings made from epoxy resins as well as organosols were investigated. The work focused on the identification of bisphenol derivatives with molecular weights between 500 and 1200 u. This mass range allowed us to obtain information about the type of lacquer used.

Experimental

Materials

(**a**) *Standards*.—BADGE (≥97%) and BFDGE (total of the 3 isomers ≥97%) were purchased from Fluka Chemie AG (Buchs, Switzerland).

(**b**) *Ammonium acetate*.—Analytical grade, obtained from E. Merck AG (Darmstadt, Germany).

(**c**) *Solvents*.—Acetonitrile for extraction of can coatings (LC grade) was purchased from Gebrüder Mächler AG (Basel, Switzerland) and for LC separation (190 nm, far UV), from Romil Ltd. (Cambridge, UK). Water was obtained from an Elgastat Maxima LC water purification unit (Elga Ltd., Bucks, UK). Deuterium oxide (99.9%) was provided by Cambridge Isotope Laboratories (Andover, MA).

(**d**) *Gases*.—Helium of 99.996% purity and nitrogen of 99.995% purity (Carbagas, Switzerland) were used.

(**e**) *Cans*.—The BADGE-based can was obtained directly from a food-can factory in Switzerland in May 1997. The PVC-based can, filled with sweet corn, was purchased in a local supermarket early in 1998. The BADGE-based can was a 3-piece can; the PVC-based can was manufactured from 2 pieces (deep-drawn can).

Sample Solutions for Ion Adduct Formation

The following sample solutions were prepared: BADGE at 10 ng/μL in (A) acetonitrile; (B) acetonitrile–water (50 + 50); (C) acetonitrile–deuterium oxide (50 + 50); (D) 10mM ammonium acetate (NH_4OAc) in acetonitrile–water (50 + 50); and (E) 10mM NH_4OAc in acetonitrile–deuterium oxide (50 + 50). The same solvents (A–E) were used to prepare sample solutions of BFDGE (containing 3 isomers of BFDGE and minor amounts of 3-ring-NOGE) and technical BADGE (containing BADGE, di-BADGE, and linear and branched tri-BADGE), all at 10 ng/μL, and unknown concentrations of the extracts of the BADGE-based and the PVC-based cans.

Stability of Sample Solutions

Solutions of BADGE, NOGE, and related compounds in acetonitrile are stable for several months when kept in the re-

frigerator. In aqueous media, BADGE and BFDGE hydrolyze slowly and compounds of higher molecular weight decompose. Therefore, water or deuterium oxide solutions were prepared immediately before use.

Apparatus

(**a**) *LC system*.—A low-pressure binary-gradient LC pump (Rheos 4000, Flux Instruments, Basel, Switzerland) was used. The samples were injected with a Valco Cheminert valve equipped with a 25 μL loop made from 0.18 mm id PEEK tubing. LC separation was performed on a C_{18} high-density phase (Nucleosil, 10 nm pore size, 3 μm particles, 125 mm column length, 3 mm id; Macherey-Nagel, Oensingen, Switzerland). The flow rate of the mobile phase was 500 μL/min. The following binary gradient was applied: A, acetonitrile, B, water; 0–0.5 min, 50% A; 0.5–4 min, 50–75% A; 4–15 min, 75–99% A; 15–30 min, 99% A; 30–31 min, 99–50% A; isocratic re-equilibration for 7 min.

(**b**) *FD system*.—A fluorescence detector (FL 3000, Thermo Separation Products, San Jose, CA) was used. Excitation and emission wavelengths were 224 and 294 nm, respectively. The total volume of the flow cell was 10 μL (8 μL illuminated volume). The flash rate of the xenon lamp was set to 100 Hz. The fluorescence detector and the mass spectrometer were coupled in series.

(**c**) *MS detection system*.—An ion trap mass spectrometer (LCQ, Finnigan MAT, San Jose, CA) was used in the positive ion mode employing atmospheric pressure chemical ionization (APCI[+]). Mass spectra were recorded in the full-scan mode (mass range, 150–2000 u). The following instrument parameters were applied: heater temperature, 400°C; nitrogen sheath gas flow, 40 arbitrary units (corresponding to ca 400 mL/min); and ionization current of corona discharge, 5 μA. Voltages of the following devices were optimized by the autotune program to achieve maximum transmission of the $[M+NH_4]^+$ ion of di-BADGE: heated capillary; tube lens offset; octapole 1 offset; inter-octapole lens; octapole 2 offset; and octapole rf amplitude. The temperature of the capillary between the ionization chamber and the first vacuum stage was held at 150°C. Ion adduct formation experiments were performed by direct injection of 50 μL of the sample solutions in the mass spectrometer with an acetonitrile flow of 500 μL/min.

(**d**) *MS^n experiments*.—MS^n experiments were performed by repeated collision-induced decays in the trap by applying a relative collision energy between 35 and 45% and an isolation width of 3 u. Samples were dissolved in acetonitrile and transferred with a syringe pump at a flow rate of 10 μL/min via a T-piece to an acetonitrile–water (75 + 25) flow of 490 μL/min. Chlorine isotope patterns were registered in MS^n spectra by isolating the parent ions within a broader mass range of 5–7 u. Direct-injection MS^n experiments saved a lot of time compared with the time required for online MS^n in a chromatographic run. However, in some cases several substances with the same nominal molecular weight were present in the extract. Therefore, additional MS^2 spectra were generated by online fragmentation of the most abundant ion after LC separation.

Extraction of Can Coatings

The can coatings were extracted by the Official Food Control Authority in Zurich (Kantonales Labor Zürich, Switzerland). Bronz et al. (5) claimed that acetonitrile is a good surrogate for oily can contents in the extraction of compounds with a molecular weight of <1000 u. The lids were opened less than halfway. The PVC-based sweet corn can was emptied and washed with warm water and detergents before extraction. The cans were laid on their sides, half-filled with acetonitrile, and stored for 24 h at room temperature. In this way, an extract of all surfaces was obtained. Typically ca 5–30 mg/dm^2 migrated from the can coatings into the acetonitrile. However, it was not our intention to achieve complete extraction. For LC separation, the acetonitrile extract was mixed with an equal volume of water. When precipitation occurred, the sample

Table 1. Influence of solvent on the formation of ions from BADGE, BFDGE, and related compounds by APCI(+)–MS[a]

Solvent	BADGE	BFDGE[b]	Compounds with M > 400 u[c]
Acetonitrile	$[M + 42]^+$ (100)	$[M + 42]^+$ (100)	$[M + 18]^+$ (100)
	$[M + 18]^+$ (5)	$[M + 18]^+$ (30)	$[M + 1]^+$ (2–10)
	$[M]^{\bullet+}$ (20)	$[M]^{\bullet+}$ (10)	
Acetonitrile–water (50 + 50)	$[M + 42]^+$ (100)	$[M + 18]^+$ (100)	$[M + 18]^+$ (100)
	$[M + 18]^+$ (90)	$[M + 42]^+$ (30)	$[M + 1]^+$ (10–20)
	$[M]^{\bullet+}$ (15)		
Acetonitrile–deuterium oxide (50 + 50)	$[M + 43]^+$ (100)	$[M + 22]^+$ (100)	$[M^* + 22]^+$ (100)[d]
	$[M + 22]^+$ (90)	$[M + 43]^+$ (30)	
	$[M]^{\bullet+}$ (15)		

[a] Relative intensities are shown in parentheses.
[b] Mean of the 3 isomers.
[c] Except BADGE + 2HCl, cyclo-di-, and cyclo-tetra-BADGE.
[d] Hydrogens of the hydroxyl groups in M* were replaced by deuterium.

was further diluted with acetonitrile–water (50 + 50) to obtain a clear solution.

Results and Discussion

Ion Adduct Formation in the APCI(+) Mode

The ions formed from BADGE, BFDGE, and related compounds in APCI(+)–MS are shown in Table 1 together with their relative intensities, which depended on the solvent. To differentiate between $[M + H_2O]^+$ and $[M + NH_4]^+$ ion adduct formation resulting in the same mass, deuterium oxide instead of water was added to the acetonitrile mixtures. $[M + 1]^+$ is the proton adduct, and $[M + 22]^+$, $[M + 42]^+$, and $[M + 43]^+$ are $[M + ND_4]^+$, $[M + \text{acetonitrile} + H]^+$ and $[M + \text{acetonitrile} + D]^+$ ion adducts, respectively. $[M + 18]^+$ is the $[M + NH_4]^+$ ion adduct from ammonium generally present in the background. It is not the $[M + H_2O]^{\cdot+}$ ion adduct, because no deuterium oxide adduct $[M + D_2O]^{\cdot+}$ is formed in acetonitrile–deuterium oxide, and the ratios between the relative intensities of $[M + 22]^+$

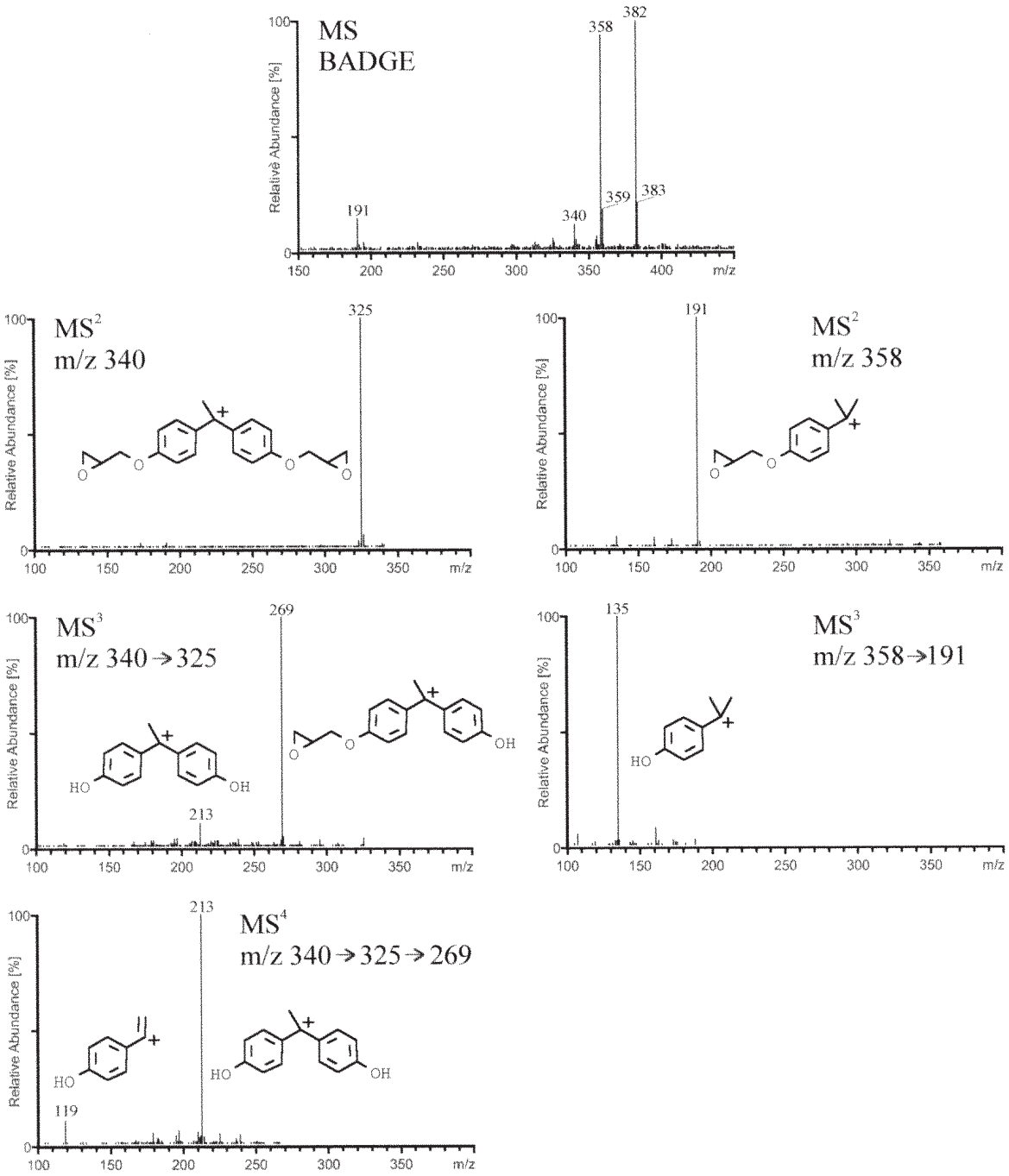

Figure 2. MS to MS^4 spectra of the main fragmentation pathways of BADGE. Structures of fragments characteristic of BADGE derivatives are shown.

and $[M + 43]^+$ are the same as the ratios between $[M + 18]^+$ and $[M + 42]^+$ in acetonitrile–water. Comparison of the relative intensities of the ion adducts in pure acetonitrile and in acetonitrile–water (50 + 50; Table 1) shows that the presence of a protonic solvent is necessary for the formation of abundant proton and ammonium adducts.

Only relatively small compounds with a molecular weight of <400 u and ≥1 epoxy group (such as BADGE and BFDGE) formed strong $[M + \text{acetonitrile} + H]^+$ ions. All other substances in technical BADGE and the can-coating extracts exclusively formed $[M + NH_4]^+$ as the base ion and a proton adduct of 10–20% relative abundance. BADGE + 2HCl and cyclo-di-BADGE were exceptions, forming $[M]^{\bullet+}$ and $[M + H]^+$, respectively, as the base ion. In addition, the MS response factors of BADGE + 2HCl and cyclo-di- and cyclo-tetra-BADGE were low compared with those of structures forming abundant $[M + NH_4]^+$ ions.

The addition of ammonium acetate resulting in a final concentration of 10mM NH_4^+ in acetonitrile–water (50 + 50) increased the intensities of the $[M + NH_4]^+$ ions by a factor of 2 and inhibited the formation of $[M + H]^+$ ions. This suppressed the ionization of cyclic compounds such as cyclo-di- and cyclo-tetra-BADGE and made a determination of the molecular weight of unidentified substances more difficult. Therefore, ammonium acetate was not used as an additive.

MS^n Fragmentation Pathways

BADGE.—A collision-induced decay (CID) of BADGE in the ion trap led to 2 different fragmentation pathways, as shown by the corresponding MS^n spectra in Figure 2. The first pathway was initiated by the loss of a methyl group from $[M]^{\bullet+}$ (*m/z* 340), generating an ion of *m/z* 325. MS^3 of *m/z* 325 resulted in the loss of 56 u (C_3H_4O) to *m/z* 269, which decayed after a second cleavage of 56 u to the bisphenol (*m/z* 213). In the next step, the loss of phenol resulted in *m/z* 119.

The second fragmentation started from the $[M + NH_4]^+$ ion adduct (*m/z* 358). Cleavage of a bond between the central carbon atom and an aromatic ring resulted in *m/z* 191, which formed *m/z* 135 by MS^3. This dominant fragmentation pathway generated the key ions characteristic of BADGE-related

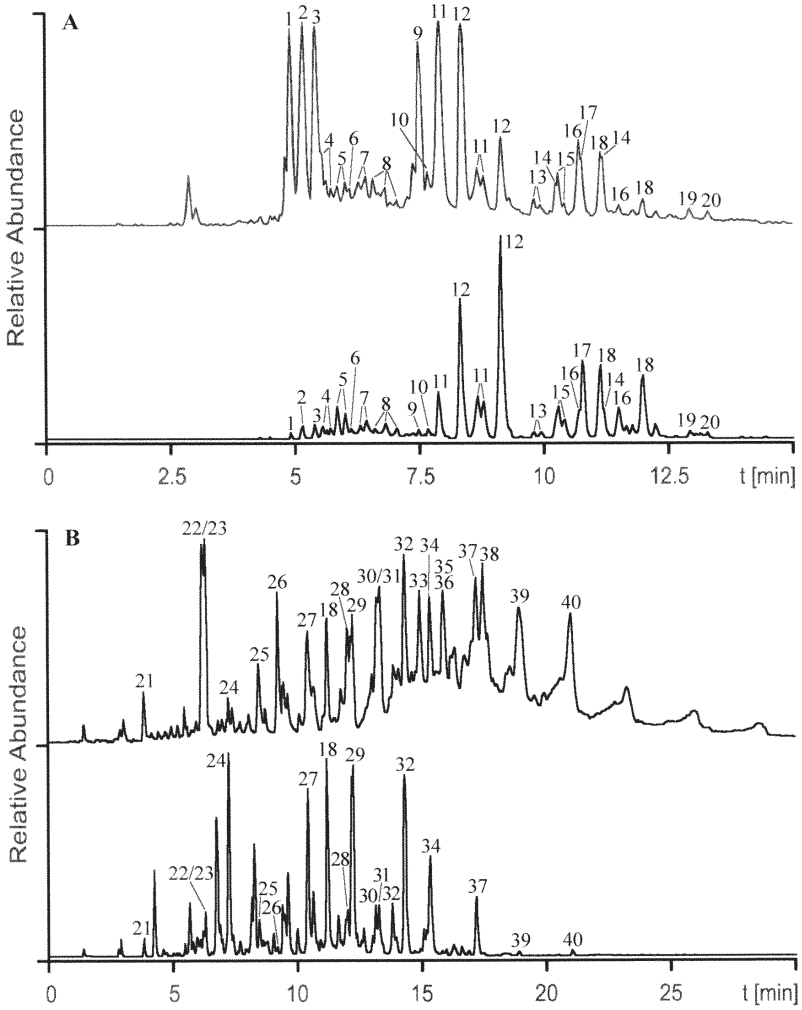

Figure 3. Chromatograms of lacquer extracts from (A) a PVC-based can and (B) a BADGE-based can. Top: FD detection; bottom: MS base peak chromatogram (range, *m/z* 150–2000). Compound identification is summarized in Tables 2 and 3.

Table 2. Pseudo-molecular ions of the compounds identified in the extract from the PVC-based lacquer and the most important fragments formed by online MS^2 of the pseudo-molecular ions

Compound[a]	MW[b]	Structure	Pseudo-molecular ions $[M+NH_4]^+$, *m/z*	Most important fragments formed by online MS^2, *m/z* (relative abundances)[c]
1	412	BADGE + 2HCl	412 + 414 + 416[d] $[M]^{\bullet+}$	Ion intensity too low for MS^2
2	376	BADGE + HCl	418 + 420[c] [M + AcCN[e] + H]$^+$	135 (20), 173 (10), 191 (10), 227 (100), 232 (10), 376 (10)
3	340	BADGE	382 [M + AcCN[e] + H]$^+$	135 (20), 173 (30), 191 (100), 232 (40), 340 (10)
4	510	3-ring-NOGE + HCl	528 + 530[d]	163 (30), 199 (50), 343 (100), 361 (70), 387 (20), 493 (20), 511 (10)
5	474	3-ring-NOGE	492	163 (50), 189 (40), 277 (30), 295 (100), 307 (60), 325 (40), 351 (30), 457 (50), 475 (10)
6	708	4-ring-NOGE + 2HCl	726 + 728 + 730[d]	305 (30), 361 (90), 397 (40), 559 (20), 691 (100), 709 (20)
7	672	4-ring-NOGE + HCl	690 + 692[d]	263 (20), 307 (30), 325 (20), 361 (60), 505 (50), 655 (100), 673 (20)
8	636	4-ring-NOGE	654	277 (30), 307 (30), 325 (100), 619 (40), 637 (20)
9	696	Di-BADGE + 2HCl	714 + 716 + 718[d]	227 (40), 511 (100), 679 (20), 697 (30)
10	530	3-ring-BADGE	548	191 (30), 381 (100)
11	660	Di-BADGE + HCl	678 + 680[d]	191 (20), 227 (20), 475 (50), 511 (100), 643 (20), 661 (40)
12	624	Di-BADGE	642	191 (20), 325 (30), 457 (20), 475 (100), 607 (20), 625 (10)
13	886	5-ring-BADGE + 2HCl	904 + 906 + 908[d]	515 (10), 551 (30), 701 (100), 737 (30)
14	980	Tri-BADGE + 2HCl	998 + 1000 + 1002[d]	511 (100), 795 (40), 981 (10)
15	850	5-ring-BADGE + HCl	868 + 870[d]	515 (20), 551 (20), 683 (10), 701 (100)
16	944	Tri-BADGE + HCl	962 + 964[d]	475 (10), 511 (20), 609 (20), 741 (20), 759 (30), 795 (100), 927 (20), 945 (40)
17	814	5-ring-BADGE	832	365 (10), 381 (10), 475 (10), 515 (60), 647 (20), 665 (100), 815 (10)
18	908	Tri-BADGE	926	325 (20), 475 (50), 569 (30), 609 (40), 741 (50), 759 (100), 873 (40), 891 (30), 909 (60)
19	1134	7-ring-BADGE + HCl	1152 + 1154[d]	515 (40), 665 (100), 701 (70), 931 (60), 967 (80), 1117 (80), 1135 (20)
20	1098	7-ring-BADGE	1116	515 (40), 649 (30), 665 (100), 913 (40), 931 (50), 949 (50), 1099 (40)

[a] *See* Figure 3A for signal assignment.
[b] MW = molecular weight.
[c] Relative abundances of fragments from different isomers may vary within ±10%.
[d] Chlorine isotope pattern.
[e] Acetonitrile.

compounds. All ions mentioned earlier were also present in the electron ionization mass spectrum of the reaction product of BADGE with phenol (4).

BFDGE.—The fragmentation of BFDGE was more complex because no central quaternary carbon atom was present to stabilize the positive charge. MS^2 of $[M + NH_4]^+$ (*m/z* 330) mainly generated *m/z* 163 and 189. Analogous to *m/z* 191 formed from BADGE, the BFDGE fragment *m/z* 163 lost 56 u (C_3H_4O) by MS^3 to form *m/z* 107. The structure of *m/z* 189 and its further fragmentation product *m/z* 133 (again loss of 56 u) could not be elucidated. All 4 daughter ions, *m/z* 189, 163, 133, and 107, are typical of NOGE-related compounds.

Fluorescence Detection versus Mass Spectrometric Detection

The response factors of compounds with various molecular weights and functional groups are similar in FD because the response is mainly dependent on the presence of the fluorophor bisphenol (5, 16). FD shows good selectivity for bisphenol structures. Therefore, an FD chromatogram gives a good im-

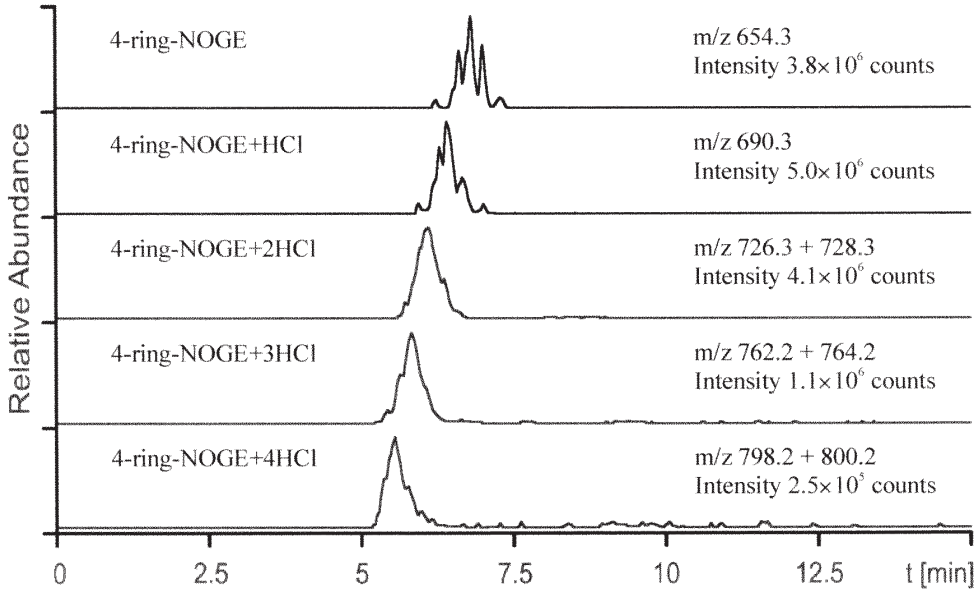

Figure 4. Mass chromatograms of the ammonium adducts of 4-ring-NOGE and its HCl-adducts in the extract from the PVC-based can. Incompletely resolved isomers are present.

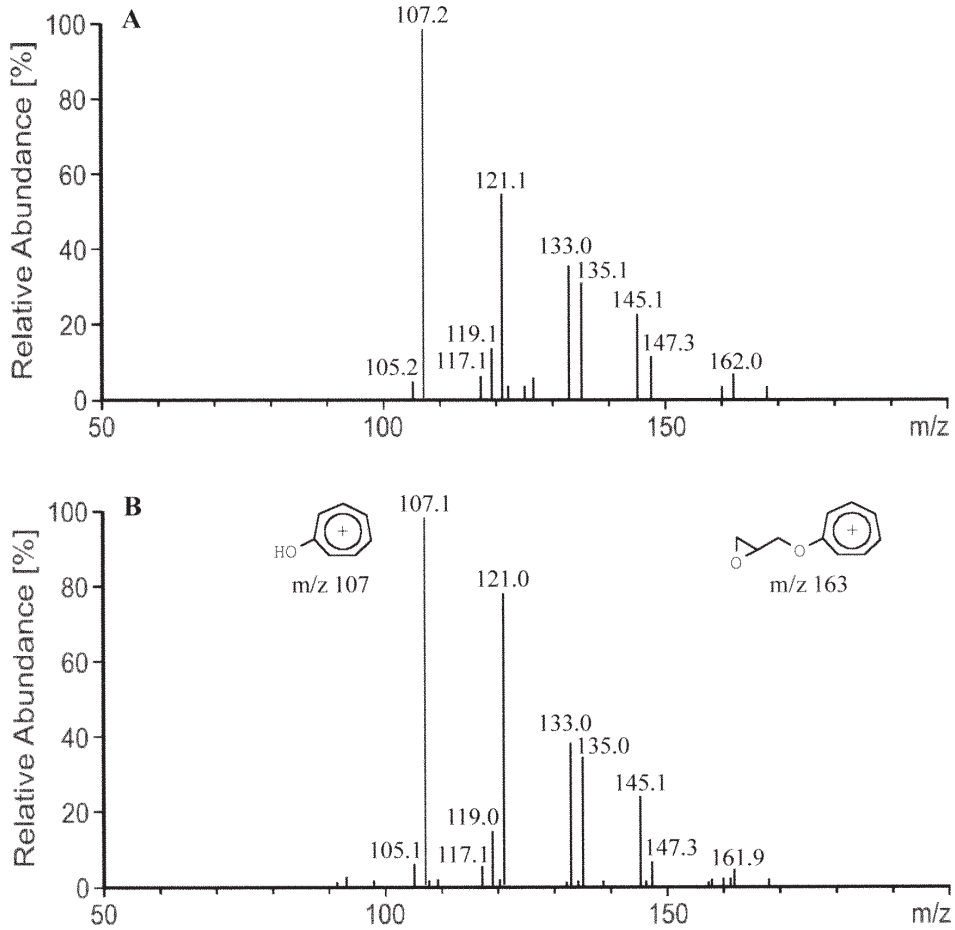

Figure 5. (A) MS^3 spectrum of 3-ring-NOGE + HCl (*m/z* 528 → 163, peak 4 in Figure 3A). (B) MS^3 spectrum of BFDGE (*m/z* 330 → 163). The tentative structures of the fragments *m/z* 163 and 107 are shown.

Table 3. Pseudo-molecular ion of the compounds identified in the extract from the BADGE-based lacquer and the most important fragments formed by on-line MS^2 of the pseudo-molecular ion

Compound[a]	MW[b]	Structure[c]	Pseudo-molecular ion $[M+NH_4]^+$, *m/z*	Most important fragments formed by online MS^2, *m/z* (relative abundances)[d]
21	476	BADGE + H_2O + BuEtOH	494	191 (10), 209 (100), 459 (10), 477 (50)
22	458	BADGE + BuEtOH	476	191 (100), 309 (20), 459 (10)
23	568	Cyclo-di-BADGE	569 $[M + H]^+$	191 (10), 265 (30), 285 (60), 325 (50), 387 (20), 419 (10), 441 (10), 457 (10), 553 (100)
24	576	BADGE + 2BuEtOH	594	309 (100), 577 (40)
25	926	Tri-BADGE + H_2O	944	475 (10), 643 (20), 759 (20), 777(30), 909 (30), 927 (100)
26	792	Di-BADGE + H_2O + tBuPhe	810	285 (10), 341 (30), 493 (100), 569 (10), 625 (60), 737 (10), 793 (20)
27	608	BADGE + BuEtOH + tBuPhe	626	191 (10), 285 (50), 309 (10), 341 (100), 553 (50), 609 (30)
28	1076	Tri-BADGE + H_2O + tBuPhe	1094	493 (20), 569 (10), 625 (30), 777 (100), 853 (10), 909 (60), 1077 (10)
29	1026	Tri-BADGE + BuEtOH	1044	309 (10), 475 (10), 593 (20), 759 (20), 877 (30), 1009 (30), 1027 (100)
30	1136	Cyclo-tetra-BADGE	1154	649 (10), 853 (20), 969 (10), 987 (20), 1137 (100)
31	1144	Tri-BADGE + 2BuEtOH	1162	309 (10), 593 (20), 609 (10), 877 (20), 1145 (100)
32	1058	Tri-BADGE + tBuPhe	1076	341 (10), 475 (20), 569 (20), 625 (100), 741 (60), 759 (70), 909 (60)
33	1476	Penta-BADGE	1494	759 (10), 1043 (20), 1233 (20), 1327 (20), 1441 (20), 1459 (40), 1477 (100)
34	1176	Tri-BADGE + BuEtOH + tBuPhe	1194	341 (10), 593 (20), 625 (20), 877 (40), 909 (30), 1177 (100)
35	1342	Tetra-BADGE + tBuPhe	1360	Ion intensity too low for MS^2
36	1594	Penta-BADGE + BuEtOH	1612	593 (10), 759 (10), 1327 (10), 1445 (10), 1577 (20), 1595 (100)
37	1208	Tri-BADGE + 2tBuPhe	1226	625 (30), 909 (100), 1209 (10)
38	1626	Penta-BADGE + tBuPhe	1644	759 (10), 909 (20), 1043 (10), 1193 (10), 1327 (20), 1477 (20), 1609 (40), 1627 (100)
39	1744	Penta-BADGE + BuEtOH + tBuPhe	1762	Ion intensity too low for MS^2
40	1776	Penta-BADGE + 2tBuPhe	1794	Ion intensity too low for MS^2

[a] *See* Figure 3B for signal assignment.
[b] MW = molecular weight.
[c] BuEtOH = 2-butoxyethanol; tBuPhe = *p-tert*-butylphenol.
[d] Relative abundances of fragments from different isomers may vary within ±10%.

pression of compound amount ratios. Work is in progress to quantitate compounds with a bisphenol backbone structure.

In MS detection, response factors depend on ammonium adduct formation, which varies strongly among molecules that differ in size or substitution. On the other hand, MS and online MS^2 detection are powerful tools for the determination of molecular weight and structure elucidation of unidentified compounds.

Separation and Structure Elucidation of Lacquer Extracts

PVC-based can.—Because 2-piece cans are coated before being shaped, a highly elastic lacquer is required. Organosols consisting mainly of PVC are usually used for this purpose. Technical mixtures of BADGE and/or NOGE are added to the polymer formulation as a scavenger for hydrochloric acid and a cross-linking agent, respectively. Approximately 103 μg migrant/mL, corresponding to 14 mg/dm^2, was extracted from the PVC-based can by acetonitrile.

The chromatograms of the extract recorded by FD and MS are shown in Figure 3A. The structures of the most abundant substances recorded by FD together with the pseudo-molecular ions and the most important fragments formed by online MS^2 are given in Table 2. Large compounds with a bisphenol backbone structure and a molecular weight of >1200 u were not present. The FD chromatogram shows

that mainly BADGE, di-BADGE, and their mono- and di-HCl-adducts were present in the extract (signals No. 1, 2, 3, and 9, 11, 12), and that NOGE derivatives were also detected. Because the can contained sweet corn in water, most of the BADGE derivatives were already extracted into the food, and the signal abundance ratios in the FD chromatogram were different from those reported for the food extract by Biedermann et al. (1).

The MS^n spectra of signals 1–20 in Figure 3A and Table 2 contained either the typical fragments *m/z* 189, 163, 133, and 107 for NOGE derivatives or *m/z* 191 and 135 for BADGE derivatives. HCl-adducts were identified by their chlorine isotope patterns. Figure 4 shows the mass chromatograms of the isomers of 4-ring-NOGE and their hydrochlorination products. The presence of ≤4 Cl atoms confirmed the former presence of the corresponding number of epoxy groups in 4-ring-NOGE.

The MS^3 spectrum of 3-ring-NOGE + HCl (*m/z* 528 → 163, peak 4 in Figure 3A) is given in Figure 5A. It shows the same fragmentation pattern as the MS^3 spectrum of BFDGE (*m/z* 330 → 163) in Figure 5B. Because no ion of *m/z* 163 is present in the MS^n spectra of BADGE derivatives, this pattern is a fingerprint for NOGE-related compounds.

Furthermore, *ortho*-substituted BADGE derivatives were identified by MS and MS^2 spectra. Signal 10 in Figure 3A is 3-ring-BADGE with a structure analogous to 3-ring-NOGE (*see* Figure 1). 5-Ring-BADGE (di-BADGE with an extra ring attached in an *ortho* position) and 7-ring-BADGE (derived from tri-BADGE) were detected as well. Because these structures contain 3 epoxy groups they can add up to 3 HCl molecules. All possible hydrochlorination products of 5-ring-BADGE were found with the corresponding chlorine isotope patterns. The presence of such compounds in can extracts has not been reported before.

BADGE-based can.—The acetonitrile extract of the BADGE-based can contained approximately 77 μg migrant/mL, which corresponds to 24 mg/dm^2. Figure 3B shows the chromatograms of the extract. The most abundant compounds in the FD chromatogram together with their pseudo-molecular ions and the most important fragments formed by online MS^2 are listed in Table 3. Only 20% of the fluorescent material consisted of compounds with a molecular weight of <1000 u (peaks 21–27 and 18 in Figure 3B). Mainly mono- and linear tri- and penta-BADGE derivatives as well as cyclo-di- and cyclo-tetra-BADGE were identified by MS^n, as expected from the reaction of BADGE with bisphenol A. The presence of fluorescent compounds with a molecular weight of ≤2000 u and the absence of HCl-adducts are indicators of an epoxy lacquer.

Structure elucidation was obtained by MS^n experiments. For example, Figure 6 shows the MS^2 spectrum of the ammonium adduct of tri-BADGE + H_2O + tBuPhe (peak 28 in Figure 3B); Δm 284 is typical for compounds with a BADGE backbone structure. The main fragmentation resulted from the cleavage of a bond between the central carbon atom and an aromatic ring of a BADGE unit, analogous to the formation of

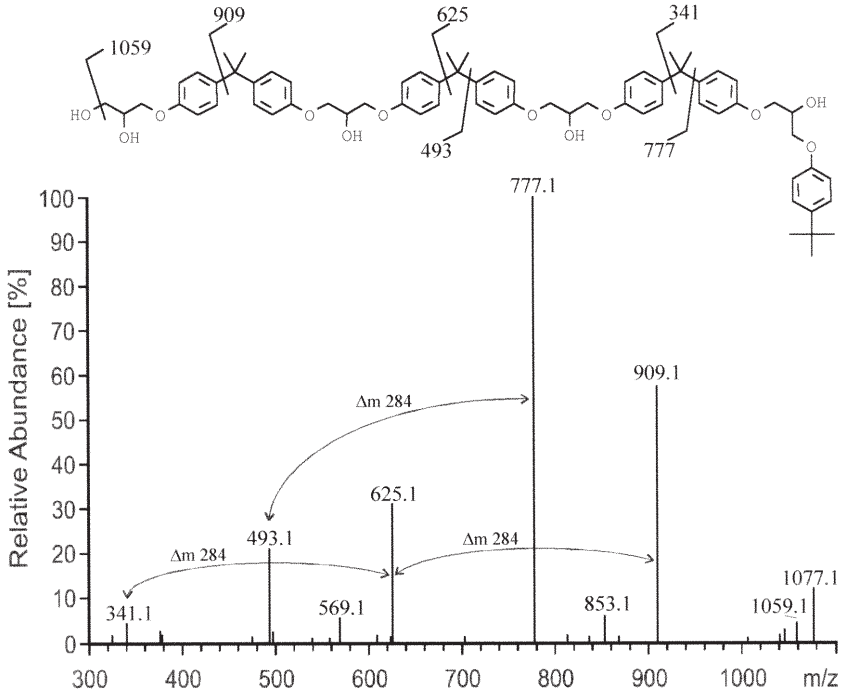

Figure 6. MS^2 spectrum of the ammonium adduct of tri-BADGE + H_2O + tBuPhe (*m/z* 1094, peak 28 in Figure 3B). Masses were assigned as follows: *m/z* 1077, $[M + H]^+$; *m/z* 853, mass 909 minus *tert*-butyl; and *m/z* 569, mass 625 minus *tert*-butyl. Further fragmentations are shown in the mass spectrum. Δm 284 is the characteristic difference between fragments of a compound with a BADGE backbone structure.

m/z 191 from BADGE (*see* Figure 2). The molecular weights of the substituents were calculated from these fragments. For example, *m/z* 341 (*see* Figure 6) minus *m/z* 191 equals 150 u, which is the molecular weight of *p-tert*-butylphenol. Furthermore, the loss of C_4H_8 from *tert*-butyl was observed. All ions in the mass spectrum shown in Figure 6 formed the characteristic BADGE fragment *m/z* 135 in consecutive MS^n spectra (*see* Figure 2).

The molecular weights of the substituents allowed the assumption that the epoxy resin was polymerized with *p-tert*-butylphenol (tBuPhe) as the chain-terminating agent. Furthermore, 2-butoxyethanol (BuEtOH) was used in the polymerization step and/or as a solvent. BADGE itself could not be detected. Cyclo-di- (peak 23) and cyclo-tetra-BADGE (peak 30) are small signals in the MS base peak chromatogram because of the lack of formation of strong ammonium adducts (*see* above).

Conclusions

Online LC/MS^n allows complete or partial elucidation of the structures of BADGE and BFDGE derivatives. Many different compounds and structural isomers that can possibly migrate into canned food were identified in 2 different can coatings. Pure BADGE and BFDGE represented only a small fraction of the material extracted from the lacquers. Organosol coatings seem to be more problematic because of the release of epoxides and chlorinated molecules of relatively low molecular weight. Furthermore, the potential hazard of epoxy polymers should not be underestimated.

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