Application Note: 521

The Analysis of Bisphenol A-diglycidyl Ether (BADGE), Bisphenol F-diglycidyl Ether (BFDGE) and Their Derivatives in Canned Food and Beverages by LC-MS/MS

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Key Words
- BADGE
- BFDGE
- LC-MS/MS
- Food Safety

Introduction

As an attempt to reduce food spoilage and prevent degradation of the container, epoxy-based lacquers or vinylic organosol (PVC) materials are commonly used as coating material in food cans. These lacquers are epoxy phenolic resins based on polymerization products of bisphenol A-diglycidyl ether (BADGE) or bisphenol F-diglycidyl ether (BFDGE). Chlorinated derivatives can be generated during the coating thermal treatment, since BADGE and BFDGE are also used as additives to remove the hydrochloric acid formed in this process. Moreover, hydrolyzed derivatives such as BADGE.2H_2O, BADGE.H_2O, and BFDGE.2H_2O can be produced during storage when the coating comes into contact with aqueous and acidic foodstuffs. The European Union has set specific migration limits (SML) for these compounds: 9 mg/kg for the sum of BADGE and its hydrolyzed derivatives and 1 mg/kg for the sum of BADGE.HCl, BADGE.2HCl and BADGE.HCl.H_2O.[1,2] The presence of this family of compounds in food has received much attention lately due to its suspected mutagenic, genotoxic, and anti-androgenic effects.[3-6]

Goal

To develop a fast and sensitive LC-MS/MS method for the simultaneous quantitative analysis of BADGE, BFDGE, and their derivatives in canned food and beverages.

Experimental

Sample Preparation

Canned Food:

The whole can content was homogenized. A sample of 3 g was mixed with 6 mL of ethyl acetate. The resulting mixture was shaken for 20 minutes and sonicated for 30 minutes in an ultrasonic bath. The mixture was then centrifuged at 4000 rpm for 15 minutes. Five (5) mL of supernatant was transferred to an 8-mL vial and evaporated to dryness under a stream of nitrogen. The extract was reconstituted in 1 mL of MeOH:H_2O (1:1) and filtered before injection (10 µL injection).

Beverages:

A 20-mL sample of beverage was degassed by sonication for 20 min. Then, 3 mL was loaded into a polymeric SPE cartridge that was previously conditioned with 3 mL of MeOH and 3 mL of H_2O. Finally, the analytes were eluted with 4 mL of MeOH. The collected fraction was evaporated to dryness and the extract reconstituted with 1 mL of MeOH:H_2O (1:1) and filtered before injection (10 µL injection).

LC Conditions

Solvent A	Formic acid-ammonium formate (25 mM, pH 3.75, 50 °C)
Solvent B	Methanol
Flow Rate	600 µL/min
Analytical Column	Fused Core™ Ascentis Express C18 150 x 2.1 mm i.d., 2.7 µm (Supelco)

The gradient method was started at 30% solvent B (0.25 min) and linearly increased to 50% solvent B in 0.75 min. The gradient was then increased to 60% of solvent B in 0.5 min, and then to 80% in 4 minutes. This composition was maintained for 0.5 min.

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MS Conditions

MS analysis was carried out on a Thermo Scientific TSQ Quantum Ultra AM mass spectrometer equipped with a heated electrospray ionization probe. The MS conditions were as follows:

Ion Source Polarity	Positive Ion Mode
Spray Voltage	4000 V
Vaporizer Temperature	475 °C
Sheath Gas Pressure (N_2)	60 units
Auxiliary Gas Pressure (N_2)	40 units
Ion Sweep Gas Pressure	2 units
Capillary Temperature	375 °C
Tube Lens	65 V
Collision Gas (Ar)	1.5 mTorr

Results and Discussion

The family of compounds studied tends to form adducts and clusters in positive ionization mode $[M+NH_4]^+$, $[M+Na]^+$, and $[M+K]^+$. The mobile phase used favored the formation of ammonium adducts ions $[M+NH_4]^+$, which dominated the full scan spectra (base peaks). The cleavage of the phenyl-alkyl bond and the α-cleavage of the ether bond were identified as the most intense and characteristic fragmentation of $[M+NH_4]^+$, and therefore selected for quantification and confirmation purposes (Table 1).

Matrix effects were evaluated by analyzing two samples free of BADGEs and BFDGEs – cola soft drink beverage and red pepper. These samples were analyzed by external and matrix-matched calibration. The results showed similar responses for both methods and matched calibration curves, indicating that no matrix effect occurred in the analysis of BADGEs and BFDGEs using the developed LC-MS/MS method.

To evaluate limits of quantification, blank samples were spiked with the studied compounds at low concentration levels (below 2.5 µg/kg) and submitted to the sample pre-treatment detailed above. The results obtained allowed the analysis of this family of compounds in beverages and canned food, given that the LOQs obtained are below (3 to 4 orders of magnitude) the specific migration limits established by the European Union (Table 2).

Good linearity ($r^2 > 0.999$) was observed for calibration curves for standard solutions ranging from 0.5 µg/kg to 5,000 µg/kg.

Run-to-run precision was evaluated by analyzing six replicates of a red pepper sample and a cola sample spiked at two concentration levels. In addition, the ion ratios (quantitative versus confirmatory) were calculated and errors (compared with standards) were always below 10%. Finally, recoveries were calculated by addition of different amounts of the studied compounds (between the LOQ and 250 µg/kg) to blank samples, which were analyzed by external calibration.

Sample Analysis

The LC-MS/MS method developed for the analysis of BADGEs and BFDGEs in canned food and soft-drinks was employed to analyze six aqueous-based canned foods and seven soft-drink samples (Figure 1). In canned soft-drink beverages only BADGE·$2H_2O$ was detected, at concentrations ranging from 2.3 µg/L to 5.1 µg/L, while other BADGEs and BFDGEs were not detected. As an example, Figure 2 shows the LC-MS/MS chromatogram of two canned soft-drinks samples where BADGE·$2H_2O$ was found. In contrast, several BADGEs were found in canned food samples. BADGE·$2H_2O$ was found in all food samples at concentrations between 2.7 µg/kg and 675 µg/kg, with the highest concentration level being

Table 1. Transitions monitored for the analysis of BADGEs and BFDGEs

Compound	Precursor ion *(m/z)*, $[M+NH_4]^+$	Quantitation		Confirmation		Ion Ratio ± SD[b]
		Product Ion *(m/z)*	CE[a] (V)	Product Ion *(m/z)*	CE[a] (V)	
BADGE·$2H_2O$	394.2	209.1	31	135.1	31	1.7 ± 0.1
BADGE·H_2O	376.2	209.1	29	135.1	29	1.9 ± 0.1
BADGE·HCl·H_2O	412.2	227.0	33	135.1	33	1.4 ± 0.1
BADGE	358.2	191.0	30	135.1	30	4.3 ± 02
BADGE·HCl	394.2	227.0	13	135.1	13	2.6 ± 0.3
BADGE·2HCl	430.2	227.0	30	135.1	30	2.0 ± 0.1
BFDGE·$2H_2O$	366.2	133.1	22	181.1	22	1.5 ± 0.1
BFDGE	330.2	163.1	12	189.1	12	1.3 ± 0.1
BFDGE·2HCl	402.1	199.1	20	181.1	20	1.7 ± 0.2

[a]CE: collision energy

[b]SD: Standard deviation (n = 5)

Table 2. MLOQs, run-to-run precision, recoveries, and ion ratio of the LC-MS/MS method

Compound	MLOQ (µg/L)	Soft-drinks and canned food			
		Precision (RSD %)		Recovery (%)	Ion ratio[c]
		Low concentration[a]	Medium concentration[b]		
BADGE·2H$_2$O	0.13 – 1.0	7	3	70 - 95	1.8
BADGE·H$_2$O	0.14 – 1.1	12	3	60 - 83	1.8
BADGE·HCl·H$_2$O	0.14 – 1.1	20	9	69 - 95	1.5
BADGE	0.16 – 1.2	12	10	80 - 86	4.3
BADGE·HCl	0.16 – 1.3	3	11	60 - 70	2.4
BADGE·2HCl	1.6 – 3.4	14	10	80 - 82	2.1
BFDGE·2H$_2$O	1.5	16	8	85 - 90	1.3
BFDGE	0.7 – 4.0	20	10	70 - 89	1.6
BFDGE·2HCl	1.6	13	4	74 - 95	1.9

[a]Low concentration level: Cola sample (0.15 µg/L to 2.0 µg/L) and red pepper (2.0 µg/kg to 15.0 µg/kg).
[b]Medium concentration level: Cola sample (1.5 µg/L to 20 µg/L) and red pepper (20 µg/kg to 150 µg/kg).
[c]Ion ratio calculated at medium concentration level.

in the asparagus sample. Other BADGEs detected in these samples were BADGE·H$_2$O at concentrations ranging from 35 µg/kg to 53 µg/kg, BADGE·HCl·H$_2$O (3.4 – 274 µg/kg) and BADGE·2HCl at concentrations between 0.9 µg/kg and 2.8 µg/kg. In contrast, the original monomer (BADGE) was not found in the samples, probably because it was easily hydrolyzed in these water-based samples. In addition, none of the BFDGEs were found, confirming the decrease in use of BFDGE-based coatings.

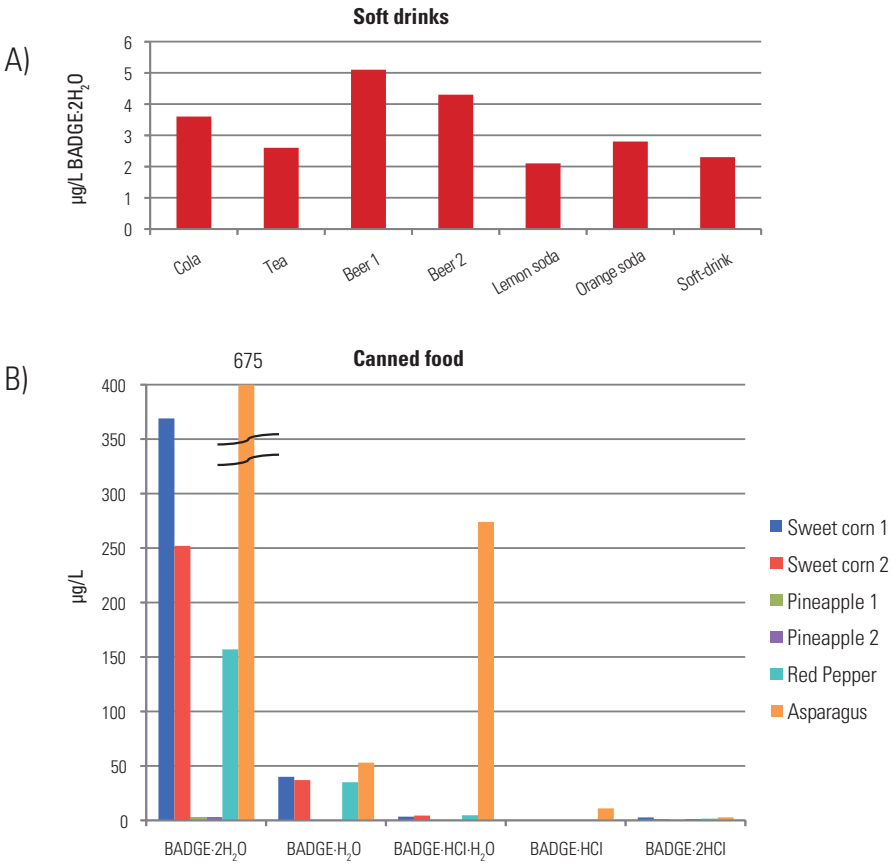

Figure 1. Canned soft-drinks (A) and food samples (B) analyzed using the developed LC-MS/MS method

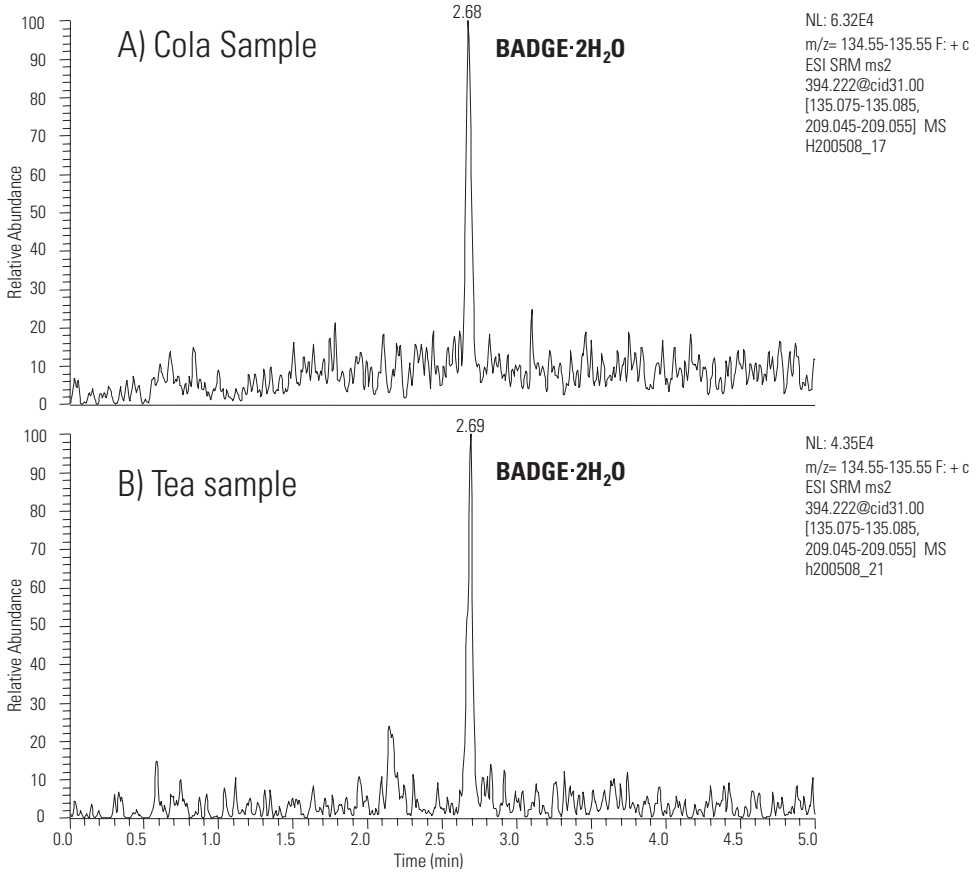

Figure 2. LC-MS/MS chromatograms for cola (A) and tea (B) samples

Conclusions

A fast and sensitive method for the simultaneous analysis of BADGEs and BFDGEs in canned food and beverages is proposed. The limits of quantification of the method vary between 0.13 and 1.6 µg/L for beverages and between 1.0 and 4.0 µg/kg for foodstuff. The method has been applied to real samples. BADGE.2H_2O was detected in all samples at levels between 2.1 and 675 µg/kg. Other derivatives of BADGE were also detected and quantified. No BFDGE or its derivatives were detected.

References

More information can be found at Gallart-Ayala, H; Moyano, E.; Galceran, M.T. *J Chrom A*, 2011, 1218, 12.

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