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Fast liquid chromatography–tandem mass spectrometry for the analysis of bisphenol A-diglycidyl ether, bisphenol F-diglycidyl ether and their derivatives in canned food and beverages

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ABSTRACT

In this work a fast liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) method using a C18 Fused Core™ column, was developed for the simultaneous analysis of bisphenol A diglycidyl ether (BADGE), bisphenol A (2,3-dihydroxypropyl) glycidyl ether (BADGE·H_2O), bisphenol A bis(2,3-dihydroxypropyl) ether (BADGE·$2H_2O$), bisphenol A (3-chloro-2-hydroxypropyl) glycidyl ether (BADGE·HCl), bisphenol A bis(3-chloro-2-hydroxypropyl) ether (BADGE·2HCl) and bisphenol A (3-chloro-2-hydroxypropyl)(2,3-dihydroxypropyl ether) (BADGE·HCl·H_2O) and bisphenol F diglycidyl ether (BFDGE), bisphenol F bis(2,3-dihydroxypropyl) ether (BFDGE·$2H_2O$), bisphenol F bis(3-chloro-2-hydroxypropyl) ether (BFDGE·2HCl). The LC method was coupled with a triple quadrupole mass spectrometer, using an ESI source in positive mode and using the $[M+NH_4]^+$ adduct as precursor ion for tandem mass spectrometry experiments. The method developed was applied to the determination of these compounds in canned soft drinks and canned food. OASIS HLB solid phase extraction (SPE) cartridges were used for the analysis of soft drinks, while solid canned food was extracted with ethyl acetate. Method limits of quantitation ranged from 0.13 $\mu g\,L^{-1}$ to 1.6 $\mu g\,L^{-1}$ in soft drinks and 1.0 $\mu g\,kg^{-1}$ to 4.0 $\mu g\,kg^{-1}$ in food samples. BADGE·$2H_2O$ was detected in all the analyzed samples, while other BADGEs such as BADGE·H_2O, BADGE·HCl·H_2O, BADGE·HCl and BADGE·2HCl were also detected in canned foods.

1. Introduction

Epoxy-based lacquers or vinylic organosol (PVC) materials are commonly used for coating the inside of food cans, big storage vessels and food containers to reduce food spoilage and to prevent degradation of the food can. These lacquers are epoxy phenolic resins based on polymerization products of bisphenol A-diglycidyl ether (BADGE) or bisphenol F-diglycidyl ether (BFDGE), so these coatings can release these compounds as well as oligomers and derivatives which can migrate into the packed foods. Chlorinated derivatives may be generated during the thermal coating 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, BFDGE·$2H_2O$ and BFDGE·H_2O can be produced during storage when the coating comes into contact with aqueous and acidic foodstuffs. The presence of this family of compounds has received attention lately due to the suspected mutagenic, genotoxic and anti-androgenic effects of these compounds [1–4]. With regard to toxicity of these compounds the European Food Safety Authority (EFSA) indicates that BADGE and its chlorohydrins do not raise concern for carcinogenicity and genotoxicity *in vivo*, but more studies on toxicity are needed for the other BADGE derivatives [5]. However in the literature there is information about cytotoxic effects of epoxy-BADGEs [6]. In addition, BADGEs containing chlorine atoms could also be toxics, for instance a study on the toxicity of BADGE·2HCl indicated that this compound showed estrogenic activity probably due to the presence of halogen atoms in its chemical structure [7]. Regarding legislation, the European Union (EU) has set specific migration limits (SML) of 9 $mg\,kg^{-1}$ for the sum of BADGE and its hydrolyzed derivatives and 1 $mg\,kg^{-1}$ for the sum of BADGE·HCl, BADGE·2HCl and BADGE·HCl·H_2O [8]. The use and/or presence of BFDGE in the manufacture of materials and articles intended to be in contact with food is prohibited and in consequence its presence in food is undesirable.

Bisphenol A diglycidyl ether, bisphenol F diglycidyl ether and their hydrolyzed derivatives are traditionally analyzed by gas chromatography coupled with mass spectrometry (GC–MS) or by liquid chromatography with fluorescence detection (LC–FLD) [9–13]. Liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) is also used in the analysis of BADGEs and BFDGEs [14–17]. These methods use conventional analytical columns (2.1 or 4.6 mm I.D.) using reversed-phase C18 stationary phases with

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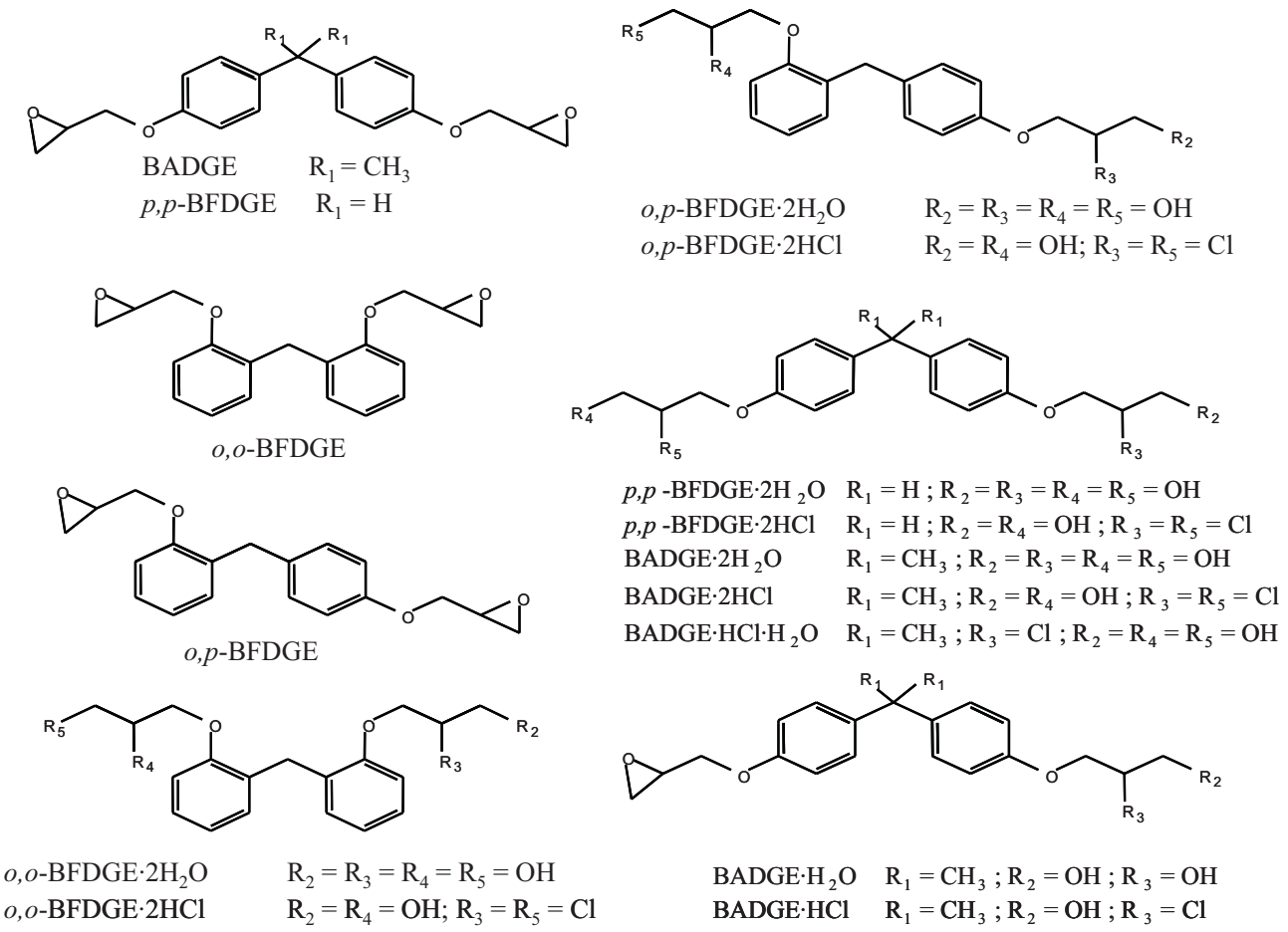

Fig. 1. Chemical structures of bisphenol A diglycidyl ethers and bisphenol F diglycidyl ethers.

particles of 3.5–5 μm, provide long analysis times (>25 min) when both BADGEs and BFDGEs are analyzed in the same run. Recently, ultra-high performance liquid chromatography (UHPLC) has been used to analyze BADGEs in canned food, with the greater chromatographic efficiency used to shorten analysis times [17]. Since the complexity of food matrixes usually requires extensive sample treatment, liquid–liquid extraction (LLE) [9,12,18–20] and solid phase extraction (SPE) [17,21] are the procedures most commonly used for the analysis of this family of compounds in canned foods, while other techniques such as pressurized liquid extraction (PLE) [22] have been scarcely employed.

The aim of this study was to develop a fast LC–MS/MS method for the analysis of bisphenol A-diglycidyl ether, bisphenol F-diglycidyl ether and their derivatives in canned food samples and soft-drink beverages after a simple sample treatment procedure. The applicability of a porous shell particle column was evaluated in order to provide short analysis times and high chromatographic efficiencies for the analysis of these compounds.

2. Experimental

2.1. Chemicals and reagents

Bisphenol A diglycidyl ether (BADGE), bisphenol A (2,3-dihydroxypropyl) glycidyl ether (BADGE·H_2O), bisphenol A bis(2,3-dihydroxypropyl) ether (BADGE·$2H_2O$), bisphenol A (3-chloro-2-hydroxypropyl) glycidyl ether (BADGE·HCl), bisphenol A bis(3-chloro-2-hydroxypropyl) ether (BADGE·2HCl) and bisphenol A (3-chloro-2-hydroxypropyl)(2,3-dihydroxypropyl ether) (BADGE·HCl·H_2O) standards of analytical grade were obtained from Sigma–Aldrich (Steinheim, Germany). Bisphenol F diglycidyl ether (BFDGE), bisphenol F bis(2,3-dihydroxypropyl) ether (BFDGE·$2H_2O$), bisphenol F bis(3-chloro-2-hydroxypropyl) ether (BFDGE·2HCl) (all of them, mixtures of *ortho–ortho*, *ortho–para* and *para–para* isomers) were also obtained from Sigma–Aldrich (Steinheim, Germany). The chemical structures of the compounds studied are given in Fig. 1.

LC–MS grade methanol (MeOH), acetonitrile (ACN) and water were purchased from Riedel-de Haën (Seelze, Germany). Ammonium formate (≥99.0%) and ethyl acetate were obtained from Fluka (Steinheim, Sweden) and formic acid (98–100%) from Merck (Darmstadt, Germany). Stock standard solutions (200 mg kg^{-1}) were individually prepared by weight in methanol and stored at 4 °C. Intermediate solutions were prepared weekly from the stock standard solution by appropriate dilution in MeOH:water (1:1). Calibration standard solutions ranging from 0.5 μg kg^{-1} to 5000 μg kg^{-1} were prepared daily. Mobile phases were filtered through 0.22 μm Nylon membrane filter (Whatman, Clifton, NJ, USA) and sample extracts were filtered through 0.22 μm pore size Ultrafree-MC Centrifugal Filters (Millipore, Bedford, USA). OASIS HLB cartridges (60 mg) purchased from Waters (Mildford, MA, USA) were used for solid phase extraction (SPE).

Nitrogen (99.98% pure) supplied by Claind Nitrogen Generator N_2 FLO (Lenno, Italy) was used for the API source. High-purity Argon (Ar_1) purchased from Air Liquide (Madrid, Spain) was used as a collision-induced gas (CID gas).

Table 1
Tandem mass spectrometry transitions for SRM.

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).

2.2. Instruments, LC and MS conditions

A liquid chromatograph (Accela system; Thermo Fisher Scientific, San José, CA, USA) equipped with a low-pressure quaternary pump, an autosampler and a column oven was coupled with a triple quadrupole mass spectrometer. The chromatographic separation was performed on a Fused Core™ Ascentis Express C18 column (150 × 2.1 mm i.d., 2.7 μm particle size) from Supelco (Bellefonte, PA, USA), using as mobile phase methanol (solvent A) and 25 mM formic acid–ammonium formate buffer at pH 3.75 (solvent B) at 600 μL min^{-1}, at a column temperature of 50 °C. The gradient elution program started at 30% of solvent A (0.25 min), followed by a linear gradient up to 50% of solvent A in 0.75 min, then a second linear gradient up to 60% of solvent A in 0.5 min and finally a third linear gradient up to 80% of solvent A in 4 min. This composition was then maintained for 0.5 min.

The liquid chromatographic system was coupled with a triple quadrupole mass spectrometer TSQ Quantum Ultra AM (Thermo Fisher Scientific, San José, CA, USA) equipped with a heated-electrospray ionization source (H-ESI I) working in positive mode. Nitrogen (purity > 99.98%) was used as a sheath gas, ion sweep gas and auxiliary gas at flow rates of 60, 20 and 40 a.u. (arbitrary units), respectively. The ion transfer tube temperature was set at 375 °C, the vaporizer temperature at 25 °C and the electrospray voltage at 4 kV. When data were acquired in low-resolution selected reaction monitoring (SRM) mode, a resolution of 0.7 *m/z* full width half maximum (FWHM) on Q1 and Q3 and a scan width of 0.01 *m/z* were used. In highly selective selected reaction monitoring (H-SRM), mode quadrupole Q1 operated at a mass resolution of 0.1 *m/z* FWHM with a scan width of 0.01 *m/z*, whereas Q3 operated at low resolution (0.7 *m/z* FWHM). Argon, used as collision gas at 1.5 mtorr, and the optimum collision energy (CE) selected for each transition are indicated in Table 1. Ammonium adducts $[M+NH_4]^+$ were used as precursor ions in tandem mass spectrometry and two transitions for each compound and a dwell time of 10 ms were chosen for quantitative analysis and confirmation purposes (Table 1).

To optimize source working conditions and to carry out multiple-stage mass spectrometry experiments, a standard solution (1 mg L^{-1}) prepared in methanol was infused at a flow-rate of 3 μL min^{-1} using the syringe pump integrated in the TSQ instrument and was mixed with the mobile phase (600 μL min^{-1}, 60:40, v/v, MeOH:formic acid–ammonium formate at pH 3.75) by a Valco zero dead volume tee piece (Supelco, Alcobendas, Spain).

2.3. Sample treatment

A total of six canned food and seven canned beverage samples were purchased at local supermarkets (Barcelona, Spain) and processed using two sample treatments: (i) canned food and (ii) canned beverages.

(i) For canned food samples of vegetables and fruits, the whole can content was homogenized using Ultra-Turrax TR-50 (Staufen, Germany). A subsample of 3 g was weighed into a 15-mL centrifuge tube, and 6 mL of ethyl acetate was added as extraction solvent. The resulting mixture was shaken for 20 min in a rotatory shaker and for 30 min in an ultrasonic bath. Then the mixture was centrifuged at 4000 r.p.m. for 15 min using a Selecta Centronic centrifuge (Selecta, Barcelona, Spain). Five milliliters of the supernatant was transferred to an 8 mL vial and evaporated to dryness under nitrogen stream. Then, the extract was reconstituted in 1 mL of MeOH:water (1:1) and filtered before injection of 10 μL of it into the LC–MS/MS system.

(ii) The seven canned soft-drink beverages included soda, beer, cola, tea and a tonic drink, all of them were carbonated except tea. They were stored unopened until analysis at 4 °C. Twenty milliliters of beverage samples was degassed by sonication for 20 min. In order to obtain an extract free of sugars and other matrix components 3 mL of beverage sample was loaded into the OASIS HLB SPE cartridge, which was previously conditioned with 3 mL of MeOH and 3 mL of water. The analytes were eluted with 4 mL of MeOH. The collected fraction was evaporated to dryness and the extract was reconstituted with 1 mL of MeOH:water (1:1) and filtered before injection of 10 μL of it into the LC–MS/MS system.

Supelco Visiprep and Supelco Visidry SPE (Supelco) vacuum manifold were used for SPE and solvent evaporation.

A soft-tonic beverage and a red pepper sample both packed in glass were submitted to the sample treatment detailed above and analyzed by the LC–MS/MS method. As they were shown to be free of BADGEs, BFDGEs and their derivatives, they were used to study the matrix effects and to evaluate the quantitative method. Analytes were determined in canned food and beverage samples by external calibration.

3. Results and discussion

3.1. Liquid chromatography–mass spectrometry

In this study, a fast LC–MS/MS method was developed for the analysis of BADGE, BFDGE and their hydrolyzed derivatives in canned food and beverages. In a preliminary study, two short columns, a Fused Core™ (Ascentis Express C18 50 × 2.1 mm i.d., 2.7 μm) column and a totally porous sub-2 μm particle size column (Acquite BEH C18 50 × 2.1 mm i.d., 1.7 μm), were evaluated for the separation of BFDGEs isomers. MeOH:ammonium formate/formic acid (25 mM, pH 3.75, 50 °C) gradient elution at 600 μL min^{-1} was used in both cases. As can be seen in Fig. 2, both columns provided similar resolution and efficiencies for the separation of these isomers, although the Fused Core™ column showed a lower back-

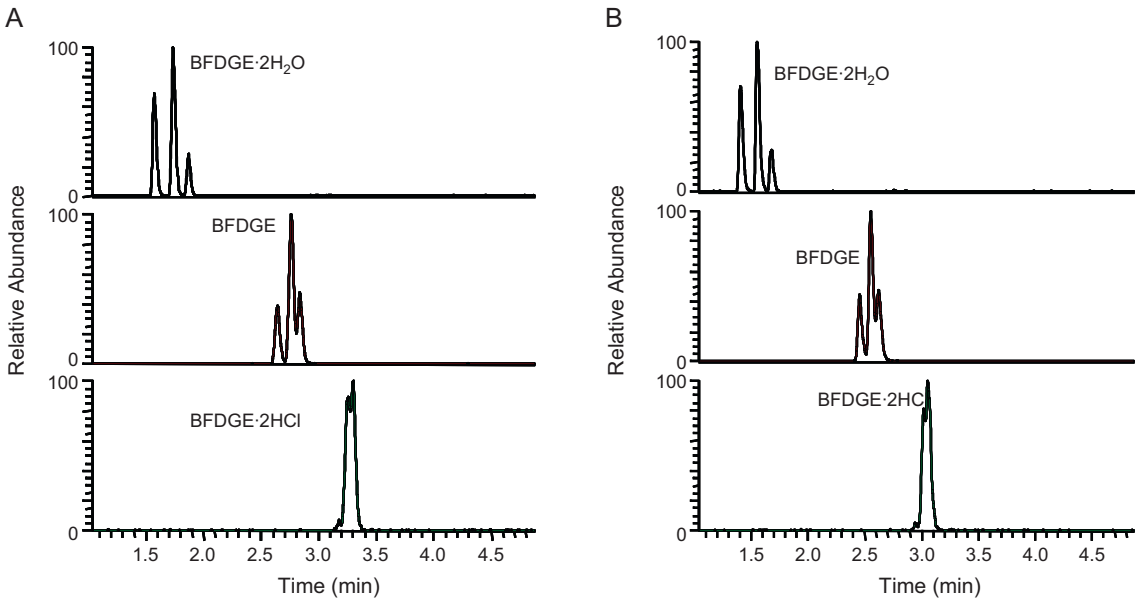

Fig. 2. LC–MS/MS chromatogram of BFDGE·$2H_2O$ isomers, using (A) Acquity BEH C18 column (50 mm × 2.1 mm i.d. 1.7 μm particle size) and (B) Ascentis Express C18 column (50 mm × 2.1 mm i.d. 2.7 μm particle size). MeOH:25 mM formic acid–ammonium formate buffer at pH 3.75, linear gradient elution from 40% to 80% MeOH in 3 min and 600 μL min^{-1} as flow rate.

pressure of 200 bar against 513 bar for the sub-2 μm column. The Fused Core™ column was selected for further experiments, as its low backpressure permitted the increase of the column length to 150 mm, which allowed the separation of BADGEs, BFDGEs and their hydrolyzed derivatives.

Several mobile phase compositions and gradient elution programs were tested using the 150 mm Fused Core™ C18 column and the whole set of compounds. The best separation was obtained in less than 5 min with MeOH:formic acid–ammonium formate buffer (25 mM, pH 3.75, 50 °C) and the gradient elution indicated in the experimental section at a flow rate of 600 μL min^{-1}. Fig. 3A shows the chromatogram obtained for a standard solution under these conditions. It should be mentioned that acetonitrile as organic modifier in the mobile phase was also evaluated, but MeOH-based mobile phases produced, for most of the compounds, higher responses in electrospray. This can be seen in Fig. 3, where the chromatograms using both MeOH and ACN are shown. Moreover, in agreement with the results published by Lintschinder and Rauter [18] the elution order for BADGE·HCl/BADGE·2HCl and BFDGE/BFDGE·2HCl changed when ACN was used instead of MeOH, probably due to the different proton donor/acceptor characteristics of the two solvents. However, ACN provided better chromatographic resolution between BFDGE isomers (Fig. 3B). Thus, MeOH can be proposed as an organic modifier to improve the sensitivity of the method, although ACN can be used in a second analysis if positive samples are detected and if it is important to know the distribution of BFDGE isomers.

The fast liquid chromatography separation was coupled to the triple quadrupole mass spectrometer, using an ESI source in positive mode. This family of compounds tends to form adducts and clusters in positive ionization mode, $[M+NH_4]^+$, $[M+Na]^+$ and $[M+K]^+$, as described in a previous work [23]. The mobile phase used

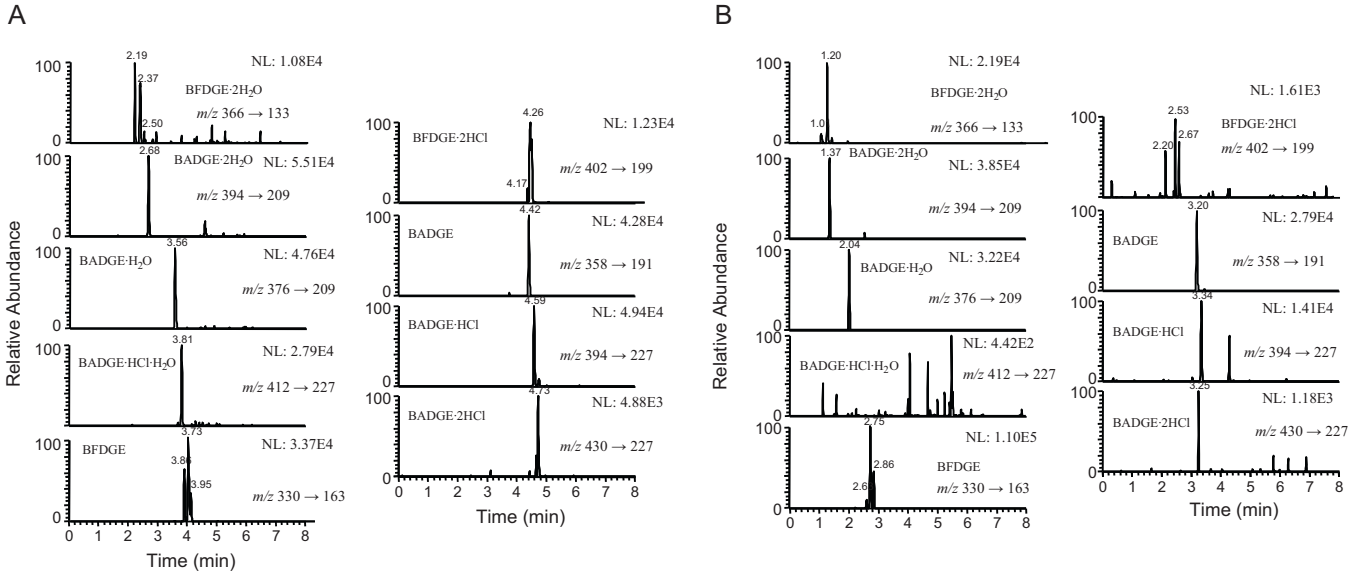

Fig. 3. LC–MS/MS chromatograms of BADGEs and BFDGEs, using (A) methanol: 25 mM formic acid–ammonium formate buffer at pH 3.75 gradient elution and (B) ACN: 25 mM formic acid–ammonium formate buffer at pH 3.75 gradient elution.

Table 2
Levels of BADGEs detected in cemented needles ($\mu g\,L^{-1}$).

Compound	Needle 1	Needle 2	Needle 3	Needle 4	Needle 5	Needle 6
BADGE·2H$_2$O	20.2	17.9	45.9	21.0	<LOQ[b]	<LOQ[b]
BADGE	n.d.[a]	37.6	15.4	36.0	n.d.[a]	n.d.[a]
BADGE·HCl·H$_2$O	<LOQ[b]	<LOQ[b]	<LOQ[b]	<LOQ[b]	n.d.[a]	n.d.[a]
BADGE·H$_2$O	n.d.[a]	63.3	35.2	74.1	n.d.[a]	n.d.[a]
BADGE·HCl	n.d.[a]	<LOQ[b]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]

[a] n.d., not detected.
[b] <LOQ, below the instrumental limit of quantitation.

favored the formation of ammonium adduct ions $[M+NH_4]^+$, which were the base peak of the full-scan spectra. The two most intense and characteristic fragmentations in tandem mass spectrometry provided by the $[M+NH_4]^+$ for BADGEs and BFDGEs were the cleavage of the phenyl–alkyl bond and the consecutive cleavages of the phenyl–alkyl bond and the α-cleavage of the ether bond [23], which were selected for quantification and confirmation (Table 1).

Instrumental quality parameters such as limit of detection (LOD), limit of quantitation (LOQ), run-to-run precision, ion-ratio precision and linearity were studied in selected reaction monitoring (SRM). Limits of detection (LODs), based on a signal-to-noise ratio of 3 and limits of quantitation (LOQs), based on signal-to-noise 10 were estimated by the injection of 10 μL of standard solutions at low concentration levels (down to 200 ng kg^{-1}). LODs from 0.15 μg kg^{-1} for BADGE, BADGE·2H$_2$O, BADGE·H$_2$O, BADGE·HCl·H$_2$O, BADGE·HCl and BFDGE to 8 μg kg^{-1} for BADGE·2HCl, BFDGE·2H$_2$O and BFDGE·2HCl and LOQs from 0.5 μg kg^{-1} for BADGE, BADGE·2H$_2$O, BADGE·H$_2$O, BADGE·HCl·H$_2$O, BADGE·HCl and BFDGE to 2.5 μg kg^{-1} for BADGE·2HCl, BFDGE·2H$_2$O and BFDGE·2HCl were obtained. Good linearity ($r^2 > 0.999$) was observed for calibration curves for standard solutions ranging from 0.5 μg kg^{-1} to 5000 μg kg^{-1} using 6 calibration levels. Run-to-run precision ($n = 5$) was evaluated at two concentration levels (0.5 μg kg^{-1} and 5000 μg kg^{-1}) and the relative standard deviations (RSDs) based on concentration were lower than 10%. From these results it can be concluded that SRM mode provides good selectivity and is robust enough to be used as acquisition mode for the analysis of BADGEs and BFDGEs by LC–MS/MS.

3.2. Feasibility of the method

In this study, to ensure good quantitation results, blank samples were analyzed to evaluate possible contamination sources. Some BADGEs, BADGE·2H$_2$O, BADGE·H$_2$O, BADGE·HCl·H$_2$O, BADGE and BADGE·HCl, were detected in the analysis of blank samples. To identify the source of contamination, all sample treatment steps were studied and some of the compounds were detected after transferring the extracts into the injection vials by syringes with metal needles. To evaluate the contamination, 1 mL of MeOH:water (1:1) was transferred to 2 mL injection vials from different suppliers by several syringe metal needles and then analyzed by LC–MS/MS. BADGE, BADGE·2H$_2$O and BADGE·H$_2$O were detected at μg L^{-1} level in most of the needles (Table 2), while in two of them only BADGE·2H$_2$O was detected at a concentration below LOQ. In addition, the needles were divided into three parts, each exposed to 1 mL of MeOH:water (1:1), and then the solution was analyzed. The results indicated that the contamination came from the adhesive used to cement the needles, probably an epoxy-resin based on BADGE. These results are consistent with those of Watabe et al. [24], who detected BPA in cemented syringe needles at similar concentration levels to these BADGEs. To prevent contamination in further studies, only Pasteur pipettes were used to transfer the extracts.

Shorter chromatographic run times and simplified sample clean-up often lead to matrix suppression effects in electrospray ionization. In this study, matrix effects were evaluated by means of two matrix samples (free of BADGEs and BFDGEs) selected as representative of the analyzed samples: a cola soft-drink beverage and red pepper (one of the most complex vegetable samples), both in glass. 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 quantitation, cola and red pepper samples were spiked with the studied compounds at low concentration levels (below 2.5 μg kg^{-1}) and submitted to the sample treatments detailed above in Section 2.3. The developed LC–MS/MS method using SRM acquisition mode provided a good method limit of quantitation (MLQ) (Table 3): between 0.13 μg L^{-1} and 1.6 μg L^{-1} in cola samples and between 1.0 μg kg^{-1} and 4.0 μg kg^{-1} in red pepper. This allowed the analysis of this family of compounds in canned food and beverages, since these values are 3–4 orders of magnitude lower than the specific migration limits (SML) established by the European Union (EU) [8].

Run-to-run precision was evaluated by analyzing six replicates of a red pepper sample and a cola sample spiked at two concentration levels. The low concentration level ranged from 0.15 μg L^{-1} to 2.0 μg L^{-1} (depending on the compound) for the cola sample and from 2.0 μg kg^{-1} to 15.0 μg kg^{-1} for the red pepper, while the medium level was ten times higher for both samples. The relative standard deviations (RSDs) based on concentration provided similar results for both sample matrices (cola and red pepper), ranging from 3 to 20% (Table 3). In addition, the ion ratios (quantitative versus confirmatory transitions) were calculated and errors (compared with standards) were always below 10%.

Finally, recoveries were calculated by the addition of different amounts of the studied compounds (between LOQ and 250 μg kg^{-1}) to blank samples (cola and red pepper), which were analyzed by external calibration. The slope of the calculated amount versus the added concentration provided average recoveries ranging from 70% to 95% (Table 3).

In addition, to avoid false positive and false negative results, the use of enhanced mass resolution acquisition mode (H-SRM) was evaluated, since selectivity can be increased by filtering chemical background noise [25,26]. Cola and red pepper blank samples spiked at low concentration levels (<2.5 μg kg^{-1}) were analyzed by H-SRM on Q1 (Q1: 0.1 m/z FWHM, Q3: 0.7 m/z FWHM) mode, with cleaner chromatograms and limits of detection 2–10 times better than those obtained using SRM mode. As an example Fig. 4 shows the LC–MS/MS chromatograms of a red pepper sample spiked at low concentration level with BADGE and BADGE·2H$_2$O. As can be seen an improvement of 3 times was obtained in H-SRM on Q1 mode if compared with SRM.

3.3. Sample analysis

The LC–MS/MS method developed for the analysis of BADGEs and BFDGEs in canned food and soft-drinks has been employed to analyze six aqueous based canned foods and seven soft-

Table 3
MLOQs, run-to-run precision, recoveries and ion ratio of the LC–MS/MS method.

Compound	Cola					Red pepper				
	MLOQ ($\mu g\,L^{-1}$)	Run to run		Recovery (%)	Ion ratio[c]	MLOQ ($\mu g\,kg^{-1}$)	Run to run		Recovery (%)	Ion ratio[c]
		Low concentration[a]	Medium concentration[b]				Low concentration[a]	Medium concentration[b]		
BADGE·2H_2O	0.13	7	3	95	1.8	1.0	13	4	70	1.7
BADGE·H_2O	0.14	12	3	83	1.8	1.1	20	5	60	1.8
BADGE·HCl·H_2O	0.14	20	9	95	1.5	1.1	7	7	69	1.3
BADGE	0.16	12	10	80	4.3	1.2	4	5	86	4.4
BADGE·HCl	0.16	3	11	70	2.4	1.3	9	5	60	2.5
BADGE·2HCl	1.6	14	10	82	2.1	3.4	8	6	80	1.9
BFDGE·2H_2O	1.5	16	8	85	1.3	1.4	16	8	90	1.5
BFDGE	0.7	20	10	70	1.6	4.0	17	6	89	1.6
BFDGE·2HCl	1.6	13	4	95	1.9	1.5	7	8	74	2.0

[a] Low concentration level: cola sample (0.15–2.0 $\mu g\,L^{-1}$) and red pepper (2.0–15.0 $\mu g\,kg^{-1}$).
[b] Medium concentration level: cola sample (1.5–20 $\mu g\,L^{-1}$) and red pepper (20–150 $\mu g\,kg^{-1}$).
[c] Ion ratio calculated at medium concentration level.

drink samples (Table 4). Samples were prepared as described in Section 2.3 and analyzed by triplicate. In canned soft-drink beverages only BADGE·2H_2O was detected, at concentrations ranging from 2.3 $\mu g\,L^{-1}$ to 5.1 $\mu g\,L^{-1}$, while other BADGEs and BFDGEs were not detected. In contrast, several BADGEs were found in canned food samples. BADGE·2H_2O was found in all food samples at concentrations between 2.7 $\mu g\,kg^{-1}$ and 675 $\mu g\,kg^{-1}$, with the highest concentration level being in the asparagus sample. Other BADGEs detected in these samples were BADGE·H_2O at concentrations ranging from 35 $\mu g\,kg^{-1}$ to 53 $\mu g\,kg^{-1}$, BADGE·HCl·H_2O (3.4 – 274 $\mu g\,kg^{-1}$) and BADGE·2HCl at concentrations between 0.9 $\mu g\,kg^{-1}$ and 2.8 $\mu g\,kg^{-1}$. 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 lesser use of BFDGE-based coatings. As an example, Fig. 5 shows the LC–MS/MS chromatograms obtained for the asparagus samples in which BADGE·2H_2O, BADGE·H_2O, BADGE·HCl·H_2O, BADGE·HCl and BADGE·2HCl were detected. These samples were also analyzed by LC–MS/MS, using H-SRM on Q1. No false positives/negatives were detected in the samples analyzed.

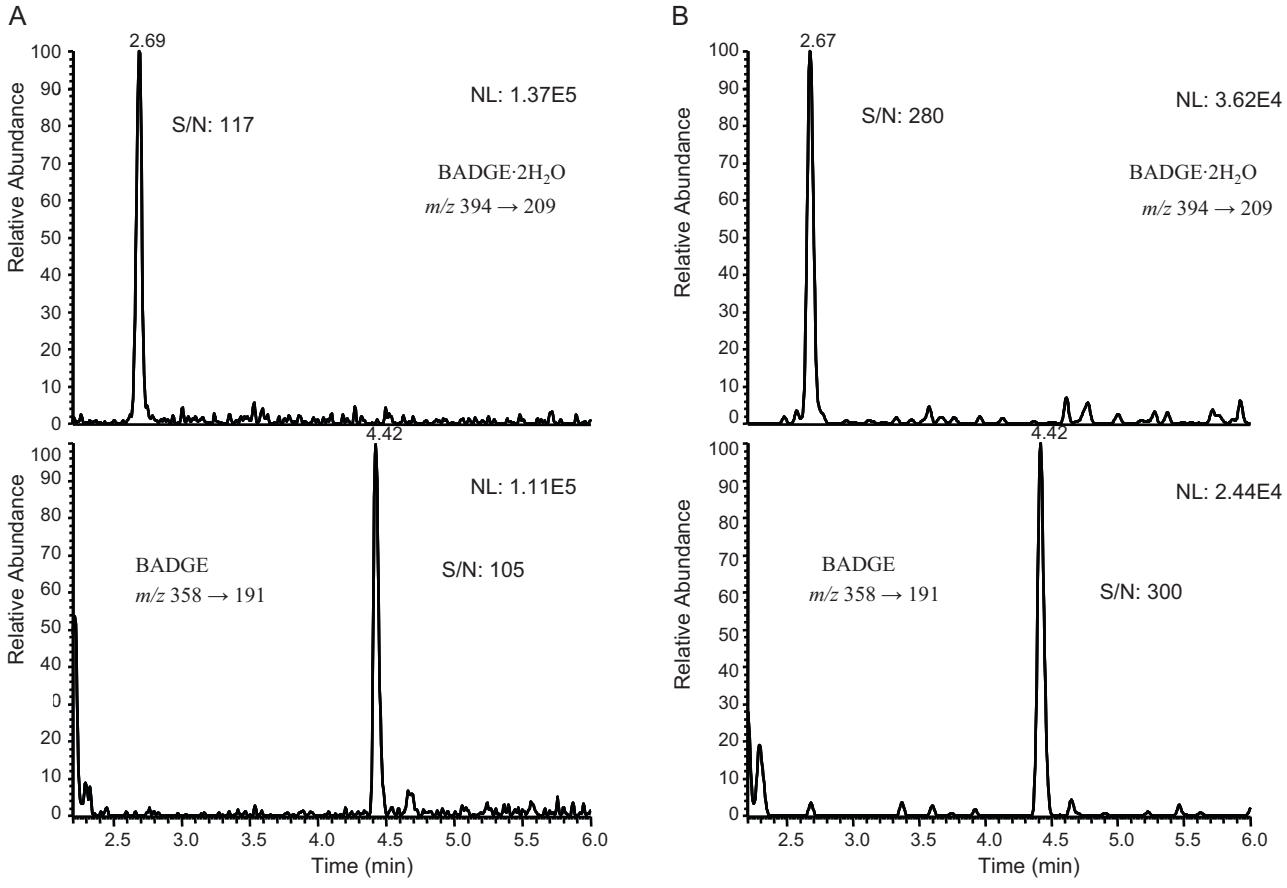

Fig. 4. LC–MS/MS chromatograms of a red pepper sample spiked with BADGE·2H_2O and BADGE analyzed using (A) SRM acquisition mode and (B) H-SRM on Q1 acquisition mode.

Table 4
Canned soft-drinks and food samples analyzed using the developed LC–MS/MS method.

Samples	BADGE·2H$_2$O (μg kg^{-1}) ± SD[a]	BADGE·H$_2$O (μg kg^{-1}) ± SD[a]	BADGE·HCl·H$_2$O (μg kg^{-1}) ± SD[a]	BADGE·HCl (μg kg^{-1}) ± SD[a]	BADGE·2HCl (μg kg^{-1}) ± SD[a]
Soft-drinks					
Cola	3.6 ± 0.4	n.d.	n.d.	n.d.	n.d.
Tea	2.6 ± 0.2	n.d.	n.d.	n.d.	n.d.
Beer 1	5.1 ± 0.6	n.d.	n.d.	n.d.	n.d.
Beer 2	4.3 ± 0.5	n.d.	n.d.	n.d.	n.d.
Lemon soda	2.1 ± 0.1	n.d.	n.d.	n.d.	n.d.
Orange soda	2.8 ± 0.1	n.d.	n.d.	n.d.	n.d.
Soft-drink	2.3 ± 0.3	n.d.	n.d.	n.d.	n.d.
Canned food					
Sweet corn 1	369 ± 18	40 ± 1	3.4 ± 0.7	n.d.	2.7 ± 0.3
Sweet corn 2	252 ± 19	37 ± 6	4.4 ± 0.8	n.d.	1.1 ± 0.1
Pinapple 1	2.8 ± 0.1	n.d.	n.d.	n.d.	n.d.
Pinapple 2	3.1 ± 0.6	n.d.	n.d.	n.d.	0.9 ± 0.1
Red pepper	157 ± 25	35 ± 7	4.7 ± 1.0	n.d.	1.6 ± 0.1
Asparagus	675 ± 100	53 ± 11	274 ± 40	11 ± 1.5	2.8 ± 0.2

BADGE, BFDGE, BFDGE·2H$_2$O and BFDGE·2HCl were not detected in the analyzed samples.
[a] SD, standard deviation (standard deviation was calculated for triplicate analysis, $n = 3$).

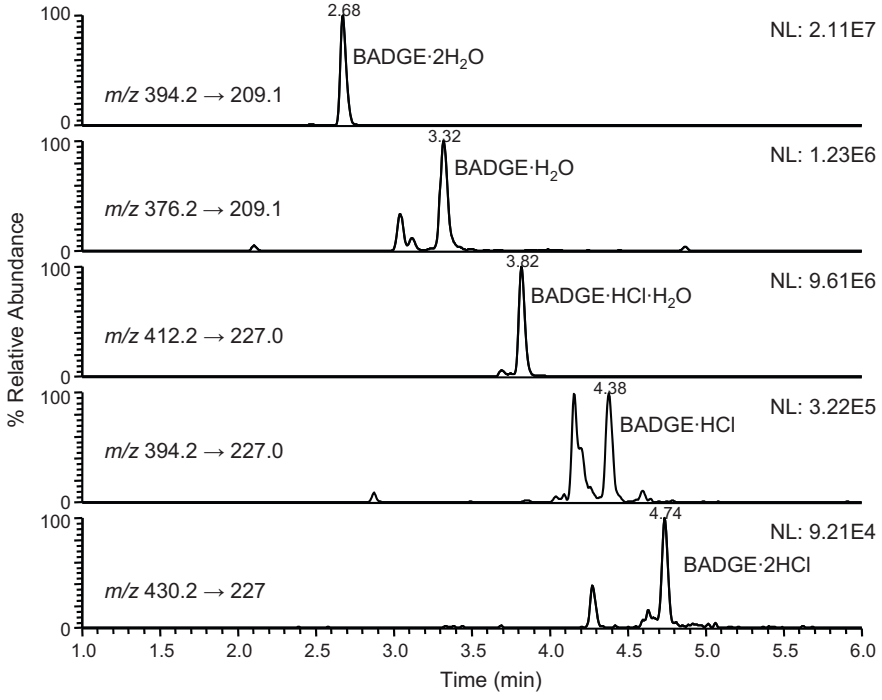

Fig. 5. LC–MS/MS chromatogram in SRM acquisition mode for the analysis of BADGEs in asparagus sample.

4. Conclusions

In this paper a fast liquid chromatography–tandem mass spectrometry (LC–MS/MS) method is proposed for the simultaneous analysis of BADGEs and BFDGEs in canned food samples and soft drinks. Highly efficient separation, in less than 5 min, was obtained by using a Fused Core™ column at 600 μL min^{-1}. Good limits of quantitation below 1.6 μg L^{-1} in soft drinks and below 4.0 μg kg^{-1} in canned food were obtained.

The LC–MS/MS method developed was used to analyze these compounds in several soft drinks and canned foods. BADGE·2H$_2$O was always present in the analyzed samples at μg kg^{-1} level in soft drinks, whereas in canned food concentrations rose up 675 μg kg^{-1} (asparagus sample). Moreover, the other compounds BADGE·H$_2$O, BADGE·HCl·H$_2$O, BADGE·HCl and BADGE·2HCl were also detected in the canned food samples at concentrations lower than BADGE·2H$_2$O. The absence of both false positives and false negatives was confirmed by the use of H-SRM acquisition mode.

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