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Determination of bisphenol A diglycidyl ether and its hydrolysis and chlorohydroxy derivatives by liquid chromatography–mass spectrometry

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Abstract

European Legislation establishes that the sum of the migration levels of bisphenol A diglycidyl ether (BADGE), its hydrolysis (BADGE·H_2O and BADGE·$2H_2O$) and chlorohydroxy (BADGE·HCl, BADGE·2HCl and BADGE·H_2O·HCl) derivatives shall not exceed the limit of 1 mg/kg in foodstuffs or food simulants. A reversed-phase high-performance liquid chromatographic (RP-HPLC) method combined with mass spectrometry detection using atmospheric pressure chemical ionisation (APCI) is developed for the separation, quantification and identification of the interesting compounds. Quantification of the analytes was carried out in the single ion recording mode, once their characteristic masses were selected from their full spectra, by using an external calibration. The optimised method was suitable for the migration evaluation of these compounds in different samples.

Keywords: Bisphenol A diglycidyl ether

1. Introduction

Epoxy resins are used to make internal surface coatings for food cans (sea products, vegetables, beer, soft drinks, powder milk), big storage vessels (wine, water) and various types of food containers. Bisphenol A epoxy resins are mainly condensation products of bisphenol A with epichlorhydrin [1]. If the reaction process or cure conditions were not applied in a proper way, and finished coating is not correctly crosslinked, components of bisphenol A epoxy resins and reaction products formed could migrate to food [2], and in some cases react with food components giving new compounds.

Recent studies about the toxicity of these compounds, [3–5] have demonstrated that the genotoxic effect of bisphenol A diglycidyl ether (BADGE) was stronger than the genotoxic effect of BADGE·H_2O and BADGE·$2H_2O$. Regarding BADGE·HCl, its genotoxic effect was comparable to those obtained to BADGE·H_2O. These studies support the hypothesis that the degree of toxicity of epoxy compounds depends mainly of the concentration of unreacted epoxy groups, although the case of BADGE·2HCl is different because it does not present any epoxy group, but its genotoxicity is probably due to the presence of Cl groups.

BADGE can also be used in organosols in order to remove hydrochloric acid, which results in formation of BADGE·HCl, BADGE·2HCl and BADGE·H_2O·HCl [6]. BADGE may easily hydrolyse in contact with aqueous and acidic food forming BADGE·H_2O and BADGE·$2H_2O$ [7] (Fig. 1).

European Legislation establishes that the sum of the migration levels of BADGE, its hydrolysis (BADGE·H_2O and BADGE·$2H_2O$) and chlorohydroxy (BADGE·HCl, BADGE·2HCl and BADGE·H_2O·HCl) derivatives shall not exceed the limit of 1 mg/kg in foodstuffs or in food simulants or 1 mg/6 dm^2 in the material tested (conventional conversion factor that expresses the relationship between the surface area of materials and the quantity of foodstuffs in contact therewith [8,9].

The most widely used techniques for the analysis of BADGE are normal- and reversed-phase high-performance liquid chromatography NP-HP (LC and RP-HPLC) with fluorescence detection, [10–18] as well as its hydrolysis and chlorohydroxy derivatives [12,16,19–21]. Several

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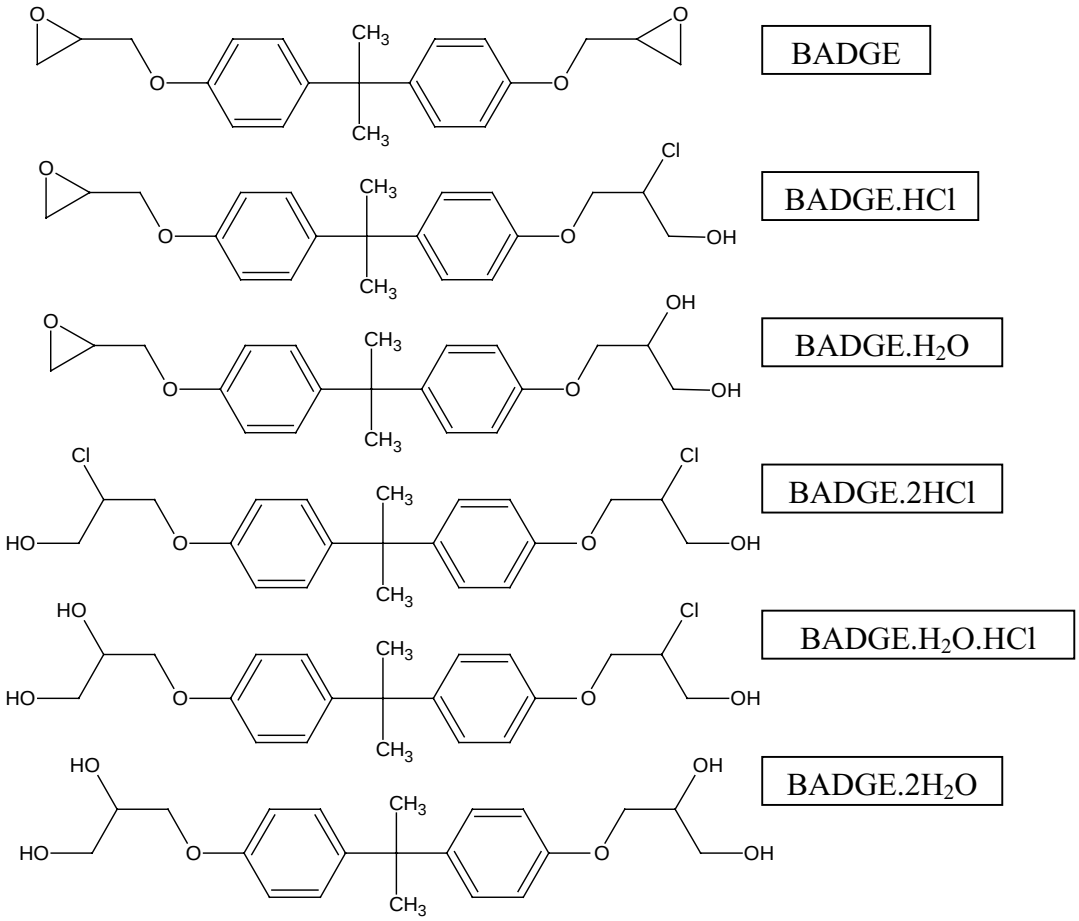


Fig. 1. Chemical structures of BADGE and its derivatives.

researches have applied gas chromatography (GC) coupled with mass spectrometry detection to determine these compounds [22–25]. Most of the times this technique was used just to confirm positively the presence of these compounds after HPLC quantification with fluorescence detection [11,14,21,26].

Some works are focused on the identification of these substances and other oligomers of higher molecular mass using HPLC with detection by atmospheric pressure chemical ionisation (APCI) [27–30], thermospray mass spectrometry (TSP) [31] and electrospray ionisation (ESI) [29,32]. The characterisation of BADGE hydrolysis products has also been described by other authors [33]. Very few works are focused on the quantification using an HPLC–MS technique [34], but this last case is limited to BADGE and BADGE·2H_2O.

The objective of this work is to develop a method that allows both the quantification and positive identification of BADGE and its derivatives using an RP-HPLC–APCI-MS technique, after its majority characteristic masses were selected. This will facilitate the migration studies in complex food or in food simulants, once this way possible interferences are minimised.

2. Experimental

2.1. Chemicals and reagents

Acetonitrile was HPLC grade, supplied by Merck (Darmstadt, Germany) and ultrapure water was prepared using a Milli-Q filter system (Millipore, Bedford, MA, USA). Standards of bisphenol A diglycidyl ether (CAS No. [1675-54-3], (≥97%)), bisphenol A (2,3-dihydroxypropyl) glycidyl ether (BADGE·H_2O, CAS No. [76002-91-0], (≥97%)), bisphenol A bis(2,3-dihydroxypropyl) ether (BADGE·2H_2O, CAS No. [5581-32-8], (≥97%)), bisphenol A (3-chloro-2-hydroxypropyl) glycidyl ether (BADGE·HCl, CAS No. [13836-48-1], (≈95%)), bisphenol A bis(3-chloro-2-hydroxypropyl) ether (BADGE·2HCl, CAS No. [4809-35-2], (≥99%)) and bisphenol A (3-chloro-2-hydroxypropyl) (2,3-dihydroxypropyl) ether (BADGE·H_2O·HCl, CAS No. [227947-06-0], (≥98%)) were all purchased from Fluka (Buchts, Switzerland).

Individual stock solutions of all compounds containing 1000 mg/l, were prepared in acetonitrile and were kept at −20 °C. Intermediate standards solutions in 90% (v/v) acetonitrile and water were prepared by dissolving

appropriate amounts of all compounds to yield a concentration of 10 mg/l. Calibration solutions in water and 90% (v/v) acetonitrile were prepared from these intermediate solutions. Intermediate solutions in 90% acetonitrile were kept at 4 °C and intermediate solutions in water were kept at −20 °C.

2.2. Equipment

The HPLC–MS system comprised a Spectra-Physics Series P200 liquid chromatograph equipped with a 50 μl injection loop Rheodyne, and a Fisons VG Platform mass detector (VG Biotech, Altrincham, UK), controlled by VG Mass Lynx software (all from SP Thermo Separation Products, Altrincham, UK). The column was a Kromasil 100 C_{18} (15 cm × 0.4 cm i.d., 5 μm particle size) (Teknokroma).

2.3. Chromatographic conditions

Elution conditions were developed in a previous article [16]. The mobile phase was acetonitrile–water (30:70, v/v) in an isocratic mode for 2 min, followed by a gradient to 80% acetonitrile for 18 min, another gradient to 100% acetonitrile for 3 min and finally an isocratic elution during 7 min. The flow rate was 1.0 ml/min. Detector settings: positive or negative APCI mode, probe temperature 500 °C, ionisation source temperature 130 °C, cone voltage +30 or −30 V, electron multiplier voltage 700 V, drying gas nitrogen at 425 l/h, APCI sheet gas nitrogen at 175 l/h, full-scan mode detection range *m/z* 100–500 and selected ion recording (SIR) (Table 1).

2.4. Samples

Conventional empty three-piece type cans (with easy-open lids) were provided by the industry. Cans and lids were extracted separately and both were extracted only for the surface intended to be in contact with foodstuffs. Cans were completely filled with acetonitrile and stored for 4 h at 40 °C. They were covered to prevent evaporation. In this way, an extract of all surfaces was obtained. For the extraction of the lids, special glass cells were used: the lids were located inside the cap cell which is recovered with teflon and turning over the cells once they were closed. Therefore, only the internal surface of the lid was in contact with the acetonitrile. These cells were kept for 4 h at 40 °C. The exposure conditions were the same for all cans tested.

For the HPLC analysis, 0.1 ml of water was added to 0.9 ml of the extract obtained and it was filtered through a PTFE 0.45 μm, 13 mm syringe filter, and injected into the chromatograph. In any case no step of cleaning up was applied.

Table 1
Selected ions for quantification

Analyte	APCI (+)		APCI (−)	
	Selected ion (+*m/z*)	Other ions	Selected ion (−*m/z*)	Other ions
BADGE	382[a]	191		
BADGE·HCl	418[a]	382		
BADGE·2HCl	382[b]	191		
BADGE·H_2O	400[a]	209		
BADGE·H_2O·HCl			393[c]	283, 227
BADGE·$2H_2O$			375[c]	301, 227

[a] Fragment corresponding to $[M + CH_3CNH]^+$.
[b] Unknown fragment.
[c] Fragment corresponding to $[M - H]^-$.

3. Results and discussion

3.1. Mass spectrometry

The first part of this work was focused in stabilising the optimums detector conditions. Analysis by this technique is greatly influenced by the cone voltage, which determines the degree of fragmentation of analyte ions. Cone voltages of 10, 30 and 50 V (in (+) and (−) mode) were evaluated for each substance in order to set a cone voltage that allowed to obtain selective ions for each compound. Probes temperatures of 250 and 500 °C were tested and although it did not greatly affect the degree of fragmentation, it was finally set at 500 °C due to the low volatility of all compounds.

Although full scan mode gives more information, it was chosen the SIR mode for the quantification, since this way the sensitivity was improved (about 50 times).

Once detector conditions were optimised and to establish the SIR parameters for the quantification, initial tests on concentrate standard solutions of 10 mg/l were carried out using the full scan mode to obtain the maximum information from their mass spectra (Fig. 2). BADGE, BADGE·H_2O and BADGE·$2H_2O$ were characterised by other authors [33] using the APCI technique. Thus, for BADGE and BADGE·H_2O, APCI (+) was used, and fragments corresponding to the clusters comprising these analytes and a molecule of acetonitrile were observed to be the most abundant ($[M + CH_3CNH]^+$) (Fig. 2A and D). For BADGE·$2H_2O$, APCI (−) was used and the fragment corresponding to the $[M - H]^-$ was selected to quantify due to its selectivity (Fig. 2F).

For BADGE·HCl the major fragment corresponds to the $[M + CH_3CNH]^+$ (Fig. 2B) which coincides with other authors [27]. The case of BADGE·2HCl is more difficult to explicate. It could be explained if the molecule loses its Cl atoms and hydroxyl groups of the chlorohydrin act as a nucleophilic reagents, giving rise to a conjugated acid from which the epoxide arises by the elimination of a proton [35]. So the formed molecule would act as BADGE, forming a cluster with a molecule of acetonitrile.

BADGE·H_2O·HCl was determined using the APCI (−) mode instead of (+) mode due to the impossibility of undergoing a chromatographic separation from BADGE·H_2O.

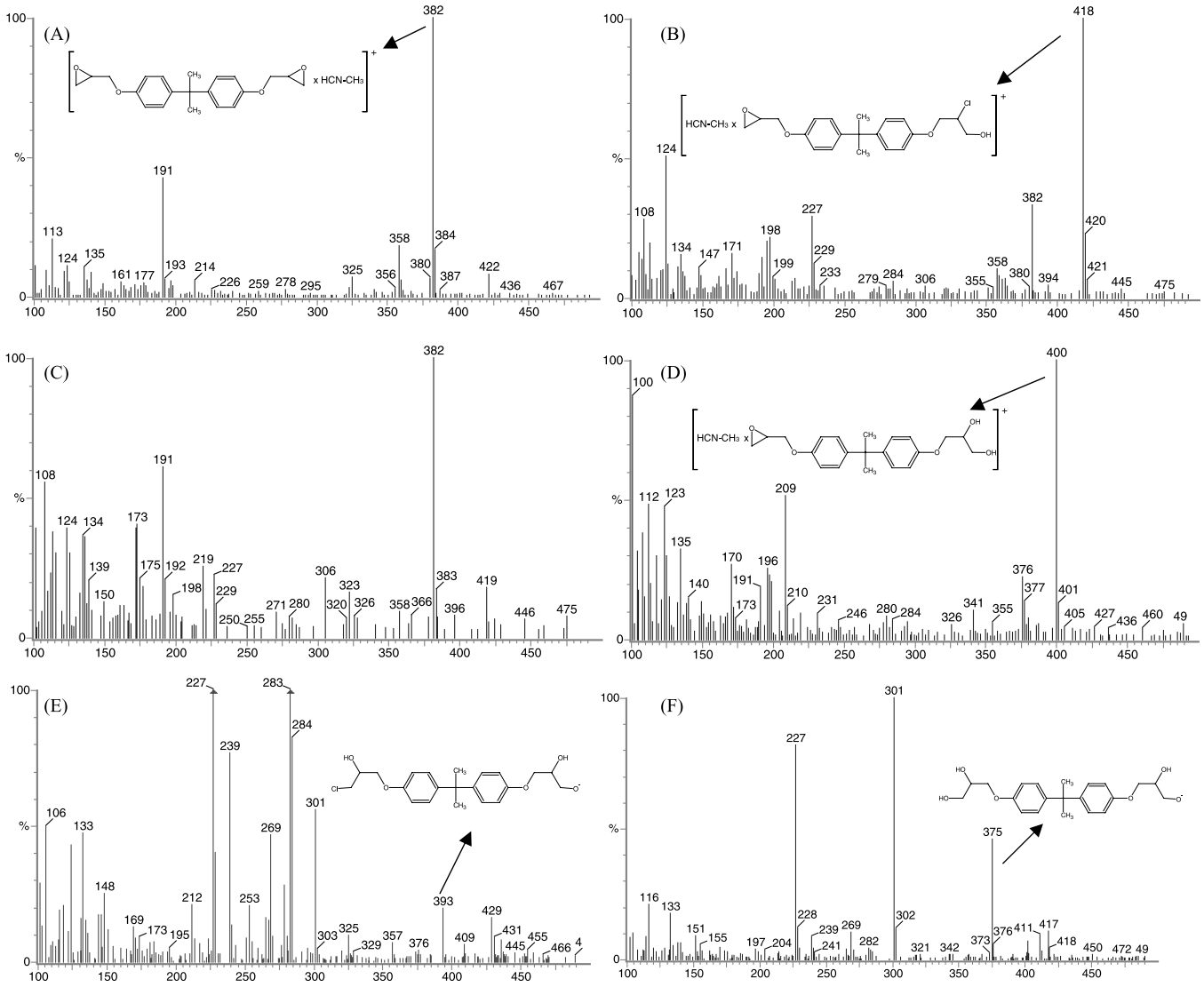


Fig. 2. (A) MS spectra of BADGE; (B) BADGE·HCl; (C) BADGE·2HCl; (D) BADGE·H_2O; (E) BADGE·H_2O·HCl; and (F) BADGE·$2H_2O$.

Hence a very selective fragment of BADGE·H_2O·HCl corresponding to its $[M - H]^-$ (Fig. 2E) ion was selected to determine it.

Furthermore, for the substances that were determined using the APCI (−) mode, other fragments (Table 1) were present in a more relative abundance, they were not selected because they were common to more species. Table 2 shows possible structures of these fragments.

3.2. Chromatography

Chromatographic protocol has been optimised previously in order to improve the separation of these substances. Not all BADGE derivatives have response in the same ionisation mode, so two injections of each sample were always necessary in negative and positive mode. Figs. 3 and 4 show chromatograms acquired in both positive and negative mode.

3.3. Method validation

The method was calibrated using series of standards (mixtures of all substances) in 90% acetonitrile of known concentrations. The relationship between known concentrations and measured areas was assessed by linear regression (five calibrations points), and the linearity obtained indicates that the method is appropriate for quantification of these compounds (Table 3). Detection limits (DLs), (defined as signal three times the height of the noise level) were calculated in accordance with American Chemical Society [36] and are shown in Table 3. The lower DL corresponds to BADGE, and although in other works [6,17,22,28] have reached lower levels employing fluorescence detection, in this case, no positive confirmation is necessary. Additionally, for the substances with a higher DL, it has been observed that if other ions are syntonized it could be reached a lower DL (about three times less in case of BADGE·H_2O·HCl).

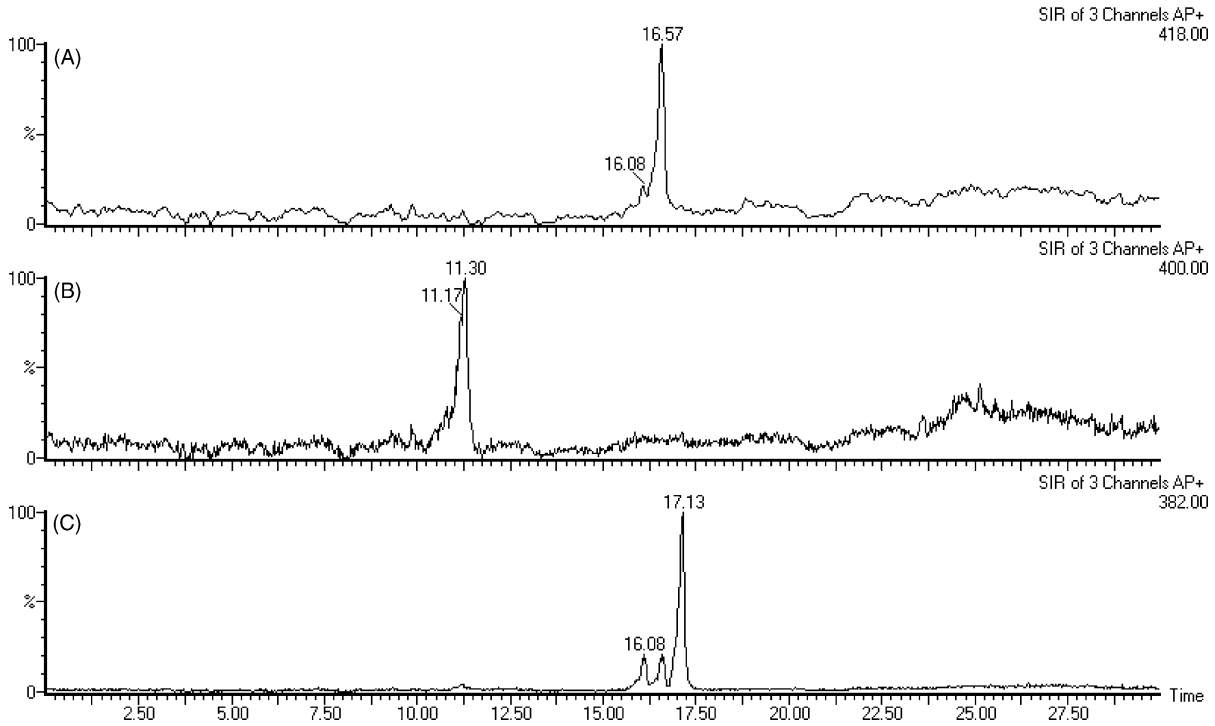


Fig. 3. (A) MS chromatograms in APCI (+) of BADGE·HCl; (B) BADGE·H_2O; (C) and BADGE (t_R: 17.3) and BADGE·2HCl (t_R: 16.0), at a concentration of 0.8 mg/l. Time scale in minute.

The method was also evaluated in water instead of 90% acetonitrile. For all substances correlation coefficients were higher than 0.99 and the DLs were similar except for BADGE·$2H_2O$ and BADGE·H_2O·HCl, which were 0.1 mg/l for both substances.

In both cases (for 90% acetonitrile and water), comparing the established SMLs in the European Legislation [8], the detection limits provide well enough performance although with other techniques lower values can be obtained.

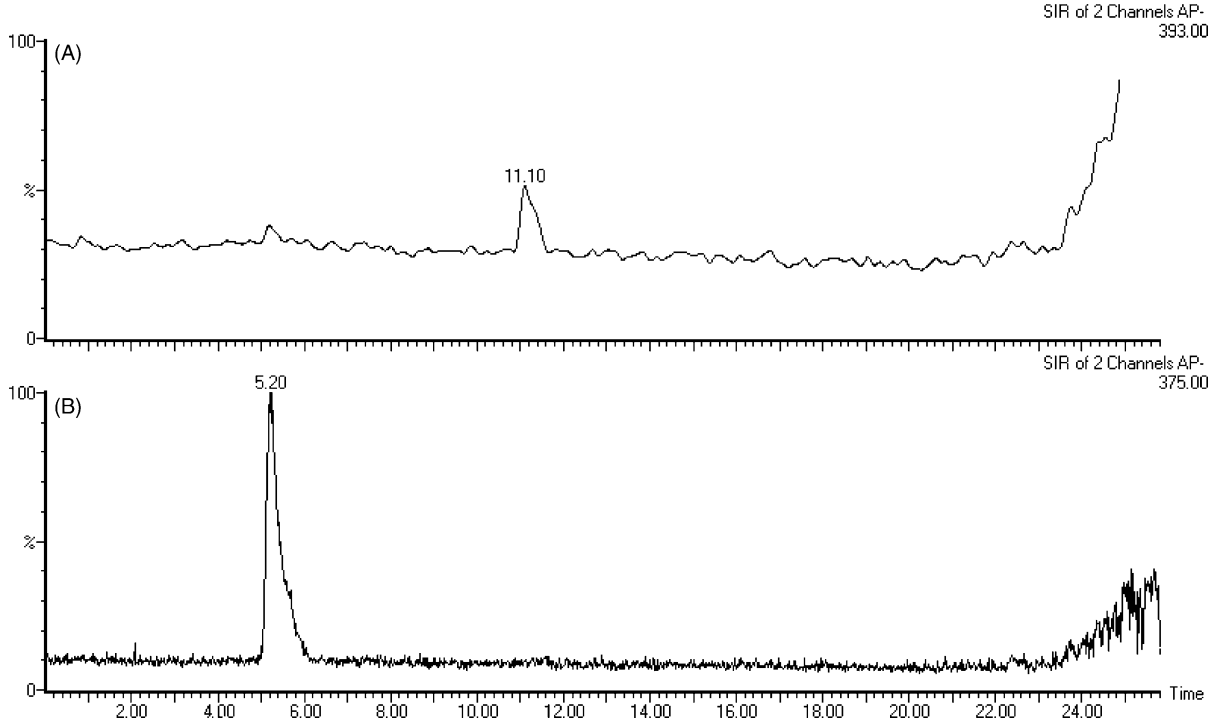
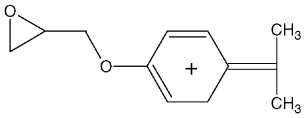
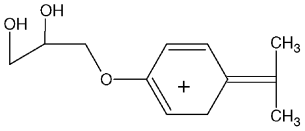
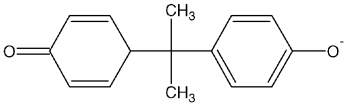
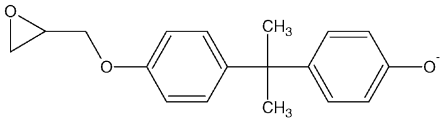
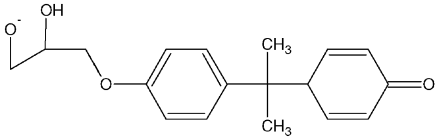


Fig. 4. MS chromatograms in APCI (−) of (A) BADGE·H_2O·HCl and (B) BADGE·$2H_2O$, at a concentration of 0.8 mg/l. Time scale in minute.

Table 2
Possible structures of other fragments

Ion	Structure	APCI
191		(+)
209		(+)
227		(−)
283		(−)
301		(−)

Precision was estimated as relative standard deviation (R.S.D.) analysing six independent solutions at the level of 1 mg/l, obtaining for all compounds a value equal to 5% (Table 3).

Once the operating conditions had been optimised, screening analyses were performed with empty cans (eight different samples) analysing them by duplicate and extracting them with acetonitrile as it has been described previously. No peaks of interest were detected in any case, which demonstrates the safety of the cans tested. A similar extracting procedure has been used by other authors [22] to test empty cans. Acetonitrile helps to test this type of materials and seems to be a good extractor [27] once this simple sample procedure could give an idea of the security of cans prior its use as a packaging material for foodstuffs.

In conclusion, the developed method is appropriate for the simultaneous analysis of all these compounds in acetonitrile extracts and in water (official food simulant). This procedure is also a powerful technique that allows the positive confirmation of the presence of these compounds, not being necessary another technique for its quantification.

Table 3
Method validation parameters

	Linear range (mg/l)	Linearity (r^2)	Detection limit (mg/l)	R.S.D. (%)
BADGE	0.1–2.4	0.9975	0.05	3.9
BADGE·HCl	0.4–2.4	0.9970	0.1	4.1
BADGE·2HCl	0.4–2.4	0.9902	0.1	3.9
BADGE·H_2O	0.4–2.4	0.9980	0.1	4.3
BADGE·H_2O·HCl	0.8–2.4	0.9903	0.4	5.0
BADGE·$2H_2O$	0.4–2.4	0.9975	0.1	4.0

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