ELSEVIER

Journal of Chromatography A, 864 (1999) 137–144

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Use of solid-phase microextraction for the analysis of bisphenol A and bisphenol A diglycidyl ether in food simulants

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Received 21 June 1999; received in revised form 10 September 1999; accepted 10 September 1999

Abstract

A new method has been developed to simultaneously analyse bisphenol A (BPA) and bisphenol A diglycidyl ether (BADGE) in aqueous based food simulants. The method consists on direct immersion solid-phase microextraction (SPME) of the analytes from the liquid matrix and subsequent chromatographic analysis by gas chromatography–mass spectrometry. Using the proposed method, a whole analysis (including chromatographic step) can be completed in less than 40 min, with minimum sample handling. The SPME method shows good analytical performance for simultaneous BPA and BADGE analysis, except for BADGE determination in the aqueous alcohol (simulant C) solution. Detection limits ranging from 0.1 to 2.0 ng/g for BPA and from 13 to 15 ng/g from BADGE were obtained, with a linear range from the low-ng/g to several-μg/g range for BPA and from 0.1 μg/g to 40 μg/g for BADGE. A possible optimisation method has been also developed and introduced.

Keywords: Food simulants; Bisphenol diglycidyl ethers

1. Introduction

Bisphenol A (4,4′-isopropylidenediphenol, CAS No. 80-05-7, commonly named BPA) is a principal component of both polycarbonate and epoxy resins. It is also used in flame-retardants, and in unsaturated polyester, polysulphone, polyetherimide and polyacrylate resins. Many foodstuff containers are made of some of these resins including many for oven and microwave cooking.

Bisphenol A diglycidyl ether [2,2 bis-(4-hydroxyphenyl) propane-bis (2,3-epoxypropyl) ether, CAS No. 1675-54-3, also known by the acronym BADGE] is commonly used as a precursor of epoxy-based coatings of food cans and storage vessels and for acrylic/epoxy adhesives.

When a polymeric material is in contact with food, especially at high temperatures (e.g., microwaved food or heated processed canned food) the ability of BPA and BADGE to migrate from the polymer to the food has been described [1–5]. Moreover, BPA has been proved, in vitro, to be estrogenically active [6–9] and BADGE has been classified as tumorigen, mutagen and primary irritant by the National Institute for Occupational Safety and Health [10] and in Occupational Health Services Inc. Material Safety Data Sheets [11]. In animals, compounds related to BADGE have been shown to produce chromosomal aberration [12] and to have carcinogenic activities [13,14].

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0021-9673/99/$ – see front matter
PII: S0021-9673(99)00987-5

The EU Commission established a specific migration limit (SML) for BPA of 3 mg per kg of food or food simulant [15] and of 0.02 mg/kg for BADGE. More recently, the EU Commission and the Scientific Committee for Food proposed to increase the SML for BADGE up to 1 mg/kg [16,17].

Migration testing is usually carried out by working with food simulants rather than with foods. Foods are complex mixtures of variable composition, and their analysis presents some analytical and practical drawbacks. So, food simulants have been selected to model the various categories of foods (aqueous, acidic, alcoholic and fatty) to simplify migration testing, and to allow a plastic to be assessed for its suitability for a whole class of foods in a range of applications [18,19].

Analytical methods for determining BPA and BADGE residues in solid and liquid matrices have been proposed. High-performance liquid chromatography (HPLC) followed by fluorescence detection with or without a prior extraction step, depending on matrix characteristics, is reported in the literature for determination of BPA [3,20,21]. BADGE determination is also usually carried out by HPLC–fluorescence detection [5,22,23].

These methods have quite good analytical performance, but a big drawback for them is that they need a separate analysis by mass spectrometry (MS) to obtain structural confirmation, especially for BPA. This is due, for example, to the coelution phenomena observed for BPA and the diol-epoxide formed by hydrolytic opening of BADGE under reversed-phase (RP) HPLC–fluorescence detection [24].

To overcome these problems, the use of inclusion complexes followed by spectrofluorimetric analysis [25]; the use of liquid–liquid extraction, [$^2H_8$]BPA as internal standard and cool on-column injection [26] or chemical derivatization [27] followed by gas chromatography (GC) have been proposed for BPA analysis in water. GC–MS and HPLC–MS–MS have been also proposed for BADGE quantitation, but only for solid matrices, as microwave susceptors, pizza or canned foodstuffs [2,28].

Solid-phase microextraction (SPME) is an extraction method that utilises a small fused-silica fibre, coated with a suitable polymeric stationary phase for analyte isolation and preconcentration from a matrix [29–32]. After the sorption step, the whole extraction device is transferred to a GC system, and the fibre is inserted into a hot injector, where thermal desorption of trapped analytes takes place. The technique is fast, cheap, easy to use and completely solvent-free.

In this paper the simultaneous analysis of BPA and BADGE in aqueous based food simulants [distilled water (simulant A), 3% (w/v) acetic acid–water solution (simulant B) and 10% (v/v) ethanol–water solution (simulant C)] using SPME followed by GC–MS is described. The analytical performance of the method and some considerations about the SPME procedure are also discussed.

2. Experimental

2.1. Gas chromatographic analysis

The chromatographic analysis was performed on a Hewlett-Packard 6890 gas chromatograph (Wilmington, DE, USA) equipped with a 5973 mass-selective detector. A HP-5 MS (30 m×0.25 mm, 0.25 μm film thickness) capillary column was used.

The temperature program was as follows: initial oven temperature: 200°C, hold 2 min, linear temperature gradient 10°C/min to 270°C and hold 15 min. A post-run step was performed after each analysis by raising the temperature up to 280°C for 5 min.

The carrier gas was helium, C-50 quality, supplied by Carburos Metálicos (Barcelona, Spain). All the work was carried out in constant flow mode set at 1.1 ml/min.

Quantitation of the analytes was carried out in the selected ion monitoring (SIM) mode, once their characteristic masses were selected from their full spectra. The selected masses were m/z 228 and 213 for BPA and m/z 340 and 325 for BADGE analysis. In both cases, they correspond to the M^+ (molecular ion) and to $[M-CH_3^+]$, respectively.

2.2. Solid-phase microextraction

2.2.1. SPME apparatus

The SPME apparatus consists of a manual reusable syringe assembly, supplied by Supelco (Bellefonte, PA, USA). The scheme of the apparatus has been described elsewhere [30,33]. The microextraction fibres tested in this study were coated with

poly(dimethylsiloxane) (PDMS) of 100 and 7 μm thickness; 85 μm film thickness of polyacrylate (PA) and 65 μm thickness of Carbowax–divinylbenzene (CW–DVB), all of them purchased from Supelco. The fibres were conditioned before their first use according to manufactor's specifications.

2.2.2. SPME procedure

Simulant solutions containing BPA and BADGE were prepared by adding the appropriate mass of the undiluted stock standard solution to a 20-ml glass vial and diluting to 12 g with the current food simulant. The appropriate salt amount [NaCl for distilled water; Na_2HPO_4 to 3% acetic acid simulant and $(NH_4)_2SO_4$ to 10% ethanol as simulant] was then added to make a solution of 7.5% (w/w) in each case.

The vial, crimped with a PTFE faced septum, was then magnetically stirred to allow salt dissolution and temperature equilibration. Then, sampling was performed through the septum by total immersion of the SPME fibre into the stirred solution for a predetermined time and temperature. After sampling, the SPME device was transferred to an standard split–splitless injection port on the GC instrument where thermal desorption of the analytes was carried out.

The analysis of BPA and BADGE in simulant C (10%, v/v, ethanol) represents the extraction of a fully degassed simulant, by using sonication for 10 min prior to extraction, and allowing to cool at room temperature. The reason is that bubble deposition on the fibre reduces the coating surface and does not allow a full contact between the stationary phase and the liquid matrix.

Blank analyses of each simulant were performed daily at the beginning of the day to check fibre interferences.

2.3. Reagents

Bisphenol A (>99%) was obtained from Aldrich (Madrid, Spain). BADGE (>98%) was supplied by Central Science Laboratory of Ministry of Agriculture, Fisheries and Food (Norwich, UK). Standard stock solutions (~500 μg/g) containing these compounds were made up in acetonitrile. Optimisation and calibration solutions were prepared in each simulant from this standard solution.

All the solvents used were from Merck (Darmstadt, Germany), Suprapur quality. Distilled water was Milli-Q quality purified with a Millipore purification system (Bedford, MA, USA). Sodium chloride, ammonium chloride and ammonium sulphate were also from Merck (analytical-reagent grade). Sodium hydrogenphosphate (>99%) was supplied by Probus (Barcelona, Spain).

3. Results and discussion

3.1. SPME optimisation

The selection of the operating conditions in SPME is still a difficult task. To our knowledge, most of developers optimise extraction methods one parameter at a time, making the procedure tedious and not very effective. Only two different ways to optimise SPME have been found in the scientific literature [34,35]. So, a possible optimisation procedure was developed and introduced [36,37].

The method is a composite design which consists of a fractional replicate of a 2^n full factorial design for quantitative variables optimisation superposed on a Latin squares for qualitative optimisation. The experimental design matrix is already described and it has been applied to the SPME of pesticides in ethanol–water mixtures used as food simulants [37].

Quantitative factors where those for which a continuous variation is possible for all the experimental range, whereas qualitative factors are those for which only discrete values are suitable. Table 1 shows the experimental variables and the variation range selected.

This way, six experimental variables (four quantitative and two qualitative) were studied in the present work. Different matrices were generated for each single simulant by modification of the test columns. Matrix determination was carried out in duplicate. Fig. 1a–c show typical chromatograms corresponding to analyte determination in each simulant.

Evaluation of the matrix was performed by comparison of the average of each single result with the average of all the determinations, noted as 100%. Then, a positive influence implies a coefficient higher than 100% whereas a negative tendency is

Table 1
Factors selected and levels in the experimental design

Variable	Level	
	High (+)	Low (−)
Quantitative factors		
(I) Sorption temperature[a]	50°C	Room temperature
(II) Desorption temperature[b]	Max. temp. −10 °C	Min. temp. +10 °C
(III) Sorption time	20 min	5 min
(IV) Salt concentration	7.5% (w/w)	0% (w/w)
Qualitative factors		
(V) Salt identity (pH)	(1) Na_2HPO_4 (2) NH_4Cl (3) NaCl (4) $(NH_4)_2SO_4$	
(VI) Fibre identity	(A) 65 μm CW–DVB (B) 7 μm PDMS (C) 100 μm PDMS (D) 85 μm PA	

[a] Room temperature was maintained at 20±1°C in all the experiments.
[b] Maximum and minimum temperatures selected according to manufactor's specifications.

defined by a lower coefficient. Table 2 shows the optimum values found in each simulant for the ranges studied. BADGE was not detected in any case working with simulant C, which is a quite surprising achievement.

The absence of BADGE could be attributed to several reasons. Firstly, its degradation in ethanol. However, the half-life of BADGE in 15% (v/v) ethanol is about two-times longer than in distilled water and more than seven-times longer than in acetic acid [38], so not analyte degradation could be reasonably expected. Secondly, working with SPME, one must keep in mind that this is a distribution process described by a fibre coating/sample matrix distribution constant for each analyte that is strongly influenced by matrix characteristics [37]. The higher the distribution constant, the better extraction performance for an analyte in a matrix.

As BADGE solubility is much higher in ethanol than in the others simulants tested, the fibre coating/sample matrix distribution relationship for this analyte is displaced to the matrix. So, the value of the constant diminishes making quantitative extraction in this particular situation not possible. On the other hand, the presence of ethanol reduces the performance of the fibre, as was previously demonstrated [37,39].

As Table 2 shows, the optimum values found for the range studied were near identical between the food simulants tested. SPME conditions are related to analyte itself and to changes on the analyte properties due to matrix influence. The results shown in Table 2 indicate that no degradation or chemical transformations of the analytes are induced by the simulant characteristics. This is consistent with the results presented by Paseiro et al. [38], which established half-lives ranging from 8.1 to 69.8 h for BADGE in the selected food simulants.

Concerning the optimum values found, a brief discussion on them could be interesting. The sorption temperature was fixed at the maximum value tested. At a given time before the equilibration is attained, the concentration in the fibre is greater at a higher temperature than a lower temperature, because the rate of extraction is greater, as was previously established [32]. However, the limit of sorption temperature is established by analyte characteristics: thus, the shelf-life for BADGE in simulant C reduces from 8.1 h at 40°C to 2.0 h at 60°C [38]. So, temperatures higher than 50°C could lead to significant BADGE degradation and produce significant quantitation errors.

Several different coatings, covering all the polarity range were tested. In all the cases the 85 μm PA

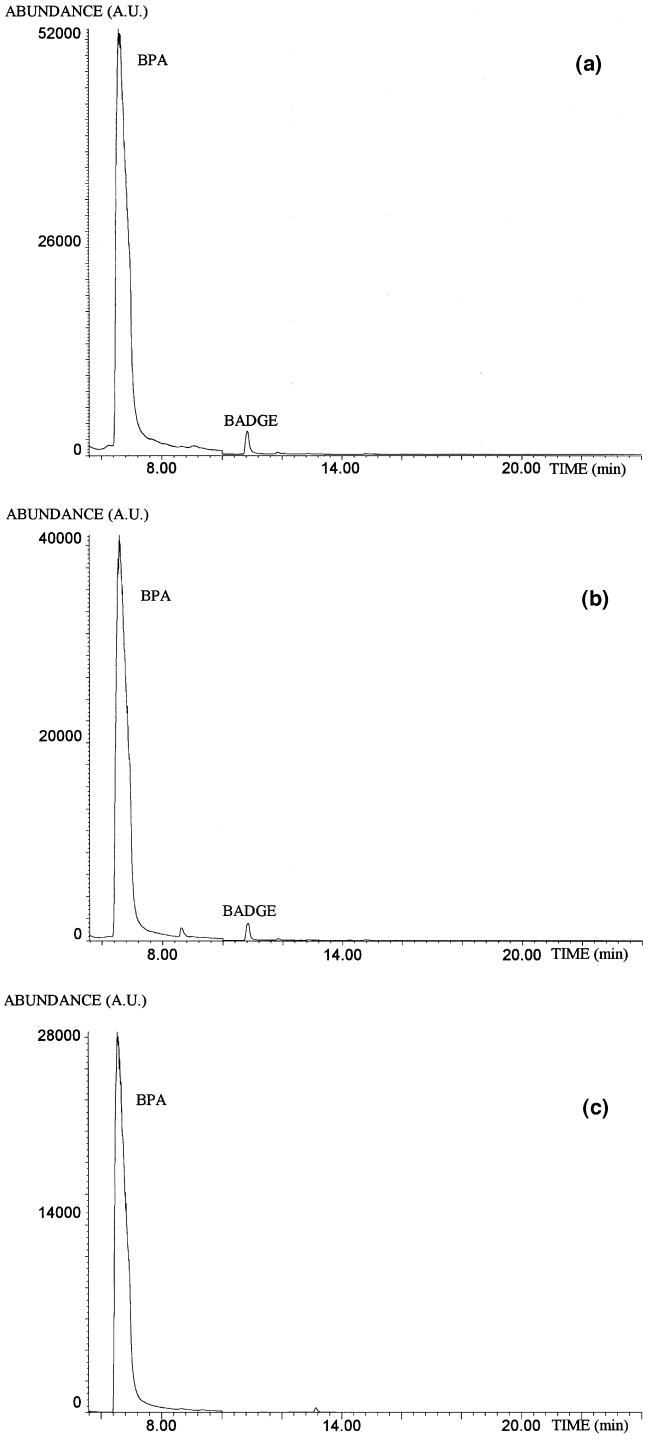

Fig. 1. Chromatograms obtained after SPME from (a) simulant A (distilled water); (b) simulant B (3%, w/v, acetic acid) and (c) simulant C (10%, v/v, ethanol). Peak identification: 1=BPA; 2=BADGE. (Spiking levels: simulant A: 1 ng/g BPA; 15 ng/g BADGE; simulants B and C: 10 ng/g BPA, 20 ng/g BADGE).

Table 2
Optimum SPME conditions found for the selected food simulants

Simulant	I	II	III	IV	V	VI
Simulant A (distilled water)	+	+	+	+	3	D
Simulant B 3% (w/v) acetic acid	+	+	+	+	1	D
Simulant C 10% (v/v) ethanol	+	+	+	+	4	D

fibre was selected as the most useful. This fact is related to the analytes polarity; PA has a medium polarity between PDMS and CW–DVB, and its polarity is similar to the target analytes. Nevertheless, if the aim of the study were the migration study of BADGE in these simulants, the selection of the coating must be revised.

Different studies showed that the only substance remaining after 10 days at 40°C (established migration conditions [40]) is the second BADGE hydrolysis product (opening of the two oxyrane rings) in the simulant B whereas in simulants A and C both hydrolysis products remaining (opening of one and two oxyrane rings). So, this fact must be taken into account in order to select the appropriate fibre coating, and a new optimisation study must be carried out.

As a general rule, the highest desorption temperature amenable for the analytes and the fibre coating should be used to provide a fast transfer of the analytes to the column. In this case, temperature was limited by polyacrylate coating characteristics and fixed at 300°C. No analyte degradation was observed.

Sample introduction was performed in the splitless mode. After the desorption of the target analytes (splitless time 2 min) the fibre was kept on the hot injector (10 min) to clean the possible unknown high affinity analytes sorbed in the fibre. Anyway, this point was checked by performing a blank fibre desorption without prior extraction every five single runs, showing that no carryover phenomena were produced in any simulant.

Sorption time was limited to 20 min to allow an analysis to take place in a reasonable and practical period of time. It is logical to think that longer periods of time will lead to higher analyte response until the equilibrium was reached, but the proposed time is enough to achieve good analytical performance, as was described below. So, 20 min was selected as a ''practical'' optimum extraction time.

With respect to salt addition, the amount extracted increases with increasing salt concentration in the simulant, which is related to the ionic strength of the solution and equilibrium displacement to the fibre. The pH effect was taking into account by changing the salt identity, because the pH was different since the hydrolysis of NH_4Cl supplies an acidic pH, whereas NaCl and $(NH_4)_2SO_4$ have neutral reaction and Na_2HPO_4 provides an alkaline pH.

As shown in Table 2, the maximum sorption is reached at neutral pH. So, the presence of Na_2HPO_4 is necessary in the case of simulant B (acetic acid) to neutralise the acidic pH. This nearly neutral pH is expected to prevent to some extent the hydrolysis of BADGE, providing the best analytical performance in its analysis.

3.2. Analytical performance

3.2.1. Linear range

Spiked solutions of BPA and BADGE in the different simulants were prepared by diluting the

Table 3
Linear range

Simulant	Analyte	Linear range (μg/g)	r^2
A (distilled water)	BPA	0.01–8	0.9980
	BADGE	0.1–40	0.9987
B (3% acetic acid)	BPA	0.02–10	0.9971
	BADGE	0.1–40	0.9960
C (10% ethanol)	BPA	0.02–3	0.9997
	BADGE[a]	Not applicable	Not applicable

[a] For a detailed explanation on BADGE's behaviour see the text.

Table 4
Precision and limits of the method

Simulant	Analyte	RSD (%) (intra)	RSD (%) (inter)	DL (ng/g)	QL (μg/g)
A (distilled water)	BPA	3.5	10.6	0.1	0.01
	BADGE	6.5	22.5	13	0.1
B (3% acetic acid)	BPA	5.7	12.4	1	0.02
	BADGE	7.7	23.6	15	0.1
C (10% ethanol)	BPA	4.5	12.6	2	0.02
	BADGE	Not applicable	Not applicable	Not applicable	Not applicable

appropriate mass of the standard solutions to 12 g with the respective food simulants. Three replicates of these solutions were prepared and measured. Table 3 shows the linear range found for each simulant and the regression coefficient obtained, expressed as r^2.

As can be seen, a wide and convenient linear range was obtained for both of the analytes working with simulants A and B. The method performance becomes only acceptable for BPA and not suitable for BADGE analysis in simulant C.

3.2.2. Precision

Six independent solutions of each simulant were extracted. The analyses were performed in duplicate and the relative standard deviation (RSD, %) within the series and between them was calculated. The results are shown in Table 4. As can be seen, the intercomparison results are not very good. So, it is necessary to prepare a calibration graph for each series of analysis.

3.2.3. Detection and quantitation limits

Detection limits were calculated by extracting diluted solutions of the analytes in each food simulant. The criteria were selected according to IUPAC and ACS definition, as follows:

Detection limit (DL): $A_s - A_b = 3S_b$

Quantitation limit (QL): $A_s - A_b = 10S_b$

were A_s is the average of sample signal (area); A_b is the average of blank signal (area) and S_b is the standard deviation of blank signal (area). The obtained limits of the method are presented in Table 4.

Comparing with the established SML in the European Legislation for BPA and BADGE (3 and 0.02 μg/g, respectively), the detection and quantitation limits provide well enough performance. It is interesting to point out the concentration behaviour of the SPME technique.

4. Conclusions

The method described here is strongly recommended for determining BPA and BADGE in simulants A and B, and for analysis of BPA in simulant C. It shows good analytical characteristics, having a linear range from the low-ng/g to several-μg/g range for BPA and from 0.1 to 40 μg/g for BADGE. The detection limits (0.1–2 ng/g for BPA and 13–15 ng/g for BADGE) adhere to the EU regulations. Moreover, this method overcomes a practical drawback of the proposed HPLC methods for BPA analysis, because it does not need further structural confirmation.

Further work will be focused to extent the range of application of the method to the hydrolysis derivatives from BADGE in order to perform migration tests in these aqueous simulants, as well as to develop an analytical method to work with fatty simulants, in which the direct immersion method proposed here is not suitable.

Acknowledgements

The authors gratefully acknowledge the collaboration of Mr. M. Philo and Dr. L. Castle from Central Science Laboratory (Ministry of Agriculture,

Fisheries and Food). This work has been financed by the Spanish Project ALI98-0686 from the Comisión Interministerial de Ciencia y Tecnología (CICYT) and the Spanish Acciones Integradas between Spain and United Kingdom HB1996-0200 from the Ministerio de Educación y Ciencia (MEC).

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