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Determination of bisphenol-type contaminants from food packaging materials in aqueous foods by solid-phase microextraction–high-performance liquid chromatography

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Abstract

A fast screening method consisting of off-line solid-phase microextraction coupled to HPLC and fluorescence detection, suitable for the analysis of several bisphenol derivatives and their degradation products in aqueous solution, has been developed. Detection limits of 0.7 ng ml^{-1} for 2,2-bis[4-(glycidyloxy)phenyl]propane, 0.9 ng ml^{-1} for bisphenol A bis(3-chloro-2-hydroxypropyl)ether, 1.1 ng ml^{-1} for 2,2-bis(4-hydroxyphenyl)propane and 2.4 ng ml^{-1} for bisphenol F diglycidyl ether have been achieved working in the linear range 10–500 ng ml^{-1}. The good analytical features achieved make the proposed method an interesting option for the direct determination of these compounds in aqueous canned food such as peas, tuna, olives, maize, artichokes or palm hearts. Both the optimization process and the results, including the analysis of real samples, are given and discussed.

Keywords: Food analysis; Bisphenol A; Bisphenol F

1. Introduction

Among the coating varnishes or lacquers used in cans for foodstuff packaging purposes, the most popular are those based on vinylic organosols (novolacs), which can include in their composition epoxy resins such as BADGE (bisphenol A diglycidyl ether) [1] and BFDGE (bisphenol F diglycidyl ether) [2]. These resins, obtained from the monomers bisphenol A (BPA) and bisphenol F (BPF), respectively, act as hydrochloric acid scavengers during the heat treatment of the coating procedure. Nevertheless, and despite the obvious advantages derived from their use [3], recent studies have shown that these compounds have both mutagenic and cytotoxic properties [4,5].

Several analytical procedures have been developed for the determination of bisphenol A, bisphenol F and their derivatives, such as BADGE or BFDGE, as well as some of their hydrolysis products [6,7]. Depending on the matrix, sample preparation includes liquid–liquid extraction [8–10], solid-phase extraction [11], solvent extraction [12–15] or, as in our case, solid-phase microextraction [16,17], requiring an extra derivatization step [8,17]. Further determination is usually achieved by means of chromatographic techniques, mainly HPLC [18–21] with MS [22–24] or, more frequently, fluorescence [12–15,23–25] detection. Although GC–MS can be satisfactorily applied to the determination of bisphenol A [9,16,19], the method seems to be limited

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for BADGE itself and its derivatives, due to their low volatility.

As the concentration of BPA, BPF and their derivatives in canned food is expected to be very low, preconcentration of analytes is required. In this sense, one of the best systems to extract these compounds from aqueous solutions is the already mentioned solid-phase microextraction (SPME), which has been shown to be very efficient [16]. Although the desorption process is usually carried out by thermal release in the injection port of a gas chromatograph, SPME can also be satisfactorily used for trapping and further determining non-volatile compounds. In this case, an HPLC system can be used for their analysis, the desorption process being performed by means of a suitable solvent instead of thermally.

This paper reports the analysis of several bisphenol derivatives in aqueous food simulants, including 2,2-bis(4-hydroxyphenyl)propane (bisphenol A; BPA), bisphenol A diglycidyl ether (BADGE), bisphenol F diglycidyl ether (BFDGE), bisphenol A bis(3-chloro-2-hydroxypropyl) ether (BADGE-2HCl) and their hydrolysis degradation products. Due to the high sensitivity achieved, SPME has been used off-line coupled to HPLC with fluorescence detection. Experimental SPME conditions, which include fiber type, sample volume, sorption and desorption time, temperature and ionic strength, have been optimized. For HPLC, both static and dynamic injection modes have been tested.

Once the optimum conditions had been determined, the analysis of several commercial canned samples of asparagus, peas, tuna, olives, maize, artichokes and palm hearts was carried out. In addition, and to check for the presence of possible matrix effects, spiked samples were prepared and studied. Results are presented and discussed.

2. Experimental

2.1. Apparatus

An HPLC (Kontron Instruments, Model 322; Milan, Italy), with a Waters (Milford, MA, USA) XTerra MS C_{18} column (10 cm×4.6 mm, 5 μm) and a fluorescence detector (Waters, Model 474), was operated at 1.2 ml/min with water–acetonitrile (50:50), isocratic mode. The injection volume was 20 μl. The selected wavelengths were 275 nm (excitation) and 305 nm (emission). Data collection was provided by a personal computer interfaced to the detector using PC Integration Pack software (Kontron).

The SPME fiber holder designed for use with Varian 8100/8200 autosamplers or with a HPLC interface was purchased from Supelco (Bellefonte, PA, USA). The interface used (only for dynamic mode, although both static with the same mobile phase or different solvents, or dynamic modes are possible) was supplied by Supelco and has been described elsewhere [26,27].

The microextraction fibers used were coated either with poly(dimethylsiloxane) (PDMS) of 100 μm thickness, an 85 μm film thickness of polyacrylate (PA), a 60 μm thickness of poly(dimethylsiloxane)–divinylbenzene (PDMS–DVB) or a 65 μm thickness of Carbowax (CW), all supplied by Supelco. The fibers were conditioned by introducing them into a 20 ml vial filled with mobile phase for 1 h. After conditioning, a blank fiber desorption was carried out in all cases, in order to check the performance of the procedure. No chromatographic peaks were detected in any case.

2.2. Reagents

All reagents used were HPLC grade and purchased from Merck (Darmstadt, Germany). BPA, CAS No. [80-05-7] was from Aldrich (Madrid, Spain), BADGE 97.0%, CAS No. [1675-54-3], bisphenol A bis(2,3-dihydroxypropyl) ether (BADGE-diol) ~97%, CAS No. [5581-32-8], BADGE-2HCl 99.0%, CAS No. [4809-35-2], BFDGE ~97%, CAS No. [2095-03-6], and bisphenol F bis(2,3-dihydroxypropyl) ether (BFDGE-diol) ~97.0%, CAS No. [72406-26-9] were from Fluka (Madrid, Spain). Chemical structures are presented in Fig. 1.

Standard solutions containing all the compounds (concentrations of about 150, 75, 15, 7.5 and 1.5 μg ml^{-1}, respectively) were prepared weekly in acetonitrile. All water samples were prepared by spiking this standard solution into high-purity water produced by a Milli-Q system (Millipore, Bedford, MA,

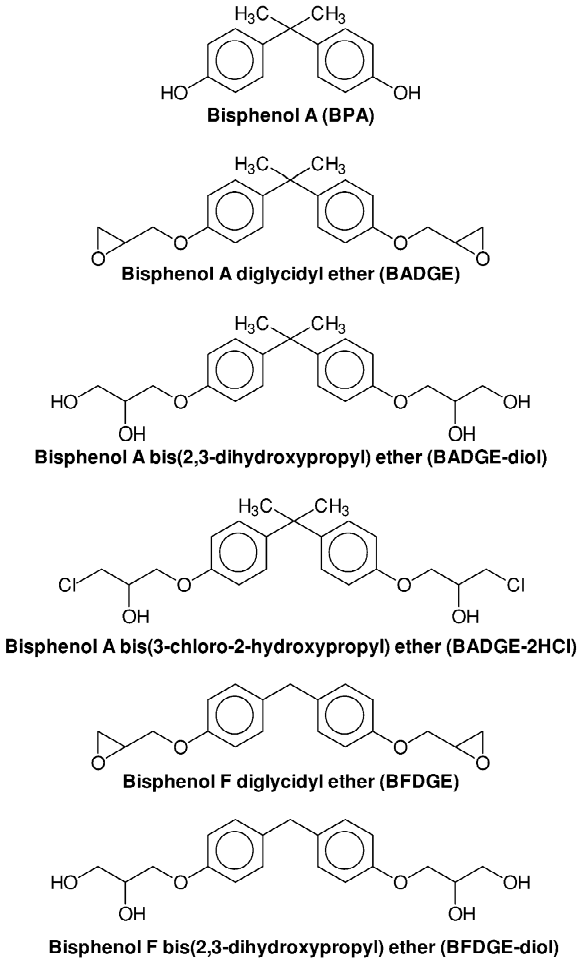

Fig. 1. Structures of analytes determined in this study.

USA). Sodium chloride of analysis quality was supplied by Merck.

2.3. SPME procedure

Aliquots of 10 ml were extracted from 20 ml glass vials sealed with hole caps and PTFE-lined septa. After the addition of a magnetic stirrer bar to the vial (600 rpm), the fiber was exposed to the aqueous phase for 20 min. After extraction, the fiber was extracted for 5 min by means of a 200 μl capacity microvial containing 150 μl of mobile phase (static desorption mode), or placed in the desorption chamber of the SPME–HPLC interface (dynamic desorption mode, held for 3 min).

2.4. SPME optimization

Despite the number of variables which can potentially influence the SPME procedure, most workers optimise extraction methods one parameter at a time. Based on knowledge acquired in one of our previous studies [16], this is the strategy we adopted in this study.

2.5. Preparation of food samples

As the main objective of this work was to serve as a first screening method for the determination of bisphenol-type contaminants, sample handling was reduced to a minimum, as follows. A reasonable quantity of the aqueous phase in contact with canned foods was filtered through 0.45 μm pore size nylon 66 syringe filters. Then 10 ml was placed in a 20 ml glass vial, 0.75 g of NaCl and a magnetic stirrer were added, followed by the recommended SPME procedure described above. No clean-up step was applied to any of the foodstuffs under study.

3. Results and discussion

Due to the large number of implied variables, and in order to obtain the optimum conditions for the determination of bisphenol derivatives in food simulants and real canned samples, a sequential, systematic procedure was followed. The first step consisted of HPLC program optimization (carried out by direct injection of standards). The HPLC column used (Waters Xterra MS C_{18}) showed greater separation enhancement capabilities, compared with other conventional C_{18} phases. Fig. 2 shows the results obtained, which can be considered as being very good for these substances. With respect to the extraction phase, four SPME fibers of different polarity were evaluated, by extracting 1000 ng ml^{-1} aqueous solutions containing all the standards: 100 μm PDMS (red), 85 μm PA (white), 65 μm CW (orange) and 60 μm PDMS–DVB (brown). The best results were obtained, in general, with the most polar fibers. The PDMS fiber did not extract BFDGE-diol, and the polyacrylate fiber did not extract any of the hydrolysis products. For the Carbowax and PDMS–DVB fibers, the first gave significantly higher results

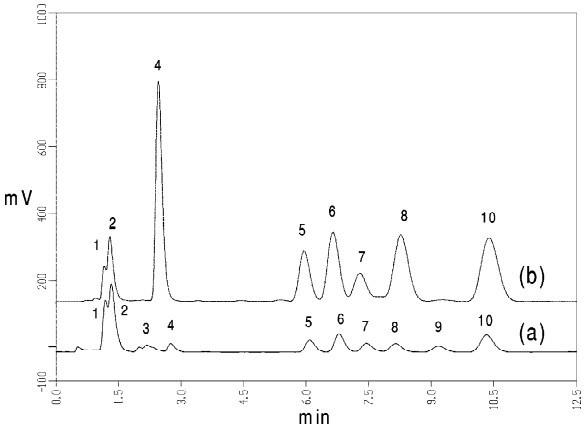

Fig. 2. HPLC chromatograms obtained for a 100 ng ml^{-1} standard solution: (a) direct injection; (b) after static SPME procedure. Peaks: 1=BFDGE-diol; 2=BADGE-diol, first peak; 3=BADGE-diol, second peak; 4=bisphenol A; 5, 6 and 7=BFDGE, different isomers; 8=BADGE-2HCl; 9=BADGE-2HCl hydrolysis product; 10=BADGE.

in all cases. Consequently, the Carbowax fiber was selected for further analysis. Table 1 summarizes the results obtained, where the recovery was calculated by comparison with a direct injection of a standard solution, the concentration of which was calculated by taking into account the theoretical value corresponding to a quantitative extraction.

The presence of 7.5% NaCl in the aqueous phase improved the sorption process by, on average, 17%. Since no pH change occurs, the most likely reason for this is an increase of the ionic strength, giving a higher fiber/aqueous phase partition coefficient.

With respect to the desorption stage, two possibilities were tested: the first consisted of the off-line, static mode, by immersing the fiber in a microvial (200 μl capacity, filled with 150 μl of solvent). In order to obtain maximum performance, we investigated the optimum acetonitrile percentage, which was varied from 30 to 100% (in 10% increments), was investigated and a maximum recovery value corresponding to 50% acetonitrile–water was found. The second desorption mode made use of the commercially available SPME–HPLC interface in dynamic mode. Obviously, in this case the mobile phase was used as solvent, keeping the fiber in contact with the liquid for 3 min. In all cases, the static mode gave larger areas in the chromatograms. Nevertheless, a blank analysis performed regularly did not show significant memory effects (maximum observed: 0.8% in the subsequent injection, when extracting a 1000 ng ml^{-1} standard aqueous solution), therefore the main reasons for the dynamic mode being less efficient than the static mode appear to be: (1) the reconcentration effect in the latter, and (2) the desorption kinetics in the dynamic mode [although the introduction of analytes into the HPLC system takes place in a short time (several seconds, probably less than a half minute), it is not immediate, giving broader peaks than in the static mode]. With respect to the desorption time in the static mode, up to 15 min were tested, but 5 min was demonstrated to be sufficient to attain quantitative desorption (calculated by injecting a standard solution with the same concentration as could be expected by assuming both 100% sorption and desorption in the fiber). Therefore, 5 min was used thereafter to avoid unnecessary time expenditure.

Table 1
Relative overall performance of the SPME procedure for the different compounds according to fiber type (in percent)

	100 μm PDMS	85 μm PA	65 μm CW	60 μm PDMS–DVB
Bisphenol A	58	7	90	6
BADGE	65	92	44	89
BADGE-2HCl	8	94	32	85
BFDGE	49	86	27	92
BADGE-diol	81	–	35	65
BFDGE-diol	–	–	52	8

Analytical characteristics are shown in Table 2. As can be seen, good linearity, detection and quantitation limits are achieved. The only weak point of the method is the low reproducibility, which may be caused by hydrolysis of these substances, as can be seen in the HPLC chromatograms.

Finally, once the operating conditions had been defined, SPME screening analyses were performed with real canned samples, all coated internally with varnish. Figs. 3 and 4 show the chromatograms obtained in the analysis of tuna and maize. No peaks of interest were detected in any case (considering the detection limits of about 1 ng ml^{-1}), thus demonstrating the safety of the analyzed packages. In

Table 2
Analytical characteristics of the optimized SPME–HPLC method

	Linear range (ng ml^{-1})	Linearity (R^2)[a]	RSD (%)[b]	Detection limit (ng ml^{-1})	Quantitation limit (ng ml^{-1})
Bisphenol A	10–500	0.9991	22	1.1	3.8
BADGE	10–500	0.9106	17	0.7	2.5
BADGE-2HCl	10–500	0.9959	32	0.9	2.8
BFDGE	10–500	0.9957	14	2.4	7.2

[a] Five calibration points in the range 10–500 ng ml^{-1}.
[b] Evaluated at the 100 ng ml^{-1} level for each compound in water, four replicates.

addition, and in order to check for possible matrix effects, samples were spiked (at the 100 ng ml^{-1} level). Compared with synthetic standard aqueous samples, a noticeable, general decrease in the area of the peaks was observed, indicating the presence of interference from the matrix. The calculated recoveries for these compounds were in all cases between 7 and 65%, depending on the food sample. Therefore, the standard addition procedure should be used for quantitation purposes. As a reference, both standard aqueous solutions and samples were injected directly (without SPME) into the HPLC system, showing no peaks, which indicates the high concentration factor (more than 100 times) achieved with the SPME procedure.

4. Conclusions

The following conclusions can be drawn from this work.

(1) Static off-line desorption of SPME fiber in a microvial is a very appropriate technique for the analysis of non-volatile compounds trapped on a SPME fiber. This procedure does not require a special and expensive interface or equipment for the use of SPME in HPLC systems.

(2) The developed procedure is a powerful and fast technique for direct screening of non-volatile migrants in aqueous foodstuffs containing complex liquid matrices.

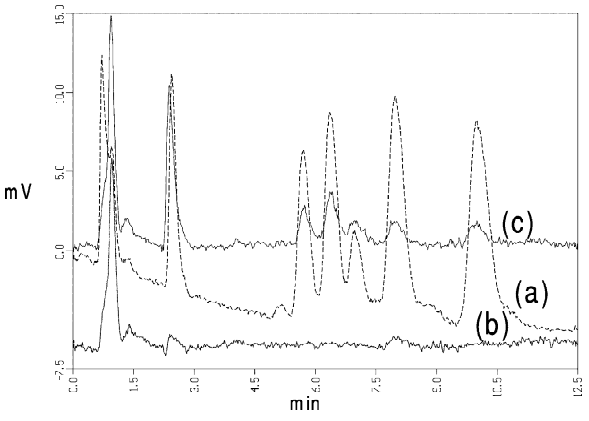

Fig. 3. SPME–HPLC chromatograms (static mode) from: (a) 100 ng ml^{-1} standard solution; (b) canned tuna; (c) spiked canned tuna (at 100 ng ml^{-1}). Peaks: 1=BFDGE-diol; 2=BADGE-diol, first peak; 3=BADGE-diol, second peak; 4=bisphenol A; 5, 6 and 7=BFDGE, different isomers; 8=BADGE-2HCl; 9=BADGE-2HCl hydrolysis product; 10=BADGE.

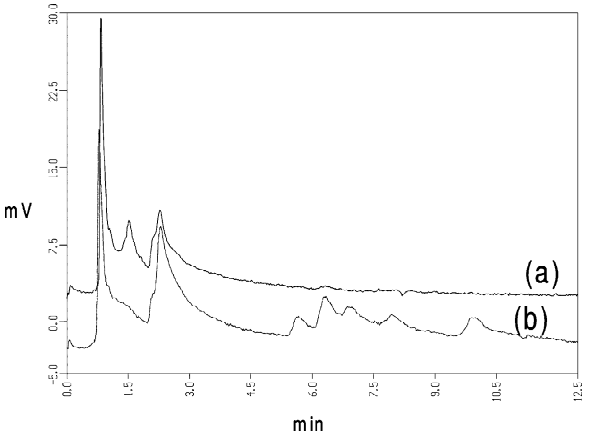

Fig. 4. SPME–HPLC chromatograms (dynamic mode) from: (a) canned maize; (b) spiked canned maize (at 100 ng ml^{-1}). Peaks: 1=BFDGE-diol; 2=BADGE-diol, first peak; 3=BADGE-diol, second peak; 4=bisphenol A; 5, 6 and 7=BFDGE, different isomers; 8=BADGE-2HCl; 9=BADGE-2HCl hydrolysis product; 10=BADGE.

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