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Use of solid-phase microextraction followed by on-column silylation for determining chlorinated bisphenol A in human plasma by gas chromatography–mass spectrometry

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Abstract

In this study, a solid-phase microextraction (SPME) method based on poly(acrylate)-coated fibres has been developed for detection and quantification of chlorinated bisphenol A in human plasma due to the need for an assessment of human exposure to them. After desorption of the analytes for 7 min at 300 °C, they were directly derivatized in the GC injector port by injection of 2 μL of diluted bis(trimethylsilyl)trifluoroacetamide (BSTFA). The formation of trimethylsilylate derivatives improves the selectivity, sensitivity and performance of the chromatographic properties obtained when the analytes are directly separated. Quantification was carried out using single-ion monitoring (SIM). The respective chloroderivative molecular ions appear at 406, 440, 474 and 508 *m/z*; whereas the base peaks corresponding to a loss of a methyl group in all cases appear at 391, 425, 459 and 493 *m/z* for mono-, di-, tri- and tetrabisphenol A, respectively. Deuterated bisphenol A (BPA-d_{16}) was used as an internal standard. The method was applied to the determination of Cl-BPA, Cl_2-BPA, Cl_3-BPA and Cl_4-BPA at very low concentration levels in plasma. Recovery efficiencies were close to 100% in all cases.
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1. Introduction

The use of chlorine for disinfecting waste waters or drinking water has become widespread in this century. Recently, however, the increasing presence and variety of aquatic contaminants has raised the question of the chemical fate of these contaminants when subjected to aqueous chlorination [1]. In fact, the production of organochlorine compounds in chlorinated water, including mutagenic and carcinogenic substances, has been well established [2,3].

4-4′-Isopropylidenediphenol commonly named bisphenol A (BPA) is a monomer widely used in the manufacture of epoxy and phenolic resins, polycarbonates, polyacrilates and corrosion-resistant unsaturated polyester-styrene resins. It may be found in a diverse range of products [4] and migrates at ppb levels from polycarbonate products or the epoxy coating on cans to water during thermal processes [5], also from PVC hoses to water at room temperature and at neutral pH. These migrations are thought to be a significant source of human exposure. In addition, BPA that has migrated from plastics to tap water may react with residual chlorine in the tap water that had been added as a disinfectant, producing chlorinated BPA congeners [6]. Human exposure to and environmental contamination with BPA is well studied and documented [7,8]. Also it has been reported that BPA was not persistent based on its rapid biodegradation in acclimated wastewater treatment plants and receiving waters. In contrast, polychlorinated BPAs have been recently identified and a biodegradation test using activated sludge revealed that they are not easily biodegraded [9]. In addition, Kuruto-Niwa et al. [10] found that chlorinated BPAs were more cytotoxic

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than BPA. Therefore, the determination of these compounds in biological samples is essential due to the need for an assessment of human exposure to them.

To our knowledge, the determination of bisphenol A in different biological matrices has been accomplished by gas chromatography–mass spectrometry [11] or high-performance liquid chromatography equipped with ultraviolet, fluorescence [12], mass spectrometry [13,14] or electrochemical [15,16] detection. But there are no methods to date for the analysis of chlorinated bisphenol A congeners in human fluids.

In a previous paper we have reported a methodology for the determination of chlorinated bisphenol A by gas chromatography–mass spectrometry in wastewater samples, following liquid–liquid extraction [17]. To address the determination of these chemicals in biological matrices we have chosen the solid-phase microextraction (SPME) technique in combination with GC–MS due to the advantages that this extraction process exhibit over conventional techniques (LLE or SPE).

SPME is a two-step process conducive to the simultaneous extraction and preconcentration of analytes from sample matrices. In the first step, a fused-silica fibre coated with a polymeric stationary phase is exposed to the sample matrix where the analyte partitions between the matrix and the polymeric stationary phase. In the second step, the fibre/analyte is transferred to the analytical instrument for desorption, separation and quantification. This is a fast, simple and inexpensive technique, which does not require any organic solvents and is easily automated. Since SPME was first reported in 1990 by Arthur and Pawliszyn [18], a wide variety of applications have been developed in different matrices such as environmental samples [19,20], biological matrices [21] or food [22,23] in conjunction with both liquid and gas chromatography or linked directly to a detector without a separating column.

In addition, SPME allows the derivatization of the analytes to take place in the sample matrix, in the SPME fibre coating or in the GC injector port [24,25], increasing the versatility of the technique.

In this paper, SPME coupled with derivatization of chlorinated BPAs in the GC injector port is presented for the determination of the analytes in human plasma. The method is very simple, reducing the potential for analyte loss during the extraction and derivatization processes.

2. Experimental

2.1. Apparatus

All chromatographic measurements were performed with a Hewlett Packard system comprising a 5890 Gas Chromatograph fitted with an HP 7673 Autosampler, a split-splitless injector for capillary columns and a 5971 Mass Spectrometer. The mass spectrometer was calibrated every day before use with Perfluorotributylamine (PFTBA) as a calibration standard. The column was a HP1 fused silica capillary (30 m × 0.25 mm i.d. × 0.25 μm film thickness) bonded with methyl silicone gum phase. The carrier gas used was helium (purity 99.999%) at a flow-rate of 1.5 mL min^{-1}.

Commercially available l00 and 30 μm PDMS, 65 μm PDMS/DVB, 75 μm Carboxen/PDMS, 65 μm CW/DVB and 85 μm polyacrylate fibres housed in manual SPME holder were purchased from Supelco (Bellefonte, PA, USA). A SPME inlet guide and pre-drilled Thermogreen LB-2 septa from Supelco were used.

A magnetic stirrer/temperature-controlled oil bath (Agimatic-N, Selecta, Spain) was used during the extraction process.

2.2. Chemicals

All reagents were of analytical reagent grade unless stated otherwise and reverse osmosis quality water was used throughout. Stock solutions of monochloro, dichloro, trichloro bisphenol A (synthesised in our laboratory) and tetrachlorobisphenol A (supplied by Sigma–Aldrich) containing 100 μg mL^{-1} were prepared in ethanol 99% (v/v) (Panreac). The solutions were stored in dark bottles at 4 °C, remaining stable for at least 3 months. These solutions were used to spike the water samples.

A standard solution of 100 mg L^{-1} of bisphenol A-d_{16} (supplied by Sigma–Aldrich) in ethanol was used as internal standard after adequate dilution to a final concentration of 50 μg L^{-1}.

N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) supplied by Fluka, was used as the silylation reagent. Sodium chloride (Panreac) was used for the extraction procedure. Zinc sulphate (Merck) and sodium hydroxide (Panreac) were used to precipitate the proteins of the sample.

2.3. Preparation of samples

Plasma samples from healthy volunteers were collected and stored at −20 °C until analysed. Prior to analysis, 5 mL of plasma samples were spiked with appropriate stock solution amount of monochloro, dichloro, trichloro and tetrachlorobisphenol A as well as the internal standard. Next the plasma samples were diluted to 15 mL with de-ionised water and the proteins were precipitated by adding 1.5 mL of $ZnSO_4$ solution (10%) and 1.0 mL of 0.1 M NaOH [26]. The solutions were centrifuged for 10 min at 5000 rpm. The supernatant liquid was filtered through a 0.2 μm Millipore membrane filter to remove any remaining particulate matter and acidified with hydrochloric acid (0.1 M) to pH 3.0–3.5. Finally, they were brought up to 20 mL with de-ionised water.

2.4. Solid-phase microextraction procedure

The fibres were conditioned before their first use according to manufacturers specifications. A fibre blank was run after

the conditioning process to confirm that there were no peaks that could be assigned to compounds introduced during the manufacture of the fibre.

The SPME process was carried out by introducing 20 mL of aqueous samples containing the chlorinated bisphenol A compounds and 50 $\mu g\,L^{-1}$ of bisphenol A-d_{16} into 25 mL vials. After addition of 0.1 g of sodium chloride the vial was crimped with PTFE faced septum and magnetically stirred to allow salt dissolution and temperature equilibration. Sampling was performed through the septum by total immersion of the SPME fibre for 40 min into the solution while being stirred at 100 rpm and maintained at 40 °C.

After sampling, the SPME device was transferred to the injection port on the GC instrument, where thermal desorption of the analytes was carried out at 300 °C with the split closed for 7 min and the total gas flow rate fixed at 100 mL min^{-1}.

2.5. Derivatization and gas chromatography analysis

For the derivatization experiment, a 1 mL vial of bis(trimethylsilyl)trifluoroacetamide (BSTFA) is diluted with 1 mL of dichloromethane. After desorption of the analytes in the GC injector port, 2 μL of diluted BSTFA is injected and left to react for 1 min.

The splitless mode was used, with the split closed for 8 min, during desorption and subsequent derivatization of the analytes. The GC–MS parameters used were: injector temperature, 300 °C; detector temperature, 300 °C and oven temperature, programmed from 50 °C (8 min) to 300 °C at 30 °C min^{-1}, remaining constant for 7 min. The selected ions of the compounds for SIM mode operation were: *m*/*z*'s 371 and 386 for silylated BPA-d_{16}; 391 and 406 for silylated Cl-BPA; 425 and 440 for silylated Cl_2-BPA; 459 and 474 for silylated $C1_3$-BPA and 493 and 508 for silylated Cl_4-BPA, where the first value corresponds to the base peak attributed to loss of a methyl group whereas the second one corresponds to the molecular ion peak of each compound. The concentrations of these compounds were calculated by the internal standard method.

3. Results and discussion

3.1. Solid-phase microextraction optimisation

Solid-phase microextraction is optimised by adjusting parameters that impact analyte adsorption and desorption. The primary parameters influencing analyte adsorption into the stationary phase are fibre type, extraction time, ionic strength, temperature and agitation.

The commercially available SPME fibres PDMS, PDMS/DVB, CAR/PDMS, CW/DVB and PA were tested for the extraction of the analytes from the aqueous samples. The higher extraction yield were obtained for the more polar coatings PA and PDMS-DVB, being similar for dichloro-, trichloro- and tetrachloro bisphenol A and much higher

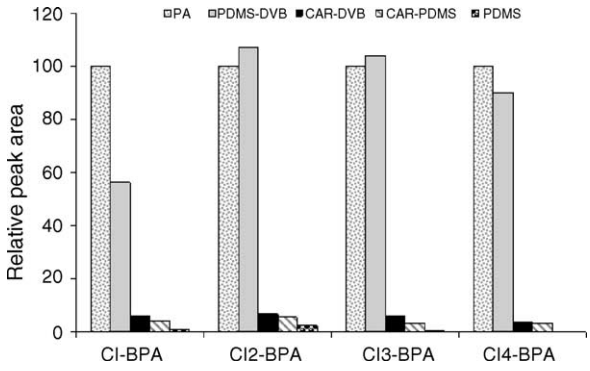

Fig. 1. Extraction yield obtained for different SPME fibers.

with PA for monochloro bisphenol A as is shown in Fig. 1. Therefore, the compounds extraction was carried out with PA fibre.

Extraction time profiles were studied extracting aqueous samples of 10 $\mu g\,L^{-1}$ in Cl-BPA and Cl_2-BPA, 20 $\mu g\,L^{-1}$ in Cl_3-BPA and Cl_4-BPA and 50 $\mu g\,L^{-1}$ of BPA-d_{16} in the time interval between 10 and 60 min (Fig. 2). After 40 min, monochloro- and dichloro-BPA reach the maximum signal but the extraction yield of trichloro and tetrachloro-BPA increase markedly for longer time. An extraction time of 40 min was fixed as a compromise between analysis time and sensitivity.

The role of the ionic strength of the matrix was investigated using sodium chloride. The amounts tested for a sample volume of 20 mL varied from 0.0 to 1.0 g of NaCl. The results obtained show an strong increment in the extraction yield for Cl_3-BPA and Cl_4-BPA when the NaCl amount increases from 0.0 to 0.1 g. The peak area corresponding to Cl_2-BPA increases as well in this interval but for Cl-BPA the obtained signal does not change significantly. For higher NaCl amounts the obtained signal decreases for dichloro-, trichloro- and tetrachloro-BPA and the extraction yield of monochloro-BPA is practically constant for the interval tested. Thus, 0.1 g of NaCl was selected to obtain an adequate salting-out effect.

The effect of the temperature was monitored by extracting samples between 30 and 80 °C. Responses were higher at 40 °C for all the compounds studied, decreasing above this

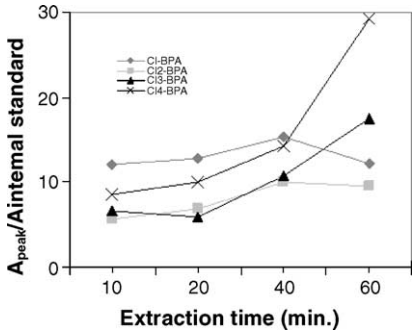

Fig. 2. Extraction time profiles.

Table 1
Analytical parameters

Parameter	BPA-Cl	BPA-Cl_2	BPA-Cl_3	BPA-Cl_4
N	7	7	7	7
Slope (L μ^{-1})	2.94×10^{-2}	8.64×10^{-3}	3.46×10^{-3}	3.45×10^{-3}
S_b	4.98×10^{-4}	1.25×10^{-4}	1.05×10^{-4}	1.13×10^{-4}
Intercept	-2.49×10^{-3}	-6.26×10^{-4}	-3.14×10^{-4}	-1.25×10^{-4}
S_a	6.18×10^{-3}	1.78×10^{-3}	4.01×10^{-3}	4.44×10^{-3}
Determination coefficient (%)	99.68	99.90	99.72	99.52
Linear dynamic range ($\mu g\,L^{-1}$)	0.8–25.0	0.8–25.0	4.5–50.0	5.0–50.0
$CC_{\alpha,0.05}$ ($\mu g\,L^{-1}$)	0.5	0.5	2.7	3.0
$CC_{\beta,0.05}$ ($\mu g\,L^{-1}$)	0.8	0.8	4.5	5.0
Relative standard deviation (%)	4.2	4.5	3.9	3.8
$S_{y/x}$	5.73×10^{-3}	1.75×10^{-3}	1.08×10^{-3}	1.47×10^{-3}
Lack-of-fit test, P_{lof} (%)	40.5	32.5	29.2	28.9

Table 2
Relative abundances of the ions in the chlorinated cluster

Composition	M	$M+2$	$M+4$	$M+6$	$M+8$
Theoretic values					
Cl_1-BPA	3.00	1.00			
Cl_2-BPA	9.00	6.02	1.07		
Cl_3-BPA	27.00	26.70	8.87	0.95	
Cl_4-BPA	80.80	108.00	53.20	11.00	0.98
Experimental values					
Cl_1-BPA	3.00	1			
Cl_2-BPA	9.00	6.02	1.07		
Cl_3-BPA	27.0	26.7	8.87	0.95	
Cl_4-BPA	80.8	108	53.2	11.0	0.98

temperature. Extraction is basically limited by mass transfer with higher efficiency the higher the temperature; however, adsorption is an exothermic process and when the temperature is increased the overall effect above a certain temperature is negative. Extraction were carried out at 40 °C.

The optimum stirring rate was determined in the range between 60 and 500 r.p.m. Extraction increases with increased stirring speed from 60 to 100 r.p.m. decreasing for higher speed. Extraction were carried out at 100 r.p.m.

Extraction time and temperature are the primary factors governing SPME-GC desorption. The desorption temperatures monitored ranged from 270 to 300 °C. According to our results, the peak area of all chloroderivatives studied increase with an increasing desorption temperature. Carryover or memory effect is a problem frequently encountered when using the SPME method. A second desorption performed at 300 °C after the initial one was used to determine whether the analytes remain on the fibre. The carryover is less than 1% for all compounds at 300 °C.

Desorption time was investigated within a range of 5–8 min, by leaving the fibre in the injector for a progressively longer period of time and maintaining the injector temperature at 300 °C. The analytes desorbed reach a maximum after 7 min.

3.2. Derivatization of the analytes

In earlier work, BSTFA was selected as the most adequate silylation reagent for chlorinated-BPA [17]. The optimisation of the derivatization method was carried out by applying the experimental design methodology. The effect of varying the concentration of BSTFA, volume of BSTFA and reaction time was tested on the analytical response corresponding to a mixture of Cl-BPA Cl_2-BPA Cl_3-BPA and Cl_4-BPA previously extracted and desorbed in the injector port. The three factors were simultaneously optimised by application of a 2^3 central composite design plus face centred (with

Table 3
Recovery assay in human plasma samples

Added ($\mu g\,L^{-1}$)	BPA-Cl		BPA-Cl_2		BPA-Cl_3		BPA-Cl_4	
	Found	%R	Found	%R	Found	%R	Found	%R
1.00	0.95	95.0	0.95	95.0	–	–	–	–
1.00	1.03	103.0	1.05	105.0	–	–	–	–
1.00	0.97	97.0	0.98	98.0	–	–	–	–
10.0	9.86	98.6	10.50	105.0	10.90	109.0	10.35	103.5
10.0	10.10	101.0	9.84	98.4	9.65	96.5	9.92	99.2
10.0	9.45	94.5	9.75	97.5	9.85	98.5	9.72	97.2
25.0	24.30	97.2	25.90	103.6	25.70	102.8	25.40	101.6
25.0	25.40	101.6	23.50	94.0	24.60	98.4	23.90	95.6
25.0	24.10	96.4	26.10	104.4	24.80	99.2	26.80	107.2
50.0	–	–	–	–	47.90	95.8	50.80	101.6
50.0	–	–	–	–	52.60	105.2	47.90	95.8
50.0	–	–	–	–	48.80	97.6	51.90	103.8

three centred points). Considering the results obtained from the established response surface, the following optima values were found: a 50:50 (v/v) BSTFA:DCM concentration, volume 2 μL BSTFA:DCM and a reaction time of 1 min. The application of experimental designs was carried out using Statgraphics [27].

3.3. Analytical performance

Calibration graphs for samples treated according to the analytical procedure described above were made using SIM mode. The calibration graphs are linear for the concentration range 0.8–25.0 $\mu g\,L^{-1}$ for mono and dichlorobisphenol A, 4.5–50.0 $\mu g\,L^{-1}$ for trichlorobisphenol A and 5.0–50.0 $\mu g\,L^{-1}$ for tetrachlorobisphenol A. Linearity of the calibration graphs was tested according to the *Analytical Methods Committee* [28]; the lack-of-fit test was applied to two replicates and three injections of each standard. The results for the intercept (*a*), slope (*b*), correlation coefficient (R^2) and probability level of the lack-of-fit test (P_{lof} (%)) are summarised in Table 1. Thus, the data yield shows good linearity within the stated ranges. The precision, determined as relative standard deviation (R.S.D.), was measured for a concentration of 10.0 $\mu g\,L^{-1}$ in each analyte by performing 10 independent determinations.

A fundamental aspect which needs to be examined in the validation of any analytical method is its limit of detection in order to determine if an analyte is present in the sample. In this paper, criteria for method performance have been proposed that include the decision limit, CC_α, and the detection capability, CC_β [29]. The decision limit is the limit from which it can be decided that a sample is contaminated with an error probability of α. The detection capability is the smallest content of the analyte that may be detected, identified and/or quantified in a sample with an error probability of β. Decision limit and detection capacity which are better adjusted to a statistical evaluation are implemented. Thus, CC_α ($\alpha = 5\%$) and CC_β ($\beta = 5\%$) were calculated and the results obtained are also summarised in Table 1.

4. Application to real plasma samples

The proposed method was applied to spiked plasma samples ($n = 9$) from healthy volunteers treated according to the *Preparation of Samples* described above.

In order to confirm the identity of the analytes in the human plasma samples, a isotope pattern of chlorine containing molecules is shown in Table 2. The theoretic and experimental relative abundance of the ions in the chlorinated clusters show a good agreement in the mass spectrum of all chlorinated-BPA's.

The concentration of each bisphenol A chlorinated derivative was determined by interpolation from the standard calibration curve within their linear dynamic range. Table 3 shows recovery results and a representative chromatogram of plasma samples spiked with 10 $\mu g\,L^{-1}$ of each analyte is depicted in Fig. 3.

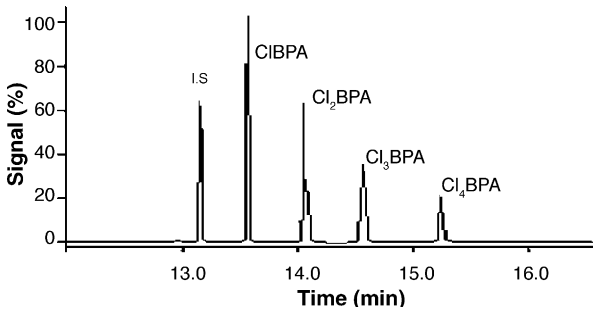

Fig. 3. Representative chromatogram of a plasma sample spiked with 10 $\mu g\,L^{-1}$ of each analyte and the IS.

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