

Determination of Bisphenol A in Water by Micro Liquid-Liquid Extraction Followed by Silylation and Gas Chromatography-Mass Spectrometry Analysis

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Abstract

A method for the determination of bisphenol A according to the European Union guideline, which establishes a limit of 0.1 ng/mL for organic pollutants in water, is proposed. The method involves a micro liquid-liquid extraction using dichloromethane followed by a silylation step. Identification and quantitation are performed with gas chromatography-mass spectrometry, using an HP-5MS column. The retention time is 7.02 min. Quantitation is carried out using single-ion monitoring (SIM) at m/z 73, 357, and 372. A clean-up is not necessary using SIM mode. Deuterated anthracene ($^2\text{H}_{10}$ -anthracene) is used as an internal standard. The method is applied to the determination of bisphenol A at very low concentration levels (10.0–250.0 ng/L) in different types of natural water samples. The detection limit obtained is 0.4 ng/L. Recovery efficiencies are close to 100% in all cases.

Introduction

There is a concern that chemicals in our environment affect human health by disrupting normal endocrine function (1,2). The controversy involves a group of chemicals that can interact directly with steroid hormone receptors. Recently, laboratory tests have shown that bisphenol A (BPA) can mimic the female hormone estrogen. The global production of BPA is well over one million tons per year (3); this chemical is used extensively in the industrialized world because it is the monomer used in the manufacture of a wide range of plastic products. It is present in a diverse range of manufactured articles (e.g., plastics, lacquer-coated cans, tooth fillings, dental materials, etc.) and thus leaches into the open environment.

The recommendations of the Oslo and Paris conventions for the prevention of marine pollution include the need to assess the risk of groundwater pollution with BPA and the risk of

drinking water becoming contaminated by this chemical (3). In a previous paper, we proposed a gas chromatography-mass spectrometry (GC-MS) method with a detection limit of 0.6 ng/mL for the determination of BPA in natural water (4). However, the European Union established a limit for organic pollutants in water of 0.1 ng/mL. In order to improve the sensitivity and selectivity of our method, we propose a new GC-MS method for the determination of trace levels of BPA in water by combining two methodologies: the use of a high-preconcentration step, such as micro liquid-liquid extraction, and the amplification of the analytical signal by an adequate derivatization reaction, such as the silylation of BPA.

Analysis of trace amounts of organic compounds in water usually requires an adequate preconcentration step. This may be a solvent-extraction or a solid-phase-extraction method. In most methods, the solvent phase requires further concentration, which may cause serious losses of compounds. Thus, methods of analyses that avoid the solvent concentration step are preferable for the recovery of trace organics in water (5–7). In addition, the use of micro methods in which the amount of organic solvents is very small reduces the damage to the environment and the analysis time employed.

The use of chemical derivatives to improve analysis has been a common practice in the GC-MS analysis of organic pollutants (8–10). The silylation reaction is one of the most widely used procedures for this purpose. Through this reaction, an active hydrogen of the analyte is replaced by an alkylsilyl group. The silyl derivatives improve their chromatographic properties, volatility, polarity, and thermal stability. The use of quantitative GC-MS in conjunction with selected ion monitoring (SIM) has led to the development of derivatization reactions yielding products that form intense high-mass ions. When such derivatives are used, sensitivity is increased by the resulting concentration of the ion current into fewer mass fragments.

In this paper, we propose the formation of trimethylsilyl derivatives from BPA by using trimethylchlorosilane and hexa-methyl-disilazane as reagents following micro liquid-liquid extraction

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with dichloromethane and subsequent GC-MS analysis.

Experimental

Apparatus and software

All chromatographic measurements were performed with a Hewlett Packard system comprising a 5890 GC fitted with an HP 7673 autosampler, a split-splitless injector for capillary columns and a 5971 MS with an EI of 70 eV as the ionization source and quadrupole mass filter. This system was modified to enhance its sensitivity and could be used in scan (complete spectrum) and SIM modes. The MS was calibrated every day before use with per-fluorotributylamine (PFTBA) as a calibration standard. The column was an HP-1 fused-silica capillary (30 m \times 0.25-mm i.d., 0.25- μ m film thickness) coated with methyl silicone gum phase. The computer was an HP-UX Chemsystem with a spectral library of more than 140,000 compounds and was used to control the entire chromatographic system. The carrier gas used was helium (purity 99.999%).

The micro liquid-liquid extraction procedure was carried out using the equipment shown in Figure 1. An IKA Labortechnik Eurostar basic stirrer was used to shake the samples mechani-

cally in a separatory funnel. A home-made microcolumn was attached to the end of the funnel to dehydrate the samples.

A Statgraphics 6.0 software package (11) was used for the statistical analysis of data.

Reagents

All reagents were of analytical reagent grade unless stated otherwise.

A stock solution of 4,4'-isopropylidenediphenol commonly named bisphenol A (CAS number 80-05-7, Aldrich) containing 50 mg/L was prepared in a 100 mL volumetric flask, by dissolving 5.0 mg of the compound in ethanol 96% (v/v) (Panreac). This solution was used to spike the water samples.

A standard solution of 100 mg/L of $^2\text{H}_{10}$ -anthracene in *n*-hexane supplied by Cromlab (Barcelona, Spain) was used as an internal standard after adequate dilution with *n*-hexane to a final concentration of 3 mg/L.

Sigma SIL-A (mixture of trimethylchlorosilane [TMCS], hexamethyldisilazane [HMDS], and pyridine in a ratio of 1:3:9) was used as the silylation reagent.

Sodium chloride, sodium sulfate, and dichloromethane (panreac) were used for the microextraction procedure.

Basic procedure

One-hundred fifty grams of NaCl and 2-3 drops of 1:1 HCl were added to a 500-mL water sample containing 10.0-250.0 ng/L of bisphenol A. The solution was transferred to a separation funnel, and 3.5 mL of dichloromethane was added. The mixture was mechanically shaken for 1 min at 1500 rpm, and the underlying organic phase was allowed to drip into a glass funnel filled with anhydrous sodium sulfate in order to dehydrate it. The organic phase was then directly transferred to a 2-mL reaction vial and evaporated to dryness under nitrogen. The vial was stoppered because silyl derivatives tend to be sensitive to moisture. Sigma SIL-A (50 μ L) was added, and the mixture was mechanically shaken for 1 min at room temperature. The sample was spiked with 50 μ L of $^2\text{H}_{10}$ -anthracene internal standard solution before analysis; then it was ready to be injected into the GC-MS. A calibration graph was constructed in the same way using solutions of bisphenol A of known concentrations.

Treatment of water samples

Water samples were filtered through a cellulose acetate filter with a pore size of 0.45 μ m (Millipore HAWP 04700), collected in a glass bottle that had been cleaned carefully with hydrochloric acid, and stored at 4°C until analysis. The usual precautions were taken to avoid contamination (12). Analysis was performed with the least possible delay.

Natural water samples containing an adequate amount of bisphenol A were acidified with hydrochloric acid (pH 3-5) and treated as described under Basic procedure.

GC-MS analysis

A 2- μ L aliquot of the extract was injected by the autosampler using the splitless injection mode with the split valve closed for 2 min. The GC-MS parameters used were as follows: injector temperature, 200°C; detector temperature, 280°C; and oven temperature, programmed from 150°C (2 min) to 270°C at

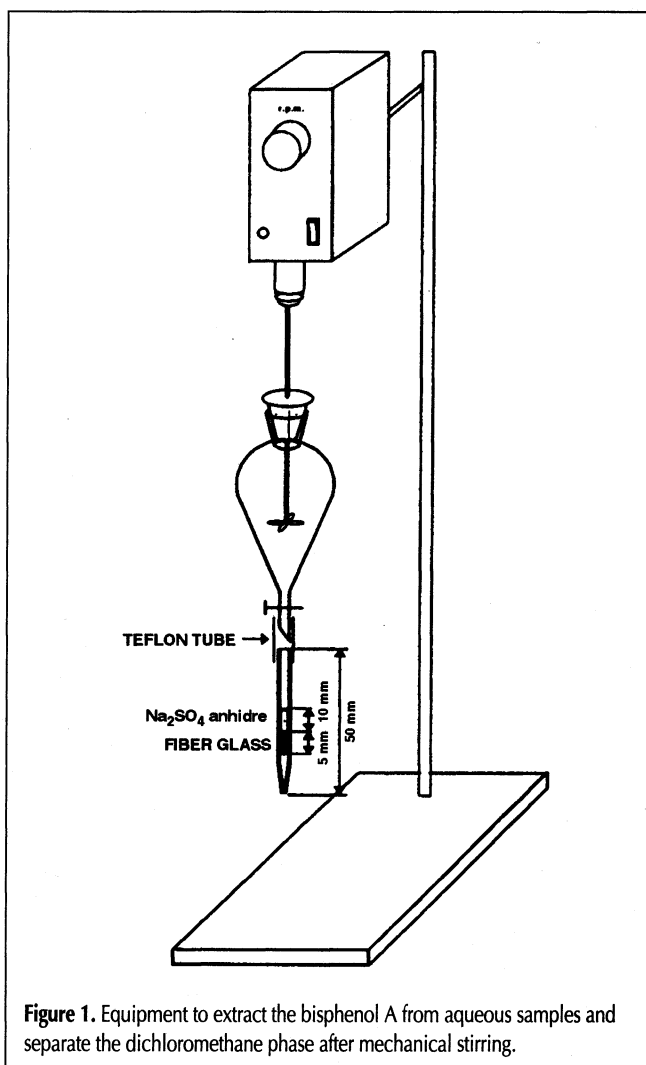
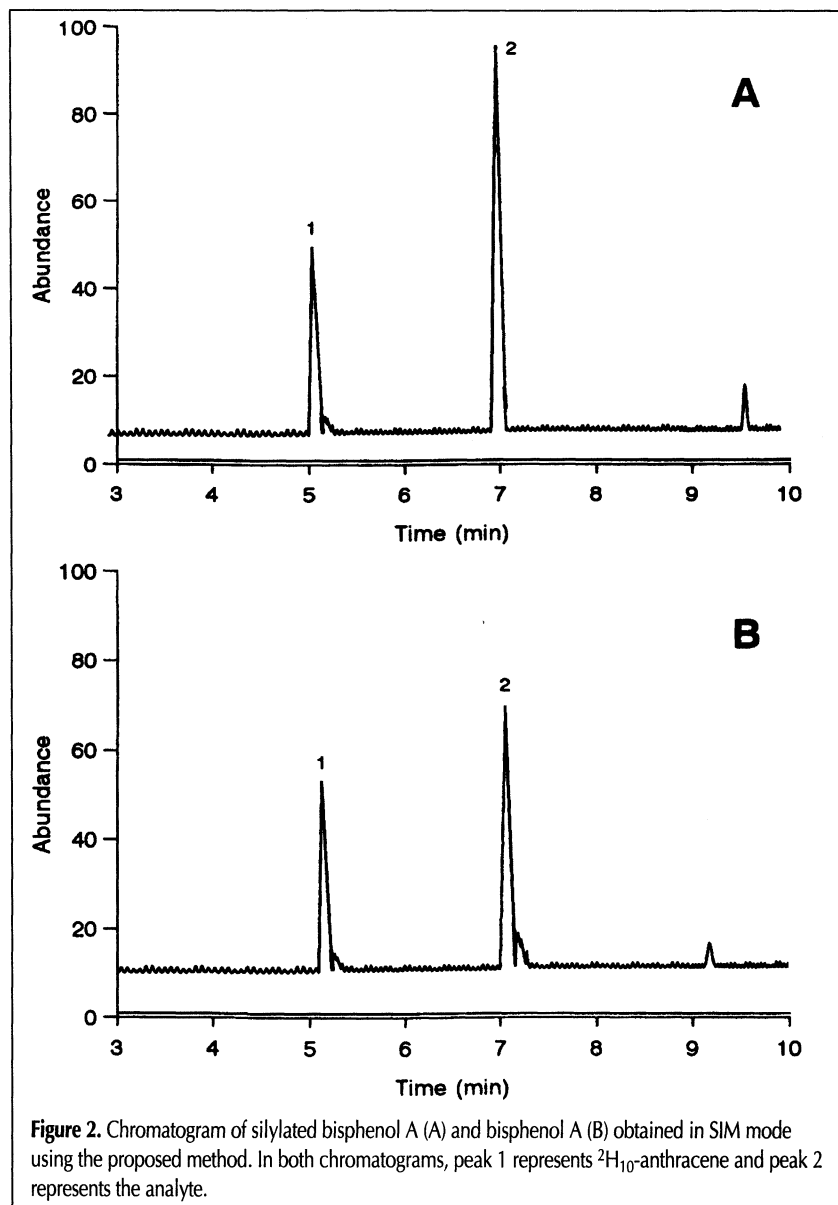


Figure 1. Equipment to extract the bisphenol A from aqueous samples and separate the dichloromethane phase after mechanical stirring.



30°C/min, remaining constant for 7 min. The selected ions of the compound for SIM mode operation were m/z 73, 357, and 372. The concentrations of the environmental estrogenic pollutant BPA were calculated by the internal standard method.

Results and Discussion

With regard to the extraction method, a micro liquid-liquid extraction procedure was selected. We studied six different solvents: *n*-hexane, iso-octane, ethyl-ether, dichloromethane, trichloro-methane, and carbon tetrachloride. Dichloro-methane was selected as the most adequate. The effect of ionic strength was studied using NaCl. The most relevant effect was the increase in the volume of organic phase recovered as ionic strength increased. Consequently, to minimize the solubility of dichloromethane in water and allow a greatly reduced volume of organic solvent to be used, the saturation of the water samples with NaCl is recommended before the dichloromethane is added.

The ratio of aqueous/organic phase (500:3.5 mL) was fixed because it enables an easily handled amount, approximately 500 μL , of organic phase to be recovered.

The extraction efficiency of BPA remained constant and maximum for pH values lower than 6.5, decreasing sharply for higher values. This behavior could be attributed to a drop in the extraction efficiency because the dissociated form remains in the aqueous phase. For this reason, we decided to acidify the solutions by adding 2–3 drops of (1:1) hydrochloric acid before the microextraction step was carried out. In all cases, the measured pH should be lower than 6.5.

In order to achieve high reproducibility, extraction was carried out using a mechanical mixing device (Figure 1). The maximum efficiency is obtained for a stirring speed higher than 1500 rpm for 1 min.

Although the volatility and thermal stability presented by bisphenol A make it suitable for detection and quantitation by GC-MS, we propose a derivatization procedure to improve the selectivity, sensitivity, and performance of the chromatographic properties obtained when the analyte is directly injected. Trimethylsilylation of organic compounds containing labile hydrogen atoms is extensively used in analytical chemistry. As the silylation reagent, we selected the mixture formed by TMCS, HMDS, and pyridine as polar solvent in a ratio 1:3:9. Hexamethyldisilazane

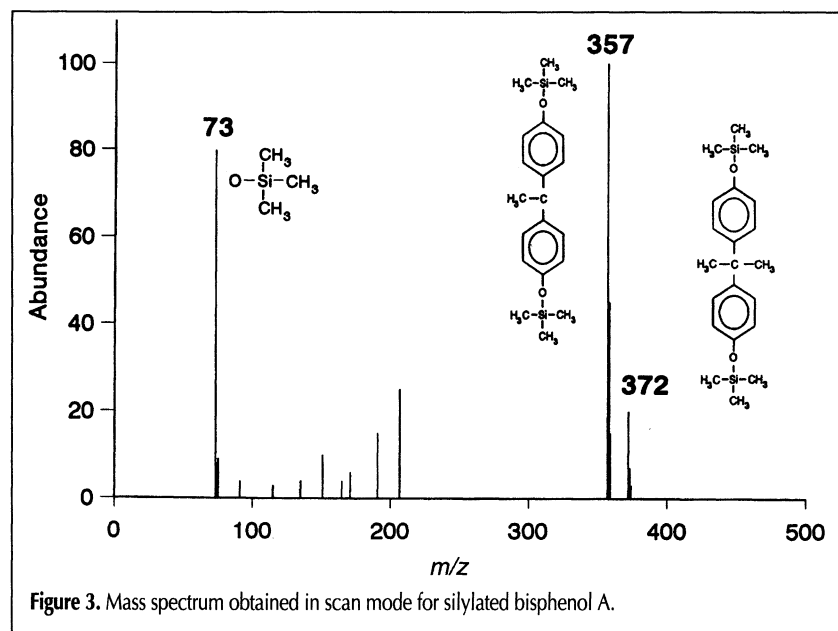


Figure 3. Mass spectrum obtained in scan mode for silylated bisphenol A.

shows a low silyl donor ability, and TMCS is often added to increase the silyl donor strength. The reaction takes up to 10 min at room temperature.

The chromatogram obtained with the described conditions is shown in Figure 2. Only 8 min were necessary to complete the analysis. The retention time obtained is the same whether BPA is injected directly in the chromatograph or previously derivatized. This was due to the fast temperature program rate (30°C/min).

When the silyl derivative of BPA is injected into the chromatograph, the peak shape is improved and, consequently, so is the chromatographic performance. Asymmetrical peaks were obtained when BPA was directly analyzed; this may be attributed to adsorption effects in the column because polar samples tend to adsorb on active surfaces of the stationary phase.

In SIM analysis, a high mass number and a high intensity were chosen in order to obtain good sensitivity and to reduce the likelihood of interferences.

The mass spectrum obtained in scan mode for this compound is shown in Figure 3. The molecular ion appeared at m/z 372, whereas the base peak corresponding to loss of a methyl group appeared at m/z 357. A relevant peak corresponding to trimethylsilyl derivatives appeared at m/z 73. Other specific peaks showed a lower abundance. Because of its higher abundance, we selected m/z 357 as a target ion and 73 and 372 as qualifier ions for the SIM mode analysis of bisphenol A. For the internal standard $^2\text{H}_{10}$ -anthracene, the ions used were those corresponding to m/z 187 and 188.

Analytical parameters

Calibration graphs for samples treated according to the Basic procedure described previously were made using SIM mode. They are linear for the concentration range 10.0–250.0 ng/L of bisphenol A. In order to check the linearity of the calibration graph according to the Analytical Methods Committee (13), the lack-of-fit test was applied for 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 summarized in Table I. Thus, the data yield shows good linearity within the stated range. The precision determined as relative standard deviation was measured for a bisphenol A concentration of 100 ng/L by performing 10 independent determinations.

There is no agreement yet about how to obtain the detection limit (DL) and quantitation limits (QL) from the blank standard deviation in GC. Frequently, the IUPAC recommendations are

not strictly followed (14,15). We believe that the method we proposed (16) to calculate DL and QL is more in line with the IUPAC recommendations. It can be assumed that if the chromatographic peak shape is a Gaussian-type, then the estimation of base width (W_b) for 99.73% of the peak-area is $W_b = 6\sigma = 2.548 W_{0.5h}$, where $W_{0.5h}$ is the half-width of the peak. Extrapolation of the graph of $W_{0.5h}$ at different concentrations of analyte can give us an adequate, statistically significant idea of the width of the base for "zero concentration". The blank signal for each analyte can be determined by integration over the baseline of the chromatograms taking a width $t_R \pm 0.5W_{b0}$ where t_R is the retention time of the analyte and W_{b0} has been evaluated as explained previously. It relies on studying the blank standard deviation in a time interval corresponding to the peak width at its base extrapolated to zero concentration. Detection limits that are better adjusted to a statistical evaluation are implemented. Thus, DL and QL were calculated, and the results obtained are also summarized in Table I.

Applications of the method

To check the accuracy of the proposed method, a recovery study was carried out on various types of water samples. River water from Loja (Granada, Spain), sea water from Motril (Granada), and underground water from a well in the aquifer of the fertile plain of Granada were analyzed after adequate additions of the chemical.

The results obtained are summarized in Table II, showing that the predicted concentrations are acceptable and, fortunately, that the waters analyzed prior to our deliberate spiking with BPA are free of this contamination within the low limit detected by our method.

Conclusion

A method for the determination of BPA in water samples according to the EU guideline is proposed (DL < 0.1 ng/mL). A micro liquid-liquid extraction procedure in conjunction with a silyl derivatization was used for the analysis by GC-MS. The detection limit for water samples was 0.4 ng/L.

Table I. Analytical Parameters

Parameter	Value
Intercept (a)	-0.0376
Slope (b) (ng/L)	0.0076
Correlation coefficient (r^2)	0.9899
Lack of fit test (p -value)	0.165
Linear dynamic range (ng/L)	10.0–250.0
Detection limit (ng/L)	0.4
Quantitation limit (ng/L)	1.2
Precision (RSD) (%)	10.9

Table II. Recovery Study of Bisphenol A in Water Previous Silylation

Water sample	Added (ng/L)	Found* (ng/L)	Recovery (%)
River water	50.0	52.0	104.0
	100.0	111.8	111.8
	200.0	219.5	109.75
Sea water	50.0	49.1	98.2
	100.0	104.9	104.9
	200.0	196.6	98.3
Underground water	50.0	51.6	103.2
	100.0	109.9	109.9
	200.0	207.6	103.8

* Data based on the average obtained from three determinations.

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Atlas of Chromatograms

Analysis of Acrylate Monomers on Steel Capillary Column GC Separations

