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DERIVATIZATION AND GAS CHROMATOGRAPHY–LOW-RESOLUTION MASS SPECTROMETRY OF BISPHENOL A

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SUMMARY

Gas chromatography–mass spectrometry (GC–MS) has been investigated for identification of bisphenol A (BPA) at ppb levels in biological samples. Initially a limit of detection (LOD) of 600 ppb BPA was established by direct selected-ion monitoring (SIM) GC–MS. Next, standard solutions of BPA and extracts of powdered milk were derivatized with two different reagents – *N*,*O*-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS), and bromoacetonitrile (BAN). Compounds were separated on a 30 m × 0.25 mm i.d. fused-silica capillary column coated with a 0.25 μm film of 5% diphenyl 95% dimethylpolysiloxane. LOD of BPA and the derivatization products were calculated from the signal-to-noise ratio. Detection limits for BPA were very dependent on the derivatization agent used – 57 ppb for BSTFA + 1% TMCS and 367 ppb for BAN. The procedure was used to confirm the presence of BPA in extracts of powdered milk. Identification of BPA was based on comparison of retention times and peak-intensity ratios of selected ions from mass spectra of BPA derivatization products obtained by GC–MS of standard solutions and milk extracts.

INTRODUCTION

Over many years a large number of industrial chemicals have been introduced into the environment. Some of these chemicals have hormonal activity and can modulate the endocrine system, thereby affecting the health, reproduction, and development of humans and wildlife [1–4]; they are, therefore, commonly referred to as endocrine disruptors.

Bisphenol A (BPA), a recognised estrogenic endocrine-disrupting chemical, is widely used as a monomer in the manufacture of polycarbonate plastic, which is used as a lining in most food and beverage cans, as a dental sealant, and as an additive in other widely used consumer products [5–11]. The level of human exposure to BPA is not insignificant, because microgram quantities of BPA have been detected in liquid from canned vegetables [12]. Because, recent in-vitro studies have shown that the estrogenic effects of BPA can occur at concentrations as low as 1 pM, or 0.23 ppt [2], sensitive analytical methods are required to identify and determine trace levels of this compound in environmental and biological matrices.

Numerous methods have been developed for identification of BPA in complex environmental and biological extracts [13–15]. Most are based on chromatography, including gas chromatography (GC) and liquid chromatography (LC) [16]. Because gas chromatographic analysis of low-volatility polar compounds, for example phenolic and acidic compounds, results in poor sensitivity and in peak tailing [17], derivatization methods have been extensively used to improve gas chromatographic accuracy, reproducibility, and sensitivity. Derivatization by methylation [18], acetylation [19–22], and silylation [7,13,23,24] have frequently been used for identification and quantification of traces of BPA.

The objective of our study was to compare the efficiency of two methods of derivatization to increase the limit of detection of BPA in biological matrices.

EXPERIMENTAL

Chemicals and Reagents

Bisphenol A (BPA), *N*,*O*-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS), and bromoacetonitrile (BAN) were obtained from Sigma–Aldrich. Hexane, acetone, and chloroform were purchased from Mallinckrodt, and potassium carbonate was from J.T. Baker. All chemicals and reagents used in this work were analytical or research grade and were used without further purification.

Extracts of two powdered milk samples from Poznań supermarkets were prepared in the Department of Analytical Chemistry UAM in Poznań [25].

Derivatization of BPA Standards

Method 1 – Trimethylsilylation (BSTFA + 1% TMCS) [7]

BPA standard solution (200 µL) was placed in a vial (1 mL) and evaporated to dryness under nitrogen at 60°C. Silylating agent (BSTFA containing 1% TMCS; 100 µL) was added to the residue and the vial was vortex mixed and heated at 80°C for 30 min. After cooling, the derivatized solution was evaporated to dryness and the residue was redissolved in 100 µL chloroform. This solution (1 µL) was analysed by GC–MS.

Method 2 – Cyanomethylation (BAN) [18]

BPA standard solution (200 µL) was placed in a vial (1 mL) and evaporated to dryness. The residue was dissolved in 200 µL acetone and 20 µL bromoacetonitrile and 100 mg K_2CO_3 were added. The solution was heated at 60°C for 60 min in a heating block. After cooling to room temperature 1 µL was analysed by GC–MS.

GC–MS Conditions

Gas chromatography (GC) was performed with a Perkin–Elmer Autosystem XL GC, equipped with an autosampler, mass spectrometric detection, and split-splitless injection. Compounds were separated on a 30 m × 0.25 mm i.d. fused-silica capillary column coated with a 0.25 µm film of Rtx-5MS (5% diphenyl 95% dimethylpolysiloxane). Helium was used as carrier gas at a flow rate of 1 mL min^{-1}. The injector was operated in the splitless mode at 250°C. The oven temperature was maintained at 150°C for 2 min after injection then programmed at 30° min^{-1} to 270°C, which was held for 5 min. Retention times were measured with an accuracy of 0.01 min by use of a Turbomass Data System (Perkin–Elmer).

The transfer line to the mass spectrometer was set at 300°C. The electron-impact ionization MS source, electron energy 70 eV nominal, was used at 300°C. Acquisition of mass spectra was initiated immediately after sample injection. The dwell time was set at 80 ms and the multiplier potential was 450 V. Full-scan mass spectra between 100 and 500 *m*/*z* were acquired once every second. Confirmation of the identities of BPA and its derivatives was performed in selected ion monitoring mode (SIM), after selection of characteristic masses.

Application to Powdered Milk

Extracts of powdered milk were derivatized the same way as BPA

standard (Methods 1 and 2, described above). After derivatization 1 μL of the sample was analyzed by GC–MS.

RESULTS AND DISCUSSION

From among the derivatization methods used for GC–MS of BPA only two (BSTFA containing 1% TCMS, and BAN) were chosen because of their availability, simplicity of use, and the time consumed. The procedures used for derivatization of BPA were the same as those described by Kuo and Ding [7] and Shin et al. [18].

In the first step, BPA standard was derivatized with both derivatization reagents. The GC–MS chromatograms obtained from BPA and its derivatives are presented in Fig. 1. The results obtained (Figs 1A and 1B; Table I) show that the retention time of BPA (6.727 min) not differ substantially from that of the bis(trimethylsilyl) ether derivative of BPA (6.757 min), in contrast with that of the cyanomethyl derivative of BPA (Fig. 1C). This means that identification of BPA based solely on retention time could be questionable after derivatization with BSTFA. In the next step, therefore, the full-scan EI mass spectra of the chromatographic peaks were studied. As shown in Fig. 2, the normal electron-impact ionization (EI) mass spectrum of BPA (Fig. 2A) is characterized by a molecular ion at *m*/*z* 228. The most abundant fragment ion (213 *m*/*z*), corresponds to loss of CH_3 from the molecular ion, as reported by Chang et al. [6].

As shown in Fig. 2B, the EI mass spectrum of the bis(trimethylsilyl ether) of bisphenol A is similar to that of the unmodified compound. The molecular ion is present at *m*/*z* 372 and direct loss of CH_3 from the molecular ion yields the most abundant ion at *m*/*z* 357 [7].

The molecular ion (*m*/*z* 306) and the diagnostic ions (*m*/*z* 211, 251, and 291) in the EI mass spectrum of the cyanomethyl ether of bisphenol A (Fig. 2C) indicate that bisphenol A was converted to the corresponding bis(cyanomethyl) ether by reaction with bromoacetonitrile. The base peak (*m*/*z* 291) is formed by loss of a CH_3 group from the molecular ion [18]. The ions at *m*/*z* 251 and 211 are formed by loss of one or two CH_2CN groups, respectively, from the base peak, *m*/*z* 291.

The retention times of BPA and its derivatization products (BSTFA and BAN) and the peak-intensity ratios of selected ions in the corresponding mass spectra were determined. The results (Table I) show that the standard deviation of the retention times of BPA and its derivatives is 0.005 and that relative standard deviations are <0.1. The precision

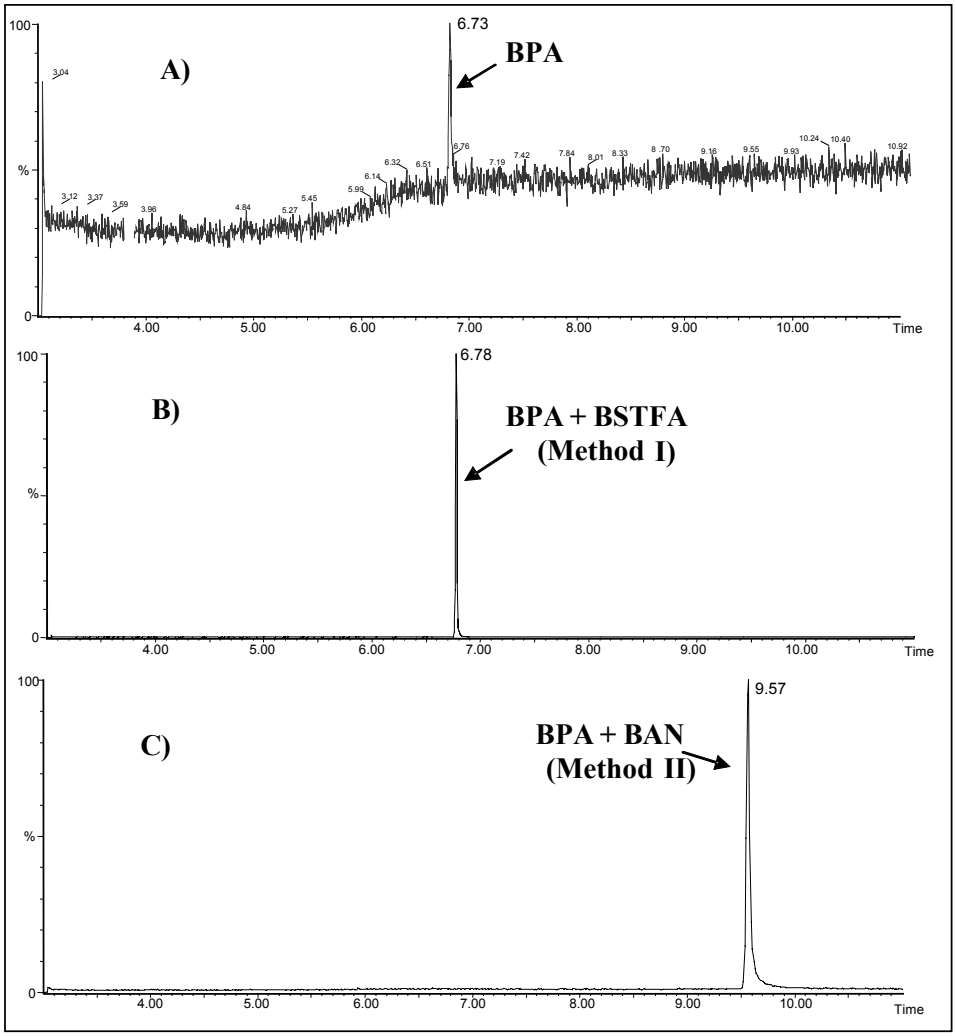

Fig. 1

GC–MS (full scan) chromatograms obtained from (A) a standard solution of BPA, (B) the bis(trimethylsilyl) ether derivative of BPA, and (C) the cyanomethyl derivative of BPA

of the ratio of the confirmatory ions was satisfactory, because for all the mass spectra examined *RSD* did not exceed 7.1%. Limits of detection (LOD) of 57 ppb (BSTFA + 1% TMCS) and 367 ppb (BAN) were obtained at a signal-to-noise ratio of 3.

The proposed derivatization procedures were used to confirm the presence of BPA in two extracts of powdered milk in which the com-

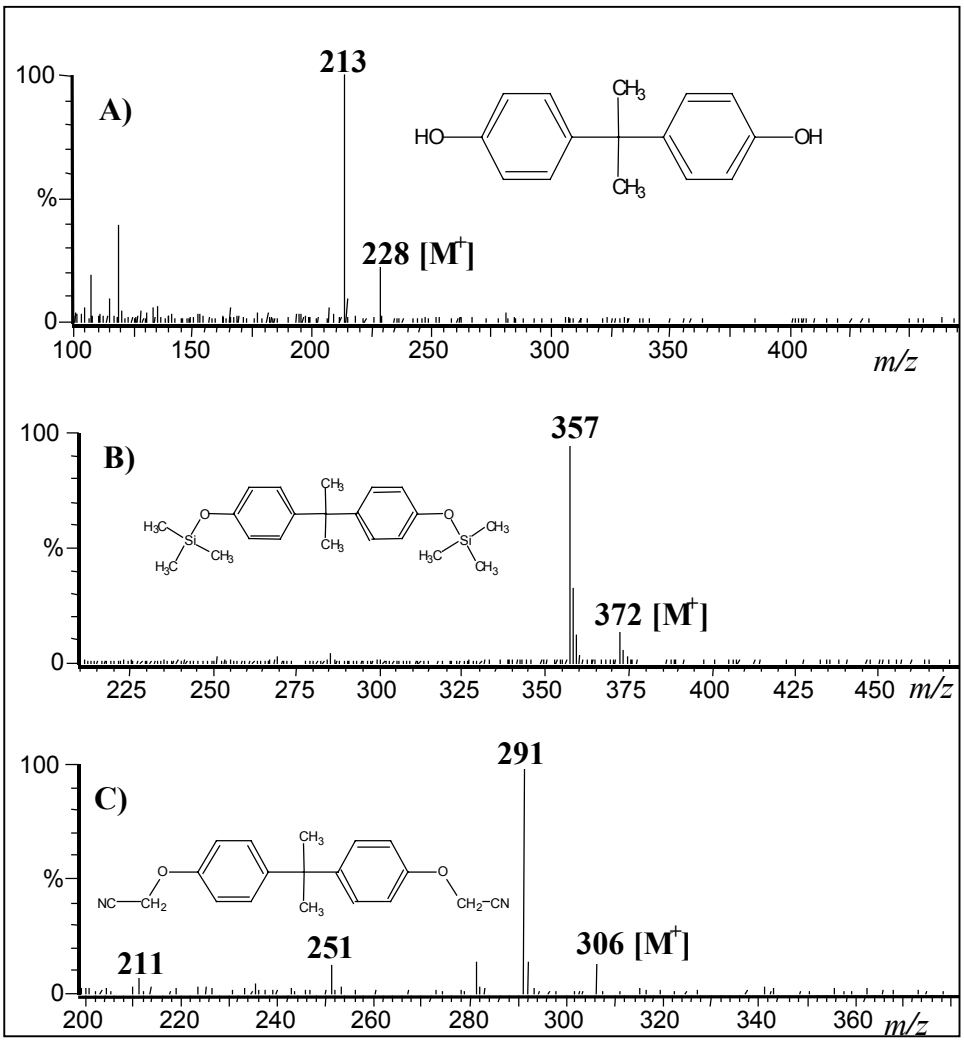

Fig. 2

Full-scan EI mass spectra of (A) BPA, (B) the bis(trimethylsilyl)ether derivative of BPA, and (C) the cyanomethyl derivative of BPA

pound had previously been identified by gas chromatography with flame ionization detection (GC–FID) [26]. The samples were analyzed by GC–MS directly and after derivatization with BSTFA and BAN. As shown in Fig. 3, the BPA peak in the TIC chromatogram was almost undetectable. After derivatization, the peak of the bis(trimethylsilyl)ether derivative was readily visible, both in the TIC chromatogram (Fig. 3A) and when SIM

Table I

Characteristics of the data used for identification of BPA standard and its BSTFA and BAN derivatives ($n = 6$)

		BPA	BSTFA derivative of BPA	BAN derivative of BPA
Retention time (min)	$\bar{x} \pm \Delta x$	6.727 ± 0.013	6.757 ± 0.006	9.527 ± 0.013
	S	0.005	0.005	0.005
	RSD (%)	0.08	0.08	0.05
Ions monitored (m/z)		213, 228	372, 357	211, 251, 291, 306
Ion ratio	Calculated	(228/213)	(372/357)	(306/291)
	$\bar{x} \pm \Delta x$	0.1306 ± 0.006	0.0695 ± 0.0035	0.1111 ± 0.0082
	S	0.005	0.0035	0.0078
	RSD (%)	4.03	5.00	7.07

was used (Fig. 3B). The same results were obtained for the cyanomethyl derivative of BPA. The retention times and the calculated peak-intensity ratios of selected ions are listed in Table II.

Confirmation of the presence of BPA in the milk extracts was based on the following requirements:

1. The retention time of the corresponding peaks in the chromatograms obtained from the extracts (non-derivatized and derivatized) should be within the confidence interval of the retention times of BPA standard and its derivatives.
2. The calculated peak-intensity ratios of selected ions from mass spectral SIM of the corresponding peaks in the extract chromatograms should not differ by more than 10% from those obtained from BPA standard and its derivatives.

The results listed in Table II show both criteria were fulfilled for the extract of milk sample 1, which confirms the presence of BPA in the powdered milk. For sample 2, the retention times of the peaks corresponding to BPA and its derivatives in the chromatograms obtained from the extract were within the corresponding confidence intervals from Table II, but only one peak intensity ion ratio in the mass spectrum obtained from the extract proved the presence of the bis(trimethylsilyl)ether derivative of BPA. This was because the LOD of the BAN derivative of BPA is an order of magnitude lower than that of the BSTFA derivative.

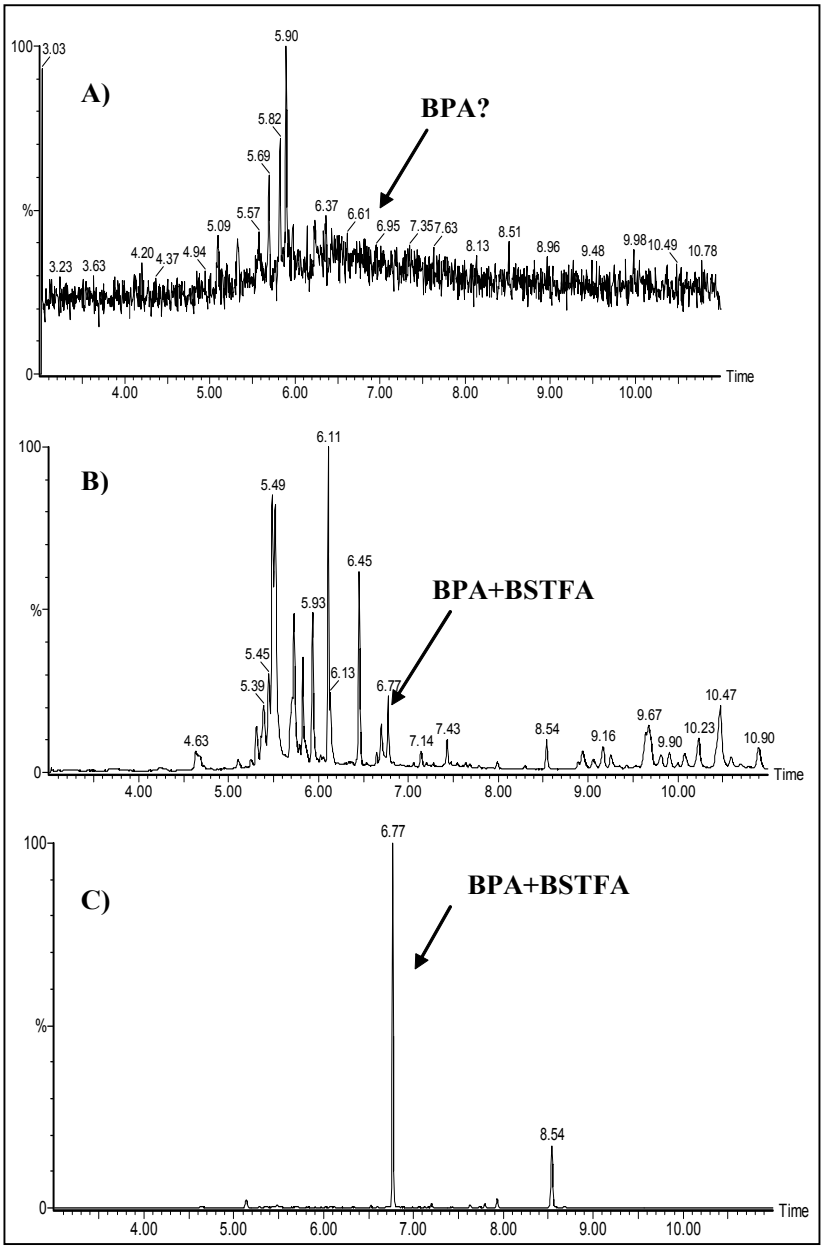

Fig. 3

GC–MS chromatograms obtained from (A) an extract of a milk sample 1, (B) the bis(trimethylsilyl)ether derivative of BPA in an extract obtained from milk sample 1 (TIC), and (C) the bis(trimethylsilyl)ether derivative of BPA in an extract obtained from milk sample 1 (SIM)

Table II

Criteria used for identification of BPA in extracts of powdered milk

Analyte	Retention time (min)			Ions ratio in mass spectrum		
	Sample 1	Sample 2	BPA standard*	Sample 1	Sample 2	BPA standard**
BPA	6.73	6.73	6.72–6.74	0.123	0.1807	0.117–0.144
BSTFA derivative	6.77	6.76	6.75–6.77	0.072	0.070	0.063–0.077
BAN derivative	9.54	9.54	9.52–9.54	0.099	0.094	0.099–0.122

* Corresponds to the confidence interval of retention times (Table I)
** Corresponds to ($\overline{x} - \Delta x$, $\overline{x} + \Delta x$) (Table I)

CONCLUSION

The results obtained in our experiments show that BSTFA is more useful than BAN for confirming the presence of BPA in extracts of biological samples.

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