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Determination of bisphenol A in river water and body fluid samples by stir bar sorptive extraction with in situ derivatization and thermal desorption-gas chromatography–mass spectrometry

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Abstract

A new method, based on stir bar sorptive extraction (SBSE) with in situ derivatization and thermal desorption (TD)-gas chromatography–mass spectrometry (GC–MS) is described for the determination of trace amounts of bisphenol A (BPA) in river water, urine, plasma, and saliva samples. The derivatization conditions with acetic acid anhydride and the SBSE conditions such as sample volumes and extraction time are investigated. Then, the stir bar is subjected to TD followed by GC–MS. The detection limits of BPA in river water, urine, plasma, and saliva samples are 1–5, 20, 100, and 20 pg ml^{-1} (ppt), respectively. Calibration for BPA was shown to be linear with a correlation coefficient of >0.99. The average recoveries of BPA in all samples are higher than 95% (R.S.D. < 10%) with correction using an added surrogate standard, $^{13}C_{12}$-bisphenol A. This simple, accurate, sensitive, and selective analytical method may be applicable to the determination of trace amounts of BPA in liquid samples.

Keywords: Stir bar sorptive extraction; Derivatization, GC; Bisphenol A

1. Introduction

Bisphenol A (BPA), 2,2-bis(4-hydroxyphenyl)propane, is a chemical contained in polycarbonate plastics and epoxy resins and is included in various products used in industry. In 1993, Krishnan et al. [1] documented that BPA was released from polycarbonate flasks during autoclaving and exhibited estrogenic activity. The effects of BPA on the environment and human health have become a controversial issue. In Japan, the River Environment Management Foundation of the Ministry of Construction was reported that concentrations of the environmental pollution substance in major rivers [2]. Moreover, the Health Sciences Research conducted by the Ministry of Health, Labour and Welfare of Japan revealed that risk assessment, human exposure, and toxicological studies have been conducted and analytical techniques for endocrine disruptors have been developed by many researchers [3]. That BPA has contributed significantly to the environmental problem is evidenced by a number of studies indicating the effects of BPA in the "low dose" range, including its effects at the ppt level [4–6]. Therefore, in order to assess environmental and human exposures to BPA, a reliable sensitive analytical method is required. Many analytical methods are available for the determination of BPA in environmental water [7–10]. It was reported that a significant amount of BPA in resin-based components and sealants used in dentistry was released into saliva [11,12]. There are also a number of reports of the measurement of BPA in human urine [13,14], serum, and plasma samples [15–21] by different analytical methods in an attempt to assess human exposure. Thus, the measurement of BPA in various liquid samples is performed by different analytical methods. In this regard, the determination of BPA in liquid samples by only one analytical method is desired. However, to our knowledge, no such a study has been performed so far.

Many analytical methods for the determination of BPA in various samples have been reported. The enzyme-linked immunosorbent assay (ELISA) has been recently reported to be a sufficiently sensitive technique for the determination of BPA in human serum samples [20,21]. However, we found

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that ELISA may give erroneous results due to non-specific binding to the antibody, as evidenced by the overestimation of trace amounts of BPA in human semen samples [22]. Other reliable methods of analysis include liquid chromatography (LC) with fluorescence detection (FD) [8,19], electrochemical detection (ED) [7,14–16], and mass spectrometry (MS) [15,17,22]. However, the LC methods have low resolution and are influenced by the sample matrix, frequently. On the other hand, gas chromatography–mass spectrometry (GC–MS) was initially used for the determination of phenol compounds including BPA, even though derivatization was required [9,10,13,18]. The derivatization leads to sharper peaks and hence to better separation of and higher sensitivity for the phenols. However, the derivatization procedure requires much time and effort. In order to avoid this problem, in situ derivatization has been developed, which involves the simple addition of a reagent into a liquid sample.

Recently, a new sorptive extraction technique that uses a stir bar coated with polydimethylsiloxane (PDMS) was developed [23]. The technique is known as stir bar sorptive extraction (SBSE) and its main advantage is its wide application range that can include volatile aromatics, halogenated solvents, polyaromatic hydrocarbons, polychlorinated biphenyls (PCBs), pesticides, preservatives, odor compounds, and organotin compounds [24–33]. In addition, SBSE has been applied successfully in biological samples [29–33]. An SBSE method that employs in situ derivatization was used in the analysis of phenolic compounds [31–33].

The aim of this study was to determine trace amounts of BPA in various liquid samples by SBSE with in situ derivatization and thermal desorption (TD)-GC–MS. The developed method was applied to river water, and human urine, plasma, and saliva samples.

2. Experimental

2.1. Materials and reagents

Bisphenol A of environmental analytical grade, bisphenol A diacetate of analytical grade and acetic acid anhydride for trace analysis were purchased from Kanto Chemical Inc. (Tokyo, Japan). $^{13}C_{12}$-Bisphenol A ($^{13}C_{12}$-BPA) was obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). *Escherichia coli* β-glucuronidase (144 U ml^{-1}) was purchased from Fluka Inc. (Buchs, Switzerland). Other reagents and solvents were of pesticide or analytical grade and purchased from Wako Pure Chemical Inc. (Osaka, Japan). The water purification system was a Milli-Q gradient A 10 with an EDS polisher (Millipore, Bedford, MA, USA).

2.2. Standard solutions

A concentrated solution (1.0 mg ml^{-1}) of BPA was prepared as required by the addition of purified water. Calibration was performed daily for all samples with a surrogate standard.

2.3. Instrumentation

Stir bars coated with a 500-μm-thick layer (24 μl) of PDMS (Twister^{TM}: a magnetic stirring rod is placed in a glass jacket and coated with PDMS) were obtained from Gerstel (Mülheim an der Ruhr, Germany). The stir bars were conditioned for 4 h at 300 °C in a flow of helium. The stir bars could be used more than 50 times with appropriate re-conditioning. For the extraction, 10 and 100 ml headspace vials from Agilent Technologies (Palo Alto, CA, USA) and GL Science (Tokyo, Japan) were used. TD was performed using a Gerstel TDS 2 thermodesorption system equipped with a Gerstel TDS A autosampler and a Gerstel CIS 4 programmable temperature vaporization (PTV) inlet. GC-MS was performed using an Agilent 6890N gas chromatograph with a 5973N mass-selective detector (Agilent Technologies).

2.4. TD-GC–MS conditions

The TDS 2 temperature was programmed to increase from 20 °C (held for 1 min) to 280 °C (held for 5 min) at 60 °C min^{-1}. The desorbed compounds were cryofocused in the CIS 4 at −150 °C. After the desorption, the CIS 4 temperature was programmed to increase from −150 to 300 °C (held for 10 min) at 12 °C s^{-1} to inject the trapped compounds onto the analytical column. Injection was performed in the splitless mode. The separations were conducted on a DB-5MS fused silica column (30 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies). The oven temperature was programmed to increase from 60 to 300 °C (held for 4 min) at 15 °C min^{-1}. Helium was used as the carrier gas at a flow rate of 1.2 ml min^{-1}. The mass spectrometer was operated in the selected ion-monitoring (SIM) mode with electron ionization (ionization voltage: 70 eV). For SIM, three ions were monitored (*m*/*z* 213 and 228 for the acyl derivative of BPA and *m*/*z* 225 for the acyl derivative of $^{13}C_{12}$-BPA. The underlined number is the *m*/*z* of the ion used for quantification). A blank run of the stir bar was always performed after an analysis, and memory effects were not detected. Therefore, satisfactory analysis was possible under these thermal desorption conditions.

2.5. Environmental water and body fluid samples

River water samples were obtained from two sites in Tama River, Tokyo, Japan and stored at 4 °C prior to use. Urine, plasma, and saliva samples were originally collected from three healthy volunteers (22–25-year old) and stored at −80 °C prior to use.

2.6. Sample preparation by SBSE with in situ derivatization

One ml of human urine, 200 μl of plasma, and 500 μl of saliva samples were buffered with ammonium acetate (200 μl, 1.0 M, pH 6.8). After *Escherichia coli* β-glucuronidase (15 μl, 144 U ml^{-1}; Fluka Chemie AG, Buchs, Switzerland) was added, each of the samples was sealed in a glass tube and gently mixed. Quantitative glucuronidase hydrolysis for releasing the free BPA was accomplished by incubating at 37 °C for 3 h. After enzymatic deconjugation, the samples were filled with purified water to a total volume of 2 ml. This treatment was sufficient to deconjugate the glucuronidase of glucuronidated BPA [13,14]. Then 2, 10 or 50 ml of river water sample, or 2 ml of urine, plasma or saliva sample was placed in a headspace vial with a surrogate standard. To the 2, 10 or 50 ml sample were added sodium carbonate (10.6, 53.0 or 265.0 mg) and sodium hydrogen carbonate (8.4, 42.0 or 210.0 mg) as a pH adjustment agent (pH 10.5), and acetic acid anhydride (40, 200 or 1000 μl) as a derivatization reagent. A stir bar was added to each vial and the vial was crimped with a Teflon-coated silicone septum. SBSE was performed at room temperature for 10–180 min while stirring at 500 or 1000 rpm. After the extraction, the stir bar was removed with forceps (due to the magnetic attraction effect), rinsed with purified water, dried with lint-free tissue, and placed in a glass TD tube. The TD tube was then placed in the TD unit. Then, the stir bar was thermally desorbed in the TD-GC–MS.

3. Results and discussion

3.1. Derivatization

In electron ionization-MS analysis of standard solutions of BPA and $^{13}C_{12}$-BPA in the scan mode, *m/z* 213 and 225 were observed as the major signals, and *m/z* 228 and 240 were observed as the minor signals, respectively. After the derivatization, an acyl derivative of BPA and an acyl derivative of $^{13}C_{12}$-BPA were obtained with *m/z* 213 and 225 as the major signals. The mass spectra of these compounds are shown in Fig. 1. The acyl derivative of BPA was identified as BPA diacetate by coincidence of the retention time and mass spectrum with that of an authentic standard.

3.2. Time for and efficiency of SBSE with in situ derivatization

Table 1 shows $\log K_{o/w}$ and the theoretical recoveries of the compounds investigated in this work. The $K_{o/w}$ values were calculated from the log *P* predictor, which is available from Interactive Analysis Inc. (Bedford, MA, USA). Theoretical recoveries are calculated by the following equation:

$$\text{Theoretical recovery} = \frac{K_{o/w}/\beta}{(1 + K_{o/w}/\beta)}$$

where $\beta = V_w/V_{PDMS}$; V_{PDMS} is the volume of PDMS; and V_w is the volume of water. The theoretical recoveries by SBSE were calculated on the basis of a 2, 10 or 50 ml sample volume and a stir bar with a phase thickness of 500 μm (24 μl PDMS). The results revealed that, when sample volume was increased, the recovery of BPA was decreased. However, the recovery did not change markedly even if the sample volume was increased.

Table 1
$\log K_{o/w}$ and theoretical recoveries of BPA and BPA diacetate by SBSE

Compound	$\log K_{o/w}$[a]	Sample volume (ml)	Theoretical recovery (%)
Bisphenol A	3.50	2	97
		10	88
		50	60
Bisphenol A diacetate	4.48	2	100
		10	99
		50	94

[a] $\log K_{o/w}$ values for BPA and BPA diacetate as predicted by "IA log *P* predictor" as well as theoretical recoveries.

An important parameter affecting SBSE was the extraction time. To optimize the extraction time, a 10 ng ml^{-1} standard solution of BPA was used. The extraction time profiles (equilibration curves) of acyl derivative of BPA in 2, 10, and 50 ml standard solutions using SBSE with in situ derivatization and BPA in 2 ml standard solution using SBSE without derivatization were determined by TD-GC–MS, and are shown in Fig. 2. The acyl derivative of BPA reached equilibrium after approximately 45, 90, and 120 min, respectively, for the case with in situ derivatization. This was a proof that extraction time became longer, when sample volume was increased. On the other hand, BPA reached equilibrium after approximately 60 min for the case without the derivatization. These conditions were, therefore, used for the determination of BPA in liquid samples. When sample volume was set to 2 ml, acyl derivative of BPA with a high $\log K_{o/w}$ reached equilibrium (full equilibration) the more rapidly, whereas BPA with a low $\log K_{o/w}$ reached equilibrium more slowly. In addition, the peak area of acyl derivative of BPA was larger than that of BPA at equilibrium. Therefore, it was thought that high-sensitivity analysis by derivatization was possible.

3.3. Comparison of analytical figures of merit

The mass spectrometer was operated in the SIM mode. For SIM, three ions were monitored (*m/z* 213, 228 for BPA and acyl derivative of BPA, and *m/z* 225 for $^{13}C_{12}$-BPA and the acyl derivative of $^{13}C_{12}$-BPA). The underlined number is the *m/z* of the ion used for determination.

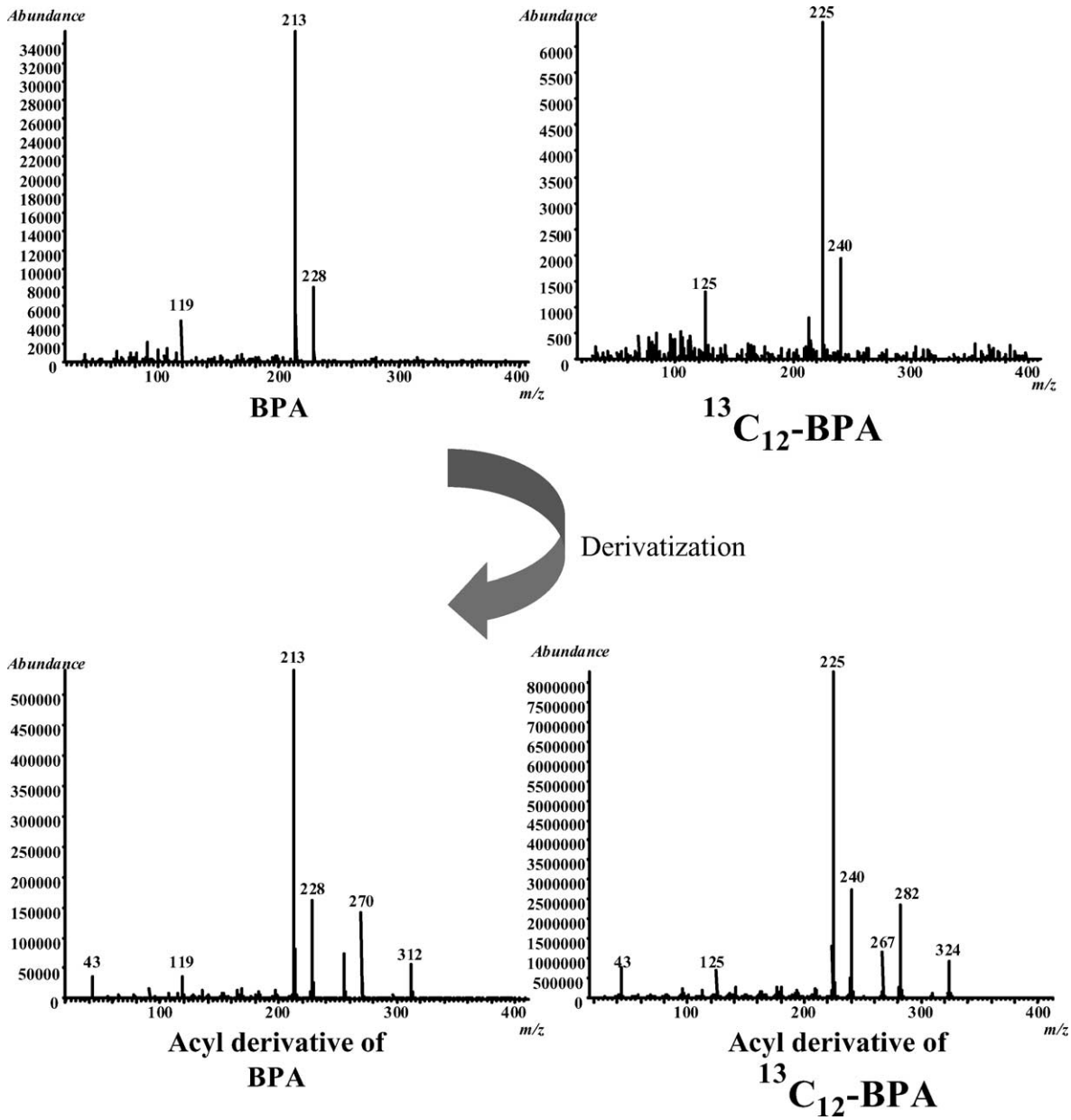

Fig. 1. Mass spectra of BPA, $^{13}C_{12}$-BPA, acyl derivative of BPA and acyl derivative of $^{13}C_{12}$-BPA.

When sample volume was 2 ml, the calculated detection limits (LODs) of BPA in river water sample with in situ derivatization and that without the derivatization were 5 and 500 pg ml^{-1}, respectively, by SBSE–TD-GC–MS when the ratio of the compound's signal to the background signal (*S*/*N*) was 3. In addition, the calculated limits of quantification (LOQs) when *S*/*N* >10 were 20 and 2000 pg ml^{-1} for BPA in river water sample with in situ derivatization and that without the derivatization, respectively. The in situ derivatization method exhibited approximately 100-fold higher sensitivity than the method without derivatization. Moreover, when sample volume was increased to 10 and 50 ml, LODs of acyl derivative of BPA were 2 and 1 pg ml^{-1}, respectively, for samples with in situ derivatization. On the other hand, the LODs of acyl derivative of BPA in urine, plasma, and saliva samples were 20, 100, and 20 pg ml^{-1}, and the LOQs of acyl derivative of BPA were 100, 500, and 100 pg ml^{-1}, respectively. The peak area ratios with respect to each surrogate standard were plotted and the response was found to be linear over the calibration range with correlation coefficients (r^2) higher than 0.99. The validation results are summarized in Table 2.

The recovery and precision of the method were assessed by replicate analysis ($n = 6$) of various samples spiked at the 1.0 ng ml^{-1} level with the surrogate standard. Non-spiked and spiked samples were subjected to SBSE with in situ derivatization and TD-GC–MS. The recoveries were calculated by subtracting the results for the non-spiked samples

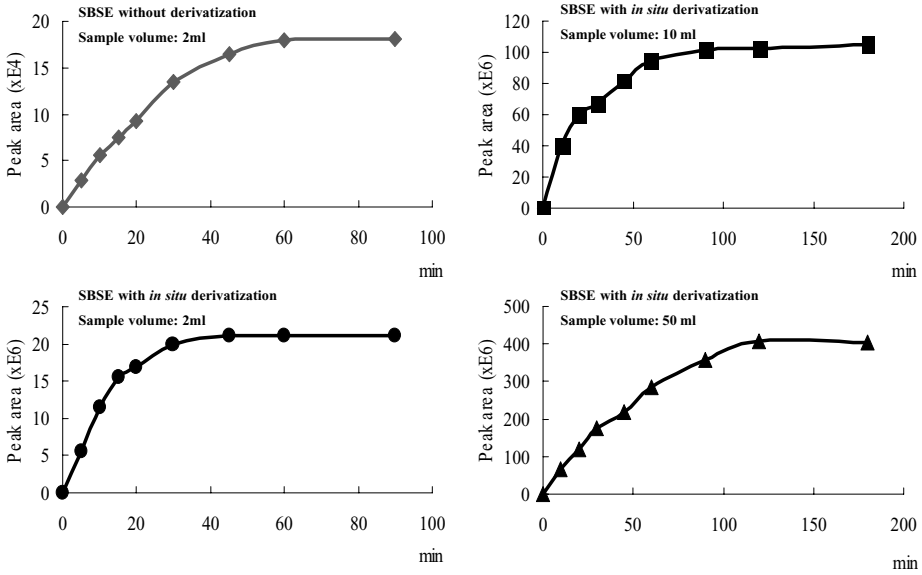

Fig. 2. Extraction time profiles of BPA in water samples using PDMS stir bar. A PDMS stir bar and derivatization reagents or none are added to 10 ng ml^{-1} standard solutions and stirring is commenced for 10–90 or 180 min at room temperature (25 °C) in a glass vial. The extract is then analyzed by TD-GC–MS.

from those for the spiked samples. The results were obtained by using calibration curves of standard solutions with surrogate standards. The average recovery was higher than 95% (R.S.D. < 10%) for various samples (Table 3). Consequently, the method recovery appeared not to be influenced by the type of liquid sample analyzed. Therefore, the method enables the precise determination of standards and can be applied to the determination of trace amounts of BPA in river water, and human urine, plasma, and saliva samples.

3.4. Determination of BPA in environmental water and body fluid samples

Two river water samples the volumes of which were set to 2 ml were analyzed using the present method. The re-

Table 2
Comparison of analytical figures of merit of SBSE methods with in situ derivatization and without derivatization

SBSE method	Sample	Sample volume (ml)	BPA		
			LOD[a]	LOQ[b]	r^2
With in situ derivatization	River	2	5	20	0.999 (0.02–10)[c]
		10	2	10	0.999 (0.01–10)
		50	1	5	0.998 (0.005–10)
	Urine	1	20	100	0.999 (0.02–10)
	Plasma	0.2	100	500	0.999 (0.02–10)
	Saliva	0.5	20	100	0.999 (0.02–10)
Without derivatization	River	2	500	2000	0.997 (2–100)

[a] LOD, limit of detection (pg ml^{-1}).
[b] LOQ, limit of quantification (pg ml^{-1}).
[c] Values in parentheses are the linear ranges of the calibration curves (ng ml^{-1}).

Table 3
Recoveries of BPA in various spiked samples by SBSE with in situ derivatization

Compound	Sample	Sample volume (ml)	BPA	
			Recovery (%)	R.S.D. (%)[a]
BPA	River	2	104.6	3.8
		10	98.0	4.0
		50	97.6	4.7
	Urine	1	95.2	6.3
	Plasma	0.2	99.3	9.6
	Saliva	0.5	100.7	9.3

[a] The recoveries and precision were also examined by replicate analysis ($n = 6$) of various samples spiked at the 1.0 ng ml^{-1} level.

Table 4
Concentrations of BPA in various samples by SBSE with in situ derivatization

	Sample	BPA (pg ml^{-1})[a]	
		β-Glucuronidase	None
A	River water	–	47
B	River water	–	39
C	Urine	450	N.D.
	Plasma	N.D.	N.D.
	Saliva	N.D.	N.D.
D	Urine	220	N.D.
	Plasma	N.D.	N.D.
	Saliva	N.D.	N.D.
E	Urine	410	N.D.
	Plasma	N.D.	N.D.
	Saliva	N.D.	N.D.

[a] N.D. indicates BPA concentrations in river water, urine, plasma, and saliva samples lower than 20, 100, 500, and 100 pg ml^{-1}, respectively.

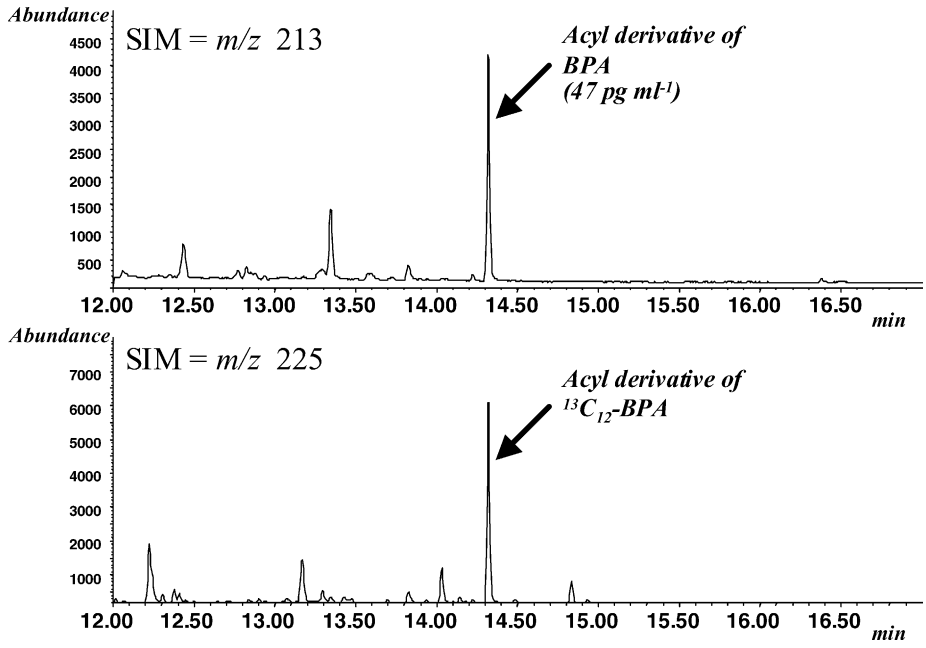

Fig. 3. Chromatograms of acyl derivative of BPA and surrogate standard in river water samples. A PDMS stir bar and derivatization regents are added to 2 ml of river water sample, surrogate standard and stirring is performed for 45 min at room temperature (25 °C) in a glass vial. The extract is then analyzed by TD-GC–MS.

sults of BPA determination in the river water samples are shown in Table 4. BPA (39 and 47 pg ml^{-1}) was detected by this method. Typical chromatograms of the river water samples are shown in Fig. 3. The BPA levels in the river water samples were very low and might not be determined quantitatively by SBSE–TD-GC–MS without derivatization. However, the combination of SBSE with in situ derivatization and TD-GC–MS led to the successful determination of

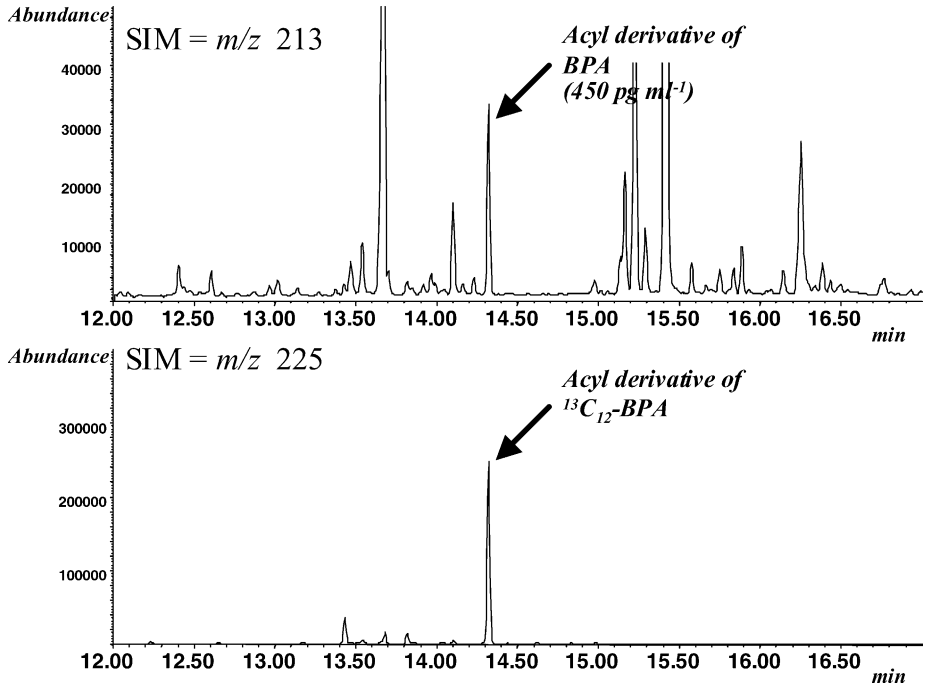

Fig. 4. Chromatograms of acyl derivative of BPA and surrogate standard in human urine samples. One ml of human urine sample was buffered with ammonium acetate. After *E. coli* β-glucuronidase was added, the samples was sealed in a glass tube and gently mixed. Quantitative glucuronidase hydrolysis for releasing the free BPA was accomplished by incubating at 37 °C for 3 h. After enzymatic deconjugation, the samples were filled with purified water to a total volume of 2 ml. A PDMS stir bar, surrogate standard and derivatization regents are added to 2 ml of human urine sample and stirring is performed for 45 min at room temperature (25 °C) in a glass vial. The extract is then analyzed by TD-GC–MS.

trace amounts of BPA in the river water samples. A previous study revealed morphological abnormalities in milt in approximately 30% of male carp downstream of sewage treatment plants in the Tama River, Japan during 1997–1998 [34]. Subsequently, elevated levels of vitellogenin were observed in male carp [2]. Therefore, this river is a good study area for assessing estrogenic activity in the environment in Japan. In addition, the determination of BPA in Tama River water samples by LC–MS with off-line SPE was conducted [35]. A concentration range of N.D. (<0.6) to 700 pg ml^{-1} BPA was detected. However, that SPE method required a large sample volume (4–20 l) for acquiring high sensitivity. In this study, SBSE with in situ derivatization and TD-GC–MS led to the successful determination of trace amounts of BPA in a small sample volume (2 ml).

The urine, plasma, and saliva samples collected from three healthy volunteers were analyzed using the present method. The results are shown in Table 4. BPA could not be detected in either of the samples by this method before β-glucuronidase deconjugation. On the other hand, BPA concentrations of 220–450 pg ml^{-1} were detected in human urine samples after β-glucuronidase deconjugation. The typical chromatograms of human urine samples are shown in Fig. 4.

Investigations of BPA metabolism in vivo [36,37] and in vitro [38] suggest that BPA absorbed by the intestine is glucuronidated in the liver and excreted as BPA glucuronide in the urine. Moreover, it has been reported that the concentration of BPA in human urine samples was 110–510 pg ml^{-1} after β-glucuronidase deconjugation [13]. That range is almost the same as that obtained by our method. In our study, analyses of urine, plasma, and saliva samples collected from the same healthy human volunteer led to the detection of BPA glucuronide from urine and not plasma and saliva. Hence, urinary BPA glucuronide may be a good marker for estimating human exposure levels.

4. Conclusions

The determination of trace amounts of BPA in river water and body fluid samples using SBSE with in situ derivatization and TD-GC–MS was described. The proposed method has many practical advantages including simplicity of the extraction method; avoidance of organic solvents and high sensitivity, and can be applied to various liquid samples. The detection limits of BPA in river water, urine, plasma, and saliva samples were 1–5, 20, 100, and 20 pg ml^{-1}, respectively. In addition, the present method showed good linearity and high correlation coefficients using surrogate standards. In addition, the recoveries were 95–105% with good precision (R.S.D.: 3.8–9.6%) for various samples spiked at the 1.0 ng ml^{-1} level. This simple, accurate, and highly sensitive method is expected to have potential applications in various liquid samples.

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