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Molecularly imprinted solid phase extraction using stable isotope labeled compounds as template and liquid chromatography–mass spectrometry for trace analysis of bisphenol A in water sample

Migaku Kawaguchi, Yoshio Hayatsu, Hisao Nakata, Yumiko Ishii, Rie Ito, Koichi Saito, Hiroyuki Nakazawa *

Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan

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Abstract

We have developed a molecularly imprinted polymer (MIP) using a stable isotope labeled compound as the template molecule and called it the "isotope molecularly imprinted polymer" (IMIP). In this study, bisphenol A (BPA) was used as the model compound. None imprinted polymer (NIP), MIP, dummy molecularly imprinted polymer (DMIP) and IMIP were prepared by the suspension polymerization method using without template, BPA, 4-*tert*-butylphenol (BP) and bisphenol A-d_{16} (BPA-d_{16}), respectively. The polymers were subjected to molecularly imprinted solid phase extraction (MI-SPE), and the extracted samples were subjected to liquid chromatography–mass spectrometry (LC–MS). Although the leakage of BPA-d_{16} from the IMIP was observed and that of BPA was not observed. The selectivity factors of MIP and IMIP for BPA were 4.45 and 4.43, respectively. Therefore, IMIP had the same molecular recognition ability as MIP. When MI-SPE with IMIP was used and followed by LC–MS in the analysis of river water sample, the detection limit of BPA was 1 ppt with high sensitivity. Moreover, the average recovery was higher than 99.8% (R.S.D.: 3.7%) by using bisphenol A-$^{13}C_{12}$ (BPA-$^{13}C_{12}$) as the surrogate standard. In addition, the IMIP were employed in MI-SPE of BPA in river water sample by LC–MS. The concentration of BPA in the river water sample was determined to be 32 pg ml^{-1}. We confirmed that it was possible to measure trace amounts of a target analyte by MI-SPE using IMIP.

Keywords: Isotope molecularly imprinted polymer (IMIP); Molecularly imprinted solid phase extraction (MI-SPE); Bisphenol A (BPA); Liquid chromatography–mass spectrometry (LC–MS)

1. Introduction

Molecularly imprinted polymers (MIPs) as man-made mimics of antibodies have been developed for the selective recognition of a target molecule [1–3]. The molecularly imprinted solid phase extraction (MI-SPE) method is useful for the selective pretreatment and enrichment of analyte in complex matrices [4–14]. However, because MIP is prepared by using the target analyte as template molecule, leakage of a trace amount of the imprinted molecule remaining in the MIP has hindered the accurate and precise assay of the target analyte. In order to solve this problem, a structural analogue is imprinted and used in combination with chromatographic separation [15–18], the so-called "dummy molecularly imprinted polymer" (DMIP). However, DMIP is inferior to MIP in terms of selectivity for the target analyte.

In this regard, it has been reported that novel MIP using a stable isotope labeled compound as the template molecule by typical multi-step swelling and polymerization method [19]. The isotope labeled compound molecularly imprinted polymer (IMIP) was subjected to on line MI-SPE, and the extracted sample was subjected to liquid chromatography–mass spectrometry (LC–MS). Although the leakage of isotope labeled compound from the IMIP was observed and that of analyte was not observed. Moreover, it was expected that the selectivity for a target analyte of IMIP was higher than that of DMIP. In this study, we try to develop IMIP by suspension

* Corresponding author. Tel.: +81 3 5498 5763; fax: +81 3 5498 5062.
E-mail address: nakazawa@hoshi.ac.jp (H. Nakazawa).

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polymerization. In addition, because there are many stable isotope labeled compounds containing deuterium or ^{13}C, it is possible to use one stable isotope labeled compound as the template molecule of IMIP, and another stable isotope as the surrogate standard. Thus, the realization of a highly precise analytical method with high selectivity is possible by using two stable isotope labeled compounds.

In this study, bisphenol A (BPA) was used as the model compound. BPA is widely used for the production of epoxy resins and polycarbonates, and is recently suspected to be an endocrine disruptor. In vitro assays have revealed that BPA binds to estrogen receptor and causes a weak estrogenic activity [20]. A trace amount of BPA is eluted from plastic packages and tableware, polluting foods and drinks [21]. In addition, we already reported that BPA concentrations of 39–47 pg ml^{-1} in river water samples was detected by using stir bar sorptive extraction (SBSE)–thermal desorption (TD)-gas chromatography–mass spectrometry (GC–MS) [22]. As the low-dose effect of BPA cannot be completely denied, molecular recognition materials that can selectively extract BPA are desired from the viewpoint of environmental conservation.

In this study, we prepared none imprinted polymer (NIP), MIP, DMIP and IMIP, all of which can recognize BPA, by the suspension polymerization method using without template, BPA, BP and stable isotope labeled BPA (bisphenol A-d_{16}, BPA-d_{16}), respectively. The prepared polymers were evaluated by determining their selectivity factors using an HPLC system. Then, the NIP and MIPs were employed in MI-SPE of BPA in river water sample by LC–MS using bisphenol A-$^{13}C_{12}$ (BPA-$^{13}C_{12}$) as a surrogate standard.

2. Experimental

2.1. Materials

The chemicals for polymer syntheses were bisphenol A (BPA) (purity > 95%), 4-*tert*-butylphenol (BP) (> 95%), 4-vinypyridine (4-VP) (stabilizer: 4-*tert*-butylpyrocatechol. abt. 0.3%), ethylene glycol dimethacrylate (EGDMA) (> 97%), 2,2′-azobisisobutyronitrile (AIBN) (> 98%), completely and partially hydrolyzed poly(vinyl alcohol) (average degree of polymerization: 900 to 1100) from Wako Pure Chemical Inc. (Osaka, Japan), bisphenol A-d_{16} (BPA-d_{16}) (98%) from Aldrich (Steinheim, Germany), bisphenol A-$^{13}C_{12}$ (BPA-$^{13}C_{12}$) (99%) from Cambridge Isotope Laboratories Inc. (Andover, MA, USA), and toluene (> 99.5%) from Kanto Chemical Inc. (Tokyo, Japan). The chemical structures of BPA, BP, BPA-d_{16} and BPA-$^{13}C_{12}$ are shown in Fig. 1. The monomers were purified prior to use via standard procedures in order to remove the stabilizers. Reagents other than those mentioned above were purchased from Wako Pure Chemical Inc. (Osaka, Japan). The water purification system used was a Milli-Q gradient A 10 with an EDS polisher (Millipore, Bedford, MA, USA).

2.2. Apparatus

The liquid chromatography–mass spectrometry (LC–MS) with quadrupole system was an Agilent LC-MSD Superior Line (Agilent Technologies, Palo Alto, USA) equipped with an electrospray ionization (ESI) source. The injection volume was 5.0 μl in the needle washing mode. A Mightysil RP-18 GP (2.0 mm i.d. × 100 mm, 5 μm) analytical column with a guard column (Mightysil RP-18 GP, 2.0 mm i.d. × 5 mm, 5 μm) from Kanto Chemical Industries, Ltd. was used for separation at 40 °C. The separation was carried out using a mobile phase of 0.01% (v/v) acetic acid in water/acetonitrile (65:35, v/v) at a flow rate of 0.2 ml min^{-1}. The working conditions for the electrospray MS were as follows: the drying nitrogen gas was set at a temperature of 350 °C and was introduced into the capillary region at a flow rate of 12 l min^{-1}, and the capillary was held at a potential of 3500 V relative to the counter electrode in the negative ion mode. The fragmentor voltage was 140 V during the chromatographic run. The chromatographic monitoring mode was gain 1.0. When working in the selected ion monitoring (SIM) mode, the *m/z*

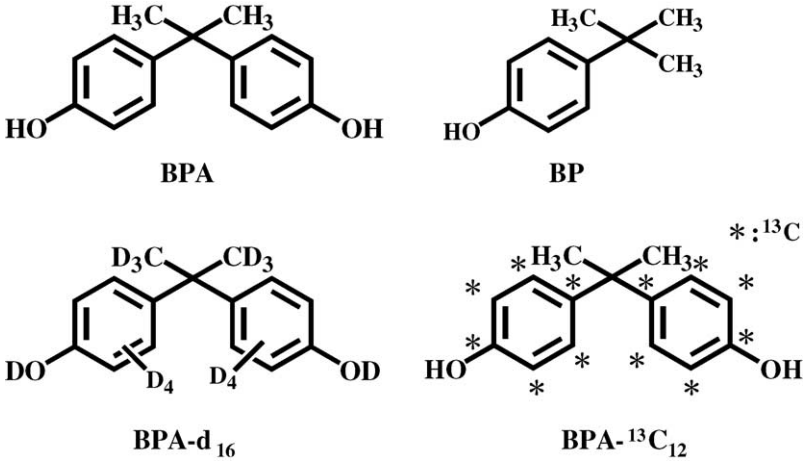

Fig. 1. Chemical structures of BPA, BP, BPA-d_{16} and BPA-$^{13}C_{12}$.

227, 239 and 241 ions were assigned to the $[M-H]^-$ of BPA, BPA-$^{13}C_{12}$ and BPA-d_{16}, respectively.

Stock solutions of BPA (1000 μg ml^{-1}) were prepared in methanol. The internal standard calibration was established with BPA-$^{13}C_{12}$ as surrogate standard. The calibration curve showed linearity in the concentration range of 0.5–100 ng ml^{-1}. Quantitative analysis was performed in the SIM mode in order to maximize sensitivity.

The HPLC system for evaluation of the molecular recognition ability of MIPs for BPA consisted of an LC-10AS pump, a CTO-6A column oven, an SPD-10AS spectrophotometer, and a C-R6A integrator (Shimadzu, Kyoto, Japan). The flow rate was maintained at 1.0 ml min^{-1}. The detection was performed at 210 nm. All separations were carried out at 40 °C using the column oven. The eluent used was sodium dihydrogen phosphate and disodium hydrogen phosphate (pH 5.1; 20 mM) acetonitrile (3:7, v/v). The amount loaded was set to 200 ng.

2.3. River water samples

River water sample was obtained from a site in Tama River, Tokyo, Japan. The sample was filtered and stored at 4 °C prior to use.

2.4. Preparation of NIP and MIPs by suspension polymerization

One group has reported a preparation method for MIP using EGDMA as the cross-linker, 4-VP as the functional monomer, and BPA as the template molecule [23]. The development of DMIP using 4-*tert*-butyphenol, a structural analogue of BPA, has been reported as well [24]. In this study, we prepared NIP, MIP, DMIP and IMIP, all of which can recognize BPA, by the suspension polymerization method using without template, BPA, BP and BPA-d_{16}, respectively.

In 18 ml of toluene was dissolved 228 mg (1 mmol) of BPA, 150 mg (1 mmol) of BP or 244 mg (1 mmol) of BPA-d_{16}. Then, 631 mg (6 mmol) of 4-VP, 5946 mg (30 mmol) of EGDMA and 125 mg of AIBN were added. The details are shown in Table 1. This organic phase was poured into 50 ml of water in which poly(vinyl alcohol) (partially hydrolyzed: 0.26 g, completely hydrolyzed: 0.14 g) was dissolved. The mixture was stirred (500 rpm) at 60 °C for 6 h and at room temperature for 14 h. The resultant particles were filtered to remove particles of inappropriate size for chromatography or MI-SPE. Three filters (45, 75 and 125 μm) were used to divide the size of NIP and MIPs. The polymer (45–75 and 75–125 μm) was washed with methanol (10 ml, five times) and dried at room temperature over night.

2.5. Evaluation of NIP and MIPs

Five grams of dried particles (45–75 μm) was packed into a stainless-steel column (4.6 mm i.d. × 150 mm) by the slurry packing technique using methanol/2-propanol (2:1, v/v) as the slurry solvent and methanol as the packing solvent to evaluate their retention characteristics.

The NIP and MIPs were evaluated by HPLC. The retention factor was calculated using the equation $k = (t_R - t_0)/t_0$, where t_R and t_0 are the retention times of retained and unretained solutes, respectively. The retention time of unretained solute, t_0, was measured by injecting a solution the organic modifier content of which was slightly different from that obtained using the equation $S = k_{imprinted}/k_{non\text{-}imprinted}$, where $k_{imprinted}$ and $k_{non\text{-}imprinted}$ are the retention factors of a solute on molecularly imprinted and NIP, respectively. The selectivity factor S was used for evaluating the molecular recognition ability of MIPs for BPA.

2.6. MI-SPE method

Two hundred milligrams of dried particles (75–125 μm) was packed into a 6.0 ml polypropylene SPE column. The column was fitted with a stopcock and a reservoir at the bottom end and the top end, respectively. The MI-SPE column was rinsed with 10 ml of methanol and 10 ml of water. The water sample (100 ml) was adjusted to pH 2 by adding 1 M HCl and surrogate standard (BPA-$^{13}C_{12}$) was added. The sample was loaded onto the column filled with the polymer at a flow rate of approximately 10 ml min^{-1}. After the sample loading, the column was washed with 10 ml of acetonitrile/water (1:9, v/v) adjusted to pH 2 by adding 1 M HCl. Finally, the analyte was extracted three times with 5.0 ml of methanol, and the extracted sample was analyzed with the LC–MS system.

3. Results and discussion

3.1. Preparation and evaluation of NIP and MIPs

We prepared NIP, MIP, DMIP and IMIP by the suspension polymerization method using no template molecule, BPA, BP and BPA-d_{16}, respectively. Table 2 shows the retention factors

Table 1
Molar amounts of template molecules, functional monomer and cross-linker used for the preparation of NIP, MIP, DMIP and IMIP

Polymer	Template molecule		Functional monomer		Cross-linker	
	Type	Amount (mmol)	Type	Amount (mmol)	Type	Amount (mmol)
NIP	None	–	4-VP	6	EGDMA	30
MIP	BPA	1	4-VP	6	EGDMA	30
DMIP	BP	1	4-VP	6	EGDMA	30
IMIP	BPA-d_{16}	1	4-VP	6	EGDMA	30

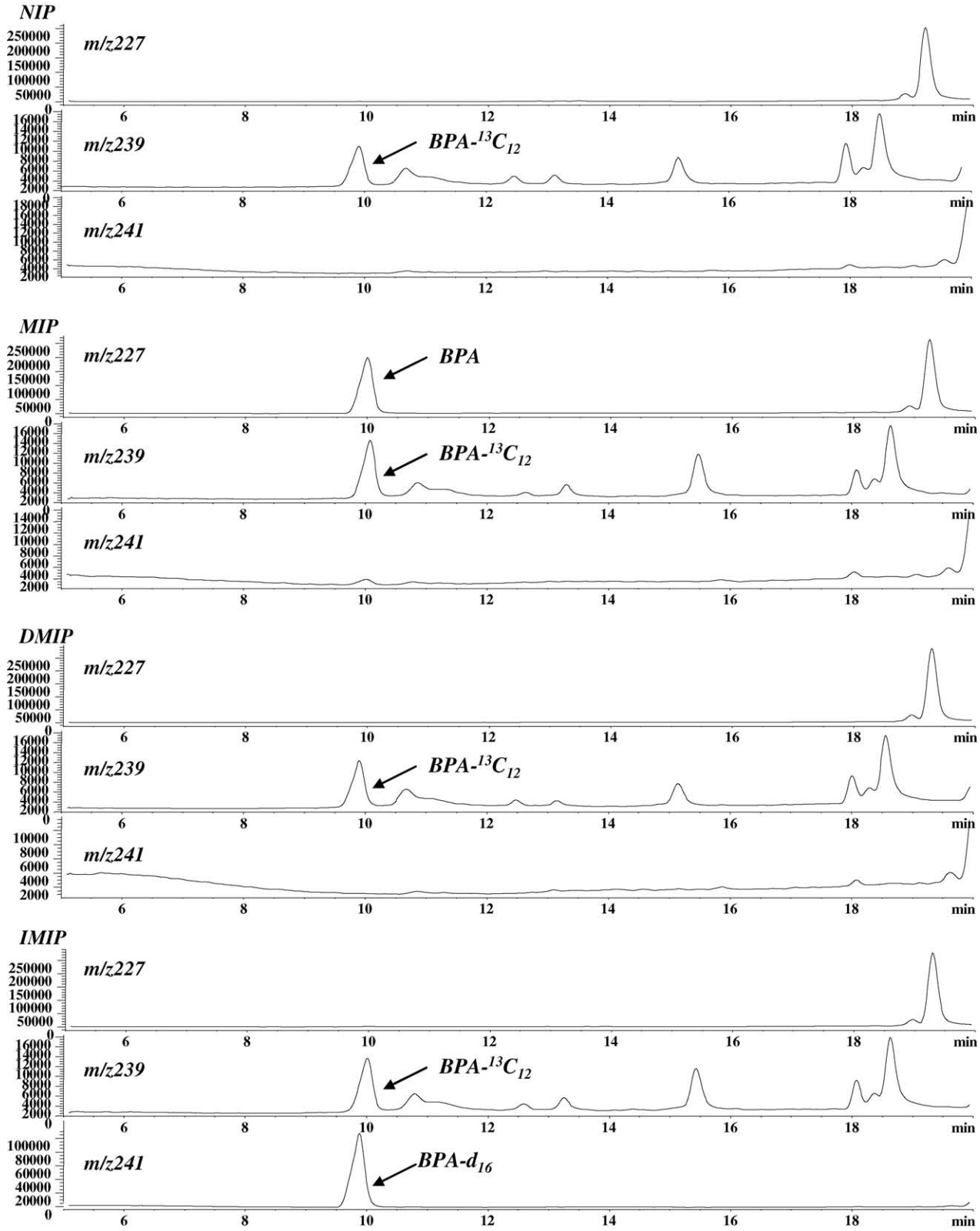

Fig. 2. LC–MS chromatograms of BPA, BPA-$^{13}C_{12}$ and BPA-d_{16} subjected to MI-SPE using NIP, MIP, DMIP and IMIP.

Table 2
Retention factors and selectivity factors for BPA on NIP, MIP, DMIP and IMIP

Polymer	BPA	
	$k_{imprinted}$	S^a
NIP	0.33	1.00
MIP	1.47	4.45
DMIP	0.70	2.12
IMIP	1.46	4.43

[a] S is the selectivity factor, $k_{imprinted}/k_{non\text{-}imprinted}$.

and the selectivity factors for BPA of the respective polymers. The difference in selectivity factor between MIP and DMIP was ascribable to the difference in the number of interaction sites. The two phenol groups of BPA might interact with the two pyridyl groups of MIP by forming hydrogen bonds. On the other hand, there is only one such interaction site for BP. Moreover, there was almost no difference in selectivity factor between MIP and IMIP. Therefore, IMIP has the same molecular recognition ability as MIP.

Most of the hitherto reported MIPs have been prepared as bulk blocks; therefore, they require tedious and time-consuming procedures prior to column packing, such as

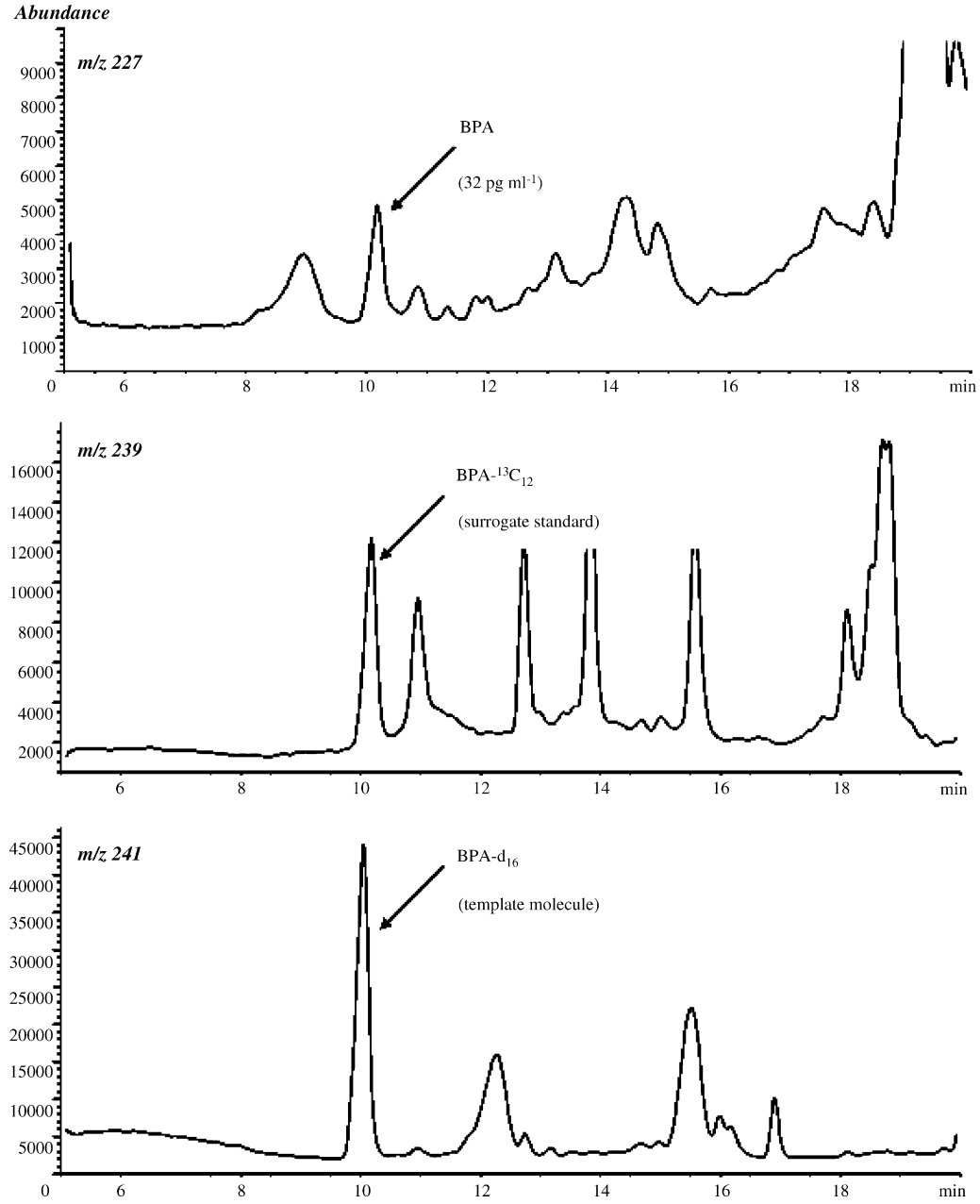

Fig. 3. SIM chromatograms of BPA, BPA-$^{13}C_{12}$ and BPA-d_{16} in river water sample subjected to MI-SPE using IMIP by LC–MS.

grinding and sieving. Furthermore, the resultant particles are not optimal for column use because of irregularities in size and shape. In this study, we performed molecular imprinting with the suspension polymerization method to obtain a bead-shaped synthetic affinity-type sorbent that is ready for column packing.

3.2. Comparison of analytical figures of merit

BPA-$^{13}C_{12}$ (final concentration: 100 pg ml^{-1}) was added to 100 ml of water. Then, the added sample was prepared by MI-SPE using each of NIP, MIP, DMIP and IMIP. The extracted sample was evaporated completely with an evaporator and then adjusted with 1.0 ml of methanol. The obtained sample was analyzed by LC–MS. Typical chromatograms of the water samples containing BPA-$^{13}C_{12}$ (100 pg ml^{-1}) are shown in Fig. 2. In MI-SPE using MIP, leakage of BPA from the MIP was observed. Therefore, it was suggested that BPA concentration was overestimated by MI-SPE using MIP. On the other hand, in MI-SPE using DMIP or IMIP, no BPA was detected. Therefore, the MI-SPE using IMIP was superior to that using MIP.

The recovery and the repeatability precision of the method were assessed by replicate analysis ($n = 6$). The average recoveries were 75.3% (R.S.D.: 15.7%), 99.3% (R.S.D.: 14.3%), 85.2% (R.S.D.: 16.1%) and 98.9% (R.S.D.: 15.6%) by the MI-SPE method using NIP, MIP, DMIP and IMIP, respectively (Table 3). The reason to show the high recovery of NIP is thought to be a hydrophobic interaction. MIP and IMIP had the highest recoveries, followed by DMIP and NIP. Therefore, IMIP has the same molecular recognition ability as MIP.

3.3. Application of MI-SPE using IMIP for determination of BPA in river water sample

MI-SPE using IMIP was applied to the analysis of river water sample. The calculated detection limit (LOD) of BPA in the river water sample was 1 pg ml^{-1} by LC–MS when the ratio of the compound's signal to the background signal (S/N) was 3. In addition, the calculated limit of quantification (LOQ) when S/N > 10 was 5 pg ml^{-1} for BPA in the river water sample. The peak area ratios with respect to each surrogate standard (BPA-$^{13}C_{12}$) were plotted and the response was found to be linear over the calibration range with correlation coefficients (r) higher than 0.99. The recovery and the precision of the method were assessed by replicate analysis ($n = 6$) of river samples spiked with the surrogate standard at 100 pg ml^{-1}. Non-spiked and spiked samples were subjected to LC–MS with MI-SPE, and the recoveries were calculated by subtracting the results for the non-spiked samples from those for the spiked samples. The results were obtained by using internal standard calibration curve with surrogate standard. The average recovery was higher than 99.8% (R.S.D.: 3.7%) for the river water samples. Therefore, the method enables the precise determination of standards and can be applied to the determination of trace amounts of BPA in river water samples.

Table 3
Recoveries of BPA-$^{13}C_{12}$ in spiked water samples

Polymer	BPA-$^{13}C_{12}$	
	Recovery (%)	R.S.D. (%)[a]
NIP	75.3	15.7
MIP	99.3	14.3
DMIP	85.2	16.1
IMIP	98.9	15.6

[a] The recovery and precision were also examined by replicate analysis ($n = 6$) of water samples.

River water sample was analyzed using the present method. The concentration of BPA in the river water sample was determined to be 32 pg ml^{-1}. Typical chromatograms of the river water sample are shown in Fig. 3. We already reported that BPA concentrations of 39–47 pg ml^{-1} in river water samples were detected by SBSE–TD-GC–MS [22]. That range is almost the same as that obtained by present method. It was therefore possible to measure trace amounts of a target analyte in river water samples by MI-SPE using IMIP.

4. Conclusion

A MIP that uses a stable isotope labeled compound as the template molecule, the so-called IMIP, was developed by suspension polymerization. The IMIP was applied to MI-SPE and the extracted sample was measured by LC–MS. Although the leakage of BPA-d_{16} from the IMIP was observed and that of BPA was not observed. On the other hand, leakage of BPA was observed by MI-SPE using MIP which was used BPA as template. The selectivity factors of MIP and IMIP for BPA were 4.45 and 4.43, respectively. Therefore, IMIP was found to have the same molecular recognition ability as MIP. When MI-SPE with IMIP was used and followed by LC–MS in the analysis of river water sample, detection limit of BPA was 1 ppt with high sensitivity. Moreover, the average recovery was higher than 99.8% (R.S.D.: 3.7%) by using bisphenol A-$^{13}C_{12}$ (BPA-$^{13}C_{12}$) as the surrogate standard. In addition, the IMIP were employed in MI-SPE of BPA in river water sample by LC–MS. The concentration of BPA in the river water sample was determined to be 32 pg ml^{-1}. The principle of IMIP is expected to be applicable to other analytes as well. It is now possible to determine trace amounts of a target analyte by LC–MS in combination with MI-SPE using IMIP. The number of reports of MI-SPE using IMIP is expected to increase in the near future.

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