Quantification of Brominated Polycyclic Aromatic Hydrocarbons in Environmental Samples by Liquid Chromatography Tandem Mass Spectrometry with Atmospheric Pressure Photoionization and Post-column Infusion of Dopant

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A sensitive method for the quantification of brominated polycyclic aromatic hydrocarbons (BrPAHs) in environmental samples is yet to be developed. Here, we optimized the analytical conditions for liquid chromatography tandem mass spectrometry with atmospheric pressure photoionization and post-column infusion of dopant (LC-DA-APPI-MS/MS). We then compared the sensitivity of our developed method with that of conventional gas chromatography high-resolution MS (GC-HRMS) by comparing the limits of quantification (LOQs) for a range of BrPAHs. Finally, to evaluate our developed method, 12 BrPAHs in sediments and fish collected from Tokyo Bay, Japan, were analyzed; 9 common PAHs were also targeted. The LOQs of the developed analytical method were 14 – 160 times lower than those of GC-HRMS for the targeted BrPAHs. The developed analytical method is a sensitive approach for determining the concentrations of BrPAHs in sediment and fish samples.

Keywords Polycyclic aromatic hydrocarbons, brominated polycyclic aromatic hydrocarbons, LC-MS/MS, APPI, dopant

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Introduction

Brominated or chlorinated polycyclic aromatic hydrocarbons (Br/ClPAHs) are compounds in which one or more hydrogen atoms have been substituted with bromine or chlorine atoms. These halogenated PAHs are structurally similar to dioxins (*e.g.*, polyhalogenated dibenzo-*p*-dioxins, dibenzofurans, and polyhalogenated biphenyls), and some have higher carcinogenicity and mutagenicity than their parent PAHs.[1] Like PAHs,[2-5] halogenated PAHs have been ubiquitously detected in environmental samples.[6-12] Ohura *et al.*[13] reported that the total concentration (sum of individual concentrations) of 11 BrPAHs and 20 ClPAHs in surface sediment collected from the Yellow Sea was in the range of 5.5 – 250 and 290 – 1200 ng g^{-1}, respectively. Jin *et al.*[14] reported that the total concentration of 19 BrPAHs and 19 ClPAHs in haze in China was 24 and 260 pg m^{-3}, respectively. The total toxic equivalency concentration, calculated based on the toxicity relative to benzo[*a*]pyrene (BaP), of 20 ClPAHs (36 – 1210 pg g^{-1}) was higher than that of polychlorinated dibenzo-*p*-dioxins (0.039 – 29 ng g^{-1}) and dibenzofurans (0.034 – 5.5 ng g^{-1}) in sediment cores collected from Tokyo Bay, Japan.[15,16] Together, these findings suggest that halogenated PAHs are emerging pollutants.

Currently, the prevalences of BrPAHs in the environment are much less understood than those of ClPAHs. One reason for this is the lack of a suitable analytical method for determining the concentrations of BrPAHs in environmental samples. Although gas chromatography–mass spectrometry (GC-MS) is traditionally used for the analysis of PAHs, and of halogenated PAHs in general, high-molecular-weight compounds undergoing GC analysis can thermally decompose and adsorb onto the GC inlet and column.[17] In addition, the high molecular weight of bromine and the weakness of the carbon–bromine bond could result in higher limits of quantification (LOQ) for BrPAHs compared with ClPAHs. An alternative approach for the analysis of polar organic compounds is liquid chromatography–mass spectrometry (LC-MS)[18-21] with electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) for ionization of the target compounds; however, the low polarity of PAHs means that they cannot be ionized by ESI and APCI.[22] Thus, a sensitive method for the determination of BrPAH concentrations in environmental samples is needed.

Atmospheric pressure photoionization (APPI) is a soft ionization technique that can ionize molecules that cannot be ionized by ESI or APCI.[23] In APPI, ultraviolet light emitted from a krypton lamp photochemically ionizes target compounds or dopants added to indirectly improve the ionization of the target compounds *via* subsequent gas-phase reactions.[24] For the analysis of PAHs, toluene and anisole are suitable dopants that can be introduced into the sample stream to create a source of charge carriers that then react with neutral target molecules *via* proton transfer and charge-exchange reactions.[25] Itoh *et al.*[25] have reported that a 99.5:0.5 (v/v) toluene/anisole mixture reduces the limit of detection for PAHs by 3.8 – 40 times. When coupled with liquid chromatography tandem mass spectrometry

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Table 1 Polycyclic aromatic hydrocarbons (PAHs) and brominated PAHs (BrPAHs) targeted in the present study

	Compound	Abbreviation
BrPAHs	2-Bromofluorene	2-BrFle
	9-Bromofluorene	9-BrFle
	2-Bromoanthracene	2-BrAnt
	1-Bromoanthracene	1-BrAnt
	9-Bromophenantherene	9-BrPhe
	9-Bromoanthracene	9-BrAnt
	3-Bromofluoranthene	3-BrFlu
	1-Bromopyrene	1-BrPyr
	4-Bromopyrene	4-BrPyr
	7-Bromobenz[*a*]anthracene	7-BrBaA
	2,7-Dibromofluorene	2,7-Br_2Fle
	1,4-Dibromophenantherene	1,4-Br_2Phe
	2,4-Dibromoanthracene	2,4-Br_2Ant
	1,5-Dibromoanthracene	1,5-Br_2Ant
	9,10-Dibromoanthracene	9,10-Br_2Ant
	1,6-Dibromopyrene	1,6-Br_2Pyr
	1,8-Dibromopyrene	1,8-Br_2Pyr
PAHs	Fluorene	Fle
	Phenanthrene	Phe
	Anthracene	Ant
	Fluoranthene	Flu
	Pyrene	Pyr
	Benz[*a*]anthracene	BaA
	Chrysene	Chr
	Benzo[*b*]fluoranthene	BbF
	Benzo[*a*]pyrene	BaP

(LC-MS/MS), APPI provides increased sensitivity for high-molecular-weight PAHs compared with conventional ionization methods.[26] Furthermore, the mass spectra obtained using APPI are much simpler to interpret, both for compound identification and quantification, compared with the spectra obtained using conventional ionization methods.[27] Although APPI has the potential to become the standard ionization method for the analysis of PAHs, the optimal APPI conditions for the analysis of BrPAHs are currently unknown.

Here, we optimized the APPI conditions for the analysis of BrPAHs by LC-MS/MS with post-column dopant infusion (LC-DA-APPI-MS/MS). We then compared the LOQs obtained using our approach with those obtained by GC–high-resolution MS (GC-HRMS), which is the current standard method for the analysis of halogenated PAHs. Finally, we used our approach to evaluate the concentrations of 12 BrPAHs in real-world marine environmental samples.

Experimental

Chemicals and materials

In the present study, 17 BrPAHs and 9 PAHs were targeted (Table 1). Analytical standards for the target compounds were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) or Sigma-Aldrich (St. Louis, MO, USA), or were prepared by organic synthesis in our laboratory. Isotope-labeled phenanthrene-$^{13}C_6$, fluoranthene-$^{13}C_6$, chrysene-$^{13}C_6$, and bromobenz[*a*]anthracene-$^{13}C_6$ as recovery standards and fluoranthene-d_{10} as an internal standard were obtained from Cambridge Isotope Laboratories (Andover, MA, USA). Hexane, and dichloromethane (residual-pesticide-analysis grade) for extraction and clean-up were obtained from Wako Pure Chemical (Osaka, Japan). A 50-mL KOH silica gel column filled with 12 g of 2% KOH silica gel (dioxin-analysis grade) was obtained from Wako Pure Chemical. An activated-carbon cartridge (Carboxen 1016, 200 mg) was obtained from Supelco (St. Louis, MO, USA). Reduced copper was obtained from Wako Pure Chemical.

To evaluate our approach for the analysis of BrPAHs in real-world environmental samples, sediment and fish samples (*Lateolabrax japonicus* and *Pseudopleuronectes yokohamae*) were collected from 10 stations in Tokyo Bay, Japan, in 2016 – 2017; the samples were stored in the dark at –18°C until analysis.

Clean-up procedure

The BrPAHs and PAHs in the samples were extracted by Soxhlet extraction for 16 h using 250 mL of dichloromethane spiked with the recovery standards. The determinations of BrPAHs and PAHs in the extracts were performed according to an established method.[28,29] The silica-gel column with reduced copper (5 g) was washed with 20 mL of 10% dichloromethane/hexane, and the activated carbon cartridge was washed with 120 mL of toluene. The column and cartridge were connected in series, and the sample extract was loaded onto the column. After loading the extract, the column was washed with 20 mL of 10% dichloromethane/hexane. After washing, the cartridge was removed and back-flushed with 120 mL of toluene to extract the target compounds. The collected toluene fraction containing the target compounds was spiked with the internal standard and concentrated to 100 μL for further analysis.

Analytical methods for LC-MS/MS

A liquid chromatograph (Thermo Fisher Scientific Vanquish, Thermo Scientific Inc., Waltham, MA, USA) coupled to a tandem mass spectrometer (TSQ Quantiva, Thermo Scientific Inc.) was used to determine the concentrations of PAHs. A Fusion 101 syringe pump (Chemyx, Stafford, TX, USA) was integrated into the system for post-column dopant infusion. A Fusion 101 syringe pump was also used for the injection of standard solutions used to optimize the MS parameters, such as quantitative ion, reference ion, collision energy, and lens settings, using the Xcalibur data acquisition (Ver. 1.3, Thermo Scientific Inc.) and interpretation software (Ver. 4.2, TraceFinder, Thermo Scientific Inc.). Standard solutions were diluted to 1.0 μg mL^{-1} with a solvent mixture containing methanol, water, toluene, and anisole (80:10:5:5, v/v) and injected into the MS system at 10 – 20 μL min^{-1}. The positive ion mode was used. The Q1 and Q3 resolutions were both 0.7 Da. The optimal MS parameters for each compound were determined individually. Sheath gas (arbitrary unit), auxiliary gas (arbitrary unit), and sweep gas (arbitrary unit) were optimized by manual adjustment to 50, 15, and 0, respectively. The temperature of the ion transfer tube and vaporizer was optimized in the present study. Toluene, anisole, and acetone were used as dopants. The dopant injection volume was 2 μL min^{-1}.

A Poroshell 120 SB-C18 column (2.1 mm i.d. × 150 mm length, 2.7 μm, Agilent Technologies Japan, Ltd., Tokyo, Japan) was used for the separation in the LC. The flow rate was 0.25 mL min^{-1}. Water (Eluent A), and methanol (Eluent B) were used as the mobile phases. The gradient program was as follows: isocratic at 50% Eluent B for 3 min, 50 to 100% Eluent B in 10 min, isocratic at 100% Eluent B for 4 min, 100 to 50% Eluent B in 0.1 min, and then isocratic at 50% Eluent B for 3 min. The column temperature was kept at 50°C.

The MS/MS was operated under the selected reaction monitoring (SRM) mode. Peaks were identified by comparing the retention times of samples with those of the standards if the signal-to-noise ratio was >3, and they were quantified if the target/qualifier ion ratio was within 15% of the theoretical value.

Table 2 Mass spectrometry parameters and method performances for liquid chromatography tandem mass spectrometry analysis

Compound	Polarity	Quantitative ion (m/z)	Reference ion (m/z)	Collision energy/V (for quantitative ion)	Collision energy/V (for reference ion)	Correlation coefficient for calibration curve	Intermediate precision (n = 10)	
							Retention time, %RSD[a]	Peak area of quantifier ion, %RSD[a]
2-BrFle/9-BrFle	Positive	244 > 165	246 > 165	24	23	0.999	0.032	5.6
2-BrAnt	Positive	256 > 176	258 > 176	41	40	0.998	0.036	6.9
1-BrAnt/9-BrPhe	Positive	256 > 176	258 > 176	41	40	1.00	0.12	13
9-BrAnt	Positive	256 > 176	258 > 176	41	40	0.997	0.036	10
3-BrFlu	Positive	282 > 201	280 > 201	38	38	0.998	0	14
1-BrPyr	Positive	282 > 201	280 > 201	38	38	0.999	0.090	11
4-BrPyr	Positive	282 > 201	280 > 201	38	38	0.996	0	11
7-BrBaA	Positive	306 > 226	308 > 226	55	45	0.997	0.055	11
2,7-Br_2Fle	Positive	324 > 243	326 > 245	24	23	1.000	0.070	5.9
1,4-Br_2Phe/2,4-Br_2Ant	Positive	336 > 176	338 > 176	45	44	0.997	0	15
1,5-Br_2Ant	Positive	336 > 176	338 > 176	45	44	0.998	0.066	15
9,10-Br_2Ant	Positive	336 > 176	338 > 176	45	44	0.997	0.063	11
1,6-Br_2Pyr/1,8-Br_2Pyr	Positive	360 > 200	362 > 200	55	55	0.999	0.066	13
Fle	Positive	166 > 163	166 > 115	51	46	0.999	0.084	32
Phe	Positive	178 > 152	178 > 176	34	41	0.998	0.041	15
Ant	Positive	178 > 152	178 > 176	34	41	1.00	0.040	20
Flu	Positive	202 > 200	202 > 151	50	55	1.00	0	37
Pyr	Positive	202 > 200	202 > 151	50	55	1.00	0.066	37
BaA	Positive	228 > 226	228 > 200	44	55	0.999	0.099	8.5
Chr	Positive	228 > 226	228 > 200	44	55	1.00	0.023	16
BbF	Positive	253 > 250	253 > 225	55	55	1.00	0	14
BaP	Positive	253 > 250	253 > 225	55	55	0.999	0.034	7.7
^{13}C-Phe	Positive	184 > 158	184 > 157	33	33		0.088	8.2
^{13}C-Flu	Positive	208 > 206	208 > 207	48	48		0	10
^{13}C-Chr	Positive	234 > 232	234 > 206	44	41		0.038	13
^{13}C-BrBaA	Positive	312 > 232	314 > 232	47	45		0.043	10
Flu-d	Positive	212 > 208	212 > 210	52	39		0	12

a. RSD: Relative standard deviation.

Analytical methods for GC-HRMS

A GC-HRMS (JMS-700, JEOL, Tokyo, Japan) was used to provide data for comparing with our developed approach. Gas chromatographic separation was accomplished with a 60-m BPX-DXN fused silica capillary column (0.25 mm i.d., Kanto Chemical Co., Inc., Tokyo, Japan). A 2-μL aliquot of the sample was injected to the system in the splitless mode at 280°C. The temperature of the column oven was kept at 130°C for 1 min, raised at a rate of 5°C min^{-1} to 250°C, raised at a rate of 10°C min^{-1} to 320°C, and then held for 18 min. The MS was operated in the electron-impact selected ion monitoring mode at a resolution >10000 (10% valley). Peaks were identified by comparing the retention times of samples to those of standards if the signal-to-noise radio was >3, and they were quantified if the target/qualifier ion ratio was within 15% of the theoretical value.

Results and Discussion

Analytical method optimization

Table 2 shows the quantitative and reference ion transitions and collision energies determined in the present study. The most abundant ion transition was selected for quantification. For the PAHs examined, the transition from the molecular cation $[M]^+$ to the $[M-2]^+$ ion resulted in higher signal intensities for fluorene (Fle) (166 > 163), fluoranthene (Flu) (202 > 200), pyrene (Pyr) (202 > 200), benz[*a*]anthracene (BaA) (228 > 226), and chrysene (Chr) (228 > 226). The transition of phenanthrene (Phe) was $[M]^+$ to $[M-26]^+$. $[M+H]^+$ was monitored as the parent ion in the benzo[*b*]fluoranthene (BbF) (253 > 250) and BaP (253 > 250) analyses. Previously, in their analysis using LC-DA-APPI-MS/MS, Hollosi and Wenzl[22] used the same ion transitions for BaA and Chr as those determined in this study, but they used different transitions for BbF (252 > 250) and BaP (252 > 250). Hutzler *et al.*[26] also used similar ion transitions for BaP (253 > 252) and BaA (228 > 226) in LC-DA-APPI-MS analysis. The ion transitions from both of these studies were in good agreement with those determined in the present study.

For the BrPAHs, the quantitative ion transitions of BrFle (*i.e.*, 2-bromofluorene/9-bromofluorene), BrPhe/Ant (2-bromoanthracene, 1-bromoanthracene/9-bromophenantherene, and 9-bromoanthracene), BrFlu/Pyr (3-bromofluoranthene, 1-bromopyrene, and 4-bromopyrene), BrBaA (7-bromobenz[*a*]-anthracene), and Br_2Fle (2,7-dibromofluorene) were 244 > 165, 256 > 176, 282 > 201, 306 > 226, and 324 > 243, respectively. The implication is that these quantitative ions were generated by debromination of the parent molecule ($[M-Br]^+$). The quantitative ion transitions of Br_2Phe/Ant (1,4-dibromophenantherene/2,4-dibromoanthracene, 1,5-dibromoanthracene, and 9,10-dibromoanthracene) and Br_2Pyr (1,6-dibromopyrene/1,8-dibromopyrene) were 336 > 176 and 360 > 200, respectively. The implication is that these quantitative ions were also generated by debromination of the parent ion $[M-2Br]^+$. Moukas *et al.*[30] measured polybrominated diphenyl ethers and brominated diphenyl ether (BDE) using LC-APPI-MS/MS. Diphenyl ethers with four to six bromine atoms were ionized in negative ion mode with parent ions corresponding to $[M–Br+O]^-$. Diphenyl ether with three bromine atoms (BDE 28) was ionized in the positive ion mode to generate the precursor ion $[M]^+$. The ion transitions of the BrPAHs determined in the present study were markedly different from those reported for the BDEs.

In the GC-MS/MS analysis, the fact that the ion transitions of BrFle, BrPhe/Ant, BrPyr, and BrBaA were 165 > 115, 256 > 176, 282 > 200, and 308 > 226, respectively, indicated that debromination (*i.e.*, $[M–Br]^+$ was converted to $[M]^+$).[31] The present results are consistent with the report by Ohura *et al.*[1] that certain BrPAHs can be photodegraded and debrominated. The ion transitions of the BrPAHs were similar between GC-MS/MS and LC-DA-APPI-MS/MS.

The effect of the ion source temperature (range, 240 – 320°C) on the BrPAH peak intensity is shown in Fig. 1. The signal intensities of three- and four-ring compounds with low molecular weight, such as 2-BrFle/9-BrFle, 2,7-Br_2Fle, 2-BrAnt, and 3-BrFlu, were 2 – 3 times larger at the lowest ion source temperature (240°C) than at the highest ion source temperature (320°C).

We also examined three commonly used dopants (toluene, anisole, and acetone) for their ability to increase the sensitivity of LC-APPI-MS/MS for the analysis of BrPAHs.[22,24,25] The effects of the dopant type on the signal intensity are shown in Fig. 2. The relative peak intensity was calculated as a value with respect to the signal of 100% anisole dopant. Although using toluene or acetone alone as the dopant did little to increase the signal intensity (data not shown), using a 1% or 50% mixture of anisole in toluene or using 100% anisole increased the signal intensity. Itoh *et al.*[25] and Smith *et al.*[32] have also reported that anisole is suitable for the analysis of PAHs using LC-DA-APPI-MS. These results are in reasonably good agreement with the present results.

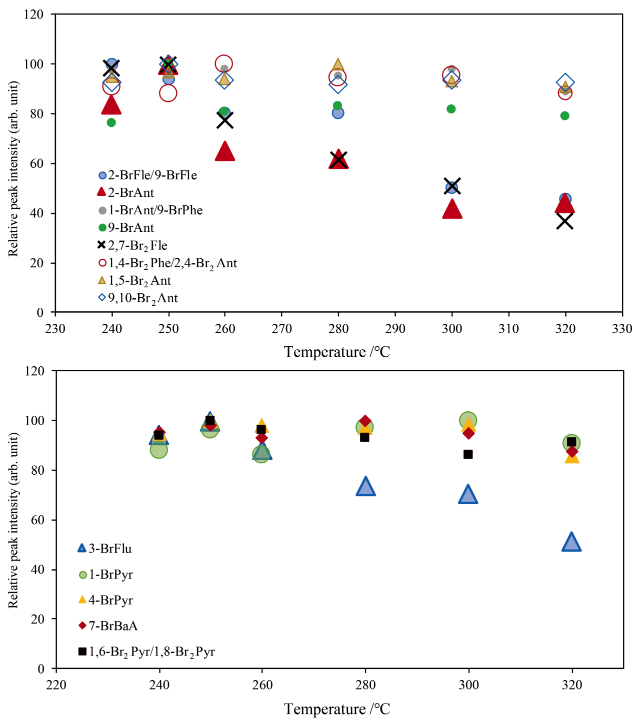

Fig. 1 Effect of the ion source temperature on the peak intensities for brominated polycyclic aromatic hydrocarbons.

Sensitivity of LC-DA-APPI-MS/MS vs. GC-HRMS

The LOQs of our analytical method were compared with those of GC-HRMS, which is the conventional approach for analyzing halogenated PAHs (Fig. 3). For the BrPAHs, the LOQs of our LC-DA-APPI-MS/MS approach were 14 – 160 times lower than those of GC-HRMS (*e.g.*, BrFle (14 times lower), BrAnt (28 – 49 times), BrPyr (130 – 160 times), BrFlu (76 times) and BrBaA (23 times)).

Comparing the LOQs of our approach for BrPAHs and their parent PAHs, the LOQs of BrAnt (150 – 190 times lower), BrFlu (210 times), and BrPyr (220 – 240 times) were significantly lower than those of the parent compounds. These results suggest that BrPAHs are more easily ionized than their parent compounds. This may be attributed to the fact that BrPAHs are more polar than PAHs. In addition, the fact that the dibrominated PAHs (*i.e.*, Br_2Fle, Br_2Phe, and Br_2Ant) had lower LOQs than the corresponding monobrominated PAHs (*i.e.*, BrFle, BrPhe, and BrAnt) suggested that BrPAHs containing a larger number of bromine atoms are more easily ionized. Adding bromine atoms to a PAH likely improves absorption of the light energy emitted by the krypton lamp of APPI and thereby improves the ionization of the compound.

BrPAHs and PAHs in sediment and fish samples

To evaluate the use of our LC-DA-APPI-MS/MS approach for determining the concentrations of BrPAHs and PAHs in real-world samples, samples of sediment and fish collected from Tokyo Bay, Japan, were examined.

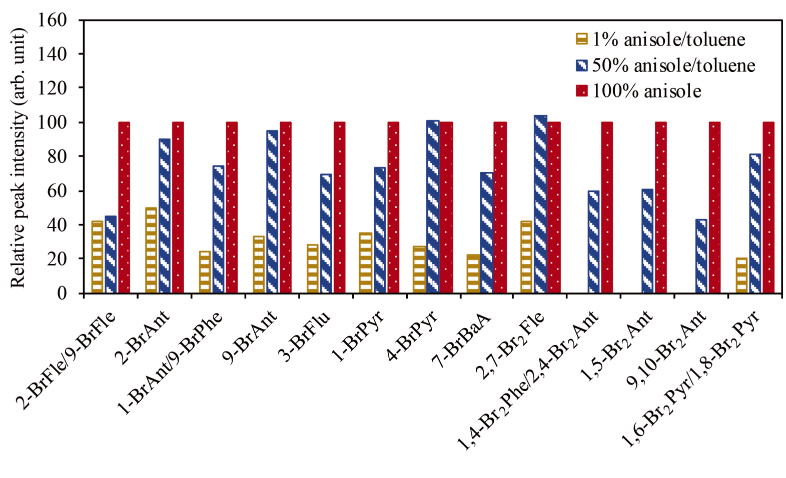

Fig. 2 Effects of the dopant on the peak intensities for brominated polycyclic aromatic hydrocarbons.

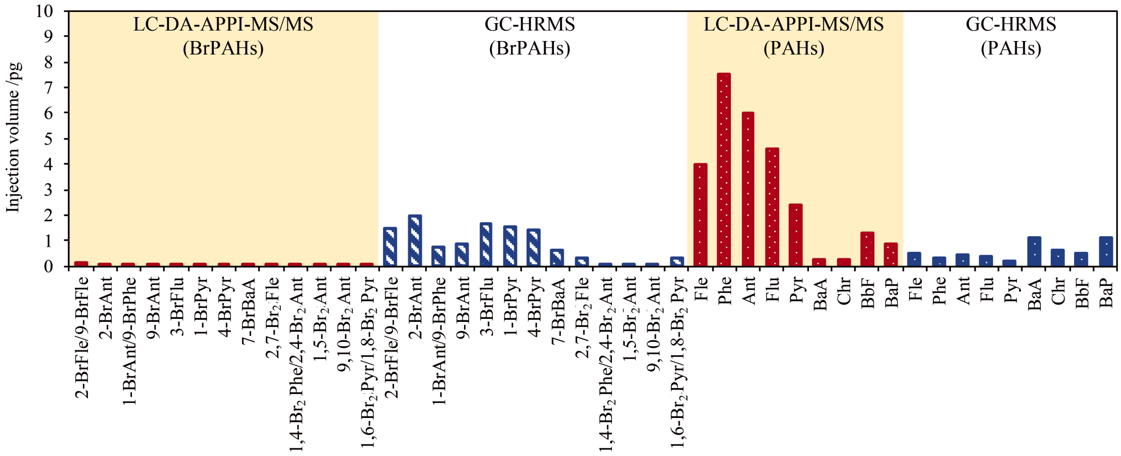

Fig. 3 Comparison of the sensitivities of the analysis of polycyclic aromatic hydrocarbons (PAHs) and brominated PAHs (BrPAHs) by liquid chromatography tandem mass spectrometery in the atmospheric pressure photoionization mode with a post-column infusion of the dopant (LC-DA-APPI-MS/MS) *versus* gas chromatography with high-resolution MS (GC-HRMS).

Table 3 Concentrations of brominated polycyclic aromatic hydrocarbons (BrPAHs) and PAHs in sediments collected from 10 stations in Tokyo Bay, Japan (ng g^{-1})

Compound	St1	St2	St3	St4	St5	St6	St7	St8	St9	St10	LOQ[a]
2-BrFle/9-BrFle	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.0011
2-BrAnt	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.00039
1-BrAnt/9-BrPhe	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.00017
9-BrAnt	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.00032
3-BrFlu	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.00022
1-BrPyr	0.042	0.035	0.020	0.026	0.031	0.012	0.020	0.029	0.031	0.015	0.00010
4-BrPyr	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.00011
7-BrBaA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.00027
2,7-Br2Fle	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.00023
1,4-Br_2Phe/2,4-Br_2Ant	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.00008
1,5-Br_2Ant	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.00014
9,10-Br_2Ant	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.000090
1,6-Br_2Pyr/1,8-Br_2Pyr	0.054	0.068	0.048	0.049	0.057	0.044	0.053	0.064	0.051	0.043	0.000090
∑BrPAHs	0.096	0.103	0.068	0.075	0.088	0.056	0.073	0.093	0.082	0.058	
Fle	0.71	1.3	2.4	3.7	0.30	0.42	0.91	2.6	2.1	1.9	0.040
Phe	19	40	72	81	32	22	47	77	73	53	0.075
Ant	14	24	37	33	19	17	27	38	37	27	0.060
Flu	110	120	200	190	160	130	190	220	230	170	0.046
Pyr	130	160	200	210	180	130	200	220	240	150	0.024
BaA	60	58	78	8.0	100	55	61	86	97	43	0.0026
Chr	46	52	71	73	60	54	86	82	88	71	0.0025
BbF	200	270	280	250	300	230	240	280	290	170	0.013
BaP	130	130	140	160	160	130	170	190	200	130	0.0090
∑PAHs	710	860	1100	1000	1000	770	1000	1200	1300	820	

a. LOQ: Limit of quantification.

Quality assurance and quality control data for our analytical method are given in Table 2. The calibration curves for the BrPAHs and PAHs were linear over the ranges of 0.3 – 10 ng mL^{-1} (0.3, 1, 3, 10 ng mL^{-1}) ($r > 0.996$) and 1 – 1000 ng mL^{-1} (1, 3, 10, 100, 300, and 1000 ng mL^{-1}) ($r > 0.998$), respectively. The LOQs of BrPAHs and PAHs in sediment and fish samples were calculated as 3 times the standard deviation from 5 injections of blank level samples that had a signal-to-noise ratio of 3 – 10. Relative standard deviations for the retention times for each peak and the peak area of quantifier ions in the intermediate precision test ($n = 10$) were within 0.04% (median) (range, 0 – 0.12%) and 12% (5.6 – 37%), respectively. The average accuracies were 96% (median) (range, 73 – 130%). The LOQs for the BrPAHs and PAHs in the sediment and fish samples are listed in Tables 3 and 4, respectively. The recoveries of the recovery standards spiked into each of the sediment samples were 51 – 79% (phenanthrene-$^{13}C_6$), 67 – 120% (fluoranthene-$^{13}C_6$), 86 – 110% (chrysene-$^{13}C_6$), and 73 – 110% (bromobenz[*a*]-anthracene-$^{13}C_6$); those in the fish samples were 75 – 92% (phenanthrene-$^{13}C_6$), 86 – 90% (fluoranthene-$^{13}C_6$), 68 – 75% (chrysene-$^{13}C_6$), and 82 – 83% (bromobenz[*a*]anthracene-$^{13}C_6$).

Figure 4 shows LC-DA-APPI-MS/MS chromatograms for BrPyr and Br_2Pyr in sediment collected from Tokyo Bay. Conventional GC-HRMS was unable to detect BrPAHs in the

Table 4 Concentrations of brominated polycyclic aromatic hydrocarbons (BrPAHs) and PAHs in fish collected from Tokyo Bay, Japan (ng g^{-1})

Compound	Fish species: *Lateolabrax japonicus*	Fish species: *Pseudopleuronectes yokohamae*	LOQ[a]
2-BrFle/9-BrFle	<LOQ	<LOQ	0.0026
2-BrAnt	<LOQ	<LOQ	0.0010
1-BrAnt/9-BrPhe	<LOQ	<LOQ	0.00042
9-BrAnt	<LOQ	<LOQ	0.00079
3-BrFlu	<LOQ	<LOQ	0.00054
1-BrPyr	<LOQ	<LOQ	0.00025
4-BrPyr	<LOQ	<LOQ	0.00027
7-BrBaA	<LOQ	<LOQ	0.00067
2,7-Br_2Fle	<LOQ	<LOQ	0.00056
1,4-Br_2Phe/2,4-Br_2Ant	<LOQ	<LOQ	0.00020
1,5-Br_2Ant	<LOQ	<LOQ	0.00035
9,10-Br_2Ant	<LOQ	<LOQ	0.00023
1,6-Br_2Pyr/1,8-Br_2Pyr	<LOQ	<LOQ	0.00023
∑BrPAHs	<LOQ	<LOQ	
Fle	1.5	<LOQ	0.10
Phe	17	1.2	0.19
Ant	1.5	0.73	0.15
Flu	0.80	<LOQ	0.12
Pyr	0.067	<LOQ	0.060
BaA	<LOQ	<LOQ	0.0065
Chr	<LOQ	<LOQ	0.0062
BbF	<LOQ	<LOQ	0.033
BaP	<LOQ	<LOQ	0.022
∑PAHs	21	1.9	

a. LOQ: Limit of quantification.

sediment samples, and so was considered to not be sensitive enough to determine the concentrations of BrPAHs in environmental samples (data not shown). The results indicated that our approach was sensitive enough to determine the concentrations of BrPAHs in environmental samples. The concentrations of the target BrPAHs and PAHs in sediments collected from Tokyo Bay are given in Table 3. The total concentration (sum of individual concentrations) of PAHs and BrPAHs in the sediments was in the range of 710 – 1300 and 0.056 – 0.010 ng g^{-1}, respectively. Horii *et al.*[15] reported that the concentrations of PAHs in sediments collected from Tokyo Bay were in the range of 110 – 1200 ng g^{-1}. Ohura *et al.*[13] reported that the respective concentrations of PAHs and BrPAHs in sediment were 170 – 4500 and 0.0055 – 0.25 ng g^{-1} (Yellow Sea); 350 – 1500 and 0.015 – 0.10 ng g^{-1} (Negombo, Sri Lanka); and 1400 – 3000 and 0.020 – 0.16 ng g^{-1} (Kandy, Sri Lanka). These previous results are reasonably consistent with the present results.

In the present study, the concentrations of Pyr were in the range of 130 – 240 ng g^{-1}, respectively, and the concentrations of 1-BrPyr and 1,6-/1,8-Br_2Pyr were in the range of 0.012 – 0.042 and 0.043 – 0.068 ng g^{-1}, respectively. The concentrations of Pyr were about 3100 – 10000 and 2400 – 4300 times higher than that of BrPyr and Br_2Pyr.

Table 4 shows the concentrations of BrPAHs and PAHs in fish from Tokyo Bay. The total concentration of PAHs in *Lateolabrax japonicus* (Japanese sea bass) and Pseudopleuronectes yokohamae (aka *Limanda yokohamae*; marbled flounder) was 21 and 1.9 ng g^{-1}, respectively. Among the PAHs, Fle (*P. yokohamae*: 1.5 ng g^{-1}; *L. japonicus*: <LOQ), Phe (17 and 1.2 ng g^{-1}), Ant (1.5 and 0.73 ng g^{-1}), Flu (0.80 ng g^{-1} and <LOQ), and Pyr (0.067 ng g^{-1} and <LOQ) were detected in the fish. Takeuchi *et al.*[33] reported that the concentrations of Phe, Ant, Flu, and Pyr in *Acanthogobius flavimanus* (yellowfin goby) collected from Tokyo Bay were 0.77, 0.11, 0.17, and 0.12 ng g^{-1}, respectively.

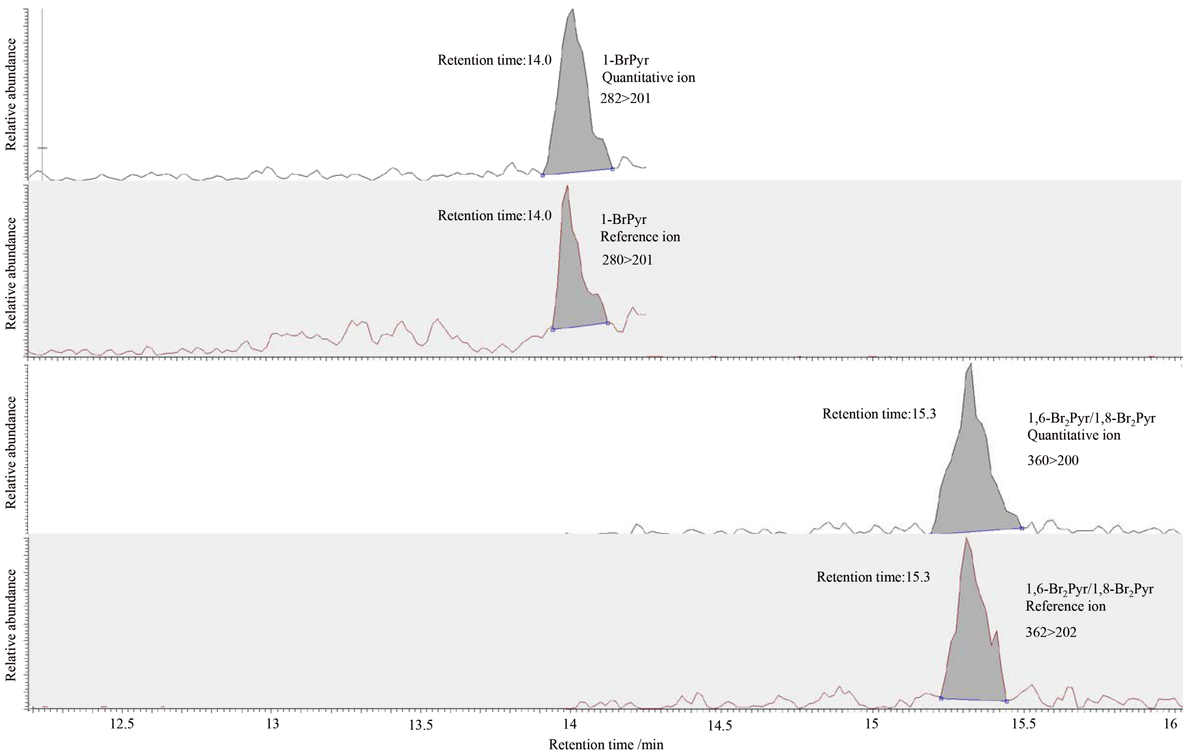

Fig. 4 Chromatograms of bromopyrene (BrPyr) and dibromoanthracene (Br_2Pyr) in sediment collected from Tokyo Bay.

Liang *et al.*[34] reported that the concentrations of Phe, Ant, Flu, and Pyr in the muscle tissues of tilapia (*Sarotherodon mossambicus*), a freshwater fish, collected from Mai Po Marshes (Hong Kong) were in the range of 16 – 23, 2.7 – 3.7, 9.9 – 13, and 8.2 – 11 ng g^{-1}, respectively. These previous studies show that PAH concentrations vary among different species. The concentrations of PAHs in the present study were most comparable with those reported by Takeuchi *et al.* in *A. flavimanus*.[33]

The concentrations of the target BrPAHs in the fish samples studied were lower than the LOQs. Wickrama-Arachchige *et al.*[35] reported that the concentrations of PAHs and BrPAHs in tuna fish species (three individuals of *Thunnus albacares* and three individuals of *Katsuwonus pelamis*) collected from the Indian Ocean near Sri Lanka were in the ranges of 238 – 2023 and 4.73 – 776 ng g^{-1}, respectively. These previously reported PAH concentrations are 100 times higher than those obtained in the present study, and the BrPAH concentrations are higher than the LOQs of our present analytical method. These inconsistent findings may be a result of differences in the concentrations and the accumulation pattern of PAHs in different fish species. The concentrations of BrPAHs in the environment should be lower than those of PAHs according to the formation mechanism of halogenated PAHs.[36,37] Bioconcentration of BrPAHs could be different in different fish species, and that in tuna could be higher than that in *L. japonicus* and P. yokohamae. The low LOQs of the present method for the detection of BrPAHs in fish samples suggests that the concentrations of BrPAHs in fish collected from Tokyo Bay are low. However, to obtain bioconcentration factors for BrPAHs, a more sensitive analytical method is required.

Conclusion

Here, we developed a sensitive analytical method for the quantification of BrPAHs using LC-DA-APPI-MS/MS. Our method was 100 times more sensitive at detecting BrPAHs compared with the corresponding PAHs, and was successfully used to determine the concentrations of BrPAHs and PAHs in sediment and fish samples.

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