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Environmental analysis of chlorinated and brominated polycyclic aromatic hydrocarbons by comprehensive two-dimensional gas chromatography coupled to high-resolution time-of-flight mass spectrometry

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ABSTRACT

A method for the analysis of chlorinated and brominated polycyclic aromatic hydrocarbon (Cl-/Br-PAHs) congeners in environmental samples, such as a soil extract, by comprehensive two-dimensional gas chromatography coupled to a high resolution time-of-flight mass spectrometry (GC × GC–HRTOF-MS) is described. The GC × GC–HRTOF-MS method allowed highly selective group type analysis in the two-dimensional (2D) mass chromatograms with a very narrow mass window (e.g. 0.02 Da), accurate mass measurements for the full mass range (*m/z* 35–600) in GC × GC mode, and the calculation of the elemental composition for the detected Cl-/Br-PAH congeners in the real-world sample. Thirty Cl-/Br-PAHs including higher chlorinated 10 PAHs (e.g. penta, hexa and hepta substitution) and ClBr-PAHs (without analytical standards) were identified with high probability in the soil extract. To our knowledge, highly chlorinated PAHs, such as $C_{14}H_3Cl_7$ and $C_{16}H_3Cl_7$, and ClBr-PAHs, such as $C_{14}H_7Cl_2Br$ and $C_{16}H_8ClBr$, were found in the environmental samples for the first time. Other organohalogen compounds; e.g. polychlorinated biphenyls (PCBs), polychlorinated naphthalenes (PCNs), and polychlorinated dibenzofurans (PCDFs) were also detected. This technique provides exhaustive analysis and powerful identification for the unknown and unconfirmed Cl-/Br-PAH congeners in environmental samples.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs); some of them known to be carcinogenic or mutagenic, as well as polychlorinated-dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) are organic pollutants largely produced in the combustion of organic compounds. Chlorinated or brominated PAHs (Cl-/Br-PAHs) are compounds with one or more chlorines or bromines added to the PAHs. In past decades, Cl-/Br-PAHs have been detected in environmental samples such as fly ash [1], urban air [2], snow [3], automobile exhaust [4], kraft pulp mill wastes [5,6] and sediment [7,8]. However, analytical methods documented in most research papers were not focused on the analysis of Cl-/Br-PAH congeners [3–7], for reasons including the lack of individual and purified analytical standards. Therefore, information about Cl-/Br-PAH congeners in the environment has been limited.

Recently, toxicities of Cl-PAHs have been investigated and reported on by several groups [9–11]. In 2009, the potencies of 19 individual Cl-PAHs and 11 individual Br-PAHs in inducing aryl hydrocarbon receptor (AhR)-mediated activities, relative to the potency of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), were determined in vitro by use of a recombinant rathepatoma cell (H4IIE-*luc*) assay by Horii et al. [11]. They indicated that several Cl-PAHs induced AhR-mediated activity, and also a structure–activity relationship for AhR mediated potencies of Cl-PAHs. The relative potencies of lower-molecular-weight Cl-PAHs, such as chlorophenanthrene and chlorofluoranthene, tended to increase with increasing chlorination of the compounds. Their study indicated that we have to understand the occurrence and toxicity of not only reported Cl-PAHs but also unconfirmed highly chlorinated PAHs to know precisely the risk of human exposure to Cl-PAHs.

For the analysis of Cl-/Br-PAHs, GC coupled with quadrupole mass spectrometer (GC–QMS) or a high resolution mass spectrometer (GC–HRMS) in selected ion monitoring (SIM) mode, has been used. Horii et al. have indicated the existence of highly substituted Cl-PAHs, which have no analytical standards, in the fly ash samples from the results of GC–QMS analysis based on monitoring of molecular ions and the isotope ions (M, $(M+2)^+$, or $(M+4)^+$). However, the information from SIM with GC–QMS was very limited for the posi-

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Table 1
Abbreviations of Cl-/Br-PAH standards and analytical performance of GC × GC–HRTOFMS.

	Compounds	Formula	Abbreviation	*m/z*	Linearity (r^2)	Range (pg)	Repeatability[a] (RSD %, n = 6)	LOD (pg)[b]
1	9-Monochlorofluorene	$C_{13}H_9Cl$	9-ClFle	200.0394	0.9974	0.5–40	22	0.44
2	9-Monochlorophenanthrene	$C_{14}H_9Cl$	9-ClPhe	212.0393	0.9981	0.1–10	15	0.39
3	2-Monochloroanthracene	$C_{14}H_9Cl$	2-ClAnt	212.0393	Σ = 0.9973	Σ = 0.1–10	Σ = 5.0	Σ = 0.08
4	9-Monochloroanthracene	$C_{14}H_9Cl$	9-ClAnt	212.0393				
5	3,9-Dichlorophenanthrene	$C_{14}H_8Cl_2$	3,9-Cl_2Phe	246.0003	0.9999	0.5–40	15	0.24
6	9,10-Dichlorophenanthrene	$C_{14}H_8Cl_2$	9,10-Cl_2Ant	246.0003	Σ = 0.9993	Σ = 0.1–10	Σ = 11	Σ = 0.22
7	1,9-Dichlorophenanthrene	$C_{14}H_8Cl_2$	1,9-Cl_2Phe	246.0003				
8	9,10 Dichlorophenanthrene	$C_{14}H_8Cl_2$	9,10-Cl_2Phe	246.0003	0.9977	0.1–10	4.3	0.09
9	3-Monochlorofluoranthene	$C_{16}H_9Cl$	3-ClFlu	236.0392	0.9915	0.1–40	12	0.26
10	8-Monochlorofluoranthene	$C_{16}H_9Cl$	8-ClFlu	236.0392	0.9989	0.1–10	12	0.28
11	1-Monochloropyrene	$C_{16}H_9Cl$	1-ClPyr	236.0392	0.9998	0.1–10	9.1	0.16
12	3,9,10-Trichlorophenanthrene	$C_{14}H_7Cl_3$	3,9,10-Cl_3Phe	279.9613	0.9993	0.5–40	16	0.23
13	3,8-Dichlorofluoranthene	$C_{16}H_8Cl_2$	3,8-Cl_2Flu	270.0003	0.9998	0.5–40	15	0.24
14	3,4 Dichlorofluoranthene	$C_{16}H_8Cl_2$	3,4-Cl_2Flu	270.0003	0.9994	0.5–40	16	0.18
15	6-Chlorochrysene	$C_{18}H_{11}Cl$	6-ClChr	262.0549	0.9999	0.1–40	17	0.27
16	7-Chlorobenz[a]anthracene	$C_{18}H_{11}Cl$	7-ClBaA	262.0549	0.9975	0.1–40	14	0.24
17	6,12-Dichlorochrysene	$C_{18}H_{10}Cl_2$	6,12-Cl_2Chr	296.0160	0.9970	0.1–40	19	0.24
18	7,12-Dichlorobenz[a]anthracene	$C_{18}H_{10}Cl_2$	7,12-Cl_2BaA	296.0160	0.9996	0.1–40	18	0.21
19	6-Monochlorobenzo[a]pyrene	$C_{20}H_{11}Cl$	6-ClBaP	286.0549	0.9982	0.5–40	16	0.13
A	2-Monobromofluorene	$C_{13}H_9Br$	2-BrFle	243.9888	0.9942	0.5–20	13	3.2
B	9-Monobromophenanthrene	$C_{14}H_9Br$	9-BrPhe	255.9888	0.9995	0.1–20	11	2.3
C	9-Monobromoanthracene	$C_{14}H_9Br$	9-BrAnt	255.9888	0.9983	0.5–40	4.4	0.78
D	9,10-Dibromoanthracene	$C_{14}H_8Br_2$	9,10-Br_2Ant	333.8993	0.9915	0.5–40	27	0.81
E	1-Monobromopyrene	$C_{16}H_9Br$	1-BrPyr	279.9888	0.9992	0.5–40	18	2.0
F	7-Monobromobenz[a]anthracene	$C_{18}H_{11}Br$	7-BrBaA	306.0044	0.9902	1–40	28	0.26
G	7,11-Dibromobenz[a]anthracene	$C_{18}H_{10}Br_2$	7,11-Br_2BaA	383.9149	Σ = 0.9524	Σ = 5–40	Σ = 15[c]	–
H	7,12-Dibromobenz[a]anthracene	$C_{18}H_{10}Br_2$	7,12-Br_2BaA	383.9149				
I	4,7-Dibromobenz[a]anthracene	$C_{18}H_{10}Br_2$	4,7-Br_2BaA	383.9149	Σ = 0.9619	Σ = 5–40	Σ = 15[c]	–
J	5,7-Dibromobenz[a]anthracene	$C_{18}H_{10}Br_2$	5,7-Br_2BaA	383.9149				
K	6-Monobromobenzo[a]pyrene	$C_{20}H_{11}Br$	6-BrBaP	330.0044	0.9535	5–40	22[c]	–

[a] Repeatability was assessed by replicate analyses (n = 6) of 1 pg for Cl-PAHs, 10 pg for Br-PAHs except for 5 Br-PAHs (G, H, I, J and K).
[b] The LODs were estimated by triplication of the standard deviation of values obtained from six analyses for 1 pg of Cl-PAHs and 10 pg of Br-PAHs except for 5 Br-PAHs (G, H, I, J and K).
[c] Repeatability was assessed by replicate analyses (n = 3) of 40 pg.

tive identification of the highly substituted Cl-PAHs, since Cl-PAHs might have co-eluted with matrices by one-dimensional separation, and the selectivity of GC–QMS was not enough in this case [1]. To search for the occurrence of highly chlorinated and brominated PAHs congeners in the environment, exhaustive analysis with high selectivity and the capability of total profiling of Cl-/Br-PAHs is needed. For this purpose, even GC–HRMS has limitations, since the numbers of monitored ions are limited due to the slow acquisition speed of magnetic sector-type mass spectrometers.

In the last decade, comprehensive two-dimensional gas chromatography (GC × GC) coupled with mass spectrometry (MS) has been widely applied in environmental analysis. The GC × GC–MS method can yield many practical advantages, e.g. large separation power, high sensitivity, high selectivity, group type separation and total profiling. Also, because of the aforementioned benefits, minimizing sample preparation procedures and speeding up analysis for the detection of minor compounds in environmental samples can be provided. In 2006, Panić and Górecki reviewed GC × GC in the environmental analysis and monitoring [12]. They indicated that the main challenge in environmental analysis is that the analytes are usually present in trace amounts in very complex matrices. In overcoming this hurdle, GC × GC–MS is a very powerful and attractive system that has been successfully applied for the many kinds of environmental pollutants, such as PCDDs, PCDFs, polychlorinated biphenyls (PCBs) [13,14], polychlorinated naphthalenes (PCNs) [15], nonyl phenol (NP) [16–18], benzothiazoles, benzotriazoles, benzosulfonamides [19], pharmaceuticals and pesticides [20]. In one such paper, Hoh et al. suggested that GC × GC coupled with high speed TOF-MS (50 Hz) with unit-mass resolution has the potential to lower costs and allow for the faster analysis of minor environmental pollutants, such as PCDD/Fs over the current predominant method [14]. They separated the most important PCDD/F congeners from PCB interferences using GC × GC–TOF-MS in less than 1 h. Mass spectral deconvolution software also helped to enhance the identification capability. The method allowed for the detection of TCDD at a level as low as 0.25 pg. However, GC × GC–TOF-MS with unit resolution may not be selective enough for the detection of minor compounds in highly complex matrix samples.

An ideal data acquisition rate for GC × GC is more than 100 Hz to maximize its large separation power. Therefore, the high speed TOF-MS with a unit-mass resolution has been widely used as the best candidate MS for GC × GC. On the other hand, several researchers have reported the applicability of moderate acquisition rate instruments, such as Q-MS (e.g. 20 Hz) as the next best candidate MS for GC × GC, even with the limited mass range and lack of sufficient data acquisition rate to reproduce the GC × GC peak shape. A few years ago, GC × GC coupled with a high-resolution TOF-MS (HRTOF-MS) that allowed accurate mass measurement (mass measurement with uncertainties of a few mDa) using the acquisition rate of 20–25 Hz was applied for environmental analysis. Čajka et al. summarized the advantages of HRTOF-MS as the acquisition of spectral data across a wide mass range without a decrease in detection sensitivity, a high mass resolution that provides power to resolve the target analyte against interference, and mass measurement accuracy that permits estimation of the elemental composition of the detected ions [21]. These are the significant advantages for the investigation of unknown compounds in environmental samples. Also, HRTOF-MS is capable of determining not only target compounds but also non-target compounds in the complex matrix samples. Thus, the use of GC × GC–HRTOF-MS is very important in environmental analysis even with the moderate data acquisition rate. In 2007, Ochiai et al. characterized nanoparticles in roadside atmospheric samples with thermal

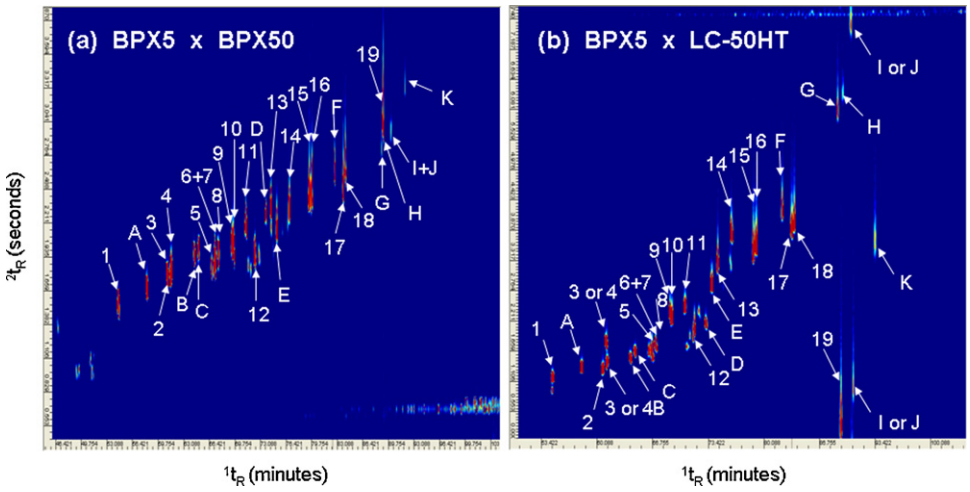

Fig. 1. GC × GC–HRTOF-MS 2D chromatogram of 19Cl-/11Br-PAH analytical standards (a) 1st column: BPX5, 2nd column: BPX50, (b) 1st column: BPX5, 2nd column: LC-50HT. Abbreviations are shown in Table 1.

desorption (TD) – GC × GC–HRTOF-MS [22]. They showed the accurate mass detection capability of the HRTOF-MS to plot the two-dimensional (2D) extracted ion chromatograms with 0.05 Da windows. This approach helped with compound class visualization and identification for the minor compounds in the matrix-rich environmental samples. Also, the elemental composition for fifty compounds, including oxygenated polycyclic aromatic hydrocarbons and nitrogen-containing polycyclic aromatic hydrocarbons, were calculated from the accurate mass molecular ions and subsequently identified. The TD–GC × GC–HRTOF-MS which allowed the high sensitivity and high selectivity analysis was a valuable technique for the characterization of environmental samples such as nanoparticles, which comprised a very small mass but included a number of minor and unknown organic compounds.

In the following year, Hashimoto et al. reported a GC × GC–HRTOF-MS application for PCDDs and PCDFs analysis with a resolving power of 5000, acquisition range of m/z 35–500 and acquisition rate of 25 Hz [23]. The benefits of using HRTOF-MS were clearly shown to discriminate against interferences for analysing real-world environmental samples such as fly ash and flue gas samples from municipal waste incineration (MWI). All congeners with a TCDD toxic equivalency factor (TEF) were isolated from the other isomers. Furthermore, they reported quantification results using GC × GC–HRTOF-MS for a certified reference material and crude extracts of fuel gas emitted from MWIs. The results fairly agreed with those obtained by GC–HRMS. Therefore, GC × GC–HRTOF-MS allowed that all congeners with TEF were quantified by only one injection, while the existing method requires several measurements using different GC columns.

The objective of this paper was to develop an effective method for the exhaustive analysis of Cl-/Br-PAH congeners in a soil extract using GC × GC–HRTOF-MS. GC × GC–HRTOF-MS provided highly sensitive and selective analysis for Cl-/Br-PAH congeners in the complex matrix. Identification of Cl-/Br-PAH congeners in the soil extract was performed by group type separation using mass spectrometry with a 0.02 Da wide window, formula calculation with

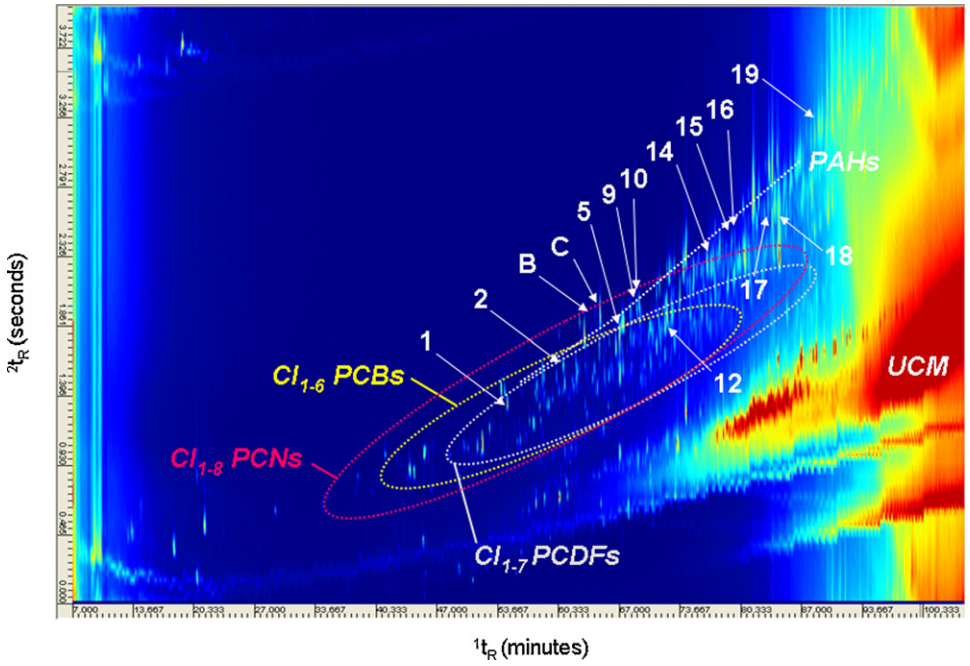

Fig. 2. GC × GC–HRTOF-MS 2D total ion chromatogram of a soil extract by BPX5 × BPX50. *Abbreviations are shown in Table 1.

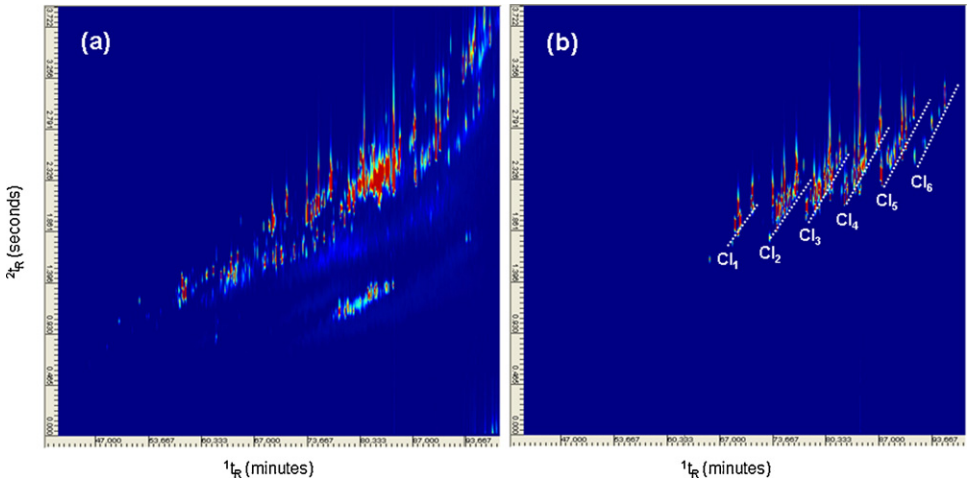

Fig. 3. Comparison of group type separation using the 2D mass chromatograms obtained using the GC × GC–HRTOF-MS of a soil extract (sum of selected ions for mono to hexa Cl-PAHs; *m/z* 236.0392, 270.0003, 303.9654, 337.9239, 371.8834 and 405.8444). (a) 1.0 Da wide window and (b) 0.02 Da wide window.

accurate mass measurements, and comparison of mass spectra of Cl-/Br-PAH congeners with those of the isotope model.

2. Experimental

2.1. Chemicals

19 individual Cl-PAHs and 11 individual Br-PAHs were used for the analysis. Abbreviations of individual Cl-PAHs and Br-PAHs analysed are shown in Table 1. Standards of 2-monochloroanthracene, 9-monochloroanthracene, and 9,10-dibromoanthracene were purchased from Aldrich (St. Louis, MO). Standards of 9-monobromoanthracene, 9-monobromophenanthrene and 7-monobromobenz[a]anthracene were purchased from Tokyo Chemical Industry (Tokyo, Japan). 9-monochlorophenanthrene was obtained from Acros Organics (Geel, Belgium). The remaining compounds were synthesized by the authors following published procedures [2,9,24]. The purities of the synthesized standards of Cl-/Br-PAHs were > 95% (determined by GC with flame ionization detection on the basis of chromatographic peak areas). All standards were mixed together and used for the analysis. The concentration of all compounds was 100 ng/ml in isooctane.

2.2. Samples

The soil sample was collected at a former chlor-alkali plant in Tokyo, Japan. The air dried soils (1.067 g) were extracted using Soxhlet apparatus with toluene. The toluene extract was diluted up to 25 ml with *n*-hexane. The 20 ml of the solution was diluted up to 25 ml with hexane. This process was done twice. The 15 ml of the solution was diluted again up to 25 ml. A further 1 ml of solution was extracted and we ultimately diluted the solution up to 50 ml. As a result, the 25 ml extract of the soil was diluted in total by about 5.5 times (Actual figure: 5.425). One microliter of the extract was used for the analysis without any clean up.

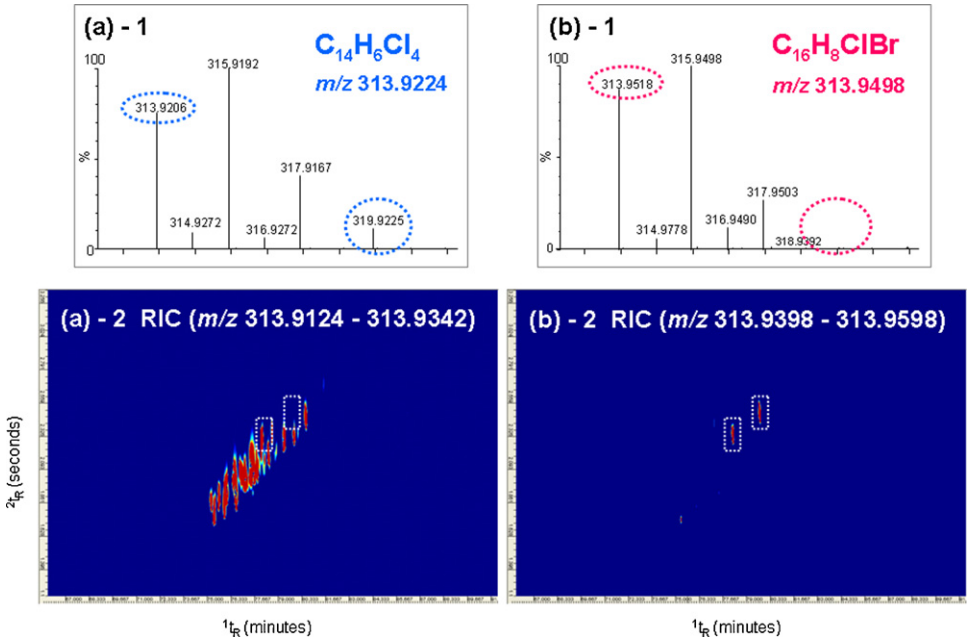

Fig. 4. The difference of isotope patterns between two peaks in the soil extract; (a)-1 $C_{14}H_6Cl_4$ and (b)-1 $C_{16}H_8ClBr$ and GC × GC–HRTOF-MS 2D exact mass chromatogram of a 0.02 Da wide windows (a)-2 $C_{14}H_6Cl_4$; *m/z* 337.9224 and (b)-2 $C_{16}H_8ClBr$; *m/z* 313.9498.

Table 2
The results of identification for Cl-/Br-PAHs in the soil extract obtained by GC × GC–HRTOF-MS.

No.	^1t_R[a] (min)	^2t_R[b] (s)	Formula	Measured *m/z*	Theoretical *m/z*	Mass error (ppm)
1	61.32	1.68	$C_{14}H_9Cl$[c]	212.0383	212.0393	−4.7
2	67.06	1.91	$C_{14}H_8Cl_2$[c]	245.9987	246.0003	−6.5
3	72.27	2.05	$C_{14}H_7Cl_3$[c]	279.9616	279.9613	1.1
4	77.26	2.05	$C_{14}H_6Cl_4$	313.9206	313.9224	−5.7
5	82.00	2.33	$C_{14}H_5Cl_5$	347.8816	347.8834	−5.2
6	87.87	2.70	$C_{14}H_4Cl_6$	381.8399	381.8444	−12
7	89.60	2.42	$C_{14}H_3Cl_7$	415.8089	415.8054	8.4
8	70.93	2.19	$C_{16}H_9Cl$[c]	236.0382	236.0393	−4.7
9	73.73	2.23	$C_{16}H_8Cl_2$[c]	269.9983	270.0003	−7.4
10	78.46	2.14	$C_{16}H_7Cl_3$	303.9614	303.9613	0.3
11	85.47	2.56	$C_{16}H_6Cl_4$	337.9221	337.9224	−0.9
12	89.95	2.75	$C_{16}H_5Cl_5$	371.8843	371.8834	2.4
13	93.67	2.84	$C_{16}H_4Cl_6$	405.8445	405.8444	0.2
14	98.81	3.19	$C_{16}H_3Cl_7$	439.8043	439.8054	−2.5
15	79.74	2.51	$C_{18}H_{11}Cl$[c]	262.0538	262.0549	−4.2
16	84.08	2.88	$C_{18}H_{10}Cl_2$[c]	296.0149	296.0160	−3.7
17	88.54	2.93	$C_{18}H_9Cl_3$	329.9760	329.9770	−3.0
18	92.08	2.93	$C_{18}H_8Cl_4$	363.9382	363.9380	0.5
19	96.14	3.16	$C_{18}H_7Cl_5$	397.9005	397.8990	3.8
20	86.54	2.93	$C_{20}H_{11}Cl$[c]	286.0529	286.0549	−7.0
21	91.22	3.16	$C_{20}H_{10}Cl_2$	320.0169	320.0160	2.8
22	95.22	3.26	$C_{20}H_9Cl_3$	353.9761	353.9770	−2.5
23	97.75	3.40	$C_{20}H_8Cl_4$	387.9361	387.9380	−4.9
24	101.09	3.63	$C_{20}H_7Cl_5$	421.8972	421.8990	−4.3
25	64.39	1.77	$C_{14}H_9Br$[c]	255.9874	255.9888	−5.5
26	74.26	2.09	$C_{14}H_8Br_2$[c]	333.8998	333.8993	1.5
27	72.60	2.14	$C_{16}H_9Br$[c]	279.9889	279.9888	0.4
28	70.86	2.05	$C_{14}H_8ClBr$	289.9491	289.9498	−2.4
29	75.40	2.05	$C_{14}H_7Cl_2Br$	323.9108	323.9101	−2.2
30	79.74	2.47	$C_{16}H_8ClBr$	313.9518	313.9498	6.4

[a] First column retention time (min).
[b] Second column retention time (s).
[c] Confirmation with authentic compound was performed.

Table 3
The results of identification for organohalogen compounds in the soil extract obtained by GC × GC–HRTOF-MS.

No.	^1t_R[a] (min)	^2t_R[b] (s)	Formula	Measured *m/z*	Theoretical *m/z*	Mass error (ppm)	Compound group
1	38.17	0.74	$C_{10}H_7Cl$	162.0248	162.0236	7.4	PCNs
2	45.85	0.93	$C_{10}H_6Cl_2$	195.9852	195.9847	2.6	PCNs
3	51.45	0.98	$C_{10}H_5Cl_3$	229.9469	229.9457	5.2	PCNs
4	57.78	1.21	$C_{10}H_4Cl_4$	263.9060	263.9067	−2.7	PCNs
5	64.32	1.40	$C_{10}H_3Cl_5$	297.8690	297.8677	4.4	PCNs
6	69.85	1.49	$C_{10}H_2Cl_6$	331.8277	331.8288	−3.3	PCNs
7	76.79	1.91	$C_{10}HCl_7$	365.7895	365.7898	−0.8	PCNs
8	82.20	2.33	$C_{10}Cl_8$	399.7516	399.7508	2.0	PCNs
9	51.25	1.07	$C_{12}OH_7Cl$	202.0179	202.0185	−3.0	PCDFs
10	58.12	1.30	$C_{12}OH_6Cl_2$	235.9805	235.9796	3.8	PCDFs
11	63.52	1.40	$C_{12}OH_5Cl_3$	269.9406	269.9406	0.0	PCDFs
12	69.79	1.63	$C_{12}OH_4Cl_4$	303.9028	303.9016	3.9	PCDFs
13	74.46	1.72	$C_{12}OH_3Cl_5$	337.8625	337.8627	−0.6	PCDFs
14	79.26	1.86	$C_{12}OH_2Cl_6$	371.8243	371.8237	1.6	PCDFs
15	83.33	2.05	$C_{12}OHCl_7$	405.7852	405.7847	1.2	PCDFs
16	70.39	1.91	$C_{16}OH_9Cl$	252.0332	252.0342	−4.0	PC-Benzonaphthofurans
17	76.20	2.09	$C_{16}OH_8Cl_2$	285.9937	285.9952	−5.2	PC-Benzonaphthofurans
18	81.07	2.23	$C_{16}OH_7Cl_3$	319.9571	319.9562	2.8	PC-Benzonaphthofurans
19	85.60	2.37	$C_{16}OH_6Cl_4$	353.9165	353.9173	−2.3	PC-Benzonaphthofurans
20	89.67	2.47	$C_{16}OH_5Cl_5$	387.8769	387.8783	−3.6	PC-Benzonaphthofurans
21	93.20	2.70	$C_{16}OH_4Cl_6$	421.8372	421.8393	−5.0	PC-Benzonaphthofurans
22	42.98	0.88	$C_{12}H_9Cl$	188.0406	188.0393	6.9	PCBs
23	49.44	0.98	$C_{12}H_8Cl_2$	221.9996	222.0003	−3.2	PCBs
24	56.71	1.16	$C_{12}H_7Cl_3$	255.9596	255.9613	−6.6	PCBs
25	62.38	1.30	$C_{12}H_6Cl_4$	289.9233	289.9224	3.1	PCBs
26	67.99	1.49	$C_{12}H_5Cl_5$	323.8817	323.8834	−5.2	PCBs
27	70.65	1.63	$C_{12}H_4Cl_6$	357.8459	357.8444	4.2	PCBs
28	74.52	1.68	$C_{12}H_3Cl_7$	391.8096	391.8054	11	PCBs
29	58.51	1.21	$C_{14}OH_{11}Cl$	230.0510	230.0498	5.2	Alkylated-PCDFs
30	65.32	1.35	$C_{14}OH_{10}Cl_2$	264.0106	264.0109	−1.1	Alkylated-PCDFs
31	70.39	1.49	$C_{14}OH_9Cl_3$	297.9709	297.9719	−3.4	Alkylated-PCDFs
32	75.59	1.63	$C_{14}OH_8Cl_4$	331.9326	331.9329	−0.9	Alkylated-PCDFs
33	80.73	1.81	$C_{14}OH_7Cl_5$	365.8933	365.8940	−1.9	Alkylated-PCDFs
34	67.87	1.86	$C_{12}H_5OCl_2Br$	313.8921	313.8901	2.0	Others
35	74.93	2.09	$C_{12}H_4SCl_4$	319.8800	319.8788	1.2	Others

[a] First column retention time (min).
[b] Second column retention time (s).

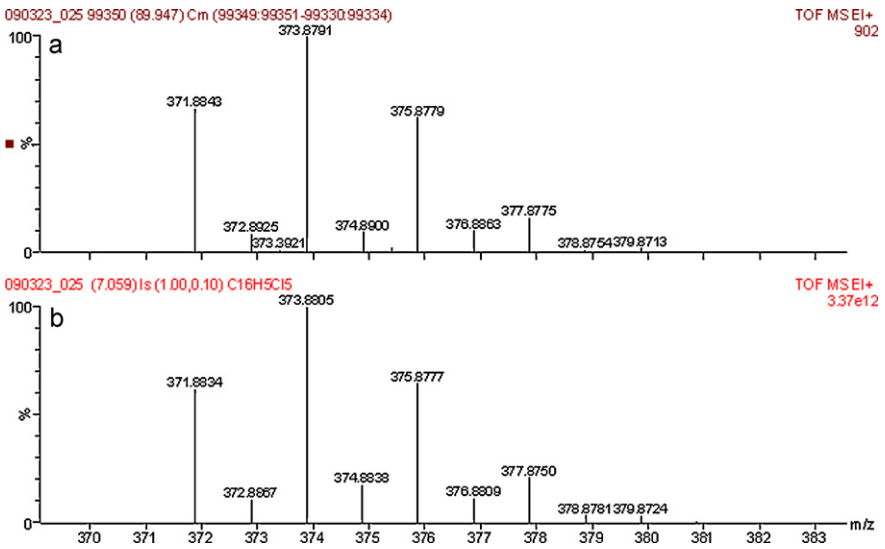

Fig. 5. The comparison of (a) isotope pattern of a compound in the soil extract with (b) theoretical isotope pattern of $C_{16}H_5Cl_5$.

2.3. GC × GC column sets

BPX5 (30 m × 0.25 mm i.d., 0.25 μm film thickness, SGE International) was used for the first column. For the evaluation of the optimum column set for the Cl-/Br-PAHs analysis, two options for the second column were tested; BPX50 (50% Phenyl Polysilphenylene-siloxane, 1 m × 0.10 mm i.d., 0.10 μm film thickness, SGE International (BPX5 × BPX50)) and LC-50HT (liquid crystal polysiloxane, 1 m × 0.10 mm i.d., 0.10 μm film thickness, J&K Scientific Inc., Canada (BPX5 × LC-50HT)), specially made for this study.

2.4. GC × GC–HRTOF-MS

Analyses were performed with a GERSTEL CIS 4 programmed temperature vaporization (PTV) inlet (GERSTEL, Mulheim an der Ruhr, Germany) and a Zoex KT2004 loop type modulator (Zoex corporation, Houston, TX, USA) installed on an Agilent 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a Waters GCT Premier time-of-flight mass spectrometer (Waters, MA, USA). MassLynx software (Waters) was used for the raw data analysis. GC Image software (ZOEX) was used for the data analysis in contour plots (2D chromatogram). A 1 μL-sample was injected into a PTV inlet with a quartz baffled liner at 30 °C and the inlet was programmed from 30 °C to 350 °C (held for 5 min) at 720 °C min^{-1} to inject compounds onto the analytical column. Injection was performed in the splitless mode with a 2 min splitless time. During the injection, the GC was held at the initial temperature of 50 °C. The GC was programmed from 50 °C (held for 2 min) to 350 °C (held for 2 min) for BPX5 × BPX50, and to 300 °C (held for 10 min) for BPX5 × LC-50HT, at 3 °C min^{-1}, respectively. Helium was used as a carrier gas supplied at 1.5 ml min^{-1}. The modulation period

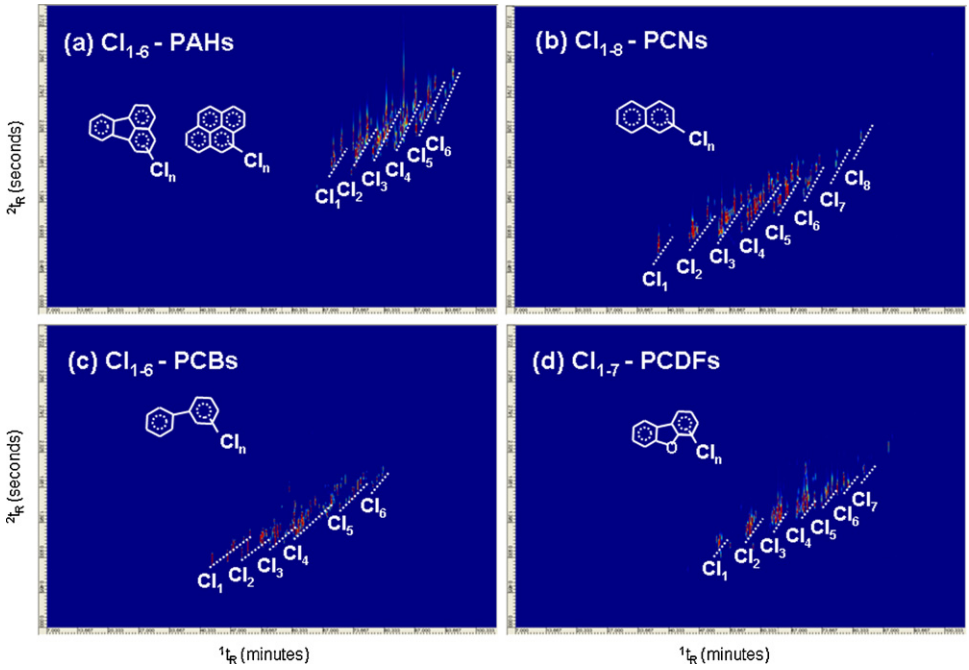

Fig. 6. GC × GC–HRTOF-MS 2D exact mass chromatogram of a 0.02 Da wide windows (a) Cl-PAHs, (b) PCNs, (c) PCBs and (d) PCDFs.

was 4 s for BPX5 × BPX50, and 8 s for BPX5 × LC50-HT. The modulator hot gas temperature was programmed from 220 °C (held for 2 min) to 350 °C at 3 °C min^{-1} (held for 58.67 min) and the hot gas duration time was 300 ms. A HRTOF-MS was operated at a multi-channel plate voltage of 2900 V, a pusher interval of 40 μs (resulting in 25,000 raw spectra per second) and a mass range of m/z 35–600 using electron ionization (EI; electron-accelerating voltage: 70 V). The resolving power was 6215, calculated using full width at half maximum (FWHM) at m/z 218.9856 of perfluorotributylamine (PFTBA). The data acquisition speed was 20 Hz (maximum data acquisition speed of a Waters GCT Premier time-of-flight mass spectrometer). A column background ion (m/z 281.0517 or m/z 355.0705) was used for single lock mass calibration after the sample analysis.

3. Results and discussion

3.1. Evaluation of GC × GC column sets

Two GC × GC column sets were tested by analysing a mixture of 19 Cl-PAHs and 11 Br-PAHs. In this study, a normal column set (e.g. non-polar × polar) was evaluated because it provided a wider separation space for aromatic compounds compared with a reversed column set (e.g. polar × non-polar). BPX50 was evaluated for the second column because the maximum operating temperature is very high (370 °C) and some researchers have successfully used this column as the second column for PAH analysis by GC × GC–MS [22,25]. The column set can analyse a wide range of PAHs (from phenanthrene to benzo [g,h,i] perylene) with no wraparound in 4 s. On the other hand, LC-50 is a novel liquid crystal polysiloxane based column, and the stationary phase is highly effective in isomer-specific separation and analysis of environmental pollutants, e.g. PAHs, PCBs and PCNs. A number of researchers have used this column as the second column for the GC × GC, and excellent separation was obtained for the congeners of environmental pollutants. However the maximum operating temperature (270 °C) is occasionally problematic for the analysis of high-boiling compounds. Recently, a high temp LC-50 column; LC-50HT (maximum operating column temperature: 300 °C) was developed. In this study, the new LC-50HT was evaluated for the analysis of Cl-/Br-PAHs congeners.

Fig. 1 shows a 2D total ion chromatogram (TIC) obtained by two column sets; BPX5 × BPX50 and BPX5 × LC-50HT with GC × GC–HRTOF-MS. For BPX5 × BPX50, all Cl-/Br-PAHs were eluted regularly on the 2D TIC with no wraparound in the second dimensional separation and group type separation was successfully achieved (Fig. 1(a)). The high maximum operating temperature (370 °C) and the phenyl structure retention mechanism of the second dimensional column (BPX50) were keys to providing these results. Moreover, the separation space was deemed to be enough for Cl-/Br-PAHs and sample matrices. On the other hand, BPX5 × LC-50HT did not yield a structured chromatogram for Cl-/Br-PAHs, and the group type separation was not easy because the retentive nature of the liquid crystal phase was extremely strong for late eluting compounds (e.g. 19, I, J and K) (Fig. 1(b)). It was assumed that Cl-/Br-PAHs, including unknown higher substituted Cl-/Br-PAHs, would not elute without wraparound with keeping its separation and the constant oven temperature program (3 °C/min), even if a shorter second column (e.g. 0.7 m) was used. The wraparound is expected to be a problem in the analysis of matrix-rich environmental samples since the target compounds could be overlapped by the co-eluting matrix. In actual fact, an environmental sample was analysed by BPX5 × LC-50HT. The higher boiling Cl-PAHs, such as 6-ClBaP, were overlapped by the unresolved complex mixtures (UCM) in the sample and it was a problem for identification. Furthermore, a secondary oven for the LC-50HT column was not evaluated because the oven temperature reached 300 °C at 85.33 min and some of the Cl-/Br-PAHs eluted after that, for example 6-monochlorobenzo[a]pyrene; 89.02 min and 6-monobromobenzo[a]pyrene; 91.89 min. In this case, the temperature offset by the secondary oven is not viable for the LC-50HT column, since its maximum operating temperature is 300 °C. The separation of Cl-/Br-PAHs was much better than that of BPX50. For example 4,7-Br_2BaA and 5,7-Br_2BaA were separated on the 2D TIC. This result was not achieved by the use of the column set BPX5 × BPX50.

In this study, the column set BPX5 × BPX50 was selected because of the higher priority for the group type separation of Cl-/Br-PAH congeners in environmental samples over the individual separation on the 2D TIC.

3.2. Analytical performance of GC × GC–HRTOFMS method for Cl-/Br-PAHs

Linearity, repeatability and limit of detection (LOD) with 19Cl-/11Br-PAHs were evaluated for the GC × GC–HRTOFMS (Table 1). Correlation coefficients (r^2) at five levels between 0.1 pg and 40 pg were in the range of 0.9973–0.9999 for Cl-PAHs, and in the range of 0.9902–0.9995 for Br-PAHs except for the late eluting Br-PAHs, e.g. 7,11-dibromobenz[a]anthracene (G), 7,12-dibromobenz[a]anthracene (H), 4,7-dibromobenz [a]anthracene (I), 5,7-dibromobenz[a]anthracene (J) and 6-monobromobenzo[a]pyrene (K). The correlation coefficients (r^2) of 5 Br-PAHs were in the range of 0.9524–0.9619. The repeatability of selected ion response (RSD %, $n = 6$) was in the range of 4.3–22% for Cl-PAHs at 1 pg, and 4.4–28% for Br-PAHs at 10 pg except for 5 Br-PAHs (G, H, I, J and K). For 5 Br-PAHs, the repeatability of selected ion response (RSD %, $n = 3$) was in the range of 15–22% at 40 pg. The LODs were estimated by triplication of the standard deviation of values obtained from six analyses for 1 pg of Cl-PAHs and 10 pg of Br-PAHs except for 5 Br-PAHs. The LODs of Cl-PAHs in the range of 0.08–0.44 pg was obtained. The LODs of Br-PAHs ranged from 0.26 pg to 3.2 pg. The linearity and LODs were acceptable for most of the analytes, however the repeatability were more than RSD 10% in most cases. Therefore, the use of internal standards would be required for more reliable quantification.

3.3. Identification of Cl-/Br-PAHs congeners and other organohalogen compounds in the soil extract

Fig. 2 shows the 2D TIC of a soil extract obtained by GC × GC–HRTOF-MS. The hundreds of compounds such as Cl-/Br-PAHs, PAHs, PCNs, PCBs and PCDFs were clearly separated from the UCM. More than 1000 compounds were detected on the 2D TIC, even if no sample clean up procedure was done. Using 19Cl-/11Br-PAH standards, the existence of 19 Cl-PAHs and 3 Br-PAHs was confirmed in the soil extract and some of them are indicated on the 2D TIC. Ohura et al. analysed the same sample by GC coupled with the tandem mass spectrometer (GC–MS/MS) and quantified these 19 Cl-PAHs [26]. The range of the Cl-PAH concentrations was from 1 to 210 μg/g dry weight and total Cl-PAHs concentration was 970 μg/g dry weight. The concentrations were extremely high compared with those of other samples reported before, such as the Tokyo bay sediment core; 2.6–187 pg/g (total 584 pg/g) [8], Saginaw River watershed sediment; 2.8–186 pg/g (total 1140 pg/g) [8], and fly ash from the some waste incinerations; total <0.06–6990 ng/g dry weight [1]. In actual fact, this soil sample was collected at a former chlor-alkali plant site in Tokyo. In the recent study, the high concentrations of Cl-PAHs in marsh sediment collected near a former chlor-alkali plant were also reported by Horii et al. [8]. They suggested that the chlor-alkali process was a source of Cl-PAHs in the environment. Additionally, 16 priority EPA PAHs in this soil

extract were analysed. The range of the concentrations were from 5.8 to 374 μg/g dry weights and total 16 PAHs concentration was 2050 μg/g dry weights. The total concentrations of 16 PAHs were almost two times higher than those of 19 Cl-PAHs.

To search for the existence of highly chlorinated PAH congeners in the soil extract, mass chromatography with a 0.02 Da wide window for Cl-PAHs were performed. Fig. 3 shows the 2D mass chromatograms of mono to hexa chlorinated fluoranthene or pyrene (Cl_1–Cl_6-PAHs, sum of m/z 236.0392, 270.0003, 303.9654, 337.9239, 371.8834 and 405.8444) with (a) a 1 Da wide window, (b) a 0.02 Da wide window, and the results of the identification are indicated. The 2D mass chromatogram of a very narrow mass window allowed greater selectivity and more detailed group type analysis than that of a 1.0 Da wide window. On the 2D mass chromatogram with a 0.02 Da window, no peaks were found except for peaks that eluted linearly in each Cl-PAH group, although the interferences were found in the 2D mass chromatogram with a 1 Da wide window. In each group of Cl_1–Cl_6-PAHs in Fig. 3(b), 15 isomers were detected on average. All peaks were identified if they had a specific accurate mass spectrum of a molecular ion and an isotope pattern for each Cl-PAH. In addition, the elemental compositions were calculated from the accurate mass molecular ion in the raw chromatogram with MassLynx software (Waters). For the current study, 1 μL of the sample was injected in splitless mode to detect as many of the Cl-/Br-PAH congeners as possible. However, the dynamic range of the HRTOF-MS is narrow; it is about two or three orders of magnitude, and so the signals of the molecular ion for the major compounds were saturated. Therefore, a sliced peak that had an unsaturated molecular ion signal was selected from all sliced peaks of a compound (2–4 sliced peaks per a compound after modulation) for the calculation of the elemental composition. A single lock mass calibration with a column background ion (m/z 281.0517 or m/z 355.0705) was performed after the sample analysis. The closest column background peak to a target peak was used for the calibrations. The m/z 281.0517 was used for the calibration of the target compounds whose molecular ion was lower than m/z 350, and m/z 355.0705 was used for the calibration of the target compounds whose molecular ion was higher than m/z 350.

Fig. 4(a)-1 and (b)-1 shows the difference of isotope patterns between two peaks in the soil extract obtained by GC × GC–HRTOF-MS. The 2D mass chromatogram of Cl-PAHs with a 0.05 Da window was initially used for the identification. First, the positions of the Cl-PAHs were marked by this 2D mass chromatogram. Then the mass spectra of the peaks were evaluated on the 2D TIC. The mass spectra were carefully evaluated if they had specific isotope patterns for Cl-PAHs. However, the different isotope patterns from that of Cl-PAHs were found in the peaks on the 2D mass chromatogram with a 0.05 Da window. As a result of the calculation of the elemental composition, the candidate compound for the peak (a) was $C_{14}H_6Cl_4$ and the peak (b) was $C_{16}H_8ClBr$. The theoretical mass difference between (a) $C_{14}H_6Cl_4$ (m/z 313.9224) and (b) $C_{16}H_8ClBr$ (m/z 313.9498) was only 0.0274 Da. Therefore, the narrower range; a 0.02 Da wide window was used for the mass chromatogram of Cl-PAHs. Fig. 4(a)-2 and (b)-2 shows two 2D mass chromatograms of $C_{14}H_6Cl_4$ (m/z 313.9224) and $C_{16}H_8ClBr$ (m/z 313.9498) with 0.02 Da wide windows, respectively. Two peaks in Fig. 4(b)-2 were eluted in the same region as the peaks in Fig. 4(a)-2, but they were clearly separated using the 2D mass chromatograms with a 0.02 Da wide window.

Since a NIST library search was not available for the identification of these unknown compounds such as higher chlorinated PAHs, manual identification was performed for all compounds on the 2D mass chromatograms of the target Cl-/Br-PAHs. The representative results of identification of Cl-/Br-PAHs in the soil extract were shown in Table 2. The first column retention time (^1t_R), the second column retention time (^2t_R), candidate formula, measured m/z value, theoretical m/z value and mass error (ppm) were listed. Fig. 5(a) shows an isotope pattern with a peak in the soil sample data and (b) shows a theoretical isotope pattern of $C_{16}H_5Cl_5$. The isotope patterns showed a high degree of similarity. For all of the compounds in Table 2, isotope patterns of the peak were confirmed if they showed a similar pattern compared with the theoretical pattern. In total, thirty Cl-/Br-PAHs, including 11 compounds identified using our analytical standards, were identified in the soil extract. For chlorinated anthracene or phenanthrene ($C_{14}H_{10}$) and fluoranthene or pyrene ($C_{16}H_{10}$) congeners, very small amounts of hepta chlorinated PAHs, were found in the soil sample. Also, for chlorinated benz[a]anthracene or chrysene ($C_{18}H_{12}$), and benzo[b]fluoranthenes or benzo[k]fluoroanthene or benzo[a]pyrene ($C_{20}H_{12}$) congeners, penta chlorinated PAHs, were found in this sample. For Br-PAHs, brominated anthracene or phenanthrene ($C_{14}H_9Br$ and $C_{14}H_8Br_2$), and $C_{16}H_9Br$ were detected in this sample. Moreover, some ClBr-PAHs were found in the sample. For the 30 ClBr-PAHs, the mass errors (ppm) were in the range of −7.4 to 3.8 ppm with a root mean square of 4.1 ppm, except for $C_{14}H_4Cl_6$ (−12 ppm) and $C_{14}H_3Cl_7$ (8.4 ppm) that existed in very trace amounts. To our knowledge, highly chlorinated PAHs, such as $C_{14}H_3Cl_7$ and $C_{16}H_3Cl_7$, and ClBr-PAHs, such as $C_{14}H_7Cl_2Br$ and $C_{16}H_8ClBr$, were found in the environmental samples for the first time. It suggested that there are a number of unconfirmed and highly substituted Cl-/Br-PAHs in the environmental samples as results of various reactions by chlorine, bromine and aromatic precursors (e.g., chlor-alkali processes, municipal waste incineration and automobile exhaust) [8,27]. Recently, Yamamoto et al. reported that Cl-PAHs might have been formatted from brine electrolysis by graphite electrode abundantly contained pitch in the past [28]. This soil sample was collected at the former chlor-alkali plant, therefore, the high concentration of highly substituted Cl-PAHs in this soil sample might have been formatted by the same process.

Fig. 6 shows 2D mass chromatograms of (a) Cl-PAHs, (b) PCNs, (c) PCBs and (d) PCDFs with 0.02 Da wide windows of the soil extract. For other organohalogen compounds, the highly selective group type separation could also be performed with a very narrow mass window, and highly sensitive detection for the congeners of these pollutants in the complex matrix sample was possible. Table 3 shows the results of the identification of other organohalogen compounds in the soil extract. Thirty five compounds were listed in the table, including PCNs, PCDFs, PCBs, polychlorinated benzonaphthofurans (PC-Benzonaphthofurans), mixed chlorine and bromine furans, and halogenated organosulfur compound. For the 35 organohalogen compounds, the mass errors (ppm) were in the range of −6.6 to 7.4 ppm with a root mean square of 3.7 ppm, except for $C_{12}H_3Cl_7$ (PCB, 11 ppm). Other organohalogen compounds, such as brominated dioxin, brominated biphenyls, and chlorinated diphenyl ethers, were also searched for, but were not found in this soil sample.

4. Conclusion

The combination of GC × GC and HRTOF-MS can provide a very powerful system for the exhaustive analysis and powerful identification of Cl-/Br-PAH congeners and other organohalogen compounds in complex environmental samples. This is the first study for the identification of highly chlorinated PAHs (mono- through hepta chloro-substituted PAHs) in a real-world environmental sample by GC × GC–HRTOFMS. The proposed method provides many useful advantages for the identification of unknown Cl-/Br-PAHs, such as total ion monitoring (m/z 35–600) with accurate mass measurement in GC × GC, highly selective group type analysis in the 2D mass chromatograms with a 0.02 Da wide window and the calculation of the elemental composition from the

accurate mass of molecular ion, even with the moderate data acquisition speed (20 Hz). GC × GC–HRTOF-MS could detect more than 1000 compounds including Cl-/Br-PAH congeners and other organohalogen compounds in the complex real-world samples with only one injection. Additionally, this soil extract data has great possibilities in helping the post target analysis, because full spectrum acquisition with exact mass measurement was performed. In a future study, the standards of more highly substituted Cl-PAHs found in this current study are expected to be synthesized and examined for toxicity and quantified in various sample types to know the occurrence and effect of highly substituted Cl-PAHs on the environment and humans.

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