Optimisation of pre-treatment and ionisation for GC/MS analysis for the determination of chlorinated PAHs in atmospheric particulate samples

Yuta Kamiya, Fumikazu Ikemori and Takeshi Ohura

Graduate School of Agriculture, Meijo University, Nagoya 468-8502, Japan; Nagoya City Institute for Environmental Science, Nagoya 457-0841, Japan

(Received 6 January 2015; accepted 26 April 2015)

Chlorinated polycyclic aromatic hydrocarbons (ClPAHs) have been discovered to represent ubiquitous environmental pollutants in the last decade. In the present study, sample pre-treatment and ionisation conditions associated with the gas chromatography/mass spectrometry (GC/MS) analysis of ClPAHs were evaluated. The optimal pre-treatment of ambient air particulate samples was achieved using fractionation over silica gel with 10% dichloromethane in n-hexane as the eluent. The optimised condition of GC/MS with electron impact ionisation permitted analysis of all target ClPAHs. Not all target ClPAHs were detected using GC/MS with negative chemical ionisation, although this technique exhibited greater sensitivity for several of the compounds compared to electron impact ionisation. The analytical method was applied to the survey of ClPAHs in atmospheric particulate matter obtained close to an industrial site and in a standard sample of tunnel dust. Fourteen and eighteen species of ClPAHs were detected in the industrial air samples and tunnel dust, respectively, confirming the capability of the method. The compositions of ClPAHs were significantly different between air samples and tunnel dust. It suggests that alternative emission sources rather than vehicle exhaust could play a significant role in the air.

Keywords: chlorinated polycyclic aromatic hydrocarbon; air pollution; clean-up; tunnel dust; emission source

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are well-known environmental mutagens/carcinogens and are widely distributed throughout the environment [1–6]. As a result, efficient analytical methods for the analysis of PAHs have been established based on various analytical techniques, including US EPA Method 610 based on gas chromatography (GC) and US EPA 8310 using high-performance liquid chromatography (HPLC). The development of such analytical methods has made a significant contribution to our understanding of the presence of PAHs in the environment.

Chlorinated PAHs (ClPAHs) are a subcategory of PAH derivatives and are also recognised as potent environmental mutagens/carcinogens [7]. Although the biological toxicities of ClPAHs are not yet fully understood, the widespread environmental occurrence of ClPAHs has led to concern regarding the potential exposure risks [8]. Ohura and co-workers have performed detailed investigations of the atmospheric and sedimentary occurrences of ClPAHs and demonstrated that these compounds are just as widely
distributed in the environment as PAHs [9–15]. However, such studies of ClPAHs in the environment have been limited in comparison to the body of work concerning PAHs, possibly because of the lack of readily available ClPAH standards and/or limited information regarding analytical methods for ClPAH analysis. Recently, novel analytical techniques for ClPAHs have been developed to address this deficit. Ieda et al. [16] reported the qualitative analysis of ClPAHs using comprehensive two-dimensional GC/time-of-flight mass spectrometry (GC×GC/TOF-MS), and demonstrated that highly chlorine substituted PAHs (HCl-PAHs, more three chlorine substituted PAHs) were present in soil samples. Using state-of-the-art FT-ICR (Fourier transform ion cyclotron resonance), Fernando et al. [17] found both HCl-PAHs and high molecular weight (HMW)-ClPAHs (MW > 300) in a composite soil sample. These findings provide new insight into ClPAHs and suggest that both HCl-PAHs and HMW-ClPAHs are persistent in the environment. In these studies, the concentrations of ClPAHs in the environment were approximately one to three orders of magnitude lower than PAH concentrations. A total of 20 different ClPAHs were identified, containing three to five aromatic rings substituted with one to three chlorine atoms. To evaluate the potential emission sources, environmental occurrences and behaviours of these diverse ClPAHs, it will be necessary to develop analytical methods capable of determining multiple ClPAHs, including H-ClPAHs and HMW-ClPAHs, with high sensitivity.

GC/MS is one of most common analytical techniques for the determination of organic pollutants. The sensitivity and selectivity of a mass spectrometer will vary depending on the ion source and the ionisation mode and, although electron impact ionisation (EI) is the most common, chemical ionisation (CI) sometimes offers greater selectivity. The negative chemical ionisation (NCI) mode has been shown to be effective for the analysis of organochlorine compounds, but there have been no reports with regard to the sensitivity and selectivity of GC/MS in the NCI mode during ClPAH analysis. This study, therefore, had several goals: (1) the synthesis of H-ClPAHs and HMW-ClPAHs as new ClPAHs standard compounds, (2) the optimisation of pre-treatment processes in preparation for ClPAH analysis, (3) the establishment of analytical conditions of the detection of 24 ClPAHs using GC/MS in either the EI or NCI modes and (4) the application of this optimised method to survey airborne particles for ClPAHs. Based on the resulting data, the present report also discusses possible sources of ClPAHs.

2. Experimental
2.1. Chemicals

Pyrene (Py) and benzo[a]pyrene (BaP) were purchased from Kanto Chemicals (Tokyo, Japan) and Sigma Aldrich (St. Louis, MO), respectively. N-chlorosuccinimide (NCS) and sulphuryl chloride were used as chlorinating agents, both special grade, and were purchased from Wako Pure Chemicals (Osaka, Japan).

The target compounds consisted of 24 different ClPAHs as listed in Table 1. Detailed synthetic procedures for 20 of these ClPAHs have been described in previous reports [9,10]. The other four compounds were newly synthesised in this study, and the details are provided in the following section. Deuterated PAHs were used as internal standards. These were phenanthrene-d\(_{10}\) (Phe-d\(_{10}\)), fluoranthene-d\(_{10}\) (Fluor-d\(_{10}\)) and perylene-d\(_{12}\) (Pery-d\(_{12}\)), purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). Solvents used for extraction and clean-up were pesticide analysis grade, purchased from Wako Pure Chemical (Osaka, Japan) or Kanto Chemical (Tokyo, Japan).
Table 1. CIPAH results obtained from GC/MS analysis.

| No. | CIPAH                        | Abbreviation | Rings | Mw   | RT<sup>a</sup> (h:m:s) | Q1<sup>b</sup> | Q2<sup>c</sup> | LOD<sup>d</sup> | Q1  | Q2  | LOD  |
|-----|-----------------------------|--------------|-------|------|-------------------------|---------------|---------------|----------------|-----|-----|------|
| 1   | 9-Chlorophenanthrene        | 9-ClPhe      | 3     | 212.67 | 00:09:25               | 212           | 214, 176      | 0.07           |     |     |      |
| 2   | 2-Chloroanthracene          | 2-ClAnt      | 3     | 212.67 | 00:09:30               | 212           | 214, 176      | 0.07           | 212 | 214 | 4.36 |
| 3   | 9-Chloroanthracene          | 9-ClAnt      | 3     | 212.67 | 00:09:36               | 212           | 214, 176      | 0.07           | 212 | 214 | 4.4  |
| 4   | 3,9-Dichlorophenanthrene    | 3,9-ClPhe    | 3     | 247.12 | 00:11:35               | 246           | 248, 176      | 0.11           |     |     |      |
| 5   | 9,10-Dichloroanthracene     | 9,10-Cl2Ant  | 3     | 247.12 | 00:11:48               | 246           | 248, 176      | 0.12           | 246 | 248 | 0.36 |
| 6   | 1,9-Dichloroanthracene      | 1,9-ClPhe    | 3     | 247.12 | 00:11:48               | 246           | 248, 176      | 0.12           | 246 | 248 | 0.36 |
| 7   | 9,10-Dichlorophenanthrene   | 9,10-Cl2Phe  | 3     | 247.12 | 00:11:59               | 246           | 248, 176      | 0.13           |     |     |      |
| 8   | 3-Chlorofluoranthene        | 3-ClFluor    | 4     | 236.70 | 00:12:42               | 236           | 238, 200      | 0.09           | 236 | 238 | 0.3  |
| 9   | 8-Chlorofluoranthene        | 8-ClFluor    | 4     | 236.70 | 00:12:46               | 236           | 238, 200      | 0.09           | 236 | 238 | 0.12 |
| 10  | 1-Chloropyrene              | 1-ClPy       | 4     | 236.70 | 00:13:28               | 236           | 238, 200      | 0.11           | 236 | 238 | 1.08 |
| 11  | 3,9,10-trichlorophenanthrene| 3,9,10-Cl3Phe| 3     | 281.56 | 00:14:13               | 280           | 282, 210      | 0.23           | 280 | 282 | 1.92 |
| 12  | 1,3-Dichlorofluoranthene    | 1,3-Cl2Fluor | 4     | 271.14 | 00:14:54               | 270           | 272, 200      | 0.22           | 270 | 272 | 0.02 |
| 13  | 3,8-Dichlorofluoranthene    | 3,8-Cl2Fluor | 4     | 271.14 | 00:15:34               | 270           | 272, 200      | 0.24           | 270 | 272 | 0.03 |
| 14  | Dichloropyrene              | Cl2Py        | 4     | 271.14 | 00:16:20               | 270           | 272, 200      | 0.48           | 270 | 272 | 0.49 |
| 15  | 3,4-Dichlorofluoranthene    | 3,4-Cl2Fluor | 4     | 271.14 | 00:16:25               | 270           | 272, 200      | 0.3            | 270 | 272 | 0.09 |
| 16  | 6-Chlorochrysene            | 6-CIChry     | 4     | 262.73 | 00:17:51               | 262           | 264, 226      | 0.09           |     |     |      |
| 17  | 7-Chlorobenz[a]anthracene   | 7-CIBaA      | 4     | 262.73 | 00:18:01               | 262           | 264, 226      | 0.12           | 262 | 264 | 0.18 |
| 18  | Trichloropyrene             | Cl3Py        | 4     | 305.59 | 00:18:44               | 304           | 306, 234      | 0.48           | 304 | 306 | 1.48 |
| 19  | 6,12-Dichlorochrysene       | 6,12-Cl2Chry | 4     | 297.18 | 00:20:29               | 296           | 298, 226      | 0.28           | 296 | 298 | 0.17 |
| 20  | 7,12-Dichlorobenz[a]anthracene| 7,12-Cl2BaA| 4     | 297.18 | 00:20:36               | 296           | 298, 226      | 0.3            | 296 | 298 | 1.09 |
| 21  | Tetrachloropyrene           | Cl4Py        | 4     | 340.03 | 00:20:59               | 340           | 338, 268      | 0.26           | 340 | 338 | 0.96 |
| 22  | 6-Chlorobenzoflu[ap]pyrene  | 6-CIBaP      | 5     | 286.75 | 00:23:21               | 286           | 288, 250      | 0.22           | 286 | 288 | 0.66 |
| 23  | Dichlorobenzoflu[ap]pyrene  | Cl2BaP       | 5     | 321.20 | 00:26:15               | 320           | 322, 250      | 0.68           | 320 | 322 | 1.09 |
| 24  | Trichlorobenzoflu[ap]pyrene | Cl3BaP       | 5     | 355.64 | 00:29:15               | 354           | 356, 284      | 3.3            | 354 | 356 | 1.18 |

Note: <sup>a</sup>RT: retention time, <sup>b</sup>Q1: quantitation ion, <sup>c</sup>Q2: confirmation ion, <sup>d</sup>LOD: limit of detection (pg), <sup>e</sup>ND: not detected.
2.2. Synthesis of ClnPy and ClnBaP

The chlorinations of Py and BaP were performed to obtain the corresponding ClPAH standards since these precursors represent typical PAHs and are relatively abundant in the environment. Tri- and tetrachlorinated Py were obtained by adding NCS (4000 mg) to Py (200 mg) dissolved in chloroform (30 mL), and the mixture was held at 60°C for 72 h. Following the reaction, a white precipitate was observed in the reaction container. GC/MS analysis of this material indicated a mixture of trichloropyrene (Cl$_3$Py) and tetrachloropyrene (Cl$_4$Py). The precipitate was subsequently dissolved in toluene, followed by recrystallisation from methanol/water. Recrystallised powders were obtained by adjusting the water content in the solvent mixture, producing Cl$_4$Py with 95% purity as determined by its GC/MS spectrum peak area. Cl$_3$Py was obtained in 99% purity by further addition of water.

Dichlorinated BaP was synthesised by adding NCS (100 mg) to BaP (100 mg) dissolved in propylene carbonate (5 mL) and maintaining the mixture at 100°C for 2 h. The reaction mixture was subsequently fractionated by HPLC using a Shim-pack PREP-ODS column with methanol as the eluent. The fractions corresponding to the dominant peak were isolated and analysed by GC/MS, showing that Cl$_2$BaP was obtained in 99% purity.

Trichlorinated BaP was synthesised by adding sulphuryl chloride (1.67 mg) to BaP (110 mg) dissolved in chloroform (10 mL). The mixture was maintained at room temperature for several seconds, and a precipitate was observed. The product was isolated by filtration and analysed by GC/MS, showing that Cl$_3$BaP was obtained at 99% purity. It should be noted that the conformations of the synthesised ClPAHs were not determined via $^1$H-NMR because of poor solubility in CDCl$_3$ and/or the generation of unreadable $^1$H-NMR spectrum.

2.3. Comparison of clean-up processes

The application of the appropriate clean-up procedure can be vital to achieve high sensitivity when analysing environmental pollutants, and it is typically accomplished using column chromatography with silica gel and activated carbon [18]. In the present study, the clean-up of matrices containing various ClPAHs and PAHs was assessed using four column packings: silica gel (Supelclean™ LC-Si SPE tube, Sigma-Aldrich Co. Ltd, St Louis, MO), sulphoxide (Supelclean™ Sulfoxide SPE tube, Sigma-Aldrich), activated alumina (Wako Pure Chemical, Osaka, Japan) and activated carbon (dual-layer carbon reversible tube, Sigma-Aldrich).

In the case of silica gel, the packed column (1 g) was prewashed and conditioned with 20 mL of n-hexane. For sulphoxide, the packed column (3 g) was prewashed with 20 mL of acetone and conditioned with 40 mL of n-hexane. For alumina, the powder was first activated for more than 8 h at 130°C and then stored at room temperature in a desiccator. A portion of activated alumina (1 g) was then packed in a column and prewashed/conditioned using 20 mL of n-hexane. For activated carbon, a packed column was prewashed with 20 mL of toluene and conditioned with 40 mL of n-hexane.

The clean-up performance trials was carried out using a mixture of 1-ClPy (abbreviations of each ClPAH are noted in Table 1, 18.6 ng), 7-ClBaA (14.4 ng) and 6-ClBaP (14.1 ng) as typical ClPAHs, together with benz[a]anthracene (BaA) (28 ng) as a representative PAH and Fluor-d$_{10}$ (78 ng) and Pery-d$_{12}$ (50 ng) as internal standards (clean-up spike), all dissolved in 200 μL of n-hexane. A 200 μL aliquot was transferred onto each test column (n = 6) and eluted using 40 mL of n-hexane in the case of silica gel,
sulphoxide and activated alumina stationary phases. The activated carbon column was eluted with 30 mL of 10% acetone in n-hexane or 10% DCM in n-hexane, then the column was reversed and eluted with 40 mL of toluene. The eluate was fractionated into 2 mL portions and each portion was concentrated to approximately 500 μL under a gentle stream of nitrogen gas at 40°C. Phe-d_{10} (67 ng) was added to each concentrated fraction as second internal standard (syringe spike) and the resulting mixture was analysed by GC/MS (EI mode).

2.4. Air samples and tunnel dusts

Sampling of ambient air particulates was conducted at an industrial harbour site in Nagoya, Japan, located within 5 km of various chemical and industrial plants. Particles were captured on pre-combusted (450°C, 2 h) quartz fibre filters (QFF, 20.3 × 25.4 cm, Tokyo Dylec Co. Ltd., Tokyo, Japan) using a high volume air sampler (HV-1000 F; SIBATA Co. Ltd., Tokyo, Japan) operating at a constant flow rate of 1.0 m³/min. Sampling was conducted continuously over three-day sessions in all four seasons: from 17 May 2011 to 20 May 2011 (spring), 23 August 2011 to 26 August 2011 (summer), 29 November 2011 to 2 December 2011 (autumn) and 21 February 2012 to 24 February 2012 (winter). After sampling, the QFFs were separately wrapped in aluminium foil and sealed, then stored in a freezer at −35°C until extraction. The tunnel dust used was a certified reference material in tunnel dust (NMIJ CRM7308-a) that was purchased from the National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan [19].

Extraction and pre-treatment of those air samples and tunnel dust were conducted as follows: After collecting air samples, the QFFs were cut in half and inserted into 9 mL stainless steel ASE 300 extraction cells. Prior to extraction, Fluor-d_{10} (32 ng) and Pery-d_{12} (38.4 ng) were spiked onto the QFFs as internal standards. The QFF samples were extracted three times with dichloromethane at 150°C and 1500 psi using an ASE 300 accelerated solvent extraction system (Dionex Corporation, CA). During extractions, the oven heat up time was 7 minutes, static time was 5 minutes, flush volume was 120% and purge time was 60 seconds. The extracted samples were concentrated to approximately 1 mL on a rotary evaporator, applying a gentle stream of N₂ at 40°C. The concentrated solutions were subsequently passed through silica-gel columns as a cleaning step (see Section 3.1 about selection of column). Each silica-gel column was prewashed with 20 mL of n-hexane and the target compounds were eluted with 15 mL of 10% dichloromethane in n-hexane. The eluted solutions were concentrated to approximately 50 μL under a gentle stream of N₂ at 40°C and then added to 100 μL of inertness and refractory solvent, isoctane. Phe-d_{10} (67 ng) was finally added to each solution as syringe spike. The extraction and clean-up procedures applied to the tunnel dust samples (200 mg) were similar to the above procedures described for the QFFs. During the pre-treatment process, samples were protected from light to avoid photochemical degradation and were stored in a freezer at −35°C while awaiting GC/MS analysis.

Recoveries of the internal standards spiked into individual samples were 102 ± 8% for Fluor-d_{10} and 72%±13% for Pery-d_{12}. None of the target ClPAHs was detected in procedural blank samples. The instrumental limits of detection (LODs) of ClPAHs were calculated as three times the standard deviation of six replicate analyses of a diluted standard solution (0.8 ng/mL for 9,10-Cl₂Ant to 6.9 ng/mL for 2-ClAnt), which ranged from 0.03 (9-ClPhe) to 1.41 pg/m³ (Cl₃BaP) based on passing air volumes of 700 m³ through the QFFs. For statistical analysis purposes, the concentrations of non-detect samples were considered to be zero.
2.5. GC/MS conditions

Both CIPAHs and PAHs were analysed using a JMS-Q1000GC quadrupole MS (JEOL, Tokyo, Japan) in conjunction with a 7890A (Agilent Technologies) GC and an InertCap 5 MS/NP capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness, GL Science Inc., Tokyo, Japan). Helium was used as a carrier gas at a flow rate of 1.0 mL min⁻¹. During CIPAH analysis, the oven temperature was initially held at 100°C for 2 min, then increased from 100°C to 200°C at 25°C/min with no hold and finally ramped from 200°C to 300°C at 5°C/min then held at 300°C for 15 min. The injector and GC/MS transfer line were kept at 300°C and 280°C, respectively. These parameters were held constant throughout the evaluation of optimum GC/MS conditions.

The MS system was run in both EI and NCI modes to compare the performance of the two ionisation techniques. In both cases, the MS was operated in the selected ion monitoring (SIM) mode, and the ion current was held at 200 μA. The ion source temperatures applied for EI and NCI were 300°C and 150°C, respectively. The sample injection volume was 1 μL, using a pulsed splitless mode.

3. Results and discussion

3.1. Comparison of ionisation modes in GC/MS analysis of CIPAHs

The ionisation mode applied during the GC/MS analysis is potentially the most important factor affecting the sensitivity of the method. In the EI mode, the electron energy is generally set to 70 eV, although lower values can result in higher sensitivity by reducing cleavage of the molecular ions. The quantification (Q1) and confirmation ions (Q2) of the target CIPAHs were initially determined using the full scan mode (Table 1). Comparing the sensitivities of the method for the various CIPAHs as a function of the electron energy from 26 to 70 eV, all CIPAHs were detected, and it was determined that the sensitivities were slightly enhanced by increasing the energy, contrary to expectations (Fig. S1 in Supplemental data). That is, ionisation of CIPAHs will need sufficient energy of 70 eV. In addition, the effect of the detector voltage was evaluated by varying the voltage from −1000 to −1300 V, from which it was determined that the highest sensitivity (the highest signal-to-noise ratios) for most CIPAHs was obtained at −1150 V (Fig. S2). Hence, the optimum EI electron energy and detector voltage for CIPAH analysis were 70 eV and −1150 V, respectively.

GC/MS analysis operating in the NCI mode could be an effective tool for the detection of halogenated compounds, including CIPAHs, because this technique exhibits especially good selectivity and sensitivity for compounds with high electron affinity [20]. Thus, in the current project, the analysis of CIPAHs was also performed using GC/MS with NCI, applying essentially the same GC conditions as during analysis in the EI mode. The optimum CI gas (methane) flow rate for CIPAH analysis was first determined. Comparing the chromatograms obtained at various flow rates, the sensitivity was found to increase with increasing flow rates up to 60% and was then essentially constant up to a value of 70%. The optimum ion source temperature was also investigated, and sensitivity was observed to decrease as the temperature was raised from 140°C to 300°C. In addition, a comparison of results at various detector voltages showed that 1200 V gave the highest sensitivity. Although highly sensitive detection of some CIPAHs was possible using the NCI mode under the above analytical conditions, 9-CIPhe, 3,9-Cl₂Phe, 9,10-Cl₂Phe, 6-CICChry and 6,12-Cl₂Chry were not detected even when increasing the amount of analyte injected. The chromatograms obtained for the target CIPAHs using the optimised ionisation conditions are presented in Figure 1.

As noted, not all CIPAHs were detected by GC/MS when applying the NCI mode, while the EI mode was able to detect all the target compounds. On the other hand, the
NCI exhibited higher sensitivity for some of the ClPAHs compared to the EI mode. The sensitivities of the method for each ClPAH was determined based on the LOD values calculated from the standard deviations of triplicate analyses of diluted standard solutions. These LODs for the EI and NCI modes ranged from 0.07 (9-ClPhe) to 3.3 pg (Cl3BaP) and from 0.02 (1,3-Cl2Fluor) to 4.4 pg (9-ClAnt), respectively (Table 1). For some ClPAHs, the LOD values compare favourably to those reported for GC-high resolution MS [21]. Therefore, the sensitivities of the present GC/MS method with both EI and NCI mode could be sufficiently applied to analyse ClPAHs, assuming appropriate clean-up of the samples. When using EI, it was observed that the sensitivity decreased with increasing analyte molecular weight, although this same trend was not seen in the NCI data. This suggests that the ionisation efficiency in the NCI mode may be affected by molecular structure rather than molecular weight. Therefore, EI mode is better to be used to detect more compounds.

3.2. Comparison of clean-up columns for ClPAHs analysis

Optimisation of the sample clean-up process was performed using four different stationary phases: silica gel, sulphoxide, alumina and activated carbon.

The elution of all test compounds from a silica gel column was achieved by using 12 mL of 100% n-hexane (Figure 2A-a), and the recovery values ranged from 83 (Pery-d_{12}) to 97% (1-ClPy). When 10% DCM in n-hexane was used as the elution solvent, all test compounds were eluted when applying 10 mL of the solvent (Figure 2A-b), and the recovery values ranged from 87 (Fluor-d_{10}) to 100% (Pery-d_{12}). Comparing the elution profiles of n-hexane and 10% DCM in n-hexane, it is evident that the test compounds
were stripped from the column more rapidly by the 10% DCM solvent (Figure 2A). Nonetheless, pure n-hexane appears to be superior to 10% DCM in n-hexane as an elution solvent because the 10% DCM solvent also elutes contaminants from the column.

The elution of all test compounds from a sulphoxide column was achieved using 22 mL of 100% n-hexane (Figure 2B-a), and the recovery values ranged from 82 (Fluor-d$_{10}$) to 116% (6-ClBaP). In contrast, 12 mL of 10% DCM in n-hexane (Figure 2B-b) stripped off all test compounds and generated recovery values ranging from 82 (Fluor-d$_{10}$) to 125% (Pery-d$_{12}$). Once again, the 10% DCM in n-hexane was superior to the 100% n-hexane as an elution solvent. Based on these results, it would be desirable to first use 4 mL of 100% n-hexane for the removal of contaminants, followed by approximately 12 mL of 10% DCM in n-hexane to obtain the target analytes.

The elution of all test compounds from the activated alumina column was achieved with 2 mL of 100% DCM after 40 mL of 100% n-hexane (Figure 2C-a), whereas all compounds were eluted using only 8 mL of 10% DCM in n-hexane (Figure 2C-b). The recovery values obtained with 10% DCM in n-hexane ranged from 89 (BaA) to 107% (Pery-d$_{12}$). The clean-up of CIPAHs using an activated alumina column should therefore be performed while paying close attention to the elution conditions.

Figure 2. Elution profiles of selected compounds from various columns using different solvents. Stationary phases: (A) silica gel, (B) sulphoxide, (C) activated alumina and (D) activated carbon. Eluents: (a) n-hexane or (b) 10% dichloromethane (DCM) in n-hexane, except in (D) where toluene was used after a prewash with (a) 10% DCM in n-hexane or (b) 10% acetone in n-hexane.
attention to the adjustment of the elution solvent because the elution profiles vary considerably depending on the composition of the solvent.

Activated carbon is typically used in reverse phase columns, often in the final clean-up step of aromatics such as dioxins. All test compounds were back-eluted from an activated carbon column using 16 mL of toluene following a prewash with 30 mL of 10% DCM in n-hexane (Figure 2D-a). The recovery values ranged from 91 (Fluor-d<sub>10</sub>) to 144% (6-ClBaP). In addition, a similar elution profile was obtained with 16 mL of toluene when instead applying 10% acetone in n-hexane as the prewash solvent (Figure 2D-b), and the recovery values ranged from 89 (Fluor-d<sub>10</sub>) to 113% (6-ClBaP). There were no differences in the elution profiles between these two prewash solvents.

### 3.3. Application to survey of ambient particles and tunnel dust

The clean-up procedures developed above were evaluated by performing a clean-up of the ambient air samples. Comparing the GC/MS chromatograms of CIPAHs in pre-treated samples obtained from each column, no significant differences were observed even when applying a combination of each column (Fig. S3). As such, the pre-treatment of CIPAHs in air samples obtained from suitably large sampling volumes could be accomplished using solely silica gel columns. This pre-treatment process was thus applied to the survey of CIPAHs associated with particles found in atmospheric samples from industrial air in Japan.

A total of 14 of the 25 target CIPAHs were detected in the air samples throughout the four seasons when using the optimised GC/MS procedure in the EI mode (Table 2). The concentrations of total CIPAHs ranged from 28.2 to 144.4 pg/m<sup>3</sup>, with an annual mean concentration of 94.4 pg/m<sup>3</sup>. This mean value was approximately three times greater than values determined in our previous study conducted in a Japanese city [9], suggesting that the high level of CIPAHs observed in the present study could be due to the presence of significant local industrial emission sources. With regard to the individual CIPAHs, 6-ClBaP was the most abundant, followed by 1-ClPy and Cl<sub>2</sub>BaP. It was interesting to find that the newly synthesised target compound Cl<sub>2</sub>BaP was present at relatively high levels among the CIPAHs found in air, although the possible sources remain unclear.

To date, there has been no information available regarding the principal sources affecting the ambient concentrations of CIPAHs. Herein we investigated the occurrence of CIPAHs in tunnel dust, which has been reported as one possible source of ambient CIPAHs [22]. The tunnel dust used in this study was a certified reference material (NMIJ CRM7308-a, AIST). Nineteen species of CIPAHs were detected in the tunnel dust, and the total CIPAH concentration was 914 ng/g (Table 2). The total CIPAH concentrations (based on the total suspended particle concentrations) in the air samples ranged from 1225 to 3654 ng/g with a mean concentration of 2047 ng/g, indicating that the CIPAH concentration in the tunnel dust was significantly lower than the levels in air (Table 2). Furthermore, a comparison of the individual CIPAH compositions showed that the dust and air samples exhibited very different profiles. While 6-ClBaP accounted for 52% of the total CIPAH concentration in the air samples, 2-ClAnt was dominant (25% of the total) in the tunnel dust, which contained only 2% 6-ClBaP (Figure 3A). In addition, three-ring compounds accounted for 44% of the CIPAHs in the tunnel dust, whereas the contributions of three-ring CIPAHs were as low as 13% in the air samples (Figure 3B). Thus, the CIPAHs profile of the tunnel dust greatly differed from those of the air samples. These findings seem to indicate that vehicle exhaust is not the major contributor of CIPAHs found at the sampling site and, therefore, alternative emission sources must play a
Table 2. Concentrations of ClPAHs in air near an industrial site, Nagoya, Japan\textsuperscript{a} and in a standard tunnel dust sample.

| ClPAHs                  | Concentration (pg/m\textsuperscript{3}) | Concentration (ng/g) | Tunnel dust (ng/g, n = 3) |
|-------------------------|----------------------------------------|----------------------|---------------------------|
|                         | May  | August | November | February | Mean | May  | August | November | February | Mean | SD | Mean  | SD   |
| 9-ClPhe                 | 11.44| 0.44   | 3.87     | 1.98     | 4.43 | 154.67| 19.55   | 122.61   | 39.72    | 84.14 |    | 102.70| 3.59 |
| 2-ClAnt                 | 1.62 | 1.43   | 1.91     | 2.57     | 1.88 | 21.98 | 63.62   | 39.14    | 52.36    | 44.28 |    | 225.28| 4.62 |
| 9-ClAnt                 | 1.56 | 1.55   | 1.65     | 1.51     | 1.57 | 20.72 | 68.41   | 38.48    | 30.03    | 39.41 |    | 4.18  | 0.19 |
| 3,9-Cl\textsubscript{2}Phe | 1.33 | 0.67   | 3.83     | nd       | 1.46 | 18.14 | 29.30   | 74.72    | nd       | 30.54 |    | 13.14 | 0.77 |
| 9,10-Cl\textsubscript{2}Ant+1,9-Cl\textsubscript{2}Phe | nd\textsuperscript{b} | nd | 0.21   | 0.48   | 0.17 | nd | nd | 7.72 | 9.95 | 4.42 | 18.00 | 0.43 |
| 9,10-Cl\textsubscript{2}Phe | 3.18 | 1.93   | 3.13     | 2.27     | 2.63 | 43.15 | 85.95   | 78.16    | 46.11    | 63.34 |    | 30.69 | 0.67 |
| 3-ClFlu                 | 4.82 | nd     | 3.09     | 3.76     | 2.92 | 65.48 | nd      | 75.79    | 77.66    | 54.73 |    | 73.24 | 3.25 |
| 8-ClFlu                 | nd   | nd     | nd       | nd       | nd   | nd   | nd      | nd       | nd       | nd   |    | nd   |    |
| 1-ClPy                  | 13.98| 2.81   | 13.12    | 14.51    | 11.11| 187.63| 124.49  | 297.47   | 292.63   | 225.55|    | 164.79| 2.81 |
| 3,9,10-Cl\textsubscript{3}Phe | nd  | nd     | nd       | nd       | nd   | nd   | nd      | nd       | nd       | nd   |    | 5.20  | 0.33 |
| 1,3-Cl\textsubscript{2}Flu | nd  | nd     | nd       | nd       | nd   | nd   | nd      | nd       | nd       | nd   |    | 7.33  | 0.68 |
| 3,8-Cl\textsubscript{2}Flu | nd  | nd     | nd       | nd       | nd   | nd   | nd      | nd       | nd       | nd   |    | 15.09 | 2.20 |
| Cl\textsubscript{2}Py    | 6.72 | 3.97   | 5.64     | 4.11     | 5.11 | 92.92 | 176.47  | 129.45   | 83.26    | 120.53|    | 18.29 | 3.87 |
| 3,4-Cl\textsubscript{2}Flu | nd  | nd     | 1.50     | nd       | 0.38 | nd   | nd      | 20.07    | nd       | 5.02  |    | 10.13 | 1.27 |
| 6-ClChry                | nd   | nd     | nd       | nd       | nd   | nd   | nd      | nd       | nd       | nd   |    | 44.14 | 3.35 |
| 7-ClBaA                 | 7.11 | 2.45   | 9.09     | 4.90     | 5.89 | 95.17 | 103.82  | 189.41   | 100.53   | 122.23|    | 146.47| 9.15 |
| Cl\textsubscript{2}Py    | nd   | nd     | nd       | nd       | nd   | nd   | nd      | nd       | nd       | nd   |    | nd   |    |
| 6,12-Cl\textsubscript{2}Chry | nd  | nd     | nd       | nd       | nd   | nd   | nd      | nd       | nd       | nd   |    | 12.08 | 0.96 |
| 7,12-Cl\textsubscript{2}BaA | nd  | nd     | nd       | nd       | nd   | nd   | nd      | nd       | nd       | nd   |    | 9.82  | 0.82 |
| Cl\textsubscript{3}Py    | nd   | nd     | nd       | nd       | nd   | nd   | nd      | nd       | nd       | nd   |    | nd   |    |
| 6-ClBaP                 | 54.21| 12.95  | 82.97    | 49.85    | 49.99| 723.89| 552.96  | 2047.15  | 947.35   | 1067.84|    | 13.84 | 0.67 |
| Cl\textsubscript{2}BaP   | 7.11 | nd     | 12.32    | 5.81     | 6.31 | 92.83 | nd      | 458.17   | nd       | 166.29|    | nd   |    |
| Cl\textsubscript{3}BaP   | nd   | nd     | 2.02     | nd       | 0.51 | nd   | nd      | 75.18    | nd       | 18.80 |    | nd   |    |
| Total ClPAHs            | 113.08| 28.19 | 144.37   | 91.75    | 94.35| 1516.58| 1224.57 | 3653.51  | 1793.79  | 2047.11|    | 914.43| 12.10|

Note: \textsuperscript{a}The number of samples analysed in May, August, November and February are 3, 2, 3 and 2, respectively. \textsuperscript{b}nd: not detected.
significant role. This also implies that the particulate CIPAHs in ambient air could vary between different sampling locations.

4. Conclusion
In the present study, we first synthesised four species representing more highly CIPAHs or HMW-CIPAHs as new analytical standards. In addition, we assessed the effects of clean-up processes and ionisation modes in a series of analytical trials determining CIPAHs using GC/MS. The pre-treatment of CIPAHs in air samples could be accomplished using solely silica gel columns with 10% dichloromethane in n-hexane as the eluent. In addition, GC/MS with EI mode is better to be used to detect more CIPAHs. A subsequent survey of CIPAHs associated with particulates was conducted using the optimised analytical method, and it was demonstrated that this method was capable of the detection of CIPAHs in air samples, thus allowing testing for the presence of these compounds in the environment. The specific profile obtained from air samples was compared to that of tunnel dust, and the results suggested that CIPAHs in ambient air resulted from industrial processes rather than traffic sources. This newly optimised analytical method for CIPAHs requires no specific pre-treatment and could further expand the analysis of CIPAHs and increase our understanding of the presence of such compounds in the environment.

Disclosure statement
No potential conflict of interest was reported by the authors.
Funding
This work was supported in part by the Environment Research and Technology Development Fund [Rbf-1103] of the Ministry of the Environment, Japan, and by the Ministry of Education, Culture, Sports, Science and Technology, via a Grant-in-Aid for Scientific Research (C) [No. 26340015].

Supplemental data
Supplemental data for this article can be accessed at http://dx.doi.org/10.1080/03067319.2015.1048439

References
[1] S.O. Baek, R.A. Field, M.E. Goldstone, P.W. Kirk, J.N. Lester and R. Perry, Water Air Soil Pollut. 60, 279 (1991). doi:10.1007/BF00282628
[2] C.E. Bostrom, P. Gerde, A. Hanberg, B. Jernstrom, C. Johansson, T. Kyrklund, A. Rannug, M. Tornqvist, K. Victorin and R. Westerholm, Environ. Health Perspect. 110, 451 (2002). doi:10.1289/ehp.02110s3451
[3] T. Ramdahl, Environ. Sci. Technol. 17, 666 (1983). doi:10.1021/es00117a008
[4] T. Wenzl, R. Simon, J. Kleiner and E. Anklam, Trends Anal. Chem. 25, 716 (2006). doi:10.1016/j.trac.2006.05.010
[5] K. Ravindra, R. Sokhi and R. Van Grieken, Atmos. Environ. 42, 2895 (2008). doi:10.1016/j.atmosenv.2007.12.010
[6] K.H. Kim, S.A. Jahan, E. Kabir and R.J. Brown, Environ. Int. 60, 71 (2013). doi:10.1016/j.envint.2013.07.019
[7] P.P. Fu, L.S. Von Tungeln, L.-H. Chiu and Z.Y. Own, J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 17, 71 (1999). doi:10.1080/10590509909373510
[8] J.-L. Sun, H. Zeng and H.-G. Ni, Chemosphere 90, 1751 (2013). doi:10.1016/j.chemosphere.2012.10.094
[9] T. Ohura, A. Kitazawa, T. Amagai and M. Makino, Environ. Sci. Technol. 39, 85 (2005). doi:10.1021/es040433s
[10] T. Ohura, S. Fujima, T. Amagai and M. Shinomiya, Environ. Sci. Technol. 42, 3296 (2008). doi:10.1021/es703068n
[11] T. Ohura, ScientificWorldJournal 7, 372 (2007). doi:10.1100/tsw.2007.75
[12] Y. Horii, T. Ohura, N. Yamashita and K. Kannan, Arch. Environ. Contam. Toxicol. 57, 651 (2009). doi:10.1007/s00244-009-9372-1
[13] K. Kakimoto, H. Nagayoshi, Y. Konishi, K. Kajimura, T. Ohura, K. Hayakawa and A. Toriba, Chemosphere 111, 40 (2014). doi:10.1016/j.chemosphere.2014.03.072
[14] J. Ma, Z. Chen, M. Wu, J. Feng, Y. Horii, T. Ohura and K. Kannan, Environ. Sci. Technol. 47, 7615 (2013). doi:10.1021/es400333h
[15] T. Ohura, K. Sawada, T. Amagai and M. Shinomiya, Environ. Sci. Technol. 43, 2269 (2009). doi:10.1021/es803633d
[16] T. Ieda, N. Ochiai, T. Miyawaki, T. Ohura and Y. Horii, J. Chromatogr. A 1218, 3224 (2011). doi:10.1016/j.chroma.2011.01.013
[17] S. Fernando, K.J. Jobst, V.Y. Taguchi, P.A. Helm, E.J. Reiner and B.E. McCarry, Environ. Sci. Technol. 48, 10656 (2014). doi:10.1021/es503428j
[18] R. Barro, J. Regueiro, M. Llompart and C. Garcia-Jares, J. Chromatogr. A 1216, 540 (2009). doi:10.1016/j.chroma.2008.10.117
[19] N. Itoh, K. Inagaki, T. Narukawa, Y. Aoyagi, I. Narushima, M. Koguchi and M. Numata, Anal. Bioanal. Chem. 401, 2909 (2011). doi:10.1007/s00216-011-5399-z
[20] A.G. Harrison, Chemical Ionization Mass Spectrometry, 2nd ed. (CRC Press, Boca Raton, FL, 1992).
[21] Y. Horii, G. Ok, T. Ohura and K. Kannan, Environ. Sci. Technol. 42, 1904 (2008). doi:10.1021/es703001f
[22] U.L. Nilsson and C.E. Oestman, Environ. Sci. Technol. 27, 1826 (1993). doi:10.1021/es00046a010