Development of LC-DA-APPI-MS/MS method for determination of nitrated polycyclic aromatic hydrocarbons and its diurnal variations during transboundary air pollution events

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Introduction

Nitrated polycyclic aromatic hydrocarbons (NPAHs) are widespread pollutants in the atmosphere and toxicities of some NPAHs have been assigned much higher than those of parent polycyclic aromatic hydrocarbons (PAHs)[1]. The NPAHs are generated by incomplete combustion of fuel and biomass and also formed from the heterogeneous reactions between parent PAHs and atmospheric oxidants. The distinction and quantification of the NPAHs sources are important to know dynamics and health effects of NPAHs in the environment. Previous observation of NPAHs in the atmosphere indicated that vehicle exhaust and secondary formation affect the diurnal variations of concentrations of NPAHs[e.g.2]. In Japan, effects of transboundary air pollution from the Asian continent have been paid attention recently due to decreasing in a primary local emission of air pollutant. Therefore, we focused on diurnal variations of NPAHs during transboundary air pollution event to know the secondary formation of NPAHs from long-range transported PAHs.

Prior to the atmospheric sample analysis, we developed a method for quantifications of NPAHs using a liquid chromatography with dopant assisted atmospheric pressure ionization tandem mass spectrometry (LC-DA-APPI-MS/MS). Gas chromatography (GC) and LC techniques have been employed for the determination of NPAHs. The LC technique has an advantage in the analysis of thermally degradable NPAHs since GC analysis requires injection at high temperature. Therefore, LC methods have been developed for the determination of NPAHs, and researchers who employ MS/MS detection have been increasing due to high selectivity. Compared with ionization method and LC mode, the highest sensitivity for the measurement of dinitropyrene was obtained by normal phase LC mode and APPI detection with toluene as dopant[3]. Most recently, Smith et al. demonstrated that mixture of aryl and methoxybenzenes was effective dopant to enhance sensitivities of PAHs in reverse phase LC mode with APPI-MS/MS detection[4]. We applied these dopants to NPAHs analysis and optimized dopant and eluent compositions for reverse phase LC-DA-APPI-MS/MS.

Materials and methods

1) Reagents and materials

5-Nitroacenaphthene (5-NAce), 9,10-Dinitroanthracene (9,10-DNAnt), 9-Nitroanthracene (9-NAnt), 2-Nitoroanthracene (2-NAnt), 3-Nitrofluoranthene (3-NFR), 6-Nitrochrysene (6-NChr), 7-Nitrobenz[a]anthracene (7-NBaA), 1,3-Dinitropyrene (1,3-DNP), 1,6-Dinitropyrene (1,6-DNP), 1,8-Dinitropyrene (1,8-DNP), 1-Nitropyrene (1-NP), 2-Nitropyrene (2-NP), and 6-Nitrobenzo[a]pyrene (6-NBaP) were obtained from AccuStandard (New Haven, CT, USA) and 2-Nitrofluoranthene (2-NFR) from Chiron AS (Trondheim, Norway). Stable isotopes of some NPAHs were purchased from Cambridge Isotope Laboratories (Andover, MA, USA) and used as clean-up (5-NAce-d, 9-NA-d, 3-NFR-d, 6-NChr-d, and 1-NP-d) and syringe spikes (2-Nitrofluorene-d).

Mixtures of PAHs (FR, Fluoranthene; Py, Pyrene; BaA, Benz[a]Anthracene; Chr, Chrysene; BbF, Benzo[b]Fluoranthene; BkF, Benzo[k]Fluoranthene; BeP, Benzo[e]Pyrene; BaP, Benzo[a]Pyrene; Per, Perylene; IndP, Indeno[1,2,3-cd]pyrene; and B(ghi)P, Benzo[ghi]perylene) and deuterated PAHs solutions were obtained from Wellington Laboratories (Guelph, Ontario, Canada)

The standard reference material SRM 2975 (diesel particulate matter collected from a diesel-powered forklift engine) was obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA).

2) *LC-MS/MS*

LC-MS/MS measurements were carried out with a Waters ACQUITY UPLC system and Waters XEVO TQ MS tandem quadrupole mass spectrometer (Waters, Milford, MA, USA) equipped with APPI source. An Inertsil Phenyl HP column (50 mm × 2.1 mm i.d., 3 μm particle size, GL Sciences, Tokyo, Japan) and an Inertsil ODS-P HP column (250 mm × 2.1 mm i.d., 3 μm particle size, GL Sciences, Tokyo, Japan) were connected tandem for measurement of NPAHs. The temperature of the Inertsil Phenyl and Inertsil ODS-P columns was 40°C and 20°C, respectively. The connected columns were eluted with a gradient of 70 % methanol (eluent A) and methanol (eluent B) (0% B for 2min, 0–100% B in 13 min, 100% B for 15 min) at a flow rate of 200 μL min^{-1}. Dopant was supplied by another single-piston pump (Reagent Manager, Waters) at a flow rate of 70 μL min^{-1} and combined with the eluent before entering the ion source using a tee. The optimized dopant composition was toluene/anisole (99.5:0.5, v/v). The anisole was distilled before use. The injection volume was 10 μL. The sensitivities of NPAHs were compared with different dopant (n = 3): toluene, anisole, toluene/anisole (99.5:0.5, v/v), bromobenzene (BrBz)/2,4-difluoro anisole (DFA) (99.5:0.5, v/v), chlorobenzene (ClBz)/3-(trifluoromethyl)anisole (TMFA) (99.5:0.5, v/v), and isoprene.

Analysis of PAHs was conducted on the Inertsil ODS-P column at 20°C with water/methanol gradient and BrBz/DFA (99.5:0.5, v/v) as dopant.

3) *Sample*

Atmospheric aerosols with aerodynamic diameter less than 2.5 μm ($PM_{2.5}$) were collected on pre-combusted quartz filter by high volume air sampler equipped with an impactor (TE-231; Tisch Environmental, Village of Cleaves, OH, USA) at a flow rate of 1,130 L min^{-1}. The sampling site was located in Osaka which is metropolis in west Japan. The $PM_{2.5}$ samples were collected during transboundary air pollution from the Asian continent[5]; 14–18 April 2014, 26 May–3 June 2014, 10–13 November 2014, and 23–27 February 2015. The sampling time interval was 6, 12, or 24 h. Concentrations of $PM_{2.5}$, NO, and NO_2 in the atmosphere were calculated from monitoring data of 9–14 stations in Osaka city.

4) *Extraction and pre-treatment*

A half of the quartz filter or 20 mg of NIST sample was extracted with 200 mL of dichloromethane by sonication for 20 min. The clean-up spike was added to each sample before extraction. Extraction was repeated twice with 150 and 50 mL of dichloromethane and combined extracts were evaporated. The concentrated extract was washed with 2 M NaOH and aqueous layer was discarded. Residual organic layer was exchanged with *n*-hexane after washed with water and dried over sodium sulfate. The *n*-hexane solution was purified by SPE on silica cartridge (Sep-Pak Plus Silica, Waters). PAHs fraction was eluted with *n*-hexane, then syringe spike and dimethyl sulfoxide (DMSO) were added. The PAHs fraction was evaporated to under nitrogen, the remaining dissolved in 1 mL of methanol. NPAHs fraction was eluted from silica cartridge with *n*-hexane/dichloromethane (7:3, v/v), and path through an aminopropyl SPE cartridge (Sep-Pak Plus NH_2, Waters). The syringe spike and DMSO were added to the eluate, and then the NPAHs fraction was evaporated and dissolved in 0.1 mL of methanol. The PAHs and NPAHs fractions were filtrated (0.2 μm) and analyzed by LC-DA-APPI-MS/MS.

5) *Matrix effect and limit of detection (LOD)*

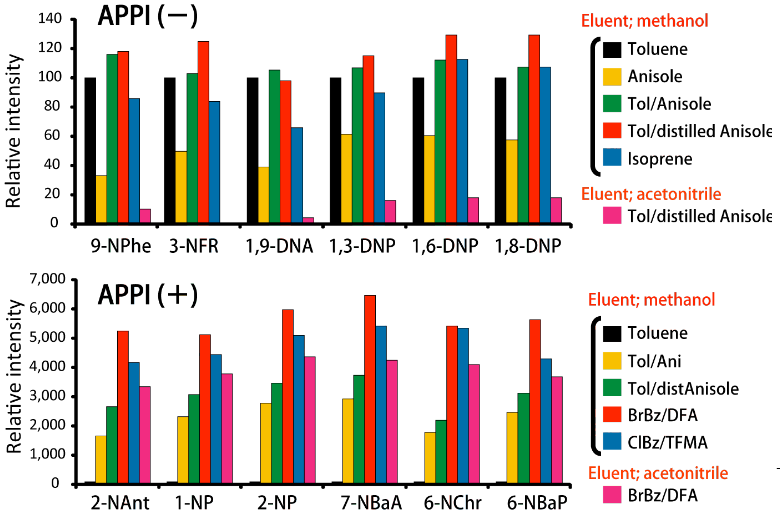
Matrix effects on ionization efficiency were calculated for NPAHs comparing standard solutions and $PM_{2.5}$ extracts spiked with the same account of analytes after purification to exclude an effect of sample treatment (n = 2). Responses of spiked NPAHs were defined as difference between the responses of spiked sample and those of unspiked sample. LOD corresponded to the analyte concentration for which the area was equal to three times the standard deviation of repeated analysis of 1 or 5 ng mL^{-1} standard solution (n = 5).

Results and discussion:

1) *Effects of dopant on the sensitivity of NPAHs*

The effects of the dopant on relative intensities of selected NPAHs are shown in Figure 1, in which the chromatographic peak area of each analyte obtained with the various dopants is normalized to the value obtained with the toluene dopant. When methanol was used as an eluent for APPI in negative ion mode, BrBz/DFA and ClBz/TMFA showed low ionization efficiencies. The toluene/anisole mixture provided higher sensitivities for the NPAHs in negative mode APPI. Removal of impurity in anisole by distillation resulted in the increase in the sensitivities for the NPAHs. In addition, our result clearly shows that methanol is remarkably effective eluent for APPI in negative ion mode compared to acetonitrile.

Differences in the sensitivities for the NPAHs in positive mode APPI between methanol and acetonitrile were not significant. Similar to the result of a comparison study of dopant for PAHs by Smith et al.[4], the BrBz/DFA mixture provided the highest sensitivities for the NPAHs in positive mode APPI. We selected methanol and the toluene/anisole mixture as eluent and dopant, respectively, because our mass spectrometer showed less sensitivity in negative ion mode.



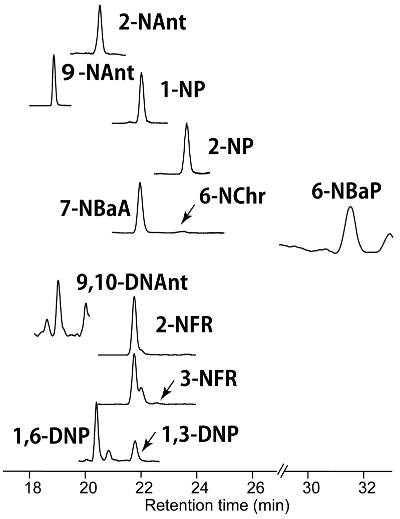


Fig. 1 Effects of dopants on relative intensities of NPAHs.

Fig. 2 Chromatograms of NPAHs in a $PM_{2.5}$ extract.

2) Method performance

Chromatograms of NPAHs in a $PM_{2.5}$ extract obtained by the developed method are described in Figure 2. Twelve NPAHs were detected selectively. Because the Inertsil ODS-P column could not separate 1-NP and 2-NFR, we connected the Inertsil Phenyl and the Inertsil ODS-P columns in series for chromatographically separation of these NPAHs. The LODs of NPAHs were 0.091–1.7 ng mL^{-1}. The calculated matrix effects of NPAHs were -14–1.8% and were sufficiently low.

The quantification results of SRM 2975 are shown in Table 1. The levels of NPAHs measured were in agreement with certified and reference values of NIST. Relatively lower contents of some NPAHs in this study compared with the data published by NIST may be resulted from a difference in extraction method. The developed method was sensitive enough to detect the NPAHs in 20 mg of SRM 2975 except for 2-NFR, and low coefficient of variation (< 7%) indicated an accuracy of the method.

Table 1: Contents of NPAHs in SRM 2975.

Analyte	NIST ($\mu g\ g^{-1}$)	This study ($\mu g\ g^{-1}$)[c]
9-NAnt	3.07 ± 0.28[b]	1.92 ± 0.096
2-NFR	0.231 ± 0.032[a]	Not detected.
3-NFR	3.80 ± 0.24[a]	2.96 ± 0.081
6-NChr	2.45 ± 0.33[a]	1.80 ± 0.059
7-NBaA	3.57 ± 0.32[a]	3.20 ± 0.15
1,3-DNP	1.12 ± 0.21[b]	1.30 ± 0.088
1,6-DNP	2.35 ± 0.51[b]	3.02 ± 0.16
1,8-DNP	3.10 ± 0.95[b]	2.44 ± 0.044
1-NP	35.2 ± 2.2[a]	36.5 ± 0.41
6-NBaP	1.36 ± 0.40[b]	1.21 ± 0.024

[a] Certified value. [b] Reference value.
[c] Mean value ± standard deviation ($n = 3$).

Table 2: Summary of concentrations of $PM_{2.5}$, PAHs, and NPAHs.

Date YY/MM/DD	$PM_{2.5}$ ($\mu g\ m^{-3}$)	ΣPAHs ($pg\ m^{-3}$)	ΣNPAHs ($pg\ m^{-3}$)
14/04/14–18	15.0–55.4 (33.4)	1,263–6,488 (3,525)	28–504 (211)
14/05/26–06/3	4.8–63.3 (36.2)	637–6,414 (2,274)	22–630 (132)
14/11/10–13	6.9–29.1 (15.7)	919–8,956 (3,542)	19–255 (108)
15/02/23–27	17.8–38.3 (28.3)	2369–7,908 (5,213)	96–225 (160)

* Minimum–Maximum (Mean).

3) *Diurnal concentrations of NPAHs and PAHs in Osaka city during transboundary air pollution*

Summary of the concentrations of $PM_{2.5}$, a sum of PAHs (∑PAHs), and a sum of NPAHs (∑NPAHs) are shown in Table 2. The concentrations of ∑NPAHs ranged within values previously observed by some researchers[6]. Maximum of ∑PAHs was higher in Autumn and Winter compared with Spring. In contrast, the relatively higher value of the maximum of ∑NPAHs was observed in Spring, suggesting the differences in concentrations of gas phese PAHs or in the formation rate of NPAHs with the season.

Figure 3 shows the temporal variations of concentrations of NPAHs, PAHs, $PM_{2.5}$, NO, and NO_2 on May 26–June 3, 2014, at Osaka city. Preliminary analysis revealed that the increase in the concentrations of $PM_{2.5}$ during May 27–June 2 was the effect of transboundary air pollution from the Asian continent[5]. The relatively high concentrations of PAHs and the ratios of FR/Py (1.2) and BaP/BeP (0.57) during this period indicated that the PAHs were originated from coal combustion and transported long distance[7,8]. Interestingly, diurnal variations in the concentrations of NPAHs differed from those of PAHs. It appears that the concentrations of secondary NPAHs were increased at night time; this trend was pronounced after the remarkable increase in the concentrations of PAHs. Mean value of a ratio of 2-NFR/2-NP (47) indicated that NO_3 initiated pathway was dominant formation pathway of 2-NFR[9]. Our results suggested that portions of the PAHs originated from the transboundary air pollution were nitrated at night time mainly via the NO_3 initiated pathway.

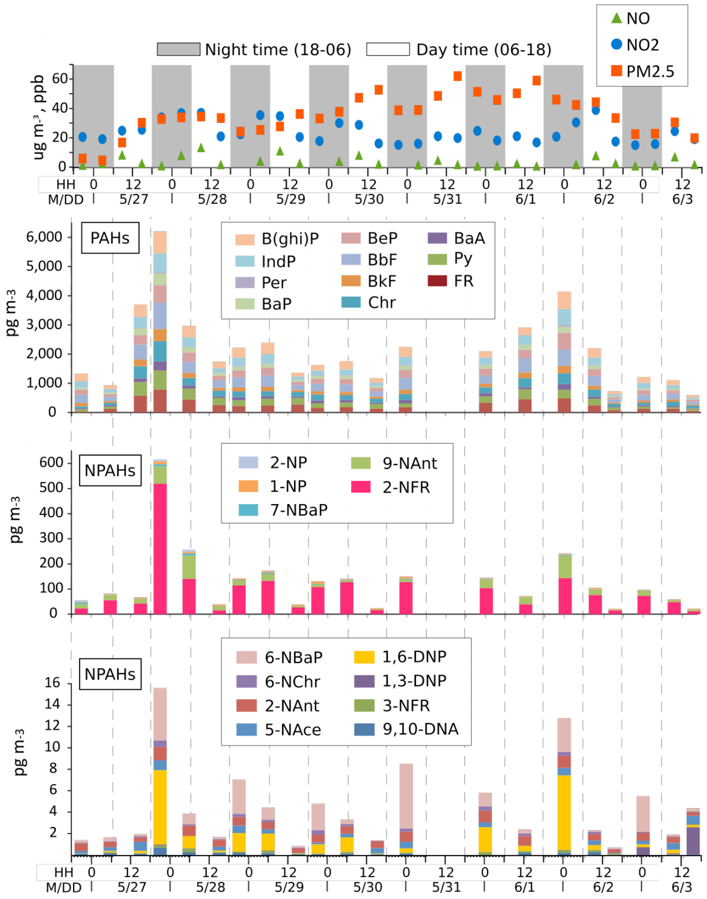


Fig. 3 Concentrations of PAHs, NPAHs, $PM_{2.5}$, NO, and NO_2 in May 26–June 3, 2014 at Osaka, Japan.

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