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Simultaneous Determination of Polycyclic Aromatic Hydrocarbons and Their Nitro-derivatives in Airborne Particulates by Using Two-dimensional High-performance Liquid Chromatography with On-line Reduction and Fluorescence Detection

Yaowatat Boongla[1)], Walaiporn Orakij[1)], Yuuki Nagaoka[1)], Ning Tang[2),3)], Kazuichi Hayakawa[3)] and Akira Toriba[2),*]

[1)]Graduate School of Medical Sciences, Division of Pharmaceutical Sciences, Kanazawa University, Kakuma-machi, Kanazawa, Ishikawa 920-1192, Japan
[2)]Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan
[3)]Institute of Natural Science and Environmental Technology, Kanazawa University, Kakuma-machi, Kanazawa, Ishikawa 920-1192, Japan

*Corresponding author. Tel: +81-76-234-4457, E-mail: toriba@p.kanazawa-u.ac.jp

ABSTRACT

An analytical method using high-performance liquid chromatography (HPLC) with fluorescence (FL) detection was developed for simultaneously analyzing 10 polycyclic aromatic hydrocarbons (PAHs) and 18 nitro-derivatives of PAHs (NPAHs). The two-dimensional HPLC system consists of an on-line clean-up and reduction for NPAHs in the 1st dimension, and separation of the PAHs and the reduced NPAHs and their FL detection in the 2nd dimension after column-switching. To identify an ideal clean-up column for removing sample matrix that may interfere with detection of the analytes, the characteristics of 8 reversed-phase columns were evaluated. The nitrophenylethyl (NPE)-bonded silica column was selected because of its shorter elution band and larger retention factors of the analytes due to strong dipole-dipole interactions. The amino-substituted PAHs (reduced NPAHs), PAHs and deuterated internal standards were separated on polymeric octadecyl-bonded silica (ODS) columns and by dual-channel detection within 120 min including clean-up and reduction steps. The limits of detection were 0.1-9.2 pg per injection for PAHs and 0.1-140 pg per injection for NPAHs. For validation, the method was applied to analyze crude extracts of fine particulate matter ($PM_{2.5}$) samples and achieved good analytical precision and accuracy. Moreover, the standard reference material (SRM1649b, urban dust) was analyzed by this method and the observed concentrations of PAHs and NPAHs were similar to those in previous reports. Thus, the method developed herein has the potential to become a standard HPLC-based method, especially for NPAHs.

Key words: Polycyclic aromatic hydrocarbon, Nitropolycyclic aromatic hydrocarbon, HPLC, Fluorescence detection, Airborne particulate matter

1. INTRODUCTION

Airborne particulate matter (PM) is produced by the combustion of organic materials and from atmospheric gaseous reactions. Airborne PM has been classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC) (IARC, 2016). Polycyclic aromatic hydrocarbons (PAHs) and their nitro-derivatives (NPAHs) are hazardous chemicals commonly found in PM, with many of these compounds having potential carcinogenic and/or mutagenic properties (IARC, 2016, 2013; Choi *et al*., 2010). The IARC has categorized several PAHs and NPAHs such as benzo[*a*]pyrene (BaP) as Group 1 (carcinogenic to humans), 1-nitropyrene (1-NP) as Group 2A (probably carcinogenic to humans) and several other PAHs and NPAHs as Group 2B (possibly carcinogenic to humans) (IARC, 2016). Additionally, NPAHs are of specific concern because dinitropyrenes (DNPs) have been shown to exhibit the strongest direct mutagenic effects of this class of compounds (Bandowe and Meusel, 2017). The presence of NPAHs in the ambient atmosphere has been shown to contribute to the mutagenicity of PM (Hayakawa *et al*., 1995).

PAHs and NPAHs are mostly emitted by incomplete combustion processes from residential heating, vehicle exhaust, and coal and wood burning (Bandowe and Meusel, 2017; Cheruiyot *et al*., 2015; Chuesaard *et al*., 2014; IARC, 2013; Tang *et al*., 2009). Atmospheric NPAHs are produced not only by primary sources such as diesel engine exhaust, but also secondary reactions of their parent PAHs in the atmosphere (Bandowe and Meusel, 2017). NPAHs are generally found in the atmosphere at very low concentrations (several pg/m^3~ in total) which are about 1-3 orders of magnitude lower than their parent PAHs (Bandowe and Meusel, 2017). Therefore, considerable efforts have been expended to determine NPAHs in environmental samples (Hayakawa *et al*., 2017, 2016a; Cvačka *et al*., 1998). Numerous studies focused on only the quantitative determination of PAHs in PM samples and, specifically, the 16 priority PAHs listed by U.S. Environmental Protection Agency (EPA) have been widely determined and discussed (Cheruiyot *et al*., 2015). However, analysis of interrelationship between PAH and NPAH concentrations as well as individual components is useful for source identification. For example, 1-NP is an important marker for automobile exhaust and the ratio of 1-NP to PAHs has been used to determine the contribution of car exhaust to urban PM samples (Hayakawa *et al*., 2016a; Chuesaard *et al*., 2014; Tang *et al*., 2009). Several NPAHs formed in the atmosphere via reactions of their parent PAHs with OH or NO_3 radicals have been used as markers for atmospheric reactions (Bandowe and Meusel, 2017; Jariyasopit *et al*., 2014; Tang *et al*., 2014; Ciccicoli *et al*., 1996). The analysis of both PAHs and NPAHs is essential to discuss sources and health effects of airborne PM such as $PM_{2.5}$.

Currently, PAHs in the environmental matrix are mostly analyzed by gas chromatography-mass spectrometry (GC-MS) with electron impact ionization (EI) mode (Hayakawa *et al*., 2017; Ma *et al*., 2016; Nyiri *et al*., 2016; Bandowe *et al*., 2014), and high-performance liquid chromatography with fluorescence detection (HPLC-FL) (Hayakawa *et al*., 2017, 2016a; Toriba *et al*., 2003). Although the selected ion monitoring of GC-MS is a powerful tool for identifying each analyte, PAH isomers with the same monitoring ions should be separated on a column. Benzo[*k*]fluoranthene (BkF) and benzo[*b*]fluoranthene (BbF) are representative of PAHs that are difficult to separate using GC-MS (Quintas *et al*., 2008). HPLC-FL provides an alternative method for the quantification of PAHs in environmental PM samples because of the high fluorescence quantum yield and detection specificity of PAHs and good separations of the isomers on reversed-phase columns such as polymeric-type octadecyl-bonded silica (ODS) columns (Toriba *et al*., 2003).

GC-MS(/MS) methods for determining atmospheric NPAHs are typically accompanied by detection with negative ion chemical ionization (NICI) mode (Albinet *et al*., 2014; Bandowe *et al*., 2014; Kawanaka *et al*., 2007). However, it is difficult to simultaneously analyze NPAHs and PAHs because PAHs are not detectable in NICI mode (Keyte *et al*., 2016; Bandowe *et al*., 2014). HPLC methods for analysis of NPAHs entail reduciton of the analytes, separation by reversed phase columns and detection via fluorescence or chemiluminescence (HPLC-FL or HPLC-CL) (Ohno *et al*., 2009; Tang *et al*., 2005a, 2003; Cvačka *et al*., 1998). In typical HPLC protocols, manual reduction of NPAHs to their corresponding amino-derivatives can be performed (Hayakawa *et al*., 1995, 1992; Kamiura *et al*., 1991) or reduction can be achieved through a column packed with platinum/rhodium (Pt/Rh)-coated alumina (Hayakawa *et al*., 2017, 2016a; Ohno *et al*., 2009; Tang *et al*., 2005a, 2003). Reduction is required to obtain molecules with florescent properties. A reduction column provides efficient reduction of NPAHs and is commonly applied to HPLC-based methods for analysis of NPAHs. The resulting amino-derivatives can be separated on reversed-phase column(s) and then detected by an FL detector or a CL detector after the reaction with peroxy oxalate esters such as (2,4,6-trichlorophenyl) oxalate and hydrogen peroxide as post-column reagents (Hayakawa *et al*., 2017, 2016a; Cvačka *et al*., 1998). While FL detectors suffered from poor performance for the detection sensitivity for NPAHs in PM samples, CL detection has been successfully used for their analysis. However, the CL system is complicated by the extra equipment needed for post-column CL reagents, which consume an especially large amount of acetonitrile. Furthermore, the CL detection cannot be applied to PAH analysis because the fluorescence characteristics of PAHs are inadequate for sensitive detection (Hayakawa *et al*., 2016a). Murahashi *et al*. (1994) reported an HPLC method for simultaneously determining PAHs and NPAHs with one injection. However, two independent flow passes were required to detect PAHs and NPAHs with FL and CL detectors, respectively. The sensitivity of FL detectors has improved remarkably over the last 20 years. At present, FL detection of NPAHs is more sensitive and saves solvent compared to CL detection. The HPLC-based method also enables a large-scale injection (100 μL or more), simultaneous analysis of PAHs and NPAHs, and can be used for the analysis of samples with a small sampling volume limited by collecting instruments such as personal samplers.

The aim of this study is to develop an HPLC system for simultaneous determination of PAHs and NPAHs using only a FL detector based on our previous HPLC-

CL method. At the same time, this method simplifies the HPLC system, shortens the total analytical time and reduces solvent consumption. First, we examined characteristics of reversed phase columns to effectively remove sample matrix and collect PAH and NPAH fractions. A two-dimensional HPLC method consisting of clean-up, reduction, column-switching, separation and FL detection steps was developed for determining 10 PAHs and 18 NPAHs in airborne particulates such as $PM_{2.5}$. The developed system allows for the application of crude extract of environmental samples without any complicated pretreatment before injection. The performance and potential of the method were validated using actual $PM_{2.5}$ samples and standard reference material (SRM1649b, urban dust).

2. MATERIALS AND METHODS

2.1 Reagents and Chemicals

The USEPA 610 PAHs mix, a mixture of 10 PAHs (10 μg/mL in acetonitrile) including fluoranthene (Flu), pyrene (Pyr), benz[*a*]anthracene (BaA), chrysene (Chr), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), dibenz[*a,h*]anthracene (DBA), benzo[*ghi*]perylene (BghiP) and indeno[1,2,3-*cd*]pyrene (IDP) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Three internal standards for PAHs, pyrene-d_{10} (Pyr-d_{10}), benzo[*a*]anthracene-d_{12} (BaA-d_{12}) and benzo[*a*]pyrene-d_{12} (BaP-d_{12}) were purchased from Wako Pure Chemicals (Osaka, Japan). 1,6-Dinitropyrene (1,6-DNP), 1,3-dinitropyrene (1,3-DNP), 1,8-dinitropyrene (1,8-DNP), 2-nitroanthracene (2-NA), 9-nitroanthracene (9-NA), 9-nitrophenathrene (9-NPh), 2-nitrofluorene (2-NF), 2-nitrofluoranthrene (2-NFR), 3-nitrofluoranthrene (3-NFR), 1-nitropyrene (1-NP), 7-nitrobenz[*a*]anthracene (7-NBaA), 6-nitrochrysene (6-NC) and 6-nitrobenz[*a*]pyrene (6-NBaP) were purchased from AccuStandard, Inc. (New Haven, CT, USA) (100 μg/mL in toluene). 1-Nitrofluoranthrene (1-NFR), 2-nitropyrene (2-NP), 1-nitroperylene (1-NPer) and 3-nitroperylene (3-NPer) were supplied from Chiron AS (Trondheim, Norway) (0.1 mg/mL in toluene), and 4-nitropyrene (4-NP) was from Tokyo Chemical Industry (Tokyo, Japan). 6-Nitrochrysene-d_{11} (6NC-d_{11}) was for an internal standard for NPAH analysis and was purchased from Cambridge Isotope Lab. Inc. (Andover, MA, USA). Chemical structures of the analytes and their abbreviations are presented in Fig. 1. All solvents and other chemicals used were of analytical-reagent or

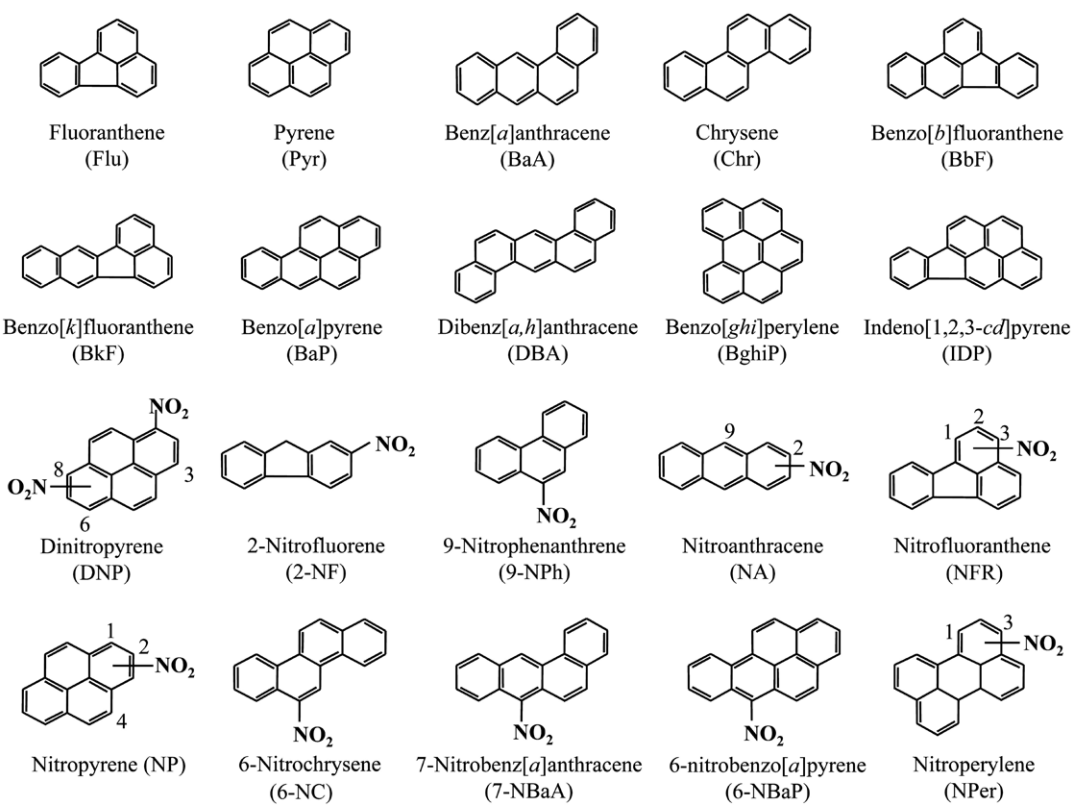

Fig. 1. Structures of the 10 PAHs and 18 NPAHs analyzed in this study.

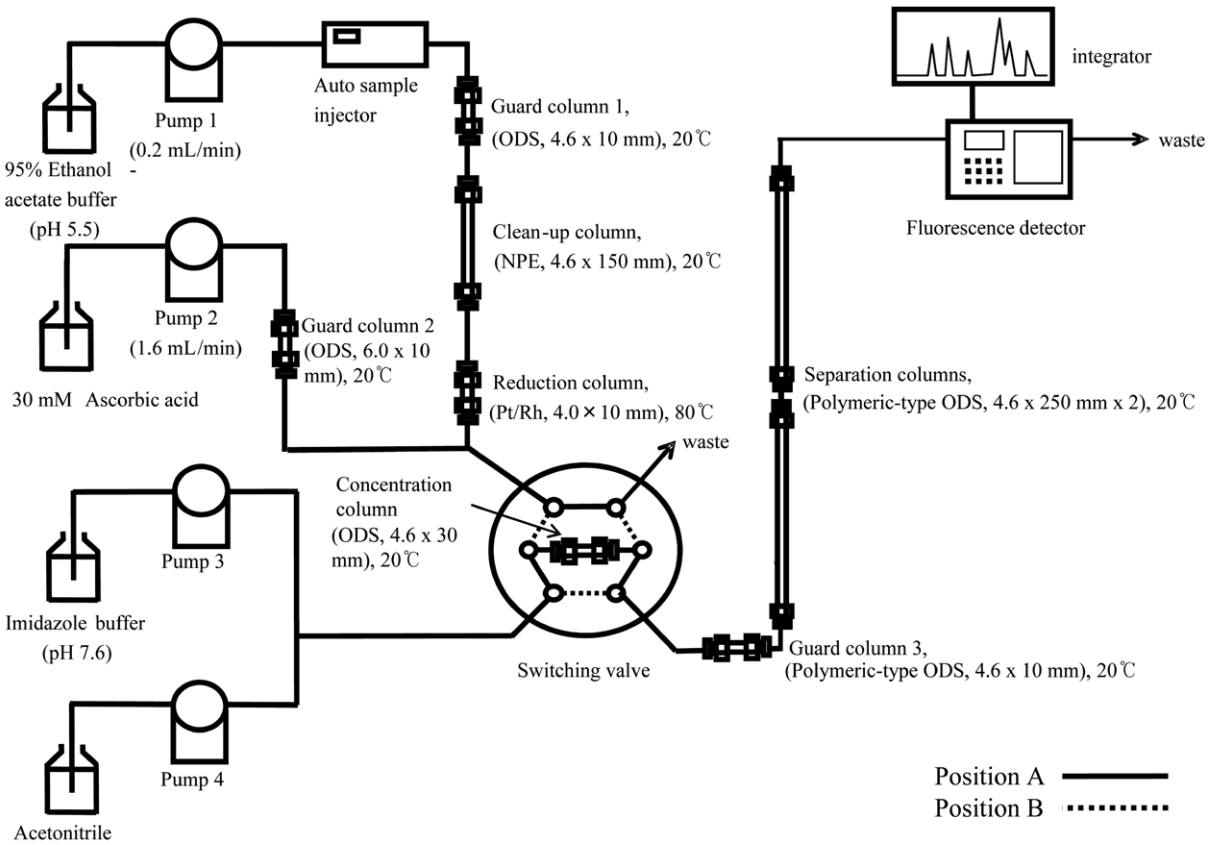

Fig. 2. Schematic diagram of the developed HPLC-FL method.

HPLC grade. Water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). The standard reference material of urban dust (SRM 1649b) was purchased from National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). The SRM sample is an atmospheric particulate material collected in an urban site with certified concentrations of some PAHs and NPAHs (NIST, 2016; Albinet *et al*., 2014; Schantz *et al*., 2012).

2.2 HPLC Systems and Conditions

A schematic diagram of the HPLC-FL system used for the simultaneous determination of PAHs and NPAHs is shown in Fig. 2. The system consists of 4 LC20AD pumps (Pump 1-4), a SIL-20AC auto sample injector, a degasser (DGU-20A5), a CMB-20A system controller and an integrator (LCsolution software), a CTO-20 AC column oven, a six-port switching valve, and a RF-20Axs fluorescence detector (All from Shimadzu, Kyoto, Japan). The injected sample was eluted through a clean-up column (Cosmosil, 5NPE, 150 × 4.6 mm i.d., 5 μm, Nacalai Tesque, Kyoto, Japan) with its guard column 1 (10 × 4.6 mm i.d.) and then NPAHs were reduced to their amino-derivatives by using a reduction column (NPpak-RS, 10 × 4.6 mm i.d., JASCO, Tokyo, Japan) at 80°C (0-15.4 min, switching valve position A). The mobile phase for the clean-up and reduction columns was ethanol/acetate buffer (pH 5.5) (95/5, v/v) at a flow rate of 0.2 mL/min. A fraction of the amino-derivatives and unchanged PAHs eluted from the reduction column with the mobile phase was mixed with 30 mM ascorbic acid through the guard column 2 (Asahipak ODP-50G-6A, 10 × 6.0 mm i.d., 5 μm, Shodex, Tokyo, Japan) at a flow rate of 1.6 mL/min and was then trapped on the concentration column (Spheri-5 RP-18, 30 × 4.6 mm i.d. 5 μm, Perkin Elmer, MA, USA) with a switching time of 15.4-33.0 min (position B). The concentrated fraction was passed through two separation columns (Inertsil ODS-P, 250 × 4.6 mm i.d., 5 μm, GL Sciences, Tokyo, Japan) with their guard column 3 (10 × 4.6 mm i.d.) in tandem (33.0-120.0 min, position A). All columns except the reduction column were maintained at 20°C. A gradient elution of the separation columns was performed using 10 mM imidazole buffer (pH 7.6) as eluent A and acetonitrile as eluent B. The gradient conditions (B concentration and

Table 1. Major interactions of the examined columns.

Stationary phase	Abbreviation	Major interaction
Octadecyl group (monomeric type)	$5C_{18}$-MS-II	Hydrophobic interaction
Octadecyl group (polymeric type)	$5C_{18}$-AR-II	Hydrophobic interaction
Phenylethyl group	PE	Hydrophobic and π-π interactions
Nitrophenylethyl group	NPE	Hydrophobic, π-π and dipole-dipole interactions
Pentafluorophenyl group	PFP	Hydrophobic, π-π and dipole-dipole interactions
Naphtylethyl group	πNap	Hydrophobic and π-π interactions
Pyrenylethyl group	PYE	Hydrophobic, π-π and dispersion interactions
Pentabromobenzyl group	PBr	Hydrophobic and dispersion interactions

flow rate) for the separation of amino-derivatives of NPAHs and unchanged PAHs were as follows: 0-15.4 min (B conc. 20%, 0.5 mL/min), 15.4-33.0 min (B conc. 65%, 0.5-0.8 mL/min), 33.0-65.0 min (B conc. 65-80%, 0.8-1.0 mL/min), 65.1-86.4 min (B conc. 80-100%, 1.0-1.8 mL/min), 86.5-120.0 min (B conc. 100%, 1.8 mL/min). Finally, the separated analytes were detected with their optimum excitation (Ex.) and emission (Em.) wavelengths by the dual-channel FL detector. The optimum wavelength used for each PAHs and NPAHs were as follows: Channel 1: 0-52.5 min (Ex. 369 nm, Em. 442 nm; 1,6-DNP), 52.5-56.1 min (Ex. 345 nm, Em. 430 nm; 9-NPh), 56.1-59.2 min (Ex. 260 nm, Em. 490 nm; 9NA), 59.2-63.2 min (Ex. 265 nm, Em. 488 nm; 2-NA, 2-NFR), 63.2-66.5 min (Ex. 360 nm, Em. 430 nm; 4-NP, 1-NP), 66.5-69.5 min (Ex. 338 nm, Em. 438 nm; 2-NP), 69.5-75.2 min (Ex. 300 nm, Em. 475 nm; 7-NBaA), 75.2-80.35 min (Ex. 227 nm, Em. 540 nm; 1-NPer), 80.35-82.75 min (Ex. 331 nm, Em. 391 nm; Pyr-d_{10}, Pyr), 82.75-84.3 min (Ex. 420 nm, Em. 475 nm; 6-NBaP), 84.3-88.7 min (Ex. 227 nm, Em. 540 nm; 3-NPer), 88.7-91.0 min (Ex. 265 nm, Em. 381 nm; Chr), 91.0-103.5 min (Ex. 295 nm, Em. 420 nm; BbF, BkF), 103.5-120 min (Ex. 268 nm, Em. 397 nm; DBA); Channel 2: 0-52.5 min (Ex. 395 nm, Em. 454 nm; 1,8-DNP, 1,3-DNP), 52.5-56.2 min (Ex. 285 nm, Em. 370 nm; 2NF), 56.2-63.0 min (Ex. 273 nm, Em. 437 nm; 1-NFR), 63.0-66.5 min (Ex. 300 nm, Em. 530 nm; 3-NFR), 66.5-78.0 min (Ex. 273 nm, Em. 437 nm; 2-NP, 6-NC-d_{11}, 6-NC), 78.0-82.75 min (Ex. 289 nm, Em. 450 nm; Flu), 82.75-86.0 min (Ex. 283 nm, Em. 513 nm; 6NBaP), 86.0-112.6 min (Ex. 264 nm, Em. 407 nm; BaA-d_{12}, BaA, BaP-d_{12}, BaP, BghiP), 112.6-120 min (Ex. 300 nm, Em. 500 nm; IDP).

For column evaluation, a simple HPLC-FL system was used to determine the retention times of the PAHs and NPAHs on the candidate clean-up columns. The system consisted of two HPLC pumps for gradient elution, two column ovens for the tested column, the reduction column and FL detector. Eight reversed-phase columns, 2 octadecyl ($5C_{18}$-MS-II and $5C_{18}$-AR-II)-, phenylethyl (PE)-, pentafluorophenyl (PFP)-, nitrophenylethyl (NPE)-, naphthylethyl (πNAP)-, pyrenylethyl (PYE)- and pentabromobenzyl (PBr)-bonded silica columns (Cosmosil columns, 150 × 4.6 mm i.d., 5 μm, all from Nacalai tesque, Kyoto, Japan) were examined (Table 1). A gradient elution using water (eluent A) and methanol (eluent B) was carried out (B, 70-100% liner gradient for 60 min, 100% isocratic after 60 min) at a flow rate of 1.0 mL/min. To evaluate the performance of the columns, the retention factor (k) was considered to be a factor for parameter calculation. The switching index can be difiened by Eq. (1).

$$\text{Switching index} = (k_{max} - k_{min}) / k_{mean} \quad (1)$$

where k_{max}, k_{min} and k_{mean} are the maximum, minimum and mean of retention factors (k) of all target compounds, respectively. Using the index, the overlap and length of elution times for each column were evaluated.

2.3 Particulate Samples and Extraction Procedure

$PM_{2.5}$ samples were collected on a quartz fiber filter (2500QAT-UP, Pall Life Sciences, Ann Arbor, MI, USA) by a high-volume air sampler (Model HV-700F, Shibata Sci. Tech., Saitama, Japan) with an impaction plate for a 50% cutoff point of 2.5 μm ($PM_{2.5}$) for 24 h at a flow rate of 1000 L/min. The $PM_{2.5}$ samples were collected at an urban site in Kanazawa, Japan from November 5 to 17, 2016 and they were analyzed to determine the accuracy and precision of the developed method. The filters were stored at −20°C until analysis. The commercially available urban dust (SRM 1649b) was from atmospheric particulate material collected in the Washington, DC area in 1976 and 1977 (NIST, 2016). After the addition of internal standards, a mixture of Pyr-d_{10}, BaA-d_{12} and BaP-d_{12} (25, 12 and 13 ng, respectively) for PAH quantification and 6-NC-d_{11} (1.8 pg) for NPAH quantification, the samples were ultrasonically extracted with dichloromethane (DCM) for 15 min. One-fourth of the $PM_{2.5}$ filters was cut into small pieces and extracted with 75 mL of DCM. The SRM samples (3 mg of the powder) were extracted with 5 mL of DCM. The extraction procedure was

repeated 3 times. After adding 30 μL of dimethylsulfoxide (DMSO) to the extract, the DCM in the extract was completely evaporated. The resulting DMSO solution was mixed with 270 μL of ethanol. Finally, the solution was filtered through a centrifugal filter (Ultrafree-MC, 0.45 μm Millipore) and then an aliquot (100 μL) of the solution was injected into the developed HPLC-FL system.

2.4 Calibration, Sensitivity, Accuracy and Precision

The developed method was validated with calibration curves, the limit of detection (LOD), the limit of quantification (LOQ), precision and accuracy. The concentrations of PAHs and NPAHs were quantified from the peak area ratios of the analytes to the deuterated internal standards. The internal standards used for determining PAHs and NPAHs were as follows: 6-NC-d_{11} for all of NPAHs, Pyr-d_{10} for Pyr and Flu, BaA-d_{12} for BaA, Chr, BbF, and BkF, and BaP-d_{12} for BaP, DBA, BghiP, and IDP. Calibration curves of each PAHs and NPAHs were prepared by plotting five points (n = 5) between the lowest concentration of 0.01-1 μg/L for PAHs and 0.005-5 μg/L for NPAHs, and at the highest concentration of 500 μg/L for PAHs and 10 or 100 μg/L for NPAHs. The LOD and LOQ were determined from lowest concentration at which the signal-to-noise (S/N) ratio was higher than 3 and 10, respectively, with precision less than 15% through the entire treatment of spiked blank samples. The intra-day and inter-day accuracy and precision were examined by repetitive determination of the $PM_{2.5}$ samples spiked with standards at a constant concentration for each analyte, together with non-spiked samples. The accuracy was expressed as the ratio of the quantified concentration to that of the known concentration of the spiked analyte. The precision was calculated as the relative standard deviation (SRD, %) of the replicates. To evaluate the intra-day precision, the spiked samples and non-spiked samples were prepared four times per day. The inter-day precision was determined using independent experiments repeated on four consecutive days. All extracts were analyzed on the same day as the samples were extracted.

3. RESULTS AND DISCUSSION

3.1 The Clean-up Column Evaluation

The two-dimensional HPLC system consists of clean-up, reduction, column-switching, separation and FL detection steps (Fig. 2). A high percentage of ethanol is necessary for the reduction step using the Pt/Rh column and acetonitrile decreases the reduction efficiency (Hayakawa *et al*., 2001). On the other hand, acetonitrile is an effective solvent for clear separation of all targets including similar isomers (Tang *et al*., 2005a) and to decrease column pressure. To eliminate opposing solvent effects, the two-dimensional system was able to switch solvent source to minimize the solvent effects of the 1st dimension. The clean-up column can be incorporated into the 1st dimension for a partial purification to remove hydrophilic matrix in a sample through the column-switching based on differences in the elution times from the column between the analytes and the sample matrix. Since PAHs and NPAHs have a wide range of hydrophobicities, ODS columns have little effect on removing substances that may interfere with the detection of analytes and require a long time for the elution of all analytes. This is especially true for low abundance NPAHs and consequently, the CL system required laborious pretreatments such as washing sample extracts with sodium hydroxide and sulfuric acid (Tang *et al*., 2003). Furthermore, our previous CL method required 58 min to trap the analytes on the concentration column and then 108 min to separate only NPAHs (total 166 min) (Tang *et al*., 2005a). The retention characteristics of 8 reversed-phase columns were evaluated to find a more effective column than the conventional ODS column. The characteristics of the stationary phases are listed in Table 1. The ideal characteristics of a clean-up column are a short elution band and large retention factors for all analytes. A shorter band can decrease the loading time to the concentration column and strong retention can increase the specificity of clean-up column. To satisfy these conditions, analytes with low logP values such as DNPs need to be retained with other interactions in addition to hydrophobic interaction, whereas the retention of the analytes with high logP values, such as IDP, needs to be suppressed.

The retention times of PAHs and NPAHs on each column were determined with methanol : water as the mobile phase, because of limitations in the reduction column and incompatibility between acetonitrile and π-π interaction (Snyder *et al*., 2004). The k values of the PAHs and NPAHs are listed in Table 2. The hydrophobicity of NPE, PFP and PBr phases is much smaller than that of ODS phases ($5C_{18}$-MS-II and $5C_{18}$-AR-II) and similar to that of the PE phase (Kimata *et al*., 1992). Nevertheless, mean k values of the three phases for NPAHs were comparable to or higher than those of the ODS phases. A non-substituted PAH (Pyr), nitro-substituted PAHs (1-, 2- and 4-NPs) and dinitro-substituted PAHs (1,3-, 1,6- and 1,8-DNPs) were eluted from the 6 tested columns in the following order: Pyr < NPs < DNPs, showing a reversal of the elution order in the ODS columns. The strong retention of NPAHs on NPE

Table 2. Capacity factor (k values) of PAHs and NPAHs in the examined columns.

Compound	Examined reversed-phase column							
	$5C_{18}$-MS-II	$5C_{18}$-AR-II	PE	πNap	PYE	NPE	PFP	PBr
PAHs								
Flu	8.27	9.76	2.71	5.97	8.47	4.14	6.73	17.57
Pyr	8.88	10.56	2.83	6.15	8.64	4.44	7.37	18.93
Chr	11.09	13.31	4.33	8.31	12.38	5.65	7.92	20.47
BaA	11.24	13.38	4.62	8.35	11.97	5.74	7.94	21.54
BbF	13.59	16.26	5.48	10.22	15.15	7.58	9.82	21.72
BkF	13.89	16.60	5.59	10.11	15.95	7.80	9.62	26.40
BaP	14.43	17.36	5.66	10.59	16.66	8.28	10.50	28.66
DBA	16.01	19.02	6.90	12.12	17.66	11.20	11.08	29.90
BghiP	16.85	20.46	6.62	12.34	21.20	9.31	11.96	42.62
IDP	16.89	20.44	6.67	12.59	19.93	10.43	12.98	36.10
NPAHs								
9-NA	5.08	6.05	2.61	6.58	11.00	4.62	5.68	13.19
2-NF	4.57	5.78	2.50	7.52	17.32	3.81	4.18	14.91
2-NA	5.82	6.97	3.08	8.41	17.97	4.92	5.42	18.64
9-NPh	5.16	7.43	2.83	7.35	13.83	4.97	5.77	16.19
4-NP	7.82	9.54	4.54	10.34	20.48	7.13	8.46	24.49
3-NFR	7.99	9.59	4.12	10.71	22.86	6.62	7.90	24.15
1-NFR	8.16	10.18	4.03	9.81	19.59	7.22	9.09	24.29
2-NFR	8.14	9.87	4.72	10.84	22.04	7.02	7.87	24.53
1-NP	7.37	9.42	4.04	9.92	21.29	7.07	7.91	24.70
2-NP	8.59	10.52	4.69	11.11	23.15	7.02	8.28	25.83
7-NBaA	9.36	11.18	5.39	11.24	19.23	8.17	9.00	22.43
6-NC	10.08	12.20	5.96	12.99	23.28	8.65	9.28	26.23
1-NPer	9.87	12.20	6.21	13.23	24.03	10.24	8.86	27.22
1,6-DNP	7.73	7.32	5.66	17.25	29.34	9.69	7.48	31.58
1,3-DNP	7.72	9.81	6.87	19.04	30.22	11.11	9.79	31.40
1,8-DNP	6.69	8.64	5.84	17.20	30.38	11.35	10.38	32.05
6NBaP	12.28	15.06	6.94	14.67	27.75	11.54	11.64	32.13
3NPer	12.25	15.32	7.10	16.60	36.97	11.52	10.90	41.11
k_{max}	16.89	20.46	7.10	19.04	36.97	11.54	12.98	42.62
k_{min}	4.57	5.78	2.50	5.97	8.47	3.81	4.18	13.19
k_{mean}	9.85	11.94	4.95	11.13	19.95	7.76	8.71	25.68
k_{max}-k_{min}	12.32	14.69	4.60	13.07	28.50	7.73	8.80	29.42
Switching index	1.25	1.23	0.93	1.17	1.43	1.00	1.01	1.15

and PFP phases compared to the PE phase indicated the presence of strong dipole-dipole interactions (Kimata *et al*., 1992). In particular, PAHs and NPAHs were strongly retained on PYE and PBr phases, indicating strong dispersive interactions between the aromatic species and the stationary phases in addition to their hydrophobic and π-π interactions (Turowski *et al*., 2001).

To evaluate the length of the elution band and the distribution of retention times, switching indexes were calculated for each column (Table 2). A small value indicates a short elution band and strong retention of the analytes. Although the PE column showed the smallest switching index (0.93), the retention of the analytes on the column was considerably weaker than the other columns. Taking into consideration the separation of the analytes from a hydrophilic matrix, the NPE column (switching index: 1.00) was selected as the clean-up column for the switching column system. Finally, ethanol/acetate buffer (pH 5.5, 95/5, v/v) at a flow rate of 0.2 mL/min was used for the clean-up column as the mobile phase, taking into account the conditions required for the reduction step (Hayakawa *et al*., 2001). The performance of the NPE column was maintained under the conditions because the switching index showed 1.05, the same value as observed in the conditions for the column evaluation. All the analytes were retained for over 15 min and eluted for 17.6 min (switching time: 15.4-33.0 min).

3.2 Separation and Detection of Analytes in the 2nd Dimension

After column switching, 10 PAHs and 18 amino-derivatives of NPAHs were separated on the separa-

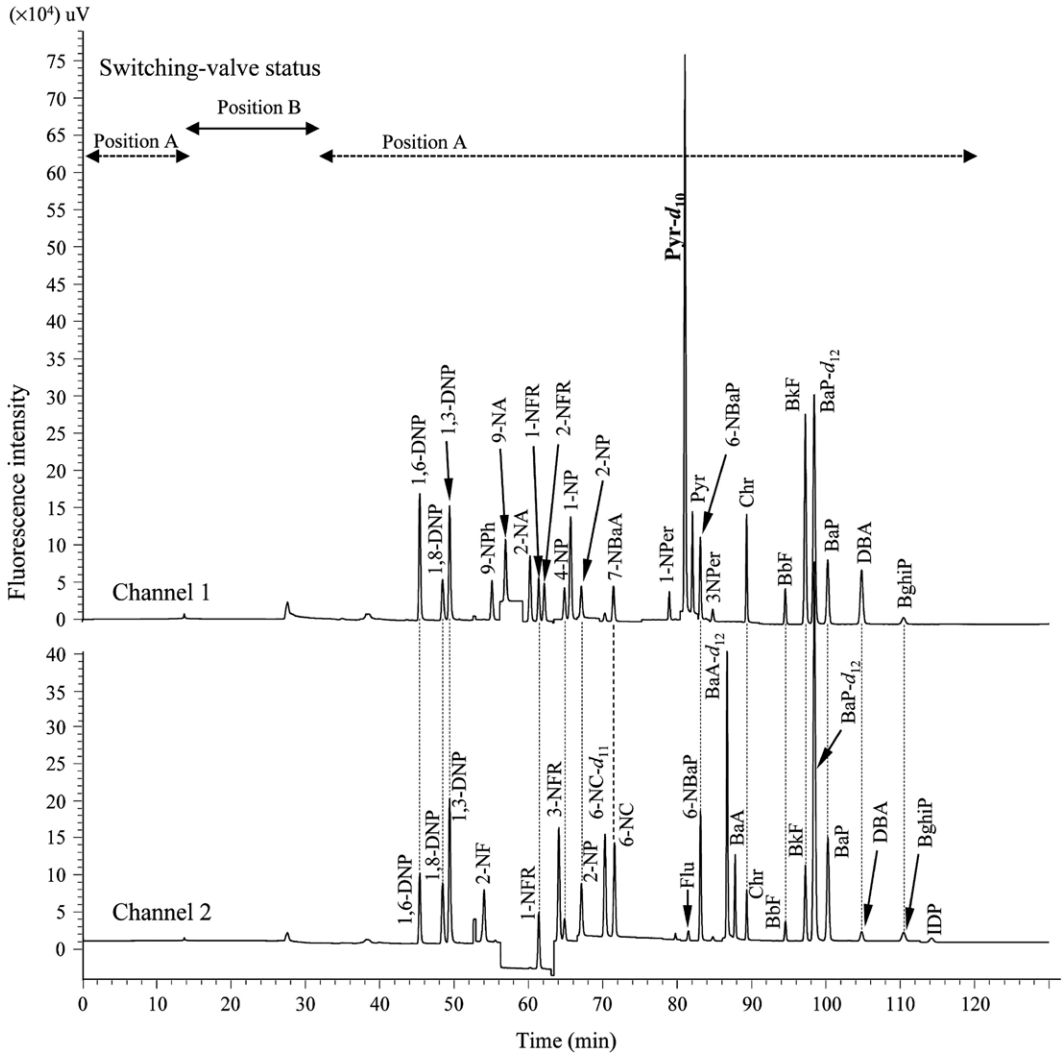

Fig. 3. Representative standard chromatograms of PAHs and NPAHs measured by the developed HPLC-FL method. Injected amounts: Channel 1; 1,6-DNP, 750 pg; 9-NPh, 3 ng; 9-NA, 3 ng; 2-NA, 500 pg; 2-NFR, 1 ng; 4-NP, 12.5; 1-NP, 500 pg; 7-NBaA, 1 ng; 1-Nper, 10 ng; Pry-d_{10}, 11 ng; Pyr, 1 ng; 3-NPer, 10 ng; Chr, 1 ng; BbF, 1 ng; BkF, 1 ng; DBA, 1 ng; Channel 2; 1,8-DNP, 1 ng; 1,3-DNP, 1 ng; 2-NF, 500 pg; 1-NFR, 1 ng; 3-NFR, 5 ng; 2-NP, 4 ng; 6-NC-d_{11} 6 ng; 6-NC, 1 ng; Flu, 1 ng; 6-NBaP, 6 ng; BaA-d_{12}, 6 ng; BaA, 1 ng; BaP-d_{12}, 7 ng; BaP, 1 ng; BghiP, 1 ng; IDP, 1 ng.

tion columns which consist of 2 polymeric-type ODS columns (4.6 mm i.d. ×250 each, 5 μm) in tandem. Fig. 3 shows typical chromatograms of a standard mixture of target PAHs, NPAHs and deuterated standards, which were all well separated by gradient elution. The reduced NPAHs were eluted from the columns faster than non-substituted PAHs. Three deuterated PAHs (Pyr-d_{10}, BaA-d_{12}, BaP-d_{12}) and the amino-derivative of 6-NC-d_{11} were separated from the non-deuterated compounds with sufficient resolution ($R_s > 2.85$). In general, stable isotope-labeled compounds are excellent internal standards for mass spectrometric detection, but not for optical detection methods such as FL detection. However, deuterated PAHs can be separated from the non-deuterated analogues with baseline resolution on polymeric-type ODS columns and have nearly the same fluorescence characteristics (Toriba *et al*., 2003). Furthermore, we have successfully applied deuterated PAHs, 1-NP and hydroxylated PAHs to HPLC-FL methods for environmental and biological samples (Ohno *et al*., 2009; Toriba *et al*., 2007). Total analytical time for simultaneously determining PAHs and NPAHs was 120 min including 33 min for clean-up and reduction steps in the 1st dimension. After the 1st dimension, the 2nd dimension required 52 min and 82 min to separate NPAHs (elution time: 45.0-85.0 min) and PAHs

Table 3. Limits of detection (LOD), limits of quantification (LOQ) and calibration curves of PAHs and NPAHs by the proposed HPLC-FL method.

Compound	LOD[a] (pg/injection)	LOQ[b] (ng/L)	Calibration range (μg/L)	Linearity (r^2)	LOD[c] (HPLC-CL) (pg/injection)
PAHs					
Flu	4.0	130	1-500	0.9999	–
Pyr	0.4	12	0.05-500	1.0000	–
BaA	0.4	13	0.05-500	1.0000	–
Chr	0.6	18	0.05-500	1.0000	–
BbF	0.7	24	0.05-500	1.0000	–
BkF	0.1	4.3	0.01-500	1.0000	–
BaP	0.3	8.7	0.01-500	1.0000	–
DBA	0.4	14	0.05-500	1.0000	–
BghiP	5.0	170	1-500	1.0000	–
IDP	9.2	310	1-500	0.9999	–
NPAHs					
1,6-DNP	0.4	12	0.05-10	0.9960	0.3
1,8-DNP	1.1	37	0.05-10	0.9957	0.3
1,3-DNP	0.5	15	0.05-10	0.9947	0.3
2-NF	0.5	17	0.05-10	0.9959	210
9-NA	9.3	310	0.5-10	0.9942	0.9
9-NPh	2.6	87	0.1-10	0.9983	27
2-NA	0.1	2.2	0.005-10	0.9928	2.2
1-NFR	1.6	52	0.1-10	0.9948	–
2-NFR	0.7	23	0.05-10	0.9957	–
3-NFR	25	820	1-100	0.9953	5.2
4-NP	27	890	1-100	0.9974	7.4
1-NP	0.1	3.1	0.005-10	0.9960	2.5
2-NP	1.9	64	0.1-10	0.9954	37
7-NBaA	1.0	34	0.05-10	0.9978	2.7
6-NC	0.5	17	0.05-10	0.9951	41
1-NPer	87	2900	5-100	0.9976	1.2
6-NBaP	8.9	300	0.5-10	0.9970	0.6
3-NPer	140	4500	5-100	0.9946	0.6

[a]Limit of detection (S/N = 3), [b]Limit of quantification (precision < 25%, S/N = 10), [c]Tang *et al*., 2005

(elution time: 79.0-115.0 min), respectively. The analysis time for NPAHs was reduced by half compared to the HPLC-CL method (Tang *et al*., 2005a) and was followed by PAH analysis.

Moreover, we focused on the separation of nitrofluoranthrenes and nitropyrenes which display similar chromatographic behavior on reversed-phase columns. Specifically, 2-NFR and 2-NP are well-known secondary products formed via atmospheric reactions of their parent PAHs and have been frequently found in PM samples (Tang *et al*., 2014; Hayakawa *et al*., 2011). It is essential to separate these two compounds from the other detectable isomers (1-NFR, 3-NFR, 1-NP and 4-NP) in real samples. The 6 compounds were successfully separated on the 2 ODS columns in tandem which required a high number of theoretical plates (Fig. 3). Dual-channel fluorescence detection was used to avoid frequent changes of the detection wavelengths or separate co-eluted or closely eluting peaks. The two-channel program was set based on optimal excitation and emission wavelengths of the analytes (Šoustek *et al*., 2008; Cvačka *et al*., 1998). Remarkably, 6-NC and 7-NBaA were successfully separated by their specific excitation and emission wavelengths, not by the separation columns as they had identical retention times (Fig. 3). The signal arising from one analyte was not detected on the other channel. These results suggest that the proposed HPLC-FL system is suitable for the identification of all target compounds.

3. 3 LOD, LOQ and Calibration Curve

The concentrations of the analytes which establish S/N ratio of 3:1 and 10:1 were considered to be the LODs and LOQs, respectively. The LODs and LOQs for PAHs and NPAHs are listed in Table 3, indicating that the developed method is sensitive enough to measure the analytes in the PM samples (1.1 pg/m^3 for 3-NPer which showed the lowest sensitivity). Compared to previous HPLC-CL method (Tang *et al*., 2005a), the LODs of 2-NF, 9-NPhe, 2-NA, 1-NP, 2-NP 7-NBaA

Table 4. Precision and accuracy of the PAH determinations. (n = 4)

Compound	Spiked conc. (pg/m^3)	Observed (mean ± SD, pg/m^3)[a]		Accuracy (%)[b]		Precision (RSD%)	
		Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
Flu	0	133 ± 4.8	129 ± 6.3			3.6	4.9
	140	269 ± 9.1	264 ± 7.3	98	96	3.4	2.8
Pyr	0	93 ± 10	86 ± 4.4			11	5.1
	140	229 ± 9.1	224 ± 7.9	97	99	4.0	3.5
BaA	0	25 ± 0.7	25 ± 1.4			2.8	5.9
	140	162 ± 6.4	157 ± 7.6	98	94	4.0	4.9
Chr	0	50 ± 2.4	49 ± 4.3			4.9	8.9
	140	186 ± 6.1	181 ± 10.2	98	95	3.3	5.6
BbF	0	50 ± 5.3	54 ± 9.6			10	18
	140	175 ± 5.3	181 ± 16.3	89	90	3.1	9.0
BkF	0	20 ± 1.9	19 ± 2.1			9.4	11
	140	151 ± 9.7	144 ± 15.4	93	89	6.4	11
BaP	0	31 ± 0.1	30 ± 3.0			0.3	10
	140	156 ± 4.8	152 ± 6.7	90	88	3.1	4.4
DBA	0	6.5 ± 0.6	7.0 ± 0.5			9.0	6.9
	140	132 ± 3.5	131 ± 4.5	90	89	2.6	3.5
BghiP	0	41 ± 1.6	40 ± 4.7			4.0	12
	140	163 ± 7.3	161 ± 4.4	87	86	4.5	2.8
IDP	0	44 ± 2.7	41 ± 4.5			6.1	11
	140	178 ± 3.3	167 ± 8.4	96	90	1.8	5.0

[a]The recovered concentrations were calculated as the difference between the concentrations of the spiked samples and mean concentration of non-spiked samples.
[b]Accuracy is expressed as the percentage of accuracy [(mean observed concentration/spiked concentration) × 100].

and 6-NC were substantially improved, whereas the sensitivities of 9-NA, 1- and 3-NPer were decreased. The sensitivities of the PAHs were higher than those previously reported because of instrumental improvement (Toriba *et al*., 2003). Calibration curves were constructed from the peak area ratio of the analyte to deuterated internal standards and good linearity ($r^2 > 0.9999$ for PAHs and > 0.99 for NPAHs) was observed for all calibration curves, as shown in Table 3.

3. 4 Accuracy and Precision

The extraction method of PAHs and NPAHs in PM samples involved ultrasonic extraction with DCM, evaporation and redissolving steps, and then the crude extract was directly injected to the HPLC system. The analysis of the crude extracts is difficult to perform with GC-MS. The recoveries of the deuterated internal standards (Pyr-d_{10}, BaA-d_{12}, BaP-d_{12} and 6-NC-d_{11}) were 80-119% through the entire pretreatment. Known amounts of PAHs and NPAHs were added to $PM_{2.5}$ samples, and their quantification accuracy and precision were evaluated (Tables 4 and 5). The accuracies were 86-98% and 87-109% for the PAHs and NPAHs, respectively. The precision was good with a RSD of 15% or less for all analytes. Both intra- and inter-day accuracy and precision were satisfactory for the simultaneous determination of all PAHs and NPAHs in the $PM_{2.5}$ samples. Fig. 4 shows typical chromatograms resulting from a crude extract of the spiked $PM_{2.5}$ samples. The sample matrix did not interfere with the identification and quantification of the analytes in the chromatograms. The results suggest effective fractionation of the analytes from the sample matrix by the clean-up column and column-switching in the 1st dimension.

3. 5 Quantitative Determination of Atmospheric Samples

The concentrations of PAHs in the suburban $PM_{2.5}$ samples used for the validation study (the concentrations of non-spiked samples in Table 4) were slightly higher than those in a background site located in the Noto peninsula without any major sources near the station (Tang *et al*., 2014). In contrast, the PAH levels

Table 5. Precision and accuracy of the NPAH determinations. (n = 4)

Compound	Spiked conc. (pg/m^3)	Observed (mean ± SD, pg/m^3)[a]		Accuracy (%)[b]		Precision (RSD%)	
		Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
1,6-DNP	0	0.03 ± 0.00	0.03 ± 0.00			7.8	6.6
	1.39	1.36 ± 0.11	1.40 ± 0.18	96	99	8.3	13
1,8-DNP	0	0.16 ± 0.01	0.15 ± 0.01			3.5	4.1
	1.39	1.49 ± 0.14	1.49 ± 0.16	96	96	9.4	11
1,3-DNP	0	–[c]	–				
	1.39	1.49 ± 0.07	1.47 ± 0.09	107	106	4.5	6.2
2-NF	0	< LOQ[d]	< LOQ				
	1.39	1.52 ± 0.01	1.46 ± 0.11	109	105	0.7	7.6
9-NPh	0	0.28 ± 0.02	0.26 ± 0.04			8.2	14
	2.78	2.95 ± 0.15	2.75 ± 0.14	96	90	5.2	5.1
9-NA	0	5.13 ± 0.18	4.93 ± 0.14			3.5	2.8
	2.78	7.68 ± 0.33	7.80 ± 0.36	92	103	4.3	4.7
2-NA	0	–	–				
	1.39	1.42 ± 0.07	1.44 ± 0.12	102	104	5.2	8.3
1-NFR	0	0.01 ± 0.00	0.01 ± 0.00			7.8	7.0
	1.39	1.22 ± 0.05	1.26 ± 0.11	87	90	4.0	8.3
2-NFR	0	1.67 ± 0.14	1.68 ± 0.15			8.6	9.0
	11.1	12.5 ± 0.71	13.2 ± 1.24	97	104	5.7	9.4
3-NFR	0	0.84 ± 0.12	0.85 ± 0.13			14	15
	6.94	7.33 ± 0.14	7.21 ± 0.37	93	92	1.9	5.1
4-NP	0	0.09 ± 0.00	0.08 ± 0.01			4.4	13
	2.78	2.77 ± 0.11	2.66 ± 0.18	96	93	3.9	6.6
1-NP	0	0.45 ± 0.01	0.46 ± 0.02			2.2	3.8
	2.78	3.14 ± 0.24	3.06 ± 0.24	97	93	7.6	7.8
2-NP	0	0.76 ± 0.03	0.79 ± 0.04			4.5	5.6
	12.5	12.6 ± 0.46	12.4 ± 0.74	95	93	3.6	5.9
7-NBaA	0	–	–				
	1.39	1.37 ± 0.03	1.34 ± 0.06	99	96	2.1	4.2
6-NC	0	–	–				
	0.28	0.26 ± 0.02	0.28 ± 0.04	92	101	6.2	14
1-NPer	0	–	–				
	43.1	39.5 ± 3.19	38.4 ± 2.31	92	89	8.1	6.0
6-NBaP	0	–	–				
	2.78	2.66 ± 0.13	2.62 ± 0.18	96	94	4.9	7.0
3-NPer	0	–	–				
	43.1	37.4 ± 1.61	37.6 ± 1.43	87	87	4.3	3.8

[a]The recovered concentrations were calculated as the difference between the concentrations of the spiked samples and mean concentration of non-spiked samples.
[b]Accuracy is expressed as the percentage of accuracy [(mean observed concentration/spiked concentration) × 100].
[c]Not detected
[d]less than limit of quantification

were lower than samples collected at a road side in Kanazawa city (Tang *et al*., 2005b) and the other Japanese cities (Hayakawa *et al*., 2016b; Naser *et al*., 2008). PAHs are commonly found in $PM_{2.5}$ fractions and PAH concentrations have been directly correlated with $PM_{2.5}$ levels (Naser *et al*., 2008). The PAH concentrations in

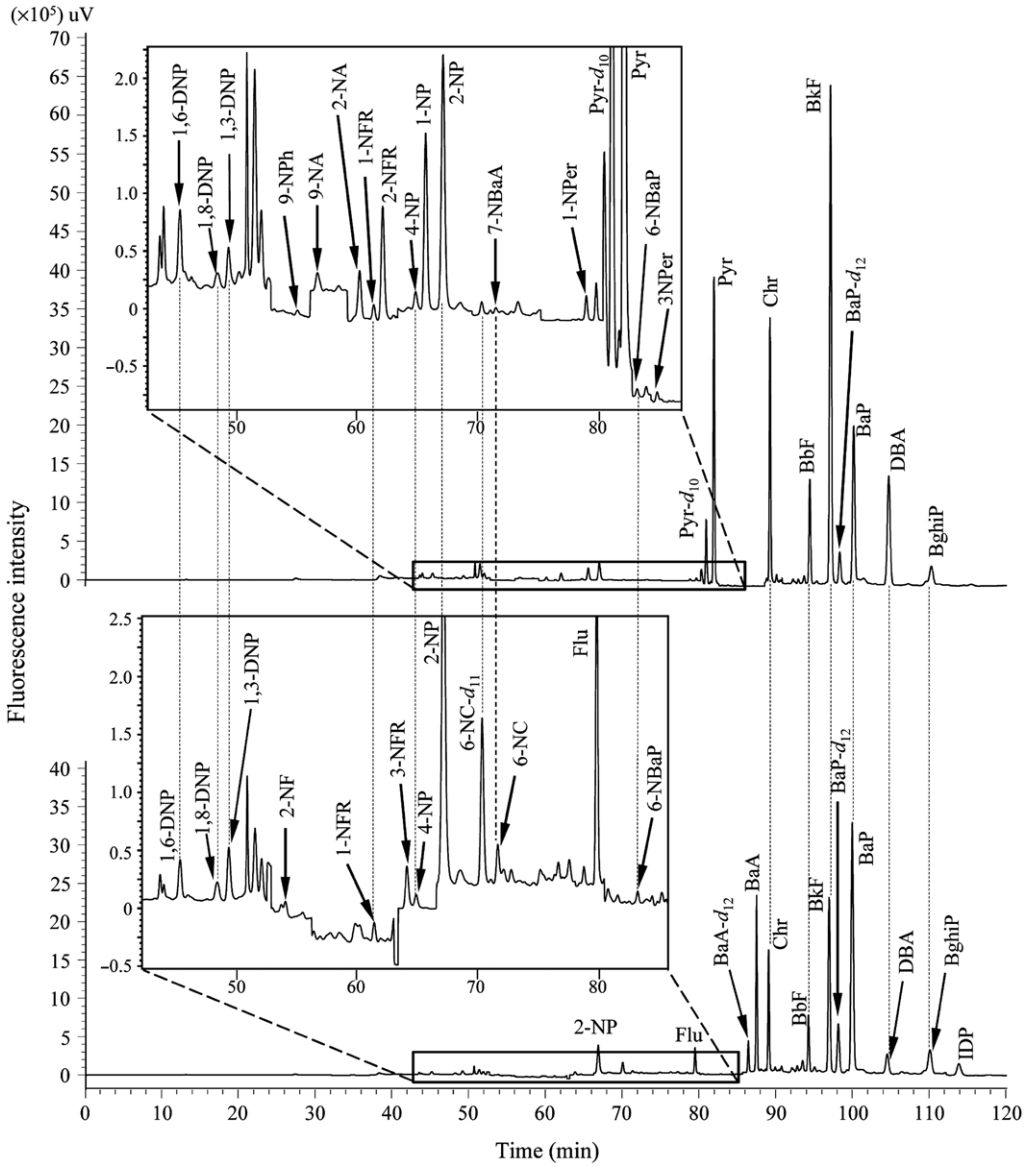

Fig. 4. Representative chromatograms resulting from a crude extract of the spiked $PM_{2.5}$ samples.

this study were reasonable, with respect to the $PM_{2.5}$ levels of 7.7 ± 2.9 μg/m^3 measured during the sampling period. Among the NPAHs, 1,3-DNP, 2-NA and NPAHs having higher molecular weight were not detected in the $PM_{2.5}$ samples (Table 5). The concentration of 1-NP in this study was significantly lower than other sites, including the background site (Hayakawa *et al*., 2016b; Tang *et al*., 2014; Naser *et al*., 2008), indicating little emission source related to automobile exhaust around the site. The 1-NP contribution to NPAHs has substantially decreased throughout Japan in recent years, in conjunction with government regulations regarding automobile emissions (Kojima *et al*., 2010).

Using the developed HPLC-FL method, we also quantified PAHs and NPAHs in an urban dust standard reference material (SRM1649b). Fig. 5 shows representative chromatograms resulting from the crude extract of SRM 1649b. The obtained concentrations of 10 PAHs and 18 NPAHs were compared with certified concentrations by NIST and data reported from other studies (Table 6). The quantified PAH concentrations showed good agreement with the certified values in the range of 90-105% (NIST, 2016). Five NPAHs were highly consistent with the certified NPAH values (92-117%), except for 9-NA, 9-NPh and 3-NFR. Several compounds showed similar concentrations with other

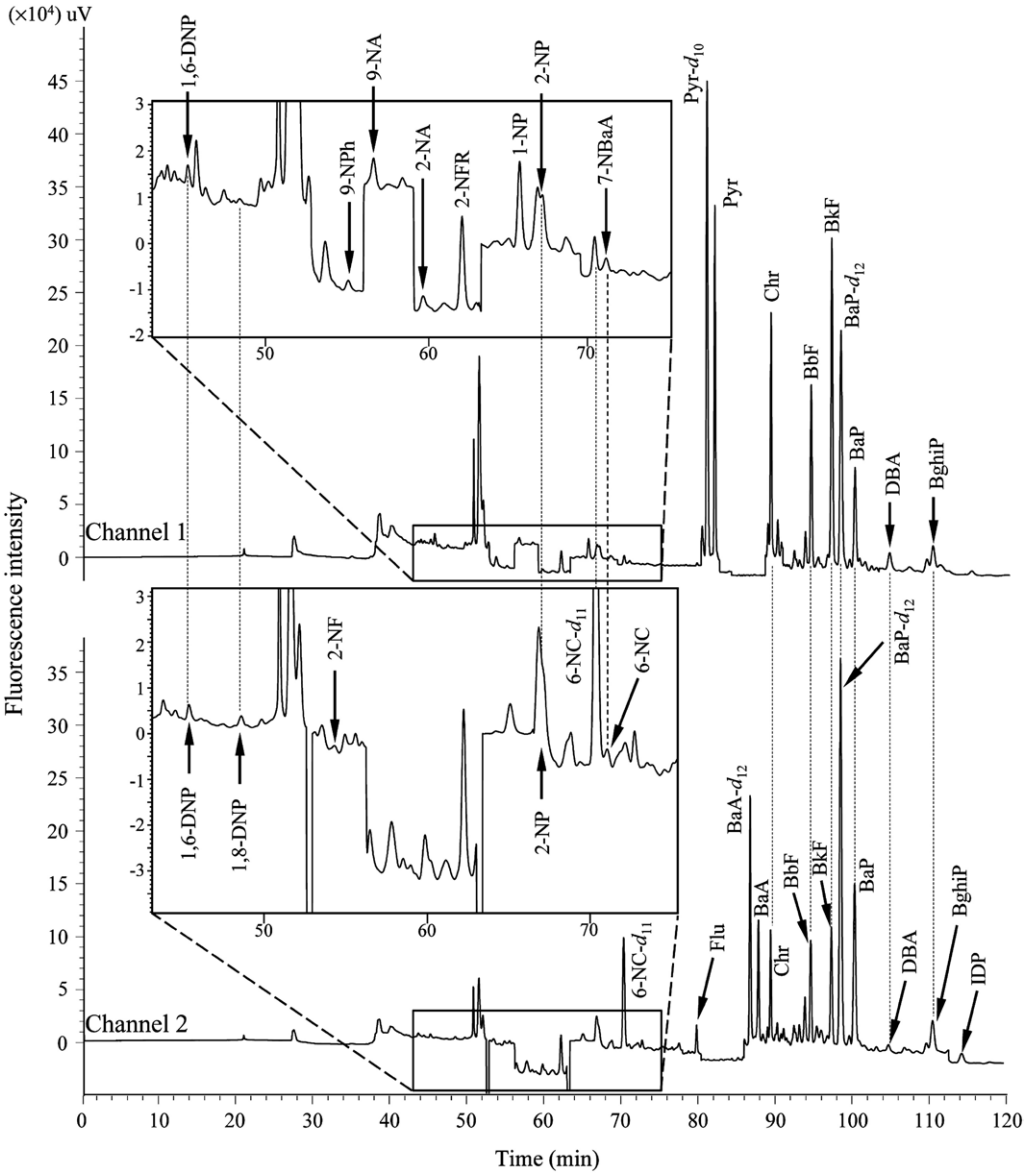

Fig. 5. Representative chromatograms resulting from the crude extract of SRM 1649b.

literature data (Albinet *et al*., 2014; Schantz *et al*., 2012). There were large variations in the reported concentrations of NPAHs from literature data (Table 6), and these differences could arise from all of the procedure of the analytical protocols employed. The NPAH concentrations determined by pressurized solvent extraction (PLE) reported by Schantz *et al*. (2012) were very close to those in the certificate. On the other hand, Albinet *et al*. (2014) used two extraction methods, PLE and the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) approaches for the NPAHs in SRM1649b. They reported concentrations of numerous NPAHs which included analytes not reported in the certificate with several other being different from the certificate data and having variations between the extraction methods. The variation of the quantified data may be from the difference among general Soxhlet extraction, PLE and sonication.

4. CONCLUSION

A two-dimensional HPLC-FL system has been developed for the simultaneous determination of 10 PAHs and 18 NPAHs. The number of the analytes was increased and a flow pass of the 2nd dimension simpli-

Table 6. Comparison of quantified concentrations of PAHs (mg/kg) and NPAHs(μg/kg) in SRM1649b in this study to the certificate and literature.

	This study	NIST certificate (2016)	Schantz *et al*. (2012)	Albinet *et al*. (2014)	Albinet *et al*. (2014)
Number of replicates	n = 3	n = 3	n = 3	n = 6	n = 6
Analytical instrument	HPLC-FL	GC-NICIMS	GC-NICIMS	GC-NICIMS (PLE)	GC-NICIMS (QuEChERS)
PAHs					
Flu	6.1	6.2	6.5	–[a]	–
Pyr	5.2	5.0	4.9	–	–
Chr	2.1	2.1	2.9	–	–
BaA	3.0	3.0	2.2	–	–
BbF	5.7	6.2	7.7	–	–
BkF	1.7	1.7	1.8	–	–
BaP	2.5	2.5	2.9	–	–
DBA	0.3	0.3	0.5	–	–
BghiP	3.6	4.0	–	–	–
IDP	2.6	2.9	2.6	–	–
NPAHs					
1,6-DNP	12.1	–	–	9.8	45.3
1,8-DNP	23.1	–	–	29.4	61.8
1,3-DNP	<LOQ[c]	–	–	nd[b]	nd
2-NF	20.1	–	–	nd	53.0
9-NA	126	243	255.0	36.1	25.7
9-NPh	44.8	1.8	1.7	11.4	15.3
2-NA	25.8	–	–	33.4	55.7
1-NFR	<LOQ	–	–	–	–
2-NFR	279	304	307.7	322.8	261.9
3-NFR	<LOQ	4.7	4.1	nd	nd
4-NP	<LOQ	–	–	13.7	27.3
1-NP	72.0	74.8	73.2	104.1	60.9
2-NP	12.7	10.9	11.1	41.0	42.0
7-NBaA	23.6	24.4	24.0	69.3	35.2
6-NC	3.9	3.6	3.3	11.6	18.8
1-NPer	<LOQ	–	–	–	–
6-NBaP	<LOQ	–	–	79.9	66.6
3-NPer	<LOQ	–	–	–	–

[a]No data, [b]Not detected, [c]Less than limit of detection

fied the whole system compared to the previous simultaneous analysis (Murahashi *et al*., 1994). The single analysis can cover particle-bound PAHs and NPAHs required for the evaluations of strong mutagenic compounds, combustion sources and atmospheric reactions. The column switching system enables direct injection of crude PM sample extract without complicated pretreatment. The simple pretreatment can shorten the total analytical time and increase recoveries of the analytes. The NPE column as the clean-up column showed short elution band and strong retention of the analytes by dipole-dipole interactions and displayed effective fractionation of the analytes from the sample matrix in the 1st dimension. Good separation of all target compounds was achieved by polymeric-type ODS columns and dual-channel FL detection in the second dimension. The time required for analysis of NPAHs was cut in half when compared to conventional HPLC-CL methods with a total time of 120 min, including analysis of PAHs. The LODs of PAHs and NPAHs were better than or comparable to those of the previous FL methods for PAHs and the CL methods for NPAHs. The FL detection can reduce solvent consumption and eliminate the requirement for pumps for the post-label reagents for the CL detection of NPAHs. This HPLC-based method also enables much larger-scale injections (100 μL or more) compared to GC-based methods, therefore, it may be applicable for samples with a small sampling volume limited by collecting instruments such as personal samplers. The quantification of PAHs and NPAHs in crude extracts of $PM_{2.5}$ samples showed good accuracy and precision and concentration deter-

minations for SRM 1649b were similar to the certificate and other values reported in the literature. Considering the above-mentioned advantages, the developed method has the potential to a standard HPLC-based method, especially for NPAH determination.

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