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Short communication

Determination of 1-nitropyrene in low volume ambient air samples by high-performance liquid chromatography with fluorescence detection

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ABSTRACT

To measure the actual exposure of a person to 1-nitropyrene (1-NP) in airborne particulate matter, it is considered more accurate to collect air samples with a portable air sampler than to sample at a fixed location. However, because the portable samplers can sample only small volumes, a sensitive method is needed to analyze the compounds that are collected on a filter. Here we describe a high-performance liquid chromatographic (HPLC) method with fluorescence detection that is sensitive and precise enough for use with portable air samplers. The developed column-switching system successfully removed the interfering substances in the samples with only a simple pretreatment. To improve the precision of the measurement, deuterated 1-NP was used as an internal standard, and it eluted immediately prior to 1-NP with sufficient resolution (R_s, 1.50). The detection limit was 0.32 fmol/injection, and the calibration range was from 2 to 100 fmol. The proposed method was applied to determining 1-NP in fine airborne particulate matter ($PM_{2.5}$) at two sites with low pollution levels. 1-NP was detected in all samples at concentrations in the low fmol/m^3 range. The proposed method has enough sensitivity and precision to determine 1-NP in the limited air volume of the portable sampler.

1. Introduction

Nitropolycyclic aromatic hydrocarbons (NPAHs) are hazardous environmental pollutants. They are produced by both primary sources such as diesel vehicle exhaust [1] and by secondary reactions of polycyclic aromatic hydrocarbons (PAHs) with nitrogen oxides and/or hydroxyl radicals in the atmosphere [2]. Several NPAHs such as dinitropyrenes exhibit strong carcinogenicity/mutagenicity [3,4]. Among NPAHs, 1-nitropyrene (1-NP) is one of the most abundant NPAHs in the atmosphere and in diesel exhaust particles and has been proposed as a chemical marker for diesel exhaust [5,6]. A large portion of NPAHs is associated with fine airborne particulates with aerodynamic diameters less than 2.5 μm ($PM_{2.5}$). In addition, fine particles ($PM_{2.5}$) themselves are of great health concern because they can easily reach pulmonary alveoli [7]. Exposure to $PM_{2.5}$ has been associated with increased human health risk such as mortality and morbidity [7].

Personal air monitoring, in which a person wears a small air sampler on the body, can more accurately characterize human exposure than stationary ambient air monitoring, because individuals have such varied activity patterns and exposures. Although previous studies have focused on atmospheric concentrations of NPAHs, there is now growing interest in personal exposure to NPAHs. Portable air samplers are typically small battery-powered devices that collect only a small volume of air (e.g., 2–4 m^3), even during a 24-h period, on a filter. However, this sample size is presently insufficient for NPAH monitoring in the general population with low exposure level. A reliable and sensitive technique for the quantification of NPAHs from the $PM_{2.5}$ in 1–2 m^3 of air sample volume is required for this approach.

Scheepers et al. have applied gas chromatography–mass spectrometry (GC–MS) or GC–MS/MS methods for personal exposure monitoring of 1-NP and its sensitivity was enough for the assessment of occupationally exposed workers [8–10]. However, these methods require laborious pretreatment steps including solid phase extraction, reduction of 1-NP to its amino analogue and derivatization prior to GC–MS (/MS) analysis. On the other hand, high-performance liquid chromatography (HPLC) methods with fluorescence, chemiluminescence and tandem MS detection have been developed for the analysis of high volume ambient air samples and standard reference materials, and these methods include on-line clean-up and reduction steps [11–14]. Therefore, HPLC methods would be more preferable for routine analysis of 1-NP for personal exposure monitoring than GC methods. The HPLC-tandem MS method is an accurate method but requires expensive instrumentation not accessible to many researchers. The objective of this study was to develop an analytical method by HPLC with fluorescence

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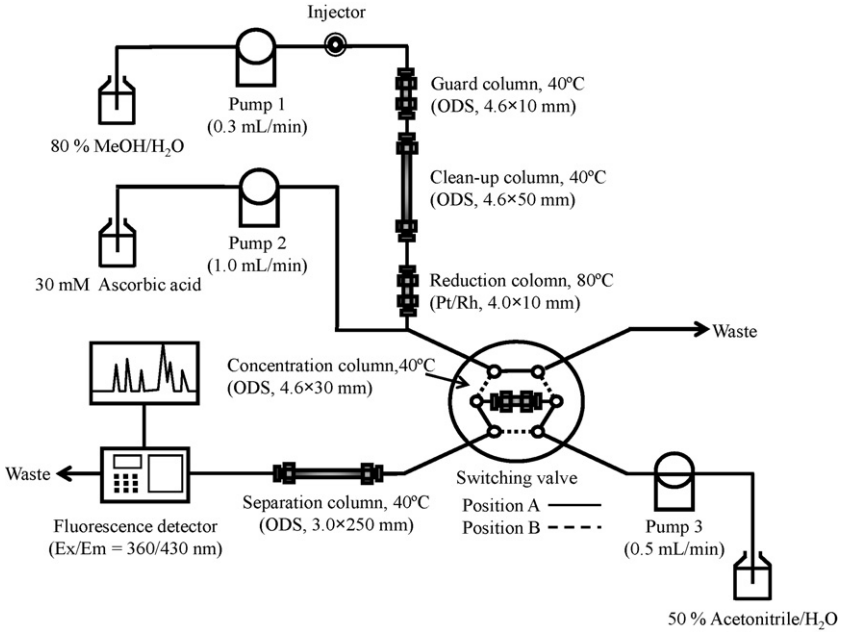

Fig. 1. Schematic diagram of the proposed HPLC system for the determination of 1-NP.

detection for determination of 1-NP in low volume ambient air samples, which would make it possible to monitor personal exposure to 1-NP.

2. Experimental

2.1. Chemicals

1-NP was purchased from Sigma–Aldrich (St. Louis, MO, USA) and perdeuterated 1-NP (1-NP-d_9) as an internal standard was obtained from C/D/N Isotopes Inc. (Quebec, Canada). Water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other chemicals and solvents used were of analytical reagent grade from Wako.

2.2. HPLC system and conditions

The HPLC system, including a FCV-12AH six-port valve (Shimadzu, Kyoto, Japan), is illustrated in Fig. 1 and consists of a LC-10AD pump (pump 1, Shimadzu), one L-6200 pump (pump 2, Hitachi, Tokyo, Japan), a LC-10AD VP pump (pump 3, Shimadzu), a Rheodyne model 7125 injector (20 μL loop), a DGU-14A degasser (Shimadzu), a 2475 fluorescence detector (Waters, Tokyo, Japan), and a CR-7A-plus integrator (Shimadzu). The system consists of five HPLC columns, a guard column (Cosmosil 5-MS-II; 4.6 mm i.d. × 10 mm, Nacalai Tespue, Kyoto, Japan), a clean-up column (Cosmosil 5C18-MS-II; 4.6 mm i.d. × 50 mm), a concentration column (Spheri-5 RP-18; 4.6 mm i.d. × 30 mm, Chemco, Tokyo, Japan), a reduction column (NPpak-RS; 4.0 mm i.d. × 10 mm, JASCO, Tokyo, Japan) and a separation column (Cosmosil 5C18-AR-II; 3.0 mm i.d. × 250 mm, Nacalai). The reduction column was kept at 80 °C in a CTO-2A column oven (Shimadzu) and the guard column, the clean-up column, the concentration column, and the separation column were kept at 40 °C in a CTO-10AS VP column oven (Shimadzu). The mobile phases and the flow rates were as described in Fig. 1. The excitation (λ_{ex}) and emission (λ_{em}) wavelengths were 360 and 430 nm, respectively. The column-switching sequence used is shown in Fig. 2. The interfering substances which elute faster or later than analytes from the clean-up column were discarded and a fraction of only the reduced analyte and internal standard were quantitatively transferred to the separation column.

2.3. Sample collection

According to our previous report [15], airborne particulate matter, both $PM_{2.5}$ and $PM_{10-2.5}$ fractions were collected. Residential indoor and suburban outdoor filter samples were collected for 24 h (about 2 m^3) in a house in Hakusan city (Ishikawa, Japan) and on the veranda of a building of Kanazawa University (Kanazawa, Ishikawa, Japan), respectively. The residential indoor samples ($n = 3$) were collected in the primary living area, not bedroom area where the participant spent the most time. The suburban outdoor samples ($n = 4$) were collected on a veranda away from the roadway and parking lot. The filter samples of the $PM_{2.5}$ fraction were used to determine 1-NP.

1. Sample extraction
   Filter sample (particles < 2.5 μm ($PM_{2.5}$) on a 20 mm Emfab filter)
   + Internal standards (1-NP-d_9)
   + DMSO (20 μL)
   Extracted with benzene/ethanol (2 mL) × 2
   Evaporated
   + Acetonitrile (80 μL)
   Sample solution (ca. 100 μL)

2. HPLC analysis (time events for the column-switching procedure)
   a) Injection step (0 min, position A):
   20 μL of the sample solution is injected.
   b) Clean-up and reduction step (0-12 min, position A):
   The analytes were separated on the clean-up column from interfering substances and then reduced to the corresponding amino-derivatives by the reduction column.
   c) Trapping step (12-16 min, position B):
   The reduced analytes were trapped on the concentration column with decreasing the content of methanol in the eluate.
   d) Detection step (16-40 min, position A):
   The enriched substances were eluted and separated on the separation column.

Fig. 2. Schematic diagram of the analytical procedures.