Direct Determination of NO₂-Substituted Pyrenes by Liquid Chromatography–Atmospheric Pressure Chemical Ionization–Mass Spectrometry

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Polycyclic aromatic compounds (PACs) are widespread as environmental contaminants, and many of them have a high mutagenicity and carcinogenicity, suggesting that these compounds pose risks to human health.¹ In particular, NO₂-substituted polycyclic aromatic hydrocarbons are strongly mutagenic. Furthermore, nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) may be generated in large quantities during combustion and ubiquitously exist in various environmental media.²

Gas chromatography–mass spectrometry (GC/MS) is an excellent and powerful tool for the identification of PAHs because of its high separation efficiency. However, it was found that the partial decomposition of nitro-PAHs occurs in the injector, the column and the interface of the GC-MS.³ Therefore, methods based on HPLC coupled with an UV detector have been extensively used for the analysis of PACs in environmental samples.⁴ However, the sensitivity and selectivity of LC-UV detection are low, and it is difficult to separate minute amounts of these compounds from environmental contaminants.

On the other hand, LC–fluorescence or chemiluminescence detection showed high sensitivity and selectivity for nitro-PAHs.⁵ In particular, TCPO–H₂O₂ chemiluminescence is the most sensitive HPLC detection technique in nitro-PAHs analyses.⁶ These techniques require the reduction of the nitro-PAHs to the aminated PAHs as a pretreatment. In order to analyze the nitro-PAHs in various samples, it is important to establish a direct detection method of the nitro-PAHs without prerelution of the nitro-PAHs to the aminated PAHs, because there are some cases when the NH₂-substituted PAHs exist in an environmental medium, such as biological samples.⁷

The LC/MS method would supply more useful information for the analysis of PACs because of the high specificity of an MS detector. However, there have been few reports on the determination of PACs using LC/MS due to the difficulty in ionizing low polarity compounds.^{8,9} Nitro-PAHs would be detected more easily than non-substituted polycyclic aromatic hydrocarbons by LC/MS, because the nitro-group has a high polarity. In fact, some of the nitro-PAHs have been detected by negative ion chemical ionization, because of the electron-withdrawing effect of the nitro group.¹⁰

In this report, we have presented a method for the direct determination of high mutagenic nitro-PAHs, 1-NP, 1,3-DNP,

1,6-DNP and 1,8-DNP using LC/APCI-MS without pretreatment. The detection technique has been applied to the analysis of urban soil, and 1-NP has been identified.

Experimental

All solvents were of HPLC grade, while the other chemicals were of analytical reagent grade. Four nitropyrenes, 1-NP, 1,3-DNP, 1,6-DNP and 1,8-DNP, were used as the target compounds. 1-NP was obtained from Wako Pure Chemical (Osaka, Japan). 1,3-DNP, 1,6-DNP and 1,8-DNP were purchased from Tokyo Kasei (Tokyo, Japan). Ultra-pure water was produced using a Milli-Q system (Millipore, Bedford, USA).

Liquid chromatography was carried out on an HPLC apparatus equipped with a Waters Associates (Milford, MA, USA) Alliance 2690 HPLC. A NUCLEOSIL 120-5C18 column (5 µm particle size, 4.6 mmφ × 150 mm i.d.) was used for the LC separation of the nitropyrenes. The mobile phase was water–methanol (25 + 75, v/v), and the flow rate was 1 ml min⁻¹. The column temperature was 45°C.

A Waters Associates (Milford, MA, USA) ZMD4000 mass spectrometer equipped with an atmospheric-pressure chemical ionization (APCI) interface was employed for the LC/MS analysis in the SCAN or selected ion-monitoring (SIM) mode. The operational conditions are described as follows: ionization, APCI-negative ionization; APCI probe temperature, 400°C; source block temperature, 130°C; capillary voltage, 3.0 kV; cone voltage, –25 V; desolvation gas flow rate, 400 l h⁻¹ and cone gas flow rate, 100 l h⁻¹ of nitrogen. When working in the SIM mode, the [M][–] (*m/z* 247 and 292 for 1-NP, 1,3-, 1,6- and 1,8-DNP) ions were monitored in the negative ion mode depending on the target analytes.

Two soil samples were taken from the side of a street with heavy traffic in Osaka city. The samples were prepared to determine the four nitropyrenes with LC/APCI-MS. The preparation of the soil sample for LC/APCI-MS analysis was based on the method of Hayakawa *et al.* using H₂SO₄ and NaOH to decompose the environmental matrix as described in Ref. 6. Each soil sample (10 g) was extracted twice with 30 ml of benzene–ethanol (3:1, v/v), ultrasonically homogenized, and then filtered. The filtrate was cleaned by extraction with 60 ml of 5% (w/v) sodium hydroxide, followed by 60 ml of 20% (w/v) sulfuric acid, and then twice with 60 ml of water. The benzene phase was evaporated to dryness, and the residue was dissolved in 1 ml of acetonitrile. The solution was then concentrated to

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Table 1 Detection limits and calibration equation for the nitropyrenes obtained using LC/APCI-MS

Analyte	Monitoring ion (m/z)	Retention time/min	Detection limit ^a	Calibration equation ^b	Correlation coefficient (r^2)
			Injection (pg)		
1-NP	247	11.47	25	$y = 3216x$	0.9942
1,3-NP	292	12.43	25	$y = 1481x$	0.9995
1,6-NP	292	8.98	5.0	$y = 4755x$	0.9998
1,8-NP	292	10.38	5.0	$y = 3974x$	0.9997

a. Calculated as three times the baseline noise. b. Least-squares regression equation.

Fig. 1 LC/APCI-MS chromatograms of the nitropyrenes (200 pg each). a) monitoring m/z 292, b) monitoring m/z 247, c) sample (m/z 247).

100 μ l. A 20 μ l aliquot was injected into the LC/APCI-MS.

Results and Discussion

In the case of the mass spectral investigation by ESI through flow injection of the nitro-PAHs solution (1.0 μ g/ml), there were no peaks assignable to the nitro-PAHs in either the negative or the positive ion modes. On the other hand, the $[M]^-$ peaks attributed to the radical anions of all the nitropyrenes (m/z 247 and 292) were observed as the only peak by APCI-MS through FIA in the negative ion mode. The mass spectra of nitropyrenes by FIA in the positive ion mode showed no peaks corresponding to the products of the reduction and elimination of the nitro-group. In the cases of 1-aminopyrene, 1,3-aminopyrene, 1,6-aminopyrene and 1,8-aminopyrene in the positive ion mode, the $[M+H]^+$ ions were observed. These facts indicate that nitropyrenes do not form amino derivatives of pyrene at the interface of the LC/APCI-MS. Consequently, the detection of the nitro-PAHs by APCI-MS makes the identification and quantification of the nitro-PAHs simpler than detection by GC/MS.

The nitropyrene ions were detected by APCI-MS after separation of the nitropyrenes through a reversed-phased ODS column. Figures 1a and 1b show the chromatograms of the 10 ng ml^{-1} nitropyrenes standard solution using selected ion monitoring of m/z 292 and 247 ions. The separation is achieved

in 14 min and the peaks are sharp and well-resolved. The retention times and the instrumental detection limits are summarized in Table 1. The instrumental detection limits calculated as three times the standard deviation of the base line were 25, 25, 5.0 and 5.0 pg injected 1-NP, 1,3-DNP, 1,6-DNP and 1,8-DNP, respectively. The calibration equations were obtained for the nitropyrenes using a series of standard solutions over the concentration range of 5 – 100 ng ml^{-1} . The equations and correlation coefficients (r^2) are summarized in Table 1. The sensitivities of the nitropyrenes by LC/APCI-MS were due to the electron affinity of the compounds.

Two soil samples were prepared for LC/APCI-MS analysis as described in the experimental section. No peaks of 1,3-DNP, 1,6-DNP and 1,8-DNP were observed. On the other hand, the peak of 1-NP was obtained using the SIM of the m/z 247 ion. The chromatogram for 1-NP in the sample is shown in Fig. 1c. The peak of 1-NP was clearly detected without interference from the peaks of contaminants in the soil samples. The retention times of 1-NP for the sample and standard solution were consistent, even if the elution pattern of the LC changed. From this result, the peaks of the samples were certain to be the peak of 1-NP.

The 1-NP level of the samples determined by this method was 0.14 and 0.96 ng g^{-1} . It is well known that diesel exhaust particles contain ng g^{-1} levels of the nitro-PAHs.¹ The determined levels of these samples were almost the same level as that of the diesel exhaust particles.

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