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# Electrospray mass spectrometric determination of 1-nitropyrene and non-substituted polycyclic aromatic hydrocarbons using tropylium cation as a post-column HPLC reagent

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A method is presented for the electrospray mass spectrometric determination of 1-nitropyrene and non-substituted polycyclic aromatic hydrocarbons (PAHs) using tropylium cation as a post-column HPLC reagent. In the method, the analytes form a π–π complex with the tropylium cation by mixing with the tropylium solution after the separation of 1-nitropyrene and PAHs by reversed-phase liquid chromatography. The complexes then transfer the cations of the polycyclic aromatic compounds (PACs) and the tropylium compounds by collision-induced dissociation (CID) at the ion transfer region of the electrospray system. The generated PAC cations are detected by mass spectrometry. With the proposed method, 1-nitropyrene and PAHs could be simultaneously determined, and the detection limits (S/N = 3) in the selected-ion monitoring mode were 0.83 ng of the injected 1-nitropyrene and 0.67–1.24 ng of PAHs.

## Introduction

It is well-known that many polycyclic aromatic compounds (PACs) are mutagenic and carcinogenic.[1] In particular, $NO_2$-substituted polycyclic aromatic hydrocarbons (nitro-PAHs) are strongly mutagenic. For example, when tested in *Salmonella typhimurium* (strain TA98) in the absence of exogenous metabolizing enzymes (*i.e.*, −S9), 1-nitropyrene (1-NP) is reported to be 200 times more mutagenic than benzo[*a*]pyrene (+S9).[2] Furthermore, nitro-PAHs may be generated in large quantities during combustion and exist ubiquitously as environmental contaminants.[3] In order to evaluate the risk of nitro-PAHs in the environment, the levels in various media have been extensively studied, and many methods for the determination of PACs have been studied and used.[4,5]

Gas chromatography mass spectrometry (GC-MS) is an excellent and powerful tool for the identification of PACs because of its high separation efficiency.[6] However, it was found that the partial decomposition of unstable species of PACs, such as nitro-PAHs, occured in the injector, the column and the interface of the GC-MS.[7] This effect makes the identification and quantification of PACs difficult. These problems are overcome by using high performance liquid chromatography (HPLC), because the thermal decompositions and reactions of PACs are avoided by carrying out HPLC at room temperature. Therefore, methods based on HPLC coupled with a fluorescence detector have been extensively used for the analysis of PACs in environmental samples.[8]

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 of ionizing low polarity compounds.[9,10]

In a previous report, my group presented a method for the determination of non-substituted PAHs (PAHs) by liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS).[11] In the method, PAHs were detected by monitoring the PAH-tropylium complexes, which formed on mixing of PAHs with the tropylium cation after the separation of PAHs using HPLC. The tropylium cation ($TR^+$) has long been known as a strong π-acceptor. It recognizes PAHs by π–π interactions and almost quantitatively forms the [PAH-$TR^+$] 1 : 1 cation complex.[12] The complexes were pre-charged in solution and showed good sensitivity in ESI-MS. However, it is difficult to detect the tropylium complexes of some species of PACs, in which the interactions with the tropylium cation are weak. For example, the tropylium complex of 1-NP is unstable thermodynamically and difficult to detect by ESI-MS.

In this work, the PACs formed the π–π complex with tropylium cations on mixing with the tropylium solution after the separation of five PACs, 1-NP, pyrene, benzo[*a*]pyrene, perylene and coronene, by reversed-phase liquid chromatography. The complexes then transferred the cations of the PACs and the tropylium compounds by collision-induced detection (CID) at the ion transfer region of the electrospray system. The generated PAC cations were detected by mass spectrometry. The π–π complexes do not need to be stable enough to pass the electrospray interface in this method. Using this method, 1-NP and PAHs were determined simultaneously by LC-ESI-MS.

## Experimental

### Materials

All solvents were of HPLC grade and other chemicals were of analytical-reagent grade. Five PACs, 1-NP, pyrene, benzo[*a*]-pyrene, perylene and coronene, were used as target compounds. 1-NP, perylene and pyrene were obtained from Wako Pure Chemical (Osaka, Japan). Coronene and tropylium tetrafluoroborate were purchased from Tokyo Kasei (Tokyo, Japan) and benzo[*a*]pyrene was from Nacalai Tesque (Kyoto, Japan). Ultra pure water was produced with a Milli-Q system (Millipore, Bedford, USA).

### HPLC conditions

Liquid chromatography was carried out on an HPLC apparatus equipped with a Hewlett-Packard HP-1100 system (Yokogawa,

Tokyo, Japan). A Hewlett Packard Zorbax Eclipse XDB-C18 column (5 μm particle size, 150 × 2.1 mm id) was used for the LC separation of the PAC. The column temperature was 30 °C. Acetonitrile–water (70 : 30, v/v) was used as the mobile phase and the flow rate was 0.2 ml $min^{-1}$. For LC-ESI-MS, the post-column addition of 1.0 μg $ml^{-1}$ of the tropylium cation solution (acetonitrile–water 10 : 90, v/v) at 0.1 ml $min^{-1}$ was carried out using a Shimadzu LP-6A liquid delivery pump. The mobile phase was mixed at a T junction (Yokogawa 0100-0782, stainless-steel) with the tropylium cation solution.

### LC-ESI-MS

Flow injection analysis (FIA)-ESI-MS was performed using a Hewlett-Packard HP-1100 MSD system. Acetonitrile–water (50 : 50, v/v) was used as the mobile phase and the flow rate was 0.20 ml $min^{-1}$. The working conditions for ES were the following: the drying nitrogen gas temperature was set at 340 °C, and the gas was introduced into the capillary region at a flow rate of 12 l $min^{-1}$. The capillary was held at a potential of 3500 V relative to the counter electrode for the positive-ion mode with a mass to charge range of 150–400. The fragmentor voltage was set at 100 V. When optimizing the fragmentor voltage, the voltage was varied between 30 and 200 V. The injection volume of the sample solution in ESI-MS was 10 μl. For LC-ESI-MS, the fragmentor voltage was set at 70 V from 0 to 8.2min and 180 V from 8.2 to 60 min. The injection volume of the sample solution in ESI-MS was 50 μl. When working in the selected ion monitoring mode (SIM), the $[M + H]^+$ and $[M]^+$ ions (*m/z* 248, 202, 252 and 300 for 1-NP, pyrene, perylene, benzo[*a*]pyrene and coronene) were monitored depending on the target analytes.

## Results and discussion

### Detection of PAHs

In the case of the mass spectral investigation by ESI through flow injection of the PAH–acetonitrile solution (1.0 μg $ml^{-1}$ of pyrene, benzo[*a*]pyrene, perylene and coronene), there were no peaks assignable to the PAHs. On the other hand, the injection of the solution of PAHs containing the same molar amount of $TR^+$ (1.0 μg $ml^{-1}$) gave $[M + 91]^+$ peaks corresponding to the [PAH-$TR^+$] complex, and $M^{+\cdot}$ peaks attributed to the PAH radical cations as the main peaks. The intensities of the $M^{+\cdot}$ peaks were stronger than those of the $[M + 91]^+$ peaks, when the fragmentor voltage was at 100 V. For example, in the case of pyrene, the peak of the pyrene radical cation was about ten times stronger than that of the [pyrene-$TR^+$].

In order to establish the optimum fragmentor voltage for the detection of the PAH complexes, the signals of the complex and radical cation *versus* fragmentor voltage for pyrene were studied. At first, acetonitrile–water (50 : 50, v/v) was used as the mobile phase. When higher fragmentor voltages were used, more of the pyrene radical cation peak was observed and a decrease in the intensity of the [pyrene-$TR^+$] occurred. This fact indicates that charge transfer may potentially compete with complex formation and collision-induced dissociation in the electrospray interface as shown in Scheme 1. Such charge transfer reactions are often significant.[11,12] The [Pyrene-$TR^+$] complex showed a maximum at 60 V as can be seen in Fig. 1. On the other hand, the $M^{+\cdot}$ of pyrene showed a maximum at 180 V, and the peak intensity of the pyrene radical cation at 180 V was about six times stronger than that of [Pyrene-$TR^+$] at 60 V. Hence, radical cations of the PAHs were selected as the monitored ions to detect the PAHs, and the optimum fragmentor voltage was determined to be 180 V for the present method. When methanol–water (50 : 50, v/v) was used as the mobile phase, the peak intensity of the [Pyrene-$TR^{+\cdot}$] ion was stronger than that observed in the acetonitrile–water mobile phase. This result would be due to the coulomb stabilization between the complex and methanol. Acetonitrile–water was selected as the mobile phase, because the peak intensity of $M^{+\cdot}$ in acetonitrile–water was stronger than that in methanol–water.

Under these conditions (fragmentor voltage, 180 V; mobile phase, acetonitrile–water), the mass spectra of coronene (1.0 μg $ml^{-1}$) in the presence of the same molar amount of $TR^+$ by FIA showed the radical cation species (*m/z* 300), but there were no peaks corresponding to the [coronene-$TR^+$] complex (*m/z* 391) as shown in Fig. 2.

### Detection of 1-nitropyrene

In the case of the mass spectral investigation by ESI through flow injection of the 1-NP–acetonitrile solution (1.0 μg $ml^{-1}$), there were no peaks assignable to 1-NP. On the other hand, for the injection of the solution of 1-NP containing the same molar amount of $TR^+$ (1.0 μg $ml^{-1}$), the $M + H^+$ peak attributed to the protonated 1-NP was observed as the main peak. The $M + H^+$ peaks could not be observed in the absence of $TR^+$. This protonation of 1-NP did not occur directly; it proceeded through the formation of [1-NP·$TR^+$] complex as shown in Scheme 2.

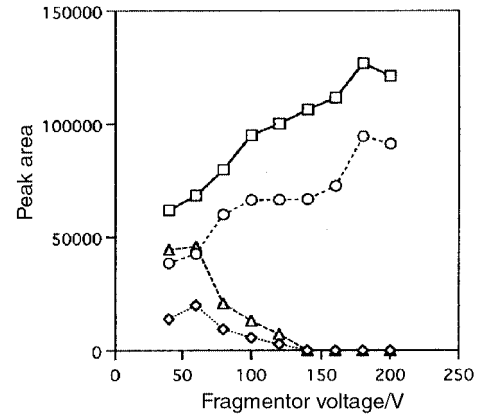



**Fig. 1** Variation in peak of $[M^{+\cdot}]$ and [M + 91] ions *versus* the fragmentor voltage (V) for pyrene: □ $[M^{+\cdot}]$ (mobile phase; acetonitrile–water); ○ $[M^{+\cdot}]$ (methanol–water); ◇ $[M + 91]^+$ (acetonitrile–water); △ $[M + 91]^+$ (methanol–water).

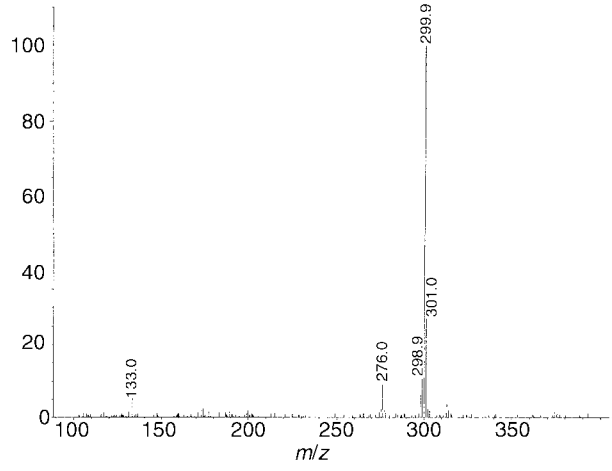


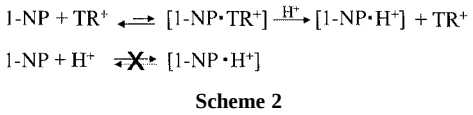

**Fig. 2** ESI-MS spectrum of coronene at fragmentor voltage of 180 V.

$$PAH + TR^+ \rightleftharpoons [PAH\cdot TR^+] \xrightarrow{CID} PAH^{+\cdot} + TR^{\cdot}$$

**Scheme 1**

Protons can attack the cation complex more easily than neutral 1-NP, because of the resemblance in their polarity. Therefore, the addition of $TR^+$ may lead to the protonation of 1-NP and the ability to detect it by LC-ESI-MS.

In order to establish the optimum fragmentor voltage for the detection of 1-NP, the signals of $[M + H]^+$ *versus* drift voltage for 1-NP were studied. The $[M + H]^+$ ion showed a maximum at 70 V as can be seen in Fig. 3. Hence, the optimum fragmentor voltage for 1-NP was determined to be 70 V. The mass spectra of 1-NP (1.0 μg $ml^{-1}$) in the presence of the same molar amount of $TR^+$, by FIA, showed the protonated 1-NP (*m/z* 248), but there were no peaks corresponding to the products of the reduction and elimination of the nitro-group as shown in Fig. 4. This result indicates that detection by ESI-MS using the proposed method makes the identification and quantification of 1-NP simpler than detection by GC-MS.




**Scheme 2**

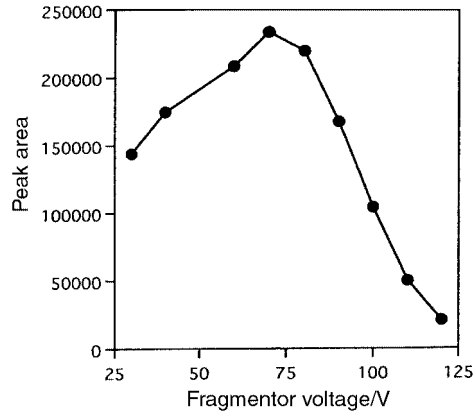



**Fig. 3** Variation in peak area of $[M + H]^+$ ions *versus* the fragmentor voltage (V) for 1-nitropyrene.

## LC-ESI-MS

Radical cations of PAHs and protonated 1-NP were detected by ESI-MS with the addition of $TR^+$ solution after the separation of PACs through an ODS column. The fragmentor voltage was changed after 8.2 min. From 0 to 8.2 min, the voltage was 70 V for 1-NP and from 8.2 to 60 min was 180 V for other PAHs. Fig. 5 shows the chromatograms of 1-NP, pyrene, perylene, benzo[*a*]pyrene and coronene using the selected ion modes, *m/z* 248, 202, 252 and 300, each peak of PACs being detected separately by the selected ion modes. Thus, the combination of HPLC and ESI-MS provides further high specificity.

Calibration curves were obtained for 1-NP, pyrene, perylene, benzo[*a*]pyrene and coronene using a series of standard solutions over the concentration range from 1.0 to 25 μM, as

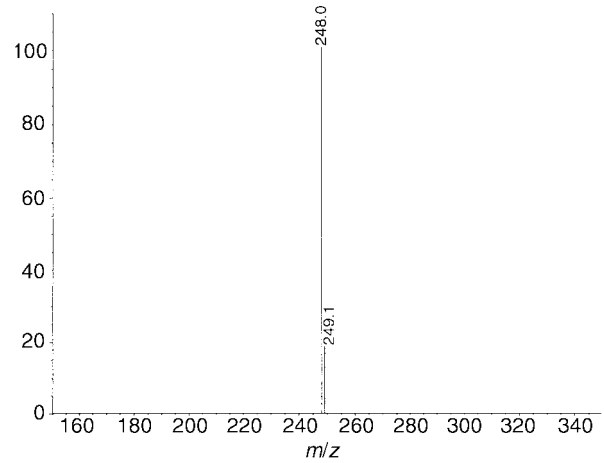



**Fig. 4** ESI-MS spectrum of 1-nitropyrene at fragmentor voltage of 70 V.

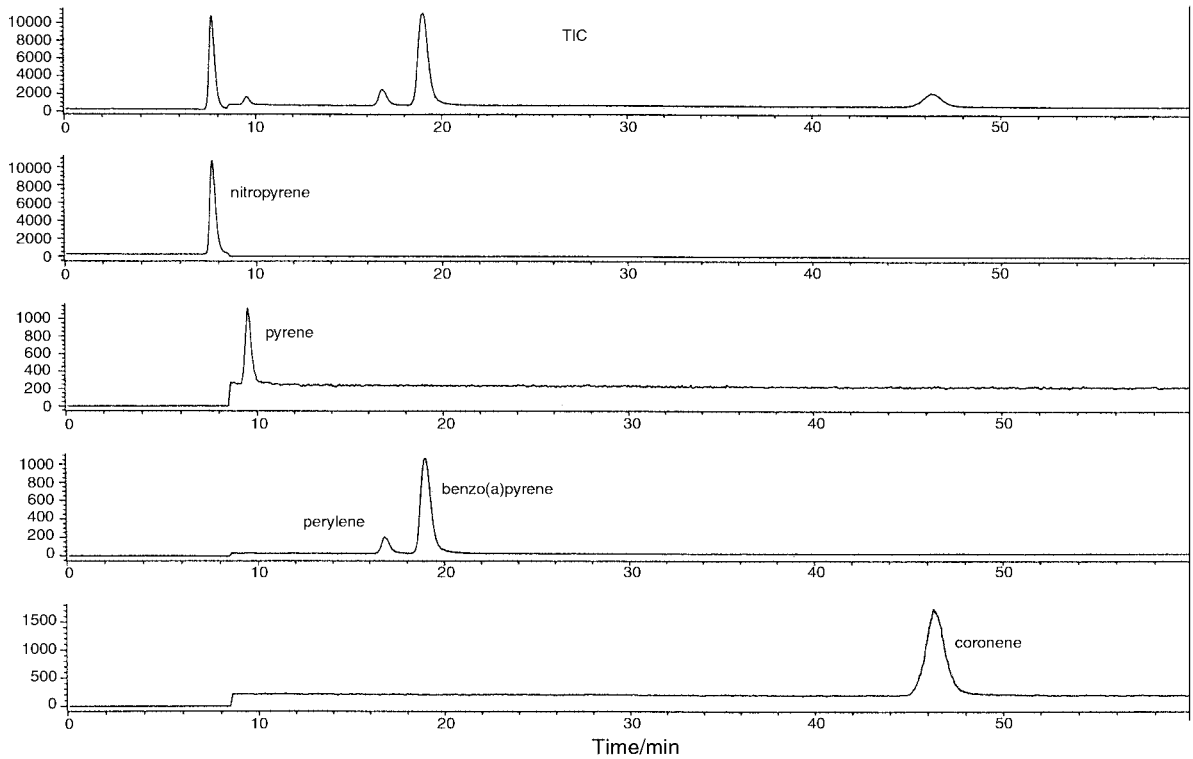



**Fig. 5** LC-ESI-MS chromatograms of 1-nitropyrene and PAHs for the injection (50 μl) of the standard solution by selected-ion monitoring; *m/z* 248, 202, 252, 391.

**Table 1** Detection limits and calibration equation for 1-nitropyrene and PAHs obtained using LC-ESI-MS

| Analyte | Monitor ion (*m/z*) | Retention time/min | Detection limit[a] Conc/μM | Detection limit[a] Injection/ng | Calibration equation[b] | Correlation coefficient ($R^2$) |
|---|---|---|---|---|---|---|
| 1-Nitropyrene | 248 | 7.66 | 0.67 | 0.83 | $y = 8201x - 3732$ | 0.999 |
| Pyrene | 202 | 9.48 | 1.2 | 1.2 | $y = 832.8x - 489.9$ | 0.996 |
| Perylene | 252 | 16.8 | 0.76 | 0.96 | $y = 2368x - 1218$ | 0.999 |
| Benzo[*a*]pyrene | 252 | 19.0 | 0.39 | 0.67 | $y = 16450x - 2885$ | 1.00 |
| Coronene | 300 | 46.3 | 0.35 | 0.68 | $y = 4560x - 706.4$ | 0.998 |

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

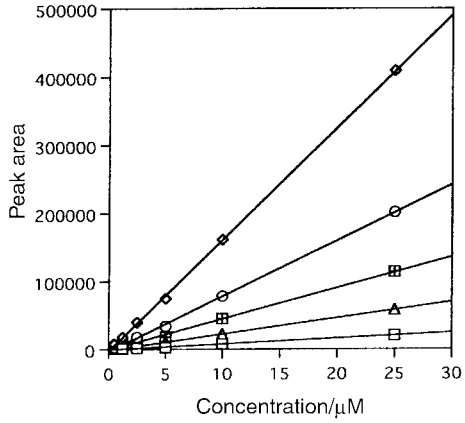



**Fig. 6** Calibration graphs for 1-nitropyrene and PAHs in the range of 1–25 μM: ○ 1-nitropyrene; □ pyrene; ◇ benzo[*a*]pyrene; △ perylene; ⊞ coronene.

shown in Fig. 6. The detection limits and calibration equations are summarized in Table 1. The detection limits, defined as three times the noise, in the selected-ion monitoring mode were 25–115 ng for the PAHs injected.

## Conclusions

The simultaneous determination of 1-NP and PAHs by LC-ESI-MS has, in the past, not been particularly well reported. The present method makes electrospray-mass spectrometric determination of PACs possible using $TR^+$ as a post-column reagent.

The detection of 1-NP by LC-ESI-MS in this method did not need the reduction and elimination of the nitro-group. Therefore, quantification of 1-NP is simple and easy compared to that using GC-MS. The next step of this work is to expand the analytical method to the detection of other nitro-PAHs and to apply the procedure to the determination of PACs in various media, such as diesel exhaust particulates.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