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DECOMPOSITION OF NITROPYRENE DURING GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC ANALYSIS OF AIR PARTICULATE AND FLY-ASH SAMPLES

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SUMMARY

1-Nitropyrene was found to decompose during gas chromatographic-mass spectrometric (GC-MS) analysis. Decomposition was observed both on the column and after the column. The decomposition product was tentatively identified as aminopyrene. Extracts of an air particulate sample and an electrostatically precipitated fly-ash sample from a municipal incinerator were examined for the presence of 1-nitropyrene. The compound was identified in the air particulate extract at the low ppm level, but was not detected in the fly-ash sample. A stable isotope standard was employed to compensate for the lability of the 1-nitropyrene in the GC-MS system to assure positive identification and quantification of the low levels present in air particulates.

INTRODUCTION

There is an increasing awareness that the mutagenicity of airborne particulate matter cannot be attributed solely to the polycyclic aromatic hydrocarbons (PAHs) present. Direct-acting mutagens, that is, compounds which unlike the PAHs are mutagenic for *Salmonella typhimurium* strains without activation by a mammalian metabolic system, have been found in airborne particulates¹, diesel particulate extracts^{2,3} and fly-ash^{4,5}. The presence of nitroaromatics in airborne particulates has been implicated through the use of bacterial strains of differing sensitivity to nitroaromatics¹. By fluorescence quenching after thin-layer chromatography, 3-nitrofluoranthene and 6-nitrobenzo[*a*]pyrene have been identified in atmospheric samples⁶. 1-Nitropyrene has been quantified in diesel particulate extracts^{2,3,7} and has been found to account for up to 30% of the direct-acting mutagenicity of a diesel exhaust extract².

In this study we have examined pooled air particulate sample extracts and

pooled municipal incinerator fly-ash extracts for the presence of 1-nitropyrene using gas chromatography-mass spectrometry (GC-MS). Nitropyrene was present in the air particulate sample and undetectable in the fly-ash sample. Decomposition of nitropyrene in the GC-MS system made positive identification and quantification difficult at the low levels present in the environmental samples. The use of deuterated (d_9) 1-nitropyrene as an internal standard allowed the positive identification and quantification of 1-nitropyrene.

EXPERIMENTAL

Sample collection and extraction

The air particulate sample was a pooled extract from two urban sampling sites in southern Ontario. The samples were supplied by the Ontario Ministry of the Environment, Air Resources Branch. The particulates were collected on 8×10 in. glass-fiber filters using a standard Hi-Vol technique⁸. The filters were extracted overnight with benzene using a Soxhlet extraction apparatus. The dry weight of the combined extracts was 20.2 mg.

The fly-ash sample was a pooled extract from eleven samples of fly-ash from a municipal incinerator in Ontario. The methods of collection and extraction have been described previously⁹. The samples were of electrostatically precipitated fly-ash and were collected on different days. About 20 g of each fly-ash sample were extracted with benzene in a Soxhlet apparatus. The dry weight of the combined extracts was 16.3 mg.

Analysis

A 1-nitropyrene and a deuterated (d_9) 1-nitropyrene standard were received from Ford Motor Company (Dearborn, MI, U.S.A.). 1-Aminopyrene was purchased from Aldrich (Milwaukee, WI, U.S.A.). The high-performance liquid chromatographic (HPLC) separation procedure described by Schuetzle *et al.*² was used to fractionate the samples. A 10.7-mg aliquot of the air particulate sample was fractionated and an 8.9-mg aliquot of the fly-ash extract was separated on a semi-preparative scale Porasil 10- μ m normal-phase column. A 1-nitropyrene standard was used to determine the time during the gradient program when the fraction should be collected for nitropyrene analysis.

The GC-MS analyses were performed using an HP5992 GC-MS-calculator system equipped with an x-y plotter and floppy disk. Packed column runs were carried out on a 6-ft. column packed with high-performance Aue packing¹⁰. A membrane separator interfaced the packed column to the MS system. Analysis by capillary GC was carried out on a 25-m fused silica OV-101 column with splitless injection. An HP59916A glass capillary effluent splitter interfaced the capillary column to the MS system. The mass spectrometer was tuned using the manufacturer-supplied program AUTOFUNE.

RESULTS AND DISCUSSION

The electron-impact fragmentation pattern of 1-nitropyrene as reported by Schuetzle *et al.*² is given in Table I. These abundances are in good agreement with the

TABLE I
RELATIVE ABUNDANCES (%) OF IONS IN 1-NITROPYRENE AND 1-AMINOPYRENE

m/z	1-Nitropyrene				1-Aminopyrene	
	Schuetzle et al. ²	480 ng injected	130 ng injected	35 ng injected		
247	100	90,90,93	88,82	59,58	30,31	—
201	100	100,100,100	100,100	72,70	39,41	2,2
200	55	74,75,74	77,72	62,55	39,34	3,3
217	40	49,61,50	60,71	100,100	100,100	100,100
189	40	56,56,54	59,60	62,65	61,50	35,39
216	—	6,7,5	9,10	22,18	24,25	21,20
108.5	—	3, <5,2	5,6	20,15	24,22	23,26
94	—	5,7,5	8,10	21,18	33,34	31,34

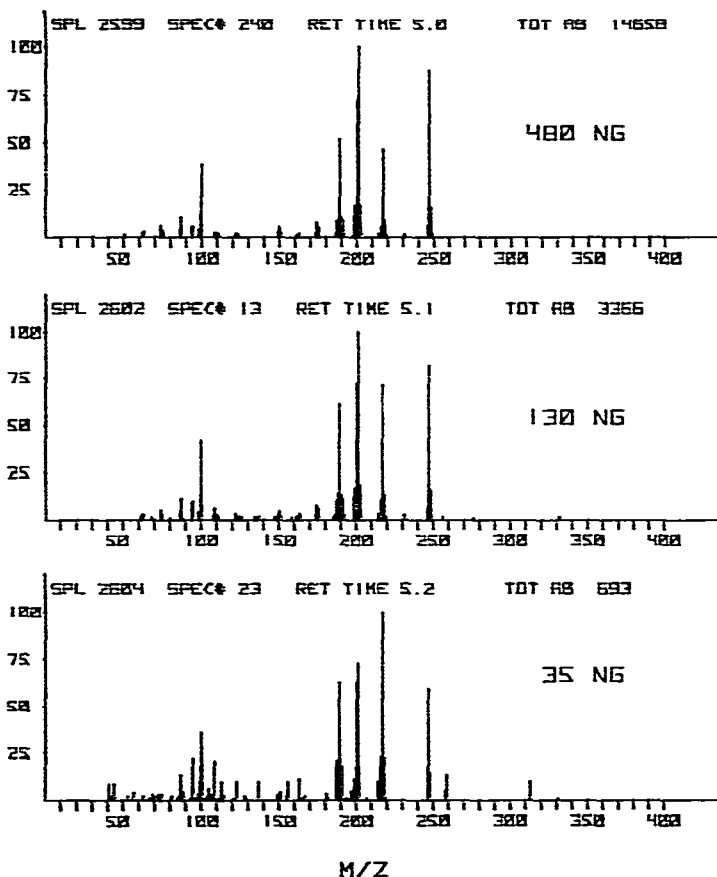


Fig. 1. Mass spectra for three amounts of 1-nitropyrene. The small peaks at $m/z = 259$ and 313 of the 35-ng injection are due to the background.

spectrum identified by Yu and Hites¹¹ as nitropyrene or nitrofluoranthene in a diesel particulate extract sample. Our spectra of high concentrations of 1-nitropyrene agree well with these published spectra. However, it was found that when smaller amounts of 1-nitropyrene were injected these relative abundances were not observed.

Fig. 1 shows the spectra obtained for 1-nitropyrene for three levels of compound injected. The 1-nitropyrene was introduced by on-column injection on a packed column. The spectra were taken at the peak maxima, with the lowest valley between peaks used for background subtraction. The ions at $m/z = 217$, 216, 108.5 and 94 increased in relative abundance as lower amounts of 1-nitropyrene were injected. Fig. 2 shows that these ions are all present in the spectrum of 1-aminopyrene. As can be seen from Table I, for the 15–35-ng 1-nitropyrene injections, the relative ion abundances of m/z 217/216, 217/108.5 and 217/94 all approach the values found in 1-aminopyrene spectra. This suggests that the spectra in Fig. 1 are combined spectra of 1-nitro- and 1-aminopyrene with the 1-aminopyrene to 1-nitropyrene ratio increasing with decreasing amounts of 1-nitropyrene injected. If, as suggested by the data in Table I, the presence of ions at $m/z = 216$, 108.5 and 94 is due to 1-aminopyrene, then the $m/z = 108.5$ peak is due to the doubly charged molecular ion of 1-aminopyrene.

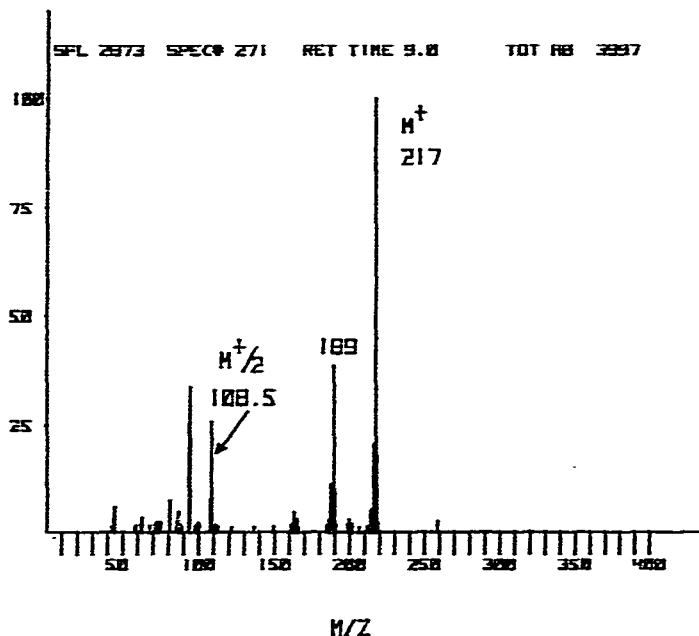


Fig. 2. Mass spectrum of 1-aminopyrene.

Possible explanations for the presence of 1-aminopyrene are that it is an impurity in the standard 1-nitropyrene or that the nitropyrene decomposes to 1-aminopyrene somewhere in the GC-MS system. Fig. 3 shows a selected-ion monitoring (SIM) trace of a co-injection of 1-nitropyrene and 1-aminopyrene. As the two compounds are separated on the packed column employed, a 1-aminopyrene impurity in

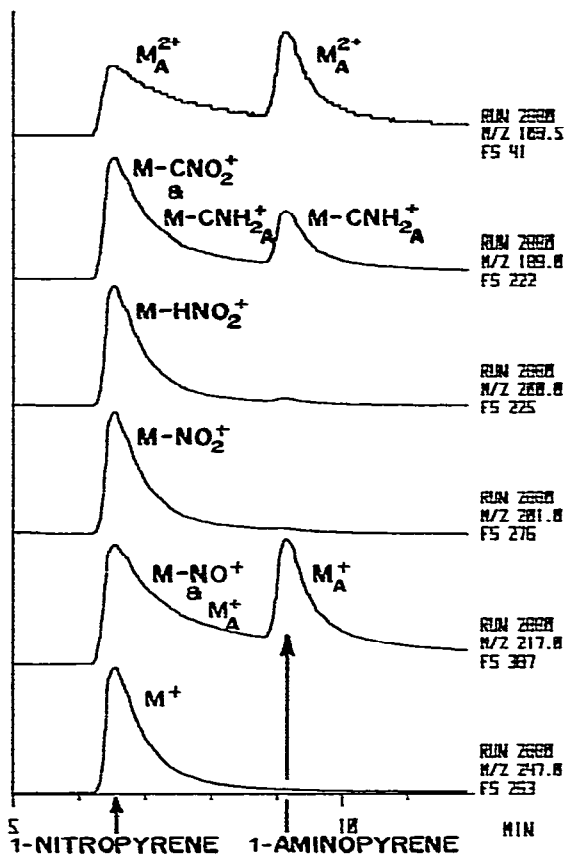


Fig. 3. SIM data for a co-injection of 1-nitropyrene (101 ng) and 1-aminopyrene (97 ng). Aue 6-ft. packed column. Conditions: injection port temperature, 250°C; initial column temperature, 220°C for 3 min, then programmed at 4°C/min to 250°C. Subscript A indicates ion of 1-aminopyrene. FS = full-scale value.

the standard could not explain the 1-aminopyrene present in the spectra in Fig. 1. Decomposition in the GC-MS system would explain the observed spectra. For decomposition on the column, the longer retention time of the compound as it decomposed to 1-aminopyrene would produce a tailing peak. It can be seen in Fig. 3 that the ions characteristic of 1-aminopyrene, *i.e.*, $m/z = 217$, 189 and 108.5, are indeed tailing peaks. The ions at $m/z = 247$, 201 and 200, which are present only in 1-nitropyrene, produce more nearly symmetrical peaks.

In addition to on-column decomposition of 1-nitropyrene to 1-aminopyrene, it would appear that decomposition is occurring at the GC-MS interface or in the mass spectrometer source. The $m/z = 247$ peak is due entirely to the 1-nitropyrene, so the peak maximum of this peak gives the retention time of nitropyrene on the column. In Fig. 3 the high relative abundance of the $m/z = 217$ ion in comparison with the literature spectra of 1-nitropyrene indicates some 1-aminopyrene to be present. As the peak maximum of the $m/z = 217$ ion is coincident with that of $m/z = 247$, this indicates that the compound traveled through the column as nitropyrene and decomposed after the column at the interface or in the MS source. The appearance of a

peak at $m/z = 108.5$ with the same retention times maximum also suggests decomposition after the column, as this peak is the doubly charged molecular ion peak seen in the spectrum of 1-aminopyrene. Suggested identities of the fragment ions are given in Fig. 3.

The same change in spectral pattern with concentration occurred using a fused silica capillary column (OV-101) with splitless injection. Fig. 4 shows the $m/z = 247$ (M)⁺ and $m/z = 217$ ions for a SIM run with 18 ng of 1-nitropyrene standard. Note that no molecular ion peak is present, suggesting that the 1-nitropyrene is entirely decomposed.

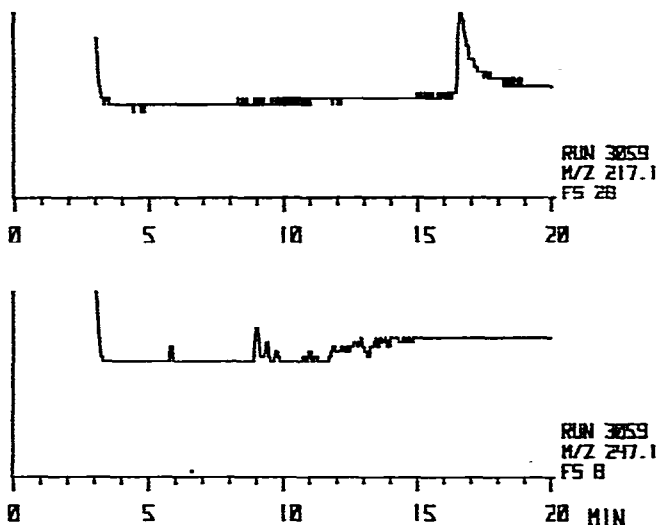


Fig. 4. SIM data for 18 ng of a 5.5 ng/ μ l standard of 1-nitropyrene. $m/z = 247$ is the molecular ion, (M)⁺. 25-m OV-101 capillary column; splitless injection. Conditions: injection port temperature, 250°C; initial column temperature, 150°C, then programmed at 5°C/min to 250°C.

Fig. 5 shows the mass spectrum of a deuterated (d_9) 1-nitropyrene standard ($M^+ = 256$). Fig. 6 shows a SIM analysis of a co-injection of a large amount (*ca.* 1 μ g) of this deuterated 1-nitropyrene with 18 ng of 1-nitropyrene standard. A distinct (M)⁺ peak for 1-nitropyrene is now present at $m/z = 247$ and the abundances of both $m/z = 247$ and 217 are increased (compare with Fig. 4). Fig. 7 shows a SIM run of 1-nitropyrene- d_9 , monitoring the (M)⁺, ($M - NO$)⁺ and ($M - CNO_2$)⁺ peaks for both 1-nitropyrene and 1-nitropyrene- d_9 . The very low full-scale value for the $m/z = 247$ ion shows that there is no interference of the 1-nitropyrene- d_9 with the molecular ion of 1-nitropyrene, that is, the $m/z = 247$ abundance in Fig. 6 is due entirely to the 1-nitropyrene. The $m/z = 217$ abundance, however, is in part from the deuterated standard.

By injecting a large amount of deuterated standard, the fraction decomposing is small. As the active sites causing decomposition do not distinguish deuterated from non-deuterated, the fraction of 1-nitropyrene decomposing is also small and the $m/z = 247$ peak reflects the actual concentration. The estimated minimum detectable amount of 1-nitropyrene, that is, the minimum amount of 1-nitropyrene which when

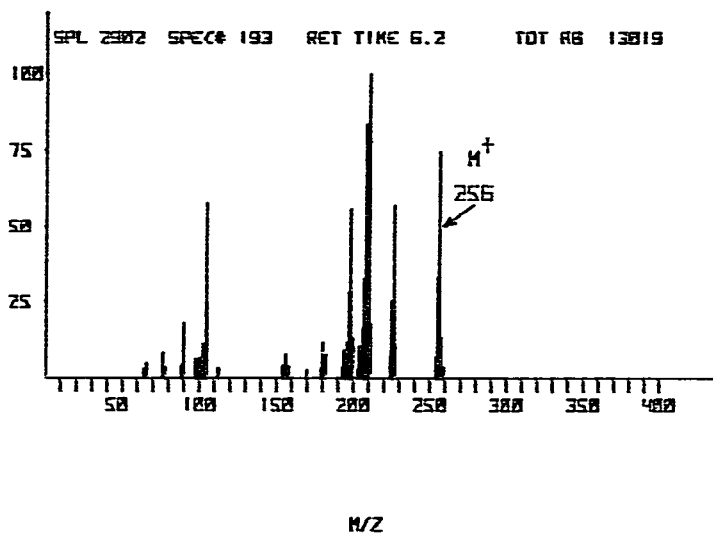


Fig. 5. Mass spectrum of 1-nitropyrene-d₉.

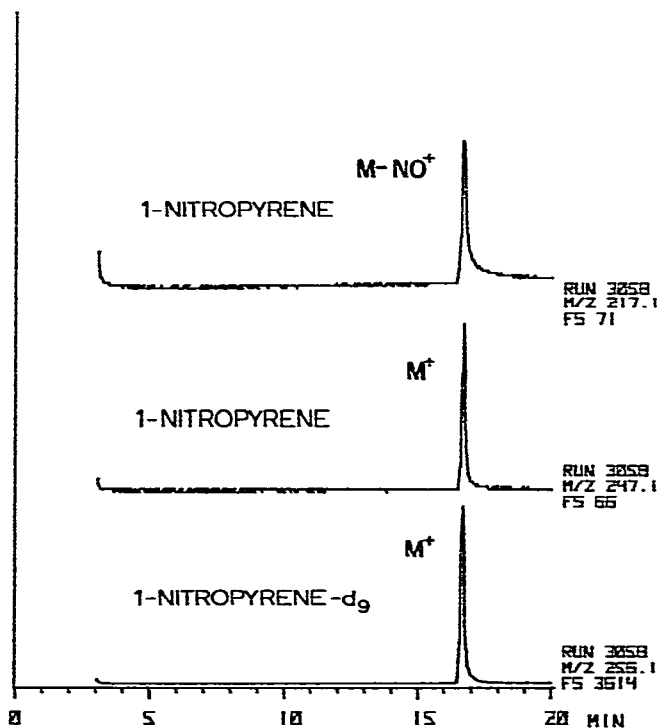


Fig. 6. SIM data for 18 ng of 1-nitropyrene standard co-injected with ca. 1 μ g of 1-nitropyrene-d₉. Column and conditions as in Fig. 4.

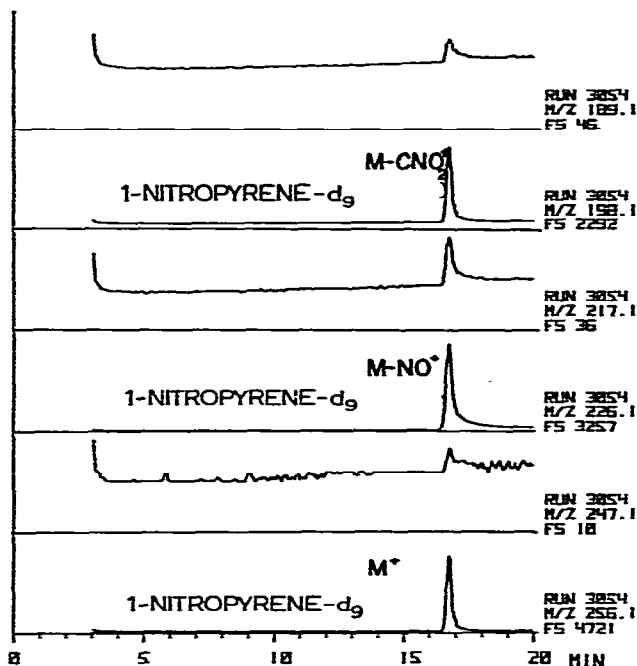


Fig. 7. SIM data for ca. 1 μg of 1-nitropyrene- d_9 , monitoring the $(\text{M})^+$, $(\text{M} - \text{NO})^+$ and $(\text{M} - \text{CNO}_2)^+$ ions for 1-nitropyrene- d_9 and 1-nitropyrene. Column and conditions as in Fig. 4. Note the insignificant $m/z = 247$ abundance.

co-injected with 1 μg of 1-nitropyrene- d_9 would give a discernible $m/z = 247$ peak, is 3 ng.

The HPLC fraction of the air particulate sample in which 1-nitropyrene would be eluted was examined by GC-MS-SIM. Fig. 8 shows the air particulate fraction injected alone (bottom two ion traces) and co-injected with 1-nitropyrene- d_9 . The appearance of a peak at $m/z = 247$ which coincides with a peak at $m/z = 217$ and matches the retention time of the $(\text{M})^+$ peak of $m/z = 256$ for 1-nitropyrene- d_9 identifies this peak as 1-nitropyrene. One or more of the three earlier peaks seen on the $m/z = 217$ ion trace could be isomers of nitropyrene or nitrofluoranthene with no discernible $(\text{M})^+$ peak.

The average response (area of $m/z = 256$ per ng) for four injections of 1.1 μg of 1-nitropyrene- d_9 was 435 area counts. The standard deviation of the four numbers was 60. This relatively large standard deviation may be attributable to the difficulties with reproducibility in the splitless injection mode and to variable adsorption effects. The spectral pattern as evidenced by the ratio of $m/z = 256/226$ remained constant.

For the air particulate sample, the molecular ion was used for quantification as there is no interference from the deuterated standard. Using the co-injected deuterated standard as an internal standard to correct for non-uniform adsorption and injection, corrected response (area of $m/z = 247$ per ng) values for duplicate injections of the 5.5 ng/ μl of 1-nitropyrene standard were 382 and 399. By comparing Figs. 6 and 8, it can be seen that the 5.5 ng/ μl standard closely corresponds to the amount of 1-nitropyrene in the air particulate sample injections. Therefore, the average value

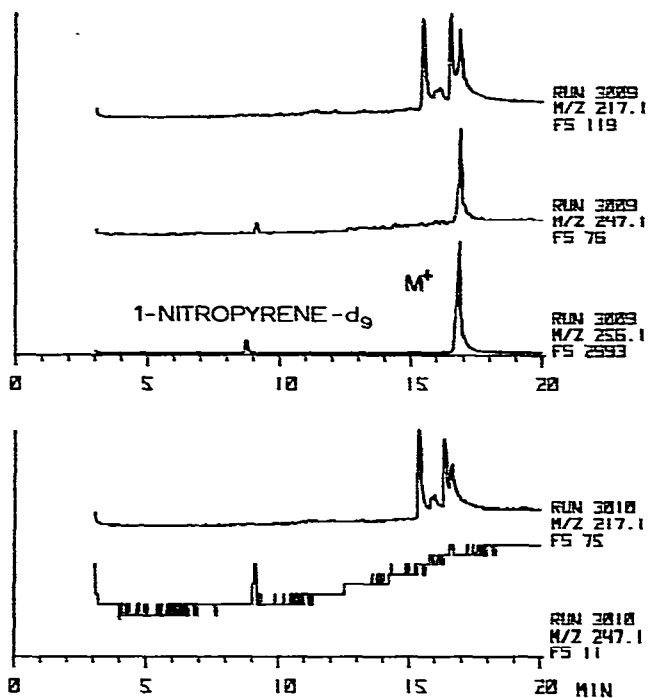


Fig. 8. SIM data for an air particulate extract sample, injected alone (lower two traces) and co-injected with $ca. 1 \mu\text{g}$ of 1-nitropyrene- d_9 (upper three traces). Column and conditions as in Fig. 4. Each injection was 3% of the air particulate HPLC fraction.

of 390 (area of $m/z = 247$ per ng of nitropyrene) was used to calculate the concentration of 1-nitropyrene in the air particulate sample injections. Accounting for dilution volumes and the total air particulate extract weight, the 1-nitropyrene concentration in the extract is 60 and 68 ppm (w/w) for the duplicate quantifications.

For the HPLC fraction of the fly-ash extract in which 1-nitropyrene would appear, if present, no discernible peaks at $m/z = 247$ or 217 appear at the retention time for 1-nitropyrene. Co-injection of the 1-nitropyrene- d_9 standard produced only a small interference peak at $m/z = 217$. Owing to a high background from the fly-ash for $m/z = 247$, the detection limit for 1-nitropyrene in this sample is about 20 ppm (w/w) of extract.

Although 1-nitropyrene was not detected in this precipitated fly-ash from a municipal incinerator, the presence of 1-nitropyrene in the exhaust gases from the incinerator cannot be ruled out. Fisher *et al.*⁴ have reported that ash samples collected from the hoppers of an electrostatic precipitator in a coal-fired power plant were not mutagenic, whereas fly-ash collected from the smokestack of the power plant were found to be mutagenic.

The decomposition of 1-nitropyrene in the GC-MS system makes positive identification and quantification difficult. The use of a deuterated internal standard allowed the quantification of 1-nitropyrene in an air particulate sample.

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