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Three capillary gas chromatography/mass spectrometry (GC/MS) procedures were developed to confirm the presence of *N*-nitrosamines in workplace air sample extracts, which were previously analyzed by gas chromatography with a thermal energy analyzer. In the first procedure, high resolution ion monitoring of NO^+ was used to confirm the presence of eight *N*-nitrosamines and to screen the samples for other *N*-nitroso compounds. In the second procedure, high resolution monitoring of parent ions was used for confirmation of specific *N*-nitrosamines; and in the third procedure, full-scan GC/MS was used for identification of compounds above the level of 2 ng per injection. Detection limits of the three procedures and retention time precision of both selected ion monitoring techniques are given. Typical examples in the use of these techniques for confirmation of *N*-nitrosamines in sample extracts also are described.

Introduction

The potential hazards of nitrosamines to man have been recognized by the scientific community for many years. Of almost 100 *N*-nitroso compounds that have been investigated, over 80 have been found to be carcinogenic in a wide variety of laboratory animals.[1] Nitrosamines have been identified in foods — particularly those processed with nitrate or nitrite — and in a variety of alcoholic drinks; they are frequently present as workplace contaminants in the

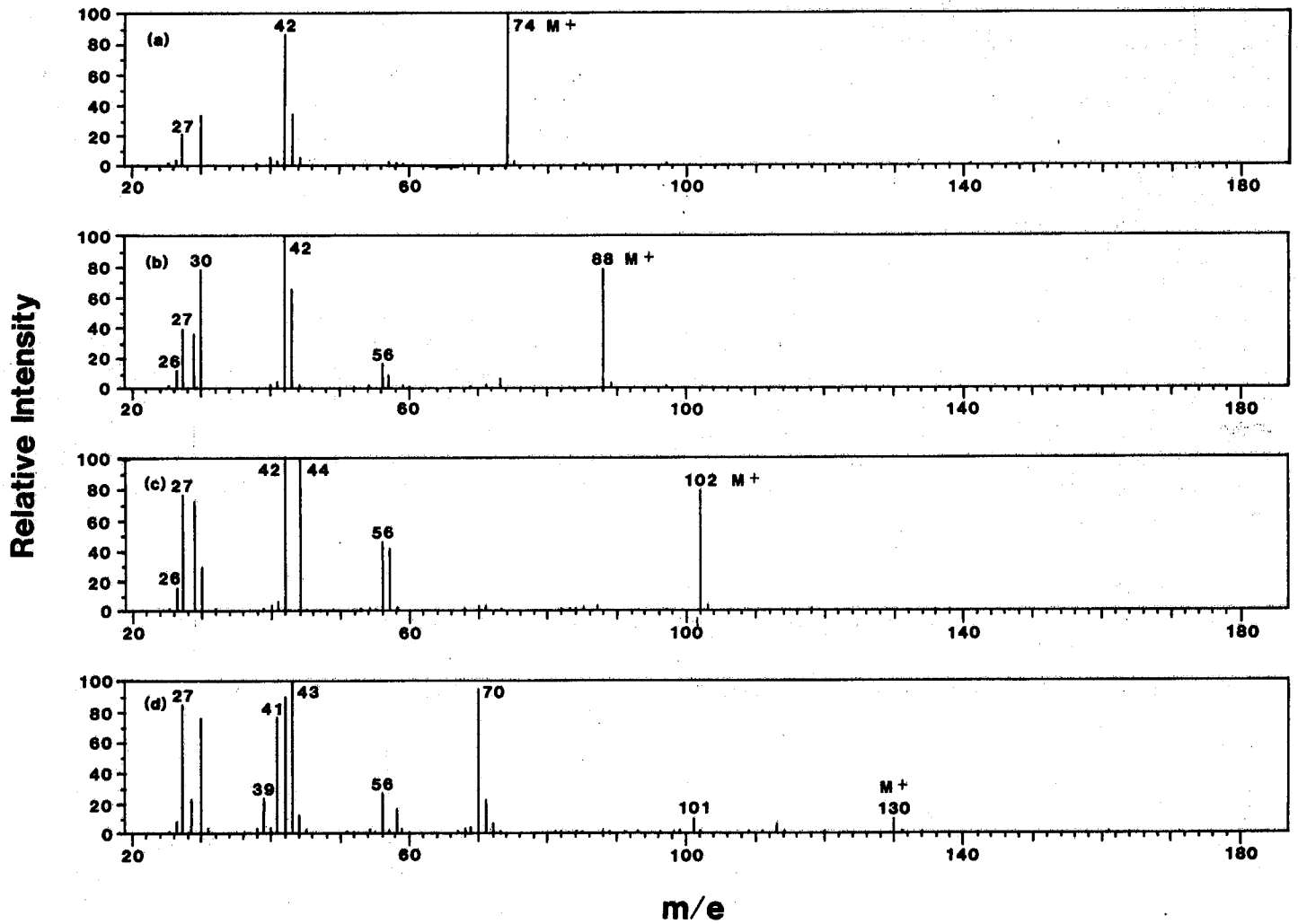

Figure 1 — Mass spectra of nitrosamine: standards: a) *N*-nitrosodimethylamine; b) *N*-nitroso-*N*-methylethylamine; c) *N*-nitrosodiethylamine; d) *N*-nitrosodipropylamine.

TABLE I
Operating Parameters for GC/MS/HRMID Procedure

N-Nitrosamine	Group	Time (s) Start	Time (s) End	Ions Monitored[A] Molecular	Ions Monitored[A] Fragment
Dimethyl	1	180	236	74.0480	-
N-Methylethyl	1	180	236	88.0637	-
Diethyl	2	236	207	102.0793[B]	-
Dipropyl	3	270	330	130.1106[C]	70.0657
Dibutyl	4	330	415	158.1419[D]	84.0813[D]
Piperidine	4	330	415	114.0793	-
Pyrrolidine	5	415	445	100.0637[C]	69.0578[B]
Morpholine	6	445	470	116.0586	86.0606

[A]Dwell time was 200 ms, except where indicated. The CF^+_3 reference ion at m/e 68.9952 was monitored for 200 ms.
[B]Dwell time = 300 ms.
[C]Dwell time = 100 ms.
[D]Dwell time = 150 ms.

rubber, tanning and metal working industries. Other potential sources of exposure include cosmetics, tobacco smoke, cutting oils, synthetic fuels and various industrial processes.[2]

Previous technology for detection and measurement of nitrosamines was developed primarily for analysis of food and biological samples.[3] Many of these analytical procedures utilize chemiluminescence techniques for analysis of *N*-nitrosamines.[4] The more generally accepted procedure employs a thermal energy analyzer interfaced to a gas chromatograph or liquid chromatograph.[5-6] Recent interest is in the development of methods that are sensitive and specific for assessment of volatile nitrosamines in workplace air.

In NIOSH studies of worker exposure to nitrosamines, air was monitored with the ThermoSorb/N sampler.[7] Samples thus obtained were analyzed by gas chromatography with detection by a thermal energy analyzer.[8] The thermal energy analyzer is a highly sensitive detector for *N*-nitroso compounds, but it lacks the specificity required for unequivocal identification of these substances in complex environmental samples.[2] It has been recommended that the most reliable procedures involve the use of mass spectrometry incorporating high resolution monitoring of NO^+ ions for all *N*-nitrosamines and high resolution monitoring of parent ions for specific compounds.[9]

This report describes three procedures employing gas chromatography/mass spectrometry (GC/MS) that we have developed for confirmation of eight *N*-nitrosamines in samples showing positive responses by GC/thermal energy analysis. The first procedure utilized high resolution selected ion monitoring of NO^+ coupled with capillary gas chromatography for confirmation. Since NO^+ is a characteristic fragment ion of all *N*-nitrosamines, it also was used to screen the sample for the presence of other *N*-nitroso compounds where analysis conditions were not established previously with standards. The second procedure incorporated capillary gas chromatography along with high resolution multiple ion detection for identification of specific *N*-nitrosamines. In this technique, the parent ion and (in some instances) a characteristic fragment ion were selected for monitoring. In the third procedure, the mass spectrometer was operated in the full scan mode, which provided a complete mass spectrum of the compound under study.

Experimental

Solvents and Standards

Dichloromethane, methanol and acetone were "distilled in glass" UV grade (Burdick and Jackson, Muskegon, Mich.) and used without further purification. Standard solutions containing eight nitrosamines were prepared in acetone with high purity (at least 97%) compounds purchased from Alpha Products (Danvers, Mass.). The compounds studied were: *N*-nitrosodimethylamine, *N*-nitroso-*N*-methylethylamine, *N*-nitrosodiethylamine, *N*-nitrosodipropylamine, *N*-nirtosodibutylamine, *N*-nitrosopyrrolidine, *N*-nitrosopiperidine and *N*-nitrosomorpholine.

Samples

A known volume of air was drawn through the tubes with a personal sampling pump to collect field samples on ThermoSorb/N sorbent tubes. Samples were prepared for analysis by "backflushing" the ThermoSorb/N air sampler with 1.8 to 2.0 mL of a 3:1 mixture of dichloromethane and methanol, and collecting the eluent in vials. Five microliters of the solution was analyzed by GC/thermal energy analysis. GC/MS analyses were performed on 1 μL aliquots.

TABLE II
Detection Limits Obtained for the Three GC/MS Procedures[A]

N-Nitrosamine	Detection Limits (ng per injection) Full Scan GC/MS	GC/MS Screening	GC/MS/HRMID
Dimethyl	2	0.01	0.002
N-Methylethyl	2	0.01	0.002
Diethyl	2	0.01	0.002
Dipropyl	3	0.01	0.004
Dibutyl	3	0.01	0.004
Piperidine	2	0.02	0.004
Pyrrolidine	2	0.02	0.004
Morpholine	2	0.02	0.004

[A]Injections of serial dilutions of the nitrosamine mixture were used to obtain these values.

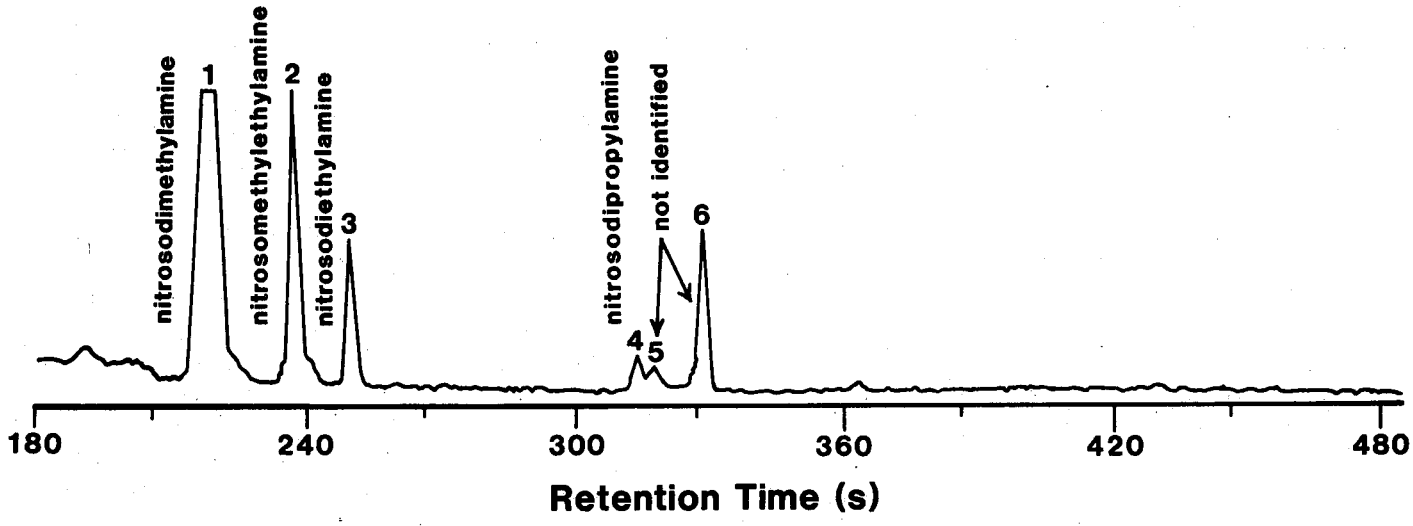

Figure 2 — Single ion chromatograms of m/e 29.998 obtained from analysis of Thermosorb/N sample extract.

Full Scan Gas Chromatography/Mass Spectrometry Procedure

The GC/MS system was comprised of a Hewlett-Packard Model 5840 gas chromatograph interfaced to a VG Micro-Mass 7070 HS mass spectrometer and a VG Model 2250 data system. The chromatograph was equipped with a 25-m long by 0.21-mm i.d. fused silica capillary column coated with Carbowax 20M to a film thickness of 0.25 μm (SGE, Austin, Tex.). The column was connected to the MS source housing's capillary inlet system. The column temperature was programmed from 55°C to 200°C at 15°C/min; the carrier gas was helium flowing at 46 cm/sec. The chromatograph was operated in the splitless mode. GC interface and mass spectrometer source temperatures were maintained at 210°C and 250°C, respectively. These GC conditions were maintained for all three procedures. The mass spectrometer resolution was set to 2000 (10% valley definition) by adjustment of both source and collector slits to provide 50% ion transmission. Electron impact measurements were made at 70 eV with the emission current maintained at 500 μA. The mass spectrometer was scanned exponentially from 340 to 25 atomic mass units (amu) every 2 sec. Mass spectral data were acquired by the data system and stored on a 5.2 megabyte cartridge disc.

The Full Scan EI mass spectra were acquired from the analysis of eight nitrosamine standards. Representative spectra are shown in Figure 1. These spectra were normalized and then added to a nitrosamine mass spectral library on the VG data system. Identification of unknown compounds was made with the interactive search facility that compared unknown spectra to library spectra. The library search results for each unknown were weighted mathematically by the search algorithm for purity, mixture and reverse fit factors.

GC/MS Screening Procedure

The mass spectrometer was adjusted to a resolution of 4000 (10% valley) and operated in the selected ion recording (SIR) mode with the use of voltage switching. The mass spectrometer accelerating voltage was calibrated over a mass range of 25 to 50 amu with the use of perfluorokerosene. During analysis, the mass spectrometer and data acquisition were controlled automatically by a set of operating parameters that were entered into a method file of the SIR program. They included the following: the accurate masses of the characteristic NO^+ ion at m/e 29.9980 and a system background reference ion (CHO^+) at m/e 29.0027; the ion dwell time of 100 and 200 ms, respectively; the data acquisition start time at 3 min and the end time at 8 min.

GC/MS/High Resolution Multiple-Ion Detection (HRMID)

GC/MS/HRMID analyses were performed with the mass spectrometer operating in the SIR mode with the use of voltage switching. Mass spectrometer resolution was maintained at 4000, and the accelerating voltage was calibrated over a mass range of 68 to 171 amu with the use of perfluorokerosene. Table I shows the operating parameters that were used for computer control of the mass spectrometer and data acquisition during analysis of eight nitrosamine standards. The molecular ions and other characteristic ions are arranged into six groups with each group containing no more than two ions plus the perfluorokerosene reference ion (CF^+_3) at m/e 68.9952. During analysis, groups were loaded from the method file into the SIR acquisition program in the order shown. For example, in Table I the first group of ions to be monitored were 74.080, 88.0637 and 68.9952, respectively. Beginning at 180 sec into the chromatographic run, these ions were monitored one at a time in a cycle, each one for its specific dwell time. At 236 sec into the run, the next group of ion parameters was loaded. Group switching continued in the same manner until all six groups were completed.

Results and Discussion

Retention Time Precision

The within-day precision of retention times for the selected ion monitoring procedures was evaluated to determine the

reliability of retention times for predicting compound identifications. In general, the overall standard deviation of the retention times was within 2 sec over a concentration range of 0.02 to 2 ng/μL. The procedures were evaluated on different days; this fact probably accounts for the more than 4 sec difference in the average retention times. Since day-to-day precision of retention times was worse than within-day precision, it was essential that a standard be run every 4 hr to assure reliable retention times during sample analysis.

Detection Limits

The sensitivities of the three GC/MS procedures were determined for eight nitrosamine standards. Detection limits for both selected ion monitoring techniques were based on a 3:1 signal-to-noise ratio, while in the full scan mode it was based on the amount of material required for an identifiable spectrum.[10] A summary of the results is presented in Table II. In general, the data show that the detection limit was about 2 ng for the full scan GC/MS; 10 to 20 pg for the screening procedure; and 2 to 4 pg for the GC/MS/HRMID procedure. Limit values are given on a per injection basis. GC/MS/HRMID was more sensitive than the screening procedure because fragmentation of *N*-nitrosamines exhibited parent ions that were two to three times more intense than the NO^+ ion.

Sample Analysis

Ambient air from a rocket fueling facility was sampled with ThermoSorb/N tubes, which then were returned to the laboratory for analysis. After GC/thermal energy analysis indicated *N*-nitroso compounds were present in several sample extracts and gave quantitative data, further confirmation of specific *N*-nitrosamines was carried out with the GC/MS screening procedure.

Figure 2 shows a single ion chromatogram of m/e 29.998 (NO^+) that was obtained from one of the analyses. Retention times of Peaks 1 through 4 were 211, 232, 246 and 312 sec, respectively. These retention times were within 2 sec of the standards. Retention times of Peaks 5 and 6 did not match any of the other standards but did indicate the presence of other *N*-nitroso compounds.

In a final identification step, the extract was reanalyzed with the use of full scan GC/MS. Figure 3a is the total ion chromatogram obtained from the analysis. Figure 3b is the background corrected mass spectrum of the peak at Scan 34. Scan 34 was identified as *N*-nitrosodimethylamine by its

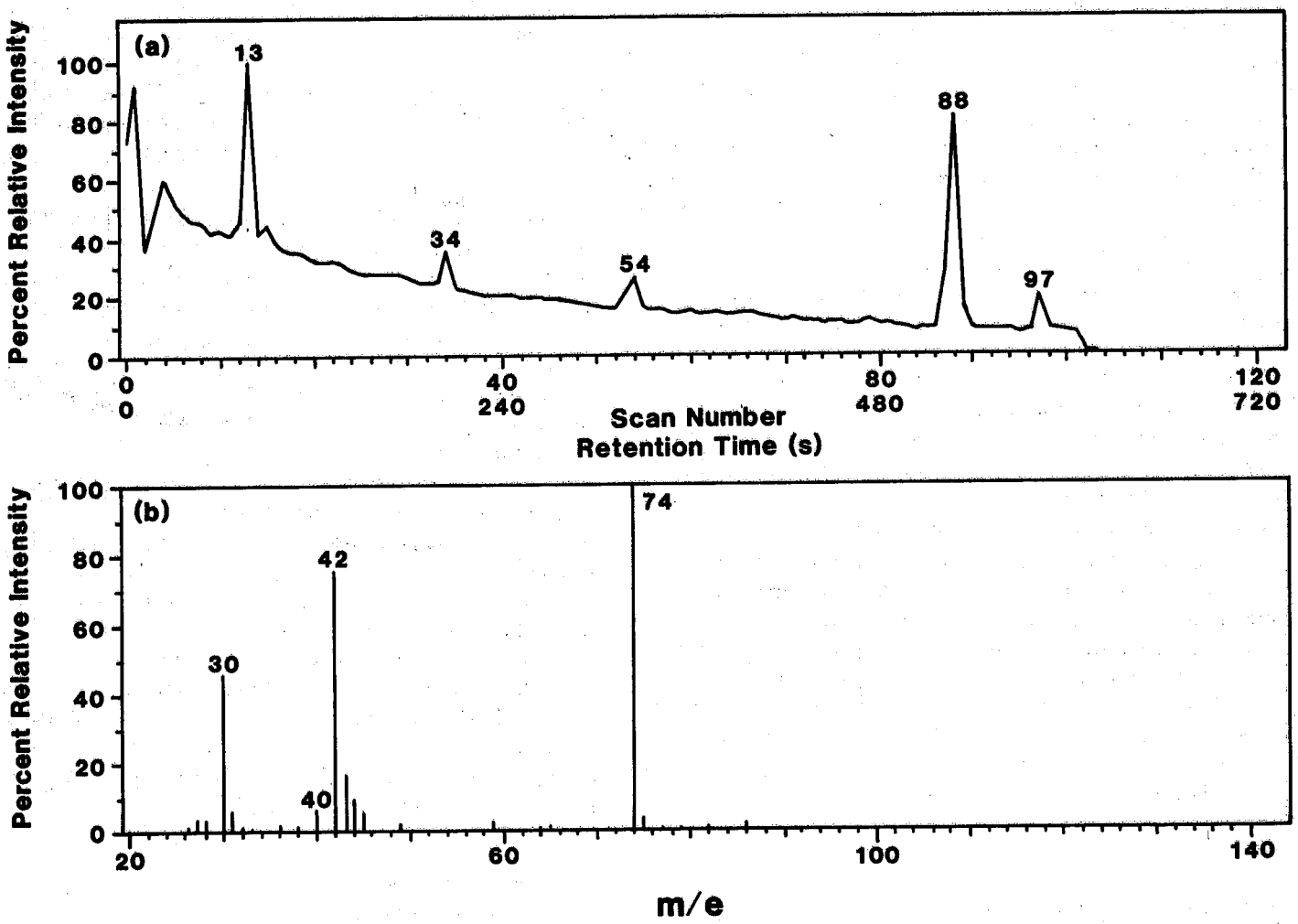

Figure 3 — a) Total ion chromatogram from full scan GC/MS analysis of sample extract; b) Background corrected mass spectrum at scan 34, identified as *N*-nitrosodimethylamine by the library search facility.

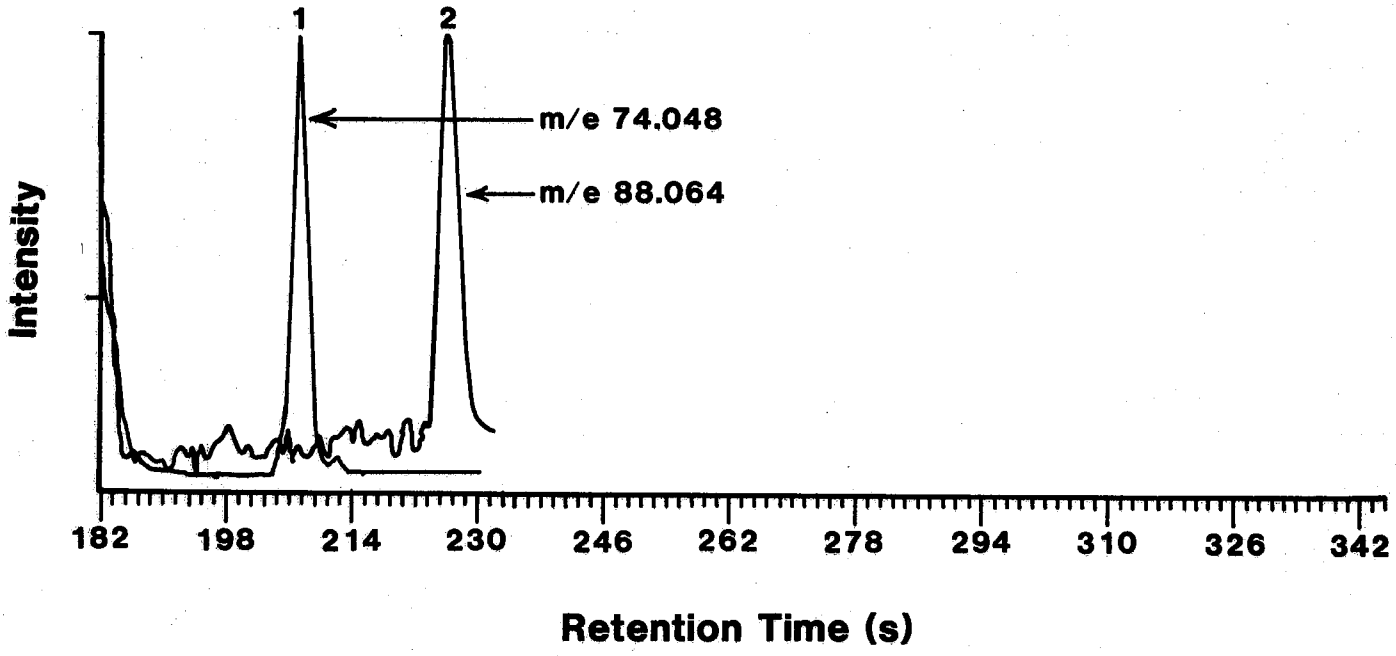

Figure 4 — GC/MS/HRMID analysis of a Thermosorb/N sample extract, showing chromatograms obtained by monitoring the masses of the molecular ions of (1) *N*-nitrosodimethylamine (m/e 74.048) and (2) *N*-nitroso-*N*-methylethylamine (m/e 88.064). The peaks appear at the retention times, 105 sec and 225 sec, characteristic of the nitrosamine.

retention time and the computer search facility, which compared the mass spectrum with standard spectra in the nitrosamine library. The levels of the other suspected nitrosamines were below the detection limit of this procedure. The remaining peaks shown in Figure 3a were identified as siloxanes.

GC/MS/HRMID analysis was used to confirm the presence of *N*-nitrosodimethylamine and *N*-nitroso-*N*-methylethylamine in a second group of ThermoSorb/N air samples that originated from the same workplace as the previous samples. Ion current profiles obtained from one of the analysis are shown in Figure 4. These profiles were recorded while the parent ions of *N*-nitrosodimethylamine and *N*-nitroso-*N*-methylethylamine (m/e 74.048 and m/e 88.064, respectively) were monitored during the expected retention times of the compounds of interest. Confirmations were based on exact mass of the molecular ions and retention times that matched to within 1 sec of the retention times of *N*-nitrosodimethylamine and *N*-nitroso-*N*-methylethylamine (205.6 and 225.8 sec, respectively).

Both of the selected ion monitoring procedures were used to confirm *N*-nitrosodimethylamine in several sample extracts over a concentration range from 0.005 to 0.2 ng/μL. Assuming a 100-L sample, this range corresponds to an air concentration of 0.10 to 4 $\mu g/m^3$. Based on the data obtained from these analyses, the useful range for confirming *N*-nitrosodimethylamine in workplace air samples was estimated at 0.1 to 2.5 $\mu g/m^3$ for the screening procedure and 0.02 to 2.5 $\mu g/m^3$ for the GC/MS/HRMID procedure. Since the detection limits of the other seven *N*-nitrosamine standards are similar (see Table II), it is reasonable to assume that these compounds can be confirmed at about the same levels.

The method of choice for identifying *N*-nitroso compounds in sample extracts was determined during GC/thermal energy analysis. When *N*-nitroso compounds were detected above the 2 ng level, samples were characterized further with a full-scan procedure. Both selected ion monitoring procedures were used to confirm *N*-nitroso compounds below the level of 2 ng per injection.

Conclusion

This paper reports three GC/MS procedures for confirmation of the indentities of *N*-nitrosamines in workplace air sample extracts. The limits of detection of the procedures, ranging from 2 to 2000 pg per injection, were sufficient for typical workplace environmental samples. The data obtained from the analysis of several samples indicate that these GC/MS procedures provide a highly reliable approach for confirmation of *N*-nitrosamines in workplace air samples.

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