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MICRODETERMINATION OF NITRATE BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY TECHNIQUE WITH MULTIPLE ION DETECTOR

YULIN L. TAN

Health and Safety Laboratory, HSC, U.S. Energy Research and Development Administration, 376 Hudson St., New York, N.Y. 10014 (U.S.A.)

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SUMMARY

A simple, sensitive method for the determination of nitrate in water by gas chromatography (GC) and mass spectrometry (MS) with a multiple ion detector (MID) has been developed. The principle of the method is the nitration of 1,3,5-trimethoxybenzene in sulfuric acid. In this medium, nitration is followed by the hydrolysis of the ether groups, yielding a simple nitrobenzene as the final product. This is then analyzed by GC-MS and detected with MID. Hexamethylbenzene serves as an internal standard. The interference of nitrite and chloride is prevented by using sulfamic acid and mercuric sulfate, respectively.

INTRODUCTION

Nitrogen plays an important biological role in living organisms. Nitrate ion is one of the principal nutrients for aquatic life. In recent years, increasing concern has been focused on the formation of carcinogenic nitrosoamine from various nitrogen oxides^{1,2}. The analysis of nitrate in environmental samples is, therefore, of vital importance particularly as it relates to ecology and public health.

The determination of low nitrate levels in unpolluted environmental samples has been hampered by the lack of a simple, sensitive analytical method. As part of the global baseline studies carried out in our laboratory, a spectrophotometric method has been previously reported³. Following the spectrophotometric method, a simpler and more sensitive method using a gas chromatographic-mass spectrometric (GC-MS) system and a multiple ion detector (MID) has been developed and is described here. The method employs the nitration of 1,3,5-trimethoxybenzene (TMB) followed by GC-MS analysis of nitrobenzene.

EXPERIMENTAL

Reagents

Spectroanalyzed benzene from Fisher Scientific (Pittsburgh, Pa., U.S.A.) was used as the organic solvent. TMB and HMB (99% pure) were purchased from Aldrich

(Milwaukee, Wisc., U.S.A.) and K & K (Plainview, N.Y., U.S.A.), respectively. Eastman-Kodak electronic grade nitrobenzene was used. All other chemicals used are of reagent grade.

The nitrate solution was prepared as follows. 0.137 g of sodium nitrate was dissolved in doubly deionized water and diluted to 1 l as the stock solution. Fresh working solutions were prepared by diluting the stock solution in proper proportions.

HMB internal standard was prepared by dissolving 0.01 g of HMB in 100 ml of benzene, and diluting 0.3 ml of this solution to 100 ml with benzene to give an internal standard of 300 $\mu\text{g/l}$.

TMB reagent was made by dissolving 0.1 g of TMB in 100 ml of benzene with 300 $\mu\text{g/l}$ of HMB internal standard.

Mercuric sulfate (5%) was prepared by dissolving 5 g of mercuric sulfate in 100 ml of 10% (v/v) sulfuric acid, and 5% sulfamic acid by dissolving 5 g of sulfamic acid in 100 ml of doubly deionized water. The latter solution must be made fresh every 3 months.

Sample preparation

The aqueous nitrate sample (10 ml) is pipetted into a 50-ml stoppered Erlenmeyer flask. Sulfamic acid reagent (0.01 ml), 0.01 ml of mercuric sulfate reagent are added, and then 20 ml of sulfuric acid are slowly added from a buret. Mix, place the flask in a cold water bath and cool to room temperature. Add, by pipet, 2 ml of TMB reagent with internal standard. Shake the mixture for 5 min. Wait for the phases to separate. Inject the upper benzene layer into the GC-MS system for analysis. A series of analyses should include a doubly deionized water blank with the same reagents from the same bottles so that any slight impurities in the reagents are cancelled out as the reagent blank.

Instrumentation

A Hewlett-Packard 5710A GC and 5980A MS system interfaced with a membrane and a 5974A MID were employed for the analysis. The GC parameters and MS and MID settings were listed in Table I. The chemical ionization mode in MS was

TABLE I
INSTRUMENT CONDITIONS

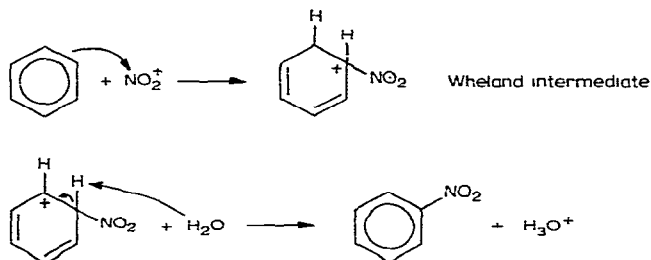
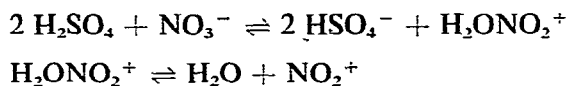
<i>Parameter</i>	<i>Description</i>
GC column	Packed, 3 ft. \times 2 mm I.D. glass, 2% SP-2300 or Chromosorb W HP (100-120 mesh)
Oven temperature	110° to 180° at 32°/min
Injection port temperature	250°
Auxiliary temperature	250°
Carrier gas*	Methane, 12 ml/min
Sample size	1 μl
MS gain	11
Masses on MID	124.1 and 163.3
MID gain	For mass 163.3, 100; for mass 124.1, 100 for 50 $\mu\text{g/l}$ NO_3^- and below, 10 for 75 $\mu\text{g/l}$ NO_3^- and above

* In obtaining the mass spectra in the electron impact mode, helium at a flow-rate of 40 ml/min was used as carrier gas instead.

used. The mass settings on MID of 124.1 and 163.3 were for detecting $M+1$ ions of nitrobenzene and HMB, respectively. A 5- μ l Hamilton syringe with Chaney adaptor was used for sample injections.

RESULTS AND DISCUSSION

The nitration of aromatic ring is an electrophilic substitution reaction. The electrophilic agent is the nitronium ion, NO_2^+ , generated from the nitrate ion and sulfuric acid. The sulfuric acid, being an extremely strong acid, protonates the nitrate ion to protonated nitric acid, H_2ONO_2^+ , which loses water to form NO_2^+ . The NO_2^+ ion attacks the aromatic ring to form a Wheland intermediate which in turn releases a proton to yield the final product. The full mechanism of the reaction can be written as



The rate determining step of the electrophilic substitution reaction is the formation of the Wheland intermediate. Benzene itself is a molecule of average reactivity as aromatic rings go. Ross *et al.*⁴ have nitrated benzene in a sulfuric acid–water (10:1) medium at room temperature and Glover and Hoffsommer⁵ in a sulfuric acid–water (3:1) medium at $75^\circ \pm 5^\circ$. In the present study, with the intention of carrying out an easier reaction, TMB was chosen to be nitrated. The three methoxy groups on the benzene ring, being electron donating, activate the molecule toward electrophilic substitution. This could be explained by the Wheland intermediate which contains an excess of a positive charge. The electron donating groups stabilize the positive charge, lower the energy of the intermediate and lead to a faster reaction. Consequently, nitration was carried out quantitatively in a less vigorous condition, *viz.* a sulfuric acid–water (2:1) medium at room temperature. The final product was found to be nitrobenzene which is discussed below. There are two possibilities for this result. One is the nitration of benzene, the other is the nitration of TMB forming 1-nitro-2,4,6-trimethoxybenzene (NTMB) followed by hydrolysis of NTMB. Based on the foresaid reasoning, the latter is most plausible.

The gas chromatogram of the reaction mixture in benzene is shown in Fig. 1. The first peak is confirmed to be nitrobenzene by retention time and mass spectra in both the electron impact (Fig. 2) and the chemical ionization (Fig. 3) modes. The mass spectrum in Fig. 2 matches well with the published spectrum of nitrobenzene⁶. Tesch

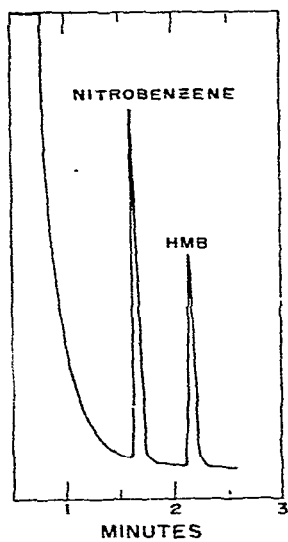


Fig. 1. Gas chromatogram of nitration mixture.

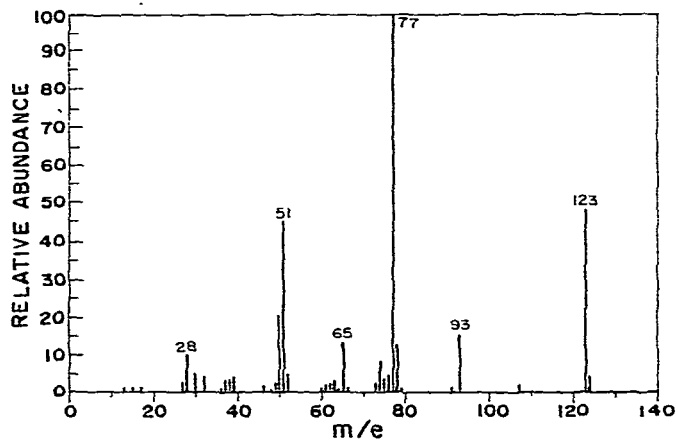


Fig. 2. Mass spectrum of nitrobenzene in electron impact mode.

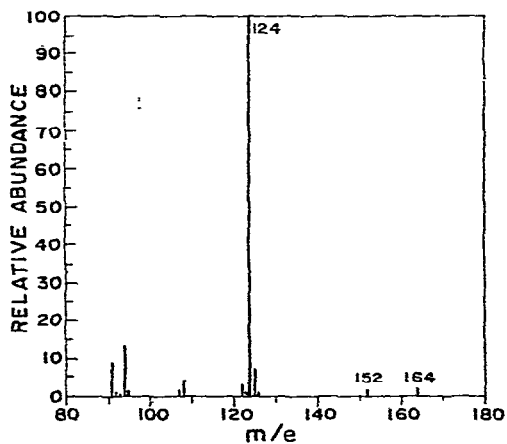


Fig. 3. Mass spectrum of nitrobenzene in chemical ionization mode.

*et al.*⁷ found NTMB to be the product of nitration of TMB under their experimental conditions. However, the lack of the characteristic peaks of $(M-CH_3)^+$, $(M-CH_3-CO)^+$, $(M-CH_2O)^+$, $(M-OCH_3)^+$ and $(M-CHO)^+$ of methyl phenyl ether indicates that the NTMB is further hydrolyzed under the experimental conditions used here. Furthermore, the $M+1$, $M+29$ and $M+41$ peaks of masses 124, 152 and 164 in the chemical ionization mode in Fig. 3 provide the positive proof of molecular weight of 123 (nitrobenzene).

The hydrolysis of the three methoxy groups on TMB is a favorable phenomenon since it leads to the formation of nitrobenzene as the final product. Compared to NTMB, nitrobenzene is smaller and simpler. It elutes at a lower temperature from the GC instrument so that any taxing effect on the membrane separator at higher temperature can be avoided. It can also be detected at a lower mass by MS which in turn yields higher sensitivity.

The signals of the $M+1$ ions for nitrobenzene and HMB are shown in Fig. 4. The MID readings at mass 124.1 of nitrobenzene are calibrated against that at 163.3 of HMB, the internal standard. Linear response on the MID, corresponding to the nitrate concentration, is observed as shown in Fig. 5. Projecting from these signals, a nitrate concentration as low as a few $\mu\text{g/l}$ could be observed. This is much more sensitive than the existing methods^{3-5,7}. Unfortunately, the scattering along the straight line is $\pm 15 \mu\text{g/l}$ which interferes with the readings at low levels.

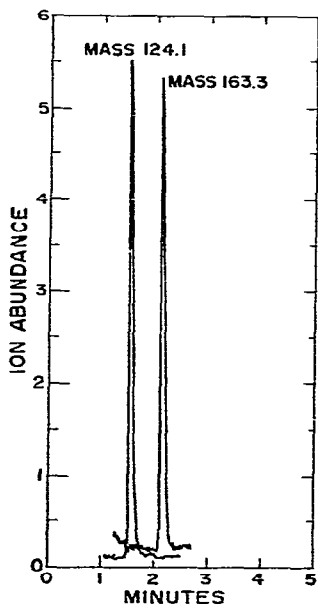


Fig. 4. MID response of nitrobenzene and HMB with $20 \mu\text{g/l NO}_3^-$, $300 \mu\text{g/l HMB}$.

The precision of MID was tested with HMB and pure nitrobenzene in benzene. Linear response was observed down to MID readings of 0, 0.1, 0.2, 0.4 and 0.6 at a gain of 100. This excludes the possibility of instrumental irregularity. Contamination while carrying out the sample preparation is then the suspect, particularly since nitric

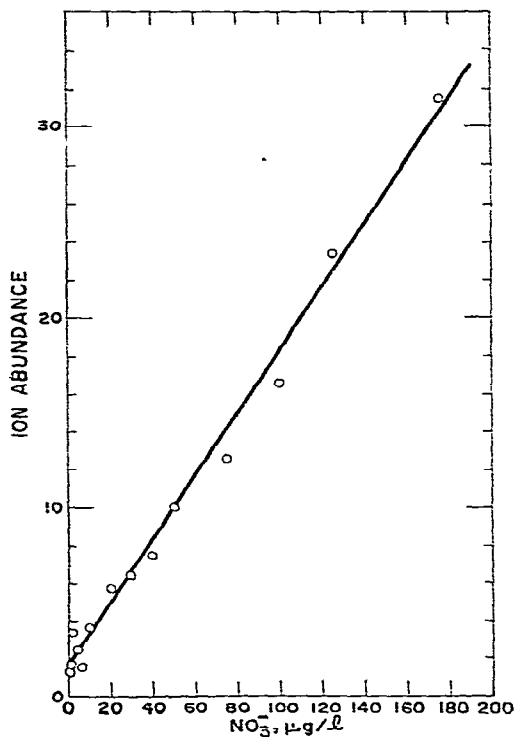


Fig. 5. Nitrate standard curve.

acid is used extensively in our laboratory for ashing and apparatus washing. Nitrogen oxides are evolved into the atmosphere during these processes. These oxides will very likely be picked up when sulfuric acid is being mixed with the aqueous nitrate sample and thus causes the contamination. The method performed under a cleaner environment should yield better accuracy, and analysis of a few $\mu\text{g/l}$ of nitrate could be possible.

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