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GAS CHROMATOGRAPHIC METHOD FOR DIRECT MEASUREMENT OF TRACE LEVELS OF VOLATILE ALIPHATIC AMINES IN AQUEOUS SAMPLES

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Volatile aliphatic amines in environmental samples are generally determined by gas chromatographic methods. Although these methods are the most sensitive, there are significant interferences when trace levels are to be measured in complex aqueous samples. A gas chromatographic method using low oven temperature (30°C) and a packing consisting of 10% Carbowax 20M with 2% KOH on 80/100 Chromosorb W AW was developed to minimize these problems. The method has been used to measure these amines in bacterial broth cultures from direct, unfiltered injections of the medium with little or no interference by other compounds.

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PREFACE

Manufacture of new propellant formulations based on some of the ammonium nitrates is planned. Waste streams from the manufacturing, load, assembly, and packing facilities involved may unavoidably contain significant concentrations of these materials. Assessment of the most efficient treatment methods necessary to minimize adverse environmental impact is now being completed. The gas chromatographic method presented here was developed to simplify the detection of volatile, aliphatic amines in complex, aqueous samples thereby making that assessment easier.

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INTRODUCTION

Volatile aliphatic amines are often present in environmental and biological samples.[1,2,3,4] One source of input to the environment is waste water from munitions manufacturing facilities. In order to better assess the impact that these materials have on the environment and to estimate the best methods for treating these wastes, we have undertaken biodegradation studies of trimethylammonium nitrate (TMAN) and isopropylammonium nitrate (IPAN), and their expected breakdown products, dimethylamine (DMA) and methylamine.

Gas chromatographic (GC) methods for quantitation of these compounds are generally the most sensitive, however, there are significant interferances when using complex aqueous samples. Recently Dunn *et al.*,[5] Dalene *et al.*,[6]

[1] S. R. Dunn, M. L. Simenhoff, and L. G. Wesson, Jr. 1979. Gas Chromatographic Determination of Free Mono-, Di, and Trimethylamines in Biological Fluids. Anal. Chem. 48: 41-44.

[2] A. Hobson-Frohoc. 1979. The Quantitative Determination of Trimethylamine in Egg. J. Food Technol. 14: 441-447.

[3] C. S. Ough, C. E. Daudt, and E. A. Crowell. 1981. Identification of New Volatile Amines in Grapes and Wines. J. Agric. Food Chem. 29: 938-941.

[4] J. M. Thomas and M. Alexander. 1981. Microbial Formation of Secondary and Tertiary Amines in Municipal Sewage. Appl. Environ. Microbiol. 42: 461-463.

[5] See reference 1, p. 1.

[6] M. Dalene, L. Mathiasson, and J. A. Jönsson. 1981. Trace Analysis of Free Amines by Gas-Liquid Chromatography. J. Chromatog. 207: 37-46.

DiCorcia *et al.*,[7] and Kuwata *et al.*,[3] have all developed improved GC methods for quantitation of these compounds at trace levels in aqueous samples. We found, however, that these approaches did not meet our needs because we wished to make direct injections of the bacterial broth cultures used in our studies. "Solvent" peaks interfered with the peaks of interest for all of these methods. Traditional approaches taken with samples of this sort are either to selectively extract the compounds of interest or to form chemical derivatives.[9] Quantitative extraction or derivatization of volatile amines, especially in complex mixtures, is cumbersome and we believed that it was important to keep the method as simple as possible to avoid the losses that often accompany excessive manipulation.

Detection limits for these compounds are generally reported near the lower nanogram levels and vary depending on the packing and, more importantly, the type of detector.[10]

This paper reports the development of a new procedure for quantitation of low nanogram levels of the lower molecular weight, volatile, aliphatic amines that can be used directly with complex aqueous samples with little or no interference from other compounds.

[7] A. DiCorcia, R. Samperi, and C. Severini. 1979. Improvements in the Gas Chromatographic Determination of Trace Amounts of Aliphatic Amines in Aqueous Solution. J. Chromatog. 170: 325-329.

[3] K. Kuwata, Y. Yamazaki, and M. Uebori. 1980. Determination of Traces of Low Aliphatic Amines by Gas Chromatography. Anal. Chem. 52: 1980-1982.

[9] Supelco, Inc. 1979. Amine Analysis. Bulletin 737C.

[10] L. Mathiasson, P. Lövkvist. 1981. Comparison of Column Packings for Trace Analysis of Free Amines by Gas-Liquid Chromatography. J. Chromatog. 217: 177-181.

MATERIALS AND METHODS

Chemical and Column Packings: Solutions of TMAN and IPAN (both 50% w/v) were obtained from Aberdeen Proving Ground, MD. Dimethylamine (DMA) (100%) and methylamine (MA) (40%) were purchased from Eastman Kodak, Rochester, NY. Stock standards, 1000 ng/uL, 100 ng/µL, and 10 ng/µL were prepared in both distilled water and half-strength (4 g/liter) nutrient broth (Difco, Detroit, MI). GC column packings, 10% Carbowax 20 M with 2% KOH on 80/100 Chromosorb W AW, 4% Carbowax 20 M and 0.8% KOH on Carbopak B, and 5% KOH on trimethylchlorosilane (TMCS) washed 80/100 Chromosorb 102 (manufactured according to Kutwata, *et al.*[11]) were purchased from Supelco Co., Inc., Bellefonte, PA.

Gas Chromatograph and Column Conditioning: A Hewlett Packard GC model 5840 A with a dual flame ionization detector (FID) was used for all analyses. Initially, a 2-m silanized glass column packed with 5% KOH on TMCS washed 80/100 Chromosorb 102 (Column A) was conditioned at 200°C and 10 mL/min nitrogen carrier flow over 3 days. After conditioning and with the oven still hot, 10 µL portions of distilled, deionized water were injected 30 times during 30 minutes. This procedure converts any potassium carbonate formed on the column by reaction of CO_2 with the packing back to KOH. It also "washes out" water soluble decomposition products of the packing formed during conditioning.[12] Initial results indicated that this conditioning protocol was insufficient for use in the detection of low ppm levels of the low molecular weight aliphatic amines. Therefore, an additional 48-hr conditioning period, using the same conditions as before, followed by another series of 30 water injections was added to the conditioning procedure.

[11] See reference 8, p. 6.

[12] See reference 9, p. 6.

For later analyses silane treated glass columns, 2-m (Column B) and 3-m (Column C), were packed with 10% Carbowax 20 M and 2% KOH on 80/100 Chromosorb W AW and conditioned at 170°C with 45 mL/min nitrogen carrier flow 24 to 48 hours. Following conditioning, 10 µL portions of water were injected as above.

Operating Conditions: Initial operating conditions used with Column A, as recommended by Kuwata, *et al.*[13] for isothermal analysis, were: injection temperature 170°C; oven temperature 150°C; FID temperature 200°C; nitrogen carrier flow 50 mL/min. Initial operating conditions used with Column B were: injection temperature 150°C; oven temperature 90°C; FID temperature 200°C; and nitrogen carrier flow 50 mL/min. Final operating conditions for Column C, conditioned as above, are: injection temperature 150°C; oven temperature 30°C; FID temperature 230°C; nitrogen carrier flow 45 mL/min. Two 5 µL injections of water are made 10 minutes before the first daily injection, and subsequent injections can be made at approximately 8-minute intervals. When necessary (about once per week during periods of constant use), we recondition the column overnight at 170°C with 20 mL nitrogen carrier flow, followed by water injections, as explained above. All samples injections were 2 µL.

[13] See reference 8, p. 6.

RESULTS

In our search for a suitable method for detection of low concentrations of aliphatic amines by direct injection of aqueous samples, we first attempted to reproduce the results obtained by Kuwata *et al.*[14] for their isothermal runs using Column A (5% KOH on 80/100 Chromosorb 102). Retention times were: MA, 0.76; DMA, 1.00; TMAN, 1.33; water, approximately 4 min. Injection of amine standards in nutrient broth or in aerobic sludge cultures gave similar results, however, column performance deteriorated. Persistent "ghost peaks" from components in these standards interfered with integration of the amine peaks of interest. In addition, the retention time of the water peak slowly decreased with column age and occasionally would overlap an amine peak. We attempted to solve these problems by modifying the isothermal operating conditions and later by temperature programming. Kuwata *et al.*[15] suggested a programmed temperature increase of 30°C per minute. With our Column A, this, as well as slower rates of temperature increase (15°C and 8°C per minute) were not successful and it became necessary to pack another column A. After conditioning, the water and DMA peaks overlapped. It was possible to shift their relative position slightly by varying oven temperature (70°C to 200°C), carrier flow (15 mL/min and 60 mL/min), or both. Despite these changes, sufficient resolution was not obtained.

[14] See reference 8, p. 6.

[15] *Ibid.*

A third column of this type was prepared for dual column compensation of the temperature programmed runs. The compensation, however, proved to be insufficient to produce even a relatively stable baseline due to a build-up of contaminants. At this point, Column B packed with 10% Carbowax 20 M and 2% KOH on 80/100 Chromosorb W AW was studied. After runs at the operating conditions described, lower oven temperatures were tried when interference between water and amine peaks was noted. Temperatures down to 30°C, the lowest stable temperature that could be maintained on this GC, were tried due to the low boiling points of these amines. At temperatures below 60°C the water peak was substantially reduced. At 30°C, the amine peaks retained good form while the water peak had been virtually eliminated, however, there was insufficient resolution of the MA and DMA peaks. The 30°C isothermal runs were repeated using Column C, a 3 m glass column with the same packing material. Initially there was no apparent improvement, however, the resolution improved after several injections. While working with Column C we concluded that the retention times of these compounds at this temperature were affected by the amount of moisture on the column. Ordinarily, Carbowax is not recommended for use as liquid phase when operating temperatures will be below 60°C because it freezes to a waxy solid thus effecting column performance.[16] Figure 1 shows chromatographic runs before and after two sequential 5 µL injections of water, followed by a 10 min equilibration to allow the baseline to stabilize. The 2 µL samples contained equal concentrations (30 ng/µL in dH_2O) of TMAN, DMA, and MA.

[16] Supelco, Inc. 1982. Chromatographic Supplies Catalog #20. p. 75.

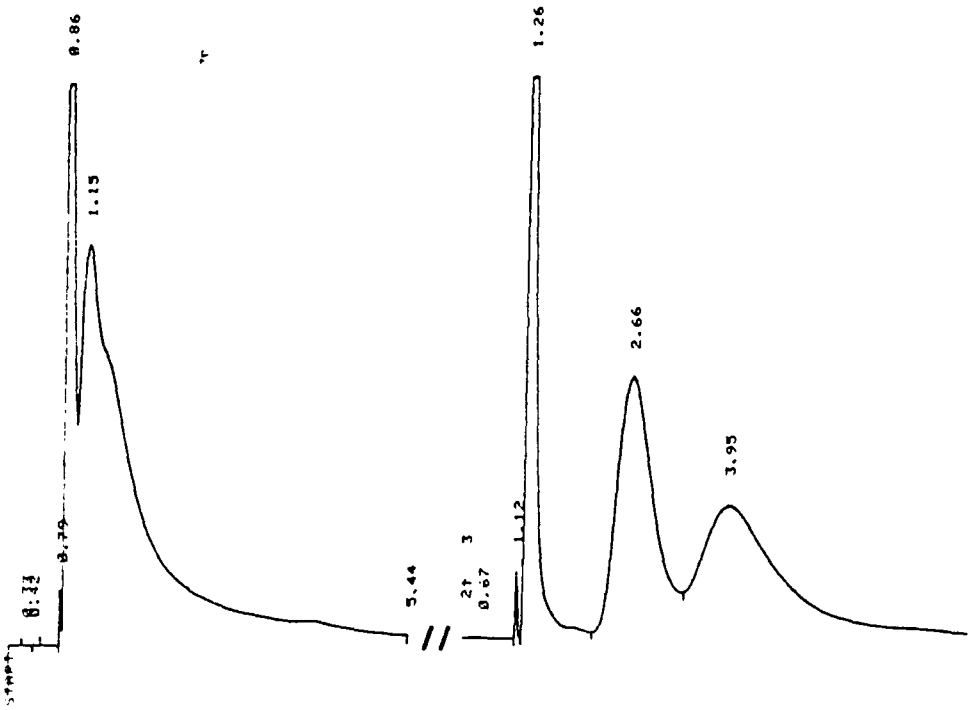

Figure 1. Gas chromatographic runs of a mixed standard both before and after two sequential 5 µL injections of water. (See text for details).

The effect of the preinjection of water diminishes if the column is not used within approximately 20 to 30 minutes. By timing sample injections 7 to 9 minutes apart, the retention times of the amines remain relatively stable and "drying-out" of the column is inhibited. When samples are injected more frequently, retention times gradually decrease and integration of the peaks is eventually affected by a lack of resolution.

An experiment was designed to clarify and quantify these relationships. A series of 2 µL injections of a mixed 30 ng/µL standard of TMAN, DMA, and MA

in distilled water were made at each of three different injection intervals (5, 10, and 15 min). The change in retention time for a compound was plotted against the minutes of spacing between injections and both linear and quadratic regression lines were plotted. An "ideal spacing" (the injection spacing for a 2-μL injection at which there is no change in retention time) was obtained from each regression line by setting the change in retention time equal to 0 and solving the equation for the injection spacing. Table 1 gives these data along with the correlation coefficient relating injection spacing to the change in retention time for each compound. In addition, the GC areas obtained from this experiment were subjected to analysis of variance.

TABLE 1

Correlation of the Retention Time to the Time Interval Between Injections

Compound	Correlation Coefficient	"Ideal Spacing" (minutes)*	
		linear estimate	quadratic estimate
TMAN	-0.73	8.9	7.5
DMA	-0.94	8.7	8.0
MA	-0.94	8.6	8.1

* Optimum interval of time between injections for a 2 μL injection (calculated from the linear and quadratic regression lines as described in the text).

The results of this analysis showed that there were no successive changes among the areas from injections within a spacing group (P = 0.911, 0.323, and 0.448 for TMAN, DMA, and MA, respectively), but that there were significant differences between the injection spacing groups (P = 0.014, 0.052, and 0.010 for TMAN, DMA, and MA, as above). This further emphasizes the need for consistency of injection spacing. With injection spacing between 5 and 10 minutes, the change in the area averages 2 percent per minute (the area decreased with increased spacing). After conditioning or long periods without injections (20 to 30 minutes, or more), one or two 5-μL injections of water may be needed to restore the separation. Average retention times for these three compounds from 18 injections made at various times is shown in Table 2 along with that for IPAN.

TABLE 2

Average Retention Time with Standard Deviation

for some Volatile Amines*

Compound	Average (minutes)	Standard Deviation
TMAN	0.58	± 0.03
IPAN	1.31	± 0.17
DMA	1.57	± 0.28
MA	2.73	± 0.52

* Method as described in the text.

The greater consistency of the TMAN retention time as seen in Table 2 has allowed its use as an "internal standard" for retention time in some of our assays. The TMAN and DMA retention times have a positive correlation factor of 0.926 with the slope of the regression line equal to approximately 10. TMAN and MA retention times have a positive correlation of 0.870 with a slope of approximately 20. Precision and accuracy data along with detection limits are given in Table 3 for TMAN, DMA, MA, and also IPAN. Detection limits were obtained from our calibration data by the method of Hubaux and Vos[17] using N-2 degrees of freedom and the corresponding two-tailed $t_{.05}$ value. The final run conditions used for the method are as given in materials and methods.

TABLE 3

Precision and Accuracy Data for some Volatile Amines

Compound	Detection Limit* (ng)	Accuracy** x 10^{-5}	Standard Error of Estimate
TMAN	11.4	13.60	0.060
DMA	29.6	-1.96	0.022
MA	32.8	7.95	0.080
IPAN	15.4	5.45	0.056

* Calculated from our distilled water calibration data by the method of Hubaux and Vos (See reference 17) using N-2 degrees of freedom and the corresponding two-tailed $t_{.05}$ value.

** Slope of the regression line of the mean ratio of spiked distilled water vs. spiked 4 g per liter nutrient broth.

[17] A. Hubaux and G. Vos. 1970. Decision and Detection Limits for Linear Calibration Curves. Anal. Chem. 42: 849-855.

Dalene *et al.*[18] have shown that "spiking" samples with ammonia reduced adsorption of amines to column and packing surfaces in certain cases. This method was evaluated with several concentrations (0, 10, 30, and 100 ng/μL) of MA made up in 4 g per liter nutrient broth with and without 500 ng/μL ammonia. Use of the ammonia under the conditions of our assay resulted in the appearance of several peaks (in addition to the ammonia peak) that sometimes interfered with the integration of the amine peak. Also, at some concentrations, the use of ammonia appeared to degrade the shape of the MA peak.

We also made a brief comparison of the packing in Column C to one of the GC packings more traditionally used for aliphatic amines, 4% Carbowax 20 M and 0.8% KOH Carbopak B. In general, the sensitivity at 30°C was less than that found for the packing used in our method. At 90°C the sensitivity of the Carbopak-based material improved, but, as with the other packings, there was interference by the solvent peak.

During the course of our biodegradation studies we also noted that methanol could be estimated by this method in low ng/μL concentrations. The retention time of methanol was variable, but averaged approximately 4.5 minutes.

[18] See reference 6, p. 5.

DISCUSSION

A method for the direct determination of volatile amines in the low mg per liter range in aqueous solution on a 10% Carbowax 20 M with 2% KOH on 80/100 Chromosorb W AW column using a FID has been developed and successfully used on a complex biological matrix.

The elution order of the series of methylamines is TMAN, DMA, and MA. This is the reverse of the order that would ordinarily be expected and, as stated by Dunn *et al.*[19] minimizes the effects of tailing because this is also the order of increasing tendency to hydrogen bond. Although the retention times produced by the method are variable, this is not a problem in practice. Injection of a mixed standard solution both before and after running a series of samples provides the reference points needed to calibrate the retention times expected for the runs.

Preliminary experiments indicated that by doubling the injection volumes to 4 µL, the minimum detectable concentration for TMAN in distilled water was halved. However, the calculations on variation of retention time with injection spacing presented in the results are based on 2-µL injections. Routine use of a 4-µL injection would require the estimation of a new "ideal spacing" to effect stable retention times and areas. The 2-µL injection volume was chosen as our standard because we make direct injections of bacterial broth cultures and need to balance the sensitivity of the assay against the life span of the column. Life span of our columns has ranged from 5 to 7 months with daily use.

[19] See reference 1, p. 5.

Detection limits were calculated by the method of Hubaux and Vos.[20] We believe that detection limits specified in this way are more realistic, reliable, and better defined than when estimated by less rigorous procedures. Our detection limits would probably improve significantly with a nitrogen specific detector.

"Spiking" samples with ammonia to reduce amine adsorption to the column and packing suggested by Dalene *et al.*[21] appears to be less successful for complex, aqueous samples than for the samples used in her study. However, Dunn *et al.*[22] reported that the use of an ammonia column conditioner was successful for these types of samples. We believe that with further development, Dunn's approach may be useful with our method as well.

[20] See reference 17, p. 14.

[21] See reference 6, p. 5.

[22] See reference 1, p. 5.

CONCLUSIONS

In summary, our low temperature method for aliphatic amines is useful in those instances where a simple, rapid, accurate estimate of the volatile amines present in an aqueous sample is needed. We have found this method particularly helpful because it allows us to quantitate these amines in our bacterial broth cultures from direct, unfiltered injections of the medium. Although the material injected for our studies is an extremely complicated mixture, we have found no significant interferences by other peaks. The fact that we were able to estimate the concentration of methanol in the ppm concentration range suggests that the other volatile alcohols could be estimated in this way as well. Although some optimization of the running conditions may be necessary for various classes of samples, use of the 10% Carbowax and 2% KOH packing material, in conjunction with low GC oven temperatures, should greatly simplify the analysis of complex aqueous samples for the volatile amines.

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