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THE USE OF A MOLECULAR SEPARATOR IN THE DETERMINATION OF TRACE CONSTITUENTS BY COMBINED GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

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SUMMARY

A method combining gas chromatography and mass spectrometry for the detection and estimation of traces of volatile nitrosamines in extracts is described. A membrane separator is used and details of its construction and performance are given. By peak matching against a reference compound a detection limit of 2 mg/l of the nitrosamines is achieved. By incorporating a peak cutting system into the gas chromatograph the detection limit is lowered to 0.2 mg/l, which represents a limit of 0.2 μg/kg on the original material.

INTRODUCTION

Combined gas chromatography and mass spectrometry (GC–MS) is used extensively for the analysis of multi-component mixtures. A recent survey of the literature revealed that the most frequently used interface is the Watson–Biemann separator. This may be attributed partly to the fact that the device is satisfactory, although not necessarily ideal, for the vast majority of analyses, and that it is commercially available from several mass spectrometer manufacturers. The separator does, however, have a number of potential disadvantages, which become significant, for example, when the analysis of trace constituents is attempted. The separator has a large surface area and volume, within which losses by adsorption and band spreading may occur. The separator functions on the basis of Graham's law, and hence the efficiency is relatively low for compounds of low molecular weight.

The separator introduced by LLEWELLYN AND LITTLEJOHN[1] offers advantages in that the dead volume and surface area are small, and sample enrichment is achieved predominantly as a result of partition within a silicone rubber membrane, and not by diffusion. In common with the Watson–Biemann separator, the original membrane separator required intermediate pumping with a rotary pump. A single-stage membrane separator has more recently been described by HAWES *et al.*[2], which has the added advantage that the membrane is pumped directly by the mass spectrometer pump system, so that no sample is lost through an intermediate stage. The high pres-

sure side of the membrane is vented to atmosphere (and is often connected to a flame ionisation detector), so that connecting the gas chromatograph to the mass spectrometer does not result in any change in carrier gas flow rate within the column. Changes do occur when a Watson–Biemann separator is used. Potential disadvantages are that significant residence times within the membrane may occur, particularly if too thick a membrane is employed. A compromise is necessary since a workable ion source pressure may not be obtainable with a thin membrane in a single-stage separator. Irreversible adsorption may occur on the membrane, resulting in substantial or complete loss of sample. The maximum operating temperature of the system is governed by the stability of the GC stationary phase as with all interface systems, and also by the thermal stability of the membrane.

A membrane separator has been constructed in the laboratory, and its use for the transfer of nanogram quantities of materials eluted from a GC column to a mass spectrometer has been evaluated. Bearing in mind the limitations mentioned above, it has been found to be significantly more useful for this work than a Watson–Biemann separator. A description of the apparatus and the results of the study are described below.

APPARATUS

The mass spectrometer is an AEI model MS902 double focusing instrument having a maximum scan speed of 10 sec per decade mass range, and fitted with peak matching facilities. The gas chromatograph is a Philips Research instrument, fitted with a flame ionisation detector. The membrane separator is housed in the GC oven, and is connected to the mass spectrometer with 0.5 mm I.D. stainless steel tubing, contained within 12 mm flexible metal tubing to ensure uniform heating. The tubing

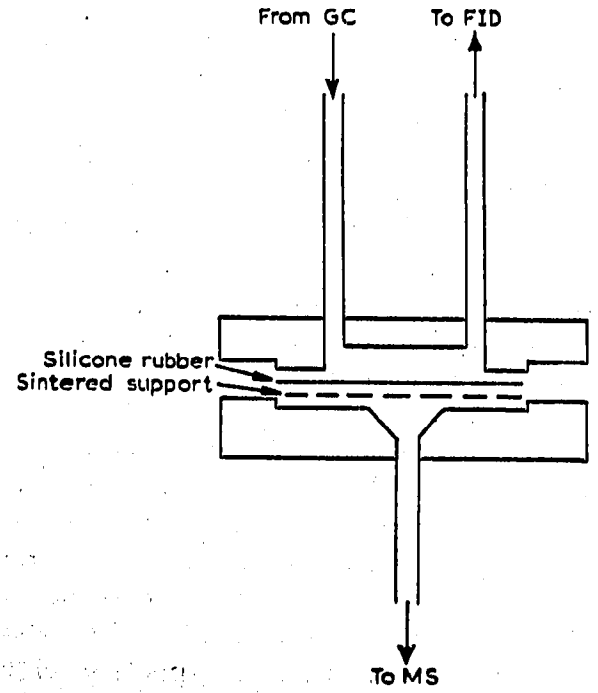


Fig. 1. Diagram of membrane separator.

is wrapped in heating tape and the line temperature controlled with a variable voltage transformer.

The transfer line contains a stainless steel bakeable bellows on/off valve (Nupro type H), so that the mass spectrometer can if necessary be isolated from the GC, and both instruments used independently. The temperature of the complete interface system is monitored at several points with chromel–alumel thermocouples.

The separator consists of a sheet of 0.05 mm × 21 mm diameter silicone rubber sheet (available from Sandev Ltd., Harlow, Essex) supported on a metal sintered disc (Perkin-Elmer filter disc) clamped between stainless steel flanges (see Fig. 1). About 8 sq.mm of membrane come into direct contact with GC effluent. The separator is assembled by placing the sinter and rubber on the lower flange, the perimeter of the rubber is coated with a silicone rubber moulding paste (Esco Rubber type SR600), and the upper flange, which is fitted with a gold 'O' ring, is placed on the membrane, and the assembly clamped together. The paste is allowed to polymerise at room temperature, after which the separator is heated for several days at 200° in a stream of nitrogen. It is then tested for leaks simply by confirming that no gas flows at the exit tube to the mass spectrometer, and for free passage across the membrane surface to the venting line. The separator is then connected to the mass spectrometer with the GC inlet and venting lines blanked off, to check that there is no leakage between the flanges to atmosphere. The GC column and detector are connected, and a final leakage test is made by noting the ion source pressure. The whole system is baked at 220° until an acceptable background spectrum is obtained. A pressure about one order of magnitude higher than is normally obtained in the ion source is usual with a new separator. It has been found that after prolonged use (several weeks) the instrument pressure will slowly decrease to a minimum. The separator used in the present work has been in continuous operation between 130° and 200° for ten months, and has shown no signs of deterioration.

DISCUSSION

Much of the work carried out on the GC–MS system has involved the detection of traces of volatile nitrosamines, and as an example of the value of the system, it is this application which will be considered in detail.

For the satisfactory recording of the spectra of GC effluent, at least partial resolution of the constituents is necessary. However, the separation of trace constituents, from extraneous material present in substantially larger amounts in extracts, is difficult to achieve. Capillary columns may be of some value[3], but they have not been used in the present work, firstly because the sample loading required to obtain reasonable amounts of the constituents under study in the ion source would result in gross overloading of the column, and secondly because a quantitative estimate of the amounts of nitrosamines was required. Additional criteria were that the initial qualitative analysis of an extract for the presence of nitrosamines must be complete in a reasonable time (less than 1 h per sample), and that all the nitrosamines must be resolved from each other. For a standard mixture of volatile dialkyl and heterocyclic nitrosamines these conditions can be satisfied and a chromatogram of such a mixture, in water, is shown in Fig. 2a. Operating conditions are given in Table II. No difficulty was encountered in obtaining a complete spectrum for each nitrosamine. A chromato-

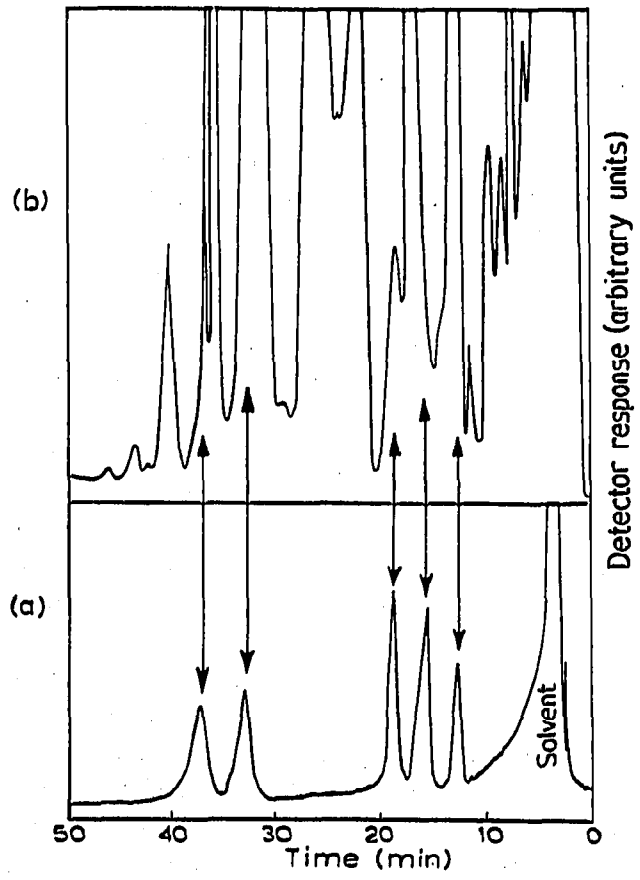


Fig. 2. Comparison of chromatograms of nitrosamine standard solution, and an extract. (a) 5-μl standard (50 ng per nitrosamine). Attenuation $\times$ 10. (b) 5-μl extract. Attenuation $\times 10^3$.

gram of an extract run under identical conditions is shown in Fig. 2b, from which it can be seen that nitrosamines are completely masked. A spectrum run at the point at which diethylnitrosamine is expected is clearly useless, particularly when it is realised that the detection of 50 ng of each nitrosamine requires a sensitivity increase of 2×10^3 over that used to obtain the spectrum illustrated (Fig. 3). The detection of traces of known materials can, however, be achieved even under such conditions of gross interference by monitoring, under high resolution, a characteristic and abundant ion in the spectrum, rather than by attempting to run a complete low resolution

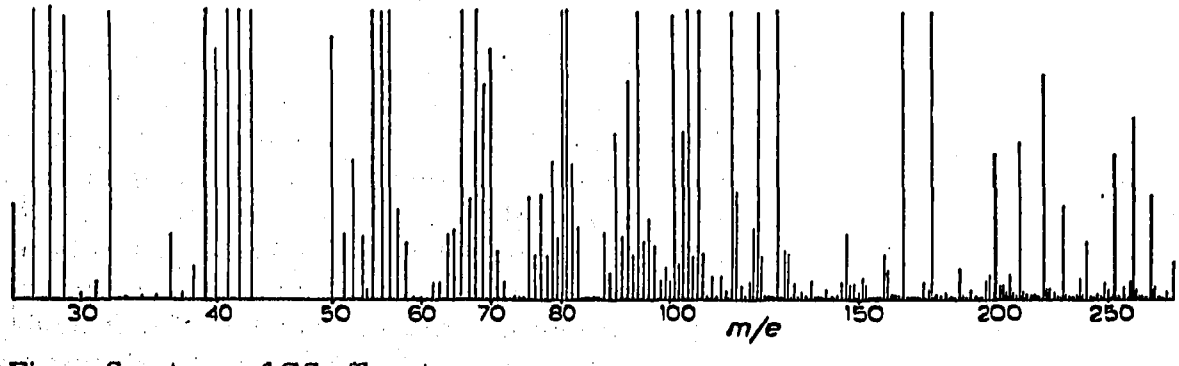


Fig. 3. Spectrum of GC effluent.

spectrum for characterisation. For the detection of a series of nitrosamines the appropriate parent ion was chosen. Since the retention time of each nitrosamine can be determined using standard mixtures, the analysis of an extract for several nitrosamines can be carried out in a single run, provided that the interval between the elution of successive nitrosamines is sufficient to allow the mass spectrometer to be set for the appropriate parent ion.

The resolution necessary for such a procedure will depend on the extent of interference expected from other compounds of the same nominal mass, eluted at the same time. Account must also be taken of fragment ions in assessing the resolution. Resolution requirements will be more stringent the higher the molecular weight of the nitrosamine, but against this the probability of interference will decrease with retention time.

The coincident elution of volatile nitrosamines with other compounds present in foodstuffs is obviously widespread, so that GC analysis alone[4], even using nitrogen-specific detectors[5], will not give unequivocal results. Even GC–MS analysis must be used with caution. Bearing in mind that for trace analysis the maximum possible sensitivity is required, a resolving power was used which would unequivocally detect the presence of nitrosamines in mg/l amounts.

The GC detection limit, using a standard solution of dimethylnitrosamine in water, with a flame ionisation detector is 1 mg/l (= 5 ng). With a rubidium sulphate thermionic detector a similar value is obtained, and with a Coulson coulometric detector the limit is 0.5 mg/l (ref. 6). Some workers[7,8] have improved the detection limit by converting nitrosamines to the corresponding nitramines and detecting by electron capture. A limit of 30 μg/l (= 16 pg) has been quoted[7]. Even with a specific detector, the limits are substantially higher when extracts are analysed, and the probability of erroneous results due to interference is still high. A sample which gives a positive result for nitrosamines by GC should be subjected to GC–MS analysis for confirmation.

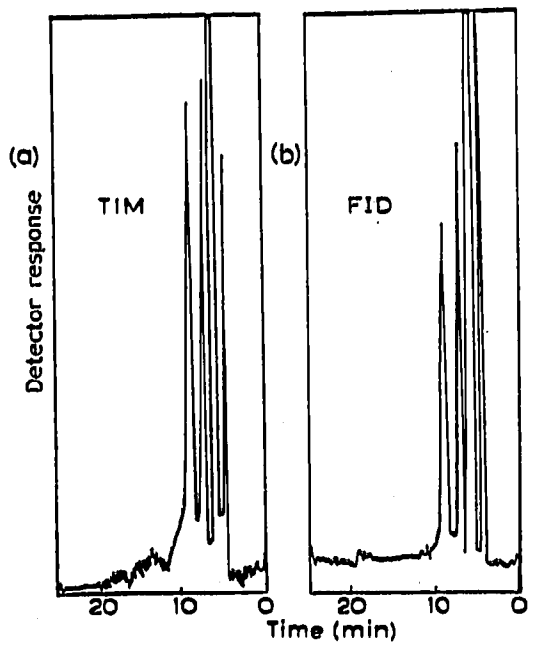


Fig. 4. Comparison of (a) total ion monitor and (b) flame ionisation detector responses (0.05 μl ketone mixture).

TABLE I

LIST OF NITROSAMINES STUDIED

Nitrosamine	*Abbreviation*	*Molecular weight*	*Base peak*	*Relative intensity*	*Reference mass*	*Ratio*
Dimethyl	DMN	74.0480	74	100.00	73.9968	1.000692
Methylethyl	MEN	88.0637	88	100.00	92.9952	1/1.05600
Diethyl	DEN	102.0793	102	100.00	100.9984	1.010702
Dipropyl	DPN	130.1106	70	24.73	130.9920	1/1.006774
Ethylbutyl	EBN	130.1106	58	34.68	130.9920	1/1.006774
Dibutyl	DBN	158.1419	84	14.74	156.9969	1.007293
Piperidyl	PIPN	114.0793	42	91.26	113.9967	1.000725
Pyrrolidyl	PYRN	100.0366	41	92.86	99.9936	1.000701

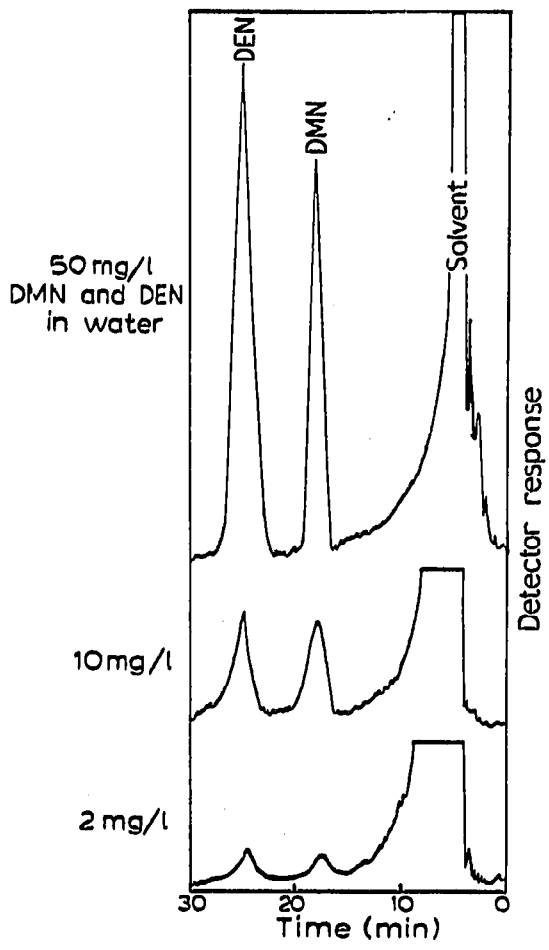
RESULTS

Preliminary experiments were carried out to show that the membrane separator did not suffer any memory effects, at least for the components under study, and that resolution losses between the GC column and the mass spectrometer were negligible. Fig. 4 shows a comparison between the flame ionisation detector response and total ion monitor response, for a trial four-component ketone mixture. Satisfactory spectra were obtained by scanning at 10 sec per decade mass in the region of the effluent peak maxima. Similar experiments were carried out using a series of nitrosamines (molecular weight 74 to 158), and satisfactory results were obtained. The detection of trace amounts of nitrosamines to the 50 mg/l level was satisfactory, but below this difficulty was encountered. However, treatment of the separator and transfer line to the mass spectrometer with bis(trimethylsilyl)ether resulted in a significant improvement and by peak matching 2 mg/l amounts of nitrosamines could be detected under the conditions quoted in Table II. A list of nitrosamines studied, with their accurate masses and the mass of the appropriate fragment ion of the reference compound

TABLE II

OPERATING CONDITIONS FOR GC–MS ANALYSIS OF NITROSAMINES

Gas chromatograph	Philips Research Model
Carrier gas	Helium
Flow rates	10 ml min^{-1} and 15 ml min^{-1}
Temperatures	
injection port	160°
column	130° and 160°
detector	175°
transfer line	160°
Column	5.1 m × 2 mm I.D. stainless steel, 15% FFAP on 80–100 BS mesh a.w. Chromosorb W
Sample size	5 μl
Mass spectrometer	MS 902
Accelerating voltage	8 kV
Trap current	100 μA
Electron beam voltage	60 eV (tuned for maximum monitor response)
Multiplier voltage	2 kV
Ion source pressure (helium)	3 × 10^{-6} mm Hg
Resolution (10% valley)	7000 or 12,000



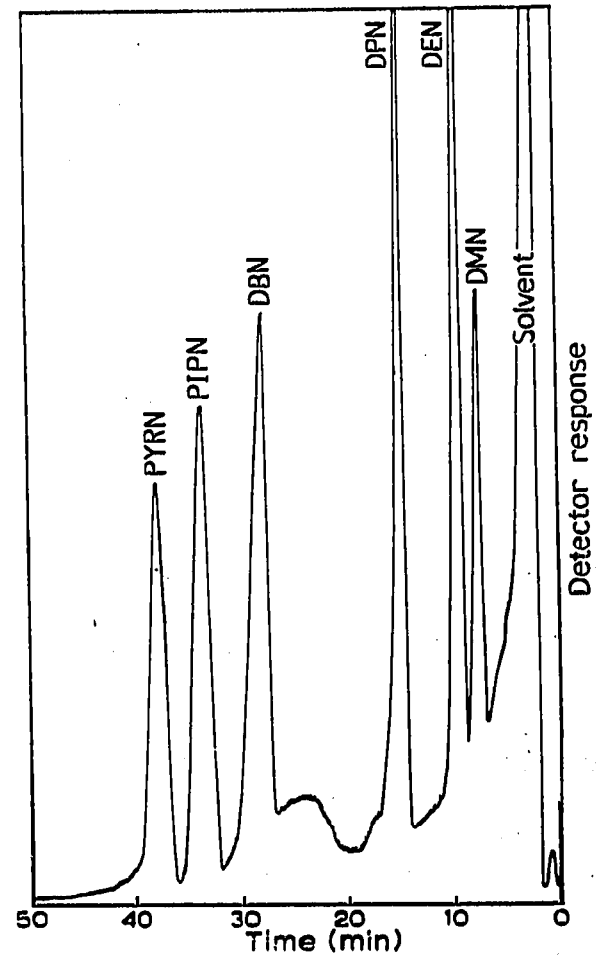


Fig. 5. Chromatograms of standard dimethyl- (DMN) and diethylnitrosamine (DEN) solutions 130°).)

Fig. 6. Chromatogram of a standard nitrosamine solution (160°). For abbreviations, see Table I. Concentration: 33 mg/l of each nitrosamine in water.

(heptacosafluorotributylamine) is given in Table I. Relative intensities of the parent ions are also given[9].

Parent ion monitoring for the nitrosamines requires that all the nitrosamines are well resolved from each other in the gas chromatograph (except ethylbutyl from dipropyl), since a finite time is involved in setting the mass spectrometer for each ion in turn. Further, although nitrosamines can be detected even under a solvent peak, sensitivity is suppressed. It was found necessary to carry out the analysis of a mixture for the first three nitrosamines (Table I) under different GC conditions to that for the remainder of the nitrosamines. Where a large number of samples were to be analysed this was found to effect more economy of time than the temperature programming of each mixture for the analysis of all the nitrosamines in a single run.

Experimental conditions are given in Table II.

The two sets of conditions given for carrier gas flow rate and column temperature refer to the conditions used for the analysis of early and late members of the series, respectively. Chromatograms of standard mixtures of nitrosamines of various concentrations are shown in Figs. 5 and 6. Calibration of the mass spectrometer was carried out using standard mixtures in water, and calibration curves covering the

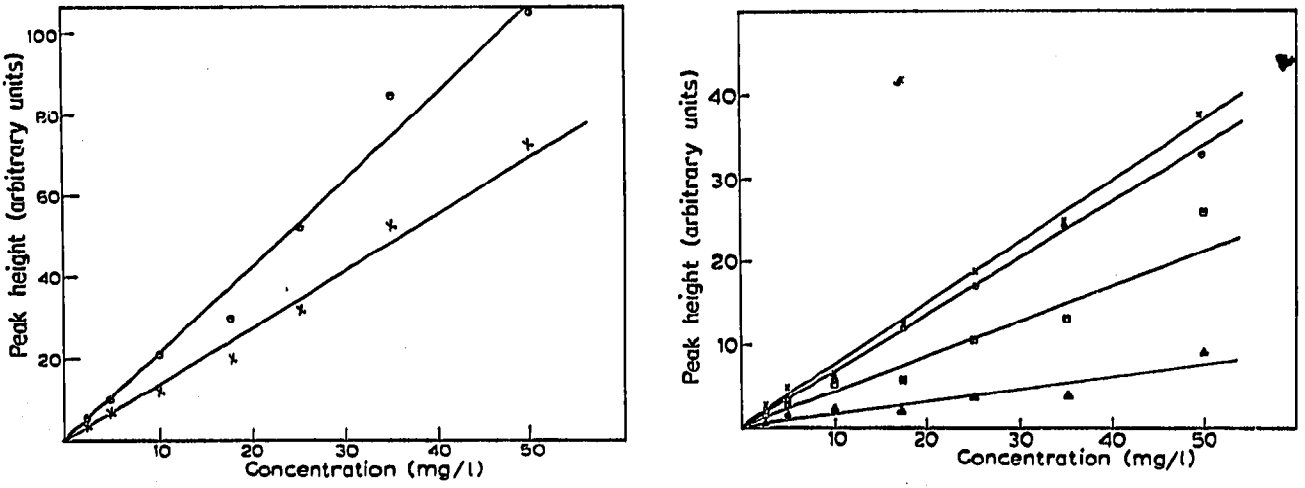


Fig. 7. Calibration of mass spectrometer for dimethyl- (○) and diethylnitrosamine (×) (GC at 130°).

Fig. 8. Calibration of mass spectrometer for higher nitrosamines (GC at 160°). □, Dipropyl-; △, dibutyl-; ×, piperidyl-; and ○, pyrrolidylnitrosamine.

range 2 to 50 mg/l are shown in Figs. 7 and 8. It has been found, however, that there are significant variations in mass spectrometer response with time even under standard operating conditions, and calibration must be carried out regularly. Fig. 9 shows the variation of response to a 5-μl sample of 10 mg/l dimethylnitrosamine in water over a period of four months.

Under normal operating conditions a detection limit of 2 mg/l for dimethylnitrosamine in an extract is regularly attainable, using a resolution of 7,000. The limits are similar for the other nitrosamines. For a 5-μl injection this is equivalent to 10 ng. Extracts of solid samples are concentrated by three orders of magnitude prior to analysis, and hence the detection limit on the original material is 2 μg/kg. A resolution of 12,000 is used in cases of doubt arising from masking of the parent ion due to excessive amounts of extraneous material in the same region. The detection limit is increased to about 5 μg/kg in such cases.

A substantial improvement in the absolute detection limit could be achieved by introducing a greater volume of extract into the chromatograph, but this has several drawbacks, *viz.* overloading of the column, rapid deterioration of the column and ion

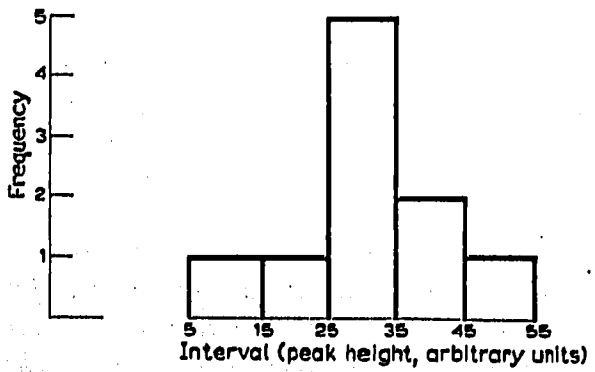


Fig. 9. Variation of mass spectrometer response to dimethylnitrosamine.

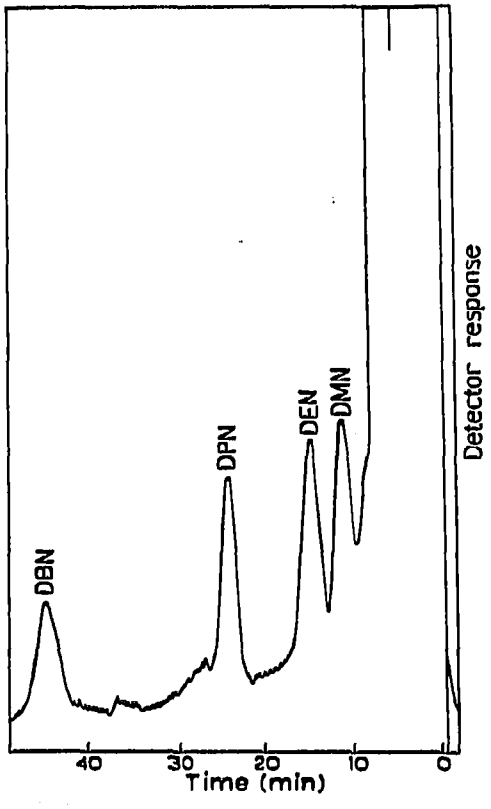


Fig. 10. Chromatogram of a 50-μl standard nitrosamine mixture containing 10 mg/l of each nitrosamine using a peak cutting system. For abbreviations, see Table I.

source, and an unacceptable increase in source pressure. To overcome this, a peak cutting system[10] has been built and incorporated into the chromatograph, in which the first column is a preparative scale column, and the second an analytical scale column. Detection at the exit of the first column is by means of a micro-volume katharometer. It is thus possible to inject say 50 μl of extract on to the preparative column and to resolve the nitrosamines from the solvent which is vented to atmosphere, at the end of the preparative column. The remainder of the extract is passed on to the second column for further GC resolution, and is detected by the mass spectrometer in the manner described above. Materials of excessively long retention

TABLE III

OPERATING CONDITIONS FOR PEAK CUTTING

Carrier gas flow rate	23 ml min^{-1}
Temperatures	
injection	175°
columns	175°
detectors	175°
transfer line	160°
Columns	
first	1.8 m × 4 mm I.D. stainless steel, 15% Carbowax 20 M on 60–80 BS mesh a.w. Chromosorb W
second	5.4 m × 2 mm I.D. stainless steel, 5% Carbowax 20 M on 80–100 BS mesh a.w. Chromosorb W

time may be backflushed. A chromatogram obtained using this system is shown in Fig. 10 under the conditions given in Table III. The limit of detection is lowered by about one order of magnitude for all the nitrosamines studied.

CONCLUSIONS

The membrane separator is a satisfactory device for the transfer of trace quantities of nitrosamines from a GC column to a mass spectrometer. Volatile nitrosamines can be unequivocally detected by peak matching with a reference compound under high resolution. By conventional GC separation a detection limit in solution of 2 mg/l can be routinely obtained, and by using a peak cutting device amounts as low as 0.2 mg/l can be detected. For sample extracts concentrated by three orders of magnitude this is equivalent to 0.2 μg/kg in the original material. The detection limits are of the same order both for standard samples and extracts. Quantitative estimates of the amounts of nitrosamines present can be obtained, but frequent calibration of the system is necessary to obtain even moderate precision.

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REFERENCES

1 Varian Technical Information Quarterly, Spring 1966.
2 J. E. HAWES, R. MALLABY AND V. P. WILLIAMS, *J. Chromatogr. Sci.*, 7 (1969) 690.
3 K. HEYNS AND H. ROPER, *Z. Lebens. Unters. Forsch.*, 145 (1971) 69.
4 R. KADAR AND O. G. DEVIK, *Acta Chim. Scand.*, 24 (1970) 2943.
5 W. A. AUE, C. W. GEHRKE, R. C. TINDLE, D. L. STALLING AND C. D. RUYLE, *J. Gas Chromatogr.*, 5 (1967) 381.
6 N. T. CROSBY, private communication.
7 N. P. SEN, *J. Chromatogr.*, 51 (1970) 301.
8 J. ALTHORPE, D. A. GODDARD, D. J. SISSONS AND G. M. TELLING, *J. Chromatogr.*, 53 (1970) 371.
9 *MSDC Spectra*, Her Majesty's Stationary Office, London, 1971. 3829–3845.
10 D. R. DEANS, *Chromatographia*, 1 (1968) 18.