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A METHOD FOR THE DETECTION OF TRACES OF NITROSAMINES USING COMBINED GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

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SUMMARY

A gas chromatograph and high-resolution mass spectrometer, coupled via a membrane separator, are used for the analysis of samples for traces of nitrosamines. The nitrosamines are detected by parent ion monitoring with a detection limit of 1 mg/l on injected material. The gas chromatograph incorporates a pressure-programming and peak-cutting device which is described in detail. Overall analysis time is substantially shorter than for isothermal or temperature-programmed runs.

INTRODUCTION

A number of methods for the detection of volatile nitrosamines in foodstuffs are currently used. In view of the carcinogenic nature of nitrosamines, detection limits of the order of 1 mg/kg are desirable in the concentrated extracts from foodstuffs. Under favourable conditions this level can be approached by conventional gas chromatography (GC) using a flame ionisation detector, provided that the extract is free from extraneous matter of similar retention time. In practice this is very difficult to achieve and it is preferable to employ a nitrogen-selective GC detector, as examples of which the thermionic and Coulson conductimetric are commonly used[1]. A more reliable analysis and a lower limit of detection can be achieved by preparing a derivative of the nitrosamine prior to the GC examination. Methods for the preparation and detection of nitramines[2,3] and polyfluorinated amides[4] have been published.

The most reliable method to date is analysis by combined GC and high-resolution mass spectrometry (MS)[5,6]. Using a silicone membrane separator, detection limits of the order of 2 mg/l can be reached for volatile nitrosamines, whilst the incorporation of preparative-scale columns and peak cutting units lowers the limits to 0.2 mg/l (ref. 6). The present paper describes a more rapid but equally reliable procedure for the determination of volatile nitrosamines, using carrier gas pressure-programming techniques[7,8] in combination with a silicone membrane separator.

EXPERIMENTAL AND RESULTS

The pressure-programming unit is based on the design of SCOTT[9] but has been modified to incorporate the following facilities: (1) a means of re-setting the initial

column inlet pressure without the need for careful manual adjustment, (2) a more rapid attainment of pressure equilibrium between runs, and (3) a peak-cutting system.

Items (1) and (2) reduce turn-round time to about 3 min. Item (3) is to prevent solvent and other extraneous matter reaching the ion source of the mass spectrometer and reduces the frequency of source cleaning and the possibility of suppression. An alternative method to peak cutting is to close the GC–MS isolating valve until after elution of the solvent, but when using a separator which does not require intermediate pumping such as the membrane separator used in this work, this is unsatisfactory as re-opening the tap causes a violent pressure surge in the MS. A diagram of the programming and cutting apparatus is shown in Fig. 1, in which column 1 is 2.4 m × 2 mm I.D., containing 15 % Carbowax 20M, and column 2 is 5.4 m × 2 mm I.D., containing 5 % Carbowax 20M. The stationary phase is supported on 80–100 mesh AW Chromosorb W on both columns. The mass flow controller FC1 is a Brooks controller Type 8744 fitted with a digital revolution counter Type 8513. NV1 is a Brooks needle control valve 8501. The pressure regulators R1, R2 and R3 are Watts Type 15-2. The pressure-adjusting stem has been removed from R2 and replaced by the supply from FC1.

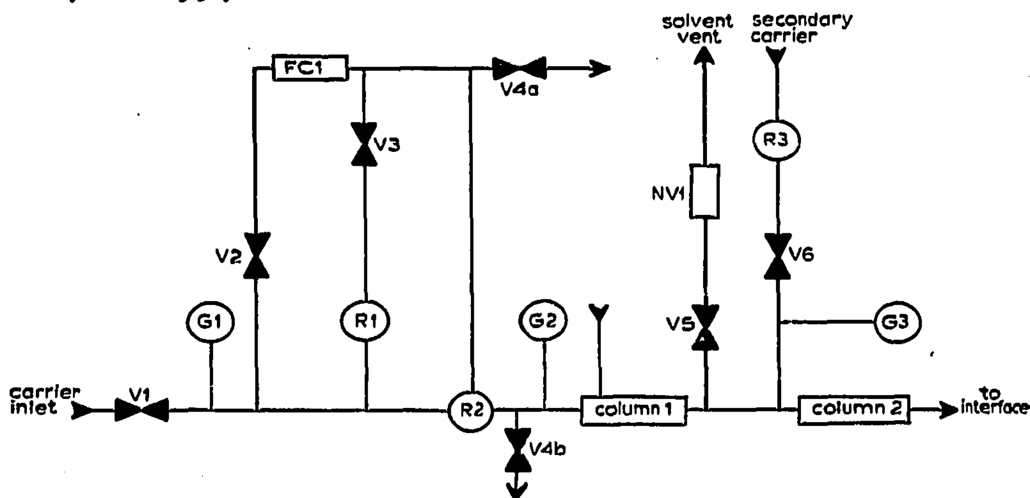

Fig. 1. Pressure-programming and cutting system.

Symbol	*Description*	*Function*
FC1	Mass flow controller	Rate of programming
G1	Pressure gauges	Supply pressure
G2		First column pressure
G3		Second column pressure
NV1	Needle valve	Flow-rate balance
R1	Pressure regulators	Initial pressure limit
R2		Programming regulator
R3		Secondary carrier regulator
V1	On/off valves	Primary carrier supply
V2		Programme start
V3		Initial pressure reset
V4 a, b		Pressure release at completion of run
V5		Solvent vent
V6		Secondary carrier supply

Initial isobaric conditions are set using pressure regulator R1, with on/off valves V1 and V3 open. All other valves are closed. The column pressure is noted on gauge G1. The line pressure is set to the maximum necessary for the programme. Pressure at G3 is noted, valves V1 and V3 are closed and V6 opened. Regulator R3 is adjusted until the pressure at G3 has reached its former value. In a correctly balanced system there will be negligible detector baseline shift as V1 is opened and closed. With V1, V3, V5 and V6 open, the solvent needle valve NV1 is adjusted until no fraction of the solvent reaches column 2. The programming rate is controlled by FC1. The operating procedure is as follows: V1 and V3 opened, and V3 closed when column pressure is reached. V5 and V6 are opened, the sample is injected and after solvent elution both are closed. The programming is initiated by opening V2, and will proceed at the rate determined by FC1 until pressure G1 is reached. On completion of the run V2 is closed and V4 opened until G2 registers zero. V3 is opened for the next sequence.

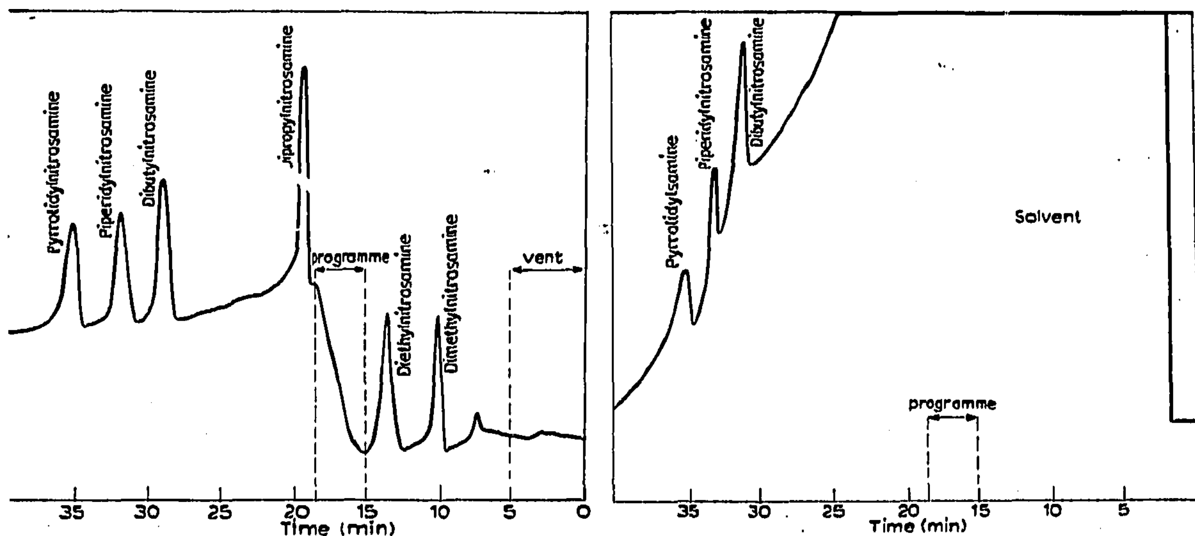

Fig. 2. Chromatogram of nitrosamines using pressure programming and peak cutting.

Fig. 3. Chromatogram of nitrosamines using pressure programming only.

A chromatogram illustrating the use of the apparatus is shown in Fig. 2. Fig. 3 shows a chromatogram of the same mixture of nitrosamines run under identical conditions, in which the peak-cutting system is not used: all nitrosamines are eluted on the tail of the solvent with complete suppression of MS sensitivity for dimethyl- and diethylnitrosamine. The programme is designed to run between the elution of diethyl- and dipropylnitrosamine. MS operating conditions and the procedure for determining the nitrosamines by parent ion monitoring have previously been published[6]; the GC operating conditions are given in Table I.

Total analysis time is compared with published data in Table II. Temperature equilibrium between two successive runs under the conditions used by TELLING *et al.*[5] was determined by placing thermocouples in the GC oven and in Celite packed in a glass column. The establishment of flow equilibrium between runs was measured by taking flow-rate measurements at the second column exit at 30-sec intervals after

TABLE I

GC OPERATING CONDITIONS

Gas chromatograph	Philips Research model
Detector	FID
Carrier gas	Helium
Flow-rate	6–25 ml/min (see Fig. 5)
Temperatures, °C	
injection port	160
columns	145
detector	175
transfer line	160
membrane separator	145

TABLE II

ANALYSIS TIMES

Nitrosamine	*Procedure (time, min)*			
	Temperature programme according to ref. 5	*Isothermal according to ref. 6*		*Pressure programme*
		130°	*160°*	
Dimethyl	7	(7)	18	11
Diethyl	12	(10)	24	15
Dipropyl	23	16	—	20
Dibutyl	40	28	—	30
Piperidyl	42	35	—	32
Pyrrolidyl	44	38	—	36
Equilibrium	15	—	—	3
Total analysis time	59	38	24	39

opening valve V3. A constant flow-rate is reached after 3 min (see Fig. 4). The change in column flow-rate during pressure programming is shown in Fig. 5. The effect of separator temperature on ion source pressure is shown in Fig. 6, for various flow-rates embracing those used for the pressure programming.

Using the technique of characteristic ion monitoring, samples are analysed for each nitrosamine only over the period during which the nitrosamine is expected to be eluted from the GC, based on the retention time of standards. It is therefore particularly important that sensibly constant retention times should be attainable using the pressure-programming system. Two standard analyses were carried out per day, and on any given day the retention times of each nitrosamine did not vary by more than 15 sec. Variations over four months' continual operation have been recorded and are given in Table III for a 10 mg/l solution of nitrosamines in water. Quantitative calibration was simultaneously carried out and substantial variations in the response of the mass spectrometer to standard solutions have been observed over the same period of time. Estimates of the amounts of nitrosamines in extracts of food are likely to be somewhat worse than the values obtained for the standards (Table III), since material simultaneously eluted may suppress MS response to varying extents depending on the degree of contamination.

For a 5-μl sample the detection limit is typically 1 mg/l, which represents 1 μg/kg of original material, using the extraction procedure adopted by this laboratory[10].

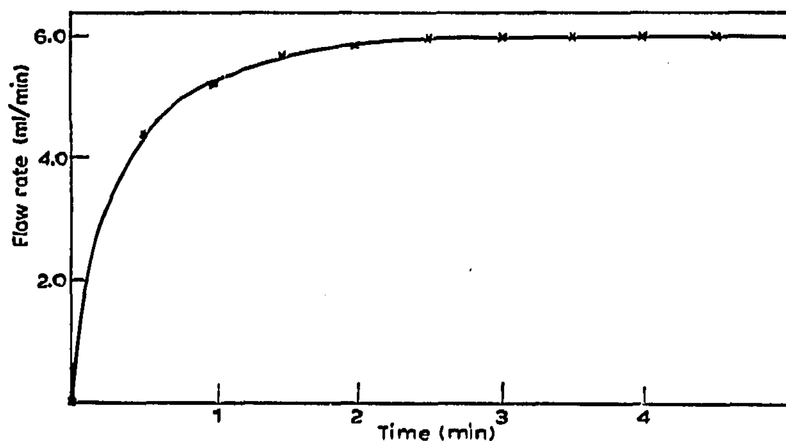

Fig. 4. Establishment of equilibrium prior to run.

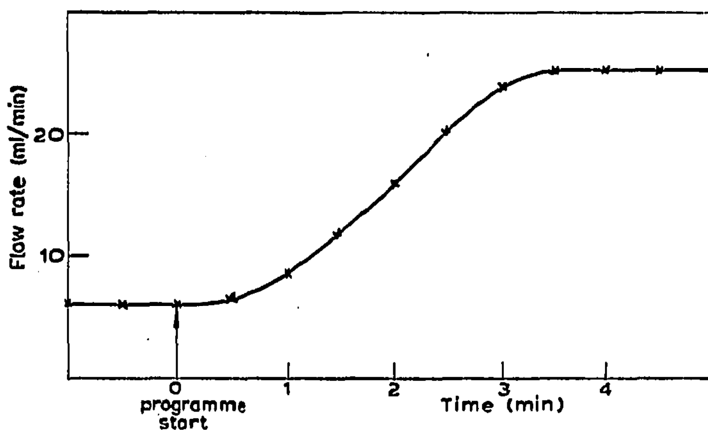

Fig. 5. Carrier gas programming.

TABLE III

VARIATION IN RETENTION TIME AND RESPONSE FOR STANDARD SOLUTIONS, OVER A PERIOD OF 4 MONTHS (28 RUNS)

Nitrosamine	*Mean retention time (min)*	*Standard deviation (min)*	*Mean response*[a]	*Standard deviation*[a]
Dimethyl	11.4	0.3	5.5	1.3
Diethyl	14.7	0.5	4.1	1.3
Dipropyl	19.5	0.6	0.87	0.34
Dibutyl	29.5	1.0	0.24	0.08
Piperidyl	32.4	1.1	1.6	0.41
Pyrrolidyl	36.0	1.4	1.6	0.62

[a] Arbitrary units.

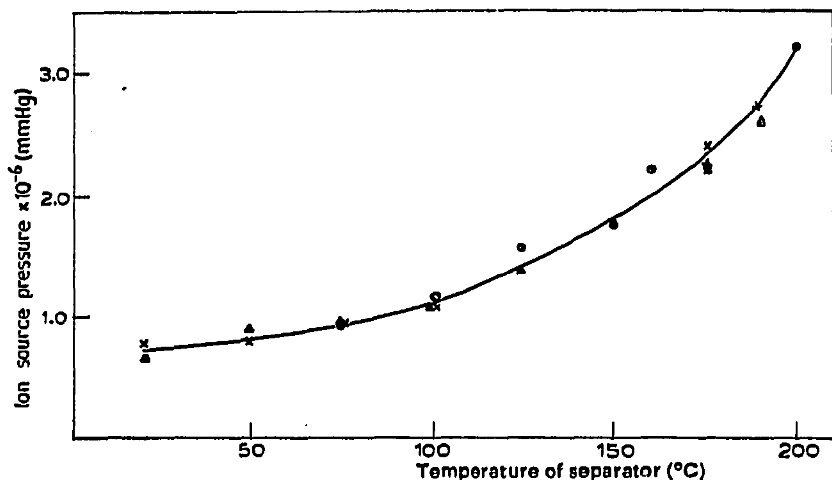

Fig. 6. Effect of separator temperature on source pressure at various column flow-rates. Carrier gas flow-rate: ×, 0.25 ml/min; ○, 15 ml/min; and △, 30 ml/min.

Although it is accepted that GC–MS is the most reliable procedure for the unambiguous detection of nitrosamines, it should be recognised that there is a very small probability that false positive results arising from retention and mass coincidence with an interferant can be obtained. It is well known that the great majority of trimethylsilyl derivatives will give rise to a particularly abundant fragment ion of *m/e* 73[11,12]. Accurate mass measurement shows this to have a mass of 73.0473, which corresponds to the trimethylsilyl group. The other isotopes of Si will give rise to fragment ions of mass 74.0469 and 75.0442 with abundances of 4.7 % and 3.1 % with respect to ^{28}Si. The parent ion (and base peak) of dimethylnitrosamine has a mass of 74.0480, so for complete resolution between this and $Me_3{}^{29}Si$ a resolving power of 70,000 is necessary, although a mismatch is detected at a much lower resolving power. During the analysis of some extracts from food, fragment ions corresponding to the trimethylsilyl group were detected using a resolving power of 12,000 but they were not eluted from the GC coincidentally with dimethylnitrosamine (relative retention 1.3). It was established that the interferant was present in the sample extract and did not result from interaction between the sample and the interface system, which had previously been silanised. It is desirable to repeat analyses on any samples giving positive results after conversion to nitramines or other derivatives. An adaption of the nitramine conversion procedure of SEN[2] is used, and dimethylnitramine is detected by monitoring the parent ion (*m/e* 90.0429).

DISCUSSION

For repetitive GC analysis in which the constituents for separation have widely differing retention times, the analysis time per run may be shortened by temperature programming. This procedure is used by TELLING *et al.*[5] for the detection of volatile nitrosamines by GC–MS. In this laboratory the analysis of nitrosamines has until recently been carried out using two distinct isothermal runs, one for dimethyl- and

diethylnitrosamines, and the other at a higher temperature to cover other dialkyl- and some heterocyclic nitrosamines[6]. Pressure programming offers several advantages over both isothermal and temperature-programming techniques. Pressure equilibrium between successive runs can be achieved far more rapidly than temperature equilibrium. A pressure programme can be chosen such that dimethylnitrosamine is eluted well clear of the solvent peak maximum and most extraneous material of relatively short retention time, thus minimising suppression of the MS response. The time interval between each nitrosamine can be sufficient to allow resetting of the MS when detection is by characteristic ion monitoring, and can at the same time be consistent with a short overall analysis time. The success of pressure programming relies on the use of a column exhibiting a shallow HETP/u curve.

An investigation into the performance of a silicone membrane separator has shown that ion-source pressure rises markedly with membrane temperature, but is virtually independent of carrier gas flow-rate[13]. The membrane separator is therefore more suited to pressure programming than temperature programming.

CONCLUSIONS

Pressure programming can be used satisfactorily with a combined GC–MS system incorporating a membrane separator. Difficulties associated with the use of temperature programming in such a system are eliminated with no attendant disadvantages. Retention times are sufficiently reproducible to enable the characteristic ion monitoring of a variety of materials in an extract to be carried out.

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