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DUAL COLUMN GAS CHROMATOGRAPHIC SYSTEM FOR USE IN MASS SPECTRAL DETERMINATION OF NITROSAMINES

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SUMMARY

A packed gas chromatographic column and a support coated open tubular (SCOT) column are connected in series. Between the columns are two micro-volume switching valves, one enabling solvent to be vented. Short retention nitrosamines are passed through both columns, whereas longer retention nitrosamines by-pass the SCOT column by means of the other switching valve.

INTRODUCTION

The presence of traces of volatile nitrosamines in cured meat and related products is now established[1–5] and techniques for their detection on a routine basis are available[6–12]. It is generally accepted that confirmation of the presence of nitrosamines must be carried out using combined gas chromatography and mass spectrometry (GC–MS) and papers describing suitable procedures have been published[9,11–13]. In view of the high capital and running costs of a mass spectrometer, it is essential that the analysis time for nitrosamine determination is kept to a minimum. In addition, the spectrometer must not be contaminated by extraneous eluted material from food extracts, which would preclude the immediate use of the spectrometer for other work. In a previous communication from this laboratory[13], rapid analysis time and minimal contamination was achieved using a combined pressure-programming and GC peak venting system. An alternative and equally effective means of satisfying these two criteria, without the need to build ancillary GC apparatus, is described below.

EXPERIMENTAL

A Pye Model 104 gas chromatograph, interfaced to an AEI MS 902 mass spectrometer with a silicone membrane separator[9] is used. The chromatograph is fitted with two flame ionisation detectors (D1 and D2). One is connected to the separator to monitor material entering the mass spectrometer, and the other although not mandatory is useful for monitoring vented material. It also offers a convenient means of destroying potentially harmful compounds which could otherwise enter the labora-

tory air. Two stainless-steel columns (C1 and C2) are connected in series, between which are placed two micro-volume four-port switching valves (V1 and V2). Details of the arrangement are shown in Fig. 1. Column C1 is 1.8 mm I.D. × 1.6 m, packed with 15% Carbowax 20M on 80–100 BS mesh Chromosorb W AW DMCS. Column C2 is a SCOT column, 0.5 mm I.D. × 30 m, containing Carbowax 20M. Each switching valve can be operated in two modes, one shown by the full line and the other by the pecked line, on Fig. 1. In the parallel reference circuit, restrictors R1 and R2 are short lengths of compressed steel tubing exerting back pressures equal to C1 and C2, respectively. This ensures that in whatever mode the valves are operated, the flow-rates within the GC columns and reaching the separator and flame detectors are unchanged. Equal flow-rates of helium are introduced at positions I and II. Position I is the sample injection port. Helium is also introduced at positions III and IV, as a make-up gas to optimise flame detector performance. Note that the make-up gas to detector D1 must be introduced after the separator to maintain a high transfer efficiency to the mass spectrometer.

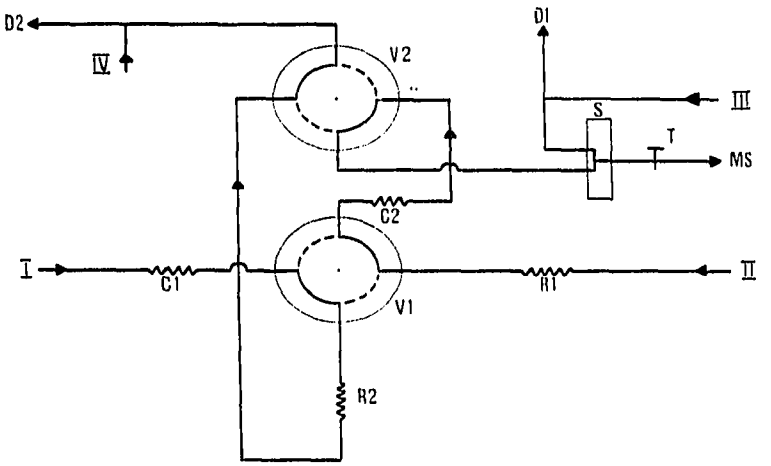

Fig. 1. Diagram of flow system. I–IV = Helium inlets; C1, C2 = columns; V1, V2 = switching valves; D1, D2 = flame ionization detectors; S = GC–MS interface; MS = mass spectrometer; T = GC–MS isolating tap; R1, R2 = restrictors.

It has previously been shown that with a single-column system connected to the separator, the mass spectrometer source pressure is typically 2×10^{-6} torr. Incorporating the micro-valves results in an unacceptably high pressure which is attributed to leakage from the atmosphere across the valve faces. Since there is little chance of eliminating such leaks, the valves were built into sealed chambers, pressurized with helium. Thus only helium is able to leak into the GC–MS system, giving rise to an acceptable pressure. Valves with this facility are available commercially (Carle, Fullerton, Calif., U.S.A.; Switching Valve type 2011P; distributed in Great Britain by Techmation, Edgware).

There are four possible modes of operation of the system, the appropriate mode being manually selected outside the GC oven. These are summarized below: Mode A, Column C1 to vent (detector D2); Mode B, Columns C1 and C2 to detector

TABLE I

GC OPERATING CONDITIONS

Chromatograph	Pye Model 104
Detectors	flame ionization
Carrier gas	helium
Carrier gas flow-rate, ml/min.	4
Make-up gas flow-rate, ml/min.	15
Temperatures, °C	
Injection port	160
Columns	140
Detectors	200
Transfer line	160
Separator	140
Sample size, µl	5
Detection limit (mass spectrometer), µg/ml	1

D1 and mass spectrometer; Mode C, Column C1 to detector D1 and mass spectrometer; Mode D, Columns C1 and C2 to vent (detector D2). Fig. 1 shows the system in Mode A.

In a typical run the valves are set to Mode A so that no solvent or other extraneous material reaches the mass spectrometer, but the flow is monitored by flame detector D2. After solvent elution, Mode B is selected and the compounds are resolved on columns C1 and C2 prior to reaching the flame detector D1 and the mass spectrometer. For long retention materials or for large amounts of a single compound which would overload the SCOT column (C2), Mode C may be used, in which material passes only through column C1 before reaching the mass spectrometer. Mode D is used for GC runs in which the mass spectrometer is not required, leaving it free for other work.

GC operating conditions are given in Table I, and retention and mode changing data in Table II. Mass spectrometer operating conditions and details of the detection procedure for mass spectrometry have previously been published[9].

TABLE II

ANALYSIS TIME DATA

Event	*Time after injection*
Mode A	zero
Solvent elution	0 min 30 sec
Select Mode B	2 min 0 sec
N-Nitrosodimethylamine elution	7 min 0 sec
N-Nitrosodiethylamine elution	9 min 0 sec
N-Nitrosomethyl-*n*-propylamine elution	11 min 0 sec
N-Nitrosodipropylamine elution	15 min 0 sec
Select Mode C	18 min 0 sec
N-Nitrosopiperidine elution	21 min 30 sec
N-Nitrosopyrrolidine elution	26 min 30 sec
Select Mode A	28 min 0 sec

DISCUSSION

Nitrosamines have been found in cured meat products up to the μg/kg level and all current techniques for their detection require a substantial concentration and clean-up of the substrate extract prior to analysis. The procedure used in this laboratory involves steam distillation of an aqueous suspension of the comminuted foodstuff followed by solvent extraction and evaporation[14]. This results in a thousandfold increase in the concentration of the nitrosamines. Chromatography of such an extract shows many compounds, some of which are present in amounts greatly exceeding those of the nitrosamines, and in many instances only partially resolved from them. Such interferants can result in undesirable contamination of the mass spectrometer, although they do not adversely affect the ability to confirm the presence of nitrosamines in a mass spectrometer operating under high resolution.

The use of capillary columns to obtain better separation of nitrosamines from extraneous material appears attractive, but has the disadvantage that the lower sample capacity of these columns results in a correspondingly poorer detection limit. This may be as much as two orders of magnitude higher than that obtained using a packed column. Support coated open tubular (SCOT) columns offer a superior performance to packed columns, but with a less restrictive sample capacity than capillary columns. Column overloading is caused predominantly by the solvent, rather than by material co-eluted with the nitrosamines. By injecting on to a packed column, venting the solvent, and allowing the remaining constituents of the extract to pass on to a SCOT column, good resolution of the nitrosamines from other co-extracted compounds may be achieved, without any adverse effect on the detection limit. An additional advantage accrues from the use of a two-column system. Retention times of the lower nitrosodialkylamines are somewhat shorter than those of the volatile heterocyclic nitrosamines, which thus dictate overall GC–MS analysis time. Temperature programming, which is used by some workers[15], is excluded on the grounds that there is a significant equilibration time between successive runs. Pressure programming, favoured by this laboratory[13], virtually eliminates the inter-run equilibration period but requires specially built ancillary equipment. Using a two-column system, N-nitrosodimethylamine and other low retention nitrosodialkylamines are passed through both columns, whereas long retention nitrosamines are allowed to pass only through the first column.

Most work has been centred on N-nitrosodimethylamine, N-nitrosodiethylamine, N-nitrosodipropylamine, N-nitrosopiperidine, and N-nitrosopyrrolidine. The apparatus described herein is designed predominantly for the determination of these compounds, but has general applicability in the volatile organic trace analysis field.

In the standard procedure used in this laboratory for nitrosamine determination[16] N-nitrosodipropylamine is incorporated as an internal standard to check recoveries from the clean-up process. The nitrosamines are detected in the mass spectrometer by parent ion monitoring as previously described[9], necessitating a time difference of at least 2 min between the elution of each nitrosamine for resetting the mass spectrometer. The GC conditions used in the present work satisfy these criteria.

It has become apparent during the examination of a wide range of foodstuffs that by far the most frequently occurring nitrosamines are N-nitrosodimethylamine and N-nitrosopyrrolidine. The use of N-nitrosomethyl-*n*-propylamine as an alter-

native internal standard enables the same reference fragment to be used in the mass spectrometer for peak matching purposes for the detection of the standard itself, the N-nitrosopyrrolidine and if necessary N-nitrosodiethylamine. Thus the mass spectrometer requires less frequent resetting during such a run.

CONCLUSIONS

The dual column system described herein enables volatile nitrosamines to be resolved and rapidly eluted from a gas chromatograph, prior to on-line mass spectrometric detection. The GC peak switching facility enables large volumes of extracts of foodstuffs to be injected without adversely affecting the performance of the SCOT column and mass spectrometer.

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REFERENCES

1 N. T. Crosby, J. K. Foreman, J. F. Palframan and R. Sawyer, *Nature (London)*, 238 (1972) 342.
2 T. Fazio, R. H. White and J. W. Howard, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 1157.
3 N. P. Sen, *Food Cosmet. Toxicol.*, 10 (1972) 219.
4 N. P. Sen, B. Donaldson, J. R. Iyengar and T. Panalaks, *Nature (London)*, 241 (1973) 473.
5 A. E. Wasserman, W. Fiddler, R. C. Doerr, S. F. Osman and C. J. Dooley, *Food Cosmet. Toxicol.*, 10 (1972) 681.
6 A. E. Wasserman, in W. Davis (Editor), *N-Nitroso Compounds Analysis and Formation*, International Agency for Research on Cancer Scientific Publication, No. 3, Lyon, 1972, p. 10.
7 J. W. Rhodes and D. E. Johnson, *J. Chromatogr. Sci.*, 8 (1970) 616.
8 T. G. Alliston, G. B. Cox and R. S. Kirk, *Analyst (London)*, 97 (1972) 915.
9 T. A. Gough and K. S. Webb, *J. Chromatogr.*, 64 (1972) 201.
10 G. M. Telling, *J. Chromatogr.*, 73 (1972) 79.
11 J. M. Essigman and P. Issenberg, *J. Food Sci.*, 37 (1972) 684.
12 T. A. Bryce and G. M. Telling, *J. Agr. Food Chem.*, 20 (1972) 910.
13 T. A. Gough and K. S. Webb, *J. Chromatogr.*, 79 (1973) 57.
14 J. F. Palframan, J. MacNab and N. T. Crosby, *J. Chromatogr.*, 76 (1973) 307.
15 G. M. Telling, T. A. Bryce and J. Althorpe, *J. Agr. Food Chem.*, 19 (1971) 937.
16 K. Goodhead and T. A. Gough, *Food Cosmet. Toxicol.*, in press.