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SELECTIVE DETECTION OF N-NITROSAMINES BY GAS CHROMATOGRAPHY USING A MODIFIED MICROELECTROLYTIC CONDUCTIVITY DETECTOR IN THE PYROLYTIC MODE

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SUMMARY

The pyrolysis of nitrosamines in a modified Hall microelectrolytic conductivity system provides the basis for a highly selective and sensitive determination method. Under the conditions specified, the response of this detector system to nitrosamines is at least 10^7 times greater than that for *n*-hexane. The influence of several operational parameters on the response of the detector to nitrosamines was studied. For open-chain nitrosamines, the detection limit is 50 pg and the response is linear up to at least 200 ng.

INTRODUCTION

The carcinogenic properties of N-nitroso compounds are well established[1]. In recent years, a number of methods for their isolation and detection in foods have been developed[2–6]. Three types of nitrogen-specific detectors have been applied for screening by gas–liquid chromatography (GLC): the alkali flame-ionization detector (FID)[7], the Coulson electrolytic conductivity detector (CECD) in pyrolytic[4] and reductive modes[7] and the so-called thermal energy analyzer[8]. Positive findings obtained by these methods should be confirmed by gas–liquid chromatography–high-resolution mass spectrometry (GLC–MS).

This paper reports on the determination of volatile nitrosamines using a modified microelectrolytic conductivity detector (Hall detector, Model 310, Tracor Instruments, Austin, Texas, U.S.A.)[9]. As will be shown, this system is considerably more sensitive and selective towards nitrosamines than is the earlier described Coulson detector[10].

EXPERIMENTAL

Chemicals

The purity of nitrosamines used as analytical standards was checked by gas chromatography. Dichloromethane (analytical grade; Merck, Darmstadt, G.F.R.) was purified by column chromatography on silica gel–calcium oxide and by distilla-

tion; *n*-hexane was purified by column chromatography on silica gel and distillation. Standard solutions were prepared by dissolving nitrosamines (about 100 mg) in 2 ml of dichloromethane and making up to volume with *n*-hexane.

Gas chromatography

A Hewlett-Packard 7620A instrument was fitted with a 3 m × 1/8 in. O.D. stainless-steel column, packed with 10% Carbowax 20M–terephthalic acid on 80–100-mesh Gas-Chrom Q. A 4 cm × 1/8 in. O.D. stainless-steel pre-column, filled with silylated glass fibres, was used. The chromatographic column was conditioned prior to use for 35 h at 215° with a helium flow-rate of 36 ml/min.

The temperature of the injection block was maintained at 200° and the column temperature was programmed at 10°/min from 100° to 210°. The carrier gas was helium (flow-rate 60 ml/min), purified through a two-stage Hydro-Purge gas filter (Applied Science Labs., State College, Pa., U.S.A.).

Samples of 1–5 μl were injected. The solvent peak was vented through the four-port valve of the detector unit (Fig. 1).

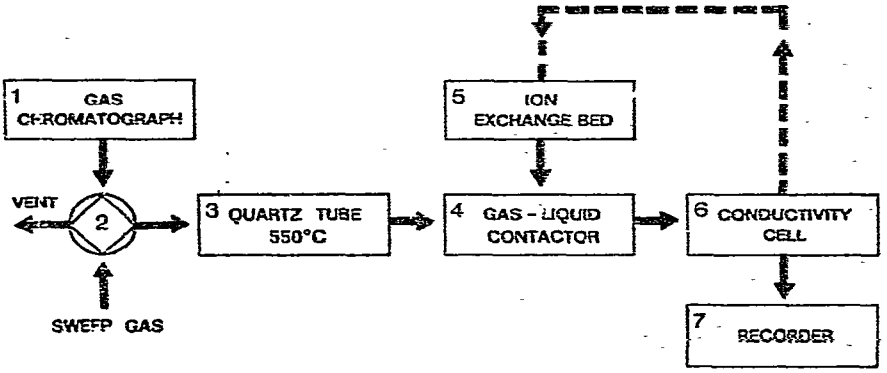

Fig. 1. Block diagram of the modified microelectrolytic conductivity detector.

Detector

The system consists of a low-dead-volume conductivity cell connected to a modified pyrolysis furnace from a Coulson detector (Tracor Instruments). Nitrosamines present in the effluent from the gas chromatograph (1) enter the quartz tube (3) via a four-port valve (2) and are subjected to gas-phase pyrolysis at 550°. The pyrolysis products are swept by the carrier gas stream into the gas–liquid contactor (4), where they are dissolved in deionized water which is continually recycled through an ion-exchange column (5). The signal from the electrolytic conductivity cell (6) is amplified and recorded on a strip-chart recorder (7). Pyrolysis products produce a detector signal when they are readily soluble in water and when they change its electrolytic conductivity. The GC column is connected to the detector by a glass-lined stainless-steel capillary (20 cm × 0.7 mm I.D., S.G.E., Melbourne, Australia). The temperature of the capillary is 200° and that of the four-port valve is 270°.

The quartz tube (6 mm O.D., 1 mm I.D., 30 cm long) is held at 550° in a furnace (21 cm long). A plug of 100 mg of granulated potassium carbonate, placed at the outlet end of the quartz tube between silanized glass-wool, is held at 450° by

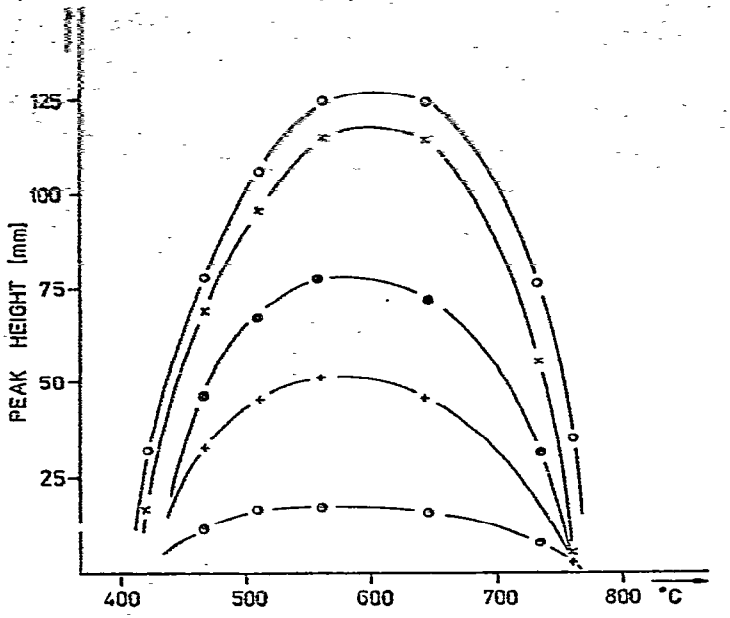

Fig. 2. Effect of furnace temperature on the detector response for open-chain and heterocyclic N-nitrososamines; 10 ng/μl of each nitrosamine injected. ○, N-nitrosodimethylamine (NDMA); ×, N-nitrosodiethylamine (NDEA); ●, N-nitrosodi-*n*-propylamine (NDPA); +, N-nitrosodi-*n*-butylamine (NDBA); ⊙, N-nitrosopiperidine (N-pip).

an additional heating device (4 cm long). The outlet of the quartz tube is connected to the conductivity cell by a 1-cm length of PTFE tubing (0.6 mm I.D., 1.7 mm O.D.). The helium sweep gas flow-rate is 60 ml/min.

Water flow through the conductivity cell is exactly adjusted to values of 0.3 to 1.8 ml/min by a micrometer-controlled (Mitutoyo, Tokyo, Japan) needle valve. It is circulated by a centrifugal pump (No. 480, Eheim, Deizisau, G.F.R.) through a mixed-bed ion-exchange resin column (18 g of AG50 1-X8, 20–50 mesh, Bio Rad Labs., Richmond, Calif., U.S.A.).

RESULTS AND DISCUSSION

The influence of the furnace temperature on the detector response for five nitrosamines is shown in Fig. 2. It can be seen that the optimal temperature is between 550° and 650°. Within this range, obviously the most efficient fragmentation of nitrosamines in the gas phase to electrolytically conducting products is achieved. Fig. 3 shows the influence of the flow-rate of water on the response of the conductivity cell. A decreased flow-rate of water results in an increased response. A flow-rate of 0.7 ml/min was found to be optimal. Values below 0.7 ml/min further increase the detector response (Fig. 3), but also result in baseline noise and peak tailing. The value of 0.7 ml/min also agrees with the flow-rate found to be optimal for the Hall detector in the reductive mode[11].

Linearity of the detector response was established for the range of 1–200 ng

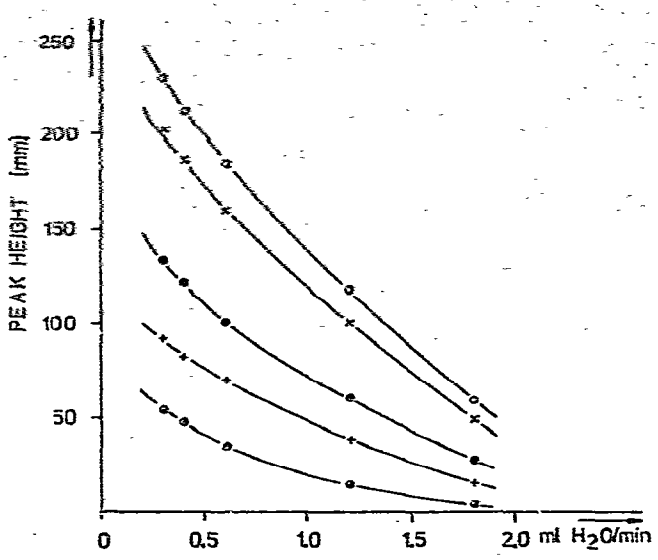

Fig. 3. Effect of flow-rate of water through the conductivity cell on the detector response; 10 ng/μl of each nitrosamine injected. ○, NDMA; ×, NDEA; ●, NDPA; +, NDBA; ⊙, N-pip.

with five nitrosamines (Fig. 4). In Table I, mean values for peak areas and retention times are listed, together with standard deviations and coefficients of variation from seven consecutive isothermal separations of six N-nitroso compounds.

A comparison of the detector response for each nitrosamine, relative to N-nitrosodimethylamine (NDMA) (P, Table I), with the relative nitrogen content of each molecule (M, Table I) reveals that there is no clear relationship between the two parameters. Evidently, pyrolytic fragmentation of these compounds to electrolytically conducting products is strongly influenced by their molecular structure. This finding is in accordance with the results obtained with a CECD in the pyrolytic mode[12]; in the reductive mode, however, the detector response reflects more clearly the relative nitrogen content of a particular nitrosamine[7].

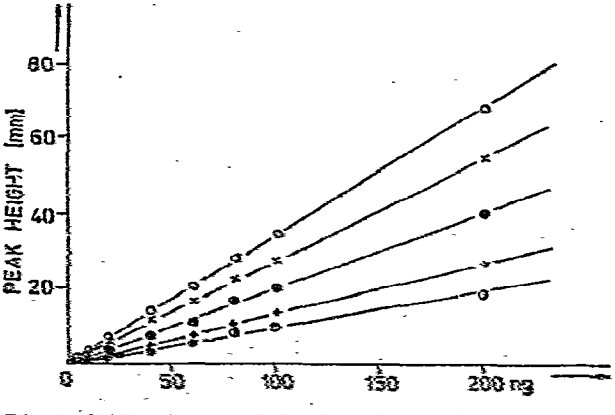

Fig. 4. Calibration graph for five nitrosamines; 1 μl of each standard solution injected. ○, NDMA; ×, NDEA; ●, NDPA; +, NDBA; ⊙, N-pip.

TABLE I

CHROMATOGRAPHIC RESULTS FOR N-NITROSAMINES

$\bar{X}_1$ = mean retention time (sec); $\bar{X}_2$ = mean peak area (mm²); S_1 and S_2 = standard deviations; V_1 and V_2 = coefficients of variation; P = 100 (peak area of given compound)/(peak area of NDMA); M = 100 (mol. wt. of given compound)/(mol. wt. of NDMA).

N-Nitroso derivatives	$\bar{X}_1$	S_1	V_1	$\bar{X}_2$	S_2*	V_2	P	M
Dimethylamine	113	0.44	0.39	120	7.3	6.1	100	100
Diethylamine	145	0.44	0.30	106	6.7	6.3	87	73
Di-*n*-propylamine	230	0.5	0.22	100	4.9	4.9	82	57
Di-*n*-butylamine	451	0.5	0.11	64	2.0	3.1	53	48
Piperidine	506	0.33	0.07	32	1.0	3.2	26	65
Pyrrolidine	561	0.53	0.09	17	1.3	7.7	14	74

* Standard deviation with the FID was 1 for NDMA.

The detection limit, estimated from the minimal amount of nitrosamine detectable at twice the noise level, was about 50 pg for straight-chain dialkylnitrosamines. For heterocyclic compounds, it was considerably higher (0.5 ng for N-nitrosopiperidine and 1 ng for N-nitrosopyrrolidine). A smaller response for heterocyclic nitrosamines was also found by Rhoades and Johnson[13] and Essigman and Issenberg[14], using a CECD in the pyrolytic mode.

The response for heterocyclic nitrosamines could be improved, however, when a stainless-steel capillary (1/16 in. I.D.), heated at 400°, was used to connect the GLC column with the detector, instead of the described glass-lined capillary (Fig. 5A and 5B). Hot metal surfaces obviously contribute to the catalytic pyrolysis of heterocyclic nitrosamines. Fig. 5 shows that the relative peak heights of heterocyclic nitrosamines

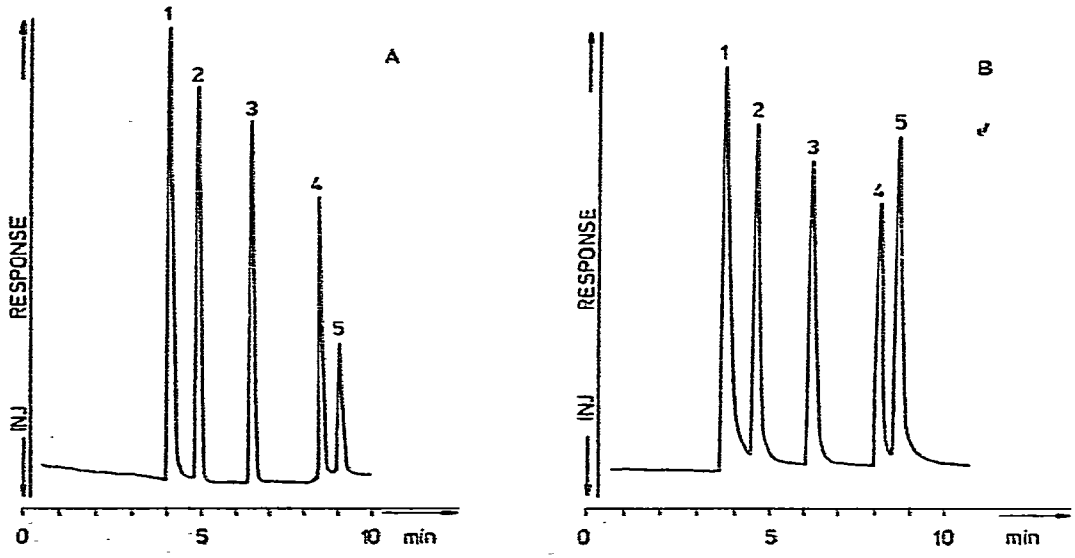

Fig. 5. Influence of temperature and type of capillary on the detector response. (A) Standard equipment, GLC column and detector connected by glass-lined capillary, held at 200°. (B) GLC column and detector connected by stainless-steel capillary held at 400°. Peaks: 1 = NDMA; 2 = NDEA; 3 = NDPA; 4 = NDBA; 5 = N-pip.

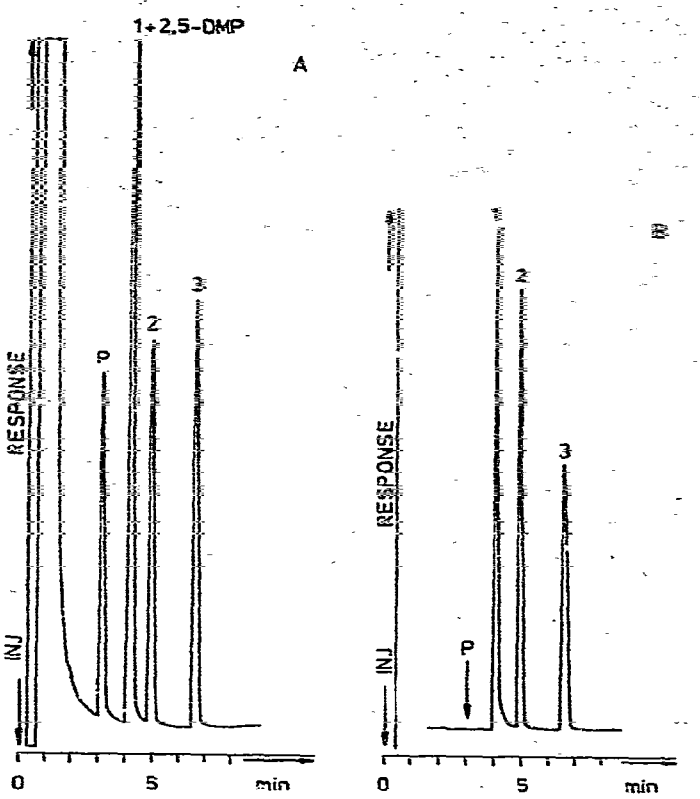

Fig. 6. Comparison of the detector response for nitrosamines and pyrazines. (A) FID detection. (B) Detection by microelectrolytic conductivity detector. Peaks: P = pyrazine; 2,5-DMP = 2,5-dimethylpyrazine; 1 = NDMA; 2 = NDEA; 3 = NDPA.

under these conditions are higher, but tailing of the peaks also increases considerably.

The selectivity of the system for the detection of nitrosamines can be expressed as the ratio of the amount of a given nitrosamine to that of a straight-chain hydrocarbon, when both give equal chromatographic signals. For NDMA and *n*-hexane, this ratio was found to be $1:10^7$. In the trace analysis of nitrosamines using less selective methods, GLC peaks caused by substituted pyrazines have sometimes been wrongly attributed to nitrosamines[15,16]. Pyrazines are most likely to be expected as interfering compounds, especially when heated foods are analyzed. For instance, 2,5-dimethylpyrazine and NDMA have identical retention times (Fig. 6). We therefore tested the response of the detector for two representative compounds, pyrazine and 2,5-dimethylpyrazine. Using the conditions under which 100 pg of NDMA produced a detector signal of 30 μV, 10 μg of each pyrazine still gave no detectable signal.

Fig. 7a and 7b shows as an example a comparison between reductive detection (pyrolysis in hydrogen at 820°) and pyrolytic detection. It can be seen that analysis of complex food extracts is facilitated if the detector is used in the pyrolytic mode. The chromatogram of an extract from a food sample, recorded in the reductive mode, suffers heavily from interfering peaks; the peak of NDMA, added at a concentration of 6 μg/kg (ppb) to the sample, is almost undetectable because of interfering substances.

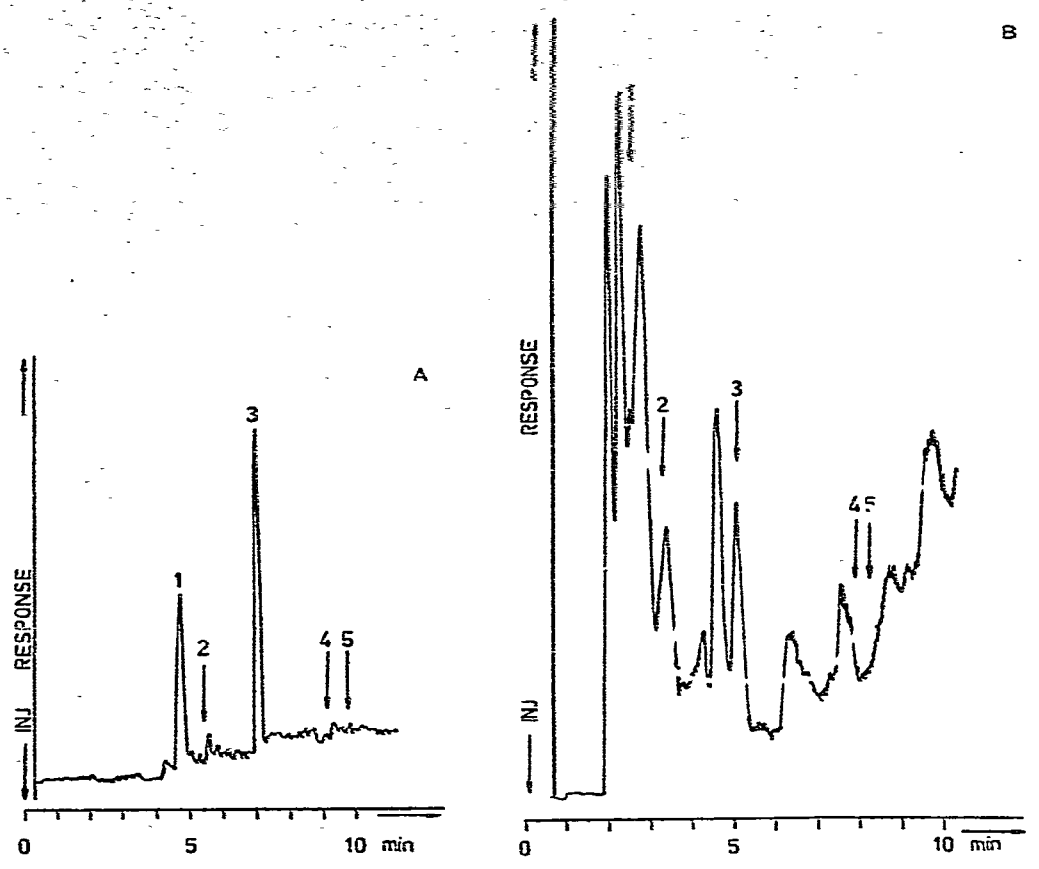

Fig. 7. Comparison of detector response in (A) pyrolytic mode (550°) and (B) reductive mode (820°). 5 μl of concentrate of mixed barley and wheat, fortified with 6 ppb of NDMA and 10 ppb of NDPA. Peaks: 1 = NDMA; 2 = NDEA; 3 = NDPA; 4 = NDBA; 5 = N-pip.

Pyrolytic detection, however, gives a clean chromatogram with prominent peaks of two nitrosamines (NDMA and NDPA), added at concentrations of 6 and 10 ppb, respectively, to the original sample. The extract was prepared simply by steam distillation under reduced pressure, extraction of the distillate with dichloromethane and concentration of the extract in a Kuderna–Danish evaporator; the final solution was made up in *n*-hexane[6]. It is obvious from Fig. 7 and also from a large series of similar chromatograms obtained from a wide variety of food extracts prepared in the same way[6] that, by the use of the detector in the pyrolytic mode, additional clean-up of the concentrate to be analyzed can be avoided.

The system has been in continuous use for over 18 months. For proper maintenance, it is advisable to replace the potassium carbonate plug in the quartz tube after about 2 months' use and to clean the quartz tube at the same interval with chromic acid, distilled water and acetone; after cleaning, the tube must be reconditioned at 750° in a helium stream for about 1 h. It is also advisable to exchange the water in the conductivity cell every week and to replace the mixed-bed ion-exchange

resin monthly. From time to time, the small piece of PTFE tubing that connects he quartz tube with the cell should be cleaned or replaced.

Dichloromethane is not a suitable solvent, because it causes bleeding of the stationary phase into the detector and pyrolyzes to hydrochloric acid at the working temperature. It is also necessary for the carrier gas and food extracts to be free from moisture, because even trace amounts of water spoil the potassium carbonate scavenger.

CONCLUSION

By modifying a commercially available microelectrolytic conductivity detector (Hall detector), we obtained a nitrogen-specific detection system with superior properties for nitrosamine analysis. A built-in four-port valve allows the solvent peak to be vented to the atmosphere before it can enter the detection system.

The dead volumes of the connections between the gas chromatograph and the heated quartz tube, and between the tube and the conductivity cell, were reduced as far as possible. This resulted in better sensitivity and a lower time constant of the system, giving narrower peaks. A built-in micrometer allowed reproducible adjustment of the flow-rate of water through the cell.

The modifications described render the system highly sensitive and selective for nitrosamine detection and permit uninterrupted long-term use.

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REFERENCES

1 H. Druckrey, R. Preussmann, S. Ivankovic and D. Schmähl, *Z. Krebsforsch.*, 69 (1967) 103.
2 G. M. Telling, *J. Chromatogr.*, 73 (1972) 79.
3 J. W. Howard, T. Fazio and J. O. Watts, *J. Ass. Offic. Anal. Chem.*, 19 (1970) 269.
4 N. P. Sen, J. R. Iyengar, B. A. Donaldson, T. Panalaks and W. F. Miles, in P. Bogovski and E. A. Walker (Editors), *N-Nitroso compounds in the Environment*, IARC, Lyon, Scientific Publications No. 9, 1974, p. 49.
5 K. Goodhead and T. A. Gough, *Food Cosmet. Toxicol.*, 13 (1975) 307.
6 G. Eisenbrand, E. v. Rappard, R. Zappe and R. Preussmann, *Proceedings of IVth International Conference on the Analysis and Formation of N-Nitroso Compounds, Tallinn*, IARC, Lyon, Scientific Publications, 1975, in press.
7 J. F. Palframan, J. Macnab and N. T. Crosby, *J. Chromatogr.*, 76 (1973) 307.
8 D. H. Fine and D. P. Roumbehler, *Proceedings of IVth International Conference on the Analysis and Formation of N-Nitroso Compounds, Tallinn*, IARC, Lyon, Scientific Publications, 1975, in press.
9 R. C. Hall, *J. Chromatogr. Sci.*, 12 (1974) 152.
10 D. M. Coulson, *J. Gas Chromatogr.*, 3 (1966) 285.
11 B. P. Wilson and W. P. Cochrane, *J. Chromatogr.*, 106 (1975) 174.
12 P. Issenberg and S. R. Tannenbaum, in P. Bogovski, R. Preussmann and E. A. Walker (Editors), *N-Nitroso Compounds—Analysis and Formation*, IARC, Lyon, Scientific Publications No. 3, 1972, p. 31.

13 J. W. Rhoades and D. E. Johnson, *J. Chromatogr. Sci.*, 8 (1970) 616.
14 L. M. Essigman and P. Issenberg, *J. Food Sci.*, 37 (1972) 684.
15 R. Kadar and O. G. Devik, *Acta Chem. Scand.*, 24 (1970) 2943.
16 K. Heyns, H. Koch and H. Röper, in P. Bogovski, R. Preussmann and E. A. Walker (Editors), *N-Nitroso Compounds—Analysis and Formation*, IARC, Lyon, Scientific Publications No. 3, 1972, p. 46.